Histol Histopathol (2019) 34: 13-24

**http://www.hh.um.es**

**Histology and Histopathology**

*From Cell Biology to Tissue Engineering*

## *Review*

# Microenvironment in breast tumorigenesis: Friend or foe?

**Diana Martins[1,2,3,4] and Fernando Schmitt[1,2,5]**

[1]I3S, Instituto de Investigação e Inovação em Saúde, University of Porto, [2]IPATIMUP, Institute of Molecular Pathology and Immunology of University of Porto, [3]School of Allied Health Technologies, Polytechnic of Porto, Porto, [4]Polytechnic Institute of Coimbra, ESTESC-Coimbra Health School, Department of Biomedical Laboratory Sciences, Coimbra and [5]FMUP, Faculty of Medicine, University of Porto, Porto, Portugal

**Summary.** It is now widely accepted that the tumor microenvironment is a pathologically active niche that shapes tumor nature, evolution and response to treatment. Close interactions between cancer cells and stroma are known to regulate several cancer pathways and thus the determination of different tumor-stromal interactions could be an important step in invasiveness. The breast cancer microenvironment is a complex combination of several different cell types and molecules and a key contributor to tumor development and progression. The microenvironment includes fibroblasts, macrophages, immune cells, tumor-infiltrating lymphocytes, endothelial cells and angiogenic vascular cells, whereas stromal cells surround and interact with tumor cells. Recent data demonstrate significant gene expression alterations in microenvironment cells during disease progression and several stromal cell types are implicated in promoting the "hallmarks of cancer", which can be explored as targets for cancer therapy. Besides identifying new therapeutic targets, the microenvironment has also been implicated in chemotherapy resistance, suggesting that the crosstalk between cancer and its microenvironment is a promising strategy to target breast cancer.

**Key words:** Breast cancer, Tumor stroma, Microenvironment

*Offprint requests to:* Prof. Fernando Schmitt, Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Rua Júlio Amaral de Carvalho 45, Porto 4200-135 Portugal. e-mail: fschmitt@ipatimup.pt

DOI: 10.14670/HH-18-021

## Introduction

Cancer has been referred as a "wound that does not heal" and this concept has been redefined at the molecular level as the role of the tumor microenvironment in cancer progression is clarified (Dvorak, 1986). A tumor is much more than clusters of transformed cells (Bissell and Radisky, 2001), and these tumor cells can only develop in a microenvironment composed of altered extracellular matrix and several non-transformed cells, such as fibroblasts, immune cells, myoepithelial and epithelial cells that play a role in the initiation and progression of the neoplasms (Radisky et al., 2001; Tlsty and Hein, 2001).

Epithelial-mesenchymal interactions in tissue differentiation demonstrated that embryonic mesenchyme strongly influences the terminal differentiation of both embryonic and adult epithelia (Sakakura et al., 1976). In cell culture, normal mammary epithelial cells in a laminin-rich three-dimensional matrix, form acini with a central lumen, become responsive to lactogenic hormones and produce milk proteins enhancing the influence of the ECM and other components of the microenvironment in mammary duct morphogenesis (Maller et al., 2010). Early studies demonstrated that the normal mammary micro-environment is capable of "reverting" the neoplastic phenotype of breast cancer cells by inducing cellular differentiation (DeCosse et al., 1973, 1975). Moreover, the changes in microenvironment accompany tumor formation, and increased fibroblast proliferation and ECM remodelling are often found adjacent to cancer cells (Ronnov-Jessen et al., 1996). The tumor micro-

environment is not just a passive bystander that simply reacts to the transformed cells, but rather interacts with epithelial cells and has been recognized as a major regulator of carcinogenesis (Hanahan and Weinberg, 2011) playing a key role in defining tumor behavior and patient outcome (Beck et al., 2011). Gene expression changes occur in cancer-associated stroma and are known to be implicated in the prognosis, as well as in cancer progression (Ma et al., 2003, 2009; Finak et al., 2008; Farmer et al., 2009). Specifically, evidence from gene expression profiling suggests that the stroma co-evolves with the epithelial compartments during progression (Chang et al., 2004). Several *in vitro* and *in vivo* studies, using diverse experimental systems, have demonstrated that the growth, survival, polarity and invasive behaviour of breast cancer cells can be modulated by myoepithelial and various stromal cells, and several genes have been implicated in this process (Radisky et al., 2001; Gudjonsson et al., 2003; Kenny and Bissell, 2003; Shekhar et al., 2003). The influence of stromal cells in the epithelial component occurs due to the secretion of several ECM proteins, cytokines, growth factors, proteases and protease inhibitors, constituting an extensive network of cross talks between cancer cells and the host (Creighton et al., 2005).

