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Apoptosis induced by endoplasmic reticulum stress depends on activation of caspase-3 via caspase-12

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Abstract

Recently, endoplasmic reticulum (ER) dysfunction has been implicated in neuronal death in patients with Alzheimer’s disease. Treatment of human neuroblastoma cells with ER stress inducers causes apoptotic death. We confirmed that ER stress inducers specifically targeted the ER to cause apoptotic morphological changes. We also found that caspase-3, and not caspase-9 (a known mitochondrial apoptotic mediator), was mainly activated by ER stress. We generated the neuroblastoma cells that stably expressed caspase-12 and analyzed its influence on caspase-3 activation and vulnerability to ER stress. Cells expressing caspase-12 were more vulnerable to ER stress than cells expressing the empty vector, concomitant with increased activation of caspase-3. These findings suggested that activation of ER-resident caspase-12 indirectly activates cytoplasmic caspase-3 and might be important in ER stress-induced neuronal apoptosis.

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The endoplasmic reticulum (ER) is an organelle that ensures correct protein folding and assembly by expressing numerous molecular chaperones [3,7]. Under various conditions, such as glucose starvation, disturbance of intracellular calcium homeostasis, inhibition of protein glycosylation, or exposure to free radicals, unfolded proteins accumulate in the ER lumen, a process called ER stress. Excessive ER stress induces cell death [6,17]. Neuronal death in some neurodegenerative disorders, especially Alzheimer’s disease (AD), is reported to be due to ER dysfunction. Familial AD-linked presenilin-1 mutants or presenilin-2 splice variants which are expressed in sporadic AD brains, and increase neuronal vulnerability to ER stress [5,13,14]. Thus, the ER may be important for regulating intracellular apoptotic signals in neurons, but the mechanisms of ER stress-induced cell death are poorly understood.

Caspase-12, one of the caspase family of proteases, is localized to the ER and is specifically activated by ER stress [10], with several possible mechanisms being suggested [9,12,18]. Caspase-12-deficient cells are resistant to ER stress, but not non-ER stress [10], and such evidence suggests that caspase-12 is a major mediator of ER-stress-induced apoptosis.

We found that activation of ER resident caspase-12 causes activation of cytoplasmic caspase-3, and not mitochondria-related caspase-9, during ER stress-induced apoptosis.

When cells die from ER stress, it is believed that apoptosis occurs, but whether these cells show typical morphological features of apoptosis is unclear. Therefore, we analyzed the morphological and biochemical changes during cell death after ER stress in SK-N-SH, human neuroblastoma cells.
As shown in Fig. 1, chronic ER stress (30 h of exposure to 1 μg/ml tunicamycin (Tm), a N-linked glycosylation inhibitor) caused apoptotic morphological changes, including nuclear fragmentation, chromatin condensation, and cell shrinkage. Interestingly, prior to these changes, swelling of the ER lumen and dissociation of ribosomes from rough ER were observed by electron microscopy. These studies indicated that ER stress-induced cell death was a type of apoptosis.

Caspases are critical mediators of apoptosis in mammalian cells [16], so we measured several caspases activities using synthetic fluorometric substrates (50 μM Ac-YVAD-AMC, Ac-DEVAD-AMC, Ac-VEID-AMC, Ac-IETD-AMC, and Ac-LEHD-AMC for caspase-1, caspase-3, caspase-6, caspase-8, and caspase-9, respectively). After 24 h of treatment with 1 μg/ml Tm or 0.5 μM thapsigargin (Tg), an ER Ca²⁺-ATPase inhibitor, caspase-3 activity was significantly higher than that of other caspases (Fig. 2a, upper). Activation of caspases requires cleavage of pro-caspases, e.g. pro-caspase-3 is cleaved into smaller subunits under apoptotic conditions [1]. To confirm the role of caspase-3 in ER stress-induced apoptosis, we assessed pro-caspase-3 cleavage in SK-N-SH cells treated with 1 μg/ml Tm by western blotting using an anti-caspase-3 antibody that detected both uncleaved and cleaved caspase-3 (Fig. 2a, bottom). Cleaved caspase-3 was observed under ER stress conditions. Next, we examined the effects of specific caspase inhibitors on apoptosis (Fig. 2b). After exposure to 1 μg/ml Tm with or without each caspase inhibitor (5 μM), cell death was quantified from LDH release into the medium. When cells were treated with both Tm and a caspase-3 inhibitor, death was significantly reduced, suggesting that caspase-3 was a major caspase activated by ER stress. Similar results were observed after treatment with Tg (Fig. 2b, right).

To clarify the link between activation of ER resident...
caspase-12 and cytoplasmic caspase-3, we assessed caspase-3 activation in SK-N-SH cells with stable expression of caspase-12 or empty vector (mock). Caspase-3 activation was significantly greater in caspase-12-expressing cells than in mock-expressing cells after treatment with 1 mg/ml Tm for 10 h (Fig. 3a). Cleavage of pro-caspase-3 was also increased in caspase-12-expressing cells (Fig. 3a). To investigate whether increased caspase-3 activation in caspase-12-expressing cells was accompanied by vulnerability to ER stress, we compared LDH release by caspase-12-overexpressing cells with mock-expressing cells. After treatment with 1.0 μg/ml Tm for 20 h, culture medium was harvested to assay LDH activity by the manufacturer’s protocol (KYOKUTO, Japan). Data are the mean of four independent experiments.

This study showed that activation of exogenous murine caspase-12 led to activation of cytoplasmic caspase-3, rather than caspase-9, in ER stress-induced apoptosis. We also showed by electron microscopy that ER stress inducers cause ER swelling prior to mitochondrial disruption. These findings suggested that cross-talk between these ER and cytoplasmic caspases is important for ER stress-induced apoptosis in SK-N-SH cells, rather than ER-mitochondrial cross-talk [4,15]. This possibility was recently supported by Rao et al. [11]. There have been several reports on the molecular mechanism of caspase-12 activation [9,12,18], so caspase-12 may play a role in apoptosis after ER stress.

Human caspase-12 has not been cloned yet and there is controversy about its expression [2,12]. Consequently, we investigated murine caspase-12 as a substitute for endogenous human caspase-12-like protease. Future isolation and functional analysis of human caspase-12 may lead to better understanding of ER stress-induced apoptosis in human cells.

References


