An Insight into the Cellular and Molecular Mechanisms Underlying the Metastasis of Breast Cancer

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Abstract- **Metastasis is defined as the phenomenon of invasion and migration of cancer cells away from the original tumor to nearby tissues and other organs. Cancer metastasis is a complicated process and is the most harmful feature of cancer. Breast cancer is the most prevalent malignancy of the world and accounts for majority of cancer-associated deaths among women worldwide. In breast cancer metastasis, the cells separate from the original breast tumor and travel to remote organs such as the brain, liver, lungs, and bones via blood vessels or lymph nodes. Moreover, the preferred metastatic location of the disseminated breast cancer cells depends on their specific clinical subtypes. 10%-15% of the total number of patients affected by breast cancer develop the aggressive form of the disease causing the tumor cells to migrate to distant organs within three years of developing the primary tumor. When compared to the primary tumor, metastatic breast cancer is quite diverse in characteristics making it tedious to assess the risk factors responsible for promoting metastasis and discover an efficient and effective treatment approach. However, the formation of micro metastases at remote sites after ten years of initial diagnosis is quite natural. Therefore, the need to understand the basics of the metastasis process of breast cancer arises. In this review, a narrative description of the cellular and molecular mechanisms of breast cancer metastasis along with the emerging options of different therapeutic measures is provided.**

Keywords: **Breast cancer, Metastasis, Invasion, Angiogenesis, Circulating tumor cells.**

I. INTRODUCTION

Cancer metastasis is a complicated process and it describes the most harmful feature of cancer. It is the most vital hallmark of cancer that describes the phenomenon of invasion and propagation of cancer cells away from the original tumor to nearby tissues or other organs.As per the reports from World Cancer Research Fund International, breast cancer is the most prevalent malignancy in the world (12.5%) followed by lung (12.2 %) and colorectal (10.7%) malignancies respectively. In most of the instances of breast malignancies, it has been reported that the majority of the deaths linked to this disease are mainly attributed to the metastasis of the tumor instead of theoriginal tumor. 10%-15% of the total number of patients affected by breast cancer develop the aggressive form of the disease causing the tumor cells to migrate to distant organs within three years of developing the primary tumor [Fig.1] [1]. However, the formation of micrometastases at remote sites after ten years of initial diagnosis is quite natural [2]. This is the reason why breast cancer patients are at threat of developing metastasis throughout their entire lifetime. When compared to the primary tumor, metastatic breast cancer is quite diverse making it tedious to assess the risk factors responsible for promoting metastasis and discover an efficient and effective treatment for the condition [3].

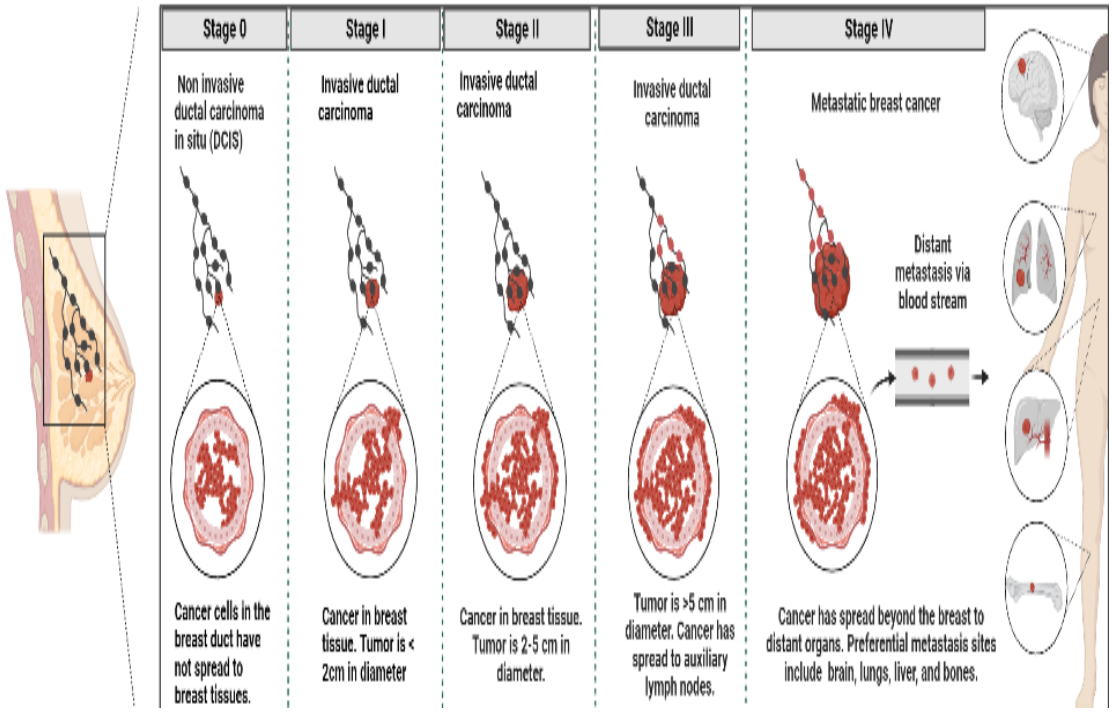

Figure 1: Stages of breast cancer metastasis. Stage 0 corresponds to the localized tumor which is non-invasive. Eventually, under favourable conditions, the tumor grows in size and invades the auxiliary lymph nodes by passing through stages 1 to 3. After the tumor has successfully spread beyond the breast tissues, it enters stage 4 and gains the ability to metastasize to nearby tissues and distant organs among which the preferred ones are the brain, lungs, liver, and bones.

