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Hypercholesterolemia changes HDL-miRNA signature and enhances HDL-miR126-3p and -5p delivery to endothelial cells modulating genes involved in vascular health

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INTRODUCTION: High-density lipoproteins (HDLs) are associated to cardiovascular protection and have been recognized to transport miRNAs (non-coding RNA sequences) and deliver them to recipient cells with functional targeting capabilities. However, the impact of hypercholesterolemia as a cardiovascular risk factor on HDL-bound miRNAs and miRNA cell transfer remains poorly understood.

PURPOSE: We sought to investigate the effect of diet-induced hypercholesterolemia on HDL-miRNA profile and miRNA delivery to endothelial cells.

METHODS: Four-month-old pigs were fed during 10days a normocholesterolemic (NC; N=10) or a hypercholesterolemic (HyC; N=10) diet reaching cholesterol levels of 38.5 [25.8 - 41.0] mg/dL and 245.5 [166.2 - 291.2] mg/dL, respectively (P<0.0001). HDL particles were isolated from blood samples of all pigs by ultracentrifugation, purified and quantified. We performed a differential HDL-miRNA expression profiling (n=149 miRNAs) between NC- and HyC- HDLs following the multipanel qPCR technique. We then carried out culture studies in porcine aortic endothelial cells (PAEC) to determine whether the identified differentially expressed miRNAs were delivered to endothelial recipient cells. Finally, by implementing bioinformatic analyses (Ingenuity Pathways Analysis; IPA) we identified those functional networks and potential gene targets modulated by the miRNA candidates.

RESULTS: Five microRNAs were found to be differentially transported between NC- and HyC- HDL particles (p<0.05). Particularly, HyC-HDL carried high levels of miR-126-5p, miR-126-3p and miR-30b-5p (2.7x, 1.7x and 1.3x respectively) while levels of miR-103a-3p and miR-let-7g-5p were found to be reduced (-1.6x, -1.4x, respectively) vs. NC-HDL. Only miR126 (both -3p and -5p) was found to be enhanced in endothelial cells upon HDL treatment (50µg/mL). Interestingly, miR-126-3p and -5p levels were found to be 3-fold higher in those endothelial cells incubated with HyC-HDL as compared to NC-HDL (p<0.05), an effect that persisted despite HDL removal. IPA analyses revealed that miR126 regulated 101 transcripts involved in lipid metabolism (PPARa, PIK3R2, ALG10B, B4GALT4, DENND1B, LRP10 and PRKAB2), insulin sensitivity (IRS1, many SLC family genes) apoptosis (CRK, E2F1, KANK2, TNFRSF10B, TPD52L1, MYC, MDM4, PAWR, RNF217, RYBP, CASP3 and LPAR2), inflammation and immunity (VCAM1, EFDH2, HOXA9, IKZF1, CD84 and IL21R), clot formation (F8A family, and NOX5) and angiogenesis (EGFL7, VEGFA, ADAM9, ETS1, and HSD11B1). No changes were detected in endothelial cells scavenger receptor B1 (SRB1) gene levels upon HyC- or NC- HDL treatment.

CONCLUSIONS: Our results collectively suggest that hypercholesterolemia induces changes in HDL-miRNA signature and enhances HDL-miR126 delivery to endothelial cells likely modulating key processes related with vascular health.
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