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Review

Caspase-independent apoptosis in yeast

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ABSTRACT

Apoptosis is a highly regulated cellular suicide program crucial for metazoan development. Yeast counterparts of central metazoan apoptotic regulators, such as metacaspase Yca1p, have been identified. In spite of the importance of Yca1p in yeast apoptotic process, many other factors such as Aif1p, orthologs of EndoG, AMID and cyclophilin D play important roles in caspase-independent apoptotic pathways. This review summarized recent progress about studies of various intrinsic and extrinsic apoptotic stimuli that may induce yeast cell death via caspase-independent apoptosis.

1. Introduction

Apoptosis has been first recognized during vertebrate development as part of a natural process to remove superfluous or used-up cells [1,2]. The term apoptosis was coined by Kerr et al. in 1970s, which was defined as an active, orderly cell death process involving membrane blebbing, shrinkage of the cytoplasm and nucleus, and disintegration of the cells into apoptotic bodies surrounded by an intact cell membrane; a more chaotic way of dying was named as necrosis, which is violent and characterized by cytoplasmic swelling, membrane rupturing and organelle dissolution [3]. Worth noting is that with the recent development of our knowledge about cell death, some kinds of cell death cannot fit the simple dichotomy-apoptosis or necrosis.

The introducing of an invertebrate model organism, *Caenorhabdtis elegans*, resulted in the great discovery of an executor of apoptosis-caspase [4], which belongs to a family of cytosolic cysteine proteases, and upon activation, functions as a caspase cascade, cleaving crucial death substrates after Asp residues [5]. Largely dependent on in vitro systems, the biochemical pathways of caspase activation during apoptosis were elucidated [6]. However, numerous clues indicate that the process of caspase activation is not the sole determinant of life and death decisions in programmed cell death-PCD [7], and later Xiang et al. clearly demonstrated a caspase-independent cell death [8]. Now, accumulating pieces of evidence show that apoptosis-inducing factor (AIF) [9], AIF-homologous mitochondrion-associated inducer of death (AMID) [10] and endonuclease G (EndoG) [11,12] can all induce apoptotic cell death in a caspase-independent manner. Compared with caspase-dependent apoptotic cell death, our knowledge about the mechanism of caspase-independent cell death is still at a very initial stage.

For a long time, it has been imagined that apoptosis was limited to multicellular organisms only, and thought there was no apoptotic machinery in yeast. This idea was challenged by the discovery of an apoptotic phenotype in a yeast strain carrying a *CDC48* mutation [13]. Like mammalian cells, yeast cells (*Saccharomyces cerevisiae*) undergoing apoptosis display characteristic markers such as DNA cleavage, apoptosis-typical chromatin condensation, externalization of phosphatidylserine, and cytochrome *c* release from mitochondria [13–15]. The past 10 years have seen the discovery of several yeast orthologs of crucial apoptotic regulators [16–28]. These findings firmly established that yeast and metazoan apoptosis are in essence the same cellular program and lay the foundation of using yeast as a tool for apoptosis research. In this review we will summarize the recent progress on caspase-independent apoptosis in yeast. Unless otherwise specified, yeast as described in this review is meant to be *S. cerevisiae*.

2. Caspase-independent yeast death induced by heterologous expression of proapoptotic genes

In the early studies of yeast death, yeast had long been assumed to lack apoptotic processes, and therefore contain no apoptotic machinery components such as caspase or Bcl-2 family members [29,30]. To overcome the difficulty of elucidating the components and pathways that are often interwoven with each other in mammalian cells, some

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groups intended to reconstitute the cell killing pathway of mammalian elements in the budding yeast [29,31,32]. So yeast has been (and still often is) employed as "clean room" for investigating the interaction of proteins involved in apoptosis and PCD in general [33].

Ced-3, Ced-4, and Ced-9 are key components of a cell suicide program in *C. elegans*. Ced-4 facilitates the proteolytic activation of the caspase, Ced-3, while Ced-9 opposes Ced-3/Ced-4 killing. One interesting example is that without caspase Ced-3, Ced-4 was by itself lethal when expressed in *S. cerevisiae*, revealing an intrinsic killing activity of Ced-4 to yeast. Furthermore, only Ced-3 but not Ced-4 toxicity was attenuated by coexpression of caspase inhibitor CrmA, an inhibitor of caspases derived from cowpox virus, and p35. Thus, besides its Ced-3- and Ced-9-dependent action in *C. elegans*, it seemed that Ced-4 has an additional caspase-independent killing mechanism in yeast. It has been reported that the cell killing effect of Ced-4 may be mediated by its homomerization [34].

