Long Noncoding RNA PTTG3P Promotes the Development of Childhood Asthma by Targeting the miR-192-3p/CCNB1 Axis

Bing Dai

Shengjing Hospital of China Medical University

Feifei Sun

Shengjing Hospital of China Medical University

Xuxu Cai

Shengjing Hospital of China Medical University

Chunlu Li

Shengjing Hospital of China Medical University

Fen Liu

Shengjing Hospital of China Medical University

Yunxiao Shang (✉ sxy15088706914@163.com)

Shengjing Hospital of China Medical University

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Abstract

Background: Increasing studies have suggested that long non-coding RNAs (lncRNAs) affect the regulation of immune responses, airway inflammation, and other pathological processes of asthma. In this study, we investigated the functions of the lncRNA PTTG3P in the progression of childhood asthma.

Methods: A competitive endogenous RNA network PTTG3P/miR-192-3p/CCNB1 was identified via bioinformatics analyses. Real-time qPCR and western blot were used to quantify gene and protein expression levels, respectively. Cell counting kit-8 and transwell assays were used to evaluate the proliferation and migration abilities of bronchial epithelial cells (16HBE). Double luciferase reporter gene assay was used to validate the predictive targets in PTTG3P, miR-192-3p, and CCNB1.

Results: PTTG3P was highly expressed in the peripheral blood of children with asthma. Knocking down PTTG3P could inhibit the epithelial-mesenchymal transition (EMT), proliferation, and migration of 16HBE cells. Mechanistically, PTTG3P promoted childhood asthma progression by targeting the miR-192-3p/CCNB1 axis.

Conclusions: Childhood asthma development can be stemmed by targeting the PTTG3P/miR-192-3p/CCNB1 axis. This study provides potential diagnosis and treatment biomarkers for childhood asthma.

1. Introduction

Bronchial asthma is one of the most common chronic respiratory diseases worldwide. In the vast majority of patients with asthma, the early symptoms begin in childhood, especially early school age, making asthma the most common chronic respiratory disease in childhood [1]. In recent years, the incidence of childhood asthma has been increasing yearly, which seriously affects the healthy growth of children and exerts heavy mental pressure and economic burden on the affected families and society [2, 3]. Therefore, clarifying the pathogenesis of childhood asthma and seeking targets for the diagnosis and treatment of childhood asthma are vital.

Long non-coding RNAs (lncRNAs) are a class of RNA molecules of > 200 nucleotides. They exist widely in eukaryotes and affect the regulation of various intracellular processes, but they cannot encode proteins [4]. Compared with protein-coding mRNAs, they are generally expressed with a low abundance, have a highly conserved secondary structure, and exhibit great tissue cell-type specificity [5]. lncRNAs are a potential molecular marker for the diagnosis and prognosis of many diseases, and the study of their functional mechanism provides a target for disease treatment. Current studies have conducted sequencing analysis on whole blood samples or peripheral blood T lymphocytes of adult patients with asthma and have found that lncRNA is differentially expressed (DE) in asthma patients, suggesting that lncRNAs influence the regulation of immune responses, airway inflammation, and other pathological processes of adult asthma from the perspective of epigenetics [6–9]. However, research on the function and mechanism of most asthma-related lncRNAs remains limited. Therefore, analysis of the differential expression profiles of lncRNAs in childhood asthma and the search for lncRNAs potentially related to the

progression of childhood asthma would provide a new direction and therapeutic target for preventing and treating childhood asthma.

Salmena et al. [10] first proposed the ceRNA hypothesis. LncRNAs can act as molecular sponges of endogenous miRNAs, playing a similar role as “sponges”, by competitively binding the miRNAs and thereby regulating the expression of protein-coding genes [11]. This cRNA network, formed by lncRNA/miRNA/mRNA interaction, is prevalent in the biological processes of many diseases, including liver metastasis, epithelial-mesenchymal transition (EMT), inflammatory formation, and chemoradiotherapy resistance. In this study, we found a ceRNA network—PTTG3P/miR-192-3p/CCNB1—that promotes childhood asthma development.

