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Review

Proangiogenic hematopoietic cells of monocytic origin: roles in vascular regeneration and pathogenic processes of systemic sclerosis

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Summary. New blood vessel formation is critical, not only for organ development and tissue regeneration, but also for various pathologic processes, such as tumor development and vasculopathy. The maintenance of the postnatal vascular system requires constant remodeling, which occurs through angiogenesis, vasculogenesis, and arteriogenesis. Vasculogenesis is mediated by the *de novo* differentiation of mature endothelial cells from endothelial progenitor cells (EPCs). Early studies provided evidence that bone marrow-derived $CD14^+$ monocytes can serve as a subset of EPCs because of their expression of endothelial markers and ability to promote neovascularization *in vitro* and *in vivo*. However, the current consensus is that monocytic cells do not give rise to endothelial cells *in vivo*, but function as support cells, by promoting vascular formation and repair through their immediate recruitment to the site of vascular injury, secretion of proangiogenic factors, and differentiation into mural cells. These monocytes that function in a supporting role in vascular repair are now termed monocytic pro-angiogenic hematopoietic cells (PHCs). Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by excessive fibrosis and microvasculopathy, along with poor vascular formation and repair. We recently showed that in patients with SSc, circulating monocytic PHCs increase dramatically and have enhanced angiogenic potency. These effects may be induced in response to defective vascular repair machinery. Since $CD14^+$ monocytes can also differentiate into fibroblast-like cells that produce extracellular matrix proteins, here we propose a new hypothesis that aberrant monocytic PHCs, once mobilized into circulation, may also contribute to the fibrotic process of SSc.

Key words: Angiogenesis, Endothelial progenitor cells, Monocytes, Scleroderma, Vasculogenesis

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Introduction

Postnatal blood vessel formation is important for tissue repair and regeneration, but the regulation of this critical process is not fully understood. Maintenance of the postnatal vascular system requires constant remodeling in response to injury and senescence. This may occur by synergic effects of three distinct processes: (i) angiogenesis, which refers to the formation of new blood vessels that sprout from preexisting vessels by a process involving the proliferation and migration of mature endothelial cells (ECs); (ii) vasculogenesis, which refers to the *de novo* differentiation of mature ECs through the recruitment and differentiation of endothelial progenitor cells (EPCs); and (iii) arteriogenesis, which refers to the remodeling of nascent vessels via the recruitment of mesenchymal cells, such as pericytes and smooth muscle cells (Fisher et al., 2006). Since the first description of EPCs as circulating primitive cells that contribute to postnatal vasculogenesis (Asahara et al., 1997), numerous *in vitro* and *in vivo* studies have been carried out to clarify the mechanisms of postnatal vascular formation and repair, as well as the contribution of EPCs to the pathogenesis of vascular diseases, and to develop potential therapeutic strategies that promote tissue regeneration or attenuate pathologic neovascularization. However, a great deal of controversy about EPCs and their roles in postnatal vascular

formation has arisen because of discrepancies in how EPCs are defined (Watt et al., 2010).

The major problem in defining EPCs derives from the lack of specific markers. In the landmark paper by Asahara et al, EPCs were characterized using EC marker-positive cells, which were selected as a cell fraction from peripheral blood mononuclear cells that was enriched in cells expressing CD34 or vascular endothelial growth factor (VEGF) receptor type 2 (VEGFR-2). These cells contributed to the revascularization and salvage of ischemic hind limbs in animal models (Asahara et al., 1997). Currently, it is widely accepted that there are at least two types of EPCs that can be discriminated based on their surface antigen expression, proliferation potential, and time of emergence in the cell culture system (Prater et al., 2007). The first subset is endothelial colony-forming cells (ECFCs) or late-outgrowth EPCs, which are regarded as "true EPCs," based on their potential for clonogenic expansion *in vitro* and their ability to form vessels *in vitro* and *in vivo* (Prater et al., 2007). Circulating precursors of ECFCs have not been identified yet, but they are known to express CD34 and CD31, and to lack the expression of CD133, CD45, and CD14 (Estes et al., 2010).

