Identification of lncRNA–mRNA Regulatory Network Associated With Isolated Systolic Hypertension And Atherosclerotic Cerebral Infarction

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Research

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Abstract

Background: Increasing evidences uncover that lncRNAs play an important role in Isolated systolic hypertension (ISH). However, a systematic lncRNA-mRNA regulatory network is still absent in isolated systolic hypertension and atherosclerotic cerebral infarction patients (ISH & ACI).

Aim: This research aims to establish a lncRNA-mRNA co-expression network in patients with ISH & ACI, to probe into the potential functions of lncRNA in those patients.

Design and Setting: Expression profiles of lncRNA and mRNAs are collected and compared respectively from 8 patients with ISH and 8 patients with ISH & ACI by RNA-seq data.

Methods: Differentially expressed lncRNAs and mRNAs were screened out via high-throughput sequencing in the plasma of ISH/ACI patients and control ISH patients. Then, a lncRNA-mRNA interaction network was built using the Pearson correlation coefficient by Cytoscape software. The expression levels of the hub genes and lncRNAs were verified by qRT-PCR in another 10 ISH/ACI patients and 10 control patients.

Results: 2768 differentially expressed lncRNAs and 747 differentially expressed mRNAs were identified. 2 hub genes (CD226 and PARVB) and 11 lncRNAs were identified in the lncRNA-mRNA interaction network. qRT-PCR and cell assay results verified that lncRNAs ENST00000590604 and CD226 are highly expressed in patients of ISH & ACI. CD226 was associated with vascular endothelial cells growth and stability through platelet activation and focal adhesion pathway.

Conclusion: We established a novel mRNA-lncRNA interaction network. lncRNAs ENST00000590604 and CD226 might be the potential biomarkers of ISH & ACI.

How This Fits In

Occurrences of cerebral infarction are closely related to hypertension and wide pulse pressures have become an independent predictor for cardiovascular morbidity. However, a systematic lncRNA-mRNA regulatory network is still absent in isolated systolic hypertension and atherosclerotic cerebral infarction patients.

This paper indicates that lncRNAs ENST00000590604 and CD226 might be the potential biomarkers of ISH&ACI patients. In a clinical context, these lncRNAs can be adopted as biomarkers for precaution of ACI in ISH patients.

Introduction

Isolated systolic hypertension (ISH) is a major risk factor for cardiovascular morbidity and mortality, and the most common form of hypertension in the elderly population [1, 2]. With an ageing population, the prevalence of hypertension, particularly ISH, is rapidly rising [2]. Besides, ISH is associated with

cerebrovascular diseases, such as Isolated Systolic Hypertension and Atherosclerotic Cerebral Infarction patients (ISH&ACI) [2].

However, the underlying molecular mechanisms of ISH&ACI have not been elucidated. Therefore, we seek the key molecules involved in ISH&ACI and identify potential diagnostic biomarkers and therapeutic targets for ISH&ACI.

Long non-coding RNAs (lncRNAs) is a class of lncRNA longer than 200 nucleotides. lncRNAs are involved in many cellular processes, including chromatin remodeling, transcription modulation, and interacting with other RNAs to modulate gene expression [3]. With the rapid technological advances in high-throughput sequencing, the research of lncRNAs has made remarkable progress [4]. A lot of experimental evidence shows that lncRNAs play an important role in the development of hypertension and might be the potential key regulators of hypertension [5, 6]. lncRNAs TUG1/miR-145-5p/FGF10 regulates the proliferation and migration of vascular smooth muscle cell in hypertension by activating the Wnt/ β -catenin pathway [7]. Down-regulation of MRAK048635_P1 is probably an important factor for vascular remodeling in essential hypertension [8]. Nevertheless, the relationship between lncRNAs and ISH has not been reported.

In this paper, 946 up-regulated and 1822 down-regulated differentially expressed lncRNAs, and 288 up-regulated and 519 down-regulated differentially expressed mRNAs were identified between ISH&ACI cases and ISH controls, respectively. The GO and KEGG enrichment analyses revealed that differentially expressed lncRNAs and mRNAs were significantly enriched in platelet activation. According to the lncRNA-mRNA co-expression network, 2 hub genes (CD226 and PARVB) were identified and 9 lncRNAs were predicted to target these 2 hub genes. Then, the lncRNA-mRNA pairs were verified by real-time PCR in 20 individual samples. Besides, flow cytometer was performed to evaluate proliferation and apoptosis rate and real-time PCR was performed to validate the lncRNA-mRNA pairs in Human Brain Microvascular Endothelial Cells (HBMECs).

