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Proteomic study on CUMS-induced senile depression mice's frontal lobe cortex and the regulating effect of KTLD formula

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Abstract. – OBJECTIVE: The aim of this study was to investigate the protein expression of chronic unpredictable mild stress (CUMS)-induced senile depression in SAMP-8 mice's frontal lobe cortex and the regulating effect of the kidney tonifying and liver dispersing (KTLD) formula.

MATERIALS AND METHODS: A total of 15 male SAMP-8 mice were randomly divided into control, CUMS, and KTLD groups. CUMS and KTLD mice were subjected to CUMS for 21 days. Control group mice were kept to normal feeding. At the same time as molding, the herbal gavage (KTLD formula, 19.5 g/kg/d) was given from the beginning of the stress stimulation, while the control group and the CUMS group mice were given the same volume of saline for 21 days. Open-field testing (OFT) was used to assess the mice's depression levels. Isobaric tags for relative and absolute quantification (iTRAQ) were used to identify differentially expressed proteins (DEPs) in mice's frontal lobe cortex. Bioinformatics analysis including Gene Ontology (GO); Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and protein-protein interaction (PPI) networks were utilized to study the DEPs connections.

RESULTS: Results revealed that mice with senile depression experienced more anxiety and depression than control mice, whereas KTLD mice had the opposite experience. Biological processes including transport, regulation of transcription, and DNA-templated were identified in both KTLD and CUMS. The KEGG enrichment study of the DEPs in KTLD revealed their involvement in the MAPK signaling pathway, glutamatergic synapse, dopaminergic synapse, axon guidance, and ribosome. KEGG pathway enrichment showed that the mechanism of senile depression and the pathway of KTLD are closely related to axonal conductance and ribosomes. According to the PPI analysis, disease-related proteins regulated by KTLD revealed that some proteins, such as GLOI1 and TRRAP, have potential interactions. This provides fresh insight into how KTLD works to cue senile depression.

CONCLUSIONS: KTLD treats senile depression *via* multiple targets and pathways, which may include regulations of 467 DEPs. Proteomics showed significant changes in protein levels in geriatric depression and after KTLD intervention. Senile depression involves the cross-linking and modulation of signal pathways, presenting a pattern of multiple pathways and multiple targets. According to a protein pathway enrichment and protein interaction model of KTLD in senile depression, KTLD is capable of treating senile depression *via* multiple pathways and targets.

Key Words:
Senile depression, SAMP-8 mice, Kidney tonifying and liver dispersing formula, Proteomics study.

Introduction

Senile depression is an aging-related disorder of the mind, manifested by symptoms such as depressed mood, cognitive decline, slowed thinking, sleep disturbance, and in severe cases, suicidal tendencies[1]. According to epidemiological and statistical surveys[2], 6.8% of Chinese people report having experienced depression at some point in their lives. The aging of Chinese population is increasing, and proportion of older adults will reach 28% in 2040. That depression will become a common psychiatric disorder in the elderly population. Selective serotonin reuptake inhibitors (SSRI), the primary treatment option, have achieved good clinical results. The therapeutic effect decreases gradually under long-term medication and often triggers the aggravation of

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the underlying disease in the elderly[3,4]. Therefore, there is an urgent need to find psychotherapy medications for the elderly that are effective and have no adverse effects.

Monoamine neurotransmitter hypothesis is recognized to be an essential mechanism leading to depression and the reduction of monoamine neurotransmitters such as 5-serotonin (5-HT), dopamine (DA), and norepinephrine (NE). NE reuptake inhibitors also play an important anti-depressant role[5,6]. The cytokine hypothesis links depression with inflammatory factors and immune correlates, which one of the critical hypotheses of depression[7]. Panax quinquefolium hydroalcoholic leaf extract effectively reversed depressive behavior in mice by regulating tumor necrosis factor-α and neuroinflammation[8]. BDNF is abundant in hippocampus and prefrontal, involved in forming plasticity in the central nervous system. BDNF modulates multiple signaling pathways, including phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways[9]. It was demonstrated[10] that ginsenosides reversed depressive behavior in CUMS mice by increasing the content of BDNF in hippocampus and attenuating immunoreactive neurons in nucleus accumbens.

In literature, there is evidence supporting the fact that prefrontal cortex plays a leading role in regulating emotion and cognition. In the understanding of depression, a large numberof therapeutic studies[11-14] centered on the prefrontal cortex are being conducted.