In breast cancer metastasis, the cells separate from the original breast tumor and travel to remote organs such as the brain (4%-10%), liver (15%-33%), lungs (21%-32%), and bones (30%-60%) via blood vessels or lymph nodes. Moreover, the preferred metastatic location of the disseminated breast cancer cells depends on their specific clinical subtypes [4]. Metastasis can be explained by two spatial-temporal models known as the linear model and parallel model of metastasis respectively [5,6]. The linear model of metastasis states that the progressive accumulation of favorable genetic modifications in cancer cells within the original tumor enables them to expand and survive through clonal evolution. This leads to the distinction of a small subpopulation of tumor cells with acquired properties of the original tumor. These cells are more potent in invading other tissues and organs when isolated from the original tumor and the metastasis occurs when the primary tumor has attained a significant volume. In comparison to the linear model, breast cancer cells normally prefer to disseminate to farther organs by the parallel model of metastasis [5,7]. The parallel model of metastasis implies that breast cancer cells disseminate at the initial stage of neoplasm development. It also states that the dissemination of tumor cells in the bloodstream and the acquisition of genetic alterations in the disseminated cells in the bloodstream are independent of the development of the primary tumor [5,7].

Breast tumor progression is tightly associated with tumor angiogenesis or neo-angiogenesis where new blood vessels are originated from the previously existing ones in order to nourish and oxygenate the tumor cells while at the same time removing cellular wastes. Angiogenesis also provides the escape route for the tumor cells to form metastasis [8]. Metastasis initiates with the disarrangement of connections of the cell-to-cell extracellular matrix (ECM) via adhesion proteins like integrins resulting in the detachment of cancer cells from nearby cells and from the cells of the basement membrane [9,10]. Upon gaining this attribute, tumor cells start to dislocate and invade the surrounding tissues through the utilization of proteolytic enzymes. These enzymes degrade the ECM and provide a passage for the cells to migrate and invade farther tissues and organs [11]. The migrated tumor cells attach to the exterior side of blood vessels, employ a specific class of proteases known as matrix metalloproteinases (MMPs) to degenerate the basement membrane and penetrate into the bloodstream by passing throughthe endothelial cells. After the completion of successful intravasation into the lumen of blood or lymphatic vessel, cells need to spread to other organs. For that, they need to gain resistance against anoikis (cell death caused by detachment of cells) and hydrodynamic forces exertedby the blood and lymph to maintain their existence independent of anchorage [12]. Cancer cells require to tolerate and escape the immune surveillance which removes them and must elude from the apoptotic signals in order to exist [13]. These circulatory tumor cells attach to the endothelial side of the lymphatic orblood vessel and drift into the nearby stoma in between the cells of endothelium and basement membrane thus completing the extravasation step at the site of metastasis [14]. After extravasation is complete, cancer cells with carcinogenic potential arrive within the tissue parenchyma and form small clumps or minute colonies of disseminated cancer cells known as micrometastases [15]. After the tumor cells form micrometastases at the secondary site (bone, liver, lungs, brain, etc), they grow

and colonize into macroscopic tumors [Fig.2]. Colonization is termed as the most complex step ofmetastasis as the success rate of this step is entirely dependent upon the environment of the secondary site and if it is able to provide the newly arrived cancer cells with the essential growth and survival factors that the primary tumor site provided them initially.

Since metastasis is a complicated process, therefore, to overcome all the obstacles and most importantly, to survive in a detached state and invade distant tissues, and produce new tumors at the secondary site, neoplastic cells need to acquire different properties [16]. If any of the steps is hindered or affected, the entire metastasis process will come to a halt.

