A new strategy for therapeutic angiogenesis using dual-transgene selection plasmid vector in a mouse model of hindlimb ischemia

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Critical limb ischemia (CLI), as a severe manifestation of peripheral artery disease, is emerging as a major concern for aging society worldwide. Cell based gene therapy has been involved in a treatment for CLI to induce angiogenesis in ischemic area. Despite the many studies demonstrated its efficacy as promising results, claudication and amputation along with cardiovascular events are critical complications that need advanced therapy. In the present study, we have established a simplified method to improve the transduced gene effect based on cell-based gene therapy using the selection plasmid vector with sorting system.

We constructed the selection plasmid vector by incorporating vascular endothelial growth factor (VEGF) coding sequences as a target gene into pEF1/HisC vector, and subsequently cloned enhanced green fluorescence protein (EGFP) as a selection marker gene with its CMV promoter. Dual-gene expressing MSCs were collected solely via flow cytometry sorting system and the sorted cells were injected into the ischemic hindlimb of mouse. BALB/c mice are divided into three groups; intact MSCs (MSC injection, n=6), unsorting (PAM-ABP/pEF1/HisC:VEGF:EGFP-MSCs without sorting, n=6), and sorting (PAM-ABP/pEF1/HisC:VEGF:EGFP-MSCs with sorting, n=6). MSC treatment soon after ischemia induction does not appear to be salvaging of ischemic hindlimb therefore, cells were injected 24hrs after hindlimb ischemia.

By using the selection plasmid vector (pEF1/HisC:VEGF:EGFP), both VEGF and EGFP genes were expressed evenly in cardiomyocytes (H9C2 cells) and mesenchymal stem cells (MSCs) in vitro. A significant improvement of blood perfusion was displayed at day 21 after hindlimb ischemia induction compared to that in a typical cell-based gene delivery, which means without sorting system, or intact MSCs control. In immunohistochemistry, mouse VEGF was detected in intact MSCs with low expression level while over-expression of VEGF was identified in sorting group. Control intact MSCs group exhibited significant fibrosis, whereas significantly attenuated in the sorting group.

These results suggest that the selection plasmid vector together with sorting system efficiently improved therapeutic angiogenesis in ischemic hindlimb by augmenting the presence of transduced VEGF gene in the based MSCs. Furthermore, this selection plasmid vector could be a practical use in a wide range of disease, which use cell-based gene therapy as a treatment tool, by simply replacing the target gene or based cell source.