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Role of the Tumor Microenvironment in Breast Cancer

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Key Words

Breast cancer · Cancer-associated fibroblasts · Immunoediting · Tumor-associated macrophages · Tumor microenvironment

Abstract

In recent years, it has been shown that breast cancer consists not only of neoplastic cells, but also of significant alterations in the surrounding stroma or tumor microenvironment. These alterations are now recognized as a critical element for breast cancer development and progression, as well as potential therapeutic targets. Various components of the breast cancer microenvironment, such as suppressive immune cells, soluble factors and altered extracellular matrix, act together to impede effective antitumor immunity and promote breast cancer progression and metastasis. Stromal cells in the breast cancer microenvironment are characterized by molecular alterations and aberrant signaling pathways, some of which are prognostic of clinical outcome. Several new therapies targeting stromal components are in development or undergoing clinical trials. We focus herein on the composition of the breast cancer microenvironment and concomitant molecular alterations, the specific interplay between various cell types and cancer cells, and the clinical implications of these findings.

Introduction

Emerging evidence suggests that tumors consist not only of neoplastic cells but also present a significantly altered surrounding stroma. Indeed, this tumor microenvironment is now recognized as a critical element for tumor development and progression, as well as a measurable parameter of response to treatment. Many studies have demonstrated significant epigenetic alterations leading to aberrant gene expression in the cells of the tumor microenvironment [1], and gene expression signatures derived from tumor stroma are predictive of clinical outcome [2]. In view of these recent findings, there is increasing interest in the breast cancer microenvironment as a prognostic factor as well as a potential therapeutic target, and new treatments directed against stromal components are in development. This review focuses on the composition of the breast cancer microenvironment, the alterations in molecular signatures of cells comprising this microenvironment, their interplay with cancer cells, and the possible clinical implications of these findings.

Composition

The breast cancer microenvironment can be considered at local (intratumor), regional (in the breast) and distant (metastatic) levels [3], each of which encompass multiple

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cell types such as fibroblasts, leukocytes, adipocytes, and myoepithelial and endothelial cells. They also include components [e.g. extracellular matrix (ECM)], soluble factors (e.g. cytokines, hormones, growth factors and enzymes) and physical properties (e.g. pH and oxygen content) [3].

Local Microenvironment

Cross-talk between epithelial and stromal cells is essential for the normal development and differentiation of the mammary gland. Physiological stroma maintains epithelial polarity and inhibits uncontrolled cell growth and neoplastic transformation [4]. For example, myoepithelial cells have been recognized as natural tumor suppressors in the breast and function as gatekeepers of tumor formation, since they produce the basement membrane (BM) and represent a physical barrier around luminal epithelial cells [5]. Studies in breast cancer xenograft models suggest that the loss of myoepithelial cells promotes the transition of ductal carcinoma in situ (DCIS) to invasive carcinoma [6]. Two models of the in situ-to-invasive carcinoma transition have been proposed: the 'escape' and the 'release' models [5]. The 'escape' model proposes that genetic changes in tumor epithelial cells enables them to invade tissue adjacent to the ducts, while the 'release' model suggests that an abnormal microenvironment leads to disruption of the BM and spread of the tumor epithelial cells into the stroma [5]. It is probably a combination of these two models that generates the key event of the in situ-to-invasive transition in breast cancer, emphasizing the necessity of changes in both the epithelial and stromal compartments for tumor formation and progression.

