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Cathepsin B links oxidative stress to the activation of NLRP3 inflammasome

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ABSTRACT

Oxidative stress-mediated activation of NLRP3 inflammasome in microglia is critical in the development of neurodegerative diseases such as Alzheimer's disease (AD), Parkinson disease (PD). However, the mechanism underlying oxidative stress activates NLRP3 inflammasome remains exclusive. Here we demonstrated cathepsin B (CTSB) as a regulator of the activation of NLRP3 inflammasome by H_2O_2·H_2O_2 induced IL-1β secretion in NLRP3 inflammasome-dependent manner·H_2O_2 treatment increased CTSB activity, which in turn activated NLRP3 inflammasome, and subsequently processed pro-caspase-1 cleavage into caspase-1, resulting in IL-1 β secretion. Genetic inhibition or pharmacological inhibition of CTSB blocked the cleavage of pro-caspase-1 into caspase-1 and subsequent IL-1 β secretion induced by H_2O_2. Importantly, CTSB activity, IL-1β levels and malondialdehyde (MDA) were remarkably elevated in plasma of AD patients compared to healthy controls, while glutathione was significantly lower than healthy controls. Correlation analyses showed that CTSB activity was positively correlated with IL-1β and MDA levels, but negatively correlated with GSH levels in plasma of AD patients. Taken together, our results indicate that oxidative stress activates NLRP3 through upregulating CTSB activity. Our results identify an important biological function of CTSB in neuroinflammation, suggesting that CTSB is a potential target in AD therapy.

1. Introduction

Accumulating data have recognized microglia activation as a key regulator of neurodegenerative diseases such as Alzheimer's disease (AD) [1–5]. Activated Microglia express elevated Interleuk-1β (IL-1β) in senile plaques in the brain of patients with AD [6], and higher IL-1β levels are found in the cerebrospinal fluid of patients with AD and Parkinson's disease [7]. IL-1β is a major proinflammatory cytokine that plays a critical role in the pathogenesis of neurodegenerative diseases [8]. IL-1β production is mainly mediated by the activation of NOD-like receptor containing a pyrin domain 3 (NLRP3) inflammasome, which contributes to the pathology in APP/PS1 mice [9]. The NLRP3 inflammasome is activated by signals derived from microorganisms or metabolic dysregulation [10]. Activated NLPR3 inflammasome triggers the cleavage of procaspase-1 into caspase-1, which in turn regulates the production of pro-IL-1β and subsequent maturation and release of IL-1β. IL-1β is produced from its inactive precursor pro-IL-1β after stimulation [11]. However, the central mechanism underlying activation of NLRP3 inflammasome is poorly understood.

Several studies have suggested three proposed models to activate NLRP3 inflammasome. 1) Lysosomal rupture. Phagocytosis of particles or live pathogens leads to lysosome rupture, releasing CTSB or a protein modified by CTSB [12,13]. Phagocytosis inhibitors can block NLRP3 activation by particulates. However, this hypothesis cannot explain all stimuli. 2) The channel model. The decrease of intracellular potassium (K^+) concentration leads to NLRP3 activation through ion channels or pore-forming bacterial toxins [14]. 3) Reactive oxygen species (ROS) model. Cellular redox mediates the activation of NLRP3 inflammasome [15–19]. All activators of NLRP3 are involved in ROS generation [20]. Blocking ROS generation by mitochondria abolishes NLRP3 activation by artificial induction of mitochondrial ROS [21], however, NADPH-deficient cells or cells derived from the patients with defective NADPH oxidase subunits have normal, or even increased, caspase 1 activity [22]. Although a recent study provides a mechanistic insight that ROS released thioredoxin-interacting protein (TXNIP) from thioredoxin complex, allowing TXNIP to activate NLRP3, promote the processing of pro-caspase-1 cleavage to caspase-1 and subsequent maturation of IL-1β [23], but TXNIP deletion or knockdown partially impairs caspase-1 activation. In addition, Bauernfeind et al. reported that ROS inhibitors block priming, but not activation of NLPR3 inflammasome [24]. These

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controversial observations suggest that other regulators mediated by ROS are involved to initiate NLRP3 inflammatory response.