2. Materials And Methods

2.1 Specimen collection

Twenty-six children each with asthma and in good health, respectively, in Shengjing Hospital of China Medical University (Shenyang, China) from August 2016 to February 2017 were included. For the diagnostic criteria for asthma, we referred to the Guidelines for the Diagnosis and Prevention of Bronchial Asthma in Children revised by the Respiratory Group of Pediatrics Society of Chinese Medical Association in 2016. Exclusion criteria for childhood asthma included an acute respiratory infection or glucocorticoid treatment within 4 weeks before the hospital visit. Inclusion criteria for the healthy control group were as follows: 1. No definite abnormality found in routine physical examination indicators in the laboratory 2. No history of underlying diseases or drug therapy before blood collection; 3. Matching mean age and sex composition with asthma group. Approximately 5 ml of venous blood was collected from all subjects, along with clinical data and laboratory test results. This study was approved by the ethics committee of Shengjing hospital, China Medical University, and all patients signed written informed consent. The clinical information of the patients is presented in Table 1.

Table 1

Basic information about the subjects.

	Chip analysis group		qRT-PCR test group	
	Asthm (n=3)	control (n=3)	Asthm (n= 26)	control (n=26)
Age (year)	8.00±1.76	7.80±1.28	7.53±2.16	6.83±1.83
gender	2/1	2/1	17/9	18/8
FEV1/FVC (%)	66.39±10.34*	84.21±7.03	68.09±13.68**	86.99±6.54
FEV1 (L)	2.48±0.83*	3.42±0.58	2.31±0.73**	3.32±0.48
FEV1 (%)	77.36±14.24*	101.45±10.42	80.36±6.49**	100.40±1.12
FVC (L)	3.52±0.69*	3.90±0.72	3.38±0.87**	3.83±0.56
FVC (%)	0.90±0.21	1.17±0.17	1.03±0.18	1.08±0.15
PEF (L)	5.52±2.10*	7.91±1.83	5.36±1.91**	8.97±2.37
FeNO (ppb)	47.47±20.07*	13.28±8.74	60.42±12.56**	15.33±7.24
IgE CIU/mL)	187.00±98.80*	58.32±6.43	198.33±104.23**	56.52±9.39

Note: Values are presented as means ± SD unless otherwise stated. Abbreviations: FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak expiratory flow; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; SD, standard deviation. *p<0.05, ** *p<0.01.

2.2 Arraystar human lncRNA/mRNA expression profile microarray analysis

Peripheral blood mononuclear cell samples (PBMCs) were isolated by Ficoll density gradient centrifugation within 3 h of collecting the 5 ml-venous blood samples. Six PBMC samples (3 asthma patients and 3 healthy control patients) were selected and analyzed by lncRNA/mRNA expression microarray. The chip used Arraystar Human LncRNA Microarray V4.0 (Arraystar, Rockville, MD, USA), and the detection and data processing of the gene chip were assisted by Kangcheng Biological Engineering Co. Ltd. (Shanghai, China). The GeneSpring GX software V12.1 software (Agilent Technologies, Palo Alto, CA, USA) was used for quantile standardization of the original data.

2.3 Differential expression analysis and gene enrichment analysis

“Limma” package was used to select the DE lncRNAs and mRNAs between patients with asthma and healthy patients (control) based on the screening criteria of p<0.05 and log₂|FC|>2. Then, the DE mRNAs were imported into the DAVID 6.8 database (<https://david.ncifcrf.gov/>) for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. Thus, the functional level and biological pathway analyses results of genes were obtained. A p<0.05 indicated statistical significance.

2.4 Protein-protein interaction network analysis

The DE mRNAs were imported into the String database (<https://string-db.org/>) to obtain the interaction between proteins expressed by genes. Subsequently, we visualized this interaction and selected the five genes with the highest connectivity as hub genes.

2.5 Construction of the ceRNA network

Pearson correlation analysis was used to obtain the correlation coefficient between DE lncRNAs and hub genes. $p < 0.05$ and $r > 0.9$ were selected as screening criteria. Hub gene-related lncRNAs were screened out, and the target miRNAs of the lncRNAs were searched through the lncRNA Base database (<http://starbase.sysu.edu.cn/starbase2/mirLncRNA.php>). The related gene pairs between hub mRNAs and miRNAs were searched through the miRWalk database (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>). Then, the miRNA-mRNA pairs and the lncRNA-miRNA pairs were intersected to obtain the lncRNAs-miRNA-mRNA gene pairs.

