Distinct Hippocampal Expression Profiles of Long Non-coding RNAs in an Alzheimer’s Disease Model

Bo Yang1 · Zi-an Xia1,2 · Bingwu Zhong2 · Xingui Xiong1 · Chenxia Sheng2 · Yang Wang1 · Wei Gong2 · Yucheng Cao2 · Zhe Wang2 · Weijun Peng2

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Abstract Alzheimer’s disease (AD), the most prevalent form of dementia worldwide, is a complex neurodegenerative disease characterized by the progressive loss of memory and other cognitive functions. The pathogenesis of AD is not yet completely understood. Although long non-coding RNAs (lncRNAs) have recently been shown to play a role in AD pathogenesis, the specific influences of lncRNAs in AD remain largely unknown; in particular, hippocampal lncRNA expression profiles in AD rats are lacking. In this study, microarray analysis was performed to investigate the hippocampal expression patterns of dysregulated lncRNAs in a rat model of AD. A total of 315 lncRNAs and 311 mRNAs were found to be significantly dysregulated in the AD model (≥2.0 fold, \( p < 0.05 \)). Then, quantitative real-time PCR was used to validate the expression of selected lncRNAs and mRNAs. Bioinformatics tools and databases were employed to explore the potential lncRNA functions. This is the first study to comprehensively identify dysregulated hippocampal lncRNAs in AD and to demonstrate the involvement of different lncRNA expression patterns in the hippocampal pathogenesis of AD. This information will enable further research on the pathogenesis of AD and facilitate the development of novel AD therapeutics targeting lncRNAs.

Keywords Alzheimer’s disease · Long non-coding RNAs (lncRNAs) · Microarray · Hippocampus · Expression profiles

Introduction

Alzheimer’s disease (AD) is considered to be the most common cause of dementia; AD is a progressive neurodegenerative disease characterized by the accumulation of amyloid-\(\beta\) (A\(\beta\)) plaques and neurofibrillary tangles, synaptic and neuronal loss, and cognitive decline [1]. In the USA alone, an estimated 5.2 million individuals aged 65 and older have AD, and this number is expected increase to 13.8 million by 2050 [2]. Unfortunately, no currently available therapeutic strategies for AD slow or stop the neuronal damage that causes AD symptoms and eventually results in death [3, 4]. Thus, there is an urgent need for novel strategies of improving our mechanistic understanding of AD, which could lead to the discovery of novel therapeutic targets.

Long non-coding RNAs (lncRNAs), a subclass of ncRNAs, are most commonly defined as the transcripts of more than 200 nucleotides that structurally resemble mRNAs but have no protein-coding capacity [5]. With the development of techniques to detect lncRNA, accumulating evidence indicates that lncRNAs participate in a wide variety of important biological phenomena, such as imprinting genomic loci, influencing chromosome conformation and allosterically regulating enzymatic activity [6]. Moreover, multiple lines of evidence have linked lncRNA mutations and dysregulation with diverse human diseases, ranging from different types of cancer and neurodegeneration to gynaecological diseases [7–9].
The role of IncRNAs in AD has attracted considerable attention. Recent studies have further confirmed the involvement of certain IncRNAs in AD [10–13]. In addition, with recent advancements in transcriptome-wide profiling, numerous AD-associated IncRNAs have been discovered. Furthermore, the dysregulation of IncRNA expression in post-mortem tissue samples from AD patients [14, 15] and transgenic AD animals [16] has been investigated. Despite these findings, the expression patterns, targets, and functions of IncRNAs involved in the pathogenesis of AD remain largely unknown [17]. Therefore, further research is of great importance.

In addition, as a crucial component of the medial temporal lobe memory circuit, the hippocampus is affected early in AD and displays synaptic and intraneuronal molecular remodelling against a pathological background of extracellular Aβ deposition and intracellular neurofibrillary tangle formation in the early stages of AD [18]. Moreover, behavioural studies have long suggested that the hippocampus plays a critical role in learning and memory, which depend on functional and structural changes occurring in the hippocampus, such as long-term potentiation and synaptic remodelling [19].

