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Review article:

APOPTOSIS: MOLECULAR MECHANISMS AND PATHOGENICITY

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ABSTRACT

Apoptosis triggered by exogenous and endogenous stimuli such as ultraviolet radiation, oxidative stress, and genotoxic chemicals is a crucial phenomenon within biological systems. DNA damage activates and stabilizes p53 in nucleus and cytoplasm and regulates other proteins that stimulate intrinsic and extrinsic apoptotic pathways. Apoptosis is morphologically distinct from that of necrosis and both the phenomena depend on the types, developmental stages, physiological environment of tissues and the nature of death signal. Malfunctioning of apoptotic pathway may cause human diseases like cancer, neurodegenerative and autoimmune disorders. Recently, potent apoptosis-inducing compounds associated with human health have been recorded that prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses. Certain photosensitizing drugs are being employed in photodynamic therapy to induce apoptosis for the treatment of cancer and non-cancerous cells. This review emphasizes the molecular mechanisms of apoptosis, associated diseases and certain therapeutic agents implicated in the elimination of malignant cells.

Keywords: apoptosis, apoptosis-inducing drugs, caspase, necrosis, pathogenesis, photodynamic therapy, ultraviolet radiation

INTRODUCTION

In addition to cell-cycle arrest and repair machinery, the damaged cells, where damage is beyond repair, may induce an apoptotic (programmed cell death; PCD) response that is highly cell-specific and is the most common form of physiologic cell death in multicellular forms. The concept of PCD was first developed by plant biologists in 1920s (Jones, 2001). In unicellular organisms the mechanisms of PCD is less understood in comparison to metazoan, however some workers assume it as unicellular origin with a later adoption by multicellular life (Ameisen, 2002). Apoptosis play an essential role in survival of the organisms and is considered to be an imperative component of various processes including normal cell turnover, proper development and functioning of the immune system, multiplication of mutated chromosomes, hormone-dependent atrophy, normal embryonic development, elimination of indisposed cells and maintenance of cell homeostasis (Reed & Tomaselli, 2000; Elmore, 2007). DNA damage and production of the predominant lesions such as cyclobutane pyrimidine dimers (CPDs), 6–4 photoproducts (6–4PPs) and certain other lesions (Sinha & Häder, 2002; Kumari et al., 2008) as a result of UV radiation (UVR), ionizing radiation (IR), oxidative stress, replication or recombination errors as well as from en-

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vironmental and therapeutic genotoxins is one of the principal triggers of apoptosis (Nagata, 1997; Norbury & Zhivotovsky, 2004; Batista et al., 2009).

UVR (mostly UV-B; 280–315 nm) is one of the most potent carcinogens that affect the normal life processes of all organisms ranging from bacteria to human (Häder & Sinha, 2005; Friedberg et al., 2006). UV radiation is a DNA damaging agent that activates and stabilizes p53 in nucleus as well as in cytoplasm and regulates a number of genes which stimulate two major apoptotic pathways: the intrinsic and the extrinsic pathways (Haupt et al., 2003). A key tumor suppressor p53, susceptible to DNA damage induced apoptosis (DDIA) (Clarke et al., 1993) is functionally conserved in multicellular animals such as *Caenorhabditis elegans* (*Cep-1*) to human cells (Schumacher et al., 2001), but is apparently absent from unicellular species (Norbury & Zhivotovsky, 2004). The apoptotic regulators in *C. elegans* such as Egl-1, Ced-9, Ced-4, and Ced-3 show homology with mammalian apoptotic proteins such as BH3, Bcl-2, Apaf-1, and Casp-9 respectively, which indicates a highly conserved cell death mechanism that exists in worms and mammals (Jiang & Wang, 2004) (Figure 1). Activation of p53 by DNA damage can lead to apoptosis by transcriptional activation of pro-apoptotic members of Bcl-2 family, such as Puma, Noxa, Bim, Bid, Bik, Bak, Bax, Apaf-1, Bmf, Hrk, Pag608, Drs, Fas and Gadd45 (Vogelstein et al., 2000; Vousden and Lu, 2002; Tian et al., 2007). This diverse family includes multidomain pro-apoptotic (e. g. Bax, Bak), anti-a programmed cell death (PCD apoptotic (e. g. Bcl-2, Bcl-XL, Mcl-1) as well as a number of BH3 only (e. g. Puma, Noxa, Bid, etc.) proteins that together regulate mitochondrial permeability (Cory & Adams, 2002). However, the relevance of transcriptional activation in p53 apoptosis in human cancer is highly controversial. Kakudo et al. (2005) have shown that p53 mediated transactivation does not play a major role in p53 dependent apoptosis. The study of mouse fibroblasts treated with different doses of UV and γ irradiations has also revealed the primary response mediated by p53 transcriptional activation as anti-apoptotic, whereas pro-apoptotic response requires an additional, transcriptional-independent mechanism (Speidel et al., 2006). Generally senescent cells are resistant to apoptosis induced by genotoxic stress and the study of pre-senescent primary human diploid foreskin fibroblasts has also revealed that pass aging cells results in progressive acquisition of resistance to UV-induced apoptosis in the presence of BCL-2- family proteins (Rochette & Brash, 2008).

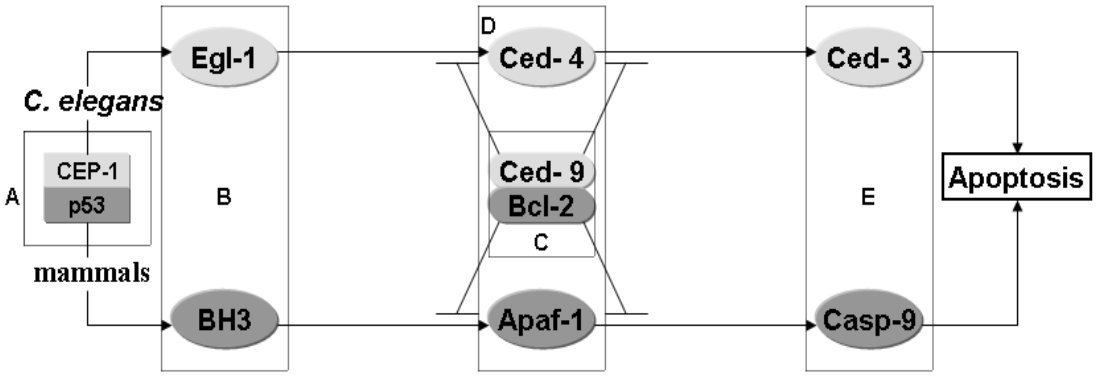

Figure 1: Programmed cell death in *C. elegans* and mammals showing highly conserved cell death machinery (in box), **A** (CEP-1/p53), **B** (Egl-1/BH3), **C** (Ced-9/Bcl-2), **D** (Ced-4/Apaf-1) and **E** (Ced-3/Casp-9).

Recently, Wu et al. (2008) have confirmed that both p53 transcription-dependent and independent pathways contribute to UV-induced apoptosis. In cells, the level of p53 is maintained through its MDM2-mediated degradation. Several lines of evidence show that transcriptional-independent p53 apoptosis (Erster et al., 2004; Yu, 2006) is directly linked to the intrinsic or mitochondrial apoptotic pathway (Chipuk et al., 2004; Leu et al., 2004), where it interacts with anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-XL) and stop their action to promote the activities of BH3-only proteins (Bak/Bax). Pro-apoptotic Bax/Bak are essential regulators of the mitochondrial or intrinsic pathway of apoptosis (Ranger et al., 2001). Bak resides permanently on the outer mitochondrial membrane (OMM), whereas Bax is normally found in the cytosol of healthy or UV-unirradiated cells, which translocates to the outer mitochondrial membrane during apoptosis (Gross et al., 1998). Wu et al. (2007) have demonstrated that translocation of Bax by the incidence of UV irradiation is a Bid-independent event, inhibited by over-expression of Bcl-xL and delayed by the p53 inhibitor. P53 plays an important role in Bax conformational change induced by UV radiation. The molecular mechanisms of Bax activation is not clearly understood, however, Chen et al. (2007) have shown the role of BimL in Bax activation during UV irradiation-induced apoptosis. Confocal microscopic studies has revealed the cytoplasmic distribution of BimL before UV irradiation, but after UV irradiation BimL translocated to mitochondria, although how Bim activates Bax to induce apoptosis, directly or indirectly is not clear (Marani et al., 2002; Zhu et al., 2004). The transcriptional factor E2Fs (E2F1-3) may also induce apoptosis either dependent or independent of p53. In later case E2Fs directly activates p53 related protein p73 (Irwin et al., 2000). p73-induced apoptosis is mediated by induction of a BH3-only protein PUMA, which leads to Bax mitochondrial translocation and release of cytochrome-c and triggers cell death (Ramadan et al., 2005). Recently, it has been found that TFDP3 is a negative regulator of E2F-1 induced apoptosis (Tian et al., 2007; Qiao et al., 2007). Some cells express Fas or TNF receptors that can lead to apoptosis *via* ligand binding and protein cross-linking. Other cells have a default death pathway that must be blocked by a survival factor such as a hormone or growth factor. Even though, a number of key apoptotic proteins have been recognized; the molecular mechanisms of action of these proteins remain to be elucidated. It seems that apoptosis is a synchronized and frequently energy-dependent course of action that involves the activation of a set of cysteine proteases known as "caspases" and a complex cascade of events that link the initiating stimuli to the ultimate demise of the cell. Apoptosis has been recognized as a typical and important mode of "programmed" cell death, which involves the genetically determined elimination of cells (Guicciardi & Gores, 2009). However, several other forms of PCD have been illustrated and quite a number of other forms of PCD may yet be discovered (Formigli et al., 2000; Sperandio et al., 2000; Debnath et al., 2005; Qiao et al., 2007). The main objective of this review is to provide an overview of existing knowledge on the molecular mechanisms of apoptosis.

