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Review Article

Breast Carcinoma: From Initial Tumor Cell Detachment to Settlement at Secondary Sites

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Metastasis represents a multistep cascade of cancer cell alterations accompanied by structural and functional changes within the tumor microenvironment which may involve the induction of a retrodifferentiation program. Major steps in metastatic developments include (A) cell detachment from the primary tumor site involving epithelial-mesenchymal transition (EMT), (B) migration and invasion into surrounding tissue, (C) transendothelial intravasation into the vasculature of blood and/or lymphatic vessels as circulating tumor cells (CTCs), (D) dissemination to distant organs, and (E) extravasation of CTCs to secondary sites as disseminated tumor cells (DTCs). This article highlights some aspects of the metastatic cascade with a focus on breast cancer cells. Metastatic steps critically depend on the capability of cancer cells to adapt to distant tissues and the corresponding new microenvironment. As a consequence, increasing plasticity and developmental changes paralleled by acquisition of new cancer cell functionalities challenge a successful therapeutic approach.

1. Introduction

Breast cancer accounts for the most common type of cancer in women. The leading cause of cancer death results from metastasis and not from the primary tumor itself [1, 2].

Breast cancer metastasis is characterized by a multistep cascade. This metastatic process can be subdivided into 5 steps which are discussed in principle and involve the following: (A) tumor cells including breast cancer stem-like cells are liberated from the primary tumor tissue potentially undergoing epithelial-mesenchymal transition (EMT), (B) tumor cells migrate and infiltrate neighboring tissue, (C) tumor cells cross endothelial barrier and enter blood and lymphatic vessels as circulating tumor cells (CTCs), (D) tumor cells attach at secondary sites after circulation to escape blood and lymphatic vessels as disseminated tumor cells (DTCs), and (E) tumor cells migrate to distant tissue and form metastases [3–5] (see Figure 1). Especially for the first step of the metastatic cascade, the tumor microenvironment (TME) has a tremendous impact whereby direct and indirect interactions contribute to further development and heterogeneity of the breast tumor, including progression and initiation of metastasis. The TME harbors several cell populations such as a variety of different immune cells, pericytes in perivascular niches, mesenchymal stroma/stem cells (MSC), tumor-associated fibroblasts, adipocytic cells and endothelial precursors, and mature cells. Moreover, soluble factors like cytokines, chemokines, growth factors, hormones, metabolites, and components of the extracellular matrix (ECM) additionally contribute to tumor maturation and diversification. Of interest, particular interaction of MSC with breast cancer cells favors the establishment of a putative carcinoma stem cell niche for generation of cancer stem cell-like cells (CSCs) or tumor-initiating cells (TICs) [6–11]. Although various studies consider CSCs as TICs [12, 13], other work discriminated this interchangeability by stem cell markers, for example, CD133-expressing CSCs in the colon or $CD24^{low}/CD44^{high}$ and $ALDH1^{high}$ expression by breast cancer CSCs representing different functional characteristics [as reviewed in [14]]. Moreover, tumor growth and gene expression profiles in CSCs of metastases are significantly altered as compared to a TIC in the primary tumor which could be more appropriately described as originating tumor cell [as reviewed in [15, 16]]. Cellular processes for a successful

development of metastases are performed by several strategies and diversifications which can vary within different tumor entities. Accordingly, the present work mainly focuses on formation of breast cancer metastases.

(A) Tumor Cells Escape from the Primary Tumor Site. The beginning of metastasis is represented by detachment of individual cells from the primary tumor site [17, 18]. Since detached epithelial and endothelial cells undergo anoikis due to incorrect ECM/cell attachment, detached cancer cells display a certain resistance to anoikis [19]. In order to escape apoptosis tumor cells alter phenotype and functionality including loss of cell polarity and changes in cell-to-cell and cell-matrix adhesion and an increase in migratory potential. These functional and structural alterations are achieved via induction of EMT in the cancer cells [20, 21]. However, there is evidence that EMT is not essential for metastasis. In vivo mice studies revealed that lung metastases exhibit non-EMT cancer cells, that inhibition of EMT influences lung metastasis formation, but that additionally EMT cells show chemoresistance and are the cause for recurrence [22]. Similar results have been reported with pancreatic ductal adenocarcinoma whereby EMT suppression did not affect metastasis but led to induction of chemoresistance [23].

