Cell Death Mode Switch from Necrosis to Apoptosis in Brain

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In brain ischemia, cell destructive necrosis occurs in the core, which in turn links to cell death expansion in the vicinity. Apoptosis, on the other hand, occurs in the surroundings of the core, called the penumbra, several days later. As cells showing apoptosis disappear by microglial phagocytosis in the brain, cell death induced by ischemic stress should eventually be terminated. Thus, the authors propose the hypothesis that the cell death mode switch in the event of brain ischemia is an in vivo self-protective mechanism. The authors attempt to overview the current understanding of the molecular mechanisms of necrosis and apoptosis in relation to the ATP hypothesis, and also introduce novel mechanisms for an in vitro cell death mode switch.

Key words necrosis; apoptosis; cell death mode switch; ischemia; ATP

INTRODUCTION

Several billions of neurons in the brain form complex networks and manifest various functions which involve survival and plasticity, such as memory and learning. As neurons are postmitotic cells, they should survive for a long period of life by virtue of many self-protective mechanisms in the brain against various stresses, or in order to maintain their functions. Representative self-protective mechanisms in the brain are mediated by neurotrrophic factors or cytokines working in neuron-neuron and neuron-glia communities.1–3) Neurogenesis might be added to such self-protective mechanisms. The existence of neuronal stem cells in the adult brain has recently been reported, and their physiological roles have been discussed in relation to the formation of neuronal circuits and in neuroprotection against brain injury.4–7) The third mechanism for self-protection would be through a cell death mode switch.8,9) Following ischemic stress of the brain, necrosis occurs first in the ischemic core, while apoptosis occurs several days later at the region surrounding the core, called the penumbra.10,11) Since necrotic cells induce secondary damage in surrounding cells, while apoptotic cells do not, a switch in the mode of cell death from necrosis to apoptosis could play a role in ameliorating the spread of cell death in the brain.12) Here, we overview the current status as to molecular basis of mechanisms of necrosis and apoptosis, and propose a new hypothesis for the role of a cell death mode switch in the self-protection of the ischemic brain.

1. MORPHOLOGICAL DIFFERENCE BETWEEN NECROSIS AND APOPTOSIS

Cell death in most cells, including neurons, has been classified into apoptosis and necrosis from a morphological characterization.13) In scanning electron microscopic (SEM) observation, a cultured cortical neuron in the presence of serum has a rough cell surface and neurites (Fig. 1). As early as 6 h after the start of serum-free starvation stress, neurons in the low-density culture had numerous pores throughout the cell.8) By 12 h, all cell structures except for the nuclei were lost. In the transmission electron microscopic (TEM) observation, the cells lost cytoplasmic electron density, and mitochondrial swelling was accompanied with cristae destruction (Fig. 1). These features represent typical necrosis. When high glucose is added to the serum-free culture, the neuron shows smooth cell surface in the beginning, and later has some blebbings on the surface in SEM analysis.9) In TEM analysis, on the other hand, the cell has a split nuclei and chromatin condensation, but there is no significant damage in the mitochondria. All these features represent typical apoptosis.

2. PROPOSED MECHANISMS FOR APOPTOSIS

For the mechanisms of apoptosis, three major pathways have been proposed.14–17) All these mechanisms include caspase-3 activation and nuclear fragmentation as the final step (Fig. 2). The first mechanism is through mitochondrial pathways, where the release of apoptosis-initiation factors from mitochondrial permeability transition pores (mPTP), constituted of adenine nucleotide translocase (ANT) and voltage-dependent anion channel (VDAC), plays a key role in the successive apoptosome formation and caspase-3 activation.15,18–20) Such factors include cytochrome c,21) AIF,22) Smac/DIABLO,23) EndoG,24) and HtrA2/Omi.25) Released cytochrome c forms an apoptosome, together with pro-caspase-9, Apaf1 and ATP to catalyze pro-caspase-9. Thus, the activated caspase-9 further activates caspase-3, which in turns causes DNA fragmentation through an activation of caspase-activated DNase (CAD).26,27) Caspase-3 also catalyzes poly (ADP-ribose) polymerase (PARP), and thereby inhibits the ATP consumption to DNA damage (Fig. 2). This process also plays a role in maintaining the apoptosome formation. The opening of mPTP is positively and negatively regulated by various Bcl-2 family proteins. Bak and Bak, pro-apoptotic proteins, stimulate, while Bcl-2 and Bcl-xl, anti-apoptotic ones, inhibit the open state of mPTP.28,29) Thus, the expression ratio of these pro-apoptotic proteins to anti-apoptotic
ones in the mitochondria, and translocation of these proteins within the mitochondrial membrane, are the major causes of the induction of apoptosis. Several other Bcl-2 family proteins also regulate the mPTP opening. Bad and Bim, other pro-apoptotic proteins, stimulate the opening by absorbing Bcl-2 and Bcl-xL.30 Bad is phosphorylated by Akt, which is the major downstream kinase of many growth factors, and phosphorylated Bad loses the interaction with Bcl-2 and Bcl-xL.31—33 This mechanism could explain growth factor deprivation-induced apoptosis. Regarding Bim, its unphosphorylated form is sequestered to microtubules, while the phosphorylated one is detached from microtubules.34 The free Bim, on the other hand, is transported to the mitochondria for heterodimerization with Bcl-xL.35 As it is known that some growth factors enhance the expression of Bcl-2 and Bcl-xL,36—38 this mitochondrial pathway could be closely related to the actions of growth factors.