Caspase (cysteine protease)

Organization

Caspases (c: cysteine protease mechanism, aspase: ability to cleave after aspartic acid) are aspartate-directed cysteine proteases that play a key role in the initiation and execution of apoptosis or PCD, necrosis and inflammation, failure of which may cause tumor development and several autoimmune diseases (Danial & Korsmeyer, 2004; Ghavami et al., 2009; Green et al., 2009). Structural details of several caspases (such as Casp-1, 2, 3, 7, 8 and 9) (Yan & Shi, 2005) have been described and it was found that in most cases, the functional caspase unit is a homodimer, with each monomer having a large (~17-21 kDa) and a small (~10-13 kDa) subunit (Nicholson,

1999; Tait & Green, 2008) (Figure 2). Components of the proteolytic mechanism, including the active site Cys and His residues, are harboured within the large subunit while aspartate (Asp) at the P1 site (P1Asp) is obscured in a cavernous pocket, expressed as S1 site, are derived from both the large as well as small subunits and the S2, S3, and S4 subsites are contributed by the small subunit and thus both the small as well as large subunits determine the active site (Earnshaw et al., 1999). Recently, it has been found that the active site is made up of five protruding loops (L1-L4 and L2') which are conserved in all caspases. Loop L1-L4 are present on one monomer and L2' on the nearby monomer. All the four loops together decide the sequence specificity of the substrates and the active site conformation is stabilized by the L2' loop from the adjacent monomer (Yan & Shi, 2005). Caspases are synthesized as zymogens where N-terminal prodomain is followed by the sequence programming of a large and subsequently a small subunit. The crystal structure of active caspase has revealed its tetramer structure (resulting from combination of two procaspase zymogens) containing two large subunits (homodimer) and two small subunits (heterodimer) folded into a dense canister having a central six-stranded β-sheet flanked by five helices. In the tetramer which is formed by the prodomain-facilitated dimerization of caspase, two of these cylinders align in a head-to-tail configuration, so that the two active sites are at opposite ends of the molecule (Figure 3) (Nicholson, 1999; Boatright & Salvesen, 2003; Yan & Shi, 2005). The initiator caspases contain death domain (DD) such as a caspase activation and recruitment domain (CARD) (e. g. caspases-1, 2, 4 and 9) or a death effector domain (DED) (e. g. caspases-8 and 10) (Figure 2) which facilitates caspases to act together with other molecules that direct their activation. The DED has been found to be involved in interactions with DEDs of signaling adapter proteins such as MORT1/FADD and TRADD (Hsu et al., 1995; Boldin et al., 1996). The CARD protein interaction motif is conserved among multiple key apoptotic regulators such as caspases, adaptor molecules RAIDD and Apaf-1, and inhibitors of apoptosis c-IAP1 and c-IAP2 (Ho & Hawkins, 2005).

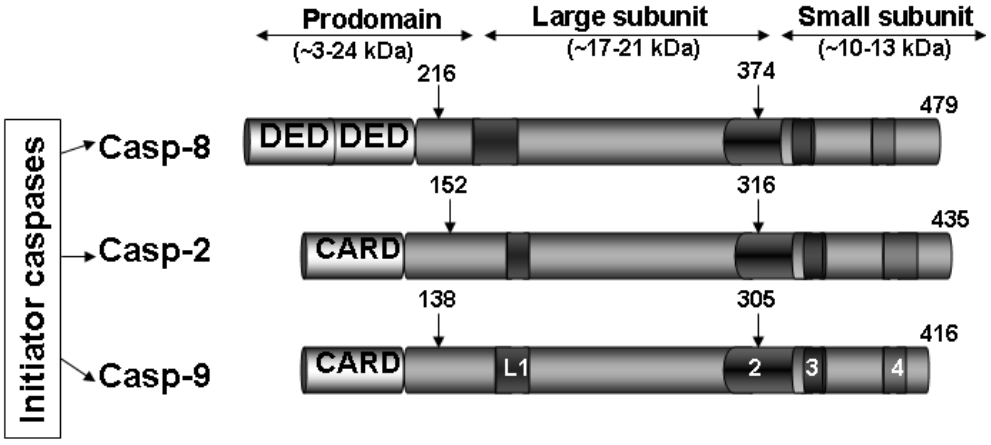

Figure 2: Graphic illustration showing the organization of mammalian initiator proteases (procaspases) having either CARD (e. g. caspase-2 and 3) or two DEDs (e. g. caspase-8) associated with long prodomain. The proteolytic cleavage (shown by arrow) takes place at specific sites during the production of the active enzyme. The active site of a caspase is determined by the four loops (L1-4). Monomeric form of caspases are generally formed by two subunits, i. e., a large (~17-21 kDa) and a small (~10-13k Da) subunit.

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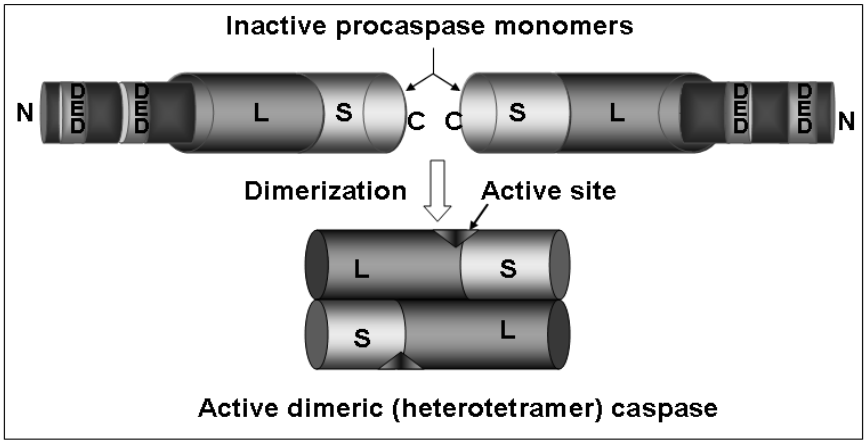

Figure 3: Diagrammatic view of initiator procaspase-8 activation. The zymogens of initiator procaspase exist as suppressed monomers. The prodomain of caspase-8 consists of two DED. The large and small subunits are shown by 'L' and 'S' respectively. The dimerization of monomeric units of caspase results into active dimeric caspase due to their proteolytic activation. The active dimeric caspase has two active-sites whose conformation is conserved in all known caspases.

Activation

Generally, caspases occur in the cells in an inactive form (i. e., procaspase) and once activated can trigger other caspases leading to amplification of apoptotic signaling pathway followed by cell death due to their proteolytic activity. Caspases are widely expressed in an inactive proenzyme (zymogens) form in most cells and are stimulated by an autoproteolytic mechanism or by meting out by other proteases and once activated can often activate other procaspases, allowing initiation of a protease cascade. Some pro-caspases can also aggregate and autoactivate. This proteolytic cascade, in which one caspase can activate other caspases, amplifies the apoptotic signaling pathway and thus leads to rapid cell death. At least 14 mammalian caspases (caspase- 1 to 14) have been recognized that can be categorized into three functional groups such as initiators (e. g., casp-2, 8, 9 and 10), effectors (e. g., casp-3, 6 and 7) and the prototypical member of a subclass of caspases involved in cytokine activation termed inflammatory caspases (casp-1, 4, 5, 11, 12 and 13) (Table 1) (Cohen, 1997; Rai et al., 2005; Elmore, 2007; Martinon & Tschopp, 2007; Pop & Salvesen, 2009). The term 'inflammatory' has been given as the main caspase-1 substrates recognized to date are proIL-1β (interleukin-1β) and proIL-18, two related cytokines that play critical roles in inflammation (Fischer et al., 2002). The specific substrates of mouse caspase-11 and caspase-12 and human caspase-4 and caspase-5 are not yet known, hence the specific functions of these caspases are still an open issue. However, Martinon & Tschopp (2007) have tried to discuss the current role of these caspases. The molecular mechanisms concerned with the activation of these inflammatory proteases have revealed the significant role for the NLR (NOD-like receptors) family members, NALPs (NALP1, 2 and 3), NAIP (neuronal apoptosis inhibitory protein) and IPAF (ICE protease-activating factor) proteins that promote the assembly of multiprotein complexes termed inflammasomes (Figure 4A), which are essential for activation of inflammatory caspases (Martinon & Tschopp, 2007). It has been found that caspase-11 regulates apoptosis and cytokine maturation during septic shock, caspase-12 intervenes endoplasmic-specific apoptosis and cytotoxicity by amyloid-β. The gene for caspase 12 is found on chromosome 11 in humans in a locus with other inflammatory caspases (Fischer et al., 2002). Caspase-13 (also known as Evolutionarily Related Interleukin-1β Converting Enzyme": ERICE) was identified in cattle and was originally reported as a human caspase that could be

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activated by caspase 8 (Humke et al., 1998) but later on caspase-13 was established as a bovine gene (Koenig et al., 2001). The exact function of caspase-14 is still not known, however, its occurrence has been found very specifically in the skin and supposed not to be involved in apoptosis or inflammation, but plays an important role in skin cell development, maintaining the balance of moisture in the skin, and protection against UV-B rays (Denecker et al., 2007, 2008). It has been concluded that the caspase-14-activating protease is not a caspase but most likely an epidermis-specific serine protease with elastase-like properties (Denecker et al., 2007). Recently, it has been observed that inflammatory caspases are essential in promoting a pro-inflammatory microenvironment and influencing increased epithelial cell death in the target tissues of Sjögren's syndrome (SjS) before disease onset (Bulosan et al., 2009).