EMT can be initiated by autocrine and paracrine signals involving TGF-β and Wnt or by activation of receptor tyrosine kinases via binding and trans-signaling of growth factors such as epidermal growth factor or fibroblast growth factor [24]. In general, EMT induction leads to activation of EMT-associated transcription factors including Twist1, Slug, Zeb1/2, and Snail1/2 which promote downregulation of, for example, E-cadherin. Consequently, tumor cells lose cell-to-cell adhesion and reduce cell-cell junctions [25]. Moreover, mesenchymal markers like fibronectin, vimentin, and N-cadherin become activated which leads to a more mesenchymal-like phenotype with enhanced migration and increased cell-to-stroma interactions [26–28].

The acquisition of mesenchymal marker expression has also been reported in several studies addressing interactions between mesenchymal stroma/stem cells (MSC) and cancer cells including breast and ovarian cancer [29]. Indeed, MSC represent a heterogeneous cell population with multiple subpopulations displaying stem cell-like properties [30, 31]. Whereas MSC reside in a variety of different tissues within the organism including bone-marrow, adipose tissues, peripheral blood, dental pulp, and perivascular niches of several other tissues, predominant properties include differentiation capacity along phenotypes of the mesenchymal lineage and potential cross-germline maturation. Previous studies suggested that MSC from birth-associated tissues such as umbilical cord exhibit superior properties including a higher expansion rate and engraftment capacity as compared to MSC derived from adult tissue sources [as reviewed in [32]]. Moreover, MSC exhibit immune-modulatory functions at sites of tissue damage and injury. Thereby, local tissue-associated MSC contribute to wound healing, damage repair, and tissue regeneration and homeostasis by secretion of anti-inflammatory cytokines, chemokines, metabolites, and a large variety of growth factors which in parallel can stimulate endothelial cells for vessel formation and subsequent neovascularization. These multiple functionalities of MSC and their crosstalk with various adjacent cell populations which depends on the state of activation and the age of MSC also apply to cellular interactions between MSC and neighboring tumor cells, considering invasive cancer growth as a permanent wound [32–34]. Therefore, MSC also play an important role during tumor cell interaction. For example, breast cancer cells acquire CD90 during coculture with umbilical cord-derived MSC involving gap junctional intercellular communication and Notch signaling which represents one of the characteristic mesenchymal markers of MSC [35]. Moreover, interactions between MSC and cancer cells proceed bidirectionally since vice versa, acquisition of some epithelial-like markers including epithelial cell adhesion molecule (EpCAM) can be detected in MSC following coculture with ovarian cancer cells [36]. Furthermore, MSC acquire increased expression of ECM proteins such as fibronectin and laminin during certain chemotherapeutic treatment and thereby promote tumor cell protection and a distinct chemoresistance [37].

In addition to the downregulation of epithelial traits and upregulation of certain mesenchymal markers in cancer cells, release of ECM-degrading enzymes including matrix metalloproteinases (MMPs) facilitates liberalization from the originating tumor tissue, migration, and subsequent invasion into tumor-neighboring tissue such as epithelial cell layers and eventually blood or lymphatic vessels (Figure 1).

Together, interactions between MSC and tumor cells can facilitate a mesenchymal-like transition of cancer cells to support EMT and metastatic potential.

(B) Tumor Cells Migrate and Infiltrate Neighboring Tissue. Tumor cells can migrate either individually and/or collectively through adjacent connective tissue. Single cell movement can be performed slowly via a mesenchymal type of migration. Alternatively, tumor cells display a faster movement via so-called amoeboid cell invasion. By contrast, leader cells of collectively migrating cancer cells exhibit a mesenchymal migration phenotype whereby inner cells of the collectively migrating unit retain their epithelial phenotype [3, 38].