The cells originally identified as EPCs in various assays are in fact hematopoietic lineage cells that display pro-angiogenic properties, and are now termed pro-angiogenic hematopoietic cells (PHCs). PHCs include several different circulating cell types that are identified in the literature as circulating angiogenic cells (CACs), circulating endothelial precursors, monocytic EPCs, early-outgrowth EPCs, and colony-forming unit (CFU)-ECs. They are hematopoietic progenitors derived from the bone marrow (BM) that fall into at least two distinct major subsets: $CD14^+$ monocytic PHCs (the dominant population) and $CD14^-$ non-monocytic PHCs, which are primitive cells positive for CD34, CD133, and VEGFR-2 (Peichev et al., 2000). Currently, it is generally accepted that PHCs do not give rise to ECs, but rather work as pro-angiogenic support cells (Richardson and Yoder, 2011).

In this review, we focus on the vascular regenerative functions of PHCs originating from the monocytic lineage and their potential roles in the pathogenesis of systemic sclerosis (SSc), a multisystem connective tissue disease characterized by excessive fibrosis and widespread microvasculopathy.

Pro-angiogenic capacity of $CD14^+$ monocytes

EC-like features of $CD14^+$ monocyte-derived cells have been reported ever since Asahara et al.'s 1997 paper was published. Fernandez et al. described a subset of $CD14^+$ monocytes that become adherent within 24 hours of the culture and change their morphology to that of EC-like cells with Weibel-Palade bodies (Fernandez et al., 2000). When cultured with multiple pro-angiogenic growth factors, these $CD14^+$ monocytes gradually lose their expression of hematopoietic markers, such as CD14 and CD45, and display an up-regulated expression of EC markers, including von Willebrand factor (vWF), CD144, CD105, CD34, CD36, acetylated low-density lipoprotein-receptor, endothelial nitric oxide synthase, VEGF receptor type 1 (VEGFR-1), and VEGFR-2 (Fernandez et al., 2000; Schmeisser et al., 2001). In these reports, the cultured EC-like cells formed tubular structures in three-dimensional gel cultures that consisted of short sprouts from the EC-like colonies.

Subsequently, the *in vivo* functional capacity of monocytes was evaluated using animal models for neovascularization. In a study by Urbich et al, peripheral blood-derived $CD14^+$ monocytes were incubated on a fibronectin-coated plate under pro-angiogenic conditions for 4 days, and the recovered adherent monocytes were transplanted into the hind-limb ischemia mouse model (Urbich et al., 2003). The transplanted monocyte-derived cells were incorporated into the vascular structure and promoted neovascularization. In another study, peripheral blood- or BM-derived $CD34^-CD14^+$ monocyte lineage cells accelerated re-endothelialization in a monocyte chemoattractant protein 1 (MCP-1)-dependent manner in a rat model for balloon-injured artery (Fujiyama et al., 2003). These findings together suggest that a subset of $CD14^+$ monocytes can differentiate into the endothelial lineage and contribute to *in vivo* neovascularization and vascular repair (Urbich and Dimmeler, 2004).

A specific marker for this unique monocyte subset has not been identified, but the expression of VEGFR-2 in circulating $CD14^+$ monocytes is essential for their capacity to differentiate into the EC lineage (Elsheikh et al., 2005). Upon vascular injury, a subset of $CD14^+$ monocytes is mobilized into the circulation, adheres to the injured endothelium, and differentiates into EC-like cells, although whether or not monocyte-derived EC-like cells are integrated properly into the endothelium and serve as fully functional ECs has not been confirmed.

Circulating $CD14^+$ monocytes as a primary source of PHCs

The cultivation of circulating mononuclear cells in medium favoring endothelial differentiation has been used to identify EPCs and to expand circulating EPCs. In these cultures, it is difficult to determine which precursor cells give rise to the EPCs, because the starting cell population is heterogeneous, and cellular phenotypes change over time in culture. In the original protocol by Asahara et al. peripheral blood mononuclear cells were cultured on fibronectin for 7 days (Asahara et al., 1997). Currently, CACs are described as the cell type of origin for these cultured cells (Hirschi et al., 2008). Typically, these cells do not form colonies in culture, but they have EC features, including the ability to bind Ulex lectin Europeus Agglutinin-1, to take up acetylated low-density liproprotein, and to express CD31, CD105,

VEGFR-2, and vWF. The vast majority of the cells recovered in these cultures express both CD45 and CD14, indicating their monocytic origin.

