MICRORNAS MIR-147-3P AND MIR-298-5P ARE NOD-DRIVEN REGULATORS OF ENDOTHELIAL CYTOKINE EXPRESSION

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Background

The cytosolic nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors NOD1 and NOD2 belong to the class of pattern recognition receptors (PRR) and mainly sense bacterial cell wall components leading to an inflammatory response via NFκB and MAPK.

Objective

Although the involvement of NOD1 and NOD2 in experimental atherosclerosis has already been investigated, not much is known about the NOD-dependent microRNA regulation and its role in vascular inflammation. Hence, we investigated the microRNA expression profile in response to specific NOD1 and NOD2 activation in murine primary lung endothelial cells (ECs) by performing a TaqMan Low Density Array (TLDA). Subsequently, validated microRNAs were screened for inflammation-related target genes using different *in-silico* analysis software. Afterwards, using transfection experiments the regulation of these target genes was investigated.

Results

Purity control of ECs after two-step magnetic bead-based cell isolation

NOD1- and NOD2-dependent induction of proinflammatory cytokines

NOD-regulated microRNAs: Identification

Validation of NOD-regulated microRNAs

In-silico target gene analysis

Summary and outlook

Using TaqMan Low Density Array, we identified a pattern of significantly regulated miRNAs in murine primary lung endothelial cells following NOD1 and NOD2 stimulation. Transfection experiments with miR-147-3p and miR-298-5p revealed a decrease in TNF-α and IL-6 mRNA levels. Finally, we uncovered a novel, direct interaction of miR-298-5p with the 3'UTR of the IL-6 mRNA by luciferase reporter assays. We here identified miR-147-3p and miR-298-5p in murine pulmonary endothelial cells as being down-regulated by NOD stimulation thereby enabling the induction of TNF-α and IL-6 mRNA.