Interestingly, when comparing the transcriptional profiles of neoplastic and stromal cells during breast cancer progression, the more robust changes in gene expression were observed in the stroma (Knudsen et al., 2012). By isolating multiple cell types from normal breast, *in situ* and invasive lesions and analysing their gene expression profiles, it has been demonstrated that dramatic gene expression changes occur in all cell types during breast cancer progression, from tumor epithelial, endothelial and myoepithelial cells, to fibroblasts and leucocytes (Knudsen et al., 2012; Vargas et al., 2012). Moreover, major alterations were associated with processes such as proliferation, ECM remodelling and epithelial to mesenchymal transition (Knudsen et al., 2012; Vargas et al., 2012). Other genetic aberrances also happen in breast tumor stroma, such as gene copy number changes, loss of heterozygosity (LOH), microsatellite instability (MSI) and point mutations both in tumor suppressor genes and oncogenes (Kurose et al., 2001, 2002; Fukino et al., 2004, 2007).

The presence of gene expression alterations, but the lack of genetic abnormalities in *in situ* and invasive breast lesions, suggests that other molecular mechanisms, such as epigenetic changes, including DNA methylation and chromatin modification, could be responsible for the abnormal phenotypes of cancer associated stromal cells (Vargas et al., 2012). Indeed, the analysis of genome-wide methylation profiles identified alterations in DNA methylation patterns both in stroma fibroblasts and DCIS myoepithelial cells, suggesting that the phenotypic changes observed in tumor stromal cells are at least, partially due to epigenetic modifications (Hu et al., 2005).

## The role of microenvironment in breast tumorigenesis

The prevailing model of breast cancer progression is still tumor epithelial cell-driven, since tumor cells have acquired genetic changes and demonstrate genomic instability, where the cells with the most aggressive phenotype were selected through clonal selection (Knudsen et al., 2012). However, this model has been questioned due to several aspects. For example, the presence of genetic alterations in tumor stroma, although controversial, raises the possibility that clonal selection can also occur in non-epithelial cells, with the tumor microenvironment playing an active role in driving tumor progression (Vargas et al., 2012). In addition, the identification of global gene expression changes and epigenetic alterations, together with the fact that the genetic background of the host influences metastatic behaviour, emphasizes the concept that progression is a "driving force" with several key players, rather than a tumor epithelial cell centred event (Ma et al, 2009).

The breast cancer microenvironment (Fig. 1) is a complex combination of several different cells and molecules with a known key role in breast progression (Ma et al, 2009; Place et al., 2011). Recent data also suggest that stromal cell types are implicated in promoting the "hallmarks" of cancer cells (Hanahan and Coussens, 2012). The stromal component has an abundance of inflammatory cells and activated fibroblasts both expressing extracellular matrix (ECM) components and growth factors that support survival and proliferation of tumor cells in a paracrine fashion (Bergamaschi et al., 2008). In fact, in breast carcinomas, the presence of tumor infiltrating lymphocytes (TILs) is significantly associated with high expression of Ki67, suggesting that the immune response plays an important role in tumor progression (Polónia et al., 2017). Accordingly, growth factors, cytokines, chemokines and matrix metalloproteinases secreted by stromal cells lead to the recruitment of macrophages, endothelial precursor cells and regulatory lymphocytes, which sustain tumor progression (Cirri and Chiarugi, 2012; Criscitiello et al., 2014). The constituents of the stroma has also been correlated with clinical outcomes and response to therapy in breast cancer (DeNardo et al., 2011). The expression of ECM genes, both in neoplastic and in adjacent stromal cells, may classify breast cancers into different subgroups with different clinical outcomes (Bergamaschi et al., 2008; Triulzi et al., 2013). When analysing gene expression alterations within tumor stroma, the authors identified "outcome-linked" clusters that were independent of tumor grade, size, hormone receptor and lymph node status (Finak et al, 2008). A 26-gene signature that predicted clinical outcome independently of ER or HER2 tumor status was derived, implying distinct stromal subtypes different from breast tumor subtypes (Finak et al, 2008). In another study, the authors analyzed the gene expression profiles of reactive

tumor stroma from biopsies obtained prior to ER-negative tumors and obtained a signature that predicted clinical response to neoadjuvant chemotherapy (Farmer et al., 2009), suggesting that microenvironment influences patient outcomes and stromal gene expression signatures. Altogether, these observations argue in favor of a non-tumor epithelial cell centered model for breast cancer progression, emphasizing the importance of the microenvironment during cancer progression and for clinical outcome.

