Bioinformatics analysis of a long non-coding RNA and mRNA regulation network in rats with middle cerebral artery occlusion based on RNA sequencing

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Abstract. Long non-coding RNAs (lncRNAs) have been proven to be critical gene regulators of development and disease. The main aim of the present study was to elucidate the lncRNA-mRNA regulation network in ischemic stroke induced by middle cerebral artery occlusion (MCAO) using RNA sequencing (RNA-seq) in rats. lncRNA expression profiles were screened in brain tissues to identify a number of differentially expressed lncRNAs (DELs) and genes (DEGs) by RNA-seq. Reverse transcription-quantitative polymerase chain reaction was performed to further confirm the lncRNA expression data. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used to mine mRNA functions, and a lncRNA-mRNA network was constructed. Additionally, *cis*- and *trans*-regulatory gene analyses of DELs were predicted. A total of 134 DELs (fold change >2, false discovery rate <0.05) and 1,006 DEGs (fold change >2 and P<0.05) were identified. Eighteen lncRNAs were predicted to regulate heme oxygenase 1, mitotic checkpoint serine/threonine kinase B, chemokine ligand 2 and DNA Topoisomerase IIα, amongst other genes. These genes are all associated with a cellular response to inorganic substances, alkaloids, estradiol, reactive oxygen species, metal ions, oxidative stress, and are associated with metabolic pathways, chemokine signaling pathways, malaria, Parkinson's disease, the cell cycle and other GO and KEGG pathway enrichments. The present study identifies novel DELs and an lncRNA-mRNA regulatory network that may allow for an improved understanding of the molecular mechanism of ischemic stroke induced by MCAO.

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Introduction

Stroke, universally acknowledged as a cerebrovascular accident, may result in lasting brain damage, long-term disability or even mortality (1,2). A multitude of biological processes are implicated in ischemic stroke, including oxygen deprivation, neuronal necrosis and an intense inflammatory response (3,4). MicroRNAs (miRNAs), long non-coding RNAs (lncRNAs) and even circular RNAs (circRNAs) contribute to RNA-mediated networks (5-8) that regulate notable cellular events through a variety of complicated mechanisms (9,10). These networks have been implicated in ischemic stroke in previous studies (5-10); however, there remain gaps in current knowledge in this regard, and novel ncRNAs need be mined in order to provide a better understanding of the precise molecular mechanisms involved in ischemic stroke.

lncRNAs have been proven to be critical gene regulators of development and disease (11-13). lncRNAs may also perform functions through competitively binding to miRNAs known as competitive endogenous RNAs (14). Washietl *et al* (15) systematically analyzed the conservatism of human lncRNA and other six mammalian lncRNA and identified that ~54% human lncRNA loci may be mapped to that of a rat. A previous study has demonstrated that significantly differentially expressed lncRNAs (DELs) may contribute to the stabilization of mRNA expressions in stroke (7). Stroke-induced lncRNAs may also interact with chromatin-modifying proteins and modulate genes associated with ischemic brain damage (16,17). Furthermore, lncRNA BC088414 was revealed to be involved with apoptosis-associated genes following hypoxic-ischemic brain damage (8). Similarly, another study suggested that lncRNA C2dat1 may modulate calcium/calmodulin-dependent protein kinase II expression to promote neuronal survival following cerebral ischemia (10). Although a host of lncRNAs have been identified by massive parallel sequencing, to date, little

is known on functional RNA molecules and RNA-mediated regulation networks in ischemic stroke.

The main aim of the present study is to elucidate the lncRNA-mRNA regulation networks in ischemic stroke induced by middle cerebral artery occlusion (MCAO) using RNA sequencing (RNA-seq) in rats.

Materials and methods

MCAO model and tissue preparation. A focal cerebral ischemia model induced by MCAO, prepared as previously described (18), was prepared using 20 7-week-old male Sprague-Dawley rats of a specific pathogen-free grade (weighing 200±20 g), purchased from the experimental animal center of Anhui Medical University (Anhui, China). The study protocol was ethically approved by the Committee on the Ethics of Animal Experiments of Anhui University of Chinese Medicine (approval no. 2012AH-036-03). In brief, the animals were fasted overnight but allowed *ad libitum* access to water. They were then anesthetized with chloral hydrate (350 mg/kg, intraperitoneal injection). A 4-0 silicon-coated monofilament nylon suture with a round tip was inserted through an arteriectomy in the common carotid artery just below the carotid bifurcation and then advanced into the internal carotid artery ~18 mm distal to the carotid bifurcation until a mild resistance was felt. Following 2 h of MCAO, the filament was removed to allow reperfusion. As a control, control-operated rats underwent identical surgery but did not have the suture inserted. Four days subsequent to MCAO, the left hemispheres were collected and immediately frozen in liquid nitrogen.

RNA-seq. RNA-seq was performed by Ao-Ji Bio-Tech (Shanghai, China). Briefly, total RNA was extracted using an RNeasy Mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. The RNA quality control was performed using Nanodrop 2000 and Agilent 2100, and mainly depended on the concentration, purity and integrity of the RNA. Ribosomal RNA was removed from total RNA using Ribo-Zero rRNA removal beads (Illumina, Inc., San Diego, CA, USA). Libraries were constructed according to the standard TruSeq protocol (19). Purified cDNA libraries were prepared for cluster generation and sequencing on an Illumina HiSeq 2500 (Illumina, Inc.) according to the manufacturer's protocol. Subsequently, data analyses were performed *in silico*.

lncRNA annotation. Quality control of the RNA-Seq reads was conducted using FastQC (v0.11.3) (The Babraham Institute, Cambridge, UK). Reads were trimmed using the software seqtk (github.com/lh3/seqtk) for known Illumina TruSeq adapter sequences, poor reads and ribosome RNA reads. Trimmed reads were aligned to the rat genome (Rn6) using Hisat2 (version 2.0.4) (20). Transcripts were assembled using Stringtie (v1.3.0) (20,21). Transcripts constructed from Stringtie were compiled together by gffcompare (v0.9.8) (20,21). Transcripts detected in at least five samples (half of the total number) were considered to be bona fide transcripts. Transcripts, with the exception of those with just one exon and shorter than 200 base pairs, were further analyzed for the identification of lncRNAs. Transcripts with class codes 'i,' 'u,' and 'x,' were considered to be potential novel long transcripts. Pfam (22), Coding Potential Calculator (CPC) (23) and Coding-Non-Coding Index (CNCI) (24) were used to estimate the coding potential of each novel transcript. Transcripts with a Pfam score <0, CNCI <0 and CPC non-significant were considered to lack coding potential. Transcripts were compared with annotation databases, including NONCODE (v4) (http://www.noncode.org) and Ensembl (25). The matched transcripts were considered to be known lncRNAs, and others were considered to be novel lncRNAs. All lncRNAs were quantified using Stringtie. According to the positional association between lncRNA and mRNA in the genome, lncRNA may be classified into six types: Bidirectional, exonic_antisense, exonic_sense, intergenic, intronic_antisense and intronic_sense (26).

The lncRNA-mRNA coexpression network. Initially, the DELs and differentially expressed genes (DEGs) were analyzed using EdgeR (27). For DEGs, log2| [fold change (FC)] |>1 and P<0.05 were used as the cutoff values. Meanwhile, log2| (FC) |>1 and false discovery rate (FDR) <0.05 were used as the threshold for DELs. Hierarchical clustering of DELs was performed based on mean signals using a Euclidean distance function. In addition, a volcano plot was generated. The Pearson's correlation coefficient (PCC) between lncRNAs and mRNAs was calculated (cutoff value, PCC>0.9, P<0.05) and the lncRNA-mRNA regulatory network was structured using Cytoscape 2.8.3 (28).

