

ROLE OF CASPASE IN PROGRAMMED CELL DEATH IN MULTICELLULAR ORGANISMS

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ABSTRACT

Apoptosis or programmed cell death is a regulatory process in a multicellular organism that involves aspartate specific cysteine rich protease called caspase are members of the interleukin-1 β -converting enzyme family. Apoptosis is induced via two main routes involving either the mitochondria (the intrinsic pathway) or the activation of death receptors (the extrinsic pathway). Both pathways converge to induce the activation of caspases the final executioners of cell death, although, it should be noted that caspase-independent forms of apoptosis have been reported. Ultimately, apoptotic cells are ingested by neighboring cells and phagocytes, preventing inflammation and tissue damage that might ensue upon cell-lysis. The activation and function of caspases, involved in the delicate caspase-cascade system, are regulated by various kinds of molecules, such as the inhibitor of apoptosis protein, Bcl-2 family proteins, calpain and calcium.

KEY WORDS: Apoptotic protein, Bcl-2 family proteins, Calpain, Interleukin-1 β converting enzyme, Cysteine rich protease-caspase.

1. INTRODUCTION

Programmed cell death, or apoptosis, is an important regulatory process that is required to maintain the integrity and homeostasis of both developing and adult multicellular organisms²⁸.

The diverse developmental and pathological stimuli that can induce apoptosis, including receptor-signaling, DNA-damage, growth factor deprivation and several stress signals, all converge on common effector mechanisms in which the key component is the activation of the cell-suicide proteases, the caspases. These enzymes mediate highly specific cleavage of cellular substrates, which results in a series of characteristic morphological changes and the subsequent demise of the

apoptotic cell. Inappropriate apoptosis is involved in many human diseases, including neurodegenerative diseases, ischemic damage, autoimmune disorders and cancer. Thus, deciphering the precise role of caspases in diverse physiological and pathological conditions is of fundamental importance. Apoptosis is a major form of cell death, characterized by a series of distinct morphological and biochemical alterations. Apoptotic cell death occurs in two phases: first a commitment to cell death, followed by an execution phase characterized by dramatic stereotypic morphological changes in cell structure, suggesting the presence in different cells of common execution machinery. Apoptosis is characterized by

condensation and fragmentation of nuclear chromatin, compaction of cytoplasmic organelles, dilatation of the endoplasmic reticulum (frequently in a sub plasma lemmal distribution), a decrease in cell volume and alterations to the plasma membrane resulting in the recognition and Phagocytosis of apoptotic cells, so preventing an inflammatory response

In apoptosis, a biochemical cascade activates proteases that destroy molecules that are required for cell survival and others that mediate a program of cell suicide. During the process, the cytoplasm condenses mitochondria and ribosome's aggregate, the nucleus condenses, and chromatin aggregates. After its death, the cell fragments into "apoptotic bodies," and chromosomal DNA is enzymatically cleaved to 180-bp inter nucleosomal fragments. Other features of apoptosis are a reduction in the membrane potential of the mitochondria, intracellular acidification, generation of free radicals, and externalization of phosphatidyl-serine residues.

The destruction of cell is induced by enzymes executors of proteins they belong to a group of enzymes known as cysteine proteases and exist within the cell as inactive pro-forms or zymogens. These zymogens can be cleaved to form active enzymes following the induction of apoptosis. Induction of apoptosis via death receptors typically results in the activation of an initiator caspase such as caspase 8 or caspase 10. These caspases can then activate other caspases in a cascade. This cascade eventually leads to the activation of the effector caspases, such as caspase 3 and caspase 6. These caspases are responsible for the cleavage of the key cellular proteins, such as cytoskeletal proteins, that leads to the

typical morphological changes observed in cells undergoing apoptosis.

2. CASPASES AND CHROMATIN BREAKDOWN

One of the hallmarks of apoptosis is the cleavage of chromosomal DNA into nucleosomal units. The caspases play an important role in this process by activating DNases, inhibiting DNA repair enzymes and breaking down structural proteins in the nucleus.

