

Molecular chaperones and hypoxic-ischemic encephalopathy

Cong Hua¹, Wei-na Ju², Hang Jin², Xin Sun^{2,*}, Gang Zhao^{1,*}

1 Department of Neurosurgery, The First Hospital of Jilin University, Changchun, Jilin Province, China

2 Department of Neurology, The First Hospital of Jilin University, Changchun, Jilin Province, China

How to cite this article: Hua C, Ju WN, Jin H, Sun X, Zhao G (2017) Molecular chaperones and hypoxic-ischemic encephalopathy. *Neural Regen Res* 12(1):153-160.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Abstract

Hypoxic-ischemic encephalopathy (HIE) is a disease that occurs when the brain is subjected to hypoxia, resulting in neuronal death and neurological deficits, with a poor prognosis. The mechanisms underlying hypoxic-ischemic brain injury include excitatory amino acid release, cellular proteolysis, reactive oxygen species generation, nitric oxide synthesis, and inflammation. The molecular and cellular changes in HIE include protein misfolding, aggregation, and destruction of organelles. The apoptotic pathways activated by ischemia and hypoxia include the mitochondrial pathway, the extrinsic Fas receptor pathway, and the endoplasmic reticulum stress-induced pathway. Numerous treatments for hypoxic-ischemic brain injury caused by HIE have been developed over the last half century. Hypothermia, xenon gas treatment, the use of melatonin and erythropoietin, and hypoxic-ischemic preconditioning have proven effective in HIE patients. Molecular chaperones are proteins ubiquitously present in both prokaryotes and eukaryotes. A large number of molecular chaperones are induced after brain ischemia and hypoxia, among which the heat shock proteins are the most important. Heat shock proteins not only maintain protein homeostasis; they also exert anti-apoptotic effects. Heat shock proteins maintain protein homeostasis by helping to transport proteins to their target destinations, assisting in the proper folding of newly synthesized polypeptides, regulating the degradation of misfolded proteins, inhibiting the aggregation of proteins, and by controlling the refolding of misfolded proteins. In addition, heat shock proteins exert anti-apoptotic effects by interacting with various signaling pathways to block the activation of downstream effectors in numerous apoptotic pathways, including the intrinsic pathway, the endoplasmic reticulum-stress mediated pathway and the extrinsic Fas receptor pathway. Molecular chaperones play a key role in neuroprotection in HIE. In this review, we provide an overview of the mechanisms of HIE and discuss the various treatment strategies. Given their critical role in the disease, molecular chaperones are promising therapeutic targets for HIE.

Key Words: nerve regeneration; hypoxic-ischemic encephalopathy; molecular chaperones; excitatory amino acid; cellular proteolysis; oxygen radicals; inflammation; apoptosis; reviews; neural regeneration

Introduction

Hypoxic-ischemic encephalopathy (HIE) is a disease that occurs when the brain is subjected to hypoxia and ischemia. Neonates suffer from HIE most frequently due to birth asphyxia. HIE can also result from pathological conditions, such as cardiac arrest, the most common cause of HIE in adults (Chan et al., 2014). Other causes of HIE include shock, cerebrovascular events, diffuse cerebral vasospasm, severe intracranial hypertension, carbon monoxide (CO) poisoning, and status epilepticus (Yang et al., 2016). The cerebral ischemia and hypoxia in HIE perturbs energy metabolism, leading to neurodegeneration and neurological deficits, resulting in a poor prognosis. It is a debilitating neurological disease in desperate need of effective treatment.

Although asphyxia in newborns and cardio-cerebrovascular events in adults both give rise to HIE, their pathogenesis differ substantially. In general, in neonates, the cessation of respiration initially causes hypoxemia, leading to a reduction in cardiac output, which finally results in cerebral ischemic

and hypoxic injury (Liu et al., 2015). In comparison, adults primarily suffer brain ischemia as a result of cardiac arrest or cerebrovascular disease, and cerebral hypoxia is secondary to the reduced regional cerebral blood flow (Biagas, 1999). Furthermore, the severity of brain injury caused by hypoxia and ischemia varies according to the maturity of the neuron. A previous study demonstrated that the immature brain has a stronger capacity to resist hypoxia and ischemia than the mature brain (Wang et al., 2009), although the mechanisms underlying this ability remain unknown. The mechanisms underlying the death of immature and mature neurons during hypoxia and ischemia are greatly different (Zhu et al., 2009). Immature neurons can initiate the intrinsic apoptotic machinery upon ischemia, while this ability weakens gradually as the brain matures (Hu et al. 2000a, b; Liu et al., 2004a, b; Blomgren et al., 2007). Studies are needed to compare the responses of the brain at different maturities to hypoxia and ischemia. Such studies should provide molecular targets for the treatment of hypoxic-ischemic brain damage in adults

***Correspondence to:**
Gang Zhao, M.D., Ph.D.
or Xin Sun, M.D., Ph.D.,
1035722011@qq.com or
sjnksunxin@163.com.

orcid:
0000-0001-5472-7828
(Gang Zhao)

doi: 10.4103/1673-5374.199008

Accepted: 2016-12-26

and neonates.

Parcellier et al. (2003) found that heat shock proteins (HSPs), which are ubiquitous and highly conserved proteins that are induced in response to a wide variety of physiological and environmental insults, are induced in HIE. These proteins, which play essential roles in cellular housekeeping, help cells survive otherwise lethal conditions.

In this review, we describe the mechanisms of HIE and the various treatment approaches, with a focus on the molecular chaperones, which are promising therapeutic targets for brain injury in HIE.

Mechanisms of Hypoxic-Ischemic Brain Injury

The processes leading to neural injury after hypoxic-ischemic brain injury include excitatory amino acid (EAA) release, cellular proteolysis, free radical generation, nitric oxide (NO) synthesis, inflammation, and abnormal protein aggregation (Li et al., 2015; Yao et al., 2016; Zhao et al., 2016). Hypoxic-ischemic insult to the brain leads to neuronal depolarization and massive EAA (e.g., glutamate [Glu]) release, as well as a reduction in the activity of neurotransmitter reuptake pumps on presynaptic astrocytes (Zanelli et al., 2015). This leads to the accumulation of Glu in the synaptic cleft, which in turn triggers the opening of N-methyl-D-aspartate (NMDA) receptor channels and calcium channels, leading to excessive calcium influx into neurons. This calcium influx activates nitric oxide synthetase (NOS), leading to abnormally high NO synthesis. NO reacts with oxygen free radicals generated by mitochondria upon reoxygenation following hypoxia, attacking enzymes associated with oxidative phosphorylation and electron transport (Blanco et al., 2017). Calcium also activates other enzymes, including calpains and esterases (Zhong et al., 2016). Together, these processes cause the necrosis and apoptosis of neurons *via* the activation of cell death cascades (Johnston et al., 2011).

