Original Article

Screening of long non-coding RNAs markers in plasma of children with chronic gastritis

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Abstract

Objective: The study aimed to detect and analyze long non-coding RNAs (lncRNAs) in plasma of children diagnosed with chronic gastritis, and to explore their biological functions and involved signaling pathways.

Methods: The plasma samples were collected from six children that were diagnosed with chronic gastritis by physical examination, gastroscopy, and pathological examination and six healthy children. The plasma samples were assayed for determining the expression profiles of lncRNA based upon the gen chip detection. The specific expression of lncRNA in plasma of children with chronic gastritis was analyzed and its biological functions were speculated.

Results: Five lncRNAs (RP11-697M17.1, RP11-388M20.9, AFAP1-AS1, BC062758, and XLOC001406) were significantly up-regulated, and five lncRNAs (UNQ697, BX571672.5, CYP4F35P, ANKRD20A5P, and AL832737) were observed to be significantly down-regulated. The lncRNAs RP11-697M17.1 and UNQ697 were detected with the highest up-regulation and down-regulation, respectively. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that the up-regulated lncRNAs were significantly enriched in 20 signaling pathways such as phosphoinositide-3-kinase–protein kinase B (PI3K-Akt) pathway, and the down-regulated lncRNAs target genes were significantly enriched in 20 signaling pathways such as the metabolic pathway.

Conclusion: The analysis of the lncRNA expression profiles in plasma of children with chronic gastritis revealed that the lncRNA RP11-697M17.1 and lncRNA UNQ697 may act as plasma markers for predicting chronic gastritis in children.

Keywords: RP11-697M17.1; UNQ697; Chronic gastritis; Plasma markers

Introduction

Chronic gastritis is an inflammation of the stomach lining that is caused by a combination of various factors, including the Helicobacter pylori (H. pylori) infection. It can lead to various conditions, such as
hernia, nausea, pain etc. In the current scenario, the common methods for the treatment of chronic gastritis include diet therapy, symptomatic treatment, and eradication of *H. pylori*. However, an effective approach for the treatment of chronic gastritis is the eradication of *H. pylori* that can markedly reduce its recurrence rate. The long non-coding RNAs (lncRNAs) are a group of non-coding transcripts that are greater than 200 nucleotides (nt) in length but lack the protein-coding capacity. Recently, studies have reported that lncRNAs are widely implicated in various physiological and pathological processes in the body, and also involved in regulation of the progression of various diseases. Specific lncRNAs, such as *H19* and HOX transcript antisense RNA (*HOTAIR*), have been shown to play important roles in digestive tract diseases. For example, *H19* induces the production of microRNA-675 (miR-675) by the tumor suppressor runt-related transcription factor 1, and regulates the gastric cancer cell proliferation. Moreover, the overexpression of *HOTAIR* may be involved in tumor escape mechanisms. These observations strongly suggest that lncRNAs are a molecular etiology of digestive tract diseases. The plasma RNA has been identified as a novel non-invasive diagnostic biomarker. It has been reported that the circulating RNA in the blood is encapsulated within the vesicles, such as exosomes enabling it to persist for a longer time. Furthermore, it is more heterogeneous than endoscopy for detection of the disease. Previously, the circulating biomarkers have been screened mainly in adults for the detection of digestive tract diseases, and screening of patients. The plasma RNA has been identified as a novel non-invasive diagnostic biomarker. It has been reported that the circulating RNA in the blood is encapsulated within the vesicles, such as exosomes enabling it to persist for a longer time. Furthermore, it is more heterogeneous than endoscopy for detection of the disease. Previously, the circulating biomarkers have been screened mainly in adults for the detection of digestive tract diseases. In the present study, we report the analysis of expression profile of lncRNAs and screening of specific lncRNA markers in the plasma of children that were diagnosed with chronic gastritis.

