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PROGRAMMED CELL DEATH IN LOWER EUKARYOTES

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Abstract

Studies on Progarmmed Cell Death (PCD) or apoptosis scenarios in lower eukaryotes suggested conservation of downstream cell death machinery with mitochondria and reactive oxygen species (ROS) generation as central regulator of apoptosis. The studies so far also point out, to an extent, the mimicking of mammalian intrinsic pathway of apoptosis. Given the intricate connection of apoptosis with cellular biology (epigenetic modifications, DNA replication and mRNA turnover), future perspective is to give detailed understanding at system biology, mechanism of pathogenesis and human health.

Key words: Cell Death, Lower Eukaryotes

Introduction

In eukaryotes, PCD is a genetically regulated cell death mechanism for the elimination of damaged or unwanted cells. It plays an important role in the development, cellular homeostasis and maintenance of the integrity of organisms. The basic molecular machinery regulating PCD in mammals was also conserved in yeast *Saccharomyces cerevisiae*, including the yaest caspase Yca1p, Apoptosis Inducing Factor1 (Aif1p), HtrA2/Omi (Nma111p), and AMID (Ndi1p), and the anti-apoptotic proteins Cdc48p and Bir1p [1]. Apoptosis in yeast may be initiated by the accumulation of ROS in response to ageing, or exposure to external stimuli such as H_2O_2 or acetic acid. Various morphological and biochemical events observed in apoptotic cells are chromatin fragmentation and its condensation, externalization of phosphatidylserine to the outer leaflet of the plasma membrane, mitochondrial fragmentation, cytochrome *c* release, cytoskeletal perturbations and histone H2B phosphorylation. Similar to mammalian PCD, ROS found to act as an important regulator of yeast PCD [2, 3].

The mRNA metabolism and its regulating mechanism has been very well conserved in all eukaryotes from yeast to humans. Two major pathways of mRNA decay exist in eukaryotes. Both pathways are initiated by poly(A) shortening of the mRNA. In the 5' to 3' decay pathway, the Dcp1p/Dcp2p decapping enzyme complex can hydrolyze the cap structure following deadenylation by Pan2p and Ccr4p enzymes and the mRNA is subsequently degraded from the 5′ end by Xrn1 nuclease. In the 3' to 5' decay pathway, deadenylated mRNAs are degraded in a 3' to 5' exonucleolytic manner and require two multiprotein complexes: the exosome containing various 3′-exonucleases and the Ski complex consisting of RNA helicase Ski2p, Ski3p, Ski7p and Ski8p. In the 5' to 3' pathway, the heterooctameric Lsm1p-7p-Pat1p complex (made of seven Sm-like proteins, Lsm1p through Lsm7p and Pat1p) interacts with several decay factors and activates decapping in vivo. Poly A binding protein (Pab1p) when bound to the 3’ poly(A) tail promotes interaction of eukaryotic translation initiation factor (eIF4E) to the 5’cap of mRNA and protect the 5’ end from decapping (4).

Inappropriate regulation of apoptosis or mRNA turnover results in several human diseases. Apoptotic studies in yeast suggested interconnecting mechanism between mRNA metabolism and apoptosis. Expression of a truncated form of Kluyveromyceslactis LSM4 (Kllsm4Δ1) in a S. cerevisiae strain leads to increased mRNA stability and caspase mediated-apoptotic death. The disruption of *YCA1* in the *Kllsm4Δ1* mutant background improved cell viability without affecting mRNA stability. Strains lacking genes encoding proteins of mRNA metabolism were oversensitive to oxidative stress. These included genes encoding rRNA helicases (*DBP3*, *DBP7*), rRNA processing factors (*NOP12*, *NSR1*), mRNA deadenylases (*CCR4*, *POP2*) and several mitochondrial RNA splicing components [1, 3]. Moreover processing bodies (P-bodies), specific cytoplasmic foci for mRNAs decapping and decay, may play important roles during cellular responses to stress in that their size and number increased in several stress conditions such as oxidative stress, glucose deprivation, osmotic stress, ultraviolet light, and late stage of growth [2, 5, 6].

Yeast strains lacking factors for 5' to 3' mRNA decapping and decay (*DCP2* and *LSM1*), mutants defective in 3' to 5' cytoplasmic exosome function (*SKI2*) or deadenylation (double deletion of *CCR4* and *PAN2*) were found to undergo PCD with various biochemical and morphological features of eukaryotic apoptosis in the mid-log phase cultures such as externalization of phosphatidyl serine, activation of metacasapase, ROS accumulation and DNA fragmentation [7].