Mesenchymal movement encompasses a five-step migration cycle characterized by pseudopod protrusion at the leading edge, formation of focal contacts, focalized proteolysis, actomyosin contraction, and detachment of the trailing edge [as reviewed in [39]]. This type of invasion mainly arises in cells which undergo EMT from epithelial cancers or connective tumor tissue. In contrast to amoeboid migration, mesenchymal invasion is protease dependent since it involves a plethora of soluble, secreted, and surface proteases including matrix metalloproteinases (MMPs), kallikreins, serine proteases, and cathepsins to enzymatically remodel and cleave ECM components such as collagen, fibronectin, and laminin to enable movement through the ECM [39, 40]. Cathepsins are found both extracellularly and intracellularly in lysosomes and are represented by different amino acids at active sites such as serine (cathepsin G), aspartic acid (cathepsin D), and cysteine (cathepsins B, F, H, K, and L) [41].

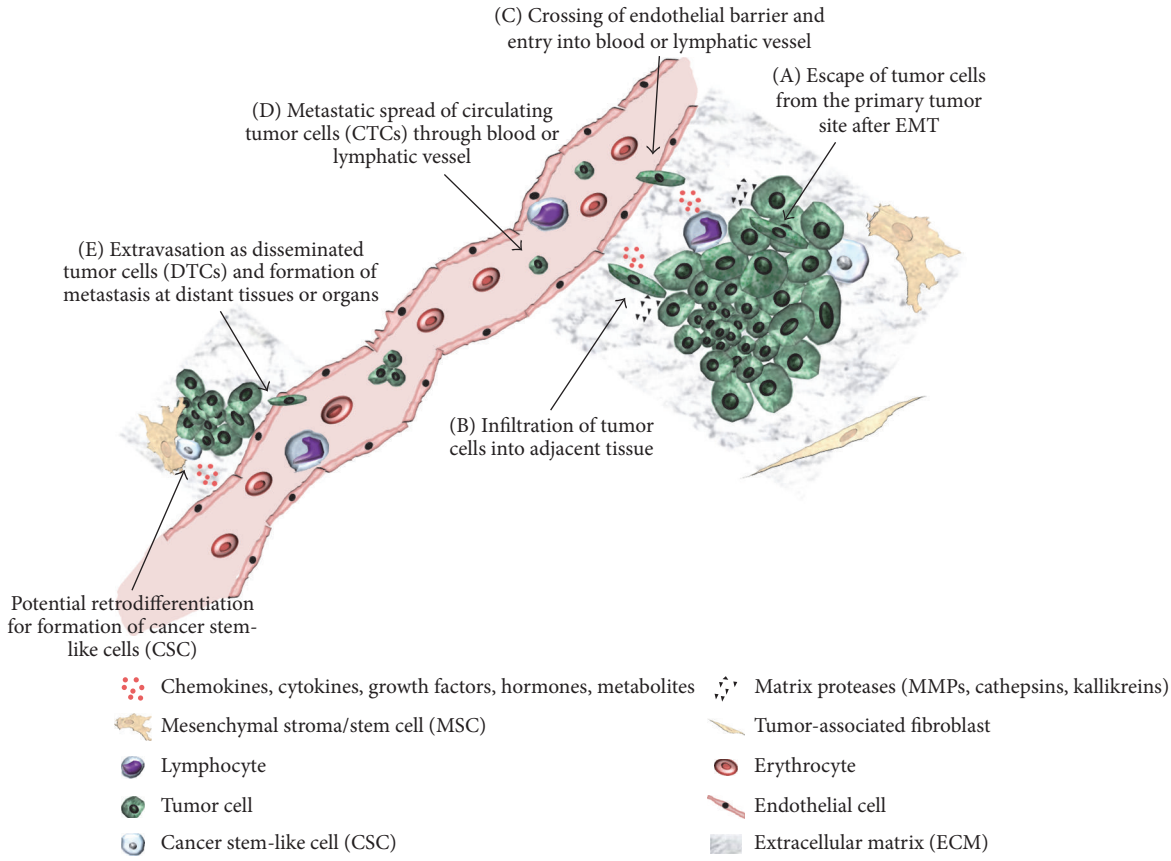

Figure 1: *Schematic view of metastatic process*. Metastatic cascade starting from primary tumor site to distant sites via (A) escape of tumor cells from the primary tumor site, (B) infiltration of tumor cells into adjacent tissue, (C) crossing of endothelial barrier and entry into blood or lymphatic vessel, (D) metastatic spread of circulating tumor cells (CTCs) through blood or lymphatic vessel, and (E) extravasation as disseminated tumor cells (DTCs) and formation of metastasis at distant site (modified according to [9]).

Whereas secreted cathepsins cleave extracellular ECM components, intracellularly located cathepsins degrade endocytosed ECM proteins like collagen in lysosomes [42–46]. For instance, inhibition of cathepsin B reduced ECM degradation and decreased inflammatory breast cancer invasion in vitro [47]. Besides proteolytic enzymes like MMPs and cathepsins, kallikreins represent a further protease family of ECM remodeling proteins. Evidences suggest that kallikreins are involved in tumor progression and distinct kallikreins are applied as tumor marker in cancer diagnosis, for example, kallikrein hK3 also known as prostate-specific antigen [48, 49]. Moreover, the human kallikreins hK2 and hK4 can induce activation of the urokinase plasminogen activator system resulting in ECM degradation and/or activation of tumor cell spreading [48, 50].