Recent studies reported that oxidative stresses are associated with CTSB activity. CTSB expression is upregulated by oxidative stress in the retinal pigment epithelium /choroid of the mouse [25]. Gemcitabine and 5-fluorouracil, which generate excessive ROS, triggered CTSB release in myeloid-derived suppressor cells to activate the NLRP3 inflammasome [26]. Moreover, CTSB has been reported to accumulate in microglia surrounding senile plaques where oxidative stresses are remarkably elevated, and inhibition of CTSB improved memory and decreased amyloid plaques in a mouse model of Alzheimer's disease [27–30]. Furthermore, CTSB is also found to be colocalized with Aβ in amyloid plaques in AD brains [31–33] and is elevated in cerebral spinal fluid of AD patients [34]. In addition, CTSB was involved in the processing and secretion of IL-1β in chromogranin A-treated microglia [35]. Therefore, we hypothesized that ROS might activate NLRP3 inflammasome through regulating CTSB activity in microglia. To test this, we utilized BV-2 cells, a murine microglial cell line, which was v-raf/v-myc-immortalized murine neonatal microglia [36]. This cell line is a well-accepted alternative to primary microglia [37]. Therefore, we treated BV2 cells with H_2O_2 to mimic oxidative stress in microglia.

Here we demonstrated CTSB mediates H_2O_2-induced NLRP3 activation and IL-1β maturation·H_2O_2-induced microglial IL-1β release is dependent on NLRP3 activation. CTSB is required for H_2O_2-mediated NLRP3 activation. Genetic and pharmacological inhibition of CTSB dramatically impaired H_2O_2-induced NLRP3 activation, the processing of pro-caspase-1 to caspase 1, IL-1β release and subsequent cell death. Importantly, CTSB activity are positively correlated with oxidative stresses and IL-1β levels in plasma of AD patients. These findings identify a novel role for CTSB in linking oxidative stress to NLRP3 activation.

2. Results

2.1. H_2O_2 induced IL-1 β secretion in dose and time dependent manners

To examine whether H_2O_2 treatment created oxidative stress status in microglia, BV2 cells were treated with H2O2. MDA is a member of Oxidized Low Density Lipoproteins (ox-LDL), a marker of oxidative stress, therefore we measured MDA level. MDA level was significantly elevated by H_2O_2 (Fig. 1A), indicating that H_2O_2 may establish oxidative stress in microglia. Next, we confirmed whether H_2O_2 may induce IL-1β secretion in microglia. As shown in Fig. 1B, ELISA assay showed that exposures to H_2O_2 (1–5 mM) induced IL-1β secretion in a dose-dependent manner. Moreover, IL-1β secretion was increased over time in response to H_2O_2 treatment (Fig. 1C). Western Blotting demonstrated that H_2O_2 stimulation triggered the release of IL-1 β (Fig. 1D). These data suggest that H_2O_2 induced oxidative stress and IL-1β release.

2.2. H_2O_2-induced IL-1 β secretion was dependent on NLRP3 inflammasome

NLRP3 inflammasome is a major regulator of IL-1β release. Therefore, we examined whether the expression of NLRP3 inflammasome was required for H_2O_2-induced IL-1β release. We first knockdown NLRP3 expression by siRNA. Western Blotting analysis showed that NLRP3 was remarkably silenced by NLRP3 siRNA in BV2 cells (Fig. 2A). NLRP3 silencing abolished the processing of pro-caspase-1 cleavage to caspase-1 induced by H_2O_2 (Fig. 2B). Consistent with blocking of pro-caspase-1 cleavage, subsequent IL-1β secretion was blocked by NLRP3 knockdown (Fig. 2B). Trypan Blue staining showed that alive cells were significantly higher in NLRP3 knockdown than siScr after H_2O_2 treatment (Fig. 2C). These results indicated that NLRP3 expression was required for H_2O_2-induced IL-1 β secretion and cell death.

