

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1700260> since 2019-04-29T12:26:44Z

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

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

A. Mazzeo, E. Beltramo, C. Gai, T. Lopatina, M. Trento, M. Porta;
Dept. of Medical Sciences, University of Turin, Torino, Italy.

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 \pm 5.9, disease duration 28.0 \pm 12.8), age- and gender-matched with 7 healthy controls (CTR, gender: 3F/4M, age: 41.0 \pm 10.6) and 7 diabetic subjects without retinopathy (noDR, gender: 3F/4M, age: 46.1 \pm 11.7, disease duration: 27.3 \pm 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-1 α 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.

Clinical Trial Registration Number: CS/236

Supported by: EFSD/Lilly - MIUR

Disclosure: A. Mazzeo: Grants; EFSD/Lilly Fellowship 2016 - Italian Ministry of Education, Universities and Research.