Identification of a hippocampal IncRNA-regulating network in a natural aging rat model

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**Abstract**

**Background:** Dysregulation of long noncoding RNA (lncRNA) expression is related to aging and age-associated neurodegenerative diseases, and the lncRNA expression profile in the aging hippocampus is not well characterized. In the present investigation, the changed mRNAs and IncRNAs were confirmed via deep RNA sequencing. GO and KEGG pathway analyses were conducted to investigate the principal roles of the clearly dysregulated mRNAs and IncRNAs. Subsequently, through the prediction of miRNAs via which mRNAs and IncRNAs bind together, a competitive endogenous RNA network was constructed.

**Results:** A total of 447 lncRNAs and 182 mRNAs were upregulated, and 385 lncRNAs and 144 mRNAs were downregulated. Real-time reverse transcription-polymerase chain reaction validated the reliability of mRNA and lncRNA sequencing. KEGG pathway and GO analyses revealed that differentially expressed (DE) mRNAs were associated with cell adhesion molecules (CAMs), the p53 signaling pathway (SP), phagosomes, PPAR SP and ECM—receptor interactions. KEGG pathway and GO analyses showed that the target genes of the DE IncRNAs were related to cellular senescence, the p53 signaling pathway, leukocyte transendothelial migration and tyrosine metabolism. Coexpression analyses showed that 561 DE IncRNAs were associated with DE mRNAs. A total of 58 IncRNA–miRNA–mRNA target pairs were confirmed in this IncRNA–miRNA–mRNA network, comprising 10 mRNAs, 13 miRNAs and 38 IncRNAs.

**Conclusions:** We found specific IncRNAs and mRNAs in the hippocampus of natural aging model rats, as well as abnormal regulatory ceRNA networks. Our outcomes help explain the pathogenesis of brain aging and provide direction for further research.

**Keywords:** Aging, mRNAs, lncRNAs, Hippocampus

**Introduction**

As the global population ages, problems related to aging are sparking immense attention [1, 2]. Brain aging processes are highly complex phenomena. Many basic and clinical investigations have shown that the hippocampus is the main part of the brain involved in aging and dementia [3]. Changes in aging brains comprise changes in the transcription and epigenetics of coding and non-coding genome areas. Among noncoding transcripts, long noncoding RNAs (lncRNAs) have recently emerged as essential regulators of the molecular pathways underlying age-related phenotypes. Previous investigations have also demonstrated that numerous alterations in lncRNA expression occur during aging [4, 5], and it remains unknown whether the lncRNA-modulating network in the hippocampus is altered and how it changes.
IncRNAs are a type of RNAs that are over 200 nucleotides in length, and they lack a complete open reading frame, featuring no or little protein-coding capability. IncRNAs modulate gene expression via various mechanisms, such as RNA–DNA interactions, RNA–protein interactions and RNA–RNA base pairing [6]. In the last twenty years, it has been indicated that IncRNAs, featuring specific spatiotemporal expression patterns across different species, are broadly involved in many biological pathways, including posttranscriptional processing, transcription control, chromatin remodeling and epigenetic regulation [7, 8]. Recent investigations have shown that hundreds of IncRNAs undergo significant changes during the aging process in many organisms, including rhesus monkeys [9] and C. elegans [10]. Additional investigations have confirmed that IncRNAs are involved in the pathogenesis of various age-related disorders, such as liver cancer [11], colorectal cancer [12], vascular aging [13], and Parkinson's disease [14], implying that IncRNAs play a role in the growth and aging of various tissues and organs. Previous studies have shown that, at the epigenetic level, IncRNAs in the hippocampus are closely related to various age-related neuropsychiatric diseases [15], suggesting that IncRNAs in the hippocampus may play an important role in brain aging.

The rat model of natural aging is an ideal animal model for aging research. It can accurately and completely reflect the aging state of the body and the characteristics of human aging [16]. Our previous studies showed that, compared with young rats, the expression of P16 protein and senescence-associated β-galactosidase (sa-β-gal) in tissues and organs. Previous studies have shown that, at the epigenetic level, IncRNAs in the hippocampus are closely related to various age-related neuropsychiatric diseases [15], suggesting that IncRNAs in the hippocampus may play an important role in brain aging.

