The Non-apoptotic Function of Caspase-8 in Neurodegenerative Diseases

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Abstract

With the development of society and the deepening of the aging degree, the problems of neurodegenerative diseases are increasing, which has aroused widespread concern in society. Neurodegenerative diseases are seriously related to people's quality of life. Microglia are innate immune effector cells in the central nervous system and play an important role in the physiological processes of the central nervous system. The activation of microglia and inflammation-mediated neurotoxicity play an important role in some neurodegenerative diseases. The form of programmed cell death is apoptosis, caspase-8 is the apical component of the cell death pathways, and activated caspase-8 can drive classical caspase-dependent apoptosis. Recently, caspase-8 has also been implicated in intracellular inflammatory pathways. In particular, caspase-8 can participate in the synthesis and processing of IL-1β through canonical and non-canonical pathways. Therefore, in this review, we will discuss the related knowledge of caspase-8, which is initially associated with apoptosis, in mediating neuroinflammation in neurodegenerative diseases.

Keywords

Apoptosis, caspase-8, neurodegenerative diseases

1. Introduction

The main feature of the organism is homeostasis, through the homeostasis control system can maintain the balance of tissue and cell level. Programmed cell death is a crucial intracellular mechanism for maintaining homeostasis in multicellular organisms, and is widely used to remove harmful cells. Apoptosis is generally considered as a programmed cell death machinery, which does not cause inflammation. However, it plays a crucial role in regulating the growth, development, and immune response of organisms and in eliminating redundant or abnormal cells. Apoptotic cell death has been divided into intrinsic and extrinsic pathways. The internal pathway of apoptosis is triggered by internal stimuli to destroy the mitochondrial membrane and the release of cytochrome C, the external pathway of apoptosis is triggered by the oligomerization of the transmembrane protein of the death receptor superfamily, both of which can activate effector cysteine aspartic proteases. Caspases are an evolutionarily conserved family of cysteine-dependent endoproteases, which is highly homologous with the cell death gene CED-3 of Caenorhabditis elegans and are involved in programmed cell death and inflammation. Structurally, all caspases contain a large subunit and a small subunit, forming a catalytic pocket responsible for the function of the enzyme. Traditionally, caspases have been thought to be associated with apoptosis, a homeostatic and non-lysed regulatory cell death model that clears old and injured cells. In recent years, the mechanism by which inflammatory caspases promote pyroptosis has been revealed, which is related to the secretion of the inflammatory cytokines interleukin (IL)-1β and IL-18. Based on the described functions and domain architecture, the members of the caspase family are classified as inflammatory or apoptotic, which consist of sequentially variable-sized amino-terminal domains.
Apoptotic caspases are functionally divided into promoters (caspases-2, -8, -9 and -10) and effectors (caspases-3, -6 and -7). Caspases-1, -4, -5, -11, and -12 are classified as inflammatory caspases and share a card domain at the N-terminal end. Among them, the human genome encodes caspase-1, caspase-4 and caspase-5, the mouse genome encodes caspase-1, caspase-11 and caspase-12, human caspase-4/5 and murine caspase-11 are homologues (Figure 1).

According to the World Population Aging Report issued by the United Nations, with the rising trend of population aging, it is estimated that the population over 60 years old will increase to 2.1 billion by 2050 [1]. A disease that cannot be ignored in an aging population is a neurodegenerative disease. Neurodegenerative diseases are characterized by the gradual loss of selectively vulnerable neuronal populations, which leads to motor and cognitive impairments. Neurodegenerative diseases are genetically susceptible, and environmental factors are also issues that affect the occurrence and development of the disease. Neurodegenerative diseases not only bring pains in life to individuals, but also bring huge economic burdens to families, and even affect the development of human society. Therefore, more and more researches focus on the mechanism research and treatment of neurodegenerative disease to improve people's living standards. So far, researchers have discovered many neurodegenerative diseases, but Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis have received more attention. In this review, the function of caspase-8 and its role in degenerative diseases of the nervous system are clarified to provide a theoretical basis for the study of this disease.

Figure 1. The functional classification of mammalian caspases. Caspase-1, -4, -5, -11 and -12 are inflammatory caspases. Apoptotic caspase is divided into initiator (caspase-2, -8, -9 and -10) and executioner (caspase-3, -6 and -7).