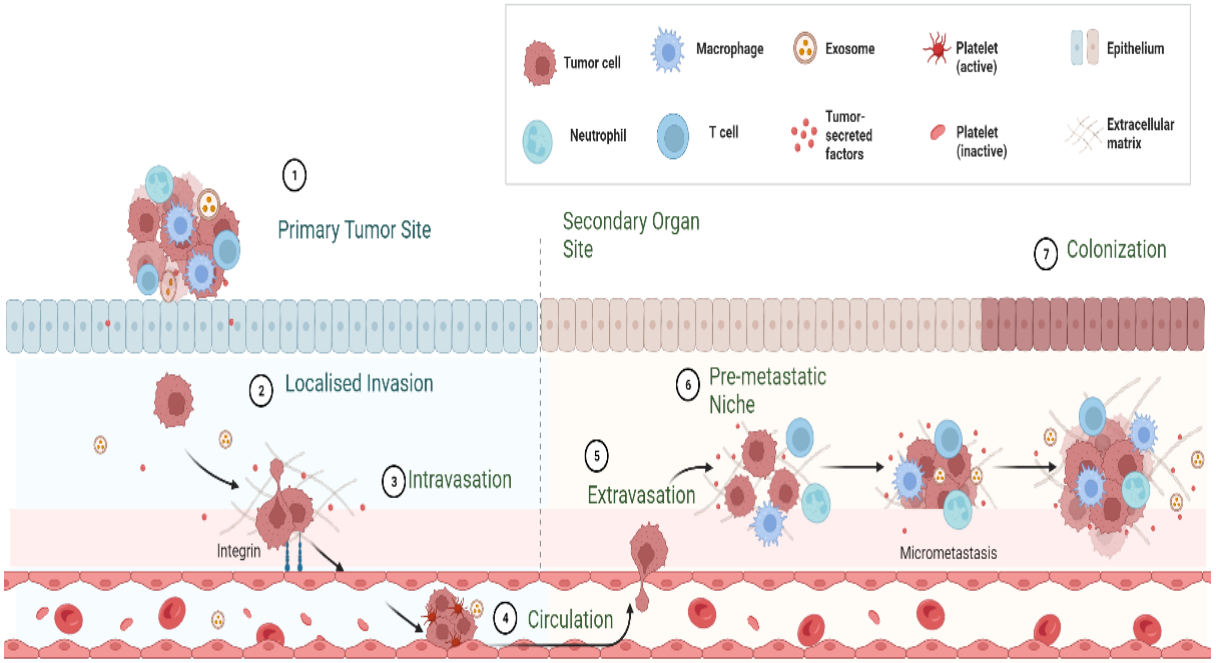

Figure 2 : Overview of the breast cancer metastasis process. After the primary tumor is formed, the tumor cells get detached from the original tumor by undergoing EMT, enter the circulation by crossing the endothelial cells (intravasation) and subsequently enter the blood or lymphathic circulation. Then they undergo extravasation and colonize a new organ where they proliferate and eventually form secondary metastases.

II. MECHANISM OF BREAST CANCER METASTASIS

The complete understanding of the mechanism of metastasis is still fragmentary. English surgeon, Stephen Paget proposed that the formation of metastasis at a specific secondary organ site is due to the reliance of cancer cells (the seed) upon the secondary organ (the soil) [17]. As per the hallmarks of cancer proposed by Hanahan and Weinberg in 2000, thepotential of a cancer cell to infiltrate and metastasize is a key factor in deciding the hostile features of the disease and can be a favorable molecular target for suitable drug discovery [18].

A. Tumor angiogenesis

Angiogenesis or neoangiogenesis, the rapid development of new blood vessels from previously existing ones, is the first event in the metastatic cascade after the primary tumor is formed. This process is necessary for the supply of adequate oxygen and nourishment for the growth of tumors. When the physiological process of angiogenesis takes place in normal situations like wound healing or pregnancy, a proper balance is always maintained between pro-angiogenic and anti-angiogenic factors whereas cancer cells frequently lose this equilibrium favoring uncontrolled angiogenesis [19]. There are several steps involved in promoting angiogenesis in a tumor and producing subsequent endothelial tubes, which are controlled primarily by hypoxia. In hypoxic environments, the hypoxia-inducible factor-1α (HIF-1α) is stabilized and forms a heterodimer with HIF-1β [20]. The transcription of several genes that help human cancer cells to adapt to a low oxygen environment as well as that of those genes that are involved in anaerobic metabolism, angiogenesis, and resistance to apoptosis is triggered by HIF-1 [21] which is composed of HIF-1α and HIF-1β. Additionally, it also stimulates the production of the lysyl oxidase enzyme which aids in the maturation of collagen and CXCR4, the chemokine receptor that mediates organ-specific migration of cancer cells [22].

Recent data suggest that neutrophils actively contribute to carcinogenesis by releasing MMPs such as MMP-9 and growth factors such as oncostatin M, TGF-β, and platelet-derived growth factor (PDGF) that cause angiogenesis [23,24]. Cancer cells around the stromal cells promote angiogenesis by synthesizing pro-inflammatory and pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF), interleukin -8 (IL-8), and tumor necrosis factor-α (TNF-α). Out of several pro-angiogenic factors, the most potent one is VEGF [25]. VEGF regulates vascular permeability which helps in the formation of new blood vessels and causes an increase in the number of endothelial cells. During the process of angiogenesis, VEGF activates its two receptors in endothelial cells known as VEGFR-1 and VEGFR-2 which lead to increased vascular permeability, endothelial cell motility, cell proliferation,

and survival [Fig 3] [26,27]. It has been observed in breast cancer cells that the VEGF pathway helps to increase cancer cell viability through ERK and AKT signals [28]. ERK and AKT enter the surrounding tissues and attach to the cells in the endothelium of the existing blood vessels situated close to the primary breast tumor. With the help of proteases, the activated endothelial cells start their expansion and degenerate the surrounding stroma. Integrins promote the adhesive-nonadhesive interactions of tumor cells with stromal cells, mediating the cancer cells to migrate through the degraded stroma [29]. The moving endothelial cells produce new, immature vessels that go through the vascularization process and produce a whole vascular network of blood vessels providing the tumor cells with a rich supply of blood. The immature vessels formed are characterized by aberrant structural dynamics, more permeability when compared to normal vessels, and a deficit supply of smooth muscle cells [30]. Similarly, the newly formed lymphatic vessels are expanded, leaky,discontinuous,fluid-engored,and dilated.

