Expression of LncRNA-BCYRN1 in pediatric asthma and related factors of disease induction

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ABSTRACT

The study aimed to investigate the expression of LncRNA-BCYRN1 in serum of pediatric asthma patients and the related factors of disease induction. For this purpose, a total of 95 patients were randomly selected from the outpatient department of pediatrics of a hospital affiliated with the medical university from October 2018 to October 2019, including 25 intermittent episodes, 25 mild persistent episodes, 20 moderate persistent episodes, and 20 severe persistent episodes. In addition, serum samples from 30 healthy control children were selected for comparison. The expression levels of LncRNA-BCYRN1 and the inflammatory factors IL-4, IL-12, IL-37, 25-(OH)D in serum were detected by real-time quantitative fluorescence PCR(qrt-PCR), and the influence of the expression of LncRNA-BCYRN1 on the prognosis of children and its correlation with the expression of TNF-α and IgE were analyzed.

Results: the expression of LncRNA BCYRN1 was positively correlated with the expression of TNF-α and IgE (r=0.712, 0.748, all P<0.001). Serum IL-4 levels in each group were 21.06±2.93pg/ml, 23.88±3.91pg/ml, 25.85±4.02pg/ml, and 17.74±1.77pg/ml, respectively. Serum IL-4 levels in each group were higher than those in the normal control group, and the difference was statistically significant (P<0.001). The levels of IL-12, IL-37 and 25-(OH)D in all the affected groups were decreased compared with the normal control group, while the serum levels of IL-37 were negatively correlated with IL-4 and positively correlated with IL-12 and 25-(OH)D. Conclusion: serum IL-4 level of children in the disease group was increased, indicating hyperexpression of Th2 cell function, and serum IL-12 and IL-37 levels were decreased, suggesting decreased Th1 cell function, indicating Th1/Th2 imbalance in children with asthma. The low serum vitamin D level of children in the disease group was negatively correlated with serum IL-4 level and positively correlated with IL-12 and IL-37 levels, which may be the trigger factor of inflammation in asthmatic diseases and should be supplemented in clinical treatment. The expression level of LncRNA-BCYRN1 is related to the severity of pediatric asthma, which can be used as a prognostic indicator and has a certain effect on the prognosis of clinical pediatric asthma.

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Introduction

positive More than 80% of asthma cases occur before the age of 5, and childhood asthma has become one of the most common chronic respiratory diseases among children (1). Asthma is a chronic non-specific airway inflammatory disease, which is induced by multiple factors and involved by multiple cells and cytokines, and is prone to recurrent attacks, with obvious individual differences. The main symptoms were recurrent airway obstruction, bronchial hyperreactivity, and inflammatory cell infiltration of the airway, which were mainly manifested as recurrent wheezing, chest tightness, cough, chest urgency, and shortness of breath (2, 3). It has become an urgent task for researchers to search for the risk factors of asthma to avoid the inducing factors and provide a basis for the effective prevention and control of the occurrence and attack of asthma and improve the quality of life of children with asthma (3).

In the United States, the incidence of childhood asthma and wheezing symptoms is as high as 9.4%, and there are about 8 million patients. The annual number of asthma-related deaths is as high as 9000. The highest incidence of asthma is about 9.6% in children aged 7-12 in Hong Kong, and 10.3% in Beijing, seriously affecting children's physical and mental health. Recent studies have shown that non-coding RNA (ncRNAs) play an important role in the

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pathogenesis of asthma (1). Long non-coding RNA (LncRNA) is a non-coding RNA with a length of more than 200 nucleotides, and it is a kind of RNA with a length of more than 200 nucleotides, without the protein-coding ability (2). LncRNA has a variety of biological functions, including X chromosome silencing, chromosome modification, transcriptional activation, intracellular transport, cell differentiation, proliferation and apoptosis, etc. LncRNA can regulate the expression of related genes at the transcriptional, post-transcriptional and protein levels (3). Existing studies on LncRNA have mainly focused on LncRNA as a biomarker for cancer, and LncRNA has been found to be a biomarker for early screening, diagnosis, prognosis and evaluation of cancer (4). Recent studies have shown that changes in the expression of LncRNA can lead to the occurrence of diseases and are related to the prognosis of diseases, and some lncRNAs are disease and tissue-specific, so they are expected to become molecular markers for the early diagnosis of diseases (5). LncRNA will provide a molecular marker in the body fluid that can be screened early for the diagnosis of this disease without pathological examination, which will provide important significance for the diagnosis of asthma disease (6).