Particularly MMPs play an essential role in cancer cell invasion by reorganization of the ECM which consists of basement membranes and interstitial matrix. For example, MMP2 and MMP9 degrade collagen type IV, the major component of basement membranes representing thin structures at the basolateral site of epithelium and endothelium [51, 52]. Moreover, MMP7 was suggested in juvenile human mammary epithelial cells (HMEC) to form a ternary complex together with the growth factor precursor form pro-HB-EGF and anchorage to the hyaluronan receptor CD44. Subsequent cleavage releases the soluble sHB-EGF which binds to the dimerized form of the ErbB4 surface receptor to relay intracellular signaling via the cleaved 4ICD intracellular receptor domain and phosphorylation of Erk1/2, thereby activating Fra-1 for nuclear translocation and repression of tropoelastin production. Vice versa, senescent HMEC express reduced MMP7 protein whereby formation of the ternary complex with HB-EGF and CD44 is disrupted. The resulting failure to activate Fra-1 via the missing ICD induces tropoelastin formation, subsequent cleavage by lysyl oxidases, and extracellular formation of elastin fibers. Consequently, signaling in senescent HMEC with altered MMP7 levels has been attributed to increased fibrosis as a prerequisite for breast cancer development [53–55].

Besides MMP2, MMP7, and MMP9, transmembrane collagenase MT1-MMP/MMP14, which is particularly associated with cell protrusions such as invadopodia, represents a key enzyme in remodeling ECM by driving invasion of tumor cells via degradation of collagen type I and thereby facilitating dissemination of cancer cells [40, 56–59]. Moreover, this MMP was demonstrated to protect invading breast carcinoma cells from collagen type I induced apoptosis [60].

In contrast to mesenchymal cell invasion, hallmarks of amoeboid cancer cell movements are protease independency with total loss of cell polarity, lower adhesiveness due to missing focal contacts, and reduced capability to remodel the ECM. Furthermore, amoeboid migrating tumor cells grow in suspension and exhibit roundish cell morphology [39, 61, 62]. These characteristics allow amoeboid-like invading tumor cells to move much faster through adjacent tissue compared to mesenchymal movement [63, 64].

