The role of matrix metalloproteinase-9 in pro-inflammatory factors-induced brain inflammation and neurodegenerative diseases

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Inflammation is a central pathogenic mechanism of various neuropathies including neurodegenerative diseases. In chronic neurodegenerative diseases such as Alzheimer's disease (AD) or Parkinson's disease (PD), the pathology is associated with an abnormal inflammatory response, characterized by the activation of several cell populations in the brain such as neuroglial cells. The relationships between inflammation and the development of these neuropathies involve complex molecular networks and processes. Recent evidence suggests that brain inflammation may impact on local inflammation in the brain diseases leading to over-production of several inflammatory mediators such as matrix metalloproteinases (MMPs), which may in turn influence functions including migration or apoptosis. Moreover, elevated levels of several pro-inflammatory factors including cytokines, peptides, pathogenic structures, per-oxidants in central nervous system (CNS) have been detected in the patients with brain disorders such as AD or PD. These pro-inflammatory factors exert as potent stimuli in brain inflammatory responses through up-regulation of diverse inflammatory mediators, in particular MMP-9. The expression of MMP-9 by these factors may be due to integration of the signaling networks that augment brain inflammation by recruiting immune cells and leading to neurodegenerative disorders. In this review, we discuss the mechanisms underlying the intracellular signaling pathways (e.g., calcium, protein kinase Cs, reactive oxygen species, or mitogen-activated protein kinases) involved in the expression of MMP-9 induced by pro-inflammatory factors in brain resident cells. Understanding of signaling transduction mechanisms involved in the expression of MMP-9 proteins and genes may provide helpful therapeutic managements for brain injury, inflammation, and neurodegenerative disorders.


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Introduction

In general, inflammation is a protective reaction to various cell and tissue injury. The purpose of this process is to remove the detrimental agents and injured tissues, thereby benefiting tissue repair. When this helpful reaction is uncontrolled, the effect initiates extravagant cell and tissue damage that results in normal tissue destruction and chronic inflammation[1-3]. Moreover, the
brain inflammatory diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD), are characterized by intracellular signaling state imbalance and chronic inflammation, a major cause of cell damage and death. Several of the well-known inflammatory mediators such as matrix metalloproteinase-9 (MMP-9) are associated with diverse signaling molecules activated by pro-inflammatory factors such as cytokines, peptides, pathogenic structures, and per-oxidants [4, 5]. These signaling molecules, including calcium, protein kinase Cs (PKCs), reactive oxygen species (ROS), or mitogen-activated protein kinases (MAPKs) are widely recognized as the key mediators of cell survival, proliferation, differentiation, and apoptosis [4, 5]. Excessive activation of various signaling molecules by pro-inflammatory factors is usually thought to be responsible for tissue injury associated with a range of brain injury, inflammation, and degenerative diseases such as AD[5]. Moreover, brain cells, neuronal cells especially, are susceptible to the injurious effects of various stresses. Several studies have shown that brain cells like microglia and astrocytes induce and release diverse inflammatory mediators in response to pro-inflammatory factors[5-7]. In central nervous systems (CNS), following the stimulation of pro-inflammatory factors, integration of various signaling molecules to trigger inflammatory responses through activation of different transcription factors, including nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) [5-7]. Therefore, this review will focus on many general aspects of pro-inflammatory signaling molecular regulation and summarize the current progresses regarding the occurrence and effects of these signals on CNS, and their involvement in the expression of inflammatory target protein MMP-9 in response to various pro-inflammatory factors during brain inflammation. Moreover, the pharmacological interventions which protect against MMP-9-mediated neuroinflammation and neurodegenerative diseases will be discussed.

