IJPSR (2012), Vol. 3, Issue 07 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March, 2012; received in revised form 29 April, 2012; accepted 24 June, 2012

APOPTOSIS SIGNALING; A DRUG DESIGN IN CANCER THERAPY: A REVIEW

Md. Ataur Rahman*

Department of Pharmacology, College of Medicine, Institute of Natural Medicine, Hallym University, Chuncheon, Gangwon-do, 200-702, South Korea

Keywords:
Apoptosis,
Mitochondria,
Caspases,
Anticancer,
Drug

Correspondence to Author:

Md. Ataur Rahman

Ph. D Scholar, Department of Pharmacology, College of Medicine, Institute of Natural Medicine, Hallym University, Chuncheon, Gangwon-do, 200-702, South Korea

ABSTRACT

Apoptosis plays an important role in development, growth, differentiation, altered gravity conditions, tissue homeostasis, immune defense, and cancer to control cell number in tissues and to eliminate individual cells that threaten the animal's survival. The purposes of this review are to describe the signaling pathways and the cellular changes that occur with apoptosis. Currently, two pathways for activating apoptosis have been studied in detail. One starts with ligation of a death ligand to its transmembrane death receptor, followed by recruitment and activation of caspases in the death-inducing signalling complex. The second pathway involves the participation of mitochondria, which release caspase-activating proteins into the cytosol, thereby forming the apoptosome where caspases will bind and become activated. The novel agents include those targeting for the design of more effective and selective therapeutic strategies. This review providing an overview of the recent understanding of apoptotic signaling pathways, the main mechanisms by which cancer cells resist apoptotic insults, and discuss some recent attempts to target for restoring efficient cell death signaling in cancer cells to develop a potential anticancer drug.

INTRODUCTION: Apoptosis plays a central role both in development and in homeostasis of metazoans [1]. Cells die by apoptosis in the developing embryo during morphogenesis or synaptogenesis and in the adult animal during tissue turnover or at the end of an immune response. Because the physiological role of apoptosis is crucial, aberration of this process can be detrimental.

Thus, unscheduled apoptosis of certain brain neurons contributes to neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, whereas the failure of dividing cells to initiate apoptosis after sustaining severe DNA damage contributes to cancer [2]. Extensive research in the cancer field has explored numerous pathways in cancer progression to elucidate effective measures to target these cancer cells. Though cancer therapeutics favor a multiple approach where individual treatment varies between surgery, radiation, chemicals, antibodies and/or cells of the immune system, the effectiveness of the treatment differs largely between individuals and the cancer background [3]. In this regard, improving therapeutic efficacy and selectivity and overcoming drug resistance are the major goals in developing anti cancer agent's today [4].

To fulfill these goals, a thorough understanding of apoptotic signaling pathways and how tumor cells become apoptosis is imperative, because they provide directions to unravel novel therapies and key targets to surpass or supplement current cancer treatments. Therefore, in this review I would like to summarize the apoptotic signaling pathways and how cancer cells resist apoptotic insults to cancer therapy.

Apoptotic Pathway: Apoptosis is characterized by cell shrinkage, blebbing of plasma membrane, maintenance of organelle integrity, condensation and fragmentation of DNA, followed by ordered removal of phagocytes [5]. It works like a "suicide" program and it causes minimal damage to surrounding tissues. Apoptosis has been subclassified into two types of death pathways, namely, the extrinsic pathway and the intrinsic mitochondria-mediated pathway. These two processes however, are not exclusive and evidence suggests that they can be linked and that molecules in one pathway can influence the other [6]. Moreover, recent evidences support non-apoptotic roles for many effectors of the apoptotic signaling pathways. For instance, caspase-2, the most conserved member of the caspase family, also plays a role in cell cycle regulation, DNA repair, and tumor suppression[7].

1. **Extrinsic pathway:** The extrinsic or receptor mediated death pathway requires effective engagement between the death receptors found on the surface of the cell membranes and their respective ligands [8] (**Fig. 1**). The receptor-mediated pathway involves death receptors from the tumor necrosis factor (TNF) superfamily such as TNF, CD95 (Fas) and TNF-related apoptosis inducing ligand (TRAIL) receptors. These receptors have an extracellular domain to engage the ligands and an intracellular cytoplasmic domain that is also referred to as the death domain. This death domain is responsible for transmitting the death signal from the surface to the intracellular signaling pathways [9].

   Activation of CD95 or TNF receptors often leads to receptor clustering and intracellular recruitment of proteins into a death-inducing signaling complex (DISC), which then activates an initiator caspase, procaspase-8. Activated caspase-8 then triggers the execution phase of apoptosis via the activation of the downstream effector caspase, caspase-3 [10]. The activated caspases can also induce mitochondrial damage and reinforce the death signal by facilitating the egress of death amplifying proteins from the mitochondrial inter-membranous space[11-14].