### *The role of myoepithelial cells in breast tumorigenesis*

The major diagnostic criterion that has been used to differentiate *in situ* from invasive carcinomas is the presence or absence of an intact myoepithelial cell layer (Damiani et al., 1999). Myoepithelial cells form a continuous layer of cells that surrounds the luminal epithelial cells and separates them from the basement membrane and the stroma (Polyak and Hu, 2005), and their presence is assessed by performing immunohistochemical analyses against myoepithelial cell-specific genes, such as smooth muscle actin (SMA), p63 or CD10 (Damiani et al., 1999). However, it remains largely unknown what leads to the disappearance of the myoepithelial cells in invasive tumors and how this contributes to tumor progression (Polyak and Hu, 2005). The exposure of myoepithelial cells to low concentrations of carrageenan's (sulphated polysaccharides used in commercial food preparation) leads to cell death, but if the myoepithelial cells are destroyed by these exogenous chemicals or by other environmental agents, such as mechanical factors, immune reaction or loss of renewal capacity is not known (Tobacman, 1997; Man and Sang, 2004).

Myoepithelial cells have been recognized as "natural tumor suppressors" and function as gatekeepers of tumor progression (Barsky and Karlin, 2005; Gudjonsson et al.,

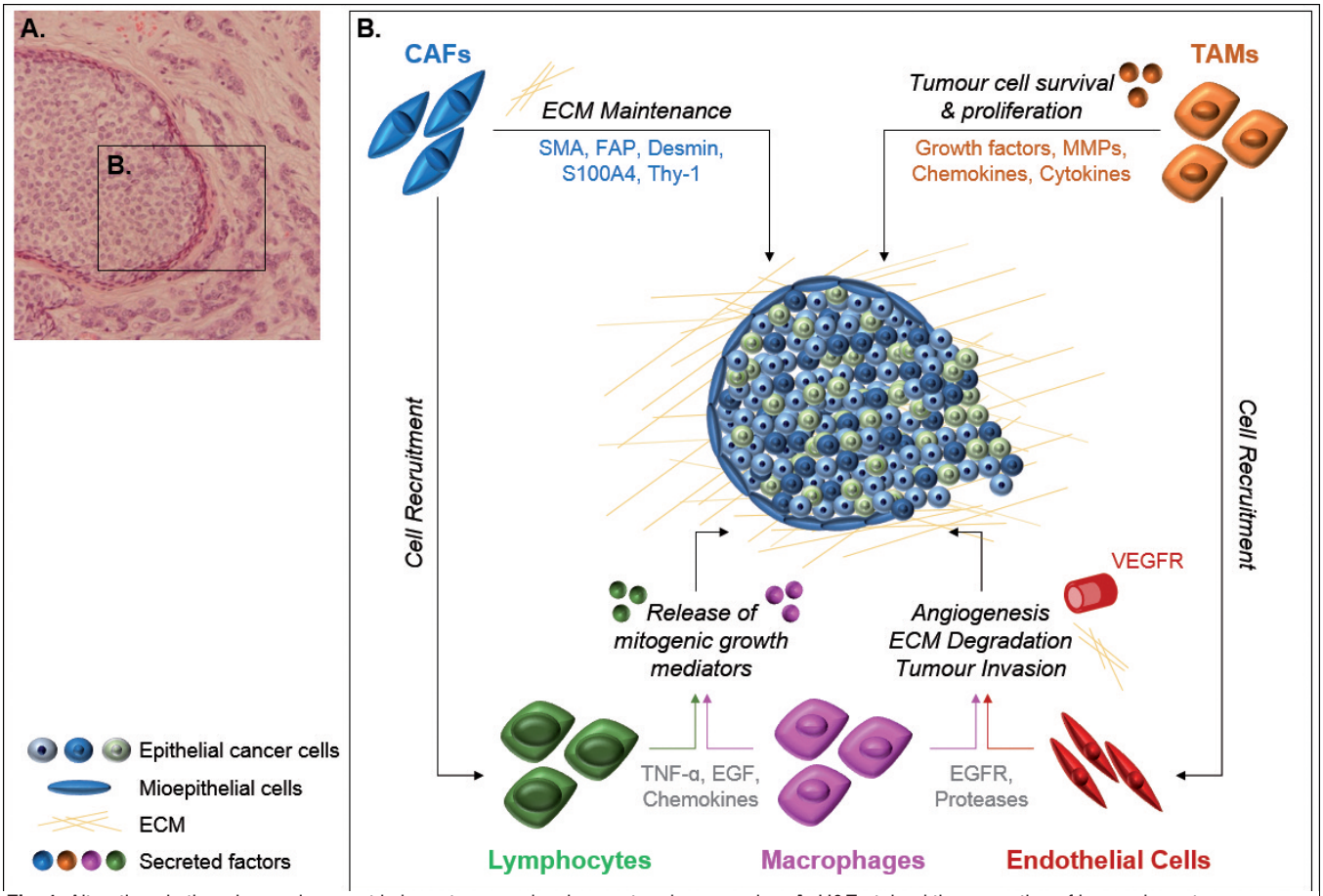


**Fig. 1.** Alterations in the microenvironment in breast cancer development and progression. **A.** H&E-stained tissue section of human breast cancer, showing *in situ* and invasive components of breast carcinoma. **B.** Hypothetical model summarizing the importance of the microenvironment surrounding breast carcinoma using the model DCIS to IDC. The model highlights the crosstalk between tumor cells, the immune system and stromal components as key players in breast carcinogenesis. x100.