Normal myoepithelial cells have also been shown to suppress growth, invasion and angiogenesis of breast cancer cells [7]. In cancer-associated stroma, however, myofibroblasts and fibroblasts seem to promote tumorigenesis and metastatic spread through complex paracrine signaling [5], creating a receptive microenvironment and influencing cancer progression and metastasis. In many aspects, the associated tumor stroma resembles a healing wound with proliferation of fibroblasts and ECM remodeling but lacking physiological controls [8]. Accordingly, numerous studies have shown that inflammatory cytokines such as interleukin (IL)-6 promote breast cancer progression and metastasis by acting on breast cancer stem cells [9]. For example, the chemokine (C-X-C motif) ligand (CXCL)12 is physiologically important for initiating tissue regeneration and repair by recruiting C-X-C chemokine receptor type 4 (CXCR4)-positive stem and progenitor cells [10]. CXCR4/CXCL12 signaling in the breast cancer microenvironment results in increased migration and proliferation of stromal cells as well as secretion of matrix metalloproteinases (MMP) and consecutive tissue remodeling. Additionally, numerous studies have demonstrated that CXCL12 directly stimulates tumor cell migration and invasion as well as epithelial-mesenchymal transition via CXCR4 expression on cancer cells [10–12]. Thus, CXCR4 overexpression by breast cancer cells is associated with lymph node metastases and poorer clinical outcome [13].

Metastatic Microenvironment

Metastasis is a complex process in which circulating tumor cells extravasate through the capillary wall to colonize a new microenvironment [3]. Here, tumor cells either enter a 'dormant' state that can last for years or start to form micrometastases. Of note, circulating chemokines and cytokines from the primary tumor recruit bone marrow-derived cells that are then released into the circulation and subsequently create a so-called premetastatic niche even before tumor cell mobilization [14, 15]. Interestingly, co-traveling of fibroblasts together with cancer cells to metastatic sites has also been observed [16].

The formation of bone metastases is likely the most widely studied and best understood example of metastases, involving complex interactions between cancer cells and local cells such as osteoblasts, osteoclasts and hematopoietic stem cells [3]. Breast cancer cells secrete various cytokines and growth factors that promote the production of receptor activator nuclear factor κβ ligand (RANKL), which, in turn, leads to osteoclast activation and increased bone resorption [3]. Breakdown of bone then releases tumor-promoting factors, resulting in further bone destruction, representing a self-sustaining cycle [14]. Recently, RANKL has also been associated with the formation of lung metastases via CD4+ regulatory T cells (T_{reg}), suggesting that participation of distinct immune cells may be necessary for the formation of metastases [17]. Interestingly, animal studies have shown the possibility of multidirectional metastases in breast cancer, with dissemination of cancer cells not only from the primary tumor to the bone but also from bone to other distant sites and even back to the site of origin, suggesting that the bone microenvironment might be a key coordinator in the metastatic process [3, 18].

Molecular Alterations in the Breast Cancer Microenvironment

An innovative study by Allinen et al. [19] isolating each cell type comprising normal breast tissue, DCIS and invasive carcinoma showed that extensive changes in gene ex-

pression occurred in all cell types during cancer progression. The highest number of differently expressed genes was found in myoepithelial cells from normal breast tissue and DCIS, confirming dramatic differences in the microenvironment between normal breast tissue and in situ lesions. These investigators also showed that most of the differentially expressed genes encode secreted proteins and receptors. Two highly overexpressed genes encode the chemokines CXCL14 and CXCL12, which, in turn, bind to CXCR4 on cancer cells and contribute to their proliferation and migration [19]. In a similar study, Ma et al. [20] confirmed that the most significant gene expression alterations in the stromal compartment occur in the transition from normal breast tissue to DCIS. Additionally, they also showed elevated expression of several ECM-degrading proteases during the transition from DCIS to invasive carcinoma, suggesting that these proteases might play a role in the destruction of the normal BM. Since genetic alterations have only been detected in cancer cells [19, 21], aberrant gene expression in tumor-associated stromal cells are at least partly due to epigenetic changes, a hypothesis supported by the detection of significant changes in DNA methylation patterns of stromal cells in breast cancer [1, 22]. A major proportion of these aberrantly methylated genes encode transcription factors important for development and differentiation [1]. Since it has been postulated that tumor-associated myofibroblasts and fibroblasts develop from bone marrow-derived stem cells that are specifically recruited to the microenvironment of developing tumors [23], their epigenetic changes might be directly induced by factors produced by the tumors [5]. Thus, the tumors are active participants in shaping their microenvironment and ensuring favorable conditions. In addition to epigenetic changes, active cytokine and chemokine signaling is of significant importance.

