Breast Cancer Microenvironment and the Metastatic Process

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5.1 Introduction

Breast cancer is the most frequently diagnosed cancer, the second leading cause of cancer-related death in women worldwide, and the leading cause of cancer-related death in less developed countries [1]. Overall prognosis is favorable with 85% survival chances in developed countries [2]. Most of the remaining 15% of patients succumb to sequelae of metastasis, the spread of cancer from one part of the body to another (Dictionary of Cancer Terms, NCI), as their disease becomes drug resistant. In poorer countries, women are more likely to die of their metastases because they are diagnosed at later stages of the disease and have less access to costly treatments [3–5].

In general, breast cancer cells that escape from the primary tumor take the lymphatic and/or venous route and home most frequently to the bones, the lungs, the brain, and the liver. The WHO distinguishes more than 20 different histologic subtypes [6], and global gene expression profiling has revealed at least 5 molecular subtypes of breast cancer [7–9]. Some of these subtypes have distinct clinical features and show different metastatic behaviors. Luminal/estrogen receptor-positive (ER^+) tumors disseminate more frequently to the bones than TN tumors do, and human epidermal growth factor receptor 2-positive ($HER2^+$) and TN tumors have a predilection for the brain [10]. Lobular carcinomas typically metastasize to the peritoneum and the ovaries [11].

Metastasis is a complex pathological process, and seven distinct steps have been defined at the cellular level. It begins with increased cell motility and cell migration followed by stromal invasion. Subsequently, tumor cells intravasate into lymphatic and/or blood vessels; they adapt to survive in the circulation; they extravasate, colonize distant organs, and eventually, sometimes after many years of dormancy, grow to overt metastases [12–15].

For decades, most research efforts concentrated on the cancer cell-intrinsic factors that drive this process. In recent years, the tumor microenvironment (TME) defined as the normal cells, molecules, and blood vessels that surround and feed a carcinoma (Dictionary of Cancer Terms, NCI) has moved to the center of attention. Both at the primary and at the metastatic sites, numerous noncancerous cells, cancer-associated specialized cell types, and matrix molecules interact with the tumor cells and affect their biological properties [16–18]. Heterotypic cell contacts, exosomes, cytokines, and other soluble factors produced by cancer and stromal cells are now known to support tumor initiation, progression, and metastatic spread [19]. In conventional breast cancer xenograft models, breast cancer cell lines are typically injected either subcutaneously or orthotopically in the mammary fat pad of immunocompromised mice [20]. Intriguingly, the site of injection affects metastatic capability with increased metastasis observed upon orthotopic engraftment [21, 22].

Throughout the multistep metastatic process, tumor cells rely on epithelial-mesenchymal transition (EMT) [12, 23]. This well-characterized process is repeatedly required during development as for the formation of the mesoderm from epithelial epiblasts during gastrulation, neural crest formation, and formation of muscle cell precursors from epithelial somite walls. In adulthood, it has a role in wound healing [12, 24–27]. All these processes have in common that epithelial cells dedifferentiate, lose cell adhesion, become more migratory, and acquire stem cell properties. Cancer cells hijack this developmental program to detach from the epithelial tissues of which they are part, to reach vessels, and to

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acquire the self-renewal properties and the cellular plasticity important in the metastatic process [28]. At the distant organ, the reverse process called the mesenchymal-to-epithelial transition (MET) is commonly found at metastases [27, 29]. Intriguingly, recent in vivo studies of murine tumors are arguing for a role of EMT and MET transitions in breast cancer drug resistance [30].

Here, we review how the cancer microenvironment affects the spread of cancer cells to distant sites and discuss how the microenvironment at the distant sites contributes to colonization and to the growth of the metastases. The important role of the normal tissue microenvironment as a barrier to tumorigenesis has been extensively described elsewhere [31]. Most of our insights into the metastatic process stem from experimental models, which do not distinguish between different clinical subtypes. Hence, wherever possible, we refer to clinical observations that provide clues of subtype-specific properties.