2005) due to their inhibitory effect on various neoplastic phenotypes, including tumor cell growth, invasion and angiogenesis (Sternlicht and Barsky, 1997; Lakhani and O'Hare, 2001; Deugnier et al., 2002). This phenotype was identified through the ability of myoepithelial cells to inhibit the growth and invasion of breast cancer cells in co-culture *in vitro* assays and inhibit tumor growth *in vivo* xenograft assays (Sternlicht and Barsky, 1997; Shao et al., 1998; Nguyen et al., 2000). These effects have been attributed to paracrine factors secreted by myoepithelial cells that exert their effects on the tumor epithelial cells. Such factors include ECM proteins, protease inhibitors and several growth factors (Polyak and Hu, 2005). Myoepithelial cells also express extracellular matrix structural proteins and accumulate extracellular matrix instead of degrading it, perhaps explaining DCIS lesions that do not invade (Adriance et al., 2005). To better understand the role of myoepithelial and stromal cells in the transition from DCIS to IDC, the MCF10DCIS xenograft model, which forms DCIS-like lesions that spontaneously progress to IDC, was co-injected with normal myoepithelial cells: it was observed that the presence of these myoepithelial cells efficiently suppressed the growth of MCFDCIS xenografts and prevented the transition to invasive carcinoma. Hence, these results suggest that the loss of myoepithelial cells may promote DCIS to IDC. Also, by comparing epithelial, myoepithelial, infiltrating leucocytes, fibroblasts and myofibroblasts, the myoepithelial cells from normal breast tissue and DCIS yielded the highest number of consistently differentially expressed genes (Allinen et al., 2004). Many of the genes specific for normal myoepithelial cells were absent or dramatically downregulated in DCIS myoepithelial cells, suggesting that these cells are less differentiated and likely have lost some of the functions of normal myoepithelial cells. In agreement with this, the gene expression and DNA methylation profile of DCIS-associated myoepithelial cells suggest loss of differentiation markers and elevated levels of pro-angiogenic and invasive genes (Hu et al., 2005). More importantly, tumor associated myoepithelial cells express higher levels of several basement membrane degrading enzymes including metallo-proteases, compared to their normal counterparts (Allinen et al., 2004). Regarding the progression, many of the genes involved in the normal myoepithelial cell differentiation and function were downregulated, including the ones encoding laminin and oxytocin receptors, whereas genes that promote tumorigenesis were increased, including CXCL12 and CXCL14.

Although in DCIS the ducts are still enclosed by the altered myoepithelial cells surrounded by the basement membrane, dramatic changes have already occured creating a favourable tumor microenvironment for cancer progression. With the loss of normal or the gain of tumor-associated myoepithelial cells, *de novo* autocrine and paracrine signalling can occur, leading to degradation of the basement membrane and consequent invasion of the tumor epithelial cells into the stroma and thus transition into IBC, a more severe clinical entity.

### *Stromal key components in tumor microenvironment*

Cancer-associated fibroblasts (CAFs) constitute an abundant non-neoplastic cell type within tumors (Marusyk et al., 2016). Contrary to normal fibroblasts, which are responsible for the maintenance of the extracellular environment through the production and remodelling of the ECM, CAFs differ from the resident fibroblasts of normal tissues in terms of both molecular signatures and functional impact on adjacent epithelial cells (Sotgia et al., 2012). CAFs are themselves heterogeneous with a subset of them sharing markers of contractility with myofibroblasts, such as alpha smooth muscle actin (αSMA), fibroblast activation protein (FAP), desmin, S100A4 and Thy-1 (Xing et al., 2010; Place et al., 2011), which reflects their acquired motility function. CAFs activation is irreversible and in breast cancer almost 80% of stromal fibroblasts acquire an activated phenotype that manifests by secretion of elevated levels of growth factors, cytokines and metalloproteinases (Jezierska-Drutel et al., 2013). The origin of CAFs has been actively investigated: one possibility is that they are derived from native interstitial fibroblasts whose phenotype has been modified by aberrant signalling from neighbouring tumor epithelial cells. Alternatively, CAFs may have differentiated from bone-marrow-derived mesenchymal stem cells that were recruited to the tumor though endocrine stimulation by tumor-derived factors (Place et al., 2011). CAFs are commonly found in the cancer stroma (Jezierska-Drutel et al., 2013) and promote tumor formation (Orimo et al., 2005) and metastasis (Karnoub et al., 2007) in human breast cancers. They are essential for ECM deposition and remodelling through the synthesis of several ECM components and of ECM-degrading proteases, such as matrix metalloproteinases (MMPs) (Fig. 1). Gene-expression profiling of laser capture-microdissected tumor stroma from 53 breast cancer patients allowed the identification of a stroma-derived prognostic signature, an independent prognostic factor that strongly predicts clinical outcome (Finak et al., 2008). Nowadays, it is also widely accepted that the tumor microenvironment plays an important role in defining and reprogramming cancer cell metabolism (Morandi and Chiarugi, 2014). In breast tumorigenesis, the oxidative stress promoted by the tumor cells induces degradation of Cav-1 by CAFs, which induces a metabolic reprogramming of stromal cells. Consequently, cancer cells induce upregulation of MCT4 by stromal fibroblasts, resulting in invasiveness of the tumor (Witkiewicz et al., 2009, 2012; Sotgia et al., 2012; Martins et al., 2013).