2. **Intrinsic pathway:** The intrinsic or mitochondria pathway of cell death can be activated by a variety of receptor-independent stimuli such as radiation, free radicals, viral infections and serum/growth factor withdrawal (Fig. 1). Initially, it was demonstrated that these triggers invariably result in changes in the inner mitochondrial membrane permeability due to the opening of the mitochondrial permeability transition (MPT) pore. The major consequences of this change of permeability are the loss of the mitochondrial transmembrane potential ($\Delta\Psi_m$), the release of pro-apoptotic proteins and the arrest of the bioenergetic function of the organelle.

   The proteins that are released can be broadly classified into two categories. The first category comprises of proteins that can activate the caspase-dependent pathway. This group includes proteins such as cytochrome *c* (cyt *c*) and Smac/ DIABLO (second mitochondria-derived activator of caspases).

   The holocytochrome *c* induces Apaf-1 oligo merization, leading to the activation of caspase-9. This active cyt*c*/Apaf-1/caspase-9 complex forms the apoptosome and activates the executioner caspases-3 and -7 resulting in the dismantling of the cell through nuclear fragmentation [10, 15, 16]. Smac/DIABLO binds to IAPs (inhibitor of apoptosis proteins) and deactivates them, thus preventing the IAPs from arresting the apoptotic process, permitting apoptotic progression.

   The second group includes other pro-apoptotic proteins such as apoptosis inducing factor (AIF) and endonuclease G (Endo G). In some models, the release of these proteins is a late event in apoptosis, which occurs once the cells are committed to die. Following the release of AIF, it translocates to the nucleus where it promotes DNA fragmentation. Both AIF and Endo G act in a caspase-independent manner to execute cell death [17]. For example, in an ovarian stem cell model, Endo G was shown to mediate caspase-independent cell death in response to chemotherapeutic agents [18]. Alternatively, AIF has also been recently proposed to participate in a different form of cell death, namely programmed necrosis [19].

