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Extracellular Matrix Glycoprotein Thrombospondin 1: An Overlooked Pathological Mediator in Vasculopathy of Systemic Sclerosis-Secondary Raynaud's Phenomena

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Abstract

Systemic sclerosis (SSc) is an autoimmune connective tissue disorder characterized by a widespread vasculopathy, autoimmunity, and fibrosis of the skin and internal organs such as the lung and kidneys. Raynaud's phenomenon is the earliest recognized symptom of SSc. Unlike the primary Raynaud's disease, the condition responds well to management with non-pharmacological measures and pharmacological agents, Raynaud's phenomenon presented complex and progressive vasculopathy in the superficial vasculature embedded under the skin. The mechanisms underlying the development and progress of Raynaud's phenomenon are still unclear. Repeated Raynaud's phenomenon attacks are characteristic of ischemia-reperfusion episodes with different durations. What occurred to the superficial vascular bed highly possibly replicates at the vasculature in the vulnerable internal organ(s) at a later time and a distinctive pace. Elevated extracellular matrix glycoprotein thrombospondin 1 (TSP1) levels are found in circulation and throughout the skin in patients with SSc. TSP1-mediated vascular pathologies have been extensively investigated in multiple conditions, including hypertension, pulmonary arterial hypertension, and renal ischemia-reperfusion injury. TSP1 vasculopathy in these conditions is evidenced by a variety of function-modulating and vascular remodeling effects, specifically enhancing vasoconstrictive tone, platelet hyperaggregation, and inflammatory cell infiltration, inducing capillary rarefaction and promoting intimal thickening. In addition, the well-known anti-angiogenic property of TSP1 significantly impairs the self-repair and self-renewal capacity of growth factor- or stem cell-based regenerative therapies. Thanks to the growing knowledge of TSP1 vasculopathy in affected internal organs and adverse effects on regrowth, it is proposed that pathological levels of TSP1 may exert a similar pattern of multifaceted effects on the vasculature in SSc and regenerative therapies under investigation.

Keywords: Systemic sclerosis; Raynaud's phenomenon; Thrombospondin 1; Dysfunction; Remodeling; VEGF; EPC; AD-SVF; MSC; Pro-angiogenic; Anti-angiogenic; Ischemia-reperfusion

Abbreviation

AD-SVF: Adipose-derived stromal vascular fraction; CCB: Calcium channel blocker; EC: Endothelial cell; eNOS: Endothelial nitric oxide synthase; EPC: Endothelial progenitor cell; ET-1: Endothelin-1; EndoMT: Endothelium-to-mesenchymal transition; ECM: Extracellular matrix; HIF:

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Hypoxia inducible factor; ICAM: Intercellular adhesion molecule; IRI: Ischemia-reperfusion injury; MSC: Mesenchymal stromal cell; NC: Nailfold capillaroscopy; NO: Nitric oxide; PDE5: Phosphodiesterase 5; PDGF: Platelet-derived growth factor; PRP: Primary Raynaud's phenomenon; PGE1: Prostaglandin E1; PGI2: Prostaglandin I2; ROS: Reactive oxygen species; SRP: Secondary Raynaud's phenomenon; sGC: Soluble guanylyl cyclase; SSc: System sclerosis; TGF: Transforming growth factor; TNF: Tumor necrosis factor; VCAM: Vascular cell adhesion molecule; VEGF: Vascular endothelial growth factor; VSMC: Vascular smooth muscle cell; NVC: Videocapillaroscopy; vWF: von Willebrand factor

