Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Long non-coding RNA expression profiles in different severity EV71-infected hand foot and mouth disease patients

Jun Meng [a, 1], Zhenyu Yao [b, 1], Yaqing He [a], Renli Zhang [a], Hong Yang [a], Xiangjie Yao [a], Long Chen [a], Hailong Zhang [a], Jinquan Cheng [a, *]

[a] Shenzhen Center for Disease Control and Prevention, Shenzhen, Guangdong, China
[b] Department of Translational Medicine R&D Center, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, China

ARTICLE INFO

Article history:
Received 18 September 2017
Accepted 25 September 2017
Available online xxx

Keywords:
Enterovirus 71
Long non-coding RNA
Hand foot and mouth disease
Inflammation
Innate immunity

ABSTRACT

Enterovirus 71 (EV71) is associated with the severe hand foot and mouth disease (HFMD) outcomes, however the host-virus interaction mechanism and the pathogenesis remain poorly understood. Long non-coding RNAs (lncRNAs) are involved in variety physiological and pathological processes, but the functions of lncRNAs in EV71 infection remain elusive. Here we profiled the expression of lncRNAs in peripheral blood mononuclear cells (PBMCs) from EV71-infected mild patients, severe patients as well as the healthy controls, and identified 8541 lncRNAs were differentially expressed. Focused on the dynamic changed lncRNAs, we performed systematic bioinformatics analysis with Series Test of Cluster (STC) algorithm, Gene Ontology (GO) analysis, pathway analysis and lncRNA-mRNA co-expression network analysis, and revealed the potential functions and related pathways of these lncRNAs were associated with immunity and inflammation during the clinical process of EV71-infected HFMD. Among the significant dynamic changed lncRNAs, ten lncRNAs were screened whose expression were further validated in EV71-infected mild patients, severe patients and healthy control. These results shed light on the potential roles of lncRNAs in EV71-infected HFMD, especially in distinguishing the mild and severe cases for early diagnose and treatment, moreover, provide deeper insight into the mechanism of EV71-induced immune and inflammatory responses, as well as the pathogenesis of the imbalanced inflammation in severe EV71 infection.

1. Introduction

Enterovirus 71 is well known as one of the major causative agents of Hand, foot and mouth disease (HFMD), which usually initiates self-limiting acute febrile disease followed by papulovesicular rashes on the buccal mucosa and palms, soles and buttocks [1]. However, EV71 infection is also associated with severe neurological manifestations, including aseptic meningitis, acute flaccid paralysis and brainstem encephalitis, resulting in autonomic dysregulation, fulminant pulmonary edema, myocardial dysfunction, shock and severe sequelae, even death [2]. Accumulating evidences propose that the mutant of the virus, as well as the age, immune status and genetic profiles of the host may be involved in the clinical phenotypes exhibited by EV71-infected individuals [3], nevertheless, what determines the clinical outcome of EV71-induced HFMD is poorly understood.

The long non-coding RNAs (lncRNAs) are non-protein-coding transcripts at least 200 nucleotides, which play versatile roles in diverse physiological and pathological processes [4]. In 2009, the potential function of lncRNAs in innate immunity were identified for the fist time [5]. Since then, increasing number of lncRNAs have been identified to participated in innate immune system and the related immune and inflammatory responses, such as lincRNA-COX2 [5], Lethe [6], PACER [7] and lnc-DC [8]. The innate immune system is the first defense line of the host to recognize and clear the invasion pathogens. The tightly regulation of innate immune system is closely associated with the elimination efficiency of pathogen infection. Although, many lncRNAs have been uncovered exhibiting flexible manners in regulation of innate immunity, the function of most of lncRNAs are still unknown and need to be discovery.

* Corresponding author.
E-mail address: jinquancheng01@163.com (J. Cheng).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2017.09.141
0006-291X/

Application of genome-sequence and microarray technologies have facilitated the deciphering of dramatic changes in the host transcriptome upon virus infection, and the function of lncRNAs in host-virus interaction attract more attentions [9]. Virus-inducible non-coding RNA (VINC) is the first reported viral infection related lncRNA [10]. There were widespread differential regulation of lncRNAs during SARS-CoV infection [11]. Moreover, the lncRNAs have distinctive kinetic expression profiles in type I interferon receptor and STAT1 knockout mice infected with SARS-CoV, including unique signatures of lncRNAs expression associated with lethal infection [11]. The expression of lncRNA Tmevpg1 (also known as NeST) was increased and essential for the host persistence of Theiler's virus [12]. In EV71-infected Rhabdomyosarcoma (RD) cells, more than 4800 lncRNAs differentially expressed [13], however, whether lncRNAs play essential roles in host-EV71 interaction is still lack in vivo and clinical evidences, which is needed further exploration.