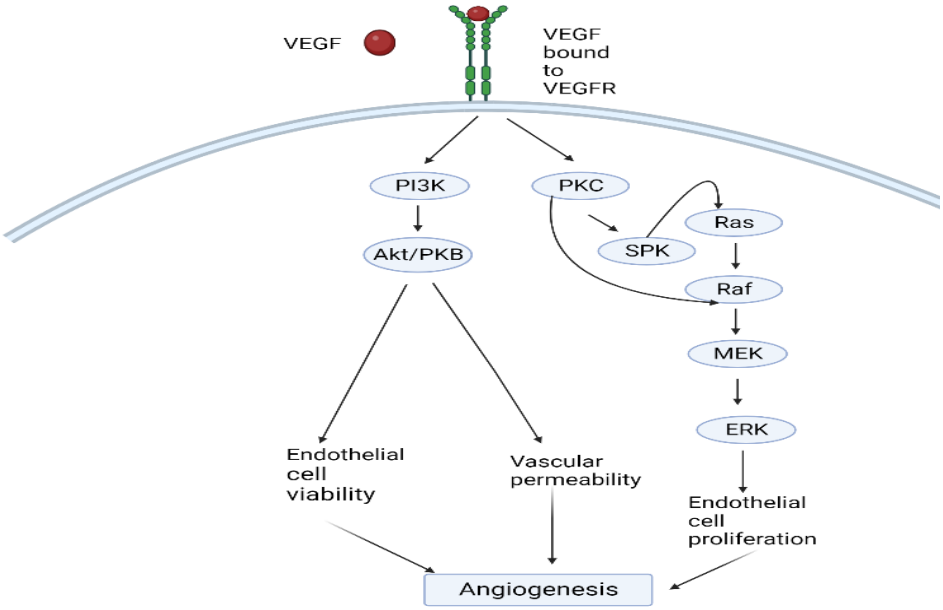

Figure 3: Role of VEGF in promoting angiogenesis in breast cancer. VEGF activates its receptors VEGFR 1 and VEGFR2 which produce the ERK and AKT signals. These signals attach to the cells in the endothelium of the existing blood vessels situated close to the primary breast tumor which, in turn, lead to increased vascular permeability, endothelial cell motility, cell proliferation, and survival.

B. Localized invasion and migration from the primary tumor

Metastasis of breast cancer cells begins during the process of localized invasion and migration where the tumor cells invade their local environment by breaking free from molecular constraints that connect nearby cells. They can travel alone or in large clusters through the stromal microenvironment [31]. Pre-cancerous neoplasia and malignant tumors are distinguished by invasion through the basement membrane which contributes to a stiffer environment through increased collagen deposition, fiber thickness, and linearized fiber architecture [32,33]. During the advancement of metastasis, a positive feedback loop is created by the contractility of cancer cells and the stiffness of the matrix [34]. The epithelial tissues, where most of the malignancies start, need to undergo epithelial-to-mesenchymal transition (EMT) in order to break their tight cell adhesion and invade the surrounding tissues. Some of the prominent characteristics of EMT include loss of epithelial attributes (cell-cell junctions and cell polarity) due to loss of E-cadherin and cytokeratin expression, and development of a spindle-shape fibroblastic morphology because of increased expression of markers like vimentin and N-cadherin. In most instances, it has been observed thatindividual breast tumor cells leave the primary tumor and undergo EMT that is mediated by molecules like TGF-β, MAP kinases, VEGF, and transcription regulators such as WNT, Notch, Hedgehog, Twist, and Snail (SNA, SNAI1) [35, 36]. It has been reported in EMT that the expression of a mesenchymal marker, vimentin, in cells of the epithelial layer of thebreast tumor indicates a shorter life expectancy of patients after the operative phase [37].

Cadherins represent a broad category of adhesion molecules that are primarily responsible for maintaining tight intercellular adhesion interactions by interacting with the cytoskeleton's actin component via cytosolic proteins known as catenins. Any change in the expression levels of cadherins or catenins leads to a decrease in adhesion levels among the cells, which has been reported in several metastatic phenotypes [38]. E-cadherin acts as the caretaker of the epithelial phenotype of the cells by convening sheets of epithelial cells and maintaining the inactivity of the cells within these sheets [39]. Transcription factors such as those belonging to Snail/Slug family, SIP1/ZEB2, EI2/E47, Twist, and δEF1/ZEB1 function by recognizing specific DNA sequences known as E-box sequences. E-box sequences are located at the promotor region of E-cadherin where these transcription factors recruit co-factors and histone deacetylases, thus acting as master molecular switches [40]. These transcription factors are also involved in controlling several signaling pathways including Wnt/β-catenin, Notch, PI3K-AKT, cadherin, and TGF-β [41]. Sufficient evidence has been

reported that they are linked to poor prognosis of breast cancer. Snail is one of the crucial transcription factors involved in breast cancer EMT and is the first transcription factorresponsible for suppressing the expression of E-cadherin. In addition to inhibiting the expression of E-cadherin, Snail also aids in suppressing the expression of other epithelial molecules which includes mucin-1 and occludins, and promotes the expression of those genes associated with invasive and mesenchymal phenotypes [42].

Out of all the EMT-inducing molecules, TGF-β plays the most significant role by acting as a tumor suppressor during the preliminary stages of tumor growth by triggering the arrest of cell growth and ultimately leading to cell apoptosis. But during the later stages of tumor growth, the inhibitory properties of TGF-β are repressed and exposure of breast cancer cells to TGF-β has been associated with increased conversion of epithelial phenotypes to mesenchymal phenotypes [Fig.4] [43]. The adhesion and debonding interactions of cancer cells with matrix components are mediated mainly by integrins like α5β1 and avβ6 is crucial for local or regional migration. In MCF-7 human breast tumor cells it was experimentally verified that overexpression of the integrin α5β1 increased the rate of invasion by tumor cells and induced resistance to doxorubicin by upregulating the functions of mTOR, AKT, and ERK1/2 protein kinases. As the cancerous cells migrate, they mechanically redesign the ECM through a series of cell expansions and contractions and degrade the matrix by MMPs. The intratumoral bloodvessels that are marked by the enhanced permeability and leaky expression help the tumor cells to get into the systemic circulation [44].

