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Exploiting the Extrinsic and the Intrinsic Apoptotic Pathways for Cancer Therapeutics

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Abstract

Cell death or apoptosis is a cellular process that occurs in physiological and pathological conditions. Cancer is a scenario where there is too little apoptosis. Apoptotic extrinsic and intrinsic signaling pathways are twomajor mechanisms of apoptosis. Defects can occur at any point along these pathways, leading to malignant transformation and resistance to anti-cancer drugs. The understanding of the extrinsic and intrinsic apoptotic signaling mechanisms has provided the basis for novel targeted therapies that can stimulate death in cancer cells and or sensitize them to established cytotoxic agents and radiation therapy. This review focuses on the current knowledge of the extrinsic and intrinsic pathways to apoptosis in normal and diseased cells and the agents developed to target them.

Keywords: Apoptosis; Cancer therapeutics; Extrinsic pathways; Intrinsic pathways

Introduction

Cancer is a primary cause of death worldwide. The World Health Organization estimates that by 2030 there will be 13 million cancer related deaths [1]. The underlying etiology of cancer was first attributed to acceleratedand dysregulatedcell proliferation that led to cellular increase and accumulation of tissue mass. Later, it had been found that some cancers were caused by the lack of physiological cell death (also referred to as cellular suicide or apoptosis) rather than an increased rate of cell proliferation [2]. Nowadays, the dysregulation ofthe two well-defined apoptotic signaling pathways; the extrinsic/death receptors and the intrinsic/mitochondrial pathway,is considered a major clinical problem to overcome in the treatment of cancer.

The notion that apoptosis might impact cancer malignant phenotype dated back to the early 1970s where pathologists observed that radiation and chemotherapy can yield cell death with morphological characteristics of apoptosis [3]. Kerr et al. [4] were the first to raise the possibility that a large percentage of cell loss from tumors was due to apoptosis. Since then, many investigators have ventured into targeting various aspects of apoptosis to trigger tumor cell deathandenhance cancer therapies [5,6]. Several target molecules aimed at the two-main apoptotic signaling pathways; the extrinsic pathway and the intrinsic pathway led to the development of various agents including natural and synthetic compounds, antibodies, antisense, and silencing RNA. Specific Histone Deacetylase (HDAC) inhibiters (HDIs) have also emerged as a new target for cancer therapy [7] since HDIs can induce apoptosis via the extrinsic and the intrinsic pathways [8].

This review provides a general overview on how the disruption of normal function of the extrinsic and the intrinsic apoptotic pathways promotes the incidence of cancer and the strategies directed at theses pathways in cancer therapeutics.

Mechanisms of apoptosis regulation

Different stresses originated either from outside or inside the cell trigger cell death by apoptosis [9]. As indicated above, to date, there are two major apoptotic pathways: the extrinsic and the intrinsic pathway. These two pathways are tightly linked with some molecules from one pathway able to influence the other pathway [10]. The extrinsic and intrinsic pathway proteins abbreviations and alternate nomenclature are summarized in Table 1 and 2.

The extrinsic apoptotic pathways: The extrinsic apoptotic signaling pathways or death receptor pathways are mediated by transmembrane death receptors, which depend on ligand-receptor interactions for cell death effectors activity [11]. These receptors are part of the Tumor Necrosis Factor (TNF) receptor gene super family. They share cysteine-rich extracellular domains and a cytoplasmic death. The best-characterized ligands and corresponding death receptors are; FasL/Fas

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Table 1: Extrinsic pathway proteins, abbreviations, and other nomenclature.