The activation of an effector caspase, such as caspase-3, 6 or 7 is performed by an initiator caspase, such as caspase-2, 8, 9, or 10, through internal cleavages to separate the large and small subunits (Yan & Shi, 2005). The activation of these initiator caspases depends significantly on the commitment and establishment of recruitment platforms e. g., the death inducing signaling complex (DISC) (Figure 4B) for caspase-8 and caspase-10, the PIDDosome (Figure 4C) (having adaptor RAIDD and p53-induced protein with a death domain; PIDD) for caspase-2, and the well explained apoptosome (Figure 4D), for caspase-9 (Peter & Krammer, 2003; Tinel & Tschopp, 2004; Martinon & Tschopp, 2007).

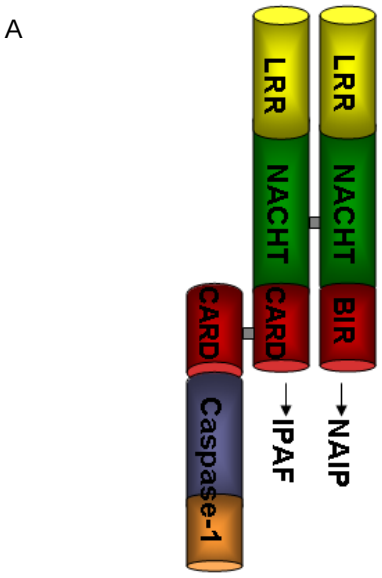

Figure 4: Diagrammatic representation of initiator caspase activation complexes.
(A) Caspase-1-activating inflammasomes associated with IPAF and NAIP may also be part of the complex.

Table 1: Common known caspases involved in apoptosis (or programmed cell death)

Cysteinyl aspartic acid-protease (caspase)		
Type	name	synonyms
Initiator (or Apical)	Caspase-2	ICH1, Nedd2
	Caspase-8	FLICE, MACH1, MCH5, FADD-like Ice
	Caspase-9	MCH6, ICELAP6
	Caspase-10	FLICE2, MCH4
Effectors (or executioner)	Caspase-3	CPP32, YAMA
	Caspase-6	MCH2
	Caspase-7	MCH3, CMH, ICELAP3
Inflammatory	Caspase-1	ICE
	Caspase-4	ICH2, TX, ICErII
	Caspase-5	ICErIII, TY
	Caspase-11	-
	Caspase-12	-
	Caspase-13	ERICE
	Caspase-14	MICE

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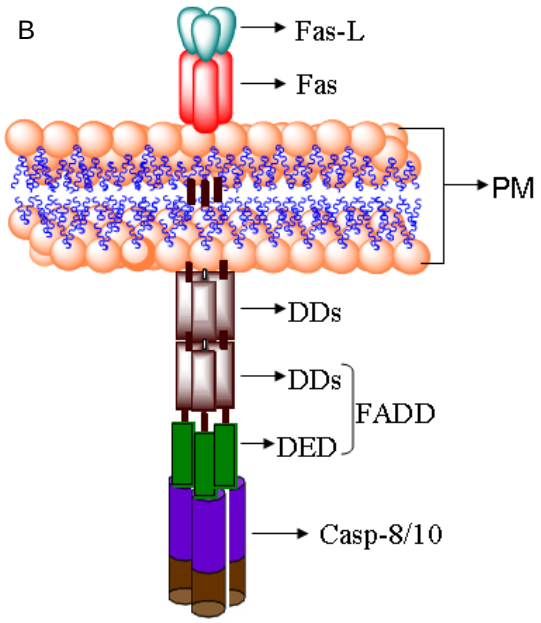
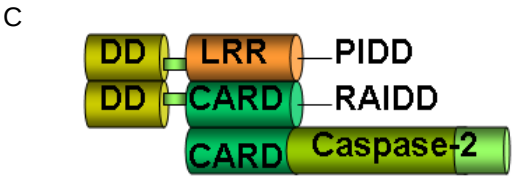
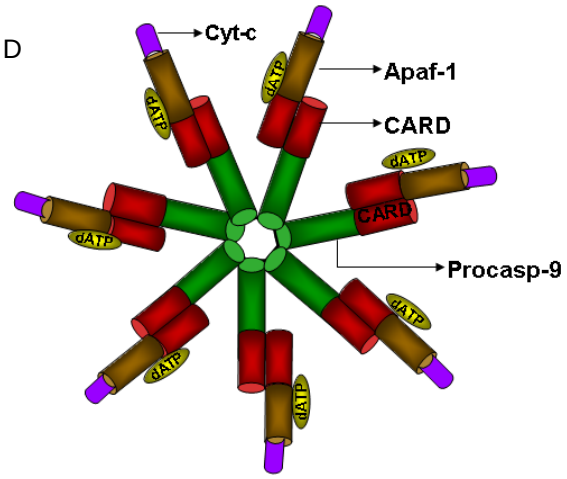


Figure 4: Diagrammatic representation of initiator caspase activation complexes.
(B) Death-inducing signaling complex (DISC): It consists of death ligands (e. g., FAS-L, TNF) linked with their receptor (e. g., FAS, TNF-R1) and includes FADD associated with DED of procaspase-8/10.



(C) PIDDosome: it consists of p53-related DD containing protein PIDD, RAIDD (having leucine-rich region, LRR) and procaspase-2.



(D) Apoptosome: It is heptameric array of Apaf-1 molecules, linked with cytochrome c in dATP dependent manner allowing the binding of procaspase-9 by means of their CARD.

These recruitment platforms are multi-protein complexes consisting of a variety of molecules accumulated on a central scaffold protein for example apoptosome scaffold protein Apaf-1, which is comprised of three domains such as ligand sensing, oligomerization and caspases recruitment domain. Apaf-1 possesses a CARD for caspase-9 recruitment, a NB-ARC domain for oligomerization and a WD repeat that senses the release of cytochrome c from the mitochondria and leads to apoptosis by the activation of apoptosome (Jiang & Wang, 2004; Pop et al., 2006).

Caspase inhibition

In mammals, inhibition of caspase is associated with the proteins belonging to IAPs (inhibitors of apoptosis) family (e. g. cIAP-1, cIAP-2, ILP-2, survivin, XIAP, Livin, BIRC, NAIP) that are differentiated by the presence of three baculoviral IAP repeats (BIR) and in some, a RING-finger motif at the C-terminus (Verhagen et al., 2001). The IAPs family proteins inhibit the function of some initiator (e. g. casp-9) and effector caspases (casp-3, and 7) by different processes (Deveraux et al., 1998; Sun et al., 1999; Kasof & Gomes, 2001), although several initiator as well as effector caspases (such as casp-1, 2, 6, 8 and 10) are resistant to inhibition by XIAP, c-IAP1 and c-IAP2 (Ho et al., 2005). The action of caspase-8 has been found to be regulated by a family of proteins known as FADD-like ICE (FLICE)-inhibitory proteins (FLIPs). It has been found that FLICE-inhibitory proteins (FLIPs) derived from a virus (v- FLIPs) are capable to bind with FADD and casp-8 by homotypic interactions and block the recruitment of casp-8 to the DISC leading to casp-8 inactivation (Thome et al., 1997). Regulation of caspase-9 activity can also be achieved by the release of mitochondrial proteins Smac /DIABLO and HtrA2 /Omi (Verhagen et al., 2000, 2002; Suzuki et al., 2001). Now it has become clear that ubiquitin-mediated degradation is implicated in the regulation of IAPs and proteins with which they interact (Vaux & Silke, 2005).

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Mechanisms of apoptosis

The pathways of apoptosis are extremely complicated, where energy-dependent flow of molecular events takes place (Figure 5). Mainly two types of apoptotic pathways such as intrinsic and extrinsic (or death receptor) have been well described that involve a number of proteins (Table 2).