Besides CAFs, immune cells are also a key component of the stromal tumor microenvirnoment. In fact, immune cells constitute one of the most dynamic populations within the cancer scenario (Place et al., 2011), either by the release of mitogenic growth mediators, such as tumor necrosis factor-α, epidermal

growth factor, interleukins, heparins and chemokins, promoting proliferation (Hanahan and Coussens, 2012) or by expressing and secreting mitogenic growth factors and several proteolytic enzymes leading to evading growth suppression (Hanahan and Coussens, 2012). Indeed, several studies have shown that tumor-associated macrophages facilitate angiogenesis, ECM degradation and tumor invasion through activation of epidermal growth factor receptor signalling, secretion of proteases and paracrine signalling between tumor cells (Schedin et al., 2007; DeNardo et al., 2009). Based on the above, understanding the key players in the immune system context can therefore be important to unravel the role of immune system in tumor initiation, progression and response to treatment.

### *The role of tumor infiltrating lymphocytes (TILs)*

The importance of immune system in breast cancer is increasingly being recognized and represents one of the most promising areas regarding the development of novel anticancer treatments (Dunn et al., 2002; Schreiber et al., 2011). "Cancer immunoediting" is a dynamic process that occurs during the interactions between the immune system and the tumor cells (Mittal et al., 2014). The immune system not only protects against cancer development but also shapes the characteristics of the tumor. In the first phase – elimination phase – the immune system can suppress tumor growth through the destruction of cancer cells or inhibit their outgrowth (Hanahan and Coussens, 2012). Tumor cells that manage to survive this phase may enter in the equilibrium phase where editing occurs (Mittal et al., 2014; de Melo Gagliato et al., 2017). The escape phase represents the third and final phase where tumor progression occurs and the cells establish an immunosuppressive microenvironment.

Although breast cancer (BC) has not been considered an 'immunogenic' tumor type (Loi et al., 2014), current evidence demonstrated that the tumor immune response may influence the effectiveness of BC therapy and outcome (Shen et al., 2018), suggesting tumor-infiltrating lymphocytes (TILs) as a new hallmark of breast cancer (Shen et al., 2018). A study analyzing tumor-infiltrating CD8+ lymphocytes in breast tumors found a higher frequency of these cells in stroma surrounding the tumor, which was associated with better prognosis (Mahmoud et al., 2011). In fact, evidence supports that in addition to tumor-infiltrating cells, macrophages, natural killer (NK) and dendritic cells (DCs) are able to infiltrate tumor tissue (Schreiber et al., 2011; Dushyanthen et al., 2015). CD4+T, CD8+cytotoxic T cells, NK, M1 macrophage and DCs cells seem to play a protective role against tumor growth (Emens., 2012), whereas CD4+, M2 macrophages and myeloid-derived suppressor cells promote tumor growth (Emens., 2012). TILs are defined as lymphocytes in tumor nests and can be stromal or intratumoral (de Melo Gagliato et al., 2017). Although some studies indicate that stromal TILs are a more reproducible biomarker than intratumoral TILs, there is a high correlation between both TILs (de Melo Gagliato et al., 2017), and their morphological evaluation is attracting attention as a possible useful marker in clinical (Asano et al., 2016).