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**Materials and methods**

**Experimental animals**

Male Sprague–Dawley (SD) rats (9 months old or 14 months old, n=3) were provided and fed as prescribed by the Animal Centre of Shanghai University of Traditional Chinese Medicine (TCM), Shanghai, China. Animals were housed in an environmentally controlled feeding room (with free access to water and food, 20 ± 2 °C, 12 h light/dark cycle). The investigation was permitted by the Animal Ethics Committee of the Shanghai University of TCM. This study was carried out in strict accordance with the recommendations in the National Laboratory Animal Management Regulations of China.

**Natural aging rat model**

Fourteen-month-old rats were raised to 20 months of age.

**Tissue collection**

The rats were killed through cervical vertebral dislocation after anesthetization using pentobarbital sodium, and then the brains were rapidly excised. The gathered specimens were washed with cold normal saline. Then, the hippocampal region was divided, frozen in liquid N₂ and kept at −80 °C before use. Three samples per group were then subjected to high-throughput sequencing and RT-PCR.

**RNA extraction and library preparation**

Total RNA was extracted with an RNeasy Mini Kit (Cat#74106, Qiagen), and RNA quality was checked by applying an Agilent Bioanalyser 4200 (Agilent Technologies, Santa Clara, California, US). The qualify- ing extracted hippocampal RNA specimens satisfied the following conditions: the RNA concentration was at least 100 µg/µL; the RNA quantity was at least 1 µg; the OD260/280 value (an indicator of RNA purity) was between 1.8 and 2.2; and the RNA integrity score was at least 7 (RIN ≥ 7). The synthesized cDNA was end-repaired and then subjected to 3'adenylation. The ends of these 3’adenylated cDNA fragments were connected by utilizing adaptors. PCR Master Mix and PCR Primer Cocktail were used for PCR amplification to enrich cDNA fragments. Then, the PCR product was purified using Ampure XP beads. Sequencing libraries were generated using a VAHTSTM Total RNA-seq Library Prep Kit for Illumina (NR603, Vazyme, Nanjing, China) (VAHTSTM Stranded mRNA-seq Library Prep Kit for Illumina (NR612, Vazyme, Nanjing, China) according to the manufacturer’s instructions.

**RNA sequencing and differentially expressed RNA analyses**

Sequencing was performed on an Illumina NovaSeq platform (Illumina, San Diego, CA, USA). The Read Counts of transcripts and IncRNAs were calculated by Stringtie(version:1.3.0). And the expression of mRNAs and IncRNAs were normalized to FPKM. Then, the mRNAs and IncRNAs were used for differential expression screening and expression level calculation, and genes with |log2(Fold-change)| ≥ 1 and P value < 0.05 were considered differentially expressed (DE) genes. EdgeR (version 4.0.1) was run to screen DE genes. Each sequencing
course and analysis was performed by Shanghai Biochip Co., Ltd. (Shanghai, China).

Validation by quantitative real-time polymerase chain reaction (RT–PCR)
To verify the validity and accuracy of the RNA sequencing results, we carried out RT–PCR assessment to assess data consistency between RNA sequencing and RT–PCR. Total RNA from 50 mg of hippocampal tissue was extracted using Trizol Reagent according to the manufacturer’s protocol. First-strand complementary DNA (cDNA) was synthesized from total RNA using a First-strand cDNA Synthesis Kit (TOYOBO ReverTra Ace qPCR RT Kit). The SYBR GREEN Mix (ABI Power SYBR Green PCR Master Mix) reaction system was used for RT–PCR along with a forward primer, a reverse primer, and cDNA. The reaction process included the following steps: (1) a preincubation step at 95 °C for 10 min; (2) an amplification step involving 40 cycles of 95 °C for 15 s, and (3) different annealing temperatures and 60 °C for 1 min. A melting curve was recorded to verify the absence of primer dimers. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the endogenous control. RNA levels were assayed using the “ΔΔ Ct method” for relative expression [21]. The primers used in RT–PCR are listed in Table 1.

