Chapter 3
Pericytes in Breast Cancer

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Abstract Breast cancer is a heterogeneous disease driven not only by evolutionally diverse cancer cell themselves but also by highly dynamic microenvironment. At the center of the tumor microenvironment, tumor vasculature plays multiple roles from supporting tumor growth to providing a route for metastasis to the distant organ sites. Blood vessels in breast cancer present with perfusion defects associated with vessel dilation, tortuosity, and poor perivascular coverage (Li et al., Ultrasound Med 32:1145–1155, 2013; Eberhard et al., Cancer Res 60:1388–1393, 2000; Cooke et al., Cancer Cell 21:66–81, 2012). Such abnormal vascular system is partly due to the morphological and molecular alteration of pericytes that is accompanied by a significant heterogeneity within the populations (Kim et al., JCI Insight 1:e90733, 2016). While pericytes are implicated for their controversial roles in breast cancer metastasis (Cooke et al., Cancer Cell 21:66–81, 2012; Gerhardt and Semb, J Mol Med (Berl) 86:135–144, 2008; Keskin et al., Cell Rep 10:1066–1081, 2015; Meng et al., Future Oncol 11:169–179, 2015; Xian et al., J Clin Invest 116:642–651, 2006), the impact of their heterogeneity on breast cancer progression, metastasis, intratumoral immunity, and response to chemotherapy are largely unknown. Due to the complexity of angiogenic programs of breast cancer, the anti-angiogenic or anti-vascular treatment has been mostly unsuccessful (Tolaney et al., Proc Natl Acad Sci U S A 112:14325–14330, 2015; Mackey et al., Cancer Treat Rev 38:673–688, 2012; Sledge, J Clin Oncol 33:133–135, 2015) and requires much in-depth knowledge on different components of tumor microenvironment and how these stromal cells are interacting and communicating to each other. Therefore, understanding pericyte heterogeneity and their differential functional contribution will shed light on new potential approaches to treat breast cancer.

Keywords Pericyte · Breast cancer · Heterogeneity · Tumor microenvironment Blood vessels · Angiogenesis · Metastasis · Tumor immunity · Perivascular phenotypes · PDGFRβ · Vascular normalization

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Pericytes

Endothelial cells and pericytes are the fundamental units of blood vessels. While endothelial cells make up the inner lining of the vessel wall, pericytes are responsible for enveloping the surface of the vessels and providing structural support. Recently, pericytes have gained much attention due to their diverse roles during vessels formation, vessels maturation, and endothelial support. Pericytes can be identified not only by their distinct morphological features but also by the sets of molecular markers, namely αSMA, desmin, PDGFRβ, NG2, and RGS-5 (Bergers and Song 2005). The expression pattern of these markers can be varying in different tissues or be dynamic during various developmental stages. RGS-5, desmin, and αSMA are intracellular proteins, of which desmin and αSMA are contractile filaments, and RGS-5 is a GTPases-activating protein. Neuron-glial 2 (NG2) and platelet-derived growth factor receptor beta (PDGFRβ) are cell-surface proteins. PDGFRβ is one of the most studied molecules expressed in pericyte due to its paracrine signaling through ligand PDGF-BB to control pericyte recruitment to the growing vessels (Hellstrom et al. 1999; Enge et al. 2002). The composition of these markers is various in different tissues, potentially linked to their diverse functions in the different microenvironment. Also, two distinct types of pericytes based on their marker expression (Type 1, Nestin-/NG2+ and Type 2, Nestin+/NG2+) were shown to exert different angiogenic capacity in vitro and in vivo (Birbrair et al. 2014a). Another in vitro study using tumor-derived PDGFR-β+ perivascular progenitors showed that these progenitors could differentiate into more mature phenotype (NG2+ or αSMA+) upon culture, whereas desmin expression was only induced when they were cultured with endothelial cells together (Song et al. 2005). Such phenotypic conversion indicates that specific pericyte phenotypes are largely influenced by their local environment and different cell states. Pericyte density also varies in different parts of the body based on the unique needs and pressure the blood vessels need to withstand (Sims 2000). In particular, the highest pericyte density is observed in the central nervous system, brain, and retina, to create blood–brain barrier (Ballabh et al. 2004). Pericytes have complex ontogeny including neuro crest, bone-marrow-derived mesenchymal stem cells (BM-MSCs), or onsite proliferation based on the tissues where they are residing in and their specific functions (Armulik et al. 2011; Hall 2006). However, recent studies have suggested an alternate source of pericytes in tumor microenvironment including epithelial-to-pericytes transition (EPT) in breast cancer adding another layer of complexity (Shenoy et al. 2016).