In contrast, Hill et al. developed a semi-solid clonogenic assay, in which peripheral blood mononuclear cells that did not adhere to fibronectin within 48 hours were reseeded on fibronectin, and formed cell clusters (Hill et al., 2003). These cells are termed CFU-ECs or CFU-Hill, and express EC markers, including CD31, CD105, CD146, VEGFR-2, CD144, and vWF (Hill et al., 2003). However, unlike the CAC-derived cells, nearly all the cells within the CFU-EC clusters express the hematopoietic marker CD45, but only a tiny fraction express CD34 (Rohde et al., 2006). In addition, the depletion of $CD14^+$ monocytes from the mononuclear cells before seeding effectively prevents colony formation. CACs and CFU-ECs are primarily derived from $CD14^+$ monocytes, and thus are now categorized together as PHCs or early-outgrowth EPCs (Prater et al., 2007). Most importantly, PHCs cannot proliferate or form tubular structures *in vitro* without a co-culture with mature ECs. Several studies reported that PHCs can integrate into tubular structures and differentiate into EC-like cells *in vivo* (Elsheikh et al., 2005; Kuwana et al., 2006), but it is uncertain whether the EC-like cells can exert the full range of endothelial functioning.

PHCs are distinct from ECFCs or late-outgrowth EPCs, which appear 10-21 days after circulating mononuclear cells are plated in medium favoring endothelial differentiation (Ingram et al., 2004; Yoder et al., 2007). These cultured cells display a cobblestone morphology and express EC markers but not hematopoietic markers. Circulating precursor cells that give rise to ECFCs display clonal proliferative potential, self-renewal, and the ability to form vessels *in vivo*, compatible with features of traditional EPCs. A recent genome-wide transcriptional profiling of early- and late-outgrowth EPCs revealed strikingly different gene expression signatures between these cell populations, which provided evidence that the early-outgrowth EPCs are hematopoietic cells with a molecular phenotype linked to monocytes, whereas late-outgrowth EPCs exhibit commitment to the endothelial lineage (Medina et al., 2010). Based on these findings, it has been proposed that the term EPCs should be reserved for ECFCs (Prater et al., 2007; Watt et al., 2010; Richardson and Yoder, 2011). Whether rare ECFCs are derived from hemangioblasts in the BM or from endothelial stem cells that reside in the endothelium remains undetermined (Yoder, 2010).

Roles of monocytic PHCs in neovascularization

PHCs, whether in the monocytic or non-monocytic lineage, are no longer defined as "true EPCs," although they clearly participate in blood vessel formation and vascular repair, and thereby contribute to the maintenance of vascular homeostasis. A function in vascular regeneration was suggested for monocytic PHCs in a vascular injury model, in which green fluorescent-labeled $CD14^+$ monocytes integrated into the endothelium and improved the re-endothelialization (Elsheikh et al., 2005). Indeed, monocytic PHCs are widely accepted to function in a supporting role in vascular repair, and several different mechanisms for their involvement have been described.

First, monocytic PHCs can release a variety of potent, soluble pro-angiogenic growth factors, including VEGF, hepatocyte growth factor (HGF), granulocyte colony-stimulating factor (G-CSF), and stromal cell-derived factor-1 (SDF-1) (Rehman et al., 2003; Urbich et al., 2005). When secreted locally, these factors induce increased vascular permeability, the enhanced proliferation and migration of mature ECs, and the recruitment of progenitor and inflammatory cells from the BM.

Second, immunohistochemical studies in mouse have revealed that monocytic cells attach to the injured vascular lumen immediately after injury and change their morphology to EC-like cells; some of these cells then behave like ECs (Fujiyama et al., 2003; Elsheikh et al., 2005), although it is still unclear if monocytic PHCs are truly integrated into the vascular structures or simply localize there because of their adhesive characteristics. These EC-like cells may supplement the function of impaired ECs at the site of vascular injury, until they are replaced by mature ECs differentiated from ECFCs.

