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Invited Review

Endothelial cells and endothelial progenitor cells in the pathogenesis of systemic sclerosis

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Abstract

Systemic sclerosis (SSc) is a connective tissue disease characterized by excessive fibrosis, microvasculopathy, and autoimmunity. Endothelial cell (EC) injury and subsequent endothelial cell dysfunction is believed to be an initial event that eventually leads to a vicious pathogenic cycle. This process is further enhanced by defective angiogenesis and vasculogenesis, as the vascular repair machinery does not work properly. Endothelial progenitor cells (EPCs) are functionally and quantitatively insufficient to recover the endothelium in SSc patients. The dysfunctional ECs and EPCs not only trigger the formation of typical vascular lesions, such as progressive intimal fibrosis in small arteries and the loss of capillaries, but also promote a series of inflammatory and profibrotic processes, such as endothelial-mesenchymal transition and recruitment and accumulation of monocytic EPCs with profibrotic properties. These processes together contribute to the accumulation of extracellular matrix in the affected tissue. This review features current insights into the roles of ECs and EPCs in the pathogenesis of SSc.

Keywords: Scleroderma, systemic sclerosis, endothelial cell, progenitor

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by a combination of excessive fibrosis, microvasculopathy, chronic inflammation, and autoimmunity (1). Vascular involvement in patients with SSc mainly affects small arteries and causes reduced blood flow and tissue ischemia, leading to clinical manifestations, such as digital ulcer and pulmonary arterial hypertension (PAH). The vascular pathologies unique to SSc include progressive intimal proliferation and fibrosis in small arteries, along with the loss of capillaries. The mechanism of SSc vasculopathy is not fully understood, but increasing evidence indicates that endothelial injury and subsequent endothelial dysfunction is a primary event that triggers the subsequent formation of typical vascular lesions (2). In addition, vascular recovery barely occurs in SSc patients, which indicates a defective vascular repair process (3). Postnatal vascular repair is mediated through the collaborative effects of two distinct processes: (1) angiogenesis, i.e., the formation of new blood vessels sprouting from preexisting vessels through proliferation and migration of mature endothelial cells (ECs), and (2) vasculogenesis, i.e., the de novo differentiation of mature ECs through recruitment and differentiation of endothelial progenitor cells (EPCs) (4). This results in the activation of inflammatory and fibrotic processes in perivascular lesions, leading to complex vascular remodeling and irreversible structural changes. This review focuses on the roles of two major cell types that contribute to the homeostasis of the vascular system, ECs and EPCs, in the pathogenic processes of vasculopathy and excessive fibrosis in SSc patients.

Role of ECs in the pathogenesis of SSc

EC apoptosis as a trigger

It is believed that endothelial injury is an initial event that eventually leads to EC dysfunction in SSc patients, and can be triggered via a number of different mechanisms, including infection, ischemia-reperfusion reaction caused by the vasospasm resulting from Raynaud's phenomenon, oxidative stress through abnormal regulation of reactive oxygen species, turbulent blood flow and shear stress, and the imbalance between coagulation and fibrinolysis (5, 6). In this regard, an infection with human cytomegalovirus induces antibodies to recognize an amino acid sequence on the human cytomegalovirus-derived protein UL94, which is homologous to NAG-2, a surface molecule highly expressed on ECs. Antibodies against UL94 peptide have been shown to induce apoptosis of ECs upon engagement with the NAG-2-integrin complex (7). Another potential contributor is anti-endothelial cell antibody (AECA), which is a heterogeneous antibody family

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that reacts with various cell surface antigens on ECs (8). The mechanisms of AECA-mediated cytotoxicity against ECs include antibody-dependent cell-mediated cytotoxicity (9, 10) and have direct effects through an interaction between the Fas and Fas ligands (11, 12).