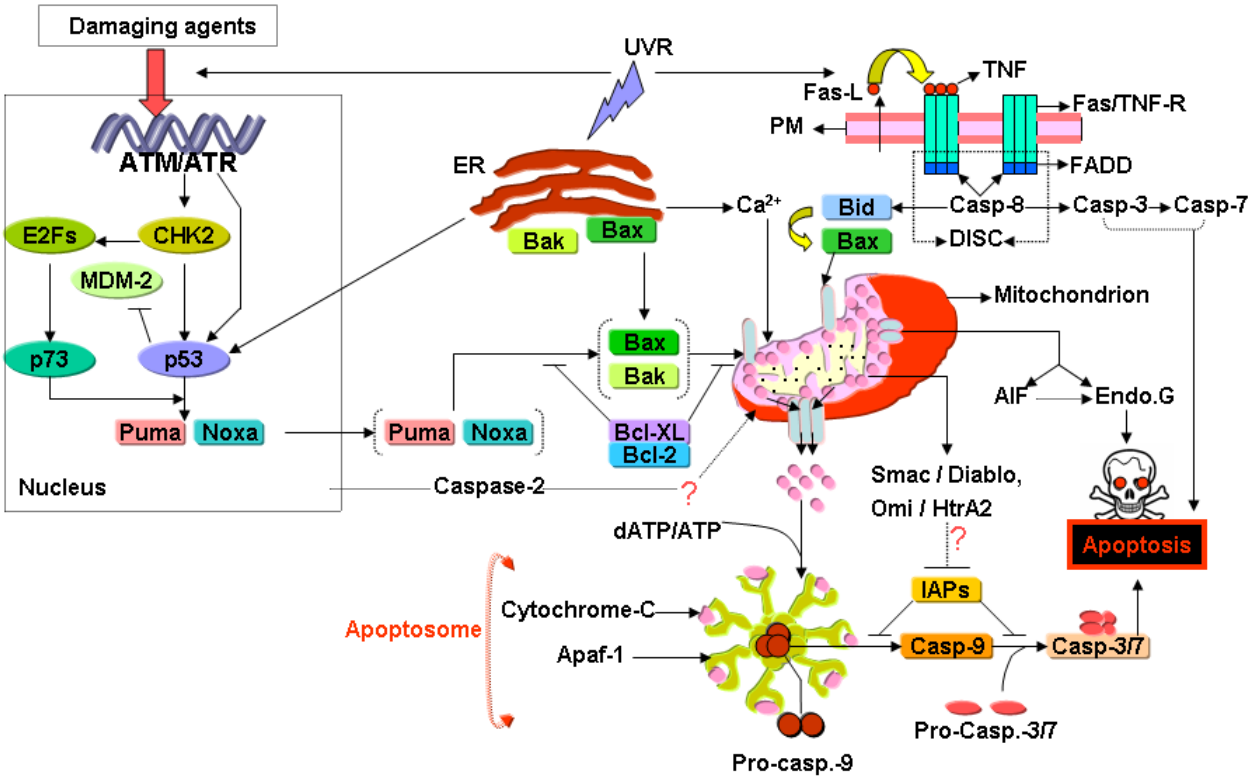

Figure 5: A generalized pathway of DNA damage-induced p53 mediated apoptosis. Various DNA damaging signals sensed by ATM/ATR and CHK2 leads to p53 accumulation by interfering the MDM 2-p53 interaction which inhibits the function of anti-apoptotic Bcl-2 family protein and stimulates the proapoptotic BH3-only as well as multidomain Bcl-2 family proteins. Bax/Bak forms oligomers on the outer mitochondrial surface through which cytochrome-C, Smac/DIABLO, Omi/HtrA2 comes out of the intermitochondrial space in the cytosol, where cytochrome c (cyt-c) binds to Apaf-1 and triggers the formation of apoptosome in dATP/ATP dependent manner, which activates casp-9. Smac/DIABLO, Omi/HtrA2 blocks the caspase inhibitory function of IAPs family proteins (e. g., survivin) and promotes the activation of downstream casp-3/7 by the proteolytic cleavage and conformational change of their precursor procaspases that leads to cell death. p53 independent pathway induces the checkpoint kinase-2 mediated signaling of E2F-1 towards the pro-apoptotic target genes encoding p73 and pro-caspases. AIF & Endo-G releasing from mitochondria may also induce cell death by DNA fragmentation, in caspase independent manner. Question mark indicates that molecular details of the action of casp-2 (involved in mitochondrial permeabilization) and IAPs is not well understood. Endoplasmic reticulum (ER) stress induces apoptosis either through direct activation of Bax/Bak or *via* p53. Damage to ER may release Ca^{2+} which may also promote apoptosis. In addition, UV-B either directly or *via* damaging nuclear DNA, activates death receptor (e. g., Fas, TNF-R) on cell surface by upregulation of death ligands (e. g., TNF). UVR may also activate death receptors in a ligand-independent manner by induction and trimerization of death receptors which than transduces death signal *via* their intracytoplasmic Fas associated death domain (FADD) and promotes apoptosis either directly or by the release of cyto-c from mitochondria.

Table 2: Some common proteins involved in extrinsic as well as intrinsic pathway of apoptosis (or PCD) (modified from Elmore, 2007).

Proteins	Acronym	Alternate name	Pathway
Apo2 ligand	Apo2L	TRAIL/TNFSF10	Extrinsic
Apo3 ligand	Apo3L	TWEAK/TNFSF12/DR3LG	Extrinsic
Apoptotic protease activating factor	Apaf-1	APAF1	Intrinsic
Apoptosis inducing factor	AIF	Apaf-3/ICE-LAP6/Mch6	Intrinsic
B-cell lymphoma protein 2	Bcl-2	Apoptosis regulator Bcl-2	Intrinsic
BCL2 like 1	Bcl-x	BCL2 related protein	Intrinsic
BCL2 related protein, long isoform	Bcl-XL	BCL2L protein, long form of Bcl-x	Intrinsic
BCL2 related protein, short isoform	Bcl-XS	-	Intrinsic
BCL2 like 2 protein	Bcl-w	Apoptosis regulator BclW	Intrinsic
BCL2 associated athanogene	BAG	BAG family molecular chaperone regulator	Intrinsic
BCL2 associated X protein	BAX	Apoptosis regulator BAX	Intrinsic
BCL2 antagonist killer 1	BAK	BCL2L7, cell death inhibitor 1	Intrinsic
BCL2 antagonist of cell death	BAD	BCL2 binding protein, BCL2L8, BCL2 binding component 6, BBC6, Bcl XL/Bcl-2 associated death promoter	Intrinsic
BCL2 interacting protein BIM	BIM	BCL2 like 11	Intrinsic
BCL2 interacting killer	BIK	NBK, BP4, BIP1, apoptosis inducing NBK	Intrinsic
BCL2 binding component 3	Puma	B lymphoid tyrosine kinase, p55-BLK, MGC10442 Puma BCL2 binding component 3 JFY1, PUMA/JFY1, p53 up-regulated modulator of apoptosis	Intrinsic
B-cell lymphoma protein 10	Bcl-10	mE10, CARMEN, CLAP, CIPER	Intrinsic
BH3 interacting domain death agonist	BID	p22 BID	Intrinsic
Bik-like killer protein	Blk	B lymphoid tyrosine kinase, p55-BLK, MGC10442 Puma BCL2 binding component 3 JFY1, PUMA/JFY1, p53 up-regulated modulator of apoptosis	Intrinsic
Cysteinyl aspartic acid-protease 8	caspase-8	FLICE, FADD-like Ice, MACH-1, MCH5	Extrinsic
Cysteinyl aspartic acid-protease-9	Caspase-9	ICE-LAP6, Mch6, Apaf-3	Intrinsic
Caspase-Activated DNAse	CAD	CAD/CPAN/DFF40	Intrinsic
Cell death regulator Aven	Aven	-	Intrinsic
Death receptor 3	DR3	TNFRSF12, Apo3, WSL-1, TRAMP, LARD, DDR3	Extrinsic
Death receptor 4	DR4	TNFRSF10A, TRAILR1, APO2	Extrinsic
Death receptor 5	DR5	TNFRS10B, TRAIL-R2, TRICK2, KILLER, ZTNFR9	Extrinsic
Death effector domain	DED	Apoptosis antagonizing transcription factor, CHE1	Extrinsic
Fatty acid synthetase ligand	FasL	Fas ligand, TNFSF6, Apo1, apoptosis antigen ligand 1, CD95L, CD178, APT1LG1	Extrinsic
Fatty acid synthetase receptor	FasR	Fas receptor, TNFRSF6, APT1, CD95	Extrinsic

Table 2 (cont.): Some common proteins involved in extrinsic as well as intrinsic pathway of apoptosis (or PCD) (modified from Elmore, 2007).

