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Functional analysis of miRNAs shuttled by extracellular vesicles from diabetic subjects reveals their role in diabetic retinopathy

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Background and aims: Extracellular vesicles (EVs) derived from mesenchymal stem cells cultured in diabetic-like conditions enter the pericytes, causing their detachment and migration, and stimulating angiogenesis. Diabetic patients have different EV patterns in comparison with healthy subjects. In particular, our data suggest a role for miR-150-5p, miR-21-3p and miR-30b-5p as putative biomarkers of the onset and development of diabetic retinopathy. The functional KEGG pathways of these 3 miRNAs showed that they are involved in pathways strictly correlated to the dysfunctions occurring in the early phases of retinopathy, such as adherents junctions, ECM-receptor interactions, TGF-β signaling. In this work, we aimed at further investigating the functional role of the 3 miRNAs on the homeostasis of retinal microvascular cells and characterizing EVs derived from diabetic subjects with/without retinopathy by mRNA content analysis.
Materials and methods: EVs were extracted from plasma of 7 type-1 diabetic subjects with severe retinopathy (DR, gender: 3F/4M, age 39.3±5.9, disease duration 28.0±12.8), age- and gender-matched with 7 healthy controls (CTR, gender: 3F/4M, age: 41.0±10.6) and 7 diabetic subjects without retinopathy (noDR, gender: 3F/4M, age: 46.1±11.7, disease duration: 27.3±14.2). As we found miR-21-3p and miR-30b-5p increased, and miR-150-5p decreased in EV of DR patients, human retinal pericytes (HRP) and endothelial cells (HMEC) were transfected with mimics or inhibitors, as appropriate, of the 3 miRNAs, to evaluate their functional role in angiogenesis (vessel-like formation assay) and migration of retinal microvascular cells. Furthermore, EV expression of genes involved in angiogenesis was measured by *Human Angiogenesis RT² Profiler PCR Array* and confirmed by qRT-PCR and Western blotting (WB).
Results: After 48 hrs from transfection, modulation of miRNA expression increases migration in microvascular cells and vessel formation *in vitro*, confirming that the 3 miRNAs are involved in angiogenesis. mRNA analysis revealed different expression of 7 genes involved in angiogenesis in the 3 groups, while subsequent qRT-PCR and WB confirmed decreased expression of angiopoietin-1 (involved in vessel stabilization) and increased expression of the pro-angiogenic HIF-1α in DR vs CTR.
Conclusion: In conclusion, the analysis of EV mRNA content reveals differences between diabetic patients with microvascular complications, and healthy controls. miR-150-5p, miR-21-3p and miR-30b-5p, differentially expressed in EVs from DR patients and controls, seem to be related to diabetic retinopathy by inducing features of retinopathy in *in vitro* models of retinal microvasculature. These miRNAs might be taken into account as potential biomarkers of the onset/development of the disease and considered as specific targets for the prevention of this complication.
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