In summary, our results showed that lncRNAs ENST00000590604 regulated the expression of CD226 through platelet activation and focal adhesion pathway. lncRNAs ENST00000590604 and CD226 might be the potential biomarkers of isolated systolic hypertension and atherosclerotic cerebral infarction patients.

Materials And Methods

2.1 Study samples

A total of 18 ISH&ACI patients were recruited from the Affiliated Hospital of Shandong University of Traditional Chinese Medicine from May 2019 to April 2020. Isolated systolic hypertension and atherosclerotic cerebral infarction were defined according to the 2018 revised edition of the Chinese guidelines for the prevention and treatment of hypertension (exposure draft) and 2015 China Cerebrovascular Disease Classification. Meanwhile, subjects conforming to the following standards were

included: 1. aged 18 years old or above, no gender limitations; 2. within 2 weeks to 6 months after the occurrence of cerebral infarction; 3. willing to participate in this experiment and cooperate with the doctors and sign the informed consent in writing. A total of 18 isolated systolic hypertension (ISH) controls were recruited from the same hospital during the same period. 8 ISH&ACI patients and 8 ISH controls were randomly selected from 18 ISH&ACI patients and 18 ISH controls for RNA sequencing, and the remaining samples were used as replication population for the validation of differentially expressed lncRNAs and mRNAs.

The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine in Shandong, China. Written informed consent was obtained from all patients or their families under the Declaration of Helsinki.

2.2 RNA extraction, library preparation, and RNA-seq

RNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Germany) according to the manufacturer's protocol. RNA was then purified and concentrated using the RNeasy MinElute Cleanup Kit (QIAGEN, Germany). RNA quality was evaluated using NanoDrop 2000 (Thermo Fisher, USA). Sequencing libraries were generated using TruSeq Stranded Total RNA LT - (with Ribo - Zero™ Gold) - Set A (Illumina, USA) following the manufacturer's recommendations. The libraries were then sequenced on the Illumina HiSeq 2500 platform and 125bp/150bp paired-end reads were generated.

2.3 Quantitative real-time PCR (qRT-PCR) validation

qRT-PCR was used to validate selected lncRNAs and mRNAs in 20 individual samples. Reverse transcription was performed using the PrimeScript™ RT reagent Kit with gDNA Eraser (Sparkjade, Shandong, China). qRT-PCR was performed using a TB Green® Premix Ex Taq™ II (Sparkjade, Shandong, China) and an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher, USA) according to standard methods. Specific primers of lncRNAs and mRNAs were listed in Supplementary Table 1. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative fold-change to the house-keeping gene (β -action).

2.4 Cell

Cell culture: HBMECs (Hunan Fenghui Biotechnology Co.Ltd,Cat.CL0116) were cultured with high glucose's (4.5 mg/ml) Dulbecco's Modified Eagle Medium (DMEM, Gibco, REF.#C11995065BT) and supplemented with 10% Fetal Bovine Serum (FBS, Gibco, REF.10099141) and 1% Penicillin/Streptomycin solution (P/S, HyClone, Cat. #SV30010) at 37°C in a humidified 5% CO₂ incubator. Then, HBMECs were intervened by Sodium pyruvate solution Ang II (Solarbio Co., Ltd., Beijing, China) and OGD/R (Oxygen-Glucose Deprivation/Reoxygenation). HBMECs of exposed into AngⅡ and normoxia were served as controls.

MTT assay: The effect of AngⅡ and OGD/R on proliferation were detected by MTT assay. HBMECs were seeded at a density of 1500 cells per well into 96-well microplates in a final volume of 100 μ L with 2×10^{-6} mol/L of AngⅡ, and they were maintained for 12, 24, 48, or 72 hours at 37°C in a humidified atmosphere