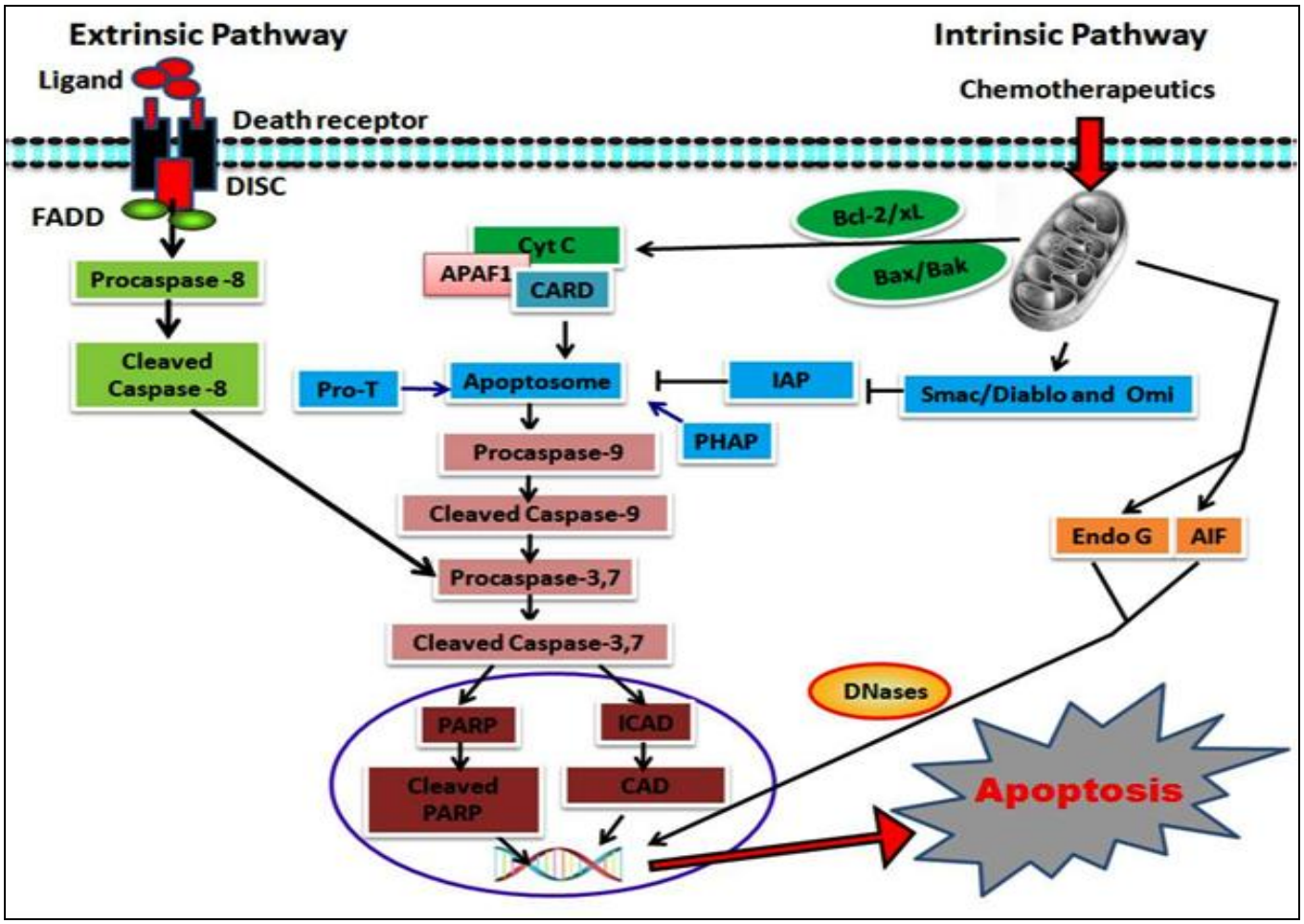

FIGURE 1: THE EXTRINSIC AND INTRINSIC PATHWAYS OF APOPTOSIS: Death receptor pathway (left) is initiated by the ligation of the ligands to their respective surface receptors. Ligation of death receptors is followed by the formation of the death-inducible signalling complex (DISC), which results in the activation of pro-caspase-8 which cleaves caspase-8 and activates pro-caspase-3, which cleaves target proteins, leading to apoptosis. The intrinsic pathway (right) is activated by death signals function directly or indirectly on the mitochondria, resulting in the formation of the apoptosome complex. This cell death pathway is controlled by Bcl-2 family proteins (regulation of cytochrome *c* release), inhibitor of apoptosis proteins (IAPs) (inhibition of caspases), and second mitochondrial activator of caspases (Smac) and Omi (negative regulation of IAPs). The apoptosome function is also regulated by the oncoprotein pro-thymosin-α (Pro-T) and the tumor suppressor putative HLA-DR-associated protein (PHAP). The intrinsic pathway might also operate through caspase-independent mechanisms, which involve the release from mitochondria and translocation to the nucleus of at least two proteins, apoptosis inducing factor (AIF) and endonuclease G (EndoG). The nuclear location of AIF is linked to chromatin condensation and the appearance of high-molecular-mass chromatin fragments, whereas the role of EndoG is still unclear.

Molecular mechanism of regulation of Apoptosis Pathway:

1. **Members of the Bcl-2 family proteins:** The Bcl-2 superfamily of apoptosis regulatory gene products includes proteins commonly ascribed as death antagonists (Bcl-2, Bcl-Xl, Bcl-W, Bfl-1, and Mcl-1), or death agonists (Bax, Bak, Bcl-Xs, Bad, Bid, Bik, Bim, and Hrk) and these proteins act mainly at the level of the external mitochondrial membrane[20]. These proteins also contain a hydrophobic carboxy terminal domain that enables them to dock onto the outer mitochondria membrane (OM), the nucleus and ER [21].

It has been shown that Bcl-2 and Bcl-X_L can protect the cells by interacting with mitochondrial proteins such as the adenine nucleotide translocase (ANT) or the voltage dependent anion channel (VDAC), thus preventing them from forming mitochondrial pores, protecting membrane integrity, and inhibiting the release of apoptogenic factors such as cytochrome *c* [22].

More recent evidence has highlighted novel functional biology of the anti-apoptotic protein Bcl-2 [23]. Overexpression of Bcl-2 was associated with a slight increase in steady state intracellular superoxide (O_2^-) production, which was linked to

increases in mitochondrial oxygen consumption and the activity of the rate limiting enzyme in the electron transport chain, cytochrome c oxidase (COX). Interestingly, pharmacological or molecular inhibition of the NADPH oxidase restored apoptosis sensitivity of Bcl-2 overexpressing cells, thus implicating the pro-oxidant activity of Bcl-2 in its anti-apoptotic function.

The altered ratios favoring the pro-survival proteins such as Bcl-X_L and Bcl-2 over the pro-apoptotic proteins such as Bak and Bax have been shown to lead to chemoresistance in cancer cells. The need to drive the ratio toward death in cancer cells has led to the development of anti-cancer compounds that can either act to suppress the pro-survival proteins such as Bcl-2 and Bcl-X_L, or mimic the pro-death BH3 only proteins such as Bid, Bim, Bad, Bmf, Noxa and PUMA to promote cell death. Another attractive drug target is the naturally occurring Bcl-2 inhibitor gossypol, which is derived from cottonseeds [24].

Gossypol, like HA14-1 was also shown to bind to the BH3 domain of Bcl-2. The anti-apoptotic effects of Gossypol have been demonstrated in head and neck cancer, colon, prostate, pancreatic and leukemia cell lines [25-27]. The second category of Bcl-2 family of proteins contains BH domains 1, 2 and 3. These proteins would include Bax and Bak. Bax is a pro-apoptotic protein that resides in the cytosol under physiological conditions.

An apoptotic trigger however, can lead to its translocation to the mitochondria and its subsequent insertion into the OMM. At the mitochondria, Bax can homodimerize or heterodimerize with other pro-apoptotic members such as Bak or truncated Bid, thus disrupting the integrity of the OMM by forming mitochondrial pores and increasing its permeability. These pores can then lead to the release of apoptogenic factors such as cyt c [28].

Some reports have also suggested that Bax engages in a close molecular cooperation with proteins from the PTPC, such as ANT and/or VDAC, to induce mitochondria membrane permeabilization (MMP) [29].