Prediction of target genes and enrichment analysis. *cis*- or *trans*-acting algorithms were used to predict the potential targets of lncRNAs. The first algorithm predicted potential target genes of *cis*-acting lncRNAs that were physically located within 10 kb upstream or 20 kb downstream of lncRNAs using liftOver genome browser (genome.ucsc.edu/cgi-bin/hgLiftOver). The second algorithm predicted potential target genes of *trans*-acting lncRNAs based on the lncRNA-mRNA complementary sequences, and predicted lncRNA-mRNA duplex energy. First, BLASTN (29) was performed to detect potential target mRNA sequences with >95% identity and E value <$1x10^{-5}$ (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, RNAplex (30) was used to calculate the complementary energy between lncRNAs and their potential *trans*-regulated target genes with RNAplex-10^{-30}. Gene Ontology (GO) (31) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (32) enrichment analyses of the identified potential target genes were performed using the Database for Annotation, Visualization and Integrated Discovery (33); and P<0.05 was considered to indicate a statistically significant difference.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from left hemisphere samples using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and reverse-transcribed using a Thermo Fisher Scientific RevertAid First Strand cDNA Synthesis kit (cat. no. K1622; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol at 42˚C for 60 min. To further confirm the expression data from RNA-seq, a cutoff value (FC>2, P<0.05) was randomly selected for qPCR verification. The expression levels of six randomly DELs (NONRATT027551.2, MSTRG.1836.1, MSTRG.4344.10,

Table I. Primer sequences.

Gene	Sequence	Polymerase chain reaction product length (base pairs)
GAPDH	F: 5'-CCTGGTATGACAACGAATTTG-3' R: 5'-CAGTGAGGGTCTCTCTCTTCC-3'	131
NONRATT027551.2	F: 5'- GGACCTGGAAGGTGAACAGG-3' R: 5'-TGAATGGGTGACCAACAGGG-3'	118
MSTRG.1836.1	F: 5'-CCATTGTCCTTCCATCCCCC-3' R: 5'-CCACCCTACCAAACTTCCCC-3'	85
MSTRG.4344.10	F: 5'-GACTTAGGCACAGTGGGTGG-3' R: 5'-ATGGCAGAGAGCGAATGGAG-3'	119
MSTRG.7720.11	F: 5'-TCCCTAGAGCAGTCCTCACC-3' R: 5'- ATCTCGGGTTCGCCTTTTGT-3'	97
NONRATT005132.2	F: 5'-CCTGACTATGGCACGTCCTC-3' R: 5'-CTGAGTCCAGTGTGCCTGTT-3'	152
MSTRG.20633.3	F: 5'-CTTTCACTCCGAGAACCCCC-3' R: 5'-GCAAGCAGGTTGGTTCCTTG-3'	117

F, forward; R, reverse.

Table II. Results of the RNA sequencing.

Sample ID	Raw reads	Clean reads	Clean ratio (%)	rRNA trimmed	Mapped reads	Mapped ratio
MCAO 1	155190870	147282901	94.90	147214274	131290423	0.824026529
MCAO 2	144450130	136930064	94.79	136768708	120311195	0.812346697
MCAO 3	151411986	142862439	94.35	142724911	125540743	0.804354762
Control 1	169303916	160544960	94.83	160466568	142289885	0.819832697
Control 2	136533930	129672892	94.97	129609605	116072061	0.828973282
Control 3	124878376	118432971	94.84	118349299	105506286	0.821661742

MCAO, middle cerebral artery occlusion.

MSTRG.7720.11, NONRATT005132.2 and MSTRG.20633.3) were assayed using a SYBRGreen flurophore (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the PikoReal real-time PCR system (Thermo Fisher Scientific, Inc.) under the following conditions: Initial denaturation at 95˚C for 30 sec, followed by 40 cycles at 95˚C for 30 sec and 60˚C for 30 sec, and a final extension step at 4˚C for 20 min. FC was determined using the $2^{-\Delta\Delta Cq}$ method (34). GAPDH mRNA was used as an internal control. The primers used are listed in Table I.

Statistical analysis. The comparisons between the MCAO group and the control group were determined using a Student's t-test for the RT-qPCR results by SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference. The PCC between lncRNAs and mRNAs was calculated using the Hmisc package in R based on the expression determined using RNA-seq (PCC>0.9, $P<0.05$). The correlation analysis between the RT-qPCR results and RNA-seq results was calculated in Excel 2013 (Microsoft Corporation, Redmond, WA, USA) with the function of CORR.

Results

lncRNA-sequencing data analysis. The present study characterized the lncRNA landscape and expression by performing deep RNA-seq experiments on three control and three MCAO tissue samples. Subsequent to the seqtk quality assessment of sequencing, >33 million total original reads for each sample were obtained, and the proportion of bases with quality values >20 was >94%. These results indicated that the quality of the sequencing results was acceptable (Table II). Subsequent to filtering out the adaptor sequence and low quality reads, the percentage of clean reads within the raw reads accounted for 94% of the total sequences in two groups. Hisat2 software was used to map the obtained clean reads to the *Rattus norvegicus* reference genome. As presented in Table II, ~97% of the