Role of astrocytes in CNS physiological and pathological processes

The CNS consists of neurons and neuroglial cells. Among neuroglial cells in the adult human brain, astrocytes constitute approximately 40% of cell population, and maintain homeostasis in normal CNS. Astrocytes have also been indicated to contribute to several functions including guidance of the neuronal development and migration during CNS development, supporter of neuronal growth, preservation of the integrity of the blood-brain barrier (BBB), and playing a part in the immune responses to brain injury or disorders [6, 7]. Moreover, as well as microglia, astrocytes display various receptors participated in innate immunity, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domains, double-stranded RNA-dependent protein kinase, mannose receptor and components of the complement system [8]. One common feature of various neurodegenerative diseases is activation of large number of astrocytes and microglia that includes the morphological changes and expression of many inflammatory mediators. Astrogliosis is characterized by astrocytic proliferation, hypertrophy of the cell body, and functional changes, when exposed to various factors including interleukin-1β (IL-1β), tumor necrosis factor (TNF)-α, and lipopolysaccharide (LPS) [9, 10]. Increasing studies have indicated that the cell-cell interactions between glial cells and neurons may be important in the regulation of brain inflammation and neurodegeneration. Recent reports also implicate that inflammation contributes to a wide variety of brain pathologies, apparently killing of neurons via glia [6, 7]. Thus, the activated glial cells are indicated to play a critical role in the progression and pathogenesis of neurodegeneration. Previous most reports have shown that microglial cells may be a major inflammatory cell of the brain [11]. The activated microglia produce several inflammatory mediators including cytokines, ROS, or COX-2/prostaglandins (PGs) as well as neurotoxic materials, which are indicated to be responsible for brain injury and disorders including trauma, AD, and neural death due to the exposure of LPS, interferon-γ, or β-amyloid [12]. However, accumulating evidence has also demonstrated the characteristic changes of astrocytes in neurodegenerative diseases [13]. To date, we have demonstrated up-regulation of several inflammatory mediators including MMP-9 by various pro-inflammatory factors such as cytokines (e.g., IL-1β), peptides [e.g., bradykinin (BK) or endothelin-1 (ET-1)], pathogenic structures (e.g., bacteria or virus), and per-oxidants [e.g., oxidized low-density lipoprotein (oxLDL)] in rat brain astrocytes [14-28]. More recent data also indicated that multiple factors, including MMP-9 from BK-challenged brain astrocytes may contribute to the neuronal cell apoptosis [29]. These results implicate that activated neuroglial cells, astrocytes especially, play a critical role in the brain inflammatory response leading to neurodegenerative diseases (Figure 1).

Role of MMP-9 in brain inflammatory responses

MMPs, a large family of zinc-mediated metalloproteinases, exert to turnover of extracellular matrix- (ECM) and physiological and pathological
processes. To date, 24 MMPs have been identified in mammals. Among these MMPs, some are membrane-type MMPs which are anchored to the cell surface and others are secreted into the extracellular space. In general, MMPs are produced as inactive proMMPs and activated through proteolytic splitting of the N-terminal domain. Among gelatinase subfamily (i.e., MMP-2 and MMP-9), the catalytic domain that contains the Zn$^{2+}$ binding site and fibronectin motif repeats of allowing to bind gelatin (a major substrate of gelatinases). The gelatinase B (MMP-9; 92 kDa) is low and usually can be induced by various pro-inflammatory factors such as cytokines. The other type of gelatinase, gelatinase A (MMP-2; 72 kDa), is usually constitutive produced and not inducible. In CNS, MMP-9 is indicated in various physiological responses, such as morphogenesis, neurite outgrowth, and injured repairing. Moreover, up-regulation of MMP-9 may exert to the pathogenesis of many CNS disorders including stroke, AD, multiple sclerosis, and malignant glioma. Several pro-inflammatory factors such as cytokines, endotoxins, or oxidative stress have been shown to up-regulate MMP-9 in brain astrocytes in vitro, implying that MMP-9 activity may be up-regulated by various proinflammatory factors during CNS inflammation. Recently, increased MMP-9 expression and activation from BK-challenged brain astrocytes has been shown to contribute to neuronal cell death in vitro. These studies suggest that up-regulation of MMP-9 by pro-inflammatory factors may be a great effect upon brain injury, inflammation, and neurodegeneration. Therefore, the inhibition of MMP-9-mediated inflammatory pathways may provide therapeutic strategies to brain inflammation and neurodegenerative diseases.

