Abnormal expression of rno_circRNA_014900 and rno_circRNA_005442 induced by ketamine in the rat hippocampus

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Abstract

Background: Recent studies have shown that circular RNA (circRNA) is rich in microRNA (miRNA) binding sites. We have previously demonstrated that the antidepressant effect of ketamine is related to the abnormal expression of various miRNAs in the brain. This study determined the expression profile of circRNAs in the hippocampus of rats treated with ketamine.

Methods: The aberrantly expressed circRNAs in rat hippocampus after ketamine injection were analyzed by microarray chip, and we further validated these circRNAs by quantitative reverse-transcription PCR (qRT-PCR). The target genes of the different circRNAs were predicted using bioinformatic analyses, and the functions and signal pathways of these target genes were investigated by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses.

Results: Microarray analysis showed that five circRNAs were aberrantly expressed in rat hippocampus after ketamine injection (fold change > 2.0, p < 0.05). The results from the qRT-PCR showed that one of the circRNAs was significantly increased (rno_circRNA_014900; fold change = 2.37; p = 0.03), while one was significantly reduced (rno_circRNA_005442; fold change = 0.37; p = 0.01). We discovered a significant enrichment in several GO terms and pathways associated with depression.

Conclusion: Our findings showed the abnormal expression of ketamine-induced hippocampal circRNAs in rats.

Keywords: Rat, Ketamine, CircRNA, Hippocampus

Background

The most commonly used antidepressants today effectively improve symptoms of depression, but require at least 2 weeks to take therapeutic effect. In addition, about two-thirds of depressive patients do not respond to the currently available antidepressants and are prone to relapse [1]. Therefore, the development of new, fast-acting antidepressants is particularly important for patients with depression-induced suicidal tendencies [2].

Recent observations suggest that sub-anesthetic doses of ketamine produce rapid therapeutic effects in depressed patients and in animal models of depression [3–6]. These effects are characterized by a rapid onset of action (within hours) after a single dose, a lasting effect (1 week), as well as an efficacy in patients resistant to traditional antidepressant drugs [7, 8].

In a prior investigation, we found that the antidepressant effect of ketamine was related to the regulation of multiple microRNAs (miRNAs) in neurons [9]. Recent studies have shown that circular RNA (circRNA) is rich in miRNA binding sites. CircRNA plays the role of an miRNA sponge in cells, thereby relieving the inhibitory effect of the miRNAs on their target genes and increasing the expression of these genes. The mechanism by...
which circRNA inhibits miRNAs to increase the expression of their target genes is called the competitive endogenous RNA (ceRNA) mechanism [10, 11].

Based on the previous reports, the aim of this study was to determine the effect of an antidepressant dose of ketamine on the expression of circRNAs in the hippocampus of rats and examine a possible circRNA-mediated mechanism for ketamine’s action. This work may provide new perspectives on the development of circRNA as a possible drug target.

**Methods**

**Animals**

These experiments were conducted in male Sprague-Dawley rats (50 days old, 150–200 g), provided by Chengdu Dashuo biological technology Co., Ltd., China (experimental animal production license: SCXK Chengdu 2013–17). These rats were housed 5 per cage in standard cages (42 × 20 × 20 cm) in a room. Animals had access to food and water ad libitum during the experiment. The room was maintained at 25–26 °C with about 65% relative humidity, on a 12-h dark/light cycle (lights on at 7 am). All experiments were performed according to National Institute of Health (NIH) guidelines and approved by the ethics committee of Southwest Medical University (Approval number: 20180306038).

**Experimental design and procedure**

After 1 week of adaptation, the rats were randomly divided into control and experimental group (12 rats/group). The rats in control group were daily injected with 0.9% saline, whereas the rats in experimental group received ketamine (15 mg/kg). All injections were done intraperitoneally for three consecutive days (the volume of injection was 1 ml/kg). The dose of ketamine used in this study was based on our previous study [9]. On day 4, approximately 24 h after the last ketamine or saline injection, the rats were sacrificed by cervical dislocation, and their hippocampus tissues were dissected out for circRNA microarray analysis (n = 3/group) and for qRT-PCR (n = 6/group). The hippocampus of 3 remaining rats per group were spare specimen according to the quality of hippocampus removed from rats.