Many studies[11] have shown that the mechanism of action of depression onset is related to the prefrontal cortex. The knockdown of Sirtuin 1 (SIRT1) mediated by AAV-Cre in the medial prefrontal cortex (mPFC) of adult male mice has shown[12] that SIRT1 inhibits neuronal excitability and leads to depressive behavior in mice. When the SIRT1 activator (SRT2104) was injected into the frontal lobe cortex of mice, it significantly reversed the depressive behavior of CUMS-induced mice.

The modulation of the active enzyme in prefrontal cortex plays a crucial role in antidepressant. Paeoniflorin has been shown to reduce neuronal apoptosis and reverse depressive-like behavior in rats by modulating miR-200a/b-3p/NR3C1 signaling in prefrontal cortex[13]. In lipopolysaccharide (LPS) and chronic stress-induced depression mice's prefrontal cortex, the level of astrocyte complement C3 was significantly increased[14]. Proteomic analysis showed that its complement system-mediated synaptic pruning mechanism is associated with neuroinflammation in depression. By blocking C3aR, the receptor for C3 enhanced synaptogenesis in the prefrontal cortex of mice, somewhat alleviating depressive symptoms. This can predictive for the onset stage of depression.

The KTLD formula is based on the composition of Zuo Gui Pill and Chaihu Shugan San, two traditional Chinese herbal medicines. By tonifying the kidney and draining the liver, it regulated neuropathy caused by aging. The molecular action of traditional Chinese medicine has the advantages of multiple targets and multiple effects. The experimental method of proteomics is the best one to find and analyze biomarkers. ITRAQ/TMT investigations are used to identify pertinent molecular pathways and protein targets, as well as to give further proof in support of illness diagnosis and medication treatment[15-17].

In this study, mice were divided into CUMS group (n=5), control group (n=5), and KTLD group (n=5). iTRAQ proteomics technology was utilized to determine the DEPs in the frontal lobe cortex of each group. The proteins expressed in mice in the CUMS group, control group, and KTLD group. The relevant DEPs were screened out, enriched by GO and KEGG pathway enrichment using bioinformatics techniques. The PPI network was used to identify the protein's interactions both regulated in KTLD and CUMS groups. In this work, we discovered KTLD's potential anti-depressant mechanism in senile depression mice.

Materials and Methods

A total of 15 male SAMP-8 mice, weighing 20±2 g, and six months old, were provided by Beijing Huafukang Biotechnology Co Specific Pathogen Free (SPF). All mice were kept adaptively for one week under contast environmental conditions, including a light/dark cycle of 24±2°C, humidity 60-70% for 12 h, with free to access food and water. All experimental procedures were licensed by the Ethics Committee of Henan University of Traditional Chinese Medicine (approved ethics code: DWLL201712101).

The CUMS Paradigm

CUMS and KTLD group mice were subjected to CUMS for 21 days. Mice were exposed to

an unexpected stressor on 21 days. These stressors included seven stimuli: water deprivation (24 hr), food deprivation (24 hr), wet bedding (24 hr), diurnal reversal (24 hr), combined mice cage (24 hr), tail pinning stimulus (1 min), and tilted mice cage (45°). Each stimulus was applied 1-3 times throughout the experiment and for three weeks.

Drugs and Treatment Plan

KTLD formula consists of Shu Di 24 g, Shan Yao 12 g, Gou Qi 12 g, Chuan Niu Xi 9 g, Tu Si Zi 12 g, Lu Jiao Jiao 12 g, Gui Ban Jiao 12 g, Chai Hu 6 g, Chen Pi 6 g, Chuan Xiong 4.5 g, Xiang Fu 4.5 g, Zhi Qiao 4.5 g, Shao Yao 4.5 g, Zhi Gan Cao 1.5 g. The herb granules were provided by China Resources Sanjiu Pharmaceutical Co. and purchased from the Third Affiliated Hospital of Henan University of Traditional Chinese Medicine(Table I).The mice were gavaged with KTLD (19.5 g/kg/d) one hour before stress stimulation every day, for a total of 21 days.The control group and CUMS group mice were given the same volume of saline.

Behavioral Experiment

Depression was assessed according to the Open Field Test (OFT) and weight change. The open field (100 × 100 × 40 cm) was divided equally into 25 square areas. The middle nine squares were defined as the central area. Mice were individually placed in the arena for 5 minutes. Walking distance and time in the central area were analyzed by using a video recorder and analyzed by software Smart 2.0 (Panlab S.L., Barcelona, Spain). Behavioral experiments were conducted on animals every week, and they were weighed four times the day before the experiment and after the CUMS model (Figure 1). All data were statistically processed.