Bcl-2 and Bcl-X_L have been shown to antagonize the apoptotic cascade by a direct interaction and sequestration of these pro-apoptotic proteins[30]. Similarly Bak, which is normally inhibited by its interaction with VDAC, can also homodimerize and result in pore formation at the mitochondria when freed. The third group of Bcl-2 family of proteins is the BH3-only proteins. The BH3-only family members include Bim, Bad, Bmf, Noxa and Puma. They act by neutralizing the anti-apoptotic proteins [31].

For instance, Bim, Puma, Bad and Bmf heterodimerize with Bcl-2 and Bcl-X_L and sequester them, thereby blocking their anti-apoptotic action at the mitochondria. While drugs that could suppress anti-apoptotic proteins have yielded some promising results, attempts to increase sensitivity by mimicking the structures of pro-apoptotic proteins has also led to the discovery of some novel BH3 mimetics. One such well understood BH3 mimetic is ABT-737. ABT-737 mimics the BH3-only protein Bad and has been found to bind and inhibit Bcl-2, Bcl-w and Bcl-X_L.

However, it displays a weak affinity to MCL-1[32]. Thus cancer cells that overexpress MCL-1, are resistant to this small molecule compound. To that end, in a small cell lung cancer model, ABT-737 was shown to sensitize cells to apoptosis induced by chemotherapeutic agents [33]. Of note, ABT-737 is highly effective against cancers with elevated expression of Bcl-2 and is an efficient apoptosis inducer in the presence of Bax and Bak [34]. Another group of BH3 peptides synthesized by Shangary et al. to mimic Bax and Bad BH3 domains demonstrated effective engagement of the intrinsic pathway with the release of cyt *c*, even in Bcl-2 and Bcl-X_L overexpressing cells [35].

BH3 peptides have also been shown to trigger oligomerization of Bax and Bak, resulting in MOMP and cyt *c* release [36]. Though the use of BH3 mimetics holds much promise, the use of BH3 peptides in cancer therapeutics has been limited due their poor cell permeability, bioavailability, solubility and metabolic stability *in vivo*. To address these issues, tagged proteins [37] or proteins with chemical modifications [38] have been employed.

2. **Apoptotic Inhibitor proteins:**The inhibitors of apoptosis (IAP) are a family of antiapoptotic proteins that directly bind to caspases, inhibiting their functional activity. Cell fate is tightly regulated by the interactions between pro and anti-apoptotic proteins which act to tweak the balance between survival and cell death [31]. In cancers, the crippling of pro-apoptotic pathways or enhancement of the anti-apoptotic pathways via the modulation of the regulatory proteins largely confers survival advantages onto the cells in the face of death triggers.

   The inhibitors of apoptosis (IAPs) family were first identified in *baculovirus* and to date, eight mammalian IAPs have been described [39]. This would include neuronal apoptosis inhibitory protein (NAIP), cellular IAP1 and IAP2 (cIAP1 and cIAP2), X-linked inhibitor of apoptosis (XIAP), Survivin, Testis-specific IAP (Ts-IAP), BIR-containing ubiquitin conjugating enzyme (BRUCE) and Livin [40].

   IAPs are characterized by the presence of 70–80 amino acid baculoviral IAP repeat (BIR) domain(s), which are important for the binding and inhibition of caspases. They play a critical role in blocking cell death by regulating the caspase cascade, and then, may influence both the intrinsic and extrinsic pathway in the cells. Of the IAPs, XIAP has been best described, possibly due to the extensive studies on its anti-apoptotic role and the plausible therapeutic benefits in targeting it. XIAPs have been shown to antagonize the apoptotic cascade via the direct inhibition of caspases and via proteasome-dependant degradation of caspases.

   In addition to these caspase inhibitory roles, it has also been found to activate nuclear factor kappa B (NF-κB) by promoting the nuclear localization of NF-κB. IAPs have been shown to be regulated by IAP binding proteins such as second mitochondrial activator of caspases (Smac/DIABLO) [41]. Smac /DIABLO normally resides in the mitochondria and upon receiving an apoptotic stimulus via the intrinsic pathway is proteolytically cleaved and released into the cytosol through the Bax/Bak channels or via Bid-induced permeabilization of the outer mitochondrial membrane.

   Subsequently, Smac/DIABLO associates with IAPs and prevents them from inhibiting caspases, thus promoting apoptosis.

3. **Apoptotic pro-survival proteins:**Upregulation of the anti-apoptotic family of proteins has been a frequent explanation for the resistance observed in cancer cells. Increased expression of the anti-apoptotic family members such as Bcl-2, Bcl-$X_L$and Mcl-1 has been often observed in cancer cells[42], where they serve to antagonize mitochondria-mediated cell death pathway.

   Specifically, Bcl-2/Bcl-X_L upregulation is clearly associated with poor prognosis in cancer [43]. The ability of these proteins to antagonize the pro-apoptotic family of proteins such as Bax and Bak has been the key mechanism by which these cells acquire resistance to apoptosis. As such, measures to target Bcl-2 or Bcl-X_L via anti-sense oligo nucleotides have been employed in clinical studies to sensitize cancer cells to apoptotic triggers [44, 45].

