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Deletion of asparagine endopeptidase reduces anxiety- and depressive-like behaviors and improves abilities of spatial cognition in mice

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ABSTRACT

Mammalian asparagine endopeptidase (AEP) is a lysosomal cysteine protease that cleaves protein substrates on the C-terminal side of asparagine. The expression and activity of AEP are closely related to many pathological conditions that include cancer, atherosclerosis and inflammation. It has been validated that the level of AEP is elevated in aged human and neurodegenerative diseases like Alzheimer's disease (AD). Mood disorder is one of the most emotional symptoms that can be seen in AD patients, which leads us to assume that AEP can modulate affective behaviors. AEP knockout (AEP KO) and wildtype (WT) mice were used in this study, and a series of behavioral tests were performed to establish a potential link between AEP and psychiatric disorders. It was demonstrated that AEP KO mice displayed lower anxiety-like behavior and more advance exploratory behavior in open-field and hole-board tests. AEP KO mice reduced depressive-like behaviors in the forced swim and tail suspension tests. Morris water maze (MWM) test showed that the abilities of spatial learning and memory were elevated in AEP-deletion mice compared with those of WT mice. Furthermore, the enhanced synaptic plasticity (LTP and DPT) as well as the increased expressions of SYP and PSD-95 proteins in hippocampus were showed in AEP KO mice. Otherwise, the level of BDNF protein was reduced and the level of NF-κB p65 protein was increased in hippocampus and frontal cortex of AEP KO mice. These data highlight the importance of studying AEP in the anxiety and depression behaviors and the spatial learning and memory.

1. Introduction

Mood disorders, including anxiety and depression, affect millions of individuals worldwide. People with mood disorders suffer distress, disability and lowered quality of life (Arvilommi et al., 2015). The suicide rate of this kind of patients is 10 times higher than that in the non-psychiatric population (Nordentoft et al., 2011). The pathomechanism of mood disorder is complex and diverse, involving the hereditary factors (Antypa et al., 2015), changes in neuroplasticity (Manji et al., 2015), and neuroinflammatory (Isgren et al., 2017). Therefore, investigating molecular and cellular mechanisms may facilitate the development of novel effective therapies for mood disorders.

Mammalian asparagine endopeptidase (AEP, also known as legumain), is a lysosomal cysteine protease that cleaves protein substrates on the C-terminal side of asparagine. The expression of AEP can be found in all tissues of animals, and is especially abundant in placenta, kidney as well as brain (Chen et al., 1997; Jinq-May et al., 1998; Shirahama-Noda et al., 2003b). The expression level of AEP is distinctly elevated in some cancer types such as breast, colon and prostate cancer (Murthy et al., 2005). It has been found that AEP promotes cancer cell migration and invasion both *in vitro* and *in vivo* (Liu et al., 2003; Meng and Liu, 2016).

The mice lacking AEP have been established by many different groups and proved to be viable and healthy (Dall and Brandstetter, 2015). The AEP knockout mice were prolific and had no gross anatomical and morphological abnormalities, although their increased body weights were not at the same rate as wild-type littermates (Shirahama-Noda et al., 2003a; Matthews and Ideussing, 2010). AEP has been showed to process a microbial antigen for class II major histocompatibility complex (MHC) presentation. There were no differences in

Abbreviations: AEP, asparagine endopeptidase; AD, Alzheimer's disease; AEP KO, AEP knockout; WT, wildtype; MWM, Morris water maze; fEPSP, field excitatory postsynaptic potential; PP, perforant pathway; DG, dentate gyrus; TBS, theta burst stimulation; LTP, long-term potentiation; DPT, depotentiation; SYP, synaptophysin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells

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processing of the invariant chain or maturation of class II MHC products in AEP-null mice compared with that in wild-type controls (Manoury et al., 1998). Furthermore, the decreased glomerular filtration function and proteinuria were found in AEP-deficient mice (Miller et al., 2011). Additionally, AEP knockout mice showed anemia and extramedullary hematopoiesis that were similar to hemophagocytic syndrome (Chan et al., 2009).

In recent years, more attention has been given to the pathophysiological effects of AEP on brain. It has been demonstrated that AEP plays a key role in stroke and neurodegenerative diseases (Ishizaki et al., 2010; Basurto-Islas et al., 2013). Researches have already showed that AEP is up-regulated in an age-dependent manner and is highly activated in human brains of Alzheimer's disease (AD). Knockout of AEP in AD mice prevents cognitive deficits by decreasing Tau hyperphosphorylation and amyloid β (Aβ) production deposition that are two typical neuropathological hallmarks in AD brains (Zhang et al., 2014, 2015).

The finding in the process of breeding mice was that AEP knockout (AEP KO) mice were extremely hyperactive over the light/dark cycle. Moreover, the AEP KO mice showed an increase in their preference for sucrose. Despite the evidence, the crucial role AEP played in the mood remains to be identified. Therefore, we investigated whether AEP affected mood related behaviors. AEP knockout mice were used in the present study. A series of behavioral tests were performed to establish a potential link between AEP and psychiatric disorders as well as spatial learning and memory.