5.2 Cancer Cell: Nonautonomous Traits and Breast Tumor Microenvironment

Cancer-associated stromal cells react to the morphological and molecular changes the tumor cells undergo by epigenetic changes and alterations of their secretome [32]. The released factors can affect tumor progression directly and/or indirectly through recruitment of other cells, nonindigenous to the breast tissue. Examples are bone marrow-derived, mesenchymal stem cells and innate and specific immune cells, which in turn participate in stimulating a vicious cycle of cell-to-cell and factor-to-cell interactions.

5.2.1 Cell Types and Secreted Factors

5.2.1.1 Fibroblasts and Mesenchymal Stem Cells

The fibroblasts are the most abundant cell type in the breast stroma [33] and can both inhibit [31, 34] and promote tumor growth [31, 33–36]. During tumor progression, fibroblasts are converted cells to activated fibroblasts or cancer-associated fibroblasts (CAFs) by transforming growth factor β1 (TGFβ1) [37], Wnt7a, and other factors secreted by the tumor cells [33, 38, 39]. CAFs are characterized by high-level expression of fibroblast-specific protein 1 (FSP1), fibroblast-activating protein (FAP), alpha-smooth muscle actin (α-SMA), and TGFβ1 [40]. They orchestrate and promote the metastatic process in two ways; first, they induce EMT through activation of TGF-β receptor signaling and extracellular matrix (ECM) remodeling [34]. Second, they recruit innate and specific immune cells to the TME and subsequently activate them. The activated immune cells, in turn, stimulate the metastatic potential of cancer cells [18, 33, 35]. More specifically, CAFs release immune-modulatory molecules including interleukins, interferons, and tumor necrosis factor α (TNFα) to attract macrophages. The tumor-associated macrophages (TAMs) are the dominant portion of the leukocyte population within the tumor [41]. They can modify the cancer cell phenotype generally leading to a more aggressive behavior in breast cancer, through factors that have not been clearly identified yet, but hypoxia has been implicated [42]. TAMs in turn, at least in models of skin carcinoma, support tumor angiogenesis [39]. Moreover, CAFs recruit regulatory T cells (Tregs) through the secretion of various chemokines including CCL5, as shown in GEMMs of mammary carcinogenesis [43].

Experiments in the TN MDA-MB-231 xenograft model showed that CAFs control organ specificity of metastases by secreting two cytokines that are mainly expressed by stromal cells in the bone marrow, the stromal cell-derived factor 1 (SDF1/CXCL12) and the insulin-like growth factor 1 (IGF-1) [44, 45]. Activation of the cognate receptor, CXCR4, on the cancer cells activates AKT thereby increasing tumor cell survival in the bone [46]. Bioinformatic analysis of primary tumor gene expression profiles revealed that Src activity correlated with late bone metastasis, and Src activity was shown in the MDA-MB-231 model to indeed enhance tumor cell survival in the bone by facilitating CXCL12-CXCR4-AKT signaling and by increasing resistance to TRAIL-induced cell death [46].

Mesenchymal stem cells (MSCs) are recruited to damaged and ischemic tissues. More recently, they were also shown to be drafted to the TME following calls by numerous factors such as growth and angiogenic factors as well as chemokines and ECM proteases, all released by cancer and stromal cells as reviewed in [47, 48]. The MSCs present in invasive human breast carcinomas can be differentiated into various cell types including fibroblasts and pericytes, and their effects on tumor growth are complex. They were shown to promote tumor growth and angiogenesis through elevated SDF1 secretion [49]. Experiments in subcutaneous breast xenografts showed that once recruited to the TME, MSCs in turn secrete the chemokine CCL5, which increases the motility and invasiveness of the cancer cells, which express the cognate receptor CCR5, and promote lung colonization [50].