Comparing gene expression profiles of tumor stroma from patients with breast cancer, Finak et al. [2] created a 26-gene prognostic predictor that predicts clinical outcome irrespective of the clinical subtype. They identified two distinct sets of involved genes reflecting hypoxia and angiogenesis that were linked to a poor outcome or indicated a Th1-like immune response linked to a favorable outcome. Additionally, a stromal gene expression signature that predicts response to chemotherapy in breast cancer has been identified [24], mainly encompassing genes associated with reactive stroma. These results strongly suggest that gene expression changes in tumor-associated stroma directly influence disease progression and outcome [2].

In view of the frequent epigenetic modulation in cells of the microenvironment, therapies specifically aimed at these epigenetic changes, such as histone deacetylase inhibitors, are currently under clinical investigation for the treatment of breast cancer in order to 'normalize' the altered tumor stroma and impede its tumor-supporting role [15].

Components of the Microenvironment and Their Interplay with Breast Cancer

Fibroblasts

The most abundant cell type in breast cancer stroma are fibroblasts, also called cancer-associated fibroblasts (CAF) [4]. They are known to secrete a variety of soluble factors, such as chemokines or growth factors, which modulate the tumor stroma and lead to enhanced tumor growth and invasion [4, 25]. Despite their regular morphology, several studies have revealed that CAF have distinct mRNA and protein expression profiles that distinguish them from fibroblasts in adjacent normal breast tissue [4]. For example, they show increased expression of genes related to development and morphogenesis, such as NOTCH2 [4, 26]. Additionally, a bidirectional signaling pathway between CAF and cancer cells suggests that CAF might influence the transcriptional profile of breast cancer cells [4]. Orimo et al. [27] showed that CAF from primary human breast cancers significantly enhanced tumor growth and angiogenesis in xenograft models. Recently, a metabolic partnership between catabolic fibroblasts and anabolic cancer cells with creation of a nutrition-rich environment has been proposed [28].

A number of origins have been proposed for CAF, including bone marrow-derived cells recruited to the tumor microenvironment [29], normal fibroblasts responding to signals generated by cancer cells [30] and an epithelial-mesenchymal transition of cancer cells [31]. As noted previously, it is now thought that the altered phenotype of CAF is mainly due to epigenetic modulation of the DNA [1]. Additionally, recent evidence suggests that the phenotype of CAF might also be influenced by intrinsic genetic variability [25]. The origin of such a genetic variability can be single nucleotide polymorphisms (SNP) [25]. SNP have been described for various genes, particularly those encoding metalloproteinases, i.e. enzymes involved in ECM modifications [32]. Holliday et al. [33] demonstrated that fibroblasts derived from women with an SNP genotype leading to high expression of MMP3 significantly promoted invasion of breast cancer cells compared with those from women lacking this SNP genotype.

While metalloproteinases produced by CAF seem to promote tumor invasion, other factors produced by fibroblasts such as caveolin-1 and podoplanin, which are associated with wound responses, have been associated with decreased nodal metastasis [4]. Analogously, loss of caveolin-1 in the fibroblast compartment of breast cancers was shown to be an independent predictor of nodal metastasis, early tumor recurrence and poor clinical outcome [34], while increased expression was associated with improved survival [35].

CAF may also play an important role in the formation of brain metastases in breast cancer patients since they are frequently found in brain metastases and also enhance the invasion, colony formation and transmigration of breast cancer cells in vitro [36].

