Involvement of LARP7 in activation of SIRT1 to inhibit NF-κB signaling protects microglia from acrylamide-induced neuroinflammation

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Abstract

**Background:** Acrylamide (AM) is a potent neurotoxin and carcinogen that is mainly formed by the Maillard reaction of asparagine with starch at high temperatures. However, the toxicity mechanism underlying AM has not been investigated from proteomic perspective, and the regulation of protein expression by AM remains poorly understood.

**Methods:** This research presents is the first to use proteomics to explore the mechanism of AM exposure-induced neuroinflammation. Target proteins were obtained by differential protein analysis, functional annotation and enrichment analysis of proteomics. Then, molecular biology methods, including Western blot, qPCR and immunofluorescence, were used to verify the results and explore possible mechanisms.

**Results:** We identified 100 key differential metabolites by proteomic analysis, which were involved in the occurrence of various biological functions. Among them, the KEGG pathway enrichment analysis showed that the differential proteins were enriched in the P53 pathway, sulfur metabolism pathway and ferroptosis. Finally, the differential target protein we locked was LARP7. Molecular biological verification found that AM exposure inhibited the expression of LARP7 and induced the burst of inflammation, while SRT1720 agonist treatment showed no effect on LARP7, but significant changes in inflammatory factors and NF-κB.

**Conclusion:** Taken together, these findings suggested that AM may activate NF-κB to induce neuroinflammation by inhibiting the LARP7-SIRT1 pathway. And our study provided a direction for AM-induced neurotoxicity through proteomics and multiple biological analysis methods.

Introduction

Acrylamide (AM), a vinyl monomer, is a well-recognized potent neurotoxin affecting nervous system(Song et al., 2017). It can be formed through a heatinduced reaction of asparagine as a source of an amino group and the reducing sugars as a carbonyl group source via the Maillard reaction(Cantrell and McDougal, 2021). With the participation of cytochrome p4502e1, AM biotransforms in the liver into glycidamide in the form of epoxide(Hogervorst et al., 2021). AM and glycidamide are neurotoxic(Hogervorst et al., 2021). Acrylamide can damage the brain and spinal cord neurons of zebrafish, causing movement disorders(Komoike and Matsuoka, 2019). There are also reports revealing that AM can inhibit neurotransmitter transmission and thereby inhibit nerve conduction(Kopanska et al., 2018). Given the increasing incidence and prevalence of acrylamide toxicity, elucidating the pathogenesis and identifying the optimal treatment modality for acrylamide-induced peripheral neuropathy is essential.

Neuroinflammation is an important marker of neurotoxicity, and its physiological manifestation is the production of a large number of inflammatory factors under noxious stimuli. Studies have successively found that the levels of inflammatory factors such as interleukin-1β (IL-1β) and interleukin-18 (IL-18) in the cerebral cortex and hippocampus of AM poisoned animals were significantly higher than those of normal animals, and were related to the severity of nerve damage. degree of
correlation (Santhanasabapathy et al., 2015; Zong et al., 2019). IL-1β belongs to the interleukin-1 family, has a strong pro-inflammatory effect, and is a major regulator of neuroinflammation in the central nervous system (Basu et al., 2004), which can induce other cytokines such as IL-6 and TNF-α, further aggravating inflammation (Palomo et al., 2015). IL-1β and IL-6 are involved in the pathogenesis of various neurological diseases (Jin et al., 2020), including viral and bacterial meningitis, multiple sclerosis, Alzheimer’s disease, and traumatic brain injury (Campbell et al., 1993).