2. Caspase-8

Caspase-8 is essential for the activation of the death cell-induced external cell death pathway. Caspase-8 was first described in 1996 and is located on chromosome 1 in mice and chromosome 2 in humans. Studies have found that it is constitutively and widely expressed in most rodent and human cells. Under normal circumstances, caspase-8 exists as inactive procaspase-8, and catalytic activity can only be obtained under proper stimulation. Caspase-8 consists of two N-terminal death effector domains (DEDs), followed by large (p18) and small (p10) protease subunits at the C-terminal end, which play a central role in apoptosis. Both caspase-8 and the adaptor protein FAS-associated death domain (FADD) contain DEDs, which mediate DED-DED homotypic interactions and coordinate the complex formation of death receptors. Activation of caspase-8 requires cleavage between large and small catalytic subunits, particularly at aspartic acid residues D374 and D384. These cleavage events stabilize the caspase-8 protein, which can then be further cleaved between the prodomain and the large catalytic subunit at D216. A growing number of studies suggest that the function of caspase-8 is related to the degree of molecular cleavage. The importance of caspase-8 is underscored by the fact that the knockout mice died around day 10.5 of the embryo.

3. The Role of Caspase-8 in Apoptotic and Necroptotic Cell Death

Traditionally, caspase-8 has been linked to apoptosis and necrotic cell death. When activated, caspase-8 acts both as a pro-apoptotic and as an inhibitor of necroptosis (Figure 2). This article does not fully consider these features of caspase-8, but we have provided some comments for readers. When death receptors [CD95, TNF receptor I (TNFR-I) or DR5] are activated, caspase-8 is recruited to the receptors by FADD, which induces the formation of the membrane-associated death-inducing signaling complex (DISC). The caspase-8 homodimer formed in this
complex leads to activation and automatic cleavage, thereby further stabilizing the active dimer. Activated caspase-8 induces cell death by directly activating effector caspase (such as caspase-3 and caspase-7) or cleaving BH3-interacting domain death agonist (BID), leading to mitochondrial dysfunction and subsequent release of cytochrome C, activating caspases-9 and subsequently activating effector caspases to perform cell death [2]. It was found that the activation of caspase-8 can be regulated by two isoforms of caspase-8, cFLIP<sub>L</sub> (cellular FADD-like IL-1β-converting enzyme-like inhibitory protein long form) and cFLIP<sub>S</sub> (short form), cFLIP inhibits caspase-8 pro-apoptotic activity through the dimerization of its DED and caspase-8. Necroptosis is a newly discovered pattern of programmed cell death, morphologically characterized by cell swelling and plasma membrane rupture. When caspase-8 is inhibited, many death receptors traditionally associated with apoptosis can also trigger necroptosis. Necroptosis can occur through a variety of receptors, such as viruses, TNF-superfamily receptors, toll-like receptors, and interferon receptors. In addition, toxins, genotoxic stress, and some anti-cancer drugs have been shown to cause necroptosis. Among them, TNFR-mediated necroptosis is the most typical pathway of necroptosis. Both receptor-interacting serine/threonine kinases 1 (RIPK1) and RIPK3 contain small protein domains called receptor-interacting protein (RIP) homotypic interaction motifs (RHIMs), which are key regulators of TNF-induced necroptosis. TNF and its homologous receptor form a large membrane receptor signaling complex. TNFR-associated death domain (TRADD) and RIPK1 are recruited first, followed by the cellular inhibitor of apoptosis (cIAPs) and TNFR-associated factor 2/5 (TRAF2/5). RIPK1 is then ubiquitinated by cIAPs and TRAF2/5, which is the key to initiating cell survival pathways. However, when the activities of both cIAPs and caspase-8 are inhibited, and RIPK1 forms a different protein complex with RIPK3 through RHIM-RHIM interactions, the necroptotic death program will start. In addition, studies have shown that the RIPK1-caspase-8 signaling pathway is the key to cell survival or cell death.

Figure 2. Role for caspase-8 (CAPS8) in inducing apoptosis and inflammation. Activation of caspase-8 results in induction of apoptosis. The formation of cFLIP-caspase-8 heterodimers inhibits apoptosis. TRIF and MyD88 signaling results in downstream NF-κB signaling events that induce inflammatory responses.