Introduction

Raynaud's phenomenon (RP) describes a symptom presenting exaggerated cutaneous vasoconstriction followed by vasodilation which typically occurs to the extremities, such as fingers and toes, in response to cold temperature or emotional change. Generally, RP patients demonstrated three phases of digital color changes, initially the white phase due to vasoconstriction or occlusion of the pre-capillary arterioles, the cyanosis phase caused by deoxygenation of isolated blood, and the red phase due to restoration of blood flow and subsequent hyperemia. This color change is often associated with pain or numbness. Different from the primary RP (PRP), also called Raynaud's disease, secondary RP (SRP) reflects a profound pathophysiology superimposed with destructive autoimmune status. A review of the classification of RP is available for a better understanding in clinical settings [1]. A high occurrence greater than 96% of RP is reported in autoimmune rheumatic disease systemic sclerosis (SSc) patients [2]. Non-invasive imaging by nailfold capillaroscopy (NC) or videocapillaroscopy (NVC) revealed morphological changes to the vascular bed to distinguish PRP and SRP. A pattern named "scleroderma pattern" was discovered in SSc-SRP patients. The presence of dilated capillaries and microhemorrhages was found in the early stage of SSc, while capillary rarefaction and atypical neoangiogenesis were more demonstrated in established disease and marked as late changes [3,4]. Each Raynaud's phenomenon attack is, in essence, an episode of ischemia-reperfusion; therefore, repeated ischemia-reperfusion injuries (IRI) not only acutely modulate vascular tone but also contribute to chronic vascular remodeling. Both pro-angiogenic and anti-angiogenic factors are upregulated in SSc in an attempt for self-repair and regeneration; however, an imbalance between these factors favors anti-angiogenic capacity resulting in failure in salvage mission and consequent drop in capillaries.

The surge of extracellular matrix protein thrombospondin 1 (TSP1) is tightly associated with IRI in other organs like the lung and kidney, and pathologies on the vasculature and surrounding tissues have been extensively explored. Furthermore, high levels of TSP1 substantially hinder the regenerative effect of growth factor and progenitor cells. High expression of TSP1 in circulation and throughout the skin is reported in SSc. An initial study revealed TSP1-induced myopathy in SSc. This mini-review briefly compares the identified vasculopathy in SSc with known TSP1 pathologies and proposes that TSP1 potentially exerts detrimental effects to the vasculature and endogenous repair attempts.

Discussion

Mechanisms underlying vasculopathy in SSc

Endothelium dysfunction, injury, and death underlie the morphological changes and clinical manifestation of RP under the setting of SSc. In SSc, vasoconstrictive endothelin-1 (ET-1) and coagulating von Willebrand factor (vWF) are significantly elevated, whereas vasodilating nitric oxide (NO) and NO generator endothelial nitric oxide synthase (eNOS) are substantially suppressed. Biogas NO activates soluble guanylyl cyclase (sGC) to produce cGMP, and NO/cGMP signaling exerts a variety of other effects, such as inhibition of vascular smooth muscle cells (VSMC) migration and proliferation and platelet hyperaggregation [5-7]. Reduced production of NO by the dysfunctional or injured endothelium is compounded by a further decrease in bioavailability of NO, scavenged by reactive oxygen species (ROS), in SSc deprived of these protective measures against pathological remodeling of the vasculature. The imbalance between these homeostasis regulators enhances vasocontraction and promotes platelet aggregation and hypercoagulation, exacerbating tissue hypoxia and leading to endothelium dysfunction and vascular damage. The finding of capillary rarefaction in late NC changes implies more profound mechanisms contributing to vasculopathy in SSc patients, including but not limited to the overproduction of ROS [8]. A review comprehensively described oxidative stress and SSc-associated vasculopathy [9]. ROS is tightly linked to vascular remodeling in terms of VSMCs, endothelial cells (ECs), and adventitial fibroblast. In particular, the resident contractile phenotype medial VSMCs and adventitial fibroblast transformed into the migratory and proliferative phenotypes to the intimal layer, causing hyperplasia and subsequent intimal thickening and luminal narrowing, which affect vasculature in more critical organs than the skin, such as the lung, the heart, and the kidney. The migrating VSMCs deposit excessive extracellular matrix (ECM), marking the affected intima with a fibrotic lesion, consequently recruiting endothelial progenitor cells (EPC) for repair.