containing 5% CO₂. After that, HBMECs were cultured in Earle's Balanced Salt Solution (EBSS, Solarbio, Cat.H2025) without glucose and fetal bovine serum and deprived-oxygen by transferring into an anaerobic incubator (0% O₂/5%CO₂/95%N₂) for 4 hours. After OGD, cells were returned to a normoxic incubator under 5% CO₂/95% air for 24 hours in complete medium. Subsequently, to monitor cell viability, 10 µl of sterile 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl-2H-tetrazolium bromide dye (MTT; Solarbio, Lot.#303H0525, Beijing, China) at a final concentration of 5mg/ml was added to each well for incubation at 37°C for 4h; this was followed by the administration of 150 µl dimethyl sulfoxide solution (DMSO; Sinopharm Chemical Reagent Co., Ltd, Cat. #30072418, Shanghai, China) per well to dissolve the formazan crystals. The optical density value (OD-value) was measured at 562nm using a Multiskan GO microplate reader (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instruction. The OD-value indicates the counts of living cells.

Apoptosis rate assay: The effect of AngII and OGD/R on apoptosis rate was assessed using Annexin V staining by Muse Cell Analyser(Germany Merck&Millipore), according to the manufacturer's direction.

qRT-PCR: To validate the sequence data, 11 DE genes related to endothelial dysfunction were chosen for qRT-PCR, including 8 lncRNAs (ENST00000565493,ENST00000606054,ENST00000590604,NONHSAT138949.2, ENST00000566942, ENST00000369385, NONHSAT217189.1, ENST00000540082) and their corresponding target mRNAs (CD226,PARVB). After being cultured in 6-well plates at a density of 6000 cells/cm², total RNAs from model cells and control cells were extracted using SPARKeasy Improved Tissue/Cell RNA Kit (HAOSAIL, Lot.YADKZ). PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Cat.#RR047A) and reverse transcription reaction according to the standard operation process provided by the manufacturer was used to synthesize the DNA template. TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (TaKaRa, Cat.#RR820A) was used to amplify qRT-PCR following the manufacturer's instructions. lncRNAs expression was normalized to GAPDH as described in the literature. Complementary DNA was synthesized using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Cat. #04379012001, Germany), and qRT-PCR was performed by using LightCycler 480 SYBR Green I Master (Roche, Cat. #04707516001), according to the manufacturer's instructions, using a LightCycler480 instrument (Roche). The primer sequences are listed in Supplementary Table 1 (Sangon Biotech, Shanghai, China). The data were calculated and presented by the 2^{-ΔΔCT} method.

2.4 Statistical analysis

All statistical analyses were performed using the R 3.6.0. Two-tailed student t-test was used for normally distributed variables and the Wilcoxon Rank-Sum test was used for abnormally distributed data. Data normality was evaluated using the Shapiro-Wilk test. Differences between case and control group were considered significant at p-value ≤0.05.

Results

3.1 Identification of DEGs and DELs

Read counts obtained from RNA-seq were normalized as fragments per kilobase of transcript per million mapped fragments (FPKM) [9]. FPKM was used to identify the differentially expressed lncRNAs and mRNAs in ISH&ACI cases (n = 8) and ISH controls (n = 8). The differentially expressed lncRNAs and mRNAs met the following cutoff criteria: p-value ≤ 0.05 and |Fold-Change| > 2 [10]. A total of 2768 differentially expressed lncRNAs (946 up-regulated and 1822 down-regulated) and 747 differentially expressed mRNAs (288 up-regulated and 519 down-regulated) were identified between ISH&ACI cases and ISH controls. Volcano plots visualized the distribution of the differentially expressed lncRNAs (Fig. 1A) and mRNAs (Fig. 1B). The hierarchical clustering plots showed clear separation and consistency in the expression profiles of ISH&ACI cases and ISH controls (Fig. 1C and Fig. 1D). (Fig. 1 Differentially expressed lncRNAs and mRNAs in ISH&ACI cases (n = 8) and ISH controls (n = 8). (A) Volcano plots of differentially expressed lncRNAs. The X-axis is log₂ Fold-Change and the Y-axis is log₁₀p-value. Red (up-regulated) and blue (down-regulated) points indicated the differential expression lncRNA. (B) Volcano plots of differentially expressed mRNAs. (C) Differentially expressed lncRNAs hierarchically clustered, each column represents a sample and every row represents a lncRNA. Red indicates high relative expression and blue indicates low relative expression. (D) Differentially expressed mRNAs hierarchically clustered.)