Defective angiogenesis

SSc patients represent clinical features that are consistent with insufficient vascular repair, but demonstrate up-regulation of a series of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF), hepatocyte growth factor, placental growth factor, and CXCL12 (13). Increased levels of circulating VEGF have been reported in SSc patients (14, 15), but it has been shown that increased VEGF isoform is actually anti-angiogenic to VEGF165-b, rather than pro-angiogenic for VEGF165. In contrast, a soluble VEGF receptor (VEGFR)-1 in circulation works as a decoy receptor for VEGF, and is decreased in SSc patients (16). Interestingly, all three forms of VEGFR were upregulated in the skin biopsies of SSc patients (17, 18), suggesting that VEGF system, which plays a central role in the development and maintenance of the blood and lymphatic vascular systems, is totally disrupted in SSc patients. In addition, reduced levels of pro-angiogenic angiopoietin-1 along with increased levels of angiopoietin-2, an antagonist of angiopoietin-1, were also observed in SSc patients. The expression of kallikreins 1, 9, 11, and 12, which are powerful modulators of angiogenesis, was down-regulated in ECs derived from the affected skin of SSc patients (19, 20). Furthermore, circulating anti-angiogenic factors, such as angiostatin, thrombospondin-1, endostatin, angiostatin, platelet factor 4/CXCL4, and pentraxin 3, were increased in SSc patients as compared to healthy controls (13, 21-23). Endostatin was increased in all phases of the disease, while angiostatin levels were elevated only in the late phase and were correlated with the severity of interstitial lung disease (ILD) (24).

Defective responses of ECs to pro-angiogenic factors in SSc patients can be explained, in part, by the down-regulated expression of their receptors and/or impaired intracellular signaling. In fact, the reduced expression of CXCR4, a receptor of CXCL12, has been found in the affected skin of SSc patients, especially in the late stage of the disease (25). The angiogenic transcription factors implicated in the pathogenesis of SSc include the Friend leukemia integration-1 (Fli1) and Fos-related antigen 2 (Fra-2). Fli1 belongs to the Ets family of transcription factors, and acts as a repressor of collagen transcription in the human skin. A sustained down-regulation of Fli1 in the SSc fibroblasts has been correlated with abnormal matrix deposition in SSc-affected skin (26). Although the Fli1 deficiency in ECs promotes migration, proliferation, and survival, it also suppresses tube formation, which suggests that Fli1 deficiency is potentially attributable to the development of both proliferative obliterative vasculopathy and the loss of vessels, which are characteristic of SSc vasculopathy (27). Fra-2 is a member of the multifunctional activator protein 1 family, and mice overexpressing Fra-2 replicate SSc phenotype, including proliferative obliterative vasculopathy (28, 29). Fra-2 expression was upregulated in SSc fibroblasts, and has been potentially correlated with an increase in the profibrotic effects of transforming growth factor-*β* (TGF-*β*) and PDGF (30). Fra-2 appears to contribute to the development of microvasculopathy by inducing EC apoptosis and reducing the migration of ECs (31).

Main Points

- Endothelial injury and subsequent endothelial dysfunction by a variety of triggers is believed to be an initial event that leads to a vicious pathogenic cycle of SSc.
- Vascular repair is mediated through two distinct processes, angiogenesis and vasculogenesis, and endothelial cells (ECs) and endothelial progenitor cells (EPCs) play critical roles in each process, respectively, but contribute collaboratively to blood vessel formation.
- In SSc, dysfunctional ECs and EPCs do not only trigger the formation of typical vascular lesions but also promote inflammation and excessive fibrosis through recruitment of monocytic cells with M2 features, activation of fibroblasts, and transdifferentiation into myofibroblasts via Endo-MT.

Roles of EC interaction with other cell types in promoting fibrosis

Dysfunctional ECs are also involved in promoting tissue fibrosis in SSc patients by cellular interactions with other cells types, including resident cells within the vascular wall and inflammatory cells infiltrated into the affected tissues. Specifically, ECs in the affected tissue recruit and activate skin fibroblasts by inducing mesenchymal-to-mesenchymal transition through the secretion of a connective tissue growth factor, TGF-β (32). On the other hand, dermal fibroblasts derived from SSc patients are known to overexpress matrix metalloproteinase (MMP)-12, which cleaves the urokinase-type plasminogen activator receptor of microvascular ECs, resulting in the failure of ECs to induce an efficient angiogenic program (33). This bi-directional interaction of ECs and fibroblasts synergistically promotes fibrosis and inhibition of angiogenesis. The altered function of microvascular pericytes has also been reported in patients with early SSc, which resulted from interactions with dysregulated ECs (34, 35). In SSc-affected skin, pericytes expressed activation markers, including PDGF receptor β, high molecular weight melanoma-associated antigen, a regulator of G protein signaling 5 (36), and secreted PDGF-BB that recruits and induces proliferation of pericyte progenitors (37). Interestingly, the co-culture of SSc-derived pericytes with microvascular ECs from healthy controls resulted in an increased production of collagen (38).

