Molecular characterization of cardioprotective exosomes secreted from postnatal cardiac Nkx2.5+ cells

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Background: A83-01, a TGFβ¹/RI inhibitor, could prominently increase cardiomyocyte survival in post-injured adult hearts via expanding cardiac Nkx2.5+ cells to produce the paracrine benefit in vivo. The present study aimed to characterize the molecular profile of the exosomes secreted from cardiac fibroblast [Exo(cfb)] and Nkx2.5+ cells treated with DMSO or A83-01 [Exo(Nkx+D) or Exo(Nkx+A)], and to compare the functional impact of the different exosomes on adult cardiomyocyte viability in vitro.

Methods: Cardiac Nkx2.5+ cells expressing GFP driven by Nkx2.5-enhancer were enzymatically isolated from mice hearts, and were further purified by flow cytometric sorter under the exclusion of hematopoietic lineages, dead cells, and sca1+ cells. Nkx2.5+ cell-secreted exosomes were extracted from culture medium by ExoQuick-TC (System Biosciences). Exosomal proteins were identified by LC-MS/MS, and exosomal miRNAs by mouse miRNA microarray (MRmiOA7.0).

Results: The molecular profiles of Exo(Nkx+D) and Exo(Nkx+A) were markedly different from those of Exo(cfb). Though ten common proteins, including thrombospondin-4, were present in Exo(Nkx+D), Exo(Nkx+A) and Exo(cfb), six proteins were exclusively in Exo(Nkx+D) and seven proteins exclusively in Exo(Nkx+A). There were 30 common miRNAs that were highly expressing in Exo(Nkx+D) and Exo(Nkx+A). Either Exo(Nkx+D) or Exo(Nkx+A) was better than Exo(cfb) in the enhancement of adult cardiomyocyte viability after treatment for 3 days in vitro.

Conclusions: The unique molecular profiles of Exo(Nkx+D) and Exo(Nkx+A) were associated with their better pro-survival effects in adult cardiomyocytes as compared to those of Exo(cfb). Stimulation or administration of cardioprotective exosomes secreted from cardiac Nkx2.5+ cells should be promising strategy to preserve post-injured myocardium.