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NON-THEMATIC REVIEW

# Regulatory T cells and breast cancer: implications for immunopathogenesis

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**Abstract** Current understanding of the role of several cancer risk factors is more comprehensive, as reported for a number of sites, including the brain, colon, breasts, and ovaries. Despite such advances, the incidence of breast cancer continues to increase worldwide. Signals from the microenviroment have a profound influence on the maintenance or progression cancers. Although T cells present the most important immunological response in tumor growth in the early stages of cancer, they become suppressive $CD4^+$ and $CD8^+$ regulatory T cells (Tregs) after chronic stimulation and interactions with tumor cells, thus promoting rather than inhibiting cancer development and progression. Tregs have an important marker protein which is FoxP3, though it does not necessarily confer a Treg phenotype when expressed in $CD4^+$ T lymphocytes. High Treg levels have been reported in peripheral blood, lymph nodes, and tumor specimens from patients with different types of cancer. The precise mechanisms by which Tregs suppress immune cell functions remain unclear, and there are reports of both direct inhibition through cell–cell contact and indirect inhibition through the secretion of anti-inflammatory mediators such as interleukin. In this review, we present the molecular and immunological aspects of Treg cells in the metastasis of breast cancer.

**Keywords** Breast cancer · Metastasis · Tregs · FoxP3

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## 1 Introduction

Breast cancer is the most common female cancer, and annually, more than one million new patients are diagnosed worldwide [1]. Breast cancer incidence has increased steadily in developed countries over the past few decades, but the mortality caused by breast cancer has decreased in recent years, partly because of improved screening techniques, surgical and radiotherapy interventions, understanding of the pathogenesis of the disease, and the use of traditional chemotherapies in a more efficacious manner [2].

Breast cancer shows some distinctive features in terms of age-specific incidence rates [3] and comprises a remarkably diverse group of diseases in terms of presentation, morphology, biological characteristics, clinical behavior [4], molecular profile, and response to therapy [5]. The degree of cellular and molecular heterogeneity in breast cancer and the large number of molecular events involved in controlling cell growth, differentiation, proliferation, invasion, and metastases [6] emphasize the importance of studying multiple molecular alterations in concert [7–14]. Clinicopathologic parameters have been validated and serve as a guide for the use of systemic therapy and prognostication. These include tumor size, lymph node stage and

histological grade, vascular invasion, histological type, and the patients' age and menopausal status [4].

In recent years, it has become evident that a subpopulation of T cells, named T regulatory cells (Tregs), plays a major role in sustaining tolerance to self-antigens. Forkhead box P3 (FoxP3)-expressing Tregs are key mediators of peripheral tolerance and suppress undesirable immune responses. It was verified that Tregs bear higher reactivity than other T cells to the selecting ligand in the thymus even after negative selection by the ligand. This broad repertoire and high self-reactivity of $CD25^{+}CD4^{+}$ Tregs, together with their high level expression of various accessory molecules, may guarantee their prompt and efficient activation upon encounter with a diverse range of self-peptide/major histocompatibility complex (MHC) complexes in the periphery, ensuring dominant control of self-reactive T cells [15].

Many studies have provided strong evidence that Tregs may express different surface molecules, reside at different locations, and express molecule increase or reduction in the cells [16]. The host immune system plays an essential role in the immune surveillance and destruction of cancer cells [17, 18]. All solid tumors are embedded in a stromal microenvironment consisting of immune cells, such as macrophages and lymphocytes, as well as non-immune cells, such as endothelium cells and fibroblasts. In this context, the present review focuses on the establishment of Tregs within the immune response to breast cancer and its implications for immunopathogenesis.

## 2 Breast cancer and immune response

The immune response to tumors is complex. Cells of the immune system can inhibit tumor growth and progression by recognizing and rejecting malignant cells, a process referred to as immunoediting. Immune responses can also promote tumor cell growth, survival, and angiogenesis by inducing oncogenic inflammation. Immunodeficiency can predispose to the development of spontaneous and virally induced cancer, and established tumors often generate immunosuppressive microenvironments that can block productive antitumor immunity, serving as a substantial barrier to effective immune therapy [19, 20].

Lymphocytes, including T cells, Tregs, and natural killer (NK) cells, and their cytokine release patterns are implicated in breast cancer primary prevention and recurrence. Cancer prognosis may be related to the immune system functional status [21].

