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REVIEW ARTICLE

Breast cancer development coordinated by changes in microenvironment setting

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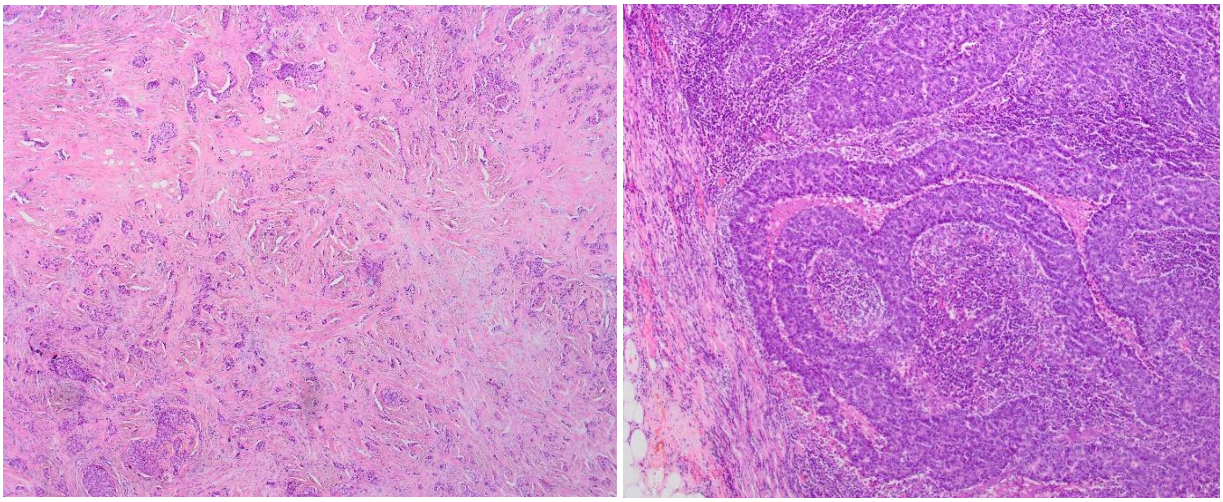
ABSTRACT

Breast cancer is the most common malignancy in women worldwide, and one of the leading causes of cancer death. This disease shows a significant heterogeneity due to its genomic and histological diversity. Breast cancer is classified by pathologic features (i.e. histological subtype, tumor grade) and gene expression profiles (i.e. molecular subtypes). There are complex mechanisms implicated in its progression and the development of chemotherapy resistance. In recent times, tumor stroma is increasingly being recognized as an important factor which influences tumor pathogenesis and progression. Tumor-stromal cells interactions are involved in many phases of tumor growth, by modulating different cellular processes. Tumor-infiltrating lymphocytes are proven to be clinically significant as they correlate with good prognosis, especially in triple-negative and HER2-positive breast cancer patients. However, tumor-infiltrating lymphocytes are just one of the many components of the tumor microenvironment, which includes fibroblasts, macrophages, adipocytes, vascular cells etc., but also non-cellular components. One of the main cellular components of the tumor microenvironment are the fibroblasts which are activated and differentiated into breast cancer associated fibroblasts. They secrete many growth factors, cytokines, and chemokines which influence tumor growth and dissemination. Tumor microenvironment could be a source of new biomarkers with a potential predictive and prognostic significance. This review highlights the tumor microenvironment as an important contributor to the process of cancer development with an overview of the main components and the potential impact on the prognosis of breast cancer. It's important to expand our understanding and knowledge of tumor-stromal signalling processes which may lead to the development of more successful and individualized therapeutic strategies.

1. Introduction

Breast cancer is the most commonly diagnosed cancer worldwide and one of the major causes of global cancer-related mortality.[1] The global burden of breast cancer is on the rise but there are variations among different countries.[2] Breast cancer is not only comprised of tumor cells, but also of the tumour microenvironment (TME). Breast cancer shows significant diversity with its different special histological subtypes and morphological patterns, but the breast cancer stroma is also very heterogeneous (*Figure 1.*). A lot of research has been done trying to find strategies to improve survival outcome. Advances in the field of medicine have enabled a better understanding of tumor progression of breast cancer and allowed the distinction of different genetic profiles of breast cancer and the use of precision-medicine strategies in its treatment.[3,4,5] Breast cancer can be divided into different subtypes (luminal A, luminal B, HER-2 positive and triple negative breast cancer) depending on their expression of estrogen receptor, progesterone receptor, HER-2 and Ki-67, which differ in their prognoses. Luminal A and B tumors usually show a good response to endocrine therapy (such as tamoxifen, fulvestrant, aromatase inhibitors). Tumors that are HER2+ have a tendency to grow faster, but specific anti-HER2 therapy has improved the patients' survival rate. Triple-negative breast cancer subtype shows a very aggressive behavior, has the worst prognosis among BC subtypes and has less specific therapeutic options than the others. The development of new treatment strategies depends on our understanding of the pathophysiology of this tumor. Tumor microenvironment consists of extracellular matrix and many different cell types, including fibroblasts, macrophages, lymphocytes and endothelial cells (*Figure 2.*). Studies have shown that cancer cells only thrive in an altered microenvironment which has an important role in tumour progression and therapy resistance.[6,7,8]



1.A. 1.B.