Endothelial-mesenchymal transition (Endo-MT)

Given the crucial role of myofibroblasts in the pathogenesis of systemic and organ-specific fibrotic diseases, such as SSc and idiopathic pulmonary fibrosis (IPF), extensive research has previously aimed at precisely identifying their cellular origin. Tissue myofibroblasts can originate from various sources, including quiescent resident tissue fibroblasts, pericytes, adipocytes, macrophages, and epithelial cells (39). Moreover, Karasek et al. (40) have demonstrated that ECs are capable of trans-differentiating toward myofibroblasts in a process called endothelial-to-mesenchymal transition (Endo-MT), by which ECs change their morphological features and acquire a myofibroblast-like phenotype. In SSc patients, evidence has shown that Endo-MT plays a role in vascular remodeling and tissue fibrosis (41). This process is mediated primarily through TGF-*β,* but the detailed intracellular pathways activated by TGF-β have not been entirely elucidated. TGF-β induces Endo-MT through both Smad-dependent and independent pathways, such as c-Abl kinase, protein kinase c-δ, and β-catenin (42). Moreover, various transcriptional regulators, such as Snail1 and Snail2, Twist, and some members of the Zeb family of proteins, are associated with the regulation of TGF-β-induced Endo-MT (43-47). In addition, many other mediators collaboratively promote Endo-MT, as shown below:

Caveolin-1 (CAV1)

CAV1, a major protein component of caveolae, plays an important role in the pathogenesis of tissue fibrosis and various fibrotic diseases by regulating the internalization, transport, and degradation of TGF-β receptors, thereby modulating TGF-β signaling (48, 49). Indeed, the gene and protein expression of CAV1 was

decreased in the affected tissues of patients with SSc and SSc-associated ILD and in the lung tissue of patients with IPF (50-52). Del Galdo et al. (50) demonstrated that *Cav1*$^{-/-}$ mice readily developed pulmonary and skin fibrosis. Restoration of CAV1 function in vitro by supplementing CAV1 with scaffolding domain peptide or overexpression of CAV1 using adenovirus helped to normalize the phenotypes of SSc fibroblasts and suppress TGF-β-induced extracellular matrix production, by inhibiting Smad3 activation and regulation of the c-Jun N-terminal kinase pathway in vitro (50-52). It has also been demonstrated that the in vivo restoration of CAV1 by transfer via adenovirus or administration of a cell-permeable CAV1 peptide prevented bleomycin-induced pulmonary fibrosis and monocrotaline-induced PAH through inhibition of the STAT3 signaling cascade (52, 53). In cultures of pulmonary ECs derived from *Cav1*$^{-/-}$ mice, Endo-MT occurred spontaneously, as evidenced by the constitutive expression of α-SMA, the high levels of production of type I collagen, and the high expression of *Snai1* and *Snai2*. These observations suggest that CAV1 deficiency may participate in the development of progressive tissue fibrosis and proliferative vasculopathy through the promotion of Endo-MT.

Endothelin-1 (ET-1)

Besides its crucial role in the development of PAH, ET-1 has been implicated in the development of organ fibrosis and is an important trigger of the fibrotic process in SSc (54-56). Recent studies have examined whether ET-1 may also play a role in the development of tissue fibrosis by inducing Endo-MT. For example, EC-derived ET-1 promotes cardiac fibrosis and heart failure in diabetic hearts through the induction of Endo-MT (56). However, ET-1 alone was unable to induce Endo-MT in murine lung EC cultures, but did enhance TGF-β-induced Endo-MT (57). The subsequent study confirmed this finding and showed that cultured human ECs induced Endo-MT *in vitro* when treated with ET-1 in the presence of TGF-β (57), indicating a potent synergistic effect of TGF-β and ET-1 on Endo-MT. Endo-MT induced by TGF-β and ET-1 primarily involved the Smad pathway, and was blocked by an ET-1 receptor antagonist, macitentan (58).

Notch pathway

Recent studies indicate that Notch pathways contribute to the pathogenesis of SSc and other fibrotic diseases (59-61), and may also be involved in the regulation of Endo-MT (62). The canonical Notch signaling can act in conjunction with TGF-β to induce Endo-MT by activating the expression of Snail and upregulate a subset of genes through recruiting Smad3 to Smad binding sites (63). However, it should be noted that Kaposi's sarcoma-associated herpesvirus was found to induce Endo-MT via Notch signaling, which was independent of the TGF-β pathway (64).