2.1. Inactivation of enzymes involved in DNA repair

The enzyme poly (ADP-ribose) polymerase, or PARP, was one of the first proteins identified as a substrate for Caspases. PARP is involved in repair of DNA damage and functions by catalyzing the synthesis of poly (ADP-ribose) and by binding to DNA strand breaks and modifying nuclear proteins. The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3.

2.2. Breakdown of structural nuclear proteins

Lamins are intra-nuclear proteins that maintain the shape of the nucleus and mediate interactions between chromatin and the nuclear membrane. Degradation of lamins by caspase-6 results in the chromatin condensation and nuclear fragmentation commonly observed in apoptotic cells.

2.3. Fragmentation of DNA

The fragmentation of DNA into nucleosomal units seen in DNA laddering assays is caused by an enzyme known as CAD, or Caspase activated DNase. Normally CAD exists

as an inactive complex with ICAD (inhibitor of CAD). During apoptosis, ICAD is cleaved by Caspases, such as Caspase 3, to release CAD.

3. PROTEIN (CASPASE) FOR APOPTOSIS AND THEIR MOLECULAR PROPERTIES

Caspases, the interleukin-1 β converting enzyme family, fourteen Caspases has been identified so far, they are aspartate specific cysteine protease; they all have a conservative penta-peptide active sites **QACXG** (X=Q,R or D) their precursor are all zymogen are known as procaspases. The N-terminal of the prodomain in procaspase contains a highly diverse structure required for Caspase activation; and they are all capable of autoactivating as well as activating other Caspases, to produce the heterodimer with big and small subunit, and two heterodimer form an enzymatic active heterotetramer. To date at least 14 different caspases have been identified, which play distinct roles in apoptosis and inflammation^{32, 22}.

Caspases are aspartate-specific cysteine proteases that are expressed as pro-enzymes containing three domains, including an NH₂ terminal, a large subunit (~20 kDa) and a small subunit (~10 kDa). Caspase activation involves the proteolytic processing between domains allowing the association of the large and the small subunit. Active Caspases function as a tetramer consisting of two heterodimers made up of a large and small subunit. A substantial body of evidence supports a cascade model for Caspase activation. Initiator Caspases such as Caspase 2, 8, 9 and 10 instigate the apoptotic cascade and lead to the activation of effector Caspases, which include Caspase 3, 6 and 7. Caspases

cause cell death by cleaving a number of cellular proteins including nuclear lamins¹⁶, DNA repair enzymes such as poly-ADP-ribose-polymerase (PARP)¹⁵ and cytoskeletal proteins such as actin²⁰, fodrin³ and gelsolin¹⁴. The fragmentation of DNA during apoptosis is caused in part by an enzyme known as caspase activated DNase (CAD). Normally CAD exists as an inactive complex with the inhibitor of CAD (ICAD). During apoptosis ICAD is cleaved by caspase 3 resulting in the release of CAD, which in turn triggers the rapid fragmentation of DNA²⁷. Caspase activity is tightly regulated by a number of endogenous caspase inhibitors such as members of the inhibitor of apoptosis protein (IAP) family, which are characterized by the presence of at least one Baculoviral IAP repeat (BIR) domain. IAPs include c-IAP1, c-IAP2, NAIP, Survivin, X-linked IAP (XIAP), Bruce, ILP-2, and Livin²⁰.

4. TYPES OF CELL DEATH

Cell death occurs by necrosis or apoptosis. These two mechanisms have distinct histologic and biochemical signatures. In necrosis, the stimulus of death (e.g., ischemia) is itself often the direct cause of the demise of the cell. In apoptosis, by contrast, the stimulus of death activates a cascade of events that orchestrate the destruction of the cell. Unlike necrosis, which is pathologic process, apoptosis is part of normal development (physiologic apoptosis); however, it also occurs in a variety of diseases (aberrant apoptosis).

5. PROPERTIES OF PROCASPASE

Procaspase unique for its proteolytic activity which has been primarily associated with the epithelial cell differentiation rather than apoptosis or inflammation, procaspase have long domain DED in procaspase-8 and procaspase-10, (Death effector domain) and CARD in procaspase-2 and procaspase-9. DED and CARD, the death domain family members, are involved in procaspase activation and downstream caspases-cascade regulation through protein-protein interaction. It contain common 3D structure known as death domain fold and six anti parallel α helix arranged in Greek key conformation.