Frontal Lobe Cortex Tissue Collection

After the studies were finished, mice's brains were dissected. To prepare for proteome analysis, frontal lobe cortex tissue was extracted on ice, washed with phosphate-buffered saline (PBS), promptly frozen in liquid nitrogen, and kept at -80°C.

Protein Preparation and TMT Labeling

The frontal cortex sample was ground and the supernant was passed to the ultrasonic crusher (Q800R, Qsonica, Newtown, CT, USA) until the tissue solution was transparent, centrifuged at 12,000 rpm for 10 min, and the whole process was performed at 4°C. Protein concentrations were determined by the micro protein-nucleic acid detector (BioDrop DuO, Biochrom, Cambridge, UK). Using an enhanced BCA (bicinchoninic acid) protein assay kit (P0010, Beyotime Biotechnology Ltd, Beijing, China). Protein samples (100 μg) were mixed with DTT solution, alkylated with iodoacetamide, and trypsinized (protein-trypsin ratio = 1:100, 12 h). Peptides were labeled by the iTRAQ8 plex kit (4381663, AB Sciex Company, Foster City, California, USA) and samples were labeled as 126 (control 1, control 3, control 5), 127 (control 2, control 4, KTLD 5), 128 (KTLD1, KTLD3, CUMS 5), 129 (KTLD 2, KTLD4), 130

Table I. Components of BuShen and Liver Recipe.

Chinese name	Botanical name	Amount (g)
Shu Di	*Rehmanniae Radix Praeparata*	24
Shan Yao	*Dioscorea oppositifolia L*	12
Gou Qi	*Lycium*	12
San Zhu Yu	*Cornus officinalis Sieb. Et Zucc*	12
Chuan Niu Xi	*Cyathula Officinalis*	9
Tu Si Zi	*Kuan Cuscuta chinensis Lam*	12
Lu Jiao Jiao	*Colla Cornus Cervi*	12
Gui Ban Jiao	*Glue of a Tortoise's and Plastron*	12
Chai Hu	*Bupleurum chinense DC*	6
Chen Pi	*Dried tangerine peel*	6
Chuan Xiong	*Ligusticum chuanxiong hort*	4.5
Xiang Fu	*Nutgrass Galingale Rhizome*	4.5
Zhi Qiao	*Fructus Aurantii*	4.5
Shao Yao	*Paeonia lactiflora Pall*	4.5
Zhi Gan Cao	*Glycyrrhizae Radix Et Rhizoma Praeparata Cum Melle*	1.5

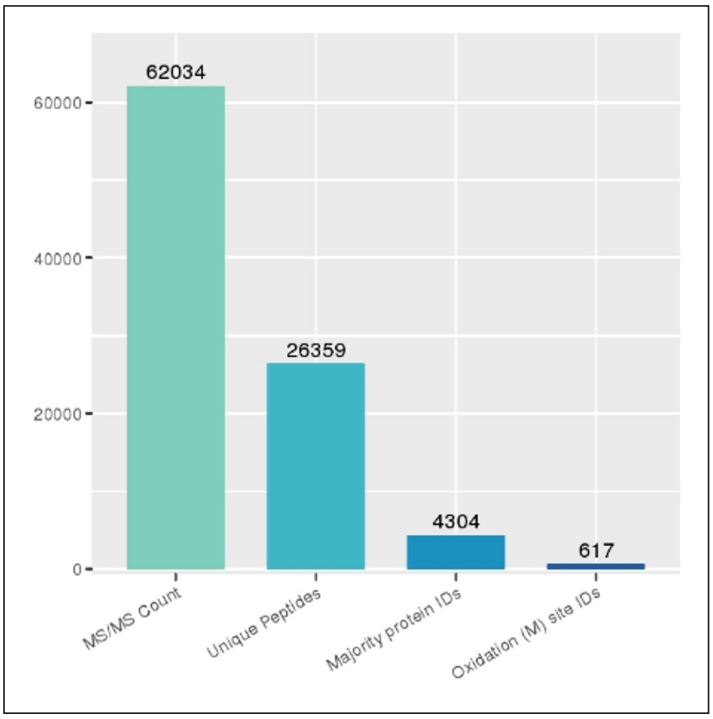

Figure 1. The number of proteins and peptides derived from the identification.

(CUMS 1, CUMS 3), 131 (CUMS 2, CUMS 4). After activation and equilibration of the desalting column, all samples were added to the desalting column and the labeled peptides were eluted, freeze-dried, then acetonitrile was removed.

