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·Minireview·

Mechanisms of lysosomal proteases participating in cerebral ischemia-induced neuronal death

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Abstract: There are three different types of cell death, including apoptosis (Type I), autophagic cell death (Type II), and necrosis (Type III). Ischemic neuronal death influences stroke development and progression. Lysosomes are important organelles having an acidic milieu to maintain cellular metabolism by degrading unneeded extra- and intracellular substances. Lysosomal enzymes, including cathepsins and some lipid hydrolases, when secreted following rupture of the lysosomal membrane, can be very harmful to their environment, which results in pathological destruction of cellular structures. Since lysosomes contain catalytic enzymes for degrading proteins, carbohydrates and lipids, it seems natural that they should participate in cellular death and dismantling. In this review, we discuss the recent developments in ischemic neuronal death, and present the possible molecular mechanisms that the lysosomal enzymes participate in the three different types of cell death in ischemic brain damage. Moreover, the research related to the selective cathepsin inhibitors may provide a novel therapeutic target for treating stroke and promoting recovery.

Keywords: lysosomes; cathepsin; necrosis; apoptosis; autophagy; cerebral ischemia

1 Introduction

Being the second most common cause of death in industrial countries and one of the major causes of death and disability, stroke has a great effect on public health and is the neurological disease which accounts for the largest number of hospitalizations. Neuronal death in cerebral ischemia influences stroke development and progression, as well as the response to cerebroprotective therapies. The acidic organelles, lysosomes, have recently been shown to implicate the neuronal cell death following cerebral ischemia. To date, there are three different types of cell death, including apoptosis (Type I), autophagic cell death (Type II), and necrosis (Type III) based on biochemical and morphological criteria[1]. Until the mid 1990s, most researchers thought that neurons injured in stroke died from necrosis. However, in the past few years, evidence of apoptosis in lower species animals of cerebral ischemia has emerged. More recently, studies have demonstrated that autophagy is also activated during cerebral ischemia.

Lysosomes are ubiquitous in all animal cells, as an acidic compartment with limiting membranes and contain various types of hydrolytic enzymes with acidic pH optima. Lysosomal proteolytic enzymes normally reside in lysosomes and are responsible for protein breakdown. Regulation of intracellular protein metabolism might cause a pathological proteolysis of cytosolic proteins and membranes leading to further cellular damage when released into the cytoplasm.

2 Lysosomes and lysosomal enzymes

Lysosomes were first described in 1955 by de Duve and his collaborators—a discovery that led to the Nobel prize award—as intracytoplasmic organelles full of acid hydrolases and surrounded by a single membrane[2]. Lysosomes,

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which are the major machinery for recycling intra- and extracellular material, have long been regarded as "suicide bags". For this purpose, lysosomes contain numerous enzymes—probably more than fifty—allowing for degradation of proteins, nucleic acids, polysaccharides, lipids and their conjugates.

Lysosomal acidic hydrolases comprise a variety of proteases, nucleases, glycosidases, sulfatases and lipases. The main class of lysosomal proteases is represented by the cathepsins. They are subdivided into three subgroups according to the amino acid of their active sites that confer catalytic activity: cysteine (cathepsins B, C, F, H, K, L, N, O, S, T, U, W and X), aspartyl (cathepsins D and E) and serine cathepsins (cathepsins A and G)[3]. Cathepsins B and L are major lysosomal cysteine proteases of neurons that might be important in the intracellular protein catabolism[4].