Target prediction
Cis and trans regulation analyses were performed to predict the underlying relationships between the altered lncRNAs and mRNAs, with 10 kb as the cutoff in the cis regulation analysis. lncRNAs may affect gene expression by playing cis and trans regulatory roles. Here, only the differentially expressed lncRNAs and mRNAs were used in the prediction to explore the potential function of lncRNAs. The mRNAs within 10 kb upstream and downstream of lncRNAs were selected as potential cis regulators. The corresponding gene sequence database was used to predict trans target genes. The complementary or similar sequences were selected by BLAST, and then the complementary binding energy between the two sequences was calculated by RNAplex to predict the trans target gene.

GO function and KEGG pathway annotation analyses
All DE genes were mapped to terms in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html) and the Gene Ontology database (http://www.geneontology.org/). GO terms and KEGG pathways with \( P < 0.05 \) were deemed as significantly enriched GO terms and KEGG terms [22].

LncRNA–mRNA coexpression analyses
For coexpression analysis of lncRNAs and genes, according to the expression levels of DE lncRNAs and genes, the Pearson correlation analysis test was used to calculate the correlation between the two expression levels [21]. A correlation coefficient (COR) of > 0.9 and a \( P \) value < 0.01 were considered to indicate a coexpression relationship.

Construction of the lncRNA–miRNA–mRNA ceRNA network
The miRbase database and the miRanda program (v. 3.3a) were used to predict the binding between these miRNA-differentially expressed mRNA/differentially expressed lncRNA sequences using the default parameters of miRanda v. 3.3a (S ≥ 150, ΔG ≤ −30 kcal/mol and demand strict 5’seed pairing), which predicts the miRNAs bound to lncRNAs or mRNAs and then determined the intersection to identify miRNAs bound to both. S refers to the single residue pair match scores of the matching area, and ΔG refers to the free energy of double chain binding [23]. Then, the miRNAs, lncRNAs and mRNAs were used to construct a ceRNA regulatory network of lncRNAs–miRNAs–mRNAs using Cytoscape software [24].

Statistical analysis
Data were analyzed using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). \( P < 0.05 \) was considered to indicate a statistically significant difference. Fold changes and \( P \) values were used to determine the statistical significance of RNA sequence data. |log2(Fold-change)| ≥ 1

Table 1 PCR primers used in this study

| Primer Name       | Sequence                              |
|-------------------|---------------------------------------|
| Cdkn1a            | F GACCTAAGCGTACCGTCCAG                 |
|                   | R CCTGTGACCGTGTCCTCCCTTC              |
| Ifi27             | F GCTGGCACCCGTTATCCAG                 |
|                   | R GCTAGAGAGGGAGGCTGCAAT               |
| NONRATT000231.2   | F AGCTGAGATGACATCCACAC                |
|                   | R CTCTACAGGATTGCCCTGCC                |
| MSTRG.548.1       | F TAGACCTAAACTGTACAAAGGTGTC           |
|                   | R GAGGTGTCTAATGTTGGCTT               |
| Mt-cyb            | F AACGCAGTTAATACCTCCGCC               |
|                   | R TGGTGTCTACTCGTTGTC                 |
| NONRATT020704.2   | F CTCTCATGCACTGCACACC                 |
|                   | R CCAAGACAGGTGACTCTCTCTTA             |
| MSTRG.6345.3      | F AGGCTCGGTTACCCGCTTATT             |
|                   | R GCTAAACTTAGCGAGACGCA               |
| GAPDH             | F GGTGCTCCTCGCAGCTTCA                |
|                   | R GGTGCCAGGTTTCTTACTC               |

The primer synthesis was completed by Shanghai Bioengineering Co., Ltd.
and P value < 0.05 were used as thresholds for DE lncRNAs and mRNAs.

Results

Expression profile of mRNAs in the hippocampus in aging rats

We employed RNA sequencing to explore the mRNA and lncRNA changes in the hippocampus that are closely related to aging. A total of 32,888 mRNAs and 25,092 lncRNAs were identified and subsequently analyzed in depth.

A total of 326 mRNAs were significantly altered in the aging rat hippocampus compared to the 9-month-old control. Among these, 182 mRNAs were upregulated, while 144 mRNAs were downregulated. The most upregulated mRNA was AY172581.16, with an FC of 78.43 compared to the 9-month control. The most downregulated mRNA was Mt-nd4l, with an FC of 0.000421554 compared to the 9-month control. The top 20 upregulated and 20 downregulated mRNAs in the aging rats are listed in Table 2. The clustering analysis and volcano plot visualization showed dramatically different expression levels of mRNAs in the aging and 9-month control groups (Figs. 1 and 2, Additional file 1).