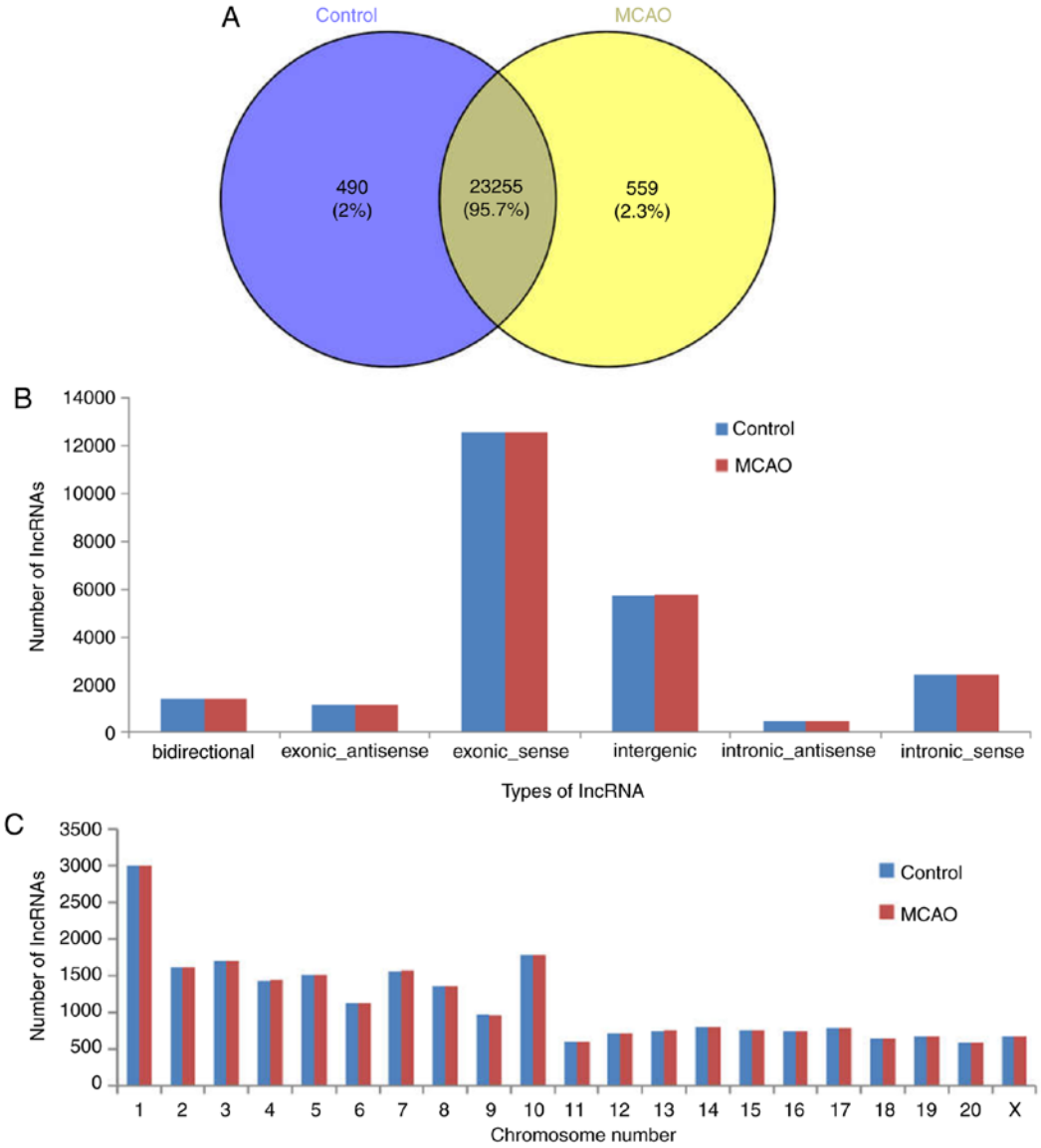

Figure 1. Class type and chromosome distribution of lncRNAs identified in the control and MCAO group. (A) Venn diagram of lncRNA in the control and MCAO groups. (B) According to the positional association between lncRNA and mRNA in the genome, lncRNAs may be classified into six types: Bidirectional, exonic antisense, exonic sense, intergenic, intronic antisense and intronic sense. (C) Number of lncRNAs on each chromosome in the MCAO and control groups. MCAO, Middle cerebral artery occlusion; lncRNA, long noncoding RNA.

trimmed reads were mapped onto the reference genome. In total, 24,304 lncRNAs were screened from six samples, and there were 23,255 shared lncRNAs detected in the MCAO and control groups (Fig. 1A). The majority of the identified lncRNAs were transcribed from protein-coding exons; others were from introns and intergenic regions (Fig. 1B). In addition, the present study analyzed the distribution of the identified lncRNAs on the rat chromosomes; 24,304 lncRNA transcripts were identified in all chromosomes, and chromosome 1 included the most lncRNAs (Fig. 1C).

Identification of DEGs and DELs. EdgeR was used to filter DEGs and DELs and differentiate their expression between the control and MCAO groups. A total of 1,007 DEGs (|FC|>2, P<0.05) were identified, including 785 upregulated genes and 222 downregulated genes. Similarly, as presented in Fig. 2, 134 DELs (|FC|>2, FDR<0.05) were identified in the MCAO group (Fig. 2A and B), including 77 upregulated and 57 downregulated DELs (Fig. 2C and D). In the present study, it was revealed that the FC values of certain DELs were equal to positive infinity and negative infinity, meaning that these lncRNAs are switched-on or off with MCAO. Essentially, positive or negative infinity indicates zero expression of the lncRNA in normal or MCAO groups. It was speculated that this may be associated with the abundance of lncRNAs and the sensitivity to RNA-seq. The top five upregulated DELs were NONRATT027551.2, MSTRG.1836.1, MSTRG.4344.10, NONRATT028102.2 and MSTRG.31500.2; the top five downregulated DELs were MSTRG.7720.11, NONRATT005132.2, MSTRG.20633.3, NONRATT020232.2 and MSTRG.1836.3.

lncRNA-mRNA network. The cutoff correlation r-values (|PCC|>0.9) and P-values (P<0.05) were selected to structure

