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Abstract Title:

Dysregulated miR-223 in small bowel tissue of infants with necrotizing enterocolitis and suppression of target gene Nuclear Factor 1A

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Background and Objectives

Necrotizing enterocolitis (NEC) is probably the most serious intestinal inflammation disease that results in high morbidity and mortality in preterm infants. In our previous micro-RNA array analysis, we profiled 80 aberrantly expressed miRNAs in NEC tissues, amongst which the level of miR-223 was significantly upregulated [Ng, Chan et al. 2015; PLoS One 10(8): e0135737]. The objectives of this study were to validate the dysregulation of miR-223 in NEC tissues by quantitative (q)PCR and to identify the direct binding target gene of miR-223.

Methods

Expression level of miR-223 was investigated in NEC tissues and surgical control tissues (Surg CTL; i.e. non-inflammatory neonatal surgical conditions) by qPCR. Potential target genes of miR-223 were selected from 6 data bases in the public domain and cross-matched with our own mRNA data base of 3472 dysregulated genes in human NEC tissues (Chan, Leung et al. 2014; Ann Surg 260: 1128-1137). The mRNA levels of selected target genes were further investigated in small bowel tissues and miR-223 mimics-transfected Caco-2 cells. The luciferase

reporter assay was applied to validate the direct binding between miR-223 and its target gene in HEK293 and Caco-2 cell lines.

Results

The expression of miR-223 was significantly increased (25.2 fold) in NEC tissues compared with Surg CTL tissues (n=10 for each group; *P*=0.0107). Nuclear Factor 1A (NFIA), Scavenger Receptor Class B Member 1 (SCARB1), and Intercellular Adhesion Molecule 1 (ICAM1) were selected as potential target genes. The expression of NFIA (0.32 fold) and SCARB1 (0.12 fold) were significantly decreased in NEC tissues compared with Surg CTL (n=10; *P*<0.001), while the expression of ICAM1 was increased (3.75 fold; *P*=0.0059). All these three genes exhibited significant negative correlation with miR-223 (*P*<0.05), which is in line with the inhibitory property of miRNA. After overexpression of miR-223 in Caco-2 cells (n = 6), expression of NFIA was significantly repressed (0.87 fold; *P*=0.0022), whilst SCARB1 and ICAM1 were unchanged. Further validation showed a direct binding between 3'-UTR of NFIA and miR-223. MiR-223 mimics significantly inhibited the luciferase activity of wild type NFIA 3'-UTR plasmid both in HEK293 (0.75 fold; n=4, *P*=0.0005) and Caco-2 (0.88 fold; n=4, *P*=0.0007) cell lines. However, miR-223 mimics did not change the luciferase activity of mutated NFIA 3'-UTR plasmid,

indicating that the interaction between miR-223 and NFIA was direct and specific. In addition, we demonstrated by Western blot analysis that miR-223 could suppress NFIA at the protein level (0.73 fold; n=5, *P*=0.0032).

Conclusion

Our study demonstrated dysregulation of miR-223 and its target gene NFIA in NEC tissues. Their interaction might play a role in NEC pathophysiology.

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