Connexin43 Down-regulation Attenuates Paracrine Effects of Human Smooth Muscle Progenitor Cells through Inactivation of NF-kappa B and Akt

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Background/Synopsis: Angiogenesis is a physiological response to tissue ischemia. Parallel lines of reports had indicated that both circulating endothelial progenitor cells (EPCs) and smooth muscle progenitor cells (SPCs) participate in post-ischemic neovascularization and contribute to tissue repair following ischemia. Our previous studies showed that down-regulation of connexin43 (Cx43) impairs angiogenic potential of EPCs.

Objectives/Purpose: However whether down-regulation of Cx43 also affects the angiogenic potential of SPCs remained unclear.

Methods/Results: Human SPCs isolated from peripheral blood expressed SMC markers, such as SMMHC, caponin, desmin and CD140B. Real-time PCR assay showed that Cx37, Cx43 and Cx45, but not Cx40, existed in the SPCs and Cx43 is the most abundant. To evaluate the role of Cx43 in cellular activities of SPCs, short interference RNA specific to Cx43 (Cx43siRNA) was used. The results showed that Cx43 expression was reduced 90% in both transcript and protein level. Gap junctional intercellular communication (GJIC), migration and proliferation of SPCs were also reduced. In mouse ischemic hindlimb injected with Cx43siRNA-treated SPCs, compared to untreated SPCs, reduced outcome, perfusion ratio and capillary density were seen, indicating that therapeutic potential of SPCs could be impaired by down-regulation of Cx43. To explore the underlying mechanism, the supernatant of Cx43siRNA-treated SPCs was added to human aortic endothelial cells (HAECs). The results showed that migration, proliferation and tube formation of HAECs were reduced. Analysis of the supernatant from the treated SPCs showed decreased levels of IL-6, IL-8 and HGF, with NF-kappa B and Akt pathway inactivation in the cells. To clarify the role of NF-kappa B and Akt pathway, betulinic acid (an NF-kappa B activator) and SC79 (an Akt activator) were applied to the Cx43siRNA-treated SPCs. IL-6, IL-8 and HGF expression could be recovered by betulinic acid, HGF expression was also recovered by SC79.

Conclusion: Gap junctions existed in human SPCs and mainly made of Cx43. Reduced expression of Cx43 lead to decreased therapeutic potential of SPCs through both NF-kappa B and Akt pathway inactivation with subsequent suppressed production of angiogenesis-related cytokines and growth factors, suggesting that Cx43 not only forms gap junction but also plays a key role in the repairing activity of SPCs.