Additionally, angiogenetic vascular endothelial growth factor (VEGF) and expression of its receptor VEGFR are elevated in SSc patients for compensatory angiogenesis and vasculogenesis [10]. However, EPC-driven repair in SSc is defective in forming a typically organized architecture of

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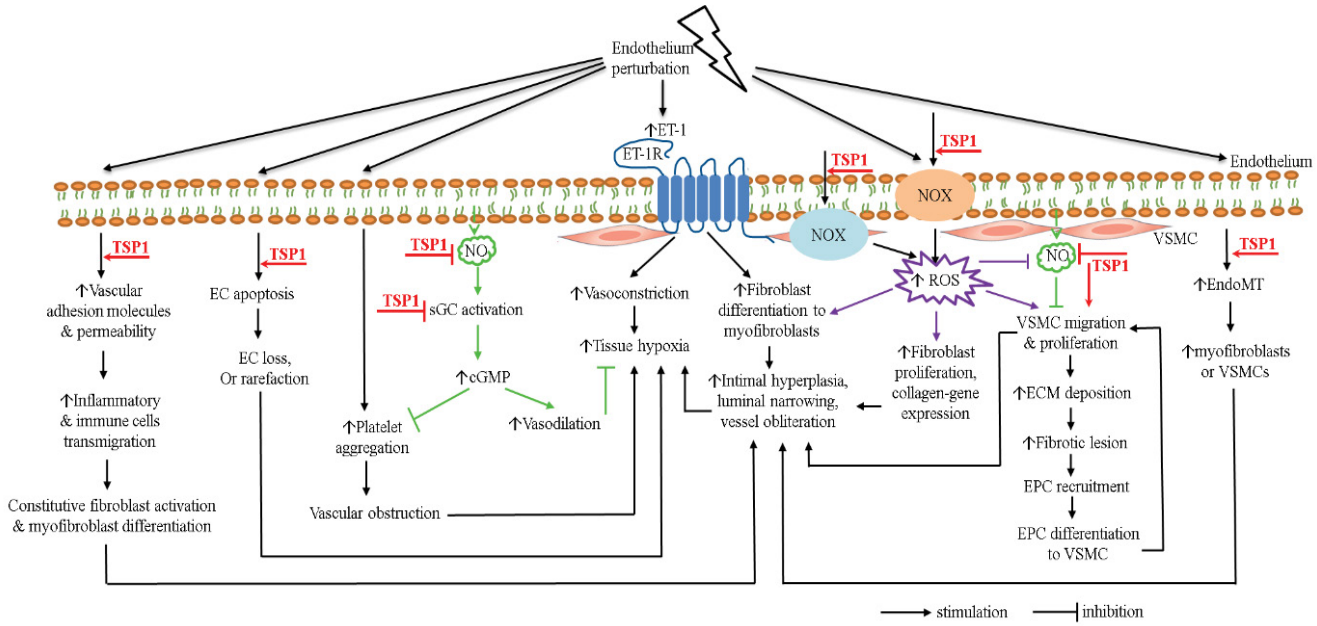

Figure 1: TSP1 may have multifaceted pathological roles in vasculopathy of SSc. Upregulated ET-1/ET-1R signaling, ROS overproduction, suppressed NO/cGMP cascade, as well as increased macrophage infiltration, VSMC migration to the intimal layer and proliferation, EC apoptosis, and EndoMT have been identified in acute vascular dysfunction and chronic vascular remodeling. Elevated circulating and tissue TSP1 in SSc may participate in the major vascular events in a time-dependent manner during different stages of SSc, leading to the clinical manifestation of SRP. Green-colored symbols: NO signaling and effects; purple-colored symbols: ROS effects; red-colored symbols: TSP1 signaling and effects; black-colored symbols: combined signaling and effects.

endothelium; VEGF-mediated neoangiogenesis produced aberrant vasculature described as "bizarre" or "bushy" capillaries as captured in NVC images [4]. Other than the compromised vascular regeneration capability in SSc, loss of functional ECs through the mechanism of endothelium-to-mesenchymal transition (EndoMT) aggravates the drop in capillaries; in that sense, ECs are transformed to VSMCs and/or myofibroblasts [11,12]. Vasculopathy in SSc is concisely summarized in Figure 1.

