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Changes in the Expression Level of S100A8/A9 in Brain Tissue and Serum of Sleep-Deprived Mice

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Abstract: To explore the expression of S100A8/A9 in the brain and serum of chronic sleep deprivation (CSR) mice. The protein expression of S100A8/A9 in the hippocampus of CSR mice and wild-type mice was detected by western blotting, the mRNA expression of S100A8/A9 in the hippocampus of CSR mice and wild-type mice was detected by RNA-sequencing, and the serum concentration of S100A8/A9 in CSR mice and wild type mice was detected by enzyme-linked immunosorbent assay (ELISA). 1) The protein expression of S100A8/A9 in the hippocampus of the CSR group was significantly lower than that in wild type group ($P<0.05$); 2) The mRNA expression of S100A8/A9 in the hippocampus of the CSR group was significantly lower than that in wild type group($P<0.05$) 3) The serum concentration of S100A9 of CSR mice was significantly higher than that in wild type group ($P < 0.0001$), but there was no significant difference in the S100A8 expression between the CSR group and the control group. The expression of S100A8/A9 in the brain and serum of CSR mice is different, and we need more studies to explore the internal mechanism.

1. Introduction

Sleep plays an important role in maintaining the normal operation of various systems of human body. Sleep disorder refers to the normal rhythm disorder of sleep, and the main manifestations of sleep disorders are lack of sleep and paroxysmal abnormalities in sleep. A survey conducted by the World Health Organization shows that there are about 27% of people with sleep disorders worldwide. People's sleep duration has been reduced by nearly 2.5 hours in ten years[1]. The animal model of chronic sleep deprivation mainly causes sleep deprivation through artificial technology,

resulting in sleep disorders, in order to study the effects of sleep loss or sleep restriction on the body. Studies have shown that chronic sleep deprivation (CSR) can trigger peripheral or central inflammation[2]. In the central nervous system, astrocytes and microglia are activated after sleep deprivation, resulting in increased release of pro-inflammatory factors and further brain damage[3,4].

S100A8/A9 proteins are members of the calcium binding protein S100. Because heterodimers are more stable than homodimers, these two proteins usually exist in the form of S100A8/A9 complexes. Studies have shown that the expression of S100A8/A9 is upregulated in inflammatory diseases, such as inflammatory bowel disease[5], systemic lupus erythematosus[6]and rheumatoid arthritis[7]. In addition, S100A8/A9 has also been confirmed to be involved in the occurrence and development of central nervous system diseases, including stroke[8], Alzheimer's disease[9] and multiple sclerosis[10].

In order to explore the role of S100A8/A9 in the occurrence and development of CSR, we detected the expression of S100A8/A9 in the hippocampus and serum of CSR and wild-type mice by Westernblot, transcriptome sequencing and ELISA.

2. Materials

2.1. Animals

C57BL/6 mice, male, 8 weeks old, weighing 25g to 30g, Chengdu Jijiaokang Biotechnology Co, Ltd.

2.2. Instrument

XR-XS107 Sleep deprivation instrument, Shanghai Xinruan Information Technology Co. Ltd.

2.3. Reagent

S100A8/A9 Antibody, NBP2-25257,Novus Biologicals,America; Mouse S100 calcium binding protein A8 (S100A8) enzyme-linked immunoassay kit, JM-11753M2, Jiangsu Jingmei Biotechnology Co, China; Mouse S100 calcium binding protein A9 (S100A9) enzyme-linked immunoassay kit, JM-11754M2, Jiangsu Jingmei Biotechnology Co, China; The Pierce BCA protein assay kit Thermo Fisher Scientific, Carlsbad, CA, USA.

3. Methods

3.1. Experimental environment

Temperature :(24±1)℃; humidity: 50% to 60%; automatic light-dark cycle for 12 hours.

3.2. Chronic sleep deprivationl

All the mice were fed in SPF laboratory for one week, then randomly divided into two groups according to the method of random number table. The mice in the WT group ate and drank freely, and the mice in the CSR group were put into the stripping instrument [11]. The speed of the instrument was 7 laps/min and rotated clockwise/counterclockwise alternately. Sleep deprivation was performed at 11:00 every day for 7 days, that is, 20 hours of daily sleep deprivation and 4 hours of normal sleep.

3.3. Western blot

The total protein was extracted from mouse hippocampal tissue.t The protein concentration was determined by BCA method, then boiled for 5 mins. Samples were run in a 8 % SDS-PAGE and transferred to PVDF membranes by wet transfer on ice.