5.2.1.2 Adipocytes

Another prevailing cell type in the TME is the adipocyte that also interacts in different ways with the tumor cells [51, 52]. Analyses of human clinical samples and *in vivo* experiments with mice have shown that adipocytes are frequently found at the invasive front of human breast tumors. Here a dialogue between the cancer cells and adipocytes occurs; the invading tumor cells are able to modify the adipocytes,

which in turn stimulate cancer cells to a more aggressive phenotype [53].

Furthermore, the fat cells promote tumor progression through systemic effects by secreting hormones, such as oestradiol (E2) and prolactin, as well as paracrine factors like the major fat tissue-derived adipokines such as leptin and adiponectin which promote breast cancer progression through activation of proliferation and survival [54–56]. A series of in vitro coculture and in vivo experiments suggest that secretion of interleukin-6 (IL-6) by adipocytes makes tumor cells more invasive and increases their metastatic potential [53]. Similarly adipocytes promote breast cancer progression through secretion of pro-inflammatory cytokines such as TNFα, which increase stem cell numbers and enhance metastasis in cell coculture and animal models [57, 58].

In this regard, the tumor-promoting effects of obesity, which are observed in postmenopausal women, relate to increased levels of E2 and of pro-inflammatory cytokines released by the adipose tissue [59, 60]. Elevated systemic E2 levels result from increased aromatase activity not only the adipocytes of the TME but in various fat depots in the body. To what extent these effects are important for the metastatic process systemically versus locally remains to be teased apart experimentally. Interestingly, when human breast adipocytes from obese patients were grafted to immunocompromised mice to model the inflammatory environment of the human breast, the mouse tumors were enriched with adipocytes secreting CCL2/IL-1β. This recruited macrophages, which in turn stimulated CCL2-associated angiogenesis [61].

5.2.1.3 Vasculature

The vasculature in the TME is another key player in the instigation of metastasis. It consists of an inner layer of endothelial cells, pericytes that wrap around the endothelial cells, and, in the case of larger vessels, smooth muscle cells. During tumor progression, the normally quiescent vasculature is activated by VEGF released by tumor and stromal cells, and new vessels sprout to sustain tumor growth [12, 62]. Critical to the metastatic process is vessel integrity, which deters cancer cell migration and prevents tumor spread into the circulation. Pericytes are responsible for vessel integrity. Consistently, many studies in mouse models have shown that low pericyte coverage is associated with invasive breast cancer, decreased survival, and lung metastasis [63–65].

5.2.1.4 Immune Cells

It was realized a long time ago that the white blood cells, which constantly patrol normal breast tissue, also closely interact with breast tumor cells [66]. Their role is ambiguous; frequently, differentiated tumor-infiltrating immune cells and "tumor-educated" macrophages further the multistep metastatic cascade by promoting tumor cell invasion, intravasation, and their survival in the bloodstream. They also assist in tumor cell arrest, extravasation, and overt growth at metastatic sites [67]. In specific clinical scenarios, however, an immune cell infiltrate is indicative of a good prognosis [68].

Studies with the MMTV-Erbb2-transgenic mouse mammary carcinoma model [43] showed that tumor-infiltrating regulatory T cells promoted lung metastasis through expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), a protein implicated in epithelial cell proliferation of the normal breast [69]. Consistently, RANKL overexpression stimulated the metastatic progression of RANK-expressing and HER2-overexpressing mammary tumors [43, 67].

Recent studies in a mouse model of invasive lobular carcinoma generated by targeted deletion of E-cadherin and p53 in the mammary epithelium indicate that neutrophils induce the release of several cytokines in the TME that increase lung metastasis without affecting primary tumor growth [70, 71]. Moreover, neutrophils support lung colonization of metastasis-initiating tumor cells in the metastatic MMTV-polyoma middle T antigen (PyMT) mammary tumor mouse model [72].