Therefore, in the present study, we applied microarray technology to analyse the expression profiles of IncRNAs and messenger RNAs (mRNAs) in the hippocampus of rats in a validated AD model. Additionally, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to predict the biological roles and potential signalling pathways of these differentially expressed IncRNAs. Moreover, an IncRNA-mRNA network analysis was conducted to further explore the potential roles of differentially expressed IncRNAs in AD pathogenesis.

Materials and Methods

Ethics Statement

All animal protocols were approved by the Central South University (Changsha, China) and were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. This investigation was conducted in accordance with ethical standards and the Declaration of Helsinki, as well as according to national and international guidelines. This research was approved by the authors’ institutional review board.

Animals and Experimental Design

A total of 20 adult, male Sprague–Dawley rats (250 ± 30 g) were purchased from the Laboratory Animal Centre of Central South University. The animals were housed under controlled conditions (12-h light/dark cycle, 25 °C, 50 ± 10 % relative humidity) with water and food pellets available ad libitum.

The animals were randomly divided into the following two groups (n = 10 in each group): (1) the AD group and (2) the control group.

Surgery

In this experiment, we utilized an intracerebroventricular (ICV) injection of Aβ1–42 oligomers into the cerebral ventricles of the animals to induce a validated AD model, as we have previously described [20]. In brief, the animals were anaesthetised with 10 % chloral hydrate (4 ml/kg) and placed in a stereotactic frame. The Aβ1–42 oligomers (5 μg*2, Sigma, St. Louis, MO, USA) were injected bilaterally into the lateral ventricles through a stainless steel cannula by using the following coordinates: 1.1 mm posterior to the bregma, 2.2 mm lateral to the sagittal suture, and 3.0 mm beneath the dura. For the control group, the vehicle was injected bilaterally into the lateral ventricles through a stainless steel cannula by using the above-mentioned coordinates.

Sample Collection

After the Morris water maze test was completed, all rats were anaesthetised with chloral hydrate and were sacrificed via decapitation. Their hippocampal tissues were stored in liquid nitrogen followed by storage at −80 °C prior to analysis.

Morris Water Maze Test

Spatial learning and memory deficits were evaluated using the Morris water maze test as we have previously described [21], with additional modifications. In brief, the test was conducted at 31–35 days after injury. Initial training was conducted on days 31–34 after injury; during this period, we trained the rats to locate a hidden, submerged platform using peripheral visual information. The rats were introduced into a pool at one of four entry points, and every entry point was used over the course of a day. The rats were given 60 s to locate the platform and were allowed to remain on the platform for 10 s before being removed. Rats that were unable to locate the platform within 60 s were placed on the platform for 10 s before being removed from the pool. On the 35th day, a probe trial was conducted, in which the platform was removed and the number of crossings over the previous platform location was recorded over one 60-s trial. An overhead video camera connected to the ANY-maze video tracking system (Stoelting Co., USA) was used to record all the trials and track the movements of the animals.
RNA Extraction

Total RNA was extracted from each hippocampal tissue sample by soaking the tissue samples in TRIzol Reagent (Invitrogen, Grand Island, NY, USA) in accordance with the manufacturer’s instructions. RNA quantity and quality were measured using a Nano Drop ND-1000, and RNA integrity was assessed by standard denaturing agarose gel electrophoresis.