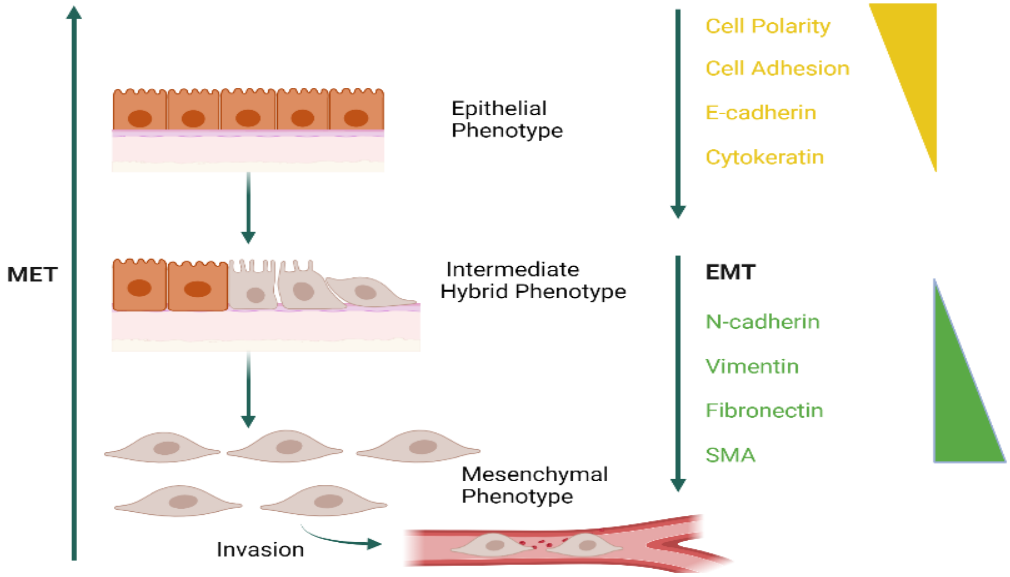

Figure 4: Mechanism of action of TGF-β in mediating EMT. TGF-β plays the most significant role by acting as a tumor suppressor during the preliminary stages of tumor growth by triggering the arrest of cell growth and ultimately leading to cell apoptosis. But during the later stages of tumor growth, the inhibitory properties of TGF-β are repressed and exposure of breast cancer cells to TGF-β has been associated with increased conversion of epithelial phenotypes to mesenchymal phenotypes. Some of the prominent characteristics of EMT include loss of epithelial attributes (cell-cell junctions and cell polarity) due to loss of E-cadherin and cytokeratin and development of a spindle-shape fibroblastic morphology because of increased expressionof markers like vimentin and N-cadherin.

C. *Intravasation*

Intravasation describes the process through which lymphatic or blood vessels are invaded by locally invasive tumor cells. In the initial stage of intravasation, cancer cells attach to the exterior side of the blood or lymphatic vessel, degrade the basement membrane using MMPs, undergo transendothelial migration, move amidst the endothelial cells bordering the capillaries, and then spread to other tissues. Daily release of several breast tumor cells intothe blood or lymphatic system is possible, but successful metastasis can only occur when these cells get arrested in the capillary beds of distant organs [45,46]. Tumor cells can migrate toward the nutrition sources, go along with the chemokine components, or enter into the tumor vasculature through vascular intravasation. Vascular intravasation can either be an active or a passive process [47]. Once cancer cells cross the epithelial as well as the vascular base membrane, they will finally come in contact with the microvasculature surrounding the tumor. This is mostly accomplished through aggressive contacts between tumor cells and the basement membrane of endothelial cells and/or through interactions mediated by integrins along with the proteolytic enzymes that degenerate the basement membrane [48]. After the malignant cells have crossed the basement membrane barrier, they can attach to the vascular endothelial cells. After this, the malignant cells then move into the systemic circulation as the endothelial cells retract.

The circulating tumor cells (CTCs) traveling alone in the bloodstream are prone to destruction compared to the cells traveling in clumps [49]. Leukocytes, the vascular endothelium, and platelets can all be affected by tumor cells. As a result of this interaction, themetastatic process may advance and cancer cells may be protected from immune destruction. A number of variables, including cytokines, chemokines, macrophages, and hypoxia play a significant role in the advancement of tumors[48]. Epidermal growth factor (EGF) secreted by macrophages encourages migration and invasion by interacting with EGF receptors expressed on tumor cells [50]. Colony-stimulating factor-1 (CSF-1) secreted by tumor cells acts as an effective chemoattractant for macrophages. CSF-

1 interacts with the CSF-1 receptors expressed on macrophages, which results in chemotaxis-based co-migration of tumor cells and macrophages away from the original tumor and regulates intravasation of cancer cells into the tissues from peripheral blood by causing positive feedback paracrine loop [50]. In addition to producing growth factors and enzymes that break down the matrix, macrophages also promote angiogenesis, invasion, and intravasation [51]. Increased levels of circulating neutrophils are a standalone indicator of a poor prognosis in cancer patients [52]. They have the capacity to secrete soluble substances that stimulate parenchymal and endothelial cancer cells and boost CTC addition to distant areas.

TNF-α, a proinflammatory cytokine exhibits antagonistic effects on the survival of cancer cells and upon the development of malignancy. TNF-α can induce apoptosis, increase anti-tumor immunity, and produce hemorrhagic necrosis at higher concentrations, but it can also initiate and advance tumors under the right circumstances [53, 54]. Normally, it is assumed that there is a relationship between cancer and inflammation at the molecular level. This helps to stimulate the synthesis of MMPs, genetic factors, and other cytokines in a variety of normal cells. Through CXCR4 signaling, the chemokine protein stromal cell-derived factor-1(SDF-1) that is secreted can promote metastasis, invasion, and intravasation [55,48]. This causes more paracrine interactions between the breast tumor cells and their microenvironment. For breast tumor cells induced by EGF, the CXCR4/SDF-1 signaling cascade in the tumor microenvironment is important. Breast cancer cells that express CXCR4 have been reported to spread more quickly by homing to lymph nodes with higher levels of SDF-1 expression [56]. In additionto this, it has also been proved that the interaction of CXCL12 and CXCR4 expressed on breast tumor cells improves the intravasative, chemoattractant, and invasive abilities of cancercells [57]. Breast cancer CTCs can widely diffuse via circulatory or lymphatic circulation following effective carcinoma cell intravasation, but they must overcome a number of obstacles to complete metastasis.

