Brain globins in physiology and pathology

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Abstract

Globins are globular proteins for either transport or storage of oxygen which are critical for cellular metabolism. Four globins have been identified in rodent and human brains. Among them, neuroglobin, cytoglobin and hemoglobin chains are constitutively expressed in normal brain, while myoglobin is only expressed in some neurological disorders. Studies on the molecular structure, expression and functional features of these brain globins indicated that they may play crucial roles in maintenance of neural cell survival and activity, including neurons and astrocytes. Their regulation in neurological disorders may help thoroughly understand initiation and progression of ischemia, Alzheimer's disease and glioma, *etc*. Elucidation of the brain globin functions might remarkably improve medical strategies that sustain neurological homeostasis and treat neurological diseases. Here the expression pattern and functions of brain globins and their involvement in neurological disorders are reviewed.

Key words: neuroglobin; myoglobin; cytoglobin; hemoglobin; brain; neuroprotection; neurodegeneration; ischemia

INTRODUCTION

Globins are porphyrin-containing proteins that bind the oxygen (O_2) reversibly and are therefore important in the respiratory system of living species (Burmester et al., 2000). These O_2-binding proteins are characterized by the typical so-called "globin-fold," which consists of a series of α-helices and a heme group (Hendgen-Cotta et al., 2014). Four globins reside in vertebrates: neuroglobin (Ngb), hemoglobin (Hb), myoglobin (Mb), and cytoglobin (Cygb) (Rahaman and Straub, 2013). Accumulated evidences indicate that in rodent and human brains, these globins are constitutively expressed or inducible by insults to the central nervous system (CNS) such as hypoxia/ischemia and neurodegeneration (Pesce et al., 2003). Their biological actions in the brain under physiological and pathological conditions have been paid special attention to, due to their roles in O_2 sensation and transport, and the high metabolic activity of the brain. Recent findings have linked several globins to intracellular signaling and oxidative stress in addition to O_2 binding (Pesce et al., 2002; Khan et al., 2008; De Henau et al., 2015), therefore expanding the knowledge about the brain globins and proposing potential novel therapeutic strategies for some neurological disorders. In this review, we summarized the expression pattern and biological functions of the major globin types in the brain.

NGB

A globin type which is predominantly expressed in human and mouse brains was identified and termed Ngb in 2000 (Burmester et al., 2000). Comparison of human Ngb with vertebrate Mb or Hb shows less than 25% amino acid sequence identity (Pesce et al., 2003). Structurally, Ngb is a monomer featuring the typical 3/3 α-helical globin fold which is like Mb. Flash photolysis studies show a high recombination rate (k(on)) and a slow dissociation rate (k(off)) for both O_2 and carbon monoxide (CO), indicating a high intrinsic affinity for these ligands (Kriegl et al., 2002; Uzan et al., 2004). However, because the rate-limiting step in ligand combination with the deoxyhexacoordinated form involves the dissociation of the protein ligand, O_2 and CO binding is suggested to be slow *in vivo* (Dewilde et al., 2001). Spectroscopic studies show that both ferrous deoxygenated and ferric Ngb forms display a hexacoordinated heme, where residues HisF8 and HisE7 are the fifth

(proximal) and the sixth (distal) Fe ligands, respectively. O_2 reversibly displaces the endogenous HisE7 heme ligand, leading to oxygenation of Ngb. The O_2 affinity for the pentacoordinated Ngb species is very high. However, because of HisE7/O_2 competition for coordination to the heme Fe atom, the actual O_2 affinity for Ngb is medium, closely similar to that of Mb (Burmester et al., 2000; Dewilde et al., 2001; Pesce et al., 2002, 2003).

