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Cellular, molecular and functional characterization of the endothelial cell migration associated pathways activated by reconstituted HDL containing human apoE3

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Introduction: Atherosclerotic coronary heart disease is a leading cause of death worldwide. HDL (High-Density-Lipoprotein) and apolipoprotein E (apoE) have atheroprotective properties and affect the functions of endothelial cells (ECs), although the exact molecular mechanisms are not yet fully characterized. Herein, we study the atheroprotective potential of reconstituted-HDL containing human apoE3 and phospholipids (rHDL-apoE3) at cellular, molecular and functional levels, focusing on EC migration.

Methods: Human Aortic ECs (HAECs) were treated with rHDL-apoE3 or PBS (negative-control), and isolated total RNA was analyzed by whole-genome microarrays (Affymetrix) followed by bioinformatical (±2-fold and ≤0.05 FDR thresholds) and high-throughput qRT-PCR analysis. Human Coronary Artery ECs (HCAECs) were treated with rHDL-apoE3 or PBS in the presence or absence of a specific inhibitor of PI3-kinase (LY294002) or DMSO, using rhVEGF as positive-control. The expression and activation of key EC migration-associated proteins was determined by western-blotting. Wound-healing assays were performed on HCAECs treated with rHDL-apoE3 or PBS or rhVEGF to measure cell migration.

Results: rHDL-apoE3 induced significant expression changes in 198 genes of HAECs involved mainly in re-endothelialization- and atherosclerosis-associated mechanisms. The most pronounced effect was observed for EC migration. In specific, 42/198 genes were involved in EC migration-related pathways: 1) PI3K/AKT/eNOS-MMP2/9, 2) Small RHO-GTPases, 3) RAS/C-RAF/MEK/ERK. Selected changes were validated by high-throughput qRT-PCR. At the protein level rHDL-apoE3 increased the expression of PIK3CG, EFNB2, FLT1 and ID1, as well as the activation of ERK1/2, AKT, eNOS and P38-MAPK in HCAECs compared to PBS-control. Consistently, rHDL-apoE3 stimulated HCAEC migration compared to PBS-control. Pre-treatment of HCAECs with the LY-inhibitor attenuated the rHDL-apoE3-induced AKT and eNOS activation.

Conclusion: rHDL-apoE3 upregulates the RAS/C-RAF/MEK/ERK, PI3K/AKT/eNOS and PI3K/AKT/RAC1-GTPase/P38-MAPK pathways at transcriptional and protein levels in primary human ECs, and activates ERK1/2, P38-MAPK as well as AKT and eNOS through PI3K-phosphorylation. Through these changes, rHDL-apoE3 induces HCAEC migration suggesting an atheroprotective role in-vitro.

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