D. Survival of breast cancer cells in circulation

Tumor cells circulate very briefly in the bloodstream after entering the vascular system. Mostof the tumor cells probably get entrapped in the microvasculature after entering the bloodstream in a short span of time. This can be attributed to the fact that the diameter of tumor cells is notably larger when compared to that of small blood capillaries[58]. Tumor cells undergo exposure to several forms of mechanical forces before as well as after arrest that includes the hydrodynamic shear stress exerted by blood flow [59] and mechanical deformation in the target organ's microvasculature [60]. The loss of a notable population of cancer cells getting into the small vessels is primarily attributed to this mechanical stress [60]. Several mechanisms guard cancer cells against cell death caused by mechanical stress including the formation of microaggregates or emboli that lead to the shielding of CTCs from mechanical stress [61]. These microaggregates are made up of cancer cells and blood platelets and are synthesized shortly after the cancer cells enter the bloodstream. Neoplasm cells induce the activation of coagulation factors and platelets by implementing various mechanisms that include exposing cancer cells to tissue factor [62,63], thus contributing to the formation of microaggregates. Breast cancer cells have also been reported to upregulate the expression of a curtailed version of pannexin-1 which improves resistance against cell death that is caused due to mechanical stress. Usually, the curtailed version of pannexin-1 is physiologically inert, but when it is co-expressed with the native form of pannexin-1, it activates the signaling pathways that lead to inhibition of apoptosis and prevents the tumor cells from lethal mechanical injuries [64].

Metastatic breast cancer cells are also prone to anoikis, a form of cell death caused due to lossof integrins-mediated anchorage to the extracellular matrix. Many mechanisms are employed by breast tumor cells to prevent cell death caused due to anoikis after the loss of anchorage mediated by Integrins. Focal adhesion kinase (FAK) recruited by endosomal integrins of separated tumor cells activates the downstream signaling cascade that helps to induce anoikis resistance [65,66]. Tumor cells are also able to inhibit anoikis by inactivation of tumor suppressor genes like phosphate and tensin homolog (PTEN) [67] or by acquiring activating mutations in pro-survival, anti-apoptotic pathways [67].

Signaling pathways like PI3K-AKT and Ras-Raf-Mek-Erk also play a major role in promoting tumor cell survival. These signaling cascades are activated by active receptor tyrosine kinases which include TrkB [68], ERBB2 [69,70], and IGF-1R [71]. These pathways help in counteracting the loss of signaling mediated by integrins and other processes that promote cell death and anoikis suppression. In addition to these, breast tumor cells employ a number of strategies to repress the anti-cancer activity of immune cells such as the natural killer (NK) cells. It has been demonstrated that cancer cells coated with activated plateletsare protected from NK cells through the downregulation of NKG2D ligand which is expressed on natural killer cells. This process assists in suppressing the activity of NK cells by releasing platelet-derived TGF-β [72,73] or by exposing inhibitory ligands on NK cells such as glucocorticoid-induced TNF-related ligand (GITRL) thus enhancing the rate of metastasis [74].

Platelets also interact with tumor cells with the help of adhesion mediated by P-selectin. Apart from platelets and NK cells, T-cells also influence cancer metastasis by expressing programmed cell death protein-1 (PD-1). When PD-1 interacts with programmed cell death ligand 1 (PD-L1) that is expressed in cancer cells, the activation, survival, proliferation, and cytotoxic secretion of T-cells are all inhibited within the tumor cells [75]. PD-L1 expression in cancer cells has been linked to increased tumor size, high grade, rapid proliferation, positive status of HER2, and negative status of estrogen receptor [76] and is inversely associated with patient survival [77]. Around 20% of TNBCs express PD-L1 [78]. Several immune checkpoint inhibitors have also proven to be effective in this aspect [79,80]. Treg cells within the breast tumor express greater amounts of CCR8 which aids in identifying a group of very activating and suppressive cells present within the breast tumor which suggests that activated Treg cells expressing CCR8 are implicated in breast cancer pathogenesis [81]. Treg cells alsosecrete RANKL which inhibits *in vitro* apoptosis of cancer cells and may contribute towards metastasis by boosting the survivance of CTCs [82]. The role of cytotoxic T-cells is relatively not much clear as it has been reported that CD8+ T cells that are specific for a particular antigen exhibit tumor-suppressive and tumor-

promoting effects as well.

D. Extravasation into the secondary site

Breast Cancer cells employ several mechanisms to exist in the antagonistic intravascular environment of the host. The metastatic potential of cancer cells eventually relies on their potential to extravasate into the nearby tissue rapidly. Cancer cell extravasation comprises of attachment of cancer cells to the endothelial side of the blood or lymphatic vessel, passage through endothelial cells and the basement membrane, regulation of the endothelial barrier, and penetration into the surrounding stroma to reach the tissues lying below it. This mode of migration through the paracellular route appears to be the most predominant mode ofextravasation by cancer cells. Extravasation of cancer cells is dependent on rearrangements ofthe cells and disruptions of cell-cell junctions present in the inter-endothelial region [83,84]. Tumor cells rarely extravasate by passing via the cells of endothelium as single cell bodies, a process called transcellular migration [85].