Dendritic Cells

Dendritic cells (DC) play an important role in the induction of antitumor responses due to their ability to cross-present antigens to CD4+ and CD8+ T cells, thus activating them to attack neoplastic cells [37]. The maturation of DC depends on the local microenvironment wherein various factors influence the formation of either tolerogenic or immunosuppressive DC [37, 38]. Tumor-associated stroma shows an abundance of immature DC with impaired capacity to stimulate antitumor immunity [39]. Additionally, tumor-associated immature DC produce proangiogenic factors and enhance endothelial cell migration, thus actively promoting tumor growth [39]. This proangiogenic property is suppressed by DC maturation [39]. In fact, it has been shown that infiltration of mature DC into primary tumor lesions is associated with fewer metastases and a better clinical outcome [40]. Of note, tumor-associated cytokines, such as vascular endothelial growth factor (VEGF), IL-10 and prostaglandin E2, can steer DC maturation towards a regulatory phenotype, which inhibits T-cell proliferation [41, 42]. Induction of DC maturation, for example through molecularly defined triggers of DC activation such as Toll-like receptor ligands and CD40 agonistic antibody, is thus a potential therapeutic strategy in cancer immunotherapy because it not only augments host immune responses but also suppresses angiogenesis [39, 43].

Macrophages

Tumor-associated macrophages (TAM) form a major cell population in breast cancer and display a characteristic phenotype oriented towards promoting tumor growth and angiogenesis, tissue remodeling and suppressing adaptive immunity [25, 44]. They originate in blood monocytes recruited at the tumor site through factors secreted by neoplastic and stromal cells, such as chemokine (C-C motif) ligand 2 (CCL2). TAM produce many tumor-promoting factors such as VEGF and cytokines and enzymes that support invasion, angiogenesis and metastasis [25, 45]. In general, macrophages can be categorized as classically (M1) or alternatively (M2) activated [45]. During normal immunological responses, most macrophages are of the M1 phenotype and involved in Th1 cytokine responses to various pathogens. The M2 phenotype, on the other hand, is associated with Th2 cytokines and is involved in wound healing and tissue remodeling [25]. Most TAM belong to the M2 phenotype and enable cancer cells to survive and disseminate through secretion of IL-10, CCL2, CCL17, CCL22 and transforming growth factor (TGF)-β [45, 46]. Via the secretion of chemokines such as CCL22, they also suppress antitumor immunity by preferentially attracting T-cell subsets devoid of cytotoxic functions, such as T_{reg} [45]. Moreover, TAM have also been implicated in the formation of metastases. By continuous matrix deposition and remodeling, TAM facilitate the invasion of the surrounding tissue and also seem to assist in the tumor cell invasion of blood vessels [45, 47]. Numerous studies have linked high TAM levels to a worse prognosis in breast cancer [48, 49], suggesting that TAM depletion or reprogramming could represent a viable therapeutic strategy.

Lymphocytes

Tumor-infiltrating lymphocytes are emerging as one of the key players in the tumor microenvironment. The majority of tumor-infiltrating lymphocytes are T cells [50] that can be divided into CD4+ helper cells, T_{reg} with a CD4+, CD25+, FOXP3+ phenotype, and effector cells, such as natural killer cells and CD8+ T cells [25].

T_{reg} normally protect against autoimmune diseases by suppressing self-reactive T cells, but in the tumor microenvironment, this translates into blocking antitumor responses [25]. They are able to suppress a wide range of immune cells, including CD8+ T cells, natural killer cells, B cells and antigen-presenting cells [9]. Recently, it has also been shown that T_{reg} produce large amounts of RANKL, which activate RANK-expressing breast cancer cells and promote metastasis [17]. Accordingly, high numbers of T_{reg} are associated with a worse prognosis in breast cancer [51, 52]. It is believed that the tumor itself recruits T_{reg} through prostaglandin E2 secretion as well as TGF-β signaling, and suppresses the functions of effector cells through the secretion of IL-10 and TGF-β to create an immunosuppressive microenvironment [53,