Activation of humoral and cellular immunity may predispose to neoplastic or cancer development [22]. Emerging from these studies is an appreciation that persistent humoral immune responses exacerbate recruitment and activation of innate immune cells in neoplastic microenvironments where they regulate tissue remodeling and pro-angiogenic and pro-survival pathways that together potentiate cancer development. Studies on advances support the hypothesis that enhanced states of local humoral and innate immune activation, in combination with suppressed cellular immunity and failed cytotoxic T cell antitumor immunity, alter cancer risk and therefore represent powerful targets for anti-cancer immunotherapeutics [23].

During the past decade, insights have been gained regarding mechanisms underlying the dynamic interplay between immune cells and tumor progression. The accumulated data indicate that the outcome of an immune response toward a tumor is largely determined by the type of immune response elicited. A tumor-directed immune response involving cytolytic $CD8^{+}$ T cells, Th1 cells, and NK cells appears to protect against tumor development and progression. If, on the other hand, the immune response involves B cells and activation of humoral immunity and/or a Th2 polarized response, the probable outcome is the promotion of tumor development and progression. This balance between a protective cytotoxic response and a harmful humoral or Th2 response can be regulated systemically by the general immune status of the individual, as well as locally by myeloid suppressor cells and T regulatory cells, and thus offers clinicians attractive targets for anticancer immune-based therapies [24].

Tregs induce immune tolerance by suppressing host immune responses against self- or non-self-antigens, thus playing critical roles in the prevention of autoimmune diseases, but they may inhibit antitumor immunity and promote tumor growth. Increasing evidence demonstrates that elevated proportions of $CD4^{+}$ Treg cells are present in various types of cancers and suppress antitumor immunity. However, less is known about $CD8^{+}$ Treg cells and their detrimental effects on immunotherapy directed toward cancer [25].

Antigen-induced suppressor T cells that were intensively studied in the 1970s and early 1980s remain to be reinvestigated in light of recent findings. Thus, the current active research of T cell-mediated self-tolerance and immune regulation is revealing Treg cell "unity" and "diversity". Further investigation of Treg cells, natural or adaptive, will make their clinical use a reality for the better control of a variety of physiological and pathological immune responses [26].

## 3 Regulatory T cells

Tregs were described in 1995 [27] reporting this cell's involvement in immune response regulation and cellular activation. Treg cells include populations that differ in

phenotype, cytokine secretion profile, and suppressive mechanism [28–30]. Several subsets of Treg cells have been identified and characterized, such as $CD8^+$ Treg cells, $CD4^+$ Treg cells, and γδ-TCR. Tregs have been reported in cancer and other diseases [25].

Studies have defined the cytokine transforming growth factor-β (TGF-β) as a critical regulator of thymic T cell development as well as a crucial player in peripheral T cell homeostasis, tolerance to self-antigens, and T cell differentiation during the immune response [31]. Two main origins have been described for $FoxP3^+$ cells whose numerical and functional importance have yet to be clarified. The first is the thymus where $FoxP3^+$ cells are generated roughly in sync with the positive selection of conventional $CD4^+$ T cells. The second is the periphery where a number of triggers induce the expression of FoxP3 in T cells. The conversion mechanism $CD25^-CD4^+$ in $CD25^+CD4^+$ in the periphery, *in vitro*, involves TGF-β in a murine model [32]. It was recently shown that indoleamine-2,3-dioxygenase (IDO) may also induce FoxP3 expression in CD4 T cells and IDO can convert human and murine $CD4^+CD25^-$ T cells to $CD4^+CD25^+FoxP3^+$ cells [33]. $CD4^+CD25^+$ Tregs are important in the control of immune responses because of their ability to suppress T cell proliferation and cytokine production [34].

$CD4^+$ Treg cells can be further divided into naturally occurring $CD4^+CD25^+FoxP3^+$ Tregs, antigen-induced $CD4^+CD25^+FoxP3^+$ Treg cells, and $CD4^+$ $FoxP3^-$ Tr1 cells [35]. Although the origin of $CD4^+$ Treg cells remains largely unknown, they may arise from antigen-experienced $CD4^+CD25^-$ T naive and effector cells in the suppressive cytokine milieu of tumor sites or expansion after antigen stimulation of naturally occurring $CD4^+CD25^+$ T cells. Unlike naturally occurring $CD4^+CD25^+FoxP3^+$ Treg cells, Tr1 cells do not express FoxP3 and are induced in peripheral tissues by a MHC/peptide stimulation in the presence of IL-10. They suppress immune responses through a cytokine-dependent mechanism [36, 37].