Expression profile of lncRNAs in the hippocampus of aging rats

With regard to lncRNAs, 1219 novel lncRNAs were identified and subsequently analyzed. These have not been reported in the past. A total of 832 lncRNAs were significantly altered in the aging rat hippocampus compared to the 9-month control. Among these, 447 lncRNAs were upregulated, while 385 lncRNAs were downregulated. The most upregulated lncRNA was MSTRG.28323.2, with an FC of 296.92 compared to the 9-month control. The most downregulated lncRNA was MSTRG.6082.3, with an FC of 0.0002 compared to the 9-month control. The top 20 upregulated and 20 downregulated lncRNAs in the aging rats are listed in Table 3. The clustering analysis and volcano plot visualization showed dramatically different expression levels of lncRNAs in the aging and 9-month control groups (Figs. 3 and 4, Additional file 1).

Expression profile validation

To verify the validity of RNA sequencing, we randomly selected the differentially upregulated mRNAs of Cdkn1a and Ifi27, the downregulated mRNA of Mt-cyb, the differentially upregulated lncRNAs MSTRG.548.1, NONRATT000231.2 and NONRATT020704.2 and the downregulated lncRNA MSTRG.6345.3 that were abundantly expressed and exhibited significant changes for detection by RT–PCR. The RT–PCR results showed that the change trends of the selected mRNA and lncRNA levels determined by RT–PCR were consistent with those determined by RNA sequencing (Fig. 5).

GO function and KEGG pathway enrichment analyses of DE mRNAs

GO analysis indicated that the most enriched mRNAs were related to negative regulation of cellular response to growth factor stimulus and long – chain fatty acid transport in the biological process category, collagen trimer, brush border membrane and protein complex involved in cell adhesion in the cellular component category, and extracellular matrix structural constituent in the molecular function category. KEGG pathway analysis showed that the top 30 differentially enriched KEGG pathways related to dysregulated mRNAs were phagosome, PPAR signaling pathway, p53 signaling pathway, ECM—receptor interaction, cell adhesion molecules, tryptophan metabolism and cell adhesion molecules (CAMs) (Fig. 6).

GO function and KEGG pathway enrichment analyses of DE lncRNAs

The target genes of lncRNAs were subjected to GO and KEGG analyses. GO analysis indicated that the most enriched target genes of lncRNAs were related to antigen processing and presentation of endogenous antigen, antigen processing and presentation of endogenous peptide antigen via MHC class Iib, regulation of skeletal muscle tissue regeneration and leukocyte migration involved in inflammatory response. KEGG pathway analysis showed that the top 30 differentially enriched KEGG pathways related to dysregulated lncRNAs were involved in cellular senescence, leukocyte transendothelial migration, the p53 signaling pathway and tyrosine metabolism (Fig. 7).

Construction of the lncRNA–mRNA coexpression network

In total, 516 DE lncRNAs and their corresponding DE mRNAs were identified (P value < 0.01 and COR > 0.9), and the lncRNA–mRNA coexpression network was constructed by Cytoscape 3.6.0 (Fig. 8). Among these lncRNAs, 52 lncRNAs may be involved in the regulation of Cdkn1a (24.445-fold change), which encodes a potent cyclin-dependent kinase inhibitor. MSTRG.548.1 (lncRNA, 5.840-fold change) and NONRATT000231.2 (lncRNA, 5.728-fold change) may be closely involved in the regulation of Cdkn1a expression.

Construction of the lncRNA–miRNA–mRNA regulatory network

Because they competitively bind miRNAs as a miRNA sponge, lncRNAs could form a ceRNA network of lncRNAs–miRNAs–mRNAs to boost the expression
of miRNA target genes. Based on the regulatory miRNA–mRNA and lncRNA–miRNA pairs, a lncRNA–miRNA–mRNA network was constructed. A total of 58 lncRNA–miRNA–mRNA target pairs were identified, including 38 lncRNAs, 13 miRNAs, and 10 mRNAs (Fig. 9).

