Microparticles: Induced Exosomes in Cardiovascular Pathology

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255 Exosomes from human cardiac-resident progenitor cells are more cardioprotective than exosomes from bone marrow mesenchymal stem cells via a pregnancy-associated plasma protein-a-dependent mechanism

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Introduction: Cardiac-derived progenitor cells (CPC) and bone marrow mesenchymal stem cells (BMSC) have been evaluated in clinical trials in patients after myocardial infarction (MI). The most beneficial cell type, however, has not been determined yet. Available evidence suggests that both cell types may exert beneficial effects in injured hearts primarily by releasing secreted factors, particularly exosomes (Exo; nanosized vesicles). Thus, a comparison of cardioprotective activities of Exo from these cellular sources is important for cell heart therapy.

Methods: Right atrial appendage and sternum BM samples were collected from patients who underwent heart valve surgery to derive CPC and BMC, respectively. Exo were isolated from culture media conditioned by these cells. They were tested in cardiomyocyte (CMC) apoptosis and angiogenesis models in-vitro, and in-vivo after MI in rats. miRNA and proteomics analyses were performed.

Results: Exo-CPC were more cardioprotective and proangiogenic than Exo-BMC both in-vitro and in-vivo, moreover Exo-CPC improved cardiac function after MI to a greater extent than Exo-BMC (LVEF% 75.37 ± 3.15% vs 58.71 ± 6.49%, p<0.01). Both Exo types were highly enriched in a set of cardioprotective miRNA (miR146a, miR210, miR132, miR181a) compared to Exo derived from human dermal fibroblast, which were not cardioprotective. Pregnancy-Associated Plasma Protein-A (PAPP-A) was identified as one of the most highly enriched proteins in Exo-CPC vs. Exo-BMC. PAPP-A on Exo-CPC induce the release of bioactive insulin-like growth factor-1 (IGF-1) that activate IGF1R, leading to the phosphorylation of Akt and ERK in recipient CMC, resulting in reduced apoptosis under stress conditions. Knocking-down of PAPP-A using siRNA abrogated benefits of Exo-CPC.

Conclusions: Exo-CPC are more cardioprotective and proangiogenic than Exo-BMC, which show some degree of activity. The mechanism of benefit of Exo-CPC involves PAPP-A-mediated activation of IGF-1 and Akt/ERK signalling pathways.

256 The human pericardial fluid is enriched with cardiovascular-expressed microRNAs and exosomes with therapeutic potential

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The pericardial fluid (PF) surrounds the heart and is contained in the pericardial sac. PF contains myocardial-derived factors. MicroRNAs (miRNAs) are negative posttranscriptional regulator of their target genes. miRNAs are released in exosome (exo) and exosomal miRNAs contribute to cell-to-cell communication. We hypothesized that the PF contains exo of myocardial origin and it represents a niche for the exchange of exo-miRNAs between heart cells. Here, we have characterized for the first time, the human PF-exo and PF-exosomal miRNAs.

PF, plasma, myocardial and vascular samples were collected as leftover from aortic valve replacement surgery. PF was submitted to a PCR-based miRNA-array (Exiqon), revealing high expression of cardiovascular miRNAs, but not miRNA-122, which was enriched in PF and PF-exo (comparison with plasma-exo). miRNA-122 was found present in the PF (9.8x10^10 ± 2.5x10^10), PF-exo were prepared and validated by electron microscopy and western blot using exosomal markers. Next, the PF-top expressed cardiovascular miRNAs and miRNA-122 (non-cardiovascular negative control) were measured (RT-PCR) in tissue samples (to confirm the miRNA cardiovascular expression in donor patients). PF and plasma and in PF-exo and plasma-exo. The functional status and proangiogenic potential of PF-exo were assessed in cultured endothelial cells (ECs) and in mouse schema model. The contribution of miRNAs in PF-exo effect was investigated in vitro.

Cardiovascular miRNAs, but not miRNA-122, were enriched in PF and PF-exo (comparison with plasma and plasma-exo), Fluorescein-tagged PF-exo were uptake by cultured ECs and improved EC survival, proliferation and tube formation. The proangiogenic miRNA let-7b-5p was highly expressed in PF-exo and could be passed to ECs, decreasing its target genes (THSB2 and TIMP2) expression in recipient ECs. MiRNA biogenesis inhibition (by Dicer silencing) in cultured ECs impaired angiogenesis, which could be restored by PF-exo. Finally, local PF-exo delivery improved post-ischemic angiogenesis and blood flow recovery in mice with limb ischemia. Plasma-exo did not show angiogenic responses either in vitro or in vivo.

In conclusion, the PF contains exosomal miRNAs that might contribute to cardiovascular homeostasis and repair.

257 Circulating microparticles of healthy origins protect against atherosclerotic vascular disease via microRNA transfer to endothelial progenitor cells

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Background: The development of atherosclerosis and cardiovascular disease is the result of multiple intermediate processes where endothelial dysfunction and vascular inflammation play key contributing roles. Cell-derived microparticles (MPs), endothelial progenitor cells (EPCs) and circulating microRNAs (miRNAs) have attracted major interest as biomarkers and potential regulators for atherosclerotic vascular disease, but their involvement in the mechanisms of inflammatory processes and vascular repair remain controversial.

Purpose: We evaluated the possible protective role of MPs and EPC-derived MPs (MP-E) of healthy origins in atherosclerosis development as well as the mechanisms responsible for their repair capacity.

Methods: The experiments were performed on hamsters divided into: (1) simultaneously hypertensive - hyperlipidemic (HH) by combining two feeding conditions for 4 months; (2,3) HH with retr-
orbital sinus injection containing MPs or MPEs, from control hamster, one dose per month for 4 months of HH diet, to prevent atherosclerosis (HHMP or HHMPE): (4) controls (C), age-matched normal healthy animals.

Results: The results showed that: (1) MP/MPE transplantation suppresses the development of atherosclerosis processes via: (i) the alleviation of dyslipidemia, hypertension, circulating EPC levels, cytokine/chemokine profiles (VEGF, IL-6, IL-8); (ii) the structural and functional remodeling within the vessel wall and heart in term of: distensibility/stiffness/pulse wave velocity of thoracic aorta, carotid wall thickness, systolic and diastolic function of left ventricle, left ventricular hypertrophy, lipid accumulation/contraction/relaxation in thoracic aorta, carotid and resistance arteries; (2) MPs operate as protective and delivery system for miRNAs in circulation — this was demonstrated by validating MPs/MPEs as intercellular carriers of functional Ago2-miRNA, Stau1-miRNA and Stau2-miRNA complexes; (3) MPs and MPEs protect against atherosclerotic vascular disease via transfer of miR-10a, miR-21, miR-126, miR-146a to circulating late EPCs. It mentioned that, the favorable effects of MPEs are similar to those of MPs.

Conclusion: The data indicate that MP and MPE transplantation can counteract HH diet-induced detrimental effects by their miRNA transfer to circulating EPCs mediating their function. These promising findings using MPs and MPEs as therapeutic tools for transferring miRNAs in an atherosclerotic animal model give hope to patients with cardiovascular disease.