Wnt pathway

Wnt contains a multigene family of secreted glycoproteins that play important roles during embryogenesis through canonical and non-canonical pathways (65, 66). Recent studies using cultured ECs have demonstrated that canonical Wnt signaling activates Endo-MT pathways (67, 68). On the other hand, the Wnt/β-catenin pathway is involved in the activation of multiple profibrotic steps in SSc pathogenesis (69-72). In fact, increased Wnt activation has been found in skin biopsies from patients with SSc, and Wnt3a-induced myofibroblast differentiation via Smad-dependent autocrine TGF-β signaling has also been observed (70). In addition, the nuclear accumulation of β-catenin in activated fibroblasts was detected in fibroblastic foci in the lungs of patients with SSc-associated ILD (73).

Hypoxia-inducible factor-1*α* (HIF-1*α*)

The transcription factor HIF-1α is a key regulator responsible for inducing a number of cellular and molecular responses to hypoxia and is dysregulated in various pathologic conditions, including SSc (74-76). The mechanisms involved in HIF-1α-induced fibrosis are very complex and may affect numerous gene expression changes, interaction with profibrotic factors (such as TGF-β and VEGF), and the induction of Endo-MT (77-79). One study has shown that the important downstream effects of HIF-1α on Endo-MT induction involve a potent activation of Snail that may ultimately lead to the development of cardiac fibrosis (80).

Roles of EPCs in pathogenesis of SSc

Defective vasculogenesis by aberrant EPCs

Since EPCs are defined as circulating primitive cells that contribute to postnatal vasculogenesis (81), many studies have been conducted to clarify the contribution of EPCs to the pathogenesis of various vascular and connective tissues diseases (82). In patients with SSc, we first reported a reduced number of circulating EPCs, compared with age- and sex-matched rheumatoid arthritis patients or healthy individuals (83). The subsequent analyses done by other groups confirmed our finding (84-86), but some showed a comparable or even increased count of EPCs in SSc patients (87-91). It is now known that these contradictory results resulted from differences in experimental protocols used for quantifying EPCs. Circulating EPCs are identified as cells expressing CD34 in combination with CD133 and/or CD309/VEGFR2 by multi-color flow cytometry, but accurate quantification is technically difficult due to the extreme rarity of this population in circulation. To overcome this limitation, flow cytometry was combined with procedures that enrich EPCs, such as sorting of CD34$^+$ cells and lineage-negative cells, in some studies (83, 90). In these circumstances, the European League Against Rheumatism Scleroderma Trials and Research (EUSTAR) proposed recommendations for the standardization of EPC research (92). We have directly compared several different protocols for quantifying circulating EPCs, and confirmed that the EUSTAR recommendations are valid when combined with an accurate quantification technique, which substantially improved the reproducibility of the results (93). Using standardized protocols, circulating EPCs were shown to be reduced in SSc patients in comparison with healthy controls. Recently, circulating lymphatic EPCs, identified by CD34$^+$CD133$^+$VEGFR3$^+$ cells, were also decreased in SSc patients, and the lower counts were associated with the current digital ulcer (94).

In terms of functional properties of EPCs, we previously reported an impaired potential of SSc-derived EPCs to differentiate into mature ECs using in vitro cultures with multiple pro-angiogenic factors (83). Another study utilizing cultured EPCs showed an impaired differentiation potential to ECs in SSc-derived EPCs, as compared to EPCs derived from healthy controls (16). We recently developed a system to evaluate the in vivo differentiation potential of EPCs, using a murine tumor neovascularization model, in which freshly isolated human CD133$^+$ cells are transplanted into the skin of mice in conjunction with syngeneic mouse tumor cells (95). Using this system, the neovascularization capacity of circulating EPCs was impaired in SSc patients, partly due to a deficiency in their vasculogenic ability. Therefore, defects in vasculogenesis observed in SSc patients are mediated through the impaired function of EPCs as well.

Studies examining the potential associations of EPC counts with clinical manifestations consistently reported an association between the presence of the digital ulcer and low EPC counts (85, 86, 88, 90). A recent prospective study revealed that low EPC counts were identified as independent predictors of the occurrence of new digital ulcer during follow-up (96) and were correlated with the late pattern of nailfold capillaroscopic findings (97). In SSc

patients, the upregulated expression of MMP-10 in EPC-derived ECs was associated with PAH, and the histologic findings of pulmonary arterial remodeling was suppressed by the blockade of MMP-10 in Fra-2-transgenic mice, a model mimicking the vascular and fibrotic aspects of SSc. These findings together suggest that defective EPC leads to the formation of digital ulcers and other vascular manifestations of SSc.