High pH Reversed-Phase Liquid Chromatography Separation and LC-MS/MS Analysis

The labeled peptide powder was dissolved using 52 µl of high pH mobile phase A (0.1% formic acid aqueous solution) and separated on a BEH column (C18 5 µm, 4.6×250 mm) on a Waters E2695 liquid chromatography system with 50 µL of the sample. One tube of fractions was collected every 1.0 min starting at minute 3. The fractions were combined into 18 fractions according to the peak pattern. Samples were lyophilized by concentration centrifuge. The High Performance Liquid Chromatography (HPLC) liquid phase separation gradient started with 0-45% buffer B (90% Acetonitrile, 0.1% Fomic acid) and was run for 35 min at 300 nL/min with a linear gradient to 80% for 2 min, maintained at 80% buffer B for 5 min, and finally returned to 5% after 2 min. Each fraction was dissolved in 5 µL of phase A (water, 0.1% FA), top-sampled with 2 µL, and analyzed by reversed-phase nanoflow LC-MS/MS, using nano-LC connected to liquid chromatograph equipped with nano upgrade (Easy-nLC-1000, Thermo Scientific, Waltham, MA, USA). Nano LC liquid phase gradient of 5% B solution (acetonitrile, 0.1% FA) lasted 40 min, 28-40% B solution for 10 min, 40-80% B solution for 4 min, and finally held in 80% B solution for six min. Mass spectrometry was performed by making a full mass spectrometer scan on Q Exactiv Plus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), with flow rate of 300 NL/min.

Protein Identification And Quantification

Raw MS/MS data were searched against UniProt/NCBI and other species-specific databases. Using MaxQuant 1.5.5.1 (Max Planck Institute of Biochemistry, Munich, Germany). The peptide false discovery rate (FDR: ≤0.01) was used as an identification criterion. The intensity was mainly used as a quantitative parameter, with the value indicating that the protein was detected, and quantitative data were available. The mean value of the labeled sample mixture was used as a reference and was based on the weighted mean of the reporter ion intensities identified for each peptide. In analyzing significant differences in quantitative results, proteins with a fold of difference meeting the 1.2-fold (up or down) and $p<0.05$ screening criteria were considered differentially significant proteins.

Bioinformatics Analysis

DEPs were analyzed by GO annotation and KEGG pathway. Using the online software DAVID (available at: https://david.ncifcrf.gov/), and functional enrichment analyses were performed for significantly altered proteins. Corrected *p*-values < 0.1 were considered significantly enriched. PPI networks were retrieved from STRING (available at: https://string-db.org/) and using Cytoscape software version 3.7.2 (available at: https://cytoscape.org/).

Statistical Analysis

All behavioral test result data were expressed as mean±SEM, and those results conformed to normal distribution were analyzed by one-way analysis of variance (One-Way ANOVA). The Fisher's least significant difference (LSD) multiple comparison method was used for comparison between groups. The above was performed with SPSS software version 26.0 (IBM Corp., Armonk, NY, USA), and $p<0.05$ was considered statistically significant.

Results

Effect of KTLD Formula on Body Weight and OFT in Senile Depression Model Mice

We used 21-day CUMS model to simulate senile depression in SAMP-8 mice. To measure depression levels, body weight and OFT were used. As shown in Table II, the body weight of the CUMS group mice was significantly reduced compared to the control group ($p < 0.05$), and the body weight of the KTLD mice was significantly increased compared to the CUMS group ($p < 0.05$); as shown in Table III and Table IV, the horizontal scores and vertical scores of the CUMS group mice were significantly decreased compared to the control group ($p < 0.05$), and the horizontal scores and vertical scores of the KTLD group mice were significantly higher ($p < 0.05$) compared to the CUMS group.

Table II. Changes in body weight of mice in each group during the experiment (n=9±S Unit: g).

Groups	Pre-experiment	1W	2W	3W
Blank control groups	27.51±1.567	27.33±1.421	26.82±1.340	26.67±1.107
Depression model groups	27.22±2.614	23.11±2.082*	21.52±1.674*	20.61±1.261*
KTLD groups	27.82±2.161	23.48±2.250*	22.94±2.195*	23.31±2.199**

*$p<0.05$ for the CUMS group compared with the control group, and **$p<0.05$ for the KTLD group compared with the CUMS group.

Table III. OFT level scores of mice in each group during the experiment (χ±S).

