Long non-coding RNA colorectal neoplasia differentially expressed correlates negatively with miR-33a and miR-495 and positively with inflammatory cytokines in asthmatic children

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Abstract

Objectives: It is reported that long non-coding RNA (lncRNA) colorectal neoplasia differentially expressed (CRNDE) targets microRNA (miR)-33a, miR-181a and miR-495 to regulate inflammation process, while few studies report their clinical application for paediatric asthma management. Therefore, this study aimed to explore the interaction of lncRNA CRNDE with miR-33a, miR-181a and miR-495, as well as their correlation with inflammation, exacerbation risk and severity in paediatric patients with asthma.

Methods: Asthmatic exacerbation children ($N = 65$), asthmatic controlled children ($N = 65$) and controls ($N = 65$) were recruited. LncRNA CRNDE, miR-33a, miR-181a and miR-495 expressions in peripheral blood mononuclear cells were detected by RT-qPCR. Besides, serum inflammatory cytokines (including TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-17) were determined by enzyme-linked immunosorbent assay (ELISA).

Results: LncRNA CRNDE, miR-33a and miR-495 expressions were different, while miR-181a expression was similar among asthmatic exacerbation children, asthmatic controlled children and controls. Moreover, lncRNA CRNDE negatively correlated with miR-33a and miR-495 in asthmatic exacerbation children and asthmatic controlled children, but not in controls. Further analyses showed that lncRNA CRNDE positively correlated with TNF-$\alpha$, IL-1$\beta$, IL-17 and exacerbation severity, while it negatively correlated with FEV$_1$/FVC in asthmatic exacerbation children. Meanwhile, miR-33a, miR-181a and miR-495 all negatively correlated with some individual inflammatory cytokines, while only miR-33a negatively correlated with exacerbation severity in asthmatic exacerbation children.

Conclusion: LncRNA CRNDE correlates negatively with miR-33a and miR-495 and positively with inflammatory cytokines in asthmatic children.
1 | INTRODUCTION

Asthma is a chronic respiratory disease commonly observed in children under 16 years, whose hallmarks are inflammation of the airway, increased mucus secretion, bronchoconstriction as well as hypersensitivity to the external stimuli.1,2 The appropriate management (including pharmacological therapy, ventilation and allergenic-specific immunotherapy) may prevent most paediatric patients with asthma from developing further chronic lung diseases, thereby impairing their long-term pulmonary function.1,3 However, some paediatric patients with asthma may experience acute asthmatic exacerbation which requires immediate medical attention.4 Therefore, identifying reliable biomarkers to prevent asthmatic exacerbation and monitor disease progression in paediatric patients with asthma is necessary.

Long non-coding RNA (lncRNA) colorectal neoplasia differentially expressed (CRNDE) located on human chromosome 16, is highly involved in cancer biology, which has been reported to be involved in promotion of cell proliferation, inhibition of cell apoptosis and assistance of epithelial-to-mesenchymal transition.5 Regarding its role in inflammation diseases, overexpression of lncRNA CRNDE activates toll-like receptor (TLR)-4/nuclear factor (NF)-κB pathway and further leads to production of inflammatory cytokines (such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6) in lipopolysaccharide (LPS) treated HK-2 cells.6 Meanwhile, upregulated lncRNA CRNDE promotes inflammatory cytokines production through the activation of NF-κB and Janus kinase/signal transduction and activator of transcription (JAK–STAT) signalling pathways in LPS treated WI-38 cells.7 As for its clinical application, lncRNA CRNDE serves as a prognostic factor for increased mortality rate in sepsis patients.8 From the previous data, lncRNA CRNDE targets several microRNAs (miRNA) (miR-33a, miR-181a and miR-495) to promote inflammation in several diseases. For instance, lncRNA CRNDE downregulates miR-181a and miR-495 to produce TNF-α, IL-1β and IL-6 in the cell model of sepsis and inflammatory bowel disease, respectively.9,10 Meanwhile, lncRNA CRNDE also downregulates miR-33a.11,12 Although lncRNA CRNDE and other above-mentioned miRNAs are heavily involved in the inflammation process of several diseases, fewer studies report their interaction and its clinical application for paediatric asthma management.

Hence, we performed this study and aimed to explore the interaction of lncRNA CRNDE with miR-33a, miR181a and miR-495, also to investigate their correlation with inflammatory cytokines and exacerbation severity in paediatric patients with asthma.