**Effects of pro-inflammatory factors on MMP-9 expression in astrocytes**

The activated neuroglial cells (i.e., astrocytes and microglia) have been found in the senile and neuritic plaque of AD, which are accompanied by inflammatory responses. Moreover, several pro-inflammatory factors and inflammatory mediators produced by intrinsic (activated glial cells) and extrinsic means (infiltrating macrophages and other leukocytes) have the potential to lead or exacerbate the neuropathological events. The MMP-9 has been indicated to be up-regulated by various stimuli, including proinflammatory cytokines, peptides, pathogens, per-oxidants, and other stress in neuronal or neuroglial cells (Figure 2).

**Interleukin-1β (IL-1β)**

IL-1β is a common inflammatory cytokine significantly elevated in neurodegenerative diseases such as AD, which play a critical role in leading and regulating the cytokine cascades during inflammatory reactions. Moreover, IL-1β is a pleiotropic cytokine and classified as a major injured biomarker. Several studies have shown that the level of IL-1β is elevated in the cerebrospinal fluid (CSF) of patients with AD, traumatic brain injury, and stroke. Thus, IL-1β plays an important role in both acute and chronic neurodegenerative diseases. In brain, IL-1β has been shown to induce the expression of several inflammatory genes like MMP-9 which may raise BBB permeability, increase immune cells infiltrating through BBB, and then cause brain inflammation and edema during brain injury. Moreover, we have indicated that IL-1β induces MMP-9 expression through a MAPKs (i.e., ERK1/2, p38 MAPK, and JNK1/2)-dependent NF-κB pathways, c-Src-dependent transactivation of PDGF/PI3K/Akt, or Ca$^{2+}$-mediated CaMKII/JNK cascade in brain astrocytes. These studies indicate that IL-1β-up-regulated MMP-9 may contribute to the CNS inflammation and neurodegenerative diseases.

**Bradykinin (BK)**

In addition to cytokines, several peptides (e.g., BK, ET-1, or relative peptides) are synthesized and released during some injuries and CNS inflammation. Moreover, astrocytes possess receptors for numerous transmitters including BK. The elevated level of BK acts an important role in the initiation of inflammatory responses in target tissues, including CNS. B2-type BK receptor (B2-BKR) has been shown to be expressed on astrocytes and the receptor is found only on type-1 astrocytes. Activation of BK receptors, a G-protein-coupled receptor (GPCR), stimulates intracellular signaling molecules, including Ca$^{2+}$, PKCs, and MAPKs in several cell types including astrocytes. Activation of these signaling pathways may lead to cell survival, proliferation, differentiation, and the expression of several inflammatory genes such as MMP-9. In brain astrocytes, BK induces the expression of MMP-9 via B2-BKR-mediated various signaling pathways, including Ca$^{2+}$, PKCs, ROS, ERK1/2, PI3K/Akt, NF-κB, AP-1. Moreover, up-regulation and activation of MMP-9 and generation of ROS from BK-challenged brain astrocytes cause neuronal cell apoptosis. These literatures suggest that BK-derived MMP-9 expression may play an important role in brain inflammation and neurodegenerative disorders.

**Endothelin-1 (ET-1)**
The endothelins (ETs), the 21-amino acid vasoconstricting peptides, are produced primarily in the endothelium, which play an important role in vascular homeostasis and have been implicated in brain inflammatory diseases. In CNS, ET-1 also plays a key role in the normal development and CNS diseases. There are some potential sources of ET-1 release in response to hypoxic/ischemic injury of the brain, including endothelial cells and astrocytes. On astrocytes, the ET type B receptor (ETB) is predominantly expressed and modulate CNS post-injury responses of astrocytes. Accumulating evidence has further implied that overexpression of ET-1 has deleterious effects on astrocytes in ischemic brain. Similarly, ET-1 causes ETB/GFAP-immunoreactive astrocyte hypertrophy, a typical characteristic of astrogliosis, leading to glial scar formation following CNS injury. Endothelial ET-1 induces cytokine production such as IL-1β released by astrocytes, which directly leads to BBB breakdown during CNS inflammation. Our previous data also indicated that ET-1-induced MMP-9 expression and astrocytic migration is mediated through ETβ-mediated ERK1/2 linking to activation of NF-κB, Elk-1, and AP-1. Moreover, we found that ET-1 triggers astrocytic migration through the tyrosine nitration of MMP-9. These findings further imply the involvement of ET-1-induced MMP-9 expression in the CNS inflammation and diseases.