Microarray Analysis

Sample labelling and array hybridization were performed according to the Agilent One-Colour Microarray-Based Gene Expression Analysis protocol (Agilent Technology), with minor modifications. In brief, mRNA was purified from total RNA after the removal of rRNA (mRNA-ONLY™ Eukaryotic mRNA Isolation Kit, Epicentre). Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3’ bias via a random priming method (Arraystar Flash RNA Labelling Kit, Arraystar). The labelled cRNAs were purified using an RNeasy Mini Kit (Qiagen). The concentration and specific activity of the labelled cRNAs (pmol Cy3/μg cRNA) were measured using the NanoDrop ND-1000. In all, 1 μg of each labelled cRNA was fragmented by adding 5 μl of 10 × Blocking Agent and 1 μl of 25 × Fragmentation Buffer and heating the mixture at 60 °C for 30 min. Then, 25 μl of 2 × GE Hybridization Buffer was added to dilute the labelled cRNA. Subsequently, 50 μl of hybridization solution was dispensed into the gasket slide, which was then assembled with the lncRNA expression microarray slide. The slides were incubated for 17 h at 65 °C in an Agilent Hybridization Oven. The hybridized arrays were washed, fixed and scanned using an Agilent DNA Microarray Scanner (part number G2505C). The hybridized arrays were washed, fixed and scanned using an Agilent DNA Microarray Scanner (part number G2505C).

Quantitative Real-Time-PCR Validation

As previously described [22], total RNA was reverse-transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Grand Island, NY, USA) in accordance with the manufacturer’s instructions. An Applied Biosystems ViiA 7 Real-Time PCR System and 2 × PCR Master Mix were used to perform quantitative real-time (qRT)-PCR (Arraystar). The reaction conditions were as follows: incubation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. The relative lncRNA and mRNA expression levels were calculated using the 2^−ΔΔCt method and were normalized to GAPDH, as an endogenous reference transcript [23]. The specific primers for each gene are listed in Table 1. The data represent the means of three experiments.

GO Annotations and KEGG Pathways

GO annotations and pathway analysis were applied to investigate the roles of all differentially expressed mRNAs, as previously described [24, 25]. In brief, GO analysis was applied to elucidate genetic regulatory networks of interest by forming hierarchical categories according to the molecular function, biological process, and cellular component aspects of the differentially expressed genes (http://www.geneontology.org). Pathway analysis was performed to explore the significant pathways of the differentially expressed genes, according to KEGG (http://www.genome.jp/kegg/).

Construction of Co-Expression Network

To identify interactions among the differentially expressed lncRNAs and mRNAs, we constructed a co-expression network based on a correlation analysis of the differentially expressed lncRNAs and mRNAs [26]. The network was constructed according to the normalized signal intensities of specific mRNA and lncRNA expression levels. Pearson’s correlation coefficients equal to or greater than 0.7 were used to identify the lncRNAs and coding genes. Then, the lncRNA-mRNA co-expression network was constructed using Cytoscape software (The Cytoscape Consortium, San Diego, CA, USA).

Statistical Analysis

All data are shown as the mean ± standard error of the mean (SEM) and were analysed using the statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Student’s t test was performed for comparisons between two groups, whereas ANOVA was performed for repeated measures. The false discovery rate was calculated to correct the p value. Differences with p < 0.05 were considered statistically significant. Fold change (FC) and Student’s t test were used to analyse the statistical significance of the microarray results. FC ≥ 2 and p < 0.05 were considered the threshold values for designating differentially expressed lncRNAs and mRNAs.

Results

Morris Water Maze Test

Spatial learning was evaluated on days 31–34 after the rats received an ICV Aβ1–42 injection, and a probe trial for spatial memory was conducted on day 35. As expected, rats injected with Aβ1–42 were less able than mice in the control group to
find the platform and learn its location by the 34th day of training, indicating poor learning performance (Fig. 1b). In addition, compared with animals in the control group, the Aβ1–42-treated animals performed poorly and failed to recollect the location of the submerged platform on the 35th day, demonstrated by significantly fewer crossings over the previous platform location (Fig. 1c).