Proteins	Acronym	Alternate name	Pathway
Fas-associated death domain	FADD	MORT1	Extrinsic
FLICE-inhibitory protein	c-FLIP	Casper, I-FLICE, FLAME-1, CASH, CLARP, MRIT	Extrinsic
High-temperature requirement	HtrA2/Omi	Omi stress regulated endoprotease, serine protease Omi protein A2	Intrinsic
Inhibitor of Apoptosis Proteins	IAP	XIAP, API3, ILP, HILP, HIAP2, cIAP1, API1, MIHB, NFR2-TRAF signaling complex protein	Intrinsic
Oncogene Myc	Myc	c-myc, Myc proto-oncogene protein	Intrinsic
Phorbol-12-myristate-13-acetate-induced protein 1	Noxa	PMA induced protein 1, APR	Intrinsic
Receptor-interacting protein	RIP	RIPK1	Extrinsic
Second mitochondrial activator of caspases/direct IAP binding protein with low PI	Smac/DIABLO	-	Intrinsic
Tumor necrosis factor alpha	TNF-*α*	TNF ligand, TNFA, cachectin	Extrinsic
Tumor necrosis factor receptor 1	TNFR1	TNF receptor, TNFRSF1A, p55 TNFR, CD120a	Extrinsic
TNF receptor-associated death domain	TRADD	TNFRSF1A associated via death domain	Extrinsic
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	14-3-3	14-3-3 eta, theta, zeta, beta, epsilon, sigma, gamma	Intrinsic

Another pathway of apoptosis has also been recognized which involves perforin/ granzyme-A or B (released by cytotoxic T lymphocytes and NK cells: perforin/granzyme induced apoptosis). It has been found that granzyme A-induced apoptosis is a caspase-independent pathway through single stranded DNA damage (Martinvalet et al., 2005). Both intrinsic as well as extrinsic pathways of apoptosis are associated and influence each other (Igney & Krammer, 2002). All the three (intrinsic, extrinsic and granzyme B-induced) pathways of apoptosis come together on the same point putting to cell death by the activation of caspase-3/7 followed by cell shrinkage, chromatin condensation and fragmentation of chromosomal DNA, degradation of nuclear as well as cytoskeleton proteins etc. (Elmore, 2007). Generally, caspases occur in the cells in an inactive form (i. e., procaspase) and once activated can trigger other caspases leading to amplification of apoptotic signaling pathway followed by cell death due to their proteolytic activity. Caspases are widely expressed in an inactive pro-enzyme form in most cells and once activated can often activate other procaspases, allowing initiation of a protease cascade. Some procaspases can also aggregate and autoactivate. This proteolytic cascade, in which one caspase can activate other caspases, amplifies the apoptotic signaling pathway and thus leads to rapid cell death.

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Extrinsic pathway of apoptosis

Increased level of p53 protein was detected in human keratinocytes after UV irradiation (Henseleit et al., 1997). Exposure of normal human keratinocytes to UV radiation induces the extrinsic apoptotic pathway, which is initiated by the cell surface death receptors, such as TNF[1] receptor (p55 or CD120a), Fas (CD95/APO-1), DR3 (Apo3 or WSL1), DR4 (TRAIL-R1) and DR5 (TRAIL-R2) (Haupt et al., 2003; Yan & Shi, 2005; Elmore, 2007). UV-B-induced apoptosis may be triggered by ligand-dependent (Caricchio et al., 1998) or ligand-independent (Rehemtulla et al., 1997) Fas clustering, with consequent caspase activation (Clément et al., 2007). Activation of death receptor is followed by the formation of death inducing signaling pathway complex (DISC), which is composed of adaptor molecule FADD (Fas-associated death domain) and caspase-8 (Boldin et al., 1996). FADD is linked with caspase-8 zymogens by means of homophilic interface with their N-terminal death effector domains (DEDs) (Boatright & Salvesen, 2003). With the formation of DISC, caspase-8 is activated, which either directly cleaves and activates the effector caspases, or indirectly activates the downstream caspases through the cleavage of BH3-only protein Bid, leading to involvement of intrinsic pathway of apoptosis (Luo et al., 1998). Naik et al. (2007) has revealed that UV radiation triggers apoptosis of skin keratinocytes mainly through the BH3-only protein Noxa. It has been shown that UV irradiation results in the activation of several members of the MAPK super family, such as ERKs, JNKs, and p38 $_{MAPK}$ (Peus et al., 1999). Assefa et al. (2000) have shown that p38 $_{MAPK}$ pathway mediates apoptosis through UV-B irradiation by inducing the release of mitochondrial cytochrome-c into the cytosol, which is followed by procaspase-3 activation. It has also been found that c-Jun N-terminal kinase1 (JNK-1) plays an important role in UV-induced apoptotic cell death (Katagiri et al., 2006a). Squamous cell carcinoma antigen (SCCA) that belongs to the serpin superfamily (SCCA1 & SCCA2) is known in humans (Schneider et al., 1995) that are strongly upregulated in the upper epidermis of the skin after UV irradiation (Katagiri et al., 2006b). Furthermore, UV-induced apoptosis was significantly decreased when SCCA was overexpressed, that strongly suggest that SCCA1 binds to JNK-1 to play a protective role against UV irradiation in keratinocytes by suppressing JNK. Recently Wang and Li (2006) have found that the ING3, a tumor-suppressor protein mainly located in the nucleus, plays a critical role in UV-induced apoptosis through modulating Fas expression, resulting in the activation of Fas/Caspase-8 pathway followed by downstream caspase cascades activation via Bid cleavage. The involvement of Caspase-10 and FLICE-like inhibitory protein (FLIP) has also been found to be involved in death-receptor-mediated apoptosis (Juo et al., 1998; Kischkel et al., 2001; Wang et al., 2001), however there is disagreement about the capability of caspase-10 to functionally alternate for caspase-8 in death-receptor signaling. FLIP is a caspase-8 homolog with certain significant differences and at low concentration has been shown to enhance Fas-induced caspase-8 activation at the DISC, most probably through heterodimerization; although at higher concentrations FLIP inhibits caspase-8 activation (Chang et al., 2002; Micheau et al., 2002).

Intrinsic pathway of apoptosis

Following DNA damage, increased level of Bax and/or decreased level of Bcl-2 may permeabilize the mitochondria to release pro-apoptotic factors, such as cytochrome-c, which trigger subsequent activation of procaspase-9, followed by downstream apoptotic effectors (Crompton, 2000). Recently, it has been found that in response to DNA damage, activation of caspase-2 is required before mitochondrial permeabilization and release of cytochrome-c (Guo et al., 2002; Lassus et al., 2002; Vakifahmetoglu et al., 2006). Activation of caspase-2 might occur without processing of the precursor molecule however, oligomerization is an important step for

caspase-2 activation (Norbury & Zhivotovsky, 2004). Expression of cyclin-D3 and caspase-2 in human cells potentially induces apoptosis, compared with expression of caspase-2 alone, which shows the involvement of cyclin-D3 in caspase-2 activation (Mendelsohn et al., 2002). After releasing cytochrome-c, it binds to apoptotic protease activating factor Apaf-1 and forms a 7-span symmetrical active complex 'apoptosome' in nucleotide dATP/ATP dependent manner (Zou et al., 1999). The apoptosome subsequently recruits procaspase-9 into its central region to form an active holoenzyme, which further activates downstream executioner caspases, such as caspase-3/7 that leads to PCD (Jiang & Wang, 2004). It has been found that in mammalian cells, caspase activity is also stimulated by one of the pro-apoptotic mitochondrial proteins Smac/Diablo, and Omi/HtrA2, which promote apoptosis by interfering the action of inhibitor of apoptosis (IAP) family protein (e. g., survivin) (Verhagen et al., 2002; Song et al., 2003), although the inhibition of apoptosis through IAPs family is not universal, as survivin is unable to prevent the induction of apoptosis in ovarian cancer cells by Smac (McNeish et al., 2005). In addition to caspase activator proteins, some other molecules unrelated to caspase activation such as AIF (apoptosis inducing factor) (Susin et al., 1999) and Endo G (endonuclease G) (Li et al., 2001) has also been found to be released from mitochondria that cause apoptosis by DNA fragmentation and subsequent chromosomal condensation. Recent evidence suggests that Bax/Bak can also be localized in the endoplasmic reticulum (ER) and gets activated in response to ER stress, leading to calcium depletion and murine caspase-12 activation (Scorrano et al., 2003). In addition to ER stress activation of Bax/Bak by Puma, Li et al. (2006) have identified Noxa as a novel BH3-only protein that is activated by ER stress. Furthermore, in mouse embryo fibroblasts (MEFs), it has been found that induction of Noxa and Puma by ER stress is largely dependent on p53 and both proteins (Puma/Noxa) contribute to ER stress-induced apoptosis.

In addition to other organisms, PCD has also been suggested in various phytoplankton species (Franklin et al., 2006) such as cyanobacteria (e. g., *Trichodesmium* sp., *Anabaena flos-aquae*) (Ning et al., 2002; Berman-Frank et al., 2004), green algae (e. g., *Dunaliella tertiolecta*) and dinoflagellates (e. g., *Peridinium gatunense*) (Vardi et al., 1999). However, it is not very clear how PCD is operating in phytoplankton and whether it has greater similarities with animal, plant, or fungal models? The presence of key apoptotic enzymes such as paracaspase (in bacteria), metacaspase (in plant/fungi) and caspases (in animals) that share common active sites (Uren et al., 2000) are considered to be highly conserved across taxa (Aravind et al., 2001), although, the presence of animal cell death regulator, such as Bcl-2 family and p53 in all the life forms is still in dispute. Furthermore, the mitochondria that play a central role in animal apoptotic pathway, its role in plant and protists is not clear (Franklin et al., 2006).