Apoptosis in *Schizosaccharomyces pombe*

Unlike *S. cerevisiae*, cell death in fission yeast *Schizosaccharomyces pombe* is caspase independent and mediated by proapoptotic Bcl-2 family members Bax and Bak [8]. Over-expression of Bax and Bak induced cell death in yeast without DNA fragmentation suggesting retention of proapoptotic roles of Bax and Bak in lower eukaryotes. This cell death is associated with prominent cytosolic vacuolization and nuclear chromatin condensation. Furthermore, it can be inhibited by the antiapoptotic counteraction of Bcl-X_L [8]. Recently, the mechanism behind cell death in *S. pombe* has been deciphered. Two gene products namely Plh 1p, a phospholipid diacyltransferase (PDAT), whereas, Dga 1p, an acyl-coA:diacylglycerol acyltransferase (DGAT) is largely essential for conversion of diacyl glycerol (DAG) and long chain fattyacids such as palmitic acid and oleic acid to triacyl glycerol (TAG), an important energy depot of nearly all eukaryotes. Deletion of these two endogenous genes (Plh 1p and Dga 1p) promoted accumulation of diacyl glycerol (DAG) and long chain fattyacids such as palmitic acid and oleic acid [9, 10]. Double deletion mutants (DKO cells) of Plh 1p and Dga 1p failed to synthesize TAG upon entry to stationary phase resulting in PCD with prominent apoptotic markers. Addition of palmitic acid and oleic acid also resulted in accumulation of DAG and triggered apoptosis by an unknown mechanism. Like budding yeast *S. cerevisiae*, apoptosis in fission yeast is also accompanied by an increase in production of ROS(ROS). The observations in *S. pombe* might indicate that the emergence of caspase-independent cell death pathways that has occurred much earlier than classical caspase-dependent apoptosis during evolution [9, 10]. The failure to maintain lipid homeostasis leads to activation of lipotoxicity in the fission yeast acoompanied by activation of cell death machinery such as metacaspase Pca1, BH3-domain protein Rad9, and diacylglycerol-binding proteins Pck1 and Bzz1. Studies as these has implications in metabolomics, human medicine and overall systems biology [11].

Apoptosis in *Candida albicans*

Candida albicans, medically relevant fungal pathogen, undergoes apoptosis when exposed to environmental stresses (acetic acid and hydrogen peroxide) and an antifungal agent (amphotericin B) [12]. The phenotypic markers of PCD were externelization of phosphatidylserine, DNA knicking, nuclear fragmentation and chromatin fragmentation, production of ROS and accumulation of cells in G_2/M phase of cell cycle [13]. Caspase mediated apoptosis was observed when *C. albicans* biofilms were treated with amphotericin B [13]. Likewise farnesol, a quorum sensing molecule, induced PCD with characteristic markers such as generation of reactive oxygen species, mitochondrial degradation, DNA knicking, caspase activation and upregulation of drug response genes [14]. These studies have got important implication for fungal adaptation to environment and survival mechanism.

PCD in *Leishmania donovani*

An unicellular protozoan *Leishmania donovani* undergoes PCD in both stationary phase as well as when cultured in the presence of amphotericin B. The various characteristic features of Leishmania PCD were loss of mitochondrial membrane potential, increase in plasmamembrane permeability, DNA nicking, nuclear condensation, formation of DNA ladder and enhanced PPL-cleavage activity that can recognize caspase inhibitors [15]. Release of Mitochondrial proteins such as cytochrome c and pro-apoptotic proteases suggest conservation of intrinsic apoptotic pathway of mammalians in protozoans [16]. Consistent with this, *o*verexpression of a mitochondrial protein peroxiredoxin suppressed peroxide induced DNA damage, promoted stabilization of mitochondrial membrane and consequently survival in *Leishmania* [17]. Moreover loss of mitochondrial membrane potential and expression of a catalytically active caspase-3 like protein was observed when cells were challenged with 3-O,28-O-disuccinyl betulin (DiSB), a potent topoisomerase type IB inhibitor. This indicated generation of ROSacts as a central regulator of apoptosis in *Leishmania donovani* [18]. Studies on *Leishmania* mutants would not only help better elucidate the downstream pathways of apoptosis in lower eukaryotes but also provide insight on the intricate connection between PCD and pathogenecity [16].

PCD in *Trypanosoma*

Alkaloids that cause loss of membrane integrity, intercalate DNA and inhibit protein biosynthesis such as isoquinoline, quinoline, indole and steroidal type (berberine, chelerythrine, emetine, sanguinarine, quinine, ajmalicine, ergotamine, harmine, vinblastine, vincristine, colchicine, chaconine, demissidine and veratridine) induced PCD in the blood streams of human protozoan parasite, *Trypanosoma brucei* [19]. Lectin ConA and proapoptotic nucleases such as TatD nuclease, a component of DNA degradation complex, and a mitochondrial nuclease endonucleaseG (endoG) triggered PCD in a caspase independent manner in *Trypanosoma brucei* [20, 21, 22]. *Trypanosoma curzi* is a protozoan parasite known to cause chagas disease was found to undergo caspase mediated PCD. Two mammalian caspase homologues such as TcMCA3 and TcMCA5 contrubute to PCD as evidenced by their caspase substrate recognition (Z-YVAD-AFC) and in vivo labeling with the caspase fluorescent inhibitor SR-VAD-FMK [23].

