Int J Clin Exp Pathol 2016;9(10):10363-10372
www.ijcep.com /ISSN:1936-2625/IJCEP0035672

Original Article

EV71 infection causes differential expression of microRNAs in colon carcinoma cells

Zhihong Nie[1*], Fanfan Cao[2*], Bojing Li[1], Lili Yuan[3]

[1]Department of Gastroenterology, Shanghai Gongli Hospital, The Second Military Medical University, Pudong New Area, Shanghai 200135, P.R. China; [2]Sino-French Cooperative Central Lab, Shanghai Gongli Hospital, The Second Military Medical University, Pudong New Area, Shanghai 200135, P.R. China; [3]Department of Gestroenterology, Shanxi Dayi Hospital, Taiyuan, Shanxi, P.R. China. [*]Co-first authors.

Received July 13, 2016; Accepted July 21, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Purpose: We aimed to clarify the mechanism of EV71 infection impacting microRNA (miRNA) expression by identifying the differentially expressed miRNAs and key target genes in human colon carcinoma cells with EV71 infection. Methods: The miRNA expression profile GSE57372 was downloaded from gene expression omnibus database. The differentially expressed miRNAs were identified and target genes were screened using miRWalk, followed by gene ontology (GO) function analysis for the target genes using DAVID. Besides, the co-regulation network was constructed using cytoscape and GO analysis was performed for the co-regulated target genes. Results: A total of 23 differentially expressed miRNAs were identified, including 16 up-regulated miRNAs and 7 down-regulated miRNAs. Total 2501 target genes were screened, which were regulated by 7 miRNAs, including 4 up-regulated miRNAs (hsa-miR-548a-3p, hsa-miR-570, hsa-miR-601 and hsa-miR-638) and 3 down-regulated miRNAs (hsa-miR-29b, hsa-miR-326 and hsa-miR-484). These genes were mainly enriched in the biological processes of neuron differentiation, cell morphogenesis involved in neuron differentiation and cellular component morphogenesis. Total 47 target genes were co-regulated, in which *NDST1*, *DLGAP2*, *PKNOX2* and *MYT1* were identified as key target genes and enriched in processes of neuron project, intracellular signaling cascade and intracellular non-membrane-bounded organelle. The hsa-miR-29b, hsa-miR-484 and hsa-miR-638 were identified as key miRNAs, which regulated all 4 key target genes. Conclusion: EV71 infection altered the expression of hsa-miR-29b, hsa-miR-484 and hsa-miR-638 which may play key roles in the progression of nervous lesions by co-regulating the expression of 4 key target genes, including *NDST1*, *DLGAP2*, *PKNOX2* and *MYT1*.

Keywords: EV71, colon cancer HT29, significant microRNA, target genes, gene ontology terms

Introduction

Enterovirus 71 (EV71) is a virus of the genus *Enterovirus* in the *Picornaviride* family, which is first isolated in California, USA [1]. EV71 is considered to be a major cause of hand, foot, and mouth disease (HFMD) which is characterized by multiple lesions on skins and mucosa of oral, hand and foot [2]. In addition, some severe neurologic complications, such as encephalitis and polio-like paralysis, also develop in patients infected by EV71 [3]. This virus is mainly transmitted by hand contact and young children are the highest risk group [4]. Recently, analysis on mechanism of EV71 infection has been performed, which may provide a new insight for both academic research and clinical treatment.

microRNAs (miRNAs) are endogenous RNA with a length of about 22-nucleotide [5] and serve as major-regulators of gene expression by pairing to the mRNAs of protein-coding genes to direct their posttranscriptional repression [6]. In recent years, miRNAs have been considered as key regulators in many biological processes [7], and the alteration of miRNA expression is highly associated with development of some diseases, such as various human cancers [8]. In case of EV71 infection, Cui et al. [9] identified cellular miRNAs and biological processes involved in the host response to EV71 infection, supporting that certain miRNAs might be essential in the host-pathogen interactions. Thus, further analysis on the miRNAs in EV71 infection is implicated for the research of treatment.

In the present study, we downloaded the miRNA microarray profile of GSE57372 from Gene Expression Omnibus (GEO) database. This dataset was obtained from colon carcinoma cell line HT29 which is isolated from a Caucasian female with colon tumor [10], and is widely used in various researches of intestinal cell function and diseases [11] including EV71 infection [12]. There are EV71 infected samples and not infected samples in this dataset. The differentially expressed miRNAs between the two groups were screened. Then the target genes of miRNAs were performed functional analysis. Besides, co-regulated network was constructed and key target genes were identified. We aimed to clarify the mechanism of EV71 infection impacting miRNA expression by identifying key miRNAs and key target genes in colon carcinoma cells.

Materials and methods

Microarray data and data preprocessing

The miRNA microarray profile of GSE57372 was downloaded from Gene Expression Omnibus (GEO) database in National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/) based on the platform of GPL14613 [miRNA-2_0] Affymetrix Multispecies miRNA-2_0 Array. The microarray profile contains 6 human colon carcinoma cells (HT29) samples, including 3 EV71 infected samples and 3 not infected samples as control.

The probe-level data were converted into gene expression measures. If multiple probes corresponded to the same gene expression value, their mean value was calculated as the gene expression value. For probes with missing values, we used KNN method of impute package in R language to supplement the missing values [13]. Quantile normalization was performed by using preprocessCore package in R language [14] and box plots were generated.