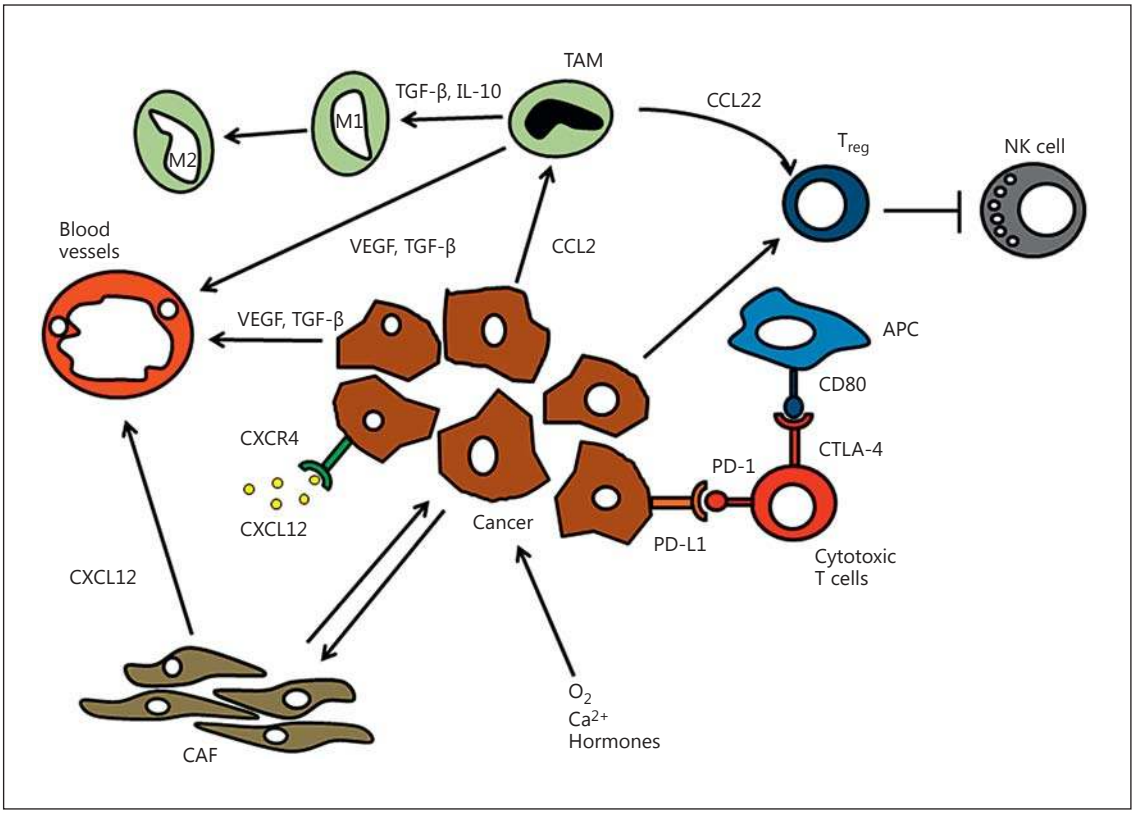

Fig. 1. Interplay between components of the tumor microenvironment and breast cancer cells. APC = Antigen-presenting cells; CTLA-4 = cytotoxic T-lymphocyte-associated protein 4; NK = natural killer.