3.2 Gene Ontology (GO) and KEGG pathway analysis

GO enrichment analysis and KEGG enrichment analysis were performed by hypergeometric test to find significantly enriched GO terms and KEGG pathways of differentially expressed lncRNAs and mRNAs. By GO enrichment analysis, differentially expressed lncRNAs and mRNAs were classified into three categories: cellular component (CC), molecular function (MF), and biological process (BP). The potential mRNAs targets of differentially expressed lncRNAs were predicted using cis-specific binding modules and trans-specific binding modules. The intersection of targets of differentially expressed lncRNAs and differentially expressed mRNAs were used to perform enrichment analysis.

As shown in Fig. 2A and Supplementary Table S2, GO Enrichment analysis identified 42 significant GO terms (p-value ≤ 0.05). In biological process, the top four GO terms were positive regulation of blood coagulation, positive regulation of coagulation, cellular response to glucagon stimulus, and platelet degranulation; In cellular component, the top four GO terms were platelet alpha granule, platelet alpha granule lumen, secretory granule lumen, and cytoplasmic membrane-bounded vesicle lumen; In molecular function, the top two GO terms were integrin binding and cell adhesion molecule binding. KEGG enrichment analysis identified 6 significant KEGG pathways (Fig. 2B and Supplementary Table S3). The 6 significant KEGG pathways were arrhythmogenic right ventricular cardiomyopathy, platelet activation, ECM-receptor interaction, hypertrophic cardiomyopathy, dilated cardiomyopathy, and Focal adhesion. The enrichment analyses showed that the intersection of targets of differentially expressed lncRNAs and differentially expressed mRNAs were mainly associated with platelet activation. (Fig. 2 GO and KEGG pathway enrichment analysis. GO enrichment contains three terms: cellular component, molecular function, and biological process. (B) the top 30 enriched KEGG pathways.).

3.3 lncRNA–mRNA co-expression network construction and hub genes analysis

We calculated the Pearson correlation coefficient using the expression value of lncRNAs and mRNAs. We identified lncRNA-mRNA co-expression with coefficient great than 0.9 and the p-value less than 0.05. According to the results of lncRNA-mRNA co-expression, we then constructed the lncRNA-mRNA network using Cytoscape software. 2 hub genes in the network were identified and the 2 hub genes were CD226 and PARVB (Fig. 3). (Fig. 3. lncRNA-mRNA co-expression network. Red indicates lncRNAs and blue indicates mRNAs. the top 2 hub genes are CD226 and PARVB.)

CD226 (also known as DNAM-1, PTA-1, or TLIISA-1) was an immunoglobulin-like glycoprotein. CD226 was expressed on the platelet membrane and was involved in platelet activation [11, 12]. Platelets played a crucial role in the control of acute hemorrhage and thrombosis, and thus were involved in atherosclerosis and myocardial infarction [13]. CD226 may participate in atherosclerotic cerebral infarction. The PARVB (also known as beta-parvin and affixin) encoded a member of the parvin family of actin-binding proteins, which played a role in cytoskeleton organization and cell adhesion [14]. PARVB was shown to be associated with coronary restenosis, hematoma volume, and spontaneous intracerebral hemorrhage [15, 16].

3.4 The validation of expression profiles of lncRNAs

qRT-PCR was performed for further validation of 9 selected differentially expressed lncRNA-mRNA pairs in 20 individual samples (Table 1). We found that the expression of CD226 and lncRNAs ENST00000590604 were significantly up-regulated in the cases compared to controls (Fig. 4). The results of lncRNAs ENST00000590604 and CD226 were consistent with the results of RNA-seq data. Hub gene CD226 was significantly upregulated in ISH&ACI patients.(Fig. 4. Relative levels of mRNAs and lncRNAs in 10 cases and 10 controls. Using qRT-PCR to calculate the relative fold-change to the house-keeping gene (β -action). From left to right are lncRNA ENST00000590604 and CD226.)