2.6 Cell culture and vectors transfection

The human bronchial epithelial cell line (16HBE cells) was purchased from the cell bank of Xiangya Medical College, Central South University (Hunan, China). The 16HBE cells were inoculated into a complete medium (DMEM high sugar medium, containing 10% fetal bovine serum, 1% NEAA, 1% L-glutamine, 1% sodium pyruvate, and 100 µg/ml penicillin-streptomycin mixture). Next, the 16HBE cells were cultivated in a 5% CO₂ incubator and fully saturated humidity at a constant temperature of 37 °C. The downregulation vectors for lncRNA SNHG15, miR-192-3p mimics, and inhibitors were designed and synthesized by GenePharma Co., Ltd. (Suzhou, China). Lipofectamine 3000 was purchased from Invitrogen (MA, USA).

2.7 Real-time qPCR

We extracted total RNA from PBMCs and 16HBE cells using TriZol Reagent (Invitrogen, USA). cDNA was synthesized using All-in-One™ First-Strand cDNA Synthesis Kit FulenGen, Guangzhou, China) and amplified using SYBR green (Applied Biosystems LLC, Bedford, MA, USA). Then, the 2-ΔΔCt method was performed to calculate the relative gene expression with β-actin and U6 serving as the internal references. The RT-qPCR primer sequences are presented in Table 2.

Table 2

RT-qPCR primer sequences.

Gene	Sequences of the primers	Annealing temperature (°C)	Product length (bp)
β-actin	F:5' GTGGCCGAGGACTTTGATTG3' R:5' CCTGTAACAACGCATCTCATATT3'	60	73
RP11-598F7.3	F:5' GAAACTGTTATTCAGGAGACAACTA3' R:5' TATTTCTTGTGCTTTGTTTTATTTT3'	60	174
NR_002734 (PTTG3P)	F:5' TCTGGTTGAGAGCGGCAATAA3' R:5' AGGATGCCTGGTTCTTCGTT3'	60	77
U6	F:5'GCTTCGGCAGCACATATACTAAAAT3' R:5'CGCTTCACGAATTTGCGTGTTCAT3'	60	89
hsa-miR-192-3p	GSP:5'GGCTGCCAATTCCATAGG3' R:5'GTGCGTGTCGTGGAGTCG3'	60	62
hsa-miR-6835-3p	GSP:5'GGGCAAAGCACTTTTCTGTCT3' R:5'GTGCGTGTCGTGGAGTCG3'	60	64
β-actin	F:5' GTGGCCGAGGACTTTGATTG3' R:5' CCTGTAACAACGCATCTCATATT3'	60	73
CCNA2	F:5' AGTGGAGTTGTGCTGGCTAC3' R:5' CACAGTCAGGGAGTGCTTTC3'	60	223
CCNB1	F:5' TGAGCCTATTTTGGTTGATACTGC3' R:5' GCAAGAATTACATCAGAGAAAGCCT3'	60	105

2.8 Western blot

RIPA lysis buffer (Beyotime, Shanghai, China) was used to extract the total protein. Protein concentration was measured with BCA Protein Quantitative Kit (Kangrun Biology Co., Ltd., Guangzhou, China). A 10% SDS-PAGE gel was used to separate 40 µg proteins, which was then transferred onto a polyvinylidene fluoride (PVDF) membrane (Kangrun Biology, Guangzhou, China). Subsequently, the membranes were blocked with 5% milk for 1.5 h at room temperature. Subsequently, the primary antibodies against GAPDH (1:10,000; Abcam, Cambridge, UK), E-cadherin (1:1000; Abcam), α-SMA (1:1000; Abcam), and vimentin (1:1000, Abcam) were added to the membrane for coculturing overnight at 4 °C. Then, the membranes were cocultured with the secondary antibody for 2 h at room temperature. Finally, the membranes were

exposed and developed with the Azure c300 Gel Imaging System (Azure Biosystems Inc., Dublin, CA, USA). The Image-Pro Plus software (www.mediacy.com › 78-products › image-pro-plus) was used to analyze the gray value of the protein bands. GAPDH was selected as the internal reference to calculate the relative expression level of the samples.

2.9 Cell counting kit-8 (CCK-8) assay

CCK-8 (Kangrun Biology, China) was used to assess the proliferation ability of the 16HBE cells. Five thousand 16HBE cells were seeded into 96-well plates. A 100 µl-DMEM medium was added to each well, and five duplicate wells were designed for each group. The 96-well plates were incubated for 24, 48, 72, and 96 h. Then, 10 µl of CCK-8 reagent was added to each well and incubated for 3 h at 37 °C. Finally, the optical density (OD) value at 450 nm was measured using a microtiter plate reader, and the absorbance was calculated.

