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Caspase 3 and Its Role in the Pathogenesis of Cancer

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Abstract

Caspases are the executioners of apoptosis, among them; caspase-3 is a frequently activated death protease, which plays vital roles in the induction, transduction, and amplification of intracellular apoptotic signals. Caspases are aspartate-specific cysteine proteases and members of the interleukin-1β-converting enzyme family. 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 Ca^{2+}. Based on the latest research, the members of the caspase family, the caspase-cascade system, and caspase-regulating molecules involved in apoptosis are reviewed. The key components of the biochemical pathways of caspase activation have been recently elucidated. The pathways involved in caspase activation are the cell surface death receptor pathway and the mitochondria-initiated pathway. Thus, Caspase-3 is essential for certain processes associated with the dismantling of the cell and the formation of apoptotic bodies, but it may also function before or at the stage when commitment to loss of cell viability is made.

Apoptosis

Apoptosis is a type of cell death synchronized by a series of signal cascades under definite situations. It has a vital role in regulating growth, development, immune response, and reimbursement of surplus or abnormal cells in organisms. It is also a principal way by which organisms can maintain a fixed number of cells for successful survival. The stimulation and completion of the process necessitate the collaboration of a series of molecules including signal molecules, receptors, enzymes, and gene-regulating proteins. Along with them, the caspase-cascade signaling system is essential in the progression of apoptosis [1].

The term 'apoptosis' was first introduced in 1972 by Kerr et al. [2] to illustrate a type of cell death related to the fragmentation of genomic DNA. In spite of DNA fragmentation, apoptosis is morphologically associated with cytoplasmic condensation, nuclear pyknosis, chromatin condensation, cell rounding, membrane blabbing, cytoskeletal collapse, and the development of membrane-bound apoptotic bodies that readily undergo phagocytosis and In digested by macrophages or neighboring cells without activation of immune response [3]. The contributing risk factors include ultraviolet or γ-irradiation, growth factor abandonment, chemotherapeutic agents, or signaling by cell Death Receptors (DRs). The essential component for the regulation and the execution of apoptosis cell death is the family of caspases [4].

Caspases

Caspases belong to the family of proteolytic enzymes. Caspases are a basic component of the apoptotic pathway [5]. These are cysteine proteases that cleave from aspartate residue in their substrates and originated their name due to this property [6]. They stay in the dormant form in the cells known as zymogens and possess a prodomain, a p20 large subunit, and a p10 small subunit. Various stimuli are involved in the activation of zymogens, which perform a cascade of events in the activated state leading to apoptosis. During activation, pre-domains are removed after the separation of large and small subunits. The large subunit contains the active site Cys285 in the catalytic dyad residues, and that active site is a part of the conserved 'QACXG' pentapeptide sequence, and His237 (caspase-1 numbering) [7]. In their substrates, caspases may bind to at least four adjacent amino acids i.e. P4-P3-P2-P1, and cleave after the C-terminal residue (P1), usually an Asp residue. Caspases are exceptionally homologous to *Caenorhabditis elegans* cell death gene CED-3. The important mechanism of the programmed cell death in *C. elegans* is three CED (Cell Death Abnormal) proteins, called CED-3, CED-4, and CED- 9. CED-9 prevents the interaction

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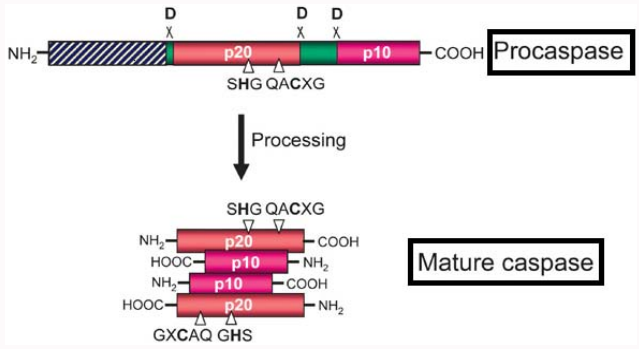


Figure 1: Procaspase activation.

of CED-4 with CED-3 in this way and plays its role as an inhibitor of apoptosis [8,9], while CED-4 is a pro-apoptotic adapter protein essential for the activation of CED-3, a cysteine protease molecule necessary for the execution phase of programmed cell death [10,11]. In mammals, the homolog's of CED-9, CED-4, and CED-3 are found in the Bcl-2 family, Apaf 1/NOD-like receptor family, and caspase (cysteinyl aspartate proteinases) family, respectively [12,13]. Until now fourteen caspases are known and all of them have some similar properties: 1) they all are aspartate-specific cysteine proteases. 2) they are capable of self-activation and also have the capability of activating other caspases, to generate a heterodimer with a big and a small subunit, and two heterodimers form an enzymatic active heterotetramer 9 [1] (Figure 1).

Classification of Caspases

Caspases can be categorized in two different ways. One method relies on their property of performing a specific activity; caspases can be divided into two subfamilies, pro-apoptotic and pro-inflammatory sub families. Pro-apoptotic caspases including caspases 2, 3, 6, 7, 8, 9, and 10 are mainly concerned with processing cell death signaling transduction, whereas pro-inflammatory caspases consisting of caspases 1, 4, 5, 11, and 12 involve in cytokine maturation during the process of inflammation. Another method of classifying caspases is to divide according to the lengths of their pro domains, which also correspond to their positions in the apoptotic signaling cascade. Accordingly, caspases are classified into three subfamilies: Initiator caspases, effect or caspases, and caspases as inflammatory mediators as shown in Table 1 [14].

Table 1: Classification of caspases.