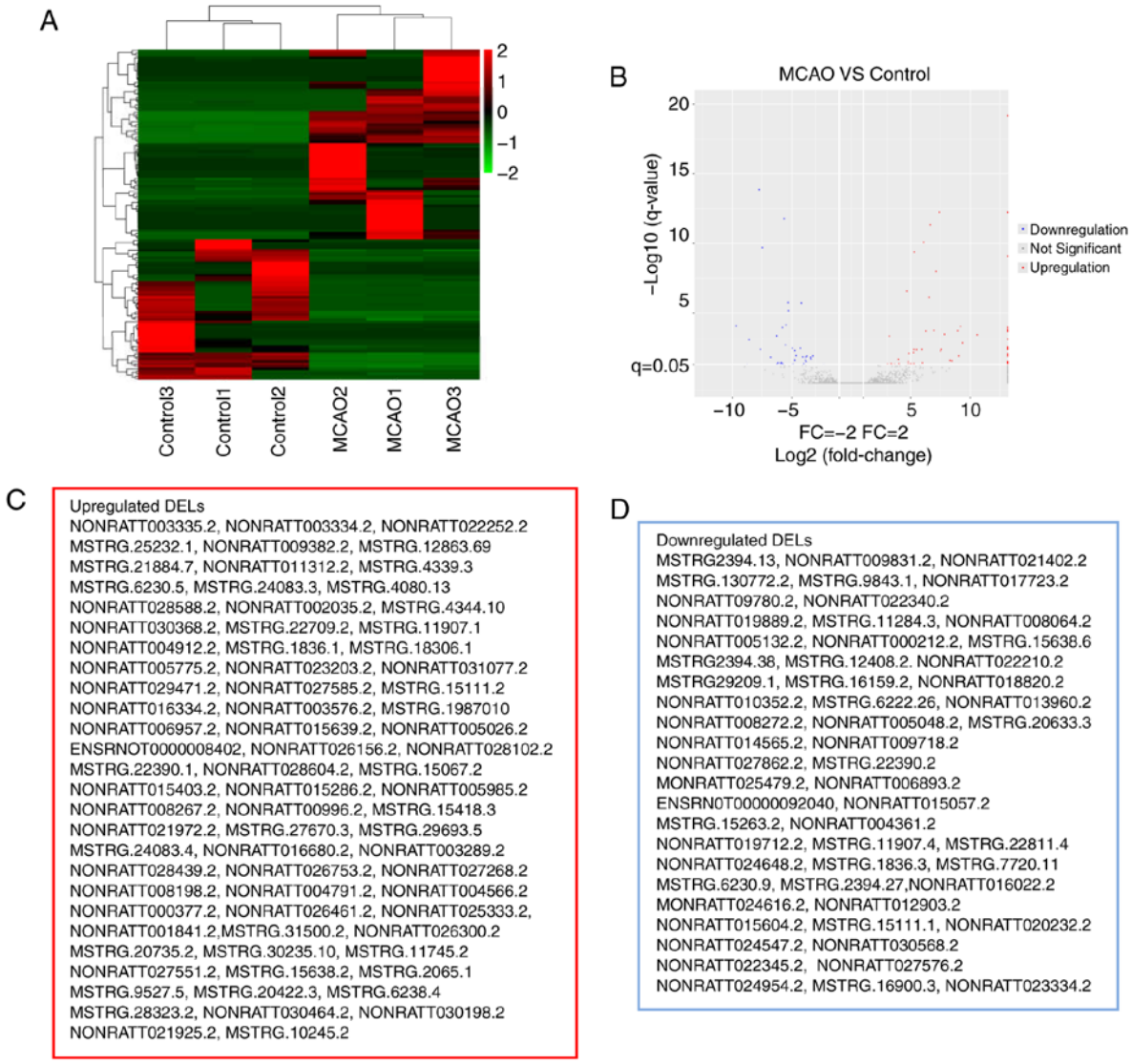

Figure 2. RNA-seq data on the differentially expressed lncRNAs between the model and control groups. (A) Hierarchical cluster of DELs between the MCAO and control groups. The color code in each heat map is linear, with green indicating the least and red indicating the greatest differentiation. The mean signals of the altered lncRNAs in each of the two groups were clustered using a Euclidean distance function. The lncRNAs with the most similar expression patterns were placed next to each other (n=3 per group). (B) A volcano plot of the RNA-seq FC and P-value of MCAO group compared with the control group. Blue and red points stand for DELs. Gray points represent lncRNAs which are not differentially expressed. (C) Upregulated and (D) downregulated DELs as exhibited in the red and blue boxes, respectively, represent the results. MCAO, Middle cerebral artery occlusion; lncRNA, long noncoding RNA; RNA-seq, RNA sequencing; FC, fold change; DEL, differentially expressed lncRNAs.

a lncRNA-mRNA co-expression network between DEGs and DELs. As Fig. 3 presents, 46 DEGs, 104 DELs and 664 edges were filtered out using Cytoscape to construct the co-expression network. The co-expression-associated top 30 GO terms and pathway terms enrichment analyses presented in Figs. 4 and 5 suggest that these DELs were associated with the cellular response to inorganic substances, alkaloids, estradiols, reactive oxygen species, metal ions and oxidative stress. In particular, the heme oxygenase 1 (HO-1) gene participates in many of these functions. A multitude of pathways were implicated, including metabolic pathways, chemokine signaling pathways, malaria, Parkinson's disease and the cell cycle. Notably, the BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) and C-C motif chemokine ligand 2 (CCL2) genes were associated with the cell cycle.

Regulatory analysis of DELs. A total of 91 *cis*-regulatory genes of 94 DELs, including 55 upregulated lncRNAs in the MCAO group were identified; 14 of the 91 *cis*-regulatory genes exhibited differential expression. A total of 13 of the DEL/*cis*-regulatory gene pairs had positive correlations as follows: NONRATT021925.2 (Rho GDP dissociation inhibitor β), NONRATT004791.2 (G protein subunit γ transducing 2), NONRATT015286.2 (periostin), MSTRG.30235.10 (LRR binding FLII interacting protein 1), NONRATT015403.2 (IQ motif containing GTPase activating protein 3), NONRATT008267.2 (kinesin family member 14), NONRATT009960.2 (non-SMC condensin I complex subunit G), NONRATT011312.2 (retinol binding protein 3), NONRATT005985.2 (DNA topoisomerase IIα), NONRATT016680.2 (ENSRNOG00000053081), NONRATT013960.2 (galanin receptor 1), NONRATT016022.2

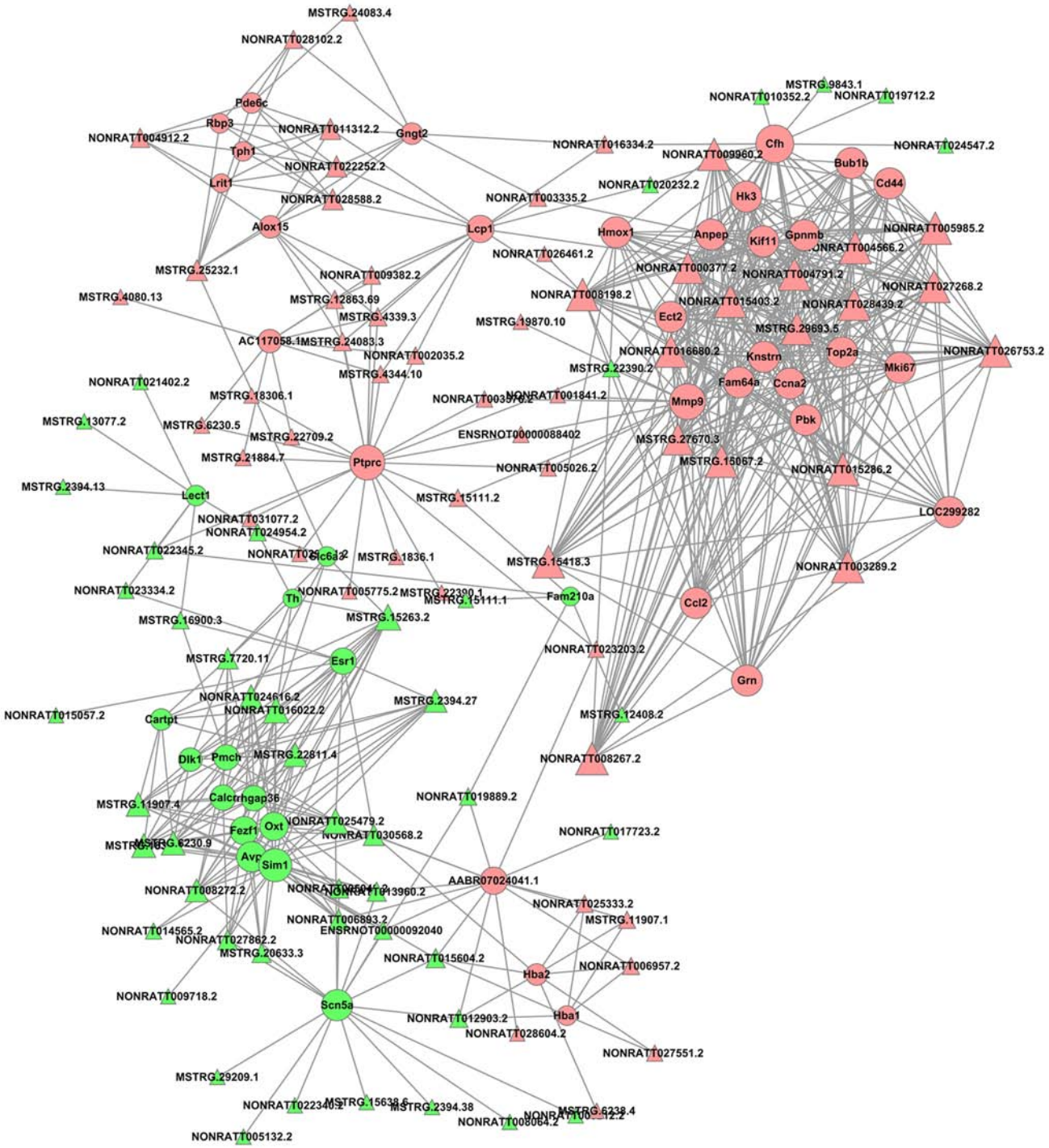

Figure 3. An lncRNA-gene-network based on Pearson's correlation coefficient. Pink nodes indicate the upregulated mRNAs or lncRNAs, and green nodes indicate the downregulated mRNAs or lncRNAs. lncRNA, long noncoding RNA.