Cytoplasmic RNA1(BCYRN1) is closely related to diseases of the respiratory system, digestive system, reproductive system and nervous system. BCYRN1 is a non-coding RNA molecule encoded by human chromosome 2p16, with a total length of about 200 nucleotides, transcribed by the polymerase (7). Initially, BCYRN1 is usually expressed only in the primate nervous system, also known as BC200, and is not detectable in normal tissue, however, this restriction is broken in tumor tissue (8). Recently, BCYRN1 expression has been found in lung cancer, esophageal cancer, cervical cancer, ovarian cancer, breast cancer and tongue cancer. BCYRN1 efficient transcription needs inside A box, B box and the participation of the upstream promoter element, with 100 bp upstream transcription complex sequence of interaction between A broad area, but in different types of cancer cells detected BCYRN1 cell level, half-life, and the activity of the promoter, and the activity of the promoter and half-life length cannot fully explain the BC200RNA differences at the cellular level, suggests there may be another mechanism involved in transcription regulation (9). Through cell fusion, serum deprivation and drug-induced experiment found that the cell cycle arrest is not an S or M, but G1 or G2, in G1 or G2 BCYRN1 expression significantly reduced, when the cell cycle arrest lifted, as cells in S phase, restore BCYRN1 expression, therefore BCYRN1 expression is crucial to the survival of the proliferation of cancer cells (10).

There were few studies on LncRNA in asthma (11-14). LncRNA-BCYRN1 is an example of the effect of mRNA sponge on the function of bronchial smooth muscle (15). However, the expressions of LncRNA-BCYRN1 in the serum of children with asthma and its relationship with clinical prognosis have not been reported at home and abroad (11). In order to further analyze and understand children with asthma in serum LncRNA - BCYRN1 expression, to analyze its relationship with asthma in early diagnosis and clinical prognosis evaluation, this paper BCYRN1 in the role and mechanism of respiratory system diseases were reviewed, in order to understand how BCYRN1 affect the incidence of respiratory disease development, as molecular markers in early diagnosis of diseases and for BCYRN1 targeted BCYRN1 treatment provide a reference or enlightenment (12).

In this study, a total of 95 patients were randomly selected from the outpatient department of pediatrics of a hospital affiliated with a medical university from October 2018 to October 2019, including 25 intermittent episodes, 25 mild persistent episodes, 20 moderate persistent episodes and 20 severe persistent episodes. In addition, serum samples from 30 healthy control children were selected for comparison. The expression levels of LncRNA-BCYRN1 and the inflammatory factors IL-4, IL-12, IL-37, 25-(OH)D in serum were detected by real-time quantitative fluorescence PCR(QRT-PCR), and the influence of the expression of LncRNA-BCYRN1 on the prognosis of children and its correlation with the expression of TNF-a and IgE were analyzed. There was no statistically significant difference in the gender composition ratio of all the children included in the experiment, and the informed consent of the children’s parents was obtained. Peripheral venous blood samples of all children included in the experiment were collected, centrifuged, and then serum samples were taken and stored in a refrigerator at -70°C. Serum levels of IL-4, IL-12, IL-37, 25-(OH)D were
measured, and the obtained experimental data were analyzed using SPSS 23.0 statistical software. The results showed that the serum IL-4 levels of children in each group were 21.06 ± 2.93 pg/ml, 23.88 ± 3.91 pg/ml, 25.85 ± 4.02 pg/ml, and 17.74 ± 1.77 pg/ml, respectively. The serum IL-4 levels of children in each group were higher than those in the normal control group, and the difference was statistically significant (P < 0.001). The levels of IL-12, IL-37 and 25-(OH)D in all the affected groups were decreased compared with the normal control group, while the serum levels of IL-37 were negatively correlated with IL-4 and positively correlated with IL-12 and 25-(OH)D. It was found that the expression level of lncRNA-BCYRN1 was related to the severity of pediatric asthma, which could be used as a prognostic indicator and play a certain role in the prognosis judgment of clinical pediatric asthma. In conclusion, this experimental study explored the role of serum IL-4, IL-12, IL-37 and 25-(OH)D levels in the pathogenesis of children's asthmatic diseases, provided a reference basis for the early detection and early intervention of bronchial asthma, and opened up a new research idea and scheme for the study of children's respiratory tract infectious diseases.