Although many authors consider FoxP3 protein the most important Tregs marker [32, 38], it does not necessarily confer a Treg phenotype when expressed in $CD4^+$ T lymphocytes [39, 40]. Using specific anti-FoxP3 monoclonal antibodies, it was shown previously that only approximately half the $CD4^+CD25^+$ population expressed FoxP3, a minority of $FoxP3^+$ cells lacked CD25 expression, and a small number were $CD8^+$ [41]. Many factors including histones, chromatin remodeling enzymes, RNA binding proteins, molecular chaperones, and transcription factors may interact directly or indirectly with FoxP3 in a dynamic manner in response to extracellular stimuli [42].

FoxP3 is a member of the forkhead/winged family of transcription factors and when it acts through NFAT (nuclear factor of activated T lymphocytes) has been postulated to control key genes to specifically drive Treg development [43].

Tregs have been characterized by the constitutive expression of FoxP3, glucocorticoid-induced TNFR family-related receptor (GITR), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and high levels of the alpha chain of the IL-2 receptor (CD25) [44]. It is known that CD127 (alpha chain of the IL-7 receptor), which are expressed in the majority of mature T cells, play an important role in their proliferation and differentiation. However, CD127 is absent in Tregs and its expression inversely correlates with Foxp3 expression and, together with the other markers, identifies over 95% of the $Foxp3^+$ cells in peripheral blood [45].

Evidence has been reported that the immune systems of patients with breast cancer were dysfunctional [46]. Regulatory T cells and IDO, an immunosuppressive enzyme, are associated with a more advanced disease in some cancers and may promote immunologic tolerance to tumors. Studies have shown that $FoxP3^+$ cells were associated with a more advanced disease in breast cancer, a finding that is proven to be true in many other cancers. IDO has been found to promote Treg differentiation and may become a suitable target to abrogate the development of T cell tolerance and to promote an effective immune response to breast cancer [47].

The identification of IL-17-producing $FoxP3^+$ Treg cells in both mice and humans suggested that Th17 and $FoxP3^+$ Treg lineages were related in ontogeny. Both lineages appeared to depend on TGF-β for their differentiation and/or maintenance, and additional cytokines may determine whether they become Th17, Treg, or dual-function effector T cells [48]. IL-17-producing $FoxP3^+$ regulatory T cells were identified in humans [49]. These authors verified that human $CD4^+Foxp3^+CCR6^-$ regulatory Tregs differentiated into IL-17 producer cells upon T cell receptor stimulation in the presence of IL-1β, IL-2, IL-21, IL-23, and human serum. This, together with the finding that the human thymus does not contain IL-17-producing Tregs, suggested that the $IL\text{-}17^+FoxP3^+$ Tregs were generated in the periphery. IL-17-producing Tregs may play critical roles in antimicrobial defense while controlling autoimmunity and inflammation.

It has been strongly suggested that the increase in functional Tregs in cancer patients was a response to the process of malignant transformation [50]. It is of interest to know whether Treg cell expansion in solid tumors is also accompanied by the expansion of naive Tregs and whether there are differences in different compartments such as the blood, secondary lymphoid organs, and bone marrow or at the tumor site.

Tumor-derived $CD4^+$ Treg cells have been extensively studied in many different types of cancer. This notion is

further supported by the fact that antigen-specific $CD4^+$ Tregs at tumor sites may significantly suppress immune responses, leading to immune tolerance of tumor cells. Despite the importance of immune cells such as T cells in immunosurveillance and control of tumor growth in the early stages of cancer, they become suppressive $CD4^+$ and $CD8^+$ regulatory Tregs after chronic stimulation and interactions with tumor cells, thus promoting rather than inhibiting cancer development and progression [51]. Neither tumor Treg nor naive Treg can suppress antitumor immunity at the effector phase of the immune response induced by adoptively transferred tumor-primed $CD4^+$ T cells. Therefore, tumor Tregs potently abrogate tumor-specific $CD8^+$ T cell responses in tumor-draining lymph nodes, thereby suppressing antitumor immunity at the early stage of the immune response induced by adoptively transferred tumor-primed $CD4^+$ T cells [52].