3 Lysosomal rupture during cerebral ischemia

3.1 Spreading of lysosomal enzyme into the cytoplasm The lysosomal membrane is known to be a physical barrier that prevents hydrolytic enzymes from digesting the cell's own cytoplasm. The spreading of hydrolytic enzymes from lysosomes into the cytoplasm due to the lysosomal membrane becoming injured or ruptured, has been suggested in different animal models of cerebral ischemia. For example, it has been well documented that increases in immunoreactivity of cathepsins B, H and L in the CA1 pyramidal neurons were observed 3 d in an experiment with a gerbil with brief forebrain ischemia, where cathepsin B was localized in an increased volume of lysosomes and autophagic vacuoles[5]. Seyfried D *et al.* demonstrated that increased cathepsin B immunoreactivity was detected exclusively within the ischemic neurons after 2 h of reperfusion following a 2-h middle cerebral artery occlusion (MCAO) in rats[6]. Benchoua A *et al.* reported that cytoplasmic activation of cathepsin B was an early event after a distal and permanent middle cerebral artery occlusion (pMCAO), and immunohistochemistry revealed the colocalization of cathepsin B with caspase-1 and caspase-11 in cells of the infarct core[7]. Tsubokawa T *et al.* showed that an initial rise in calpain and cathepsin B activity was detected as early as 2 h after the reperfusion in the peri-infarct penumbra cortex and the activation of MMP-9, calpain, and cathepsin B were extensively colocalized in both the neuronal cells and the neurovascular structures 24 h after the MCAO insult in rats[8].

3.2 Mechanism of lysosomal leakage So far, the cause and mechanisms of lysosomal rupture or destabilization are not yet completely understood. One of the molecules that have been shown to regulate lysosomal membrane permeabilization (LMP) is sphingosine. Given lysosomal (potential) detergent and lysosomotropic properties, the sphingosine accumulated in lysosomes could permeabilize lysosomal membranes and facilitate the relocation of some lysosomal proteases to the cytosol. In addition, sphingosine has been shown to cause LMP and cell death in a dose-dependent manner. Sphingosine induces partial lysosomal rupture and apoptosis at low-to-moderate concentrations (< 20 μmol/L), and extensive lysosomal rupture and necrosis at high concentration (> 20 μmol/L)[9].

Another explanation for triggering lysosomal rupture implicates the generation of reactive oxygen species (ROS). The intracellular Ca^{2+} elevation activates phospholipase A2 (PLA2). This enzyme liberates the unsaturated fatty acid arachidonic acid and thus initiates the formation of free radicals via the cyclooxygenase and lipoxygenase pathways, causing lipid peroxidation of subcellular membranes[10]. İlekel H *et al.* observed that severe ischemia reperfusion caused lipid peroxidation in cellular membranes and disruption of lysosomal membranes[11].

It has been proposed that the rise of intracellular calcium during ischemia activates μ-calpain located at the lysosomal membranes which breaks down lysosomal membranes releasing cathepsin B and L into the cytoplasm, causing degradation of cellular components[12].

The translocation of pro-apoptotic members of the Bcl-2 family into the lysosomal membrane might be an alternative mechanism of lysosomal rupture. Kagedal K *et al*. reported that Bax, a pro-apoptotic Bcl-2 member, could translocate to the lysosomal membranes in response to staurosporine treatment and promote the release of lysosomal enzymes into the cytosol[13]. On the other hand, it was shown that Bcl-2, an anti-apoptotic protein, was able to inhibit oxidative stress-induced apoptosis by blocking lysosomal rupture[14,15].

Finally, the possibility that lysosomal proteases themselves may permeate the lysosomal membrane cannot be ruled out. Indeed, after TNF–α treatment lysosomal leakage is strongly decreased in cathepsin B-deficient cells[16]. Moreover, the serine protease inhibitor Spi2A, which also inhibits cathepsin B, partially blocks lysosomal membrane

leakage[17].

The further study should clarify whether these different mechanisms play an active role in lysosomal rupture and how they are controlled.

4 Lysosome and lysosomal enzymes contribute to different modes of neuronal death in cerebral ischemia

While accumulating experimental evidences suggest the involvement of lysosomes or lysosomal constituents in some cell death programmes, the molecular mechanisms that underlie the action of these organelles or components in the death cascades are not yet fully understood. Several mechanisms have been proposed that may connect "the lysosomal pathway(s)" of cell death to other well-documented death pathways[3].

4.1 Ischemic neuronal death: necrosis and apoptosis Both necrotic and apoptotic cell death mechanisms are activated after cerebral ischemia. Studies have shown that most apoptotic neurons are distributed in the penumbra while neurons in the core region die of necrosis. In addition, both apoptotic and necrotic death pathways occur in the same cell or appear in discrete cell groups, e.g. large chromatin clump, nuclear condensation/fragmentation, and swollen cytoplasm, damaged organelles and deteriorated membranes co-exist in the same neuron after transient and pMCAO models in rats[18-20].