4. The Role of Caspase-8 in Inflammation

The inflammasome is a multimeric protein complex containing a Nod-like receptor (NLR), an adaptor protein [apoptosis-associated speck-like protein containing a CARD (ASC)] and a protease (caspase-1). The inflammasomes found in the current study include NLRP1, NLRP3, NLRP6, NLRP12, NLRC4, AIM2 and so on. Among them, NLRP1, NLRP3, NLRC4 and AIM2 are the most in-depth studies. The activation of the inflammasome consists of two signals. The "priming signal" can up-regulate the cellular level of pro-IL-1β, pro-IL-18 and inflammasome components, and the "activation signal" can initiate the activation of the inflammasome and the processing and secretion of cytokines. Receptor activation causes ASC to self-assemble into nuclei, forming a cellular complex about one micron in diameter. This multiprotein complex is often called a "speck", and caspase-1 is subsequently activated. The activated caspase-1 cleaves pro-IL-1β and pro-IL-18 to form mature cytokines and then secrete them out of the cells. Studies have shown that in addition to caspase-1, caspase-8 can hydrolyze processing pro-IL-1β protein into their biologically active forms. This was first reported by Maelfait et al., who demonstrated that the inhibition or deletion of caspase-1 had no significant effect on the processing of pro-IL-1β. However, the presence of viral protein cytokine response modifier A (CrmA) or the use of a pan-caspase inhibitor has a significant inhibitory effect. The overexpression of caspase-8 results in the enhancement of pro-IL-1β processing, and the authors show that the cleavage site of caspase-8 to pro-IL-1β is the same as that of caspase-1. Therefore, more and more scholars are investing in the research of the inflammatory function of caspase-8. Studies show that caspase-8 is in-
involved in the processing of IL-1β in two different ways, one is to directly cut pro-IL-1β, and the other is to activate the inflammasome. Several studies have shown that caspase-8 is involved in the secretion and processing of bio-
logically active IL-1β, depending on the cell type. Gringhuis et al. demonstrated that activation of dectin-1 resulted
in the recruitment of proteins CARD9, B-cell lymphoma/leukemia 10 (Bcl-10) and mucosa-associated lymphoid
tissue lymphoma translocation protein 1 (MALT1) into the scaffold complex to induce pro-IL-1β expression, and
then recruit ASC in a caspase-8 dependent manner to form a non-canonical caspase-1 independent inflammasome
in which caspase-8 mediates the processing of pro-IL-1β. Promotes the processing and secretion of IL-1β by cas-
pase-8-dependent pathways under conditions that inhibit cIAP, histone deacetylase (HDAC) and endoplasmic reti-
culum stress. Similarly, FAS signaling activates caspase-8 in macrophages and DCs, leading to the maturation
of IL-1β. Caspase-8 can also process pro-IL-1β by activating inflammasomes, such as NLRP3, NLRC4, etc..TLR3-induced NLRP3 priming depends on the caspase-8 scaffold function, FADD and caspase-8 are necessary
mediators for the priming and activation of NLRP3 inflammasome. However, studies have shown that caspase-8
can also inhibit the activation of inflammasomes and the processing of IL-1β in some cases.

5. Caspase-8 in Neurodegenerative Diseases

Long-term persistent stimulation can cause chronic inflammation, which can lead to nerve tissue damage, neu-
rodegeneration, and induce neuroinflammation. Chronic inflammation with the involvement of multiple immune
cells, including macrophages and lymphocytes. Neuroinflammation is an inflammatory response of the nervous
system that is triggered by various pathogens or toxins that induce infiltration and activation of immune cells, ulti-

mately leading to neuronal and/or axonal degeneration or death [3]. Microglia, first described in 1919, are essential
for the normal functioning of the brain. Neuropathology studies have shown that activation of microglia, the resi-
dent's immune cells of the central nervous system, plays prominent role in the pathogenesis of neurodegenerative
diseases, such as Parkinson’s disease (PD), Multiple sclerosis (MS) and Alzheimer’s disease (AD). A large number
of evidence show that caspases, especially caspase-3/7 and caspase-8, are the key regulators of microglia activation,
which can release neurotoxic pro-inflammatory factors. Therefore, it has been proposed to identify potent caspase
inhibitors that may prove to be neuroprotective agents for the treatment of several neurological diseases. Among
them, it is determined that an effective caspase-8 inhibitor may protect neurons by selectively killing or blocking
the activity of activated microglia.