Apoptosis *vs.* necrosis

Apoptosis and necrosis (accidental cell death; ACD) are two independent, sequenced as well as simultaneous processes (Zeiss, 2003) that can be induced by several stimuli such as high temperature, UV radiation, ionizing radiation, oxidative stress and cytotoxic anticancer drugs. Apoptosis is a synchronized and frequently energy-dependent process that involves the activation of a set of cysteine proteases (caspases) and a complex cascade of events, while cell death by means of necrosis does not involve gene expression and is a passive externally-driven process that results after cell death in the absence of any metabolic self-involvement (Franklin et al., 2006). The process of apoptosis is morphologically distinct from necrosis in many of its characteristics such as cell shrinkage, cytoplasmic condensation, DNA fragmentation, chromatin condensation, nuclear fragmentation, cyto-

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plasmic membrane blebbing and formation of apoptotic bodies whereas necrosis involves loss of membrane integrity, cell swelling, formation of cytoplasmic vacuoles, swollen endoplasmic reticulum, distended or ruptured mitochondria, lysosomes, lysis and release of the cytoplasmic contents into the surrounding tissue (Majno & Joris, 1995; Trump et al., 1997; Chamond et al., 1999) leading to inflammatory reaction which is absent in apoptotic cells (Kurosaka et al., 2003) (Figure 6). Recently, it has been found that alkylating DNA damage stimulates a necrotic type of PCD through the poly (ADP-ribose) polymerases (PARP) and apoptosis inducing factor (AIF) and it was observed that, similar to apoptosis, necrosis may possibly be a highly regulated cell death program (Moubarak et al., 2007). The process of necrosis may occur in a controlled fashion (Vanden Berghe et al., 2004; Saelens et al., 2005; Zong & Thompson, 2006) and analysis of necrotic pathway may provide novel opportunities to destroy apoptosis-resistant tumor cells.

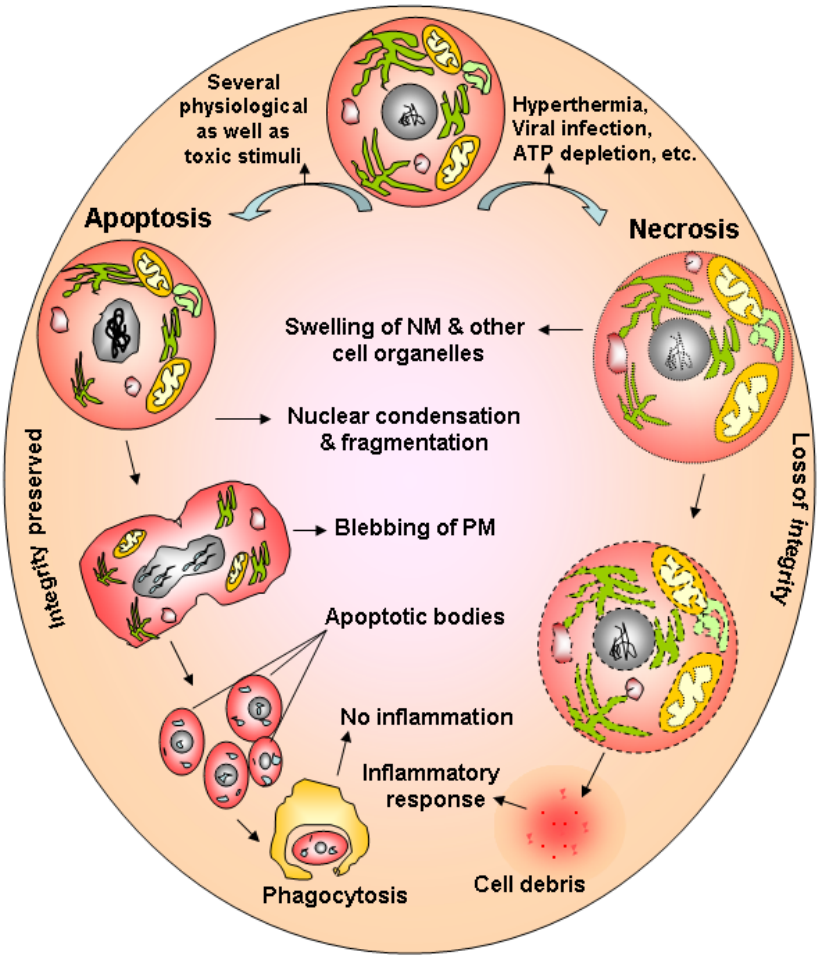

Figure 6: Diagrammatic illustration showing the morphological distinctiveness occurring during apoptosis and necrosis. The cell death by apoptosis is a normal and energy (ATP) dependent pathway caused by a number of endogenous as well as exogenous stimuli. During apoptosis, decrease in cell volume, nuclear changes with chromatin condensation, margination and fragmentation followed by blebbing and breakdown of intact cell and nuclear membranes takes place. It results into the formation of small fragmented apoptotic bodies having cytoplasmic contents surrounded by cell membrane which are removed by the process of phagocytosis in the extracellular environment avoiding the inflammatory reaction. Necrosis is an unusual and unintended process caused by external cell injury by a number of stimuli. It is characterized by the increase in cell volume followed by enlargement of cell organelles including nucleus, loss of membrane integrity and release of cellular contents which consists of certain enzymes such as hydrolases that influence the adjoining cells leading to inflammatory reaction in the adjacent tissue.

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Malfunctioning of apoptosis and pathogenesis

Improper apoptosis or malfunctioning of individual apoptotic machinery may cause several human diseases like cancer, neurodegenerative as well as several types of autoimmune disorder (Barr & Tomei, 1994; Thompson, 1995; Chun et al., 2002) (Figure 7). It has been found that unnecessary cell death and unsound regulation of caspase activity are associated with certain diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease. Augmented activities of caspases-8 and -9 have been observed in peripheral blood mononuclear cells of Alzheimer's disease patients (Tacconi et al., 2004) and in brain tissues of Alzheimer's as well as Parkinson's disease patients (Viswanath et al., 2001; Rohn et al., 2002; Yew et al., 2004). Huntington's disease, a neurodegenerative disorder, has also been found to be caused by increased activity of caspase-10 in a manner similar to caspase-8 (Miyashita et al., 2001). Mutations on Fas and Fas ligand (Fas-L) in humans may cause a complicated immune disorder like autoimmune lymphoproliferative syndrome (ALPS), a semblance of murine lymphoproliferation (lpr) and generalized lymphoproliferative disorder (gld) (Jiang & Wang, 2004).

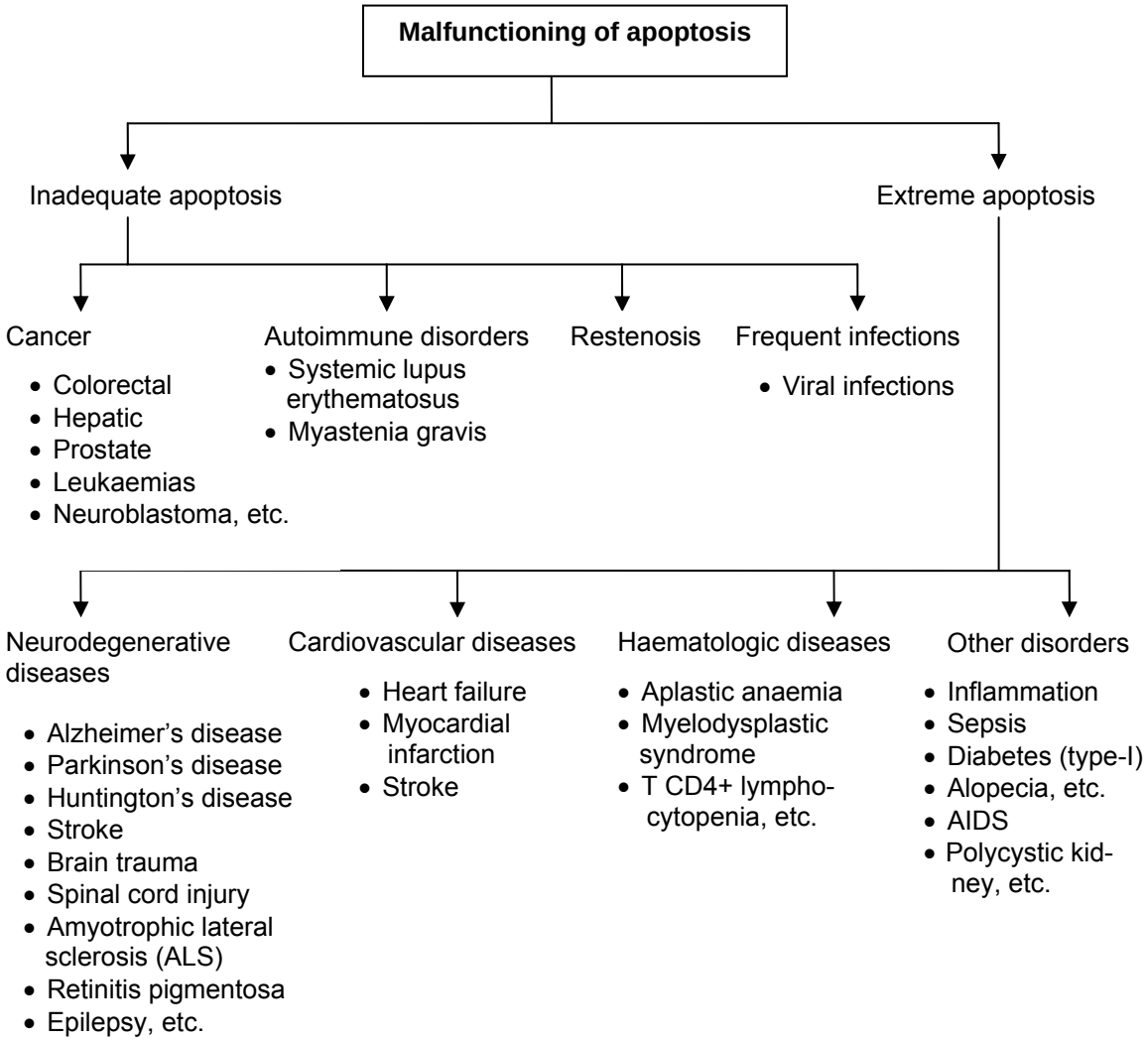

Figure 7: Some common diseases associated with malfunctioning of apoptosis or PCD (modified from references Chamond et al., 1999 and Alam, 2003)