EAA release

The major EAAs in the central nervous system include Glu and aspartic acid (Asp), and Glu plays a dominant role (Xiang et al., 2006). Excessive accumulation of Glu results in hyperactivation of Glu receptors, leading to a massive influx of Ca^{2+} and Na^+ , which produces cellular swelling and calcium overload (Chao et al., 2010). Glu is also closely associated with the concentration of reactive oxygen species (ROS). ROS trigger the opening of the mitochondrial permeability transition pore (MPTP) together with the Bcl-2 family of apoptosis-related proteins, and induce the release of cytochrome c from the mitochondrion into the cytoplasm (Yoshida et al., 1998). Cytochrome c interacts with apoptotic protease activating factor-1 (Apaf-1) to form the apoptosome, which is then followed by caspase 9 activation and the initiation of the apoptotic cascade (Prentice et al., 2015). In this mechanism of brain injury induced by EAA, called excitotoxicity, calcium overload and mitochondrial damage are two key steps in the progression to cell death (Salmina et al., 2011).

Glu homeostasis is dependent on Glu transporters, includ-

ing EAA transporters (EAATs) and cystine/Glu antiporters. EAATs play a principal role in the transport and elimination of Glu, preventing the excessive accumulation of the neurotransmitter in the synaptic cleft (Rothstein et al., 1996). However, during cerebral hypoxia and ischemia, EAATs may release Glu, resulting in its accumulation in the intercellular space (Chen et al., 2005).

Cellular proteolysis

During ischemia and hypoxia, the calcium overload impairs cellular homeostasis and leads to the activation of hydrolytic enzymes that degrade proteins (Willis et al., 2016). Many recent studies have focused on calpain (a calcium-dependent cysteine protease). The activation of calpain by the calcium influx plays an important role in EAA neurotoxicity (Siman et al., 1989). When neurons are stimulated by EAA, calpain-1 is activated and cytoskeletal proteins are hydrolyzed (Rosenkranz et al., 2012). Furthermore, calpain is involved in cell death (Blomgren et al., 2001) and participates in the endogenous apoptosis pathway (Choi, 1992). In global brain ischemia models, inhibition of calpains protects against hippocampal dysfunction and neuronal cell death (Beverly et al., 2010).

ROS generation

ROS are extremely reactive molecules that include NO, superoxide, peroxynitrite and the hydroxyl radical. NOS is strongly activated by the influx of calcium, and NO reacts with superoxide produced by mitochondrial stress to generate the highly toxic peroxynitrite anion, which in turn causes the nitration of tyrosine residues, protein damage and organellar dysfunction through lipid peroxidation (Beckman et al., 1990). NO also inhibits the activity of cytochrome oxidase, thereby affecting mitochondrial respiratory function. This leads to further increases in peroxide and peroxynitrite levels (Blomgren and Hagberg, 2006; Robertson et al., 2009).

NO synthesis

Animal experiments show that NOS is induced during ischemia and hypoxia. Inhibiting the activity of NOS reduces iron deposition and NO generation, thereby reducing the death of neurons (Lu et al., 2015). NO plays a dual role in hypoxic-ischemic brain injury. In the pathological state, NO perturbs neurotransmitter release, impairs protein synthesis and induces membrane damage. However, NO also appears to play a neuroprotective role. Rapidly increasing endothelial nitric oxide synthase (eNOS) activity elevates NO production and accelerates cerebral blood flow after hypoxic-ischemic brain injury. Indeed, Yamamoto et al. (1992) inhibited NOS activity using N-nitro-L-arginine methyl ester (a competitive inhibitor), resulting in an increase in infarct area in rats with hypoxic-ischemic brain injury.

Inflammation

Inflammation is an important component of the excitotoxic cascade. Hypoxic-ischemic brain injury activates inflammatory cells and increases ROS production and the expression

of inflammatory mediators, such as interleukin (IL)-1 β and IL-18 (Bhalala et al., 2015). Oxidative stress is a common feature of all inflammatory cascades in hypoxic-ischemic brain injury (Le Thuc et al., 2015). During inflammation, activated astrocytes, microglia and endothelial cells play a neuroprotective role. The accumulation of immune cells and the release of ROS, chemokines and cytokines leads to blood-brain barrier damage, cerebral edema, neuronal cell death, and hemorrhagic transformation (Dirnagl et al., 1999).

Protein misfolding and aggregation

Proper polypeptide folding is essential for normal protein conformation and function. When the newly synthesized polypeptide chain is misfolded, hydrophobic groups might be exposed on the surface, resulting in the aggregation of the protein (Giffard et al., 2004). Protein aggregation is toxic to cells (Taylor et al., 2002). Molecular chaperones help prevent protein aggregation, and they also facilitate protein degradation through the ubiquitin-proteasome system (Hershko and Ciechanover, 1998). In pathological conditions, abnormal proteins exhaust the cell's capacity to keep them soluble and to degrade them, and may result in their aggregation (Bence et al., 2001). After hypoxia or other severe stress, unfolded or misfolded proteins aggregate in the endoplasmic reticulum, blocking protein synthesis. Normal protein conformation is important for cellular homeostasis. Protein aggregation inhibits the functioning of the proteasome, further disrupting cell function. Protein aggregation is a feature of excitotoxic neuronal injury.

Several studies have shown that protein misfolding, aggregation and destruction of organelles are the main neuropathological changes after hypoxic-ischemic brain injury (Salminen et al., 2016; Zhao et al., 2016). Recent transmission electron microscopy (TEM) studies have revealed the presence of massive electron dense deposits in neurons undergoing delayed neuronal death after cerebral ischemia. These deposits represent aggregates of unfolded and misfolded proteins (Giffard et al., 2004; Hu et al., 2001, 2004; Liu et al., 2004a, b, 2005a, b, 2010; Zhang et al., 2006; Ge et al., 2007). Protein misfolding and aggregation in neurons are important pathogenetic features of neurodegenerative diseases, suggesting that the aggregation of misfolded proteins is the pathological basis of neuronal degeneration (Hardesty et al., 1999; Frydman, 2001).