Both migration types can be performed by single invading tumor cells. Additionally, carcinoma cells exhibit the ability to migrate collectively during metastasis. Collective invasion is characterized by maintenance of cell-to-cell adhesion and front-rear polarity within the migrating cell unit by multicellular coordination and generation of traction force for forward migration and by remodeling of ECM. The plasticity of collectively migrating tumor cells ranges from coordinated sheets, strands, and tubes to clusters [65–68]. Nevertheless, tip cells or leader cells, respectively, feature mesenchymal morphology, whereas subsequent cells are structured by epithelial cell-to-cell contacts. One major advantage of collective cell movement is the protection of inner cells, for example, from immune cell assaults [as reviewed in [39, 62]].

However, all the different tumor cell movements are not mutually exclusive. Switch from mesenchymal-to-amoeboid motility and vice versa can occur under certain conditions and is designated as mesenchymal-to-amoeboid transition (MAT) or amoeboid-to-mesenchymal transition (AMT) [69, 70]. In addition, collective to amoeboid transition (CAT) has been demonstrated in melanoma [65]. Of interest, breast cancer cells have been reported to use both migration types, single and collective cell movement, during metastatic development [71].

Even though cancer cells which are detached from primary tumor site experience a completely different microenvironment while invading through adjacent tissue, they undergo structural and functional changes that allow infiltration and forward movement to attain access to blood and lymphatic vessels.

Chemokines and further soluble factors play an important role in guiding migration of cancer cells towards vasculature and contributing to the metastatic spread [72]. Particularly, chemokine receptor CCR7 has been reported to mediate migration to lymph nodes in gastric and colorectal carcinoma [73, 74]. Breast cancer cells expressing chemokine receptors CXCR4 and CCR7 were stimulated with appropriate chemokine receptor ligands CCL12 and CCL21 leading to pseudopodia formation and higher invasiveness [75]. Additionally, siRNA knock-down of CXCR4 in breast cancer cells or neutralizing antibody against CXCR4 reduced tumor growth and inhibited metastasis [75, 76]. The CXCR4/CXCL12 axis is used by tumor cells which secrete platelet-derived growth factor (PDGF) that in turn activates endothelial cells to secrete CXCL12 leading to a chemotaxis gradient [77]. This gradient may attract CXCR4 positive tumor cells to the endothelium approaching the vasculature. Moreover, breast cancer-mediated production of the chemokine CCL5 (RANTES) by neighboring tumor-associated mesenchymal stroma/stem cells can act in a paracrine manner on the cancer cells to promote their motility, invasion, and metastasis [78].

(C) Tumor Cells Cross Endothelial Barrier and Enter Blood and/or Lymphatic Vessels. Cancer cells detached from the primary tumor site can utilize both the blood and lymphatic system to spread to secondary sites for metastasis [79]. Prerequisites for this development are neovascularization of the tumor site or lymphangiogenesis [80, 81] to provide appropriate vessel structures within the tumor microenvironment.

To enter the blood or lymphatic system and of course to leave this system again, cancer cells need to cross the endothelial barrier. Disruption of this barrier is indispensable and involves various mechanisms which cancer cells use for intra- and extravasation [82–84].

One mechanism suggests that endothelial barrier leakage occurs via tumor cell-secreted factors and receptor-ligand interactions. Metastatic melanoma cells induced endothelial cell gap formation via Src activation in endothelial cells, a nonreceptor tyrosine kinase. Further activation of Src was mediated by tumor cell-derived IL8 and VLA-4/VCAM-1 interactions leading to phosphorylation of VE-cadherin, a major component of endothelial junctions and finally to disruption of the endothelial barrier [83]. Other in vivo studies suggested a potential role for VEGF in promoting vascular permeability upstream of Src and VE-cadherin by applying VEGF and Src inhibitors that suppress tumor cell extravasation [84]. Moreover, epidermal growth factor receptor (EGFR) has been attributed to a sustaining role of tumor cell intravasation and dissemination [85].