Figure 1. Breast cancer stroma. A) Invasive breast carcinoma of no special type with medullary pattern showing prominent tumour-infiltrating lymphocyte infiltrate. B) Invasive breast carcinoma of no special type with fibrotic stroma.

Tumour microenvironment is comprised of a variety of non-cancerous cells present in the tumour. These include fibroblasts, macrophages, endothelial cells, infiltrating inflammatory cells and adipocytes together with extracellular matrix components and signalling molecules, such as cytokines and growth factors.[9,10] Breast stroma composition influences breast density, which plays a part in cancer development. There is evidence that some characteristics of the extracellular matrix (ECM) (e.g. density, stiffness, organisation) influence tumor cell growth and survival, and promote metastasis.[11] Different constituents of the tumor microenvironment have an effect on cancer development and resistance to therapy. In the following discussion we will summarise the components of tumour microenvironment and their role in tumor progression. However, it is important to emphasize that the extracellular matrix is also dynamic, and transforms in conformity with the state of tumor development, which is sequentially influenced by extracellular matrix.[12]

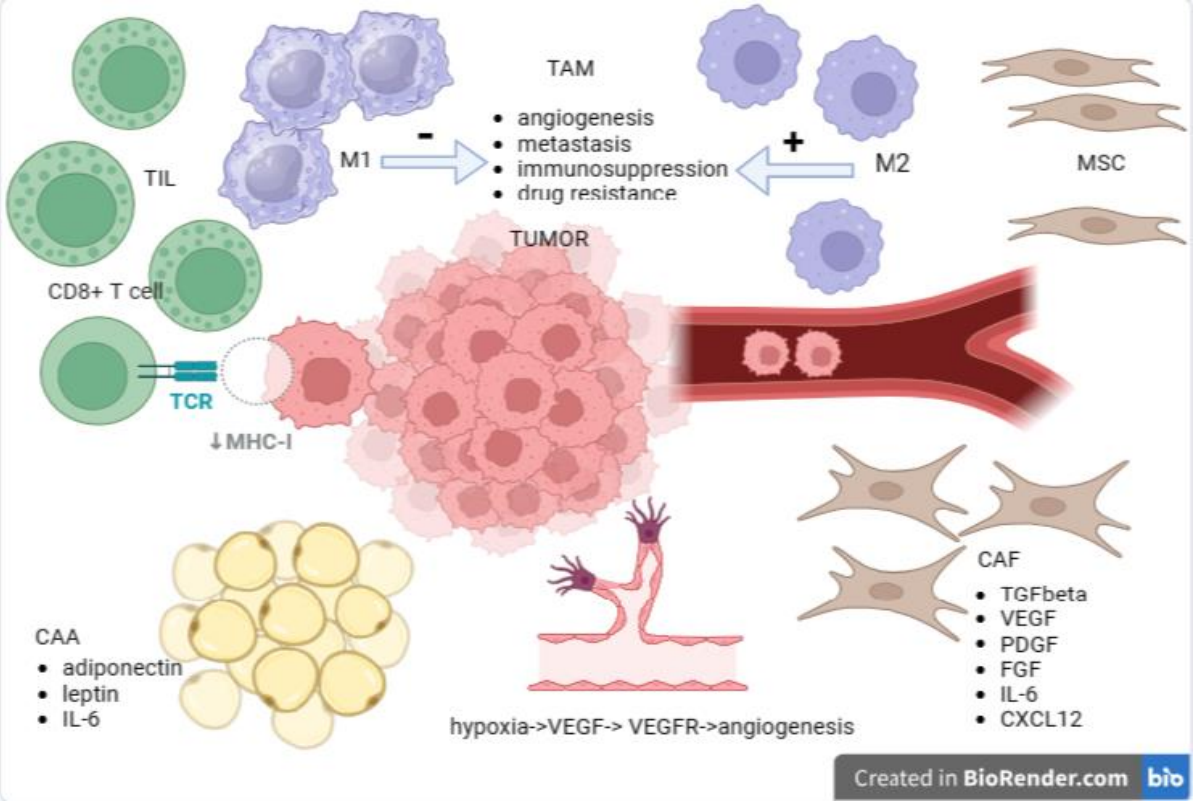


Figure 2. Tumor microenvironment. Interactions between cancer cells and TME are involved in all stages of tumor development. The most direct way cancer cells interact with the TME is through paracrine signaling (by secreting of growth factors, cytokines and chemokines by tumor cells and the stromal cells).