Subfamily	Role	Members
1	Apoptosis activator	Caspase 2
		Caspase 8
		Caspase 9
		Caspase 10
2	Apoptosis executioner	Caspase 3
		Caspase 6
		Caspase 7
3	Inflammatory mediator	Caspase 1
		Caspase 4
		Caspase 5
		Caspase 11
		Caspase 12
		Caspase 13
		Caspase 14

Molecular Properties of Caspases

The pro-caspases of the inflammatory mediator caspases and apoptosis activator caspases including all their members have a long pro-domain in pro-caspases [15]. The long prodomain contains the Death Effector Domain (DED) in procaspase-8 and procaspase-10 or the Caspase Recruitment Domain (CARD) in procaspase-2 and procaspase-9. DED and CARD are involved in pro-caspase activation and downstream regulation of caspase cascade by means of protein-protein interactions and are called the death domain family members. Alternatively, the shorter pro domains in the pro-caspases of apoptosis executioner caspases are not related to being involved with protein-protein interactions. It must be kept in mind that the upstream and downstream association is not utter and may only occur for a shorter period of time through the very initial phases of apoptosis (Figure 2).

Structure

General outline

Caspases are zymogens (inactive enzyme precursors, which need a biochemical signal to become an active enzyme) holding an N-terminal prodomain followed by a large subunit of about 20 kDa, p20, and a small subunit of around 10 kDa, p10 as presented in Figure 1 [16].

Caspase Prodomain

The large pro domains of pro-caspases encompass structural arrangements that are linked to the so-called death domain super family [17,18]. Death domains are 80- to 100-residue-long sequences associated with the transduction of the apoptotic signal cascade. This super family comprises the Death Domain (DD), the Death Effector Domain (DED), and the Caspase Recruitment Domain (CARD) [19]. Every one of these structural sequences is associated with other proteins by homotypic interactions. Every member of the death domain super family possesses similar structures that contain6 or 7 anti-parallel amphipathic α-helices. Structural resemblance elucidates the same evolutionary foundation for all recruitment domains [20]. On the other hand, the nature of the homotypic interactions contrasts within the super family. Electrostatic interactions are involved in DD

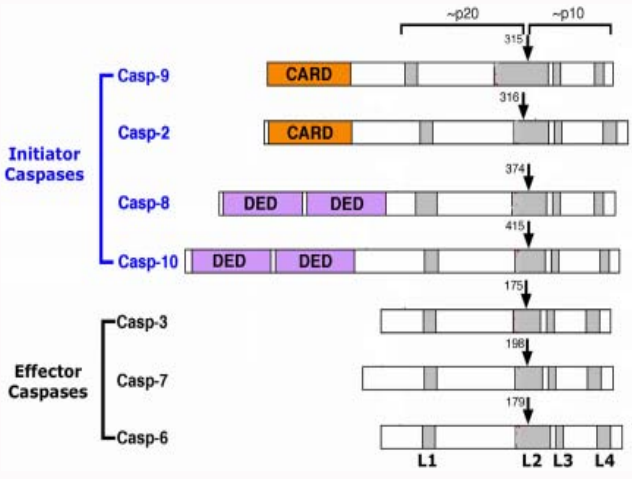


Figure 2: Apoptotic caspases in mammalian cells. The position of the first intra-chain cleavage (between the large and small subunits) is stressed by an arrow. The four surface loops, labeled L1 through L4, form an active site.

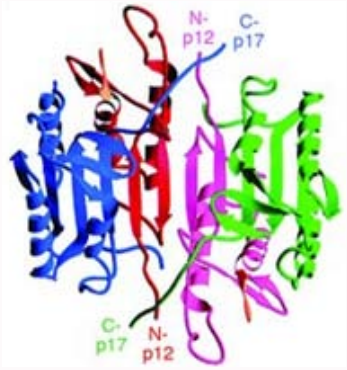


Figure 3: X-Ray structure of caspases.

and CARD, while DED interactions are hydrophobic interactions [21].

Structure of Active Caspase Heterotetramers

Active caspase forms by cleavage of a pro-caspase at the specific Asp-X, which consists of the heterotetramer p202-p102 and results in the release of the prodomain as represented in Figure 1. X-ray structures have been explained for mature caspase-1, caspase-2, caspase-3, caspase-7, caspase-8 and caspase-9 until now [22]. The overall basic structure of all caspases is alike. Each heterodimer (p10-p20) is twisted by hydrophobic interactions leading to the formation of many parallel β-sheets, containing 6 anti-parallel β-strands. Two heterodimers join by means of a 12-stranded β-sheet that is constrained by α-helices (Figure 3). In the caspase heterotetramer, the 2 heterodimers arrange in a head-to-tail configuration, in the same way, 2 active sites are to be established at opposite ends of the molecule. The architecture of the active center consists of amino acid residues from both subunits. The catalytic apparatus consists of a dyad composed of a Cysteine sulfhydryl group (Cys285) and a Histidine imidazole ring (His237) [23]. Both of them are present in the p20 subunit. The tetrahedral transition state of the cysteine protease is stabilized through hydrogen bonding with the backbone amide protons of Cys285 and Gly238. The asparagine of the substrate is to be stabilized by 4 residues: Arg179 and Gln283 from the p20 subunit and Arg341 and Ser347 from the p10 subunit.