Abbreviation	Protein full name	Other Nomenclature
Apo2L	Apo2 ligand	TNFSF10, TRAIL, TNF-related apoptosis inducing ligand
Apo3L	Apo3 ligand	TNFSF12, Apo3 ligand, TWEAK, DR3LG
caspase-8	Cysteinyl aspartic acid-protease 8	FLICE, FADD-like Ice, Mach-1, Mch5
DISC	Death inducing signaling complex	None
DED	Death effector domain	Apoptosis antagonizing transcription factor, CHE1
DR3	Death receptor 3	TNFRSF12, Apo3, WSL-1, TRAMP, LARD, DDR3
DR4	Death receptor 4	TNFRSF10A, TRAILR1, APO2
DR5	Death receptor 5	TNFRS10B, TRAIL-R2, TRICK2, KILLER, ZTNFR9
FADD	Fas-associated death domain	MORT1
Fas	Fatty acid synthetase receptor is a transmembrane receptor of the tumor necrosis factor (TNF) receptor superfamily	Fas receptor, TNFRSF6, APT1, CD95
FasL	Fatty acid synthetase ligand	Fas ligand, TNFSF6, Apo1, apoptosis antigen ligand 1, CD95L, CD178, APT1LG1
c-FLIP	FLICE-inhibitory protein	Casper, I-FLICE, FLAME-1, CASH, CLARP, MRIT
TNF-α	Tumor necrosis factor alpha	TNF ligand, TNFA, cachectin
TNFR1	Tumor necrosis factor receptor 1	TNF receptor, TNFRSF1A, p55 TNFR, CD120a
TRADD	TNF receptor-associated death domain	TNFRSF1A associated via death domain

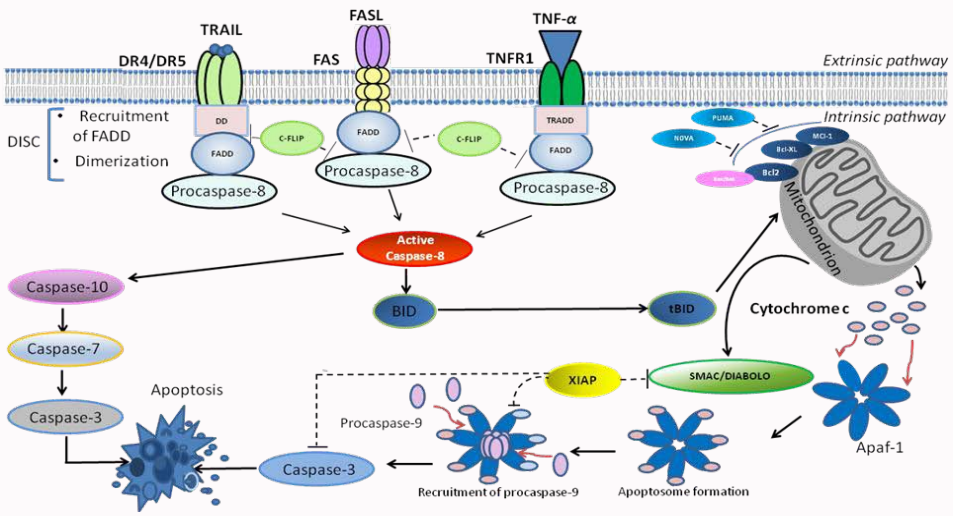

Figure 1: Mechanisms of cell death from the perspective of death receptor and mitochondrial pathways.
(1) The main extrinsic pathways to apoptosis include Fas/FasL, TRAIL/D4/D5 and TNF-α/TNFR1 and activation of effector caspases among which caspase-3 leads to excessive programmed cell death.
(2) The intrinsic pathway is initiated by various signals, essentially extracellular stimuli that activate caspase-8. Caspase-8 converts Bid into truncated Bid that initiates caspase-8 activation. Other BH3-only proteins such as Noxa and Puma engage with anti-apoptotic Bcl-2 family proteins to relieve their inhibition of Bax and Bak to activate them. Next, Bax and Bak are oligomerized and activated, leading to mitochondrial outer membrane permeabilization. Once mitochondrial membrane is permeabilized, cytochrome *c* and/or Smac/DIABLO is released into the cytoplasm, where they link with an adaptor molecule, apoptosis protease-activating factor 1, and an inactive initiator caspase, procaspase-9, within a multiprotein complex namely the apoptosome. Smac/DIABLO inhibits inhibitors of apoptosis proteins to activate caspase-9 followed by caspase-3 activation that also yields apoptotic cell death.