(CART prepropeptide) and NONRATT024616.2 (δ like non-canonical Notch ligand 1) (Table III). Additionally, 90 *trans*-regulatory genes of lncRNAs were filtered by BLASTN and RNAplex, with a negative correlation identified between ENSRNOT00000092040 and Ccl9 (Table IV).

Validation of expression of DELs by RT-qPCR. From the data in Fig. 6, NONRATT027551.2, MSTRG.1836.1 and MSTRG.4344.10 were identified to be significantly upregulated in the MCAO group compared with the control ($P<0.05$), consistent with the RNA-seq data, while MSTRG.7720.11, NONRATT005132.2 and MSTRG.20633.3 were significantly downregulated in the MCAO group compared with the control ($P<0.01$), also consistent with the RNA-seq data. These results, revealing that the RNA-seq results were consistent with the RT-qPCR results, verified that the RNA-seq results were reliable (Fig. 6).

Discussion

A host of lncRNAs have been indicated to be involved in ischemic stroke by microarray or RNA-seq studies (35,36).

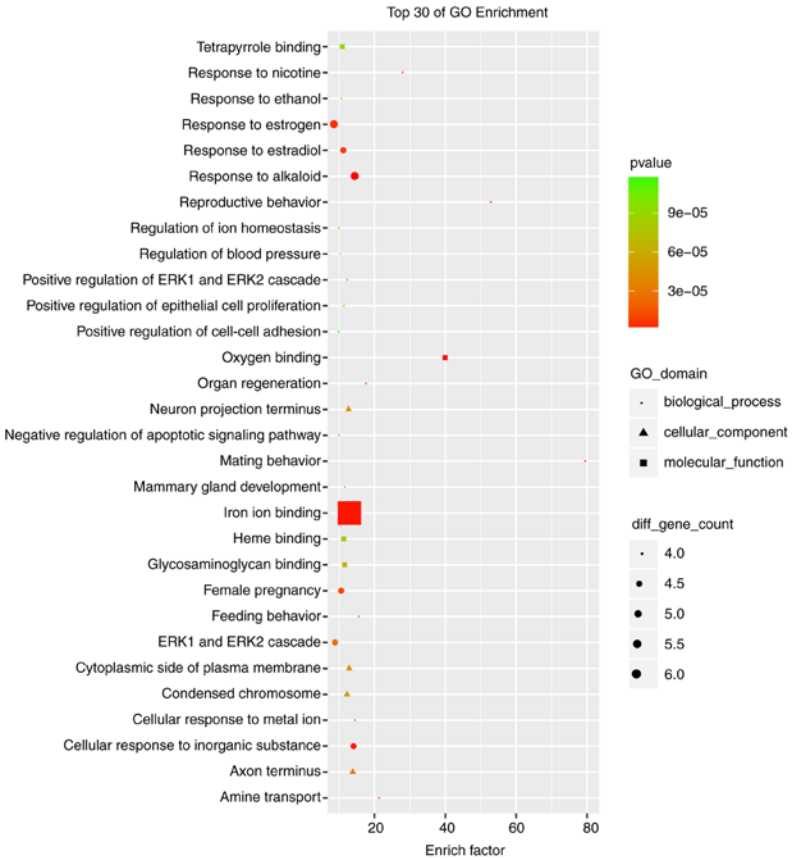

Figure 4. Top 30 significant enrichment of GO terms in the long noncoding RNA-mRNA network. GO, gene ontology; ERK, extracellular regulated kinase.

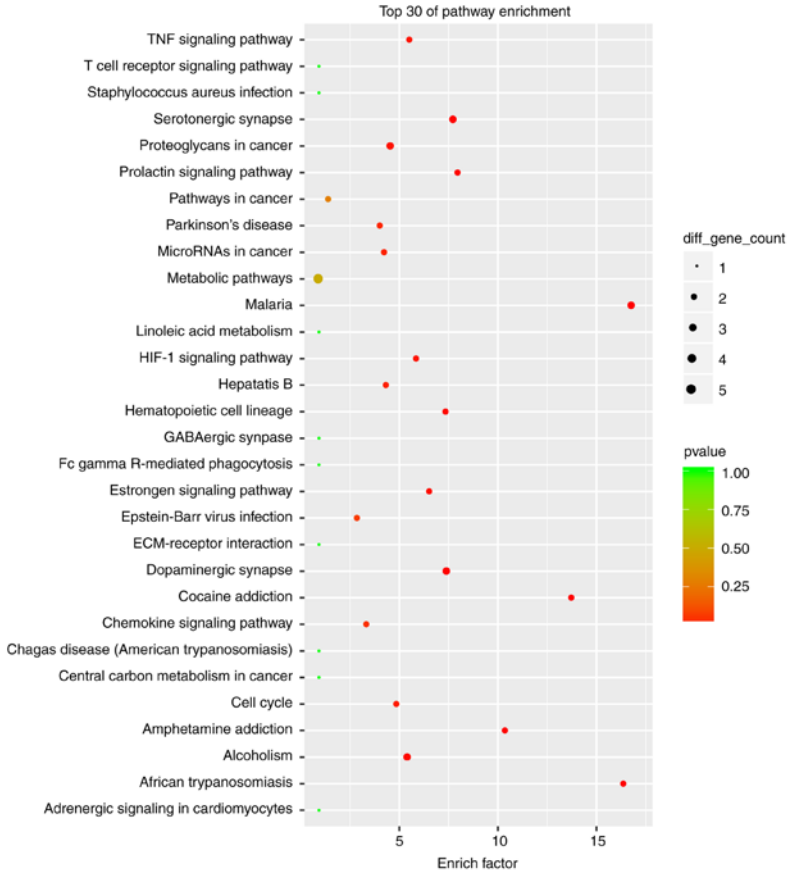

Figure 5. Top 30 significant enrichment of KEGG pathway terms in the long noncoding RNA-mRNA network. KEGG, Kyoto Encyclopedia of Genes and Genomes; TNF, tumor necrosis factor; HIF-1, hypoxia-inducible factor 1; ECM, extracellular matrix.

Table III. Differentially expressed lncRNAs mechanisms involved in *cis*-regulatory elements.