Substrate Specificity and Synthetic Peptide Inhibitors of Caspases

Caspases are particular cysteine proteases that identify 4 amino acids, namely S4-S3-S2-S1. The cleavage occurs normally after the C-terminal residue (S1), which is commonly an asparagine [24]. A list of substrate specificities of caspases is mentioned in Table 2. Interestingly the suitable S3 position is variant glutamine for all mammalian caspases. That is why the specificity of caspase cleavage can be denoted as X-Glu-X-Asp. The tetrapeptide sequence WEHD is preferred for Caspase 1, 4, and 5 (group I). Likewise, Caspases 2, 3, and 7 preferred the substrate DEXD, whereas caspases 6, 8, and 9 have a preference for the sequence (L/V) EXD. Tetrapeptide recognition sequence is present between the large and small subunits for initiator caspases as its own cleavage site, which is extremely similar to the suggested method of auto activation of initiator caspases [25].

Caspase 3

Caspase 3 is also called CPP32, YAMA (the Hindu god of Death), and Apopain. CASP3 gene code caspase 3. Caspase 3 shows a wide variety, dominantly present in cell lines of lymphocytic origin, elucidating its importance as an essential facilitator of apoptosis in the immune system. DXXD sequence is preferred for Caspase-3. Mice deficient with caspase-3 die at 1 to 3 weeks of age with characteristic morphological abnormalities. Neuronal apoptosis significantly failed in these mice resulting in hyperplasias, ectopic cell masses, and duplicated brain structures. However, the mutation does not affect other major organs. These results specify the non-redundant role of caspase-3 in neural apoptosis whereas in other tissues other isoforms can substitute for its damage. Granzyme B activates caspase-3 *in vitro*.

Table 2: The substrate specificity of caspases.

	Caspase	Substrate specificity
Group I	Caspase 1	WEHD
	Caspase 4	(W/L) EHD
	Caspase 5	(W/L) EHD
	Caspase 13	WEHD
	Caspase 14	WEHD
Group II	Caspase 2	DEHD
	Caspase 8	LETD
	Caspase 9	LEHD
	Caspase 10	LEXD
Group III	Caspase 3	DEVD
	Caspase 6	VEHD
	Caspase 7	DEVD

Activation of Caspase 3

Many times the function of caspases has been gone through in programmed cell death [26]. It is usually accepted that, in mammals, two main pathways have been involved in activating the caspase cascade, namely, the mitochondrial pathway (intrinsic pathway) and the death receptor pathway (extrinsic pathway) [27].

Intrinsic Pathway

The intrinsic pathway is started in response to a wide variety of death triggers that are formed inside the cell, such as oncogene stimulation and DNA damage. The inactivation of this pathway is generally documented as the hallmark of cancer [28]. Mitochondria mediate the intrinsic pathway, in response to apoptotic triggers. In this way, various proteins are released from the inter membrane space of mitochondria into the cytoplasm [29]. Cells undergoing mitochondrial-dependent pathways are type 2 cells. Quite a few of the fully implied proteins include cytochrome c, SMAC (Second Mitochondria-derived Activator of Caspases)/DIABLO (Direct Inhibitor of Apoptosis (IAP)-binding protein with low pI), AIF (Apoptosis-Inducing Factor), Endo G (Endonuclease G) and OMI/HTRA2 (High-Temperature-Requirement protein A2). Possibly one of the most interesting pro-apoptotic proteins is cytochrome which binds to and activates the protein APAF1 in the cytoplasm [30]. In the intrinsic pathway, Mitochondrial Outer Membrane Permeabilization (MOMP) is one of the key functions which are implicated to be carried out by Bcl-2 family members. Important characteristics of these proteins are having one or more Bcl-2 Homology (BH) domains (Figure 2) and are divided into pro- (Bax, Bak, etc.) and anti-apoptotic (Bcl-2, Bcl-XL, etc.) proteins. Pro-apoptotic members are again divided into two subgroups: The BH3-only proteins and the proteins that possess BH1, BH2, and BH3 domains (Figure 4).