Initially, macrophages were implicated in the antitumor immune reaction [73, 74]. However, recent studies indicate that TAMs promote angiogenesis, cell migration, invasion, and intravasation, thereby increasing the propensity of the cells to leave the primary site [75]. In the PyMT mammary tumor model, macrophage infiltration is seen early during tumor development when hyperplasias are present [76]. The recruitment of TAMs into TME is principally regulated by cytokines, chemokines, and growth factors secreted by both tumor and stromal cells [77]. In breast cancer patients, TAMs abundantly produce the chemokine CCL18, and its expression in cancer stroma and blood is associated with increased metastasis and reduced patient survival [78]. Mechanistically, CCL18 promotes the invasiveness of cancer cells by triggering integrin clustering and enhancing their adherence to extracellular matrix [78].

5.3 Breast Tumors in Transient and Metastatic Microenvironments

Breast cancer cells enter the lymph vessel in the TME and ultimately reach the blood vessels. Anytime after intravasation in the bloodstream, they may encounter antitumor immune cells and metabolic and oxidative stress and be exposed to extensive shear forces in the bloodstream. Once in the vessels, circulating tumor cells (CTCs) need to exit from the lumen of blood and/or lymphatic vessels and penetrate to the distant tissue. This process is highly inefficient with an estimated 1 out

of 10,000 cells successfully extravasating and homing to a distant site [16, 79, 80]. Many different cell types can be found in the blood including immune cells, fibroblasts, and platelets with important roles in the metastatic process [32].

5.3.1 Platelets

Platelets have emerged as essential "protective" escort of the carcinoma cells on the move; they appear to constitute a special type of mobile "local" microenvironment [81, 82]. Physiologically, platelets are activated when the continuity of the endothelial layer is disrupted and the underlying subendothelial matrix is exposed [83]. In the breast tumor setting, they can be activated through physical contact with tumor cells [84]. At the primary site, platelets along with macrophages and MSCs contribute to EMT of the cancer cells. In the bloodstream, platelets have dual role in the fitness of disseminated breast tumor cells both through direct physical contact and through secretion of paracrine factors both of which increase tumor cell survival and extravasation by maintaining or/and inducing an EMT status [84]. In vivo experimental evidence indicates that the paracrine effects can be ascribed to the secretion of bioactive growth factors stored in α-granules, such as EGF, PDGF, TGFβ, and VEGF, as well as inflammatory cytokines and chemokines also stored in α-granules [84, 85]. Within the peripheral tissues, activated platelets contribute to the recruitment of monocytes and granulocytes thereby helping to establish pro-metastatic and metastatic niches [83, 85, 86].

5.3.2 Metastatic TME (MTME)

Metastatic sites are generally considered inhospitable microenvironments and present a challenge to cancer cell fitness and survival [16]. Consistently, many of the CTCs that successfully extravasated from the bloodstream to a distant tissue remain dormant for months or, in the case of ER^+ tumors, even decades. Only some get reactivated and go on to form clinically apparent tumors. Several lines of evidence indicate that secreted factors, produced by tumor and stromal cells in the TME, promote the progression of tumor-specific organ colonization. Heparanase endoglycosidase secretion by primary breast tumors promotes bone resorption in a GEMM model [87]. Furthermore, tumor cell-derived exosomes may represent the postal code on a letter and lead to distinct organ colonization depending on the integrins they carry [88]. Noteworthy, the microenvironment at the distant site can induce epigenetic changes that enhance proliferation and reduce apoptosis of the metastatic cancer cells as was elegantly illustrated by xenograft mouse models [89]. Intriguingly, metastatic breast cancer cells that have lost phosphatase and tensin homolog (PTEN) are primed for the brain most probable through the induction of the chemokine (C-C motif) ligand 2 (CCL2) [89]. Moreover, immunohistochemical analysis of clinical samples for PTEN and CCL2 revealed significantly higher CCL2 expression in brain metastases than in matched primary tumors. Mechanistically, epigenetic regulation is implicated; astrocytes secrete exosomes that cause adaptive PTEN cancer cell loss [89]. Moreover, metastatic cancer cells can directly interact with stromal cells. This heterotypic dialogue with the distant stroma can provide support to the cancer cells at the early steps of colonization and promote metastasis as nicely illustrated experiments in a xenograft model of intrailiac artery injection [90]. In this model, the ER^+ breast cancer cells, MCF7, colonized efficiently the bones and made physical contacts with osteogenic stromal cells through adherens junctions. This led to increased cell proliferation of cancer cells and growth of metastasis through the activation of mTOR pathway [90]. On the other hand, cancer cells activate bone cells. For example, bone metastatic breast cancer cells promote osteolytic lesions by stimulating the formation and activity of osteoclasts via production of colony stimulating factor 1 (CSF1) as well as parathyroid hormone-related protein (PTHRP) and tumor necrosis factor-α (TNFα) [91]. The secretion of these cytokines and hormones activates the RANK pathway and inhibits the synthesis of the osteoprotegerin, thereby increasing the number and activity of osteoclasts. Finally, xenograft experiments have revealed ER^+ breast cancer-mediated systemic instigation by supplying circulating platelets with pro-inflammatory and pro-angiogenic proteins, supporting outgrowth of dormant metastatic foci [92].