1.1. Cancer associated fibroblasts

Cancer associated fibroblasts (CAFs) are cells of mesenchymal origin that are activated by the inflammation and fibrosis during tumor development.[13] These are one of the most copious cell types in tumour microenvironment, and perform many different functions, e.g. extracellular matrix deposition and modulation of immune response.[14] They are distinguished by their morphology, association with tumor cells, and absence of epithelial, endothelial or hematopoietic cell markers.[15] Cancer associated fibroblasts show some morphological differences from resting fibroblasts; they are larger, spindle-shaped with indented nuclei and branching cytoplasm, but the difference is mainly functional. They show increased proliferative, migratory, and secretory functions, higher metabolical activity and increased extracellular matrix production. Their collagen production is increased and aberrant.[16-19] Cancer associated fibroblasts are derived from different cell precursors, but their origin is not entirely clarified. They can evolve from resident fibroblasts or from mesenchymal stromal/stem cells, which express similar surface markers. The transition from fibroblasts to CAFs is mostly irreversible.[20-22] The research on this specific population of cells is impeded by the lack of a pan-specific marker, and the absence of a consensus for biomarkes to identify CAFs, which makes them very difficult to define and distinguish from other mesenchymal cells. They generally express mesenchymal biomarkers (e.g. vimentin, α-SMA, FAP, PDGFR-α). There are different phenotypes of CAFs which vary between tumor types.[23-25] Some studies of triple-negative breast cancer and ovarian cancer have demonstrated 3–4 CAF subtypes (CAF S1–S4) based on the difference of expression of fibroblast markers (FAP, integrin β1/CD29, α-SMA, S100-A4/FSP1, PDGFRβ, and caveolin-1).[25,26] It seems that these CAF subtypes are different functional fibroblastic states, and not static types.[27] As mentioned earlier, CAFs secrete more cytokines than the resting fibroblasts. Some of these are TGFβ, PDGF, FGF, HGF, VEGF, CXCL12, IL-6 etc., which are tumor-promoting.[27]

1.1.1. Transforming growth factor-β (TGF-β)

is a cytokine that regulates proliferation, migration, and differentiation of cells. It has an important role in inflammation and tissue repair. Changes in TGF-β signaling pathway is involved in cancer development. In the early stages of cancer development TGF-β has a tumor-suppressing effect by inducing cytostasis and apoptosis. But in the later stages of tumor growth, tumor cells become resistant to TGF-β tumor suppressive effect, and TGF-β starts to act as a tumor promotor by stimulating the epithelial-mesenchymal transition (EMT) which causes metastasis and therapy resistance. TGF-β also stimulates tumor growth by initiating tumor angiogenesis, activating CAFs and helping the tumor cells to escape the hosts immune response.[28] Transforming growth factor-β causes immunosuppression in the TME and promotes tumor growth, metastasis and therapy resistance. Transforming growth factor-β signaling regulates tumor metabolic reprogramming in the tumor metabolic microenvironment (TMME). Cancer is characterised by the abnormal regulation of cellular metabolism. Cancer cells and stromal cells in the TME adapt their metabolism of glucose, amino acids

and lipids. The changes in the tumor metabolism cause the accumulation of nutrients, substrates, metabolic intermediates and final metabolites intra- or extracellularly, forming a reprogrammed metabolic environment in the tumor, TMME. Investigating the role of TGF-β signaling in the TMME is important in finding new cancer therapy strategies.[29] The dual role of TGF-β, acting as a tumor suppressor and a tumor promoter is somewhat unclear. Transforming growth factor-β has a systemic immunosuppressive role. Neutralizing TGF-β stimulates CD8+ T-cell and NK-cell anti tumor activity and causes neutrophil recruitment and activation. Transforming growth factor-β also regulates inflammatory and immune cell infiltration and CAF recruitment in the TME. Understanding the role of TGF-β in mediation between cancer cells and host immunity should help in the development of effective TGF-β antagonists.[30]

1.1.2. Platelet-derived growth factor (PDGF) family is composed of four monomeric polypeptide chains (PDGFA, PDGFB, PDGFC and PDGFD) which form homodimers and heterodimers to produce biological effects. In breast tissue, PDGFR is expressed in stromal cells, but not within the normal epithelium, however in breast cancer cells PDGFRα and PDGFRβ may be upregulated which leads to autocrine signaling.[31] The PDGF/PDGFR axis is important in promoting tumorigenesis and potential target for therapy of several types of cancer.[32] Platelet derived growth factor receptors are considered to be among key regulators of the tumor microenvironment in many malignancies, such as breast cancer. In some tumors PDGFRs are activated and directly implicated in tumor cell proliferation. High stromal expression of PDGFRβ is associated with poor prognosis in breast cancer.[33]

1.1.3. Fibroblast growth factor receptor (FGFR) family incorporates four receptor tyrosine kinases (FGFR1, FGFR2, FGFR3, and FGFR4) which bind the FGF ligands. The FGF/FGFR axis has been implicated in cancer development, metastasis, and resistance to therapy. Aberrant FGF/FGFR activation also occurs in breast cancer which makes it a potential target in anti-cancer strategies.[34] Three main types of FGFR alterations in cancer are gene amplification, gene fusion and gain-of-function mutation. Fibroblast growth factors are secreted not only by CAFs but by other stromal cells as well as cancer cells, and they play a key role in the tumor microenvironment, e.g. FGF2 activates dermal fibroblasts by downregulating the TP53 gene. Fibroblast growth factors, along with VEGF and angiopoietin, are also important in angiogenesis. Since FGFs are involved in the maintenance of homeostasis, FGFR inhibitors may induce adverse effects, such as endocrine or metabolic abnormalities.[35]