## 4 Regulatory T cells: implications in breast cancer

Interleukins and cytokines are important regulators of the aetiopathogenesis of the majority of cancers [53]. The stability of a Tregs population which can downregulate FoxP3, lose regulatory activity, and, under some conditions, become memory T cells capable of recognizing self-antigens and expressing effector cell activities including the production of IL-17 and IFN gamma was reviewed [54]. They concluded that the presence of these "exTregs" in multiple inflammatory settings suggested a potential role for these cells in a variety of disease settings ranging from autoimmunity to cancer and infectious disease.

Tregs enriched in $FoxP3^+$, $GITR^+$, and $CTLA4^+$ exert a potential to suppress effector T cells in the periphery. These cells exist in markedly higher proportions within tumor-infiltrating lymphocytes, peripheral blood lymphocytes, and/or regional lymph node lymphocytes of patients with cancer. Their frequencies are suggested to be strongly related to tumor progression and inversely correlated with the efficacy of the treatment. Treg cell depletion or blockade can enhance immune protection from tumor-associated antigens that are expressed as self-antigens [55].

To determine whether intratumoral Treg accumulation and activation help in the progression of human breast carcinoma, the intratumoral expression of Foxp3 in invasive breast carcinoma was analyzed and compared with its level in ductal carcinoma *in situ* and adjacent normal tissue, with the main aim of using this factor as a tumor progression marker [56]. These authors verified that a linear association of intratumoral FoxP3 expression with invasion, size, and vascularity suggested a use for FoxP3, an indicator of Treg activity, as a marker of tumor progression and metastasis in breast carcinoma.

It has been investigated whether the expression of FoxP3 transcripts and mature protein occurred constitutively in various tumor types and demonstrated that cancer cells of various types expressed a transcript for FoxP3 as well as the mature protein [57].

The frequency of $CD4^+CD25^{high}$ in the peripheral blood of cancer patients and healthy donors was compared and demonstrated evidence of an increased $CD4^+CD25^{high}$ pool in the peripheral blood of cancer patients, which may be related to immunosuppression and tumor progress in cancer patients [58].

High-risk breast cancers, especially breast cancers at risk for recurrence, recruit high numbers of Tregs, suggesting a correlation with disease prognosis [59]. Analysis of human breast cancer samples provided strong support for an important role of the FoxP3 gene in the development of breast cancer [60].

Low-level FoxP3 mRNA expression was detectable in breast epithelium and breast cancer cell lines where FoxP3 functions as a breast cancer suppressor gene that may help understand the origin of FoxP3-expressing cells; these are breast epithelium, breast cancer cells, or Tregs. However, they demonstrated that deletion, functionally significant somatic mutations, and downregulation of the FoxP3 gene were commonly found in human breast cancer samples [61]. It has been reported that FoxP3 expression was higher in tumor tissue than in normal breast tissue [62]. However, it was strongly suggested that FoxP3 expression in breast cancer tissue indicated the tumor-infiltrating Treg cell origin. It has been suggested that the function of Foxp3 in cancer cells may depend on the nature of the breast tumor, especially concerning oncogenic pathways involved in tumor growth [63].

The clinical significance of tumor-infiltrating FoxP3-positive Tregs has been assessed in breast cancer patients with long-term follow-up [59]. These authors present the finding that high FoxP3-positive Tregs numbers represent an important marker for the identification of breast cancer patients at risk of late relapses. They concluded that the number of tumor-associated Tregs is a significant parameter for disease prognosis in both invasive and noninvasive breast tumors that can be assessed in routinely fixed tissues by immunohistochemistry to detect FoxP3-positive T cells. The authors strongly suggested that such therapy would be beneficial for a significant proportion of breast cancer patients.

A T lymphocyte inhibitory molecule named B7-H1 (also called PD-L1), expressed by antigen-presenting cells, has been shown to induce T lymphocyte anergy after linking to its T lymphocyte receptor PD-1 [64]. B7-H1 has been shown to be directly involved in the protection of cancer cells from activated T lymphocytes [65]. T cells infiltrating lymphocytes expressing the B7-H1, PD-1, and FoxP3

molecules in the microenvironment of human breast tumors and their possible association with the progression of the disease were investigated. A concurrent and abundant infiltration of different immune suppressive subsets of T lymphocytes has been shown in the microenvironment of high-risk breast cancer patients. This interesting observation suggests the development of new therapeutic modalities to target B7-H1/PD-1 and Tregs in addition to still developing immunotherapy [66].