**Table 2** Top 40 differentially expressed mRNAs determined by sequencing analysis

| Gene name     | P-value   | Fold change | log2FC | Regulation | Location       |
|---------------|-----------|-------------|--------|------------|----------------|
| AY172581.16   | 0.00000517| 78.43307244 | 6.293390211 | Up         | MT:9800–9867   |
| Sostdc1       | 0.0000565 | 37.20473726 | 5.217414425 | Up         | 6:55812747–55817066 |
| AABRO70701.2061.2 | 0.000276717 | 36.19501976 | 5.177719299 | Up         | 2:18454307–184570387 |
| Ttr           | 0.0000568 | 34.74348865 | 5.107415399 | Up         | 18:15532963–155340177 |
| Tmem72        | 0.0000164 | 30.39692823 | 4.925836364 | Up         | 14:48817514–148845267 |
| KC            | 0.0000282 | 27.60819556 | 4.787046933 | Up         | 12:9430600–987551  |
| Mifp          | 0.000000987 | 25.5258481 | 4.67388699 | Up         | 8:48437918–48443421 |
| Cldn2         | 0.0000317 | 24.44508965 | 4.61472791 | Up         | X:111122552–111137769 |
| Slco1a2       | 0.0000702 | 24.40311831 | 4.608993607 | Up         | 4:176445858–176528110 |
| Citm          | 0.00250806 | 23.73775702 | 4.569111716 | Up         | X:32118054–32153794 |
| Scl4a5        | 0.00000909 | 23.71043816 | 4.567450418 | Up         | 4:114918488–115002300 |
| LOC103690108  | 0.000276717 | 23.23285905 | 4.538094799 | Up         | 2:18454307–184570387 |
| Ttr           | 0.0000568 | 22.1951533  | 4.47217268 | Up         | 13:82479998–82535534 |
| Adipoq        | 0.00248908 | 21.02293971 | 4.393892515 | Up         | 11:81330293–81344488 |
| Steap1        | 0.000387301 | 16.99301725 | 4.086870133 | Up         | 4:114918488–115002300 |
| Colba1        | 0.0000282 | 15.79779722 | 3.981651502 | Up         | 5:124442293–124542156 |
| Igh6          | 0.000103603 | 12.57562744 | 3.652558478 | Up         | 4:85551502–8569360 |
| LOC100359515  | 0.009751355 | 0.02911514 | 5.10209043 | Down       | 4:98337367–9835237 |
| Cdcpc1        | 0.002985718 | 0.054583323 | 4.19539596 | Down       | 8:13266029–132696661 |
| Gml           | 0.0008896 | 0.061732641 | 4.01782267 | Down       | 7:116039715–116063098 |
| AABRO7043626.2 | 0.001995124 | 12.21190348 | 3.610216816 | Up         | 20:3134704–31353301 |
| Mt-nd4l       | 0.00112407 | 0.00421554 | 11.211995 | Down       | MT:9870–10166 |
| Igh6          | 0.000103603 | 0.013803597 | 6.17881192 | Down       | 11:81330293–81344488 |
| Lhx8          | 0.000915014 | 0.027012853 | 5.2102166 | Down       | 2:260574190–260596777 |
| AABRO7060872.1 | 1.62557E–05 | 0.029115265 | 5.10209043 | Down       | 4:98337367–9835237 |
| Cdcpc1        | 0.002985718 | 0.054583323 | 4.19539596 | Down       | 8:13266029–132696661 |
| AABRO7043626.2 | 0.001995124 | 12.21190348 | 3.610216816 | Up         | 20:3134704–31353301 |
| Mt-nd4l       | 0.00112407 | 0.00421554 | 11.211995 | Down       | MT:9870–10166 |
| Igh6          | 0.000103603 | 0.013803597 | 6.17881192 | Down       | 11:81330293–81344488 |
| Lhx8          | 0.000915014 | 0.027012853 | 5.2102166 | Down       | 2:260574190–260596777 |
| AABRO7060872.1 | 1.62557E–05 | 0.029115265 | 5.10209043 | Down       | 4:98337367–9835237 |