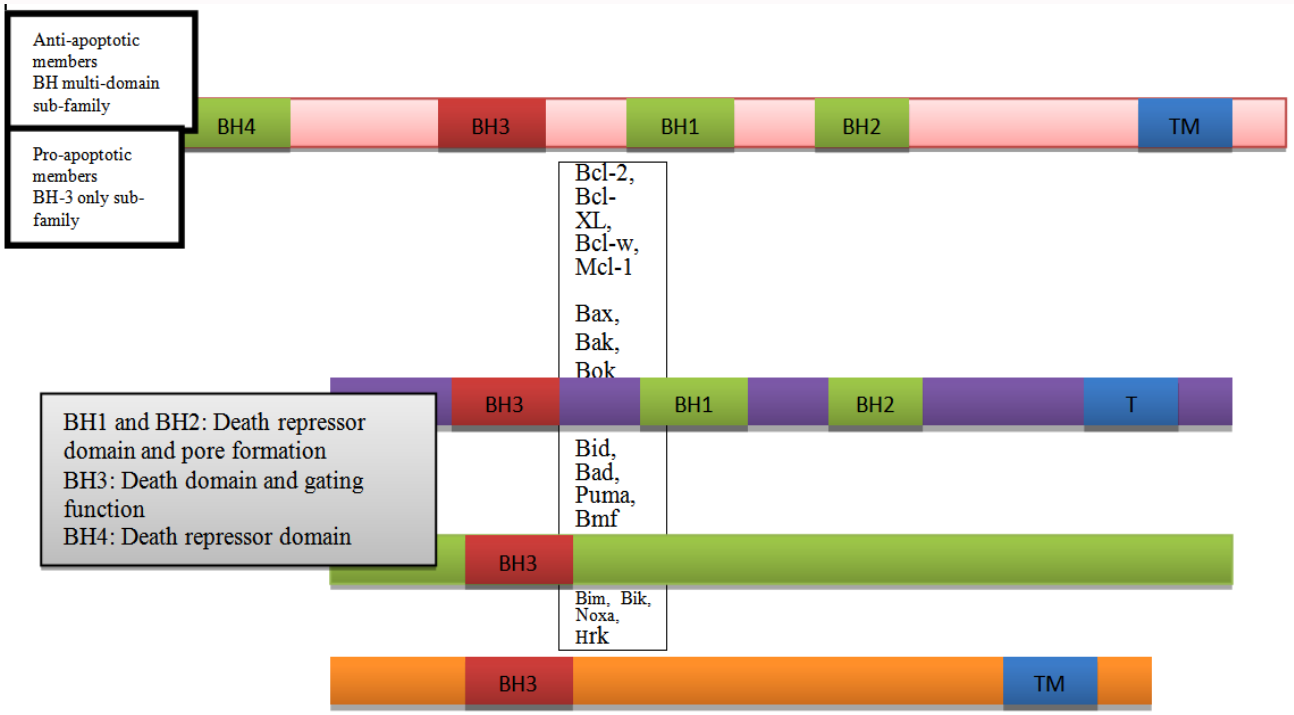


Figure 4: Bcl-2 family members.

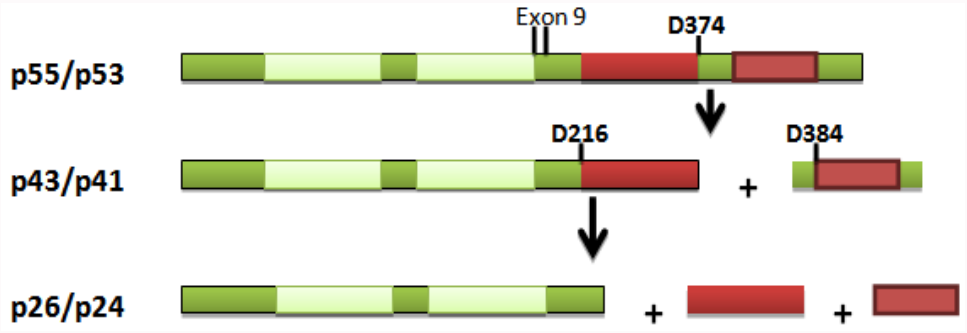


Figure 5: Activation of caspase 8.

Apoptotic stimuli cause Bax and Bak to undergo some conformational changes and are exposed to oligomerization [31] and they cause MOMP to form pores or connect with channels by disrupting the lipid bilayer, [32]. Bax and Bak are neutralized by Bcl-2 anti-apoptotic members that are positioned in the outer membrane. In this regulation, BH3-only proteins play a central role [33]. The result of MOMP is the discharge of cytochrome c into the cytoplasm, which (in the presence of dATP) causes the expansion of the Apaf1-containing macromolecular complex identified as apoptosome. As a result, this complex binds and activates procaspase-9. Mature caspase-9 remains bound to the apoptosome, recruiting and stimulating executioner caspase-3 and/or caspase-7. (Among these, XIAP is the only direct caspase-3 inhibitor) inhibit the activation of caspase-3 and -7. IAPs can be antagonized by Smac/Diablo and Omi/HtrA2, which are released from the inter membrane space when mitochondria are damaged. Apaf-1 has three characteristic domains: An N-terminal CARD, a nucleotide-binding domain, and 12-13 recurrences of WD40 near its C-terminal. CARD is accountable to binding the prodomain of procaspase-9, and therefore it is essential for procaspase-9 recruitment and activation. Nucleotide binding-domain Apaf-1 goes through oligomerization in the presence of cytochrome c and dATP. WD40 repeats are related to the interaction of Apaf-1 and cytochrome c [5].

Extrinsic Pathway

Cells that use mitochondrial independent pathways are type 1 cells. Type I cells have developed a method to evade mitochondrial functions by stimulating large amounts of caspase-8 at the DISC, thus activating caspase-3 directly, with no dependency on mitochondrial stimulation. This signaling pathway remains unaffected by Bcl-2 or Bcl-X_L [34]. Signals from a cell surface receptor trigger the extrinsic pathway. The best understood is the death receptor, Fas; Fas Ligand (FasL) that binds to their corresponding receptor leading to receptor trimerization and recruitment of definite adaptor proteins. The Fas receptor holds a Death Domain (DD) in its cytoplasmic region that interrelates with the Fas-Associated DD protein (FADD) which is an adaptor protein, developing a Death receptor-Induced Signaling Complex (DISC). Likewise, FADD retains a Death Effector Domain

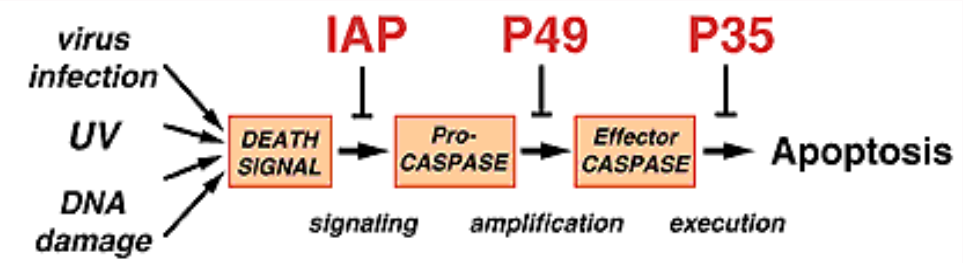


Figure 6: Diagram showing apoptosis pathway and steps inhibited by baculovirus apoptotic suppressors IAP, P49, and P35.

Table 3: Substrates of caspase 3.