2. Experimental procedures

2.1. Animals

AEP heterozygous parents on C57BL/6 background (obtained from Cyagen Biosciences Inc (Guangzhou, China) were bred to generate wild-type (WT) and AEP knockout (AEP KO) littermates. Adult, male WT and AEP KO mice (14–15 weeks, 23–25 g, n = 8 per group) were used in experiments. They were housed in specific pathogen-free environment, 4 mice per cage, under the standard conditions at 22 °C and a 12 h light-dark cycle (light on at 7:00 and off at 19:00) with food and water provided *ad libitum*. The experiments were approved by the local ethical committee at Nankai University, and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the number and suffering of the experimental animals.

2.2. Reagents

The NF-κB p65, BDNF, Synaptophysin and PSD-95 antibodies were purchased from Abcam (Abcam Cambridge, MA), and β-actin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The goat anti-mouse and anti-rabbit second antibodies (Promega Co., Wisconsin, USA) were diluted into 1:5000. The chemiluminescent substrate was purchased from Millipore (Millipore, Billerica, MA, USA).

2.3. Behavioral analysis

All the behavioral tests were carried out at about 10:00 a.m., and every mouse was tested in the same order in the different behavioral tests (Fig. 1A).

2.3.1. Open field test

The open field test was used to evaluate anxious behavior in a novel environment. The enclosed arena (40 cm L × 40 cm W × 40 cm H) was divided into center and periphery by using 16-beam infrared array, and the four squares in the middle were defined as the central zone (Salazar et al., 2012). Mice were placed individually in an open-field chamber and permitted to move freely for 5 min. The locomotor activity in the central and peripheral part of open field was tracked automatically by using a camera-driven tracker system (Ethovision 2.0, Noldus, Wagenigen, Netherlands). The number of entries into the center, the distance travelled and the amount of time spent in the center, the speed and the total distance during the testing were analyzed subsequently. The apparatus was cleaned with 70% ethanol after each test.

2.3.2. Hole-board test

The hole-board test was invented by Boissier and Simon (Boissier and Simon, 1961) to examine the response of animals in an unfamiliar environment. The hole-board in this test consisted of a white perspex panel (40 cm × 40 cm, 2 cm thick), which had 16 equidistant holes of 3 cm in diameter. The apparatus was positioned 18 cm above the floor. The protocol used in this experiment was on the basis of the previous study (Zarrindast et al., 2010). Mice were placed in one corner of the equipment and allowed to explore it freely for 5 min. The total number of head dips into the holes until both the eyes were invisible was scored during this period. The number of head-dips reflect the anxiogenic or anxiolytic behavior of animals (Tsuji et al., 2000). The equipment was cleaned with 70% ethanol and dried after each test.

2.3.3. Forced swim test

Depression-like behavior was assayed using forced swim test. In this experiment, mice were individually placed in a transparent plexiglas cylinder of 25 °C water for 6 min. Make sure that their tails could not be able to touch the bottom of the cylinder. The water was changed after each mouse. Immobility was scored when a mouse remained floating passively in water without active movements of forepaws. The immobility time was recorded during the last 4 min of the test by an investigator who knew nothing about the group status.

2.3.4. Tail suspension test

In this test, mice were suspended on the edge of a rod 50 cm above the bench surface. They were fastened for 6 min by using adhesive tape placed approximately 1 cm from the tip of tails. A small plastic cylinder was used by passing their tails to prevent the tail climbing. Mice were judged to be immobile when they hung down passively and were motionless completely. The duration of immobility was recorded during the last 5 min of the 6 min period by an observer who was blinded to the group status.

2.3.5. Morris water maze (MWM) test

Mice were trained on the Morris water maze to assess their spatial learning and reference memory. Testing was carried out based on an established hippocampus-dependent protocol (Morris, 1981). The water maze consisted of a circular pool (diameter 90 cm, height 50 cm) filled with water (23 ± 1 °C). There was a circular platform that was 9 cm in diameter and was submerged 1 cm below the water surface. The water was made opaque by the non-toxic white paint to obscure the platform from view. The surface of the pool was divided into four quadrants by two mutually perpendicular lines. The end of each line was determined as the four cardinal points: east (E), west (W), north (N) and south (S).

The test was started with spatial acquisition training. In brief, the platform stayed in the middle of the same quadrant (SW), and mice were released from four different positions (N, SE, E and NW) to locate a platform that was hidden the water surface. Mice performed four trials per day for five consecutive days. In each trial, the animal was allowed to swim with a maximum length of 60 s to find the platform and then remained on it for 10 s. The animal that failed to find the platform within 60 s was manually guided to the platform by the experimenter and allowed to stay on it for 10 s. The interval time of each trial lasted for 10–15 min. The swimming pathway was recorded by a video tracking system (Ethovision 2.0, Noldus, Wagenigen, Netherlands). The swimming speed, the total distance and the time taken to reach the platform (the escape latency) were recorded during this acquisition phase. In addition, the search strategies were analyzed