Just as normal pericytes perform a diverse function and express differential markers in different tissues, pericytes in tumor microenvironment exhibit great diversity in marker expression as well as functional contribution.

Pericyte Landscape (Investment) in Pathological Environment

Poorly invested angiogenesis in tumors results in the chaotic and disorganized vasculature that presents with tortuous, leaky, and permeable vessels often with functionally incompetent (Li et al. 2013; Eberhard et al. 2000). Under the constant

influence of cancer cells and tumor microenvironment, both endothelial cells and pericytes appear morphologically and molecularly differ from normal counterparts which, in part, leads defective vasculature (Bergers and Song 2005). Different types of tumors present strikingly different vascular architecture, including vessels size, dilation, and pericyte coverage (Morikawa et al. 2002). Notably, tumor-associated pericytes are loosely associated with the endothelial cells, with cytoplasmic processes that penetrate deep in the tumor parenchyma. Although the exact mechanism for such aberrant phenotype is still not clear, it has been proposed that abnormal expression of growth factors or signaling molecules such as VEGF, TGF-β, PDGF-BB, and Ang-2 has a significant impact on pericyte morphology, quality, and investment (Kim et al. 2016; Keskin et al. 2015; Song et al. 2005; Raza et al. 2010). In particular, PDGF-BB/PDGFRβ is one of the most well studied and prominent signaling pathways that is involved in pericyte recruitment and survivor. These signaling molecules also have been implicated in other pathological condition related to vascular abnormalities, such as diabetic retinopathy (Enge et al. 2002; Benjamin 2001; Rangasamy et al. 2011), wound healing (Lin et al. 2008), or stroke, a cerebrovascular disorder associated with blood–brain barrier (BBB) disruption (Suzuki et al. 2016).

Perivascular Signature in Breast Cancer at a Glance

In breast cancer, despite enormously enlarged and thickened vessels appearance, relatively large percentage of vessels are covered by pericytes (Eberhard et al. 2000; Bergers and Song 2005). While a great deal of heterogeneity present on pericytes in breast cancer, all these markers have been detected in human cancer tissues while it is still not clear what such heterogeneity means. Several studies have reported the mean microvascular pericyte coverage index (MPI) in breast cancer to be from 32% up to 80% by quantifying αSMA expressing pericytes (Eberhard et al. 2000; Shrivastav et al. 2016). No pan-pericyte marker can identify all pericyte populations (Armulik et al. 2011; Hall 2006) and infect, and a recent study has shown the various flavors of pericytes existing within human breast tumor tissues (Kim et al. 2016). Several studies have attempted to measure MPI using immunohistochemistry for αSMA (Eberhard et al. 2000; Shrivastav et al. 2016), NG2 (Cooke et al. 2012), PDGFRβ (Shrivastav et al. 2016; Paulsson et al. 2009), desmin (Kim et al. 2016), and CD 248 (Viski et al. 2016) using breast cancer patient's tissue samples. It is worth noting that some of these analyses include both fibroblast and pericyte as both cell types tend to express these markers. αSMA is the marker mostly explored by many investigators due to its abundance. However, it is noteworthy that αSMA expression is lacking in quiescent pericytes in normal tissues (Gerhardt and Betsholtz 2003) and hence contributes to the pathological phenotype. TGFβ, involved in smooth muscle cell maturation, is known to be responsible for ectopic expression of αSMA in tumor pericytes (Song et al. 2005). Often pericyte composition/phenotype is used to indicate the functional status of the tumor vasculature, and thus many attempts have been made to correlate it with patient outcome. However,

the results have not been consistent between analyses primarily due to different markers and methods used to quantify pericyte phenotypes. Other studies have indicated that pericyte phenotypes are differentially regulated between the primary tumor and metastases, suggesting the influence of tumor microenvironment (TME) in perivascular investment (Lyle et al. 2016). Such discordance between primary and secondary cancers also implies that perivascular phenotype in primary tumor site might be a prognostic factor for the metastasis (Jubb et al. 2011).

