Overexpression of VEGF$_{165b}$, an inhibitory splice variant of vascular endothelial growth factor, leads to insufficient angiogenesis in patients with systemic sclerosis

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Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by widespread microangiopathy, fibrosis, and autoimmunity that affects the skin and internal organs. Although in SSc there is a lack of sufficient angiogenic response to chronic tissue ischemia culminating in the loss of capillary vessels, the expression of vascular endothelial growth factor-A (VEGF) has paradoxically been shown to be upregulated in SSc skin and circulation. However, previous studies in the field did not distinguish between the proangiogenic VEGF$_{165}$ and antiangiogenic VEGF$_{165b}$ isoforms that are generated by alternative splicing in the terminal exon of VEGF pre-RNA. In the present study, we investigated whether VEGF isoform expression could be altered in skin and circulation of SSc patients. Using RT-PCR, quantitative real-time PCR, Western blotting, immunohistochemistry and confocal microscopy, we could show that the VEGF$_{165b}$ splice variant was selectively overexpressed at both the mRNA and protein levels in SSc skin. Elevated VEGF$_{165b}$ expression correlated with increased expression of profibrotic transforming growth factor-β1 (TGF-β1) and serine/arginine protein 55 (SRp55) splicing factor in keratinocytes, fibroblasts, endothelial cells, and perivascular inflammatory cells. ELISA on plasma samples revealed that circulating levels of VEGF$_{165b}$ were significantly higher in SSc patients than in control subjects. Microvascular endothelial cells (MVECs) isolated from SSc skin expressed and released higher levels of VEGF$_{165b}$ than healthy MVECs (H-MVECs). TGF-β1 upregulated the expression of VEGF$_{165b}$ and SRp55 in both SSc- and H-MVECs. In SSc-MVECs, VEGF receptor-2 (VEGFR-2) was overexpressed, but its phosphorylation and ERK1/2 downstream signaling were impaired. Recombinant human VEGF$_{165b}$ and SSc-MVEC–conditioned medium inhibited VEGF$_{165}$-mediated VEGFR-2 phosphorylation, ERK1/2 activation and capillary morphogenesis on Matrigel in H-MVECs. The addition of anti-VEGF$_{165b}$ blocking antibodies abrogated the antiangiogenic effect of SSc-MVEC–conditioned medium. Capillary morphogenesis was severely impaired in SSc-MVECs and could be ameliorated by treatment with recombinant VEGF$_{165}$ and anti-VEGF$_{165b}$ blocking antibodies. In SSc, a switch from proangiogenic to antiangiogenic VEGF isoforms may have a crucial role in the insufficient angiogenic response to chronic ischemia. The combination of proangiogenic VEGF$_{165}$ administration and VEGF$_{165b}$ neutralization might represent a potential therapeutic strategy to promote effective angiogenesis and capillary regeneration in SSc.

Keywords: Systemic sclerosis, peripheral vascular disease, angiogenesis, VEGF