Then put the membranes into 5% nonfat milk at room temperature for 2 hours, then primary antibody (S100A8/A9, 1:1000; rabbit anti-ACTB, 1:10000) was added overnight at 4 ℃. TBST washed the membranes 3 times, each for 5 mins. Then the membranes were incubated with Secondary antibodies (goat anti-rabbit, 1:10,000) at room temperature concussion for 2 hours, each for 5min. ECL chemiluminescence solution was acted on the membranes. Densitometry was performed to quantify signal intensity using ImageJ software.

3.4. Transcriptome sequencing

The mice hippocampal tissue was quickly preserved in liquid nitrogen, and the samples were sequenced by the transcriptome with reference genome on the Lianchuan Biological platform (Hangzhou Lianchuan Biotechnology Co., Ltd., China). In data analysis, there is no need for transcript splicing, only the transcriptome sequencing data are compared with the reference genome to determine the expression level of each gene.

3.5. ELISA

The blood of mice was placed at room temperature for 15 minutes and then centrifuged at 3000 rpm for 20 minutes. Collect the supernatant carefully. Set up standard hole, blank hole and sample hole to be tested, complete the steps of sample addition, incubation, washing and color development according to the operating instructions of the kit, zero the blank hole, and measure the absorbance (OD value) of each hole sequentially at 450nm wavelength. The determination was carried out within 5 minutes after adding the Terminator. The linear regression equation of the standard curve is calculated by using the concentration and OD value of the standard substance, and the OD value of the sample is substituted into the equation to calculate the concentration of the sample.

3.6. Statistical analysis

The data were analyzed by GraphPad Prism 7.0. And a paired Student t-test was performed.The measurement data were expressed by $x \pm s$. P values < 0.05 were considered statistically significant.

4. Results

4.1. Decreased expression of S100A8/A9 protein in hippocampus of CSR mice

Compared with the control group, the expression of S100A8/A9 protein in hippocampus of CSR mice decreased significantly (* $P < 0.05$).

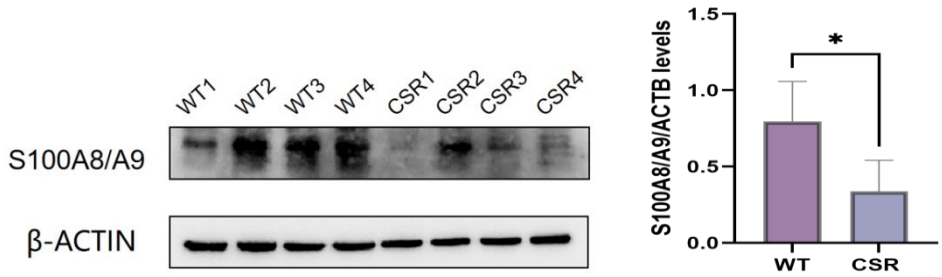

Fig.1 Comparison of S100A8/A9 protein content in hippocampus of mice in WT group and CSR group

4.2. Downregulation of S100A8/A9 mRNA in hippocampus of CSR mice

Compared with the control group, the S100A8/A9 gene in the hippocampus of CSR group was down-regulated significantly (* $P < 0.0001$).

