Down-regulation of endothelial connexin43 as a marker for endothelial dysfunction

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Connexins are single polypeptides that assemble to form paired connexon hexamers participating in gap-junctional intercellular communication. In addition, unpaired connexons at cell membrane also act as channels connecting cytosols and extracellular space. These channels' properties plus other functions of individual connexins give the molecules significant roles in endothelial cells, which mainly express connexin43 (Cx43), Cx40, and Cx37. Previous studies have shown that expression of endothelial connexins are regulated by both physiological and pathological factors, a majority of which are involved in atherogenesis, such as hypertension, hyperlipidemia, asenic, and advanced glycation end products. In vascular disorders, endothelial connexins are differentially regulated. However, down-regulation of Cx43 is a common phenomenon.

To further understand whether down-regulation of endothelial connexins activates the cells to pathological status, reduced expression of Cx43 was achieved in human endothelial cells using Cx43-specific siRNA. During the knockdown period up-regulation of coagulatory molecules and impairment of proliferation, viability, and angiogenesis occur. The processes are associated with activation of JNK signaling pathways and rectified by inhibition of the activation. These results indicate that inadequate expression of Cx43 per se impairs endothelial function via the activation of stress activated protein kinase.

In human late endothelial progenitor cells (EPC), siRNA segments specific to each of human Cx37, Cx40, and Cx43 were introduced into the EPC followed by examination of gap-junctional communication, expression profile, migration activity, and angiogenic potential. The results showed that, the mRNA transcript of Cx43 is much more than that of Cx37 and Cx40. In addition, only Cx43-specific siRNA attenuated the gap-junctional communication and migration potential of EPC. Moreover, Cx43 down-regulation decreased the expression level of vascular endothelial growth factor. Laser-Doppler perfusion imaging showed that Cx43 silencing attenuated the angiogenic potential of EPC in hindlimb ischemia mice.

To determine the contribution of gap junction to endothelial function, carbenoxolone, a gap junction blocker, was given to healthy volunteers followed by measurement of flow-mediated vasodilation (FMD), a marker of endothelial function. The results showed that carbenoxolone attenuated FMD without enhancing the release of several vasoactive molecules, including atrial natriuretic peptide, B-type natriuretic peptide, rennin, aldosterone, and cortisol.

In conclusion, consistent with previous reports that down-regulation of endothelial gap junctions is a common phenomenon in vascular disorders manifesting endothelial dysfunction, our recent studies showed that down-regulation of Cx43 per se impairs multiple functions of endothelial cells and EPC. In parallel, our clinical study demonstrated that inhibition of gap junctions attenuate FMD. These data indicate that down-regulation of Cx43 can serve as a marker of endothelial dysfunction and evaluation of the contribution of gap junction to FMD before and after administration of carbenoxolone may be useful for clinical analysis of endothelial gap junction function.