Sirtuin1 (SIRT1) is a nicotinamide adenosine dinucleotide-dependent deacetylase that can regulate DNA expression, apoptosis, and senescence by deacetylating substrate proteins, and participate in physiological or pathological processes (Jiao and Gong, 2020). SIRT1 is widely expressed in mouse and human neurons, and rodent and human nervous system anatomy studies have shown that SIRT1 is distributed in the hippocampus, prefrontal cortex, and basal ganglia regions (Ramadori et al., 2008; Zakhary et al., 2010). SIRT1 can regulate the inflammatory response of various tissues and cells and is closely related to neuroinflammation (Mendes et al., 2017; Zhang et al., 2020b). SIRT1 can deacetylate the p65 subunit of NF-κB at lysine, and the deacetylation of p65 is suspected to be involved in the inflammatory response, suggesting that the potential regulatory mechanism of SIRT1 in neuroinflammation may involve the NF-κB pathway. As a nuclear transcription factor, NF-κB can regulate the expression of cytokines related to immune, inflammatory and anti-apoptotic responses, and more importantly, NF-κB is widely regarded as the dominant factor regulating the expression of inflammatory genes. Nuclear translocation of NF-κB in the cytoplasm can induce the production of inflammatory cytokines and trigger the inflammatory response (Guzman et al., 2013), while a large number of inflammatory cytokines, such as TNF-α, IL-6 and IL-1β, can trigger and amplify local inflammation response, which in turn leads to neuroinflammation.

La ribonucleoprotein domain family member 7 (LARP7), a La-related RNA binding protein, interacts with its main target non-coding 7SK RNA and promotes its stability (Uchikawa et al., 2015) (Fig. 1). The evolutionarily conserved LARP7 plays a multifaceted regulatory role in the gene expression of health and disease by integrating into different RNPs. LARP7 regulates the transcription of RNA polymerase (pol) ii by targeting 7SK snRNA, and also acts as a cofactor to promote the methylation of the ribose 20oh group of the spliceosome u6 snRNA (u6). Decreased expression levels of LARP7 and loss of function caused by gene mutations are related to cancer and Alazami syndrome (a serious developmental disorder). It has been reported that LARP7 is associated with degenerative cognitive impairment diseases (Najmabadi et al., 2011). Researchers found enrichment of LARP7 in the nucleoli of rat neurons, and in hippocampal neurons, knockdown of LARP7 can also reduce the content of perinuclear ribosomes and protein synthesis, while knocking down LARP7 can increase neutrophil inflammation (Hoodless et al., 2016; Maraia et al., 2017). The above studies suggested that LARP7 may have a potential protective effect on tissue damage caused by inflammation. LARP7 is a master regulator that governs the DNA damage response and RNAPII (RNA polymerase II) pausing pathway (Krueger et al., 2008; Zhang et al., 2020a), but its role in neutrophil inflammation pathogenesis is incompletely understood.
Taken together, in order to reveal the protective effect of LARP7 in neuroinflammation and further explore the neurotoxicity mechanism of AM, the research group explored the relationship between AM toxicity and inflammation from the perspective of metabolomics in the previous study, and used proteomics for the first. The study found that AM exposure inhibited LARP7 expression and altered SIRT1 and NF-κB. Based on a large number of literature reports and previous research results, our group hypothesized that the molecular mechanism of AM-induced neuroinflammation may be the activation of NF-κB by inhibiting the LARP7-SIRT1 pathway (Fig. 1).

Materials And Methods

Cell culture and drug treatment

The mouse normal brain cell line BV2 was purchased from the Cell Bank of the Chinese Academy of Sciences. All cell lines were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin G, and 100 μg/ml streptomycin. The cell incubator was maintained at 37°C with a humidified 5% CO₂ atmosphere. BV2 cells were treated with 2mM concentration of AM for 24 hours as previously reported (Liu et al., 2015). Subsequently, cells were treated with different concentrations of SRT1720 (Hydrochloride) for 24 hours.

Proteomics

Protein extraction and quality inspection

Cells were collected after 24 hours of AM treatment, and the cells were immediately frozen in negative 80 refrigerator for future use, including the control group (without ACR treatment). All samples were taken out under frozen condition and placed on ice; appropriate amount of protein lysate (8m urea, 1% SDS, including protease inhibitor) was added; after ultrasonic lysis on ice, protein supernatant was extracted by centrifugation; BCA protein was quantified using standard protein provided by Thermo Scientific Pierce BCA kit, and finally SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel) electrophoresis was used to evaluate whether the quality of the sample met the requirements of follow-up experiments.

