Chapter

# Neuroimmunoendocrine Interactions in Tumorigenesis and Breast Cancer

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## Abstract

Organism homeostasis is regulated through the tri-directional relationships between immune, endocrine, and nervous systems. These relationships are established by a complex network of chemokines, cytokines, hormones (peptide and non-peptide), neurotransmitters, and neurohormones that act onto its target cells, through common receptors. Despite initial attribution of the exclusive action of each molecule group (neurotransmitters, hormones, and cytokines), to the function of one specific system (nervous, endocrine, and immune, respectively), ligand and receptor pleiotropy and redundancy showed the multidirectional communication between systems. Cancer and metabolic and autoimmune diseases get established when homeostasis is disrupted. These interactions act in different disease levels, in cancer, since initial immunosurveillance phase, until immunosubversion and metastasis, in all cases is crucial for tumor development, cancer outcome, and patient prognosis.

**Keywords:** neuroimmunoendocrine network, breast cancer, neuroimmunoregulation, endocrinoimmune regulation, tumor, cytokines, steroids, neurotransmitters

## 1. Introduction

Cancer is one of the most common health issues worldwide. According to the World Health Organization (WHO), in 2018 18,078,957 new cases and 9,555,027 related deaths were reported. Breast cancer is the second leading cancer, after lung cancer, but is the first in women incidence and prevalence [1]. An estimation made in 2009 calculated that one out of eight American women could develop breast cancer in their life course [2].

There are several risk factors associated with breast cancer. The first and most important is gender; as mentioned before, women get breast cancer more often than males. Other risk factors are early menarche, first terminal pregnancy after 30 years old, late menopause, nulliparity, no breastfeeding, overweight or obesity, family or personal history of breast cancer, alcohol abuse, consumption of hormone oral

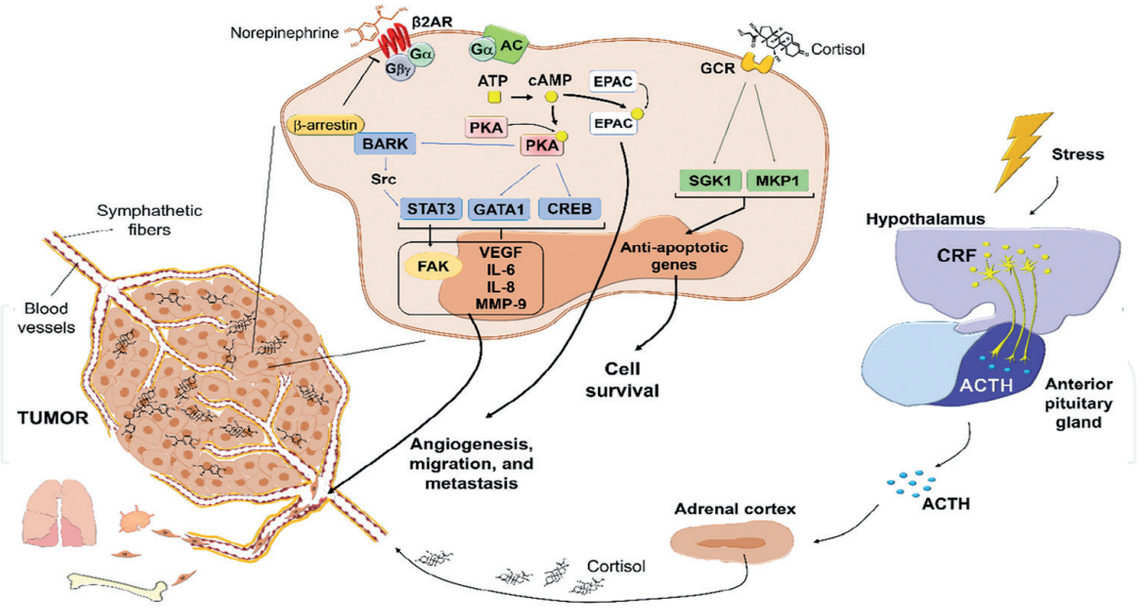


**Figure 1.**
*Nervous regulation during breast tumor growth and metastasis. Sympathetic fibers and blood vessels infiltrate tumor and are responsible for tumor communication with the nervous system. Sympathetic fibers release norepinephrine in tumor, which binds to $\beta_2$-adrenergic receptor in the tumor cell membrane and activates adenylate cyclase through G-protein-coupled receptor subunit α. AC promotes ATP-cAMP conversion, and cAMP activates protein kinase A and exchange protein activated by adenylyl cyclase. PKA phosphorylates β-adrenergic receptor kinase, CREB, and GATA1 transcription factors. BARK recruits β-arrestin, inhibits β-adrenergic signal, and activates Src kinase, which in turn activates STAT3 and downstream focal adhesion kinase. FAK enhances migration. CREB, GATA1, and STAT3 promote VEGF, IL-6, IL-8, and MMP-9 expression, enhancing angiogenesis, migration, and invasion. In the other pathway activated through cAMP, EPAC also promotes cell migration. Stress stimulates hypothalamic-pituitary-adrenocortical axis, and the hypothalamus secretes corticotropin-releasing factor (CRF) that stimulates the adrenocorticotropic hormone (ACTH) secretion into blood vessels and systemic circulation. ACTH in adrenal gland cortex stimulates cortisol release. In tumor, cortisol binds to glucocorticoid receptor (GCR) in breast cancer cells and promotes the expression of MAPK phosphatase-1 (MKP1) and serine/threonine protein kinase 1 (SGK1) and other genes related to cell survival and apoptosis protection.*

contraceptives or menopausal hormone therapy, and environmental pollution with compounds such as bisphenols and phthalates, among others [3–5].

To classify breast cancer, the actualized TNM anatomical staging system categorizes primary tumor (T), regional lymph node invasion (N), and distant metastases (M), to determine the actual stage group of breast cancer. This classification is very useful, not only for diagnosis and prognosis but also for treatment. In addition to the anatomical staging system, factor-based prognostic stage groups that include tumor grade (histological), hormone receptor status, and a multigene panel status (when is available) were added in the 2017 TNM meeting [2, 3, 6].

Hormone receptor status takes into account the presence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) in tumor mammary cells. There are some characteristics that determine the status of the tumor, such as anatomical localization, tumor grade, hormone receptor status, and, with it, the prognostic and treatment of breast cancer [6].

A normal breast is composed of mammary glands (lobules and ducts), fibrous connective and adipose tissues, blood and lymph vessels, lymph nodes, nerves, and ligaments [7]. Duct branches form each mammary gland epithelium whose caliber is decreased until it forms ductules that flow into lobes [7, 8]. The epithelium is formed by luminal epithelial cells and basal epithelial cells, also known as myoepithelial cells, adjacent to the basement membrane [9]. Proliferation and apoptosis of mammary epithelia are regulated by the extracellular matrix (ECM) signals [10]. Either lobules or ducts can become dysplastic and eventually neoplastic, a phenomenon also regulated by ECM, and adjacent cell interactions, including stromal,

vascular, fibroblasts, and immune cells, may favor the transformation and uncontrolled proliferation of cells in the breast tissue.

In breast cancer progression, there are three phases: benign disease and noninvasive and invasive cancer (**Figure 1**). By definition, benign disease and noninvasive cancer share the same condition, where transformed cells do not trespass the basal membrane but are differentiated by histological grade. An example of this is the lobular carcinoma in situ, classified as a benign tumor, with associated risk in developing carcinoma [6]. On the other hand, in the invasive cancer, cells migrate through basal membrane to stromal breast and/or adjacent tissues and organs [11].

Several interactions determine different outcomes in tumor development, including the interactions among nervous, immune, and endocrine systems with the tumor. Next, we described the overall function of these systems in breast cancer and finally the interactions between them.

## 2. System interactions in breast cancer

### 2.1 The role of the nervous system on tumor growth and metastasis

Nervous regulation during cancer is mainly mediated through the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenocortical (HPA) axis [12].

#### *2.1.1 Sympathetic nervous system*

The sympathetic nervous system regulates the organism's vital involuntary functions and is in charge of the "fight-or-flight" response in danger and stressful situations and modulates the connection between the central nervous system and immune system [13].