1.1.4. Vascular endothelial growth factor (VEGF) is mostly produced by the endothelial cells, but it is also secreted by tumor cells, some stromal cells and the immune cells of the TME. Besides its functions in angiogenesis it also has an immunosuppressive effect in cancer. There are many members of the VEGF

family, such as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F etc. which carry out their functions by binding to their receptors. Vascular endothelial growth factor is an important angiogenic factor and an immunomodulator of the TME. For example, VEGFs can suppress antigen presentation, stimulate activity of Tregs and TAMs, which promotes immunosuppression of TME. Vascular endothelial growth factors can also promote tumor development by directly interacting with tumor cells by activating tumor cell receptors through autocrine and paracrine mechanisms.[36]

1.1.5. Stromal cell-derived factor-1 (SDF-1, more commonly known as CXCL12) is a member of the family of CXC chemokines. This chemokine is expressed in many different tissues and has important roles in embryogenesis, organ development and angiogenesis. Chemokine CXCL12 binds to CXCR4 (CXC chemokine receptor 4 which is also known as CD184) and to CXCR7/ACKR3. CXCL12/CXCR4 axis also has a role in TME where it promotes tumor angiogenesis, proliferation of tumor cells, immune cells recruitment and stimulates immunosuppression. Cancer associated fibroblasts and tumor cells can both secrete CXCL12.[37] Dysregulation of CXCL12/CXCR4 and CXCL12/CXCR7 axis has been shown in many different tumors. CXCL12/CXCR4 initiates different pathways that regulate transcription, chemotaxis and survival of cells. Tumor microenvironment rich in CXCL12 can lead to immune checkpoint inhibitors therapy resistance and targeting the CXCL12/CXCR4 axis can sensitize tumors to its effect.[38]

1.1.6 Interleukin-6 (IL-6) is a member of interleukin-6/glycoprotein130 cytokine family which is a group of signaling molecules with many different functions. This cytokine family is involved in many processes, including hematopoiesis, inflammation, tissue remodeling, cell differentiation and cancer development. Each member binds to its specific receptor which starts signal transduction and results in the activation of several downstream pathways, JAK-STAT3, Ras-Raf MEK/ERK, and PI3K/AKT being the main ones. In breast cancer the most important one is JAK-STAT3. Interleukin-6 is secreted by breast cancer cells and different cells of the TME, e.g. mesenchymal stem/stromal cells, CAFs and adipocytes. Interleukin-6 plays an important role in tumor cell motility, EMT and cancer stem cell self-renewal. Interleukin-6 has been associated with resistance to endocrine therapy and trastuzumab. Antagonists of IL-6 show a potential for therapeutic use by diminishing that effect.[39] Higher levels of IL-6 have been detected in the serum of breast cancer patient and tumor site in some cancers including breast cancer, which has been associated with poor prognosis. Some studies have demonstrated a better response to treatment after downregulation of IL-6.[40]

Cancer associated fibroblasts add to tumor-promoting inflammation and fibrosis.[15] They can also induce epithelial-mesenchymal transition and promote tumor growth and cancer cell migration via IL-6.[41,42] Cancer associated fibroblasts have been shown to have a role in chemotherapy resistance. Some in vitro experiments have demonstrated that DNA

damage induced by chemotherapy actually caused increased cancer cell invasion and survival through paracrine signaling via cytokines and exosomes.[43] Transforming growth factor-β, which is a mediator of fibrosis and one of the most important factors secreted by CAFs, also mediates crosstalk between CAFs and cancer cells. Some studies have shown that the inhibition of TGFβ signaling can significantly inhibit tumor growth and metastasis.[44] Genotoxic stress induced by chemotherapy can also cause the release of inflammatory, angiogenic, mitogenic and pro-EMT factors.[45] Irradiated fibroblasts can become senescent but also convert to a CAF phenotype.[46-48] Cancer associated fibroblasts influence the tumor microenvironment secreting proinflammatory cytokines (e.g. IL-6) and stimulate immunosuppressive signaling pathways by expressing the ligands such as CXCL12, CXCL1 and G-CSF.[49-51] Cancer associated fibroblasts also prevent CD8+ cytotoxic T cell activity and recruitment partly by TGF-β and CXCL12.[52-54] The CXCL12/CXCR4 axis is significant in tumor progression and immunosuppression. Chemokine CXCL12 secreted by CAFs recruits CXCR4-positive endothelial progenitor cells and Tregs, which stimulates angiogenesis and cancer growth.[55,56] Blocking of CXCR4 signaling in CAFs using the CXCR4 inhibitor plerixafor reduced fibrosis, increased cytotoxic T cell population, decreased the number of immunosuppressive cells, and increased the effectiveness of checkpoint inhibitors.[57]