Expression of NGB in the CNS

In mouse and human CNS, Ngb is predominantly expressed in neurons. Although the vast majority of evidence to date suggests a broad expression of Ngb mRNA and protein in neurons of different brain regions, the strength of expression appears to be substantially different at the regional and cellular levels (Hankeln et al., 2004). Ngb is highly expressed in the hypothalamus, in particular in the anterior and lateral hypothalamic area (mammillary region), in the paraventricular nucleus and in the arcuate nucleus, in the dorsomedial hypothalamic nucleus and in the preoptic area. In addition, Ngb is expressed in the laterodorsal and pontine tegmental nucleus and in the anterior basomedial and posterodorsal medial amygdaloid nucleus (Cutrupi et al., 2014). Analysis on the subcellular localization of Ngb indicates that Ngb mRNA and protein were consistently detected in neuronal perikarya and processes (Reuss et al., 2002; Zhang et al., 2002; Geuens et al., 2003; Wystub et al., 2003). The presence of Ngb in axonal varicosities and terminal synapses, where mitochondria are enriched, indicates a functional involvement of Ngb in intensive metabolic processes within these neuronal cell compartments. Furthermore, Ngb expression is increased by neuronal hypoxia (Sun et al., 2001; Schmidt-Kastner et al., 2006) *in vitro* and focal cerebral ischemia (Sun et al., 2001) or global ischemia (Schmidt-Kastner et al., 2006) *in vivo*. However, the regulatory mechanisms for Ngb expression have not been thoroughly elucidated. A previous study shows putative conserved nuclear factor-kappa B (NF-κB) and early growth response protein 1 (Egr1) binding sites in the Ngb promoter region. Overexpression and knockdown of transcription factors p65, p50, Egr1 or specificity protein 1 (Sp1) increased and decreased Ngb gene expression, respectively (Liu et al., 2012). Hypoxia-inducible factor-1 alpha (HIF-1α), NF-κB and Sp1 are responsible for hypoxia-induced up-regulation of Ngb mRNA expression, although there is no consensus sequence for HIF-1 in Ngb promoter (Haines et al., 2012; Liu et al., 2012). A recent study predicts response elements in Ngb promoter, including peroxisome proliferator-activated receptor gamma (PPARγ), retinoid X receptor (RXR), zinc finger protein, X-linked (ZFX), nuclear transcription factor Y alpha (NFYA), and transcrip-

tional enhancer domain 1 (TEAD1) (Cutrupi et al., 2014). Other potential Ngb-regulating transcription factors include estrogen receptor alpha (ERα), estrogen receptor beta (Erβ), estrogen receptor 1 (ESR1), single-minded homolog 1 (SIM1), sex determining region Y-box (SOX)-3 and SOX-4 (Uzan et al., 2004).

Ngb is also found to be expressed in cultured cerebral cortical astrocytes (Chen et al., 2005) and reactive astrocytes induced by traumatic brain injury, cerebral malaria, experimental autoimmune encephalitis (DellaValle et al., 2010; Chen et al., 2015). In these CNS disorders Ngb-positive astrocytes were found within regions associated with most severe pathology and the astroglial scar. Subcellularly, Ngb was expressed primarily in the cytoplasm of astrocytes as visualization of astrocytic processes was apparent and the astrocytic nuclei were unstained. However, Ngb is not detected in astrocytes in post-stroke human brains (Jin et al., 2010). Ngb is also found in cerebrospinal fluid of female chronic regional or systemic pain patients (DellaValle et al., 2010) and glioblastoma tumor cells (Emara et al., 2009; Qin et al., 2012; Emara et al., 2014).

Neuroprotective function of NGB

Functionally, it has been demonstrated that Ngb is neuroprotective in distinct neurological disorders. In Ngb-overexpressing transgenic (Ngb-Tg) mice, the cerebral infarct size after middle cerebral artery occlusion (MCAO) is reduced by 30% compared with wild type controls (Khan et al., 2006). This reduction of infarct size is associated with enhanced expression of endothelial nitric oxide synthase in vascular endothelial cells. Wang et al. (2008) found that Ngb-Tg mice have reduced cerebral infarct size until 14 days post-MCAO, but no significant improvements in sensorimotor deficits was observed. Cai et al. (2011) delivered a fusion protein, which incorporates the 11-amino-acid human immunodeficiency virus trans-activator of transcription (TAT) protein and Ngb, to cross the blood-brain barrier and increase neuronal survival after MCAO. However, another research group indicated that Ngb is not required for neuronal survival following acute and prolonged hypoxia in Ngb-deficient mice (Hundahl et al., 2011). Instead, Ngb-deficiency appears to enhance the hypoxia-dependent response of HIF-1α and c-FOS protein while also altering the transcriptional regulation of the glycolytic pathway. These studies suggest that increase of Ngb expression may rescue neurons in hypoxia/ischemia, while other proteins (probably other globins) might compensate for Ngb when Ngb is absent. On the contrary, Raida et al. (2012b) even discovered reduced tissue infarction in Ngb-null mice in a permanent MCAO model. Therefore, the role of Ngb in hypoxia/ischemia is still obscure and further study will be

needed to clarify the temporal and spatial effects of Ngb.