**CircRNAs analysis from microarray chip**

Relevant circRNAs were analyzed according to our previous approach [12]. Hippocampus tissues (n = 3/group) were used for microarray assay to determine differentially expressed circRNA between the two groups. The microarray hybridization was performed based on the Arraystar’s standard protocols, including purifying RNA, transcribing into fluorescent cRNA, and hybridizing onto the rat circRNA Arrays (Arraystar). Finally, the hybridized slides were washed, fixed and scanned to images by the Agilent Scanner G2505C. The Agilent Feature Extraction software (version 11.0.1.1) were used to analyze the acquired array images. The raw data were normalized and data analysis was further performed with the R software Limma package (Agilent Technologies). The statistical significance of differentially regulated circRNAs between the two groups was identified through screening fold change ≥2.0, P < 0.05 and FDR < 0.05.

**Quantitative real-time PCR validation**

Hippocampus tissues (n = 6/group) were used for qRT-PCR validation. After RNA isolation, M-MLV reverse transcriptase (Invitrogen, USA) was used for synthesizing cDNA according to the manufacturer’s instructions. Subsequently, we performed qRT-PCR using the ViiA 7 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) in a total reaction volume of 10 μl, including 2 μl cDNA, 5 μl 2 × Master Mix, 0.5 μl PCR Forward Primer (10 μM), 0.5 μl PCR Reverse Primer (10 μM) and 2 μl double distilled water. The protocol was initiated at 95 °C for 10 min, then at 95 °C (10 s), 60 °C (60 s) for a total 40 cycles. β-actin was used as a reference. Results were harvested in three independent wells. For quantitative results, the relative expression level of each circRNA was calculated using 2−ΔΔCt method.

**Competing endogenous RNA analysis of differentially expressed circRNAs**

The candidate miRNA binding sites were searched on the sequences of circRNAs and miRNAs, and the circRNA-miRNA-mRNA interaction were found by the overlapping of the same miRNA seed sequence binding site both on the circRNAs and the mRNA. The miRNA-mRNA interactions were predicted by Targets can (http://www.targetscan.org/), while the miRNA binding sites were predicted by miRcode (http://www.mircode.org/).

**GO and KEGG pathway analysis**

Gene Ontology (GO) analysis (http://www.geneontology.org) were conducted to construct meaningful annotation of genes and gene products in the organisms. The ontology includes molecular functions (MFs), biological processes (BPs) and cellular components (CCs). KEGG pathway analysis were also performed to harvest pathway clusters on the molecular interaction and reaction networks in differentially regulated genes. The -log10 (p-value) denotes enrichment score representing the significance of GO term enrichment and pathway correlations among differentially expressed genes.

**Statistical analysis**

The statistical package for the social sciences (SPSS) 11.0 was selected for statistical analysis. All data were expressed as mean ± SEM. The data from the circRNA
microarray and qRT-PCR were analyzed by one-way ANOVA or multi-factorial ANOVA followed by Tukey’s post hoc test. *P* values less than 0.05 with statistically significant differences.

**Results**

The expression profile of circRNAs in the rat hippocampus using microarray analysis and secondary validation from qRT-PCR

The RNA concentration and purity of all samples meted the requirement (larger than 1.8 and an RNA concentration greater than 30 ng) for subsequent microarray detection of the circRNA expression profile.

As shown in Fig. 1, significantly different circRNAs were selected for hierarchical clustering analysis. The circRNA hierarchical clustering map not only displays circRNA expression, but also exhibits the expression change of a single circRNA from both groups. As shown in Table 1, four circRNAs were upregulated and one was downregulated in the hippocampus of rats treated with ketamine from circRNA microarray analysis (*p* < 0.05), but only two of the five circRNAs were confirmed to be differentially expressed from qRT-PCR (Table 3, *p* < 0.05). As

**Table 1** Aberrantly expressed circRNAs in rat hippocampus revealed by microarray analysis (Fold change ≥2.0, *p* < 0.05)

| circRNA         | Fold Change | Regulation | *P*-value |
|-----------------|-------------|------------|-----------|
| rno_circRNA_003460 | 5.98        | up         | 0.001     |
| rno_circRNA_014900 | 2.28        | up         | 0.003     |
| rno_circRNA_006565 | 2.11        | up         | 0.010     |
| rno_circRNA_013109 | 2.17        | up         | 0.012     |
| rno_circRNA_005442 | 2.01        | down       | 0.012     |
shown in Table 2, the primers for the five different circRNAs were designed using Primer software 5.0.