Caspase	Other names	Size and subunits	Substrates	Cleavage motif
Caspase-3	CPP32, YAMA, apopain	proenzyme: 31594 Da, active: p17 and p12	PARP DNA-PK (cs) Protein Kinase C delta Huntingtin SREBP-1 and SREBP-2 Rb DNA Frag. Factor (DFF)	DEVD - G DEVD - N DMQD - N ILVD - G DEPD - S DEAD - G DETD-S/DAVD-T

(DED) and this collects the DED-containing procaspase-8 into the DISC and at the DISCProcaspase-8 is activated. Two isoforms of procaspase-8 (procaspase-8a and procaspase-8b) were stated to be bound to the DISC. Both isoforms possess two DED domains, as well as the catalytic subunits p18 and p10. Procaspase-8a contains further 2 kDa (15 aa) fragment, that is formed by the translation of exon 9. This small fragment exists between the large catalytic subunit and the second DED producing different lengths of procaspase-8a (p55) and procaspase-8b (p53). Moreover, dimerization of two procaspase-8 molecules at the DISC is crucial for procaspase-8 activation. Procaspase-8a/b at the DISC associated with autocatalytic cleavage, for which a two-step mechanism has been demonstrated in the Figure 5. Two subunits p43/p41 and p12 are formed by the initial cleavage at Asp^{374}. In final step, cleavage takes place at Asp^{216} and Asp^{384}, which forms the active enzyme subunits p18, p10 and the prodomains p26/p24. Consequently, the caspase-8 heterotetramer $(p18/p10)_2$ is involved in the apoptotic signaling cascade.

Procaspase-8 is proteolytically cleaves and activates caspase-8, which in turn activates downstream effector caspases. In some cell types, the death receptor and mitochondrion-associated death pathways are dependent and may interact with each other [35]. The mitochondrial pathway is implicated through caspase-8-mediated cleavage of Bid, which is a pro-apoptotic member of the Bcl-2 family. Once cleaved, truncated Bid (t-Bid) translocates to the mitochondria, where cytochrome c is released in response to it. Both the extrinsic and intrinsic pathways join in at caspase-3, which with the assistance of other effector caspases (such as caspase-7 and -6), arranges the dismantling of diverse cell structures through cleavage of a specific substrate. These caspase-mediated cleavages are responsible for the phenotypic changes witnessed in the apoptotic cell. Examples of ligands and Cell Surface Death Receptors (CSDR) are: FAS/CD95 Ligand (FASL) binds to FAS receptor, TNF and lymphotoxin alpha bind to TNFR1, Apo3 Ligand (Apo3L) binds to DR3, Apo2 Ligand (Apo2L) or TRAIL binds to DR4 and DR5.

Caspase 3 Role in DNA Fragmentation

By proteolytically inhibiting DFF45 (DNA fragmentation factor-45)/ICAD (inhibitor of caspase-activated DNase) caspase-3 causes apoptotic DNA fragmentation, and active DFF40/CAD (caspase-activated DNase), the inhibitor's associated endonuclease is released. Prominently, only the DEVD cleaving caspases, caspase-3 and caspase-7, inhibit DFF45/ICAD and promote DNA fragmentation in an *in vitro* DFF40/CAD assay, signifying that granzyme B, caspase-6, and caspase-8 are responsible for DFF45/ICAD inhibition and DNA fragmentation eventually by activating caspase-3 and/or caspase-7. Together, the data suggests that caspase-3 is the primary inhibitor of DFF45/ICAD and as a result the primary activator of apoptotic DNA fragmentation. DFF40/CAD remains latent by linking to DFF45/ICAD. Instead, caspase-3 splits DFF45/ICAD at two sites, thus releasing the endonuclease, which then cuts DNA. DFF45/ICAD cleavage at the N-terminal caspase site (Asp117) is both vital and enough for DFF40/CAD stimulation. However DFF45/ICAD cleaved only at the C-terminal caspase site (Asp224), and maintaining DFF40/CAD inhibitory activity [36].

Caspase Family Protease Regulating Factors

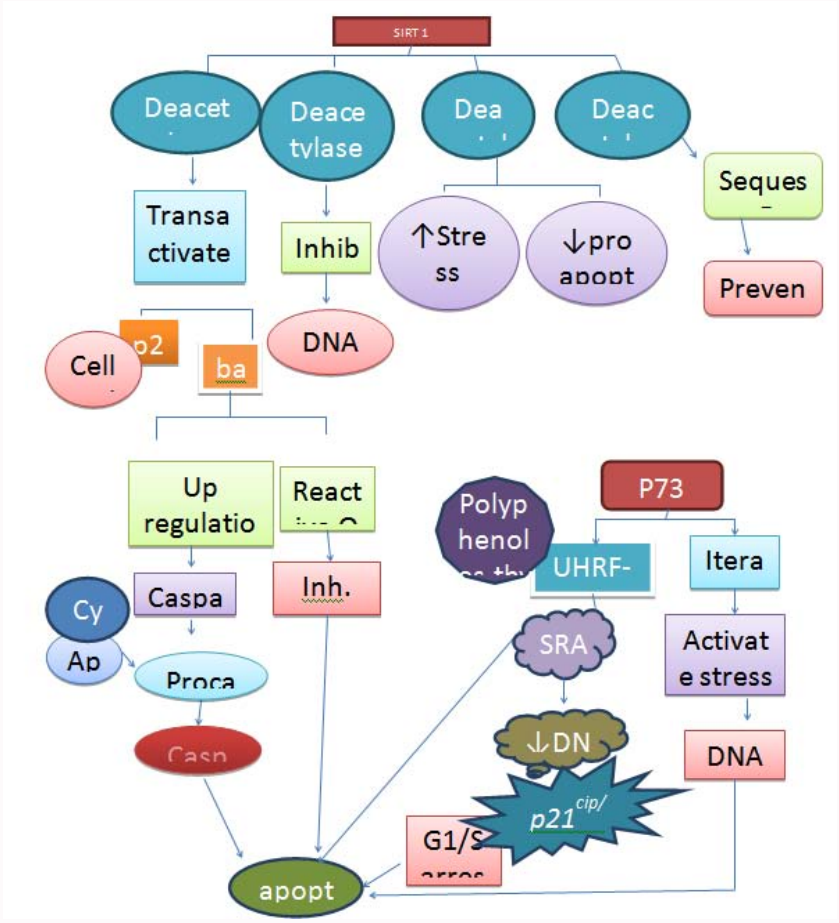
The activation and inactivation of caspases are synchronized by a variety of proteins, ions and other factors, for instance IAP, Bcl-2 family proteins, calpain, Ca^{2+}, Gran B and cytokine response modifier A (Crm A).