4.1.1 The role of lysosomes in necrosis Up to now, we know very little about the mechanism of necrosis. We usually define necrosis as caspase-independent cell death that occurs under certain normal physiological and pathological conditions. The "calpain-cathepsin" hypothesis was proposed to explain necrotic cell death after ischemic brain injury. Using a monkey model of 20 min whole brain complete ischemia, Yamashima T *et al*. provided direct evidence of CA1-specific calpain, which was activated by intracellular Ca^{2+} overload, prior to occurrence of ischemic neuronal death[12]. Furthermore, immunoelectron microscopy revealed that activated μ-calpain was localized at the vacuolated or disrupted membrane of lysosomes at the subcellular level. These data taken together suggested that excessive calpain activation in the postischemic CA1 neurons might had caused lysosomal membrane disruption which was confirmed by electron microscopy[4]. Studies with the synthetic lysosomotropic detergent MSDH indicated that the key factor in determining the type of cell death was the magnitude of lysosomal membrane permeabilization and the amount of proteolytic enzymes released into the cytosol. Of note, a necrotic cell death can be triggered by a too strong LMP[21]. However, since the identities of cathepsins involved in executing mammalian necrosis are unclear, a direct involvement of lysosomes in necrosis must also be viewed as tentative.

4.1.2 The role of lysosomes in apoptosis The role of lysosomes in what we today call programmed cell death (PCD) or apoptosis was initially suggested by Christian de Duve who nicknamed the organelles that he and his coworkers had recently discovered "suicidal bags"[2]. It is widely accepted that cysteine proteases, called caspases, are crucially important as executors of apoptosis. Among the identified caspases, caspase-3 is a potent effector of apoptosis in a variety of cell types, and promotes neuronal death during ischemic brain injury.

Recently, loss of mitochondrial or lysosomal barrier function during ischemic neuronal damage is supposed to play an important role in making certain caspases to participate in apoptosis. Oxidative stress has been demonstrated to cause destabilization of lysosomal membranes. Lysosomal enzymes may induce mitochondrial permeability transition (MPT) either directly or indirectly, through proteolytic activation of phospholipases or the Bcl-2 family members Bid, Bax and Bak. Cysteine proteases, such as cathepsins B, H and L, have been shown to cleave Bid which in its truncated form translocated to mitochondria resulting in Bax/Bak activation, while cathepsin D has been reported to activate both Bid and Bax[22-26]. Consequently, pro-apoptotic mitochondrial factors such as cytochrome c, apoptosis inducing factor (AIF) and Smac/DIABLO release into the cytosole[24,25]. Cytochrome c induces ATP- or dATP-dependent formation of a complex of proteins such as the ced-4 homolog Apaf-1, and caspase-9 (Apaf-3) that results in the proteolytic activation of procaspase-3[27,28].

Lysosomal cysteine proteases are believed to be involved in in neuronal apoptosis. For instance, Nitatori T *et al.* reported that cathepsins B, H and L underwent increased expression in the ischemic CA1 neurons experiencing delayed shrinkage and chromatin condensation, suggesting that these enzymes may participate in the cytoskeletal breakdown and DNA fragmentation, which is a characteristic of

apoptotic neuronal death following transient ischemia in gerbils[5]. Benchoua A *et al.* showed that cytoplasmic activation of cathepsin B was observed from the early stages of MCAO, and displayed an activation pattern parallel to the activation pattern of caspase-1 and caspase-11[7]. Ünal-Çevik I *et al.* demonstrated that an early and concurrent increase in caspase-3 and cathepsin-B activities was followed by an appearance of caspase-cleavage products, DNA fragmentation, which suggested that apoptotic cell death were activated in ischemic neurons after four hours in pMCAO models in mouse[19]. In addition, our lab reported that cathepsin B participated in kainic acid (KA)-induced neuronal apoptosis. Furthermore, inhibition of cathepsin B by Z-FA-FMK attenuated KA-induced striatal damage, as measured both by the amount of DNA fragmentation and by the lesion size[29]. In brief, the present studies support the hypothesis that lysosomal enzymes are involved in apoptosis induced by cerebral ischemia.

