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 (DElRs) 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 DElRs were predicted. A total of 134 DElRs (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 DElRs and an lncRNA-mRNA regulatory network that may allow for an improved understanding of the molecular mechanism of ischemic stroke induced by MCAO.

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 lncRNAs 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 (DElRs) 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 C20orf1 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 arteriotomy 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 RNA 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/Ih3/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 IncRNAs. Transcripts with class codes ‘i,’ ‘u,’ and ‘x,’ were considered to be potential novel long transcripts. Pffam (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 IncRNAs, and others were considered to be novel IncRNAs. All IncRNAs were quantified using Stringtie. According to the positional association between IncRNA and mRNA in the genome, IncRNAs 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 IncRNAs and mRNAs was calculated (cutoff value, PCC>0.9, P<0.05) and the IncRNA-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 IncRNAs. The first algorithm predicted potential target genes of cis-acting IncRNAs that were physically located within 10 kb upstream or 20 kb downstream of IncRNAs using liftOver genome browser (genome.ucsc.edu/cgi-bin/hgLiftOver). The second algorithm predicted potential target genes of trans-acting IncRNAs based on the IncRNA-mRNA complementary sequences, and predicted IncRNA-mRNA duplex energy. First, BLASTN (29) was performed to detect potential target mRNA sequences with >95% identity and E value <1x10^-3 (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, RNAplex (30) was used to calculate the complementary energy between IncRNAs and their potential trans-regulated target genes with RNAplex-10. 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,
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MSTrG.7720.11, nonraTT005132.2 and MSTrG.20633.3) were assayed using a SYB rGreen fluorophore (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

### Table I. Primer sequences.

| Gene             | Sequence length (base pairs) | Sequence length (base pairs) |
|------------------|------------------------------|------------------------------|
| GAPDH F: 5'-CCCTGTTATGCAACAGAATTG-3' 131 |
| R: 5'-CAGTGAGCGCTCTCTCTCC-3' |
| NONRATT027551.2  F: 5'-GGAACCTGGAAGGTGAAAGGG-3' 118 |
| R: 5'-GGAACCTGGAAGGTGAAAGGG-3' |
| MSTrG.1836.1     F: 5'-CCATTGGTCCTTCCCACCTCC-3' 85 |
| R: 5'-CCACCCTACAAAATCCCTCCC-3' |
| MSTrG.4344.10    F: 5'-GACTTAGGCAAGTGGTGAGG-3' 119 |
| R: 5'-GACTTAGGCAAGTGGTGAGG-3' |
| MSTrG.7720.11    F: 5'-TCCCTAGAGACGCTTCCCACC-3' 97 |
| R: 5'-ATTCGGGTTGCTGGCTTGT-3' |
| NONRATT005132.2  F: 5'-CCTGACTATGGACGTCCTCC-3' 152 |
| R: 5'-CTGAGTCAGTGGTGCCTGT-3' |
| MSTR20633.3      F: 5'-CTTCTACCTCGAGAACCCCCC-3' 117 |
| R: 5'-GCAAGCGAGTGTCTGCTTG-3' |

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.
trimmed reads were mapped onto the reference genome. In total, 24,304 IncRNAs were screened from six samples, and there were 23,255 shared IncRNAs detected in the MCAO and control groups (Fig. 1A). The majority of the identified IncRNAs 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 IncRNAs on the rat chromosomes; 24,304 IncRNA transcripts were identified in all chromosomes, and chromosome 1 included the most IncRNAs (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 IncRNAs are switched-on or off with MCAO. Essentially, positive or negative infinity indicates zero expression of the IncRNA in normal or MCAO groups. It was speculated that this may be associated with the abundance of IncRNAs 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.

**IncRNA-mRNA network.** The cutoff correlation r-values (IPCC>0.9) and P-values (P<0.05) were selected to structure...
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 gene pairs had positive correlations as follows: NONRAT021925.2 (Rho GDP dissociation inhibitor β), NONRAT004791.2 (G protein subunit γ transducing 2), NONRAT015286.2 (periostin), MSTRG.30235.10 (LRR binding FLII interacting protein 1), NONRAT015403.2 (IQ motif containing GTPase activating protein 3), NONRAT008267.2 (kinase family member 14), NONRAT009960.2 (non-SMC condensin I complex subunit G), NONRAT011312.2 (retinol binding protein 3), NONRAT005985.2 (DNA topoisomerase IIα), NONRAT016680.2 (ENSRNOG00000053081), NONRAT013960.2 (galaninreceptor 1), NONRAT016022.2