Inhibitors of Apoptosis (IAP)

IAP was first approved in insect cells infected by the baculovirus. Presently, in humans, the recognized members of the IAP family are: cIAP1, cIAP2, XIAP (X-linked mammalian Inhibitor of Apoptosis Protein), ML-IAP (Melanoma IAP), NAIP (Neuronal Apoptosis Inhibitory Protein), ILP2 (IAP like protein 2), Bruce/Apollon, surviving and living. A cell can be protected from undergoing apoptosis by IAP through down regulating the activity of caspases. The activity of mammalian IAP can be down regulated by Smac/DIABLO which is released from mitochondria. Caspases 3, 6, 7 and 9 are sensitive to IAP's and are down regulated.

Bcl-2 Family Proteins

The members of the Bcl-2 family belong to a group of important regulatory machinery in apoptosis. The members can be allocated into two classes. The Bcl-2 family members are effective modulators of cell



Relationship of caspase 3 with SIRT 1, p73, UHRF 1.

death and have an influence on the penetrability of the mitochondria. Based on their homology domain (BH), they can be grouped into two subcategories: Anti-apoptotic members and pro-apoptotic members. The BH3-only proteins cannot kill in the absence of Bax and Bak. The BH1, BH2, and BH3 region of the anti-apoptotic and maybe of the BH multi-domain sub-family produce a hydrophobic pocket which can connect with the BH3 domain of the pro-apoptotic proteins. Most of the Bcl-2 family members have a carboxy-terminal Transmembrane domain (TM) responsible for their targeting to intracellular membrane. In Bax and Bcl-w, the C-terminal tail is bound to hydrophobic pocket and should fit to permit in corporation in the membrane.

Group I family proteins all have anti-apoptotic properties, comprising of A1/Bfl1, Bcl-2, Bcl-w, Bcl-xL, Boo/Diva, Mcl-1, NR-13 and Nrf3 in mammals.

Group II family contain all pro-apoptotic proteins, including Bad, Bak, Bax, Bcl-rambo, Bcl-xS, Bid, Bik, Bim, Blk, BNIP3, Bok/Mtd, Hrk and Nip3 in mammals. The most obvious mechanism of their anti-apoptotic character is down regulation of pro-apoptotic proteins of the Bcl-2 family by linking to them.

Gran B, Crm A and p35

Gran B has the capability to excite several pro-caspases, such as procaspase-3, procaspase-7, procaspase-8, procaspase-9 and procaspase-10, to start apoptosis. In 2000, Barry et al. proposed that Bid can be cleared by Gran B to initiate mitochondrion-mediated activation pathway. And Crm A, a type of serpin from the vaccinia virus can inhibit the activity of Gran B. Crm A is a strong inhibitor of caspase-1 and caspase-8, and a weak inhibitor of caspase-3 and caspase-6, can avoid the cross-link of Fas and inactivate Gran B.

Baculovirus p35

They have the ability to link with caspases to split in this way causes the inhibition. They are powerful inhibitor of caspases ranging from caspase-1 to caspase-8 (Figure 6).

Substrates of Caspase 3

Caspase 3 is cleaved by many different substrates, especially by DNA fragmentation factor DFF-45, the actin regulatory protein gelsolin, the DNA repair enzymes poly (ADP-ribose) polymerase (PARP) and DNA-dependent Protein Kinase catalytic subunit (DNA-PK_{cs}), structural proteins such as α-fodrin, the signaling enzymes Protein Kinase Cδ (PKCδ), cytosolic Phospholipase A_2 ($cPLA_2$), sterol-regulatory element-binding proteins, and p21-Activated Kinase 2 (PAK2). It looks that cutting of these substrates and many different proteins is damaging for the cell but it is not clear that which substrates is cleaved and involved in cell death and cause apoptosis. Another unresolved problem is *in vivo*, elucidation of substrate specificities of the individual proteases. And it is more complicated because *in vitro*, more than one caspase precursor is cleaved by many caspase substrates.

DFF45/ICAD

This substrate is classified as a loss-of-function because although it causes nuclease activation as outcome at the end but reason is loss of its chaperone/inhibitor. The nuclease which causes double-strand cleavages in apoptosis, DFF40/CAD found in non-covalent complex with its endogenous chaperone/inhibitor DFF45/ICAD. Disruption of this complex occurs when caspase-3 break ICAD at $DETD_{117}$ ↓S and $DEVD_{224}$ ↓T, found in domain D1 and domain D2 and domain D3. As a result of following actions free nuclease dimerize into competent catalytic form [36-39]. Highly flexible and exposed linkers mapped with both cleavage sites as with BID. Activated CAD has crystal structure is Zn^{2+} -mediated dimer [39]. Inhibitory activity is due to central D2 domain of ICAD and it is of point that after caspase-cleavage, enzymes affinity is decreased so, inhibition is relieved. Inhibition depends not only on inhibitor destruction but in a way that proteasome destroys the NK-^{x}B inhibitor I ^{x}B but ICAD structure loses as a result of limited cleavage so that other CAD unit allow displacement so that dimerization of catalytic nuclease.