PCD in filamentous fungi

Aspergillus fumigatus was found to undergo PCD in response to hydrogen peroxide or amphotericin B treatment in a caspase independent manner with characteristic features of eukaryotic apoptosis such as accumulation of ROS species and loss of cell viability. Genome search revealed two metacaspase genes. Likewise treatment of *Aspergillus nidulans* cultures with phytosphingolipids or antifungal protein PAF or farnesol (a quorum sensing isoprenoid secreted by *Candida albicans*) triggered caspase independent cell death with characteristic features such as externalization of phosphatidyl serine, DNA degradation, loss of membrane potential and accumulation of ROS species. Both induced a strongly apoptotic phenotype and cell death [24]. Comparative genome analysis of already sequenced genomes revealed homologues of eukaryotic apoptotic machinery that is absent in yeast. The PCD machinery identified were STAND domain, poly(ADP-ribose) polymerase (PARP) that acts as caspase substrate, NB-ARC ATPases which regulate PCD in animals and plants, subunits of mitochondrial NADH ubiquinone oxidoreductase GRIM-19 and NDUFS1, TRAF3 and several APAF homologues that recruits downstream caspase-9 [24]. In *Aspergillus nidulans* caspase dependent PCD was observed at sporulation stage. Western analysis revealed cleavage of 81 kDa PARP-like protein by fungal extract high in caspase-like activity and PARP cleavage was delayed upon incubation with broad spectrum caspase 3 inhibitor (DEVD-fmk) [25].

PCD during aging

In another filamentous fungi *Podospora anserine* age dependent accumulation of ROS species concurs with activation of metacaspases, PaMCA1 and PaMCA2. Deletion PCD machinery such as PaAMID1, a homologue of a mammalian 'AIF-homologous mitochondrion-associated inducer of death' as well as the two metacaspases (PaMCA1 and PaMCA2) resulted in lifespan extension [26]. The other characteristics of *Podospora anserina* aging dependent cell death were reorganization of the mitochondrial DNA, reduction in mycelium growth r and increase in pigmentation and death of peripheral hyphae, implicating mitochondrial quality control in PCD [27].

PCD in *Plasmodium falciparum*

PCD (PCD) in *Plasmodium falciparum* is characterized by loss of mitochondrial permeability, caspase-3 activation and DNA fragmentation and suggested involvement of a novel CQ-induced pathway induced by digestive vacuole [28]. Studies with chloroquine (CQ) revealed destabilization of digestive vacuole, upstream of mitochondrial dysfunction, acts as a major route in initiating PCD in this parasite and pinpoint novels methods of treating malarial infections[29]. *P. falciparum* glutamic-acid-rich protein (PfGARP) is a 80 kDa parasitic antigen that is expressed on the cell surface of erythrocytes at early-to-late-trophozoite-stage that are recognized by plasma antibodies in children who are relatively resistant—but not those who are susceptible—to malaria caused by *P. falciparum.* The predisposition of children to malaria relies on the occurrence of pfGARP antibodies that has the ability to induce PCD in the parasite [30].

PCD in Slime mould*, Dictyostelium discoideum*

PCD is required for normal development of slime mold *Dictyostelium discoideum*. Under favorable conditions, *Dictyostelium* multiplies as a unicellular organism. Upon starvation, slime molds forms multicellular fungus-like structure called a sorocarp consisting mainly of spores and stalk cells, the latter being a result of cell death. PCD in *Dictyostelium* is accompanied by condensation of chromatin without DNA laddering and in vivo caspase activity [31]. Likewise mitochondria plays a crucial role in PCD of *Dictyostelium* contributing to cytochrome C release and also translocation of Apoptosis Inducing Factor (AIF). A *Dictyostelium* homolog of mammalian AIF (DdAIF) has been cloned and characterized it as DNA fragmentation factor suggesting evolutionary conservation of apoptotic cell death machinery in *Dictyostelium* [32]. In the caspase independent paraptosis pathway, the cell death in *Dictyostelium discoideum* is initiated by an interplay of activation of three cellular proteins such as poly(ADP-ribose) polymerase (PARP), PARG and AIF [33].

Conclusions and Perspectives

The study of PCD in lower eukaryotes and other unicellular organisms is in a nascent stage. The evidence suggests involvement of programmed death in these organisms to aid in both development and elimination of defective cells so as to decrease the load of mutants that can potentially take over, and lead to elimination of a population. Studies so far have shown that the lower eukaryotes possess features of apoptotic machinery that appear to have been conserved during evolution. Therefore, understanding PCD in lower eukaryotes is important for to make an understanding at the level of systems biology, understanding mechanism of pathogenesis and human health. Some of the areas needing research focus include: Signal transduction mechanism leading to PCD, Modulation of genes under apoptotic and non-apoptotic mode, Generation of knockout for cell death regulating genes and their pathophysiological implications inside the cell, connecting link and integrating mechanism between extra cellular signaling and intracellular signaling mechanism.

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