   However, IAPs are a class of anti-apoptotic proteins upregulated in a variety of human cancers. IAPs inhibit the activity of caspases and hence protect cells from the deleterious effects of active caspases. Smac/DIABLO, a natural antagonist of IAPs, has been shown to sensitize cells to drug- and receptor-induced apoptosis by binding to XIAP and releasing caspase-9 *in vitro*. Increased resistance to drugs that activate the extrinsic death pathway has also been observed with upregulation of proteins such as c-Flip and decoy receptors.

   c-Flip inhibits the autoproteolytic cleavage of pro-caspase-8, which is involved in DISC formation, and hence, the downstream transduction of the death signals via the extrinsic death pathway [46]. Decoy receptors can compete with death receptors for the ligands such as TRAIL and CD95, thus inhibiting/abolishing death signal transduction [47].

4. **Repression of pro-apoptotic proteins:** In addition to the upregulation of pro-survival factors, suppression of pro-apoptotic proteins also contributes to resistance against apoptosis-inducing therapeutic regimens. Thus, Bax is one key pro-apoptotic member that is frequently suppressed or mutated in cancers.

The mutations include frameshifts and/or mutations at the BH domains, which lead to a loss of function. Indeed tumors with reduced Bax expression have been found to have a poorer prognosis [48, 49]. In addition to Bax deficiencies, it has also been reported that Bak deficiencies can also lead to substantial inhibition of mitochondria-mediated apoptotic cell death. The activation of Bax and Bak is mediated via caspase-8-induced cleavage of the BH3 only protein Bid to tBid.

Activation and oligomerization of BAX or BAK have been proposed to result in the formation of a VDAC-containing pore and permeabilization of mitochondrial membranes. This leads to the release of cyt *c* and the subsequent engagement of the Apaf-1-caspase-9 apoptosome complex, which activates downstream effector caspases. In this regard, suppression of components which act downstream of the mitochondria such as cyt *c*, Apaf-1 and caspases can protect the cells from apoptotic insults. Accordingly, the expression levels of several pro-apoptotic members of Bcl-2 family, such as Bim and Puma have been shown to correlate with colon carcinoma susceptibility to chemotherapy [50].

Apoptosis and Cancer Therapy: Most drugs currently used in anti-cancer therapy kill target cells by induction of apoptosis, both by receptor-mediated and mitochondrial-mediated pathways. Disruption of the mitochondrial membrane potential, cytochrome c release and activation of different caspases have been described following treatment of cells with diverse chemotherapeutic agents [51, 52].

For example, chemotherapy-induced increase in the transcription of the p53 response gene Bax leads to cytochrome c release and caspase activity. Activation of the Fas system has been observed in different systems, e.g. induction of FasL and upregulation of Fas following treatment of different tumour cell lines with doxorubicin, cisplatin, methotrexate, cytarabine and etoposide [53-57]. In addition, treatment of CML with the death receptor ligand interferon α brings about the upregulation of Fas on CML progenitors [58]. Patients with Fas-positive AML were shown to have a better therapeutic response in comparison with Fas-negative AML patients [59]. The improved understanding of the mechanisms of apoptosis and resistance to apoptosis has provided new insights for the development of new anti-cancer agents. TRAIL, a member of the TNF family of ligands, binds to the cell surface death receptors DR4 and DR5 and is expressed by most cells; most normal cells appear to be resistant to TRAIL-induced apoptosis due to expression of decoy receptors (DcR1 and DcR2), while transformed cells are sensitive[60-62]. The dysregulation of different members of the Bcl-2 family in many cancer types led to the search for small inhibitors of this protein family.

Small molecules with high affinity for the BH3-domain on the surface of Bcl-2 induce apoptosis [63-66]. Gossypol, a natural product found in cottonseeds, interacts with the BH3-binding pocket of four anti-apoptotic proteins [67, 68]. The suppression of NF-κB is another strategy for developing new cancer therapies. This transcription factor induces expression of several anti-apoptotic genes (Bcl-2, Bcl-Xl, c-IAP-2), and NF-κB-overexpression has been found in different cancers [69, 70]. Recently, the Smac/Diablo protein was discovered. This protein binds to and inhibits the IAP-family proteins and promotes apoptosis [71].

CONCLUSION: For all these therapeutic options, the basic idea of selective activation of apoptosis in transformed cells remains the key issue and may result in the development of new therapeutic agents, more active and/or less toxic than the ones used currently. In the future, patient-specific profiles of apoptosis-related genetic alterations and genetic comparisons between chemotherapy-sensitive and chemotherapy-resistant cells will open the way for patient-specific apoptosis-based therapy with hopefully fewer adverse effects [72, 73].

Indeed, this pathway is frequently impaired in cancer cells and contributes to the development of resistance to conventional chemotherapy. Several small molecules, targeted anti/pro-oxidants and antisense oligonucleotides have been designed to activate pro-apoptotic proteins as well as to block anti-apoptotic proteins and are currently under clinical evaluation. Results of clinical trials will determine whether the promise that these strategies hold will be realized for a significant improvement in the clinical management of cancers that are refractory to conventional interventions.