6. HOW CELL DEATH OCCURS THROUGH CASPASES?

Procaspase activation: Generally, there are two Pathway through which caspases family protease can be activated.

- 1.) One is death signal-induced and another one
- 2) Stress induced
 - a) Death receptor-mediated pathway (death signal-induced)
 - b) mitochondrion-mediated pathway (stress- induced)

6.1. Death receptor-mediated procaspase activation pathway

Death receptors are cell surface receptors belonging to the tumor necrosis factor (TNF) super family, which trigger apoptosis upon ligand binding. The best characterized death receptors are Fas (CD95/Apo1)⁶, TNF receptor-1 (p55)²⁹, TRAMP (WSL-1/Apo3/DR3/LARD)^{13, 2}, TRAIL-R1 (DR4)²³ and TRAIL-R2 (DR5/Apo2/KILLER)¹⁸. Fas Ligand (CD95 ligand) binds Fas, TNF and lymphotoxin a bind to TNFR1¹, TWEAK(Apo3 ligand) binds to TRAMP¹⁹ and TRAIL (Apo2 ligand) is the ligand

for both TRAIL-R1²³ and TRAIL-R2³¹. Fas-1 and TNF-2 can be recognized by their corresponding death receptors such as TNF receptors such as TNF Receptor (TNFR-1) or Fas in the plasma membrane their binding activate the receptor.

Fas can bind to FADD and FADD aggregation and emergence of DED. The death domain can present in procaspase-8 interact with DED of Fas this result in the oligomerization of procaspase localized in the cytosolic side of the plasma membrane. Then complex is formed is called death induced signal complex or DISC.

In DISC, two linear subunit of procaspase-8 compact to each other followed by procaspase-auto-activation to caspases-8 which directly activate caspases-3 it can activated the mitochondrion mediated pathway by t-bid, a kind of pro-apoptotic protein in the cytosol into active form t-Bid activate cytochrome-c, AIF and other molecule are released from mitochondria and apoptosis will be induced.

Procaspase-10 activation is in similar way as in procaspase-8 activation. Caspases-10 function mainly in the apoptosis of lymphoid cells. It can function independently of caspases-8 in initiating Fas and TNF-related apoptosis.

Although caspases-8 and caspases-10 both interact with the DED of FADD in death receptor signaling, they may have different apoptosis substrates and therefore potentially function distinctly in death receptor signaling or other cellular processes.

6.2. Mitochondrial mediated apoptosis

The mitochondrial pathway of apoptosis begins with the permeabilisation of the mitochondrial outer membrane. The mechanisms through which this occurs remain controversial, however, it is

thought that permeabilisation can be either permeability transition (PT) pore dependent or independent⁸. The PT pore is comprised of the matrix protein cyclophilin D, the inner mitochondrial membrane protein adenine nucleotide translocator (ANT), and the outer mitochondrial membrane protein voltage dependent anion channel (VDAC)⁵. The opening of the PT pore triggers the dissipation of the proton gradient created by electron transport, causing the uncoupling of oxidative phosphorylation. The opening of the PT pore also causes water to enter the mitochondrial matrix, which results in swelling of the intermembranal space and rupturing of the outer membrane causing the release of apoptogenic proteins^{5, 8}. Released proteins include cytochrome-C³³, apoptosis inducing factor (AIF)²⁵ and endonuclease G³³. Cytochrome *c* in conjunction with apoptosis protease activating factor (APAF-1) and procaspase 9 form an 'apoptosome'³⁴. This complex promotes the activation of caspase 9, which in turn activates effector caspases that collectively orchestrate the execution of apoptosis. Mitochondrial pathway is central relying station of caspase dependent and caspase independent death pathways. It involves some Pro-apoptotic proteins like Bax and Bak that induces the mitochondrial permeabilisation and release of apoptotic molecules. (Reviewed in^{10, 21})^{7, 9, 24}. AIF²⁵ and endonuclease G³⁰ both contribute to DNA fragmentation and subsequent chromosomal condensation, which are hallmark features of apoptosis. Other proteins released upon mitochondrial outer membrane permeabilisation include Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP-associated binding protein with low pI) and Omi/HtrA2 (high

temperature requirement A2), which antagonize IAPs thereby promoting caspase activation²⁶.