1.2. Tumor-associated macrophages

Of all the cells in the tumor microenvironment, tumor-associated macrophages (TAMs) are the most prevalent; they constitute more than 50% of the tumor mass in most solid tumors.[58,59] Macrophages, as members of mononuclear phagocyte system, are the most abundant mononuclear inflammatory cells in breast cancer. They are considered to be involved in every step of tumor progression. Macrophages can differentiate into different functional phenotypes, which is determined by the signals from the tumor microenvironment. There are two categories of TAMs; tumour inhibitory (M1) and tumor promoting (M2). Tumor inhibitory M1 macrophages are classically activated macrophages that express pro-inflammatory genes, and M2 are alternatively activated macrophages that express anti-inflammatory genes. Tumour promoting M2 macrophages secrete CHI3L1, which has been associated with metastasis of breast cancer cells in vitro and in vivo.[60] Research suggests that increased M1 macrophages in the tumor microenvironment are connected with reduced tumor aggressiveness, and increased M2 macrophages are associated with tumor growth.[61] Tumor promoting M2 macrophages stimulate and facilitate angiogenesis, metastasis, immunosuppression and drug resistance.[62] A study has shown that clodronate can reduce blood vessel density in tumor tissue by depleting TAMs.[63] Tumor associated macrophages are attracted to hypoxic regions of tumors, where they release hypoxia-induced chemokines (e.g. VEGFA, endothelins and CXCL12).[64,65] Nevertheless, macrophages can also demonstrate immunosuppressive activity.[66] Tumor associated macrophages can release anti-inflammatory cytokines which suppress effector T-cell and NK-cell cytotoxicity.[67] Interaction between active T cells which express CTLA-4 receptor and TAMs expressing CD80 and CD86 resulted in

reduction in cytotoxicity and inhibition of T cell activation. Production of chemokins allows TAMs to recruit immunosuppressive cells such as Tregs to inhibit a cytotoxic T-cell response.[68] Tumor associated macrophages also influence the numbers of cancer stem cells (CSCs). M-CSF, ICAM-1 and ephrin, secreted by TAMs, can increase the survival and renewal of CSCs, which leads to tumor growth and chemotherapy resistance.[68,69] M2 phenotype TAMs can lead to resistance to therapy.[70] For example, TAMs have been linked to resistance against tamoxifen in postmenopausal women with breast cancer.[71] One possible mechanism of chemoresistance is secretion of IL-10 which upregulates BCL-2 and STAT3 expression activating IL-10-STAT3-BCL2 pathway.[72] Transforming growth factor-β facilitates epithelial-mesenchymal transition, a mechanism through which epithelial cancer cells transform their phenotype into a mesenchymal-stem cell one which results in a more aggressive behaviour.[73,74] Interleukin-6, secreted by TAMs, binds to IL-6 receptor on cancer cell membranes[75] which causes the activation of the JAK/STAT3 signaling pathway, resulting in homodimerization of two STAT3 molecules that work as a transcription factor in tumor cell nucleus.[76,77] There are studies which show correlation between STAT3 activation and the transcription of genes promoting angiogenesis, proliferation, epithelial-mesenchymal transition and cancer cell mobility in breast cancer.[75,76] TAMs also increase resistance against antiangiogenic therapies in breast cancer tissue by releasing CCL18 and VEGFA and promoting angiogenesis.[78] TAMs have also been linked to tumor cell metastasis.[79] It has been suggested that the interaction between cancer cells and TAMs are important for the cancer cell intravasation.[80] Coculture of tumor cells and macrophages increases expression of MMP2 and MMP9, which degrade the proteins in the extracellular matrix and facilitates metastasis.[81,82]

Macrophages are also recruited to the metastatic site of breast ("metastasis-associated macrophages"). These macrophages promote extravasation and growth of cancer.[83] One of the strategies to reduce the number of TAMs is to inhibit their recruitment to the tumor microenvironment.[84] Anticathepsin D antibody has been shown to inhibit TAM recruitment by lowering TGFß levels in triple-negative breast cancer.[85] In one study liposomal Zoledronic acid reduced the number of TAMs and reduced angiogenesis and breast cancer growth in triple-negative breast cancer.[86] Some studies have shown that anti-VEGF-antibody in combination with Avastin or Bevacizumab can inhibit macrophage infiltration and prevent TAMs from secreting proangiogenic factors, which improves the effect of antiangiogenic therapies.[87,88]

1.3. Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) are mononuclear immune cells that infiltrate the tumor.[89] They are more frequent in high-grade breast cancer (i.e. TNBC and HER2-positive breast cancer).[90-92] T-lymphocytes include CD4+, CD8+ and T-regulatory cells (Treg)[93] and the main component of TILs are T-lymphocytes (CD3+).[94,95] An association between high degree of TIL infiltration and better prognosis in triple-negative and HER2-positive breast cancer has been found.[96-98] High numbers of TILs are also in good correlation with pathological complete response (pCR) to neoadjuvant