**Predicted miRNAs sponged by the two circRNAs and their corresponding target genes**

As shown in Table 3, the two circRNAs 014900 and 005442 collectively sponged ten miRNAs, namely, rno-miR-466b-5p, rno-miR-6332, rno-miR-6321, rno-miR-193a-5p, rno-miR-1224, rno-miR-323-5p, rno-miR-107-5p, rno-miR-135b-5p, rno-miR-135a-5p, and rno-miR-344b-5p. Each of these miRNAs has target genes that they endogenously regulate, as shown in Table 4, the two circRNAs could indirectly regulate numerous target genes by their endogenous competition mechanism.

**GO analysis**

As shown in Fig. 2, the molecular functions (MFs) of neurons that may be regulated by the target genes of the differentially expressed circRNAs ($p < 0.05$, left: rno_circRNA_014900; right: rno_circRNA_005442). The classification of notable MFs was shown in Fig. 2a and b shows the order of these MFs by their GO analysis enrichment scores. Figure 2c shows the notable activities of neurons using fold enrichment. The detailed list of the genes identified as regulated by circRNA was shown in Additional files 1 and 2.

**KEGG pathway analysis**

Tables 5 and 6 shows the signaling pathways that may be regulated by target genes of the two differentially expressed circRNAs ($p < 0.05$). The identified pathways were involved in the regulation of central nervous system functions, such as Wnt signaling, long-term depression, PI3K-Akt signaling, dopaminergic synapse activity, mTOR signaling, p53 signaling, apoptosis, TGF-beta signaling, axon guidance, hippo signaling, and MAPK signaling.

**Discussion**

There are about 340 million patients suffering from depression around the world, and it is estimated that up to 1 million people die by depression-induced suicide every year. Therefore, depression has become a global public health problem [13]. The major clinical drawback of currently available antidepressants is their slow onset (14 days on average) and poor effects [1]. Therefore, the development of rapid-onset antidepressants is particularly

| Table 2 A list of primers used for real-time PCR |
| Gene | Bi-directional primer sequence | Annealing temperature (°C) | Primer length (bp) |
|------|--------------------------------|-----------------|-----------------|
| β-actin (Reference) | Forward: 5′ CGAGTACAACCTTCTTGCAGC 3′ Reverse: 5′ ACCATACCACCCACTCAAC 3′ | 60 | 202 |
| rno_circRNA_014900 | Forward: 5′ CTTAGTAGACCTGGAAGACCT 3′ Reverse: 5′ TAGCTTTGCTGCTGACTTAG 3′ | 60 | 124 |
| rno_circRNA_013109 | Forward: 5′ ATTAGAGCTAATAACCTTGC 3′ Reverse: 5′ TTATCTGACGACTTAAAG 3′ | 60 | 105 |
| rno_circRNA_006565 | Forward: 5′ CGACTTCCTAAGAGTGTGATT 3′ Reverse: 5′ TTTCTCTCTCGAAGCCATCCT 3′ | 60 | 54 |
| rno_circRNA_005442 | Forward: 5′ ACCCCATGAGAAAGACCCAGGTC 3′ Reverse: 5′ CTGCTCTCTTCACAAGACATC 3′ | 60 | 60 |
| rno_circRNA_003460 | Forward: 5′ CGCTAAGCTTTTCTGTGAGAG 3′ Reverse: 5′ GTAGTGGGTGTAGGGAGGAGA 3′ | 60 | 76 |

| Table 3 qRT-PCR-confirmed expression of circRNAs in rat hippocampus and the predicted target miRNAs |
| circRNA | Fold Change | Regulation | P-value | Predicted target miRNAs |
|--------|-------------|------------|---------|-------------------------|
| rno_circRNA_014900 | 2.37 up | 0.029 | rno-miR-466b-5p rno-miR-6332 rno-miR-6321 rno-miR-193a-5p rno-miR-1224 |
| rno_circRNA_005442 | 2.72 down | 0.010 | rno-miR-323-5p rno-miR-107-5p rno-miR-135a-5p rno-miR-135b-5p rno-miR-344b-5p |
important for patients suffering from major depressive disorder with suicidal tendency. The antidepressant effect of ketamine initiates quickly and is also effective in treating refractory depression [7, 8]. However, the psychosocial side effects induced by ketamine have restricted its use in the treatment of depression [14]. An in-depth understanding of the mechanism of ketamine’s rapid antidepressant effect can provide new targets for similar antidepressants.