SNS nerve fibers emerge from the thoracolumbar spinal cord, innervate different tissues, and produce norepinephrine [12, 14]. Nowadays, it is known that sympathetic nerve fibers innervate the bone marrow, thymus (primary lymphoid organs), spleen and lymph nodes (secondary lymphoid organs), and mucosa- (MALT), bronchus- (BALT), and gut- (GALT) associated lymphoid tissues [15–17]. Epinephrine arrives to the target tissue through blood circulation after being produced in the adrenal gland. Both norepinephrine and epinephrine bind with different affinities to adrenergic receptors α ($\alpha_1/\alpha_2$) and β ($\beta_1/\beta_2/\beta_3$) in target cells in different tissues and organs, such as the heart, brain, adipose tissue, mammary gland, ovaries, prostate, lymphoid tissue, bones, and different types of cancer cells [13, 14].

These adrenergic receptors are expressed differentially. In smooth muscles $\alpha_1$AR and $\alpha_2$AR can be found, although the latter also is expressed in platelets and neurons [14]. Regarding β receptors, of which noradrenaline is the main ligand, $\beta_1$AR can be found in the adipose tissue and cardiac muscle. And $\beta_2$-adrenergic receptors ($\beta_2$ARs) are expressed in tumor and immune cells, in the heart, lung tissue, and smooth muscle. At least, $\beta_3$AR can be found in the adipose tissue. Either $\beta_1$AR, $\beta_2$AR, or $\beta_3$AR activates cAMP and in turn stimulates protein kinase A (PKA) [14].

#### *2.1.2 Adrenergic signaling in tumors*

$\beta_2$AR expression has been detected in breast cancer cell lines, with different densities among them [18], and also in human breast tumor biopsies [19, 20]. Therefore, $\beta_2$AR expression should be considered if a $\beta_2$AR agonist treatment is going to be performed [21].

It is known that $\beta_2$AR signaling regulates proliferation and tumor cell invasion; this is evidenced with $\beta_2$AR blockers and the associated beneficial effect in breast cancer recurrence and bone, lung, and brain metastasis [13, 22]. Interestingly, primary TN tumor cells expressed lesser $\beta_2$AR mRNA and protein than TN brain metastatic cells from primary breast tumor; these metastatic cells exhibited increased proliferation and migration. In vivo and in vitro, invasive and metastatic potential of these cells was diminished when treated with propranolol [22].

There have been different mechanisms described that regulate invasion and metastasis through $\beta_2$AR signaling in tumor cells. Norepinephrine, epinephrine, or agonists bind $\beta_2$AR and activate adenylate cyclase (AC) through G-protein-coupled receptor subunit α ($G\alpha_s$). AC activation promotes ATP-cAMP conversion (**Figure 1**) and $Ca^{2+}$ intracellular increase [23]. In highly metastatic breast tumor cells (MDA-MB-231HM), another G-protein subunit, Gβγ, also promotes intracellular $Ca^{2+}$ augmentation through $\beta_2$AR signaling. Either through Gβγ or $G\alpha_s$, cAMP activates effector PKA and exchange protein activated by adenylyl cyclase (EPAC) and inhibits pERK1/pERK2; therefore, cell proliferation is mediated independently by ERK phosphorylation [23]. PKA phosphorylates CREB/ATF, GATA1 transcription factors, and β-adrenergic receptor kinase (BARK). BARK recruits β-arrestin which activates Src kinase, and this activates STAT3 [24]. Focal adhesion kinase (FAK) activated by STAT3 enhances migration and apoptosis resistance. CREB/ATF, GATA1, and STAT3 promote VEGF, IL-6, IL-8, and MMP-9 expression and enhance angiogenesis, migration, and invasion (**Figure 1**) [24]. Meanwhile, in breast tumor cells (MCF-7 and MDA-MB-231) treated with an EPAC inhibitor (ESI-09), migration inhibition was found associated with mislocalization of the A-kinase anchoring protein 9 (AKAP9); therefore, EPAC also promotes cell migration [25].

Another way in which $\beta_2$AR signaling stimulates tumor growth is through promoting DNA damage and p53-associated apoptosis suppression [26].

### *2.1.3 Tumor innervation*

During tumor initial innervation, nearby healthy tissue provides sympathetic fibers that infiltrate the periphery of the growing tumor [27], in response to neurotropic factors secreted by tumor cells, such as neurotrophic growth factor (NGF) [28] and brain-derived neurotrophic factor (BDNF) [29]. These factors increase nerve fiber growth and thereby tumor innervation [28]. Tumor innervation is associated with vasculature; therefore, together with nerve fibers, blood vessels go through the tumor mass [30] (**Figure 1**). Sympathetic innervation in tumor is the main catecholamine source [30, 31]; this is evidenced because its local concentration is higher than plasma [31]. In this sense, innervation is a feature of tumor microenvironment associated with tumor aggressiveness [28].

Therefore, β-receptor antagonists could have an important role in the development of new therapies that diminish metastasis risk and promote a slow tumor progression.

### *2.1.4 HPA axis*

The HPA axis also plays an important role in stress response in mammals. In the hypothalamus, the paraventricular nucleus neurons secrete corticotropin-releasing factor into hypophyseal portal blood. CRF stimulates the release of adrenocorticotropic hormone by the anterior pituitary gland, to the blood vessels and systemic circulation. When ACTH reaches adrenal glands, the cortex stimulates the corticosterone production (in rodents) or cortisol (humans) (**Figure 1**) [32].

Cortisol and corticosterone (glucocorticoids) exert their effects through glucocorticoid receptor. Different immune and breast cancer cells express this receptor, and glucocorticoids exert different effects in these cells.

GCR in breast cancer cells, especially in triple-negative lines (MDA-MB-231), promotes the expression of genes related to cell survival and apoptosis protection, for example, MAPK phosphatase-1 and serine/threonine protein kinase 1 (**Figure 1**) [33, 34]. In addition, the activation of the GCR induces the expression of genes related to cell survival, adhesion, and EMT in a premalignant breast [35]. Correspondingly, in xenograft MDA-MB-231 breast tumors in mice pre-treated with systemic dexamethasone, the paclitaxel treatment effect was inhibited [36]. Therefore, glucocorticoids protect breast cancer cells from apoptosis and enhance their survival capabilities, favoring tumor growth.

*2.1.5 Stress and tumor growth*

Stress is largely linked to cancer development. Stressor exposition and SNS activation promote noradrenaline release into the tumor that regulates tumor progression [30]. In animals under social isolation (a stress experimental model), there is an increased tumor size and reduced survival [37]. Also in other stress models, increased tumor progression and metastasis have been observed [38, 39].

During metastatic establishment, $\beta_2$AR overexpression enhances cell proliferation and invasion, to ensure metastatic establishment, and, maybe, that is why in primary tumor resection, the surgery induces stress and releases norepinephrine and epinephrine that enhance tumor metastasis. Thus, the use of treatments that antagonize the effect of these neurotransmitters may reduce metastasis [22].

Thus, nervous control of tumor growth is regulated by the sympathetic and HPA systems. The sympathetic regulation is via tumor innervation with sympathetic fibers and the adrenergic signaling in tumor, through local and peripheral catecholamine (epinephrine and norepinephrine) production and the $\beta_2$AR expression in tumor and immune cells. Meanwhile, HPA system regulates glucocorticoid production, which enhances tumor cell survival and downmodulates inflammatory response, thus enhancing tumor growth and metastasis (**Figure 1**).

## 3. Immune response in mammary tumors

Tumorigenesis usually courses a slow development during years, and the immune response depends on the different stages of the disease and the tumor microenvironment [40].

Every day, immune cells detect and destroy transformed cells, in a phenomenon called immunosurveillance. But, when transformed cells evade elimination mechanisms (immunoescape), survive and proliferate. At this point, tumor can remain in a state of dormancy, partially by the action of immune cells, but when the balance between stromal, immune, and tumor cells with their secretory products leads to local immunosuppression, the immunosubversion is established, and the tumor grows (**Figure 2**) [41, 42].