Screening of differentially expressed miRNA

After the microarray data were preprocessed, the differentially expressed miRNAs between EV71 infection group and control group were identified by using limma package in R [15] with p-value<0.05 and $|\log_2 FC \text{ (fold change)}| > 0.585$.

Prediction and functional analysis of target genes

The target genes of significant differentially expressed miRNAs were identified by using miRWalk [16], in which 10 algorithms were referenced, including DIANAmT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR4, PICTAR5, PITA, RNA22 and Targetscan. The genes that were detected by more than 5 algorithms were screened for the construction of co-regulation network. Cytocape [17] was used for visualization of the network. Besides, the target genes of all miRNAs were performed Gene Ontology (GO) biological process (BP) analysis using DAVID [18].

Co-regulation network construction and key target genes analysis

The TRANSFAC database on eukaryotic transcriptional regulation is a database comprises data of transcription factors, target genes and regulatory binding cites [19]. The University of California Santa Cruz (UCSC) Genome Browser is a web-based set of tool providing access to a database of genome sequence and annotation [20]. Transcription factors among the target genes were screened based on the TRANSFAC database [21] and the information on transcription regulation was analyzed with UCSC database. In addition, analysis of all three GO terms, including biological process (BP), cellular component (CC) and molecular function (MF), was performed for the target genes coregulated by miRNAs by using DAVID [18] with p-value<0.05.

Results

Screening of differentially expressed miRNA

As shown in **Figure 1A**, the obscuring variations in raw expression data were normalized after being preprocessed. A total of 23 significant differentially expressed miRNAs were identified, including 16 up-regulated miRNAs and 7 down-regulated miRNAs. The volcano plot was shown in **Figure 1B**.

Prediction and functional analysis of target genes

According to the 10 algorithms in miRWalk, a network of 3091 interactions were constructed

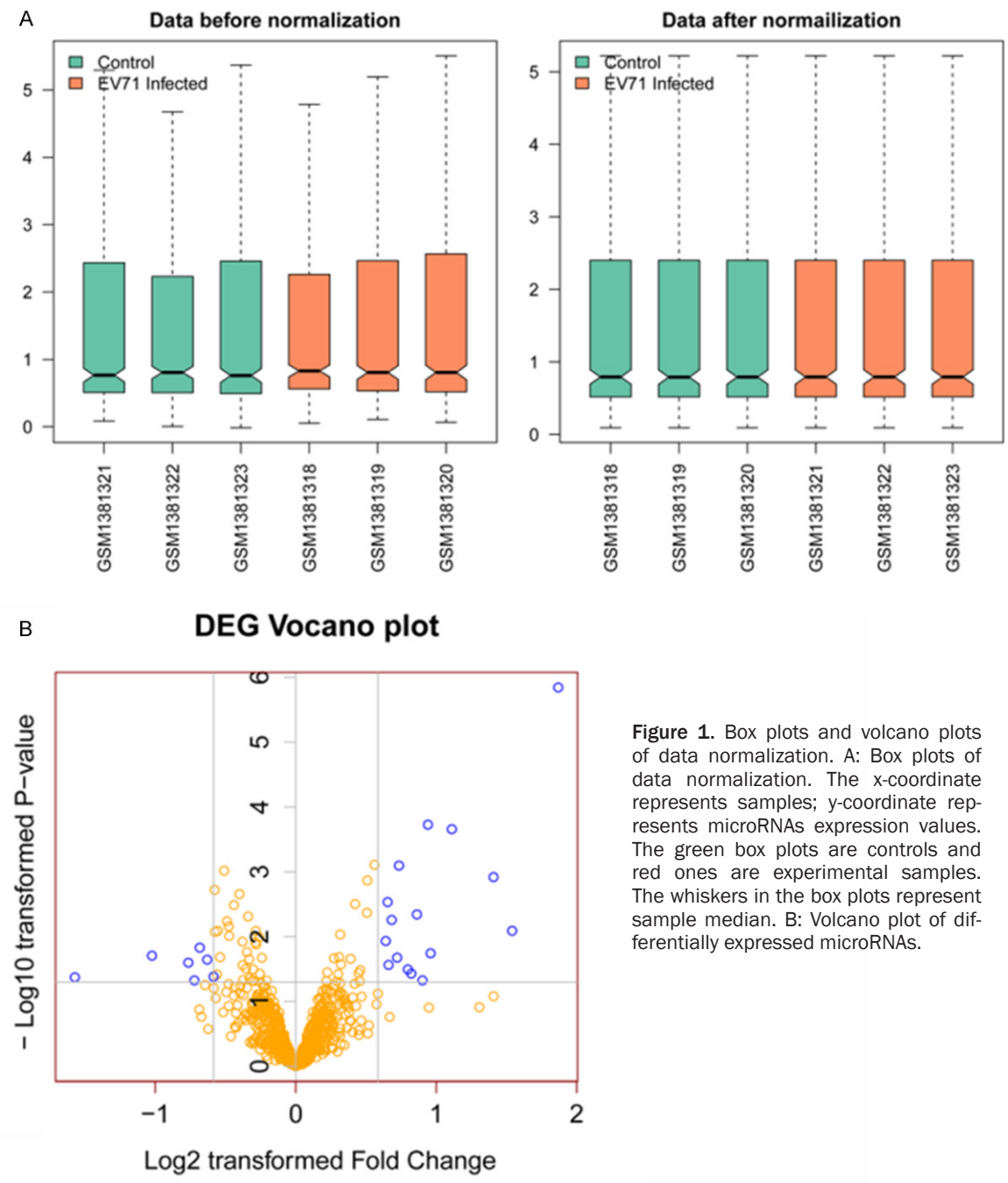

Figure 1. Box plots and volcano plots of data normalization. A: Box plots of data normalization. The x-coordinate represents samples; y-coordinate represents microRNAs expression values. The green box plots are controls and red ones are experimental samples. The whiskers in the box plots represent sample median. B: Volcano plot of differentially expressed microRNAs.