**Lipoteichoic acid (LTA)**

Bacterial infections may be involved in brain inflammation. The well-known endotoxin from Gram negative bacteria, lipopolysaccharide (LPS), regulates the expression of inflammatory proteins associated with inflammatory diseases. However, the signaling mechanisms of which activated brain cells in response to Gram-positive bacterial infection remain undefined. Brain infections by Gram-positive bacteria have occurred in bacterial meningitis and brain abscess. An amphiphilic polymer, lipoteichoic acid (LTA), is comprised in cell wall of Gram-positive bacteria. The *Streptococcus pneumoniae*, a Gram-positive bacterium, is the most common occasion of acute bacterial meningitis worldwide, implying a close relation between LTA action and brain disorders. For the initiation of LTA signaling, TLRs are thought to be responsible for LTA recognition challenged by Gram-positive bacteria like *Staphylococcus aureus* and *Streptococcus pneumoniae*. When activation of TLRs by LTA, LTA induces a serial activation such as members of IL-1 receptor associated kinase (IRAK) family and tumor necrosis factor receptor-associated factor 6 (TRAF6) by a TLR adaptor protein MyD88. Eventually, TLR signaling link to MAPKs and NF-κB, leading to regulation of several inflammatory target genes' expression such as cytokines. In CNS, astrocytes are regarded as targets in Gram-positive bacterial infection. Moreover, in CNS inflammation, TLR signals have been found to be involved in astrocytes, accompanied with up-regulation of MMP-9 with inflammatory and pro-apoptotic effects. However, the signaling mechanisms of LTA-induced brain cell responses through regulation of MMP-9 are not well characterized. Previous studies have indicated that LTA-up-regulated MMP-9 via TLR2/MyD88-mediated c-Src or Ca2+ related CaMKII-dependent transactivation of PDGFR/P3K/Akt linking to MAPKs, NF-κB, and AP-1 pathways in brain astrocytes. Recent report further demonstrated that up-regulation of MMP-9 by LTA is mediated via Nox2-derived ROS production in brain astrocytes. These data imply that targeting LTA, MMP-9, and their specific signaling molecules could yield helpful-therapeutic targets for brain inflammation or disorders upon Gram-positive bacterial infections.

**Oxidized low-density lipoprotein (oxLDL)**

Oxidative stress may cause production of several per-oxidants such as oxidized lipoprotein. Clinical studies exhibit that the AD patients reveal an increased oxidation of lipoproteins potentially toxic to neurons in CNS. Among these, the oxidized low-density lipoprotein (oxLDL) is the well-known predominantly risk factor of atherosclerosis, which may be involved in the development of the CNS disorders. In CNS, oxLDL reveals deleterious effects on several brain cell functions, such as apoptosis, capillary homeostasis, and inflammatory protein expression. Moreover, oxLDL occur in brain parenchyma and induces astrocytes to release IL-6 in patients with cerebral infarction, and might act a biomarker to reflect the level of oxidative stress. In brain astrocytes, oxLDL can induce MMP-9 expression and cell migration through P3K/Akt, MAPKs (e.g., ERK1/2 or JNK1/2), and transcription factors (e.g., Elk-1 and AP-1). Up-regulation of MMP-9 by LTA may play a critical role in the progression of CNS inflammatory diseases. These findings suggest that per-oxidants like oxLDL may play a key role in the CNS disorder progress, and also that targeting these per-oxidants-stimulated signals may provide a helpful therapeutic management for brain inflammation or neurodegenerative disorders.