### 3.1 Immune innate cells in mammary tumors

*3.1.1 Dendritic cells*

Dendritic cells are antigen-presenting cells (APCs) that recognize, uptake, process, and present antigens to different cells including T cells. In the immunosurveillance

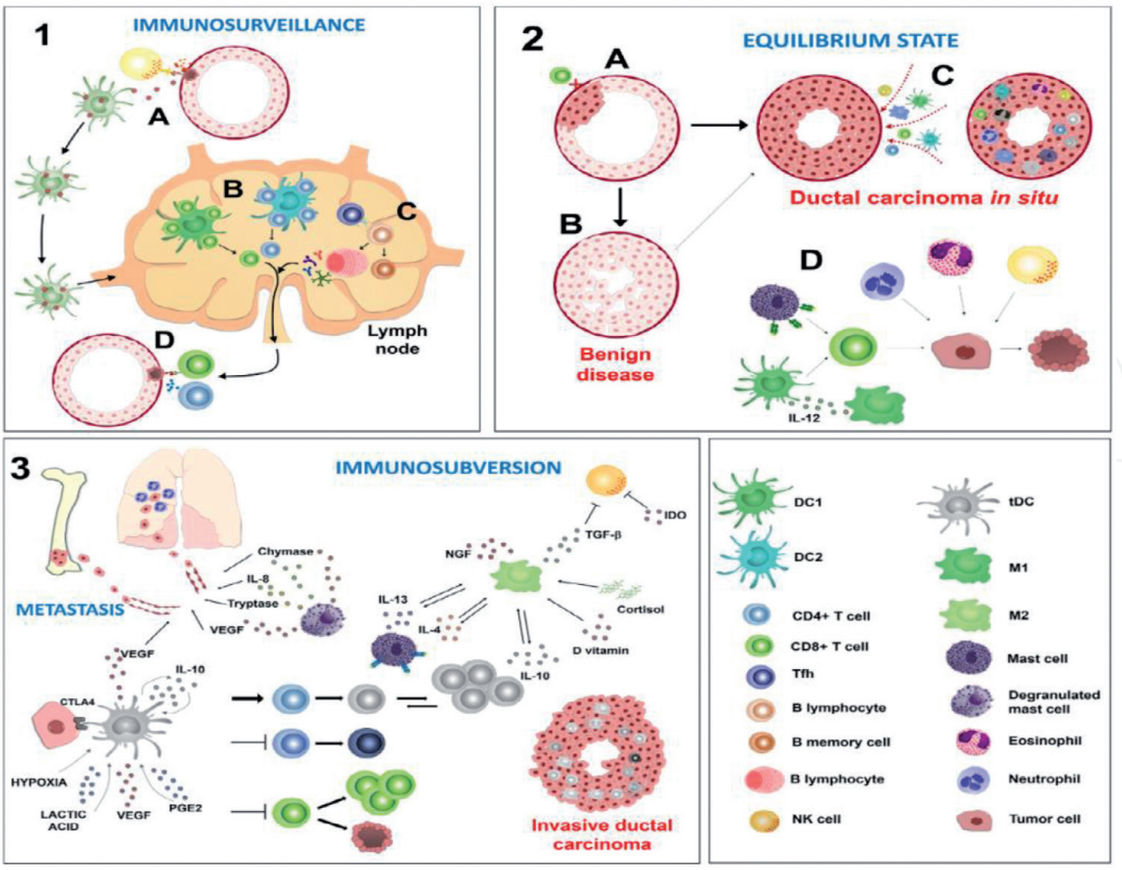


**Figure 2.**
*Immune response in breast tumor development. (1) In immunosurveillance, breast tissue-resident conventional dendritic cells (cDC) capture antigens released by transformed cell after recognition and destruction by cytotoxic cells (NK cells). cDC migrate to peripheric lymph node and as DC type 1 (DC1) or DC type 2 (DC2) present antigens to $CD8^+$ T cells or $CD4^+$ T cells, respectively. In lymph nodes T follicular cells activate B lymphocytes into plasma (antibody producer) or B memory cells. After the activation and subsequent proliferation, $CD8^+$ T cytotoxic and $CD4^+$ T helper cells migrate. Cytotoxic cells induce apoptosis to transformed cells, and T helper cells produce cytokines and chemokines to enhance cytotoxic effect. (2) In the equilibrium state, transformed cells evade immune recognition and proliferate to hyperplasia (benign disease) and eventually to ductal carcinoma in situ (DCIS). In these phases immune cells are recluted, and cytotoxic cells ($CD8^+$, neutrophils, and eosinophils) may eliminate transformed cells in an equilibrium phase. Indirectly, other cells enhance cytotoxic response, for example, M1 macrophages produce IL-12 that enhances dendritic cell antigen presentation to $CD8^+$ cells. Also mast cells MHC-$I^+$ stimulate $CD8^+$ T-cell proliferation. (3) In immunosubversion and metastasis, when invasive ductal carcinoma is established, tumor dendritic cells (tDC) are induced through IL-10, vascular endothelial factor (VEGF), prostaglandin E2 (PGE2), hypoxia, and lactic acid and by direct contact with tumor $CTLA4^+$. tDC produce IL-10, which in turn induces their own expansion as well as T regulatory cell (Tregs) proliferation and the inhibition of effector $CD4^+$ and $CD8^+$ T cells, Th1 differentiation, and $CD8^+$ T cells function. Also, IL-10, D vitamin, and cortisol activate M2. M2 macrophages produce IL-4, IL-10, IL-13 (that feedback M2 generation), NGF, and TGF-β that with IDO inhibit cytotoxic NK function. Mast cells secrete VEGF, tryptase, chymase, and IL-8 that enhance metastasis to different organs (lung and bone). Neutrophils may enhance metastasis because they form premetastatic niches that promote tumor cell migration and metastasis establishment.*

phase, dendritic cells resident in the breast tissue sense and capture different antigens released by transformed cells and then migrate to draining lymph nodes, where, as a mature cell, antigens to naïve T cells are present [43–45]. After the activation and subsequent proliferation, the $CD8^+$ T and $CD4^+$ T cells migrate to the site where transformed neoplastic cells reside. The cytotoxic response is carried out mainly by $CD8^+$ T cells and NK cells, which detect and induce apoptosis to transformed cells; meanwhile, $CD4^+$ T helper cells produce cytokines and chemokines that modulate the immune response and recruit other immune cells (**Figure 2**).

Two lineages of dendritic cells are responsible for T-cell priming. The first are DC1s that express chemokine receptor CXR1 and present antigens through MHC-I

preferentially to $CD8^+$ T cells. The second are the DC2s that express CD172a and MHC-II (high), to activate $CD4^+$ T cells [43]. DC1s can be lymphoid-resident DC1s (CD8α in mice) or migratory DC1s ($CD103^+$ in mice), being the latter mentioned as the main subset of DC that can induce a strong cytotoxic response against tumor through the activation of $CD8^+$ T cells [46]. Meanwhile, DC2s ($CD11^+$ in mouse) seem to fail in tumor antigen presentation to $CD4^+$ T cells in lymph nodes, but it is unclear why. A possibility is an inadequate process of antigen or the nature of tumor antigen. These DC2s cells can be tolerogenic, because they cannot generate an adequate activation and stimulation [43].

Three features lead to the induction of suppressive or tumor phenotype DC. The first is the presence of tumor cell neoantigens that leads eventually to immunoescape and the failure of immunosurveillance. The second is the degree of DC maturation, in which immature DC acquire a tolerogenic phenotype and generate regulatory T cells. And the third is related to the immune suppression in the tumor microenvironment mediated by other cells and soluble factors. The balance of stimulatory and suppressive signals determines tumor progression and is related to cell tumor phenotype and the interaction among cells [43].

There are many tumor microenvironment factors that suppress DC activation in vitro, for example, IL-10, vascular endothelial factor, prostaglandin E2, hypoxia, and lactic acid [47]. Another important interaction is when breast tumor and DC are in contact. Chen et al. showed a decreased expression of CD40, CD80, CD86 (costimulatory molecules), HLA-DR, and CD83 and a reduced production of IFN-γ, TNF-α, IL-1β, IL-2, IL-6, and IL-12, in lipopolysaccharide (LPS)-stimulated human DC, when co-cultured with $CTLA4^+$ breast cancer cells. These suppressive DC inhibit $CD4^+$ and $CD8^+$ T-cell proliferation, Th1 differentiation, and cytotoxic lymphocyte (CTL) function (**Figure 2**) [48].