lncRNA ID	log2FC	Q value	Up/downregulated	Type	Gene ID	Gene name	log2FC	P-value	Up/downregulated
NONRATT017723.2	-3.68	0.0125	Down	*cis*	ENSRNOG00000000633	Rhobtb1	0.85	0.1148	-
NONRATT008064.2	-6.73	0.0145	Down	*cis*	ENSRNOG00000001183	Hnf1a	1.25	0.7907	-
NONRATT000377.2	+∞	0.0492	Up	*cis*	ENSRNOG00000001492	Slc8a2	0.13	0.9091	-
NONRATT006957.2	6.69	0.0000	Up	*cis*	ENSRNOG00000001982	Cblb	0.60	0.1402	-
NONRATT009831.2	-5.72	0.0425	Down	*cis*	ENSRNOG00000002137	Aasdh	0.21	0.7048	-
NONRATT009718.2	-5.48	0.0020	Down	*cis*	ENSRNOG00000002146	Pkd2	0.29	0.5035	-
NONRATT009780.2	-3.94	0.0473	Down	*cis*	ENSRNOG00000002208	Shroom3	0.83	0.1301	-
NONRATT005026.2	6.14	0.0000	Up	*cis*	ENSRNOG00000002607	Sox9	0.25	0.5566	-
NONRATT014565.2	-2.91	0.0492	Down	*cis*	ENSRNOG00000002864	Nacc1	0.26	0.5744	-
NONRATT005048.2	-∞	0.0432	Down	*cis*	ENSRNOG00000003144	Gprc5c	0.48	0.5195	-
NONRATT024547.2	-∞	0.0082	Down	*cis*	ENSRNOG00000003955	Spata7	0.26	0.6325	-
NONRATT026753.2	3.08	0.0450	Up	*cis*	ENSRNOG00000003993	Thap2	0.26	0.7629	-
NONRATT005132.2	-8.71	0.0471	Down	*cis*	ENSRNOG00000004049	Baiap2	-0.04	0.8211	-
NONRATT026300.2	+∞	0.0469	Up	*cis*	ENSRNOG00000004628	Dazap2	0.13	0.8554	-
NONRATT026156.2	7.44	0.0000	Up	*cis*	ENSRNOG00000005332	Csdc2	-0.58	0.1263	-
NONRATT005775.2	3.69	0.0453	Up	*cis*	ENSRNOG00000005538	Psmd11	-0.03	0.7616	-
NONRATT023334.2	-4.85	0.0448	Down	*cis*	ENSRNOG00000005711	Ptprd	-0.21	0.4658	-
NONRATT021925.2	+∞	0.0000	Up	*cis*	ENSRNOG00000005809	Arhgdib	1.81	0.0074	Up
NONRATT004791.2	4.02	0.0218	Up	*cis*	ENSRNOG00000006108	Gngt2	4.95	0.0000	Up
MSTRG.22811.4	-∞	0.0388	Down	*cis*	ENSRNOG00000006966	Nfia	0.21	0.6590	-
NONRATT025479.2	-4.14	0.0000	Down	*cis*	ENSRNOG00000007610	Gdf11	0.08	0.9402	-
NONRATT020232.2	-7.72	0.0000	Down	*cis*	ENSRNOG00000007804	C1galt1	0.45	0.2729	-
NONRATT008198.2	4.83	0.0305	Up	*cis*	ENSRNOG00000007887	Elk4	0.76	0.2762	-
NONRATT022252.2	+∞	0.0305	Up	*cis*	ENSRNOG00000008099	Galnt12	0.85	0.2496	-
NONRATT024954.2	-3.98	0.0471	Down	*cis*	ENSRNOG00000008155	Dus4l	-0.17	0.7340	-
NONRATT028439.2	5.06	0.0430	Up	*cis*	ENSRNOG00000008187	Ubash3b	-0.21	0.4764	-
NONRATT022210.2	-4.24	0.0492	Down	*cis*	ENSRNOG00000008237	Unc13b	0.30	0.6027	-
NONRATT027551.2	9.08	0.0119	Up	*cis*	ENSRNOG00000008709	Arhgap32	-0.24	0.5090	-
NONRATT027576.2	-∞	0.0061	Down	*cis*	ENSRNOG00000008757	Tmem218	0.16	0.8511	-
NONRATT021402.2	-∞	0.0291	Down	*cis*	ENSRNOG00000009156	Tra2a	0.47	0.2267	-
NONRATT021972.2	+∞	0.0335	Up	*cis*	ENSRNOG00000009338	Kras	-0.11	0.6201	-
NONRATT022345.2	-5.23	0.0000	Down	*cis*	ENSRNOG00000009795	Nfib	0.04	0.9239	-
MSTRG.16900.3	-4.70	0.0248	Down	*cis*	ENSRNOG00000009882	Ppp3ca	0.07	1.0000	-
MSTRG.19870.10	+∞	0.0000	Up	*cis*	ENSRNOG00000010993	Dpm1	-0.14	0.5966	-
NONRATT008272.2	-3.96	0.0431	Down	*cis*	ENSRNOG00000011063	Dennd1b	0.20	0.7240	-
MSTRG.10245.2	6.31	0.0002	Up	*cis*	ENSRNOG00000011704	Fbxo34	-0.37	0.2745	-

Table III. Continued.

lncRNA ID	log2FC	Q value	Up/downregulated	Type	Gene ID	Gene name	log2FC	P-value	Up/downregulated
NONRATT001841.2	3.41	0.0430	Up	*cis*	ENSRNOG00000012110	Col17a1	-1.52	0.0483	Down
NONRATT002035.2	7.89	0.0003	Up	*cis*	ENSRNOG00000012324	Soga3	-0.04	0.7913	-
MSTRG.22390.2	-∞	0.0000	Down	*cis*	ENSRNOG00000012634	Fbxo10	-0.41	0.1989	-
MSTRG.22390.1	+∞	0.0000	Up	*cis*	ENSRNOG00000012634	Fbxo10	-0.41	0.1989	-
NONRATT015286.2	5.30	0.0425	Up	*cis*	ENSRNOG00000012660	Postn	3.66	0.0004	Up
MSTRG.1836.3	-7.60	0.0041	Down	*cis*	ENSRNOG00000012716	Chd2	0.06	0.9929	-
MSTRG.1836.1	9.24	0.0001	Up	*cis*	ENSRNOG00000012716	Chd2	0.06	0.9929	-
NONRATT016334.2	+∞	0.0000	Up	*cis*	ENSRNOG00000012734	Dcun1d1	-0.20	0.5140	-
NONRATT015057.2	-4.70	0.0049	Down	*cis*	ENSRNOG00000012799	Prkaa1	-0.28	0.3193	-
NONRATT000212.2	-6.12	0.0378	Down	*cis*	ENSRNOG00000013194	Rps6ka2	-0.14	0.5704	-
NONRATT030198.2	5.34	0.0000	Up	*cis*	ENSRNOG00000013213	Epha4	0.35	0.5640	-
NONRATT010352.2	-6.23	0.0004	Down	*cis*	ENSRNOG00000013353	Tmem260	0.07	0.9952	-
NONRATT029471.2	4.91	0.0082	Up	*cis*	ENSRNOG00000013557	Lancl1	-0.17	0.5373	-
NONRATT028588.2	6.05	0.0378	Up	*cis*	ENSRNOG00000013829	Chrna3	1.17	0.2523	-
NONRATT023203.2	+∞	0.0007	Up	*cis*	ENSRNOG00000013956	Rnf38	0.17	0.7841	-
MSTRG.29693.5	+∞	0.0484	Up	*cis*	ENSRNOG00000013991	Creg2	-0.69	0.2339	-
MSTRG.12408.2	-∞	0.0041	Down	*cis*	ENSRNOG00000014007	Gfod1	0.04	0.9704	-
NONRATT004566.2	3.44	0.0440	Up	*cis*	ENSRNOG00000015002	Abhd15	0.76	0.3266	-
MSTRG.15067.2	+∞	0.0090	Up	*cis*	ENSRNOG00000015334	Fcho2	0.49	0.2280	-
NONRATT003289.2	7.56	0.0052	Up	*cis*	ENSRNOG00000015717	Ptpre	0.21	0.6977	-
NONRATT013960.2	-3.81	0.0248	Down	*cis*	ENSRNOG00000016654	Galr1	-3.38	0.0001	Down
NONRATT028604.2	4.71	0.0000	Up	*cis*	ENSRNOG00000017193	Lingo1	-0.52	0.2454	-
NONRATT016022.2	-5.74	0.0001	Down	*cis*	ENSRNOG00000017712	Cartpt	-3.83	0.0001	Down
NONRATT015604.2	-5.60	0.0000	Down	*cis*	ENSRNOG00000018166	Prkab2	0.00	0.8495	-
NONRATT024616.2	-3.79	0.0143	Down	*cis*	ENSRNOG00000019584	Dlk1	-4.21	0.0000	Down
MSTRG.30235.10	8.50	0.0275	Up	*cis*	ENSRNOG00000019892	Lrrfip1	1.14	0.0479	Up
NONRATT026461.2	+∞	0.0118	Up	*cis*	ENSRNOG00000020230	Pias4	0.17	0.8050	-
NONRATT018820.2	-∞	0.0002	Down	*cis*	ENSRNOG00000020337	Sla2	0.52	0.5953	-
NONRATT004912.2	6.00	0.0041	Up	*cis*	ENSRNOG00000020658	Aarsd1	0.05	0.9927	-
NONRATT019712.2	-6.20	0.0446	Down	*cis*	ENSRNOG00000021262	Slc23a2	0.09	0.9742	-
NONRATT027268.2	5.27	0.0071	Up	*cis*	ENSRNOG00000022570	Pus7l	0.51	0.5096	-
MSTRG.12863.69	+∞	0.0304	Up	*cis*	ENSRNOG00000023661	Celf2	0.30	0.5495	-
NONRATT030368.2	5.55	0.0043	Up	*cis*	ENSRNOG00000025527	Mtcl1	0.16	0.8136	-
NONRATT027862.2	-3.75	0.0304	Down	*cis*	ENSRNOG00000027145	Rora	-0.16	0.5963	-
NONRATT015403.2	4.83	0.0409	Up	*cis*	ENSRNOG00000027894	Iqgap3	2.03	0.0137	Up
NONRATT003576.2	+∞	0.0028	Up	*cis*	ENSRNOG00000028017	Tmem109	0.36	0.4097	-