Why does protein misfolding and aggregation occur after hypoxic-ischemic brain injury? Only if the polypeptide chain folds correctly into its final 3-dimensional structure can it perform its normal biological functions. The folding of nascent polypeptide chains occurs as they exit the ribosome, in a process called co-translational folding (Siesjo and Siesjo, 1996; Hardesty et al., 1999; Frydman, 2001; Hartl and Hayer-Hartl, 2002). The hydrophobic groups of nascent polypeptide chains are exposed, which can, under the influence of hydrophobic forces, easily lead to misfolding. The normal co-translational folding process requires the following (Ito and Nagata, 2016) (**Figure 1**): (1) molecular chaperones, which assist the normal folding process; (2) auxiliary pro-

teins; and (3) energy. Under normal circumstances, when newly synthesized peptide chains misfold and aggregate, they are immediately degraded by the ubiquitin-proteasome system or by the autophagy pathway. Any abnormal protein folding or degradation may lead to protein aggregation and delayed neuronal death.

Molecular chaperones, which regulate the folding process, can identify and shield hydrophobic groups of unfolded proteins and prevent their aggregation under normal conditions. Molecular chaperones and their auxiliary proteins play important roles in the regulation of protein folding, transportation, assembly and degradation (Weiss et al., 2016). Heat shock protein 70 (HSP70) and its auxiliary protein, HSP40, play important roles in protein synthesis. The HSP70 family includes HSC70 and inducible HSP70 in mammalian cells. Under normal conditions, HSC70 is a major auxiliary protein in the co-translational folding process. Together with HSP40, HSC70 identifies the nascent peptide to assist its correct folding and transportation. When the synthesis of a domain of the polypeptide chain is completed, HSC70 dissociates and enters the next cycle, continuing to assist protein synthesis. This process is adenosine triphosphate (ATP)-dependent. If the intracellular ATP level falls below 80% of normal, such as after ischemia and hypoxia, intracellular ATP-dependent regulatory activities terminate or slow down, including co-translational folding, ubiquitin-proteasome-mediated degradation and autophagy (Siesjo and Siesjo, 1996; Huang et al., 2016), eventually leading to the aggregation of misfolded nascent peptide chains. Several recent studies have shown that the accumulation of nascent peptides after hypoxic-ischemic brain injury results in abnormal protein aggregation, irreversible protein synthesis dysfunction and irreversible organelle damage (**Figure 2**).

Apoptosis

The mechanisms of apoptosis in hypoxic-ischemic brain injury are summarized below.

Mitochondrial (intrinsic) apoptotic pathway

Under mitochondrial stress, the pro-apoptotic protein Bax increases the permeability of the mitochondrial outer membrane, thereby releasing cytochrome c, Smac/Diablo, HtrA2/Omi and apoptosis-inducing factor from the intermembrane space into the cytoplasm, leading to the formation of apoptotic bodies and DNA fragmentation (Parcellier et al., 2003; Johnston et al., 2011).

The Fas receptor mediated (extrinsic) apoptotic pathway

In hypoxic-ischemic brain injury, nuclear factor kappa B (NF- κ B) mediates microglial activation and the release of inflammatory factors (such as IL-1 β and IL-6, IL-8, tumor necrosis factor (TNF)- α , TNF- β , macrophage inflammatory protein (MIP)-1 and MIP-2) (Marcinkiewicz et al., 1995; Szaflarski et al., 1995; Hagberg et al., 1996; O'Neill and Kaltschmidt, 1997; Vodovotz et al., 1999; Bando et al., 2003; Johnston et al., 2011). Activation of the cell surface Fas re-

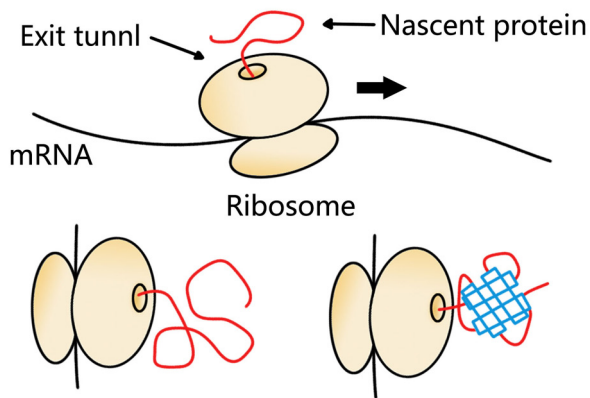


Figure 1 Co-translational folding.

Protein domains can fold into stable tertiary structures while they are synthesized by the ribosome in a process known as co-translational folding.

ceptor by its cognate ligands results in the sequential activation of caspase-8 and caspase-3, DNase activation, and DNA fragmentation (Johnston et al., 2011).

Endoplasmic reticulum stress-mediated apoptotic pathway

Hypoxic-ischemic brain injury leads to protein aggregation. High abnormal protein levels produce endoplasmic reticulum stress, which results in cell membrane dysfunction, the accumulation of intracellular calcium, and the induction of CHOP. This leads to a reduction in the expression of the anti-apoptotic protein bcl-2, which increases the permeability of the mitochondrial membrane, initiating the mitochondrial apoptotic pathway (Johnston et al., 2011). At the same time, dysfunction of the endoplasmic reticulum causes the activation of caspase-12, which sequentially activates caspase-9 and caspase-3, eventually leading to nuclear DNA cleavage and the induction of apoptosis (Morishima et al., 2002).

Treatment of Hypoxic-Ischemic Brain Injury

For nearly half a century, much attention has been given to treatment of hypoxic-ischemic brain injury associated with HIE. With advances in our understanding of pathogenesis, an increasing number of treatments have been developed.

Hypothermia

The temperature of the brain is closely related to cerebral metabolic rate and cerebral blood flow. Each 1° Celsius reduction in body temperature decreases brain metabolic rate by 6–7% (Nel et al., 2009). In addition to reducing oxygen and energy consumption, protecting the blood-brain barrier and alleviating cerebral edema, hypothermia decreases EAA release (Kim et al., 2011), glutamate antiporter expression, the generation of NO and free radicals, phosphorylation of the NMDA receptor, inflammation and apoptosis (Chao et al., 2010).

