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Galectin-3: mediator of microglia responses in injured brain

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Galectin-3 is a pleiotropic protein involved in cell activation, proliferation and migration and plays a pivotal part as an inflammatory mediator in neurodegeneration. Galectin-3 is associated with microglial activation and proliferation after ischemia. Given its putative role as a dynamic fine-tuner of microglia, activation of Galectin-3 provides molecular cues in design of new immunomodulatory strategies for stroke management. This review summarizes recent evidence on the role of Galectin-3 as a mediator of immune responses in damaged brain and mechanisms employed by Galectin-3 to affect microglial function.

Introduction

Stroke is a leading cause of death and disability worldwide. Development of an effective therapeutic strategy for stroke has been a priority for decades. Unfortunately, to date, the clinical treatments remain poorly effective. A significant pitfall in designing effective therapeutics lies with the fact that the major post-stroke events, such as inflammation, can act as a double-edged sword in the complex pathological circumstances [1]. Galectins are a 15-member family of evolutionary conserved glycan-binding proteins [2,3]; and mounting evidence suggests that galectins can act as endogenous modulators of the inflammatory response and potentially neurodegeneration [4–8]. Among them, Galectin-3 (Gal-3) is involved in cell activation, proliferation, migration and apoptosis and has been associated with immune response and inflammation.

Peripherally, Gal-3 can play a proinflammatory part by inhibiting apoptosis in inflammatory cells [9,10]. Additionally, evidence suggests that Gal-3 is instrumental for interleukin (IL)-4-mediated macrophage alternative polarization [11]. Its expression increases following acute central nervous system (CNS) injuries and contributes to insults to the brain gray matter [12]. In adult brain, Gal-3 is deemed crucial for resident microglia activation and proliferation in response to ischemic insult and potentiates the effects of trophic factors such as insulin-like growth factor (IGF)-1 [13]. In experimental stroke, Gal-3 has been implicated in tissue remodeling and neurogenesis, although the outcome of the latter is not clear in post-stroke recovery [14]. Other reports also indicate a potentially deleterious role for Gal-3 after brain injury and suggest that its role might be context dependent. Notably, in a model of global ischemia, Gal-3-dependent Toll-like receptor (TLR)4 activation was shown to induce sustained microglia activation, prolonging the inflammatory events in the brain [15]. Taken together, these findings underline the instrumental yet complex role of Gal-3 as an endogenous immunomodulator. The present review deals with Gal-3 functions on microglia biology post stroke. To this end, potential interplay of Gal-3 with various pathways and/or molecules affecting microglia activation after stroke will be discussed. The involvement of Gal-3 in post-stroke neurogenesis will be explained. Finally, special attention will be given to advancements made in designing Gal-3 modulators that might open a new avenue for accelerating post-ischemia recovery.

Gal-3 function and mechanisms of release

Gal-3, a unique chimera-type member of the galectin family, is highly expressed in different cell types. Gal-3 has three structural domains: (i) an N-terminal domain that contains a serine phosphorylation site, important for the regulation of intracellular signaling, the N-terminal is also involved in the formation of the pentamer and two important characteristics of this portion of the Gal-3 molecule include antiapoptotic activity and regulation of Gal-3 liberation

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[16,17]; (ii) a collagen-like sequence, sensitive to the matrix metalloproteinase family of proteins; and (iii) a C-terminal domain containing a single carbohydrate-recognition domain that interacts with glycosylated growth factor receptors and potentiates their signaling [9,18,19]. Gal-3 has been found in different compartments including the extracellular space, cytoplasm and nucleus [9]. Importantly, in each of these compartments it possesses distinct physiological actions. Namely, the extracellular surface-bound Gal-3 can alter the dynamics of various membrane receptors, thereby increasing the retention time of those receptors at the cell surface [19,20]. The work by Partridge *et al.* indicated that the interaction of Gal-3 with N-linked glycans attached to various growth factor (GF) receptors (GFRs), including insulin-like GF1 receptor (IGFR1), enhances GF-induced signal transduction and cellular growth. This mechanism has been proposed for the fast growth rate observed in cancerous cells and is dependent on the enzyme β1,6-*N*-acetylglucosaminyltransferase V (Mgat5). Mgat5 is upregulated in cancers and promotes substitution of *N*-glycan with poly-*N*-acetyllactosamine, the preferred ligand for Gal-3 [19]. Our *in vitro* results in primary microglia culture demonstrate that extracellular Gal-3, specifically its carbohydrate-binding site, is responsible for increased microglia ramifications and Gal-3 interaction with glycosylated GF and cytokine receptors such as IGF-1. In fact, treatment of primary microglia cultures derived from Gal-3 knockout (KO) with exogenous Gal-3 was associated with a marked increase in the levels of ramification (Fig. 1). As further shown in Fig. 1, administration of thiodigalactoside (chemical antagonist of Gal-3 that binds to the carbohydrate-binding site of galectins) abolished the Gal-3-mediated effects on microglia, thus suggesting an important role of the secreted extracellular Gal-3 on microglia morphology. Cytoplasmic Gal-3 induces proliferation, differentiation and survival, through K-Ras-mediated signaling [21]. In the nucleus, Gal-3 has been implicated in the activation of transcription factors such as cAMP response element binding protein (CREB) [22]. Furthermore, β-catenin, a canonical molecule involved in the Wnt signaling pathway, was identified as a binding partner of Gal-3 in the nucleus [9,23]. Gal-3 does not utilize the classical endoplasmic reticulum (ER)/Golgi secretion pathway [24]. Evidence suggests an alternative secretory pathway including specific vesicles and/or exosomes for Gal-3 secretion and export [2,25,26]. In both cases, once exported and released, Gal-3 can interact with several extracellular receptors. Alternatively, vesicles could directly fuse with other cells, resulting in Gal-3 uptake by the recipient cells [9]. Exosomal secretion of Gal-3 from microglia could have important roles in intercellular communication after brain ischemic insult. Interestingly, a large body of evidence suggests that exosomes mediate cell–cell crosstalk by transferring exosomal protein and RNA packages between microglia and other brain cells in neurodegenerative conditions like stroke. A modulation of Gal-3 secretion by exosomes could provide an interesting concept and potential therapeutic avenue because recent evidence shows that naturally occurring or engineered exosomes derived from the stem cells, or potentially other cell types, provide therapeutic options in stroke [27].