4.2 The role of lysosomes in autophagy It should be pointed out that lysosomes are also involved in so-called autophagic cell death. Autophagy (Greek, "self eating"), which includes macroautophagy, microautophagy, and chaperone-mediated autophagy, is an intracellular lysosome-mediated catabolic mechanism that is responsible for the bulk degradation and recycling of damaged or dysfunctional cytoplasmic components and intracellular organelles[30]. Autophagy is an evolutionarily ancient cellular response to both extracellular stress conditions (nutrient deprivation, hypoxia) and intracellular stress conditions (accumulation of damaged organelles and cytoplasmic components) that allows lower eukaryotic organisms such as yeast to survive nutrient starvation conditions by recycling. It is characterized by sequestration of bulk cytoplasm and organelles in double or multimembrane autophagic vesicles. All autophagic vacuoles eventually fuse with lysosomes, which provide hydrolases as degradating enzymes. Visualization of autophagic vesicles by an electron microscopy is the best way to show that autophagy is taking place. The involvement of autophagy in ischemic brain has only recently been described. In 1995, Nitatori T *et al.* were the first to show that the density of cathepsin B-positive lysosomes and autophagic vacuole-like structures markedly increased 3 d after ischemic insult[5]. This suggested that the cathepsin B-immunopositive lysosomes that increased in the neurons after brief ischemia in gerbils were mostly autolysosomes. Zhu C *et al.* observed that increased LC3-II (autophagosome-related marker) levels were detected as early as 8 h, much more pronounced at 24 h and 72 h after hypoxic-ischemia, and that the activation of autophagy, as judged by the recruitment of LC3-II, was three times more pronounced in the adult brains compared with those in the immature brains[31]. Adhami F *et al.* reported that many damaged neurons showed features of autophagic/lysosomal cell death during cerebral ischemia-hypoxia, e.g. cytoplasmic autophagic vacuoles and the induction of GFP-LC3 immunofluorescence[32]. Our laboratory also has observed that the induction of LC3 immunofluorescence and resembled autophagic vacuoles are seen in the damaged neurons and glia in rat models of pMCAO (unpublished data). Together, these results suggest that ischemia-hypoxia is a powerful stimulus for autophagic/ lysosomal cell death in brain. In addition, nerve growth factor (NGF) deprivation-induced sympathetic neuronal cell death and serum deprivation-induced death of PC12 cells were also found to exhibit autophagic features[33,34].

It has long been clear that not all deaths can be neatly categorized. In some situations, however, apoptosis and autophagy are observed in the same cells[35]. Furthermore, some overlaps in the control of autophagy and apoptosis exist. Ceramide, Fas-Associated protein with Death Domain (FADD), Death Associated Protein kinase (DAP), and Bcl-xL/Bcl-2 which are all involved in apoptotic control, have also been shown to modulate autophagy[36-39]. Recently, several laboratories have reported that molecules previously defined as intermediaries in the activation of apoptosis also function as intermediaries in the activation of autophagy. For instance, in neural precursor cells, deprivation of growth factors leads to an autophagic cell death, which can be blocked by the anti-apoptotic Bcl-2[40]. Ceramide, which has been considered by many researchers participating in the activation of apoptosis, is effective in establishing macroautophagy[38].

5 Neuroprotective effects of cysteine inhibitors in cerebral ischemia

Until now most of the therapeutic clinical trials based on such paradigm have yet to be proved effective for human ischemic brain injuries using agents affecting excitotoxicity and neuronal Ca^{2+} overload such as glutamate antagonists, voltage-gated Ca^{2+} channel antagonists, voltage-dependent

potassium-channel agonists, sodium-channel antagonists, GABA agonists, and free-radical scavengers[41]. Recent works suggest that cysteine protease inhibitors should be added to the list of potential new drugs for stroke.