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**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.
(CART prepropeptide) and NONRATT024616.2 (δ like non-canonical Notch ligand 1) (Table III). Additionally, 90 trans-regulatory genes of IncRNAs were filtered by BLASTN and RNAplex, with a negative correlation identified between ENSRNOT0000092040 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 IncRNAs 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.

| IncRNA ID       | log2FC | Q value | Up/downregulated | Type | Gene ID             | Gene name | log2FC | P-value | Up/downregulated |
|-----------------|--------|---------|------------------|------|---------------------|-----------|--------|---------|------------------|
| NONRAT017723.2  | -3.68  | 0.0125  | Down             | *cis*| ENSRNOG00000000633  | Rhobt1    | 0.85   | 0.1148  |                  |
| NONRAT008064.2  | -6.73  | 0.0145  | Down             | *cis*| ENSRNOG00000001183  | Hnf1a     | 1.25   | 0.7907  |                  |
| NONRAT000377.2  | +∞     | 0.0492  | Up               | *cis*| ENSRNOG00000001492  | Slec8a2   | 0.13   | 0.9091  |                  |
| NONRAT009831.2  | -5.72  | 0.0425  | Down             | *cis*| ENSRNOG00000001982  | Chlb      | 0.60   | 0.1402  |                  |
| NONRAT009718.2  | -5.48  | 0.0020  | Down             | *cis*| ENSRNOG00000002146  | Aasdh     | 0.21   | 0.7048  |                  |
| NONRAT009780.2  | -3.94  | 0.0473  | Down             | *cis*| ENSRNOG00000002208  | Shroom3   | 0.83   | 0.1301  |                  |
| NONRAT005026.2  | 6.14   | 0.0000  | Up               | *cis*| ENSRNOG00000002607  | Sox9      | 0.25   | 0.5566  |                  |
| NONRAT014565.2  | -2.91  | 0.0492  | Down             | *cis*| ENSRNOG00000002864  | Nacc1     | 0.26   | 0.5744  |                  |
| NONRAT005048.2  | -∞     | 0.0432  | Down             | *cis*| ENSRNOG00000003144  | Gprc5c    | 0.48   | 0.5195  |                  |
| NONRAT024547.2  | -∞     | 0.0082  | Down             | *cis*| ENSRNOG00000003955  | Spata7    | 0.26   | 0.6325  |                  |
| NONRAT026753.2  | 3.08   | 0.0450  | Up               | *cis*| ENSRNOG00000003993  | Thap2     | 0.26   | 0.7629  |                  |
| NONRAT005132.2  | -8.71  | 0.0471  | Down             | *cis*| ENSRNOG00000004049  | Biaiap2   | -0.04  | 0.8211  |                  |
| NONRAT026300.2  | +∞     | 0.0469  | Up               | *cis*| ENSRNOG00000004628  | Dazap2    | 0.13   | 0.8554  |                  |
| NONRAT026156.2  | 7.44   | 0.0000  | Up               | *cis*| ENSRNOG00000005332  | Csdc2     | -0.58  | 0.1263  |                  |
| NONRAT005775.2  | 3.69   | 0.0453  | Up               | *cis*| ENSRNOG00000005538  | Psmd11    | -0.03  | 0.7616  |                  |
| NONRAT023334.2  | -4.85  | 0.0448  | Down             | *cis*| ENSRNOG00000005711  | Ptprd     | -0.21  | 0.4658  |                  |
| NONRAT021925.2  | +∞     | 0.0000  | Up               | *cis*| ENSRNOG00000005809  | Arhgdib   | 1.81   | 0.0074  | Up                |
| NONRAT004791.2  | 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  |                  |
| NONRAT025479.2  | -4.14  | 0.0000  | Down             | *cis*| ENSRNOG00000007610  | Gdf11     | 0.08   | 0.9402  |                  |
| NONRAT002023.2  | -7.72  | 0.0000  | Down             | *cis*| ENSRNOG00000007804  | Cigalt1   | 0.45   | 0.2729  |                  |
| NONRAT008198.2  | 4.83   | 0.0305  | Up               | *cis*| ENSRNOG00000007887  | Elk4      | 0.76   | 0.2762  |                  |
| NONRAT022252.2  | +∞     | 0.0305  | Up               | *cis*| ENSRNOG00000008099  | Galnt12   | 0.85   | 0.2496  |                  |
| NONRAT024954.2  | -3.98  | 0.0471  | Down             | *cis*| ENSRNOG00000008155  | Dus4l     | -0.17  | 0.7340  |                  |
| NONRAT028439.2  | 5.06   | 0.0430  | Up               | *cis*| ENSRNOG00000008187  | Ubash3b   | -0.21  | 0.4764  |                  |
| NONRAT022210.2  | -4.24  | 0.0492  | Down             | *cis*| ENSRNOG00000008237  | Uncl3b    | 0.30   | 0.6027  |                  |
| NONRAT027551.2  | 9.08   | 0.0119  | Up               | *cis*| ENSRNOG00000008709  | Arhgap32  | -0.24  | 0.5090  |                  |
| NONRAT027576.2  | -∞     | 0.0061  | Down             | *cis*| ENSRNOG00000008757  | Tmem218   | 0.16   | 0.8511  |                  |
| NONRAT021402.2  | -∞     | 0.0291  | Down             | *cis*| ENSRNOG00000009156  | Tra2a     | 0.47   | 0.2267  |                  |
| NONRAT021972.2  | +∞     | 0.0335  | Up               | *cis*| ENSRNOG00000009338  | Kras      | -0.11  | 0.6201  |                  |
| NONRAT022345.2  | -5.23  | 0.0000  | Down             | *cis*| ENSRNOG0000009795   | Nfib      | 0.04   | 0.9239  |                  |
| MSTRG.16900.3   | -4.70  | 0.0248  | Down             | *cis*| ENSRNOG0000009882   | Ppp3ca    | 0.07   | 1.0000  |                  |
| MSTRG.19870.10  | +∞     | 0.0000  | Up               | *cis*| ENSRNOG000010993    | Dpm1      | -0.14  | 0.5966  |                  |
| NONRAT008272.2  | -3.96  | 0.0431  | Down             | *cis*| ENSRNOG000011063    | Dennd1b   | 0.20   | 0.7240  |                  |
| MSTRG.10245.2   | 6.31   | 0.0002  | Up               | *cis*| ENSRNOG000011704    | Fbxo34    | -0.37  | 0.2745  |                  |
| ID          | log2FC | Q-value | Type | Gene name | log2FC | P-value | Type |
|-------------|--------|---------|------|-----------|--------|---------|------|
| NONRATT010841.2 | 3.41   | 0.0370  | Up   | ENSRNOG0000000002110 | +∞     | 0.0000  | Up   |
| NONRATT018352.2 | 7.89   | 0.0000  | Up   | ENSRNOG0000000002324 | 7.60   | 0.0370  | Up   |
| MSTRG.22390.2  | 5.30   | 0.0370  | Up   | ENSRNOG0000000002634 | +∞     | 0.0000  | Up   |
| NONRATT0015286.2 | 5.30  | 0.0370  | Up   | ENSRNOG0000000002660 | 5.60   | 0.0370  | Up   |
| NONRATT01352.1 | 9.60   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.41   | 0.0370  | Up   |
| NONRATT0018352.2 | 9.24   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.05   | 0.0370  | Up   |
| MSTRG.22390.1  | 5.70   | 0.0370  | Up   | ENSRNOG0000000002716 | 5.30   | 0.0370  | Up   |
| NONRATT0015286.2 | 5.30   | 0.0370  | Up   | ENSRNOG0000000002716 | 5.00   | 0.0370  | Up   |
| NONRATT0018352.2 | 9.60   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.41   | 0.0370  | Up   |
| NONRATT0015286.2 | 5.30   | 0.0370  | Up   | ENSRNOG0000000002716 | 5.60   | 0.0370  | Up   |
| NONRATT01352.1 | 9.60   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.41   | 0.0370  | Up   |
| NONRATT0015286.2 | 5.30   | 0.0370  | Up   | ENSRNOG0000000002716 | 5.00   | 0.0370  | Up   |
| NONRATT01352.1 | 9.60   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.41   | 0.0370  | Up   |
| NONRATT0015286.2 | 5.30   | 0.0370  | Up   | ENSRNOG0000000002716 | 5.60   | 0.0370  | Up   |
| NONRATT01352.1 | 9.60   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.41   | 0.0370  | Up   |
| NONRATT0015286.2 | 5.30   | 0.0370  | Up   | ENSRNOG0000000002716 | 5.00   | 0.0370  | Up   |
| NONRATT01352.1 | 9.60   | 0.0370  | Up   | ENSRNOG0000000002716 | 9.41   | 0.0370  | Up   |
Table III. Continued.