Groups	Pre-experiment	1W	2W	3W
Blank control groups	98.78±8.955	97.11±8.022	95.67±6.324	94.78±6.723
Depression model groups	98.56±5.593	89.78±5.191*	82.44±3.940*	74.44±2.455*
KTLD groups	100.22±8.043	89.89±7.639*	86.33±6.690*	83.22±6.099**

*$p<0.05$ for the CUMS group compared with the control group, and **$p<0.05$ for the KTLD group compared with the CUMS group.

Table IV. Vertical scores of OFT for each group of mice during the experiment (χ±S).

Groups	Pre-experiment	1W	2W	3W
Blank control groups	14.56±2.789	14.11±2.088	13.11±1.167	12.00±1.414
Depression model groups	13.67±2.598	10.33±1.871*	7.44±1.333*	6.33±1.225*
KTLD groups	13.89±3.0596	10.67±1.581*	8.11±1.167*	7.89±0.928**

*$p<0.05$ for the CUMS group compared with the control group, and **$p<0.05$ for the KTLD group compared with the CUMS group.

Variation in the Proteomic Profile of CUMS in Mice Frontal Lobe Cortex

To further understand the mechanisms of age-related depression, we used iTRAQ/TMT to identify the DEPs between groups.

Based on the results of iTRAQ-LC-MS/MS analysis, a total of 4,304 proteins and 26,359 peptides were identified (peptide false discovery mice FDR $\leq$ 0.01) (Figure 2). Most of the identified proteins had molecular weights between 10-80 kDa. (To screen for protein alterations after disease and drug intervention). Screening for DEPs in the three datasets, 64 DEPs were manually selected between the CUMS group and control group including 59 up-regulated and 5 down-regulated, and 467 DEPs were selected in the CUMS group and KTLD group, of which 284 were up-regulated and 183 were down-regulated (Fold changes >1.2 and p<0.05).

Functional Annotation Enrichment of Differentially Expressed Proteins

We used the DAVID website to conduct enrichment and richness analyses of all DEPs, in order to comprehend the biological relevance, behind a significant number of DEPs between groups and the underlying mechanism of depression. After the screening, 64 DEPs were found in the CUMS group compared to the control group, including 59 up-regulated and 5 down-regulated. By GO annotation of all DEPs, in terms of biological processes, most DEPs were involved in transcription, DNA-templated (p=0.75), regulation of transcription, DNA-templated, transport (p=0.79), transport (p=0.47). In terms of cellular composition (CC), most DEPs were enriched in the cytoplasm (p=0.07), membrane (p=0.04), nucleus (p=0.10). In terms of molecular function (MF), most of the DEPs were involved in protein binding (p=0.03), metal-ion binding (p=0.15), nucleotide-binding (p=0.07), poly(A) RNA binding (p=0.00). By further GO annotation of 64 DEPs, the extracellular exosome, nucleoplasm, and intracellular ribonucleoprotein complex were important pathways. KEGG enrichment analysis showed that the proteins were mainly enriched in the Rasopathies (RAS) signaling pathway, axon guidance, and ribosome pathway (Figure 2).

Compared with the CUMS group, there were 467 DEPs in the KTLD group, of which 284 were up-regulated and 183 were down-regulated. The KTLD-regulated differential proteins were associated with biological processes (BP), including transport (p=0.00), regulation of transcription and DNA-templated (p=1.00), transcription of DNA-templated (p=1.00), and signal transduction (p=0.10). Most DEPs were enriched in the cytoplasm (p=0.00), membranes (p=0.00), and nucleus (p=0.62) by cellular components (CC) analysis.