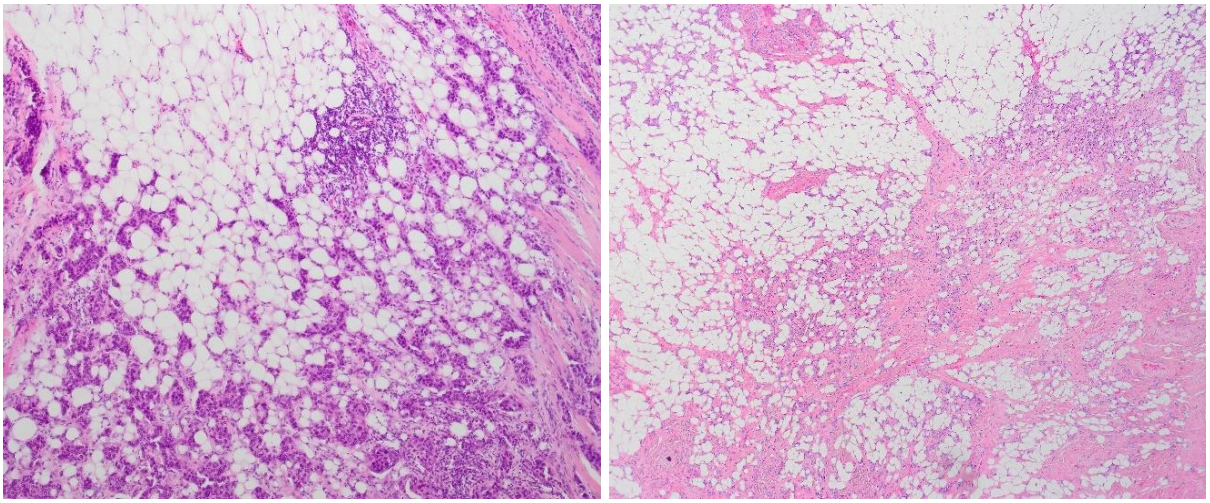
therapy in triple-negative and HER2-positive breast cancer.[99-101] Immunosuppression in tumor microenvironment may be associated with cancer cell proliferation and tumor growth. Programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) can lead to immunosuppression by blocking immune response which allows tumor cells to escape the immune system. In order to overcome this immunosuppression it is necessary to target these proteins.[102] In recent years TILs are recognized as an important prognostic and predictive biomarker, in particular in the setting of response to chemotherapy in some subtypes of breast cancer.[103] Programmed cell death ligand 1 is the ligand 1 of the programmed cell death protein 1 (PD-1), which is the main immune checkpoint present of both CD8+ and CD4+ T-cells. Programmed cell death ligand 1 is often presented by tumor cells and immune cells. When PD-1 binds PD-L1, T-cells cannot produce an effective immune response.[104] IMpassion130 study showed a clinical benefit from a combination of atezolizumab (a monoclonal antibody targeting PD-L1) to nab-paclitaxel in breast cancer patients with locally advanced and metastatic TNBCs.[105] Chemotherapy induced apoptosis of tumor cells can evoke an immune response.[106]

Regulatory T cells (Tregs) can suppress effector T cell activity and also the function of other immune cells. Elevated numbers of Tregs in breast cancer biopsies is associated with an invasive phenotype and reduced overall survival.[107-109] Transcription factor FoxP3 is essential for the development and function of Tregs. It is expressed on the surface of Tregs, and its loss of function leads to Treg deficiency. Regulatory T cells that are FoxP3 positive have been found in great numbers in tumor infiltrates and peripheral blood of cancer patients. High levels of tumor-infiltrating Tregs have been associated with poor clinical outcomes. Cytotoxic lymphocytes CD8+ and FoxP3 positive Tregs can be used as prognostic factors in breast cancer. Increased numbers of FoxP3 positive Tregs and increased Foxp3+ Treg/CD8+ T cell ratio have been correlated with more aggressive tumor behaviour, and increases in CD8+ T lymphocytes have been correlated with more favorable clinicopathologic characteristics.[110] Elevated Foxp3+ Tregs numbers in breast cancer tissue has been associated with poor recurrence free survival.[111] High numbers of intratumoral Tregs in breast cancer before chemotherapy showed shorter overall survival.[112] In some models of mammary cancer ablation of Tregs has led to reductions in primary and metastatic tumor.[113]

1.4. Cancer-associated adipocytes

There are three categories of breast adipocytes: mature adipocytes, preadipocytes, and adipose-derived stem cells (ADSCs). Cancer-associated adipocytes (CAAs) are considered a special type of adipocyte that surrounds invasive breast cancer (*Figure 3.*). Compared to normal adipocytes, CAAs show fibroblast-like phenotypes, smaller size, small and dispersed lipid droplets, overexpression of collagen VI and low expression of adiponectin and other adipokines.[114] Cancer associated adipocytes have been associated with tumor progression, metastasis and therapy resistance by producing different adipokines, e.g. leptin and adiponectin, as well as many different inflammatory mediators (chemokines and interleukins).[115,116] Adipocytes secrete more than 600 metabolites, hormones and cytokines, which are called adipokines.[114] In breast cancer

