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Review article

Lipocalin-2 as a therapeutic target for brain injury: An astrocentric perspective

Kyoungho Suk, Ph.D.

Department of Pharmacology, Brain Science & Engineering Institute, BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, 680 Gukchaebosang Street, Joong-gu, Daegu, Republic of Korea

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ABSTRACT

Lipocalin-2 (LCN2) is a member of the secreted lipocalin protein family. LCN2 is also a representative gliocalin that is primarily released by glial cells, as well as acts upon them. Astrocytes are one of the major cellular sources of LCN2 under brain injury conditions. Astrocytes secrete LCN2 to promote neuroinflammation. Studies using *Lcn2* knockout animals and cultured neural cells suggest an important role of LCN2 in regulating the development of hemorrhagic and ischemic stroke as well as other brain injuries. The clinical relevance of LCN2 is supported by studies on patients with stroke. Mechanistic studies have revealed that LCN2 is a molecular switch for determining the phenotypic fate of astrocytes under inflammatory conditions. LCN2 gene expression is regulated at the multiple levels; mostly at the transcription level, post-transcription level by microRNAs, and protein level by minor post-translational modification. Recent advances in LCN2 research strongly indicate that astrocytic LCN2 is a promising drug target for the injured brain. Future research should focus on its translational aspects, such as developing small-molecule inhibitors or neutralizing antibodies to target LCN2 for the treatment of brain injury. However, spatiotemporally complex roles of LCN2, which are either beneficial or deleterious, should be considered when targeting LCN2. The potential use of LCN2 as a biomarker for the diagnosis and prognosis of various brain disorders is also discussed.

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Abbreviation: LCN2, lipocalin-2; L-PGDS, lipocalin-type prostaglandin D synthase; ORM, orosomucoid; NGAL, neutrophil gelatinase-associated lipocalin; CNS, central nervous system; CSF, cerebrospinal fluid; MARCKS, myristoylated alanine-rich C-kinase substrate; LPS, lipopolysaccharide; BBB, blood-brain barrier; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent MCAO; BCCAO, bilateral common carotid artery occlusion; GFAP, glial fibrillary acid protein; BDNF, brain-derived neurotrophic factor; CM, conditioned media; PSD95, post-synaptic density 95; SAH, subarachnoid hemorrhage; ICH, intracerebral hemorrhage; MMP-9, matrix metalloproteinase 9; CXCL10, chemokine (C-X-C motif) ligand 10; MOG, myelin oligodendrocyte glycoprotein; ELISA, enzyme-linked immunosorbent assay; t-PA, tissue plasminogen activator; ADME, absorption distribution metabolism and excretion; CLARITY, clear lipid-exchanged acrylamide-hybridized rigid imaging/immunostaining/in situ hybridization-compatible tissue-hydrogel.

E-mail address: ksuk@knu.ac.kr (K. Suk).

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1. Lipocalins and gliocalins

Lipocalins are a broad family of secreted proteins that have a common and conserved lipocalin domain responsible for binding to diverse small hydrophobic macromolecules such as siderophore-bound iron, retinol, fatty acids, biliverdin, pheromones, porphyrin, and benzophenone (Flower, 1996; Flower et al., 2000). The lipocalin domain is composed of 160–199 amino acids with 8–9 β-strands that form a barrel structure. This family of proteins has been associated with many biological processes, such as immune responses, pheromone transport, olfaction, prostaglandin synthesis, retinoid binding, and cryptic coloration, as well as with the regulation of diverse cellular processes, including cell migration, proliferation, differentiation, cell death and survival. These proteins are evolutionary conserved and are found in various kingdoms, ranging from bacteria to plants, invertebrates, and vertebrates (Ganfornina et al., 2000).

Gliocalins form a lipocalin subfamily and are mainly produced by glia as well as an act upon glial cells. Gliocalins include lipocalin-2 (LCN2) (Lee et al., 2007, 2009), lipocalin-type prostaglandin D synthase (L-PGDS) (Lee et al., 2012), complement 8γ (C8γ), and orosomucoid (ORM) [also known as alpha-1-acid glycoprotein (α1AGp or AGP)] (unpublished data, Jo M. et al.). LCN2 is a representative member of the gliocalin subfamily. Since initial reports on its glial expression (Lee et al., 2007, 2009), LCN2 has been extensively studied in a diverse range of cellular and animal models of brain injury. LCN2 regulates multiple cellular processes, including cell viability, cell movement, cell invasion, cell differentiation, and iron transport, which are not mutually exclusive processes (Flower, 1996; Flower et al., 2000; Goetz et al., 2002; Kehrer, 2010; Kjeldsen et al., 2000; Rodvold et al., 2012). All these cellular processes are involved in neuroinflammation, in which LCN2 is known to play a central role. LCN2 is also called siderocalin since it binds to siderophores. Iron and neuroinflammation have long been implicated in diverse brain injury conditions. Therefore, the iron-transporting activity of LCN2 may be related to brain inflammation and tissue injury. Other names of LCN2 include 24p3, neutrophil gelatinase-associated lipocalin (NGAL), and 24-kDa super-inducible protein (SIP24). LCN2 is an acute phase protein whose numerous functions have been demonstrated under various physiological and pathological conditions. LCN2 has been implicated in the protection of neutrophil gelatinase from autodegradation, the protection of the central nervous system (CNS) from bacterial infection, the regulation of glial cell activity, and the modulation of neuronal activity and viability, just to name a few. In recent reviews, diverse LCN2 functions have been extensively discussed from a CNS perspective (Ferreira et al., 2015; Jha et al., 2015). New functions of LCN2 will certainly emerge through future investigation of this molecule.