Table III. Continued.

lncRNA ID	log2FC	Q value	Up/downregulated	Type	Gene ID	Gene name	log2FC	P-value	Up/downregulated
NONRATT004361.2	-5.86	0.0409	Down	*cis*	ENSRNOG00000028341	Alkbh5	0.00	0.8591	-
NONRATT012903.2	-5.26	0.0000	Down	*cis*	ENSRNOG00000031706	AABR07027388.1	-0.85	0.1761	-
MSTRG.15111.2	+∞	0.0000	Up	*cis*	ENSRNOG00000032735	Srek1	0.18	0.7203	-
MSTRG.15111.1	-7.42	0.0000	Down	*cis*	ENSRNOG00000032735	Srek1	0.18	0.7203	-
NONRATT028102.2	10.21	0.0000	Up	*cis*	ENSRNOG00000033809	Mlh1	0.76	0.1272	-
NONRATT019889.2	-4.60	0.0113	Down	*cis*	ENSRNOG00000034031	Vstm2l	-0.45	0.3771	-
NONRATT008267.2	+∞	0.0039	Up	*cis*	ENSRNOG00000037211	Kif14	5.80	0.0005	Up
NONRATT009960.2	4.06	0.0430	Up	*cis*	ENSRNOG00000038572	Ncapg	3.28	0.0054	Up
MSTRG.21884.7	+∞	0.0492	Up	*cis*	ENSRNOG00000042458	Stau2	-0.20	0.5104	-
NONRATT030464.2	5.30	0.0042	Up	*cis*	ENSRNOG00000046053	Nudt10	-0.59	0.1573	-
MSTRG.15418.3	+∞	0.0448	Up	*cis*	ENSRNOG00000048993	Metazoa_SRP	0.03	0.9881	-
MSTRG.28323.2	+∞	0.0492	Up	*cis*	ENSRNOG00000049584	AABR07070555.1	-0.13	0.8591	-
MSTRG.4080.13	+∞	0.0002	Up	*cis*	ENSRNOG00000049768	Adcy9	-0.04	0.7770	-
NONRATT025333.2	6.61	0.0000	Up	*cis*	ENSRNOG00000051719	AABR07065498.1	0.11	0.9304	-
NONRATT011312.2	+∞	0.0479	Up	*cis*	ENSRNOG00000051911	Rbp3	9.84	0.0000	Up
NONRATT024648.2	-∞	0.0492	Down	*cis*	ENSRNOG00000052540	SNORD113	0.45	0.6631	-
NONRATT005985.2	4.53	0.0490	Up	*cis*	ENSRNOG00000053047	Top2a	5.28	0.0000	Up
NONRATT016680.2	7.62	0.0041	Up	*cis*	ENSRNOG00000053081		3.59	0.0011	Up
NONRATT009382.2	8.35	0.0257	Up	*cis*	ENSRNOG00000056826	Arap2	0.21	0.6941	-
MSTRG.15263.2	-∞	0.0041	Down	*cis*	ENSRNOG00000060329	Emb	-0.06	0.7939	-
NONRATT015639.2	3.25	0.0005	Up	*cis*	ENSRNOG00000061058	Csde1	0.02	0.8739	-
NONRATT027585.2	7.19	0.0000	Up	*cis*	ENSRNOG00000061656	SNORD14	0.17	0.9160	-

lncRNA, long noncoding RNA; FC, fold change.

Table IV. Differentially expressed lncRNA mechanisms involved in t*rans*-regulatory elements.

lncRNA ID	log2FC	Q value	Up/downregulated	Type	Gene name	log2FC	P-value	Up/downregulated
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ccl9	5.19	0.0017	Up
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Homer3	1.00	0.0611	-
ENSRNOT00000088402	+∞	0.0471	Up	Trans	Aurkb	2.35	0.0723	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Galns	1.03	0.0724	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Slc39a1	0.92	0.0739	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	AC099384.2	+∞	0.1052	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Hsd3b7	0.83	0.1369	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Klrb1b	1.98	0.1413	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Me2	0.63	0.1558	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Pnma2	-0.51	0.1564	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Cass4	1.24	0.1599	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Klhl23	-0.44	0.2192	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Elac1	-0.49	0.2260	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Clcf1	1.26	0.2519	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Fzd6	0.80	0.2590	-
NONRATT009960.2	4.06	0.0430	Up	Trans	Lcorl	-0.44	0.3199	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Gpr37l1	0.40	0.3297	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Mmab	-0.35	0.3391	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Spon1	-0.28	0.3414	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ifngr2	0.44	0.3660	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Foxred2	-0.35	0.3732	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Prr22	-0.67	0.3889	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Sppl2a	0.40	0.3894	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Haus5	0.55	0.4019	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Cpvl	0.80	0.4041	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Fam120b	-0.24	0.4061	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Fbxo3	-0.21	0.4206	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ypel4	-0.24	0.4477	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Klhl26	-0.20	0.4480	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Sync	0.43	0.5076	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Lhfpl5	-0.31	0.5231	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Mrpl52	0.44	0.5306	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Cldn15	-0.39	0.5315	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Mrps35	-0.18	0.5443	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Zfp382	-0.17	0.5640	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ube2k	-0.14	0.5697	-

Table IV. Continued.