2 | MATERIALS AND METHODS

2.1 | Subjects

This study was carried out with the permission by Ethics Committee of Xingtai People’s Hospital. From October 2019 to June 2020, this study consecutively recruited 65 asthmatic exacerbation children, 65 asthmatic controlled children, and 65 healthy subjects as controls. All asthma children were required to meet the diagnosis criteria of Global Initiative for Asthma (available on www.ginasthma.org.) and have age within 1 to 16 years. All asthmatic exacerbations were diagnosed by physicians. Besides, all asthmatic controlled children had a previous physician diagnosed asthma. The asthma children were excluded from study if they had other allergic diseases, respiratory diseases, infections, autoimmune disorders, or had history of haematological system diseases or malignancies. The controls were screened out from the healthy children who underwent health examination in Xingtai People’s Hospital during the same period. The recruited controls were required to have age between 1 and 16 years, without obvious abnormality in medical examination, and have no history of asthma, infection, allergic diseases, respiratory diseases, autoimmune diseases, inflammatory diseases, or malignancies. The guardians of recruited children signed the informed consents; meanwhile, the recruited children with age ≥10 years also signed the informed consents.

2.2 | Definition

The asthmatic exacerbation was defined as an acute or subacute episode of progressive increase in asthma symptoms, associated with airflow obstruction (Exacerbations of asthma were episodes characterised by a progressive increase in symptoms of shortness of breath, cough, wheezing or chest tightness and progressive decrease in lung function, that is, they represented a change from the patient’s usual status that was sufficient to require a
change in treatment.). Moreover, the severity of asthmatic exacerbation was assessed according to The Global Strategy for Asthma Management and Prevention. The detailed category of mild, moderate and severe asthmatic exacerbation was listed in Table S1. The asthmatic controlled was defined as symptoms and signs disappeared after treatment or without treatment, and the pulmonary function recovered and maintained for at least 3 months.

2.3 | Data collection

The characteristics of the enrolled children were documented after necessary examinations and tests, which covered the age, gender, height, weight, family history of asthma, forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), FEV₁ (%predicted), eosinophil count and immune globulin E (IgE) level. With regard to asthmatic exacerbation children, the exacerbation severity was evaluated on the admission depending on the clinical manifestation and necessary tests in accordance with the guidelines of International consensus on (ICON) paediatric asthma.13

2.4 | Sample collection and determination

Collection of peripheral blood samples was carried out for the children after enrolment. Following that, the centrifugal separation and the Ficoll density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania, USA) were performed to isolate the serum samples and the peripheral blood mononuclear cells (PBMCs). The PBMCs were used for the quantitative analysis of lncRNA CRNDE, miR-33a, miR-181a and miR-495 by Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) assay. The serum samples were used for the determination of the inflammatory cytokines including tumour necrosis factor alpha (TNF-α), interleukin (IL)-1 beta (IL-1β), IL-6 and IL-17 by enzyme-linked immunsorbent assay (ELISA) using Human ELISA Kits (Shanghai Enzymelinked Biotechnology Co., Ltd, Shanghai, China).

2.5 | RT-qPCR assay

lncRNA CRNDE, miR-33a, miR-181a and miR-495 expressions were detected by RT-qPCR. In brief, total RNA from PMBCs were extracted by PureZOL™ RNA isolation reagent (Bio-Rad, Hercules, California, USA). Then reverse transcription was applied using iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, California, USA). After that, qPCR reaction was completed by SYBR® Green Realtime PCR Master Mix (Toyoobo, Osaka, Kansai, Japan). The relative expression was calculated by $2^{-\Delta\Delta C_t}$ method using GAPDH as the internal reference for lncRNA CRNDE and U6 as the internal reference for miRNA. The primer sequences were listed in the Table S2.

2.6 | Statistical analysis

Data were analysed by SPSS 21.0 software (IBM Corp., Armonk, New York, USA), and diagrams were formed using GraphPad Prism 8.01 software (GraphPad Software Inc., San Diego, CA, USA). Mean with standard deviation (SD), median with interquartile range (IQR), and count with percentage were used for descriptive analysis. One-way analysis of variance (ANOVA) Kruskal–Wallis test and Chi-square test were used for comparison among groups. Student t test, Wilcoxon rank sum test and Chi-square test were applied for comparison between two groups. Analysis of covariance (ANCOVA) was performed to detect difference of slope in different groups. Spearman’s rank correlation test was carried out for correlation analysis. P value < 0.05 was considered as statistical significance.

3 | RESULTS

3.1 | Patients’ clinical characteristics

The mean age of asthmatic exacerbation children, asthmatic controlled children and controls was 6.3 ± 2.7, 6.5 ± 2.6 and 6.6 ± 2.8 years, respectively (Table 1). There were 32 (49.2%) females and 33 (50.8%) in asthmatic exacerbation children, then 30 (46.2%) females and 35 (53.8%) males in asthmatic controlled children, and 27 (41.5%) females and 38 (58.5%) males in controls. The comparison of biochemical indexes, lung function indexes and inflammatory cytokines among three groups as well as intergroup were listed shown in Table 1.