Ngb overexpression is also protective against Alzheimer's disease (AD) (Zhu et al., 2014). Ngb level is increased in early and moderately advanced AD but declined to basal level in severe disease. In addition, in patients with AD, Ngb is detected within neurons, as well as at extracellular sites associated with amyloid-β (Aβ) deposits (Sun et al., 2013). It is also reported most recently that an age-related decline of Ngb is correlated with an increased risk of AD (Szymanski et al., 2010). Ngb overexpression decreases the levels of Aβ-induced reactive oxygen species (ROS) and lipid peroxidation in PC12 cells (Li et al., 2008). In AD transgenic mice, overexpression of Ngb decreases the levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ and improves cognitive performance (Khan et al., 2007). Chen et al. (2012) reported that the level of Ngb is negatively correlated with tau phosphorylation in mouse AD models. Overexpression of Ngb attenuates tau hyperphosphorylation at multiple AD-related sites induced by up-regulation of glycogen synthase kinase-3β (GSK-3β), a crucial tau kinase. This research implies that Ngb may attenuate tau hyperphosphorylation through activating Akt signaling pathway, implying a therapeutic target for AD.

Ngb has been found to be neuroprotective in other neurological disorders. Zhao et al. (2012) reported Ngb overexpression reduces traumatic brain lesion size in mice but does not enhance recovery of sensorimotor and spatial memory functional deficits. Elevating the neuronal Ngb prior to H_2O_2 insult enhances neuronal viability by decreasing oxidative stress and increasing intracellular adenosine triphosphate (ATP) concentration (Antao et al., 2010). Ngb expression can be up-regulated in the cerebellum of rat pups exposed to maternal epileptic seizures, implying that Ngb may also be protective against seizures (Lima et al., 2011).

However, Ngb might be harmful for human health by favoring glioma progression. Ngb is expressed in human glioma cell lines, and that expression can be significantly increased after exposure to physiologically relevant levels of hypoxia, probably maintaining tumor survival in hypoxic microenvironments (Emara et al., 2009, 2010; Qin et al., 2012). Furthermore, Ngb is up-regulated in non-small lung cancer, and Ngb promoter is hypermethylated in 30.8% of non-small lung cancer cases (Oleksiewicz et al., 2011b). 17β-Estradiol can up-regulate Ngb in a dose- and time-dependent manner in MCF-7, HepG2, SK-N-BE, and HeLa cells transfected with ERα, highlighting the pivotal role of Ngb in 17β-estradiol-induced anti-apoptotic pathways in cancer cells (Fiocchetti et al., 2014). Nonetheless, Zhang et al. (2013) proposed that Ngb acts as a tumor suppressor to control hepatocelluar carcinoma (HCC) development by linking oxygen/ROS signals to oncogenic Raf/mitogen-activated protein kinase (MAPK)/extracellular signal-

regulated kinase (Erk) signaling. Hence, the role of Ngb in tumor survival needs further investigation.

Mechanisms of NGB function

The neuroprotective efficacy of Ngb lies in the following possible mechanisms: 1) Due to its ability to bind to O_2, Ngb enhances O_2 supply to the mitochondria of the metabolically active neurons. This hypothesis is supported by the existence of Ngb in metabolically active cells and subcellular compartments, suggesting that Ngb may supply O_2 to the respiratory chain of neurons (Cai et al., 2011). 2) Ngb scavenges damaging reactive oxygen or nitrogen species (i.e., ROS/reactive nitrogen species (RNS)). Ngb can efficiently scavenge a variety of free radicals, including the 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid) cation, superoxide anion, hydrogen peroxide, and hydroxyl radical. The capacity of Ngb to scavenge the superoxide anion and hydrogen peroxide is even comparable to that of vitamin C. In addition, Ngb had Fe^{2+} chelating activity (Li et al., 2011). 3) Oxygenated derivative of Ngb (Ngb-O_2) reacts with nitric oxide (NO) rapidly to produce NO^{3-} and met-Ngb. This pathway would dispose of NO by means of a rapid reaction with Ngb-O_2, which may in turn protect cellular respiration jeopardized by the inhibitory effect of NO on cytochrome C oxidase activity (Brunori et al., 2005). 4) Ngb-cytochrome C association in mitochondria reduces the release of cytochrome C into the cytosol which triggers caspase-9-dependent apoptosis (De Marinis et al., 2013). 5) Ngb acts as a heterotrimeric Gα protein guanine nucleotide dissociation inhibitor. Ferric Ngb, which is generated spontaneously as a result of the rapid autoxidation, binds exclusively to the guanosine diphosphate (GDP)-bound form of α subunit of heterotrimeric G protein. The interaction of GDP-bound Gα with ferric Ngb liberates Gβγ, leading to protection against neuronal death (Wakasugi et al., 2003). 6) Ngb inhibits Pak1 kinase and interacts with Rho GDP dissociation inhibitor (RhoGDI) and RhoGTPase family members, inhibiting propagation of hypoxia or N-methyl-D-aspartic acid (NMDA)-induced death signal in a form of cytoskeletal reorganization and mitochondrial aggregation at the sites of raft membrane microdomains (Khan et al., 2007, 2008). Other mechanisms have also been proposed, such as Ngb stabilizing expression and DNA binding of both HIF-1α and nuclear factor erythroid 2 p45 related factor 2 (Nrf2) (Hota et al., 2012), and Ngb interacting with cystatin C to modulate intracellular transport or processing through the secretory pathway of cystatin C to protect against neuronal death (Wakasugi et al., 2005).