Table 1
-differentially expressed lncRNA-mRNA pairs

Target	lncRNAs
CD226	ENST00000565493, ENST00000606054, ENST00000590604, NONHSAT217189.1, ENST00000566942
PARVB	ENST00000565493, NONHSAT138949.2, ENST00000369385, ENST00000540082

3.5 The effects of AngⅡ and OGD/R on HBMECs

MTT assay and flow cytometer (FCM) were performed to evaluate proliferation and apoptosis rate. The results indicated that, compared with the control group, HBMECs’ growth was inhibited and apoptosis rate was increased which were treated with OGD/R (Fig. 5).(Fig. 5. The effect of OGD/R on proliferation and apoptosis rate of HBMECs. (A)HBMECs’ viability was declined after OGD/R compared with control

group (*P < 0.05, **P < 0.01 vs. Control group). (B) The apoptosis rate of OGD/R group was increased compared with control group (**P < 0.01 vs. Control group). (C) Control group apoptosis results. (D) OGD/R group apoptosis results.)

3.5 qRT-PCR validation of significant DE lncRNAs and DE mRNAs of HBMECs

The qRT-PCR results using HBMECs were consistent with the RNA-Seq results and qRT-PCR results using plasma of patients, including lncRNA ENST00000590604 and CD226 (Fig. 6). (Fig. 6. qRT-PCR validation (n = 3) of DE genes compared to RNA-Seq (n = 8). Yellow represents the expression of DE lncRNAs and DE mRNAs in the ISH complicated with stroke group veeq, blue represents the expression of DE lncRNAs and DE mRNAs in the AngⅡ and OGD/R group versus the ISH group by RNA-S control group by qRT-PCR.).

Discussion

4.1 Summary

Hypertension becomes one of the most important risk factors for cardiovascular and cerebrovascular accidents in the world today and contributes to an elevated risk of stroke, myocardial infarction, heart failure, and renal failure [17, 18]. 31.1% of adults had hypertension in the whole world [19]. The health cost of hypertension has an enormous public health burden. ISH is the most common type of hypertension in elderly men as well as young adults [20].

The emerging evidence indicates that lncRNAs play a critical role in various biological functions and disease processes [21]. For example, HOTAIR (HOX transcript antisense intergenic RNA) is one of the most well-studied lncRNAs that are overexpressed in a variety of cancers [22]. Though the significance of lncRNAs is being increasingly found in hypertension, the expression profile and the mechanism of lncRNAs remain unclear in ISH.

In this study, our RNA-seq data revealed multiple lncRNAs dysregulated in ISH&ACI. GO and KEGG enrichment analyses found that the intersection of the targets of differentially expressed lncRNAs and differentially expressed mRNAs were mainly associated with platelet activation, ECM-receptor interaction, and focal adhesion. Platelet activation signaling pathway played a critical role in the function of platelets in hemostasis and thrombosis [23]. Activated platelets aggregated at the site of endothelial cell erosion, stimulating thrombus formation and promoting atherothrombotic disease [24]. Focal adhesion signaling pathway promoted cell migration and angiogenesis [25, 26].

We also found two upregulated hub genes in ISH&ACI (CD226 and PARVB) by the lncRNA-mRNA co-expression network. CD226 was an immunoglobulin-like glycoprotein and mediated platelet and megakaryocytic cell adhesion to vascular endothelial cells [12, 27]. Researches suggested that platelet-derived miR-142-3p regulating apoptosis of endothelial cells in hypertension and the dysfunction of vascular endothelial cells played crucial roles in vascular remodeling during hypertension [28, 29]. PARVB

affected human endothelial cell shape and motility, suggesting an important role of PARVB in vessel growth and vessel stability [30, 31].

We further validated the results by qRT-PCR in another group of 20 patients. Besides, flow cytometer was performed to evaluate proliferation and apoptosis rate in HBMECs cells and real-time PCR was performed to validate the lncRNA-mRNA pairs in HBMECs cells. Here, we found lncRNAs ST00000590604 regulated the expression of CD226. CD226 was associated with vascular endothelial cells growth and stability through platelet activation and focal adhesion pathway. lncRNAs ENST00000590604 and CD226 could be the potential biomarkers for ISH&ACI and provided a new idea for the clinical treatment of ISH&ACI.

4.2 Limitations

There were several limitations:

The cases included in this research are limited in number so that expansion of sampling size is necessary for future research to generate more reliable results. Also, since no medication targeted the differentially expressed lncRNAs or mRNAs, further research on the pharmacological mechanism are still needed to unveil the essential roles of these potential biomarkers.