Protein pretreatment

Reductive alkylation and enzymatic hydrolysis of qualified protein samples were carried out. Triethyl ammonium bicarbonate buffer (TEAB) and tris-(2-carboxyethyl) phosphine (TCEP) were added to the 100 μg protein sample so that the final concentration was 100mm to react 60min at 37°C, then 40mM iodoacetamide (IAM) was added to avoid light for 40min at room temperature. Then precooled acetone (acetone: sample v/v=6:1) was added to precipitate 10000g centrifuge. Trypsin was added and hydrolyzed overnight at 37 °C, the polypeptides were obtained. The peptides were labeled with TMT reagent (ThermoFisher) added with acetonitrile and hydroxylamine. And a tube of TMT reagent was added to every 100 μg polypeptides. Each group of medium amounts of labeled products was mixed in a tube and drained by a vacuum concentrator.
Separation and Analysis of Peptides

A reversed-phase C18 column was used for high pH liquid phase separation of polypeptide samples redissolved in UPLC sample buffer (2% acetonitrile). The fraction was collected according to peak shape and time, concentrated by vacuum centrifugation, dissolved with mass spectrometry sample buffer (2% acetonitrile and 0.1% formic acid), and analyzed by liquid phase tandem mass spectrometry (liquid chromatography coupled with tandem mass spectrometry, LC-MS/MS).

Database search and data statistical analysis

Submit the original raw file off the MS machine to the Proteome Discoverer server, and check the library using the software version of Proteome Discoverer™ Software 2.4. The protein sequences of specified species in NCBI and UniProt databases or other species databases were selected to search the database. The filtering parameters were Peptide FDR ≤ 0.01. The identification information of proteins and peptides after quality control was counted. According to the expression of protein in different samples, the samples were analyzed by correlation analysis and PCA analysis. Then, according to the identification of mass spectrometry, all proteins and protein sequences were compared with major databases (Uniprot, NR, GO, KEGG, String) and subcellular localization related databases, and the annotation information of proteins in each database was obtained. Then the expression abundance of the same protein in different samples was obtained through database search and peak analysis. Through the analysis of the differential expression of proteins among samples (n=3), the differentially expressed proteins among samples were excavated and screened. Fold Change (FC) and p-value were selected as the reference criteria for the screening of differential proteins between groups. Set FC≥ 1.5 or FC≤ 0.66, p < 0.05 as the filtering parameter for differential proteins. GO enrichment analysis of the protein concentration was carried out by the software Goatools, and KEGGPATHWAY protein concentration enrichment analysis using Meiji database. All the methods were accurate Fisher test. Meiji mainly uses STRING database (http://string-db.org/) to analyze the protein interaction network, and also refers to the protein interaction network relationship in HPRD (http://www.hprd.org/), biogrid (http://thebiogrid.org), REACTOME (http://www.reactome.org) and other databases. For species with protein interactions in the database, a protein interaction network was constructed, and then networkX under Python was used to visualize the protein network.

Polymerase Chain Reaction (PCR) and Quantitative Real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated using the TRIzol reagent (ThermoFisher Scientific, USA) and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific Fisher). Reverse transcription of 2ug of total RNA was performed using Prime Script™ 1st Strand cDNA synthesis Kit (TaKaRa, Japan) according to the manufacturer’s instructions, then cDNA was subjected to PCR amplification and RT-PCR.