Recently, we examined the role of Ngb in modulating cell anabolism (Cai et al., 2016). We found increased ATP production and decreased glycolysis in Ngb-overexpressing
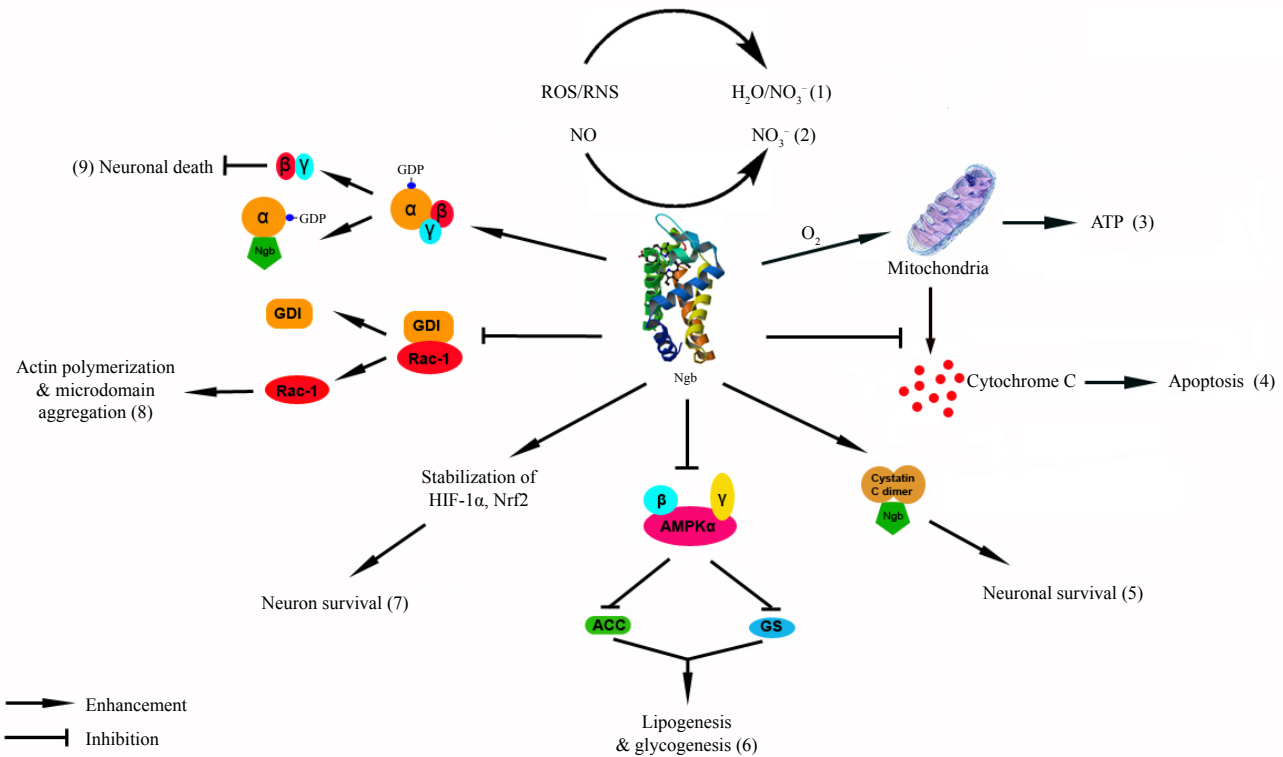
Figure 1: Currently known mechansims of neuroglobin (Ngb) functions in the brain.
Note: (1) Ngb scavenges reactive oxygen (ROS) or nitrogen species (RNS). (2) Oxygenated derivative of Ngb (Ngb-O$_2$) reacts with nitric oxide (NO) rapidly to produce NO$_3^-$. (3) Ngb enhances oxygen (O$_2$) supply to the mitochondria to promote adenosine triphosphate (ATP) production. (4) Ngb-cytochrome C association in mitochondria reduces the release of cytochrome C into the cytosol which triggers apoptosis. (5) Ngb interacts with cystatin C dimers to protect neurons. (6) Ngb inhibits AMP-activated protein kinase (AMPK) signaling to regulate lipogenesis and glycogenesis. (7) Ngb stabilizes expression of hypoxia-inducible factor-1 alpha (HIF-1α) and Nrf2 to protect neurons. (8) Ngb interacts with Rho guanosine diphosphate (GDP) dissociation inhibitor (RhoGDI) and ras-related C3 botulinum toxin substrate 1 (Rac-1) to regulate actin polymerization and raft membrane microdomain aggregation. (9) Ngb binds to the GDP-bound form of α subunit of heterotrimeric G protein to inhibit neuronal death.

