2019

Comprehensive proteomics and microRNA analyses of adult neural stem cell derived exosomes after stroke

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Recommended Citation

Liu X, Chopp M, Li C, Pan WL, Fan BY, Levin AM, Zhang RL, and Zhang ZG. Comprehensive proteomics and microRNA analyses of adult neural stem cell derived exosomes after stroke. J Extracell Vesicles 2019; 8:92-93.

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Since ASCs act predominantly through paracrine mechanisms, their secretome represents a promising cell-free alternative. Here we identified anti-hypertrophic and anti-catabolic effects of ASC conditioned medium (ASC-CM) on TNFα-stimulated human primary articular chondrocytes (CHs).

Methods: CHs were treated with 10 ng/mL TNFα and/or ASC-CM administered at a 1:5 recipient:donor cell ratio. Cell viability was assessed up to day 9. The activity, expression and/or release of hypertrophy markers (MMP-13, Collagen X and Osteocalcin), catabolic mediators (MMP-3) and cartilage-protective factors were assessed up to day 3 by enzymatic assays, qRT-PCR, Western Blot and multiplex immunoassays.

Results: ASC-CM blunted TNFα-induced hypertrophy, reducing the enhanced levels of MMP-13 activity (−61%), Osteocalcin (−37%) and Collagen X (−18%). In addition, also MMP-3 activity was diminished by −59%. We associated the observed reduction of MMP-3 and MMP-13 activity to the abundancy of TIMPs (Tissue Inhibitors of MMPs) in ASC secretome, rather than to a direct down-modulation of their expression and/or release. Moreover, ASC-CM contains high levels of OPG and DKK-1, other known chondroprotective factors.

Summary/Conclusion: ASC-CM is rich in cartilage-protective factors and exerts anti-hypertrophic and anti-catabolic effects on TNFα-stimulated CHs. These evidences open the way for its possible clinical use as a cell-free approach in contrasting osteoarthritis. We are currently investigating through a differential proteomic analysis if the recognized chondroprotective effectors are enriched in the vesicular rather than the soluble component of the secretome.

PT10.08

Epigenetic alterations in mesenchymal stem cells by osteosarcoma derived extracellular vesicles

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Introduction: Extracellular vesicles (EVs) are central to intercellular communication and play an important role in cancer progression and development. Osteosarcoma (OS) is an aggressive bone tumour, characterized by presence of malignant mesenchymal cells. Specific tumour-driving genetic alterations associated with OS development are poorly understood. The cell of origin for OS also remain unknown but cells of the mesenchymal stem cell (MSC) osteogenic lineage are likely candidates, thus indicating that MSCs and the OS stroma cells may be related cell types. Therefore, this study set out to examine the contribution of EV-mediated intercellular crosstalk of MSC and OS.

Methods: MSCs and pre-osteoblasts were treated with OS-EVs at different time points, and the epigenetic signature of OS-EVs was assessed by LINE-1 and tumour suppressor genes methylation analysis. In addition, surface markers and gene and expression of specific genes related to bone microenvironment remodelling (MMP1, VEGF-A, ICAM1) were also evaluated.

Results: Our data indicated that OS-EVs mediated LINE-1 hypomethylation in MSCs, whereas an opposite effect was seen in pre-osteoblast, indicating that MSCs but not pre-osteoblasts were more susceptible to epigenetic transformation. Thus, OS-EVs dictated the fate of MSCs by modulating the epigenetic status, and also influenced the expression of genes related to bone microenvironment remodelling.

Summary/Conclusion: Overall, this study provided evidence that epigenetic regulation may appear to be an early event in the transformation of MSCs. Elucidating the mechanisms of EV-mediated communication may lead to new avenues for therapeutic exploitation.

Funding: This research was supported by University of Helsinki project funding (WBS490302, WBS73714112), Helsinki University Hospital State funding for university-level health research (Y1014SUL05, TYH2016130), the Finnish Dental Society Apollonia, Egyptian Ministry of Higher Education (MoHE) and Selma and Maja-Lisa Selander foundation.

PT10.09

Comprehensive proteomics and microRNA analyses of adult neural stem cell derived exosomes after stroke

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Introduction: Neural stem cells (NSC) are known to facilitate healing of ischemic brain tissues. Recent studies show that NSC derived exosomes function as paracrine effectors to promote neurovascular remodelling including angiogenesis and axonal outgrowth after stroke; nevertheless, the contents of the non-stroke and post stroke NSC exosome proteome and miRNA cargo have not been determined.