Poly(ADP-ribose) Polymerase

Poly(ADP-ribose) Polymerase (PARP) is nuclear protein which is found abundantly which catalyses poly(ADP-ribose) ligation as a result of DNA strand breakage. The $DEVD_{213}$ ↓Gsite of PARP cleaved by caspase 3, and 7 as a result of bipartite nuclear localization signal, such form is produced which cannot be produced by ADP-ribose polymers as a result of DNA damage [40]. PARP is added in those substrates which are cleaved more by executioner caspase than inflammatory caspase 1 [36,41]. Substrate specificity of executioner caspases 3 and 7 perfectly matches with PARP cleavage site. Second interaction may be due to caspase 7 whose activity is less then caspase 3 at short synthetic substrate. PARP molecule modification by long, branched poly(ADP-ribose) chains are more efficient then caspase 3 homologue. Interactions between PARP and caspase-7 large subunit hinting at an exosite interaction [37]. When mice express a transgenic PARP mutant ($DEVN_{213}$ ↓G) develop which are normal caspase cleavage site. But *in vivo*, renal ischemia-reperfusion, intestinal and endotoxic shock resistance occurs representing tissue restricted defect in mice is same as failure to protein cleavage in those mice having RB cleavage which is caspase mediated [42].

PKC

Belongs to serine/threonine protein kinases family, in many growth factor receptors signal transduction takes place due to this protein and also in many cell type apoptosis is due to this protein. In the hinge region of PKC recognition sequence ($DMQD^{330}$/N for PKCδ) which is identified as caspase 3 cleavage site (CPP32), by caspase 3 inhibitors proteolysis of PKC can be blocked or anti-apoptotic proteins Bcl-2 or Bcl-x. Apoptosis can also be induced as a result of expression of catalytic domain of PKCδ in HeLa, NIH3T3, and COS1 cells, which shows that PKCδ may have important role in apoptosis. In keratinocyte maturation program PKC is central regulator. Five PKC isoforms (α, δ, ε, η, and ζ) are expressed by keratinocytes but in differentiating keratinocytes PKCη only expressed. PKCα and PKCη activation or increase in expression linked to differentiation program. In skin chemical carcinogenesis molecular target for tumor promoting phorbol models are classic and novel PKC isoforms. In human keratinocytes UV exposure on PKC isoforms was studied due to role of PKC in apoptosis in carcinogenesis and differentiation. Due to UV radiation apoptosis is inhibited which is result of PKC inhibition, as a result of PKCδ activation.

APP

Amyloid Precursor Protein (APP) produce β-Amyloid (Aβ) partial membrane spanning fragments as a result of the proteolytic enzymes beta and gamma secretases. By the action of caspases APP cleaved at $VEVA_{664}$ ↓A in cytosol and cell toxicity results due to fragment (C31) [43]. In Alzheimer disease studies transgenic mouse model show mutation in $VEVA_{664}$ ↓A site with caspases show neuronal cell protection and astrologies as compared with APP non mutated disease causing variant as compared with levels of soluble and deposited Aβ 1-40 and 1-42 [44]. In Alzheimer disease neurotoxic insult is due to points to C31 not due to Aβ but it cannot neglect the Amyloid hypothesis that cleavage of APP is due to Aβ in cytoplasm a result of caspases which form effector C31 but it is indistinct to know which caspase is accountable for APP cleavage to produce C31 as for HTT, or exactly what is mechanism of caspase activation how accumulation of C31 in nucleus takes place and how cell death occurs it is not known.

Huntingtin

In neurnal nuclei, the accumulation of N-Terminal Huntingtin (HTT) fragments is a neurodegenerative disorder called Huntington's disease. The disease pathology is caused due to expansion of poly-glutamine sequence at several caspase cleavage sites. The caspase-6 cleavage site located at $ILVD_{586}$ ↓G, which is critical for generating a toxic HTT fragment is due to transgenic mice expressing HTT with mutated potential caspase cleavage. The excitotoxic neuronal death is resistant to transgenic mice expressing a $ILVA_{586}$ ↓G cleavage site mutant [45]. Although the function of HTT is unknown, overexpression of wild-type HTT has shown a protective phenotype under models of neuronal stress [46]. The removal of HTT's protective function is not only reported in caspase cleavage of HTT, but also generates a toxic byproduct that sensitizes neurons to excitotoxic stimulation stressors. As a nature and origin of the caspase activity, which must be generated to produce the toxic fragment, which is the unknown mechanism of neurotoxicity.

RB

Tumor suppressor Retinoblastoma-associated protein (RB) phase by inhibiting transcription from E2F promoters inhibits cell cycle progression into S-phase. At $DEAD_{886}$ ↓G C-terminal region of RB cleaved by caspases through extrinsic pathway leads to apoptosis but not intrinsic pathway leads to degradation and destabilization. For Tumor Necrosis Factor Receptor (TNFR)-I-mediated apoptosis RB must be degraded. *In vitro* uncleavable sequence DEAA886 G protects fibroblasts against TNFR-I-induced apoptotic signaling as a result of germline replacement of the caspase cleavage site and *in vivo* endotoxic shock from intestinal cells. As a result of signaling and ligation of both TNFR-I and II signifies another mechanism of RB degradation [47]. In mouse intestine extrinsic pathway involve in progression of apoptosis, it is not known which RB function involve in apoptosis prevention and how cleavage of RB by caspases is involved in its degradation.

