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BURN INJURY INDUCES GELSOLIN EXPRESSION AND CLEAVAGE IN THE BRAIN OF MICE ☆

QING-HONG ZHANG,[a] JUN-CONG LI,[b] NING DONG,[a] LU-MING TANG,[c] XIAO-MEI ZHU,[a] ZHI-YONG SHENG[a] AND YONG-MING YAO[a,d]*

[a] *Department of Microbiology and Immunology, Burns Institute, First Hospital Affiliated to the Chinese PLA General Hospital, Beijing 100048, PR China*

[b] *Department of Emergency, First Hospital Affiliated to Wenzhou Medical College, Wenzhou 325000, PR China*

[c] *Department of Emergency, Second Hospital Affiliated to Wenzhou Medical College, Wenzhou 325000, PR China*

[d] *State Key Laboratory of Kidney Disease, The Chinese PLA General Hospital, Beijing 100853, PR China*

Abstract—Gelsolin is an actin filament-severing and capping protein, affecting cellular motility, adhesiveness and apoptosis. Whether it is expressed in the brain of burned mice has not yet been characterized. Mice were subjected to a 15% total body surface area scald injury. Neuropathology was examined by hematoxylin and eosin staining. Cerebral gelsolin mRNA, distribution and cleavage were demonstrated by quantitative polymerase chain reaction (QPCR), immunohistochemistry and Western blot, respectively. Cysteinyl aspartate-specific protease (caspase)-3-positive cells and activity were also measured. Burn injury could induce pathological alterations in the brain including leukocyte infiltration, necrosis, microabscess and gliosis. Compared with sham-injured mice, gelsolin mRNA was up-regulated at 8 h post-burn (pb) in a transient manner in the cortex and striatum of burned mice, while it remained at higher levels in the hippocampus up to 72 h pb. Of interest, gelsolin was further cleaved into 42 and 48 kDa (kilo Dalton) fragments as illustrated in the hippocampus at 24 h pb, and was widely expressed in the brain by activated monocyte/macrophages, astrocytes and damaged neurons. In the meantime, caspase-3-positive cells were noted in the striatum of burned mice and its activity peaked at 24 h pb. To clarify inflammation-induced gelsolin expression and cleavage in the brain, rat pheochromocytoma cells were exposed to lipopolysaccharide to show increased gelsolin expression and caspase-3-dependent cleavage. The results suggest that burn-induced cerebral gelsolin expression would be involved in the activation of both the monocytes and astroglial cells, thereby playing a crucial role in the subsequent inflammation-induced neural apoptosis following burn injury. **© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: burns, gelsolin, septic encephalopathy, lipopolysaccharide, caspase-3, apoptosis.

INTRODUCTION

Septic encephalopathy (SE) (Antequera et al., 2009) is a common feature in sepsis, occurring in ~25% of the patients often before the failure of other key organs. SE can also develop as a result of a number of systemic inflammatory response syndromes that lack an infectious etiology (e.g. acute pancreatitis, extensive burns, etc.). Patients with SE have a higher mortality rate compared with those without brain involvement. The pathogenesis of SE following burn injury is multifactorial and includes the circulatory and metabolic derangements (Semmler et al., 2008), cerebral autoregulation disturbance, and the blood–brain barrier damage (Flierl et al., 2010). The direct effect of the inflammatory process on glial cells and the resultant neuronal damage may also play a decisive role (Bozza et al., 2010; van den Boogaard et al., 2010).

Gelsolin is a highly conserved 89 kDa (kilo Dalton) actin-binding protein present in all cell types from the nervous system, including neurons (Kronenberg et al., 2010), myelin-forming cells (Tanaka and Sobue, 1994), neuronal growth cones (Tanaka et al., 1993) and choroid plexus (Matsumoto et al., 2003). Gelsolin has been reported to affect cellular configuration, differentiation, motility, adhesiveness, invasiveness and in particular, apoptosis (Kothakota et al., 1997). Divergent effect of gelsolin has been reported for apoptosis control in different *in vitro* models, due to its interaction with the regulatory pathway of apoptosis at different sites.

