Effect of inflammation on miRNA expression in pancreatic beta cells and their exosomes

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Background and aims. Inflammation is a contributor to the dysfunction of insulin-secreting pancreatic beta-cells in Diabetes Mellitus (DM). Exosomes (EXOs) have emerged as important mediators in cell communication, which carry proteins and RNA species (miRNA, mRNA, tRNAs etc). EXOs have been found under normal physiologic and disease states. Their content can reflect biological events and disease progression. In the present study, we are defining the miRNA profiles of beta cells and EXOs, with and without exposure to inflammatory cytokines. By comparing our data to literary references, it will be possible to identify miRNAs that may reflect beta cell damage and/or death.

Methods. To determine the effect of miRNA expression released by beta-cells under inflammatory and non-inflammatory conditions, pancreatic beta cell line MIN6 was cultured in presence or absence of a mixture of cytokines (IL-1β, TNF-α and IFN-γ) for 48h. Glucose Stimulated Insulin Secretion assay was performed in the cells after 48h. MIN6-derived Exosomes were isolated from the different conditions, quantified, and characterized by Nanoparticle tracking analysis (Nanosight, Malvern), flow cytometry, and western blot. Total RNA was isolated and an 800 miRNA microarray profiling was carried out in cells and exosomes to identify miRNAs with altered abundance in response to cytokine exposure. The obtained data was analyzed by nSolver software (Nanostring). A two- tailed T-Test was conducted in order to determine the significance in the up or down regulation of various miRNAs.

Results. We found that inflammation did not affect the majority of the miRNAs in MIN6 cells and exosomes. However, mmu-let-7, mmu-miR-101b, mmu-miR-125a, mmu-miR-127, mmu-miR-185, mmu-miR-2141, mmu-miR-29, mmu-miR-337-3p, mmu-miR-425, mmu-miR-487b, mmu-miR-539, mmu-miR-540-3p and mmu-miR-99b were differentially expressed (p-value < 0.05) in treated and non-treated cells. Exosomes released by treated and non-treated cells, significantly differed in the expression of

mmu-miR-129-3p, mmu-miR-2134, mmu-miR-2135, mmu-miR-328 and mmu-miR-384-3p (p-value < 0.05).

Conclusions. Our results suggest that beta-cells under inflammatory conditions modify the secretion of microRNAs associated with regulation of insulin signaling and secretion, diabetes, and beta cells. Taken together, this miRNA expression profiles revealed potential targets that could serve as a novel, non-invasive, accurate, and time-sensitive biomarker to monitor progression, and may identify molecular targets for therapeutic interventions of DM.