TME the CAA-mediated secretion of CCL2, CCL5, IL-1β, IL-6, TNFα, VEGF and leptin is increased, promoting tumor cell proliferation, invasion and angiogenesis.[117] Leptin is a hormone mainly synthesized and secreted by adipocytes. Leptin mediates proliferation, differentiation, inflammation and nutrient absorption. Increased leptin levels have been correlated with a high cancer grade, advanced tumor stage and invasive cancer subtypes in some cancers, including breast cancer. The secretion of leptin is higher in CAAs than in normal adipocytes.[118] Leptin can activate the estrogen receptor, JAK/STAT3 and PI3K/AKT signaling pathways to promote the proliferation of BC cells.[119] Adiponectin is a hormone which has a protective role in tumor progression. Cancer associated adipocytes show a decreased secretion of adiponectin.[120] Adiponectin down-regulates proliferation of cancer cells by regulating inflammatory signaling factors, such as TNFα, IL-1β, nuclear factor (NF)-κB, IL-6, IL-8 and CCL2.[121] Chemokine CCL2 is secreted by many different cells in the TME, including cancer cells, endothelial cells and fibroblasts. Chemokine CCL2 binds to receptors CCR2 and CCR4, serving as a chemoattractant for CCR2-expressing immune cells to areas of inflammation.[122] High levels of CCL2 have been connected with decreased survival in breast cancer patients.[123] Chemokine CCL5 attracts leukocytes and serves as a multifunctional inflammatory mediator. Chemokine CCL5 can be produced by many different cells, including mesenchymal stem cells, but it is highly expressed in breast cancer.[124] Breast cancer cells stimulate the secretion of CCL5 which binds to CCR5 on the membrane surface of human breast cancer cells which stimulates migration, invasion and metastasis of cancer cells.[125] Some studies have shown high levels of activation of CCL5/CCR5 axis in TNBC and HER2-positive breast cancer.[126] Interleukine-6 is a cytokine involved in many biological processes, including hematopoiesis, immune regulation and tumorigenesis. The secretion of IL-6 by adipocytes is substantially increased in obesity and cancer.[127] Interleukine-6 has been shown to be an independent poor prognostic factor for overall survival in patients with steroid-refractory metastatic breast cancer.[128] Interleukine-6 stimulates cancer cell proliferation, survival and angiogenesis by regulating the JAK/STAT3 signaling pathway.[129] Interleukine-6 also increases invasiveness of breast cancer cells and activates EMT.[130] Serum levels of IL-6 have shown positive correlation with body mass of obese women with breast cancer. Survival of obese breast cancer patients is worse than non-obese breast cancer patients.[131] Blocking of IL-6 signaling in breast cancer changes the expression of EMT regulatory genes and decreases the mobility of tumor cells.[132] Cancer associated adipocytes can enhance tumor cell proliferation and angiogenesis by different mechanisms, such as activation of ER, JAK/STAT3 and PI3K/AKT signaling pathways, and increasing cyclin D1 and VEGF/ VEGFR expression.[133] Experiments in which adipocytes were co-cultured with breast cancer cells showed they stimulate tumor progression.[134] Cancer associated adipocytes also secrete IL-6.[135] Interleukine-6 secretion influences cancer cell survival, immune suppression and drug resistance by JAK/STAT3 pathway activation.[136] Potential targets for breast cancer therapy include inflammatory factors such as CCL5 and IL-6.[114]



3.A. 3.B.

Figure 3. A) and B) Cancer associated adipocytes. CAAs surrounding invasive breast cancer.

1.1. Mesenchymal stem/stromal cells

Mesenchymal stem/stromal cells (MSCs) are multipotent spindle-shaped cells that were first described in hematopoietic bone marrow.[137,138] They are a population of stromal progenitor cells that are essential in maintaining homeostasis. They take part in tissue repair and neovascularization process after injury and have the ability of self-renewal.[139,140] They have the ability to differentiate into many stromal cell lineages, and they can migrate through the body and into the tumor.[141] Since MSCs usually migrate to areas of injury, they are also recruited to tumor microenvironment by inflammatory mediators, growth factors and cytokines. Mesenchymal stem cells usually migrate to tumors from bone marrow, they can also be recruited from surrounding adipose tissue. Mesenchymal stem cells in tumor microenvironment also have the ability to differentiate into CAFs.[142-144] Inside the tumor tissue MSCs interact with tumor cells and tumor microenvironment components either directly through gap junctions and membrane receptors or indirectly by soluble molecules.[145] Mesenchymal stem cells release endocrine and paracrine signal molecules which stimulate adjacent cells, but are also stimulated by tumor cells which results in development of an abnormal, tumor-associated phenotype.[146] In breast cancer microenvironment one of the ways in which MSCs interact with the tumor cells is by generating exosomes, which results in proliferation and migration of breast cancer cells and their resistance to drug-induced apoptosis.[147] Mesenchymal stem cell-derived exosomes contain molecules that also induce polarisation of macrophages into M2 phenotype.[148] A very interesting interaction between breast cancer cells and MSCs has also been described; breast cancer cells have been known to cannibalize MSCs within the tumor microenvironment; which results in the death of the MSC and in increased survival potential of the breast cancer cell which enters dormancy.[149] Mesenchymal stem cells also show a chemoprotective effect on breast cancer cells against certain cytotoxic drugs. Mesenchymal stem cells produce IL-6 has which causes stimulation of ERα-positive breast cancer cell proliferation.[150,151] Interleukine-6

has also demonstrated protective effect against paclitaxel and doxorubicin in ER-positive breast cancer[152,153] and against trastuzumab in Her-2 positive breast cancer.[154] Mesenchymal stem cells also secrete TGF-β which is associated with epithelial to mesenchymal transition which also contributes to the chemoprotective effect.[155]