AD is the progressive cognitive decline and memory loss, which is associated with early synaptic dysfunction
and reduced spinal density in the AD brain. The amyloid hypothesis believes that the main cause of the disease is
increased extracellular deposition of misfolded amyloid-beta (Aβ) protein, but neuroinflammation is currently con-

sidered to be a key factor in actively promoting the pathological process of AD. Caspase may play an important role
in the production of neurotoxic Aβ peptides in AD, which not only contributes to the complex proteolytic
processing of Aβ precursor protein but also leads to the increase of Aβ levels during apoptosis. In addition to their
role in amyloid processing, caspase-8 and its downstream effector caspase-3 are also involved in learning, memory
and in the control of microglial pro-inflammatory activation and related neurotoxicity. There are many and complex
inflammatory mechanisms, but the activation of microglia plays a pivotal role in the initiation of neuroinflamma-
tion. The orderly activation of caspase-8 and caspase-3 regulates the activation of microglia through a protein ki-

nase C (PKC)-δ-dependent pathway. It was reported that caspase-8 mediates Aβ-induced neuronal apoptosis in vi-

vo.

Parkinson's disease is one of the most common neurodegenerative diseases among the elderly and is characte-

rized by multiple motor dysfunctions including uncontrollable shaking, rigidity, and disturbance of balance. The
occurrence of motor symptoms is mainly due to substantial dopamine depletion, caused by the degeneration of do-
paminergic neurons in substantia nigra pars compacta. The parkin gene encodes an intracellular parkin protein con-
taining an N-terminal ubiquitin-like domain and two C-terminal RING finger domains. According to reports, le-
sions in the parkin gene are a common cause of autosomal recessive juvenile Parkinson's disease. Caspase-1 and
-8 are effective inducers of parkin cleavage, therefore, parkin activity may be impaired in the neuropathological
states with activated caspase-1 or -8. There is increasing evidence that inflammation also plays an important role in Par-
kinson's disease, and epidemiological studies have shown that long-term use of anti-inflammatory drugs can treat
idiopathic Parkinson’s disease. The activation of caspase-8 and caspase-3/7 induced by neuromelanin suggests the
non-apoptotic roles of these killing caspases in PD. Cathepsin L (CTSL) is involved in microglial-mediated neu-
roinflammation, and CTSL levels in peripheral blood mononuclear cells of patients with Parkinson's disease (PD)
are increased. By blocking the caspase-8 or NF-kB pathway, it plays a protective role in the process of neuroin-
flammation with increased expression of CTSL.
Multiple sclerosis is the most common non-traumatic disabling neurological disease among young adults, which can cause inflammatory demyelination in the brain and spinal cord white matter and axonal degeneration. Common clinical symptoms include fatigue, cramps and vision problems. In primary progressive multiple sclerosis, the formation of ASC-NLRP3-caspase-8 inflammasome complex independent of caspase-1 drives inflammation in patients. Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model of MS at present, which can simulate MS from various aspects such as symptoms and pathological changes. In experimental autoimmune encephalomyelitis, a non-classical inflammasome can be formed, IRAKM-ASC-caspase-8-NLRP3, in which IRAKM as the bridge molecule between ASC and caspase-8, which is required for the activation of caspase-8 and the production of IL-1β in microglia.

6. Concluding Remarks and Future Perspectives

In the early 1990s, scientists first discovered cysteine aspartate when studying nematodes, and named the first protease to be found caspase-1. Caspase is widely expressed, except for caspase-1 rich in monocytes/macrophages and caspase-14 limited to keratinocytes. At present, more and more evidence shows that caspase-8 plays multiple biological functions in the process of immune regulation. Not only can it promote the process of immune response by inducing the expression of inflammatory genes [4], activating IL-1β by shearing, and inducing cell pyrolysis, but also inhibit the immune response of body through necrotizing apoptosis and inhibiting the activation of the inflammasome, etc. Traditionally, caspase-8 is considered to be the promoter of the death receptor-induced apoptotic pathway. In recent years, caspase-8 has also played a role in mediating non-apoptotic inflammatory and immune effects. Studies have shown that when caspase-8 participates in non-apoptotic pathways, the possible mechanism is that caspase-8 is subjected to limited catalytic action, and caspase-8 forms a heterodimer with cFLIP. However, the mechanism by which caspase-8 is recruited into the body is involved in the crosstalk between inflammation and apoptosis signal transduction pathways, and further research is needed.

Neurodegenerative diseases are the main cause of disability, which has a great impact on the economy of human society and is currently receiving more and more attention. Its pathological features are mainly degeneration, loss and abnormal protein aggregation of central neurons. In recent years, the role of caspase-8 in inflammation has attracted attention. To alleviate the pain of patients and the pressure of social and economic, the mechanism of caspase-8 in neurodegenerative diseases and the inhibitors of caspase-8 in the treatment of related disease mechanisms need further study.

References