5.3.3 Hypoxic Conditions in the TME

As tumors grow, lack of adequate oxygen supply leads to hypoxia. Hypoxic breast cancer cells are more prone to invade and metastasize, and they also respond less efficiently to drug treatment [93]. Central in the cellular response to hypoxia is the transcription factor hypoxia-inducible factor 1α (HIF1α); it upregulates cytokines, extracellular matrix proteins, and secreted proteins such as lysyl oxidase (LOX).

The HIF1α target LOX is highly expressed in primary breast tumors and is significantly associated with metastasis in ER^- patients [94]. Moreover, LOX is a critical mediator of bone marrow cell recruitment during the formation of the pre-metastatic niche [95]. HIF1α facilitates the initiation of EMT by inducing the expression of the master mesenchymal regulator, the transcription factor TWIST [96]. Hypoxia also triggers the release of exosomes. Studies using the 4 T1-BALB/c syngeneic animal model of metastatic mammary carcinoma showed that LOX activates normal bone-constituent cells called osteoclasts, which in turn enhance bone breakdown, favoring the formation of a pre-metastatic niche where disseminated circulating breast cancer cells find the appropriate microenvironment to form metastasis [94]. Preparation of the niche [97] is a key regulator of angiogenesis [98].

5.4 Cancer Cell-Autonomous Traits

5.4.1 Genetic Aberrations

Tumor cell-intrinsic factors that contribute to metastasis include genetic and epigenetic alterations with the ensuing changes in gene expression and biological properties. Numerous somatic mutations, gene fusions, gene amplifications, and cancer predisposing single-nucleotide polymorphisms are well documented in breast cancer [55, 99–102]. Some of these are thought to drive tumorigenesis by bestowing essential tumor cell properties on normal cells [12]. However, despite many efforts to identify mutations specific to metastases that could trigger a metastatic switch, evidence for such failed to come forward. Hence a model began to prevail in which the ability to metastasize is inherent to the primary breast tumor. In line with this view, gene expression signatures were identified in primary tumors that are associated with higher likelihood to metastasize [14, 103–105].

Recent findings, however, have challenged this view. Advances in sequencing technology have led to the realization that tumors can be composed of a myriad of different subclones [106], and xenograft models combined with DNA bar coding technology have revealed unexpected dynamics during tumor evolution [107]. With the in-depth comparisons of DNA sequences from primary tumors and their matched metastases, examples of metastasis-specific mutations are beginning to emerge. The most prominent example is mutations in the estrogen receptor α (*ESR1*). They were originally identified two decades ago by S. Fuqua and colleagues who screened metastatic samples for *ESR1* mutations [108] but largely dismissed as a very rare event. This changed when deep sequencing revealed that they occur in as many as 32% of metastases [109] and in as many as 42% of circulating tumor DNA (ctDNA) samples from women with metastatic ER$^+$ disease who had been treated with aromatase inhibitors for their metastatic disease [102]. In primary breast carcinomas, these mutations either failed to be detected at all or were found in less than 0.6% only [102, 109, 110]. Most of the mutations are found at the C-terminus in the ligand-binding/AF2 domain and lead to estrogen-independent growth in vitro [111]. This suggests that metastatic cells require constitutively active ER signaling for the survival and growth in the metastatic microenvironments in the absence of estrogens.