(**Figure 2**), in which 7 differentially expressed miRNAs, including 4 up-regulated miRNAs (hsa-miR-548a-3p, hsa-miR-570, hsa-miR-601 and hsa-miR-638) and 3 down-regulated miRNAs (hsa-miR-29b, hsa-miR-326 and hsa-miR-484), and 2501 target genes were involved. In the 2501 target genes, collagen, type XI, alpha 1 (*COL11A1*), collagen, type III, alpha 1 (*COL3A1*) and hemicentin 1 (*HMCN1*) were detected by all 10 algorithms.

The top five significant GO terms for target genes were listed in **Table 1**. The target genes of up-regulated miRNAs were mainly enriched in cell morphogenesis, cellular component morphogenesis, dendrite development and regulation of transcription from RNA polymerase II promoter. The target genes of down-regulated miRNAs were significantly enriched in the processes of neuron differentiation, cell morphogenesis involved in neuron differentiation,

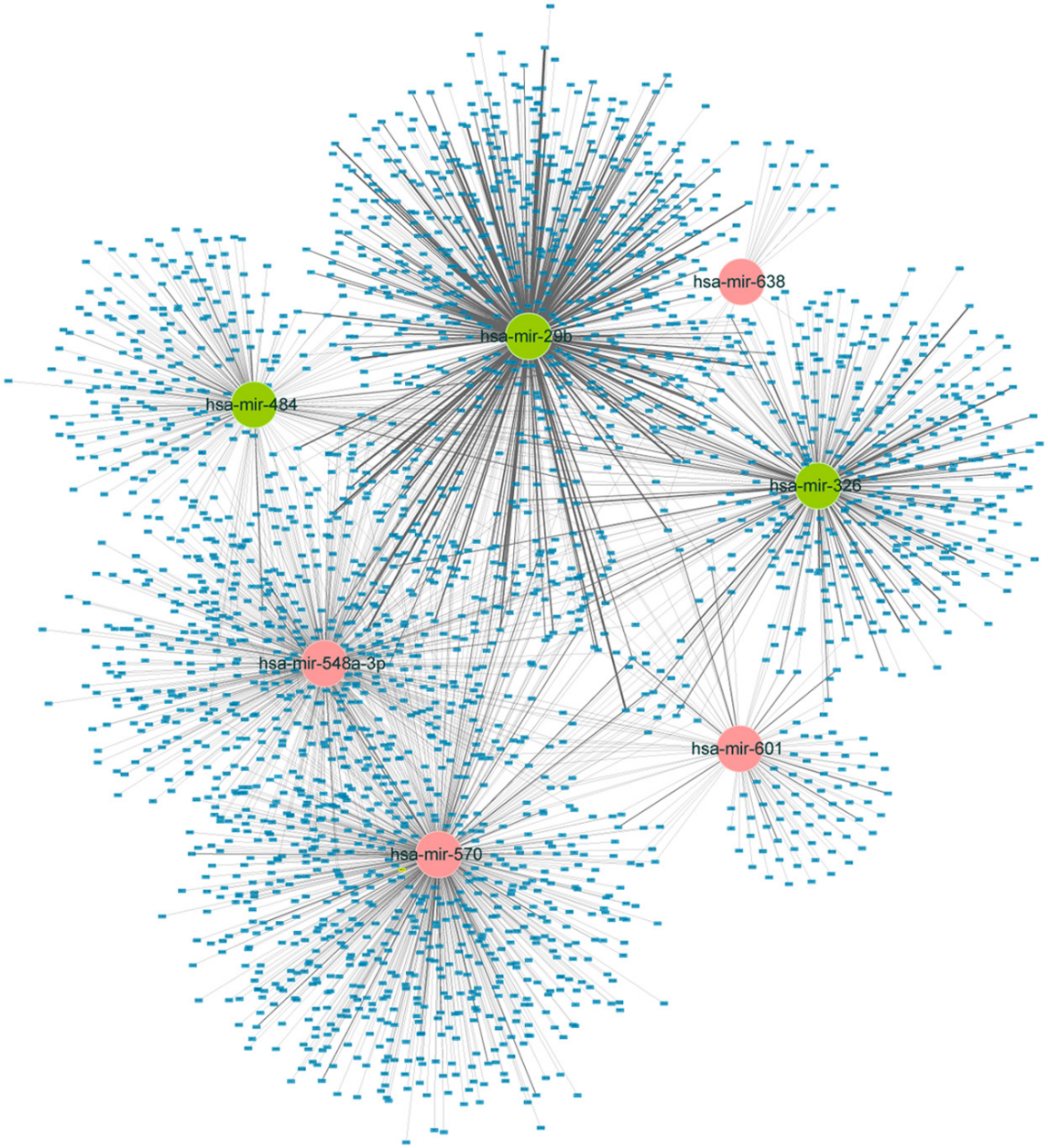

Figure 2. microRNA regulation network. Red nodes: up-regulated microRNAs; green nodes: down-regulated microRNAs; blue nodes: target genes.

intracellular transport, and cardiac muscle tissue development.