Table 1

<p>| Primer sequences of inflammatory factors |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>Forward Primer</td>
<td>TAGAAGGAAGTCAGACACCCACAGG</td>
</tr>
<tr>
<td></td>
<td>Reverse Primer</td>
<td>CACAGAAGGAAGATGGCACGACAG</td>
</tr>
<tr>
<td>IL-6</td>
<td>Forward Primer</td>
<td>AGTTGCCTTCTTTGGGACTGA</td>
</tr>
<tr>
<td></td>
<td>Reverse Primer</td>
<td>TCCACGATTTCAGAGGAAC</td>
</tr>
<tr>
<td>IL-18</td>
<td>Forward Primer</td>
<td>GGGTTCTCTCTGTTGCACTGC</td>
</tr>
<tr>
<td></td>
<td>Reverse Primer</td>
<td>CCTGATGCTGGAGGTTGCAG</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward Primer</td>
<td>AGAAGTCCCAAATGGCCTC</td>
</tr>
<tr>
<td></td>
<td>Reverse Primer</td>
<td>CCACCTGCTGGTTGATAGC</td>
</tr>
<tr>
<td>iNOS</td>
<td>Forward Primer</td>
<td>GTGTCAGTCGGTTCCAGCTC</td>
</tr>
<tr>
<td></td>
<td>Reverse Primer</td>
<td>CTGATGCTGGAGGTTGCAG</td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward Primer</td>
<td>AGAGGAAATCGTGCGAC</td>
</tr>
<tr>
<td></td>
<td>Reverse Primer</td>
<td>CAATAGTGACCTGGCCGT</td>
</tr>
</tbody>
</table>

Total RNA was extracted with the RNeasy RNA isolation kit (Qiagen) and reverse transcribed to cDNA with the SuperScript First-strand Synthesis System (Thermo Fisher Scientific). The Real-time PCR reactions were performed with Hieff® qPCR SYBR Green Master Mix (Yeasen biotech) using ABI Prism 7500 system. The primer sequences were documented in the table 1.

**Western blotting**

Radio-immunoprecipitation assay (RIPA) lysis buffer was used to extract total proteins from BV2 cells. The concentrations of protein lysates were quantified using BCA Protein Assay Reagent (Boster, Wuhan, China). The denatured proteins were separated by electrophoresis and transferred to a nitrocellulose membrane. After blocking in skim milk for 1 h, the membrane was incubated with rabbit anti-human GAPDH (Proteintech, 10494-1-AP, 1:5000), LARP7(Santa, sc-515209, 1:1000), SIRT1(Affinity, DF6033, 1:1000), P65 (Affinity, AF5006, 1:1000) and P-P65(Affinity, Catalog No:AF2006) antibodies overnight at 4°C. Subsequently, secondary antibody was incubated for 1 hour at room temperature, and the membrane was placed in a dual-color infrared laser scanning imaging system for band detection. Scan results were analyzed quantitatively using ImageJ and GraphPad Prism 8.

**Immunofluorescence assay**

Cells were seeded into 12-well plates pre-placed on treated coverslips, and drug treatments were administered 24 hours after seeding. Cells were subsequently fixed with 4% paraformaldehyde for 20 minutes, then permeabilized with ice-cold methanol and blocked in 3% BSA. Cells were then incubated
with LARP7 antibody overnight at 4 °C followed by secondary antibody for one hour in the dark. Finally, cells are imaged on a fluorescence microscope.

**Statistical analysis**

Statistical analysis was performed using the GraphPad PRISM 8 software, and results from the experiment were expressed as means ± SD. Differences between groups were determined by one-way ANOVA test or student’s t-test. P value of < 0.05 was considered as statistically significant.

**Results**

**Proteomic Results**

**Screening and visualization of differential proteins**

To further elucidate the mechanism of acrylamide-induced neurotoxicity, we used proteomics for the first time to study the neural effects of AM exposure. By intervening BV2 cells with AM, we divided the subjects into Control group (CK) and AM group (AM). Using TMT quantitative proteomics analysis, through sample correlation analysis and Principal Component Analysis (PCA analysis), it is known that the correlation of protein composition between samples within a group is high, and the protein variation between samples between groups is relatively large, which provides information for differential protein analysis (Fig.2a). By setting the parameters: up-regulated protein FC ≥ 1.5, down-regulated protein FC ≤ 0.66, P ≤ 0.05, the differential expression analysis of the proteins between the samples (N=3) was carried out. And 47 up-regulated proteins and 53 down-regulated proteins were mined and screened out; which were finally visualized in the form of volcano plots (Fig.2b).