(also called Apo1 orCD95), TNF-α/TNFR1, Apo3L/DR3 (also called Apo3), Apo2L/ DR4 (also called, Apo2, TRAILR1, Apo2L/ DR5 (also called Apo2, TRAILR2) [12-14]. Among these receptors, FasL/Fas, TNF-α/TNF1, and TRAIL models are the most studied (Figure 1). The ligation of FasL to the Fasreceptor prompts the binding of the adapter protein FADD. The ligation of TNF-α to TNF results in the binding of the adapter protein TRADD and the recruitment of FADD [15,16]. TRAIL can bind two apoptosis-inducing receptors -TRAIL-R1 (DR4) and TRAIL-R2 (DR5) – and two additional cell-bound receptors incapable of transmitting an apoptotic signal-TRAIL-R3 (LIT, DcR1) and TRAIL-R4 (TRUNDD, DcR2) sometimes called decoy receptors. Finally, a soluble receptor called Osteoprotegerin (OPG) is also capable of binding TRAIL [17]. The apoptotic signaling pathway of TRAIL is triggered by binding of trimerized TRAIL to DR4 (TRAIL1) and/or DR5 (TRAIL2) [18], followed by receptor clustering leading to the recruitment of FADDas well. In all these models, the FADD protein dimerize with procaspase-8to form a Death-Inducing Signaling Complex (DISC) known as the primary complex,leading tothe activation ofprocaspase-8 [19] (Figure 1). In Type I cells, activated caspase-8 initiates apoptosis directly by cleaving and thereby stimulating executioner caspases [20]. In Type II cells, caspase-8 must first activate the intrinsic apoptotic pathway (discussed below) to promote cell death [21]. Death receptor-mediated apoptosiscan bepreventedbycellular FLICE Inhibitory Protein (c-FLIP), which

Table 2: Intrinsic pathway proteins, abbreviations, and other nomenclature.

Abbreviation	Protein full name	Other Nomenclature
14-3-3	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	14-3-3 eta, theta, zeta, beta, epsilon, sigma, gamma
AIF	Apoptosis Inducing Factor	Programmed cell death protein 8, mitochondrial
Apaf-1	Apoptotic protease activating factor	APAF1
ATF	Activated Transcription factor	None
Bcl-B	Bcl-B selectively binds and suppresses apoptosis induction by Bax, but fails to interact with or negate apoptosis triggered by Bak overexpression. Bcl-B protein contains four Bcl-2 homology (BH) domains (BH1, BH2, BH3, BH4)[167]	None
BAD	BCL2 antagonist of cell death	BCL2 binding protein, BCL2L8, BCL2 binding component 6, BBC6, Bcl-XL/Bcl-2 associated death promoter
BAG	BCL2 associated athanogene	BCL2 associated athanogene
BAK	BCL2 antagonist killer 1	BCL2L7, cell death inhibitor 1
BAX	BCL2 associated X protein	Apoptosis regulator BAX
Bcl-2	B-cell lymphoma protein 2	Apoptosis regulator Bcl-2
Bcl-w	BCL2 like 2 protein	Apoptosis regulator BclW
Bcl-x	BCL2 like 1	BCL2 related protein
Bcl-X_L	BCL2 related protein, long isoform	BCL2L protein, long form of Bcl-x
Bcl-X_S	BCL2 related protein, short isoform	BCL2 related protein, short isoform
BID and tBID	BH3 interacting domain death agonist and truncated BID	p22 BID
BIK	BCL2 interacting killer	NBK, BP4, BIP1, apoptosis inducing NBK
BIM	BCL2 interacting protein BIM	BCL2 like 11
Blk	Bik-like killer protein	B lymphoid tyrosine kinase, p55-BLK, MGC10442
BMF	Bcl2 Modifying factor	None
CAD	Caspase-Activated DNAse-9	CAD/CPAN/DFF40
Caspase-9	Cysteinyl aspartic acid-protease-9	ICE-LAP6, Mch6, Apaf-3
HtrA2/Omi	High-temperature requirement	Omi stress regulated endoprotease, serine protease Omi protein A2
IAP	Inhibitor of Apoptosis Proteins	XIAP, API3, ILP, HILP, HIAP2, cIAP1, API1, MIHB, NFR2-TRAF signaling complex proteins
cIAP1 and cIAP2	Cellular inhibitor of apoptosis protein-1/2	cIAP1: BIRC2
Mcl-1	Anti- apoptotic Myeloid Cell Leukemia Sequence 1	TM; EAT; MCL1L; MCL1S; Mcl-1; BCL2L3; MCL1-ES; bcl2-L-3; mcl1/EAT
Noxa	Phorbol-12-myristate-13-acetate-induced protein 1	PMA induced protein 1, APR
Puma	Bcl-2 binding component 3	JFY1, PUMA/JFY1, p53 up-regulated modulator of apoptosis
Smac/DIABLO	Second mitochondrial activator of caspases/direct IAP binding protein with low PI	None
XIAP	X-linked inhibitor of apoptosis protein	Inhibitor of apoptosis protein 3 (IAP3), baculoviral IAP repeat-containing protein 4 (BIRC4)

shares sequence homology with caspase-8 and may interfere with the FADD-procaspase-8 interaction. This results in theprevention of caspase activation by competing for FADD binding, and thus, impeding the recruitment of procaspase-8 to the DISC, and its activation [22].