There is still ongoing debate about the entry mechanism of tumor cells into vessels whether it occurs actively or passively. As outlined previously, motile cancer cells can enter the blood and lymphatic system actively while mobile cells are moved by external forces, for instance, mechanical tension through the endothelium into blood and lymphatic vessels [17, 86, 87]. Motile tumor cells invade either individually or collectively. For both ways, tumor cells have to undergo significant changes involving EMT to acquire higher migratory potential concomitant with altered cell morphology and a phenotype with certain stem cell characteristics similar to CSCs or TICs [17, 88–94].

Tumor cells that have escaped the primary tumor site and entered blood or lymphatic system for metastatic spread are also defined as circulating tumor cells (CTCs) [95].

(D) Tumor Cells Disseminate to Secondary Sites as Circulating Tumor Cells (CTCs). CTCs have to overcome several obstacles that implicate blood and lymphatic vessels. Indeed, the vascular environment is completely different compared to the TME of the primary tumor site and is responsible for high mortality rates of CTCs [96]. Clinical studies about CTC occurrence in breast cancer patients revealed an averaged number of around 80 CTCs per mL blood [97] equivalent to nearly half a million of CTCs in the whole circulation. This actually demonstrates a certain inefficiency and limitation of the metastatic process whereby reasons are partially due

to the vascular environment. However, prognostic values for appearance of metastatic breast CTCs/mL by diagnostic approaches of CTC enrichment technologies using liquid biopsy could be much lower and may depend on the genomic heterogeneity of CTCs and individual patient conditions [98, 99].

First of all, fluid shear stress, the mechanical force of the blood flow, and frequent collisions with blood and immune cells represent one major hindrance for CTCs to survive and to reach distant organs [17, 100]. Nonetheless, it was shown that CTCs which underwent EMT are more resistant against these kinds of insults than tumor cells with an epithelial phenotype [96]. Collisions of CTCs with blood cells mainly result from high numbers of leukocytes, erythrocytes, and platelets affecting CTC viability [96, 101]. Moreover, CTC survival requires rescue or self-defense against immune cell assaults, particularly those from natural killer (NK) cells [102]. CTCs can evade the effective antitumoral activity of NK cells via induction of platelets to aggregate, representing a potential mechanism of tumor cells for platelet activation (tumor cell-induced platelet aggregation, TCIPA) [103, 104]. TCIPA proceeds directly via interaction of platelets with CTCs or indirectly via soluble molecules like cysteine proteases or ADP [105, 106]. Moreover, MMPs play an essential role in TCIPA and cancer cells may use this possibility to circumvent NK cell activity. For instance, in vitro studies showed that MCF7 breast cancer cells cotransfected with MMP14/β3-integrin caused platelet aggregation via introduced MT1-MMP/MMP14 and via activation of MMP2. Additionally, ADP contributed to TCIPA via stimulation of the corresponding platelet receptor $P2Y_{12}$ [107, 108]. Thus, it is feasible that tumor cell-induced platelet aggregation promotes survival of cancer cells in vascular circulation since platelets may shield CTCs from immune cell assaults and from fluid shear stress and may facilitate extravasation at distant organs resulting in enhanced metastatic potential [109–111].

In addition to TCIPA to protect from antitumoral immune cell activity, tumor cells can selectively reduce tumor-suppressive TGF-β-signaling facilitating tumor progression through the suppression of the host immune system [112, 113]. For instance, in vivo studies revealed antitumor effects through T-cell specific inhibition of TGF-β-signaling [114].

A further obstacle for CTCs is the lack/deficiency of cell-matrix interactions to provide proliferation signals and cellular stability. Nevertheless, CTCs that have undergone EMT and acquired a mesenchymal-like phenotype do not require such interactions for cell survival [17, 88, 115].