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*Correspondence to: Y.-M. Yao, Burns Institute, First Hospital Affiliated to the Chinese PLA General Hospital, 51 Fucheng Road, Haidian District, Beijing 100048, PR China. Tel: +86-1066867394; fax: +86-1068989955.

E-mail address: c_ff@sina.com (YONG-MING YAO).

Abbreviations: Abs, antibodies; AVPe, anteroventral periventricular nucleus; BCA, bicinchoninic acid; BSA, bovine serum albumin; BST, bed nucleus of striatum terminal; CA_2, CA field of the hippocampus; caspase-3, cysteinyl aspartate-specific protease-3; DAPI, 4′,6-diamidino-2-phenylindole; EDTA, ethylenediaminetetraacetic acid; GFAP, glial fibrillary acidic protein; H&E, hematoxylin and eosin; H_2O_2, hydrogen peroxide; HDB, horizontal limb diagonal band; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Iba-1, ionized calcium binding adaptor protein 1; kDa, kilo Dalton; LHb/MHb, lateral/medial habenular nucleus; LPMR, lateral posterior nucleus; LPS, lipopolysaccharide; NeuN, neuronal nuclei; pb, post burn; PBS, phosphate buffer saline; PC12, pheochromocytoma cells; QPCR, quantitative polymerase chain reaction; SE, septic encephalopathy; SFO, subfornical organ; TBSA, total body surface area; VDB, vertical limb diagonal band; VOLT, vascular organ of the lamina terminalis.

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Gelsolin was first suggested to have the pro-apoptotic functions as a major substrate of cysteinyl aspartate-specific protease-3 (caspase-3) and was shown to degrade actin filaments in a rapid, calcium-independent fashion and resulted in a 48 kDa carboxyl-terminal fragment (Kothakota et al., 1997). On the other hand, several studies have demonstrated that cytoplasmic gelsolin can inhibit apoptosis by diminishing apoptotic mitochondrial changes *via* membrane potential loss and cytochrome c release (Koya et al., 2000; Qiao et al., 2005). Under stress conditions such as homeostasis, inflammation and wound healing (Witke et al., 1995), gelsolin was required for rapid mobile response of inflammatory cells and fibroblast. In fact, overexpression of cytoplasmic gelsolin in brain parenchyma resulted in a decrease of nitric oxide production (Antequera et al., 2009) and a reduction of the ischemic brain injury (Yildirim et al., 2008), whereas gelsolin-null mice manifested a major increase in infarct size in brain ischemia compared with controls (Endres et al., 1999).

Recently, gelsolin has been demonstrated to be involved in both the peripheral and central immune responses. Increased cytoplasmic gelsolin expression was detected in lung samples of patients with idiopathic interstitial pneumonia as well as in the modeled pulmonary inflammation and fibrosis (Oikonomou et al., 2009). In contrast, plasma gelsolin was significantly decreased in the mesenteric lymph following hemorrhagic shock (Jordan et al., 2007) and sepsis (Lee et al., 2008). It was noteworthy that gelsolin level was elevated in various neurodegenerative diseases, such as Down's syndrome (Ji et al., 2009a), whereas it was decreased in the blood and cerebrospinal fluid of multiple sclerosis subjects, strongly suggesting its role in the development of neurodegeneration associated with chronic inflammation (Kulakowska et al., 2010).

Although gelsolin has been identified and biochemically characterized in the brain, the role of gelsolin in neuroinflammation and the consequent neurodegeneration, particularly in the setting of burn injury, has never been explored. Here we examine the tempospatial expression, distribution and cellular localization of gelsolin in the brain of mice under thermal injury. To confirm the hypothesis that neuroinflammation following burn injury could induce gelsolin expression and cleavage, lipopolysaccharide (LPS)-induced gelsolin expression and cleavage were determined in rat pheochromocytoma cells (PC12) *in vitro*, verifying the key role of gelsolin in burn-induced SE.