Studies have shown that Tregs might also be generated from T cell-derived tumor cells [67, 68]. It was suggested that Tregs are involved in tumor onset and progression in human primary breast cancer, possibly contributing to the poor prognosis of patients with breast cancer. FoxP3, IL-10, TGFβ1, and CCL22 mRNA expressions were significantly higher in cancer tissue than in normal tissue. FoxP3 and IL-10 mRNA expressions were significantly upregulated in progesterone receptor-negative or HER2-positive tumors [62].

In breast cancer, the Treg number is increased in the peripheral blood of breast cancer patients [69–71], and they are present within the primary tumors [69]. A recent study demonstrated a significant intratumoral infiltration of $FoxP3^+$ Tregs in high-risk breast cancer patients and those at risk of late relapse [59].

The level of mRNA expression of CTLA-4 in normal and breast carcinoma tissues has been demonstrated and showed statistically increased levels of the gene transcription in patients that correlated with disease progression [72]. Some researchers have evaluated CTLA-4 and FoxP3 transcripts, as acceptable indicators of Tregs, in the peripheral blood from women with breast cancer and found that these transcripts significantly increased even in the early stages of breast cancer [73].

By taking advantage of a highly conserved FoxP3 sequence, three haplotype-tagging single-nucleotide polymorphisms that covered 40 kb around the FoxP3 gene region were genotyped and verified that FoxP3 was a biologically relevant gene in breast cancer pathogenesis, but germline variation in their study was not meaningfully associated with risk of the disease [74].

## 5 Possible functions of the T cells in disseminated breast cancer

Adding to the complexity of metastasis, this process often follows characteristic organ distribution patterns that reflect inherent differences within the disseminating cells of distinct tumors [75, 76].

Some authors have reported that the presence of immune cells in breast tumors is unable to counteract cancer and may even contribute to tumor progression. Metastatic cancer is associated with an expansion of peripheral blood $CD4^+CD25^{high}FoxP3^+GITR^+CD152^+$ Tregs whose immunosuppressive properties do not differ from those of healthy subjects [77].

It has been suggested that homeostatic mechanisms governing the peripheral blood count of $FoxP3^+$ $CD4^+$ T cells differ fundamentally from those governing the total $CD4^+$ T cell count; this different regulation would explain, for example, why the average absolute CD4 count in patients with metastatic disease or those post-chemotherapy was lower than that for healthy volunteers, yet the absolute count of $FoxP3^+CD4^+$ T cells was not. Regulatory and non-Tregs might be regulated differently by cytokines such as IL-2 or exhibit differential susceptibility to the effects of chemotherapy or macroscopic tumor burden [78].

In 2007, the number and functional status of $CD4^+CD25^{high}$ Tregs in blood samples from patients with metastatic carcinoma was determined, and it was found that Treg numbers were significantly higher in patients with metastatic cancer compared to healthy donors [77].

In 2009, some authors studied patients diagnosed with invasive breast carcinoma who underwent primary systemic chemotherapy followed by definitive surgery and examined the correlations between the number of tumor-infiltrating FoxP3-positive cells during primary systemic chemotherapy and therapeutic effects in patients with breast cancer. They demonstrated that lymph vessel invasion was prominent in the group with a high number of FoxP3 infiltrates [79].

The use of FoxP3 as a novel, independent molecular marker of breast carcinoma outcome has been suggested, with a significant impact on important outcome measures for breast carcinomas. FoxP3 expression in tumors was associated with worse overall survival probability, and the risk increased with increasing FoxP3 immunostaining intensity. FoxP3 was also a strong prognostic factor for distant metastasis-free survival, but not for local recurrence risk [80].

Additional insight was provided into the regulatory mechanisms responsible for immunosuppression in human cancer, which may facilitate local tumor growth and metastasis. Hematogenic metastasis often represents the fatal step during the course of malignancy, which may be significantly enhanced by the suppression of blood-borne immunosurveillance mechanisms. Treg depletion may become a successful anticancer strategy, and Treg manipulation in terms of their frequency and functional activity should be added to the therapeutic to enhance tumor immunity in humans [71].