Recent many researches have shown that many transcriptional products come from the non-coding RNA (ncRNA), including the microRNA (miRNA), long non-coding RNA (IncRNA), and circular RNA (circRNA), these ncRNAs can regulate gene expression at the DNA level, pre-transcriptional level, transcriptional level, post-transcriptional level, translational level, and post-translational level [15]. Previous study from our group found that ketamine induced abnormal expression of

| Table 4 | Target genes regulated indirectly by the two differentially expressed circRNAs through miRNAs |
|---------|-------------------------------------------------------------------------------------------------|
| circRNA | Sponged miRNAs | Gene Symbol | Gene Description |
| m0_circRNA_014900 | m0-miR-193a-5p | Nova1 | neuro-oncological ventral antigen 1 |
|        | m0-miR-1224 | Clasp1 | cytoplasmic linker associated protein 1 |
|        | m0-miR-193a-5p | Rgs4, Mxi1 | regulator of G-protein signaling 4; Mix paired-like homeobox 1 |
|        | m0-miR-6332 | Usp37, RGD1566029, Zfp91, Zmiz1, Pbx1 | ubiquitin specific peptidase 37; similar to mKIAA1644 protein; zinc finger protein 91; zinc finger, MIZ-type containing 1; pre-B-cell leukemia homeobox 1 |
|        | m0-miR-466b-5p | Calccoo1 | calcium binding and coiled coil domain 1 |
|        | m0-miR-1224 | Brwd3, Nfat5, Cnot7, Camta1 | bromodomain and WD repeat domain containing 3; nuclear factor of activated T-cells 5; toneic-ty-mponse; CCR4-NOT transcription complex, subunit 7; calmodulin binding transcription activator 1 |
|        | m0-miR-6332 | Prpf4b, Hgs, Gtdc1, Dgkk | pre-mRNA processing factor 48; hepatocyte growth factor-regulated tyrosine kinase substrate; glycosyltransferase-like domain containing 1; diacylglycerol kinase kappa |
|        | m0-miR-1224 | RGD1562037, Usp24, Ssbp2 | similar to OTTHUMP00000046255; ubiquitin specific peptidase 24; single-stranded DNA binding protein 2 |
|        | m0-miR-6332 | Ssbp2, RGD1562037 | single-stranded DNA binding protein 2; similar to OTTHUMP00000046255 |
|        | m0-miR-466b-5p | Zfp91 | zinc finger protein 91 |
| m0_circRNA_005442 | m0-miR-107-5p | Eps8, Rhot1 | epidermal growth factor receptor pathway substrate 8, ras homolog gene family, member T1 |
|        | m0-miR-323-5p | Hn1 | hematological and neurological expressed 1 |
|        | m0-miR-107-5p | Ptk2, Tiarn1 | protein tyrosine kinase 2; T-cell lymphoma invasion and metastasis 1 |
|        | m0-miR-344b-5p | Slc8a1, Man2a1, Tnpo1, Rgl1 | solute carrier family 8 (sodium/calcium exchanger), member 1; mannosidase, alpha, class 2A, member 1; transportin 1; ral guanine nucleotide dissociation stimulator-like 1 |
|        | m0-miR-107-5p | Tomm6 | translocase of outer mitochondrial membrane 6 homolog (yeast) |
|        | m0-miR-135b-5p | Shisa7 | shisa family member 7 |
|        | m0-miR-135a(b)-5p | Arel1 | apoptosis resistant E3 ubiquitin protein ligase 1 |
|        | m0-miR-323-5p | Rhot1 | ras homolog gene family, member T1 |
miRNAs in the brain, and subsequent target gene and functional analysis revealed that this abnormal expression of miRNAs was closely related to the antidepressant effect of ketamine, but the therapeutic effect of antidepressants focusing on a single mechanism is poor due to the complex mechanism of major depressive disorder [9]. Therefore, we believe that a single miRNA plays relatively weak role in the regulation of a specific target gene. It was hypothesized that a substance in the body that could gather several miRNAs regulated the same specific target gene, then the regulatory action on the specific target gene would be obviously increased. It was interesting that circRNAs, a novel type of non-coding RNAs [15], were perceived as a rare curiosity to having a central regulatory role in RNA metabolism, and accumulating evidences suggested that circRNAs could function as miRNA sponges (the ceRNA mechanism), therefore, circRNA could relieve the inhibitory effect of miRNA on its target gene and increase the expression of the target gene [10–12].