Recently, whole-exome sequencing of 86 brain metastases and matched primary tumors including 21 breast tumor samples identified metastasis-specific mutations [112]. Specifically, analysis of breast cancer brain metastases revealed that 47% of genetic aberrations were not detected in the same patient's primary tumor. Patients with HER2-amplified breast cancer who developed brain metastasis under trastuzumab showed amplification and activating point mutations in epidermal growth factor receptor (*EGFR*) (L858R) and fibroblast growth factor receptor (*FGFR1*) amplification specifically in the metastatic sample but not in the primary tumor DNA. The findings from this study are of important clinical implications, as both the *EGFR* and the *FGFR* mutations are druggable. The mutations that appear only in the metastatic sites may relate to the therapeutic responses and the way the cancer cells interact with the TME [113].

Mutations in GATA binding protein 3 (GATA3) interfere with its DNA-binding ability, reducing or diminishing its binding, and are commonly found in NST luminal-like molecular subtype in human breast cancers [100] with high frequency (13%) [99, 114]. Moreover, loss of GATA3 expression in breast tumors has been linked to aggressive tumor development, poor patient survival, and increased metastatic potential [115–119]. In a spontaneous metastasis experimental model using the LM2 lung-tropic breast cancer cells that were derived from MDAMB231, increased expression of GATA3 specifically inhibited metastasis to the lungs without affecting extravasation [116]. Mechanistically, GATA3 induces *microRNA-29b* expression, which in turn inhibits metastasis by targeting a network of pro-metastatic regulators involved in angiogenesis [115].

Mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA)* are found in 40% of ER$^+$ breast cancers. A study of 292 clinical samples derived from independent international cohorts analyzed for the presence of mutations showed that they are correlated with lymph node metastasis suggesting that PI3K/AKT activation may enhance invasion of cancer cells to lymph nodes [120]. Of note, *BRCA1* germline mutations, which typically lead to aggressive breast cancers, have been linked to increased probability of cerebral metastases [121]. In particular, 67% ($n = 15$) of *BRCA1* mutation carriers were found to develop metastases in the brain compared to 0 and 6% of *BRCA2* mutation carriers ($n = 12$) and noncarriers ($n = 58$), respectively.

Mutations in genes that are implicated in metabolic processes have recently been identified and amplification of genes encoding for metabolic enzymes has been found in breast cancers. An example is the gene encoding phosphoglycerate dehydrogenase, which is frequently amplified in TN breast cancers and increases flux through the metabolic pathway of serine/glycine synthesis thereby providing advantages for bone metastatic breast cancer cells because cell proliferation is stimulated and osteoclastogenesis enhanced [122, 123]. Moreover, the proto-oncogene Neu product (ERBB2) stimulates glycolysis by AKT1-dependent and AKT1-independent pathways. Interestingly, in many breast cancer cell lines, overexpression of ERBB2, a hallmark of HER2$^+$ tumors, leads to increased glucose uptake and lactate production and decreased oxygen consumption [124]. Whether this contributes to the increased propensity of HER2$^+$ tumors to metastasize to the brain remains to be explored. It remains also to be seen how metabolic changes are related to metastasis and which are the exact molecular mechanisms that give advantages to the cancer cells to access vessels and survive in the bloodstream and establish metastases.