Currently, little is known about the mechanisms behind decreased numeric and functional aberrations in EPCs in SSc patients. In this regard, Del Papa and colleagues reported an interesting finding, i.e., EPCs in the bone marrow from SSc patients were defective in their ability to proliferate in long-term culture with pro-angiogenic factors, suggesting that EPC precursors were functionally altered before their release into the bloodstream (88). The bone marrow of patients with diffuse cutaneous SSc showed markedly reduced microvascular density and increased fibrosis (98), indicating that the dysregulated microenvironment within the bone marrow may alter the EPC differentiation process. In this regard, we recently found that EPC counts are inversely correlated with the level of circulating pentraxin 3, a multifunctional pattern recognition protein with a capacity to inhibit angiogenesis through suppression of FGF-2 (23). Pentraxin-3 is capable of inhibiting the differentiation of bone marrow stem cells into EPCs in in vitro cultures with FGF-2, indicating that exposure to a high concentration of pentraxin-3 would suppress the FGF2-mediated EPC differentiation in the bone marrow. Finally, EPCs in circulation may be attacked though autoimmune mechanisms. In this regard, Zhu and colleagues found that the sera from SSc patients were able to induce apoptosis of EPCs, which was mediated through the Akt-FOXO3a-Bim pathway (84).

Heterogeneity of EPC subsets

There is a great deal of controversy about the definitions and roles of EPCs in postnatal vascular formation (99). This is primarily because of the technical difficulty in identifying those cells due to their extreme rarity in circulation (100). The utilization of a variety of experimental procedures has resulted in a number of definitions of EPCs in the literature. Nevertheless, it is currently accepted that there are at least two EPC subsets that can be discriminated between, based on their surface antigen expression, proliferation potential, and time of emergence in the cell culture system (101). Endothelial colony-forming cells detected in cultures are lineage-restricted progenitor cells that only give rise to endothelium with a clonogenic expansion potential (101), although their circulating origin has not been identified yet. On the other hand, the cells originally identified as "EPCs" are in fact hematopoietic lineage cells that display pro-angiogenic properties, and are now termed pro-angiogenic hematopoietic cells (101). Pro-angiogenic hematopoietic cells are also heterogeneous cell population, including $CD14^+$ monocytic origin (monocytic EPCs) and $CD14^-$ cells positive for CD34, CD133, and CD309 (narrowly defined or conventional EPCs) (102), which were initially termed circulating endothelial progenitors (103). Currently, it is generally accepted that pro-angiogenic hematopoietic cells do not give rise to mature ECs efficiently, rather they work as vascular regenerating and supporting cells (104). Monocytic EPCs especially lack the capacity to proliferate or form tubular structures in the absence of mature ECs. On the other hand, conventional EPCs have typical features of progenitors, including the capacity to proliferate and to differentiate into ECs, however, their efficiency is much lower in comparison with endothelial colony-forming cells. Nevertheless, pro-angiogenic hematopoietic cells, either in a monocytic or conventional subset, are capable of promoting blood vessel formation through multiple mechanisms, including the secretion of a series of pro-angiogenic factors, including VEGF, granulocyte colony-stimulating factor (G-CSF), and stromal cell-derived factor-1 (SDF-1) (105, 106), and differentiation into other elements of the vasculature, such as pericytes and smooth muscle cells. Theoretically, pro-angiogenic hematopoietic cells play a major role in the very early phase of vascular repair by attaching to the denuded vascular endothelium immediately after injury and taking advantage of the large number of ECs in circulation (102). In the following vascular processes, endothelial colony-forming cells and pro-angiogenic hematopoietic cells work in conjunction with platelets and residential ECs to form new blood vessels.