| IncRNA ID        | log2FC | Q value | Up/downregulated | Type | Gene ID               | Gene name        | log2FC | P-value | log2FC | P-value | Up/downregulated |
|------------------|--------|---------|------------------|------|-----------------------|------------------|--------|---------|--------|---------|------------------|
| NONRAT04361.2    | -5.86  | 0.0409  | Down             | cis  | ENSRNOG000000000000  | Alkhh5           | 0.00   | 0.8591  |        |         | -                |
| NONRAT012903.2   | -5.26  | 0.0000  | Down             | cis  | ENSRNOG000000000000  | AABR07027388.1   | -0.85  | 0.1761  |        |         | -                |
| MSTRG.15111.2    | +∞     | 0.0000  | Up               | cis  | ENSRNOG000000000000  | Srek1            | 0.18   | 0.7203  |        |         | -                |
| MSTRG.15111.1    | -7.42  | 0.0000  | Down             | cis  | ENSRNOG000000000000  | Srek1            | 0.18   | 0.7203  |        |         | -                |
| NONRAT028102.2   | 10.21  | 0.0000  | Up               | cis  | ENSRNOG000000000000  | Mlh1             | 0.76   | 0.1272  |        |         | -                |
| NONRAT019889.2   | -4.60  | 0.0113  | Down             | cis  | ENSRNOG000000000000  | Vstml2           | -0.45  | 0.3771  |        |         | -                |
| NONRAT008267.2   | +∞     | 0.0039  | Up               | cis  | ENSRNOG000000000000  | Kif14            | 5.80   | 0.0005  | Up     |         | Up                |
| NONRAT009960.2   | 4.06   | 0.0430  | Up               | cis  | ENSRNOG000000000000  | Ncapg            | 3.28   | 0.0054  | Up     |         | Up                |
| MSTRG.21884.7    | +∞     | 0.0492  | Up               | cis  | ENSRNOG000000000000  | Stau2            | -0.20  | 0.5104  |        |         | -                |
| NONRAT030464.2   | 5.30   | 0.0042  | Up               | cis  | ENSRNOG000000000000  | Nudt10           | -0.59  | 0.1573  |        |         | -                |
| MSTRG.15418.3    | +∞     | 0.0448  | Up               | cis  | ENSRNOG000000000000  | Metazoa_SRP      | 0.03   | 0.9881  |        |         | -                |
| MSTRG.28323.2    | +∞     | 0.0492  | Up               | cis  | ENSRNOG000000000000  | AABR07027388.1   | -0.13  | 0.8591  |        |         | -                |
| MSTRG.4080.13    | +∞     | 0.0002  | Up               | cis  | ENSRNOG000000000000  | Adcy9            | -0.04  | 0.7770  |        |         | -                |
| NONRAT025333.2   | 6.61   | 0.0000  | Up               | cis  | ENSRNOG000000000000  | AABR07027388.1   | 0.11   | 0.9304  |        |         | Up                |
| NONRAT011312.2   | +∞     | 0.0479  | Up               | cis  | ENSRNOG000000000000  | Rbp3             | 9.84   | 0.0000  | Up     |         | Up                |
| NONRAT024648.2   | -∞     | 0.0492  | Down             | cis  | ENSRNOG000000000000  | SNORD113         | 0.45   | 0.6631  |        |         | -                |
| NONRAT005985.2   | 4.53   | 0.0490  | Up               | cis  | ENSRNOG000000000000  | Top2a            | 5.28   | 0.0000  | Up     |         | Up                |
| NONRAT016680.2   | 7.62   | 0.0041  | Up               | cis  | ENSRNOG000000000000  | Top2a            | 3.59   | 0.0011  | Up     |         | Up                |
| NONRAT009382.2   | 8.35   | 0.0257  | Up               | cis  | ENSRNOG000000000000  | Arap2            | 0.21   | 0.6941  |        |         | -                |
| MSTRG.15263.2    | -∞     | 0.0041  | Down             | cis  | ENSRNOG000000000000  | Emb              | -0.06  | 0.7939  |        |         | -                |
| NONRAT015639.2   | 3.25   | 0.0005  | Up               | cis  | ENSRNOG000000000000  | Csd1             | 0.02   | 0.8739  |        |         | -                |
| NONRAT027585.2   | 7.19   | 0.0000  | Up               | cis  | ENSRNOG000000000000  | SNORD14          | 0.17   | 0.9160  |        |         | -                |