54]. This process is called immunoediting [55] and has recently been recognized as another hallmark of cancer [54, 56]. Conversely, infiltration by CD8+ effector T cells is associated with longer breast cancer-specific survival, independent of other prognostic factors such as tumor grade, lymph node stage, tumor size, vascular invasion and HER2 status [50]. However, the effect of CD8+ cytotoxic T cells is regulated by the balance between co-stimulatory and co-inhibitory signals at so-called immune checkpoints [54, 57]. Immune checkpoint molecules such as programmed death-1 (PD-1), cytotoxic T-lymphocyte antigen 4 or B- and T-lymphocyte attenuator inhibit T-cell function, thus preventing inappropriate immune reactions and limiting the extent and duration of immune responses. PD-1 is the most extensively studied immune checkpoint receptor and is increasingly recognized as having a crucial role in immunoediting [58]. PD-1 is a co-inhibitory receptor which, through binding of its ligands PD ligand (PD-L) 1 and PD-L2, inhibits T-cell function [57]. However, this pathway can also be used by tumor cells to attenuate or escape antitumor T-cell immunity and create an immunosuppressive microenvironment, a phenomenon termed 'molecular shield' that facilitates tumor progression. PD-1 expression on tumor-infiltrating lymphocytes as well as PD-L1 expression by tumor cells has been demonstrated in multiple human tumors, including breast cancer [59–62], where it is associated with a worse prognosis. The use of this pathway by cancer cells might also explain why tumor growth is seldom controlled despite the induction of cancer-specific T cells in many trials of adoptive cell therapy, with concomitant infiltration of tumor sites [63]. Targeting PD-1 or its ligand PD-L1 with antibodies capable of inhibiting this pathway thus represents an emerging therapeutic option in breast cancer [57].

For an overview over the various cellular components of the breast cancer microenvironment and their interplay with cancer cells, see figure 1.

Extracellular Matrix

The ECM is a complex network of proteins that surrounds and stabilizes cells. It consists of three main types of proteins: structural proteins (e.g. collagen/elastin), glycoproteins (e.g. fibronectin) and proteoglycans (e.g. chondroitin sulfate) [64]. While initially regarded as a stable structure solely providing support, newer studies indicate that the ECM is surprisingly dynamic and versatile, and represents a key player in cellular processes such as cell growth, proliferation and migration [64, 65]. The ECM is often disorganized and deregulated in cancer, leading to abnormal behavior of cells through feedback regulatory mechanisms [65]. The most important contributors to altered ECM metabolism in cancer are CAF and immune cells [65].

The main protein component of the ECM is collagen. Its integrity plays a key role in cancer development, since degradation of collagen IV by proteases leads to cancer cell invasion through the BM [66]. In addition to structural roles, the ECM also guides the passage of cytokines and growth factors between cells, thus enabling intercellular communication [64]. Extracellular proteinases such as MMP maintain homeostasis of the ECM and are a second important key player in the tumor microenvironment. Altered proteinase activity is prevalent in cancer and shows an association with patient outcome [65]. In view of this, several extracellular proteinase inhibitors are in development for use in human cancer [64]. While synthetic MMP inhibitors have shown efficacy against malignant tumors in preclinical studies, the outcomes of clinical MMP inhibitor trials exceeding phase II have been disappointing [67].

In addition to changes in its biochemical properties, the architecture as well as the physical and biochemical properties of the ECM is fundamentally different in cancer [65]. Compared with normal stroma, breast cancer stroma is typically stiffer [65], which is one of the reasons that breast cancers become palpable. This increased tissue stiffness can be attributed to lysyl oxidase (LOX), which cross-links collagen fibers [65]. Studies in mouse models have shown that overexpression of LOX promotes breast cancer progression and invasiveness, whereas inhibition of LOX reduces breast cancer incidence [68]. Furthermore, up-regulation of LOX has been found in metastatic cancer sites [69]. Increased mechanical force as result of LOX activity presumably facilitates colonization of the metastatic niche by cancer cells [65]. These altered biochemical properties also play an essential role in tumor angiogenesis by facilitating vessel growth [65].

Finally, aberrant ECM also promotes tumor growth by preventing T cells from undergoing their normal differentiation and maturation, thus sabotaging the immune system in its efforts to control tumor cells [65]. For example, hyaluronan can induce T_{reg} differentiation from effector memory T-cell precursors [70].

In summary, abnormal ECM not only promotes cancer cell transformation and tissue invasion, but also helps to create a tumorigenic microenvironment that further facilitates cancer progression [65]. Determining whether abnormal ECM can be used as a therapeutic target is thus an important issue of future cancer research.