The intrinsic pathway: The intrinsic signaling pathwayimplicates intracellular signals that are controlled by the mitochondria. This signalsyieldan alteration ofthe inner mitochondrial membrane, a loss of the mitochondrial transmembrane potential, and a release of cytochrome cand Smac/DIABLO [23]. After which cytochrome c binds and activates Apaf-1 as well as procaspase-9 to form an apoptosome [24]. Causing caspase-9 activation. Smac/DIABLO can be blocked by Inhibitors Of Apoptosis (IAPs) activity [25,26]. For example, XIAP efficiently blocks the intrinsic as well as extrinsic pathways of apoptosis by binding to upstream caspase-9 and downstream caspases-3 (Figure 1).

The Bcl-2 family of proteins regulate mitochondrial apoptosis [27,28]. The family consists of more than 20 members with pro- or anti-apoptotic functions. The family is divided into three groups based on the presence of conserved Bcl-2 Homology (BH) regions vital for heterodimeric interactions among its members [29]. The anti-apoptotic proteins Bcl-2, Bcl-XL, Bcl-XS, Bcl-W, and Mcl-1 contain all four BH regions. The pro-apoptotic proteins are divided into two groups according to their number of BH3 and function.The multi-region pro-apoptotic proteins, Bax, Bak,and Bok share BH 1–3 regions, whereas the BH3-only proteins Bad, Bim, Bid, Noxa, Puma, Bik/Blk, and Bmf have homology in the BH3 region only [30]. Additional Bcl-2 family members have been described with potential pro-apoptotic (Bcl-G, Bcl-Rambo) or prosurvival (Bcl-B) capacity [31]. Based on the binding between different Bcl-2 family members, two models of Bax and Back activation have been put forward; the direct and indirect models [32]. In the direct model, BH3-only proteins such as Bid, Bim, and PUMA (named activators) bind directly to Bax andBak and Bcl-2/Bax ratio determine the sensitivity of a cell to a wide variety of apoptotic stimuli.In the indirect model, Bax and

Bakarestimulated after being displaced from pro-survival proteins by BH3-only proteins. Notably, aspects of both the direct and indirect models may be important [33]. Apoptosis take place once Bax or Bak generate an apoptotic pore in the mitochondrial outer membrane [34]. The Bax and Bak oligomerization and activation cause mitochondrial pathway activation. Furthermore, Bad heterodimerization with either Bcl-XL or Bcl-2, neutralizes either protective effect and promotes cell death [35]. Notably, the anti-apoptotic Bcl-2 family controls the activation of caspases as well. Proteins such as Bcl-2 and Bcl-XL prevent apoptotic death primarily by regulating the activation of caspase proteases [36]. For instance, Bcl-XL binds to Apaf-1 and inhibits activation of caspase-9 [37] (Figure 1).

The joint pathways: The apoptotic extrinsic and intrinsic pathways are not simply two parallel programs converging on common caspases machinery; a number of `cross-talk' happens between the two pathways. Caspase-deficient mice demonstrate that the prerequisite for different death effector molecules during apoptosis is highly variable depending on stimuli. For example, receptor-mediated activation of caspase-8 can cleave and activate Bid, [38,39]. Facilitating cytochrome c release from the mitochondria [10]. Numerous studies have reported that the spontaneous membrane binding of tBid causes the migration of soluble Bax and Bcl-XL to the membranes [40,41]. Further, serine phosphorylation of Bad is correlated with 14-3-3, a member of a family of multifunctional phosphoserine binding molecules. Once Bad are phosphorylated, it is segregated by 14-3-3 in the cytosol. When Bad is unphosphorylated, ittransfersto the mitochondria and acts to release cytochromec [42]. Downstream caspases from the extrinsic and the intrinsic pathways stimulate cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins and inhibitory subunits of endonucleases family. They also impact the cytoskeleton, cell cycle and signaling pathways, which altogether contribute to the typical morphological alterations noted in apoptosis [43].