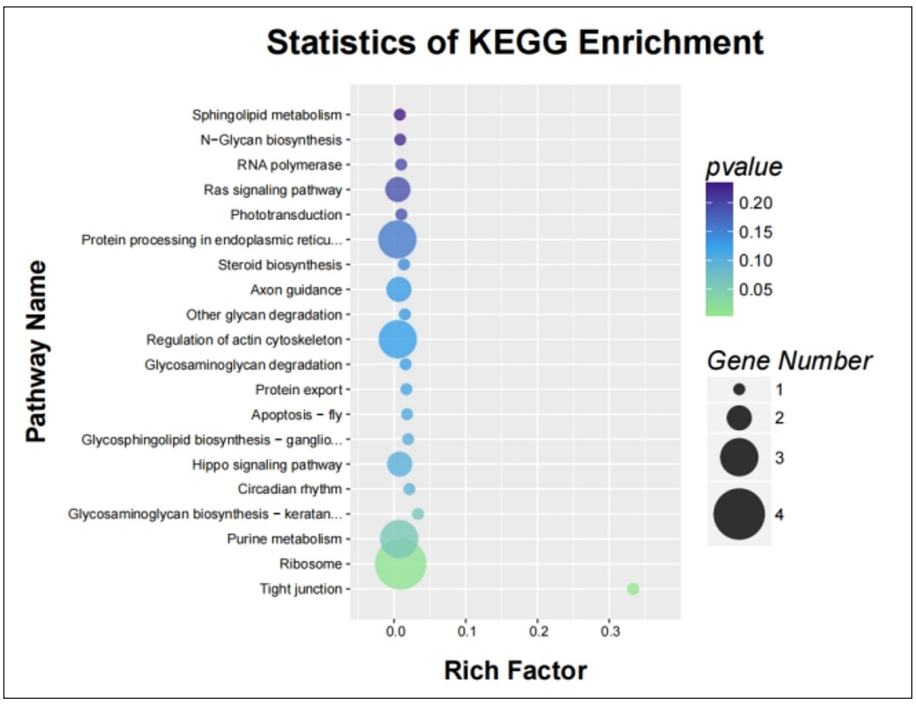

Figure 2. DEPs between control and CUM groups. The top 20 pathway enrichment of the DEPs by KEGG analysis are shown as bubble plots.

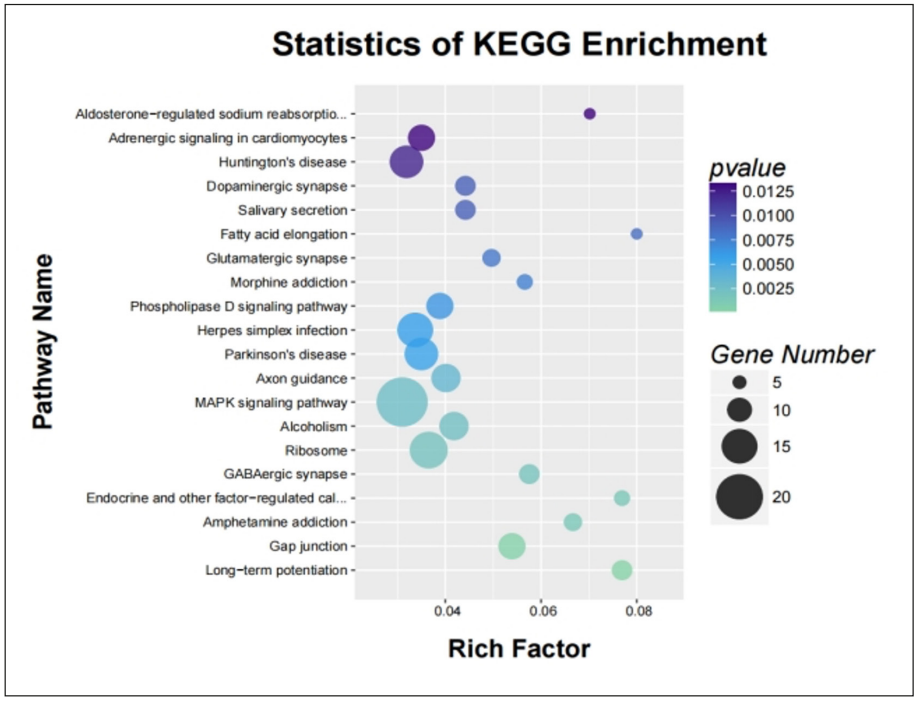

Figure 3. DEPs between CUMS and KTLD group. The pathway enrichment of the top 20 DEPs were analyzed by KEGG and shown as bubble plots.

As fot the molecular function (MF), most DEPs were involved in protein binding (p=0.00), metal-ion binding (p=0.14), nucleotide-binding (p=0.00), and poly(A) RNA binding (p=0.00). By KEGG enrichment analysis of DEPs, 467 DEPs were involved in 20 KEGG pathways, and proteins were mainly enriched on the MAPK signaling pathway, ribosome, and axon guidance pathway (Figure 3).

The pathways of DEPs in each group, transport, transcription, DNA-templated, and regulation of transcription are the same in terms of biological processes, and protein binding, metal-ion binding, nucleotide binding, and poly(A) RNA binding are the same in terms of molecular function. The KEGG enrichment analysis showed that all DEPs found in axon-guided, ribosomes were commonly enriched.