3.2 | Expressions of lncRNA CRNDE and miRNAs

lncRNA CRNDE ($P < 0.001$) (Figure 1A), miR-33a ($P < 0.001$) (Figure 1B) and miR-495 ($P = 0.003$) (Figure 1D) expressions were differed, while miR-181a expression ($P = 0.064$) (Figure 1C) was similar among asthmatic exacerbation children, asthmatic controlled children and controls.
Subgroup analysis exhibited that lncRNA CRNDE expression ($P < 0.001$) (Figure S1A) was reduced, while miR-181a ($P = 0.036$) (Figure S1C) and miR-495 ($P = 0.003$) (Figure S1D) expressions were elevated in asthmatic controlled children with treatment compared to those without treatment. Furthermore, miR-33a expression was similar between asthmatic controlled children with treatment and asthmatic controlled children without treatment ($P = 0.054$) (Figure S1D).

### 3.3 Correlation of lncRNA CRNDE expression with miR-33a, miR-181a and miR-495 expressions

In asthmatic exacerbation children, lncRNA CRNDE was negatively correlated with miR-33a ($P = 0.004$) (Figure 2A) and miR-495 ($P = 0.008$) (Figure 2C). In asthmatic controlled children, lncRNA CRNDE also negatively associated with miR-33a ($P = 0.005$) (Figure 2D).
and miR-495 ($P = 0.018$) (Figure 2F). In controls, lncRNA CRNDE was inversely correlated with miR-495 ($P = 0.007$) (Figure 2I) as well.

### 3.4 | Correlation of lncRNA CRNDE, miR-33a, miR-181a and miR-495 expressions with biochemical indexes and respiratory function indexes

LncRNA CRNDE was negatively correlated with FEV₁/FVC in asthmatic exacerbation children ($P = 0.015$), while it was positively correlated with eosinophil count in controls ($P = 0.033$) (Table 2). While these findings were weak and inconsistent among different groups of paediatric patients.

### 3.5 | Correlation of lncRNA CRNDE, miR-33a, miR-181a and miR-495 expressions with inflammatory cytokines

In asthmatic exacerbation children, lncRNA CRNDE positively correlated with all tested inflammatory cytokines (all $P < 0.05$) (Table 3), while miR-33a, miR-181a and miR-495 inversely correlated with inflammatory cytokines except for miR-181a and IL-17. In asthmatic controlled children, lncRNA CRNDE positively correlated with majority of tested inflammatory cytokines. While miR-33a, miR-181a and miR-495 negatively correlated with some inflammatory cytokines. In controls, only miR-181a was negatively correlated with TNF-α ($P = 0.039$). Furthermore, the detailed results were displayed in Table 3.

Further analysis by pooling the data in asthmatic children displayed that lncRNA CRNDE positively correlated with inflammatory cytokines, while miR-33a and miR-495 both negatively correlated with inflammatory cytokines in asthmatic children (all $P < 0.001$) (Table S3). Furthermore, miR-181a negatively correlated with TNF-α, IL-1β and IL-6 in asthmatic children. While in controls, only miR-181a negatively correlated with TNF-α. Apart from this, there was no correlation of lncRNA or miRNAs with inflammatory cytokines in controls.
3.6  |  Correlation of IncRNA CRNDE, miR-33a, miR-181a and miR-495 expressions with exacerbation severity

IncRNA CRNDE was positively correlated with exacerbation severity \( (P = 0.014) \) (Figure 3A), while miR-33a was negatively associated with exacerbation severity \( (P = 0.013) \) (Figure 3B). However, there was no correlation of miR-181a \( (P = 0.305) \) (Figure 3C) or miR-495 \( (P = 0.117) \) (Figure 3D) with exacerbation severity. Furthermore, the ANCOVA analyses displayed that difference of slope in difference groups were varied (all \( P < 0.001 \)) (Figure S2).