During focal cerebral ischemia induced by the occlusion of the middle cerebral artery, the cysteine protease inhibitors stefin A and CP-1 decreased infarct size in rats[6,42]. E64d, a μ-calpain and cathepsin B inhibitor, had significant brain protection against ischemic damage. Tsubokawa T *et al.* observed a reduction of infarction volume and brain edema, and improved neurological scores in E64d-treated rats compared with the nontreated control during 2 h of transient focal ischemia from MCAO[43]. CA-074, a specific cathepsin B inhibitor, and E-64c, an inhibitor of both cathepsin B and L, confered neuroprotection to the hippocampal subregion CA1of monkey brains undergoing transient ischemia[44]. These observations support the hypothesis that the lysosomal cathepsins contribute to cerebral injuries induced by ischemia. More recently, Luck CJ *et al.* reported that the *C. elegans* intracellular serpin, SRP-6, exhibited a prosurvival function by blocking necrosis. Minutes after hypotonic shock, SRP-6 null animals underwent a catastrophic series of events culminating in lysosomal disruption, cytoplasmic proteolysis, and death. By protecting against both the induction and the lethal effects of lysosomal injury, SRP-6 also blocked death induced by heat shock, oxidative stress and hypoxia[45].

6 Conclusions

Stroke is a leading cause of death and permanent disability, but there are currently no effective treatments. One should be noted that the landscape of ischemic brain injury mechanisms pay particular attentions to the cysteine proteases[4]. Lysosomal proteins are released into cytoplasm in different animal models of cerebral ischemia. So far, the cause and mechanisms of lysosomal rupture or destabilization are not yet completely understood. A few explanations are the intracellular Ca^{2+} elevation, the generation of free radicals and other oxidant species. Lysosomal proteins are involved in the neuronal death induced by cerebral ischemia, including necrosis, apoptosis and autophagic cell death in different possible mechanisms. These different possible mechanisms may probably overlap. Selective cathepsin inhibitors, such as CA-074 and E-64d, which can reduce infarct volume and brain water content, could be therapeutic in ischemic injury. Elucidation of the contribution of lysosomal enzymes to cerebral ischemia and underlying mechanisms will shed a new light on the "old" organelles and hopefully pave the way for the development of novel therapeutic treatments in stroke.

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References:

[1] Schweichel JU, Merker HJ. The morphology of various types of cell death in prenatal tissues. Teratology 1973, 7: 253-266.

[2] de Duve C. Lysosomes revisited. Eur J Biochem 1983, 137: 391-397.

[3] Tardy C, Codogno P, Autefage H, Levade T, Andrieu-Abadie N. Lysosomes and lysosomal proteins in cancer cell death (new players of an old struggle). Biochim Biophys Acta 2006, 1765: 101-125.

[4] Yamashima T. Implication of cysteine proteases calpain, cathepsin and caspase in ischemic neuronal death of primates. Prog Neurobiol 2000, 62: 273-295.

[5] Nitatori T, Sato N, Waguri S, Karasawa Y, Araki H, Shibanai K, *et al.* Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. J Neurosci 1995, 15: 1001-1011.

[6] Seyfried D, Han Y, Zheng Z, Day N, Moin K, Rempel S, *et al.* Cathepsin B and middle cerebral artery occlusion in the rat. J Neurosurg1997, 87: 716-723.

[7] Benchoua A, Braudeau J, Reis A, Couriaud C, Onténiente B. Activation of proinflammatory caspases by cathepsin B in focal cerebral ischemia. J Cereb Blood Flow Metab 2004, 24: 1272-1279.

[8] Tsubokawa T, Solaroglu I, Yatsushige H, Cahill J, Yata K, Zhang JH. Cathepsin and calpain inhibitor E64d attenuates matrix metalloproteinase-9 activity after focal cerebral ischemia in rats. Stroke 2006, 37: 1888-1894.

[9] Kagedal K, Zhao M, Svensson I, Brunk UT. Sphingosine-induced apoptosis is dependent on lysosomal proteases. Biochem J 2001, 359: 335-343.

[10] Imagawa DK, Osifchin NE, Paznekas WA, Shin ML, Mayer MM. Consequences of cell membrane attack by complement: release of arachidonate and formation of inflammatory derivatives. Proc Natl Acad Sci USA 1983, 80: 6647-6651.

[11] İlekel H, İlekel S, Güner G, Özdamar N. Evaluation of lipid peroxidation, cathepsin L and acid phosphatase activities in experimental brain ischemia-reperfusion. Brain Res 1999, 843: 18-24.