Finally, several lines of evidence have shown that monocytic cells contribute to arteriogenesis (Heil and Schaper, 2004). Mural cells, including pericytes and smooth muscle cells (SMCs), are essential for vessel maturation and stability, but their origin is not fully understood. In a chimeric mouse model for neovascularization, most BM-derived peri-endothelial cells were positive for CD45, CD11b (a monocyte marker), and NG2 proteoglycan (a pericyte marker) (Rajantie et al., 2004), indicating that the pericyte and monocyte lineages have a common origin. Pericyte precursors can differentiate into various mesenchymal cells, including SMCs, fibroblasts, and myofibroblasts (Diaz-Flores et al., 2009), an ability shared by circulating monocytes, which are now considered oligopotent progenitor cells (Seta and Kuwana, 2010). These findings together suggest that monocytic PHCs differentiate into EC-like cells as well as other elements of the vasculature, such as pericytes and SMCs, during the vascular repair process. In addition, monocytic PHCs comprise approximately 0.1% to 2% of peripheral blood mononuclear cells (Dimmeler et al., 2001; Elsheikh et al., 2005; Prater et al., 2007), although the frequency of monocytic PHCs varies depending on the method used to define them. Regardless, monocytic PHCs clearly predominate over non-monocytic PHCs and ECFCs in their absolute numbers in circulation (Prater et al., 2007). The potential mechanisms by which monocytic PHCs provide supportive functions in the neovascular microenvironment are summarized in Fig. 1. During this

process, the monocytic PHCs work in concert with platelets, residential ECs, non-monocytic PHCs, and ECFCs to form new blood vessels (Semenza, 2007).

Monocytic PHCs as oligopotent progenitors

Circulating $CD14^+$ monocytes exhibit heterogeneity in terms of their surface markers, phagocytic activity, and differentiation potential. They are committed precursors in transit from the BM to their ultimate sites of activity. Until recently, monocytes were believed to differentiate only into phagocytic and/or antigen-presenting cells, such as macrophages, dendritic cells, and osteoclasts. However, accumulating evidence indicates that circulating monocytes may differentiate into a variety of other cell types as well, including mesenchymal or endothelial lineage cells (Seta and Kuwana, 2010). Specifically, we described a primitive cell population termed monocyte-derived multipotential cells (MOMCs), which have a fibroblast-like morphology and a unique molecular phenotype positive for CD14, CD45, CD34, and type I collagen in culture (Kuwana et al., 2003). MOMCs include progenitors that differentiate into a variety of non-phagocytes, including bone, cartilage, fat, skeletal and cardiac muscle, neurons, and endothelium (Kuwana et al., 2003, 2006; Kodama et al., 2005, 2006).

At present, several distinct human cell populations derived from circulating $CD14^+$ monocytes have been reported to differentiate into non-phagocytes. Zhao and colleagues demonstrated that pluripotent stem cells generated from circulating monocytes by repeated stimulation with a high concentration of macrophage-colony stimulating factor and phorbol myristate acetate differentiate along several distinct cell lineages, including macrophages, T cells, epithelial cells, endothelial cells, neuronal cells, and hepatocytes (Zhao et al., 2003). Monocytic EPCs also differentiate into cardiomyocytes (Badorff et al., 2003), and monocytic EPCs residing within the circulating $CD14^+CD34^{low}$ cell population differentiate not only into endothelial cells, but also into osteoblasts, adipocytes, or neurons (Romagnani et al., 2005). Finally, fibrocytes are identified as circulating BM-derived cells, which home to sites of tissue injury, differentiate into fibroblasts, and contribute to tissue repair and fibrosis (Bucala et al., 1994). The origin of the fibrocytes is a subpopulation of circulating $CD14^+$ monocytes (Abe et al., 2001).

A variety of $CD14^+$ monocyte-derived cultured cell populations with distinct phenotypes and differentiation potentials have been reported in the literature, but their circulating precursors among the $CD14^+$ monocytes have not been identified to date. All of these cell populations can be enriched by the short-term culturing of circulating monocytes in medium containing different soluble factors and on plates coated with specific matrix proteins. Circulating fibrocytes express the chemokine receptors CCR3, CCR5, CCR7, and CXCR4 (Strieter et al., 2007), but the monocytic precursors of MOMCs are in the $CD14^+CXCR4^{high}$ population. A recent report showed that fibrocytes generated in the absence or presence of fetal calf serum exhibit different morphologies and gene expression profiles (Curnow et al., 2010).