All these hindrances of an altered microenvironment in the vascular system lead to an overall low survival rate of CTCs in the vasculature. For instance, it has been demonstrated that circulating breast cancer cells survive only a few hours in the circulation [116]. In most patients with advanced cancer (stage IV) the occurrence of CTCs is around 1 cell per billion normal blood cells [117]. Accordingly, this low number emphasizes the difficulties and challenges to detect CTCs in early stage cancer patients for timely diagnosis and therapy.

(E) Tumor Cells (CTCs) Escape Blood and Lymphatic Vessels and Migrate as Disseminated Tumor Cells (DTCs) to Secondary Sites. Extravasation starts with a reduction in blood flow velocity and a corresponding reduced CTC circulation in smaller capillaries in order to facilitate blood vessel wall attachment [118, 119]. There are two mechanisms for CTC extravasation, (1) physical occlusion and (2) cell adhesion. Whereas physical occlusion takes place in capillaries with a diameter smaller than CTCs, cell adhesion occurs in larger capillaries and requires direct binding to the endothelium [120, 121].

CTC adhesion to endothelial cells necessitates the expression of appropriate ligands and receptors on both CTCs and endothelial cells including cadherins, selectins, integrins, the hyaluronan receptor CD44, and immunoglobulin superfamily receptors [118, 122]. CD44 expressed on breast cancer cells serves as a major ligand for endothelial cell surface located E-selectin and mediates CTC adhesion to the endothelium [123]. Whereas endothelial selectin and cancer cell surface-expressed CD44 mediate CTC attachment, Rac1 and cell division control protein 42 (CDC42) promote transendothelial migration via extension of cancer cell protrusions facilitating the whole process of extravasation, for example, invadopodia which expand through the endothelial barrier [118, 124, 125]. Besides the interaction of breast cancer cell-derived integrin $\alpha v\beta 3$ with platelets, previous work demonstrated that integrin $\alpha v\beta 3$ also promotes CTC attachment to the endothelium in an activation-dependent manner [126, 127].

Following CTC attachment, metastasizing tumor cells cross endothelium by distinct mechanisms targeting endothelial junctions including VE-cadherin. Furthermore, cancer cells can incorporate into the endothelium displacing endothelial cells and disrupting the structure of the endothelium. Whereas neighbored endothelial cells maintain expression of VE-cadherin, endothelial cells in contact with cancer cells do not express VE-cadherin which may facilitate additional cancer cells to incorporate [128].

Breast cancer cells preferentially disseminate to lung, bone, liver, and brain [129, 130]. In particular, breast cancer cells with stem cell-like properties including $CD44^{high}/CD24^{low}$ subpopulations represent candidates for metastatic activities since this cell population can differentiate, escape immune surveillance, display apoptosis resistance, and sustain cell growth and self-renewal [90, 131–134]. Thus, different single cancer cell progenies acquire the capability to metastasize to distinct tissues/organs with features of an organ-specific homing whereby early dissemination of cancer cells favors metastatic capacity [2, 130, 135].

During EMT some tumor cells acquire characteristics and develop properties of CSCs [89, 90]. Nonetheless, the new microenvironment, which significantly differs between the different tissues/organs, is important for survival of extravasated CTCs and will be, most commonly, hostile for CTCs. Friendly microenvironments may represent metastatic niches which harbor stromal cell types including MSC and certain ECM proteins. Such a carcinoma stem cell niche may provide conditions via diverse tumor cell interactions or induction of a retrodifferentiation process to reprogram

tumor cells for CSC formation and protection [9, 136]. Development of breast cancer stem-like cells are associated with expression of low levels of CD24, high levels of CD44, aldehyde dehydrogenase, and the IL8-binding chemokine receptor CXCR1 [137–140].

Newly formed CSCs can be kept in a dormant/quiescent state and therefore elevate the chance for CTC survival via cell-to-cell and cell-to-matrix interactions [10, 88, 141, 142]. Although CTCs that underwent EMT exhibit a higher migratory potential and invasiveness, their proliferative capacity and ability for cell-to-cell interactions remain limited [143]. Consequently, only a subset of CTCs will survive as disseminated tumor cells (DTCs) while the majority may die or reside as dormant cells [129, 144].