HT22 cells, in parallel with inhibition of AMP-activated protein kinase (AMPK) signaling. In addition, lipid and glycogen content is increased in Ngb-overexpressing HT-22 cells. AMPK signaling is also inhibited in the brain and heart from Ngb-Tg mice. Although Ngb overexpression does not change glycogen content in whole brain, glycogen synthase is activated in cortical neurons of Ngb-Tg mouse brain and Ngb-overexpressing primary neurons. Moreover, lipid and glycogen content is increased in hearts derived from Ngb-Tg mice. These findings suggest that Ngb functions as a metabolic regulator and enhances cellular anabolism through the inhibition of AMPK signaling. This study expands the relationship between Ngb and metabolism-associated signaling. More studies should determine the interplay between Ngb and metabolism not only in neurons, but in astrocytes, cardiomyocytes and tumor cells. Known mechanisms of Ngb functions are summarized in **Figure 1**.

Of note, Ngb stability and activity might be regulated by post-translational modification. A study has shown

Ngb phosphorylation and protein-protein interactions with 14-3-3 increase during hypoxic and metabolic stress. In addition, binding to 14-3-3 stabilizes and increases the half-life of Ngb phosphorylation. Furthermore, Ngb phosphorylation by ERK2 increases the open probability of the heme pocket, which increases ligand binding (CO and nitrite) and accelerates the rate of anaerobic nitrite reduction to form NO (Jayaraman et al., 2011). Another study demonstrated that catecholamine-oxidation products, which have been implicated in neurodegenerative pathologies like Parkinson's disease (PD) and AD, can extensively modify Ngb at Cys46, Cys55, Cys120 and other amino residues. Hence, both intracellular signal proteins and metabolites might affect Ngb stability or activity (Nicolis et al., 2013).

Taken together, Ngb is beneficial for neuronal survival and functions. Positively modulating Ngb expression might be a promising therapeutic approach for neurodegenerative disorders.

Hb

Expression of Hb chains in normal CNS

Hb protein is assembled into heterotetramers, comprised of two α-globin and two β-globin polypeptides, capable of binding multiple gaseous ligands such as O_2, CO, and NO (Rahaman and Straub, 2013). Hb is mainly present in vertebrate blood erythrocytes. However, Hb α- and β-chains are also expressed in mammalian brains. In mouse, rat and human brains, these chains are located in nigral dopaminergic neurons, striatal gamma-aminobutyric acid (GABA) ergic neurons, and cortical pyramidal neurons, a subpopulation of cortical and hippocampal astrocytes and mature oligodendrocytes, suggesting that they might play a novel role in neuronal function and response to injury (Biagioli et al., 2009; Richter et al., 2009; Russo et al., 2013). Schelshorn et al. (2009) stated that Hb expression is in the cerebral cortex, hippocampus, striatum, and cerebellum of the mouse brain, but not in astrocytes and oligodendrocytes.

Potential functions of neuroanl Hb

Neuronal expression of Hb α- and β-chains is markedly decreased by rotenone, a mitochondrial toxin (Richter et al., 2009). Dopaminergic cell lines overexpressing α- and β-globin chains show changes in genes involved in O_2 homeostasis and oxidative phosphorylation, linking Hb expression to mitochondrial function (Biagioli et al., 2009). Furthermore, neuronal Hb expression is up-regulated by injection or transgenic overexpression of erythropoietin and is accompanied by enhanced brain oxygenation under physiologic and hypoxic conditions (Schelshorn et al., 2009). Hence, Schelshorn et al. (2009) proposed that neuronal Hb facilitates O_2 uptake in neurons, and might serve as an oxygen capacitator molecule. Furthermore, neuronal Hb might play a protective role against oxidative and nitrosative stress by binding to NO, since NO is the strongest known ligand of the ferrous heme iron of Hb, with a higher affinity than oxygen (Saha et al., 2014).