Table 1 Primers designed for qRT-PCR validation of candidate lncRNAs and mRNAs

| lncRNA/mRNA | Forward primer | Reverse primer | Product length | Tm(°C) |
|-------------|----------------|----------------|----------------|--------|
| BC092582    | 5′-GGAGGTGAATGCTGAGGAGGA-3′ | 5′-ATGAAGGTAGAGGCCGTGTTGTC-3′ | 60 | 60 |
| MRAK050857  | 5′-CCCCCAAGACCGTGAGGTG-3′ | 5′-GGAGACAGCGCCCTGAGAACGAG-3′ | 120 | 60 |
| MRAK088596  | 5′-AGGGTAACGAGAACAAGAGAA-3′ | 5′-CATGGTACACTAGAATGCTA-3′ | 59 | 60 |
| MRAK081790  | 5′-AAAATCGGTGAGGAGTGTAAGAATGCTG-3′ | 5′-CTTGGGCTACGATTTCCCTGT-3′ | 168 | 60 |
| MAPK10      | 5′-ATGTTAGTGATAGCCACCCCAAGAGAAG-3′ | 5′-TGCTCCCCTTTCTACGAGTTGC-3′ | 60 | 63 |
| S100a8      | 5′-GGGAATCACCATGCGGGAATTAC-3′ | 5′-GCCACACCTTATACAACACCCCC-3′ | 60 | 168 |
| GAPDH       | 5′-GCTCTCTGCTCTCCTCCTGGTCTCT-3′ | 5′-TGTAACAGGCGTCGGATAACGAG-3′ | 60 | 124 |

**Fig. 1** Spatial learning and memory was assessed in Morris water maze test. (a) Representative images of the swim paths on day 34, showing that rats in the control group were able to find the hidden platform more easily than the AD rats and indicating that the rats in the AD group (31 s) required a longer time to find the hidden platform than the rats in the control group (15 s); these results suggested that Aβ1–42 impaired spatial learning ability. (b) Escape latency. A significant difference was detected between the control and AD groups on days 32–34 (*p < 0.05). (c) Probe test. The frequency of crossing the target platform was recorded as an indicator of spatial memory. Significant differences were observed between the control and AD groups (*p < 0.05). (d) Representative images of the frequency of crossing the target platform, showing that rats in the control group had a crossing frequency of approximately 3 times, while that of rats in the AD group was reduced to approximately 1 time, suggesting that there was significantly better performance in the control group compared with the AD group; n = 6 rats/group. Data are expressed as the mean ± standard error of the mean (SEM).
| SeqID        | P value | Fold change | Regulation | Chr | Strand | Relationship         | AD-1   | AD-2   | AD-3   | Control-1 | Control-2 | Control-3 |
|-------------|---------|-------------|------------|-----|--------|----------------------|--------|--------|--------|------------|------------|------------|
| BC158567    | 0.01001 | 4.58596     | Down       | 20  | +      | sense_intron_overlap | 8.00638| 6.37337| 7.03962| 9.36119    | 9.59565    | 9.11816    |
| BC158567    | 0.01001 | 4.58596     | Down       | 20  | +      | sense_exon_overlap   | 8.00638| 6.37337| 7.03962| 9.36119    | 9.59565    | 9.11816    |
| MRAK050857  | 0.00001 | 7.65238     | Down       | 10  | -      | others               | 5.53087| 5.65205| 5.90255| 8.58667    | 8.61557    | 8.69096    |
| MRAK033976  | 0.00288 | 5.04632     | Down       | X   | -      | others               | 5.29233| 5.3179 | 5.94278| 8.28533    | 8.20126    | 8.28601    |
| MRuc009dux  | 0.00330 | 6.00053     | Down       | 4   | +      | sense_intron_overlap | 6.03729| 6.69110| 6.14928| 8.93886    | 9.46715    | 8.22693    |
| MRuc009dux  | 0.00330 | 6.00053     | Down       | 4   | +      | antisense_intron_overlap| 6.03729| 6.69110| 6.14928| 8.93886    | 9.46715    | 8.22693    |
| MRAK078857  | 0.00001 | 7.65238     | Down       | 10  | -      | others               | 5.53087| 5.65205| 5.90255| 8.58667    | 8.61557    | 8.69096    |
| MRAK033976  | 0.00288 | 5.04632     | Down       | X   | -      | others               | 5.29233| 5.3179 | 5.94278| 8.28533    | 8.20126    | 8.28601    |
| MRAK078857  | 0.00001 | 7.65238     | Down       | 10  | -      | others               | 5.53087| 5.65205| 5.90255| 8.58667    | 8.61557    | 8.69096    |
| MRAK033976  | 0.00288 | 5.04632     | Down       | X   | -      | others               | 5.29233| 5.3179 | 5.94278| 8.28533    | 8.20126    | 8.28601    |