Accumulating evidence shows that TILs play a crucial role in the outcome of breast cancer subgroups, especially the more aggressive, such as triple-negative and HER-2 positive breast cancer, in early-stage and advanced disease (Savas et al., 2016; Luen et al., 2017; de Melo Gagliato et al., 2017). Recent data show that some chemotherapies, specifically anthracyclines, use the immune system by activating CD8+ T cell responses to kill cancer cells (Stagg and Allard, 2013), suggesting that tumors rich in TILs respond better to chemotherapy (Katz and Alsharedi, 2017). Currently, systemic immunotherapy utilizes the patient's own immune system directly to eliminate neoplastic cells and is being explored as treatment for triple-negative breast cancers, as this type of breast cancer has been shown to be immunogenic (Polônia et al. 2017; Yu et al., 2017). In fact, in triple-negative, the presence of stromal TILs in tumor tissue is associated with better patient outcome after adjuvant anthracycline-based chemotherapy (Adams et al., 2014). Similarly, in HER2 positive breast cancer, the number of TILs in tumor tissue associates with a better response to trastuzumab treatment (Loi et al., 2014). Although the importance of TILs quantification in the prediction of pathological complete response post-chemotherapy, independently of the breast cancer subtype (Salgado and Loi, 2018), researchers were not able to demonstrate the same results in luminal tumors, suggesting a different immunological infiltrate (de Melo Gagliato et al., 2017).

Although higher TIL levels have been confirmed as prognostic factors in early stage breast disease, in metastatic disease, TIL levels are lower than in early stage disease (Salgado el al., 2015; Luen and Loi, 2018). As a result, in advanced disease the selection of non-imunogenic clones could occur or the development of specific immune-evasive mechanisms within the tumor or even a more suppressive tumor microenvironment (Luen et al., 2016). Therefore, incorporating and evaluating the quantity of immune response - TILs with other prognostic clinical factors, such as tumor size and nodal involvement, will allow the clinicians to estimate long-term survival rates and to improve therapeutic strategies in breast cancer patients (Salgado and Loi, 2018).

## Stroma as a therapeutic target in breast carcinoma

The interest in targeting the stroma surrounding the tumor comes not only from the identification of "drugable" targets, but also from clinical data demonstrating that stroma-derived gene expression predicts outcome in breast cancer patients (Place et al., 2011). As stromal cells work through an intricate network with cancer cells and are stable at the genomic

level, they constitute very promising therapeutic targets (Hanahan and Coussens, 2012). Currently, four types of tumor microenvironment-targeting therapies are being considered: aromatase inhibitors, inhibitors of HER family receptors, angiogenesis-modulating agents and the targeting of infiltrating immune cells. Aromatase inhibitors, which target the aromatase enzyme mostly expressed by stromal components, and transtuzumab, which inhibits receptor signaling on epithelial cells triggered by stroma-produced growth factors, have become standard therapy whereas the effectiveness of targeting the immune system or angiogenic inhibitors remain less clear.

The importance of immunity has emerged in breast cancer more recently than in other cancers. Immune response is a complex phenomenon balanced between activator and inhibitor pathways and cancer cells can maintain an immunosuppressive microenvironment that favors tumor progression (Bertucci et al., 2015). Programmed death-ligand 1 (PD-L1) is a trans-membrane protein expressed on a wide variety of cells including immune cells, epithelial and vascular endothelial cells. Recently, PD-1/PD-L1 pathway signaling was described as an adaptive immune resistance mechanism enacted by the tumor cells to evade the immune response. Several studies reported an aberrant PD-L1 expression in many tumors, often correlated with a poor prognosis, suggesting its potential role as prognostic and predictive biomarker (Ilie et al., 2016). The studies about PD-L1immunohistochemical expression in breast cancer are often discordant and not uniform, mainly due to the high molecular heterogeneity of the different tumor types and the use of different antibodies clones. However, some results showed that PD-L1 could be an important marker for prognostic stratification and for planning immune checkpoint inhibitors therapies in patients with triple negative breast carcinomas (Botti et al., 2017; Polónia et al., 2017). Anti-PD1 and anti-PDL1 monoclonal antibodies have shown favorable outcomes in patients with advanced-stage solid tumors in early-phase clinical trials (Topalian et al., 2012). Also, the approved immune checkpoint inhibitors pembrolizumab and nivolumab, both engineered antibodies that inhibit the programmed death 1 (PD-1) pathway in T cells, are the most promising clinical outcome in cancer treatment in combination with IDO1 inhibitors (Cheong et al., 2018). IDO1 plays an important role in evasion of immunosurveillance by cancer cells, through the induction of T cells and inhibition the proliferation of cytotoxic T lymphocytes that are required for the control of tumor growth (Lee et al., 2002). The other mechanism described occurs through IL-10 activation that stimulates the differentiation of the regulatory T cells that also negatively control the cytotoxic T lymphocytes (Denkert et al., 2010). Previous evidence suggests that IDO1 is a promising intracellular drug target for the development of cell permeable small molecule cancer immunotherapies (Cheong et al., 2018). Preclinical data also supports the rationale for combining trastuzumab and an anti-PDL1 monoclonal antibody in HER-2 breast carcinomas (Stagg et al., 2011). PDL1 upregulation is also described in triple negative breast carcinomas, and was associated with better survival and response to chemotherapy in this aggressive subtype, suggesting that PDL1-inhibitors could represent a promising strategy in PDL1-upregulated basal breast carcinomas (Sabatier et al., 2015).