lncRNA ID	log2FC	Q value	Up/downregulated	Type	Gene name	log2FC	P-value	Up/downregulated
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Drg1	-0.12	0.5755	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	P2rx5	-0.36	0.5770	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Xpnpep3	0.25	0.5933	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	RGD1311345	0.28	0.5950	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Acer2	0.32	0.6034	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Urgcp	0.28	0.6182	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Megf8	-0.10	0.6223	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Lrtm2	-0.14	0.6301	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	RGD1562299	0.26	0.6325	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Rpl9	0.38	0.6347	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Slc25a44	-0.10	0.6348	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ssmem1	-0.28	0.6350	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Stt3a	0.24	0.6468	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Spire1	-0.09	0.6631	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	RGD1561777	-0.13	0.6846	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Luzp1	-0.09	0.6941	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Acot2	0.26	0.7000	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Fitm2	-0.07	0.7111	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Aox4	0.32	0.7374	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Golga1	-0.04	0.7775	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Lefty2	1.23	0.7977	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Zdhhc24	-0.04	0.8075	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Zfp329	-0.03	0.8205	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Tmem101	-0.03	0.8236	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Dhh	0.28	0.8353	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ncr3	0.57	0.8354	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ppp1r15b	0.14	0.8423	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Zfyve27	-0.01	0.8439	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ints7	0.15	0.8457	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Dus1l	0.14	0.8545	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ahsa2	0.15	0.8576	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Aifl1	0.15	0.8579	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Rsg1	0.16	0.8645	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Fuom	0.15	0.8883	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Cwf19l1	0.12	0.8894	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Paqr7	0.01	0.8942	-

Table IV. Continued.

lncRNA ID	log2FC	Q value	Up/downregulated	Type	Gene name	log2FC	P-value	Up/downregulated
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Coa5	0.02	0.8985	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Cdc14b	0.13	0.8989	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Crebl2	-0.01	0.9081	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	RGD1564541	0.10	0.9210	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Slc15a1	-0.03	0.9211	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Ppm1k	0.03	0.9299	-
NONRATT025479.2	-4.14	0.0000	Down	Cis and trans	Gdf11	0.08	0.9402	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Fam20b	0.04	0.9436	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Anapc11	0.09	0.9536	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Tmem79	-0.04	0.9595	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Cwc25	0.06	0.9692	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Blvrb	0.07	0.9859	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Stk4	0.08	0.9880	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Rbm20	0.08	0.9896	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Tbc1d10b	0.06	0.9927	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Mapkbp1	0.07	0.9986	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Psma8	1.00	1.0000	-
ENSRNOT00000092040	-3.35	0.0204	Down	Trans	Rbbp8nl	0.37	1.0000	-

lncRNA, long non-coding RNA; FC, fold change.

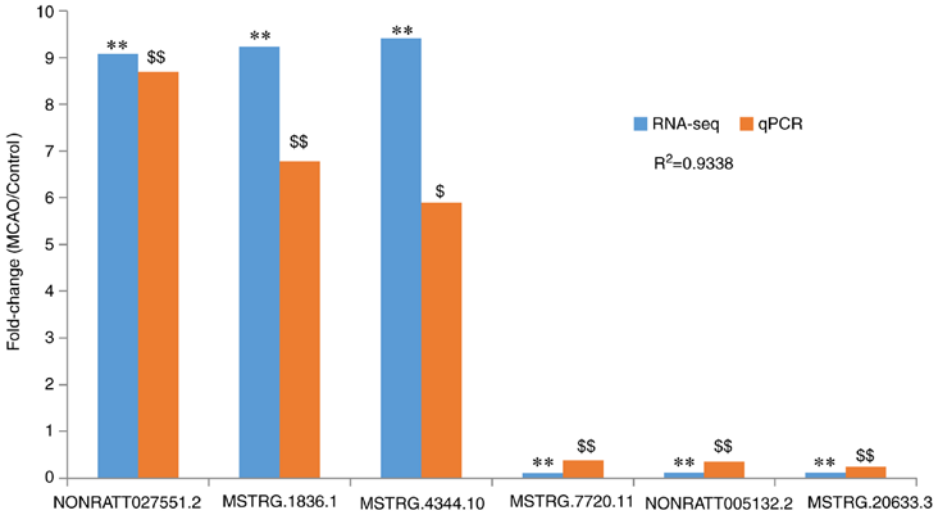

Figure 6. Validation of lncRNA RNA-seq data by RT-qPCR. Fold changes represent the comparison of the MCAO group with the control group. Blue bars indicate the fold change were detected with RNA-seq. **P<0.01 vs. the control group. The orange bars indicate the fold change detected using RT-qPCR. $^{\$}$P<0.05 and $^{\$\$}$P<0.01 vs. the control group. Comparison of the results obtained from RT-qPCR and RNA-seq revealed satisfactory consistency (R^2=0.9338). MCAO, Middle cerebral artery occlusion; lncRNA, long noncoding RNA; RNA-seq, RNA sequencing; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

Metastasis associated lung adenocarcinoma transcript 1 was identified to have a function in ischemic stroke through inhibiting endothelial cell death and inflammation (36,37). Additionally, the upregulation of H19 imprinted maternally expressed transcript may induce apoptosis and necrosis in cerebral ischemic reperfusion injury (38-40). In the present study, a total of 77 upregulated and 57 downregulated DELs (|FC|>2, P<0.05) were identified through reliable RNA-seq and validated using RT-qPCR in an ischemic stroke group induced by MCAO compared with a control group.

HO-1-mediated neurogenesis has been demonstrated to be enhanced in ischemic stroke in mice (41). HO-1 has been revealed to promote angiogenesis following cerebral ischemic reperfusion injury in rats (42). GO enrichment analysis suggested that HO-1 was associated with responses to alkaloids, cellular responses to oxidative stress and responses to reactive oxygen species. BUB1B has been reported to promote tumor proliferation in glioblastoma (43,44). Similarly, BUB1B has been implicated in tumor growth and the progression of prostate cancer (45), and overexpressed BUB1B has been demonstrated to be involved in lung adenocarcinoma in humans (46). The KEGG enrichment analysis in the present study indicated that BUB1B was associated with the cell cycle. It has previously been reported that upregulated CCL2 is associated with protection from stroke induced by hypoxic preconditioning (47), and the knockdown of CCL2 was used to successfully reverse the drug resistance of tumor cells in gastric cancer (48). In the KEGG enrichment analysis performed in the present study, CCL2 was additionally associated with the cell cycle. Furthermore, based on the data presented in Fig. 3, HO-1, BUB1B and CCL2 may be regulated by a number of novel lncRNAs, including NONRATT008267.2, NONRATT015286.2, NONRATT004791.2, MSTRG.15067.2, NONRATT003289.2, NONRATT004566.2, NONRATT005985.2, NONRATT008198.2, NONRATT028439.2, NONRATT026753.2, NONRATT027268.2, MSTRG.15418.3, NONRATT016680.2, NONRATT015403.2, MSTRG.29693.5, NONRATT009960.2, MSTRG.27670.3 and NONRATT000377.2. A previous study has suggested that the knockdown of DNA topoisomerase IIα (Top2a) may suppress proliferation and invasion of colon cancer cells (49); based on the present regulatory analysis of DELs, Top2a, as a *cis*-regulatory gene of NONRATT005985.2, may have a vital function in ischemic stroke. Overall, the analyzed data provide novel DELs and an lncRNA-mRNA regulatory network that may provide a better understanding of ischemic stroke induced by MCAO.

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Availability of data and materials

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

Authors' contributions

XD and DP conceived and designed the study. XD and QB performed the experiments. LH, CP, LX and HP analyzed the

data and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Animal Experiments Ethics Committee of The Anhui University of Chinese Medicine (Hefei, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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