4.3 Comparison with existing literature

Past research mostly focused on the differential expressions of lncRNAs and mRNAs in ACI patients and healthy subjects or in hypertension patients and healthy subjects. The differential expressions of lncRNAs in ISH&ACI patients and ISH patients have not been investigated. We initiated the probe into the differentially expressed lncRNAs and mRNAs in ISH&ACI patients and ISH patients at the transcriptive level in hope of demonstrating the potential mechanism for the progression of the disease.

4.4 Implications for Research and Practice

The findings of the experiment allow discussion of the physiological foundation for ISH&ACI from the level of lncRNAs via high-through sequencing. We drew a preliminary conclusion on the potential pathomechanism for ISH patients to lapse into ACI. Our research provided theoretical foundation for future precise diagnoses and treatments with medication.

Conclusion

We established a novel mRNA-lncRNA interaction network. lncRNAs ENST00000590604 and CD226 might be the potential biomarkers of ISH & ACI. This exploratory work generates a novel mRNA-lncRNA interaction network that was never before carried out in ISH&ACI patients. The two lncRNAs ENST00000590604 and CD226 verified as potential biomarkers for ISH&ACI were not mentioned in former literature.

Abbreviations

ISH	Isolated systolic hypertension
ISH/ACI	Isolated systolic hypertension and atherosclerotic cerebral infarction patients
lncRNAs	Long non-coding RNAs
HBMECs	Human Brain Microvascular Endothelial Cells
OGD/R	Oxygen-Glucose Deprivation/Reoxygenation
OD-value	optical density value
FPKM	transcript per million mapped fragments
KEGG	Kyoto Encyclopedia of Genes and Genomes
CC	cellular component
MF	molecular function
BP	biological process
GO	Gene Ontology
FCM	flow cytometer
HOTAIR	HOX transcript antisense intergenic RNA

Declarations

Ethics approval and consent to participate

We have obtained the ethic clearance certificate and all the participants signed the informed consent.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The author of this article has no conflict of interest

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Consent to participate

All participants signed the informed consent

Consent for publication

Not Applicable

Authors' contributions

YJ,TYN,HFX,CML,LGL,LC and JYH performed the experiments and drafted the manuscript. CYZ and YXS participated in the data interpretation and discussion. ZX contributed to software applications and data analysis. YJ and YCH designed the study and carried out important revisions of the manuscript. All authors read and approved the final manuscript.All authors read and approved the final manuscript.

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Supplemental Data

Supplemental Tables 1-3 are not available with this version.

Figures

Figure 1

Differentially expressed lncRNAs and mRNAs in ISH&ACI cases ($n = 8$) and ISH controls ($n = 8$). (A) Volcano plots of differentially expressed lncRNAs. The X-axis is log2 Fold-Change and the Y-axis is log10p-value. Red (up-regulated) and blue (down-regulated) points indicated the differential expression lncRNA. (B) Volcano plots of differentially expressed mRNAs. (C) Differentially expressed lncRNAs hierarchically clustered, each column represents a sample and every row represents a lncRNA. Red indicates high relative expression and blue indicates low relative expression. (D) Differentially expressed mRNAs hierarchically clustered.

Figure 2

GO and KEGG pathway enrichment analysis. (A) GO enrichment contains three terms: cellular component, molecular function, and biological process. (B) the top 30 enriched KEGG pathways.

Figure 3

lncRNA-mRNA co-expression network. Red indicates lncRNAs and blue indicates mRNAs. the top 2 hub genes are CD226 and PARVB.

Figure 4

Relative levels of mRNAs and lncRNAs in 10 cases and 10 controls. Using qRT-PCR to calculate the relative fold-change to the house-keeping gene (β -action). From left to right are lncRNA ENST00000590604 and CD226.

Figure 5

The effect of OGD/R on proliferation and apoptosis rate of HBMECs. (A) HBMECs' viability was declined after OGD/R compared with control group (* $P < 0.05$, ** $P < 0.01$ vs. Control group). (B) The apoptosis rate of OGD/R group was increased compared with control group (** $P < 0.01$ vs. Control group). (C) Control group apoptosis results. (D) OGD/R group apoptosis results.

Figure 6

qRT-PCR validation (n=3) of DE genes compared to RNA-Seq (n=8). Yellow represents the expression of DE lncRNAs and DE mRNAs in the ISH complicated with stroke group veeq, blue represents the expression of DE lncRNAs and DE mRNAs in the Ang $\square$ and OGD/R group versus the ISH group by RNA-S control group by qRT-PCR