Our results showed that the expression of rno_circRNA_014900 was significantly increased by ketamine, while the expression of rno_circRNA_005442 was obviously decreased. Since these two circRNAs were not investigated in previous studies on depression, we further investigated their

Fig. 2 The molecular functions (MFs) of neurons regulated by the target genes of the differentially expressed circRNAs (p < 0.05, left: rno_circRNA_014900; right: rno_circRNA_005442). a classifies the notable MFs and b shows these same MFs ordered by their GO analysis enrichment scores. c shows the notable activities of neurons that may be regulated by target genes predicted using fold enrichment.
regulated target genes and signaling pathways. Competing endogenous RNA analysis found that the rno_circRNA_014900 could sponge rno-miR-466b-5p, rno-miR-6332, rno-miR-6321, rno-miR-193a-5p and rno-miR-1224, whereas the rno_circRNA_005442 had the binding sites in rno-miR-323-5p, rno-miR-107-5p, rno-miR-135a-5p, rno-miR-135b-5p and rno-miR-344b-5p. In a prior investigation, we found that miR-206 was a critical novel gene for the expression of BDNF (brain-derived neurotrophic factor) induced by ketamine [9], our experimental results were expected to be that the circRNA identified in this study would target the miRNA-206, but structural prediction analysis showed that the miRNAs sponged by rno_circRNA_014900 and rno_circRNA_005442 did not include miRNA-206, we thought rno_circRNA_014900 and rno_circRNA_005442 did not regulate the expression of miRNA-206, how ketamine affects the expression of miRNA-206 needs to be further explored in future research. Further prediction analysis revealed that rno_circRNA_014900 and rno_circRNA_005442 may inhibit up to four miRNAs closely related to depression. For example, the predicted target genes related to depression include NOVA1 (regulated by miR-193-5p and miR-1224) [16], Rgs4 (regulated by miR-193-5p and miR-466b-5p).
[17–20], zinc-finger protein (regulated by miR-6332, miR-466b-5p, and miR-1224) [21], PBX1 (regulated by miR-466b-5p and miR-6332) [22], SLC8A1 (regulated by miR-107-5p, miR-135a(b)-5p, and miR-135b-5p) [23], PTK2 (regulated by miR-107-5p and miR-135b-5p) [24], and Tiam1 (regulated by miR-107-5p and miR-135b-5p) [25]. Therefore, we believed rno_circRNA_014900 or rno_circRNA_005442 could relieve the inhibitory effect of miRNA on its target gene and increase the expression of the target gene, the phenomenon needs to be further confirmed in future studies.

The results from GO analysis found that the target genes of the two circRNAs regulated many molecular functions (including protein phosphatase binding, SUMO binding, Wnt-protein binding, etc.), biological processes (including adherens junction assembly, neuron projection morphogenesis, neurological processes, etc.), and cellular components (including dendritic spines, AMPA-glutamate receptor binding, neuronal cell body, etc.). The results from KEGG pathway prediction showed that the signaling pathways regulated by target genes of the two circRNAs included Wnt signaling, long-term depression, PI3K-Akt
### Table 5
The signaling pathways that may be regulated by targeted genes of the differentially expressed rno_circRNA_014900 (p < 0.05)