Pericytes Phenotype Conversion

PDGF-BB/PDGFRβ signaling is one of the most studied pathways known to be crucial for pericyte recruitment, survivor, and clinical implication (Ostman and Heldin 2007). PDGFRβ gene expression levels (high vs. low) in 3455 patients with breast cancer show a correlation between high PDGFRβ expression and the recurrence-free survival probability of patients (Keskin et al. 2015). Another study has used the immunohistological analysis of PDGFRβ expression in 512 breast cancer samples. Although in this particular study PDGFRβ expression was scored in entire stroma including fibroblast and pericytes, it has shown that high PDGFRβ expression in stroma was correlated with high pathological grade, estrogen receptor negativity, and high HER2 expression, as well as shorter recurrence-free and breast cancer-specific survival (Paulsson et al. 2009). In a separate study, 75 breast cancer samples were also analyzed for PDGFRβ expression using immunohistological assay to evaluate the pericytes as a prognostic factor for lymph node metastasis and molecular subtypes. However, this study failed to show any correlation between MPI and the known prognostic and predictive factors (Shrivastav et al. 2016). To establish the significance of PDGFRβ expressing pericytes, PDGFRβ-TK (thymidine kinase) mice in which PDGFRβ+ pericytes are specifically eliminated were explored in the context of murine mammary tumor model using 4T1 cells. While primary tumor progression was repressed upon PDGFRβ+ pericytes depletion due to anti-angiogenic effects, metastatic incidences were significantly increased via increased hypoxia, vascular leakiness, and epithelial–mesenchymal transition (EMT) (Keskin et al. 2015). It is noteworthy that TK system physically eliminates all pericytes expressing PDGFRβ; therefore, instead of changing pericyte phenotype, it exerts an anti-angiogenic effect at least on the primary tumor sites.

A study mentioned above using 75 breast cancer tissues samples revealed no correlation between αSMA expression and the known prognostic and predictive factors. NG2 chondroitin sulfate proteoglycan is expressed on the surface of pericytes during vasculogenic and angiogenic processes. NG2 is often considered to be a maker for mature pericytes, and its expression is observed in the large percentage of tumor pericytes despite the abnormal phenotype and function (Armulik et al. 2011). In breast cancer, low NG2+ pericyte coverage was significantly associated with the presence of metastasis, and low NG2+/high c-Met expression was correlated with poor survival of breast cancer patients (Cooke et al. 2012). Endosialin

(CD248) is a transmembrane glycoprotein, and its expression is known to be upregulated in tumor-associated pericytes and myofibroblasts in breast cancer (Viski et al. 2016). CD248 expression in microdissected breast tumor stroma was associated with decreased recurrence-free survival, and high CD248 expression was correlated with low distant metastasis-free survival. Such observation was confirmed by a 4T1 orthotopic mammary tumor in CD248 knockout mice background. Interestingly, CD248 knockout had a significant effect on decreasing metastasis but had no effect on primary tumor growth, revealing a specific function of CD248 on intravasation process.

Another example of abnormal/pathological pericyte phenotype was described in a mouse model for melanoma, breast cancer, and rhabdomyosarcoma (detail reviewed by others (Paiva et al. 2018)). In this study, increased expression of the pluripotency gene Klf4 in pericytes induced the phenotypic switch from mature/quiescent pericytes (NG2+) to a less differentiated state with increased proliferation, migration, and extracellular matrix (ECM) (e.g., fibronectin) production, which contributes to a prometastatic fibronectin-rich environment. Pericyte-specific knockout of Klf4 decreased premetastatic niche formation and metastasis (Murgai et al. 2017). In the lung, pericytes also exhibit heterogeneity, and two different subtypes of pericyte as previously mentioned (Type 1, Nestin−/NG2+ and Type 2, Nestin+/NG2+) were shown to be present on pulmonary blood vessels. Whether or not different subtypes contribute to the formation of premetastatic niche differently is not known (Paiva et al. 2018). However, only type-1 pericytes, but not type 2, were accumulated and producing collagen at the injury site of lung contributing to pulmonary fibrosis (Birbrair et al. 2014b). On the other hand, type-2 pericytes were shown to be actively engaged in the angiogenic process during orthotopic glioblastoma progression (Birbrair et al. 2014a). Thus, it would be attractive to explorer if type-1 pericytes are mainly contributing to the formation of the premetastatic niche by converting to Klf4+ phenotype (e.g., depositing ECM) at the beginning, and type-2 pericytes will be recruited to support secondary tumor formation once tumor started to grow at the niche.