4  |  DISCUSSION

In the present study, we discovered that (a) IncRNA CRNDE, miR-33a and miR-181a expressions were varied among asthmatic exacerbation children, asthmatic controlled children and controls, (b) IncRNA CRNDE positively correlated with inflammatory cytokines and exacerbation severity in asthmatic exacerbation children, (c) IncRNA CRNDE negatively correlated with miR-33a and miR-181a in asthmatic children and (d) miR-33a, miR-181a and miR-495 all negatively correlated with some individual inflammatory cytokines, while only miR-33a negatively correlated with exacerbation severity in asthmatic exacerbation children.
From the accumulating evidence, lncRNA CRNDE activates multiple signalling pathways (such as Wnt/β-catenin signalling, Ras/MAPK signalling and PI3K/AKT signalling) to promote cell proliferation and inhibit cell apoptosis in several cancer cells (including colorectal cancer, lung cancer, breast cancer, gastric cancer and...
hepatocellular carcinoma). Meanwhile, lncRNA CRNDE severs as a prognostic factor for several cancer patients (such as liver cancer patients, lung cancer patients and clear cell renal cell carcinoma patients). However, fewer studies report the clinical value of lncRNA CRNDE in inflammation-mediated disease patients. Only one clinical study discovers that lncRNA CRNDE expression is increased, and it is positively correlated with several inflammatory cytokines (including TNF-α, IL-1β, IL-6 and IL-8), disease severity and mortality risk in sepsis patients. As for the clinical application of lncRNA CRNDE in paediatric patients with asthma, no relevant research has been conducted yet. Therefore, we performed this study and discovered that lncRNA CRNDE was positively correlated with inflammatory cytokines and exacerbation severity in paediatric patients with asthma. The possible reasons to explain these were as follows: (a) LncRNA CRNDE might activate several signaling pathways (such as NF-κB and JAK–STAT signalling pathways) to promote the production of inflammatory cytokines, thereby resulted in higher asthmatic risk. (b) As mentioned earlier, LncRNA CRNDE was positively correlated with increased inflammatory cytokines production, thereby led to impaired immune response and further resulted in increased chance of viral respiratory infection to develop exacerbation in paediatric patients with asthma. Taken together, lncRNA CRNDE was positively correlated with inflammatory cytokines and exacerbation severity in paediatric patients with asthma.

Besides, miR-33a, miR-181a and miR-495 are previously mandated to be direct targets of lncRNA CRNDE to suppress inflammation, and they are implicated in inflammation process of several diseases. For example, serum miR-33a is independently correlated with inflammation in liver transplant recipients. Moreover, miR-181a inhibits SIRT1 expression and suppress the production of inflammatory cytokines through Nrf2/NF-κB signalling pathway in LPS treated macrophages. Meanwhile, miR-495 targets ROCK1 to inhibit inflammation in LPS-treated WI-38 cells. Although lncRNA CRNDE may regulate these above-mentioned miRNAs in inflammation process, the interaction of lncRNA CRNDE with miRNAs in paediatric patients with asthma has not been determined. In the present study, we discovered that
lncRNA CRNDE was negatively correlated with miR-33a and miR-495 in paediatric patients with asthma. Besides, all the tested miRNAs (including miR-33a, miR-181a and miR-495) were negatively associated with inflammatory cytokines in paediatric patients with asthma. Furthermore, miR-33a was negatively correlated with exacerbation severity in paediatric patients with asthma. The possible reasons were: (a) lncRNA CRNDE might target miR-495/SOCS1 axis and further led to cell apoptosis in the respiratory system, thus resulted in epithelial barrier dysfunction and inflammatory cytokines production, and eventually led to higher exacerbation severity in paediatric patients with asthma.10 (b) lncRNA CRNDE might regulate miR-181a-5p/TLR4 axis and further led to inflammatory cytokines production (such as TNF-α, IL-1β and IL-6) in paediatric patients with asthma.9 (c) All these miRNAs (including miR-33a, miR-181a and miR-495) inhibited inflammation in some ways, thus higher miRNAs might suggest lower inflammatory cytokines, which further led to lower exacerbation severity in paediatric patients with asthma.9–12 Taken together, lncRNA CRNDE was negatively correlated with miR-33a and miR-495, and they correlated with inflammatory cytokines as well as exacerbation risk in paediatric patients with asthma.

There were some limitations in the present study. Firstly, the mechanisms underlying the interaction between lncRNA CRNDE with miR-33a, miR-181a and miR-495 in asthma was not discovered, and further study was needed. Secondly, our study only recruited paediatric patients with asthma; thus, the value of lncRNA CRNDE in adult patients with asthma was not explored, and further study was necessary. Finally, our study did not assess lncRNA CRNDE expression at multiple time points in recruited patients, thus its longitudinal change with disease progression in paediatric patients with asthma was not determined.

In conclusion, lncRNA CRNDE correlates negatively with miR-33a and miR-495 and positively with inflammatory cytokines in asthmatic children, indicating their potential prognostic power for asthma management.

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None.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

ETHICS STATEMENT

This study was carried out with the permission by Ethics Committee of Xingtai People’s Hospital.

AUTHOR CONTRIBUTIONS

HA conceived and designed the experiments. WL performed the experiments. XW made substantial contributions to the acquisition, analysis and interpretation of data. SS have been involved in drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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