2.3. The inhibition of Cathepsin B prevented H_2O_2-induced IL-1 β release and cell death

Oxidative stress has been reported to up-regulate CTSB activity in the retinal pigment epithelium /choroid of the mouse. Therefore we examined whether H_2O_2-induced oxidative stress might regulate CTSB expression. Western blot analysis showed that H_2O_2 increased about 1.5 fold expression of CTSB compared to media control ($P < 0.05$, Fig. 3A) and CTSB activity assay also demonstrated that H_2O_2 elevated CTSB activity ($P < 0.01$, Fig. 3B). To further examine the involvement of CTSB, CTSB first was knockdown using shRNA. CTSB expression was reduced about 50% compared to scramble control ($P < 0.01$, Fig. 3C). Active caspase-1 activity assay showed that CTSB knockdown greatly impaired active caspase-1 activity induced by H_2O_2 (Fig. 3D), CA-074Me (50 μM, a CTSB inhibitor) was used a negative control. In addition, Western Blot analysis demonstrated that CTSB inhibition abrogated H2O2-induced NLRP3 activation and blocked the processing of pro-caspase-1 cleavage into caspase-1 (Fig. 3E). As expected, IL-1β secretion was abolished due to the lack of caspase-1 caused by CTSB silencing (Fig. 3E). Annexin V/PI staining showed that H_2O_2-indcued cell apoptosis was also impaired by CTSB inhibition (Fig. 3F). Taken together, these results suggest that CTSB is critical to H_2O_2-induced IL-1β secretion and caspase-1 -dependent cell apoptosis. CTSB knockdown impaired procaspase-1 cleavage and subsequent IL-1β induced by H_2O_2.

2.4. CTSB levels and oxidative stresses were elevated in plasma of AD patients

The information of AD patients with 61–89 years was listed in Table 1. The ages of healthy controls ranged from 60 to 85 years old. The blood samples involved 16 AD cases and 16 healthy controls. These cases were from Department of Neurology, Department of Psychiatry, and Department of Medical Examination in the third affiliated Hospital of Guizhou Medical University in China. Aβ42 was elevated in AD patients' plasma (Fig. 4A). MDA levels were significantly higher in plasma of AD patients than healthy controls (Fig. 4B), however, antioxidant GSH levels were not significantly lower in plasma of AD patients than in healthy controls (Fig. 4C). CTSB content assay showed that CTSB levels are elevated in plasma of AD patients compared to healthy controls (Fig. 4D). As expected, ELISA assay showed that IL-1β were elevated in plasma of AD patients compared to healthy controls (Fig. 4E).

2.5. CTSB levels are positively correlated with oxidative stresses in plasma of AD patients

Correlation analyses showed that CTSB levels are strongly positively correlated with Aβ42 (Fig. 5A), MDA (Fig. 5B) and IL-1β (Fig. 5D). IL-1β was weakly but still significantly positively correlated with Aβ42 (Fig. 5E) and MDA. (Fig. 5F). Both CTSB and IL-1β were negatively correlated antioxidant GSH (Fig. 5C, G). As expected, GSH levels were negatively with MDA levels (Fig. 5H). Taken together, these analyses indicated that CTSB levels are positively correlated with oxidative stress in plasma of AD patients.

3. Discussion

Oxidative stresses are elevated in neurodegenerative diseases and involved in activating NLRP3 inflammasome [38,39]. However, the molecular mechanism by which how oxidative stress remains exclusive. Our results demonstrate that H_2O_2 induced IL-1β release via CTSB/NLRP3 inflammasome signaling pathway in microglia. CTSB activity is required for IL-1β release by H_2O_2. Inhibition of CTSB impaired the expression of NLRP3 inflammasome and subsequent processing of procaspase-1 cleavage as well as cell apoptosis induced by H_2O_2 treatment. Importantly, these findings are relevant with our clinical studies. CTSB activity, oxidative stresses and IL-1β levels are highly