L-PGDS has been identified as another gliocalin that modulates glial cell activity following brain injury (Lee et al., 2012). Two different types of PGDS have been characterized [lipocalin-type PGDS (L-PGDS) and hematopoietic-type PGDS (H-PGDS)] (Nagata et al., 1991; Urade and Hayaishi, 2000). L-PGDS, as one of these two distinct PGDS types, is also known as β-trace, and is one of the most abundant cerebrospinal fluid (CSF) proteins (Hoffmann et al., 1993; Watanabe et al., 1994). The L-PGDS protein has been shown to accelerate glial cell migration (Lee et al., 2012). L-PGDS gene is expressed in neurons and glial cells. The L-PGDS protein instigates morphological changes of glial cells in a manner similar to those found in reactive gliosis. L-PGDS protein induces the assembly of actin filaments and formation of focal adhesion complex through signal transduction pathways involving sequential activation of AKT (protein kinase B, PKB), Ras homolog gene family, member A (RhoA), and c-Jun N-terminal kinase (JNK). The L-PGDS protein also promotes astrocyte migration and accumulation *in vivo* around the injury sites. Stereotaxic injection of L-PGDS protein into particular regions of the animal brain, such as striatum or cortex, increased the astrocyte number near the L-PGDS-injected site. Unexpectedly, the astrocyte migration-promoting activity of L-PGDS protein was not dependent on its enzyme activity as PGD_2 synthase. Co-immunoprecipitation followed by liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis identified the myristoylated alanine-rich C-kinase substrate (MARCKS) protein as an interacting partner of the L-PGDS protein. L-PGDS interaction with MARCKS led to the enhancement of cell migration. These results indicate that L-PGDS protein is a secreted gliocalin regulating glial cell migration and accumulation following brain injury (Lee et al., 2012). These results also indicate that L-PGDS may be a critical mediator of reactive gliosis after brain injury, which could be exploited for therapeutic purposes. As this review focuses on LCN2, L-PGDS and other potential gliocalins will not be discussed in further detail.

2. Lipocalin-2 in stroke and other brain injuries

LCN2 is a marker of reactive astrocytes and is an autocrine mediator of reactive astrocytosis (Lee et al., 2009). LCN2 is associated with a variety of CNS injury conditions, such as cerebral ischemia, cerebral hemorrhage, traumatic brain injury, spinal cord injury, excitotoxic injury, stab wound, medial forebrain bundle transection, lipopolysaccharide (LPS)-induced neuroinflammation, and autoimmune neuroinflammation. All of these conditions involve reactive astrocytosis in their pathogenic mechanisms. LCN2 released from reactive astrocytes under these conditions plays distinct roles, depending on the experimental models being investigated.

2.1. Ischemic stroke models

A recent study using an experimental model of transient focal cerebral ischemia demonstrated a critical role for LCN2 in ischemia/reperfusion-induced injury in the brain (Jin et al., 2014b), which is in line with the pathological roles of LCN2 in ischemia/reperfusion injury of kidney (Mishra et al., 2003) and heart (Sickinger et al., 2013). *Lcn2* gene-deficient mice have been used to investigate the role of LCN2 in ischemic stroke. After transient middle cerebral artery occlusion (tMCAO) was experimentally induced in wild-type and *Lcn2* knockout (-/-) mice, volumes of brain infarct, neurological scores, blood-brain barrier (BBB) permeability, degree of glial activation, and expression of proinflammatory mediators were compared between the two genotypes (Jin et al., 2014b). *Lcn2* gene deficiency attenuated brain damage and neuroinflammatory parameters that were measured 24–48 h after reperfusion. Similar findings were obtained at the subacute phase of ischemia/reperfusion (5 days after reperfusion) (unpublished data, Jin M. et al.). LCN2 expression in the ischemic brain was strongly induced at 24 h after reperfusion. After tMCAO, LCN2 was primarily expressed in astrocytes and endothelial cells, while LCN2 receptor was mainly expressed in astrocytes, neurons,