**Creation and analysis of differential protein sets**

Then we created a protein set with the screened differential proteins, and divided it into two protein sets, up-regulated and down-regulated, and further analyzed the biological functions of the differential proteins. Function annotation of the differentially expressed proteins was performed to understand the function and bioprocess of the differentially expressed proteins. Among them, the GO functional annotation showed that the differential proteins under AM exposure were mainly enriched in cellular process, cellular anatomical entity and binding process, while the enrichment degree showed slight differences between up-regulated and down-regulated proteins (Fig.2c-d). Next, the KEGG pathway enrichment analysis was performed. Our previous metabolomics study found that AM significantly affected metabolism, and the enrichment pathway results of up-regulated differential protein sets, sulfur metabolism and drug metabolism, validated our study again (Fig.2e-f). Sulfur metabolism is regulated by redox-based chemical reactions, and it is likely that intracellular sources of oxidants mediate this regulation (Mistry and Brewer, 2019). Concordantly downregulated proteins were enriched in pathways and cellular locations related to p53 signaling pathway, ferroptosis, cell cycle and Human T-cell leukemia.
Effects of AM on LARP7 and inflammatory molecules

Current studies have found that LARP7 plays an important role in the human body as a key protective factor. Proteomic differential protein analysis found that AM exposure resulted in a significant down-regulation of LARP7 expression in BV2 cells compared with controls. To further understand the function of LARP7 in the pathogenic mechanism of AM, we treated BV2 cells with the same dose of AM, and detected the protein expression of LARP7 at the protein level by Western blot. The results were consistent with proteomic studies (Fig.3a) that AM inhibited the expression of LARP7. By analyzing signaling pathway of differential proteins and querying the pathogenic mechanism of AM, we speculate that LARP7 may affect neuroinflammation by affecting SIRT1-related pathways. To verify this guess, SIRT1 were detected by immunoblotting and found that AM inhibition significantly SIRT1 expression (Fig.3b). To determine whether AM modulated expression of cytokines, mRNA expression of IL-1β, IL-6, IL-18, TNF-α and iNOS were analyzed by RT-PCR. The results were illustrated that AM stimulation resulted in a burst of pro-inflammatory cytokines in BV2 microglia compared to untreated cells (Fig.3c-g). These results are consistent with those of previous studies(Elblehi et al., 2020).

Effects of SRT1720 on LARP7 and inflammatory in AM exposure

In view of the differences in protein expression caused by AM exposure and the conjecture verification results about LARP7 protein, we further studied the mechanism of LARP7. AM exposure resulted in the inhibition of SIRT1 expression, and we set out to intervene in SIRT1 to explore the mechanisms involved in LARP7 and AM exposure. SRT1720, an activator of SIRT1, was used to reverse the SIRT1 expression and observed up-regulated SIRT1 expression levels, respectively (Fig.4a). Then we examined the effect of activation of SIRT1 on the protein expression of LARP7, and the results showed that activation of SIRT1 did not reverse the expression level of LARP7 under AM treatment (Fig.4b). From this, we hypothesized that the LARP7 was located upstream of the regulatory axis and acted as a transcription factor to regulate the occurrence of biological activities. Further to the protein expressions of phosphorylated P65 (P-P65) and P65 were investigated by western blot analysis. Quantification of P-P65/total-P65 ratio revealed substantially increased P-P65 levels in AM exposure, and this result was ameliorated by SRT1720 which resulted in a relative decrease in expression (Fig.4c). Further to confirm the expression of LARP7 in AM exposure and determine its subcellular localization, immunofluorescence was performed in BV2 cells. LARP7 protein was mainly localized in the nucleus of BV2 cells and immunofluorescence staining analysis showed similar results that LARP7 was not regulated by SRT1720(Fig.4d). In contrast, SRT1720 reversed the mRNA expression of inflammatory cytokines in cells exposed to ACR (Figure 4e-i).