**Table 2** Top 40 aberrantly expressed lncRNAs in microarray analysis
The heat map and hierarchical clustering of the top 40 differentially expressed lncRNAs (a) and mRNAs (b) between AD and control hippocampal samples. The data are depicted as a data matrix, in which each row represents one lncRNA (mRNA) and each column represents one of the hippocampal samples. The relative lncRNA (mRNA) expression is depicted according to the colour scale shown at the top. Red represents high relative expression, and green represents low relative expression; −2.0, 0 and 2.0 are FCs in the corresponding spectrum. The magnitude of deviation from the median is represented by the colour saturation.

| SeqID         | P value | Fold change | Regulation | Chr | Strand | Relationship | AD-1      | AD-2      | AD-3      | Control-1 | Control-2 | Control-3 |
|---------------|---------|-------------|------------|-----|--------|--------------|-----------|-----------|-----------|------------|------------|------------|
| DQ223059      | 0.00212 | 4.47442     | Up         | 10  | +      | sense_exon_overlap | 12.99165  | 12.36331  | 12.52671  | 10.34195   | 10.93173   | 10.12288   |
| MRAK030941    | 0.00135 | 4.04066     | Up         | 4   | +      | sense_exon_overlap | 15.33834  | 14.99113  | 15.12357  | 13.02945   | 13.58079   | 12.79903   |
| uc.253+       | 0.00427 | 3.70040     | Up         | 5   | +      | intergenic      | 11.93550  | 11.18617  | 11.56148  | 9.58592    | 10.12549   | 9.30870    |
| uc.412+       | 0.00022 | 3.98847     | Up         | 10  | −      | sense_intron_overlap | 4.76540  | 4.37265   | 4.42680   | 2.42778    | 2.50436    | 2.46520    |
| XR_006990     | 0.00213 | 3.59883     | Up         | Un  | +      | intergenic      | 5.02968   | 4.64237   | 4.48417   | 3.01448    | 3.13395    | 2.46520    |

SeqID: lncRNA name. P value: P value calculated from unpaired t-test. Fold Change: the absolute ratio (no log scale) of normalized intensities between two groups (AD vs Control). Chr: chromosome no. which lncRNA is transcribed. Strand: the strand of chromosome which lncRNA is transcribed; ‘+’ is sense strand of chromosome, ‘−’ is antisense strand of chromosome. Relationship: “sense exon overlap”: the LncRNA’s exon is overlapping a coding transcript exon on the same genomic strand; “sense intron overlap”: the LncRNA is overlapping the intron of a coding transcript on the same genomic strand; “antisense exon overlap”: the LncRNA is transcribed from the antisense strand and overlapping with a coding transcript; “antisense intron overlap”: the LncRNA is transcribed from the antisense strand without sharing overlapping exons; “bidirection”: the LncRNA is oriented head to head to a coding transcript within 1000 bp; “intergenic”: there are no coding transcripts within 30 kb of the LncRNA; “others”: means other LncRNAs. AD1–3, Control1–3: Normalized Intensity of each sample (log2 transformed)
differentially expressed lncRNAs are shown in Table 2. Among the dysregulated lncRNA transcripts, S69385 was the most down-regulated, with an FC of 11.25, whereas MRAK050857 was the most up-regulated, with an FC of 7.65. The variation of lncRNA expression between the AD and control rats is shown with a scatter plot (Fig. 2a). The clustering analysis revealed the relationships among lncRNA expression patterns in different samples (Fig. 3a).

