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Circulating extracellular vesicles from diabetic and healthy subjects show different miRNA patterns

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Background and Aims: We previously demonstrated that extracellular vesicles (EV) derived from mesenchymal stem cells (MSC) cultured in high glucose and/or hypoxia are able to enter retinal pericytes, causing their detachment from substrate and migration, and stimulating angiogenesis *in vitro*.

Our present hypothesis is that circulating EV in diabetes may influence small vessel homeostasis, and that molecular differences can be identified in EV from healthy controls and diabetic subjects, with/without microvascular complications.

The aim of this study was the molecular characterization and comparative analysis of EV derived from healthy and diabetic subjects with and without microvascular complications, and their influence on pericyte detachment and angiogenesis.

Materials and Methods: EV were extracted from plasma of 4 type 1 diabetic patients with microvascular complications (nephropathy and retinopathy) (CDM, sex: 1F/3M, age: 48.0±16.3, disease duration: 22.5±10.2), 4 without complications (DM, sex: 1F/3M, age: 44.5±8.1, disease duration: 21.0±9.6) and 4 healthy age- and gender- matched controls (noDM, sex: 1F/3M, age 39.0±9.2). EV expression of surface molecules was measured by FACS. microRNA (miRNA) content was evaluated by Taqman Human MicroRNA Arrays – cards A and B, which allow detection of 754 different miRNAs. Human retinal pericyte (HRP) detachment was evaluated by cell counting after 4 and 24 hr exposure to EV obtained from MSC, CDM and noDM plasma. Proliferation and apoptosis were measured in HRP still attached to culture wells. *In vitro* angiogenesis in HRP-endothelial cell co-cultures was analysed by tube formation in Matrigel after 48 hr EV exposure.

Results: FACS analysis of surface molecules showed no significant changes between groups. Only miR-106a was increased in the DM compared with the noDM group ($p < 0.05$), while 10 miRNAs differed between the noDM and CDM groups. Five of them, with anti-angiogenic properties (miR-150, miR-155, miR-342-3p, let-7-g, miR-1243), were decreased, and 5, with pro-angiogenic properties (miR-17, miR-106a, miR-484, miR-580, miR-21*), were increased ($p < 0.05$, all). As regards CDM vs DM subjects, 7 miRNAs were significantly modulated: 2 decreased (miR-30b, miR-21*) and 5 increased (miR-139-5p; miR-342-3p; miR-150; miR-24; miR-1243) ($p < 0.05$, all).

EV from CDM subjects were able to induce HRP detachment (-19.6% after 4hrs, -31.4% after 24 hr exposure, $p < 0.05$ vs noDM), similarly to EV derived from MSC in diabetic-like conditions. HRP still attached to wells showed no sign of apoptosis. Moreover, EV from CDM subjects increased the formation of vessel-like structures *in vitro* in comparison with EV from healthy controls ($p < 0.001$).

Conclusion: These observations suggest that EV patterns may be different in diabetic patients, with or without complications, compared to healthy subjects. In particular, an imbalance between miRNAs with pro- and anti-angiogenic functions could lead to abnormal microvascular proliferation, and contribute to proliferative diabetic retinopathy. Further studies could provide predictive options for diagnostic purposes and tools for the treatment of vessel abnormalities in diabetes.