Expression of Hb in neurological disorders

Neuronal Hb expression may be altered in neurological disorders. For example, Ferrer et al. (2011) examined Hb expression in selected cell population in the brains of AD, argyrophilic grain disease (AGD), PD and dementia with Lewy bodies (DLB). They found reduced Hb α-chain and β-chain in practically all neurons with small amounts of granular or punctuate hyperphosphorylated tau deposits, and in neurons with tangles in the hippocampus and frontal cortex in AD and in the hippocampus in AGD; in ballooned neurons containing αB-crystallin in the amygdala in AD and AGD; and in about 80% of neurons

with punctuate α-synuclein deposits and in neurons with Lewy bodies in the substantia nigra pars compacta and in vulnerable neurons of the medulla oblongata in PD and DLB; and in neurons with Lewy bodies in the frontal cortex in DLB. However, the significance of the reduction of Hb in these disorders remains elusive. He et al. (2011) found that Hb α- and β-chain mRNA levels in the ipsilateral basal ganglia are significantly increased after intracerebral hemorrhage, and Hb is localized in neurons and glia cells. Exposure of neurons to hemin also up-regulates Hb α- and β-chain mRNA levels. Brown et al. (2016) observed enrichment of Hb in pyramidal neurons in internal layers of the multiple sclerosis (MS) cortex. They also discovered the interaction of neuronal Hb with subunits of ATP synthase, histones, and a histone lysine demethylase, suggesting that Hb may be a part of a mechanism linking neuronal energetics with epigenetic changes to histones in the nucleus and may provide neuroprotection in MS by supporting neuronal metabolism (Brown et al., 2016). Wu et al. (2004) found that in AD brains, Hb binds to Aβ and co-localizes in amyloid plaques and cerebral amyloid angiopathy, promoting Aβ oligomer formation. Microinjection of human Hb into the dorsal hippocampi of the APP/PS1 transgenic mice induces the formation of an envelope-like structure composed of Aβ surrounding the Hb droplets (He et al., 2011). However, the genesis of some plaques may be a consequence of sustained amyloid accretion by blood-borne Hb, due to a compromised blood-brain barrier frequently observed in aged and AD brains. Taken together, above research indicate the potential protective and detrimental effects of Hb to neurons in distinct neurological diseases.

In general, the exact role of Hb in neuronal activity has not been thoroughly elucidated. Its potential neuroprotective effect deserves further study, which could provide clues for development of novel therapeutic interventions for oxidative stress-related neuronal diseases.

Mb

Mb expression in normal CNS

Mb is a monomeric heme-bound globin known for its ability to store O_2 within cells. Mb is generally present in oxidative striated muscles and cardiac myocytes as well as in smooth muscle cells. Notably, Mb has also been traced in non-muscle cells of fish in brain, kidney, and liver and in human tumor-associated mammary cells (Cossins et al., 2009; Flonta et al., 2009). Recently Mb was found in Chelonia mydas brain, heart and liver tissues. However, the presence of Mb in healthy mammal brains has not been reported. Shonat and Koretsky (2003) generated a brain-specific Mb transgenic mouse strain in which Mb expression is under

the transcriptional control of either a human platelet-derived grown factor polypeptide B promoter sequence or a rat neuron-specific enolase promoter. They found Mb expression levels were highest in the hippocampus, cerebellum, and cerebral cortex. However, no gross morphological adaptations of neural tissue resulting from the expression were observed and no statistically significant differences in the energetic state were detected, suggesting Mb might hardly impact the homeostasis of the mouse brain. Fraser et al. (2006) reported hypoxia-inducible Mb expression in the brain of common carp, and proposed that Mb might function in the protection of tissues from deep hypoxia and ischemia as well as in reoxygenation and reperfusion injury. Hence, it will be interesting to test whether the Mb transgenic mice have higher tolerance to hypoxia and ischemic stroke.

Expression of Mb in neurological disorders

Mb expression may be influenced in the CNS under several pathological conditions. Simonetti et al. (2013) observed Mb transcript in spinal dorsal horn tissue was down-regulated by nuclear calcium signaling in spinal neurons during inflammatory pain. Additionally, Mb was detected in human medullomyoblastoma and medulloblastoma. Double immunopositivity for synaptophysin, neurofilament protein, and Mb in medullomyoblastoma cells suggests that the neuroectodermal cells may undergo differentiation into rhabdomyoblasts (Kido et al., 2009). However, the significance of Mb expression in edullomyoblastoma and medulloblastoma is still obscure. Galluzzo et al. (2009) reported that lung carcinoma cells transfected with Mb grew more slowly, were less hypoxic, and were less metastatic. So Mb is possibly a key factor on which medullomyoblastoma and medulloblastoma rely in order to modulate their expansion. Taken together, the clinical relevance of Mb is still unclear. More investigations will be necessary for interpreting the role of Mb in neuronal disorders especially in ischemic stroke.

