Dysregulation of miR223 and miR431 expression in intestinal tissues of preterm infants with necrotising enterocolitis: abridged secondary publication

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KEY MESSAGES

1. The miR-223/*NFIA* and miR-431/*FOXA1* pathways were aberrantly expressed in intestinal tissues of patients with necrotising enterocolitis.
2. The affected downstream signals could dysregulate multiple categories of cellular functions and play important roles in disease pathophysiology.

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Introduction

Necrotising enterocolitis (NEC) is a severe inflammatory disease of the gastrointestinal tract that results in high morbidity and mortality in preterm infants. Micro-RNAs (miRNAs) are a class of noncoding small RNAs (18-24 nucleotides) that exert post-transcriptional inhibition of gene expression by pairing with complementary sequences in target mRNAs. Specific miRNAs play important roles in physiologic events, and their dysregulation is associated with diverse pathologic conditions. miRNAs have been implicated in ileal and colonic mucosal as well as blood specimens of patients with Crohn disease and ulcerative colitis. This suggests that miRNAs can interfere with gut inflammation.[1-3] We reported the mRNA and miRNA expression profiles of NEC intestinal tissues, and miR-223 and miR-431 were significantly upregulated when compared with those in surgical control tissues (ie non-inflammatory neonatal surgical conditions).[4] The objectives of the current study were: (1) to validate the dysregulation of miR-223 and miR-431 in NEC tissues, (2) to identify the direct binding target genes of these two miRNAs, and (3) to investigate their functions associated with NEC pathophysiology.

Methods

Intestinal specimens were collected during surgery of infants with stage III NEC at the Prince of Wales Hospital, The Chinese University of Hong Kong, as described previously.[4] Written informed parental consents and institutional ethics approval were obtained. Surgical control tissues were obtained from infants who underwent intestinal surgery because of non-inflammatory conditions. Target genes of miR-223 or miR-431 were identified by in silico target prediction bioinformatics, luciferase assay, and Western blot analyses. Expression levels of miR-223, miR-431, and downstream regulatory genes were measured by qPCR. Functions of miR-223 and miR-431, including expressions of target genes and downstream signals, cell proliferation, and apoptosis were investigated by overexpression in Caco-2 and/or FHs74 cells upon stimulation by lipopolysaccharide or lipoteichoic acid. Regulatory networks were analysed by Metacore Analysis.

Expression levels of regulatory genes in NEC and surgical control tissues were compared using the unpaired *t* test. Correlation analyses between miRNAs and regulatory genes were performed using the Spearman correlation test. Data of luciferase reporter assay, Western blot, and cell apoptosis assays were analysed by the paired t test. The proliferation of mimic-miR or mimic control–transfected cell lines were compared using two-way ANOVA with Bonferroni correction. Statistical analyses were performed with the GraphPad Prism 5.0 software.

Results

The expression of miR-223 was significantly upregulated in NEC tissues by 25.16-fold, compared with surgical control tissues. Nuclear factor I-A (*NFIA*) was identified as the target gene of miR-223. Overexpression of miR-223 significantly regulated multiple downstream genes, including *MYOM1*, *NFIA*, *RGN*, *GNA11*, *MYLK*, *PRKCZ*, *IL-6*, and *IL-8* in Caco-2 and FHs74 cells. In addition, apoptosis

was significantly increased and proliferation was inhibited. These results suggested that upon binding with *NFIA*, miR-223 could regulate functional effectors in pathways of apoptosis, cell proliferation, G-protein signalling, inflammation, and smooth muscle contraction.

The expression of miR-431 was significantly increased by 7.05-fold in NEC tissues, compared with surgical control tissues. Forkhead box A1 (*FOXA1*) was validated as a target gene of miR-431. *IL6*, *IL8*, and *TNF* were significantly increased upon overexpression of miR-431 and lipopolysaccharide or lipoteichoic acid treatments. In addition, *HNF4A* was decreased, whereas *NFKB2* was increased. Functional analyses demonstrated that cell proliferation was significantly decreased and apoptosis was increased upon overexpression of miR-431. Overall results indicated that the miR-431/*FOXA1* axis was proinflammatory and could be associated with NEC pathology.

Discussion

Our study presented the first evidence that the miR-223/*NFIA* and miR-431/*FOXA1* pathways were dysregulated in intestinal tissues of NEC infants, and that affected downstream signals could probably reprogram the expression of effector genes in multiple categories of cellular functions. We also observed increased apoptosis and decreased proliferation of intestinal cells overexpressing miR-233 or miR-431, and/or upon exposure to bacterial toxins. Our findings strongly indicated that dysregulation of miR-223/*NFIA* and miR-431/*FOXA1* as well as downstream regulatory genes could contribute to the pathophysiology of NEC, impacted by escalated inflammation, disturbed homeostasis, increased apoptosis, and suppressed cell proliferation, and thus leading to irreparable tissue damage. Our findings provided new insights into the molecular mechanism of NEC upon regulation by miRNAs and shed lights on potential therapeutic targets for these vulnerable infants.

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Disclosure

The results of this research have been previously published in:

1. Wu YZ, Chan KYY, Leung KT, et al. Dysregulation of miR-431 and target gene FOXA1 in intestinal tissues of infants with necrotizing enterocolitis. FASEB J 2019;33:5143-52.

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