After the occlusion of a nutritive blood vessel, muscle undergoes a continuum of molecular, cellular, and extra- cellular responses that determine the fate of the ischemic tissue. During the latter phase of tissue healing, the different processes involved in new vessel formation, including angiogenesis, take place and represent an integral component of tissue remodeling, which controls the extent of ischemic injury. Angiogenesis is a complex process requiring the coordinated regulation of many activating and inhibitory pathways in which vascular endothelial growth factor (VEGF)–mediated endothelial cell (EC) migration and proliferation play an important role. VEGF acts, at least in part, through interaction of its VEGF receptor 2, also known as kinase insert domain receptor (KDR) in human or fetal liver kinase 1 (Flk1) in murine. Although the signaling pathways downstream of VEGF-mediated KDR/Flk1 activation have been analyzed in detail, the precise complex biology of this receptor has yet to be defined.

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The X-box binding protein 1 (XBP1) exists as unspliced (XBP1u) and spliced (XBP1s) forms via the action of inositol-requiring enzyme 1α (IRE1α)–mediated unconventional mRNA splicing and production of XBP1s, which can activate the unfolded protein response efficiently.1 Alternatively, XBP1s can act independently of its role in the ER stress response and is, for example, able to interact with the Forkhead box O1 transcription factor, leading to its proteasome-mediated degradation without improving the ER folding capacity.1

In a very elegant study in this issue of Circulation, Zeng et al showed, for the first time, that VEGF activates XBP1 mRNA splicing in ECs through interaction of the C-terminal domain of KDR/Flk1 with IRE1α. XBP1s then regulates AKT/GSK/β-catenin/E2F2 signal pathway, leading to EC proliferation4 (Figure). Knockdown of XBP1 or IRE1α reduces VEGF-induced EC proliferation, and global deletion of XBP1 decreases the number of vessels in XBP1-deficient embryos, in part as a result of a reduced number of CD31+ and Flk1+ cells. Furthermore, endothelium-specific deletion of XBP1 (XBP1ecko) abrogates both retinal vasculogenesis and postischemic angiogenesis in mice with surgically induced hind-limb ischemia.4 In this pathological setting, it is noteworthy that whereas reconstitution of XBP1 via adenovirus-mediated XBP1s gene transfer improves foot perfusion in XBP1ecko animals, administration of VEGF-A is unable to restore tissue perfusion in ischemic legs of XBP1ecko animals, suggesting that functional XBP1 in ECs is essential for both basal and VEGF-A–induced tissue perfusion recovery in ischemic tissues. This striking observation suggests that XBP1 may be involved in additional molecular and cellular pathways governing postischemic revascularization. Tissue reperfusion after ischemia is controlled by arteriogenesis, the appearance of new arteriolar structures, and collateral growth, the development and remodeling of preexisting arteriolar anastomoses. Both are characterized by proliferation of smooth muscle cells and production of the extracellular matrix within the vascular wall. Interestingly, a decrease in smooth muscle cells is also observed in ischemic tissue of XBP1ecko animals, suggesting that XBP1 may be involved in an interaction between ECs and smooth muscle cells. It is also likely that XBP1 is involved in VEGF-independent effects. Indeed, the authors showed that conditioned medium from nontargeted lentivirus-treated ECs could rescue proliferation of XBP1 knockdown of ECs, suggesting that soluble factors secreted by ECs may control EC proliferation in the absence of VEGF.

Alternatively, it has been shown that cellular components of the inflammatory system play a critical role in this setting.5 Hence, numerous studies have demonstrated the importance of lymphocyte and monocyte recruitment in stimulating vessel growth and remodeling in ischemic tissue,6–11 including Tie2–positive myeloid cells. Indeed, the authors used Tie2Cre mouse to generate XBP1ecko animals, and it is conceivable that hematopoietic cells contribute to the XBP1-deficient phenotype. Of note, it was shown that transplantation of bone marrow–derived cells isolated from XBP1ecko animals display reduced foot perfusion compared to transplantation of wild-type bone marrow–derived cells. These data suggest that XBP1 can directly or indirectly control bone marrow–derived inflammatory cell mobilization or recruitment to ischemic tissues and subsequently may affect postischemic revascularization.

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The IRE1α/XBP1 pathway is one of the multiple signal pathways activated by ER stress response. Interestingly, as revealed by this study, VEGF treatment does not activate other major sensor and transducer proteins on the ER membrane such as ATF6 and PERK. However, knockdown of KDR abolishes VEGF-induced IRE1α phosphorylation, XBP1 splicing, and expression of C/EBP homologous protein-10 (CHOP-10). CHOP-10 is expressed at low levels under physiological conditions but is strongly induced at the transcription level in response to major sensor and transducer proteins on the endoplasmic reticulum membrane such as IRE1, ATF6, and PERK. CHOP-10 is a transcription factor shown to inhibit the expression of the proangiogenic endothelial nitric oxide synthase (eNOS). XBP1 may also be involved in endothelial cell and smooth muscle cell interaction and likely in the control of the immunoinflammatory reaction, both of which may participate in the overall effect of XBP1 on postischemic revascularization.

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**Disclosures**

None.
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Vascular Endothelial Growth Factor and Angiogenesis: The Xbp1 Games
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