Current therapies for SSc-associated SRP and challenges

Pharmacological agents, including first-line calcium channel blockers, are administered to manage RP symptoms. However, the medications effective in primary RP patients fail to reach a similar efficacy, indicating the necessity of precise treatment by targeting a dysregulated mediator. For example, NO donor nitrates or L-arginine, cGMP-elongating phosphodiesterase type 5 (PDE5) inhibitors, or sGC activator riociguat rescued the impaired NO/cGMP signaling in SSc and demonstrated greater therapeutic benefits than those with PRP [13,14]. Excess ET-1 secreted by injured endothelium mediates pathological progression of vasculopathy in SSc through multifaceted effects; nonselective ET-1 antagonist bosentan precisely intervenes in the signaling pathways and, therefore, effectively improves the symptoms such as reducing the appearance of new digital ulcer lesions and RP attacks, significant improvement in Raynaud's condition score and visual analog pain scale [15,16]. In addition to treatment with the pharmacological agents with defined mechanisms as mentioned above, other vasodilators, including prostaglandin I2 (PGI2) class and prostaglandin E1 (PGE1), are applied to improve blood flow and reduce ischemia; however, without addressing the underneath pathology [17]. Empirical uses of botulinum toxin A failed to demonstrate clinical benefits [18].

Regenerative cellular therapies using bone marrow-borne or peripheral blood-circulating EPCs, or autologous transplantation of adipose-derived stromal vascular fraction (AD-SVF), or mesenchymal stromal cells (MSC) have been recognized and investigated for neovascularization in a limited scale of clinical trials. Two types of EPCs, early EPCs (eEPCs) and late EPCs, exist as endogenous repair and regenerative mechanisms for vascular injury or loss of vascular beds. However, EPCs in SSc are low in quantity and quality; therefore, autologous EPCs are defective for neovascularization [19,20]. For example, eEPCs isolated from SSc patients presented diminished migration due to transdifferentiation to mesenchymal-orientated cell culture characterized by high expression of CD31 and combined CD31 and αSMA [21]. 6- and 12-month follow up after local injection of autologous AD-SVF demonstrated continuous improvement in assessment categories of the Cochin Hand Function Scale, the Scleroderma Health Assessment

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Questionnaire, the Raynaud Condition Score, and hand pain, and a decrease in the number of digital ulcers as well. Meanwhile, mobility, strength, fibrosis of the hand, and quality of life showed substantial advancement compared with prior to the surgery [22-24]. However, autologous AD-SVF did not show superiority over a placebo in another small-scale clinical trial [25]. AD-SVF isolated from SSc patients has a pro-fibrotic phenotype evidenced by abnormal proliferation, differentiation potential [26]. Bone marrow MSC seems a promising alternative to other cellular therapies, including EPC and AD-SVF, by reducing necrosis, improving pain, blood flow, and healing skin lesions [27-29]. Nevertheless, similar to the stem cells mentioned above, MSCs in SSc patients exhibited a relatively low capacity to differentiate into ECs [30].