Heterologous expression of the human key apoptotic inducer Bax or Bak is lethal for yeast [29,35]. This lethality can be suppressed by coexpression of Bcl-2 family members such as Bcl-xL, Bcl-2, Mcl-1, and A1, which are anti-apoptotic in vertebrates [29], suggesting Bax/Bak induces yeast death by an apoptotic way. Indeed, Manon et al. [14] demonstrated that Bax expression resulted in the release of cytochrome *c* from mitochondria — a hallmark of mammalian cell apoptosis [6]; Ink et al. [39] showed that Bax or Bak expression in *S. pombe* induced chromatin condensation and DNA cleavage; Ligr et al. [40] and Madeo et al. [41] finally confirmed the apoptotic features of Bax-expressing yeast by TUNEL assay, Annexin V staining, detection of oxygen radical generation and chromatin condensation. This kind of yeast killing effect is not rescued by CrmA, implying that this apoptosis is caspase-independent [36]. Recently, new evidence [37,38] further indicated that yeast metacaspase *YCA1*/*MCA1* (Yor197w) is neither necessary nor facilitates Bax killing in yeast as coexpressed inducible *YCA1*/*MCA1* with Bax did not exasperate Bax toxicity; and Bax expression in a strain mutant for *YCA1* displayed the same level of killing effect. As for the downstream machinery within yeast in this system, some yeast mutant strains exhibiting greatly-reduced sensitivity to killing by Bax have been found, and most of them suggest mitochondrial involvement in modulating Bax sensitivity in yeast [42–45]. Furthermore, Pavlov et al. [46] discovered a novel high-conductance Bax-dependent channel in mitochondria of yeast and mammals. This channel was named the mitochondrial apoptosis-induced channel and is a candidate for the outer membrane pore through which cytochrome *c* and possibly other factors exit mitochondria during apoptosis. Although one mitochondrial receptor for Bax has been found to be TOM22, a core component of the mitochondrial outer membrane protein translocation pore [47], other pieces of evidence [48] are more consistent with the view, as proposed by Saito et al. [49] and Kuwana et al. [50], that Bax forms channels in the mitochondrial outer membrane independent of the presence of endogenous mitochondrial proteins.

3. Environmental stress- or drug-induced caspase-independent yeast apoptosis

Many environmental factors or drugs such as hyperosmotic stress, elevated temperature, mating-type pheromone or amiodarone, low doses of H_2O_2 or acetic acid, osmotin, SFK1, aspirin, HOCl, or merely sugar itself (reviewed in [51,52]) have been reported to be stimuli for yeast to commit cell death associated with typical hallmarks of metazoan apoptosis. However, few of them address the issue of the role of caspases on the process of apoptosis. And in special cases there are even some contradictory reports.

3.1. High temperature

Qi et al. [53] reported that when treated with high temperature (37 °C), dying *cdc13-1* cells displayed some phenotypic markers of apoptosis such as exposure of phosphatidylserine on the outer leaflet of the plasma membrane, accumulation of reactive oxygen species (ROS), and induction of caspase activity based on retention of the caspase inhibitor FITC-VAD-fmk. The study, however, fell short of using more apoptotic markers and did not investigate the role of yeast caspase and ROS in the cell death. Soon after, Wysocki and Kron [54] showed that cell death triggered by *cdc13-1* is independent of caspase-like proteases including yeast metacaspase Yca1p and reactive oxygen species but related to cell cycle arrest per se. Further, Yca1p caspase activity as assayed by cell binding of mammalian caspase inhibitors is confounded by artifactual labeling of dead yeast cells, which non-specifically bind fluorochromes. Thus, the cell death in the *cdc13-1* mutant may not be related to caspase activation. They inferred that *cdc13-1* cell death may arise as a result of loss of cell wall integrity in oversized, large-budded cells or via an active process such as induction of autolysis and/or autophagy rather than apoptosis. Nevertheless, these claims need to be established through direct evidence. Collectively, although the *cdc13-1* cell death at 37 °C may not be caspase-dependent, the type of death is not clarified and may not even be typical apoptosis.

3.2. Acetic acid

It has been reported that *S. cerevisiae* commits to apoptosis upon treatment with acetic acid [55,56], and Yca1p is indicated as an executor of acetic acid-induced apoptosis in yeast [19]. Recently, Guaragnella et al. [57] showed that yeast cells lacking metacaspase *YCA1* gene underwent the process of acetic acid-induced apoptosis in a manner similar to normal cells but at a lower rate, and although z-VAD-fmk partially inhibited caspase-like activity, it did not affect acetic acid-induced apoptosis in yeast. They suggested that Yca1p participates in acetic acid-induced apoptosis in a manner unrelated to its putative caspase-like activity.

3.3. NaCl stress

Evidence has been presented that exposure of yeast to high salinity induces apoptosis [58]. It has been observed that deletion of *SOP1*/*SRO7* gene together with its iso-gene *SRO77* brings about increased sensitivity to NaCl stress and a defective intracellular ion homeostasis [59]. Wadskog et al. [60] observed a significant increase of the proportion of wild-type cells with active caspase after exposure to NaCl stress for 4 h, and significantly improved survival rate at high salinity when *YCA1* is deleted, suggesting a general role for Yca1p in salt-induced apoptosis. However, when treated with 1.2 M salt stress, the observed caspase activity remained unaffected in *sro77*Δ mutants, similar to what was noted for *yca1*Δ cells, whereas *sro7*Δ yeast exhibited a generally increased caspase activity than that of wild-type cells. This finding, together with the observation that *sro77*Δ and *sro77*Δ *yca1*Δ mutants exhibit NaCl-induced nuclear fragmentation and strong DNA strand breakage, suggests the existence of a caspase-independent apoptotic pathway in yeast when stressed with high salt. It is clear now that the salt-sensitive phenotype of the yeast *sro7*Δ mutant results from the defective targeting of *ENA1* encoded sodium extruding ATPase to the plasma membrane [61]. But the mechanism of caspase-independent apoptosis as observed in *sro77*Δ mutants when stressed with high salt is not yet clarified.

3.4. Cu stress

Recently, Liang and Zhou [28] systematically compared the effect of Cu and Mn stress, side by side, on yeast apoptosis. They demonstrated that at sub-toxic levels both of them are beneficial to yeast cells; at moderate toxic levels, both metals induce extensive apoptosis in yeast cells. While at even higher concentration, necrosis then takes over. Mitochondria-defective yeast exhibited much reduced apoptotic