Hypothermia has been widely used to treat neonatal HIE. It also exerts neuroprotective effects on acute ischemic stroke, although there is a lack of adequate clinical research

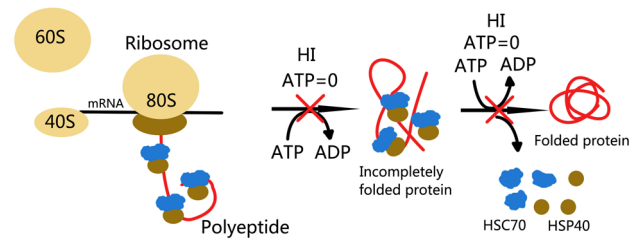


Figure 2 The relationship between protein synthesis, ATP and molecular chaperones.

HSC70 is a major auxiliary protein in the co-translational folding of proteins. In synergy with HSP40, HSC70 identifies the nascent peptide to assist its correct folding and transportation, and when the synthesis of a domain of the polypeptide chain is completed, HSC70 dissociates and enters the next cycle, continuing to assist protein synthesis. This process is ATP-dependent. ATP: Adenosine triphosphate; HSP: heat shock protein; HSC: heat shock cognate protein; ADP: adenosine diphosphate; HI: hypoxia-ischemia.

evidence (Han et al., 2015). A meta-analysis study comparing the therapeutic effects of hypothermia with systemic support treatment shows that hypothermia can increase the survival rate of children with moderate to severe HIE and promote the development of the nervous system (Tagin et al., 2012). Hypothermia therapy has also been shown to be neuroprotective after recanalization for ischemia-reperfusion injury (Han et al., 2015).

Medical treatment

Developing treatments for hypoxic-ischemic brain injury requires the continued exploration of the underlying pathogenesis. The following drug categories have been developed: anti-excitotoxic (sedative hypnotic agent, inert gas xenon, magnesium sulfate), anti-inflammatory and anti-oxidant (cromolyn sodium, N-acetylcysteine, minocycline, melatonin), erythropoietin (EPO), and growth factors (nerve growth factor, insulin-like growth factor-1, brain-derived neurotrophic factor) (Johnston et al., 2011). Here, we discuss several promising drug treatments for hypoxic-ischemic brain injury.

Inert xenon gas

The inert gas xenon is an NMDA receptor antagonist. It plays a role in neuroprotection by interfering with the excitotoxic cascade, and it also acts on ion channels, reducing the release of neurotransmitters (Johnston et al., 2011). Xenon can easily traverse the blood-brain barrier and exert a rapid effect (Baumert et al., 2016). Animal experiments have shown that xenon inhalation combined with hypothermia therapy for hypoxic-ischemic brain injury doubled the therapeutic efficacy relative to monotherapy (Johnston et al., 2011). Dingley et al. (2014) published the results of a clinical trial on the treatment of neonatal HIE using xenon combined with hypothermia, demonstrating its clinical efficacy (Johnston et al., 2011).

Melatonin

Melatonin can be used as a free radical scavenger. Addition-

ally, it can reduce inflammatory factor release and activate antioxidant enzymes, such as glutathione peroxidase, glutathione reductase and superoxide dismutase (Lee et al., 2007). Aly et al. (2015) used hypothermia in combination with melatonin to treat neonatal asphyxia in the perinatal stage and showed that melatonin can ameliorate brain injury, compared with hypothermia alone.

Erythropoietin (EPO)

EPO can play a neuroprotective role through a variety of mechanisms. Sun et al. (2005) demonstrated that EPO binds with the EPO receptor of astrocytes and microglial cells, and has an anti-inflammatory effect. Sakanaka et al. (1998) found that EPO inhibits NO-mediated apoptosis and reduces excitotoxic damage. Additionally, EPO promotes repair after injury by regulating neuronal genesis and differentiation (Lee et al., 2007). A pilot clinical study in 2015 demonstrated that EPO combined with hypothermia therapy for the treatment of hypoxic-ischemic brain injury in neonates was safe and effective (Lee et al., 2007).

Hypoxic-ischemic preconditioning

Hypoxic-ischemic preconditioning refers to a protective therapeutic strategy, in which the subject is exposed to a short-term hypoxic-ischemic stress prior to an upcoming long period of hypoxic-ischemic insult. In a study by Gustavsson et al. (2005), rats were exposed to 8% oxygen (hypoxia) for 3 hours before hypoxic-ischemic brain injury. Brain injury was substantially alleviated and long-term cognitive function was also greatly improved in rats subjected to hypoxic-ischemic preconditioning, compared with control rats (Gustavsson et al., 2005). The neuroprotective mechanisms of hypoxic-ischemic preconditioning remain unclear. However, NMDA receptors, glutamate receptors, ATP-dependent potassium channels and G-protein-coupled adenosine receptors appear to be involved in the neuroprotection conferred by hypoxic-ischemic preconditioning (Lee et al., 2007). Hypoxic-ischemic preconditioning also increases endogenous anti-oxidant and anti-apoptotic capacity, increases brain glycogen and slows down energy depletion following hypoxic-ischemic insults (Brucklacher et al., 2002).

Molecular Chaperones and Hypoxic-Ischemic Brain Injury

Molecular chaperones

The term “molecular chaperones” was first coined by Laskey (1987). Molecular chaperones were defined as nucleoplasmic that binds to histones and participates in nucleosome assembly. Later studies showed that molecular chaperones are ubiquitously present in organisms, both in prokaryotes and eukaryotes. Molecular chaperones assist in proper protein folding, assembly, transport, degradation and inhibition of protein aggregation (Brucklacher et al., 2002), helping to maintain cellular homeostasis. Molecular chaperones are also involved in DNA synthesis and transcription. Molecular chaperones participate in the regulation of the cell cycle, have anti-senescence effects, and regulate apoptosis. Parcel-

lier et al. (2003) found that when brain tissue is subjected to ischemia and hypoxia, the expression of numerous molecular chaperones is induced. Among these molecular chaperones, heat shock proteins (HSPs) are the most important (Parcellier et al., 2003).

HSPs

HSPs were first discovered in *Drosophila* exposed to high temperature, accounting for their name. HSPs are induced by the heat shock response (HSR). The HSR is an adaptive response, characterized by changes in gene expression (Brucklacher et al., 2002), following exposure to heat stress or other stressors, such as ischemia or hypoxia. HSPs prevent protein misfolding and aggregation (Brucklacher et al., 2002). More recent studies have shown that other stress states, such as nutritional deficiencies, hypoxia and chemical toxicity, induce HSP expression as well. For this reason, HSP is also considered a stress protein. Under stress conditions, such as oxidative stress and inflammation, proteins are prone to misfold and aggregate. The aggregation of proteins can lead to many diseases, such as type 2 diabetes mellitus, cardiovascular disease and neurodegenerative diseases (Brucklacher et al., 2002). HSPs assist in the proper folding of newly synthesized proteins, allowing for a proper final 3-dimensional structure. Furthermore, HSPs can help refold denatured proteins, unfold misfolded and unstable proteins, and assist in their degradation. Molecular chaperones are often classified according to their molecular weight and homology. Molecular chaperones are classified into several groups: Hsp110, Hsp90, Hsp70, Hsp60, the small HSPs and ubiquitin.