Cygb

Expression of Cygb in normal CNS

Cygb displays comparable tertiary structure and the globin fold analogous to Hb and Mb. However, it resides in a dimeric quaternary state (de Sanctis et al., 2004). Cygb protein is expressed in varying tissues types including the brain (Burmester et al., 2002). Intense Cygb staining is observed in the adult mouse piriform cortex, amygdala, hypothalamus (medial preoptic area, supra chiasmatic nucleus, lateral hypothalamus (LH), ventromedial hypothalamic nucleus, and the arcuate nucleus, habenular nuclei, laterodorsal tegmental nucleus (LDTg), pedunculopontinetegmental nucleus (PPTg), locus coeruleus, nucleus of the solitary tract and the spinal trigeminal nucleus, as well as in the hippocampus, the reticular thalamic nucleus, and the dorsal raphe nucleus (Hundahl et al., 2010). Similar results have been acquired from a human study (Hundahl et al., 2013). Of note, co-localization of Cygb and Ngb is found in the LDTg and PPTg. Most neuronal nitric oxide synthase (nNOS)-positive neurons are found to co-localize Cygb. Hundahl et al. (2014) studied neurochemical phenotype of Cygb-expressing neurons in the rat hippocampus, revealing that nearly all the parvalbumin- and hemeoxygenase 1-positive neurons co-express Cygb, and the majority of neurons expressing somastostatin and vasoactive intestinal peptide also co-express Cygb and nNOS, therefore confirming Cygb expression in different neurons. Subcellular localizations of Cygb are cytoplasm, along neurotubuli, mitochondria and the nucleus (Schmidt et al., 2004; Hundahl et al., 2010). The nuclear localization of Cygb poses the possibility that Cygb might transfer the signal into the transcriptional machinery and consequently affect gene expression. Interestingly, in embryos, Cygb expression was primarily restricted to the CNS and neural crest derivatives during the latter stages of development, suggesting it plays a fundamental role in the CNS development.

The regulation of neuronal Cygb expression under physiological condition has not been elucidated. Oliveira et al. (2015) investigated the influence of thyroid state in Ngb and Cygb metabolism in different rat brain regions and evaluated their responses in cerebellum, hippocampus and cerebral cortex. They revealed that thyroid hormone increases in Ngb gene and protein expression but decreases Cygb expression in the cortex. In the hippocampus, Ngb and Cygb protein expression are all increased. Hence, neuronal Cygb expression might be modulated by peripheral hormones.

Cygb functions in the CNS

Distinct functions of Cygb have been proposed (Oleksiewicz et al., 2011a): 1) due to its homology with Mb, Cygb might participate in transport and facilitate diffusion of intracellular oxygen (Kawada et al., 2001; Trent and Hargrove, 2002). 2) Cygb might also serve as an oxygen reservoir. 3) Cygb has nitric oxide dioxygenase activity, which is the common feature within the globin family. 4) Involvement in oxidative stress. 5) Enhancement of collagen synthesis.

The well-characterized role of Cygb in the CNS is its response to hypoxia. Cygb is up-regulated in hypoxic brains, although to a less extent in comparison with liver, hear and muscle (Fordel et al., 2004; Mammen et al., 2006). The increased Cygb was observed predominantly in regions of the brain that have previously been shown to be responsive to oxidative stress, such as hippocampus,

thalamus, and hypothalamus (Mammen et al., 2006). *In vitro* experiments have demonstrated hypoxia-driven Cygb up-regulation in neuronal HN33 cells (Fordel et al., 2004). Hypoxia-induced Cygb is likely mediated by HIF-1, which is supported by the evidences that Cygb expression in HIF-1 knockout mice is affected, and Cygb promoter region contains HIF responsive element sites (Kawada et al., 2001). In a neonatal hypoxia-ischemia brain injury model, Tian et al. (2013) reported that up-regulation of Cygb results in reduced acute brain injury. They also found that Cygb up-regulates mRNA and protein levels of vascular endothelial growth factor and increases both the density and diameter of the microvessels but inhibits activation of caspase-2 and -3, which are the potential mechanisms of Cygb-mediated neuroprotection. However, their statement is contradicted by other studies showing no significant change of Cygb expression in ischemic penumbra and in the necrotic infarct area in a mouse permanent MCAO model and in a hypoxia model (Li et al., 2006; Raida et al., 2012a). The discrepancy of their conclusions might result from different models and different extent of hypoxia/ischemia, because the increase of Cygb expression depends on duration and severity of hypoxia (Burmester et al., 2007; Fordel et al., 2007). The exact role of Cygb in hypoxic/ischemic brains needs further investigation, especially using transgenic or knockout mouse strains.