In both cases for CD248 and Klf4, their expression was abnormally upregulated in response to the tumor microenvironment and had a significant influence on metastatic behavior rather than primary tumor growth. Suggesting genes that are differentially regulated compared to normal pericyte are of great interest to understand the fundamental impact of pericyte on distant metastasis and perhaps organ-specific tropism.

More pieces of evidence are emerging to indicate a large percentage of pericytes express multiple markers rather than a single marker. Therefore, it makes more sense to define pericyte phenotype using a combination of different markers. A recent study using multispectral images of multiplex stained tissue microarray of breast cancer provides a more comprehensive understanding of perivascular heterogeneity and phenotyping. In this study, tissue microarray (TMA) was co-stained for PDGFRβ, desmin, and CD31, and imaging analysis was performed to find a significance of ratio between PDGFRβ and desmin (Kim et al. 2016). Based on two separate cohort of breast cancer samples, this study has shown that the ratio of PDGFRβ

and desmin is significantly different between subtypes of breast cancer, TNBC, and luminal, and that the high desmin to PDGFRβ ratio was considered to be a predictive factor for higher relapse-free survivor and higher breast cancer-specific survivor of patient who was treated with epirubicin but not with paclitaxel. Although the underlying mechanism remains to be determined, it provides new ways to understand the perivascular landscape and explains, in part, the discrepancy of previous different studies where a single marker was analyzed.

Origin of Pericyte in Breast Cancer

The origin of tumor pericytes has been investigated in different types of tumors, including fibrosarcoma, melanoma, and colorectal cancer. It has been shown that tumor pericytes can be recruited from local immature mesenchymal cells, bone marrow-derived cells, and onsite proliferation (Abramsson et al. 2002; Du et al. 2008; Rajantie et al. 2004). A recent study in breast cancer has proposed an alternative source of pericyte in TME through epithelial-to pericytes transition (EPT) adding another layer of complexity (Shenoy et al. 2016). In this study, MCF10DCIS cells were forced to undergo EMT, and its fate was followed in vivo and in vitro. EMT cells acquired mesenchymal phenotype (expressing PDGFR-β and N-cadherin) and physical contact with endothelium contributing tumor vasculature. Although in normal tumor context, cells undergo EMT is a small population and therefore attribute to a fraction of tumor-associated pericyte pool, it is an interesting observation to identify an alternative source of pericyte in breast cancer. A similar result was also shown in the case of glioblastoma (GBM) in which glioma stem cells (GSCs) give rise to pericytes to support vessel function and tumor growth (Cheng et al. 2013). In this case, human GBM specimens showed phenotypically switched pericyte populations containing the same mutational status with cancer cells. These studies suggest the alternative source of pericyte in the context of TME and thus new therapeutic targets. Considering what we have observed regarding pericyte heterogeneity and their functional contribution, it will be a great interest to investigate the phenotype of such converted pericytes and its correlation with the mutational status of cancer cells.

On the other hand, pericytes have been speculated for its stem cell capacities to differentiate into adipocytes and fibroblasts in different organs (Crisan et al. 2008). Cancer stem cells are often observed in perivascular niches (Calabrese et al. 2007; Pietras et al. 2008), and thus it is tempting to speculate that tumor vasculature-associated pericytes might hold mesenchymal stem cell properties although direct evidence for this proposition is still unclear. In case of renal cell carcinoma, PDGFR-β expressing pericytes were shown to transit its fate to fibroblasts (Pericyte-fibroblast transition) in response to tumor-derived PDGF-BB and contributed to tumor growth and metastasis (Hosaka et al. 2016), demonstrating phenotypic switching of pericytes in response to TME or malignancy. An interesting phenomenon has been observed in the study of breast cancer that stromal cells

(adipocytes, fibroblasts, and myoepithelial cells) gained somatic mutation on GT198, a steroid hormone receptor coactivator, independent with mutational status of cancer cells. It was suggested that the progenitor cells with GT198 mutation (GT198+) is mostly capillary pericytes and differentiated into GT198+ stroma cells collectively contributing to malignant tumor microenvironment (Yang et al. 2016). Mutant GT198 expressing cells are shown to induce VEGF expression, which in turn influence cancer cells attributing reciprocal communication between cancer cells and TME.