Breast Cancer Microenvironment as a Therapeutic Target

In the past few years, considerable effort has been made to therapeutically target different components of the tumor microenvironment. Since stromal cells are genetically stable and thus unlikely to develop chemoresistance, they represent promising therapeutic targets [71]. Currently, three types of therapies targeting the breast cancer microenvironment are in clinical practice: aromatase inhibitors (blocking the aromatase enzyme expressed in the stroma and elsewhere), angiogenesis inhibitors (e.g. VEGF inhibitors) and HER2 inhibitors (blocking HER2 signaling on cancer cells triggered by stromal growth factors) [72]. While aromatase inhibitors and HER2 inhibitors are considered standard therapies in breast cancer, the effectiveness of angiogenesis inhibitors is less clear [72].

VEGF inhibitors have been shown to extend progression-free survival in breast cancer when combined with other chemotherapeutic drugs [73] but have also been associated with significant adverse reactions [74], and studies in animal models have raised concerns that they may even enhance tumor progression and metastasis [75]. Thus, while targeting the breast cancer microenvironment is an exciting possibility, highly deleterious side effects due to disruption of homeostatic functions are frequently encountered [15].

Components of the immune system have also been investigated as possible therapeutic targets. One theory is that reestablishing an antitumor inflammatory milieu might control tumor growth. For example, tumor necrosis factor-α antagonists induce stabilization and partial response in breast cancer [76]. Other work has aimed at reprogramming TAM from the M2 to the M1 phenotype [77, 78], or at inhibiting TAM recruitment to the tumor

Table 1. Overview of microenvironment components and their therapeutic potential

Component	Mechanistics	Therapeutic potential	Clinical evidence
CAF	MP production	MP inhibitors	No conclusive clinical benefit
	TGF-β secretion	TGF-β blockers (IN-1130)	[93, 94] (mouse models)
	VEGF secretion	VEGF inhibitors (bevacizumab)	[73, 95–97]
	CXCL12 secretion	CXCR4 antagonists (byrostatin-5, AMD3100, MSX-11)	[82, 98] (in vitro and mouse models)
DC	Inhibition of T-cell proliferation	DC vaccines	[99, 100]
	T_{reg} recruitment		
	Immunosuppression		
TAM	M2 polarization	TAM inhibitors (trabectedin)	[45, 80]
	ECM remodeling through MP secretion	TAM depletion (doxorubicin)	[81]
	Angiogenesis	CSFR1 antagonists	[79] (mouse models)
	T_{reg} recruitment		
T_{reg}	Suppression of immune cells	Anti-CD25 antibodies	[101]
	RANKL production	Anti-RANKL antibodies (denosumab)	[102]
T_{eff}	T-cell exhaustion through	Anti-PD-1 antibodies	[84–88]
	PD-1/PD-L1 signaling	Anti-PD-L1 antibodies	
ECM	ECM remodeling	ECM degradation inhibitors	[103]
	Increased stiffness	LOX inhibitors (magnolol, β-aminopropionitrile)	[90, 91] (in vitro and mouse models)

T_{eff} = Effector T cells; MP = metalloproteinases.

site, which improves the efficacy of chemotherapy [79]. In breast cancer mouse models, blockade of macrophage recruitment with colony-stimulating factor-1 receptor (CSF1R) antagonists, in combination with paclitaxel, led to improved survival by slowing primary cancer development and reducing pulmonary metastasis [79]. In humans, the combination of a CSF1R inhibitor with chemotherapy is currently being tested in a phase Ib/II study in metastatic breast cancer patients (NCT01596751). Furthermore, trabectedin, a drug with selective cytotoxic effects on TAM that spares the lymphoid subset, has shown encouraging results in a phase II trial in breast cancer patients [45, 80]. An intriguing recent discovery is that overexpression of histidine-rich glycoprotein by tumor cells induces TAM conversion from the M2 to the M1 type in murine syngeneic tumor models, leading to decreased tumor growth and fewer pulmonary metastases [78]. Considering conventional chemotherapy, it seems that administration of doxorubicin leads to depletion of TAM as well as a shift in myeloid cell infiltration from immunosuppressive TAM to inflammatory monocytes in mice [81]. Additionally, since TAM infiltrate breast cancers spontaneously, recent studies have focused on these cells as natural vectors to deliver therapeutic substances into the tumor [45].