HSPs help maintain protein homeostasis after ischemia and hypoxia

After ischemia and hypoxia, or other severe stresses, unfolded proteins accumulate in the endoplasmic reticulum, inhibiting proteasomal and other cellular functions (Brucklacher et al., 2002).

The HSP90 family members include HSP90 α , HSP90 β and glucose regulated protein 9 (GRP9). Numerous HSP90 members are expressed under physiological conditions, among which 1–2% are in the cytoplasm. Under stress conditions, HSP90 expression is greatly increased (Parcellier et al., 2003), helping proteins such as steroid receptors, tyrosine kinases and serine/threonine kinases acquire their proper final conformation. The HSP70 family includes stress inducible HSP70, constitutive HSC70, mitochondrial HSP75 and GRP78. Under physiological conditions, HSP70 proteins assist in the proper folding of newly synthesized polypeptides, mediate the assembly of macromolecular complexes and the transmembrane transport of proteins (Brucklacher et al., 2002). Under stress conditions, HSP70 assists in the proper folding of unfolded and misfolded proteins and helps in their degradation (Brucklacher et al., 2002). Gupta et al. (2010) showed that HSP70 activates IRE1 α in the endoplasmic reticulum, enhances expression of transcription factor XBP1 and regulates the expression of unfolded protein response-related genes, helping cells adapt to ER stress. HSP40

is an important accessory factor for HSP70, which promotes ATP hydrolysis (Brucklacher et al., 2002).

Nearly 60% of HSP60 proteins are in the mitochondrial matrix, and 15–20% are found in the cytoplasm. Together with HSP10, HSP60 promotes the proper folding of proteins in the mitochondria, and degrades misfolded and denatured proteins in a process that consumes ATP (Parcellier et al., 2003).

Small HSP family proteins are ATP-independent molecular chaperones. The human genome encodes 10 small HSPs that range in molecular weight from 12 to 42 kDa. Small HSPs can bind to unfolded or misfolded proteins and prevent their aggregation (Brucklacher et al., 2002).

Anti-apoptotic effects of HSPs after hypoxic-ischemic brain injury

After hypoxic-ischemic brain injury, overexpression of HSP70 prevents the release of cytochrome c from mitochondria and the activation of caspase-9 by binding to Apaf-1, thereby blocking the caspase-dependent apoptotic pathway (Brucklacher et al., 2002). Overexpression of HSP70 can also block the caspase-independent apoptotic pathway by binding to apoptosis-inducing factor (AIF) (Brucklacher et al., 2002). Furthermore, overexpression of HSP70 upregulates the expression of cellular Fas-associated death domain-like interleukin-1 β converting enzyme inhibitory protein (cFLIP), inhibits caspase-8, and blocks the Fas receptor-mediated extrinsic apoptotic pathway (Brucklacher et al., 2002). Furthermore, overexpression of HSP70 inhibits NO synthetase and suppresses the production of oxygen radicals, such as NO, OH⁻, ONOO⁻ and O²⁻ (Brucklacher et al., 2002). HSP70 can inhibit JNK, activate IRE1 α , promote the production of transcription factor XPB1 and inhibit the endoplasmic reticulum stress-mediated apoptotic pathway (Brucklacher et al., 2002).

Glucose regulated proteins (GRPs), such as GRP78, are a family of highly conserved proteins that help cells cope with endoplasmic reticulum stress. Under hypoxic-ischemic conditions, cellular energy depletion causes the accumulation of unfolded and misfolded proteins in the endoplasmic reticulum, triggering the unfolded protein response and increasing GRP78 expression (Brucklacher et al., 2002). GRP78 prevents aggregation by binding to the denatured proteins, and it also binds transiently to newly synthesized polypeptides by noncovalent bonds to facilitate the proper folding of newly synthesized proteins. It is also involved in the folding and extension of proteins and the assembly of protein complexes. Additionally, GRP78 helps in the elimination of abnormal proteins by transporting them to the protein degradation system in the endoplasmic reticulum. Furthermore, GRP78 inhibits caspase-9 and caspase-12 to block the endoplasmic reticulum-mediated apoptotic pathway (Brucklacher et al., 2002).

The anti-apoptotic function of HSP60 varies based on cellular localization. Experiments in HeLa and Jurkat cells show that HSP60 in the mitochondria activates caspase-3 and induces apoptosis (Brucklacher et al., 2002). However, following ischemia/reperfusion injury to cardiomyocytes, cytosolic HSP60 binds to the pro-apoptotic proteins Bax and Bak to exert an anti-apoptotic effect (Brucklacher et al., 2002).

The anti-apoptotic function of HSP90 is controversial. The function of HSP90 varies based on the apoptotic inducers. In the monocytic cell line U937, overexpressed HSP90 increases apoptosis in cells exposed to cycloheximide and TNF- α (Brucklacher et al., 2002). However, in cells exposed to staurosporine, overexpressed HSP90 inhibits the formation of apoptotic bodies by binding to Apaf-1, thereby inhibiting apoptosis (Brucklacher et al., 2002). In addition, overexpressed HSP90 can bind to phosphorylated serine/threonine kinase Akt/PKB to protect against inactivation by dephosphorylation. Phosphorylated Akt/PKB promotes the phosphorylation of the pro-apoptotic proteins Bax and caspase-9, and blocks the mitochondrial apoptotic pathway (Brucklacher et al., 2002).

GRP94 is a member of the HSP90 family. It inhibits the activation of caspase-3 and calpain, maintains intracellular calcium homeostasis, and blocks the caspase-3-dependent apoptotic pathway to protect neurons (Brucklacher et al., 2002).