Mesenchymal-to-epithelial transition (MET), the reverse process of EMT at primary tumor sites, is deliberated to favor colonization of DTCs which can occupy distant organs as solitary cells, small preangiogenic metastases, or greater vascularized metastases [94, 129, 145]. Crucial signaling pathways to initiate MET involve protein kinase A (PKA) activation and following nuclear translocation. Subsequent PKA-mediated phosphorylation and thus activation of the histone demethylase PHF2 promote transactivation of epithelial cell-associated genes and protein products such as E-cadherin [146]. One hallmark of EMT is the downregulation of E-cadherin resulting in loss of cell adhesion [25]. Accordingly, reexpression of E-cadherin favors colonization of DTCs at distant organs or tissues. Recent studies revealed the expression of E-cadherin in metastases of E-cadherin-negative breast cancer xenografts induced by a secondary organ microenvironment [147]. Moreover, the reciprocal interplay of ZEB1/miR200 has been suggested as regulation of EMT and MET in cancer. Whereas transcription factor ZEB1 is known as potential inducer of EMT via repression of miR200 family members, miR200 has been reported to induce MET by repression of ZEB1 which subsequently leads to higher expression of E-cadherin [148–150].

In some breast cancer patients, metastases can arise after a long time, even after years or decades following first diagnosis. Since the majority of DTCs may reside as dormant cells in metastatic niches, local neovascularization together with a certain chemokine/metabolite/growth factor cocktail can stimulate reentry into the proliferative cell cycle and contributes to cancer relapse. Indeed, breast cancer cell models revealed that endothelial cells induce and maintain dormancy of DTCs in metastatic niches via thrombospondin-1, known as an inhibitor of angiogenesis and tumor growth [151, 152]. Moreover, time-lapse studies indicated that neovascular tips promote breast cancer growth and that these neovascular tips are rich in periostin and TGF-β1 suggesting an additional role for these two soluble factors in cancer relapse [151].

2. Concluding Remarks

The plasticity of tumor tissues and the continuous alterations/adaptations during liberation of tumor cells from the original tissue, transendothelial migration, and development of metastases include multiple changes in tumor cell phenotype and functionality. These variations challenge tumor cells to adapt to a new tissue environment with a certain threshold of chemokines, tissue-specific metabolites, and other soluble factors. Moreover, variations also include formation of new tumor cell populations, for example, by fusion or entosis with MSC during cellular interactions of adjacent cell types. All of these variations which are acquired in the course of metastasis contribute to the heterogeneity of the tumor and necessitate various tumor cell type-specific markers to provide potentially successful targets. Consequently, early interference with signaling pathways associated with tumor cell migration, spreading of CSCs, and formation of metastases represents a more promising therapeutic approach.

Abbreviations

TME: Tumor microenvironment
EMT: Epithelial-to-mesenchymal transition
MET: Mesenchymal-to-epithelial transition
HMEC: Human mammary epithelial cells
MMP: Matrix metalloproteinase
MAT: Mesenchymal-to-amoeboid transition
AMT: Amoeboid-to-mesenchymal transition
CAT: Collective to amoeboid transition
MSC: Mesenchymal stroma/stem cells
CTCs: Circulating tumor cells
DTCs: Disseminated tumor cells
CSCs: Cancer stem cell-like cells
TICs: Tumor-initiating cells
NK cells: Natural killer cells
TCIPA: Tumor cell-induced platelet aggregation.

Conflicts of Interest

The authors declare no financial, personal, or professional conflicts of interest.

Authors' Contributions

Catharina Melzer and Ralf Hass drafted the manuscript. The figure was designed by Catharina Melzer, Juliane von der Ohe, and Ralf Hass. The manuscript was edited by Ralf Hass. All authors have critically read and approved this work.

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