When a transformed cell escapes of the immune recognition and destruction, and starts proliferating, it recruits different immune cells and promotes a protumoral and suppressor microenvironment. Among these tumor-recruited cells are DCs, which migrate to local lymph nodes and present tumor antigens to lymphocytes. Meanwhile some recruited DC go to lymph nodes, and another subset of DC remains in tumor and developed suppressor functions through the direct inhibition of the local activation of $CD4^+$ and $CD8^+$ T cells or through suppressive cytokine production (IL-10) (**Figure 2**) [43, 49]. These DC may have an important role in lymphocyte priming in tumor, associated with the presence of tertiary lymphoid structures (TLS) in breast tumors, specially placed in stroma and with naïve T cells in tumors that are activated in situ [50, 51].

### *3.1.2 Neutrophils*

Neutrophils are polymorphonuclear (PMN) cells and the most abundant leukocyte in human. These cells are responsible for host defense to bacterial, fungal, and viral infections and support wound healing [52]. Neutrophils can phagocyte, form neutrophil extracellular traps (NETs) to eliminate invasive microorganisms, and synthesize and store in cytoplasmic granules neutrophil elastase, cathepsin G, proteinase 3, neutrophil collagenase (MMP-8), gelatinase B (MMP-9), reactive oxygen species (ROS), and antimicrobial peptides [53–55]. Through chemotactic stimuli, neutrophils arrive to the inflammation site and phagocyte the invading microorganism. Thereafter, cytoplasmic granules in the neutrophil get fused with the phagolysosome where the microorganism is destroyed [52]. Under adverse circumstances, neutrophil can release proteinases through microbursts, to the extracellular space, or produce NETs to fix the microorganisms, stop their migration, and concentrate on toxic factors [56].

Some authors mentioned a neutrophil polarization similar to classical activated macrophages (M1) and alternative activated macrophages (M2), named N1 and N2; also, neutrophils present different degrees of activation, and according to it, there are four types of PMN: naïve circulating, mildly activated, activated (acute inflammation), and highly activated (sepsis, unsuccessful phagocytosis). Among mildly activated neutrophils are tumor-associated neutrophils (TANs) that in mice express $CD11b^{+}$ and $Ly\text{-}6G^{hi}$ markers [52, 57].

Cellular cytotoxic role in cancer is traditionally associated with cytotoxic T cells, NK cells, and macrophages, and little attention is focused on neutrophils, but nowadays, reports are linking neutrophils to different stages of cancer [56]. Naïve neutrophils are recruited to the tumor, mainly by macrophages, and display the same repertory to kill a microorganism for the destruction of a tumor cell, and eventually a pro-host or pro-tumoral effect in situ is developed (**Figure 2**) [52].

The presence or absence and quantity of neutrophils within the tumor, associated with tumor type, determine the prognostic of the disease [57]. In an orthotopic murine model of breast cancer with 4T1 (metastatic cells) and 4T07 (nonmetastatic cells), more neutrophils within 4T1 tumors in comparison to 4T07 tumors were detected. Also in 4T1 tumors, higher mRNA expression of CXCL1, a neutrophil-recruiting chemokine, was detected [58]. For example, a pre-metastatic niche has been reported in remote organs, where neutrophils come together and shape a microenvironment that favored the migration of tumor cells (**Figure 2**) [59]. One of the mechanisms that shape a pre-metastatic niche could be mediated by NETs, as has been demonstrated in an experiment where neutrophils were co-cultured with 4T1 cells in a transwell chamber assay and produced more NETs than neutrophils co-cultured with 4T07 cells. In the same report, authors proved the presence of NET structures located next to 4T1 cells, which was assumed to contribute to support metastasis [58].

### *3.1.3 Eosinophils*

Eosinophils are granulocyte cells that can be found in the spleen, lymph nodes, thymus, and gastrointestinal tract [60] and are able to phagocyte and act as antigen-presenting cell in lymph nodes, through the expression of major compatibility complex and costimulatory molecules (CD40, CD80, CD86) [61]. Furthermore, eosinophils produce cytokines, chemokines, growth factors, lipid mediators, and cytotoxic granules (**Table 1**).

Eosinophils are usually related to parasitic infections, especially helminthiases, in which eosinophilia is a characteristic feature. But recently their role in cancer has become relevant. Depending on its cytokine profile production, a new classification of eosinophils has been proposed; the eosinophils that secrete Th1 cytokines (IL-8, TNF-α, and IFN-γ) are called E1, and the ones that produce Th2 cytokines (IL-4, IL-5, and IL-13) are E2. Despite this classification, in breast cancer it is unknown if the eosinophils secrete any of those cytokines, although blood eosinophilia is related to a good or poor prognostic of the disease, depending on the cancer type [79]. Related to eosinophil infiltration into the breast cancer tumor, the presence of eosinophils is one indicator of increased survival, maybe because these cells participate in host-tumor interactions and because of their cytotoxic activity [83].

### *3.1.4 Mast cells*

Mast cells originate in bone marrow, then circulate and migrate to tissues, and in nearby blood vessels mature into effector cells, in which along with DC and macrophages, are the first cells to recognize and interact with pathogens or allergens [84, 85]. Inside mast cells there are granules with preformed substances that included histamine

| | | General functions | Cancer-related features |
|---|---|---|---|
| Cytokines | IL-8 | Supports endothelial cell proliferation and survival [62] | Breast cancer tissue expresses higher concentrations of IL-8 than normal tissue[63] |
| | TNF-α | Pro-inflammatory cytokine | Chronic expression sustains breast tumor growth [64] |
| | IL-4 | Promotes a Th2 profile, B-cell differentiation, and IgE isotype switch | |
| | IL-5 | Stimulates proliferation, differentiation, recruitment, and activation of eosinophils [65] | |
| | IL-13 | Regulates IgE synthesis and mucus production [66] | A higher tumor stage correlates with higher serum levels and lymph node metastasis [67] |
| Chemokines | CCL3, CCL5, CCL11, CCL24; CXCL8, CCL7 | Chemoattractant for eosinophils | Recruitment of eosinophils to tumor |
| | CCL3, CCL5, CCL11, CCL17, CCL22, CCL23; CXCL1, CXCL5, CXCL8, CXCL9, CXCL10, CXCL11 | Chemokines secreted by eosinophils to recruit other immune cells | Recruitment of immune cells to tumor |
| Growth factors | TGF-α | | In mammary mouse tissue, overexpression of TGF-α induces hyperplasia and proliferation [68] |
| | TGF-β | Regulates cellular differentiation, proliferation, apoptosis, and migration [69] | In early stages suppresses tumor progression but in late stages favors tumor growth [69] |
| | VEGF | Promotes angiogenesis | Promotes tumor angiogenesis |
| Lipid mediators | Leukotrienes | Pro-inflammatory | Elevated levels in colon, pancreatic, and prostate cancer [70] |
| | Prostaglandin E2 | Shifts Th2 response and downregulates $CD8^+$ T-cell activity and tumor cell antigen presentation [71] | Promotes tumor growth in breast cancer Is associated with poor prognosis [72] |
| | Thromboxanes | | Overexpression is associated with poor prognosis in urothelial cancer [73] |
| | Lipoxins (lipoxin A4) | | Suppressing the polarization of B regulatory cells [74] |

| | | General functions | Cancer-related features |
|---|---|---|---|
| Cytotoxic granules | Eosinophil cationic protein | Tissue remodeling, suppression of T-cell proliferation, mast cell degranulation, and secretion of airway mucus [75] | Proliferation inhibition in colorectal carcinoma and oral squamous cell carcinoma cell lines, via osmotic lysis [76, 77] |
| | Major basic protein | Tissue damage | Cytotoxic effect in human cancer cell lines [78] |
| | Eosinophil-derived neurotoxin | Cytotoxic activity and chemoattractant of DC, monocytes, neutrophils, mast cells, and T cells [79] | Cytotoxic activity in colorectal carcinoma cell line [80] |
| | Eosinophil peroxidase | Related to inflammatory tissue injury [81] | Absent in normal breast tissue but present in breast cancer tumor stroma [82] |

*CCL5 or RANTES, regulated on activation normal T expressed and secreted; CCL3 or MIP-1α, macrophage inflammatory protein; CCL7 or MCP-3, monocyte-specific chemokine protein; TGF-α, transforming growth factor α; TGF-β, transforming growth factor β; VEGF, vascular endothelial cell growth factor; GM-CSF, granulocyte macrophage colony-stimulating factor*

**Table 1.**
*Functions and related cancer features of cytokines and chemokines.*

(vasodilator), heparin (anticoagulant), serotonin, dopamine, tryptase, and chymase. The mast cell activation stimulates the production of leukotrienes and cytokines (e.g., TNF-α) and the cell degranulation [86].

