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Large scale reverse siRNA transfection: A novel tool to dissect regulators of lipid metabolism in primary human blood-derived monocytes

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Macrophages are known key regulators in atherosclerotic plaque formation. Foam cells form in the subintimal layer by intake of low-density lipoproteins and its derivatives accumulate in the plaque and support inflammatory processes by producing cytokines and chemokines. A profound knowledge of genes, which regulate this process, and of potential differences between healthy adults and coronary artery disease (CAD) patients is essential to better understand this process.

Here, a set of 89 genes, previously described as being upregulated during the process of foam cell formation, was studied using a newly developed siRNA-based high throughput screening tool. For each of these genes three predesigned siRNAs were used for transfection. Human monocytes were isolated from human whole blood by negative bead isolation and differentiated to macrophages with monocyte colony-stimulating factor. The freshly isolated cells where seeded onto 384-well plates and reversely transfected by siRNAs. Transfection mixture and scrambled siRNAs were used as negative controls. After three days, DiI-labeled LDL was added and LDL uptake was measured by wide-field fluorescence microscopy and subsequent automatic image analysis. In the primary screening, the entire gene set was investigated in six male patients with high grade CAD and in six healthy matched control individuals. In a further confirmatory screen, ten CAD patients and ten control individuals were compared.

A main finding of the study is that human macrophages can be successfully transfected by reverse siRNA transfection, and alterations in LDL uptake can be quantified by fluorescence microscopy. Downregulation of several genes, namely *ALDH1A*, *APOC1*, *CMTM6*, *FABP4*, *EPHX1*, *WBP5* and *ZYX*, significantly altered LDL uptake. Downregulation of four of those genes, *APOC1*, *CMTM6*, *FABP4* and *WBP5*, significantly decrease LDL uptake in macrophages. *CMTM6* and *WBP5* have not been associated with foam cell formation or atherogenesis previously. Furthermore, downregulation of LDL uptake in cells derived from the CAD patient group was more pronounced than in healthy controls. In regards to potential underlying mechanisms, the MIF/CXCR4 axis but not CXCL12/CXCR4 interaction was found to promote foam cell formation through increased LDL uptake in human macrophages via a G-protein coupled mechanism.

In summary, the current study identified novel regulatory genes of cholesterol metabolism in human macrophages. These findings contribute to the elucidation of foam cell formation and atherosclerosis.