Co-regulation network construction

A target gene may be regulated by multiple miRNAs. As shown in **Figure 3**, 47 target genes were found to be regulated by more than 3 miRNAs. Among them, N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 1 (*NDST1*) was regulated by 4 miRNAs (hsa-mir-601, hsa-mir-484, hsa-mir-638 and hsa-mir-29b) and discs, large (Drosophila) homolog-associated protein 2 (*DLGAP2*) was regulated by 5 miRNAs (hsa-mir-638, hsa-mir-29b, hsa-mir-484, hsa-mir-326 and hsa-mir-548a-3p).

Key target genes analysis

By using TRANSFAC database, 3 transcription factors including PBX/knotted 1 homeobox 2

Table 1. BP Terms of target genes regulated by 7 differentially expressed microRNAs

microRNA	Term	Description	Count	*P*-value
Up-regulated miRNAs				
hsa-mir-548a-3p	GO:0006350	transcription	107	9.89E-06
	GO:0000902	cell morphogenesis	29	3.20E-05
	GO:0032989	cellular component morphogenesis	30	8.90E-05
	GO:0045449	regulation of transcription	121	1.40E-04
	GO:0031175	neuron projection development	22	1.74E-04
hsa-mir-570	GO:0016358	dendrite development	10	3.95E-06
	GO:0006357	regulation of transcription from RNA polymerase II promoter	50	4.44E-05
	GO:0045893	positive regulation of transcription, DNA-dependent	37	5.02E-05
	GO:0051254	positive regulation of RNA metabolic process	37	5.98E-05
	GO:0010557	positive regulation of macromolecule biosynthetic process	45	1.16E-04
hsa-mir-601	GO:0008219	cell death	13	0.003696468
	GO:0016265	death	13	0.003907906
	GO:0016568	chromatin modification	7	0.011474331
	GO:0030148	sphingolipid biosynthetic process	3	0.019102083
	GO:0043516	regulation of DNA damage response, signal transduction by p53 class mediator	2	0.020483938
hsa-mir-638	GO:0048568	embryonic organ development	4	0.001097795
	GO:0048562	embryonic organ morphogenesis	3	0.008827332
	GO:0030182	neuron differentiation	4	0.009143578
	GO:0045664	regulation of neuron differentiation	3	0.011426398
	GO:0030900	forebrain development	3	0.011557697
Down-regulated miRNAs				
hsa-mir-29b	GO:0048511	rhythmic process	18	1.02E-05
	GO:0009891	positive regulation of biosynthetic process	51	2.28E-05
	GO:0031328	positive regulation of cellular biosynthetic process	50	3.22E-05
	GO:0010557	positive regulation of macromolecule biosynthetic process	48	4.18E-05
	GO:0045935	positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	46	5.62E-05
hsa-mir-326	GO:0030182	neuron differentiation	31	2.25E-06
	GO:0048667	cell morphogenesis involved in neuron differentiation	20	3.30E-06
	GO:0007409	axonogenesis	19	4.17E-06
	GO:0048666	neuron development	26	4.49E-06
	GO:0000904	cell morphogenesis involved in differentiation	21	8.88E-06
hsa-mir-484	GO:0046907	intracellular transport	19	0.006751147
	GO:0009628	response to abiotic stimulus	13	0.007131466
	GO:0009314	response to radiation	9	0.008491462
	GO:0048738	cardiac muscle tissue development	5	0.009120295
	GO:0007517	muscle organ development	9	0.011512654

BP: biological process; GO: gene ontology.

(PKNOX2), myelin transcription factor 1 (MYT1) and hepatic leukemia factor (HLF) were identified among 47 coregulated target genes. After GO terms analysis for the 47 coregulated genes we found that these target genes were significantly enriched in functions associated with neuron development and differentiation (**Table 2**).

Discussion

EV71 infection is a major cause of HFMD and other neurologic complications [3]. As miRNAs have been recognized to play important roles in the development of some diseases [22], analysis of key miRNAs altered by EV71 infection may provide new insights for the nosetiology research of these diseases. In this study, we identified 23 differentially expressed miRNAs in human colon carcinoma cells with EV71 infection. The target genes of the differentially expressed miRNAs were predicted. A co-regulation network with 47 target genes, such as *NDST1*, *DLGAP2*, *PKNOX2*, and *MYT1*, and 7 significant differentially expressed miRNAs, including hsa-miR-29b, hsa-miR-326, hsa-miR-484, hsa-miR-570, hsa-miR-548a-3p, hsa-miR-601 and hsa-miR-638 was constructed. The 4