Herein, we identified there were thousands of lncRNAs differently expressed in EV71-infected HFMD mild and severe patients and the healthy controls, and focused on the dynamic changed lncRNAs to performed the systematic bioinformatics analysis with GO analysis, pathway analysis as well as the lncRNA-mRNA co-expression network analysis, to reveal the potential roles of lncRNAs in EV71-infection.

2. Materials and methods

2.1. Study design and populations

In this case-control study, peripheral blood samples were obtained from 42 HFMD patients and 20 healthy controls in Shenzhen Children's Hospital, Shenzhen Baoan District People's Hospital and Shajing Institution of Disease Prevention and Healthcare from 2015 to 2016. All of the patients were confirmed as EV71 infection, through EV71 isolation and sequence identification with clinical samples, such as stool, rectal and throat swabs. Meanwhile, the patients were diagnosed with HFMD according to the WHO Guide to Clinical Management and Public Health Response for HFMD, and were further divided into 20 mild and 22 severe patients. Healthy candidates were age sex matched with the patients, and were identified without EV71 infection and other infection disease. All of the participants were informed consent and the study was approved by the Ethics Committee of the Shenzhen Center for Disease Control and Prevention.

2.2. RNA isolation

The peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples by Ficoll paque peremium (GE Healthcare Life Science) within 2 h. The total RNA was extracted from the PBMCs using Trizol reagent (Invitrogen Corporation) and was validated with Agilent Array platform for microarray assay or store at −80 °C.

2.3. Microarray analysis

The microarray analysis was performed with the Affymetrix Human Transcriptome Array 2.0 (HTA 2.0) (Affymetrix), which contains more than 40,000 non-coding and 245,000 coding transcripts in human genome. Each transcript cloud be identified precisely by specific exon or exon-exon splice junction probes. The transcripts with $P < 0.05$ were selected, after significant and false discovery rate (FDR) analysis [14–16]. The microarray assay and bioinformatics analysis were performed by Gminix Biotechnology Company (Shanghai, China).

2.4. STC analysis

STC (Series Test of Cluster) were employed to study the gene expression dynamics profiles and to determine the profiles containing significant higher number of genes, revealing the change rule of gene expression [17]. We selected differentially expressed genes with randomized variance model corrected ANOVA. Profiles that are significant have higher probability than expected by Fisher's exact test and multiple comparison tests [18,19].

2.5. GO analysis and pathway analysis

Gene Ontology (GO) analysis was used to explore the function of differentially expressed genes, and to assign the genes to biological processes GO terms according to the annotations [20]. The pathway analysis was based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to determine the significant pathway of the differentially expressed genes [20]. The GO terms and the KEGG pathways that had $P < 0.05$ were chosen.

2.6. Construction of the lncRNA-mRNA co-expression network

The lncRNA-mRNA co-expression network was constructed to clarify the interaction between differentially expressed lncRNAs and mRNAs in Profile9. Each lncRNA-mRNA pair was analyzed with Pearson correlation to choose the significant pairs for co-expression network construction [21,22]. The lncRNA-mRNA pairs which with correlation coefficient significant more than 0.95 were selected.

2.7. Quantitative real-time PCR (qRT-PCR)

The total RNA was extracted with Trizol reagent (Invitrogen Corporation). Reverse Transcription was performed using One Step RT-PCR Kit (Takara). A lightCycler (Roche) and SYBR Quantitative real-time PCR kit (Takara) were employed for Q-PCR as described previously [23]. U6 served as the endogenous control. The primer sequences were presented in Table S1.

2.8. Statistical analysis

All statistical analysis were performed using SPSS version 17.0 software. ANOVA was used for multiple comparisons. P-values <0.05 were considered statistically significant.

3. Results

3.1. Genome-wide lncRNAs change in mild and severe EV71-infected HFMD patients and healthy control

To determine the differential regulation of lncRNAs after EV71 infection, we analyzed the expression profiles of lncRNAs and mRNAs in peripheral blood mononuclear cells (PBMCs) derived from 10 mild HFMD patients, 12 severe HFMD patients and 10 healthy candidates, which were age (34.10 ± 17.10, 33.33 ± 13.81, 35.90 ± 11.41, $P = 0.91$) and gender (male, 5(10), 6(12), 5(10)) matched. A differential expression profile of each group was obtained with comparison between healthy control, mild patients and severe patients group, which showed that the expression of 8541 lncRNAs were significantly changed ($P < 0.05$) and 5955 mRNAs were significantly changed ($P < 0.05$) (Tables S2 and S3). The Hierarchical clustering analysis demonstrated general variations in lncRNAs and mRNAs expression in the PBMCs of EV71-infected HFMD patients (Fig. 1A and B). These data indicate that the expression of large amount of lncRNAs are differently regulated in

Please cite this article in press as: J. Meng, et al., Long non-coding RNA expression profiles in different severity EV71-infected hand foot and mouth disease patients, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.09.141