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Research Report

CHMP4B, ESCRT-III associating protein, associated with neuronal apoptosis following intracerebral hemorrhage

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ABSTRACT

Charged multivesicular body protein (CHMP) represents a family of small helical proteins that contain an N-terminal basically charged region and a smaller C-terminal acidic region, which are highly conserved in all eukaryotes. CHMP4B, a core component of the endosomal sorting complex required for transport (ESCRT)-III, is requisite for endosomal sorting and other biological processes. Here, we demonstrate that CHMP4B may be involved in neuronal apoptosis in the processes of intracerebral hemorrhage (ICH). From the results of Western blot, immunohistochemistry and immunofluorescence, we obtained a significant up-regulation of CHMP4B in neurons adjacent to the hematoma following ICH. Increasing CHMP4B level was found to be accompanied by the up-regulation of Fas receptor (Fas), Fas ligand (FasL), active caspase-8, and active caspase-3. Besides, CHMP4B co-localized well with Fas and active caspase-3 in neurons, indicating its potential role in neuronal apoptosis. What's more, our *in vitro* study, using CHMP4B RNA interference in PC12 cells, further confirmed that CHMP4B might exert its pro-apoptotic function on neuronal apoptosis through extrinsic pathway. Thus, CHMP4B may play a role in promoting the brain damage following ICH.

1. Introduction

Intracerebral hemorrhage (ICH) is a devastating stroke subtype resulting from bleeding within the brain parenchyma (Andrews et al., 2012). It has an incidence of 10–30 cases per 100,000 people/year, which is increasing and expected to double by the year 2050 (Qureshi et al., 2001). Evidences during the past decades demonstrate that the fatality rate

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of ICH at 1 month is approximately 40%, which likely results from a lack of effective therapeutic schedule after ICH (Brouwers and Greenberg, 2013; Keep et al., 2012). Though multiple resources have been invested into clinical and basic researches, this high rate of mortality seems still not changed (Kuramatsu et al., 2013). Rupture of blood vessels within the brain parenchyma lead to primary and secondary injuries. The primary damage is induced by the dynamic of hematoma expansion associated with mass effect; the secondary damage comprised of many parallel pathways including cytoxicity of blood, inflammation, oxidative stress and so on together lead to irreversible injury, eventually cause the disability or even the death (Aronowski and Zhao, 2011; Zhou et al., 2014). Pathologic changes of ICH include neuronal apoptosis, astrocyte proliferation and oligodendrocyte death, which all implicated in the above processes (Bradl and Lassmann, 2010; Gong, 2001). Among them, neuronal death is regarded as one of the most crucial events which depend on complex pro-apoptotic and subtle anti-apoptotic modulation (L. Li et al., 2013). We here have only a sketchy of understanding about the underlying molecular and cellular mechanisms of ICH, lots of studies remains to be done.

Endosomal sorting complex required for transport (ESCRTs) and their associated proteins (called CHMPs) are conserved from yeast to humans, and function in the sorting of ubiquitinated cargoes into intraluminal vesicles, which are generated by inward budding of the endosomal membrane (also called multivesicular bodies (MVBs), or endosomes) (Horii et al., 2006; Katoh et al., 2003). On the way toward degradation, ubiquitinated cargoes trigger the sequential formation of protein hetero-oligomeric complexes called ESCRT I, II, and III, which then help sorting their incorporation into vesicles budding inside MVBs (Katoh et al., 2003). During the processes, charged multivesicular body protein CHMP4B is a core subunit of ESCRT-III, and facilitates the biogenesis of MVBs and implicates in playing roles in multivesicular body sorting (Hurley and Emr, 2006). Thereafter, the vesicles and their cargoes will be hydrolyzed after the MVB fusion with the lysosome (Horii et al., 2006; Hurley and Emr, 2006; Katoh et al., 2003).

In majority conditions, CHMP4B is part of the ESCRT-III, and functions in the sorting of endocytosed cell-surface receptors into MVBs, in the final abscission stage of cytokinesis, or in the budding of enveloped viruses such as HIV-1 (Hurley and Hanson, 2010; Raiborg and Stenmark, 2009). However, in pathological conditions, CHMP4B, ESCRT-III associating protein, interact with programmed cell death 6 interacting protein (PDCD6IP), also known as ALG-2 (apoptosis-linked gene 2)-interacting protein X (ALIX), an adaptor protein involved in the regulation of the endolysosomal system, might participate in involvement with autophagy-associated degenerative diseases or cell apoptosis (Mahul-Mellier et al., 2006, 2009, 2008; Sagona et al., 2014). For example, Sagona et al. (2014) indicated that CHMP4B was a novel common component of chromosome bridges and micronuclei, participating in the autophagolysosomal degradation of micronuclei during lens cell differentiation and cataract formation (Sagona et al., 2014). Mahul-Mellier et al. (2009) demonstrated that overexpression of ALIX is sufficient to induce cell death of motoneurons, whereas it prevented early programmed cell death when mutated the binding sites of CHMP4B (Mahul-Mellier et al., 2009). And the study by Mahul-Mellier et al. (2008) uncovered that ALIX and ALG-2 regulate neuronal death in ways involving interactions with proteins of ESCRT *via* forming a complex with apical caspases and with the endocytosed death receptor TNFR1 (tumor necrosis factor α receptor 1) in the presence of calcium (Mahul-Mellier et al., 2008). Additionally, there is also evidence indicated that dysfunction of ESCRT-III was associating with frontotemporal dementia linked to chromosome 3 (FTD3), which caused autophagosome accumulation and dendritic retraction before neurodegeneration in cultured mature cortical neurons, whereas its potential relationship with CHMP4B is not clear (Lee and Gao, 2009). As CHMP4B involves in multiple cellular activities, whether it takes part in the pathophysiologic processes following ICH remains to be investigated.

To date, we know little about CHMP4B, especially involving in neuronal death in the central nervous system (CNS). Since CHMP4B is indispensable for participating in cellular death, we hypothesize that CHMP4B may contribute to brain injury following ICH, and associate with neuronal apoptosis *via* caspase-dependent or -independent manner. In the present study, we for the first time investigated the expression and distribution of CHMP4B in rat basal ganglia adjacent to the hematoma after ICH. This study was performed to gain an insight into the function of CHMP4B in the adult CNS and might make a foundation for further research and be applied to clinical treatment for its role in injury and repair after ICH.

2. Results

2.1. *Expression variations of CHMP4B following ICH by western blot analysis*

Rats in the sham and ICH groups were subjected to forelimb placing and corner turn test at different survival times, respectively. Rats of ICH with significant neurological function deficits were chosen for subsequent experiments (Fig. 1A, B). Western blot was performed to investigate the temporal levels of CHMP4B in rat basal ganglia adjacent to the hematoma at various time points after ICH. CHMP4B protein level was low in the sham group, then progressively increased from 6 h after ICH and peaked at day 2 (Fig. 1C, D) ($^{*}p<0.05$). The data indicate that CHMP4B protein profiles had a temporal change after ICH.

2.2. *Expression and distribution of CHMP4B immunoreactivity*

To identify the variation and distribution of CHMP4B after ICH, immunohistochemistry was applied to detect CHMP4B at 2 days following ICH. The sham-operated group showed low CHMP4B-positive staining (Fig. 2A, B), which consisted the result from Western blot. By contrast, the number of CHMP4B in the ipsilateral group was upregulated adjacent to the hematoma (Fig. 2C, D). However, there is no significant change of CHMP4B staining in the contralateral group compared with the sham group (Fig. 2E, F). Additionally, no positive signal was observed in the negative control (Fig. 2G). The expression of CHMP4B was