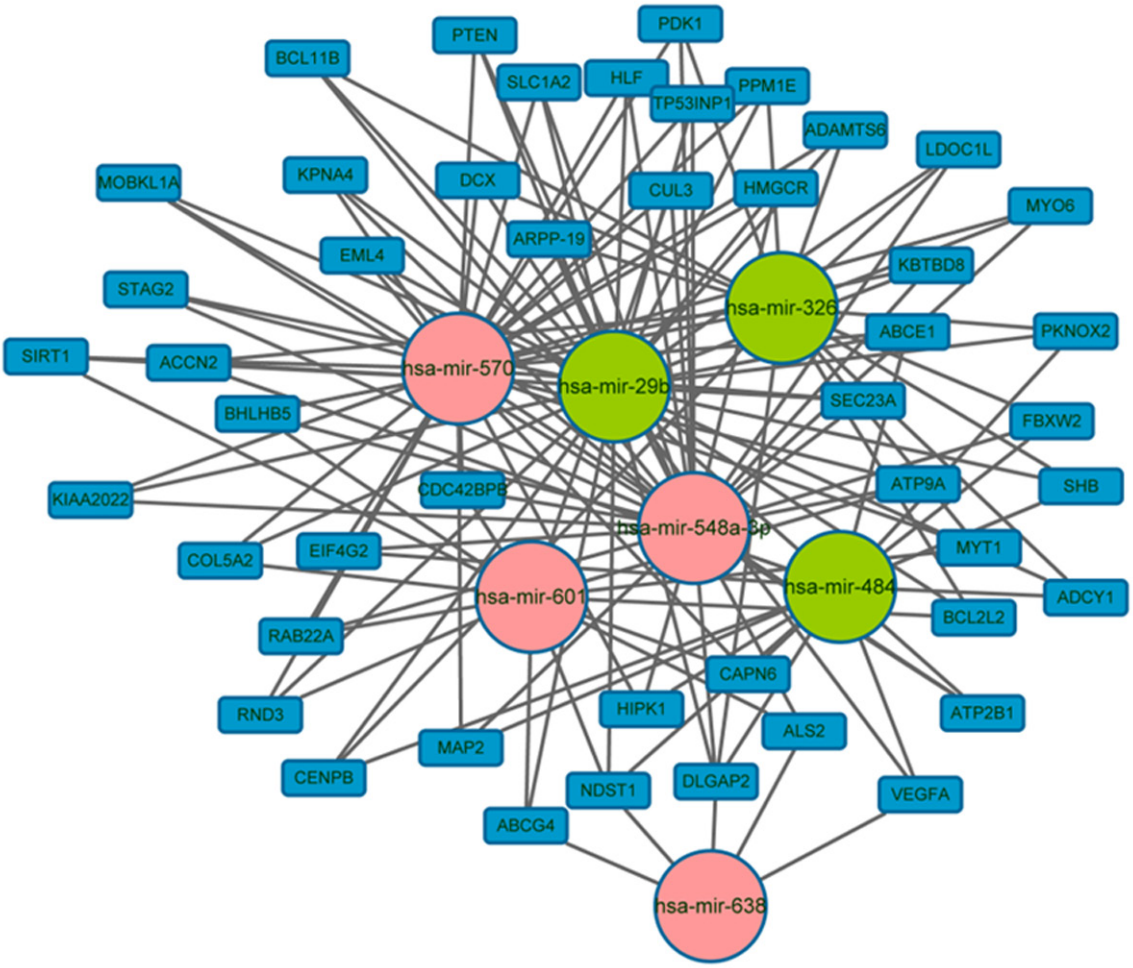

Figure 3. microRNA co-regulation network. Red nodes: up-regulated microRNAs; green nodes: down-regulated microRNAs; blue nodes: target genes.

Table 2. GO Terms of 47 co-regulated target genes

Category	Term	Description	Count	*P*-value
BP	GO:0048666	neuron development	7	5.08E-04
BP	GO:0031175	neuron projection development	6	0.001002346
BP	GO:0030182	neuron differentiation	7	0.001913688
BP	GO:0008406	gonad development	4	0.004684792
BP	GO:0030030	cell projection organization	6	0.004858244
CC	GO:0031252	cell leading edge	4	0.005769199
CC	GO:0005856	cytoskeleton	10	0.008399241
CC	GO:0030425	dendrite	4	0.009118479
CC	GO:0043005	neuron projection	5	0.01240166
CC	GO:0015630	microtubule cytoskeleton	6	0.014243266
MF	GO:0005516	calmodulin binding	4	0.007858214
MF	GO:0000166	nucleotide binding	14	0.008907333
MF	GO:0000287	magnesium ion binding	6	0.00973747
MF	GO:0005161	platelet-derived growth factor receptor binding	2	0.031740937
MF	GO:0032555	purine ribonucleotide binding	11	0.03461259

GO: gene ontology; BP: biological process; CC: cellular component; MF: molecular function.

target genes significantly enriched in the biological processes including neuron project, intracellular signaling cascade and intracellular non-membrane-bounded organelle.

Both hsa-miR-29b and hsa-miR-484 were key down-regulated miRNAs and regulated the expression of all 4 key genes (*NDST1*, *DLGAP2*, *PKNOX2*, and *MYT1*). hsa-miR-29b has been reported in various diseases, such as Alzheimer's disease [23] and lung cancer [24]. Virus infection was considered as a cause of hsa-miR-29b aberrant expression. For example, in the serum with avian-origin influenza A (H7N9) infection, hsa-miR-29b is detected to be down-regulated expressed [25]. Besides, H7N9 infection has also been found alter the expression of hsa-miR-484 which has been reported to be related with apoptosis in cardiomyocytes [26]. However, hsa-miR-29b and hsa-miR-484 have not been found to be affected by EV71 infection. Based on the analysis in this study, we speculated that hsa-miR-29b and hsa-miR-484 may be important targets of EV71 by regulating 4 key target genes.