**Discussion**

In this investigation, high-throughput sequencing revealed DE mRNAs and lncRNAs in the rat hippocampus during the aging process. In comparison to the 9-month control group, 326 mRNAs and 832 lncRNAs in the hippocampus of naturally aging rats showed
significantly changed levels. Among them, 447 lncRNAs and 182 mRNAs were upregulated, and 385 lncRNAs and 144 mRNAs were downregulated. The results showed that the RT–PCR data were almost completely consistent with the sequencing outcomes, which confirmed sequencing outcome reliability. In the process of aging, the synthesis of protein molecules related to cognitive function is reduced. Downregulation of lncRNAs may reduce the ability to protect mRNAs from degradation and reduce mRNA levels by reducing the inhibitory effect of lncRNAs on miRNAs. Upregulation of lncRNAs may upregulate the expression of protein molecules in a compensatory manner during natural aging. These DE lncRNAs and mRNAs may hold the key to the alterations in cognitive function, metabolism, function and hippocampal structure resulting from natural aging.

To better understand the potential mechanisms and biological roles of DE mRNAs in aging rats, we conducted GO and KEGG pathway enrichment analyses. GO analyses indicated that the most enriched mRNAs were associated with negative modulation of cellular response to GFS and long-chain fatty acid transport in the biological process category, collagen trimer, basal part of cell and basal plasma membrane in the cellular component category, and extracellular matrix structural constituent in the molecular function category. KEGG pathway analyses of the DE genes also revealed some key pathways associated with phagosomes, cell adhesion molecules,
ECM—receptor interactions, p53 SP, PPAR SP, tryptophan metabolism and cell adhesion molecules (CAMs). The p53 SP and PPAR SP have previously been identified to be related to aging [25, 26]. Investigations in multiple organisms have demonstrated that tryptophan metabolism is a powerful regulator of age-associated disorders and lifespan [27]. The phagosome [28] and ECM-receptor interaction [29] are the most typical pathways associated with aging. By recruiting neurotransmitter receptors, scaffolding proteins and synaptic vesicles, closed with synaptic plasticity, CAMs have been confirmed to increase synaptic strength [30].

Among noncoding transcripts, lncRNAs have recently emerged as important regulators of the molecular pathways underlying age-related phenotypes. Interestingly, DE lncRNAs have been implicated in healthy aging [31, 32] and neurodegenerative and developmental disorders [33], raising the question of whether lncRNAs play a role in human brain aging. In the investigation, GO analyses indicated that the most enriched lncRNA target genes were related to antigen processing and presentation (APP) of endogenous peptide antigens and of endogenous antigens through MHC class I b, modulation of skeletal muscle tissue regeneration and leukocyte migration associated with the inflammatory response. KEGG pathway analyses revealed that the top 30 differentially enriched pathways associated with dysregulated lncRNAs were involved in cellular senescence, leukocyte transendothelial migration, the p53 signaling pathway and tyrosine metabolism.

The mechanism of action of lncRNAs includes cis/trans gene regulation, nuclear structure organization and protein and RNA interaction and regulation [34]. In these two regulatory approaches, mRNAs and lncRNAs are coexpressed in neurons. By predicting the coexpression of mRNAs and lncRNAs, we can predict the possibility of some regulation or interaction between them. In the investigation, 561 lncRNAs related to DE mRNAs were predicted. This suggests that the lncRNAs are involved in DE mRNA regulation, but this would need to be verified through further experiments. Among these lncRNAs, 52 lncRNAs may be involved in the regulation of the

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Fig. 2  Volcano plot of differentially expressed mRNAs in the aging group and the 9-month control group. Normalized fold change and P values (aging group/9-month control group) were used to construct the volcano plots (n = 3). The y-axis and x-axis represent the P value and fold change, respectively. The red and blue dots represent significantly upregulated and downregulated mRNAs, respectively. The gray dots represent no statistically significantly altered mRNAs.
expression of Cdkn1a (24.445-fold change), a major hallmark of senescence in most cells, which encodes a potent cyclin-dependent kinase inhibitor. Analysis of the data revealed MSTRG.548.1 and NONRATT000231.2 may be closely involved in the regulation of Cdkn1a expression. Therefore, further research is necessary to better understand the regulation of these networks.