A number of studies conducted in-vitro have revealed that a broad range of receptors and ligands such as integrins, cadherins, selectins, and immunoglobulin superfamily receptors playa significant role in mediating adhesion between breast cancer cells and endothelial cells in a heterotypic fashion [86, 87]. Chemokines secreted by the target tissues promote the disseminationof tumor cells by their interaction with the receptors expressed on the tumor cells. Breast tumor cells employ the receptors CCR7 and CXCR4 and their corresponding ligands CCL21 and CXCL12 secreted by the target tissues activate these receptors and promote the dissemination of tumor cells [88]. In addition, tumor cells also activate specific genes involved in modifying the vasculature in order to improve permeability and promote extravasation. For instance, angiopoietin-like-4 (ANGPTL-4) or C-terminal fibrinogen-like domain of ANGPTL-4 (cANGPTL-4) are produced by malignant breast cells to interact with the tight and adherent junctions of the vascular endothelium in an antagonistic manner, hence promoting extravasation and migration of tumor cells [89, 90]. In lung metastases of breast cancer, it has been observed that the TGF-β/SMAD signaling pathway in tumor cells activates the expression of ANGPTL4 which in turn enhances the capacity of malignant breast cells to retent in the lungs for a longer period of time and destroy the walls of the capillaries thus forming lung metastases [90]. Several reports suggest that the expression of EGFR ligands such as heparin-binding EGF-like growth factor (HB-EGF) and epiregulin, cyclooxygenase-2 (COX-2), MMP1, and MMP2 promote extravasation of breast cancer cells [91, 92]. However, the actual mechanism is still largely unknown

The efficiency of extravasation is also dependent on the employment of myeloid cells and platelets lying closer to the tumor cells after tumor cells get seized within the vascular bed. This supports tumor cell extravasation as well as regulates their survival. Platelets induce an invasive mesenchymal-like attribute in malignant cells [93]. It has also been demonstrated by experiments that leukocytes and platelets promote extravasation by forming complex with cancer cells with the help of L or P-selectin. Overexpression of selectin ligand is correlated to poor prognosis and increased rate of metastatic progression [94]. In the lung metastasis model of breast cancer, it has been reported that E-selectin plays a pivotal role in homing the breast tumor cells to the vascular endothelium thus increasing the rate of extravasationleading to metastasis [95, 96]. Activation of endothelial cell markers like vascular cell adhesion molecule-1 (VCAM-1) which is expressed in many metastatic breast cancer cells enhances the rate of extravasation toleukocyte-rich regions like the lungs by binding to the integrins α4β7 and α4β1 expressed on tumor-associated circulating monocytes, granulocytes, and macrophages, which in turn get attached to the endothelial surface of the lungs [97]. VCAM-1 also promotes the survival of metastatic breast tumor cells by triggering the PI3K-AKT survival pathway through Ezrin [97]. This safeguards the cancer cells from apoptosis-promoting cytokines like TRAIL.

III . PRE-METASTATIC NICHE

Before metastasis occurs due to the interaction between the components of the secondary organ's stroma and the original tumor, a conducive physiological environment termed as pre-metastatic niche can be set up in the target tissues and organs. During the initial invasion phase, cancer cells tend to establish a mutual relationship with the adjacent stroma. This results in an abrupt change in many of the physiological events of the colonized stroma such as the promotion of inflammation and angiogenesis, suppression of the immune response, and the release of motility factors that enhance cell growth and survival. In response to these processes, the epithelial cells can alter their gene expression patterns to upregulate those genes that help in generating a suitable domain for the dispersed metastatic cells [98]. Many experiments have revealed that modifications at the tissue level before the seeding of cancer cells lead to the concept of the formation of a pre-metastatic niche [99].Primary tumor cells release certain factors like placental growth factor (PGF) and VEGF that act on mesenchymal stem cells in the bone marrow. This, in turn, induces the bone marrow-derived cells (BMDCs) to reach the secondary site before the arrival of infiltrating cancer cells [100]. It has been reported that the CXCL12 (ligand)/CXCR4 (chemokine receptor) axis furnishes a suitable microenvironment before the formation of bone metastases [101]. HIF-1 has also been reported in playing a significant role in breast cancer lung metastasis by inducing the formation of pre-metastatic niche andupregulating the activation of members of the lysyl oxidase family that catalyze the process of collagen cross-linking before BMDCs are recruited to the lungs [102]. Other molecules that aid in the formation of a pre-metastatic niche are periostin, VEGFR-1, and tenascin-C [100].

IV. ORGANOTROPISM

Tumors arising from different preliminary sites show unique ways of metastatic spread in a non-random way. This correlates with Paget's 'seed and soil hypothesis' which states that cancer cells (seeds) disseminate throughout the body and form secondary metastases atspecific organ sites (soil). The settlement of these cells depends on the establishment of a pre-metastatic niche. Some

metastasis models support the alternative rule of 'anatomical hypothesis' or 'mechanical entrapment' which advocates the fact that CTCs become entrapped in the vascular bed of the first organ they encounter. This entrapment of CTCs occurs because of the vessels that they encounter during their passage as the diameter of the vessels is quite small compared to that of the tumor cells [103]. But for most cancers, the formation of metastasis at distant sites cannot be elucidated by normal patterns of blood circulation. Some of the instances include the tendency of breast tumor cells to disseminate tothe brain, liver, lungs, and long bones [104].