IncRNA, long noncoding RNA; FC, fold change.
| IncRNA ID          | log2FC | Q value | Up/downregulated | Type | Gene name | log2FC | P-value | Up/downregulated |
|-------------------|--------|---------|------------------|------|-----------|--------|---------|------------------|
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Ccl9      | 5.19   | 0.0017  | Up               |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Homer3    | 1.00   | 0.0611  | -                |
| ENSRNOT0000088402 | +∞     | 0.0471  | Up               | Trans| Aurkb     | 2.35   | 0.0723  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Galns     | 1.03   | 0.0724  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Scl39a1   | 0.92   | 0.0739  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| AC099384.2| +∞     | 0.1052  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Hsd3b7    | 0.83   | 0.1369  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Klrb1b    | 1.98   | 0.1413  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Me2       | 0.63   | 0.1558  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Pnma2     | -0.51  | 0.1564  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Cass4     | 1.24   | 0.1599  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Klhl23    | -0.44  | 0.2192  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Elac1     | -0.49  | 0.2260  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Clcf1     | 1.26   | 0.2519  | -                |
| NONRATT009960.2   | 4.06   | 0.0430  | Up               | Trans| Lcorl     | -0.44  | 0.3199  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Gpr3711   | 0.40   | 0.3297  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Mmab      | -0.35  | 0.3391  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Spnb1     | -0.28  | 0.3414  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Ifngr2    | 0.44   | 0.3660  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Foxred2   | -0.35  | 0.3732  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Prr22     | -0.67  | 0.3889  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Sppl2a    | 0.40   | 0.3894  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Haus5     | 0.55   | 0.4019  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Cpvl      | 0.80   | 0.4041  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Fam120b   | -0.24  | 0.4061  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Fbxo3     | -0.21  | 0.4206  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Ypel4     | -0.24  | 0.4477  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Klhl26    | -0.20  | 0.4480  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Sync      | 0.43   | 0.5076  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Lhfpl5    | -0.31  | 0.5231  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Mrpl52    | 0.44   | 0.5306  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Clkn15    | -0.39  | 0.5315  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Mrps35    | -0.18  | 0.5443  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Zfp382    | -0.17  | 0.5640  | -                |
| ENSRNOT0000092040 | -3.35  | 0.0204  | Down             | Trans| Ube2k     | -0.14  | 0.5697  | -                |
Table IV. Continued.