Mast cells may perform both immunosuppressive and inflammatory functions depending on the interaction with the effector or regulatory immune cells [85]. For example, the expression of MHC-II in mast cells can be induced by the exposure of LPS and IFN-γ, and the interaction of MHC-II-expressing mast cells with effector T cells induces the expansion of Treg cells; meanwhile, mast cells expressing MHC-I can enhance the proliferation of $CD8^+$ T cells [87, 88]. Besides direct contact with T cell, an alternative activation mechanism could be the mast cell production of IFN. This cytokine enhances the proliferation of T cells, depending on the number of mast cells within the microenvironment, for example, at low numbers proliferation is enhanced, but in higher numbers proliferation is inhibited, in a mechanism mediated by the H1 histamine receptor [89, 90].

On the other hand, for naïve B-cell survival and activation and for plasma cell proliferation and differentiation, mast cells interact with B cell through superficial CD40L. Also for the B-cell synthesis of IgE, the secretion of IL-4 and IL-13, among others, by mast cells is necessary [85].

Despite mast cells releasing angiogenic factors, as VEGF, chymase, tryptase, heparin, fibroblast growth factor-2 (FGF-2), IL-8, TGF-β, and nerve growth factor, the inhibition of mast cell degranulation did not change the mammary tumor vascularization, but that does not mean that degranulation may enhance angiogenesis [91]. In this regard, a study informed that tryptase did not stimulate the proliferation of MDA-MB-231 breast cancer cells but indeed enhances its migration and invasion [92]. In malignant breast carcinomas, there are more tryptase-containing mast cells detected through immunohistochemistry assay than that in benign lesions [93]. Given the prominent angiogenic character of mast cells, to date, their presence has not been strongly associated with the enhancement of the tumor

vascularization, and it is not clear if comorbidities favoring increased quantities of mast cells may improve tumor vascularization and metastasis.

### *3.1.5 Macrophages*

Macrophages are mononuclear phagocytic cells that according to environment signals turn into different phenotypes. One of these phenotypes is the classically activated macrophages or M1, induced by Toll-like receptors (TLR) and IFN-γ. These cells are characterized by the expression of IL-12, the major histocompatibility complex class II (MHC-II) and TNF-α, and ROS and nitric oxide (NO) production and are associated with microorganisms and cell destruction [94]. The second phenotype is the "alternatively or selectively" activated macrophages, which are characterized by the secretion of IL-4, IL-10, IL-13, and TGF-β and the expression of arginase-1 and VEGF and are related to wound healing and humoral response [94, 95].

Macrophages, monocytes, and DC can be found in tumor microenvironment, being the macrophages the most abundant phagocytic population. Tumor-associated macrophages (TAMs) are characterized by the cell surface expression of CD68 and have been related to invasion and migration of cancer cells, being a prognostic factor in cancer [95, 96]. Some reports have shown that the density of TAMs in breast cancer samples is related to hormone receptor status, lymph node metastasis, stage, and prognosis. Higher concentrations of TAMs are associated with a poor prognosis, and the worse prognostic group is the one with a high proportion of CD163 and CD206 (M2 markers) [95].

TAMs are related to immunosuppressive features, for example, low antigen-presenting capability, low tissue remodeling activity, and low toxicity functions that promote tumor growth and metastasis [97]. These immunosuppressive TAMs function as M2 macrophages and are activated by IL-4, IL-10, IL-13, glucocorticoids, and vitamin $D_3$ [98].

### *3.1.6 NK cells*

The innate immune system recognizes and kills infected and transformed cells; NK cells are responsible for this task, through granzyme b-perforin system, TNF-related apoptosis-inducing ligand (TRAIL), and the expression of CD95 ligand [99]. NK cells produce IFN-γ, granulocyte/macrophage colony-stimulating factor (GM-CSF), and TNF [100] and are one of the main cells in antitumoral response.

Depending on the signals that NK cells receive, activation or inhibition receptors or coreceptors are expressed [101, 102].

NK cell cytotoxicity is activated by different ligands upregulated during cellular stress, also with the recognition of antibodies in the antibody-dependent cellular toxicity (ADCC) through the expression of CD16 (Fc immunoglobulin fragment low-affinity receptor) and with the detection of cells that underexpressed HLA-class I molecules [101, 103]. In immunosurveillance, tumor cells are detected and destroyed by NK cells, through these mechanisms (**Figure 2**). In immunosubversion, tumor cells evade NK cell recognition, and tumor microenvironment leads to NK cell impairment, through the inhibition of surface-activating receptor expression, such as NKp46 and NKG2D or NKp30 and NKG2D mediated by indoleamine 2,3-dioxygenase (IDO) and TGF-β1, respectively (**Figure 2**) [101, 104, 105].

In breast cancer patients, tumor NK cells possess a more prominent inhibitory phenotype than peripheral NK cells. Also depending on disease progression, for example, in late stages, NK cells lose their cytotoxic activity and express inhibitory

receptors (NKG2A); meanwhile, in early stages NK cells express activating receptors (NKp30, NKG2D, DNAM-1, and CD16). One of the stroma-derived suppressor factors that induced NK cell function impairment is TGF-β1 [101].

## 3.2 Immune adaptive cells in mammary tumors

### *3.2.1 T lymphocytes*

T lymphocytes are one of the most important cell populations in cancer. The activation of T cells is performed, firstly, through TCR stimulation with its specific antigen, presented in the context of MHC, by a dendritic cell or another professional antigen-presenting cell; secondly, with the binding of "costimulatory" molecules in the dendritic cell; and, thirdly, by the cytokine milieu and soluble factors [106, 107]. In addition, antigen presentation is performed by immature DCs, resulting in a non-responsive or anergic T cells [108]. In breast cancer tertiary lymphoid structures and germinal centers were detected next to tumor in extensively infiltrated tumors. This TLS possesses a similar structure to lymph node, including a T-cell zone with $CD3^+/CD4^+$ T cells and a germinal center with B cells and T follicular helper (Tfh) cells [109].

### *3.2.2 T helper cells*

Different factors such as the expression of transcription factors, chemokine receptors, signal transduction activators, and the chemokine and cytokine secretion regulate the effector phenotype and function of these cells [107]. Regarding human breast cancer, different effector phenotypes have been reported, for example, through flow cytometry of invasive breast tumors, Tfh, Th1, Th2, Th17, and Tregs were found [109].

### *3.2.3 T follicular helper (Tfh) cell*

The effector phenotype Tfh cell stays in the lymph node and induces activation and differentiation of affine B cells into plasma or memory cells [110]. But, in advanced stages of invasive breast cancer, $CD4^+$ Tfh cells were detected in the T-cell zone and germinal centers of TLS. This localization is may be due to their function in tumor, because Tfh cells were localized near to B cells [111].

### *3.2.4 T helper 1 (Th1)*

$CD4^+$ T helper 1 (Th1) cell differentiation and IFN-γ production are modulated by IL-12 produced by APCs (monocytes/macrophages, DCs, and even NK cells) and IFN-γ (**Figure 2**) [112–114]. Th1 cells express the transcription factor T-bet; secrete IFN-γ, TNF-α, and IL-2; and function as regulators of monocyte activation and T lymphocyte differentiation induction [115, 116]. Th1 cells are associated with early tumor phases, because of their IFN-γ production that activates $CD8^+$ cytotoxic T cells (**Figure 2**) [117]. Therefore, there is an association between improving survival and the infiltration of Th1 and $CD8^+$ T cells in breast tumors [118].

### *3.2.5 T helper 2 (Th2)*

A T helper subset related to immunosubversion and tumor progression is the $CD4^+$ T helper 2 (Th2) cells, characterized by the expression of transcription factor GATA3 and the secretion of IL-4, IL-5, IL-10, and IL-13 [121]. Th2 cells are related to

nematode response, tissue repair, and antibody production. In breast cancer, Th2 cells have been found in human mammary tumors [109], and IL-13 is reported to be present also in human breast tumors, promoting tumor development (**Figure 2**) [119].