HSP27 is a small HSP. Overexpressed HSP27 inhibits the production of oxygen radicals (Brucklacher et al., 2002), stabilizes actin microfilaments in the cells (Brucklacher et al., 2002), inhibits the release of cytochrome c, blocks the formation of apoptotic bodies, and suppresses the activation of caspases, thereby inhibiting the mitochondrial apoptotic pathway (Brucklacher et al., 2002). In addition, phosphorylated HSP27 can directly interact with Fas death domain-associated protein to inhibit the Fas-mediated apoptotic pathway (Brucklacher et al., 2002). Hsp27 exhibits neuroprotective effects in the nervous system (Kato et al., 1995; Badin et al., 2006; An et al., 2008), for example, in rat models of cerebral ischemic preconditioning (Currie et al., 2000; Dhodda et al., 2004). The inhibition of Hsp27 degradation mediated by autophagy-lysosome pathway was reported to be necessary for neuronal survival after transient global cerebral ischemia (Zhan et al., 2016). Zhan et al. (2016) also showed that post-translational modification of Hsp27 was significant in mediating neuroprotection after hypoxic post-conditioning. These findings offer a solid foundation for targeting an inherent regulatory mechanism of Hsp27 for therapeutic development and broke a new path to exploring the neuroprotective role of hypoxic post-conditioning against ischemic brain injury.

Conclusion and Future Perspective

HIE occurs when the brain is subjected to hypoxic/ischemic conditions. HIE results in neuronal death and neurological deficits and has a poor prognosis. Many treatment methods for hypoxic-ischemic brain injury have been developed over the past half century. Among these, the therapeutic use of HSPs is the most promising. HSPs play a key role in neuroprotection by maintaining protein homeostasis and by regulating apoptosis. Neurons can be protected by up-regulating the expression of HSPs or reducing the degradation of HSPs using a protein synthesis inhibitor such as cycloheximide. In the near future, HSPs may be used clinically to prevent or treat HIE in the early stage to reduce brain injury.

Author contributions: HJ performed the analysis with constructive discussion; WNJ revised the paper; XS conceived and designed the work;

CH and GZ wrote the paper. All authors approved the final version of this paper.

Conflicts of interest: None declared.

Plagiarism check: This paper was screened twice using CrossCheck to verify originality before publication.

Peer review: This paper was double-blinded and stringently reviewed by international expert reviewers.

References

- Aly H, Elmahdy H, El-Dib M, Rowisha M, Awany M, El-Gohary T, El-batch M, Hamisa M, El-Mashad AR (2015) Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study. *J Perinatol* 35:186-191.
- An JJ, Lee YP, Kim SY, Lee SH, Lee MJ, Jeong MS, Kim DW, Jang SH, Yoo KY, Won MH, Kang TC, Kwon OS, Cho SW, Lee KS, Park J, Eum WS, Choi SY (2008) Transduced human PEP-1-heat shock protein 27 efficiently protects against brain ischemic insult. *FEBS J* 275: 1296-1308.
- Badin RA, Lythgoe MF, van der Weerd L, Thomas DL, Gadian DG, Latchman DS (2006) Neuroprotective effects of virally delivered HSPs in experimental stroke. *J. Cereb. Blood Flow Metab* 26: 371-381.
- Bando Y, Katayama T, Kasai K, Taniguchi M, Tamatani M, Tohyama M (2003) GRP94 (94 kDa glucose-regulated protein) suppresses ischemic neuronal cell death against ischemia/reperfusion injury. *Eur J Neurosci* 18:829-840.
- Baumert JH, Roehl AB, Funcke S, Hein M (2016) Xenon protects left ventricular diastolic function during acute ischemia, less than ischemic preconditioning. *Med Gas Res* 6:130-137.
- Beckman JK, Howard MJ, Greene HL (1990) Identification of hydroxyalkenals formed from omega-3 fatty acids. *Biochem Biophys Res Commun* 169:75-80.
- Bence NE, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292:1552-1555.
- Bervers MB, Ingleton LP, Che D, Cole JT, Li L, Da T, Kopil CM, Cohen AS, Neumar RW (2010) RNAi targeting micro-calpain increases neuron survival and preserves hippocampal function after global brain ischemia. *Exp Neurol* 224:170-177.
- Bhalala US, Koehler RC, Kannan S (2015) Neuroinflammation and neuroimmune dysregulation after acute hypoxic-ischemic injury of developing brain. *Front Pediatr* 2:144.
- Biagas K (1999) Hypoxic-ischemic brain injury: advancements in the understanding of mechanisms and potential avenues for therapy. *Curr Opin Pediatr* 11:223-228.
- Blanco S, Hernández R, Franchelli G, Ramos-Álvarez MM, Peinado MÁ (2017) Melatonin influences NO/NOS pathway and reduces oxidative and nitrosative stress in a model of hypoxic-ischemic brain damage. *Nitric Oxide* doi: 10.1016/j.niox.2016.12.001.
- Blomgren K, Hagberg H (2006) Free radicals, mitochondria, and hypoxia-ischemia in the developing brain. *Free Radic Biol Med* 40:388-397.
- Blomgren K, Leist M, Groc L (2007) Pathological apoptosis in the developing brain. *Apoptosis* 12:993-1010.