Anti-Angiogenic (Anti-Vascular) Treatment

Angiogenesis and co-optive vascular remodeling are prerequisites of solid tumor growth. Breast cancer is one of the highly vascularized tumors with fairly high pericyte coverage (Eberhard et al. 2000) and largely dependent on vascular support for the survivor and growth. The level of neovascularization in aggressive breast cancer correlated with metastatic disease and may serve as an independent predictor for metastasis (Weidner et al. 1991). Therefore, it is a quite attractive approach to target tumor vasculature. However, anti-angiogenic treatment has been largely unsuccessful with marginal benefit (Sledge 2015; Aalders et al. 2017). Many of anti-angiogenic treatment involves targeting endothelial cells or pro-angiogenic factors, aiming to eliminate vessels and thus starving tumors. Tumor angiogenesis is accompanied by an increased level of pro-angiogenic factors such as HIf1α and VEGF (Aalders et al. 2017; Bos et al. 2001); thus, blocking VEGF pathway has been most extensively studied and considered as anti-angiogenic treatment including a monoclonal antibody against VEGF, bevacizumab (Sledge 2015). Other types of anti-angiogenic approaches include tyrosine kinase inhibitors such as sunitinib, sorafenib, imatinib, and axitinib. Anti-VEGF treatment in tumors led to a partial elimination of tumor blood vessels that are not covered by pericytes (Tolaney et al. 2015; Benjamin et al. 1999). To overcome such limitation, dual targeting of endothelial cells and pericytes has been proposed (Bergers et al. 2003). However, pericyte depletion did not provide an additive effect in some models (Nisancioglu et al. 2010; Sennino et al. 2007) or has proven to increase metastasis in breast cancer models (Meng et al. 2015; Cooke et al. 2012; Keskin et al. 2015).

In fact, low NG2+ pericyte coverage of tumor vasculature was significantly correlated with increased metastasis in clinical samples of breast cancer (Cooke et al. 2012). A separate study analyzing TNBC vs. luminal breast cancer has shown that TNBC tumor vasculature exhibits poor pericyte coverage compared to luminal tumor vasculature, suggesting lower pericyte coverage might be an indication of aggressive nature of tumor types (Kim et al. 2016). Such notion, perhaps, indicates that nonselective elimination of pericyte may not yield benefit but rather promote tumor aggressiveness and metastasis. Thus, a better understanding of pericyte heterogeneity in response to TME changes may provide insight to pericyte targeting strategy.

Vascular Normalization Using Pericyte Landscape

Despite the aberrant morphology, marker expression, and function, eliminating pericytes as a whole did not result in any beneficial effect and, in fact, did more harm by increasing metastasis. Instead, vascular normalization concept takes advantage of pericytes by only eliminating vessels that are not covered by pericytes (immature), leaving healthy pericyte covered functional vasculature (Goel et al. 2012). Several clinical trials of anti-angiogenic therapy (anti-VEGF) suggest vascular normalization phenomenon in many solid tumors. However, no significant survival benefit has been warranted so far (Mackey et al. 2012). A recent clinical trial of neoadjuvant bevacizumab and chemotherapy in breast cancer patients has shown limited efficacy despite the clear vascular normalization effect. Analysis of αSMA+ pericyte coverage in pretreatment vs. posttreatment showed significantly increased αSMA+ pericyte-associated vessels although not a clinically significant contribution to overall outcome (Tolaney et al. 2015). However, this study suggests that patient might benefit from bevacizumab treatment if sufficient numbers of vessels are initially present. Collectively, it is a plausible explanation that vascular normalization approach using bevacizumab might only benefit patients with specific vascular phenotype defined by pericyte investment.