Materials and methods

Determination of the research object

A total of 95 patients were randomly selected from the outpatient department of pediatrics of the affiliated hospital of a medical university from October 2018 to October 2019, including 25 intermittent, 25 mild sustained, 20 moderate sustained and 20 severe sustained patients. In addition, serum samples from 30 healthy control children were selected for comparison. There was no significant difference in gender composition and age between children with asthma and children in the control group (P > 0.05). There was no statistically significant difference in age and sex composition between intermittent, mild, moderate and severe persistent episodes (P > 0.05), suggesting comparability. There were no other underlying diseases and complications among the children in each group and the healthy control children. This study was approved by the medical ethics committee of our hospital, and all the children's family members signed the informed consent. The child has no other special conditions.

According to the configured reaction system, the reaction was performed in the PCR instrument. Take 1 ul extension product, add 8 ul loading, denature at 95 °C for 3min, immediately take an ice bath, and test IL-4, IL-12 and IL-37 with the up-sequencer.

Collection and preservation of specimens

4ml of peripheral blood of all children was extracted on an empty stomach and placed in a pro-coagulant vacuum vessel. Samples containing particulate matter, turbidity, lipid or red blood cell residues should be filtered or centrifuged for clarification before use. Samples with severe hemolysis, fatty blood, particulate matter, and significant bacterial contamination should not be tested. The collected blood samples were placed at room temperature and solidified naturally for about 2-4h before being centrifuged at 3000r/min for 20 minutes. Then, 2ml of the centrifuged serum was collected and placed in an EP tube. EP tubes were numbered one by one, and the relevant information was recorded and stored in a refrigerator at -70°C for the detection of IL-4, IL-12, IL-37 and 25-(OH)D levels.

Statistical analysis

SPSS 23.0 statistical software was used for data analysis. Experimental data conforming to the measurement data were described as mean ± standard deviation (x±s). The normalization and homogeneity of variance tests of the measurement data were performed first, both of which met the requirements of

Table 1. PCR reaction system and connective reaction system

<table>
<thead>
<tr>
<th>PCR reaction system</th>
<th>Junction reaction system</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA 1µl</td>
<td>PCR product 3µl</td>
</tr>
<tr>
<td>10×buffer 1.5µl</td>
<td>10×Taq DNA ligase buffer 1µl</td>
</tr>
<tr>
<td>MgCL2 1.5µl</td>
<td>/</td>
</tr>
<tr>
<td>dNTP 0.3µl</td>
<td>Taq DNA ligase(40U/ul) 0.125µl</td>
</tr>
<tr>
<td>Primers 1.15µl/quantifier /</td>
<td>/</td>
</tr>
<tr>
<td>Taq polymerase 0.3µl</td>
<td>Probe (10 p) 0.01µl</td>
</tr>
<tr>
<td>H2O Increased to 15 µl</td>
<td>H2O Increased to 10 µl</td>
</tr>
</tbody>
</table>
one-way analysis of variance (ANOVA); otherwise, the rank-sum test was used. The correlation between the two variables was analyzed by linear correlation and regression analysis. The test criteria were $a=0.05$, and $P<0.05$ was statistically significant.

Results and Discussion

Expression of BCYRN1 in tracheal smooth muscle cells in children

The relative expression level of BCYRN1 in tracheal smooth muscle cells of children with asthma in each group was significantly higher than that in the normal control group, indicating that BCYRN1 had a significant influence on the proliferation and migration of tracheal smooth muscle cells, as shown in Figure 1(Figure from WWW.baidu.com).

Figure 1. Effect of transfection of ad-bcyrn1 on migratory cells; Figure from WWW.baidu.com

Comparison of serum levels of IL-4, IL-12, IL-37 and 25-(OH)D in each group (Table 3 and Figure 2) Serum IL-4 levels were 21.48±2.16pg/ml, 23.35±3.82pg/ml, 25.52±4.16pg/ml, and 17.62 ±1.58pg/ml, respectively, in the mild, moderate, severe, and normal control groups. A completely randomized one-way analysis of variance was used to compare the levels of IL-4 between the four groups, showing a significant difference ($F=40.426$, $P<0.01$).