- Blomgren K, Zhu C, Wang X, Karlsson JO, Leverin AL, Bahr BA, Mallard C, Hagberg H (2001) Synergistic activation of caspase-3 by m-calpain after neonatal hypoxia-ischemia: a mechanism of "pathological apoptosis"? *J Biol Chem* 276:10191-10198.
- Brucklacher RM, Vannucci RC, Vannucci SJ (2002) Hypoxic preconditioning increases brain glycogen and delays energy depletion from hypoxia-ischemia in the immature rat. *Dev Neurosci* 24:411-417.
- Chan PS, McNally B, Tang F, Kellermann A; CARES Surveillance Group (2014) Recent trends in survival from out-of-hospital cardiac arrest in the United States. *Circulation* 130:1876-1882.
- Chao XD, Fei F, Fei Z (2010) The role of excitatory amino acid transporters in cerebral ischemia. *Neurochem Res* 35:1224-1230.
- Chen JC, Hsu-Chou H, Lu JL, Chiang YC, Huang HM, Wang HL, Wu T, Liao JJ, Yeh TS (2005) Down-regulation of the glial glutamate transporter GLT-1 in rat hippocampus and striatum and its modulation by a group III metabotropic glutamate receptor antagonist following transient global forebrain ischemia. *Neuropharmacology* 49:703-714.
- Choi DW (1992) Excitotoxic cell death. *J Neurobiol* 23:1261-1276.
- Currie RW, Ellison JA, White RE, Feuerstein GZ, Wang X, Barone FC (2000) Benign focal ischemic preconditioning induces neuronal Hsp70 and prolonged astrogliosis with expression of Hsp27. *Brain Res* 863: 169-181.
- Dhodda VK, Sailor KA, Bowen KK, Vemuganti R (2004) Putative endogenous mediators of preconditioning-induced ischemic tolerance in rat brain identified by genomic and proteomic analysis. *J Neurochem* 89: 73-89
- Dingley J, Tooley J, Liu X, Scull-Brown E, Elstad M, Chakkarapani E, Sabir H, Thoresen M (2014) Xenon ventilation during therapeutic hypothermia in neonatal encephalopathy: a feasibility study. *Pediatrics* 133:809-818.
- Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischemic stroke: an integrated view. *Trends Neurosci* 22:391-397
- Frydman J (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annu Rev Biochem* 70:603-647.
- Ge P, Luo Y, Liu CL, Hu B (2007) Protein aggregation and proteasome dysfunction after brain ischemia. *Stroke* 38:3230-3236.
- Giffard RG, Xu L, Zhao H, Carrico W, Ouyang Y, Qiao Y, Sapolsky R, Steinberg G, Hu B, Yenari MA (2004) Chaperones, protein aggregation, and brain protection from hypoxic/ischemic injury. *J Exp Biol* 207(Pt 18):3213-3220.
- Gupta S, Deepti A, Deegan S, Lisbona F, Hetz C, Samali A (2010) HSP72 protects cells from ER stress-induced apoptosis via enhancement of IRE1alpha-XBP1 signaling through a physical interaction. *PLoS Biol* 8:e1000410.
- Gustavsson M, Anderson MF, Mallard C, Hagberg H (2005) Hypoxic preconditioning confers long-term reduction of brain injury and improvement of neurological ability in immature rats. *Pediatr Res* 57:305-309.
- Hagberg H, Gilland E, Bona E, Hanson LA, Hahin-Zoric M, Blennow M, Holst M, McRae A, Söder O (1996) Enhanced expression of interleukin (IL)-1 and IL-6 messenger RNA and bioactive protein after hypoxia-ischemia in neonatal rats. *Pediatr Res* 40:603-609.
- Han Z, Liu X, Luo Y, Ji X (2015) Therapeutic hypothermia for stroke: Where to go? *Exp Neurol* 272:67-77.
- Hardesty B, Tsalkova T, Kramer G (1999) Co-translational folding. *Curr Opin Struct Biol* 9:111-114.
- Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295:1852-1858
- Hershko A, Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* 67:425-479.
- Hu BR, Janelidze S, Ginsberg MD, Busto R, Perez-Pinzon M, Sick TJ, Siesjö BK, Liu CL (2001) Protein aggregation after focal brain ischemia and reperfusion. *J Cereb Blood Flow Metab* 21:865-875.
- Hu BR, Liu CL, Ouyang Y, Blomgren K, Siesjö BK (2000a) Involvement of caspase-3 in cell death after hypoxia-ischemia declines during brain maturation. *J Cereb Blood Flow Metab* 20:1294-1300.
- Hu BR, Martone ME, Jones YZ, Liu CL (2000b) Protein aggregation after transient cerebral ischemia. *J Neurosci* 20:3191-3199.
- Hu BR, Martone ME, Liu CL (2004) Protein aggregation, unfolded protein response and delayed neuronal death after brain ischemia. *Maturation Phenomenon in Cerebral Ischemia V* 225-237.
- Huang CY, Pai YC, Yu LC (2016) Glucose-mediated cytoprotection in the gut epithelium under ischemic and hypoxic stress. *Histol Histopathol* 8:11839.
- Ito S, Nagata K (2016) Biology of Hsp47 (Serpin H1), a collagen-specific molecular chaperone. *Semin Cell Dev Biol* pii: S1084-9521(16)30384-6.
- Johnston MV, Fatemi A, Wilson MA, Northington F (2011) Treatment advances in neonatal neuroprotection and neurointensive care. *Lancet Neurol* 10:372-382.
- Kato H, Kogure K, Liu XH, Araki T, Kato K, Itoyama Y (1995) Immunohistochemical localization of the low molecular weight stress protein HSP27 following focal cerebral ischemia in the rat. *Brain Res* 679: 1-7.
- Kim JY, Kim N, Yenari MA, Chang W (2011) Mild Hypothermia Suppresses Calcium-Sensing Receptor (CaSR) Induction Following Forebrain Ischemia While Increasing GABA-B Receptor 1 (GABA-B-R1) Expression. *Transl Stroke Res* 2:195-201.