The most typical location for breast cancer metastasis is the bone. Due to excessive amounts of osteoclast-mediated bone reabsorption, breast cancer cells penetrate into bones, and this phenomenon is closely associated with the formation of osteolytic-sort of lesions [105]. Experimental research has revealed that in breast cancer, the SMAD pathway can be transformed into an effective metastasis-promoting factor, and signaling through this cascade helps in the formation of bone metastases [106]. Additionally, VEGF and CXCR4 expression can be independently stimulated by hypoxia (through HIF-1α) and TGF-β signaling to influence the metastasis of malignant breast cells to the bone [107].

Neoplastic breast cells that prefer to metastasize to the liver exhibit distinct transcriptional profiles [108]. According to reports, the stimulation of WNT signaling independent of β-catenin is associated with the development of liver metastases of breast cancer [109]. Another model of liver metastasis includes integrin complexes, HIFs, and lysyl oxidase as components of the hepatic microenvironment and breast cancer cells [110].

Breast cancer lung metastases generally exhibit traits of aggressive growth and invasiveness when compared to other metastatic lesions [111]. Breast cancer cells expressing the enzymes MMP-1, MMP-2, COX-2, and EGFR boost angiogenesis, release into the bloodstream, and rupture the lung capillaries thus enhancing the rate of metastasis. Numerous investigations have revealed that pyruvate carboxylase-dependent anaplerosis is more severe in breast cancer lung metastasis due to the response of the pulmonary microenvironment when compared to primary breast cancer [112].

Most of the breast cancer brain metastases are typically seen in the parenchymal brain otherwise known as the leptomeningal region [113]. In order to survive and establish brain metastases, the circulating breast cancer cells should cross the barrier separating the blood and the brain and should interact with the regional pre-metastatic niche. Molecules like CXCR4, CD44, and VEGF compromise the integrity of the endothelial layer to increase the extent of transendothelial migration of metastatic cells [114]. The tumor cells are assisted by COX-2 and HB-EGF in crossing the blood-brain barrier. Astrocytes are also reported to secrete certain substances that can result in brain metastases [115].

V . DORMANCY

Upon the clinical surveillance of breast cancer patients, it has been verified that even after many years of surgical removal of the initial tumor, there have been numerous occurrences oflate relapses [116]. This fact proves the prevalence of the phenomenon of 'metastatic dormancy' which is linked to the presence of widely dispersed tumor cells with low metabolic and proliferative activity. At the time of seeding, these cells are unable to form a secondary tumor, but they survive and develop invasive abilities as an outcome of genetic alterations and interaction with the tumor microenvironment [117]. Dormancy may provide significant obstacles to the efficiency and effectiveness of adjuvant chemotherapy as it targets active and proliferating cells but leaves dormant metastases unaffected.

VI. EMERGING TREATMENT OPTIONS AND FUTURE PERSPECTIVES

Until now, efforts made in cancer research have made the fact very simple to understand why the prevention and treatment of cancer metastasis are so complex. Targeting certain receptors that promote adhesion and dissemination of breast cancer cells are under clinical trials. One of the studies conducted reveals that cilentigitide, an inhibitor of the integrin α5β1, has shown promise toward breast cancer treatment with very few side effects and good tolerance ability, but the median survival rate is not as per expectations [118]. In a couple of trials conducted, it was experimentally verified that anticoagulants like heparin or heparin-like molecules can improve the survival rate of cancer patients [119]. Inhibitors of angiogenesis like endostatin and bevacizumab, an anti-VEGF monoclonal antibody are employed in breast cancer treatment aimed at targeting the angiogenic cascade [120]. However, due to the less effectiveness of bevacizumab, it is rarely recommended in the treatment of metastatic breast cancers [121]. EMT and anoikis resistance signaling cascades can be good targets for metastasis inhibitors. Anoikis is mainly caused due to mutations in the pro-apoptotic proteins of the Bcl-2 family. Targeting the pro-apoptotic proteins of the Bcl-2 family can be a promising step in reducing resistance to anoikis thus improving the chances of survival in metastatic breast cancer patients. Many studies suggest that antibodies like atezolizumab [Fig.5] or pembrolizumab are effective in blocking the PD-L1 or PD-1 expressed in breast tumor cells and T cells respectively [122,79]. It has also been experimentally proved and verified that anti-platelet drugs such as COX-2 inhibitors and aspirin could inhibit the interaction among the infiltrating breast cancer cells and platelets utilizing the fact that cancer cells remain in the bloodstream for a very short span of time [123].

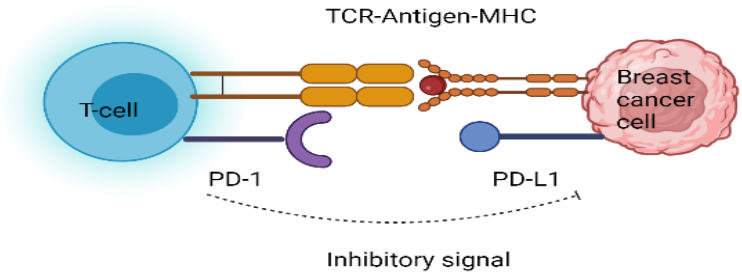

Figure 5: Atezolizumab hinders the interaction between PD-1 ligand expressed on T-cell and PD-L1 expressed on tumor cells respectively by blocking PD-L1. This improves T-cell functions against malignant cells.

VII. CONCLUSIONS

There are still a number of unanswered questions in relation to metastasis such as how, when, and where cancer cells arise from the primary tumor and if we can be able to detect disseminating cancer cells in the blood. By solving these and other closely related problems we might be able to identify the demerits of existing therapies and introduce more efficient drugs and therapeutic methods. Despite substantial advancements in the understanding of metastatic pathways, this phenomenon still represents itself as the biggest obstacle in the field of cancer therapy.

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