[12] Yamashima T, Saido TC, Takita M, Miyazawa A, Yamano J, Miyakawa A, *et al.* Transient brain ischemia provokes Ca^{2+}, PIP2 and calpain responses prior to delayed neuronal death in monkeys. Eur J Neurosci 1996, 8: 1932-1944.

[13] Kagedal K, Johansson AC, Johansson U, Heimlich G, Roberg K, Wang NS, *et al.* Lysosomal membrane permeabilization during apoptosis-involvement of Bax? Int J Exp Pathol 2005, 86: 309-321.

[14] Zhao M, Eaton JW, Brunk UT. Protection against oxidant-mediated lysosomal rupture: a new anti-apoptotic activity of Bcl-2? FEBS Lett 2000, 485: 104-108.

[15] Zhao M, Eaton JW, Brunk UT. Bcl-2 phosphorylation is required for inhibition of oxidative stress-induced lysosomal leak and ensuing apoptosis. FEBS Lett 2001, 509: 405-412.

[16] Werneburg NW, Guicciardi ME, Bronk SF, Gores GJ. Tumor necrosis factor-alpha-associated lysosomal permeabilization is cathepsin B dependent. Am J Physiol Gastrointest Liver Physiol 2002, 283: G947-G956.

[17] Liu N, Raja SM, Zazzeroni F, Metkar SS, Shah R, Zhang M, *et al.* NF-kappaB protects from the lysosomal pathway of cell death. EMBO J 2003, 22: 5313-5322.

[18] Zeng YS, Xu ZC. Co-existence of necrosis and apoptosis in rat hippocampus following transient forebrain ischemia. Neurosci Res 2000, 37: 113-125.

[19] Ünal-Çevik I, Kilinç M, Can A, Gürsoy-Özdemir Y, Dalkara T. Apoptotic and Necrotic Death Mechanisms Are Concomitantly Activated in the Same Cell After Cerebral Ischemia. Stroke 2004, 35: 2189-2194.

[20] Wei L, Ying DJ, Cui L, Langsdorf J, Yu SP. Necrosis, apoptosis and hybrid death in the cortex and thalamus after barrel cortex ischemia in rats. Brain Res 2004, 1022: 54-61.

[21] Li W, Yuan X, Nordgren G, Dalen H, Dubowchik GM, Firestone RA, *et al.* Induction of cell death by the lysosomotropic detergent MSDH. FEBS Lett 2000, 470: 35-39.

[22] Brunk UT, Neuzil J, Eaton JW. Lysosomal involvement in apoptosis. Redox Rep 2001, 6: 91-97.

[23] Cirman T, Oresiæ K, Mazovec GD, Turk V, Reed JC, Myers RM, *et al.* Selective disruption of lysosomes in HeLa cells triggers apoptosis mediated by cleavage of Bid by multiple papain-like lysosomal cathepsins. J Biol Chem 2004, 279: 3578-3587.

[24] Guicciardi ME, Leist M, Gores GJ. Lysosomes in cell death. Oncogene 2004, 23: 2881-2890.

[25] Bidère N, Lorenzo HK, Carmona S, Laforge M, Harper F, Dumont C, *et al.* Cathepsin D triggers Bax activation, resulting in selective apoptosis-inducing factor (AIF) relocation in T lymphocytes entering the early commitment phase to apoptosis. J Biol Chem 2003, 278: 31401-31411.

[26] Heinrich M, Neumeyer J, Jakob M, Hallas C, Tchikov V, Winoto-Morbach S, *et al.* Cathepsin D links TNF-induced acid sphingomyelinase to Bid-mediated caspase-9 and -3 activation. Cell Death Differ 2004, 11: 550-563.

[27] Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, *et al.* Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. Cell 1997, 91: 479-489.

[28] Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997, 90: 405-413.

[29] Wang Y, Gu ZL, Cao Y, Liang ZQ, Han R, Bennett MC, *et al.* Lysosomal enzyme cathepsin B is involved in kainic acid-induced excitotoxicity in rat striatum. Brain Res 2006, 1071: 245-249.

[30] Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000, 290: 1717-1721.

[31] Zhu C, Wang X, Xu F, Bahr BA, Shibata M, Uchiyama Y, *et al.* The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia-ischemia. Cell Death Differ 2005, 12: 162-176.