Chemokines and their receptors are involved in the control of lymphocyte, a critical component of systemic immunity. G-protein-coupled receptor (CXCR4) is a receptor of considerable biological significance, and its numerous functions suggest that it is involved in diverse

development processes. It was demonstrated that samples of peripheral blood cells of stage II samples from breast cancer patients revealed higher CXCR4 expression than the controls and other stages [81]. CXCL12 is a chemokine that binds to a CXCR4. CXCL12 is expressed in various tumors and is considered to play an important role in tumor growth and invasion [82]. Authors have investigated CXCL12 expression in human malignant mesothelioma, the chemotactic effect of CXCL12 derived from mesothelioma, and CXCR4 expression in mesothelioma tissues in relation to regulatory T cells. CXCL12 was expressed in mesothelioma cell cytoplasm from all patients, but it was not expressed in the control group. These findings suggested that CXCL12 contributed to tumor-related inflammation by inducing the accumulation of CXCR4-expressing cells with regulatory T cell markers around mesothelioma [83].

It was observed that Tregs expressed chemokine receptor CCR4 and showed demonstrable chemotactic responses to the CCR4 ligands CCL22 and CCL17 [84]. On accumulation of regulatory T cells in cancer, Tregs may be attracted by various chemokines (CCL5, CCL17, CCL22, CXCL12) to the tumor site. Cancerous cells and/or bystanding tumor-associated macrophages and myeloid-derived suppressor cells secrete these chemokines, of which Tregs possess the corresponding receptors as CCR4, CCR5, and CXCR4 [85]. The increased frequency of a new Treg subset, $CCR6^+$ Tregs, was reported, which correlated positively with the poor survival of breast cancer patients. It suggested that the $CCR6^+$ subset of Tregs might be mainly responsible for long-term immunosuppression in the tumor environment. However, successive broad screening approaches to the role of $CCR6^+$ Tregs in other tumor hosts will be worthwhile to further substantiate these initial results, which might throw a novel insight on the role of the resident unique subset of Tregs in the tumor mass and provide helpful thoughts for the designing of Treg-based immunotherapy strategy against tumors in the future [86].

Various studies have indicated a positive correlation of VEGF expression with tumor vascularity and malignancy. Activated Tregs releasing excessive levels of TGF-β1 have been suggested, which indirectly induces VEGF expression and leads to increased vascularity and tumor progression. This implies that FoxP3 levels, an indicator of Treg activity, might also be an indicator of breast tumorigenesis. Since invasion, size, and vascularity are prognostic parameters in breast cancer, the finding of a positive correlation between FoxP3 expression and these parameters suggests a role of FoxP3 as a progression marker for breast carcinoma to an aggressive tumor phenotype [56].

FoxP3 protein expression in breast cancer by immunohistochemistry has been investigated and demonstrated that high numbers of FoxP3-positive Tregs were present in high-grade tumors at increased risk of relapse [59].

Breast cancer cells disseminate through the body by direct extension, lymphatic channel invasion, and circulation through blood vessels [87]. Breast cancer preferentially spreads to the bones, lungs, liver, and brain, whereas prostate cancer almost exclusively colonizes the bones [88]. Lymph node involvement remains the most influential prognostic factor in breast cancer progression. The presence of metastatic tumor cells in a lymph node is associated with specific alterations in the T cell population [89]. Sentinel lymph nodes are the nodes nearest to a primary tumor on the direct lymphatic drainage pathway of the breast and are the typical site of earliest metastasis. It was indicated that $FoxP3^+$ Tregs increased in the microenvironment of sentinel lymph nodes along with pathologically undetectable micrometastasis and were an independent prognostic predictor in patients with node-negative breast cancer [90]. In this context, it was observed that Treg response was induced at the micrometastasis level and persisted during metastasis progression in sentinel nodes in breast cancer patients [91].

TGF-β, which is a kind of cytokine produced by Tregs, has been implicated in tumor progression. The TGF-β pathway has been implicated in many of these metastatic processes and has been shown to dramatically impact the ability of tumor cells to spread throughout the body [92–95].

Tregs were selectively recruited within lymphoid infiltrates and activated by mature dendritic cells likely through the recognition of tumor-associated antigen presentation, resulting in the prevention of effector T cell activation, immune escape, and, ultimately, tumor progression [96]. It has been suggested that FoxP3 was expressed in breast cancer cells and the expression level was associated with patient survival. They found that FoxP3 expression was associated with overall and distant metastasis-free survival, but not with local relapse, and therefore, the authors suggested that FoxP3 expression might be related to the metastatic potential of the tumor rather than to the suppression of a specific immune response [80].