5.4.2 Noncoding RNAs (ncRNAs)

Over the past years, ncRNAs have emerged as important mediators in the crosstalk between breast cancer cells and their microenvironment [125]. During breast cancer progression, expression of particular small, (miRNAs) is deregulated. When human breast cancer cell lines were injected into the tail vein of immunocompromised mice, seven miRNAs, miR-335, miR-126, miR-34a, miR-31, let-7, miR-200 s, and miR-29b, suppressed—whereas miR-10b, miR-373/520c, miR-200 s, miR-21, miR-9, and miR-103/107 promoted—several steps of the metastatic process [125, 126]. In particular, loss of expression of tumor suppressor miRNAs through epigenetic silencing lead to increased brain and lung metastases [126, 127]. Mechanistically, silencing and/or overexpression of specific miRNAs affects the expression of numerous genes, including genes involved in EMT, endothelial recruitment, anoikis resistance, invasion, and colonization. For example, silencing of miR-200 s triggers EMT via ZEB1/2-dependent repression of E-cadherin upregulation or miR-21 upregulation which is correlated with active mTOR and STAT3 signaling and increased invasion, tumor growth, and survival. miR-29, which inhibits breast cancer metastasis, for example, targets genes with established roles in collagen remodeling and angiogenesis such as VEGFA, LOX, MMP2, ANGPTL4, and PDGF. Interestingly, micro-vesicles secreted by tumor cells are enriched for miR-9 and have a direct impact on stromal cells through the regulation of the endothelial cell migration.

5.5 Conclusions and Future Perspectives

In vitro and in vivo studies have significantly advanced our understanding of the metastatic process and identified numerous pathways and molecular mechanisms that control it (Fig. 5.1). However, some limitations of the models need