**Methods**

**Ethical approval**

The study was approved by the ethics committee of Shanghai Pudong New District Zhoupu Hospital. All clinical practices and observations were conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient before the study was conducted.

**Patients**

The study included six children (8.0–12.0 years old) from Shanghai Pudong New District Zhoupu Hospital from December 2018 to August 2019. The patients were selected for the study on the basis of clinical symptoms, physical examination, gastroscopy, and pathological examination of chronic gastritis. Additionally, six blood samples were collected as control group from children that were not diagnosed with chronic gastritis by the same clinical examination.

**Plasma sample collection and preservation**

The plasma samples were collected from six children that were diagnosed with chronic gastritis, and six healthy children. The samples were collected in BD EDTA (Becton Dickinson [BD] Ethylenediaminetetraacetic acid) tubes. The plasma samples were initially centrifuged at a speed of 1500 × g at 4 °C for 30 min, and then at 3000 × g at 4 °C for 10 min. The supernatant was collected for each sample and stored in TRIzol® reagent (Qiagen, Inc. Valencia, California, USA) at −80 °C.

**Total RNA extraction and reverse transcription**

Total RNA was extracted from the plasma samples using the miRNeasy Serum/Plasma kit (REF 217184, Germany) according to the manufacturer's instructions. Total RNA concentration and purity were measured using the NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). Total RNA was then dissolved in RNase-free water and then immediately used for reverse transcription using the PrimeScript RT-PCR kit (REF RR036A, Japan) according to the manufacturer's instructions.

**lncRNA gene chip detection**

Twelve plasma samples were assayed for determining the expression profiles of lncRNA using the Agilent Human LncRNA Array v3.0 (8 × 60K, Arraystar, USA).

**GO and KEGG analysis**

Gene ontology was classified into three groups by Gene Ontology (GO) database: BP (biological process), CC (cell component), and MF (molecular
function). KEGG pathway analysis was performed to determine the involvement of differentially expressed lncRNA genes in different biological pathways. The cut off $P$ value (hypergeometric $P$ value) was set at 0.05.

Statistical analysis

Numerical data were expressed as mean ± standard deviation (SD) and measured in triplicates. The comparison between the statistically normalized data sets was performed using the t-test or the Mann-Whitney U test. For statistical significance, $P$ value was set at 0.05 and the confidence level was set at 95%.

Results

Differentially expressed lncRNAs in chronic gastritis

In gene chip detection, a specific lncRNA expression profile was obtained in children with chronic gastritis. A differential expression pattern of 2053 lncRNAs was observed in children with chronic gastritis (fold change $\geq$ 2.0, $P \leq 0.05$) as compared to the healthy control group. Out of these, 934 and 1119 lncRNAs were significantly up-regulated and down-regulated, respectively in diseased children as compared to the healthy control group (Fig. 1). Among these, five lncRNAs (RP11-697M17.1, RP11-388M20.9, AFAP1-AS1, BC062758, and XLOC001406) were observed to be significantly up-regulated (Table 1), and five lncRNAs (UNQ697, BX571672.5, CYP4F35P, ANKRD20A5P, and AL832737) were observed to be significantly down-regulated (Table 2). The lncRNAs RP11-697M17.1 (Fig. 2A) and UNQ697 (Fig. 2B) were identified with the highest up-regulation and down-regulation, respectively.

GO annotation and KEGG enrichment of differential genes

The information about GO functions was used to annotate 934 up-regulated and 1119 down-regulated genes, and each GO group was enriched separately. The results showed that 2683 up-regulated lncRNAs were enriched in BP, 435 were enriched in CC, and 675 were enriched in MF ($P < 0.05$). The GO analysis showed that 1418 down-regulated genes were enriched in BP, 261 were enriched in CC, and 428 were enriched in MF ($P < 0.05$). The functions of top 20 up-regulated and down-regulated genes are described in Figs. 3 and 4, respectively.