| PathwayID | Definition | Fisher-Pvalue | Enrichment_Score |
|-----------|------------|---------------|------------------|
| rno04510  | Focal adhesion - Rattus norvegicus (rat) | 4.05765E-06 | 5.391726 |
| rno05205  | Proteoglycans in cancer - Rattus norvegicus (rat) | 0.000263739 | 3.578826 |
| rno05200  | Pathways in cancer - Rattus norvegicus (rat) | 0.000839484 | 3.075988 |
| rno04216  | Ferroptosis - Rattus norvegicus (rat) | 0.000947662 | 3.023347 |
| rno04015  | Rap1 signaling pathway - Rattus norvegicus (rat) | 0.001242467 | 2.905715 |
| rno00564  | Glycerophospholipid metabolism - Rattus norvegicus (rat) | 0.001767738 | 2.752582 |
| rno04310  | Wnt signaling pathway - Rattus norvegicus (rat) | 0.002533546 | 2.596271 |
| rno05165  | Human papillomavirus infection - Rattus norvegicus (rat) | 0.003213976 | 2.492957 |
| rno04140  | Autophagy - animal - Rattus norvegicus (rat) | 0.00464105 | 2.333384 |
| rno05222  | Small cell lung cancer - Rattus norvegicus (rat) | 0.005031787 | 2.298278 |
| rno04070  | Phosphatidylinositol signaling system - Rattus norvegicus (rat) | 0.006461245 | 2.189684 |
| rno0561   | Glycerolipid metabolism - Rattus norvegicus (rat) | 0.00791785 | 2.101393 |
| rno04213  | Longevity regulating pathway - multiple species - Rattus norvegicus (rat) | 0.00791785 | 2.101393 |
| rno04730  | Long-term depression - Rattus norvegicus (rat) | 0.00791785 | 2.101393 |
| rno04151  | PI3K-Akt signaling pathway - Rattus norvegicus (rat) | 0.00874211 | 2.058384 |
| rno04512  | ECM-receptor interaction - Rattus norvegicus (rat) | 0.009516369 | 2.021529 |
| rno04137  | Mitophagy - animal - Rattus norvegicus (rat) | 0.009920558 | 2.003464 |
| rno04278  | Dopaminergic synapse - Rattus norvegicus (rat) | 0.01248382 | 1.903653 |
| rno04211  | Longevity regulating pathway - Rattus norvegicus (rat) | 0.01286284 | 1.890663 |
| rno04136  | Autophagy - other - Rattus norvegicus (rat) | 0.01346501 | 1.870793 |
| rno04150  | mTORC1 signaling pathway - Rattus norvegicus (rat) | 0.0138861 | 1.866825 |
| rno04115  | p53 signaling pathway - Rattus norvegicus (rat) | 0.01402018 | 1.853246 |
| rno04215  | Apoptosis - multiple species - Rattus norvegicus (rat) | 0.01492946 | 1.825956 |
| rno05214  | Glioma - Rattus norvegicus (rat) | 0.01496318 | 1.824976 |
| rno04270  | Vascular smooth muscle contraction - Rattus norvegicus (rat) | 0.02428063 | 1.61474 |
| rno01521  | EGFR tyrosine kinase inhibitor resistance - Rattus norvegicus (rat) | 0.02549069 | 1.593521 |
| rno05219  | Bladder cancer - Rattus norvegicus (rat) | 0.02580699 | 1.588263 |
| rno05224  | Breast cancer - Rattus norvegicus (rat) | 0.02850884 | 1.54502 |
| rno05166  | HTLV-I infection - Rattus norvegicus (rat) | 0.02925304 | 1.533829 |
| rno04066  | HIF-1 signaling pathway - Rattus norvegicus (rat) | 0.03188594 | 1.496401 |
| rno04068  | FoxO signaling pathway - Rattus norvegicus (rat) | 0.03490339 | 1.457132 |
| rno04144  | Endocytosis - Rattus norvegicus (rat) | 0.03599259 | 1.443787 |
| rno04720  | Long-term potentiation - Rattus norvegicus (rat) | 0.03856442 | 1.413813 |
| rno04978  | Mineral absorption - Rattus norvegicus (rat) | 0.040454532 | 1.393024 |
| rno04725  | Cholinergic synapse - Rattus norvegicus (rat) | 0.04272807 | 1.369287 |
| rno05030  | Cocaine addiction - Rattus norvegicus (rat) | 0.04327309 | 1.363782 |
| rno04010  | MAPK signaling pathway - Rattus norvegicus (rat) | 0.04346333 | 1.361877 |
| rno04120  | Ubiquitin mediated proteolysis - Rattus norvegicus (rat) | 0.04667443 | 1.330921 |
| rno00062  | Fatty acid elongation - Rattus norvegicus (rat) | 0.04768946 | 1.321578 |
| rno05211  | Renal cell carcinoma - Rattus norvegicus (rat) | 0.04775832 | 1.320951 |
| rno01522  | Endocrine resistance - Rattus norvegicus (rat) | 0.04798442 | 1.3189 |
These signaling pathways may be involved in the occurrence and development of depression, because some researches found that the Wnt signaling pathway played important roles in the depression-like behaviors [26–28], the PI3K-Akt signaling pathway was related to the rapid antidepressant-like effects of some drugs [29–33].