2.10 Transwell assay

The transwell chamber (Corning Inc., Corning, NY., USA) was used to evaluate the migration (or cell invasive) ability of the 16HBE cells. The cells were resuspended in serum-free media and seeded into the upper chamber at a density of 4×10^3 cells /well, using a volume of 200 µl. An 800 µl culture solution with 30% FBS was subsequently added to the lower chamber. After incubation for 24 h, cells were fixed, stained, and visualized under an inverted microscope. Cells that migrated to the sublayer of the microporous membrane were counted. Five fields were selected for each sample to count the number of cells, and the mean number was obtained.

2.11 Double luciferase reporter gene assay

We predicted the binding sequences between lncRNA PTTG3P and miR-192-3p, miR-192-3p, and CCNB1 from the Starbase (<http://starbase.sysu.edu.cn/>). We designed and synthesized the pSI-Check2 vector (Hanbio Biotechnology Co., Ltd Shanghai, China) including the wild-type (WT) and mutant (MT) binding sites of miR-192-3p within the PTTG3P sequence or CCNB1 3'UTR; we then named the PTTG3P-WT/MT and CCNB1-WT/MT vectors, respectively. The synthesized vectors with miR-192-3p mimic or mimic-NC were transfected into the 16HBE cells for 48 h, and the dual-luciferase reporter system (Promega, USA) was used to determine the luciferase activity.

2.12 Statistical analysis

Data are expressed as mean $\pm$ standard deviation ($\bar{X} \pm SD$). One-way analysis of variance and t-test were used to compare the differences between groups. A $p < 0.05$ indicated statistical significance. GraphPad Prism 8.0 and R 4.0.3 were used for statistical analysis.

3. Results

3.1 Differential expression analysis

A total of 397 DE lncRNAs were screened, comprising 193 upregulated and 204 downregulated lncRNAs (Figures 1A and B). There were 11 exon sense-overlapping, 24 intron sense-overlapping, 101 antisense, 23 bidirectional, and 238 long intergenic non-coding lncRNAs (Supplementary Figure 1). A total of 477 DE mRNAs were screened, comprising 398 upregulated and 79 downregulated mRNAs (Figures 1C and D).

3.2 GO and KEGG enrichment analysis

We performed GO analysis on DE mRNAs. GO analysis was performed from three perspectives: biological process, cell component, and molecular function. The results showed that upregulated DE mRNAs were mainly enriched in “cell cycle” and “protein binding” (Supplementary Figure 2), whereas the downregulated DE mRNAs were mainly enriched in “VEGF receptor binding” (Supplementary Figure 3). The upregulated DE mRNAs could be mainly related to mitotic cell cycle arrest, protein binding, and protein DNA complex. The downregulated DE mRNAs were mainly associated with vascular endothelial growth factor receptor (VEGF) binding and transforming growth factor receptor activity. KEGG pathway analysis showed that the upregulated mRNAs were enriched in cell cycle, immune regulation, p53, and other related signaling pathways (Supplementary Figure 4), whereas the downregulated mRNAs were enriched in the cell adhesion molecule signaling pathway (Supplementary Figure 5).

3.3 Protein-protein interaction network analysis

To further clarify the interrelationships between the proteins expressed by DE mRNAs, we obtained the degree of connection between DE mRNAs through the STRING database and visualized it using Cytoscape V 3.5.1 software. Supplementary Figures 6A and B show the upregulated and downregulated DE mRNAs, respectively. The five genes (CCNB1, CCNA2, CDC6, CDK1, and CHEK1) with the highest connectivity were selected as hub genes (Table 3).

Table 3

Connectivity of the hub genes.

Upregulated gene		Downregulated gene	
Gene symbol	Degree	Gene symbol	Degree
CDK1	86	NRCAM	2
CCNB1	79	SCN7A	1
CCNA2	79	CNTNAP2	1
CDC6	76	KLHL31	1
CHEK1	74	MYOM2	1
HJURP	73	SLC04C1	1
RAD51	72	NUDT12	1
MCM2	70	CLHC1	1
CDC20	70	PTEN	1