Taken together these data support the cross talk between the two main pathways to apoptosis.

Imbalance in apoptotic main mechanisms results in cancer

As described above, the extrinsic pathway is regulated at different levels that can be disrupted in tumor cells. Particularly, decreased death receptors have been implicated in the evasion of the death signaling pathways in various cancers [44] For instance, Fasreceptor is down regulated in hematomas versus normal hepatocytes, which is likely to reflect its contribution to evasion of the immune system in liver carcinogenesis [45]. The loss of Fas and the dysregulation of FasL, DR4, DR5, and TRAIL indicate a potential functional role of these death ligands and receptors during cervical carcinogenesis [46]. As the inhibiting of Fas and TRAIL signaling involves the downregulation of procaspase-8 by c-FLIP, the over expression of c-FLIP observed in carcinomas supports tumor development and progression. Other studies demonstrating reduced expression of Fas receptor involvement in treatment-resistant leukemia, [47]. And neuroblastoma [48]. Suggested that the down regulation of surface receptor expression likely to represent a mechanism of acquired drug resistance. All together, these data indicate that death receptors downregulation promotes tumor growth progression.

As stated previously, the intrinsic pathway is controlled by interactions between pro- and anti-apoptotic members of the Bcl-2 protein family. A change in the expression of either type of protein is recurrent in many human cancers. Particularly, increased Bcl-2 protein levels are generally found in many hematopoietic malignancies such as acute and chronic myeloma, [48] and prostate cancer [49]. Overexpression of Bcl-XL confers a multi-drug resistance phenotype to tumor cells and prevents them from undergoing apoptosis [50]. Similarly, mutated or downregulated Bax and Back are observed in certain cancers as well. Notably, colorectal cancers with microsatellite instability, mutations in the baxgene are very common. Miquel et al. [51] showed that dysregulated apoptosis post bax (G)8 frame shift mutations could contribute to resistance of colorectal cancer cells to anticancer treatments.

The Inhibitor of Apoptosis Proteins (IAPs) are also important regulators of cell death and cell survival. There are convincing data implicating the IAPs in cancer development [52]. IAPs known as BIRCs (BIR domain containing proteins) are a class of highly conserved proteins characterized by the presence of Baculovirus IAP Repeat (BIR) domain, a Zn2+ ion coordinating protein–protein interaction motif [53] largely known for the regulation of caspases. There are eight mammalian IAPs/BIRCs; among which cIAP-1, cIAP-2, Survivin and XIAP, [53] which are the best known for stimulation of cancer cell survival, are over expressed in breast cancer [54]. The role of IAPs in tumorigenesis is dependent of the circumstance and the cell type [55]. For instance, c-IAP2 is over expressed in pancreatic intraepithelial neoplastic lesions and pancreatic ductal adenocarcinomas and is considered an early event in the progression of pancreatic cancer [56]. Survival analysis revealed that patients co-expressing cIAP1/cIAP2 in pancreatic tumors have shorter survival rate *vs.* cases where only one or none of the proteins was expressed [56]. XIAP over expressed in esophageal cancer tissues perhaps confers resistance to apoptosis induction by activatingcaspase-3 and promoting tumorigenesis [57]. Survivin is a protein from IAP proteins which is greatly expressed in a variety of cancers but has very low expression in the corresponding normal tissues. Survivin expression is often correlated with tumor metastasis and chemo resistance [57].

Caspases are a family of cysteinyl aspartate-specific proteases that can be broadly classified into two groups: (1) those associated with caspase-1 (e.g. caspase-1, -4, -5, -13, and -14) and generally implicated in inflammatory processes, and (2) those that have a pivotal role in apoptosis (e.g. caspase-2, -3. -6, -7, -8, -9 and -10). The second group is further divided into (i) initiator caspases (e.g. caspase-2, -8, -9 and -10), primarily responsible for the initiation of the apoptotic pathway and (i) effector caspases (caspase-3, -6 and -7), responsible in the cleavage of cellular elements during apoptotic cell death [58]. As caspases remain one of the important players in the initiation and the execution of apoptosis, it is reasonable to believe that low levels of caspases expression or impairment in caspase function may lead to decreased apoptosis and carcinogenesis. Several reports point out to the important role of caspases in cancer development [7,59]. Activation of caspases is frequently impaired in human cancers, progression and therapy resistance [60]. Tumor progression toward metastatic disease was observed in 16 out of 20 primary melanomas (80%) with elevated amounts of cleaved caspase-3. Conversely, only 10 out of the 37 primary melanomas (20%) with low amounts of cleaved caspase-3 developed metastatic disease [61]. The investigators attribute the difference in tumor metastasis to increased migration and invasion, features that may be associated with non-apoptotic roles of caspases at conditions of sub-lethal activation [62]. Fong et al. [63] observed that in some instances more than one caspase can be downregulated, suggesting that this may contribute to tumor growth