The second mechanism is mediated by death receptors. Representative death receptors are homotrimeric Fas and TNFz1 receptors, which mediate death signals such as Fas ligand or TNFα, respectively, through FADD, RIP and TRADD, followed by successive activation of caspase-8 and caspase-3.39,40 A recent report has demonstrated that these receptor mechanisms also activate the mitochondrial pathway through the formation of truncated Bid (tBid) by partial cleavage of Bid.41 There is another apoptotic pathway independent of caspase-3 activation. Death receptor stimulation mediates apoptosis signal-regulating kinase 1 (ASK1) activation through RIP and TRAF-2, which are further linked to Jun N-terminal kinase (JNK) activation.42

The third one is initiated by so-called ER stress, which is caused by the accumulation of abnormal proteins in the endoplasmic reticulum (ER). The ER accumulation of misfolded proteins causes cleavage and activation of IRE1α/β and caspase-12 (caspase-4 in human).43,44 Activated IRE1α/β induces the oligomerization of TRAF-2, leading to apoptosis, as mentioned above.45—47 Activated caspase-12, on the other hand, is linked to caspase-3 activation.

3. ROLES OF CELLULAR ATP IN NECROSIS

Studies of the molecular mechanisms of necrosis remain to be fully clarified, in comparison with apoptosis. Most agreeable mechanisms would be related to an energy failure, or drastic decrease, in cellular ATP levels.48,49 Cellular ATP levels are determined by three parameters: material supply (glucose uptake), ATP synthesis (mostly in the mitochondria) and consumption (Fig. 3). Glucose uptake is mediated by active and passive glucose transporters. In peripheral cells, an insulin receptor mainly mediates the glucose uptake by recruiting glucose transporters, GLUT1 and GLUT4 (GLUT1/4), to the plasma membrane through phosphorylation.50 In neurons, on the other hand, it is reported that glucose supply is mediated by transporters constitutively expressed in the membranes, and by ones regulated by various neurotrophin receptors.51,52

The relevancy of decreased glucose uptake to necrosis induction was recently proven in experiments using cortical neurons in culture under the condition of low-density and serum-free without any supplements.8,9 The prominent cellular events which occurred were a rapid decrease in glucose uptake, measured by [3H]-2-deoxyglucose, and a decrease in
Fig. 2. Proposed Mechanisms of Three Major Pathways for Apoptosis
Details are in the text. Abbreviations: SIMPs, including AIF, cytochrome c, Smac/DIABLO and endoG; SIMPs, soluble intermembrane proteins; cyt. c, cytochrome c; AIF, apoptosis inducing factor; tBid, truncated Bid; Apaf-1, apoptosis protease activating factor-1; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; TRAF-2, tumor necrosis factor receptor-associated factor-2; RyR, ryanodine receptor/Ca channel; JNK, c-jun kinase.

Fig. 3. Roles of Cellular ATP in Necrosis
Cellular ATP levels are regulated by supply, synthesis and consumption, as indicated in the figure. Other details are in the text.
cellular ATP levels. By immunocytochemical analysis, we found that GLUT1/4 are mostly present in the cytoplasm. In other words, the membrane transport of GLUT1/4 is inhibited under this starvation condition. Although details of the mechanisms underlying decreased mitochondrial ATP synthesis remain to be determined, the generation of reactive oxygen species (ROS) and subsequent damage of mitochondrial membranes are likely involved. Most recently, it has been reported that BNIP3 opens mPTP, followed by mitochondrial influx of H2O and Ca2+. The Ca2+ influx is linked to an activation of phospholipase A2 (PLA2) and to mitochondrial membrane destruction. Lastly, the increased ATP consumption may be related to the activation of PARP. The damage to nuclear DNAs by ROS is restored by sequential steps initiated by PARP, which consumes abundant ATP molecules. The decreased ATP levels affect the activity of Na+-K+ ATPase, which also consumes 70% of cellular ATP. The accumulation of cellular Na+ due to a decreased activity of Na+-K+ ATPase causes Ca2+ influx through a Na+-Ca2+ exchanger. The decreased cellular ATP levels also affect Ca2+-Mg2+ ATPase, reducing Ca2+ efflux. Thus, elevated [Ca2+]i activates PLA2, followed by plasma membrane destruction. Osmotic problems due to damaged ion balance might be also involved in the membrane destruction. Thus, one promising strategy to inhibit necrosis would be to increase cellular ATP levels or inhibit the rapid decrease in ATP levels in the event of necrosis.