Differentially Expressed Protein-Protein Interaction Network Expressed Proteins

By comparing CUMS with DEGs of KTLD intervention, we found that 16 differential proteins were altered in the disease while simultaneously being regulated by KTLD. 16 common DEPs were changed in the CUMS group and after KTLD intervention, a PPI network graph was constructed by Cytoscape visualization analysis. A total of two clusters of protein interactions were found (GLO1, MT-Nd1, col1a1a, and TRRAP, osbpl11, Ano8) (Figure 4).

Discussion

The pathogenesis of senile depression in TCM theory is liver depressive and kidney deficiency. Zuogui Pill has the effect of tonifying the kidney and filling the essence, and Chaihu Shugan San has the effect of detoxifying the liver and relieving depression. The combined formula has the dual impact of anti-depression and anti-aging by tonifying the kidney and dispersing the liver. Therefore, the KTLD plays an influential therapeutic role in treating aging-related disorders. Still, the molecular targets have never been clarified due to the complex composition of the combined recipe.

It has been found[18-21] that Zuogui Pill and Chaihu Shugan San can show anti-neuroinflammation, regulating monoamine neurotransmitters, and increasing brain-derived neurotrophic factor (BDNF). The group pre-experiments have demonstrated that KTLD exerted a regulatory effect on serum inflammatory factors, oxidative stress and neurotransmission in the hippocampus of mice. In this study, we used a proteomic approach to explore the possible mechanisms of senile depression in mice and the antidepressant mechanisms of the frontal lobe cortex in CUMS-induced in SAMP-8 mice by KTLD. After 21 days of drug administration, behavioral data indicated that the modeling of senile depression made successful. It also demonstrated that KTLD reversed depressive behavior of CUMS-induced senile depression mice.

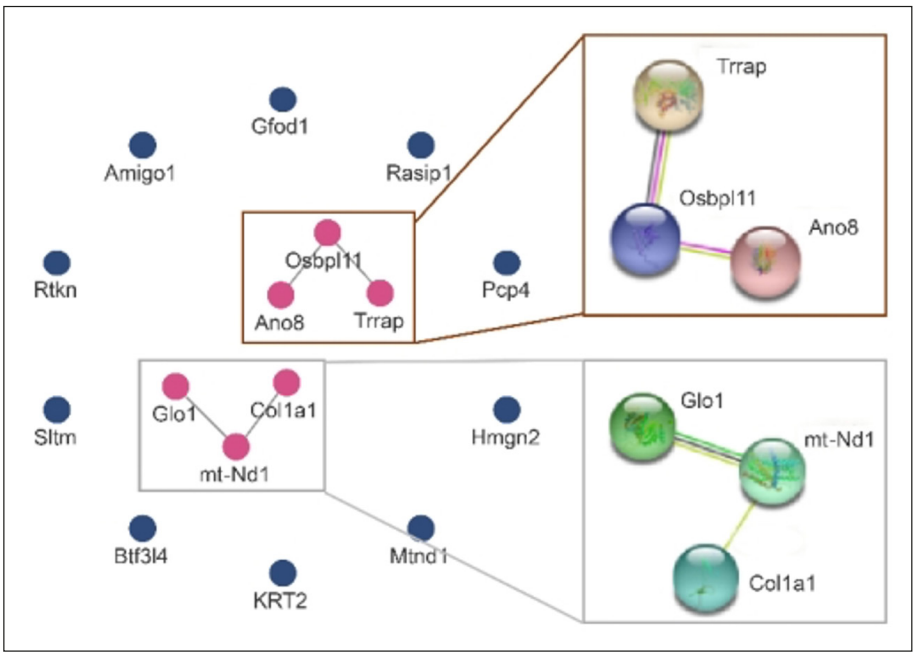

Figure 4. PPI network analyzed by string database and Cytoscape software. The nodes represent proteins and the lines represent the correlation between them.

The pathways with high levels of enrichment in genes from the KTLD group were discovered to be the MAPK signaling pathway, glutamatergic synapse, dopaminergic synapse, axon guidance, and ribosome. Inhibition of the MAPK signaling pathway regulates oxidative stress and inflammatory response in depression. It's one of the classical pathway in antidepressant disorders[22]. Clinical studies[23] have identified mechanisms by which glutamatergic drugs, instead of monoamine transmitter-based drugs, can have a positive impact on the functional nodes of depression-related brain networks by changing the functional connectivity of limbic, cognitive, and executive nodes within the brain's intrinsic connectivity network (ICN) and providing a more precise orientation for improving as well as treating depression symptoms. Musk protected brain neurons by modulating synaptic connections of dopaminergic neurons, which is similar to our findings[24].