2. EXPERIMENTAL PROCEDURES

2.1. Animal model

Adult male Balb/c mice (body weight 25–30 g, 2–3-months-old) were obtained from the Laboratory Animal Institute, Beijing, China, and housed in an animal care facility. Commercial rat chow and tap water were available *ad libitum*, and mice were maintained in a constant temperature environment with a 12-h (hours) light/dark cycle. All experimental manipulations were undertaken in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of the Chinese PLA General Hospital, Beijing, China.

The mice were randomly chosen as intact control (n = 8), sham injury (n = 32), and burn injury (n = 40) groups. Then they were anesthetized, and the dorsal and lateral surfaces were shaved. On the following day, the mice were placed on their backs and secured in a protective template with an opening corresponding to 15% of the total body surface area (TBSA), and the exposed skin was immersed in 95 °C water for 8 s (s). This procedure has been shown to produce a 15% TBSA full-thickness scald injury (Zhang et al., 2010). Sham-injured mice were subjected to all the above procedures except that the temperature of the bath was at room temperature. After immersion, the mice were immediately dried, followed by standard fluid, with the calculated volume being given immediately after thermal injury and returned to normal activity including food and water consumption.

2.2. Tissue preparation and morphological examination

Animals were terminally anesthetized with an overdose of aether. Sham and burn injury mice were sacrificed at 8, 24, 48, 72 h respectively (8–10 per time point). Brains (4–5 mice per group) were removed and were post-fixed for 24 h in 4% paraformaldehyde solution and were equilibrated in 30% sucrose in phosphate-buffered saline (PBS), sectioned at 30 μm on a freezing microtome, serially collected in PBS and finally stored in cryoprotectant solution under −30 °C. Some of the brain sections were mounted on the lysine-coated slides and underwent hematoxylin and eosin (H&E) staining. Pictures were undertaken under microscope (Olympus Optical Co., Tokyo, Japan). The rest of the brains (5–6 mice per group) were isolated by different regions and snap frozen in liquid nitrogen.

2.3. Quantitative real-time polymerase chain reaction (QPCR) for gelsolin mRNA expression

Brain of different regions (cortex, hippocampus and striatum) from the same tissues as the above (5–6 mice/time point) were used for total RNA extraction with the NucleoSpin® RNA II Kit (Macherey–Nagel INC., PA, USA) following the manufacturer's instructions, and used for cDNA synthesis with Superscript II (Promega, Beijing, China). Real-time PCR amplification was achieved in 25 μl reaction mixture containing 5 μl of cDNA sample, 12.5 μl of SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and specific primers. An ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA) with SYBR-green fluorescence was used for assay. Cycling conditions were 10 min hot start at 95 °C followed by 40 cycles of denaturation step at 95 °C for 40 s, an annealing step at 60 °C for 30 s, and an extension temperature at 72 °C for 30 s. Each sample was run in triplicate. β-Actin was used as housekeeping mRNA to normalize gelsolin transcripts abundance. Data were analyzed by using sequence Detector Systems version 2.0 software (Applied Biosystems). Gelsolin (NM_146120.3) reverse and forward primers were 5′-TTCCTTCGTGGGCTGGTT-3′, and 5′-TGATGGCTTTGGT CCTTACT-3′, respectively; β-actin reverse and forward primers were 5′-TTCCATCATGAAG TGTGACGTT-3′, 5′-CTCAGGAGGAGCAAT GATCTTG-3′, respectively. The relative concentration of mRNA was calculated using the formula $x = 2^{-\Delta\Delta Ct}$, where x fold change in the target gene at each detection time, normalized to β-actin and relative to the expression of intact brain tissue (Livak and Schmittgen, 2001) .

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