Table 2. Effects of ad-bcyrn1 transfection in tracheal smooth muscle cells on cell proliferation and migration

<table>
<thead>
<tr>
<th>group</th>
<th>n</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
<th>Number of tracheal smooth muscle cell migration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with asthma group</td>
<td>95</td>
<td>2.46</td>
<td>2.57</td>
<td>2.69</td>
<td>3.07</td>
<td>0.16, 0.11, 0.15, 19 ± 4</td>
</tr>
<tr>
<td>Normal control group</td>
<td>30</td>
<td>3.53</td>
<td>3.79</td>
<td>3.97</td>
<td>4.79</td>
<td>0.19, 0.08, 0.17, 48 ± 6</td>
</tr>
</tbody>
</table>

Serum IL-12 levels were 30.37±3.48pg/ml, 23.16±3.44pg/ml, 22.36±2.15pg/ml and 33.37±2.26pg/ml, respectively, in the mild, moderate, severe and normal control groups. The difference in IL-12 level between the four groups was significant ($F=88.372$, $P<0.01$).

Serum IL-37 levels were 21.53±2.16pg/ml, 17.15±2.37pg/ml, 17.38±2.21pg/ml, and 24.28±2.17pg/ml, respectively, in the mild, moderate, severe, and normal control groups. The difference in IL-37 level between the four groups was significant ($F=39.247$, $P<0.01$).

The expression levels of serum 25-(OH)D in each group were 17.16±3.37ng/ml, 15.17±3.58ng/ml, 12.37±3.19ng/ml and 33.37±5.92ng/ml, respectively. One-way analysis of variance with a completely random design showed that the difference of 25-(OH)D between the four groups was significant ($F=102.53$, $P<0.01$).
Table 3. Comparison of serum levels of IL-4 (A), IL-12 (B), IL-37 (C) and 25-(OH)D (D) in children in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mild sustained attack group</th>
<th>Moderate sustained attack group</th>
<th>Severe sustained attack group</th>
<th>Normal control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>35</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>A (pg/ml)</td>
<td>21.48 ± 2.16</td>
<td>23.35 ± 3.82</td>
<td>25.52 ± 4.16</td>
<td>17.62 ± 1.58</td>
</tr>
<tr>
<td>B (pg/ml)</td>
<td>30.37 ± 3.48</td>
<td>23.16 ± 3.44</td>
<td>22.36 ± 2.15</td>
<td>33.37 ± 2.26</td>
</tr>
<tr>
<td>C (pg/ml)</td>
<td>21.53 ± 2.16</td>
<td>17.15 ± 2.37</td>
<td>17.38 ± 2.21</td>
<td>24.28 ± 2.17</td>
</tr>
<tr>
<td>D (ng/ml)</td>
<td>17.16 ± 3.37</td>
<td>15.17 ± 3.58</td>
<td>12.37 ± 3.19</td>
<td>33.57 ± 5.92</td>
</tr>
</tbody>
</table>

Expression differences of LncRNA BCYRN1 in different asthma states and healthy control children

The expression of LncRNA-BCYRN1 in the serum of children with different asthma states and healthy control children was detected by PCR(QRT-PCR), as shown in Figure 3. The results showed that the expression of LncRNA-BCYRN1 in healthy control children was 1.024±0.026, significantly lower than that in children with asthma, and the expression of LncRNA-BCYRN1 increased with the progression of LncRNA-BCYRN1. The expression of LncRNA-BCYRN1 was 5.471±0.427, 8.237±0.125, and 14.873±0.768 in mild, moderate, and severe sustained attacks, respectively (P<0.01).

Expression differences of LncRNA BCYRN1 in different asthma states and healthy control children

The expression of LncRNA-BCYRN1 in asthmatic children was correlated with the expression of inflammatory factors, as shown in Figure 4. As can be seen from the figure, the expression of LncRNA-BCYRN1 in serum of children with asthma was positively correlated with the expression of inflammatory factors TNF-α and IgE (r=0.735, P<0.001). R = 0.782, P < 0.001).