Potential roles of monocytic EPCs in tissue fibrosis

When the number of circulating monocytic EPCs was examined in SSc patients using a culture system developed to enrich this cell population, circulating monocytic EPCs were found to be paradoxically increased in SSc patients as compared to age- and sex-matched healthy controls (107). Intriguingly, monocytic EPCs derived from SSc patients showed enhanced *in vitro* tubular structure formation compared with the structures seen in healthy controls. Furthermore, in a murine tumor neovascularization model, the transplantation of SSc-derived monocytic EPCs dramatically promoted tumor growth and tumor vessel formation *in vivo*, indicating that monocytic EPCs derived from SSc patients have enhanced angiogenic activity. The increased number and enhanced pro-angiogenic potency of monocytic EPCs is likely to be a compensatory response to impaired vasculogenesis due to the malfunction of conventional EPCs. Circulating monocytic EPCs are mobilized from the bone marrow and recruited to affected lesions of SSc in response to chemokines such as monocyte chemoattractant protein-1/CCL2 and SDF-1, which are upregulated in the affected skin of SSc patients (108, 109). In addition, the hypoxic condition of the affected tissues of SSc patients are known to stimulate the differentiation of monocytic EPCs through activation of HIF-1α (110). These local stimuli promote the accumulation of functionally altered monocytic EPCs into the affected lesions of SSc. Since monocytic EPCs are capable of differentiating into cells that produce extracellular matrix proteins (111-115), they might participate in the fibrotic process in the affected organs in an CCL2/CCR2-dependent amplification loop (114, 115). In this regard, the fibrotic clinical features in SSc patients were correlated with an increased proportion of $CXCR4^+$ circulating cells with monocytic and endothelial markers, which correspond to monocytic EPCs (116). Interestingly, monocytic EPCs have common phenotypic features of alternatively activated or M2 macrophages, which are appreciated increasingly as important cells that contribute to the pathogenic process of SSc (117). Recent studies have shown that circulating monocytes with combined classically and alternatively activated features are increased in SSc patients (118, 119). Furthermore, in a phase II clinical trial of tocilizumab in early, active patients with diffuse cutaneous SSc, tocilizumab treatment resulted in down-regulation of M2-associated genes in the skin and a sustained reduction of circulating CCL18, a chemokine associated with M2, in association with the improvement of skin sclerosis (120). Therefore, the pathogenic process of SSc is likely triggered by recruitment and accumulation of circulating monocytic EPCs with M2 features into the affected sites, where they acquire profibrotic properties, i.e., the production of a variety of profibrotic growth factors, cytokines, and chemokines to stimulate resident mesenchymal cells, and their own trans-differentiation into extracellular matrix-producing cells.

Summary: A potential link between aberrant EPC/EC and the pathogenic processes of SSc

Current insights raise the intriguing hypothesis that ECs and EPCs are directly involved in the pathogenesis of SSc by virtue of participating in two major pathologic aspects of the disease; vascular remodeling and excessive