REFERENCES:

1. Jacobson MD, Weil M and Raff MC: Programmed cell death in animal development. Cell 1997; 88:347–354.
2. Hanahan D and Weinberg RA: The hallmarks of cancer. Cell 2000; 100:57–70.
3. Zornig M, Hueber A, Baum W and Evan G: Apoptosis regulators and their role inTumorigenesis. Biochim. Biophys. Acta 2001; 1551:F1–F37.
4. Fulda S, Galluzzi L and Kroemer G: Targeting mitochondria for cancer therapy. Nat. Rev. Drug Discov 2010; 9: 447–464.
5. Kerr JF, Wyllie AH and Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 1972; 26:239–257.
6. Danial NN and Korsmeyer SJ: Cell death: critical control points. Cell 2004; 116:205–219.
7. Vakifahmetoglu-Norberg H and Zhivotovsky B: The unpredictable caspase-2: whatcan it do?. Trends Cell Biol 2010; 20:150–159.
8. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH and Peter ME: Two CD95 (APO-1/Fas) signaling pathways. EMBO J 1998; 17:1675–1687.
9. Ashkenazi A and Dixit VM: Death receptors: signaling and modulation. Science 1998; 281:1305–1308.
10. Hengartner MO: The biochemistry of apoptosis. Nature 2000; 407:770–776.
11. Elmore S: Apoptosis: a review of programmed cell death. Toxicol. Pathol 2007; 35:495–516.
12. Schultz DR and Harrington Jr WJ: Apoptosis: programmed cell death at a molecular level. Semin. Arthritis Rheum 2003; 32:345–369.
13. Rudin CM and Thompson CB: Apoptosis and disease: regulation and clinical relevance of programmed cell death. Annu. Rev. Med 1997; 48:267–281.
14. Fesus L, Davies PJ and Piacentini M: Apoptosis: molecular mechanisms in programmed cell death. Eur. J. Cell Biol. 1991; 56:170–177.
15. Gross A, McDonnell JM and Korsmeyer SJ: BCL-2 family members and the mitochondria in apoptosis, Genes Dev 1999; 13:1899–1911.
16. Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR and Martin SJ: Ordering the cytochromec-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J. Cell Biol. 1999; 144:281–292.
17. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CY, Sasaki T, Elia AJ, Cheng HY, Ravagnan L, Ferri KF, Zamzami N, Wakeham A, Hakem R, Yoshida H, Kong YY, Mak TW, Zuniga-Pflucker JC, Kroemer G and Penninger JM: Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature 2001; 410:549–554.
18. Alvero AB, Montagna MK, Chen R, Kim KH, Kyungjin K, Visintin I, Fu HH, Brown D and Mor G: NV-128, a novel isoflavone derivative, induces caspase-independent cell death through the Akt/mammalian target of rapamycin pathway. Cancer 2009; 115:3204–3216.
19. Boujrad H, Gubkina O, Robert N, Krantic S and Susin SA: AIF-mediated programmednecrosis: a highly regulated way to die. Cell Cycle 2007; 6:2612–2619.
20. Reed JC, Miyashita T and Takayama S: BCL-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. J Cell Biochem 1996; 60:23-32.
21. Cory S and Adams JM: The Bcl2 family: regulators of the cellular life-or-deathSwitch. Nat. Rev. Cancer 2002; 2:647–656.
22. Brenner C, Cadiou H, Vieira HL, Zamzami N, Marzo I, Xie Z, Leber B, Andrews D, Duclohier H, Reed JC and Kroemer G: Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator. Oncogene 2000; 19:329–336.
23. Chen ZX and Pervaiz S: Bcl-2 induces pro-oxidant state by engaging mitochondrial respiration in tumor cells. Cell Death Differ 2007; 14:1617–1627.
24. Pellecchia M and Reed JC: Inhibition of anti-apoptotic Bcl-2 family proteins by natural polyphenols: new avenues for cancer chemoprevention and chemotherapy, Curr. Pharm. Des. 2004; 10:1387–1398.
25. Mohammad RM, Wang S, Banerjee S, Wu X, Chen J and Sarkar FH: Nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-XL, (−)-Gossypol, enhances biological effect of genistein against BxPC-3 human pancreatic cancer cell line. Pancreas 2005; 31:317–324.
26. Bauer JA, Trask DK, Kumar B, Los G, Castro J, Lee JS, Chen J, Wang S, Bradford CR and Carey TE: Reversal of cisplatin resistancewith a BH3mimetic, (−)-gossypol, in head and neck cancer cells: role of wild-type p53 and Bcl-xL: Mol. Cancer Ther 2005; 4:1096–1104.
27. Xu L, Yang D, Wang S, Tang W, Liu M, Davis M, Chen J, Rae JM, Lawrence T and Lippman ME: (−)-Gossypol enhances response to radiation therapy and results in tumor regression of human prostate cancer. Mol. Cancer Ther 2005; 4:197–205
28. Vyssokikh MY, Zorova L, Zorov D, Heimlich G, Jurgensmeier JJ and Brdiczka D: Bax releases cytochrome c preferentially from a complex between porin and adenine nucleotide translocator Hexokinase activity suppresses this effect, Mol Biol Rep 2002; 29:93–96.
29. Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL, Prevost MC, Xie Z, Matsuyama S, Reed JC and Kroemer G: Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis, Science 1998; 281:2027–2031.
30. Yang E, Zha J, Jockel J, Boise LH, Thompson CB and Korsmeyer SJ: Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 1995; 80:285–291.
31. Reed JC: Bcl-2 family proteins. Oncogene 1998; 17:3225–3236.
32. van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW and Huang DC: The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 2006; 10; 389–399.
33. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW and Rosenberg SH: An inhibitor of Bcl-2 family proteins induces regression of solid Tumours. Nature 2005; 435:677–681.
34. Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi YX, Sneed T, Verhaegen M, Soengas M, Ruvolo VR, McQueen T, Schober WD, Watt JC, Jiffar T, Ling X, Marini FC, Harris D, Dietrich M, Estrov Z, McCubrey J, May WS, Reed JC and Andreeff M: Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006; 10:375–388.
35. Shangary S and Johnson DE: Peptides derived from BH3 domains of Bcl-2 family members: a comparative analysis of inhibition of Bcl-2, Bcl-x(L) and Bax oligomerization, induction