TSP1 vasculopathy in SSc

Elevated TSP1 has been reported as a potent anti-angiogenic mediator in SSc, as evidenced by inhibiting microvascular ECs proliferation [31-34]. In addition to the elevated levels in circulation, significantly higher expression of TSP1 throughout the skin in SSc patients was identified [33]. More than anti-angiogenic in SSc, TSP1 is correlated with EC dysfunction and myopathy via activating ECs and platelet, participating in the chronic vascular remodeling processes of fibrosis, inflammation, and reduction in capillary density. It has been established that TSP1 concurrently inhibits NO/cGMP signaling [35,36]. Exposure to TSP1 acutely suppressed eNOS activity while stimulated eNOS-driven ROS production and subsequently reduced NO bioavailability, leading to impaired endothelium-dependent vasorelaxation [37,38]. In addition to reducing the quantity of sGC activator NO, TSP1 decreased cGMP production by directly inhibiting the activity of sGC [39]. TSP1-mediated NO/cGMP signaling inhibition promoted platelet aggregation and facilitated thrombosis [40]. TSP1 is potent in inducing VSMC proliferation and migration [41]. Hypoxia in SSc induced expression of hypoxia inducible factor (HIF)-1α and increased TSP1 levels in a HIF-1α-dependent manner [42,43]. Coincidently, HIF-1α- and HIF-2α-dependent augment of TSP1 stimulated VSMC migration in the coronary and pulmonary arteries [44,45]. Both soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) are found elevated in SSc patients, and sICAM-1 is positively correlated with the inflammatory C-reactive protein (CRP), proving the involvement of inflammation in the disease [46]. TSP1 upregulated the expression of these adhesion molecules, including VCAM-1 and ICAM-1, on the endothelial cell surface to recruit leukocytes to the endothelium and participated in renal ischemia-reperfusion injury (IRI)-associated acute kidney failure [47,48]. TSP1 has been reported to contribute to fibrosis by activating latent transforming growth factor-β (TGF-β) in the kidney and liver [49-51]. Overexpression of TSP1 in scleroderma fibroblasts enhanced ECM accumulation by TGF-β signaling and promoted profibrotic M2 macrophages infiltration, as evidenced by upregulated expression of α2(I) collagen and collagen III, and the appearance of M2 macrophage marker CD163, respectively [32,52]. TSP1 is also implicated in EndoMT in animal models [53]. Suppression of TSP1 expression in an SSc animal model successfully ameliorated fibrosis, vasculopathy, and inflammation, corroborated with reduce in collagen expressions, TGF-β-dependent activation of fibroblasts, M2 macrophage infiltration, and EndoMT. In addition to the well-known anti-angiogenic property, TSP1 mediates pro-apoptotic effects by activating the CD36 or CD47 receptor [54-56]. TSP1 was not only found to be released by vascular endothelial cells under hemodynamic stasis or lack of hemodynamic force; it also reciprocally induces apoptosis under rare and transient hemodynamic stasis and frequent irregular flow, whereas laminar flow or shear stress effectively prevents apoptosis. TSP1 has been reported as an endothelial mechanosensitive death mediator in the pathogenesis of arteriosclerosis through the association between the C-terminus and the ubiquitously expressed cognate receptor CD47 [57,58]. The same pattern of apoptosis induced by a static condition or turbulent flow while reduced by laminar flow is also reported in fibroblast, confirming the critical role of TSP1 and CD47 in apoptosis in a different cell type [59]. Accumulating evidence reveals that TSP1 additionally induces pro-apoptosis and suppresses pro-survival signaling. The mediators and signaling pathways involved in TSP1-CD47 axis-mediated apoptosis other than NO/cGMP are identified. For example, in the absence of growth inducers such as VEGF, TSP1 causes a basal apoptosis rate at 16-19% of human microvascular ECs in a caspase 8-dependent manner [60]. TSP1 induces caspase 8, 3-dependent microvascular EC and vascular EC apoptosis via inducing tumor necrosis factor (TNF)-α expression and activating TNF-R1 (p55)-mediated apoptosis in brain microvasculature and pulmonary arteries, respectively [61-63]. TSP1 also inhibits the expression of pro-survival Bcl-2 while enhancing the expression of pro-apoptotic Bax and promotes caspase 3 cleavage into smaller active forms [64]. TSP1-induced EC apoptosis is reported in conditions of pulmonary arteries and cerebral microvessels [61,63].

These findings, as noted in Figure 1, collectively indicated that TSP1 is highly likely involved in both acute vascular dysfunction and injury in the early stage of the condition, and chronic vascular remodeling, leading to the progress of vasculopathy in SSc.

Adverse effects of TSP1 in regenerative therapies

Proangiogenic mediator VEGF and its receptor VEGFR2 and stromal cell-derived factor 1 (SDF-1/CXCL12) are

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upregulated in SSc to compensate for the injured and lost vasculature. However, TSP1 interrupted VEGF and VEGFR2 signaling cascades via interaction with its cognate receptor CD47 because VEGFR2 requires association with CD47 to initiate autophosphorylation of the receptor, phosphorylation of downstream signaling molecule Akt and activation of eNOS [65-67]. Other mechanisms than dissociation between CD47 and VEGFR2 were predicted in a simulation study and experimentally proved that TSP1 accelerated VEGFR2 degradation [68-70]. It partially explains why elevated VEGF/VEGFR2 in SSc did not guarantee angiogenesis and vasculogenesis as expected.