Important components of inflammatory infiltrate in several tumors are tumor-associated macrophages (TAM), which control the extracellular matrix architecture, tumor cell proliferation and migration, and survival of the tumor (Komohara et al., 2014). TAMs are represented by phenotypically distinct cells that share a common marker– the CD68 receptor – but demonstrate the expression of specific molecules (Heusinkveld and van der Burg, 2011). Previous studies have shown that the infiltration of CD68+ macrophages and high aSMA expression were significant unfavorable prognostic factors in breast carcinomas (Medrek et al., 2012; Yamashita et al., 2012). However, the presence of CD68+ macrophages in the gaps of ductal tumor structures and tumor parenchyma is negatively correlated with lymph node involvement (Buldakov et al., 2017; Mitrofanova et al., 2017; Tashireva et al., 2017). Most likely, the functions of macrophages and fibroblasts depend on their intratumoral localization and the local tumor microenvironment, which plays an important role in the polarization of these cells towards anti- or pro-tumor types (Tashireva et al., 2017). These data support the development of novel compounds that target TAMs in breast cancer therapy (DeNardo et al., 2011).

Regarding the targeting of angiogenic vascular cells, VEGF is the most known angiogenic factor with an established role in breast cancer (Criscitiello et al., 2014). VEGF induces tumor vasculature, cell survival, growth and migration through interaction with VEGF receptors, resulting in a potential antiangiogenic target. However, there is concern that inhibition of angiogenesis may enhance disease progression based on data in animal models, where treatment with anti-angiogenic agents increased invasiveness and metastatic spread (Ebos et al., 2009; Pàez-Ribes et al., 2009). Bevacizumab, is a recombinant humanized monoclonal antibody, clinically approved for VEGF inhibition and has been the most studied anti-angiogenic drug in breast cancer clinical trials (Criscitiello et al., 2014). A previous study has shown that bevacizumab, when used in combination with chemotherapy, was associated with increased risk of fatal treatment-related adverse events, in comparison with the use of chemotherapy alone (Ranpura et al., 2011). However, VEGFR antagonists are a family of receptor tyrosine kinases involved in signaling and many clinical trials have tested small molecule tyrosine kinase inhibitors that act on the intracellular domain of these receptors, thus blocking their catalytic function showing antiangiogenic effects (Curigliano and Criscitiello, 2014).

Although alterations within the microenvironment are now widely recognized in cancer progression making them attractive candidates (Place et al., 2011), both in primary tumors and metastasis, as shown previously, novel treatment strategies are urgently needed, mainly in resistance to therapy in breast cancer patients.

## Stroma and drug resistance

Response to chemotherapy can be assessed by changes in tumor size due to imaging characteristics and histopathological assessment. Tumor growth can progress, stabilize or regress in response to chemotherapy (Place et al., 2011). If the tumor has a good response to chemotherapy, characterized by decreased tumor size, it is worth hypothesizing that the tumor microenvironment actively participates in the tissue remodeling. One hypothesis suggests that classic cytotoxic therapies kill tumor cells and the stromal components reorganize and clean the necrotic debris, whereas an alternative hypothesis suggests that the microenvironment, either as an effect of chemotherapy or in response to signals derived from the damaged epithelium acquires an altered phenotype that independently inhibits tumor growth (Place et al., 2011). The crosstalk between breast cancer cells and their microenvironment has clarified tumor-associated stromal mediated mechanisms of resistance to standard therapeutics in metastatic breast cancer (Criscitiello et al., 2014). For example, resistance to aromatase inhibitors may be driven by abnormal expression of growth factor receptors (Morandi et al., 2013). Tamoxifen resistance is mediated by CAFs (Martinez-Outschoorn et al., 2014) and resistance to fulvestrant, an anti-estrogen, promotes an invasive phenotype secondary to increased epithelial expression of c-MET (Hiscox et al., 2006). A growing body of evidence suggests that tumor-associated stroma, and particularly CAFs, play a major role in resistance to both chemo and targeted therapies, including immunotherapy (Mao et al., 2013; Junttila and de Sauvage, 2013; Martinez-Outschoorn et al., 2014). ECM produced by fibroblasts can contribute to interstitial and mechanical pressures within tumors that limit proper blood flow and reduce the delivery of therapeutic agents (Provenzano and Hingorani, 2013). Stroma-derived gene signatures have also been shown to be strong predictors of clinical outcome of patients with estrogen receptor-negative breast cancer (Farmer et al., 2009). However, the mechanistic details of this protection, as well as therapeutic options to limit it, remain poorly explored.