- Le Thuc O, Blondeau N, Nahon JL, Rovere C (2015) The complex contribution of chemokines to neuroinflammation: switching from beneficial to detrimental effects. *Ann N Y Acad Sci* 1351:127-140.
- Lee MY, Kuan YH, Chen HY, Chen TY, Chen ST, Huang CC, Yang IP, Hsu YS, Wu TS, Lee EJ (2007) Intravenous administration of melatonin reduces the intracerebral cellular inflammatory response following transient focal cerebral ischemia in rats. *J Pineal Res* 42:297-309.
- Li YB, Wang Y, Tang JP, Chen D, Wang SL (2015) Neuroprotective effects of ginsenoside Rg1-induced neural stem cell transplantation on hypoxic-ischemic encephalopathy. *Neural Regen Res* 10:753-759.
- Liu C, Chen S, Kamme F, Hu BR (2005a) Ischemic preconditioning prevents protein aggregation after transient cerebral ischemia. *Neuroscience* 134:69-80.
- Liu C, Gao Y, Barrett J, Hu B (2010) Autophagy and protein aggregation after brain ischemia. *J Neurochem* 115:68-78.
- Liu CL, Ge P, Zhang F, Hu BR (2005b) Co-translational protein aggregation after transient cerebral ischemia. *Neuroscience* 134:1273-1284.
- Liu CL, Martone ME, Hu BR (2004a) Protein ubiquitination in post-synaptic densities after transient cerebral ischemia. *J Cereb Blood Flow Metab* 24:1219-1225.
- Liu CL, Siesjo BK, Hu BR (2004b) Pathogenesis of hippocampal neuronal death after hypoxia-ischemia changes during brain development. *Neuroscience* 127:113-123.
- Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE (2015) Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 385:430-440.
- Lu Q, Harris VA, Rafikov R, Sun X, Kumar S, Black SM (2015) Nitric oxide induces hypoxia ischemic injury in the neonatal brain via the disruption of neuronal iron metabolism. *Redox Biol* 6:112-121.
- Marcinkiewicz J, Grabowska A, Chain B (1995) Nitric oxide up-regulates the release of inflammatory mediators by mouse macrophages. *Eur J Immunol* 25:947-951.
- Morishima N, Nakanishi K, Takenouchi H, Shibata T, Yasuhiko Y (2002) An endoplasmic reticulum stress-specific caspase cascade in apoptosis. Cytochrome c-independent activation of caspase-9 by caspase-12. *J Biol Chem* 277:34287-34294.
- Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaesig F, Castranova V, Thompson M (2009) Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater* 8:543-557.
- O'Neill LA, Kaltschmidt C (1997) NF-kappa B: a crucial transcription factor for glial and neuronal cell function. *Trends Neurosci* 20:252-258.
- Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C (2003) Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. *Biochem Biophys Res Commun* 304:505-512.
- Prentice H, Modi JP, Wu JY (2015) Mechanisms of Neuronal Protection against Excitotoxicity, Endoplasmic Reticulum Stress, and Mitochondrial Dysfunction in Stroke and Neurodegenerative Diseases. *Oxid Med Cell Longev* 2015:964518.
- Robertson CL, Scafidi S, McKenna MC, Fiskum G (2009) Mitochondrial mechanisms of cell death and neuroprotection in pediatric ischemic and traumatic brain injury. *Exp Neurol* 218:371-380.
- Rosenkranz K, May C, Meier C, Marcus K (2012) Proteomic analysis of alterations induced by perinatal hypoxic-ischemic brain injury. *J Proteome Res* 11:5794-5803.
- Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF (1996) Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 16:675-686.
- Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, Sasaki R (1998) In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci U S A* 95:4635-4640.
- Salmina AB, Okuneva OS, Mikhutkina SV, Malinovskaya NA, Morgun AV, Zykova LD, Yudin GV, Laletin DV, Fursov MA, Frolova OV, Tagaeva GA, Bolshakova EV (2011) Perinatal hypoxic-ischemic brain injury affects the glutamatergic signal transduction coupled with neuronal ADP-ribosyl cyclase activity. *Bull Exp Biol Med* 150:583-586.
- Salminen A, Kauppinen A, Kaarniranta K (2016) Hypoxia/ischemia activate processing of Amyloid Precursor Protein: impact of vascular dysfunction in the pathogenesis of Alzheimer's disease. *J Neurochem* doi: 10.1111/jnc.13932.
- Siesjo BK, Siesjo P (1996) Mechanisms of secondary brain injury. *Eur J Anaesthesiol* 13:247-268.
- Siman R, Noszek JC, Kegerise C (1989) Calpain I activation is specifically related to excitatory amino acid induction of hippocampal damage. *J Neurosci* 9:1579-1590.
- Sun Y, Calvert JW, Zhang JH (2005) Neonatal hypoxia/ischemia is associated with decreased inflammatory mediators after erythropoietin administration. *Stroke* 36:1672-1678.
- Tagin MA, Woolcott CG, Vincer MJ, Whyte RK, Stinson DA (2012) Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis. *Arch Pediatr Adolesc Med* 166:558-566.
- Taylor JP, Hardy J, Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. *Science (New York, NY)* 296:1991-1995.
- Vodovotz Y, Chesler L, Chong H, Kim SJ, Simpson JT, DeGraff W, Cox GW, Roberts AB, Wink DA, Barcellos-Hoff MH (1999) Regulation of transforming growth factor beta1 by nitric oxide. *Cancer Res* 59:2142-2149.
- Wang X, Carlsson Y, Basso E, Zhu C, Rousset CI, Rasola A, Johansson BR, Blomgren K, Mallard C, Bernardi P, Forte MA, Hagberg H (2009) Developmental shift of cyclophilin D contribution to hypoxic-ischemic brain injury. *J Neurosci* 29:2588-2596.
- Weiss C, Jebara F, Nisemblat S, Azem A (2016) Dynamic Complexes in the Chaperonin-Mediated Protein Folding Cycle. *Front Mol Biosci* 3:80.
- Willis JD, Mazarei M, Stewart CN Jr (2016) Transgenic Plant-Produced Hydrolytic Enzymes and the Potential of Insect Gut-Derived Hydrolases for Biofuels. *Front Plant Sci* 7:675
- Xiang Y, Gao H, Zhu H, Sun N, Ma Y, Lei H (2006) Neurochemical changes in brain induced by chronic morphine treatment: NMR studies in thalamus and somatosensory cortex of rats. *Neurochem Res* 31:1255-1261.
- Yamamoto S, Golanov EV, Berger SB, Reis DJ (1992) Inhibition of nitric oxide synthesis increases focal ischemic infarction in rat. *J Cereb Blood Flow Metab* 12:717-726.
- Yang W, Zhang X, Wang N, Tan J, Fang X, Wang Q, Tao T, Li W (2016) Effects of Acute Systemic Hypoxia and Hypercapnia on Brain Damage in a Rat Model of Hypoxia-Ischemia. *PLoS One* 11:e0167359.
- Yao Y, Zheng XR, Zhang SS, Wang X, Yu XH, Tan JL, Yang YJ (2016) Transplantation of vascular endothelial growth factor-modified neural stem/progenitor cells promotes the recovery of neurological function following hypoxic-ischemic brain damage. *Neural Regen Res* 11:1456-1463.
- Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. *Cell* 94:739-750.
- Zanelli SA, Rajasekaran K, Grosenbaugh DK, Kapur J (2015) Increased excitability and excitatory synaptic transmission during in vitro ischemia in the neonatal mouse hippocampus. *Neuroscience* 310:279-289.
- Zhan L, Liu L, Li K, Wu B, Liu D, Liang D, Wen H, Wang Y, Sun W, Liao W, Xu E (2016) Neuroprotection of Hypoxic Postconditioning against Global Cerebral Ischemia through Influencing Posttranslational Regulations of Heat Shock Protein 27 in Adult Rats. *Brain Pathol* doi: 10.1111/bpa.12472.
- Zhang F, Liu CL, Hu BR (2006) Irreversible aggregation of protein synthesis machinery after focal brain ischemia. *J Neurochem* 98:102-112.
- Zhong XZ, Yang Y, Sun X, Dong XP (2016) Methods for monitoring Ca²⁺ and ion channels in the lysosome. *Cell Calcium* doi:10.1016/j.jceca.2016.12.001.
- Zhu C, Qiu L, Wang X, Xu F, Nilsson M, Cooper-Kuhn C, Kuhn HG, Blomgren K (2009) Age-dependent regenerative responses in the striatum and cortex after hypoxia-ischemia. *J Cereb Blood Flow Metab* 29:342-354.