DLGPA2 was a key target gene in this study and was mainly enriched in processes associated with neuron projection and intracellular non-membrane-bounded organelle. *DLGPA* family encode isoforms of SAP90/PSD95 associated proteins (SAPAPs), which are involved in the pathophysiology of various psychiatric disorders [27]. DLGPA2, also known as 2 (SAPAP2), is a member of DLGPA family [28]. As one of the main components of postsynaptic scaffolding proteins, DLGPA2 interacts with discs, large homolog 4 (DLG4) and SH3 and multiple ankyrin repeat domains 3 (SHANKs) to form the DLG4-DLGAPs-SHANKs complex, which plays critical roles in synaptic morphogenesis and functions [29], and are involved in the stabilization of synaptic junction and regulation of neurotransmission. Therefore, the appropriate expression of DLGPA2 is related to the function of neural communication. The mutation of DLGPAs has been reported to be associated with some diseases, such as Tourette syndrome [30], progressive epilepsy [31] and schizophrenic [32]. Thus, we speculated that the aberrant of DLGPAs caused by the coregulation of hsa-miR-29b and hsa-miR-484 may be a major cause of the neurologic complications symptom with EV71 infection.

NDST was identified as a key target gene in this study and enriched in the biological process of intracellular signaling cascade. NDST encodes a bifunctional enzyme that catalyzes both the N-deacetylation and the N-sulfation of glucosamine in heparin sulfate (HS) [33]. HS is a highly sulfated linear polysaccharide ubiquitously present on the cell surface and in the extra cellular matrix, and influences diverse biological functions, including organogenesis, growth factor, angiogenesis and cell adhesion [34]. HS influences these processes by the ionic interaction binding with a variety of proteins, which depends on the charge and structure of HS chain [35]. The NDST interaction is the first major modification in the process of HS biosynthesis and important for the subsequent interactions [36]. Thus EV71 infection may impair the expression of NDST by regulating miRNAs expression, which leads to the lesion of organogenesis and angiogenesis.

PKNOX2 and MYT1 were identified as transcription factors. *PKNOX2* was enriched in some key processes including microtubule cytoskeleton, intracellular non-membrane bounded organelle and non-membrane bounded organelle. The encoded protein PKNOX2, also known as PREP2, is a member of PREP family, which belongs to the TALE (three amino acid loop extension) class of homeodomain-containing transcription factors [37]. The TALE proteins, such as PREP1 and PREP2, are regulators of Pbx activity and form transcriptionally active complexes with Pbx [38], which play a central role in the development of organogenesis [39]. For example, during organogenesis, Pbx-1 has a potential function in determining cell fate in various tissues that depends on mesenchymal-epithelial interactions for the coordinate morphogenesis [40] and Pbx-1-deficient is related closely with the embryonic lethal phenotype [41]. Therefore, the lesion symptom on organs caused by EV71 infection may result from the disordered expression of *PKNOX2*, which was regulated by hsa-mir-29b and hsa-mir-484. *MYT1* encodes a zincfinger DNA binding protein that indicates a potential role in regulating oligodendrocyte progenitor cell proliferation [42]. It is originally identified based on binding affinity within the promoter region of the proteolipid gene, which is the most abundantly transcribed myelin gene in central nerves system [43]. Besides, MYT1 acts synergistically with neuro-

genin in the notch signaling pathway to promote the neuronal differentiation [44] and is necessary for the endocrine islet development [45]. Aberrant expression of MYT1 may cause variable oligodendrocyte development, which can contribute to demyelinating disease and leukodystrophies [46]. EV71 infection altered the expression of hsa-mir-29b, hsa-mir-484 that target *MYT1*, which may result in abnormal neuronal impulse conduction and neurologic dysfunction.

hsa-miR-638 was identified as a key upregulated miRNA and regulated the expression of *DLGPA2* and *NDST*. The main GO terms enriched by the target genes of hsa-miR-638 included embryonic organ development and neuron differentiation. hsa-miR-638 has been reported to be expressed aberrantly in some lesion cells, such as human lung cancer cells [47], lupus nephritis glomerular cells [48] and immortalized human bronchial epithelial cells exposed to benzopyrene [49]. However, the correlation between miR-638 and EV71 infection has been scarcely reported. In this study, among two target genes of hsa-miR-638, *NDST* was mainly functions in the organogenesis, while *DLGPA2* was associated with the neurologic projection. We speculated that the up-regulation of hsa-miR-638 caused by EV71 infection might alter the expression of *NDST* and *DLGPA2*, leading to the lesion symptoms of both organogenesis and nervous system.

In conclusion, EV71 infection altered the expression of hsa-mir-29b, hsa-mir-484 and hsa-miR-638. These miRNAs may play key roles in the progression of nervous lesions caused by EV71 infection by regulating *NDST1*, *DLGAP2*, *PKNOX2* and *MYT1* which were enriched in processes of neuron project and organogenesis. However, the sample size of the microarray is small, which may be a limitation of our conclusion. Besides, experiments should be performed for the verification of our conclusion.

Acknowledgements

This work was supported by Shanghai Pudong Youth Talent Project in Medicine (No.: PWRq2013-11), Shanghai Sailing Program (No.: 15YF 1410800) and Health Science and Technology Project of Pudong New Area Health and Family Planning Commission (No.: PW2015A-16).

Disclosure of conflict of interest

None.

Address correspondence to: Bojing Li, Department of Gastroenterology, Shanghai Gongli Hospital, The Second Military Medical University, 219 Miaopu Road, Pudong New Area, Shanghai 200135, P.R. China. Tel: +86-21-58858730; Fax: +86-21-58858730; E-mail: merrylbj@aliyun.com; Lili Yuan, Department of Gestroenterology, Shanxi Dayi Hospital, 99 Longcheng Avenue, Taiyuan 030032, Shanxi, P.R. China. E-mail: dangyuan831@sina.com

References

[1] Schmidt NJ, Lennette EH and Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. J Infect Dis 1974; 129: 304-309.