Interesting results from anti-angiogenic treatment have been observed in melanoma case. Subpopulations of pericytes that were characterized by distinct marker expression (high αSMA and PDGFRβ) and loose attachment to endothelial cells showed a more significant effect on combinatorial treatment using the VEGFR inhibitor PTK787 and the PDGFR inhibitor STI571 in PDGF-BB overexpressing tumor (Hasumi et al. 2007). However, desmin + pericytes that are usually more mature and tightly bound to endothelium remained intact. This study indicates that different subpopulations of pericyte responded differently not only to anti-angiogenic drug treatment but also to the intrinsic nature of cancer cell themselves, in this case, the expression level of PDGF-BB. Therefore, pericyte landscape might be a predictable marker for patients who are more likely respond to anti-angiogenic treatment. However, pericytes are well recognized for its controversial function on metastasis, and further analysis of metastatic behavior upon treatment should be followed. Such finding is in accordance with the study mentioned above in breast cancer, where the ratio between PDGFRβ+ and desmin + pericyte on treatment naïve biopsy has predictive power for patient outcome upon treatment with the specific drug (Kim et al. 2016). Despite the promising preclinical results and rational to justify anti-angiogenic therapy, the overall benefit is marginal, and the toxicity and cost are not outweighed. As pointed out by others, anti-angiogenic therapy such as bevacizumab should only be considered when we have a better idea on the predictive biomarker for sufficient benefit. Considering the emerging data on perivascular phenotype can have a profound effect on vascular functionality, perhaps pericyte landscape should be explored as a valid predictive marker for the success of anti-angiogenesis or other drugs.

Their Contribution to Breast Cancer: Friend or Foe?

Originally pericytes were proposed to be a gatekeeper ***(friend)*** of metastasis based on several studies where low pericyte coverage or depletion of pericyte leads to increased hypoxia, pro-metastatic factors, vascular leakiness, and metastasis (Gerhardt & Semb 2008; Xian et al. 2006). Therefore, simply targeting tumor pericytes will not produce many beneficial outcomes. However, increasing pieces of evidence indicate that subpopulations of tumor pericytes undergo phenotype switching by altered gene expression, leading to a pathological characteristic ***(foe)*** as we have discussed previously and summarized in Table 3.1. Thus, a better understanding of pathological phenotypes of pericyte will open up the opportunity for us to target these abnormal pericytes, which potentially leads to more efficient vascular normalization and anti-angiogenic approaches. Also, pericyte contribution to the vascular function might be context dependent. For instance, brain vasculature holds a unique structure called blood–brain barrier (BBB) of which pericyte is one of the major components. Truth hold in part in brain metastasis or brain tumor and it acts as an obstacle of drug delivery efficacy. Therefore, a specific function of the different subset of pericytes should be considered in a context-dependent manner.

We have already discussed the potential contribution of pericytes in metastasis in breast cancer. However, the newly emerging role of pericytes in the pathological/inflammatory environment gained much attention recently in the field of breast cancer. Several recent studies have shown reciprocal communication between tumor pericytes and immune components of the stroma. Regulator of G-protein signaling 5 (Rgs5) is one of the pericyte markers that are known to be expressed in PDGFRβ+ progenitor perivascular cells and overexpressed in the aberrant tumor vasculature. In RIP1-Tag5 mouse model, a large population of tumor pericytes expresses Rgs5 and PDGFRβ, representing immature progenitor status and small populations that express αSMA/NG2/desmin representing mature pericytes. In genetic deletion of Rgs-5 tumor context, pericytes phenotype was shifted toward the more mature state, αSMA/NG2/desmin leading to vascular normalization. Such phenotypic switching resulted in increased tumor infiltration by CD4+ and CD8+ T cells and immune control (Hamzah et al. 2008). This finding proposes the connection between subpopulation of pericytes (mature pericytes) and immune cells infiltration in a mouse model for pancreatic cancer, and it should be explored in breast cancer. One of the critical mechanisms by which tumors can escape from immune surveillance is the recruitment of myeloid-derived suppressor cells (MDSCs) (Gabrilovich and Nagaraj 2009). In mice defective for PDGFB retention ($PDGF\beta^{ret/ret}$), the loss of PDGFRβ+ pericytes hence decreased pericyte recruitment and enhanced intratumoral trafficking of MDSCs in IL-6-dependent manner (Hong et al. 2015). Gene expression analysis from patients with breast cancer showed that increased expression of human MDSC markers such as CD33 and S100A9 was correlated with decreased expression of pericyte marker genes. Moreover, the group of patients with low pericyte poor/MDSC rich was associated with poor long-term breast cancer-specific survival. Most recent finding in breast cancer emphasizes the importance of mutual