| IncRNA ID          | log2FC | Q value | Up/downregulated | Type | Gene name | log2FC | P-value | Up/downregulated |
|--------------------|--------|---------|------------------|------|-----------|--------|---------|------------------|
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Drg1      | -0.12  | 0.5755  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | P2rx5     | -0.36  | 0.5770  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Xpnpep3   | 0.25   | 0.5933  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | RGD1311345| 0.28   | 0.5950  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Acer2     | 0.32   | 0.6034  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Urcp      | 0.28   | 0.6182  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Megf8     | -0.10  | 0.6223  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Lrtm2     | -0.14  | 0.6301  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | RGD1562299| 0.26   | 0.6325  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Rp9       | 0.38   | 0.6347  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Slc25a44  | -0.10  | 0.6348  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Ssmem1    | -0.28  | 0.6350  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Sr3a      | 0.24   | 0.6468  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Spire1    | -0.09  | 0.6631  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | RGD1561777| -0.13  | 0.6846  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Luzp1     | -0.09  | 0.6941  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Acot2     | 0.26   | 0.7000  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Fim2      | -0.07  | 0.7111  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Aox4      | 0.32   | 0.7374  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Golg1     | -0.04  | 0.7775  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Lefty2    | 1.23   | 0.7977  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Zdhhc24   | -0.04  | 0.8075  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Zip329    | -0.03  | 0.8205  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Tmem101   | -0.03  | 0.8236  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Dhh       | 0.28   | 0.8353  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Ncr3      | 0.57   | 0.8354  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Ppl1r15b  | 0.14   | 0.8423  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Zfyve27   | -0.01  | 0.8439  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Ints7     | 0.15   | 0.8457  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Dus11     | 0.14   | 0.8545  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Ahsa2     | 0.15   | 0.8576  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Aif11     | 0.15   | 0.8579  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Rsg1      | 0.16   | 0.8645  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Fuom      | 0.15   | 0.8883  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Cwf1911   | 0.12   | 0.8894  | -                |
| ENSRNOT00000920400 | -3.35  | 0.0204  | Down             | Trans | Paqr7     | 0.01   | 0.8942  | -                |
Table IV. Continued.

| IncRNA 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  | Scl15a1    | -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             | -                 |

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

MSTRG.29693.5, NONRATT009960.2, MSTRG.27670.3 and NONRATT000377.2. A previous study has suggested that the knockdown of DNA topoisomerase IIα (Top2α) 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 IncRNA-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

Figure 6. Validation of IncRNA 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 (R²=0.9338). MCAO, Middle cerebral artery occlusion; IncRNA, long noncoding RNA; RNA-seq, RNA sequencing; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.
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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