It has been hypothesized that Treg accumulation in tumor tissue would increase in parallel with tumor progression. They found higher expression of FoxP3 mRNA in tumor tissue than in normal breast tissue, and it was observed even at the ductal carcinoma *in situ* stage and persisted at the T1 and T2,3 stages, indicating that Treg accumulation in tumor tissue was an early event in tumor development and progression [62].

Interestingly, the FoxP3 transcription factor up- or downregulates a large number of genes and has been recently reported to be expressed in tumor cells. Furthermore, FoxP3 binds to the gene region upstream of the transcriptional start site of CCR7 and CXCR4 [97], two chemokine receptors recently reported to play an important

role in cancer invasion and metastasis [98, 99]. Thus, FoxP3 expressed in breast cancer cells might influence metastasis development by modulating the expression of these chemokine receptors or of other genes encoding cell surface or secreted molecules that alter tumor cell response to the environment [80].

Nevertheless, the finding that FoxP3 can be expressed by not only tumor-infiltrating Tregs but also by tumor cells

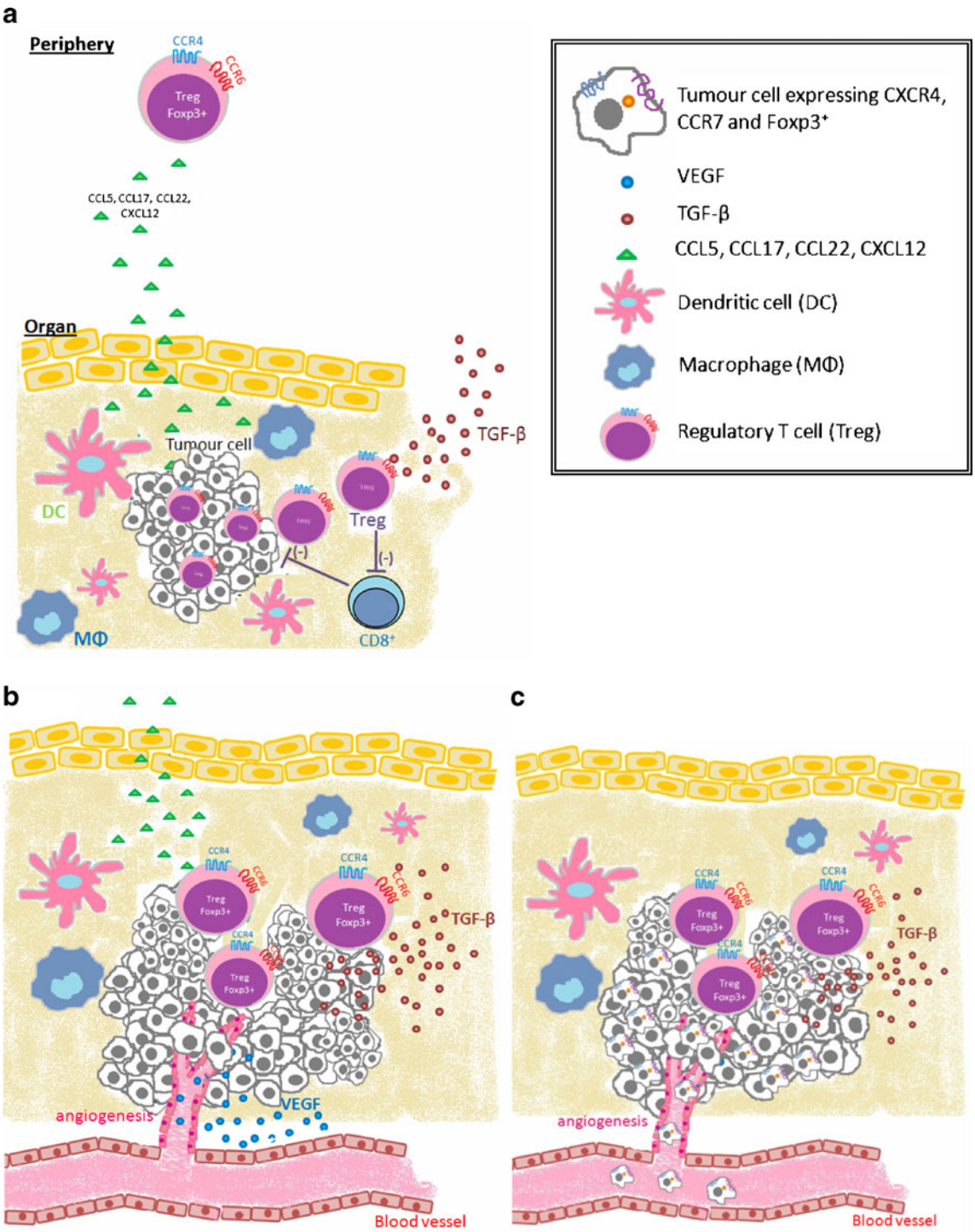


**Fig. 1** Possible role of Tregs in the tumor microenviroment. **a** Macrophages, dendritic cells, and tumor cells in the environment of tumor development secrete chemokines such as CCL5, CCL17, CCL22, and CXCL12 which attract peripheral regulatory T cells (*Treg*) to the tumor environment. **b** Tregs secrete TGF-β which plays important functions in endothelial cells and tumor cells. TGF-β induces angiogenesis by secreting VEGF from remaining endothelial cells. **c** Tumor cells express Foxp3$^{+}$ and chemokines receptors such as CXCR4 and CCR7. These modified cells (tumor cells) became able to reach the blood vessel

has two important implications. First, caution needs to be taken when interpreting gene expression data on FoxP3 expression in tumors. Increased levels of FoxP3 mRNA expression may be a result of not only an increased influx of Tregs but also the increased expression of FoxP3 directly in tumor cells. This understanding has significant importance for developing assays on the basis of FoxP3 for prognosis or drug monitoring. Second, we need to recognize that FoxP3-targeted therapy may need to be targeted at not only Tregs but also FoxP3-positive tumor cells, although the role of FoxP3 in regulating tumor cell growth remains to be clarified. The expression of FoxP3 in tumor cells indicates that FoxP3-targeted drugs must be able to penetrate the tumor bed, which is much more challenging than depleting FoxP3 in the periphery [100]. A schematic model of Tregs in breast cancer dissemination is represented, depicting their possible interplay with breast cancer cells in the microenvironment and the factors recruiting them to the cancer (Fig. 1).

