Gene Therapy for Ischemic Diseases Adenoviral-Mediated Delivery of Early Growth Response Factor-1 Gene Increases Tissue Perfusion in a Murine Model of Hindlimb Ischemia

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Tissue ischemia resulting from the constriction or obstruction of blood vessels leads to an illness that may affect many organs, including the heart, brain, and legs. In recent years, much progress has been made in the field of therapeutic angiogenesis, in which angiogenic growth factors are administered to ischemic sites to generate new blood vessels, thereby relieving ischemia. Since blood vessel formation is a complex, multistep process involving temporally and spatially regulated expression of various angiogenic factors, a combination of multiple angiogenic factors is expected to augment the therapeutic response, in comparison with the use of a single angiogenic growth factor. In this regard, we designed an alternative strategy to the combinational use of angiogenic growth factors, which is the use of transcription factor, early growth response factor-1 (Egr-1), that can induce the expression of multiple angiogenic target genes in response to several vascular stresses.

To test this hypothesis that Egr-1 gene transfer would promote the neovascularization in ischemic limbs, a constitutively active form of Egr-1 (Egr-1*) was made and evaluated *in vitro* and *in vivo*. Analyses of the transduced myocytes revealed significant upregulation of bFGF, PDGF-A, PDGF-B, IGF-II, and TGF-b1. A coculture assay of the paracrine effects of indicated that adenoviral Egr-1* promoted proliferation and migration of endothelial cells. When adenoviral Egr-1* was injected into the tibialis anterior muscle of mice, followed by explant culture in growth factor-reduced Matrigel, many capillary-like structures were observed in the Egr-1* group

compared with minimal sprouting from the LacZ group, suggesting an angiogenic potential of Egr-1*. Next we evaluated adenoviral Egr-1* in a murine model of hindlimb ischemia. Compared with slow revascularization in the control PBS or LacZ group, a rapid increase in tissue perfusion was observed in the Egr-1* group and the difference in flux ratio was statistically significant at day 7. In the injected muscle, expression of Egr-1*, upregulation of its target genes, and increased number of vessels staining positive for smooth muscle a-actin were observed. These results suggest that Egr-1 plays an important role in vascular recovery after occlusion and could be a potential target for therapeutic angiogenesis. This Egr1 gene therapy is expected to provide a treatment option for those patients with ischemic disease who are unsuited to conventional revascularizazation therapies.

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