3.4 Construction of the PTTG3P/miR-192-3p/CCNB1 ceRNA network

We obtained 49 DE lncRNA and hub gene pairs based on the Pearson correlation analysis (Figure 2A, Supplementary Table 1). We then acquired lncRNA-miRNA and miRNA-mRNA gene pairs from the lncRNA Base database and miRWalk database, respectively. After intersecting, six lncRNA-miRNA-mRNA gene pairs were obtained (Figure 2B, Table 4). Based on the analysis results, we screened the target lncRNAs most closely associated with hub genes; these were Rp11-598F7.3 and PTTG3P. Details of the two lncRNAs are shown in Supplementary Table 2. In the peripheral blood samples of 26 asthmatic children and 26 healthy control children, the relative expression levels of the two lncRNAs—Rp11-598F7.3 and lncRNA PTTG3P—were verified by qRT-PCR, using β -actin as an internal reference. Compared with that in the healthy control group, the expression level of lncRNA PTTG3P in the asthma group was increased ($p < 0.001$, Figure 3A). Conversely, that of Rp11-598F7.3 showed no statistical difference between the two groups ($p = 0.98$, Figure 3B). According to the lncRNA-miRNA-mRNA gene pairs, the miRNAs and mRNAs related to PTTG3P included miR-192-3p, miR-6835-3p, CCNB1, and CCNA2. We verified the relative expression levels of miR-192-3p, miR-6835-3p, CCNB1, and CCNA2 in the peripheral blood samples of 26 children with asthma and 26 healthy control children through qRT-PCR. Compared with that in the healthy control group, miR-192-3p expression levels in the asthma group were decreased ($p = 0.024$, Figure 3C); however, that of miR-6835-3p showed no statistical difference between the two groups ($p = 0.51$, Figure 3D). Moreover, CCNB1 expression levels were higher in the asthma group than in the healthy control group ($p < 0.0001$, Figure 3E), but CCNA2 expression levels showed no statistical difference between the two groups ($p = 0.054$, Figure 3F). Therefore, we finally selected PTTG3P/miR-192-3p/CCNB1 to construct the ceRNA network.

Table 4

lncRNAs-miRNA-mRNA gene pairs.

	miRNA	mRNA
RP11-598F7.3	hsa-miR-4256	CDK1
	hsa-miR-377-5p	CCNB1
	hsa-miR-6086	CCNB1
	hsa-miR-3192-5p	CCNB1
PTTG3P	hsa-miR-192-3p	CCNB1
	hsa-miR-6835-3p	CCNA2

3.5 Effects of PTTG3P on EMT, proliferation, and migration of the 16HBE cells

Increasing studies have shown that bronchial epithelial cells, as the first natural barrier to exposure to allergens and harmful substances, can influence the occurrence of airway remodeling through EMT upon stimulation by various environmental factors [4, 12]. The EMT process is characterized by the loss of E-cadherin, which represents the epithelial phenotype, and the acquisition of vimentin and α -SMA, which represents the mesenchymal phenotype. The bronchial epithelial cells are transformed into mesenchymal cells, and the contraction and migration of the cells are enhanced [13]. We induced EMT of 16HBE cells using TGF- β 1 with different concentration gradients and different action times and finally selected an optimal concentration of 10 ng/ml and optimal maximum use time of 48 h (Figures 4A and B). To explore the relationship between PTTG3P and the EMT process of bronchial epithelial cells, we detected the expression of PTTG3P in 16HBE cells via qRT-PCR after incubation of the cells with 10 ng/ml TGF- β 1 for 12, 24, 48, and 72 h. The results showed that, over a certain time range, PTTG3P expression levels in 16HBE cells increased with increasing TGF- β 1 treatment time. At 48 h of incubation, the expression level of lncRNA PTTG3P reached the optimal level (Figure 4C). Si-PTTG3P-362, which exhibited the highest inhibition rate, was used to inhibit PTTG3P expression in 16HBE cells (Figure 4D). Compared with that in the TGF- β 1 and si-NC+ TGF- β 1 groups, the expression level of E-cadherin was significantly increased but that of vimentin and α -SMA was significantly decreased in the si-PTTG3P +TGF- β 1 group, indicating that PTTG3P knockdown inhibits the TGF- β 1-induced EMT process in 16HBE cells (Figure 4E). Compared with that in the si-NC+TGF- β 1 group, the cell morphology in the Si-PTTG3P +TGF- β 1 group was similar to the control group, suggesting that PTTG3P knockdown alleviates the morphological changes induced by TGF- β 1 (Figure 4F). Besides, PTTG3P knockdown inhibited the proliferation (Figure 4G) and migration (Figure 4H) of TGF- β 1-induced 16HBE cells.