[2] De W, Changwen K, Wei L, Monagin C, Jin Y, Cong M, Hanri Z and Jun S. A large outbreak of hand, foot, and mouth disease caused by EV71 and CAV16 in Guangdong, China, 2009. Arch Virol 2011; 156: 945-953.

[3] Hamaguchi T, Fujisawa H, Sakai K, Okino S, Kurosaki N, Nishimura Y, Shimizu H and Yamada M. Acute encephalitis caused by intrafamilial transmission of enterovirus 71 in adult. Emerg Infect Dis 2008; 14: 828.

[4] Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P and Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis 2010; 10: 778-790.

[5] Ruby JG, Jan CH and Bartel DP. Intronic microRNA precursors that bypass Drosha processing. Nature 2007; 448: 83-86.

[6] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[7] Kurzynska-Kokorniak A, Jackowiak P, Figlerowicz M and Figlerowicz M. Human-and virus-encoded microRNAs as potential targets of antiviral therapy. Mini Rev Med Chem 2009; 9: 927-937.

[8] Giovannetti E, van der Velde A, Funel N, Vasile E, Perrone V, Leon LG, De Lio N, Avan A, Caponi S and Pollina LE. High-throughput microRNA (miRNAs) arrays unravel the prognostic role of MiR-211 in pancreatic cancer. PLoS One 2012; 7: e49145.

[9] Cui L, Guo X, Qi Y, Qi X, Ge Y, Shi Z, Wu T, Shan J, Shan Y, Wang H and Zhu Z. Identification of microRNAs involved in the host response to enterovirus 71 infection by a deep sequencing

approach. BioMed Res Int 2010; 2010: 425939.
[10] Cencič A and Langerholc T. Functional cell models of the gut and their applications in food microbiology-a review. Int J Food Microbiol 2010; 141: S4-S14.
[11] Zweibaum A, Laburthe M, Grasset E and Louvard D. Use of cultured cell lines in studies of intestinal cell differentiation and function. Comprehensive Physiology 2011.
[12] Tan CW, Chan YF, Sim KM, Tan EL and Poh CL. Inhibition of enterovirus 71 (EV-71) infections by a novel antiviral peptide derived from EV-71 capsid protein VP1. PLoS One 2012; 7: e34589.
[13] Crookston NL and Finley AO. yaimpute: An r package for knn imputation. Journal of Statistical Software 2008; 23: 1-16.
[14] Bolstad B. preprocessCore: A collection of preprocessing functions. R package version 2013; 1.
[15] Gentleman R, Carey VJ, Huber W, Irizarry RA and Dudoit S. Bioinformatics and computational biology solutions using R and Bioconductor. Springer 2005.
[16] Dweep H, Sticht C, Pandey P and Gretz N. miRWalk-database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J Biomed Inform 2011; 44: 839-847.
[17] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
[18] Dennis Jr G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 2003; 4: P3.
[19] Matys V, Fricke E, Geffers R, Gößling E, Haubrock M, Hehl R, Hornischer K, Karas D, Kel AE and Kel-Margoulis OV. TRANSFAC®: transcriptional regulation, from patterns to profiles. Nucleic Acids Res 2003; 31: 374-378.
[20] Meyer LR, Zweig AS, Hinrichs AS, Karolchik D, Kuhn RM, Wong M, Sloan CA, Rosenbloom KR, Roe G, Rhead B, Raney BJ, Pohl A, Malladi VS, Li CH, Lee BT, Learned K, Kirkup V, Hsu F, Heitner S, Harte RA, Haeussler M, Guruvadoo L, Goldman M, Giardine BM, Fujita PA, Dreszer TR, Diekhans M, Cline MS, Clawson H, Barber GP, Haussler D, Kent WJ. The UCSC Genome Browser database: extensions and updates 2013. Nucleic Acids Res 2013; 41: D64-D69.
[21] Wingender E. The TRANSFAC project as an example of framework technology that supports the analysis of genomic regulation. Brief Bioinform 2008; 9: 326-332.
[22] Kloosterman WP and Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell 2006; 11: 441-450.
[23] Satoh JI. Molecular network of microRNA targets in Alzheimer's disease brains. Exp Neurol 2012; 235: 436-446.
[24] Liu YQ, Li Y, Qin J, Wang Q, She YL, Luo YL, He JX, Li JY and Xie XD. Matrine Reduces Proliferation of Human Lung Cancer Cells by Inducing Apoptosis and Changing miRNA Expression Profiles. Asian Pac J Cancer Prev 2014; 15: 2169.
[25] Zhu Z, Qi Y, Ge A, Zhu Y, Xu K, Ji H, Shi Z, Cui L and Zhou M. Comprehensive Characterization of Serum MicroRNA Profile in Response to the Emerging Avian Influenza A (H7N9) Virus Infection in Humans. Viruses 2014; 6: 1525-1539.
[26] Wang K, Long B, Jiao JQ, Wang JX, Liu JP, Li Q and Li PF. miR-484 regulates mitochondrial network through targeting Fis1. Nat Commun 2012; 3: 781.
[27] Li JM, Lu CL, Cheng MC, Luu SU, Hsu SH, Hu TM, Tsai HY and Chen CH. Role of the DLGAP2 Gene Encoding the SAP90/PSD-95-Associated Protein 2 in Schizophrenia. PLoS One 2014; 9: e85373.
[28] Jiang-Xie LF, Liao HM, Chen CH, Chen YT, Ho SY, Lu DH, Lee LJ, Liou HH, Fu WM and Gau SS. Autism-associated gene Dlgap2 mutant mice demonstrate exacerbated aggressive behaviors and orbitofrontal cortex deficits. Mol Autism 2014; 5: 32.
[29] Welch JM, Wang D and Feng G. Differential mRNA expression and protein localization of the SAP90/PSD-95-associated proteins (SAPAPs) in the nervous system of the mouse. J Comp Neurol 2004; 472: 24-39.
[30] Crane J, Fagerness J, Osiecki L, Gunnell B, Stewart SE, Pauls DL and Scharf JM; Tourette Syndrome International Consortium for Genetics (TSAICG). Family-based genetic association study of DLGAP3 in Tourette Syndrome. Am J Med Genet B Neuropsychiatr Genet 2011; 156: 108-114.
[31] Ranta S, Zhang Y, Ross B, Takkunen E, Hirvasniemi A, de la Chapelle A, Gilliam TC and Lehesjoki AE. Positional cloning and characterisation of the human DLGAP2 gene and its exclusion in progressive epilepsy with mental retardation. Eur J Hum Genet 2000; 8: 381-384.
[32] Li JM, Lu CL, Cheng MC, Luu SU, Hsu SH and Chen CH. Genetic analysis of the DLGAP1 gene as a candidate gene for schizophrenia. Psychiatry Res 2013; 205: 13-17.
[33] Sheng J, Liu R, Xu Y and Liu J. The dominating role of N-deacetylase/N-sulfotransferase 1 in