Protective effects of Gal-3 in neuroinflammatory context after stroke

In pathophysiological conditions microglia exert distinct context- and stimuli-dependent polarization profiles. Here, we try to decipher molecular mechanisms underlying protective effects of Gal-3 within the complex microglia response mechanisms after stroke. As we discuss in detail, the interplay between the Gal-3C terminus with many GFRs and pattern recognition receptors is a pivotal step for Gal-3-induced microglia alternative activation, proliferation and regulation of cytokine production.

The majority of Gal-3 functions have been identified in the peripheral immune system. Originally identified as a marker of inflammatory macrophages, Gal-3 is reported to participate in the alternative activation of peripheral macrophages and its biological role in the brain is less well understood [2,11]. Microglia activation and proliferation are a hallmark of many types of brain injuries including stroke. The consensus today is that, once activated, microglia or macrophages can acquire a wide repertoire of immune profiles ranging from classical proinflammatory to alternative anti-inflammatory and neuroprotective phenotypes [28–30]. However, underlying mechanisms that drive the development of distinct microglial immune profiles after brain injuries remain unclear. In a search for novel immunomodulatory molecules in stroke, we investigated several molecular pathways involved in the control of the post-ischemic inflammatory cascades. One molecule that caught our attention was Gal-3. Although abundant in activated microglia [31], Gal-3 is not expressed by resting cells. Following ischemic injury there is a robust upregulation of Gal-3 in a subpopulation of activated resident microglia, suggesting its role in microglia activation in brain injury [13,31,32]. In fact, by selective ablation of proliferating microglia in mice, we showed that Gal-3 is preferentially expressed by a subset of proliferating IGF-1-expressing microglia, suggesting its role in proliferation [31]. This notion was further supported by a marked 50% reduction in the number of proliferating microglia in ischemic brains of Gal-3 KO mice [13]. Moreover, we showed that the presence of Gal-3 is instrumental for an early induction of innate immune response and TLR2 signaling post injury [13]. These findings indicate that, by orchestrating microglial activation and proliferation early after stroke, Gal-3 plays a major immunomodulatory part in the brain response to injuries. The molecular mechanism will be discussed below.

The role of Gal-3 in counteracting neuroinflammation has been demonstrated in different contexts. Gal-3 is an advanced glycation end-product (AGE) receptor (RAGE) that targets AGE for lysosomal degradation and removal (Fig. 2) [33]. Because AGE is a source of inflammation and oxidative injury in amyotrophic lateral sclerosis (ALS), deletion of Gal-3 can enhance neurodegeneration owing to AGE accumulation [34]. Gal-3 has been proposed as a negative regulator of lipopolysaccharide (LPS)-induced inflammation because macrophage-derived Gal-3 directly binds LPS, thereby inhibiting its interaction with TLRs (Fig. 2) [35]. Gal-3-deficient macrophages showed more-pronounced LPS-induced signaling and inflammatory cytokine production whereas blockade of Gal-3 binding using a neutralizing antibody augmented LPS-induced inflammatory cytokine expression by wild-type macrophages. *In vivo*, Gal-3 KO mice were more susceptible to LPS shock associated with excessive production of inflammatory cytokines and nitric oxide [35]. In the context of peripheral nerve injury, Gal-3 deficiency was characterized by significantly higher levels of the proinflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α, as well as TLR2 and TLR4 [36]. The

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