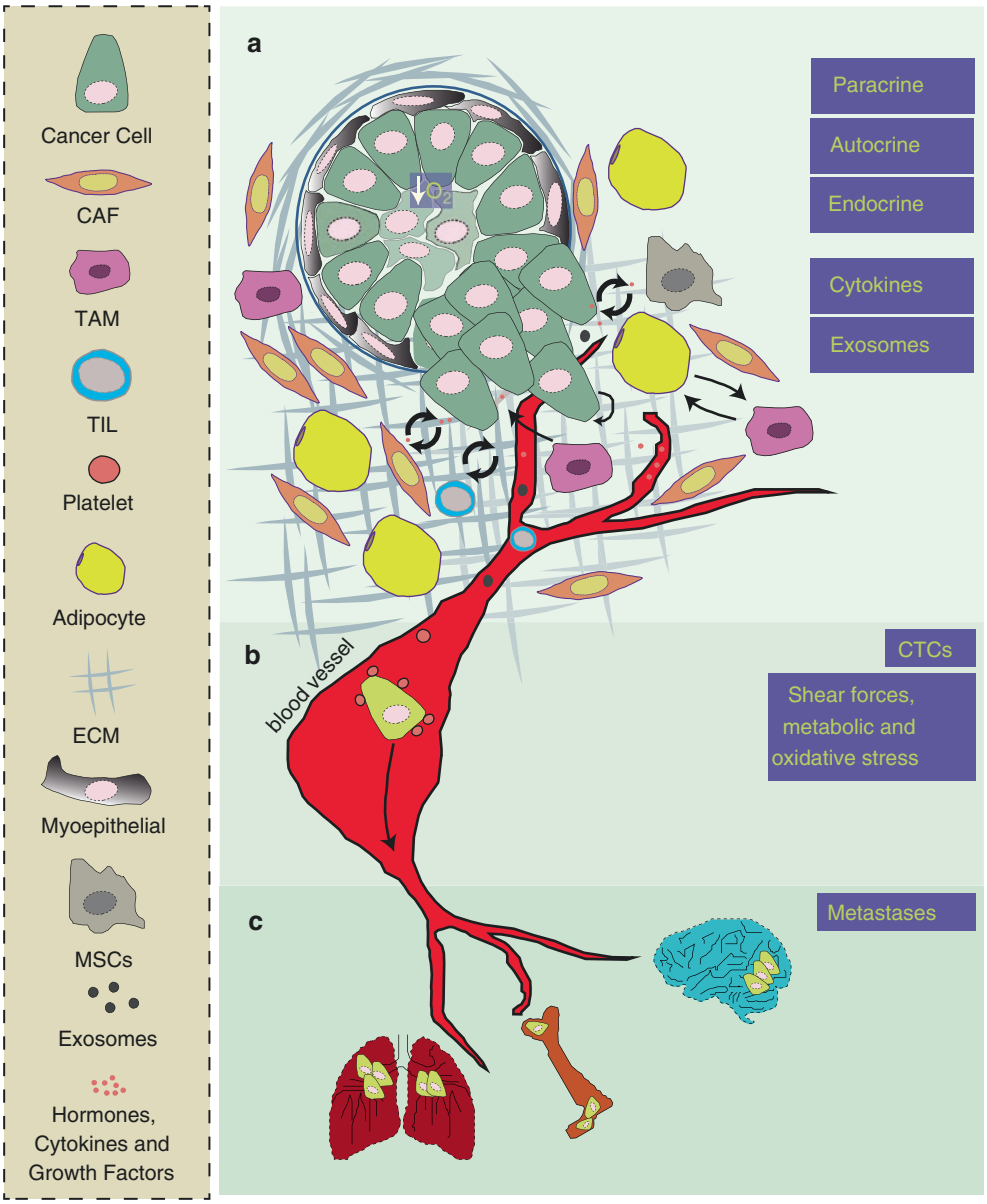

Fig. 5.1 Interactions of breast cancer cells with stromal cells and molecular factors of the tumor microenvironment: **a** Cells and molecular factors found at the primary breast tumor microenvironment and their heterotypic interactions, **b** Circulating tumor cells (CTCs) that have detached from the primary tumor travel in the bloodstream and constitute potential "seeds" for metastases, **c** Breast cancer cells that escape from the primary tumor and survive in the bloodstream frequently colonize and potentially can form metastases to distant organs

to be considered as we try to extrapolate the experimental findings to the clinical situation. The widely used animal models fail to fully reflect the heterogeneity of breast cancer with its many different histopathological and molecular subtypes. It cannot be excluded that some of the factors implicated in xenograft models and GEMMs of a particular subtype may have different roles in different breast cancer subtypes. In particular, a lot may remain to be learned about the hormone-dependent tumors because there are few preclinical models for the ER+ subtypes [128]. GEMMs are mostly ER negative, and few ER+ breast cancer cell lines grow as xenografts. We also need to consider that widely used xenograft models, in which large numbers of cells are injected either subcutaneously, directly into the bloodstream, or to a distal organ, create artifacts that affect the interpretation of the results, and they fail to recapitulate the complete metastatic process. The few ER+ cell lines that grow in vivo need to be provided with exogenous E2 [129]. This creates a nonphysiological systemic environment, which in turn impinges on the local microenvironments. A study with mice that had been xenotransplanted with MCF7 cells and were first hormone depleted by ovariectomy and subsequently hormonally stimulated suggests that ER+ micrometastases are exquisitely sensitive to E2 and progesterone [130]. We have recently demonstrated that the microenvironment is a determinant of the luminal phenotype; ER+ tumor cells grow well in the absence of exogenous hormones when they are engrafted into the milk ducts. Interestingly, in this model MCF-7 cells metastasize to the bones as well as lungs and brain [131]. As such it will be interestign to study the metastatic process in this new model.

Hence more complex models are necessary to improve our knowledge on heterotypic interactions and paracrine signaling and elucidate the role of numerous significant factors such as ECM stiffness and mechanical forces in the TME. 2D coculture, 3D coculture, organotypic slice culture, and in vivo findings need to ultimately be validated in patients.

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