Using microarray analysis, we identified 311 significantly dysregulated mRNAs in the AD rats: 191 were up-regulated, while 120 were down-regulated (≥2.0 fold, p < 0.05). The top 40 most significantly differentially expressed mRNAs are shown in Table 3. The most up-regulated and down-regulated mRNA transcripts were Ckm(NM_012530) and P518 (NM_198200), with an FC of 12.08 and 10.05, respectively. The variation of mRNA expression between the AD and control rats is shown with a scatter plot (Fig. 2b). The clustering analysis revealed the relationships among mRNA expression patterns in different samples (Fig. 3b).

Validation of the Microarray Data Using qRT-PCR

A total of six dysregulated lncRNAs and mRNAs were randomly selected to validate the microarray results using qRT-PCR. Consistent with the microarray chip data, the qRT-PCR results demonstrated that the lncRNAs MRAK088596, MRAK081790 and MAPK10 were up-regulated and that BC092582, MRAK050857 and S100A8 were down-regulated in the AD rats compared with the controls (Fig. 4).

Gene Enrichment and KEGG Pathway Analyses

To predict the functions of the lncRNAs, we employed a previously described method [27]. In brief, we first identified and then conducted a functional enrichment analysis of the mRNAs co-expressed with each of the differentially expressed lncRNAs. The enriched functional terms were used as the predicted functional terms for each given lncRNA.

As shown in Fig. 5, the GO analysis indicated that the most enriched GO terms targeted by the mRNAs co-expressed with lncRNAs were endocrine process (ontology: biological process, GO:0050886), extracellular region part (ontology: cellular component, GO:0044421) and neurokinin receptor binding (ontology: molecular function, GO:0031834). Furthermore, the KEGG pathway analysis indicated that the mRNAs co-expressed with lncRNAs were involved in the regulation of antigen processing and presentation, neuroactive ligand-receptor interaction, axon guidance, and the synaptic vesicle cycle, among others. The top 40 KEGG pathways are listed in Fig. 6.

IncRNA-mRNA Network Analysis

As shown in Fig. 7, the whole co-expression network profile consisted of 454 network nodes and 478 connections among 168 differentially expressed mRNAs and 286 differentially expressed lncRNAs. There were 200 negative and 278 positive interactions within the network. Moreover, our data showed that one mRNA may correlate with 1–66 lncRNAs and that one lncRNA may correlate with 1–2 mRNAs.
Analysing the expression profiles of lncRNAs may provide new insights into our understanding of the aetiology and pathophysiology of AD. In previous studies, D.Y. Lee et al. examined the dysregulated expression of intergenic lncRNAs (lncRNAs), a significant subgroup of lncRNAs, in a triple transgenic model of AD (3xTg-AD) [16]. X. Zhou et al. identified AD-associated lncRNAs based on post-mortem tissue samples of AD patients [14]. Additionally, M. magistri et al. identified several annotated and non-annotated lincRNAs that are differentially expressed in the hippocampus in late-onset AD (4840 Mol Neurobiol (2017) 54:4833–4846).
mRNA expression and inflammation, oxidative stress promotion and synaptic failure in numerous molecular pathogeneses, including APP overexpression, Aβ accumulation, tau hyperphosphorylation, neuroinflammation, oxidative stress promotion and synaptic failure [33, 34]. On the other hand, the lncRNA MRAK043570 is significantly associated with the PI3K-Akt, mTOR, FoxO and AMPK signalling pathways, which are altered in brains with AD [35, 36]; in addition, all these pathways are linked to the regulation of autophagy, which is strongly regarded as one of the major pathogenic mechanisms of AD [37, 38]. These findings suggest that altered lncRNAs may be involved in AD-associated signalling pathways. However, our knowledge about the potential functions of these dysregulated lncRNAs in the neuropathogenesis of AD remains limited. Thus, further investigation is of great importance.