Caspases as Targets in Drug Development

Caspases, being the key effector molecules in apoptosis, are potential targets for pharmacological modulation of cell death. First, increased levels of caspase activity are often observed at sites of cellular damage in a number of diseases, including myocardial

infarction, stroke, sepsis, and Alzheimer, Parkinson, and Huntington diseases. Inhibition of caspase activity for these diseases is predicted to be therapeutically beneficial. Second, discovery of drugs that selectively inhibit inflammatory caspases (caspase-1, -4, and -5) may help to control autoimmune diseases like rheumatoid arthritis. Finally, selective activation of caspases would be an approach in the treatment of cancer and chronic viral infections.

One of the promising strategies involves selective activation of caspases in cancer cells, leading to induction of apoptosis. An important contribution to this strategy was achieved by the approach of "forward chemical genetics" [48]. This involves screening of small molecules for their ability to perturb cellular pathways and subsequently identifying the specific targets of the active compounds. A number of potential drugs for selective induction of apoptosis were found by high-throughput screening of the compounds activating caspase-3 as a central suicide caspase. Among them are a small molecule, PETCM [α-(trichloromethyl)-4-pyridine ethanol] carbamate [49] and indolone classes of compounds [50]; and MX2167, MX116407, MX128504, and MX90745. These compounds engage different pathways; e.g., PETCM accelerates apoptosome formation by interacting with the inhibitor prothymosin-α [49]; carbamate and indolone classes of compounds promote Apaf-1 oligomerization and, thereby, apoptosome formation with the subsequent activation of caspase-3 and -9 [50].

Relationship of Caspase 3 with Other Epigenetic Proteins

SIRT1 causes the deacetylation of non-histone proteins and renders mammalian cell survival in oxidative stress condition and DNA damage at least three mechanisms. First SIRT1 causes the deacetylation of the DNA damage-repair protein Ku70. The deacetylated form of Ku70 sequesters Bax protein in the cytoplasm and prevents it from translocating to mitochondria to commence apoptosis [51,52]. Secondly SIRT1 brings about the deacetylation of FOXO family proteins culminating in dual effects on transcriptional repression of the proteins downstream pro-apoptotic target [53-59], Bim and upregulation of the stress-resistance gene GADD45 [60]. The association of SIRT1 in transcriptional regulation is by deacetylation of histones and non-histone proteins that interact with SIRT1. At third, SIRT1 causes the deacetylation of p53 and increases its ability to trans-activate its downstream target genes for instance p21 for cell-cycle arrest and Bax for the process of apoptosis [60,61]. Bax cause upregulation of death receptors CD95/Fas/APO1 or DR5 killers which leads to activation of pro-caspase 9 through Apaf1 pathway which leads to activation of caspase 3 and causes apoptosis. On the other hand, Bax produces reactive oxygen species which inhibit cell growth and causes apoptosis. p53 is definitely a SIRT1 target [61,62]. In fact the p53 tumor suppressor gene has been implicated to rouse the expression of numerous proteins concerned with cell cycle arrest and apoptosis including p21, an inhibitor of the cyclin-dependent kinase 2 and 4, hence restrains cells to traverse the next stage of cell division.

Moreover p53, p73 is a p53-related nuclear transcription factor that can bind to the p53-responsive elements, and trans-activate an overlapping set of p53-target genes concerned with G1 cell cycle arrest and apoptotic cell death [63,64]. p73 can bring on apoptosis in cancer cells exhibiting resistance to the p53-dependent apoptotic program [64,65]. It is shown that p73 is a mediator of the anticancer activity of RWPs in view of the fact that p73 was strikingly unregulated at concentrations suggesting growth arrest and apoptosis in Jurkat cells. P73 interact with C-Abl which activate Stress Protein Kinase (SAPK) lead to DNA damage which causes apoptosis. Besides UHRF1 a component of a subfamily of RING-finger type E3 ubiquitin ligases binds to specific DNA sequences, and to engage a DNA Methyl Transferase 1 (DNMT 1) to regulate gene expression. UHRF1 plays a key role in the G1/S transition by regulating topoisomerase II alpha and retinoblastoma gene expression, and plays part in the p53-dependent DNA damage process Red Wine Polyphenoles (RWPs) as well as Aroniamelanocarpa Juice (AMJ) strikingly down-regulate UHRF1 in Jurkat cells. In general, it shows that the anti-proliferative and pro-apoptotic effects of RWPs engross an up-regulation of the cell cycle inhibitor p73 and a down-regulation of the cell cycle promoter UHRF1. *Aronia melanocarpa* Juice (AMJ) like red wine polyphenoles induce pro-apoptotic pathway which is dependent on p73 as well as the dowregulation of UHRF1 in Jurkat cells [66]. UHRF 1 binds with methylated DNA. AJM acts on G2/M phases of cell cycle arrest and apoptosis of lymphoblastic leukemia Jurkat cell, Lake P53 *via* p73- pathway which causes down-regulation of UHRF1. UHRF-1 down regulation activates SRA which down regulate DNMT 1 and activate P21 and P16 which causes G1/S arrest leads to apoptosis. Pro-apoptotic activity of AMJ depends on concentration which activates caspase 3 which is major protein in p73-related pro-apoptotic pathway [67,68]. This study shows that AJM and RWPs can induce apoptosis, through a caspase 3-dependent pro-apoptotic pathway in leukemic cells in absence of p53 which is a gate keeper. This study also shows that by this pathway of AJM, we can induce apoptosis in p53 resistant patients [69].

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