As we analyzed in other study about circRNAs [12], novel therapies should include multiple genes and pathways due to the complex mechanisms of major depression disorder. Because many pathophysiological processes of stress-related depression were regulated by several miRNAs, therefore these miRNAs may be common targets of antidepressant therapies. It was not difficult to understand that the increase in relevant circRNAs expression would enhance the translation of their target genes due to miRNA sponges, a down-expression in relevant circRNAs would result in the obvious silencing of downstream target genes. Therefore, in the future, artificial circRNA drugs will be developed to restore normal transcriptional regulation.

Conclusions
In summary, we found that ketamine treatment resulted in the abnormal expression of the two circRNAs in the hippocampus of rats, and these two circRNAs may be associated with stress-related depression disorders. CircRNAs should remain the focus of researches investigating antidepressant targets because they have considerable potential in the clinical treatment of stress-related depression. As an invaluable topic for future biomedical studies, we plan to screen for specific circRNAs in the context of depression and to examine their potential value in the diagnosis and treatment of this debilitating disorder.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12888-019-2374-2.

Table 6 The signaling pathways that may be regulated by targeted genes of the differentially expressed rno_circRNA_005442 (p < 0.05)

| PathwayID | Definition | Fisher-Pvalue | Enrichment_Score |
|-----------|------------|---------------|------------------|
| rno04350  | TGF-beta signaling pathway - Rattus norvegicus (rat) | 0.002056745 | 2.68662 |
| rno04960  | Aldosterone-regulated sodium reabsorption - Rattus norvegicus (rat) | 0.002800503 | 2.551904 |
| rno05231  | Choline metabolism in cancer - Rattus norvegicus (rat) | 0.0039363 | 2.404912 |
| rno05412  | Arrhythmic ventricular cardiomyopathy (ARVC) - Rattus norvegicus (rat) | 0.00500479 | 2.300988 |
| rno04450  | Signaling pathways regulating pluripotency of stem cells - Rattus norvegicus (rat) | 0.005251424 | 2.279723 |
| rno04218  | Cellular senescence - Rattus norvegicus (rat) | 0.005747859 | 2.240494 |
| rno04360  | Axon guidance - Rattus norvegicus (rat) | 0.005747859 | 2.240494 |
| rno05225  | Hepatocellular carcinoma - Rattus norvegicus (rat) | 0.00773032 | 2.169217 |
| rno04144  | Endocytosis - Rattus norvegicus (rat) | 0.009694387 | 2.130151 |
| rno04371  | Apelin signaling pathway - Rattus norvegicus (rat) | 0.01587784 | 2.130151 |
| rno04520  | Adherens junction - Rattus norvegicus (rat) | 0.02058289 | 1.686494 |
| rno05210  | Colorectal cancer - Rattus norvegicus (rat) | 0.02501545 | 1.601792 |
| rno04973  | Carbohydrate digestion and absorption - Rattus norvegicus (rat) | 0.02580168 | 1.588352 |
| rno04010  | MAPK signaling pathway - Rattus norvegicus (rat) | 0.03810185 | 1.419054 |
| rno05140  | Hypertrophic cardiomyopathy (HCM) - Rattus norvegicus (rat) | 0.0404027 | 1.376335 |
| rno05143  | Dilated cardiomyopathy (DCM) - Rattus norvegicus (rat) | 0.04832562 | 1.315823 |
were approved by the ethics committee of Southwest Medical University in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

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Authors' contributions

J.M., T.L., D.F., H.Z., J.F., L.L., and X.W. made substantial contributions to conception and design of the study, and/or acquisition of data. J.M., T.L., D.F., and H.Z. made the first draft of the manuscript and L.L., C.Z., and X.W. critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Chinese Society for Neuroscience and Behavior recommendations for animal care. All of the experiments involving surgeries and treatments were approved by the ethics committee of Southwest Medical University (Ethics number: 20180306038).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Additional file 6. CC results for circRNA005442. The detailed list of the genes identified as regulated by the circRNA005442, CC: cellular component.
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