The discovery of the FoxP3 transcription factor as a central molecular determinant of Treg differentiation and function has made the complex biology of these cells, including maintenance of immunological tolerance to "self" and regulation of immune responses to pathogens, commensals, and tumors, the focus of intense investigation. The FoxP3 gene plays a crucial role in Treg generation, whereas the overexpression of FoxP3 results in severe immunodeficiency. Tregs may play an important role in breast cancer immunopathology due to the potent suppressive activity of both T cell activation and effector function. Comprehensive analysis of immune effector functions at different stages of tumor metastasis is fundamental to the design of effective immune intervention. Although it is known that the clinical behavior of tumors depends on the relationship between tumor cells and the host, there are reports involving molecular research which identified tumor-derived markers, but little is known about the predictive potential of host factors and their potential role in breast cancer pathogenesis. The precise mechanisms to understand how Tregs suppress immune cell functions—whether inhibition is through cell–cell contact or by indirect inhibition through the involvement of anti-inflammatory mediators in the microenviroment—remain unclear. Treg cells can avoid the anti-tumor activity of immune effector cells in breast cancer tissue, resulting in poor prognosis of breast cancer patients. Tregs exhibit potent immunosuppressive functions and are known to infiltrate primary tumors and draining lymph nodes. TGF-β1, which is one kind of cytokine produced by Treg cells, has been implicated in tumor progression. Although TGF-β1 has been reported as a multifunctional growth factor, in breast cancer, this factor could induce the expression of the vascular endothelial growth factor, which is one the most selective and potent angiogenic factors known; therefore, the TGF-β pathway has been implicated in many of these metastatic processes. Expression of FoxP3 in the tumor cells has also been verified, with expression of chemokine receptor as CXCR4 and CCR7 reported to play an important role in cancer invasion. It has inserted the significance of Tregs implicated in carcinogenesis and efforts toward the development of anticancer approaches for inhibiting the expression of FoxP3 by tumor-associated Tregs. Comprehensive analysis of immune effector functions at different stages of tumor metastasis is fundamental to the design of effective immune intervention. It could be suggested that Treg numbers could constitute an important prognostic factor for patients with breast cancer treated with primary systemic chemotherapy, and FoxP3-positive cells in tumors could be a novel therapeutic target that could improve outcomes for such patients.

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