KEGG signal pathway analysis

Based on the classification of GO annotations, KEGG pathway enrichment analysis was done for all

### Table 1
Up-regulated lncRNAs in plasma of children with chronic gastritis.

| Symbols            | Fold change | $P$     | Threshold |
|--------------------|-------------|---------|-----------|
| RP11-697M17.1      | 188.73      | 0.029   | Up        |
| RP11-388M20.9      | 76.68       | 0.032   | Up        |
| AFAP1-AS1          | 50.07       | 0.0075  | Up        |
| BC062758           | 43.63       | 0.039   | Up        |
| XLOC001406         | 34.32       | 0.026   | Up        |

### Table 2
Down-regulated lncRNAs in children with chronic gastritis.

| Symbols    | Fold change | $P$     | Threshold |
|------------|-------------|---------|-----------|
| AL832737   | 0.035       | 3.8E-05 | Down      |
| ANKRD20A5P | 0.027       | 0.04    | Down      |
| CYP4F35P   | 0.025       | 0.027   | Down      |
| BX571672.5 | 0.017       | 0.025   | Down      |
| UNQ697     | 0.0089      | 0.00049 | Down      |
identified 934 up-regulated and 1119 down-regulated genes. The up-regulated IncRNAs were mainly enriched in 20 signaling pathways, such as PI3K-Akt (Fig. 5) and the down-regulated IncRNAs target genes are significantly enriched in 20 signaling pathways, such as metabolic pathways (Fig. 6).

**Discussion**

So far, the gold standard for the diagnosis of chronic gastritis still depends upon the endoscopy and histopathological evaluation. Various studies have shown that RNA in the blood plays an important role in the processes of cell-to-cell communication i.e. the inflammatory reactions, and tissue regeneration. Circulating RNA in the blood may act as a potential biomarker for various diseases, which can be more heterogeneous than endoscopy in detecting various diseases.

In the present study, we have determined the specific expression profiles of IncRNAs in plasma of children with chronic gastritis and normal healthy
children. The results showed that 2053 lncRNAs were specifically expressed in children with chronic gastritis. It was determined for the first time that the lncRNA RP11-697M17.1, BC062758, XLOC001406, lncRNAs UNQ697, BX571672.5, CYP4F35P, ANKR-D20A5P and AL832737. In addition, lncRNA RP11-388M20.9 and lncRNA AFAP1-AS1 have been reported. It found that lncRNA RP11-388M20.9 were significantly up-regulated with increasing concentrations of Cr(VI) in human bronchial epithelial cells. In gastric cancer, overexpression of lncRNA AFAP1-AS1 promotes cell proliferation and invasion.

Studies have found that PI3K/Akt is the main signaling pathway in gastrointestinal diseases gastrointestinal...
disorders. Helicobacter pylori (HP) mediated PI3K/AKT/GSK3β signal pathways in the occurrence of gastric cancer. In addition H. pylori causes a significant increase of PI3K/Akt in gastritis. We know that metabolic activities are also very rich in gastritis. So bioinformatics predicts that metabolic pathways are enriched more, which is consistent with previous reports. In the present study, it has been detected that lncRNA RP11-697M17.1, and lncRNA UNQ697 were significantly expressed in gastritis patients as compared to the healthy control group. Moreover, that the bioinformatics’ analysis revealed that the lncRNA RP11-697M17.1, and lncRNA UNQ697 might participate in PI3K-Akt and metabolic pathways. Further, it was speculated in our study that the lncRNA RP11-697M17.1 and lncRNA UNQ697 may be involved in regulation rather than protein-coding.

One main limitation of the present study is the small sample size, where only six samples of children with chronic gastritis were included for the analysis. Further validation experiments should be performed using the large patient population to explore the role of circulating lncRNAs in gastritis disease.

Conclusively, the plasma lncRNA RP11-697M17.1, and lncRNA UNQ697 are the most promising biomarkers for the diagnosis of chronic gastritis in children.

Conflict of interest

None.

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