1.2. Endothelial cells and angiogenesis

To maintain growth and enable metastatic dissemination breast cancer needs development of blood vessels. High microvascular density has been associated with large tumor size, high histological grade, lymph node metastasis and adverse prognosis.[156-158] In breast cancer tumour microvascular density is inversely correlated with survival and tumour hypoxia is positively correlated with metastasis.[159] Endothelial cells are an important component of the tumour microenvironment, and are essential to the tumour angiogenesis.[160] Angiogenesis is a process regulated by many different growth factors, cytokines and hypoxic environment. One of the essential molecules in that process is VEGF.[161] Like other non-neoplastic, tumor associated cells in tumor microenvironment, endothelial cells also undergo changes. They are stimulated to produce wider vascular network mainly by activating VEGF-A pathway. Vascular endothelial growth factor-A is up-regulated in numerous cancers. It promotes endothelial cell growth and replication, and inhibits apoptosis. Breast cancers with low VEGF-A levels are associated with higher disease-free survival and overall survival.[162] Endothelial cells are not just passively recruited from cancer cells, they also engage in paracrine signaling, some of which go through Jag1/notch pathway.[163] Vascular endothelial growth factor is the most important pro-angiogenic factor, it is over-expressed in breast cancer, and furhermore has been associated with overall survival in breast cancer.[164,165] In advanced breast cancer patients VEGF has been associated with poor response to tamoxifen or chemotherapy.[166] There are other mechanisms that stimulate breast cancer angiogenesis, such as CL2/CCR2 signaling that stimulates tumor growth and invasion by recruiting and stimulating branching of endothelial cells, attracting and polarizing macrophages to M2 phenotype, and suppressing cytotoxic T-cell activity.[167] Normal endothelial cells express thrombospondin I which acts as a tumor suppressor. In the process of tumor angiogenesis newly formed endothelial cells express a lower concentration of thrombospondin-1.[168] As mentioned earlier, hypoxia is an important pro-angiogenic signal. Hypoxia inducible factor 1 subunit α (HIF-1α) is one of the transcription factors that causes up-regulation of proteins in hypoxic cells that promote survival and increase growth.[169] Transcription factor HIF-1α levels are more elevated in poorly differentiated than in well-differentiated breast cancers, and increased expression of HIF-1 α is also associated with increased expression of ER and VEGF.[170] Since VEGF has an established role in breast cancer and accomplishes its effect through the interaction with VEGF receptor (VEGFR), different strategies and several points of the VEGF/VEGFR pathway that could be potential targets for treatment have been investigated; especially ligand blockade. Bevacizumab, a monoclonal antibody that targets isoforms of VEGF-A, is the first and the most explored antiangiogenic drug in breast cancer clinical trials. Many clinical trials have tested tyrosine

kinase inhibitors that block the function of VEGFR[171] but despite the promising preclinical studies resistance to treatment and cardiovascular toxicities were shown.[172-175]

2. Conclusions

- Breast cancer is a heterogeneous disease due to its genomic and pathological diversity and its mortality rates remain high.
- Tumor microenvironment in breast cancer includes cancer-associated fibroblasts, immune cells, vascular and perivascular cells and adipocytes.
- Interactions between cancer cells and TME are involved in all stages of tumor development (neoplastic transformation, proliferation, invasion, metastasis).
- Fibroblasts are one of the main cellular components of the TME; they become pathologically activated and differentiated into CAFs which secrete growth factors, cytokines and chemokines.
- Tumor associated macrophages are the most abundant mononuclear inflammatory cells in breast cancer. They can be differentiated into two functional categories; tumour inhibitory (M1) and tumour-promoting (M2).
- Tumor infiltrating lymphocytes are mononuclear immune cells that infiltrate the tumor and in breast cancer they are more frequent in high-grade breast cancer. TILs have been shown to be a good prognostic and predictive biomarker in high-grade breast cancer.
- Cancer associates adipocytes have been associated with tumor progression, metastasis and therapy resistance by producing different adipokines and inflammatory mediators.
- Mesenchymal stem/stromal cells are stromal progenitor cells that are recruited to TME where they interact with tumor cells and the tumor microenvironment.
- Endothelial cells are essential to tumour angiogenesis thus maintaining tumour growth and enabling metastatic dissemination.
- Every component of TME plays an important role which opens opportunities for finding potential therapeutic targets and prognostic and predictive biomarkers in the future.

Conflict of Interest Statement:

The authors have no conflicts of interest to declare.

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