The three autosomal dominant diseases such as Muckle Wells syndrome, familial cold auto-inflammatory syndrome and chronic infantile neurological cutaneous and articular (CINCA) syndrome caused by missense mutations in the NACHT domain of NALP3 protein are closely related to autoinflammatory syndromes distinguished by periodic fever, skin rashes, amyloidosis and development of neurological complications (Martinon & Tschopp, 2007; Aganna et al., 2002; Dode et al., 2002). It has been suggested that loss of caspase-14 expression is associated with progression of ovarian cancer (Krajewska et al., 2005) and the mutation in *p53* gene may cause neoplastic diseases (Chamond et al., 1999). Thus it seems that apoptotic pathway is associated with several biological processes and plays a vital role in regulating various diseases.

Significance of apoptosis in biological systems

Apoptosis (or PCD) is an evolutionarily conserved and extremely synchronized form of cell death to facilitate the deletion of redundant, infected, injured or malformed cells during the normal life span in various biological systems which is an essential course of action in maintaining homeostasis in multicellular organisms. It is usually implicated in embryogenesis, metamorphosis, immune system and normal adult tissue remodeling as well as in a number of pathological disorders such as cancer, autoimmunity and degenerative diseases. Generally cancer cells themselves are more prone to undergo apoptosis and a comprehensive understanding of the molecular pathways that regulate apoptosis will assist in investigating novel cancer chemotherapeutic targets (Bailey et al., 2005) which in turn would offer new opportunities for the discovery and development of drugs (Alam, 2003). Stimulation of disabled p53 pathways has been suggested as a potential mode of therapy for cancer (Wen et al., 2003). A large number of non-genotoxic compounds such as Nutlin-3 (Vassilev et al., 2004), RITA (reactivation of p53 and induction of tumor cell apoptosis) (Issaeva et al., 2004), HLI98 (Yang et al., 2005) has been reported that leads to p53-dependent apoptosis (Coll-Mulet et al., 2006; Secchiero et al., 2006) by disrupting the MDM2-p53 interaction. Several other compounds such as Exotoxin-A (ETA) (Christopher et al., 2004; Chang & Kwon, 2007), Isokotomolide-A (IKA) (Chen et al., 2007), Ellipticines (Stiborova et al., 2006), MDPTQ (Shenoy et al., 2007) have recently been identified as potent apoptotic inducer compounds, that may be useful for anticancer therapy. Several recent studies have revealed a number of natural as well as synthetic anticancer drugs (Table 3) that act through the induction of apoptosis to prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses other than necrosis (Nicholson, 2000). Anticancer therapy may cause both apoptosis as well as autophagic cell death, however, there is little information about the connection between autophagy and apoptosis, but recently it has been shown that autophagy gene5 (*Atg5*) can bind to Bcl-XL and induce apoptosis, which indicates that apoptosis and autophagy could be interconnected by molecular interaction between Atg and Bcl-2 family proteins (Yousefi & Simon, 2007). UV-induced apoptotic pathways could assist in modulating the susceptibility of cells to undergo apoptosis either to improve the efficiency of cancer treatments or avoid the unwanted death of normal cells (Assefa et al., 2005). From the past few years, certain photosensitizing drugs are being employed to induce apoptosis for the treatment of cancer and other non-cancerous or abnormal cells (Dougherty, 1993; Oleinick et al., 2002; Zawacka-Pankau et al., 2008), which is termed as photodynamic therapy (PDT) induced apoptosis. Most photosensitizers for PDT are efficient producers of reactive oxygen species (ROS) such as singlet oxygen which is the dominant mechanism for PDT-induced apoptosis in cells and tissues (Oleinick et al., 2002). In many cases PDT is highly efficient in inducing apoptosis,

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though it can induce apoptosis or necrosis or both (Oleinick et al., 2002). A number of photosensitizers such as photofrin, benzoporphyrins, hypericin, m-tetrahydroxyphenylchlorin (mTHPC or Foscan), porphyrin, hematoporhyrins (Hp), protoporphyrin IX (PpIX), benzoporphyrin derivative (BPD), benzyl ester 5-ALA (Figure 8) etc. have been approved by regulatory agencies. These porphyrinogenic sensitizers may induce apoptosis through different pathways (Kralova et al., 2006; Saczko et al., 2007; Hilf, 2007; Kramer & Plaetzer, 2008; Zawacka-Pankau et al., 2008). Genetic evidence specifies that the cell surface death receptor-mediated apoptosis is significant for the functioning of a normal immune system, since mutations on Fas and Fas ligand (Fas-L) in humans may cause a complicated immune disorder like autoimmune lymphoproliferative syndrome (ALPS) (Jiang & Wang, 2004). Normally, exposure of cells to UV radiation results in increased cell death through apoptosis by increasing the activities of both the mitochondrial derived intrinsic and plasma membrane derived extrinsic apoptotic pathways (Kulms & Schwarz, 2000). A family of cysteine proteases (caspases) has been found to have an important role in this process that leads to organized destruction of the cell by a restricted cleavage of a variety of crucial cellular substrates.

Table 3: Some apoptotis-inducing natural as well as synthetic compounds used in cancer chemotherapy

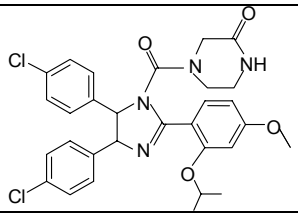
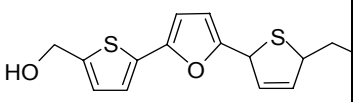
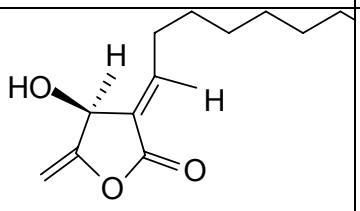
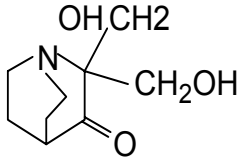
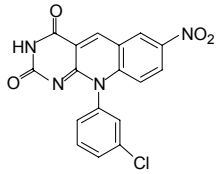
Compound	Source	Structure	Probable Mechanisms	Reference
Nutlin 3	Synthetic (cis-imidazoline derivatives)		Block MDM2 activity by disrupting the MDM2-p53 interaction	Vassilev et al., 2004; Secchiero et al., 2006
RITA	Synthetic		Block MDM2-p53 interaction and ubiquitation	Issaeva et al., 2004
ETA	*Pseudomonas aeruginosa*	-	induces cell cycle arrest and caspase-3 activation	Christopher et al., 2004; Chang and Kwon, 2007
IKA	*Cinnamomum kotoense*		Block G0/G1 cell cycle progression and also decrease the interaction of p53–MDM2	Chen et al., 2007
PRIMA-1	Synthetic (Azabicyclooctan-3-one derivatives)		reinstate sequence-specific DNA binding and reactivate mutant p53 proteins	Bykov et al., 2003
HLI98	Synthetic (7-nitro-5-deazaflavin derivatives)		inhibits HDM2 ubiquitin ligase activity	Yang et al., 2005

Table 3 (cont.): Some apoptotis-inducing natural as well as synthetic compounds used in cancer chemotherapy