In the present study, we also employed an lncRNA-mRNA network analysis to identify interactions between differentially expressed mRNAs and differentially expressed lncRNAs, as previously described [39, 40]. The co-expression network reported here was constructed based on the 315 differentially expressed lncRNAs and the 311 differentially expressed mRNAs distinguishing the AD rats from the control rats. Our results showed that a total of 168 lncRNAs and 286 mRNAs were included in the co-expression network, which consisted of 454 network nodes and 478 connections. We also found that S100a8, an mRNA, was correlated with up to 66
In addition, S100A8 is a ligand of the receptor for advanced glycation end products, which is a receptor in the immunoglobulin superfamily that also binds other ligands, including advanced glycation end products, the high-mobility group protein B1 (HMGB1), and Aβ. In addition, S100A8 could modulate APP processing towards increased β-secretase activity and the production of long, more amyloidogenic, Aβ peptides [43].

We also found that 172 lncRNAs interacted with 5 mRNAs that are mainly involved in the neuroactive ligand-receptor interaction, lysosome and renin-angiotensin system signalling pathways, which have been demonstrated to play important roles in the pathophysiology of AD and could serve as potential therapeutic targets [44–46]. The co-expression network suggests that the inter-regulation of lncRNAs and mRNAs is involved in AD and warrants further study.

Although altered lncRNAs and mRNAs were identified and their possible roles in the pathophysiology of AD were investigated in this study, several limitations should be acknowledged. First, the analysis was only performed using the hippocampus of AD animals. Global lncRNA and mRNA changes in the blood and cerebral spinal fluid of the same AD model animals should be also determined in further studies to more accurately reflect the pathophysiology of AD. Second, the present study only predicted lncRNA functions...
through investigating the functional significance of the mRNAs co-expressed with the differentially expressed lncRNAs. Third, gene expression microarrays have a limited dynamic range and lack the ability to identify novel features, such as splice isoforms or fusion transcripts. RNA-seq technology allows the discovery of previously inaccessible complexities in the transcriptome, such as allele-specific expression and novel promoters and isoforms. However, the resulting datasets are large and complex, and their interpretation is not straightforward [47]. Fourth, similar to previous studies [47, 48], we also only performed the minimal number of experiments, which may result in an underestimation of the number of altered lncRNAs and mRNAs. Larger sample sizes could achieve more optimal results. Additionally, further research should select from 8 to 10 up-regulated and 8 to 10 down-regulated lncRNAs for microarray validation and include a larger sample size than that in the present study. Future studies that overcome the limitations mentioned above are merited.

In conclusion, the present study uses microarray data to reveal for the first time that the hippocampal expression patterns of lncRNAs are significantly altered in AD. In addition, the data indicate that aberrantly expressed lncRNAs participate in several specific biological processes and are involved in related pathways that may contribute to the pathogenesis of AD. While these findings provide new found information regarding the potential role of lncRNAs in AD, further research is required to fully elucidate the detailed molecular mechanisms underlying the action of significantly dysregulated lncRNAs.

Fig. 6 KEGG pathways analysis. Top 40 pathways for the differences in lncRNA genes co-expressed in AD animals and the controls.
Fig. 7 lncRNA-mRNA-network analysis. *Blue nodes* represent dysregulated lncRNAs, *yellow nodes* represent dysregulated mRNAs. The *red lines* between lncRNAs and mRNAs indicate a negative correlation, while the *green lines* indicate a positive correlation.
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Author Contributions The work presented here was carried out in collaboration among all authors. Z.W. and W.P. conceived and designed the work and helped to coordinate support and funding. B.Y. and W.P. wrote the manuscript. Z.X., C.S., W.G. and Y.C. performed the animal experiments and helped collect the samples. B.Y. and B.Z. participated in the acquisition, analysis and interpretation of the data. X.X. and Y.W. co-worked on associated data collection and analysis. All authors reviewed and approved the final manuscript.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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