**Others**
In addition to these well-known factors, there are many factors may also contribute to neuroinflammatory responses by regulation of MMP-9 expression. Among these, TGF-β has been implicated to participate in the responses. TGF-β may bind to its receptors, TGF-βRI or TGF-βRII, which are serine/threonine kinase receptors. When the TGF-β binding, TGF-βRII can phosphorylate TGF-βRI and stimulates Smad-mediated signals and then leads to expression of several target genes [60, 61]. In addition to the Smad-mediated signals, several Smad-independent signaling components like MAPKs also involved in TGF-β-induced responses [60, 61]. In brain astrocytes, we have demonstrated that TGF-β1 induces MMP-9 expression through ROS-mediated activation of MAPKs and NF-κB pathway [27]. The results indicate that TGF-β1 may play a key role in the process of brain inflammation and neurodegenerative diseases. Moreover, increasing evidence has shown that viral infections such as Japanese encephalitis virus (JEV) may contribute to several inflammatory responses in CNS [28]. Neurotropic viruses can induce extensive neuronal dysfunction and death that cause CNS diseases. JEV belongs to the family Flaviviridae, which is a positive-sense and single-stranded RNA virus. JEV is transmitted between animals and humans by culex mosquitoes [62]. After an infected mosquito bite, JEV amplifies peripherally causing transient viremia before entering into CNS [62]. The neurons and astrocytes are the major target cells for JEV in CNS [63]. Increasing reports indicate that JEV frequently brings severe encephalitis in Eastern and Southeastern Asia. The infection with JEV is characterized with clinical manifesting with fever, headache, vomiting, signs of meningeal irritation and altered consciousness leading to high mortality [62, 63]. In CNS, JEV infection has been demonstrated to up-regulate MMP-9 gene through ROS-dependent MAPKs/NF-κB or c-Src-dependent PDGFR/P13K/Akt/MAPKs/AP-1 pathways in brain astrocytes [28, 64]. These studies concerning JEV-induced expression of MMP-9 in brain astrocytes suggest that JEV may be critical for the brain inflammation and neuro-degenerative diseases.

Conclusions

Brain glial cells maintain CNS plasticity and protect the brain for functional repair from injuries. Reactivation of glial cells may promote neuroinflammation and neurodegeneration (Figure 1) and, ultimately, the retraction of neuronal synapses, which leads to cognitive deficits [6]. Moreover, up-regulation of MMP-9 is a deleterious event in several inflammatory diseases such as AD that precedes the formation of these disease pathologies. To date, although numerous effects have been made to develop therapies based on signaling molecules or target mediators in the past years, the actual benefits to the patients have been very limited. It may be due to lack of potency, late administration and poor penetration into the brain cells [65]. Alternative strategies including searching for pro-inflammatory factors that induce MMP-9 via diverse signaling pathways are necessary to improve the efficacy of treatment (Figure 2). Moreover, increased MMP-9 by various pro-inflammatory factors, such as cytokines, peptides, pathogenic structures, per-oxidants, and other stress, serve as intracellular signals in MMP-9 regulation and signaling transduction, in addition to their detrimental effects on cellular functions. Hence, understanding what pro-inflammatory factors participate in MMP-9 expression and the mechanisms of MMP-9 regulation that might help to develop effectively therapeutic strategies for CNS diseases. First, this review focuses on glial cells, in particular astrocytes, and their effects on the CNS disorders. Next, this review summarized the interplay between MMP-9 and neuroinflammation by action of various pro-inflammatory factors contributes to neurodegeneration, thereby enhancing disease progression based on data collected from brain cells, particularly astrocytes, in in vitro and in vivo studies (Figure 2). Perhaps by retarding the activation of glial cells to decrease their neurotoxic properties and enhance their neuroprotective effects may offer potential targets for therapeutic interventions in CNS degenerative disorders. Herein, pro-inflammatory factors-induced signaling transduction pathways, including Ca²⁺-related signals, PKCs, ROS, transactivation of EGFR or PDGFR, PI3K/Akt, MAPKs, NF-κB, and AP-1 that are associated with the CNS disorders were discussed (Figure 3). Moreover, the review highlighted current progress on the association of pro-inflammatory factors-derived signaling pathways with the MMP-9 expression that may contribute to the development of the CNS inflammation and neurodegenerative diseases. Possible therapeutic strategies to target signaling molecules, transcription factors, or inflammatory mediator MMP-9 are implicated based on the updated view of pro-inflammatory factors-mediated regulation of MMP-9 in brain inflammation and neurodegenerative disorders.

Conflicting interests

The authors have declared that no competing interests exist.

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