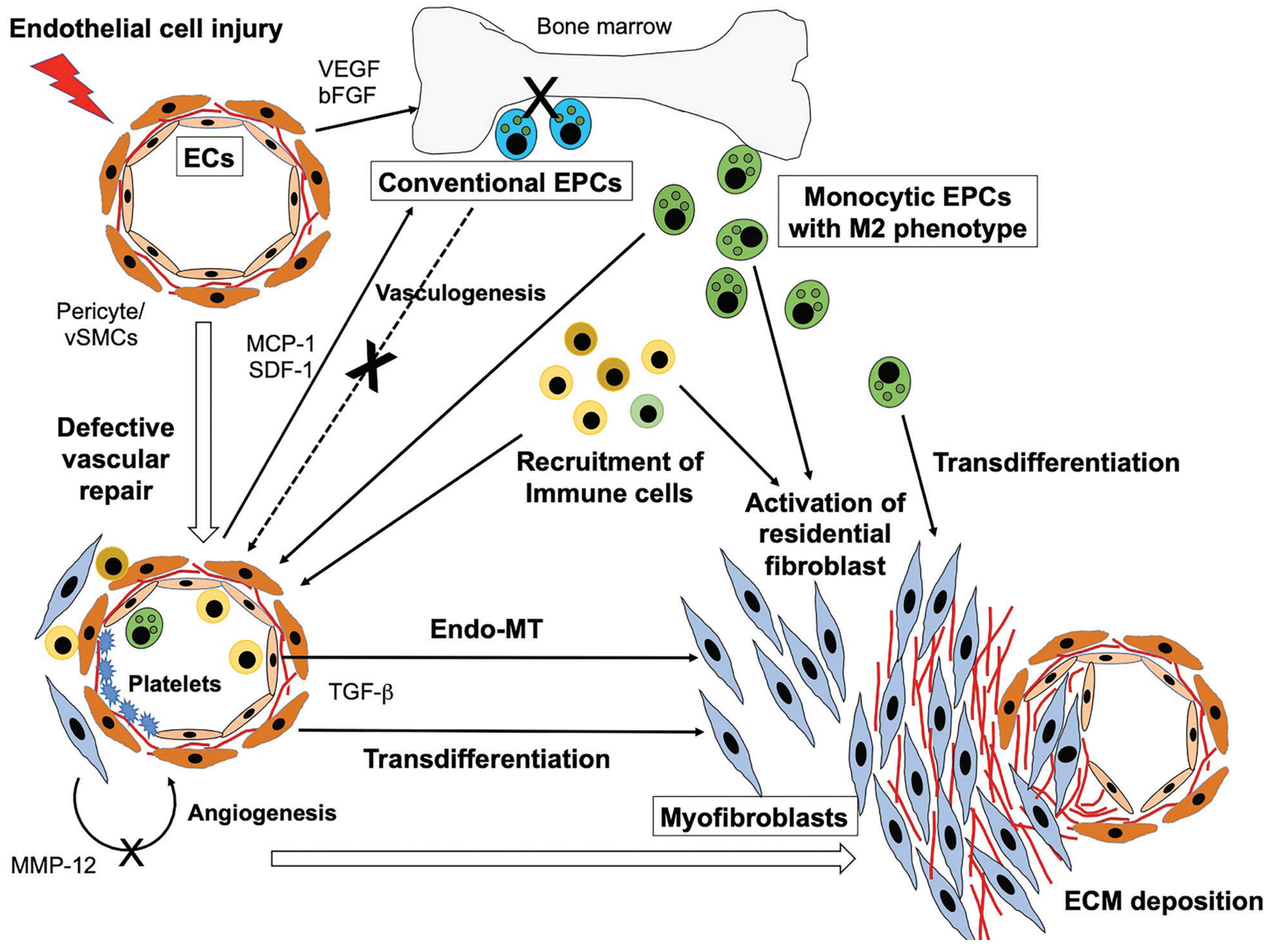

Figure 1. Roles of ECs and EPCs in pathogenesis of SSc.

A variety of triggers damage the endothelium, leading to subsequent expression of a series of pro-angiogenic factors, growth factors, and chemokines. Increased levels of these mediators promote recruitment of conventional EPCs from bone marrow, however, the vascular repair machinery is intrinsically impaired, which results in altered EC functions that induce the activation of fibroblasts by a direct interaction and their trans-differentiation into myofibroblasts via Endo-MT. Additionally, in compensation for the insufficient vascular repair process, monocytic EPCs with M2 features are recruited into circulation and are made to accumulate at the affected sites, thereby promoting ECM deposition and tissue fibrosis.

ECs: endothelial cells; vSMCs: vascular smooth muscle cells; EPCs: endothelial progenitor cells; VEGF: vascular endothelial growth factor; FGF-2: basic fibroblast growth factor-2; MCP-1: monocyte chemoattractant protein-1; SDF-1: stromal-derived factor-1; MMP12: matrix metalloproteinase 12; TGF-β: transforming growth factor β; ECM: extracellular matrix.

fibrosis (Figure 1). Specifically, in early phase of SSc, a variety of triggers damage the endothelium, leading to subsequent expression of a series of angiogenic factors, growth factors, and chemokines, including VEGF, MCP-1, and SDF-1. Normally, the denuded vessels would be rapidly fixed by a highly regulated angiogenic and vasculogenic process, but, in SSc patients, the vascular repair machinery is impaired, which results in disrupted EC functions. Dysregulated ECs promote excessive fibrosis through the activation of fibroblasts by a direct interaction and their trans-differentiation into myofibroblasts via Endo-MT. Additionally, in compensation for the insufficient vascular repair process, monocytic EPCs with M2 features are recruited into circulation instead and are made to function to enhance angiogenesis. However, this mechanism eventually fails to repair vessels because the local environment suppresses angiogenesis, a process which is mediated primarily by dysregulated ECs. Finally, monocytic EPCs accumulate at affected sites and acquire profibrotic characteristics, which enables them to participate in the progression of excessive fibrosis. Further investigation into the mechanisms underlying dysregulated endothelial homeostasis in the disease process of SSc may be key in dissecting its pathogenesis and developing novel therapeutic strategies.

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