[32] Adhami F, Liao G, Morozov YM, Schloemer A, Schmithorst VJ, Lorenz JN, *et al.* Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. Am J Pathol 2006, 169: 566-583.

[33] Ohsawa Y, Isahara K, Kanamori S, Shibata M, Kametaka S, Gotow T, *et al.* An ultrastructural and immunohistochemical study of PC12 cells during apoptosis induced by serum deprivation with special reference to autophagy and lysosomal cathepsins. Arch Histol Cytol 1998, 61: 395-403.

[34] Xue L, Fletcher GC, Tolkovsky AM. Autophagy is activated by apoptotic signalling in sympathetic neurons: an alternative mechanism of death execution. Mol Cell Neurosci 1999, 14: 180-198.

[35] Gozuacik D, Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. Oncogene 2004, 23: 2891-2906.

[36] Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, *et al.* Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004, 6: 1221-1228.

[37] Thorburn J, Moore F, Rao A, Barclay WW, Thomas LR, Grant KW, *et al*. Selective inactivation of a Fas-associated death domain protein (FADD)-dependent apoptosis and autophagy pathway in immortal epithelial cells. Mol Biol Cell 2005, 16: 1189-1199.

[38] Scarlatti F, Bauvy C, Ventruti A, Sala G, Cluzeaud F, Vandewalle A, *et al.* Ceramide-mediated macroautophagy involves inhibition of protein kinase B and up-regulation of beclin 1. J Biol Chem 2004, 279: 18384-18391.

[39] Inbal B, Bialik S, Sabanay I, Shani G, Kimchi A. DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. J Cell Biol 2002, 157: 455-468.

[40] Cardenas-Aguayo Mdel C, Santa-Olalla J, Baizabal JM, Salgado LM, Covarrubias L. Growth factor deprivation induces an alternative non-apoptotic death mechanism that is inhibited by Bcl-2 in

cells derived from neural precursor cells. J Hematother Stem Cell Res 2003, 12: 735-748.

[41] Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischemic brain injury mechanisms. Nature 1999, 399 (6738 Suppl): A7-A14.

[42] Seyfried DM, Veyna R, Han Y, Li K, Tang N, Betts RL, *et al.* A selective cysteine protease inhibitor is non-toxic and cerebroprotective in rats undergoing transient middle cerebral artery ischemia. Brain Res 2001, 901: 94-101.

[43] Tsubokawa T, Yamaguchi-Okada M, Calvert JW, Solaroglu I, Shimamura N, Yata K, *et al.* Neurovascular and neuronal protection by E64d after focal cerebral ischemia in rats. J Neurosci Res 2006, 84: 832-840.

[44] Yoshida M, Yamashima T, Zhao L, Tsuchiya K, Kohda Y, Tonchev AB, *et al.* Primate neurons show different vulnerability to transient ischemia and response to cathepsin inhibition. Acta Neuropathol 2002, 104: 267-272.

[45] Luke CJ, Pak SC, Askew YS, Naviglia TL, Askew DJ, Nobar SM, *et al.* An intracellular serpin regulates necrosis by inhibiting the induction and sequelae of lysosomal injury. Cell 2007, 130: 1108-1119.

溶酶体酶参与脑缺血性神经元死亡的分子机制

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摘要：细胞死亡有凋亡(I 型)、自噬性细胞死亡(II 型)和坏死(III 型) 三种方式。缺血性神经元的死亡影响着中风的发展进程。溶酶体是一种重要的细胞器，通过在酸性环境中降解不需要的胞外和胞内物质来维持细胞代谢的稳态。溶酶体酶包括组织蛋白酶和脂质水解酶，当溶酶体膜破裂时它们会被释放到细胞浆，会对细胞内环境产生危害，最终导致细胞结构的破坏。由于溶酶体含有催化蛋白、碳水化合物和脂质的酶，因此它们参与细胞的死亡看起来是情理之中的事情。本综述讨论了缺血性神经元死亡的最新进展, 指出在缺血性脑损伤中溶酶体酶参与三种细胞死亡方式的可能的分子机制，同时指出了选择性的组织蛋白酶抑制剂可能是治疗中风和促进康复新的治疗靶点。

关键词：溶酶体；组织蛋白酶；坏死；凋亡；自噬；脑缺血