Discussion

Neuroinflammation is pathologically associated with many neurodegenerative disorders(Leng and Edison, 2021). However, elucidation of the specific mechanism of acrylamide-induced neurotoxicity has been hampered. In biomedical studies, proteomics profiling techniques have proven to be powerful new tools for uncovering complex biological processes, which aid in exploration of novel mechanisms of
disease pathogenesis and project future approaches to personalized medicine (Aslam et al., 2017). In this study, we performed biomolecular studies of AM-treated BV2 cells using proteomics and screened for LARP7 that interacts with RNA polymerase III (Pol III) transcribed noncoding RNAs (Hasler et al., 2021). As a nuclear protein, LARP7 has been reported to be involved in various diseases, including cardiac damage and Alazami syndrome. LARP7 function in neuroinflammation has not been previously studied. We initially unveiled in this study that AM exposure resulted in the inhibition of LARP7 expression, accompanied by the downregulation of SIRT1 protein. AM exposure also significantly up-regulated the expression of pro-inflammatory cytokines such as IL-1β, IL-6, IL-18, iNOS and TNF-α. These results suggested that neuroinflammation may be the main cause of AM-induced brain injury, and the inhibition of LARP7 may be a key to triggering neuroinflammation.

According to the KEGG enrichment analysis and differential gene expression analysis of proteomics, we learned that AM exposure caused abnormal sulfur metabolism and drug metabolism, and also found that down-regulated proteins were mainly enriched in p53 signaling pathway, Ferroptosis, Cell cycle, Human T-cell leukemia virus 1 infection. Previous studies also showed that AM exposure lead to changes in inflammation-related metabolites and metabolic pathways in brain tissue (Zhao et al., 2021). This is in accordance with the results of differential protein pathway enrichment studies by proteomics. Sulfur metabolism is involved in multiple facets of cellular metabolism related to several responses to stress (Miller and Schmidt, 2020). There are multiple lines of evidence for an impaired sulfur amino acid (SAA) metabolism in autism spectrum disorder (ASD) and inflammatory diseases. And bisulfite, an end product of sulfur metabolism, abrogated ozone-induced AHR and attenuated ozone-induced neutrophil inflammation in mice (Kasahara et al., 2019). The p53 tumor suppressor gene was among the most frequently mutated genes in human cancer, which suppresses cancer formation through its role in regulating cell cycle and apoptosis (Huang et al., 2018; Tong et al., 2020). It has been shown that exposure to AM induces development of colon cancer by inhibiting the tumor suppressor gene p53-mediated mitochondria-dependent apoptosis (Zhang, 2009). This is one of the main reasons why acrylamide is listed as a carcinogen. One of the other pathways for differential protein enrichment that has to be highlighted is ferroptosis. Most organ damage and degenerative diseases are caused by ferroptosis, and the central nervous system (CNS) is the most active part of the body's oxygen metabolism, and it is easy to become the main target organ of oxidative damage. Current studies have confirmed that ferroptosis is related to oxidative metabolism (Jiang et al., 2021), and that acrylamide causes nerve damage by inducing and affecting oxidative metabolism (Yilmaz et al., 2017). Neuroinflammation and iron appear to be tightly regulated, as the inflammatory milieu is associated with iron accumulation, which is associated with NDD and neuroinflammation (Fernández-Mendivil et al., 2021). Specifically, acrylamide may cause neuroinflammation by inducing ferroptosis, contributing to triggering neuronal apoptosis. Sulfur metabolism and ferroptosis are directly or indirectly related to the occurrence of inflammation, revealing the mechanism of AM-induced inflammation from a new perspective. The functions of sirt1 are diverse and complex. It mainly interacts with different substrates such as FOXO, PGC-1α, PS3, and NF-κB, participates in glucose and lipid metabolism, cardioprotection and neuroprotection, and exerts its regulatory function on genes (Imperatore et al., 2017). Inflammatory
stimuli can induce sirt1 inhibition and transcriptional activation of P53 and P65, leading to apoptosis and neurodegeneration. Although relatively fewer studies of LARP7, several reports have pointed out the important role of LARP7 in the life process.