*3.2.6 T helper 17 (Th17)*

$CD4^+$ Th17 (T helper 17) cells are related to autoimmunity, tissue inflammation, and host defense against bacteria, fungi, viruses, and protozoa and play an important role in mucosal immunity [120]. Th17 cells produce IL-17 and IL-22 and express transcription factor RORγt [121]. Regarding Th17 cells in tumor, it is not recognized if they could be induced in tumor or be recruited from other places, but their presence has been detected in breast tumors [109]. Th17 cell tumor infiltration mediates an inflammatory microenvironment [122].

*3.2.7 Regulatory T cells (Tregs)*

Tregs are a subset of T helper cells that express Foxp3 transcription factor and have an important role in controlling inflammation and autoimmunity in mouse and man [123]. These cells normally are residents in the secondary lymphoid organs, lung, peripheral blood, gastrointestinal tract, liver, and skin and can be recruited to other tissues, under inflammatory conditions [124]. Tregs are $CD4^+$, $CD127^{low}$, $CD25^{hi}$, and also $CTLA^+$ and exert immune suppression through different mechanisms, for example, the production of tolerogenic cytokines (IL-10, IL-35, and TGF-β), the induction of arginine depletion that leads to T-cell dysfunction, the expression of suppressive molecules (CTLA-4, CD80/CD86), and the direct cytolysis through granzyme b-perforin system and through local consumption of IL-2 (with the constitutive expression of high-affinity receptor CD25) [125, 126].

IL-10 is a suppressive cytokine secreted by macrophages, NK cells, NKT cells, B cells, DCs, and $CD4^+$ T cell (specially Treg cells), that suppress inflammatory responses, prevent autoimmune diseases, and enhance tumor growth (**Figure 2**) [127–129].

*3.2.8 T cytotoxic lymphocytes (CTLs)*

$CD8^+$ cytotoxic T lymphocytes play an important role in adaptive antitumor response; therefore, when tumor immunosubversion is stablished, the cytotoxicity of $CD8^+$T cells gets compromised (**Figure 2**). During immunosurveillance, in secondary lymphoid organs (lymph nodes and spleen), APCs present tumor-associated antigens and tumor neoantigens to CTLs, which in the presence of costimulatory and cytokine signals, such as IL-12 (produced by DCs) and IFN-γ (produced by Th1 cells), undergo activation, maturation, and clonal expansion [117, 130]. After, CTLs migrate through the body, search for specific antigen, and kill the tumor antigen-specific cell through IFN-γ release and perforin and granzyme system (**Figure 2**) [117, 131, 132]. Also, through the activation of its receptor, IL-12 promotes the differentiation of effector CD8 cells and inhibits at the same time the development of memory CD8 cells [133, 134]. When effector CTL cells failed in killing target cell and are exposed to persistent antigen stimulation (in chronic infectious diseases or in tumors), CTL express inhibitory cell surface receptors, PD-1, LAG3, TIM3, TIGIT, and CTLA-4, and became exhausted $CD8^+$ T cells. During tumor immunosubversion $CD8^+$ T cell exhausted profile is generated; therefore, cytolysis or IFN-γ secretion mediated through CTLs is inhibited (**Figure 2**) [135].

Another important cytokine related to $CD8^+$ T cells is IL-2, which is described not only as a growth factor, secreted by $CD4^+$- and $CD8^+$-activated T cells, but also

as a differentiation inducer for $CD8^+$ effector cells [115]. $CD8^+$ T cells cultured with IL-2 presented an upregulation in perforin (Prf1) transcription and a suppressed expression of Bcl6 and IL-7Rα (memory $CD8^+$ markers). Meanwhile, in $CD8^+$ T cells with deficiency of IL-2 receptor (IL-2Rα or CD25), cell differentiation impairment was shown in vivo, demonstrated with granzyme B and perforin diminished expression and a poor ex vivo cytotoxicity [136].

In regard to $CD8^+$ T cell antitumor activity, an experiment in tumor from four T1 mammary gland tumor cells in syngeneic mice showed that in mice injected with IL-12, tumor growth was suppressed through an increased $CD8^+$ cell infiltration and production of IFN-γ and the induction of apoptosis of tumor cells [137]. This effect correlates with good prognosis in $CD8^+$ T cell infiltration in breast tumors in women [138]. With the time, CTLs lose their cytotoxic phenotype and acquired an exhausted phenotype that needs further characterization in breast tumors but is likely to be associated with late stages (triple-negative breast cancer).

## 4. Endocrine factors related to the development of breast cancer

Mammary gland epithelium is highly dynamic, characterized by proliferation, differentiation, and apoptosis cycles, regulated in part by hormones. Breast cancer is associated with an abnormal proliferation of epithelial cells, related to genetic mutations and epigenetic modifications in suppressor and DNA repair genes and oncogenes [139].

### 4.1 Estrogens and progesterone

During life, the mammary gland development is divided by different stages and modulated by hormones such as estrogens (17β estradiol) and progesterone. These stages are related to sexual development and includes embryonic and prepuberal phase, puberty, pregnancy, lactation, and involution. Epidermal growth factor (EGF) and estrogens that arrive through the breast stroma during puberty induce ductal elongation and branching. Meanwhile, the lobes formed by secretory epithelial cells organized in alveoli develop during gestation and probably in lactation, through the placental lactogens, progesterone, and prolactin signaling [140]. In lactation, milk secretion is promoted by the contraction of rounding epithelium smooth muscle cells mediated by oxytocin [8].

Estrogen effects are regulated through alpha and beta estrogen receptors (ERα and ERβ), both expressed in mammary normal tissue [141]. ERα signaling is responsible for ductal elongation in puberty and the stromal invasion in normal breast tissue [142].

The sharing ERα-ERβ distribution suggests that ERβ may be related to a negative ERα regulation, through antiestrogen or non-habitual effects [143]. The ERα or ERβ receptor dimerization is induced by ligand-receptor union and leads to the formation of homodimers (ERα/α, Erβ/β) or heterodimers (ERα/β). Recently, it was described that the dimer conformation is associated with their function. ERα/α is linked with proliferation induced by estrogen, and ERβ/β homodimer is linked with antiproliferative and pro-apoptotic functions; meanwhile, ERα/β effects are not elucidated as well (**Figure 3**) [144].

Proliferation in breast tumor cells is ligand-dependent in early stages and ligand-independent in late stages. In the estrogen-dependent pathway, cell proliferation is activated through cytoplasmic or membrane estrogen receptors. Intracellular signaling begins with estrogen-receptor union, their consequent translocation to nuclei, where the estrogen-receptor complex binds to specific