Stem cell-based therapies to repair injured vasculature or regenerate vasculature seem viable solutions to remedy the loss of vessels and fibrosis in SSc. While the pathophysiology of defective EPC collected from SSc patients remains unknown, findings obtained in extensive studies of IRI-induced renal failure suggested the overlooked effects of TSP1-mediated vasculopathy in SSc-specific EPC. Mechanistic studies demonstrated that TSP1 more potently inhibited late EPC-induced angiogenesis than suppression of eEPC angiogenic effect, both through binding to CD47 [71]. It seems TSP1 exerts the inhibitory effect with possibly different signaling molecules in a temporal-dependent manner; specifically, TSP1-CD47 swiftly down-regulated VEGFR2 phosphorylation within 30 minutes [65,71]; while slowly suppressing SDF-1/CXC chemokine receptor 4 pathway in days [72]. Expression of stem cell transcription factors, such as c-Myc, is suppressed by TSP1-CD47, limiting primary ECs self-renewal and thereby reducing survival under stress which is corroborated by the finding that TSP1-CD47-promoted pulmonary arterial vasculopathy and dysfunction was mediated through suppressed expression of pulmonary endothelial c-Myc [73-75]. Since c-Myc regulates CXC chemokine receptor 4 expression, TSP1 limits late EPC angiogenesis or vasculogenesis potential via c-Myc-modulated CXC chemokine receptor 4 expression [76]. Pre-clinical studies also find that eEPCs may lose the regenerative property induced by TGF-β, evidenced by aggravated senescence in eEPC itself through EndoTM [77]. Considering the association between sustained expression of TSP1 in the development of renal fibrosis, inhibited angiogenesis potential of eEPC and late EPC to stimulate regrowth of vessels can be explained by TSP1-mediated senescence of progenitor cells, in addition to interruption of VEGFR2 signaling cascade [65,71,78,79]. Furthermore, increased platelet-derived growth factor β (PDGF-β) levels in SSc may promote VSMC proliferation and migration in the presence of permissive TSP1, stabilizing newly formed capillaries to prevent further angiogenesis [80]. Pathological levels of TSP1 potently antagonize experimental progenitor cells-based angiogenetic therapies via diverse mechanisms.

Interestingly, TSP1 favors MSC proliferation through the activation of TGF-β and inhibits degradation of the growth factor via interaction with PDGF in vitro [81]. Taking into consideration of its permissive effect on VSMC proliferation and migration, it is concerned that TSP1 may have a paradoxical effect on MSC in SSc; precisely, the anti-angiogenic effect of TSP1 on endothelial cells may overwhelm the proliferation and differentiation capacity of MSCs, leading to impaired differentiation to endothelium even with the addition of VEGF [30].

Conclusion

Elevation of TSP1 and associated pathologies have been discovered in various conditions, including but not limited to pulmonary artery hypertension, renal IRI, coronary artery disease diabetic mellitus. Although an increase in TSP1 has been noted in SSc and TSP1-induced myopathy reported, TSP1 in the pathology of the early vascular manifestation of RP and progress in SSc has been overlooked, in terms of vascular dysfunction, vascular remodeling and loss, inflammation, and fibrosis. This mini-review first attempted to link the vasculopathy in RP under the setting of SSc to acute and chronic TSP1 pathologies on vascular function, morphology, death, and regeneration. TSP1 may participate in multifaceted adverse effects with the progress of the condition, as outlined in Figure 1. It is hypothesized that increased expression of TSP1 involved in the pathological vascular events through a variety of effects, as identified in the vasculature of organs such as the lung and kidney, may also play a pivotal role in RP and internal organ fibrosis in SSc. Due to the anti-regenerative effect on growth factors and stem cell therapies under investigation, TSP1 could sabotage the efficacy of these treatments.

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