

Elena Beltramo, Aurora Mazzeo, Marina Trento, Massimo Porta

Dept of Medical Sciences, University of Turin

MiRNAs shuttled by extracellular vesicles from diabetic subjects induce features of retinopathy in vitro

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.

Our purpose was the molecular characterization of EVs derived from diabetic subjects with/without retinopathy, compared with healthy controls, and their influence on small vessel homeostasis and angiogenesis.

EVs were extracted from plasma of 7 type-1 diabetic subjects with severe retinopathy (DR group), age- and gender-matched with 7 healthy controls (CTR group) and 7 diabetic subjects without retinopathy (noDR group). EV miRNA content was studied by *Taqman Human MicroRNA Arrays* and confirmed by qRT-PCR. Human retinal pericytes (HRP) and endothelial cells (HMEC) were transfected with mimics or inhibitors of differentially-expressed miRNAs to evaluate their ability in promoting tube formation. EV expression of genes involved in angiogenesis was measured by *Human Angiogenesis RT² Profiler PCR Array* and confirmed by qRT-PCR and Western blotting. EV influence on pericyte detachment, angiogenesis, migration, and permeability of the blood-retinal barrier was also investigated.

Following microarray analysis, 11 miRNAs were found to be differentially-expressed in the 3 groups. Three of them (miR-150-5p, miR-21-3p and miR-30b-5p) were confirmed by qRT-PCR. Modulation of their expression inside microvascular cells confirmed their involvement in abnormal 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 noDR and CTR. EVs from DR subjects induced pericyte detachment and pericyte/endothelial cell migration, increased the permeability of pericyte/endothelial cell bilayers and the formation of vessel-like structures, when compared with EVs from controls.

In conclusion, EVs from DR patients induce features of retinopathy in *in vitro* models of retinal microvasculature, such as migration of pericytes, formation of new vessels, increased retinal blood-barrier permeability. 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.