3.6 PTTG3P sponged miR-192-3p to upregulate CCNB1 in 16HBE cells

We hypothetically assumed that PTTG3P was involved in the EMT of bronchial epithelial cells by regulating the miR-192-3p/CCNB1 axis. To test our hypothesis, we conducted some experiments. First, we determined that the expression level of miR-192-3p in 16HBE cells decreased gradually with increasing TGF- β 1 treatment time (Figure 5A), but that of CCNB1 was reversed (Figures 5B and C). After PTTG3P knockdown, the miR-192-3p expression was significantly upregulated (Figure 5D), while that of CCNB1 was significantly decreased (Figures 5E and F) compared with TGF- β 1 and si-NC+TGF- β 1 groups. MiR-192-3p mimics were used to overexpress miR-192-3p levels in 16HBE cells and the overexpression efficiency was detected (Figure 5G). Confirming our hypothesis, overexpression of miR-192-3p was found to inhibit the TGF- β 1-induced EMT process in 16HBE cells (Figures 5H and I) and downregulate the expression level of CCNB1 (Figures 5J and K). The dual-luciferase reporter gene assay was used to verify whether miR-192-3p could be combined with PTTG3P and CCNB1 3'UTR terminals. pSI-check2-PTTG3P and pSI-check2-CCNB1-3'UTR plasmids were constructed using pSI-check2 vectors. After transfection with miR-192-3p mimics and negative miRNA controls for 48 h, double luciferase kits were used for detection. Compared with the miRNA-negative control group, miR-192-3p significantly downregulated pSI-check2-PTTG3P (Figure 5L) and pSI-check2-CCNB1-3'UTR (Figure 5M) luciferase activity, suggesting that miR-192-3p can directly bind to PTTG3P and the 3'UTR terminal of CCNB1. Next, we performed a rescue experiment using a miR-192-3p inhibitor to explore whether PTTG3P functioned in 16HBE cells by regulating miR-192-3p expression. miR-192-3p inhibitor was found to reverse the effects of PTTG3P silencing on the protein expression levels of CCNB1, E-cadherin, vimentin, and α -SMA, indicating that PTTG3P functioned by negatively regulating the expression of miR-192-3p in 16HBE cells (Figure 5N).

4. Discussion

Bronchial asthma is the most common chronic nonspecific inflammatory disease of the airway in childhood. Recent studies have reported that lncRNAs were DE in patients with asthma and hence may play a key regulatory role in the pathogenesis of asthma [14–17]. Therefore, screening out the DE lncRNAs in childhood asthma and exploring their functions and related molecular mechanisms are vital for the diagnosis, treatment, and prognosis of childhood asthma.

In this study, PBMCs from childhood asthma were used as research objects. lncRNA/mRNA expression profiles were analyzed using gene microarray, and DE lncRNAs/mRNAs were screened. PBMCs include lymphocytes (which make up the majority) and monocytes. Recently, many studies have shown that PBMCs significantly influence innate immune activation, acquired immune regulation, autoimmune diseases, and other physiological and pathological processes [18]. Additionally, peripheral blood samples are noninvasive, easily accessible, and stable; besides, a lymphocyte separation solution can be used to easily isolate PBMCs from peripheral blood samples. Therefore, PBMCs have been widely used in basic experiments and clinical research [19]. Subsequently, we created a pTTG3p/miR-192-3p/CCNB1 ceRNA

network through a series of bioinformatics analyses. A dual-luciferase reporter gene assay verified that miR-192-3p could be combined with PTTG3P and CCNB1 3'UTR terminals.

PTTG3P is a pituitary tumor transforming gene (PTTG). It is located on chromosome 8q13.1 and shows high homology with the PTTG gene [20]. Presently, research on PTTG3P focuses on the field of tumors. Studies have shown that PTTG3P promotes the occurrence of liver cancer by upregulating PTTG1, thereby activating P13K/Akt and its downstream signals, including a variety of cell cycle regulators and EMT-related factors [21]. PTTG3P promotes cervical cancer cell growth by regulating CCNB1-mediated cell proliferation, and it also plays a certain role in enhancing cell invasion and inducing EMT [22]. MiR-192-3p is the precursor of miR-192. Studies have shown that the expression level of miR-192 in peripheral blood of patients with asthma is significantly reduced after inhaled allergen stimulation [23]. Cyclin B1—the protein encoded by CCNB1 mRNA—is a Cyclin family protein. Cyclin B1 is a cellular structural protein involved in mitosis and a key cell cycle regulator that promotes cell transformation from the G2–M phase [24]. Most studies on Cyclin B1 focus on tumor-related fields. Evidence has shown that Cyclin B1 may achieve high expression levels in pituitary adenoma by promoting the EMT process and can enhance the invasion and migration abilities of cells [25].