Since circulating $CD14^+$ monocytes change their morphology, gene expression profiles, and function over

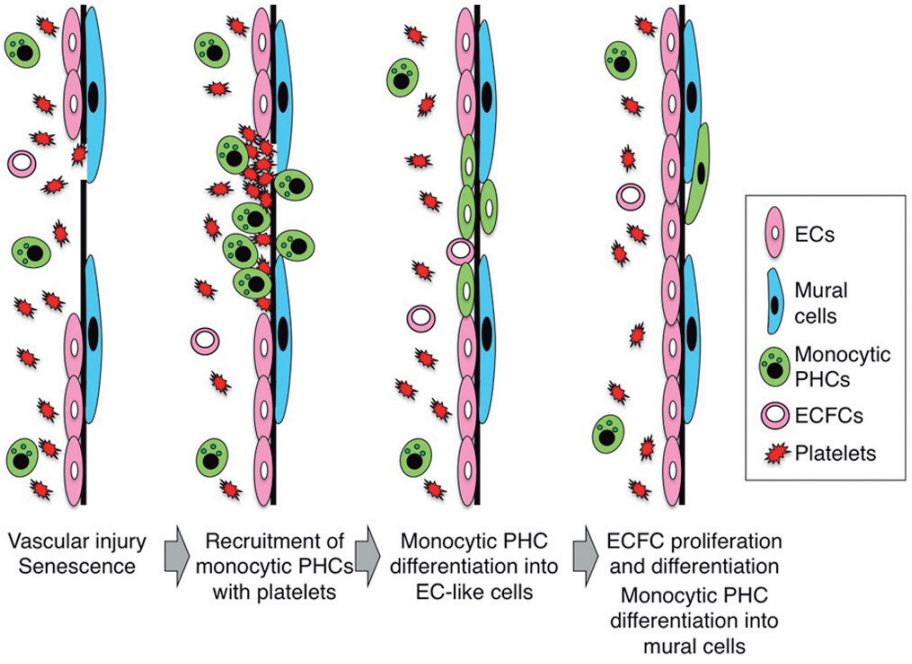

Fig. 1. Potential roles of monocytic PHCs in neovascularization. Monocytic PHCs are recruited to the site of vascular injury, differentiate into EC-like cells, and function as ECs by being incorporated into the vascular structure until ECFCs differentiate into mature ECs. Monocytic PHCs also provide supportive functions by releasing angiogenic factors, chemokines, and proteases to enhance the proliferation, migration, and maturation of the cells required for vascular regeneration. In the late phase of vascular recovery, monocytic PHCs differentiate into mural cells.

time *in vitro* (Seta and Kuwana, 2010), the heterogeneity among culture-enriched monocytic cells does not necessarily indicate that they originate from different circulating precursors. In other words, it is possible that culturing the same $CD14^+$ monocyte precursors in different conditions can generate cell progenies with different characteristics. In fact, monocyte-derived oligopotent cells have common characteristics, including a spindle shape, the expression of CD34 when cultured on fibronectin or type I collagen, and a low proliferative capacity (Seta and Kuwana, 2007). These characteristics are shared by monocytic PHCs, which are oligopotent for differentiation into mesenchymal cells other than EC-like cells. It is possible that monocytic PHCs and other monocyte-derived primitive cells, such as MOMCs and fibrocytes, are all derived from circulating $CD14^+$ precursors.

Roles of monocytic PHCs in neovascular responses in SSc

Given the critical role of monocytic PHCs in postnatal vascular formation and repair, alterations in their numbers and/or functions may contribute to the pathogenic processes of various vascular diseases. In this regard, we focused on SSc, which is characterized by excessive fibrosis and microvascular abnormalities. SSc vasculopathy mainly affects small arteries and causes reduced blood flow and tissue ischemia, leading to clinical manifestations such as digital ulcers and pulmonary arterial hypertension (LeRoy, 1996). Two types of vascular pathology are progressive intimal proliferation and fibrosis, and the loss of capillaries. The mechanism of SSc vasculopathy is not fully understood, but increasing evidence indicates that an endothelial injury is a primary event in the pathogenesis of scleroderma (Guiducci et al., 2007). The persistent increase in pro-angiogenic factors, such as VEGF, platelet-derived growth factor, and SDF-1 observed in SSc patients indicates a strong pro-angiogenic response to vascular damage (Liakouli et al., 2011). Nailfold capillaroscopic findings reveal giant capillaries in the early phase of the disease, and the loss of capillaries and vascular disorganization in the late phase (Herrick and Cutolo, 2010). Severe capillary loss may result from vascular damage, but there is almost no evidence of vascular recovery. In addition, the formation of abnormal blood vessels like giant and bushy capillaries indicates an inadequate vascular repair process. These findings together suggest that, in patients with SSc, the vascular repair machinery does not work properly, and the disease progresses toward irreversible structural changes, despite the strong neovascular push. Thus, impaired angiogenesis and vasculogenesis were proposed in an intriguing hypothesis to explain the pathogenesis of SSc vasculopathy (Manetti et al., 2010).