## Exosomes and microenvironment in breast tumorigenesis

Exosomes are 40-100nm diameter membrane vesicles of endocytic origin, which are secreted by various kinds of cells (Jia el al., 2017). Studies on the role of exosomes involved in tumorigenesis and cancer progression have grown exponentially over the last years, including immune suppression, angiogenesis, cell migration and invasion (Valadi et al., 2007; Iero et al., 2008). As exosomes are capable of delivering specific proteins and nucleic acids to recipient cells in the tumor microenvironment or at specific distant sites, cancers have used exosomes as a tool by which cancer cells can transfer malignant phenotype to normal cells, and establish a local and distant microenvironment to help cancer cell growth (Greening et al., 2015). So, tumor cell-derived exosomes act as a vehicle for the transfer of exosomal miRNAs and protein between epithelial cells, stromal cells, macrophages, and fibroblasts, manipulating the tumor microenvironments to establish a premetastatic niche, which is more permissive for cancer invasion and migration (Vlassov et al., 2012; Dutta et al., 2014). For instance, cancer-derived extracellular miR-122 converts the metabolic environment to a premetastatic niche, by suppressing cells glucose uptake and reprograming systemic energy metabolism (Fong et al., 2015). Recently, exosomes have been proved to be involved in breast cancer organ-specific metastasis. By injecting mice with exosomes taken from breast-cancer cells, the authors suggest that exosomes express different cell-adhesion receptor proteins –integrins and these molecules can target different organs. For example, exosomal $\alpha v\beta 5$ integrin directs to the liver, whereas $\alpha 6\beta 4$ promoted homing to the lung (Hoshino et al., 2015; Liu and Cao, 2016).

As breast cancer cells escape chemotherapy through several mechanisms, resistance to chemotherapy drugs remains an essential issue in the management of patients. In fact, exosomes are emerging as local and systemic mediators of oncogenic information and can play an important role in cancer progression (Jia et al., 2017). In fact, drug-resistant breast cancer cells may spread resistance capacity and alter chemo-susceptibility in cells through intercellular transfer of exosomal miRNAs, including miR-100, miR-222, and miR-30a (Chen et al., 2014a,b; Ma et al., 2014). The intercellular transport of P-glycoprotein (P-gp) was known to be involved in drug resistance via drug efflux across membranes (Dong et al., 2014; Yamagishi et al., 2013). Exosomes from doxorubicin-resistant breast cancer cells can lead to resistance capacity by delivering miRNAs, such as miR-100, miR-222, and miR-30a (Chen et al., 2014a,b), and therefore, exosomal miRNAs can be a mechanism for transmission of drug resistance to sensitive cells (Mao et al., 2016). Besides miRNAs, exosomes derived from stromal fibroblasts can also tranfer noncoding RNA to breast cancer cells and contribute to treatment resistance through expanding therapy-resistant tumor initiating cells (Boelens et al., 2014). Based on the above, exosomes could potentially be used to identify patients who are likely to develop metastatic disease, and the process of exosome production could yield new targets for cancer therapy (Jia et al., 2017).

## Conclusions and challenges

Despite the major diagnostic and therapeutic innovations, breast cancer still remains the leading cause of cancer death for women worldwide. Novel treatment strategies are extremely important especially for metastatic disease. Such novel therapies will have to take into account that the breast microenvironment is a complex niche constituted by different cell types, as well as the proteins they secrete and the supportive ECM (Place et al., 2011). Alterations within the microenvironment are now recognized as key events in breast carcinogenesis, making them suitable candidates for therapeutic modulation (Place et al., 2011). However, there are some obstacles regarding stromal-target therapy. One major obstacle is the disruption of homeostatic function in normal tissues caused by anti-cancer therapies (Place et al., 2011) due to the inability of current treatment strategies to distinguish normal from neoplasic tissue. Another important challenge is the tumor microenvironment behavior during chemotherapy-induced strategies, suggesting that the major obstacle of aggressive forms of breast cancer treatment still relies on cytotoxic agents and new insights into these alterations may identify important pathways and/or molecules with less cytotoxicity or in combination trials.

Another concept particularly important is personalized cancer therapy, which is based on the concept that detailed molecular characterization of the patient's tumor and its microenvironment will enable tailored therapies to improve outcomes. Although the translation of this concept in cancer treatment could be very attractive, there are numerous challenges to overcome before delivering on the promise of personalized cancer therapy. Such challenges include tumor heterogeneity and molecular evolution, the current lack of effective drugs against most genomic aberrations and technical limitations of molecular tests.

In conclusion, the role of the tumor microenvironment in breast cancer initiation and progression is still under-recognised. Further efforts should be made to explore the precise mechanisms and associated signalling molecules to identify promising therapeutic targets for reduction of pro-tumorigenic events and to treat metastatic disease in breast carcinoma.

*Acknowledgements:* This article is a result of the project NORTE-01-0145-FEDER-000003, supported by Norte Portugal Regional Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

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Accepted July 6, 2018