Figure 3. Expression differences of LncRNA-BCYRN1 in different asthmatic states and healthy control children

Figure 4. Correlation between serum LncRNA-BCYRN1 expression and inflammatory factors in asthmatic children

Bronchial asthma is a common disease in clinical practice; the main clinical manifestations are as follows: chest tightness, cough, wheezing, expiratory dyspnea, etc (16). In recent years, there has been a trend of increasing frequency, which has attracted extensive attention from the medical community (17). Currently, asthma is a global chronic disease, which has a serious impact on public health and quality of life. It can occur at multiple ages (18). Currently, the incidence of asthma in children has been increasing year by year (19). Children are the future of the country and the family, so it is of great clinical value to control and reduce the incidence probability, control the fatality rate and improve the quality of life of children with asthma (20). The incidence and mortality of asthma diseases have not been suppressed, and on the contrary, they are on the rise, especially in children (21). After epidemiological methods, for the overall situation and trend of the above diseases have a certain degree of understanding, the understanding was studied for the trigger asthma risk factors of the disease can help prevent these diseases, prevention, only such ability can carry on the strict and effective on the whole face of asthma control and prevention (22).

The relationship between respiratory tract infection and asthma disease is relatively complex (22, 23). Currently, respiratory tract infection diseases that may
cause asthma mainly include Mycoplasma pneumoniae, parainfluenza virus, pneumococcosis virus, rhinovirus and respiratory syncytial virus (23). Respiratory tract infection for the first independent risk factors of acute asthma attack, its mechanism is considered related to the following factors: infection can damage the respiratory epithelium, damage its integrity, making increased permeability, resulting in the formation of airway hyperresponsiveness, causes the body to produce specific IgE, increased sensitivity to allergens, also can make the respiratory excitatory cut or M cholinergic receptors overactive, elevated reactive cholinergic nerve, etc. (24). Mycoplasma is one of the important pathogens of childhood pneumonia or other respiratory tract infections. Respiratory virus infection is the main cause of acute asthma attacks in children, especially children under 5 years old. Viral infection can directly destroy the epithelial cells of the respiratory tract, leading to the hyperresponsiveness of the airway. On the other hand, multiple inflammatory mediators can be produced, which eventually lead to the imbalance of Th1 and Th2, thus inducing asthma. The pathogenesis of asthma is very complex. Among the immune factors, the imbalance of Th1 and Th2 cells can promote the abnormal increase of total IgE or allergen-specific IgE in vivo, which is also an important mechanism for the pathogenesis of asthma (25-27).

In this study, serum samples of subjects in each group were collected, and the expression levels of lncRNA-BCYRN1 in the serum of children with asthma and healthy control children were detected by real-time quantitative PCR. The influence of the expression of lncRNA-BCYRN1 on the prognosis of children with asthma was analyzed, and the inflammatory regulation mechanism of LncRNA in children with asthma was discussed. The results showed that the expression of lncRNA-BCYRN1 in the serum of asthmatic children was significantly higher than that of the control group. The expression level of lncRNA-BCYRN1 gradually increased with the aggravation of the disease, and the expression of lncRNA-BCYRN1 increased with the aggravation of the disease. The expression of lncRNA-BCYRN1 was positively correlated with the expression of inflammatory factors TNF-a and IgE.

In subsequent studies, BCYRN1 was found to be abnormally expressed in a variety of cancers and to be highly expressed in the brain of Alzheimer’s disease patients. In this study, the expression of BCYRN1 in tracheal smooth muscle cells of children in each asthma group was up-regulated compared with that in the normal control group. In vitro experiments, fluorescence quantitative PCR was also used to detect the up-regulated expression of BCYRN1, indicating the abnormal expression of BCYRN1 in the proliferation of tracheal smooth muscle cells. By transfection si-bcyrn1 into tracheal smooth muscle cells, it was found that down-regulation of BCYRN1 could reverse the survival, proliferation and migration of tracheal smooth muscle cells induced by pdgf-bb. In vivo experiments, down-regulating the expression of BCYRN1 reduced the inspiratory resistance and expiratory resistance of the asthma model. The specific regulatory molecular mechanisms of BCYRN1 in the proliferation and migration of tracheal smooth muscle cells need to be further studied, possibly by regulating the expression of important proteins related to proliferation and migration. At present, the research on the relationship between the abnormal function of tracheal smooth muscle cells and long-stranded non-coding RNA is still at the initial stage. With further understanding, long-stranded non-coding RNA can be used as a biological indicator for disease diagnosis and prediction in the future. This study showed that the abnormal expression of BCYRN1 could affect the proliferation and migration of tracheal smooth muscle cells, providing new evidence for the functional role of BCYRN1 in tracheal smooth muscle cells, and further providing an experimental basis for the development of BCYRN1 as a new target for the development of therapeutic drugs for asthma.

Acknowledgments

None.

Interest conflict

The authors declare no conflict of interest.

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