Acting as molecular sponges for miRNAs via shared miRNA response elements (MREs), ceRNAs are groups of noncoding RNAs, miRNAs and other RNAs competing with miRNAs at the posttranscriptional level, thus modulating downstream molecular pathways and regulating mRNA expression. The ceRNA network links the role of protein-encoding mRNAs to the role of noncoding...
RNAs. In a target transcript, miRNAs binding to MREs decrease miRNA suppressor activity against other target genes. In theory, all transcripts containing one or more MREs can play a role as ceRNAs. Hence, the ceRNA modulation theory predicts a widespread posttranscriptional modulation pattern of gene expression. An in-depth investigation of the ceRNA regulatory mechanisms will help elucidate the disorder-related pathogenesis.

The lncRNA FLJ46906 binds to the transcription factors AP-1 and NF-κB and modulates the expression of aging-related genes [35]. Meanwhile, the lncRNA NEAT1 is involved in neurodegeneration, and suppression of this lncRNA in the hippocampus enhances memory in elderly mice by repressing neuronal histone methylation [36]. Previous investigations have shown that lncRNAs modulate target gene expression through the ceRNA network and are involved in the development of age-associated disorders [37, 38]. lncRNA-ES3 suppresses miR-34c-5p expression through direct interaction, and knockdown of this lncRNA inhibits the senescence of vascular smooth muscle cells, regulated through the lncRNA-ES3/miR-34c-5p/Bcl-2 modifying factor axis [39]. The lncRNA NONMMUT 055,714 constitutes a miRNA-7684-5p sponge that protects against postoperative cognitive dysfunction [40].

In this study, 58 lncRNA–miRNA–mRNA target pairs were confirmed, comprising 10 mRNAs, 13 miRNAs and 38 lncRNAs in rat hippocampal tissue. miR-214-3p, miR-378a-3p, miR-34c-5p, miR-24-3p, miR-222-3p, miR-221-3p, miR-31a-5p, miR-150-5p,
miR-127-3p, miR-672-5p, and miR-34a-5p were identified to be related to aging processes in previous investigations. After comparing all total RNAs acquired from 13 old and 15 young individuals for validating the FC by employing quantitative RT-PCR, miR-24-3p was confirmed as a novel candidate aging biomarker [41]. By applying miR-378a-3p to study ‘aging miRNA’ profiles, patients were classified into two distinct groups presenting obviously different outcomes for some clinical/biological aging parameters [42]. In neurons of the cortex and hippocampus, miR-150-5p has previously been identified as deregulated in brain tissues in AD models [43]. Functional luciferase assays implied that mir-31a-5p in the hippocampus can modulate the expression of the interleukin 1 receptor antagonist and Mt1a [44]. miR-34a-5p was confirmed as an exosomal transfer RNA for inducing cardiac senescence-related injury, and suppressing miR-34a-5p in macrophages decreased the exosome PD-1 suppressor-induced prosenescent impact in cardiomyocytes [45]. Circulating miR-127-3p is a potential biomarker for differential diagnoses in frontotemporal dementia [46]. The results revealed that miR-31a-5p acts as an important regulator in the age-associated bone marrow microenvironment through the influence of osteoblastic and osteoclastic differentiation and that it
Fig. 6 GO function and KEGG pathway classification (A and C) and enrichment (B and D) analyses of DE mRNAs. The enrichment value (− log10 (P value)) was calculated and visualized to show the top 30 enriched GO terms (B), and top 30 enriched pathways (D).
Fig. 6 continued

KEGG Classification

Top 30 of Pathway Enrichment

Gene_number

p_value

Rich Factor
Fig. 7  GO function and KEGG pathway classification (A and C) and enrichment (B and D) analyses of DE lncRNAs. The enrichment value (− log10 P value) was calculated and visualized to show the top 30 enriched GO terms (B), and top 30 enriched pathways (D).
Fig. 7 continued

KEGG Classification

- Sensory system
- Nervous system
- Immune system
- Excretory system
- Environmental adaptation
- Endocrine system
- Digestive system
- Development
- Circulatory system
- Xenobiotics biodegradation and metabolism
- Nucleotide metabolism
- Lipid metabolism
- Glycan biosynthesis and metabolism
- Global and overview maps
- Energy metabolism
- Carbohydrate metabolism
- Biosynthesis of other secondary metabolites
- Amino acid metabolism
- Translation
- Transcription
- Replication and repair
- Folding, sorting and degradation
- Signaling molecules and interaction
- Signal transduction
- Membrane transport
- Transport and catabolism
- Cellular community
- Cell motility
- Cell growth and death