Through the verification experiments, we can know that the neuroinflammation caused by AM exposure may be related to the inhibition of LARP7 expression. By exploring the expression of P-P65 when LARP7 is inhibited, we can conclude that LARP7 activates the NF-κB inflammatory pathway. SRT1720 can activate sirt1 for neuroprotection(Khader et al., 2017). As we all know, if the activation of sirt1 has no effect on LARP7, it means that sirt1 is not an upstream regulatory protein of LARP7, and LARP7 may regulate the expression of sirt1. This conclusion has now been confirmed(Yu et al., 2021). The treatment results of SRT1720 also reiterated that LARP7 can regulate the protein expression of sirt1. The detection results of mRNA expression levels of cytokines also confirmed that LARP7 mediates the expression of sirt1 to guide the changes of cytokines. Therefore, we speculate that acrylamide can induce neuroinflammation by downregulating LARP7 and activating Sirt1/NF-κB pathway.

Conclusion

In this study, new factors of AM-induced neuroinflammation were explored through proteomic detection methods and molecular biological experiments. To gain an in-depth understanding of the role of LARP7, future research will focus on animal experiments to verify the molecular mechanism of LARP7. We will also further explore the regulatory role of LARP7 in Sirt1/NF-κB, so as to fully understand the molecular mechanism of AM-induced neuroinflammation.

Declarations

Author contributions

Material preparation, data collection and analysis were performed by Jinxiu Guo, Hongjia Xue, Wenxue Sun, Shiyuan Zhao and Junjun Meng. The first draft of the manuscript was written by Jinxiu Guo, Haitao Zhong and Pei Jiang supervised the study.

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Data availability
The data used to support the findings of this study are available from the corresponding author upon request.

**Ethics approval**

We did not use any human specimens and animal in this research.

**References**


15. Jin K, J Lu, Z Yu, Z Shen, H Li, T Mou, et al. Linking peripheral IL-6, IL-1β and hypocretin-1 with cognitive impairment from major depression. J Affect Disord. 2020; 277: 204-211.


**Figures**

**Figure 1**
Possible neuroprotective mechanism of LARP7 against acrylamide-induced neurotoxicity. Acrylamide affects SIRT1/NF-κB-induced cytokine release and causes neuroinflammation by downregulating LARP7 expression.

Figure 2

AM exposure shifts the expression of proteins. a Principal Component Analysis (PCA) for Proteomics. b Volcano plot of differentially expressed proteins. c-d Go annotation analysis of downregulated expressed proteins(c) and upregulated expressed proteins(d). e-f KEGG pathway enrichment analysis of downregulated expressed proteins(e) and upregulated expressed proteins(f). g-h Chord plots of GO functional significance enrichment analysis of differential proteins (downregulated proteins(g) and upregulated proteins(h)).

Figure 3

Effects of AM on LARP7 and inflammatory molecules. a AM exposure downregulates the expression of LARP7. b Alterations of SIRT1 following LPS treatment. c-g Alteration of relative mRNA expression of inflammatory factors by AM.

Figure 4

Effects of SRT1720 on LARP7 and inflammatory in AM exposure. a SRT1720 reverse the SIRT1 expression. b Regulation of LARP7 activity by SRT1720. c SRT1720 affects the relative expression of P-P65. d Localization of LARP7 and the regulation of SRT1720 by immunofluorescence staining. e-h SRT1720 reversed the mRNA expression of inflammatory cytokines.