Multiple agents to target the CXCL12/CXCR4 axis are currently being developed. Among these, the anti-CXCR4 drug AMD3100 (Plerixafor®) is the most studied and has been shown to decrease the metastatic potential of different types of tumors, including breast cancer, in animal models [10, 82]. AMD3100 is currently being tested in phase I/II trials as a therapeutic option in acute myeloid leukemia, but has not yet been tested in breast cancer patients [83]. Another CXCR4 inhibitor, MSX-122, is currently being tested in a phase I trial for advanced malignant diseases [10, 83], but potential side effects on the stem cell compartment in normal tissue may prevent successful clinical use [10]. Another strategy targeting immune cells is blockade of the PD-1/PD-L1 pathway. Multiple recent phase I clinical trials investigated the effects of fully human anti-PD-1 and anti-PD-L1 antibodies in patients with various types of advanced solid cancers [84–87]. Of note, success was documented in cancers that have long been considered resistant to immunotherapy, such as non-small cell lung cancers. In addition, some of these responses were durable, suggesting that targeting the PD-1/PD-L1 signaling pathway is likely to develop into an important treatment modality for patients with advanced malignancies. Interestingly, preliminary results of an ongoing Phase Ia trial testing an anti-PD-L1 antibody (MPDL3280A) in a small cohort of metastatic triple negative breast cancer patients showed objective responses in 24% and complete response in 10% of patients, respectively [88]. Therefore, further de-

fining the importance of the PD-1/PD-L1 signaling pathway in breast cancer is of significant clinical relevance, since it will lead to important insights into whether antibody therapies targeting this pathway will be of clinical use in selected breast cancer patients.

Strategies aimed at depleting T_{reg} are also being investigated. Since T_{reg} are highly dependent on IL-2 for their survival, neutralization of IL-2 with specific antibodies can substantially reduce the number of T_{reg} and their suppressive activity, a concept that has been proven by Curiel [89] in patients with advanced carcinomas, including breast carcinoma [54].

Strategies to reduce the stiffness of tumor stroma are also being investigated. For example, two tested LOX inhibitors have shown promising results in breast cancer cell cultures and mouse models [90, 91].

Table 1 gives an overview of the various components of the breast cancer microenvironment and their therapeutic potential. In addition to drugs being developed against novel targets in the microenvironment, the observed antitumor efficacy of several older therapeutic agents seem to be mediated through the microenvironment [72]. For example, it is now recognized that bisphosphonates have direct antitumor effects through modulation of angiogenesis and immune cells [72, 92]. Furthermore, following administration of classic cytotoxic chemotherapies, the microenvironment seems to acquire an altered phenotype in response to signals derived from the killed cancer cells [72], and it is speculated that this altered phenotype independently inhibits tumor growth. For example, cells of the innate immune system can be activated by proteins secreted by dying cells, such as SIN3A-associated protein 130 [45].

Concluding Remarks

The tumor microenvironment is increasingly recognized as a key player in tumor progression and as a promising therapeutic target in breast cancer. Composed of various cellular elements as well as ECM and soluble growth factors, it represents a complex network of cellular signaling and distinct tissue properties. Suppressive immune cells, soluble factors and altered ECM act together to impede effective antitumor immunity and promote breast cancer progression and metastasis. The surrounding inflammation induced during natural tumor progression is possibly responsible for the failure of the immune system to effectively restrain breast cancer expansion. Therefore, new therapeutic strategies aim at 'normalizing' the surrounding stroma as well as at modulating the immune system and enhancing antitumor activity. To achieve this, the identification of protumorigenic signaling pathways is critical and requires further study.

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