of cytochrome c release, and activation of cell death. Biochemistry 2002; 41:9485–9495.
36. Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, BA Sullivan, Green DR and Newmeyer DD: BH3 domains of BH3-only proteins differentially regulate Baxmediated mitochondrial membrane permeabilization both directly and indirectly. Mol. Cell 2005; 17:525–535.
37. Denicourt C and Dowdy SF: Medicine Targeting apoptotic pathways in cancer cells. Science 2004; 305:1411–1413.
38. Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL and Korsmeyer SJ: Activation of apoptosis *in-vivo* by a hydrocarbonstapled BH3 helix, Science 2004; 305:1466–1470.
39. Birnbaum MJ, Clem RJ and Miller LK: An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J. Virol. 1994; 68:2521–2528.
40. Duckett CS, Nava VE, Gedrich RW, Clem RJ, Van Dongen JL, Gilfillan MC, Shiels H, Hardwick JM and Thompson CB: A conserved family of cellular genes related to the baculovirus iap gene and encoding apoptosis inhibitors. EMBO J 1996; 15:2685–2694.
41. Duckett CS: IAP proteins: sticking it to Smac. Biochem. J 2005; 385:e1–e2.
42. Reed JC: Dysregulation of apoptosis in cancer. J. Clin. Oncol 1999; 17:2941–2953.
43. Schattner EJ: Apoptosis in lymphocytic leukemias and lymphomas. Cancer Invest 2002; 20:737–748.
44. Yamanaka K, Rocchi P, Miyake H, Fazli L, So A, Zangemeister-Wittke U and Gleave ME: Induction of apoptosis and enhancement of chemosensitivity in human prostate cancer LNCaP cells using bispecific antisense oligonucleotide targeting Bcl-2 and Bcl-xL genes. BJU Int. 2006; 97:1300–1308.
45. Zangemeister-Wittke U, Leech SH, Olie RA, Simoes-Wust AP, Gautschi O, Luedke GH, Natt F, Haner R, Martin P, Hall J, Nalin CM and Stahel RA: A novel bispecific antisense oligonucleotide inhibiting both bcl-2 and bcl-xL expression efficiently induces apoptosis in tumor cells. Clin Cancer Res 2000; 6:2547–2555.
46. Micheau O, Thome M, Schneider P, Holler N, Tschopp J, Nicholson DW, Briand C and Grutter MG: The long form of FLIP is an activator of caspase-8 at the Fas death-inducing signaling complex. J. Biol. Chem 2002; 277:45162–45171.
47. Ashkenazi A: Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat. Rev. Cancer 2002; 2:420–430.
48. Jeong SH, Lee HW, Han JH, Kang SY, Choi JH, Jung YM, Choi H, Oh YT, Park KJ, Hwang SC, Sheen SS, Oh YJ, Kim JH and Lim HY: Low expression of Bax predicts poor prognosis in resected non-small cell lung cancer patients with non-squamous histology. Jpn. J. Clin. Oncol 2008; 38:661–669.
49. Schuyer M, van der Burg ME, Henzen-Logmans SC, Fieret JH, Klijn JG, Look MP, Foekens JA, Stoter G and Berns EM: Reduced expression of BAX is associated with poor prognosis in patients with epithelial ovarian cancer: a multifactorial analysis of TP53, p21, BAX and BCL-2. Br. J. Cancer 2001; 85:1359–1367.
50. Sinicrope FA, Rego RL, Okumura K, Foster NR, O'Connell MJ, Sargent DJ and Windschitl HE: Prognostic impact of bim, puma, and noxa expression in human colon carcinomas. Clin. Cancer Res. 2008; 14:5810–5818.
51. Vermeulen K, Strnad M, Havlicek L, Van Onckelen H, Lenjou M, Nijs G, Van Bockstaele D and Berneman ZN: Plant cytokinin analogues with inhibitory activity on cyclin dependent kinases (CDK) exert their antiproliferative effect through induction of apoptosis initiated by the mitochondrial pathway: determination by a multiparametric flow cytometric analysis. Exp Hematol 2002; 30:1107–1114.
52. Kroemer G and Reed JC: Mitochondrial control of cell death. Nat Med 2000; 6:513–519.
53. Muller M, Strand S, Hug H, Heinemann EM, Walczak H, Hofmann WJ, Stremmel W, Krammer PH and Galle PR: Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. J Clin Invest 1997; 99:403–413.