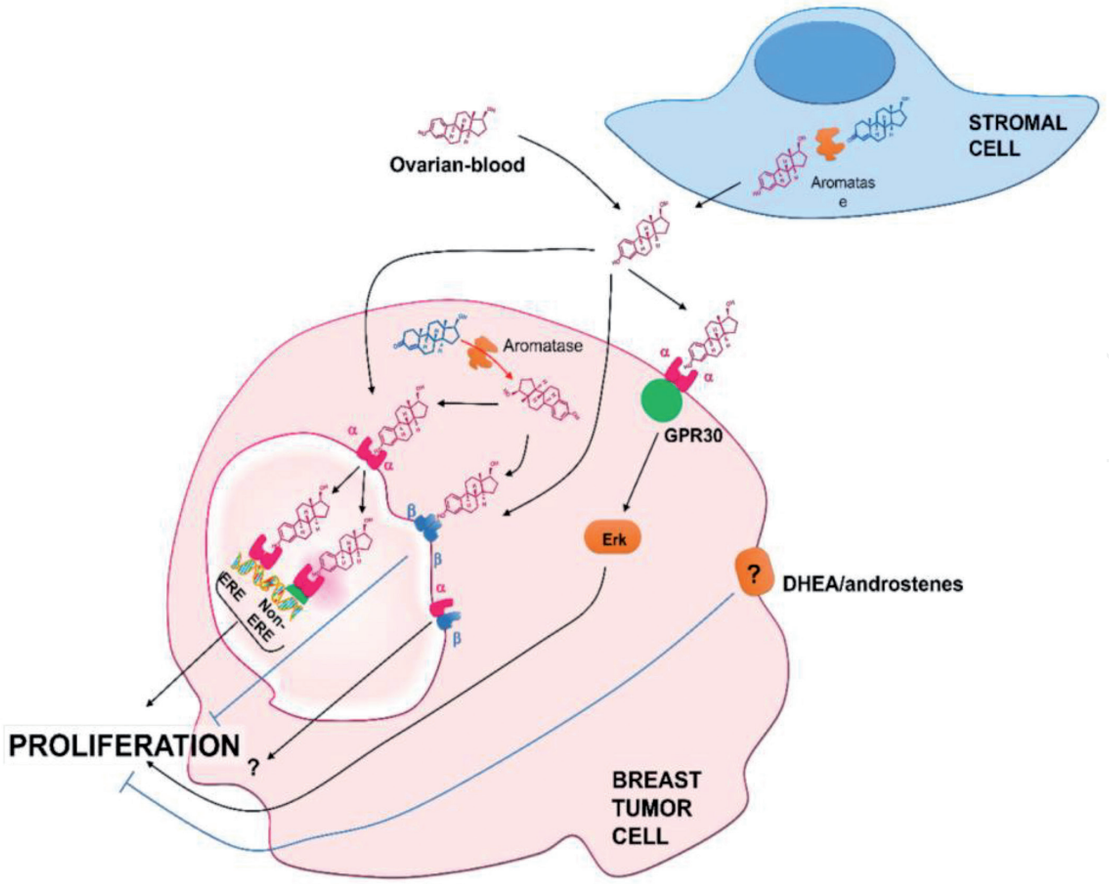


**Figure 3.**
*Endocrine interactions in breast cancer. In the estrogen-dependent pathway, cell proliferation is activated through cytoplasmic or membrane estrogen receptors. Intracellular signaling begins with estrogen-receptor union, their consequent translocation to nuclei, where the estrogen-receptor complex binds to specific estrogen-response elements (ERE) in estrogen-responsive genes, in a non-ERE way, functioning as gene transcription co-regulator. Furthermore, estrogen-membrane ER signals through GPR30 and Erk to elicit proliferation. Estrogen sources to breast tumor cell are intracrine, endocrine (blood supply), and paracrine (stromal adjacent cells). Dehydroepiandrosterone (DHEA) and other androstenes inhibit tumor cell proliferation.*

estrogen-response elements in estrogen-responsive genes or to other transcription factors, such as AP1 or Sp1, in a non-ERE way, functioning as gene transcription co-regulator [145, 146]. Furthermore, estrogen-membrane ER signals through GPR30 and Erk to elicit proliferation (**Figure 3**) [145]. Meanwhile, the estrogen-independent pathway is mediated through ligand binding and activation of growth factor receptors (GFRs), such as epithelial growth factor receptor (EGFR), insulin-like growth factor receptor (IGF), and HER-2, among others. This activation promotes ER phosphorylation and activation through PI3K/AKT and Ras/Raf/MAPK pathways [145, 147].

On the other hand, progesterone exerts its action through two receptors (PRA and PRB), both signaling and activating gene transcription [148]. ERα and PR are co-expressed in the mammary gland in 15–30% of epithelial cells [149]. Meanwhile, estradiol and progesterone drive epithelial mammary gland proliferation directly through the hormone-receptor union and have been proposing a second control mechanism in which epithelial cells sense hormone concentrations through their estrogen and progesterone receptors and, in consequence, secrete or not growth factors to promote nearby cell proliferation [148].

In breast cancer staging, ER and/or PR expression loss is associated with more aggressive tumors, with self-sufficiency of growth signals independently of estrogen or progesterone receptors. Additionally, positive ERα tumor is related to a better prognostic, as well as ERβ tumor expression [150].

Besides the receptor's presence in tumor cells, another important issue to consider is the hormone levels within the tumor. Intratumoral estradiol concentration in normal breast tissue was lower than in ductal carcinoma in situ and invasive ductal carcinoma [151].

In this regard, aromatase is an important enzyme responsible for the production of estrogens, estrone, and estradiol, through the aromatization and conversion of androstenedione and testosterone [152]. Invasive ductal carcinoma expresses a higher amount of aromatase mRNA than DCIS and normal breast tissue, and both epithelial and stromal cells expressed aromatase mRNA [151]. Therefore, tumor cells have different sources of estrogen, called endocrine (ovary), intracrine, and paracrine, which enhance cell proliferation of tumor target cells.

### 4.2 Androgens

Meanwhile estrogens stimulate mammary gland development, and androgens inhibit it. For example, estrogen treatment in prostate cancer patients promotes mammary gland growth and ingestion of androgens by athletes or transsexuals and produces mammary gland atrophy [153].

Androgens as testosterone (T) and dihydrotestosterone (DHT) exert their effects through the union to their androgen receptor (AR). This receptor has been colocalized with ER and PR in mammary gland epithelia, but not in adjacent stromal cells; therefore, androgen-mediated proliferation is regulated in the mammary epithelium [154]. The androgen receptor (AR) has been reported to be present in 80% of primary breast tumors, and its presence is associated with a favorable response to endocrine treatment and a better prognostic, especially if ER is also present [155].

In this sense, the union of BRCA1 gene product with AR activates AR functions; therefore, the mutation of this BRCA1 may interfere with AR antiproliferative functions and allow cell proliferation [156].

### 4.3 Adrenal steroids

Dehydroepiandrosterone, an estrogen and testosterone precursor [157], is an adrenal steroid which is metabolized into active metabolites, such as Δ5-androstene-3β, 17β-diol (17β-androstenediol), Δ5-androstene-3β17α-diol (17α-androstenediol), and Δ5-androstene-3β,7β,17β-triol (17β-androstenetriol) [158]. DHEA is able to activate ERα, ERβ, AR, and glucocorticoid receptor (GR); meanwhile, its metabolites showed a weaker activation [159]. All of androstene hormones (DHEA, 17β-androstenediol, 17α-androstenediol, and 17β-androstenetriol) have been shown to have an antiproliferative effect in cellular lines, including breast cancer (**Figure 3**) [160]. Only DHEA presented a protective effect in vivo, but the other androstenes have not been tested in vitro [160]; therefore, there is a promising search field around androstenes and the development of breast cancer.

## 5. Tumor development and neuroimmunoendocrine interactions

The relation between nervous and immune systems is importantly transduced through $\beta_2$AR and GCR localized in immune cells, such as B lymphocytes, T lymphocytes, NK cells, and macrophages, which regulate cytokine production, molecule expression, development, survival, proliferation, circulation, and cell recruitment [161]. Meanwhile, interactions among endocrine and immune or nervous systems are regulated through the hormone receptor expression (ER, PR, and AR) and the effects driving in immune and/or nervous cells (**Figure 4**).

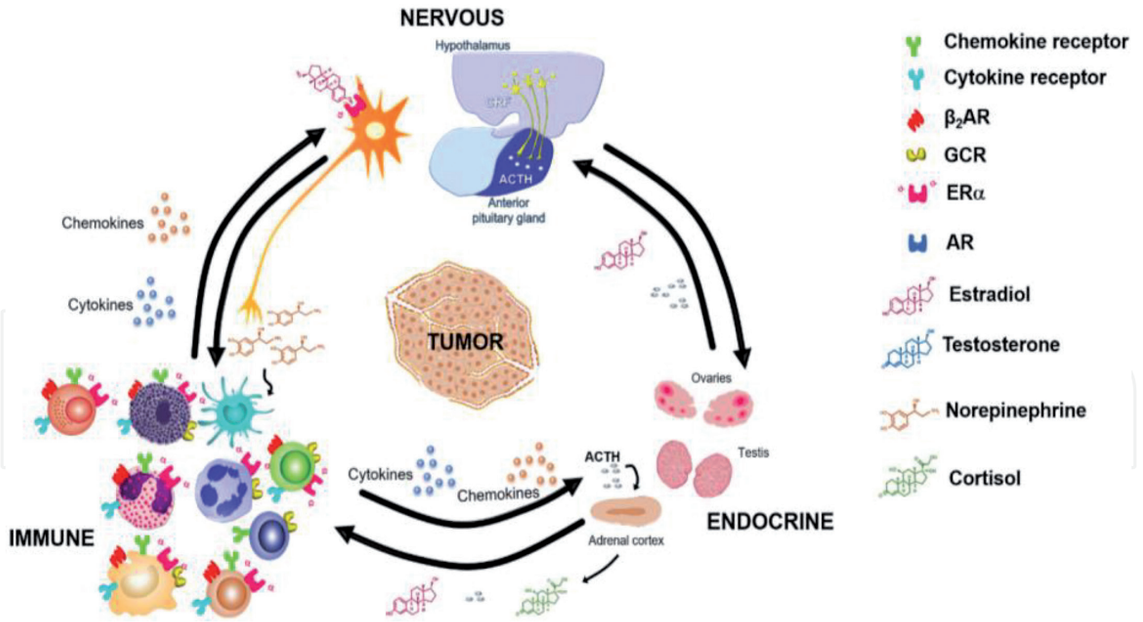


**Figure 4.**
*Neuroimmunoendocrine interactions in breast cancer. Bidirectional interactions among nervous, immune, and endocrine systems are stablished by soluble factors and receptors in cells of the three systems.*