Table 3.1 Functional contribution of various pericytes in breast cancer

Friend				Foe			
Pericyte (marker)	Functional contribution			Pericyte (marker)	Functional contribution		
	IM	MET	VF		IM	MET	VF
NG2	Increase tumor immunity	Inhibit metastasis	Less hypoxia Better perfusion	CD248	NS	Increase intravasation	No effect
PDGFRβ	Suppress MDSC recruitment	Inhibit metastasis	Less hypoxia Better perfusion	Kfl4	NS	Metastatic niche formation	No effect
Desmin	Increase tumor immunity	Inhibit metastasis	Less hypoxia Better perfusion	Rgs-5	Immunosuppressive	Increase metastasis	Induce hypoxia Leaky vessels
αSMA[a]	?	?	?	GT198[b]	NS	NS	Leaky vessels
				EPT[c]	NS	Promote tumor growth	Less permeable vessels

IM tumor immunity, *MET* metastasis, *VF* vascular function, *NS* not studied
Pericyte phenotype is color-coded based on Fig. 3.1
[a]αSMA expression in pericytes of capillary beds is abnormal and a hallmark of pathological pericytes and its contribution to tumor microenvironment has not been explored in breast cancer
[b]Mutant form GT198 expression is the marker for malignant pericyte that can manifest the tumor microenvironment by affecting other stromal cells
[c]Tumor-specific type of pericyte arose by "Epithelial to pericyte phenotypic transition"