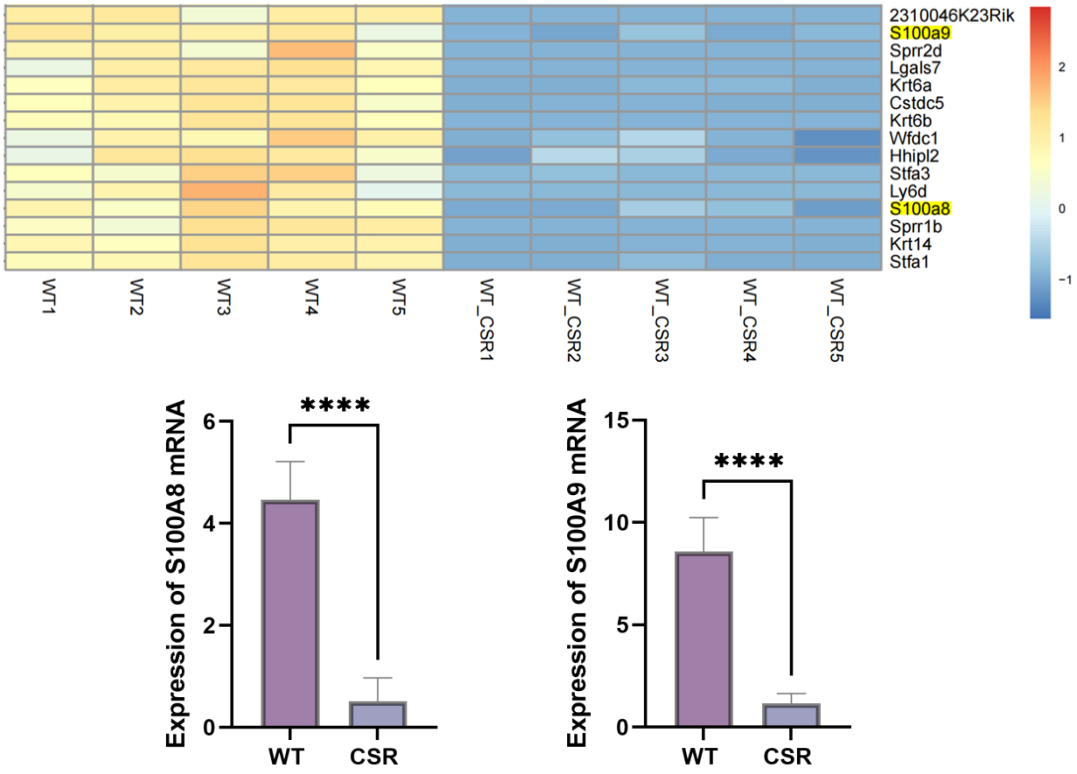

Fig.2 Comparison of S100A8/A9 mRNA expression in WT group and CSR group

4.3. S100A8/A9 expression in serum in CSR group

Compared with the control group, the content of serum S100A8 in CSR group had no significant change, but the content of serum S100A9 increased significantly (* $P < 0.05$).

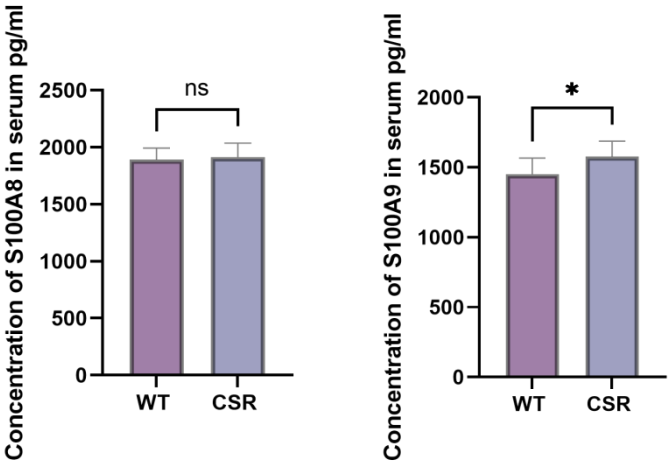

Fig.3 Comparison of S100A8/A9 expression in serum in WT group and CSR group

5. Discussion

S100 protein family is a group of calcium-binding proteins with similar structure and function, in which S100A8 and S100A9 are calcium-binding cytoplasmic molecules with two characteristic calcium-binding EF hand-shaped structures, which are connected by a central hinge region, and the two ends have different affinity with calcium, forming a classical helix-ring-helix ligand[12], usually in the form of heterodimer. S100A8/A9 is a kind of pro-inflammatory factor, which mainly depends on myeloid cell inflammation and autoimmune state. S100A8/A9 participates in the migration of inflammatory cells and the metabolism of arachidonic acid (AA) [14], regulating neutrophil chemotaxis[15], promoting monocyte and macrophage migration [16], and inducing the secretion of multiple inflammatory cytokines[17].

S100A8/A9 is secreted largely in infection-induced inflammation. After bacterial infection, neutrophils, macrophages and monocytes strongly secrete S100A8/A9, which regulates the inflammatory process by inducing inflammatory cytokines, reactiving oxygen species (ROS) and nitric oxide (NO). In metabolic inflammatory diseases such as gout[19] and diabetes[20], S100A8/A9 levels are detected to be elevated in both serum and inflammatory sites. S100A8/A9 is also upregulated in inflammation caused by autoimmune diseases, such as strong expression in the subsynovium of patients with psoriatic arthritis[21].

It has been confirmed that the serum concentration of S100A8/A9 in patients with active systemic lupus erythematosus[22], antigen-induced arthritis[23] (AIA), glomerulonephritis[24], inflammatory bowel disease [25] and other non-central nervous system inflammatory diseases is higher than that in healthy people. For the inflammation of central nervous system diseases, the increase of serum S100A8/A9 concentration was reported in patients with ischemic stroke[26], acute cerebral hemorrhage[27], acute arteriosclerotic cerebral infarction[28], stroke and other diseases. Chronic sleep deprivation can cause inflammation in the central nervous system, and the increase of serum S100A8/A9 concentration in CSR mice is consistent with the above results. However, the expression of A100A8/A9 protein in the hippocampus of CSR mice was lower than that of wild-type mice. The sequencing results showed that the expression of S100A9 gene in CSR mice was downregulated, which was contrary to the serum concentration. At present, it is not clear whether this result is related to the acute degree of the disease or whether S100A8/A9 passes through the blood-brain barrier, and more research work is needed to explore the mechanism.

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