Methods: NSC derived exosomes were purified from conditioned media of cultured NSCs harvested from the subventricular zone of non-ischemic and ischemic rats, respectively. Liquid chromatography mass spectrometry (LCMS) and miRNA array were employed to comprehensively characterize the protein and miRNA contents of NSCs and their derived exosomes after stroke. Bioinformatic analyses were performed using Ingenuity Pathway Analysis (IPA).
Results: Exosome markers including CD63, CD9, Alix and size distribution (50–200nm) were verified with Western blot, transmission electron microscopy (TEM) and Nanosight, respectively. In total, proteomics analysis yielded 2409 and 1770 proteins identified in ischemic NSC and NSC derived exosomes, respectively. Bioinformatics analysis identified that 52, 39 and 31 proteins in the NSCs-derived exosomes were related to regulating neuronal cell proliferation, migration and differentiation, respectively. In addition, 318 miRNAs were identified in ischemic NSCs with 26% of miRNAs (84 miRNAs) overlapped with parent NSCs. Gene ontology analysis showed that up- and down-regulated miRNAs with the fold change above 1.5 were highly related to inflammation, invasion, cell proliferation, cell cycle, cell death, differentiation, etc. The top three upregulated miRNAs were validated in ischemic NSC-exosomes using real-time RT-PCR.
Summary/Conclusion: Collectively, the results of our proteomic and miRNA analysis, to our knowledge, demonstrate for the first time that NSC derived exosomes contain a robust profile of protein and miRNA effectors. These data provide a platform for beginning to understand the mechanism by which NSCs are activated after cerebral ischemia, and may lead to a deeper mechanistic understanding of their role in tissue repair after neural injury.
Funding: NIH RO1 DK102861, American Heart Association (AHA) grant 18IPA34170331, NINDS RO1 NS075156 and RO1 NS088656.

PT10.10

Anion exchange chromatographic isolation of iPSC-MSC derived extracellular vesicles ameliorated allergic asthma in mice

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Introduction: The extracellular vesicles (EVs) derived from mesenchymal stem cells have been shown to elicited similar therapeutic effects to their patent cells in many diseases. However, the difficulties in the large-scale preparation of high-purity EVs largely limited its clinical application in the future. We sought to apply a novel anion chromatography for the isolation of iPSC-MSC EVs, and explored the effects and mechanisms of iPSC-MSC EVs in the therapy for asthma.
Methods: The EV-enriched supernatants were collected for the isolation of the iPSC-MSC EVs using the anion chromatography. The morphologies of EVs were characterized by transmission electron microscope, the markers of EVs were assayed by western blot and flow cytometry. The anti-inflammatory effects of the EVs were determined using the macrophage assay. Also, the uptake activities of macrophages on RPF-iPSC-MSC EVs were determined. Finally, the asthma mouse model was developed and the iPSC-MSC EVs were administrated intravenously. The lung pathology, the levels of inflammatory cytokines in the bronchoalveolar lavage fluids (BALF) and the polarization of lung macrophages were evaluated.
Results: We successfully obtained concentrated iPSC-MSC EVs after the isolation and the final concentration of EVs was about 200 μg/mL (Bradford) and 10–$15*10^{11}$/mL (Nanosight). The iPSC-MSC EVs were morphologically intact and were positive for the markers including CD9/63/81, Alix and TSG101. Most of the preparations of iPSC-MSC EVs could significantly decreased the level of IL-6 in the macrophage assay. The Raw 264.7 macrophages began to uptake iPSC-MSC EVs at 4 h and the uptake activities peaked at 12 h and then receded at 24 h. Also, our *in vivo* study showed that splenic macrophages started to uptake iPSC-MSC EVs at 4 h and the uptake activities were augmented at 24 h. In addition, the iPSC-MSC EVs significantly reduced the inflammatory infiltration, the epithelial goblet cell numbers, the levels of inflammatory cytokines and inflammatory cells in the BALF as well as the polarizations of pulmonary macrophages.
Summary/Conclusion: Our results showed that the anion exchange chromatography was a promising method for the large preparation of iPSC-MSC EVs, which could possibly be an alternative therapy for asthma in the future.
Funding: NSFC (81322012, 81373174, 81471832, 81671882 and 81770984)

PT10.11

Characterization and miRNA expression profiles of exosomes from HLA homozygous haplotype dental pulp cells and iPS cells

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