To test this hypothesis, several studies have been conducted to quantify the circulating $CD14^-CD34^+$ $CD133VEGFR^+$ EPCs, which are now regarded as a non-monocytic subset of PHCs, in patients with SSc. We first reported that there is a reduced number of non-monocytic PHCs in SSc patients (Kuwana et al., 2004). In subsequent analyses by other groups, some confirmed our finding (Zhu et al., 2008; Mok et al., 2010), but

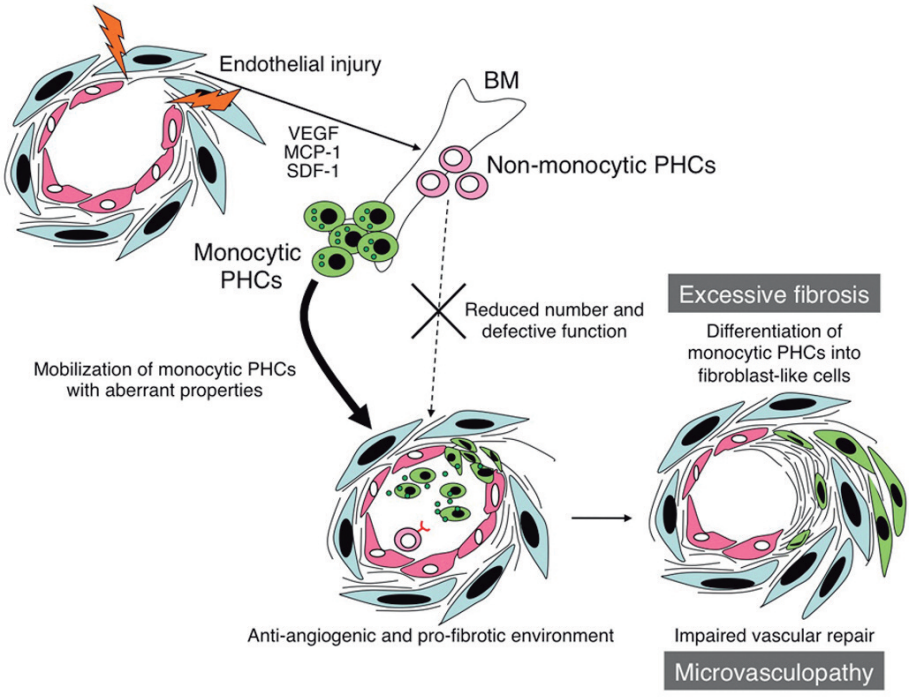

Fig. 2. Potential roles of monocytic PHCs in the pathogenesis of SSc. Growth factors and chemokines produced at the site of endothelial injury mobilize a variety of progenitor cells, including monocytic PHCs. The strong anti-angiogenic environment at the affected site prevents adequate vascular repair, leading to microvasculopathy. In the pro-fibrotic environment, accumulated monocytic PHCs differentiate into fibroblast-like cells and promote excessive fibrosis.

others showed an increase in non-monocytic PHCs in SSc patients (Del Papa et al., 2006; Avouac et al., 2008). Thus, the effect of SSc on the number of circulating non-monocytic PHCs remains a matter of debate (Kuwana and Okazaki, 2012). On the other hand, there is little information on the roles of monocytic PHCs in SSc vasculopathy.

We recently evaluated the number of monocytic PHCs in SSc patients using a culture system previously developed to enrich for MOMCs (Yamaguchi et al., 2010). The MOMCs enriched in this culture can differentiate into EC-like cells and promote blood-vessel formation *in vitro* and *in vivo* (Kuwana et al., 2006), and thus correspond to monocytic PHCs. Unexpectedly, we observed a paradoxical increase in monocytic PHCs in SSc patients compared with healthy controls. Intriguingly, the monocytic PHCs derived from SSc patients showed enhanced *in vitro* tubular structure formation compared with those from healthy controls. Furthermore, in a murine tumor neovascularization model, the transplantation of SSc-derived monocytic PHCs dramatically promoted tumor growth and tumor vessel formation *in vivo*, indicating that monocytic PHCs have enhanced angiogenic activity in SSc patients, an effect that has also been observed in a chick embryo chorioallantoic membrane assay (Ribatti et al., 1998) and in the SCID mouse skin xenograft model (Liu et al., 2005), in which the normal tissue surrounding an SSc skin graft showed a prominent increase in new blood vessel formation. The increased number and enhanced angiogenic potency of the monocytic PHCs are likely to be compensatory responses to damaged vessels.