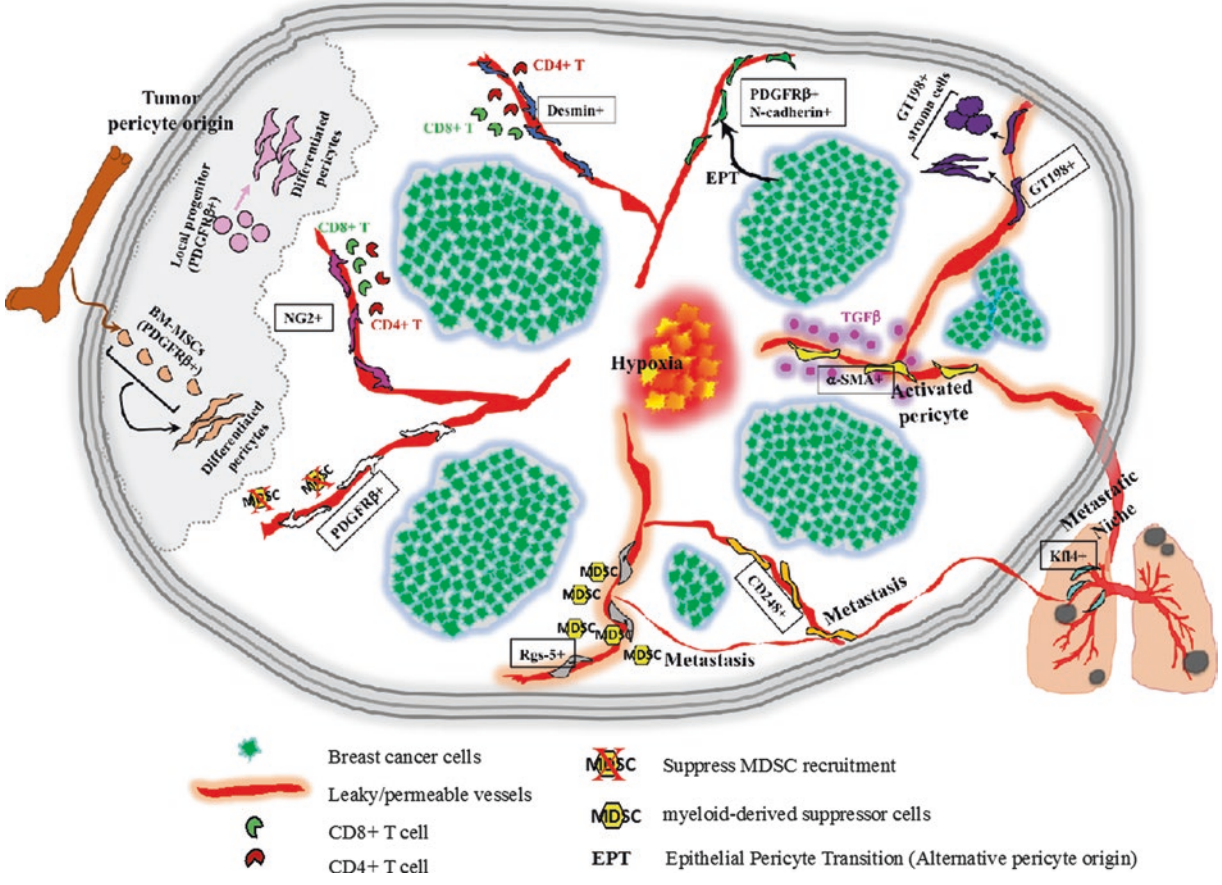

Fig. 3.1 Various pericyte phenotypes and their functional contribution in the context of breast cancer. Each phenotype is defined based on studied marker expression. In general, pericytes with ectopic expression of markers (e.g., Klf4, Rgs-5, GT198) confer adverse effects on tumor progression, metastasis, intratumoral immunity, and vascular function (Table 3.1). Details and references are described in the main text

regulation of tumor vascular normalization and tumor immunity. In this study, bioinformatic analysis data indicated that gene expression related to vascular normalization correlate with immune-stimulatory pathways and such hypothesis was further validated in a various model system. Loss of mature pericyte (NG2+ pericyte) leads to reduced T lymphocytes infiltration into orthotopic breast tumor, E0771. In reverse, T lymphocytes deficiency in genetically engineered mice (CD4KO, CD8KO, TCRKO) resulted in decreased pericyte coverage, increased vessels permeability, and increased circulating tumor cells, suggesting reciprocal regulatory loop between perivascular phenotype and tumor immunity (Tian et al. 2017). A different functional contribution of pericyte in tumor immunity reiterates that we should pay more attention to the type of pericytes we are looking after.

What Do We Do Now? Future Direction

It is clear now that we know more than ever how pathological pericytes are different from normal counterpart and their potential function in the context of the TME. However, the future depends on how we use such knowledge to benefit patients with cancers.

It is not a matter of presence or absence of these cells. It, perhaps, depends on their phenotype or landscape on a larger scale. Considering the marginal benefit of anti-angiogenic approaches, it is probably not a good idea to eliminate pericytes or vasculature as a whole. To this end, vascular normalization concept is closer to what we want to accomplish in which immature, leaky, and nonfunctional vessels without appropriate pericyte coverage will be eliminated. However, we cannot assume that all the left pericytes will contribute to normal vascular structure/function as some of these pericytes are abnormal or malignant themselves. It has been shown that ectopic expression of Klf4, Rgs-5, αSMA, CD248, and mutant GT198 in tumor-associated pericytes can spread malignancy to the primary tumor site as well as metastatic organs. We cannot afford to keep these pericytes around. Therefore, it will be a safer approach to specifically target these genes or gene products rather than target pericytes as a whole. Increasing pieces of evidence show a particular type of pericytes is differentially contributing or affecting tumor immunity. By understanding what flavor of pericytes are responsible for the good vascular structure and immune-stimulatory effect, we might be able to kill two birds with one stone by improving vascular perfusion and intratumoral immunity.

Perivascular heterogeneity is largely recognized and appreciated in breast cancer filed. A recent study using TMA from the different patient cohort with breast cancer has shown promising results in which pericyte phenotype can be a potential predictor for successful response to the specific type of drug. Although the underlying mechanism remains elusive, such results add a promising approach to map out personalized treatment. An additional approach might include reverting malignant pericyte phenotype to beneficial phenotype by molecular conversion. In this case, we do not have to kill anything. We just need to correct the problem.

The fact that different subtypes of breast cancer, namely TNBC and luminal, displayed significantly different perivascular phenotype might implicate that such perivascular landscape is either an intrinsic property of the particular type of cancer or heavily influenced by the distinct tumor microenvironment. Either way, we should consider identifying a connection between the properties of cancer cells and pericyte phenotypes.

We have accumulated enough pieces of evidence to be finally convinced that tumor-associated pericytes are heterogeneous and should not be considered as a single-cell population, as it can be a Jekyll or a Hyde at any moment depends on their phenotype, environment, and perhaps influence by cancer cells.

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