4. CELL DEATH MODE SWITCH FROM NECROSIS TO APOPTOSIS

As mentioned above, appropriate levels of cellular ATP are essential in apoptosis mechanisms, while the loss of ATP causes necrosis. Thus, it is evident that ATP plays a key role in the cell death mode determination. This view is supported by report of using oligomycin, which inhibits mitochondrial ATP synthesis. When oligomycin was added to a cell which had been stimulated by Fas ligand, the cell death mode switched from apoptosis to necrosis. Although these findings suggest that the loss of ATP may cause necrosis, it remains to be determined whether ATP is the only component to determine the cell death mode or to cause apoptosis. Many investigators have attempted to create situations which induce necrosis or apoptosis in the cell. Apoptosis has been induced by serum- or growth factor-deprivation, as well as by the use of specific reagents, such as etoposide or staurosporin. On the other hand, necrosis could be experimentally induced only by the addition of reagents such as 4-hydroxy-2-nonenal (HNE) or oligomycin. As such reagents to induce apoptosis and necrosis have non-specific targets, the clear characterization of such cell death mode switch has remains to be determined. This issue has recently been solved in the primary culture of neurons under a serum-free condition without any supplements, as shown in Fig. 4. Cortical neurons from embryonic rats in such starvation stress showed necrosis in the low-density culture (1×10^5 cells/cm²), and apoptosis in the high-density one (5×10^5 cells/cm²). In the low-density culture, there is a rapid decrease in cellular ATP levels and glucose uptake, presumably through a decreased membrane translocation of GLUT1/4. The density-dependent difference in cell death modes is attributed to the action of conditioned medium (CM) factors, which inhibit necrosis through a reversal of decreased cellular ATP levels, while apoptosis is induced through protein kinase C (PKC)-mediated mechanisms. Quite similar observations were also found when high glucose was added to the low-density of culture, causing the reversal of decreased ATP levels. High glucose treatment showed an induction of apoptosis through the PKC-mediated induction of Bax, as well as the inhibition of necrosis through PKC-mediated membrane translocation of GLUT1/4, and following the reversal of decreased ATP levels. The addition of pyruvate inhibited the necrosis through a reversal of decreased ATP levels, but did not induce apoptosis. This finding suggests that the ATP level is not the only factor to determine the cell death mode, and high glucose might have unidentified signaling mechanism to induce apoptosis through an activation of PKC. Several reports have

![Fig. 4. Cell Death Mode Switch from Necrosis to Apoptosis by CM Factors or High Glucose Treatment](image)

Details are in the text. From ref. 8.
also demonstrated that the inhibition of necrosis is independent of ATP mechanisms. Insulin inhibits necrosis without changing the cellular ATP levels in cortical neurons under the serum-free starvation condition, as mentioned above.\textsuperscript{65) Nefiracetam, a cognition-enhancer, inhibits both necrosis and apoptosis through an activation of Ca\textsuperscript{2+} channels in retinal neuron-neuroblastoma hybrid N18-RE105 cells.\textsuperscript{66)}

5. CONCLUSION

Necrosis is found in several diseases, such as Alzheimer’s disease, Parkinson’s disease, brain ischemia and traumatic brain injury.\textsuperscript{10,11,59,60) However, necrosis has not been a major target for neuroprotection, since the molecular basis of its mechanisms has not been demonstrated. In brain ischemia, the cell destructive necrosis occurs in the core, which in turn is linked to cell death expansion in the vicinity. Several days later, apoptosis occurs in the surroundings of the core, called the penumbra. As the cells showing apoptosis are phagocytosed by microglia in the brain, the cell death expansion would then be terminated. Therefore, this cell death mode switch from necrosis to apoptosis would be an important self-protective mechanism of the brain. The identification of molecules which cause this cell death mode switch in the CM factors, as mentioned above, would provide a promising target for the development of medicines, to minimize ischemia-induced brain damage. We have preliminary observations that the topical use of glucose to the ischemic retina or brain showed significant neuroprotection, possibly through this cell death mode switch.\textsuperscript{61) Through the successful characterization of necrosis, several new candidates to inhibit necrosis through ATP-dependent and independent mechanisms have been proposed. The novel hypothesis of a cell death mode switch for neuroprotection may create new fields of drug development to protect against ischemic brain injury.

REFERENCES AND NOTES

32) Zha J., Harada H., Yang E., Jockel J., Korsmeyer S. J., Cell, 87, 619—
628 (1996).