Despite the robust pro-angiogenic responses, appropriate blood vessel formation does not occur in patients with SSc. The neovascular process consists of a sequence of highly regulated events, including angiogenesis, vasculogenesis, and arteriogenesis, which are tightly controlled by pro- and anti-angiogenic signals (Semenza, 2007). In this regard, the SSc-affected tissues, such as skin and lungs, exhibit dysregulated endothelial features. In microvascular ECs isolated from the skin of SSc patients, metalloproteinase (MMP)-12 is over-expressed and cleaves urokinase-type plasminogen activator receptor, causing inhibition of the invasion/migration capacities of ECs (D'Alessio et al., 2004; Margheri et al., 2006). Furthermore, the reduction of tissue kallikreins 9, 11, and 12, which exert a mitogenic effect on ECs, and the up-regulation of anti-angiogenic kallikrein 3 were reported in SSc skin (Giusti et al., 2005). In addition, in SSc lesions, ECs lose their expression of VE-cadherin, which is required for vascular tube formation (Fleming et al., 2008). Finally, selective up-regulation of the anti-angiogenic VEGF b isoform was observed in the circulation and skin of SSc patients, indicating a switch from the pro-angiogenic to the anti-angiogenic VEGF isoform in these patients (Manetti et al., 2011). These dysregulated endothelial features at the site of SSc organ involvement are responsible for the disease-related defects in angiogenesis and prevent vascular repair. Together, these data suggest that the balance between pro- and anti-angiogenic responses favors anti-angiogenesis in SSc patients.

Pathogenic roles of monocytic PHCs in SSc

Current data on the functions of monocytic PHCs provide strong hints about their roles in the pathogenesis of SSc. Circulating monocytic PHCs are mobilized from the BM and recruited to SSc-induced lesions in response to chemokines such as MCP-1 and SDF-1, which are up-regulated in the affected skin of SSc patients (Distler et al., 2001; Cipriani et al., 2006). In addition, the hypoxic condition of the affected tissues of SSc patients appears to potentiate the in situ differentiation of circulating monocytic cells into EC-like cells (Bellik et al., 2008). Thus, functionally altered monocytic PHCs accumulate at SSc lesions.

Since monocytic PHCs are oligopotent in terms of their capacity to differentiate into mesenchymal lineage cells (Badorff et al., 2003; Kuwana et al., 2003; Kodama et al., 2005; Romagnani et al., 2005), they may differentiate into fibroblast-like cells, produce collagens and other extracellular matrix proteins, and participate in the fibrotic process. In this regard, recent lines of evidence indicate that $CD14^+$ monocytes are involved in fibrogenesis. For instance, fibrocytes derived from $CD14^+$ monocytes home to the site of tissue injury and contribute to tissue repair and fibrosis by differentiating into myofibroblasts that express αSMA (Abe et al., 2001). In addition, $CD14^+$ circulating monocytes acquire the ability to produce extracellular matrix components, such as type I collagen, in an MCP-1/CCR2-dependent amplification loop (Sakai et al., 2006). Furthermore, an enhanced profibrotic phenotype of circulating $CD14^+$ monocytes was reported in SSc patients with interstitial lung disease (Mathai et al., 2010). Another report described a correlation between fibrotic clinical features and the increased proportion of $CXCR4^+$ circulating cells with monocytic and endothelial markers in SSc patients (Campioni et al., 2008). Therefore, monocytic PHCs may acquire pro-fibrotic characteristics and contribute to the promotion of fibrosis at sites affected by SSc that have a strong anti-angiogenic and pro-fibrotic environment (Fig. 2).

Conclusions

In summary, monocytic PHCs contribute to postnatal blood vessel formation and vascular repair, mainly through their immediate recruitment to the site of vascular injury, their secretion of a variety of pro-angiogenic factors, and their differentiation into mural cells. These cells are also oligopotent; that is, they can differentiate into various cell types in the mesenchymal lineage. This unique feature raises the intriguing hypothesis that monocytic PHCs are involved in the pathogenesis of SSc by participating in two major

pathological features, microvasculopathy and excessive fibrosis. Understanding the roles of monocytic PHCs in the progression of SSc may be key to dissecting its pathogenesis and to developing novel therapeutic strategies for this intractable condition.

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