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DKK3 SECRETED BY MESENCHYMAL STROMAL CELLS PREVENTS FIBROBLAST-TO-MYOFIBROBLAST TRANSITION *

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Abstract

It is well known that mesenchymal stromal cells (MSCs) prevent the development of fibrosis in a paracrine manner; still, the underlying mechanisms have not yet been studied. Using proteomic analysis, we identified the DKK3 protein, a regulator of the Wnt signaling pathway, in the MSC secretome. We also demonstrated that the DKK3 protein in the MSC secretome suppresses the canonical Wnt signaling pathway in fibroblasts and prevents myofibroblast differentiation.

Mesenchymal multipotent stem cells (MSCs) regulate tissue renewal and regeneration in a paracrine manner. In humans, the formation of a scar, or fibrous tissue, is the prevailing outcome of healing of damaged tissue. Fibrous tissue is formed by myofibroblasts which originate predominately from resident tissue fibroblasts under the influence of profibrotic stimuli. Dysregulation of fibroblast-to-myofibroblast differentiation can lead to fibrosis, a pathological condition where healthy functional tissue is excessively replaced by scar tissue. We have recently shown that soluble proteins secreted by MSCs prevent the fibroblasts-to-myofibroblasts transition [1, 2], but the mechanisms of this have not yet been studied.

Here, we isolated a fraction of soluble proteins from the total secretome of MSCs and studied its protein contents using semi-quantitative proteomic analysis. We found that soluble proteins secreted by MSCs are enriched in proteins capable of regulating fibroblasts-to-myofibroblasts transition through interaction with TGF-β, Wnt and Notch canonical signaling pathways. Using real-time PCR, we showed that the soluble proteins fraction of MSCs secretome desreases the basal expression level of the *AXIN2* gene, a transcriptional target of the canonical Wnt signaling pathway, but does not alter expression levels of TGF-β and Notch transcriptional targets in fibroblasts at the early stages (6 hours) in the model of fibroblasts-to-myofibroblasts transition. We demonstrated by Western blotting that the soluble proteins fraction of MSCs secretome reduces fibroblasts nuclear content of beta-catenin, a canonical Wnt signaling pathway mediator, in a model of fibroblasts-to-myofibroblasts transition. According to our proteomic analysis results, among proteins abundant in the soluble proteins fraction of MSCs secretome there is DKK3 protein. Literature data suggest that DKK3 can downregulate canonical Wnt signaling pathway in target cells [3, 4]. We depleted DKK3 protein from the soluble proteins fraction of MSCs secretome by immunoprecipitation method. We found that depletion of the DKK3 dramatically reduces the ability of the soluble proteins fraction of MSCs secretome to reduce the level of expression of the *AXIN2* gene in fibroblasts in a model fibroblasts-to-myofibroblasts transition, and also diminihes its ability to effectively prevent the fibroblasts-to-myofibroblasts transition. The data obtained reveal mechanisms of MSCs paracrine effects on the fibroblasts-to-myofibroblasts transition as well as pave the way for the development of potential antifibrotic drugs based on the MSCs secretome.

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