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

Mirko Manetti[1], Serena Guiducci[2], Eloisa Romano[2], Claudia Ceccarelli[2], Marco Matucci-Cerinic[2], Lidia Ibba-Manneschi[1]

[1] Department of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy
[2] Department of Biomedicine, Division of Rheumatology, University of Florence, Florence, Italy

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 $VEGF_{165}b$ 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 $VEGF_{165}b$ splice variant was selectively overexpressed at both the mRNA and protein levels in SSc skin. Elevated $VEGF_{165}b$ 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 $VEGF_{165}b$ were significantly higher in SSc patients than in control subjects. Microvascular endothelial cells (MVECs) isolated from SSc skin expressed and released higher levels of $VEGF_{165}b$ than healthy MVECs (H-MVECs). TGF-β1 upregulated the expression of $VEGF_{165}b$ 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 $VEGF_{165}b$ 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-$VEGF_{165}b$ 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-$VEGF_{165}b$ 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 $VEGF_{165}b$ neutralization might represent a potential therapeutic strategy to promote effective angiogenesis and capillary regeneration in SSc.

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