Airway remodeling is an important feature of asthma and may lead to progressive impairment of lung function and irreversible airflow limitation; this is closely related to the clinical severity and prognosis of asthma [26]. Increasing evidence suggests that EMT, as a new source of fibroblasts, is involved in airway remodeling in patients with asthma [27]. EMT is a phenomenon whereby polarized epithelial cells attached to the basement membrane differentiate into fibroblasts to repair tissues damaged by chronic inflammatory stimulation. However, EMT also aggravates the degree of subepithelial fibrosis, which essentially affects the occurrence and development of airway remodeling in asthma [28]. Therefore, how to regulate the EMT process of bronchial epithelial cells to reduce airway remodeling has become the focus of research on asthma prevention and treatment. Previous studies have shown that TGF- β 1 has various biological functions and is a potent fibrogenic cytokine that can induce EMT in bronchial epithelial cells [29]. As an important inducer of EMT in epithelial tissues, TGF- β 1 is widely used in cytological experiments in vitro, mainly for inducing morphological and phenotypic transformation of numerous epithelial cells. In our study, the expression level of PTTG3P was significantly increased in a TGF- β 1-induced EMT model of bronchial epithelial cells. Subsequent knockdown experiments further confirmed that PTTG3P knockdown partially reversed the EMT process of the TGF- β 1-induced 16HBE cell, suggesting that PTTG3P promotes the EMT process of bronchial epithelial cells. Finally, a series of experimental studies was done to confirm that PTTG3P influenced the EMT process of bronchial epithelial cells by regulating the miR-192-3p/CCNB1 axis.

In conclusion, our research confirmed that targeting the lncRNA PTTG3P reversed the EMT process of TGF- β 1-induced 16HBE cell in vitro by regulating the miR-192-3p/CCNB1 axis. PTTG3P, miR-192-3p, and CCNB1 may be potential biomarkers for the diagnosis and treatment of childhood asthma.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shengjing hospital, China Medical University, and all patients signed written informed consent. Consent for publication.

Not Applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files

Competing interests

The authors declare that they have no competing interests

Author Contributions

Bing Dai, Feifei Sun, Xuxu Cai, Chunlu Li, Fen Liu, Yunxiao Shang conceived and designed the study. Bing Dai, Feifei Sun Yunxiao Shang developed the methodology. Bing Dai, Feifei Sun, Xuxu Cai, Yunxiao Shang analyzed and interpreted the data. Bing Dai, Yunxiao Shang wrote, reviewed, and/or revised the manuscript.

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Figures

Figure 1

Differential expression of mRNAs in the peripheral blood of a patient with childhood asthma. (A) Cluster heat map of DE lncRNAs. Red, green, and gray points represent upregulated, downregulated, and unaltered expression, respectively. Horizontal and vertical lines represent the clustering of lncRNAs and the clustering of samples, respectively. (B) Volcano maps of DE lncRNAs. Red, green, and gray points represent upregulated, downregulated, and unaltered expression, respectively. Horizontal and vertical lines

represent the clustering of lncRNAs and the clustering of samples, respectively. (C) Cluster heat map of DE mRNAs. (D) Volcano maps of DE mRNAs.

Figure 2

Conduction of a ceRNA network. (A) lncRNA-mRNA gene pairs. Red and purple points represent hub mRNAs and lncRNAs associated with hub mRNAs, respectively. (B) lncRNAs-miRNA-mRNA gene pairs. Red, purple, and green points represent mRNAs, lncRNAs, and miRNAs, respectively.

Figure 3

Relative expression levels of lncRNA, miRNA, and mRNA were verified via qRT-PCR. (A) PTTG3P. (B) RP11-598F7.3. (C) miR-192-3p. (D) miR-6835-3p. (E) CCNB1, (F) CCNA2. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 4