Cygb might also function in the cerebellum. Beltran-Parrazal et al. (2010) discovered a significant increase of Cygb protein in rats after being exposed to chronic mild carbon monoxide during prenatal and postnatal periods, suggesting Cygb may protect cerebellar cells from the chronic presence of CO exposure during prenatal and postnatal development. However, whether Cygb is present in adult mammal cerebellum has not been examined.

Cygb can be found in gliomas. Emara et al. (2010) showed that Cygb transcript and protein are expressed in GBM cell lines. Cygb protein is significantly increased in these cell lines after hypoxia. Furthermore, they demonstrated that Cygb is detected in all human brain tumors, including grades I–IV astrocytomas, and ependymoblastomas, gangliogliomas and oligodendrogliomas. Xu et al. (2013) conducted a retrospective analysis showing that Cygb loss may contribute to tumor recurrence and a worse prognosis in gliomas. Thus, Cygb may serve as an independent predictive factor for prognosis of glioma patients. Fang et al. (2011) found that knockdown of Cygb expression sensitizes human glioma cells to oxidative stress induced by chemical inhibitors of the electron transport chain, increases cellular radiosensitivity, and decreases the doubling time of glioma cell lines, suggesting a putative tumor suppressor function. Indeed, Cygb was firstly identified as a tumor suppressor

in some tumors (Shivapurkar et al., 2008; Kawada, 2013). Its anti-tumor function has been further confirmed by a study on the Cygb-deficient mice (Thuy le et al., 2011). However, other studies showed that Cygb also harbors oncogene functions (Oleksiewicz et al., 2013). Therefore, further research is in need to explore the functional aspects of Cygb in gliomas.

Human Cygb has been identified in the cytoplasmic eosinophilic inclusions of protoplasmic astrocytes of the neocortex, usually in the clinical setting of epilepsy and/or psychomotor retardation (Hedley-Whyte et al., 2009), and in the hyaline deposits of putaminal neurons and glia in a patient with hereditary ferritinopathy (Powers, 2006). The potential pathogenic importance of Cygb in neuro-degenerative diseases may be related to the unbalanced protective effect of this globin against reactive nitrogen and oxygen species. However, the potential roles of Cygb in other neurological disorders such as AD, PD, MS and traumatic injury have not been documented so far. A Cygb knockout mouse strain has been established in Osaka City University in Japan (Thuy le et al., 2011). Utilization of this Cygb-deficient model might elucidate the elusive role of Cygb in neurological disorders.

In conclusion, up-regulation of Cygb expression might be an efficacious approach to treat hypoxia/ischemia-induced brain damage and glioma. However, elaborated preliminary studies are still needed to completely disclose the mechanisms by which Cygb exerts its function.

PERSPECTIVES

Globins reside not only in peripheral tissues but also in the CNS. Their co-expression in the brain suggests their multiple roles in brain under both physiological and pathological conditions. Most studies have indicated that they are relatively beneficial for maintaining neuronal activity and survival. Therefore promotion of brain globin expression and/or function might be promising therapeutic strategies for treating corresponding neurological disorders. Although the above-mentioned studies are inspiring in explaining brain globin neuroprotection mechanisms, most of them are based on indirect or correlative experimental data (Ascenzi et al., 2014). Thus, a better understanding of the molecular mechanisms by which these globins function bears fundamental and translational significance, with potential implications for the development of nerve globin-targeted therapeutics against stroke and other neurological disorders.

Another question about brain globins is whether they are detrimental to neural cells when their expression, structure or activity is dysregulated. In the periphery, impaired production of Hb incurs thalassaemia and porphyria.

Generation of highly oxidizing species ferryl Mb initiates renal failure (Hendgen-Cotta et al., 2010). Cygb increases hepatocyte necrosis in an induced liver damage model (Teranishi et al., 2015). Whether similar detrimental effects exist in the brain needs further investigation, especially on Ngb and Cygb which are relatively abundant in the brain.

Author contributions
LKX wrote the manuscript. SHY proof-read the manuscript. Both of them read and approved the final version of the manuscript.
Conflicts of interest
The authors declare no conflicts of interest.

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