forming domain structures in heparan sulfate. J Biol Chem 2011; 286: 19768-19776.
[34] Ledin J, Ringvall M, Thuveson M, Eriksson I, Wilén M, Kusche-Gullberg M, Forsberg E and Kjellén L. Enzymatically active N-deacetylase/N-sulfotransferase-2 is present in liver but does not contribute to heparan sulfate N-sulfation. J Biol Chem 2006; 281: 35727-35734.
[35] Lindahl U and Li JP. Interactions between heparan sulfate and proteins-design and functional implications. Int Rev Cell Mol Biol 2009; 276: 105-159.
[36] Grobe K, Ledin J, Ringvall M, Holmborn K, Forsberg E, Esko JD and Kjellén L. Heparan sulfate and development: differential roles of the N -acetylglucosamine/N-sulfotransferase isozymes. Biochim Biophys Acta 2002; 1573: 209-215.
[37] Haller K, Rambaldi I, Daniels E and Featherstone M. Subcellular localization of multiple PREP2 isoforms is regulated by actin, tubulin, and nuclear export. J Biol Chem 2004; 279: 49384-49394.
[38] Meyers RA. Encyclopedia of molecular cell biology and molecular medicine. Wiley Online Library; 2005.
[39] Ferretti E, Villaescusa JC, Di Rosa P, Fernandez-Diaz LC, Longobardi E, Mazzieri R, Miccio A, Micali N, Selleri L, Blasi F and Ferrari G. Hypomorphic mutation of the TALE gene Prep1 (pKnox1) causes a major reduction of Pbx and Meis proteins and a pleiotropic embryonic phenotype. Mol Cell Biol 2006; 26: 5650-5662.
[40] Laurent A, Bihan R, Omilli F, Deschamps S and Pellerin I. PBX proteins: much more than Hox cofactors. Int J Dev Biol 2008; 52: 9-20.
[41] Manley NR, Selleri L, Brendolan A, Gordon J and Cleary ML. Abnormalities of caudal pharyngeal pouch development in Pbx1 knockout mice mimic loss of Hox3 paralogs. Dev Biol 2004; 276: 301-312.
[42] Nielsen JA, Berndt JA, Hudson LD and Armstrong RC. Myelin transcription factor 1 (Myt1) modulates the proliferation and differentiation of oligodendrocyte lineage cells. Mol Cell Neurosci 2004; 25: 111-123.
[43] Vana AC, Lucchinetti CF, Le TQ and Armstrong RC. Myelin transcription factor 1 (Myt1) expression in demyelinated lesions of rodent and human CNS. Glia 2007; 55: 687-697.
[44] Quan XJ, Denayer T, Yan J, Jafar-Nejad H, Philippi A, Lichtarge O, Vleminckx K and Hassan BA. Evolution of neural precursor selection: functional divergence of proneural proteins. Development 2004; 131: 1679-1689.
[45] Gu G, Wells JM, Dombkowski D, Preffer F, Aronow B and Melton DA. Global expression analysis of gene regulatory pathways during endocrine pancreatic development. Development 2004; 131: 165-179.
[46] Levine JM, Reynolds R and Fawcett JW. The oligodendrocyte precursor cell in health and disease. Trends Neurosci 2001; 24: 39-47.
[47] Rui W, Bing F, Hai-Zhu S, Wei D and Long-Bang C. Identification of microRNA profiles in docetaxel-resistant human non-small cell lung carcinoma cells (SPC-A1). J Cell Mol Med 2010; 14: 206-214.
[48] Lu J, Kwan BC, Lai FM, Tam LS, Li EK, Chow KM, Wang G, Li PK, Szeto CC. Glomerular and tubulointerstitial miR-638, miR-198 and miR-146a expression in lupus nephritis. Nephrology 2012; 17: 346-351.
[49] Li D, Wang Q, Liu C, Duan H, Zeng X, Zhang B, Li X, Zhao J, Tang S and Li Z. Aberrant Expression of miR-638 Contributes to Benzo (a) pyrene-induced Human Cell Transformation. Toxicol Sci 2011; 125: 382-91.