Class
- Cellular Processes
- Environmental Information Processing
- Genetic Information Processing
- Metabolism
- Organismal Systems

Top 30 of Pathway Enrichment

- Vitamin B6 metabolism
- Viral myocarditis
- Ubiquinone and other terpenoid quinone biosynthesis
- Tyrosine metabolism
- Type I diabetes mellitus
- Sphingolipid metabolism
- SNARE interactions in vesicular transport
- Small cell lung cancer
- Salmonella infection
- Porphyrin and chlorophyll metabolism
- P53 signaling pathway
- Non-homologous end-joining
- Nicotinate and nicotinamide metabolism
- Mismatch repair
- Lipoic acid metabolism
- Leukocyte transendothelial migration
- Histidine metabolism
- Graft-versus-host disease
- Glycosphingolipid biosynthesis globoseries
- Glycosaminoglycan biosynthesis keratan sulfate
- Gioma
- Fatty acid elongation
- Fatty acid degradation
- Cellular senescence
- Caffeine metabolism
- Autoimmune thyroid disease
- Arginine biosynthesis
- Allograft rejection
- ABC transporters
- 2-Oxocarboxylic acid metabolism

q_value
- 0.5
- 0.4
- 0.3
- 0.2

Gene_number
- 5
- 10
- 15
- 20

Rich Factor
- 2.5
- 5.0
- 7.5
- 10.0
can be a potential therapeutic target for age-associated osteoporosis [47].

Therefore, we speculated that the DE IncRNAs associated with aging in this study could hold the key to hippocampal senescence through the ceRNA network. Our research is only the beginning, and there remain many challenges to be addressed in the future. The mechanism by which DE IncRNAs regulate brain aging through the ceRNA network will be further verified. We will validate the ceRNA network and then search for meaningful pathways and biological processes based on the GO and KEGG results, identify the target mRNAs, and study the IncRNAs and miRNAs regulating the target mRNAs using gene knockdown or siRNA techniques in future work.

**Conclusions**

In this study, we found specific IncRNAs and mRNAs in the hippocampus of natural aging model rats, as well as abnormal regulatory ceRNA networks. However, our current research has some limitations. A small specimen size can cause improper prediction of DE mRNAs
and DE lncRNAs. In future research, a larger specimen size to verify our current outcomes is needed. Further experimental studies are also needed to compare the DE genes differences between male and female rats. In this study, 58 lncRNA–miRNA–mRNA target pairs were confirmed in the hippocampus, which may be involved in brain aging. Nonetheless, experiments are required to verify how lncRNAs modulate mRNAs via miRNAs. To date, this is the first high-throughput sequencing analysis of the expression profiles of lncRNAs and mRNAs in natural aging rats. Our results are helpful for understanding possible mechanisms of natural brain aging and offering a promising target to address aging.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12868-022-00743-7.

Additional file 1: The dramatically differentially expressed mRNAs in the aging and 9-month control groups.
Additional file 2: The dramatically differentially expressed lncRNAs in the aging and 9-month control groups.

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Author contributions
LQ and XL contributed equally to the work. SZ and CZ designed the study. XL, DLI, XYJ analyzed the data; SYZ and XL conducted the animal experiments and recorded the experiments; SYZ, SHS, SML, GNL and BZM performed the research; LQ wrote the main paper text; XL, DH, LH prepared Figs. 1–9 and revised the paper; SZ and CZ supervised the research and edited the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated during the current study are available in the GSA repository (CRAO07007, https://ngdc.cnpc.ac.cn/gsaas/p4q61kSb).

Declarations

Ethics approval and consent to participate
The humane care and use of animals in this study was permitted by the Ethics Committee of Shanghai University of TCM (PZSHUTCM190524004). This study was carried out in strict accordance with the recommendations in the National Laboratory Animal Management Regulations of China. This study is reported in accordance with ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors have stated no conflicts of interest.

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