In mice treated with selective $\beta_2$AR agonists, such as clenbuterol or salbutamol, to stimulate these receptors, the lymph node egress of $CD4^+$, $CD8^+$ T lymphocytes, and antigen-primed T cells and B cells is retarded and is associated with lymphopenia. This lymphocyte retention is mediated through CXC chemokine receptor 4 (CXCR4) in T cells and B cells and is thought to explain the reduction of T-cell-mediated inflammation and lymphopenia [161]. CXCR4 is also expressed in monocytes and dendritic cells. CXCR4 ligand is the stromal cell-derived factor 12 (CXCL12), and both are linked to breast cancer metastasis. CXCR4-CXCL12 union promotes cell migration and adhesion and angiogenesis [162].

In lymph nodes with breast cancer metastasis, an increased level of CXCR4 transcript was detected compared to nonmetastatic lymph nodes. Also, a higher mRNA expression was found in breast cancer tumor stages III–IV than in stages I–II. Interestingly, in tumor tissues with HER-2 expression, CXCR4 transcription levels are also more elevated than in HER-2-negative tumors; therefore, there is a positive correlation between CXCR4 and HER-2 expression in breast tumors [163]. This phenomenon is explained because HER-2 enhances and impedes CXCR4 degradation [164]. CXCR4-CXCL12 axis is evolved in organ-directed metastasis, mainly associated with a higher CXCL12 expression in lymph nodes and lung, liver, and bone tissues where breast cancer metastasis is very common. CXCL12 acts as a chemoattractant for breast cancer cells that express CXCR4, promoting the arrival of these cells [165, 166].

Another group of immune cells, in which $\beta_2$AR produces an inhibitory effect, is the DCs. The stimulation of $\beta_2$AR with salbutamol inhibited the NF-κB transcription [167]. This transcription factor is required for DC antigen presentation; for the expression of CD80, CD86, and CD40 (costimulatory molecules); and for IL-12 secretion [168]. Also, salmeterol ($\beta_2$AR agonist) diminishes the IL-1, IL-6, and TNF-α production, in LPS-activated DCs [169].

On the other hand, in human monocytes primed during 16 hours with IFN-γ and stimulated with LPS, the addition of salbutamol diminished the IL-12 and TNF-α secretion, but not the IL-1α, IL-1β, or IL-10 production. Also, in neonatal $CD4^+$ lymphocytes, the Th1 cell differentiation in vitro was inhibited; instead, these $CD4^+$ T cells, stimulated with $\beta_2$AR agonists, produce IL-4 (Th2 cytokine), but not

IFN-γ (Th1 cytokine) [168]. Meanwhile in rats, adrenaline or metaproterenol ($\beta_2$AR agonist) in physiologic doses inhibited NK cell activation [170].

Either by directly action on tumor or immune cells, sympathetic signals regulate tumor progression. In this regard, as mentioned before, immune cell recruitment is a crucial step in immunosurveillance and immunosubversion. Sympathetic innervation in distant organs such as bone marrow promotes noradrenaline secretion that activates bone marrow-resident cells and promotes immune cell development and trafficking [171] and the posterior cell recruitment to tumor microenvironment mediated through tumor chemokine release and chemokine receptor expression in immune cells [166]. In this sense, tumor primary macrophage recruitment [39] and tumor cells increasing cytokine pro-inflammatory expression [21] are $\beta_2$AR mediated and influencing tumor progression [37, 39].

Despite the differences in $\beta_2$AR breast tumor cell expression that as an example in MB-231 cell line is higher than in MB-231BR cell line, the treatment with a $\beta_2$AR agonist (terbutaline or norepinephrine) modulates VEGF secretion through cAMP-PKA pathway, which is diminished in MB-231 cell line and augmented in MB-231BR cell line (metastatic to mouse brain). Meanwhile, IL-6 production in both cell lines was increased, in a cAMP-dependent and PKA-independent pathway [21]. These differences in VEGF secretion are maybe associated with the brain metastatic potential of MB-231BR cell line, because VEGF enhances blood vessel neoformation for tumor growth.

As mentioned before, immunosuppressive TAM works as M2 macrophages and, in this sense, has been found that epinephrine induces M2 macrophage polarization, in RAW 264.7 cells. This polarization is regulated through $\beta_2$AR. Also, in breast tumors co-expression of $CD163^+$ (M2 macrophages human marker) and $\beta_2$AR cell has been demonstrated; thus, macrophages in tumor microenvironment are influenced by adrenergic signals [37]. The relationship between M1 or M2 macrophages and hormone receptor status in breast cancer is due to the development of the disease. Hollmen et al. reported that when a cell line positive for the estrogen receptor (ER) is co-cultured with human monocytes, they acquire an M1 phenotype; meanwhile, the co-culture of them with ER- breast cancer cell line induced an M2 phenotype. The above indicates that ER governs the changes of the macrophages phenotype [172]. $ER^+$ breast cancer is related with an "early" development and a better prognostic, maybe associated with a M1 acute phase inflammatory response that effectively controls tumor progression. Meanwhile, TNBC usually presents a worse prognosis, and the presence of M2 exerts an immunosuppressive intratumoral effect that allows breast tumor growth and metastasis. Therefore, the macrophage phenotype is due to microenvironmental conditions and is associated consequently with tumor staging and prognosis.

Overexpression of HER-2 is correlated with $\beta_2$AR expression levels in breast tumor samples. In this sense, in MCF-7 cells overexpressing HER-2 (MCF-7/HER-2), $\beta_2$AR expression was also elevated, in an autocrine way through MCF-7/HER-2 epinephrine secretion. Interestingly, $\beta_2$AR activation with epinephrine, with norepinephrine, or with $\beta_2$AR agonists (isoproterenol and salmeterol) induces HER-2 expression in MCF-7 breast cancer cells. These findings are important because in $HER\text{-}2^+$ breast cancer cells, the activation of this surface tyrosine kinase may improve epinephrine secretion, through ERK signaling. Epinephrine may upregulate either $\beta_2$AR expression or HER-2 [173].

Concerning to breast cancer, in more aggressive tumors (TNBC), an increased amount of $Foxp3^+$ lymphocytes can be found in than less aggressive tumors ($ER^+$ or $HER^+$ tumors); this higher Treg tumor infiltration is also related to an increased risk of recurrence and a poor prognosis [174].

Ali et al. reported that in $ER^-$ and $HER^+$ breast tumors, $CD8^+$ cell infiltration in tumor was associated with a reduction (28%) of mortality risk, but if these cells were in the tumor stroma, the risk reduction was lower (21%). A similar risk reduction (27%) was found in $ER^+/HER^+$ breast tumors [138]. The $CD8^+$ T-cell presence in tumor is associated then with the induction of tumor cell apoptosis that improve the prognosis and in some point is still acting as an effector-killing cell rather than a memory cell.

## 6. Conclusion

In breast cancer development, tumor cell proliferation is extensively studied, and almost all the treatments are encouraged to diminish it. Tumor cell interactions with other immune, nervous, tumor, and stromal cells, through the production of soluble factors and the expression of receptors, are the drivers of this proliferation. These relations may be driven inside the tumor or across the organism in distant places that respond to tumor signals. Therefore, elucidating not only molecular mechanisms but interactions among cells may enhance the development of new and more effective therapies against breast cancer.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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