Angiogenesis and vasculogenesis in systemic sclerosis

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In addition to inflammatory infiltrates and an accumulation of extracellular matrix proteins, vascular changes are a hallmark in the pathogenesis of systemic sclerosis (SSc). Consistent with the ongoing endothelial cell apoptosis, several markers of EC damage are up-regulated in the serum of SSc patients. Surprisingly, vascular endothelial growth factor (VEGF), a very potent angiogenic molecule, is overexpressed in SSc patients despite the insufficient angiogenesis. VEGF can protect patients from fingertip ulcers, but a prolonged overexpression of VEGF might have paradoxical effects leading to the formation of irregular vessels similar to that observed in SSc. Besides defective angiogenesis, recent studies suggest that vasculogenesis is also impaired in SSc patients with reduced numbers and functional defects of endothelial progenitor cells.

Introduction

Systemic sclerosis (SSc) is a chronic connective tissue disorder of an unknown aetiology that affects the skin and a variety of internal organs. The most obvious histopathological hallmark of SSc is an excessive accumulation of extracellular matrix components, which is caused by an overproduction of extracellular matrix components by activated fibroblasts. Other histopathological characteristics of SSc are perivascular inflammatory infiltrates and alterations of the capillary network, which precede the development of fibrosis. The capillary network of SSc patients shows a reduced density and an irregular chaotic architecture. These changes result in a decreased capillary blood flow causing a lack of nutrients and severe tissue hypoxia [1], which can manifest clinically as skin ulcers.

Endothelial cell apoptosis

Several markers of endothelial cell (EC) activation and apoptosis such as endothelin-1, soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), thrombomodulin and von Willebrand factor protein are increased in the blood of SSc patients [2, 3]. Besides serological parameters, evidence for EC activation and apoptosis can also be observed morphologically. Capillary changes in SSc patients can be analysed in vivo by nailfold capillary microscopy. In contrast to the structurally intact architecture in healthy individuals, the capillary network in SSc patients shows rather chaotic alterations with reduced density of capillaries, microhaemorrhages and giant and bushy capillaries. Giant and bushy capillaries are interpreted as frustrate attempts of angiogenesis. Ultrastructurally, a multiplication of the basal membrane of microvascular ECs has been observed as a marker for EC damage [4]. Further evidence for an important role of EC damage in the pathogenesis of SSc are derived from studies on the University of California at Davis line 200 (UCD 200) chicken. In this animal model, ECs undergo apoptosis before inflammatory infiltrates or accumulation of extracellular matrix occur [5]. These findings in the UCD 200 chicken were confirmed in skin biopsies of SSc patients. Autoantibodies against ECs might play an important role in the induction of apoptosis in ECs, since increased titres of anti-EC antibodies have been detected in UCD 200 chickens and in SSc patients.