6.3. Mitochondrial-mediated Procaspase-activation pathway of caspases-8

Apart from being recruited to form a DISC complex after auto-activation, procaspase-8 could also be activated through a cytochrome *c*-dependent pathway. After cytochrome *c* is released from mitochondria to the cytosol, caspase-6 is the only cytosolic caspase with the ability to activate procaspase-8, which depends solely on procaspase-6 activation by pro-domain cleaving. It means that, in the cytochrome *c*-dependent pathway, the activation of procaspase-8 requires neither the interaction with FADD nor the formation of a DISC Complex.

6.4. Mitochondrial-mediated procaspase-activation pathway of caspase-9

When cellular stress (DNA damage) occurs, pro-apoptotic protein is activated, which will in turn induce the opening of the pores of mitochondria. As a result, cytochrome *c* is released to the cytosol. Other components like dATP or ATP, apoptotic protease activation factors-1 (Apaf-1) oligomerizes. Combination of Procaspase-9, dATP, cytochrome-*c* and oligomerized Apaf-1 form a complex known as Apoptosome.

7. THE APOPTOSOME

There are a number of other mechanisms, aside from activation of the death receptors, through which the caspase cascade can be activated. Granzyme B can be delivered into cells by cytotoxic T lymphocytes and is able to directly activate caspases 3, 7, 8 and

10. The mitochondria are also key regulators of the caspase cascade and apoptosis.

Release of cytochrome C from mitochondria can lead to the activation of caspase 9, and then of caspase 3. This effect is mediated through the formation of an apoptosome, a multi-protein complex consisting of cytochrome C, Apaf-1, pro-caspase 9 and ATP.

Activated caspases-9 can in turn activate procaspase-3 and procaspase-7. The activated caspases-3 will then activate procaspase-9 and form a positive feedback activation pathway.

8. DOWNSTREAM SUBSTRATE OF CASPASE

Once activated, apoptosis activator caspases such as caspase-2, caspase-8 or caspase-10 will activate other downstream substrate including caspase-3,-6,-7. In addition, activated caspase-8 in turn induces the cleavage of bid to t-bid and translocates to the intermembrane mitochondrial space and release the cytochrome-c which triggers the apoptosis.

The activated executioner caspases can subsequently cleave distinct cellular proteins such as PARP [poly(ADPribose)polymerase], lamin, fodrin, and also Bcl-2, leading to the characteristic morphological changes. The downstream substrates of inflammatory mediator caspases, such as caspase-1, -4 and -5, include pro-IL-1 β , pro-IL-18, IL-1F7b and NOD-LRR (nucleotide-binding oligomerization domain-leucine-rich repeat) members such as Ipaf (interleukin-1 β -converting-enzyme protease-activating factor), LRR and pyrin proteins, etc.¹¹

9. CASPASE-3,-6,-7

Caspase-3, a key factor in apoptosis execution, is the active form of procaspase-3. The latter can be activated by caspase-3, caspase-8, caspase-9, caspase-10, CPP32 activating protease, granzyme B (Gran B), and others. The downstream substrates of caspase-3 include procaspase-3, procaspase-6, procaspase-9, DNA-PK, PKC γ , PARP, D4-GDI (D4 GDP-dissociation inhibitor), steroid response element-binding protein, U1-70kD, inhibitor of caspase activated deoxyribonuclease (ICAD) and so on. Because all substrates of caspase-3 contain DEVD sequences in common, artificially synthesized tetrapeptides Ac-DEVE-AMC and Ac-DEVE-CHO are usually used as the specific substrate and inhibitor of caspase-3, respectively. Caspase-6 and caspase-7 are highly homologous to caspase-3. Procaspase-6 can be activated by caspase-3 but not Gran B. Caspase-6 can also activate procaspase-3 by a positive feedback pathway. The substrates of caspase-6 include PARP, lamin and procaspase-3. Procaspase-7, whose substrates include PARP, procaspase-6 and steroid response element-binding protein, can be activated by GranB⁴.