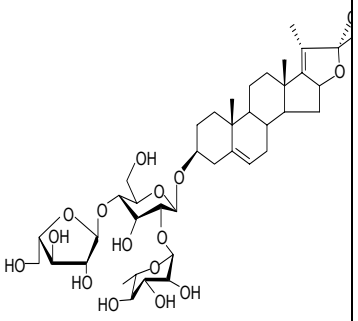
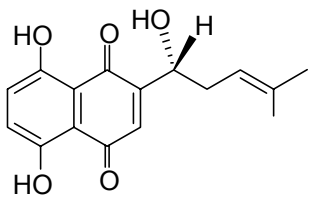
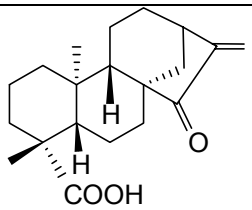
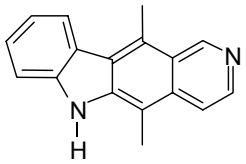
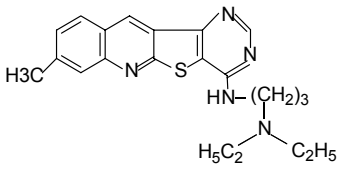
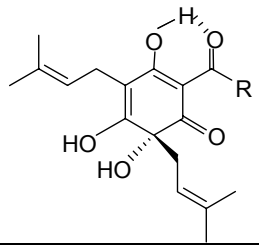
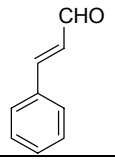
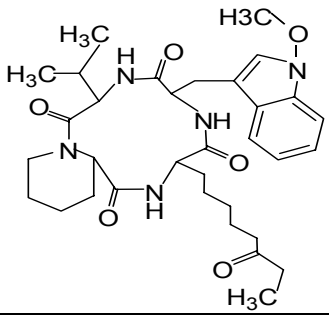
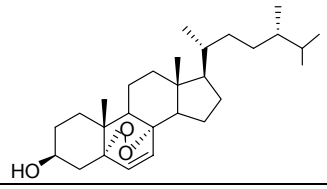
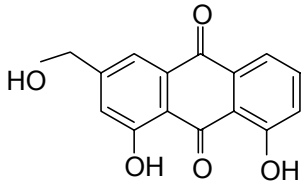
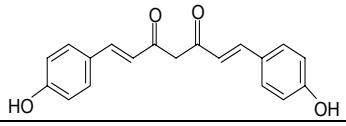
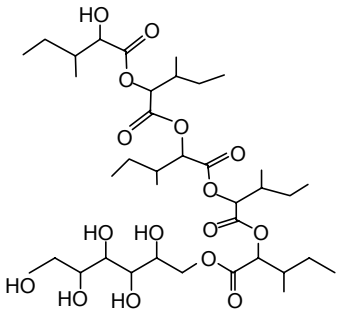
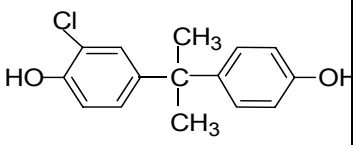
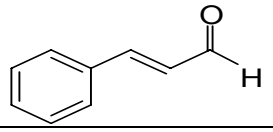
Compound	Source	Structure	Probable Mechanisms	Reference
PD	*paris polyphylla*		acts on mitochondria and elicits dissipation of transmembrane potential (Δψm), generation of ROS, and release of cytochrome c and AIF	Cheung et al., 2005
Shikonin (Human Colorectal Carcinoma Cells)	*Lithospermum erythrorhizon*		loss of mitochondrial membrane potential, reactive oxygen species (ROS) generation, cytochrome c release, and subsequent induction of pro-caspase-9 and -3 processing	Hsu et al., 2004
EOKA	*Espeletia schultzii; Espeletia grandiflora*		activates caspace-3 and caspace specific cleavage of PARP, and also induces nucleosomal DNA fragmentation as well as reduction of Bcl-2 protein level	Yarimar et al., 2008
Ellipticine	*Ochrosia borbonica, Excavatia coccinea*		accumulation of dephosphorylated mutant p53	Kuo et al., 2005; Stiborova et al., 2006
MDPTQ	Quinoline derivative		increase in ROS followed by loss of membrane potential (Δψm)	Shenoy et al., 2007
Hop Bitter Acids (e. g. humulone)	*Humulus lupulus* L.		Fas activation and mitochondrial dysfunctions	Chen & Lin, 2004
Cinnamaldehyde	*Cinnamomum osmophloeum*		loss of Δψm and release of cytochrome c into cytosol, by the imbalance of Bcl-2 family of proteins	Huang et al., 2007

Table 3 (cont.): Some apoptotis-inducing natural as well as synthetic compounds used in cancer chemotherapy

Compound	Source	Structure	Probable Mechanisms	Reference
Apicidin	*Fusarium* sp.		induces Fas and Fas-L, cytochrome *c* release into the cytosol, and activation of casp-9 and 3	Kwon et al., 2002
EDS (epidioxysterols)	*Meretrix lusoria*		induces chromatin condensation, and sub-G1 cell population	Pan et al., 2007
Aloe-emodin	*Aloe vera*		blocks casein kinase II activity and phosphorylation of Bid and induces Aif and cytochrome c	Chen et al., 2007
Curcumin	*Curcuma longa*		induces the production of ROS and downstream activation of JNK as well as activation of caspases 9, 3, and 8	Johnson & Mukhtar, 2007
Hormonemate	*Hormonema dematioides*		induces caspae-3 activity	Filip et al., 2003
ClBPAs	Synthetic (derivatives of bisphenol A)		induces DNA fragmentation followed by activation of caspase 3, 8 and 9	Mutou et al., 2008
Cinnamaldehyde	*Cinnamomum cassia*		induces the ROS-mediated mitochondrial permeability transition and resultant cytochrome c release	Ka et al., 2003

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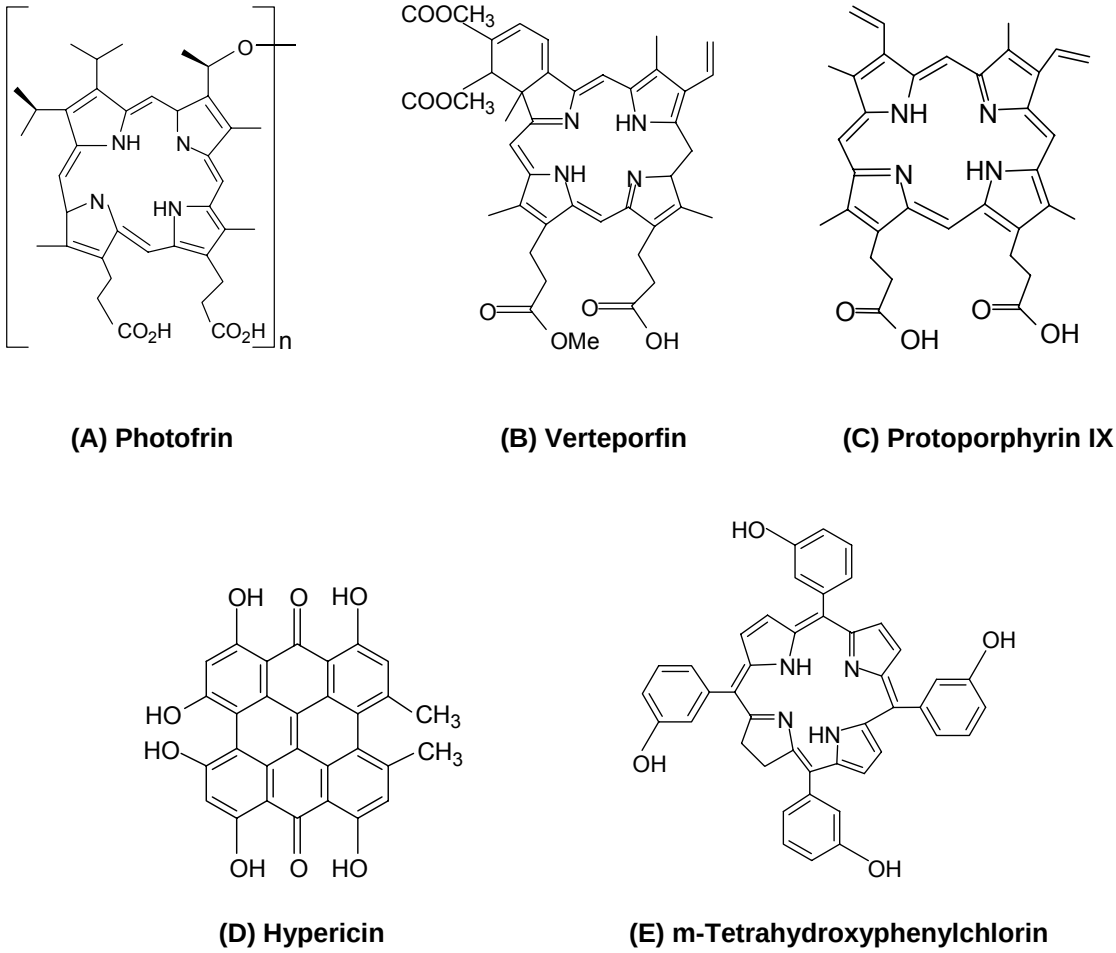

Figure 8: Structure of some photosensitizers used in PDT-induced apoptosis. (A) Photofrin, (B) Verteporfin (benzoporphyrin derivative), (C) Protoporphyrin IX (D) Hypericin and (E) m-Tetrahydroxyphenylchlorin (mTHPC or foscan)

CONCLUSION

Apoptosis is an exceedingly complicated phenomenon, with energy-dependent flow of molecular events accomplished by two types of pathways such as intrinsic and extrinsic that involves the activation of a set of cysteine proteases known as "caspases". The process of cell death by means of apoptosis (PCD) and necrosis (ACD) is accompanied by a number of distinctive morphologic and metabolic changes. Apoptosis plays a significant role in survival by maintaining the homeostasis in multicellular organisms as well as in the management of many diseases, since malfunctioning of apoptotic pathway may cause several human diseases like cancer, neurodegenerative as well as several types of autoimmune disorder. Presently, large numbers of synthetic as well as natural compounds have been found that are pharmacologically highly effective against certain diseases through inducing the apoptosis of target cells such as cancerous cells. These compounds may promote the development of novel remedy based on the inflection of apoptosis. As of now, the basic mechanisms of apoptosis have been established, but its implications for therapeutic purposes have still to be worked out.

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