Insufficient angiogenesis

The term angiogenesis describes the formation of new vessels by sprouting of differentiated ECs from pre-existing vessels. Hypoxia is a major stimulus for angiogenesis in order to compensate for the lack of oxygen [6]. However, sufficient angiogenesis does not occur in SSc patients despite severe tissue hypoxia [7]. Vascular endothelial growth factor (VEGF) is a central regulatory factor for the formation of new vessels, which controls several steps of angiogenesis. VEGF increases the vascular permeability, stimulates the migration and proliferation of ECs and induces tube formation. Even subtle changes in the concentration of VEGF have profound effects on angiogenesis. A reduction of the concentration of VEGF by 50% leads to vascular malformation and lethality in early embryonic stages. Considering the insufficient angiogenesis in SSc patients, one would expect a downregulation of angiogenic factors or an overproduction of angiostatic factors. To date, no angiostatic molecule has been identified to be consistently up-regulated in SSc [7]. Surprisingly, VEGF is strongly overexpressed in the skin of SSc patients [1]. In agreement with this finding, serum levels of VEGF are significantly increased in SSc patients throughout different disease stages [7]. The serum levels of VEGF correlate significantly with the development of fingertip ulcers. SSc patients with fingertip ulcers have increased serum levels of VEGF compared with healthy individuals, but they are not as high as those in SSc patients without fingertip ulcers [7]. These data indicate that VEGF can have protective effects in SSc patients, if the levels of VEGF exceed an individual threshold. Besides the levels of VEGF, the time kinetics of its expression appears to be critical. If the up-regulation of VEGF is too short, the newly formed vessels are unstable [8]. On the other hand, a prolonged overexpression of VEGF has also unfavourable effects, because the vessels fuse in an uncontrolled manner and form a chaotic vessel network with giant capillaries, a picture strikingly resembling the disturbed capillary network in SSc [8]. Moreover, isolated microvascular ECs from SSc patients show an impaired response to VEGF and other growth factors in the Matrigel capillary morphogenesis assay [9]. Recent data suggest that the impaired ability to form capillaries in the Matrigel assay might be in part caused by matrix metalloproteinase 12 (MMP-12)-mediated cleavage of the urokinase-type plasminogen activator receptor (uPAR) on ECs [9]. The urokinase-type plasminogen activator (uPA)–uPAR system modulates the concentration of VEGF by 50% leads to vascular malformation and lethality in early embryonic stages. Considering the insufficient angiogenesis in SSc patients, one would expect a down-regulation of angiogenic factors or an overproduction of angiostatic factors. To date, no angiostatic molecule has been identified to be consistently up-regulated in SSc [7]. Surprisingly, VEGF is strongly overexpressed in the skin of SSc patients [1]. In agreement with this finding, serum levels of VEGF are significantly increased in SSc patients throughout different disease stages [7]. The serum levels of VEGF correlate significantly with the development of fingertip ulcers. SSc patients with fingertip ulcers have increased serum levels of VEGF compared with healthy individuals, but they are not as high as those in SSc patients without fingertip ulcers [7]. These data indicate that VEGF can have protective effects in SSc patients, if the levels of VEGF exceed an individual threshold. Besides the levels of VEGF, the time kinetics of its expression appears to be critical. If the up-regulation of VEGF is too short, the newly formed vessels are unstable [8]. On the other hand, a prolonged overexpression of VEGF has also unfavourable effects, because the vessels fuse in an uncontrolled manner and form a chaotic vessel network with giant capillaries, a picture strikingly resembling the disturbed capillary network in SSc [8]. Moreover, isolated microvascular ECs from SSc patients show an impaired response to VEGF and other growth factors in the Matrigel capillary morphogenesis assay [9]. Recent data suggest that the impaired ability to form capillaries in the Matrigel assay might be in part caused by matrix metalloproteinase 12 (MMP-12)-mediated cleavage of the urokinase-type plasminogen activator receptor (uPAR) on ECs [9]. The urokinase-type plasminogen activator (uPA)–uPAR system modulates
extracellular matrix degradation and the adhesion of endothelial cells to the extracellular matrix during angiogenesis.

**Defective vasculogenesis**

In contrast to angiogenesis, vasculogenesis describes the formation of new vessels by circulating endothelial progenitor cells (EPCs), independent from pre-existing vessels. The release of EPCs from the bone marrow into the blood can be stimulated by cytokines and angiogenic growth factors such as GM-CSF or VEGF [10]. Different combinations of a variety of cell surface markers such as CD34, CD133, VEGFR-2, VE-cadherin, von Willebrand factor, c-kit and CD146 have been used to identify EPCs. Other groups characterized EPCs by double staining with labelled low-density lipoprotein and lectin. Since the different methods for the characterization of EPCs might identify distinct subsets and maturation stages of EPCs, results of studies with different characterization methods are difficult to compare. However, recent data suggest that vasculogenesis might be impaired in SSc. The number of circulating EPCs is significantly reduced in patients with diffuse SSc compared with healthy controls and patients with rheumatoid arthritis [11]. In addition to reduced numbers, the capability of EPCs from SSc patients to differentiate into endothelial cells in vitro was reduced. Further studies are needed to confirm these findings and to characterize the proposed functional defects in more detail.

### References


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