10. OTHER DOWNSTREAM SUBSTRATES OF CASPASES

The downstream substrates of Caspases, such as PARP, DNA-PK and U1-70kD, are also involved in DNA repair. Once these substrates have been inactivated by the cleavage of caspases, DNA degradation will ensue. Caspase-activated deoxy-ribonuclease (CAD) is a kind of constitutive, magnesium-dependent endonuclease that can be activated by caspases. CAD plays an important role in DNA degradation in the apoptosis of mammals. In normal

cells, CAD resides in the nucleus, binding with its specific inhibitor, ICAD, to form a complex. ICAD is not only the inhibitor but also the molecular chaperone of CAD, essential for the proper folding of CAD. In apoptosis, caspase-9 damages the nuclear pores in an unknown fashion so that caspase-3 can enter the nucleus to cleave ICAD. This releases the CAD from the complex, which can result in DNA degradation. Lamin A and fodrin are essential components of the nuclear skeleton and cytosolic skeleton, respectively. The cleavage of lamin by caspases in apoptosis can lead to the condensation of chromatin and the decomposition of the nuclear membrane. The cleavage of fodrin by caspases in apoptosis can result in apoptotic body formation.

When all kinds of caspase substrates are activated, the cell will go through a series of changes, including the activation of related genes, a decrease in DNA damage repair ability, the activation of zymogens or inactivation of enzymes, cytoskeleton disassembly, and chromatin fragmentation.

11. ROLE OF CASPASE-2

Caspase-2 is the earliest identified caspase in mammals. This enzyme is unique for its features of both initiator and effector caspases. Caspase-2 appears to be necessary for the onset of apoptosis triggered by several insults, including DNA damage, administration of TNF, and different pathogens and viruses³⁵. Both caspase-2 and caspase-9 are similar to CED-3 in *C. elegans*, all of them with a CARD. Caspase-2 widely distributes in most tissues and cell types. It can be found in the nucleus as well as the cytoplasm, with a considerable portion in the Golgi complex.

12. REGULATING FACTORS RESPONSIBLE FOR CONTROLLING CASPASE FAMILY PROTEASE

The activation and inactivation of caspases are regulated by various proteins, ions and other factors, such as IAP, Bcl-2 family proteins, calpain, Ca²⁺, Gran B and cytokine response modifier A (Crm A).

13. IMPORTANCE OF APOPTOSIS

Apoptosis is vital in normal embryonic genesis and development, the differentiation of immune cells, autoimmunity, tumor genesis and nervous system injuries. Caspase family proteases are key factors in apoptosis, and the related research can help us to obtain the essence of the above phenomena at the molecular level and enable us to make breakthroughs in the therapy of tumors, immune system diseases and nervous system diseases using the artificial control of apoptosis.

14. CONCLUSION

Apoptosis or programmed cell death is a regulatory process in a multicellular organism that involves aspartate specific cysteine rich protease called caspase are members of the interleukin-1 β -converting enzyme family.

Apoptosis is induced via two main routes involving either the mitochondria (the intrinsic pathway) or the activation of death receptors (the extrinsic pathway). Both pathways converge to induce the activation of caspases that result in ultimately cell death by phagocytes cells that prevent from inflammation and tissue

damage that might ensue upon cell-lysis and timed cell death is necessary for the cells otherwise, Inappropriate apoptosis is involved in many human diseases, including neurodegenerative diseases, ischemic damage, autoimmune disorders and cancer. Thus, deciphering the precise role of caspases in diverse physiological and pathological conditions is of fundamental importance.

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TABLE.1

Sub family	Role	Members
I	Caspases activator	Caspases-2, 8, 9, 10
II	Apoptosis executioner	Caspases-3, 6, 7
III	Inflammatory mediator	Caspases-1,4, 5, 11, 12, 13

Table.1 shows caspases are divided into three sub-families, as Sub-family of members of caspase family are based on their homology in amino acid sequences.