54. Friesen C, Herr I, Krammer PH and Debatin KM: Involvement of the CD95 (APO-1/FAS) receptor/ligand system in drug-induced apoptosis in leukemia cells. Nat Med 1996; 2: 574– 577.
55. Friesen C, Fulda S and Debatin KM: Deficient activation of the CD95 (APO-1/Fas) system in drug-resistant cells. Leukemia 1997; 11:1833–1841.
56. Fulda S, Sieverts H, Friesen C, Herr I and Debatin KM: The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. Cancer Res 1997; 57:3823–3829.
57. Fulda S, Friesen C, Los M, Scaffidi C, Mier W, Benedict M, Nunez G, Krammer PH, Peter ME and Debatin KM: Betulinic acid triggers CD95 (APO-1/Fas)- and p53-independent apoptosis via activation of caspases in neuroectodermal tumors. Cancer Res 1997; 57:4956–4964.
58. Selleri C, Sato T, Del Vecchio L, Luciano L, Barrett AJ, Rotoli B, Young NS and Maciejewski JP: Involvement of Fasmediated apoptosis in the inhibitory effects of interferon-alpha in chronic myelogenous leukemia. Blood 1997; 89:957–964.
59. Min YH, Lee S and Lee JW: Expression of FAS antigen in acute myeloid leukemia is associated with therapeutic response to chemotherapy. Br J Haematol 1996; 93:928–930.
60. Ashkenazi A and Dixit VM: Death receptors: signaling and modulation. Science 1998; 281:1305–1308
61. Landowski TH, Qu N, Buyuksal I, Painter JS and Dalton WS: Mutations in the Fas antigen in patients with multiple myeloma. Blood 1997; 90:4266–4270.
62. Medema JP, de Jong J, Peltenburg LT, Verdegaal EM, Gorter A, Bres SA, Franken KL, Hahne M, Albar JP, Melief CJ and Offringa R: Blockade of the granzyme B/perforin pathway through overexpression of the serine protease inhibitor PI-9/SPI-6 constitutes a mechanism for immune escape by tumors. Proc Natl Acad Sci USA 2001; 98:11515–11520.
63. Wang JL, Zhang ZJ, Choksi S, Shan S, Lu Z, Croce CM, Alnemri ES, Korngold R and Huang Z: Cell permeable Bcl-2 binding peptides: a chemical approach to apoptosis induction in tumor cells. Cancer Res 2000; 60:1498–1502.
64. Lugovskoy AA, Degterev AI, Fahmy AF, Zhou P, Gross JD, Yuan J and Wagner G: A novel approach for characterizing protein ligand complexes: molecular basis for specificity of small-molecule Bcl-2 inhibitors. J Am Chem Soc 2002; 124:1234–1240.
65. Wang JL, Liu D, Zhang ZJ, Shan S, Han X, Srinivasula SM, Croce CM, Alnemri ES and Huang Z: Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc Natl Acad Sci U S A 2000; 97:7124–7129.
66. Degterev A: Identification of small-molecule inhibitors of interaction between BH3 domain and Bcl-Xl. Nat Cell Biol 2001; 3:173–182.
67. Pellecchia M and Reed JC: Inhibition of anti-apoptotic Bcl-2 family proteins by natural polyphenols: new avenues for cancer chemoprevention and chemotherapy. Curr Pharm Des 2004; 10:1387–1398.

68. Qiu J, Levin LR, Buck J and Reidenberg MM: Different pathways of cell killing by gossypol enantiomers. Exp Biol Med (Maywood) 2002; 227:398–401.
69. Karin M, Cao Y, Greten FR and Li ZW: NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002; 2:301–310.
70. Rayet B and Gelinas C: Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999; 18:6938–6947.
71. Du C, Fang M, Li Y, Li L and Wang X: Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000; 102:33–42.
72. Siegel RM, Chan FK, Chun HJ and Lenardo MJ: The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. Nat Immunol 2000; 1:469–474.
73. Schmitt CA and Lowe SW: Apoptosis is critical for drug response *in vivo*. Drug Resist Updat 2001; 4:132–134.

How to cite this article:
Rahman Md. A: Apoptosis Signaling; A Drug Design in Cancer Therapy: A Review. *Int J Pharm Sci Res*, 2012; Vol. 3(7): 1946-1954

****