The standard molecular targets of KEGG in the control, the CUMS groups, and the KDLT group were axon guidance and ribosome. Changes in axonal morphology are involved in the neural origin and plasticity of the frontal lobe cortex, which is one of the bases of learning and memory, and in adapting the frontal lobe cortex to external stress stimuli. They have an essential role in the mechanism of depression[25]. Meranzin hydrate, a bioactive compound absorbed from the traditional Chinese medicine Chaihu Shugan San, showed that the mechanism of antidepressant action of Meranzin hydrate may be highly related to the axon-directed, mMAPK signaling pathway[26]. Based on KEGG analysis, meranzin hydrate has an antidepressant effect through axon-directed, mMAPK signaling pathways.

By comparing hippocampal transcription in patients with significant depression and animal models, it was demonstrated[27] that model animals and patients with major depression shared 20.9% to 41.6% of co-expressed genes.And axon guidance signaling was the most abundant pathway. It was found[28] that Zuo Gui Pill can potentially promote axonal regeneration by regulating Camp/PKA signaling pathway, and KTLD is identical to this pathway. Ribosomal p70 S6 kinase 1 (S6K1) plays a vital role in the development and plasticity of neurons. It is related to the mechanism of depression, which needs to be further investigated[29]. The neurobiological basis of refractory depression in rats, and antidepressant-related targets are associated with ribosomal proteins, inflammatory responses, and transcriptional/epigenetic regulation[30].

The cross between the differential protein associated with senile depression and the differential protein after the KTLD intervention demonstrates that KTLD may be used to treat senile depression by modulating the differential protein gene. Glyoxalase 1 (GLO1) and translational/transcriptional domain-associated protein (TRRAP)are critical targets in the network. GLO1 plays a significant role in the metabolism of methylglyoxal (MG), involved in the oxidative stress response. It can adequately mitigate protein inactivation, DNA

glycosylation, and cellular dysfunction caused by dihydroxy stress[31]. The expression of GLO1 in the brain and the accumulation of MG are associated with depression, anxiety, and other disorders of the mind[32]. One of the particular biomarkers for monitoring and predicting depression in tests on individuals, with serious depression and considerable mania is GLO1, which has been clinically established[33]. GLO1 activity decreases with age and increases mitochondrial ROS production, associated with developing aging-related diseases[34].

TRRAP contributes to Foxo3-induced cell arrest and apoptosis when interacting with $FoxO_3$[35]. The Tip60/TRRAP complex promotes anti-oxidative stress by upregulating the expression of FOXO transcription factors. Therefore, TRRAP, as a member of the MOZ, Ybf2/Sas3, Sas2 and Tip60 (MYST) family of acetyltransferases, is essential for delaying aging and related diseases[36]. Contrarily, TRRAP-histone Tip60's acetylation regulates the loading of repair proteins and DNA double-strand breaks in a reparative manner[37]. Findings from transcriptomic, epigenomic, and proteomic analyses[38] suggest that TRRAP deletion may contribute to neurodegenerative diseases in old mice and that human neuropathies.

Conclusions

In this study, we used quantitative proteomics methods to reveal and analyze the therapeutic effect of KTLD on CUMS-induced senile depression mice and the effect on frontal lobe cortex protein expression profiles in mice subjected to CUMS. The present data demonstrated that KTLD could regulate depressive behaviors induced by CUMS, and identified 467 DEPs, including 284 upregulated and 183 down-regulated. Enrichment analysis of KEGG indicated that axonal conductance and ribosomes might be affected in CUMS mice frontal lobe cortex. The interaction of the two proteins (GLO1, MT-Nd1, col1a1a, and TRRAP, osbpl11, Ano8) showed the probable mechanism of KTLD in depression treatment.

Founding
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Conflict of Interests
The authors declare no competing financial interests.

Authors' Contributions
Zhijing Zhang wrote the paper; Yuexuan Liu analyzed the data, and is the co-first author; Jianping Yao and Shujuan Ma conceived and designed the experiments; Mei-Ying Huang and Xian Li performed the experiments; Ziyi Guo and Di Li visualized the data. All authors reviewed the manuscript.

Ethics Approval
All experimental procedures were licensed by the Ethics Committee of Henan University of Traditional Chinese Medicine (approved ethics code: DWLL201712101).

Informed Consent
Not applicable.

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