Effects of lncRNA PTTG3P on EMT, proliferation, and migration of 16HBE cells. (A) EMT markers were detected after inducing 16HBE cells with different concentrations of TGF-β1 (*, #, and & indicate comparisons between each group and 0 ng/ml, 2.5 ng/ml, and 5 ng/ml, respectively; $p < 0.01$). (B) EMT markers were detected after 16HBE cells were induced with 10 ng/ml TGF-β1 for different times (*, #, and & indicate comparisons between each group with 0, 12, and 24 h, respectively, $p < 0.01$). (C) The relative expression level of PTTG3P was detected after 16HBE cells were induced with 10 ng/ml TGF-β1 for different durations (*, #, &, and \$ indicate comparisons between each group with the 0, 12, 24, and 48 h

groups, respectively; $p < 0.05$). (D) The relative expression level of PTTG3P in each group after siRNA transfection (Compared with control and negative control (NC) groups; $*p < 0.05$, $**p < 0.005$). (E) Western blotting was used to detect the effect of PTTG3P knockdown on EMT markers in TGF- β 1-induced 16HBE cells, $*p < 0.05$. (F) Morphological changes of the 16HBE cells in each group after knockdown of PTTG3P (inverted light microscope, 200X). (G) At 24, 48, 72, and 96 h after cell inoculation, CCK8 assay was used to detect cell proliferation ability in each group (Compared with TGF- β 1 and si-NC+TGF- β 1 groups, $*p < 0.05$). (H) Histogram of crystal violet staining results (200X) and the number of 16HBE cells passing through the transwell chamber (* and # represent comparisons with TGF- β 1 and si-NC+TGF- β 1 groups, $p < 0.05$).

Figure 5

LncRNA PTTG3P sponged miR-192-3p to upregulate CCNB1 in 16HBE cells. (A) The relative expression level of miR-192-3p was detected after the 16HBE cells were induced with 10 ng/ml of TGF- β 1 for different durations (*, #, and & indicate comparisons with the 0, 12, and 24 h groups, respectively; $p < 0.05$). (B) TGF- β 1 induced change in CCNB1 protein expression in 16HBE cells at different time points (*, #, and & indicate comparisons with the 0, 12, and 24 h groups, respectively; $p < 0.05$). (C) TGF- β 1

induced change in CCNB1 mRNA expression in 16HBE cells at different time points (*, #, and & indicate comparisons with the 0, 12, and 24 h groups, respectively; $p < 0.05$). (D) Expression of miR-192-3p in each group (* and # indicate comparisons with blank control ($p < 0.0001$) and si-NC+TGF- β 1 groups ($p < 0.005$), respectively). (E) Western blotting was used to detect the effect of PTTG3P knockdown on CCNB1 protein expression in TGF- β 1-induced cells (* and # indicate comparisons with TGF- β 1 and si-NC+TGF- β 1 groups, respectively; $p < 0.05$). (F) CCNB1 mRNA expression in each group (* and # indicate comparisons with blank control ($p < 0.0001$) and TGF- β 1 group and si-NC+TGF- β 1 groups ($p < 0.005$)). (G) The relative expression level of miR-192-3p in each group (*Compared with blank control and miRNA negative control groups, $p < 0.005$). (H) Effects of miR-192-3p overexpression on the morphology of 16HBE cells (Inverted light microscopy, 200X) (I) Western blotting was used to detect the effect of miR-192-3p overexpression on the expression of E-cadherin, α -SMA, and vimentin in TGF- β 1-induced cells, * $p < 0.01$. (J) The effect of miR-192-3p overexpression on CCNB1 protein expression in TGF- β 1-induced cells was detected by western blotting (* and # indicate comparisons with the blank control and miRNA NC+TGF- β 1 groups, respectively; $p < 0.05$). (K) The effect of miR-192-3p over-expression on CCNB1 mRNA in TGF- β 1-induced 16HBE cells was detected by qRT-PCR (* and # indicate comparisons with the blank control ($p < 0.0001$) and miRNA NC+TGF- β 1 ($p < 0.005$) groups, respectively). (L) Dual-luciferase reporter gene analysis showed that miR-192-3p had a targeting relationship with PTTG3P (*, compared with miR-NC transfection group; $p < 0.05$). (M) Dual-luciferase reporter gene analysis showed that miR-192-3p had a targeting relationship with CCNB1 (*, compared with miR-NC transfection group; $p < 0.05$). (N) The protein changes of CCNB1, E-cadherin, α -SMA, and vimentin in each group were detected by western blot. 1. Blank control; 2: the TGF - beta 1; 3: si- PTTG3P +TGF- β 1; 4: miR-192-3p inhibitor +TGF- β 1; 5: si- PTTG3P + miR-192-3p inhibitor +TGF- β 1 groups (*, #, &, and \$ indicate comparisons with the blank control, TGF- β 1, si-PTTG3P +TGF- β 1, and miR-192-3p inhibitor+TGF- β 1 groups, respectively; $p < 0.05$).

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