Table 3: Summary of the treatment strategies targeting the extrinsic and the intrinsic pathway molecules discussed in this review.

Protein targeted	Drug names and effects
Agents that target the death receptor family of proteins	· Agents against Fas/FasL
	-ABT-510 has anti-angiogenic activity
	-Anti-APO-1 and anti-Fas antibodies induce apoptosis in different types of cancer cells by binding to a receptor on the surface of the cells they kill.
	-Histone Deacetylase Inhibitors (HDIs): Apicidin stimulates apoptosis through Fas/FasL expression in human acute promyelocytic leukemia cells.
	· Agents against TRAIL
	-TRAIL suppresses the growth of colon and breast xenografts without the side effects observed with TNF and FasL.
	-TRAIL combination with standard chemotherapeutic agents resulted in increased apoptosis in mutant KRAS non-small cell lung carcinoma cells and in significant inhibition of tumor growth in a prostate cancer in vivo model.
	-HGS-E
	TR1 human agonistic TRAIL-R1 monoclonal antibody is a potent antitumor agent with favorable pharmacokinetic characteristics on a broad range of human malignancies.
	-TRAIL-R2-specific antibodies and recombinant TRAIL can synergize to kill cancer cells as well.
Agents that target the Bcl-2 family proteins	BH3 mimetics
	-Gossypol is a BH3 mimetics and natural compound that inhibited anti-apoptotic Bcl-2, Bcl-x_L and Mcl-1 proteins. Levo gossypol (AT-101, Ascenta) tested in patients with small cell lung cancer were disappointing. But, apogossypol (Burnham Institute) seemed to better target Bcl-2 and Mcl-1 and has single-agent activity in Bcl-2–transgenic mice and has better efficacy with less toxicity than gossypol.
	-ABT-737exhibits cytotoxicity in lymphoma, small cell lung carcinoma cell line and primary patient-derived cells and caused regression of established tumors with a high percentage of cure in mice. This agent inhibits Bcl-2, Bcl-X_L, and Bcl-W proteins and binds the anti-apoptotic Bcl-2 family. It shows low activity to Bcl-B and no effects to Mcl-1 and BFL-1. It does not inhibit the pro-survival proteins Mcl-1, Bcl-B, Bfl-1 (A1).
	-GX15-070 (obatoclax, Gemin X) blocks most anti-apoptotic Bcl-2 family of proteins. It has a relatively low affinity for Bcl-2, Bcl-X_L, Bcl-w, and Mcl-1 versus ABT-737. GX15-070 combined with PI3-kinase/mTOR-inhibitor BEZ235 inhibit chronic lymphocytic leukemia cells.
	-A-1210477 is a potent and selective Mcl-1 inhibitor over other Bcl-2 family members.
	-BH3 mimetics e.g. ABT-737, ABT-263, GX15-070, and A-1210477 have entered in clinical trials.
	-HA14-1 inhibits the binding of Bcl-2 and Bcl-X_L to Bax and Bakand induce apoptosis in glioma cells and colon cancer cells.
	- Silencing the Bcl2 anti-apoptotic proteins/genes
	-G3139 (Oblimersen sodium, Genasense, Genta Inc., Berkeley Heights, NJ) silencing resulted in decreased Bcl-2 protein translation.
	-Silencing proto-oncogene Bmi-1 in MCF breast cancer cells downregulated Bcl-2 expression making the cells susceptible to doxorubicin.
	-Antisense Bcl-$X_L$oligonucleotide and bispecific antisense bcl-2/bcl-xL oligonucleotide simultaneously downregulates Bcl-2 and Bcl-X_L expression, induces apoptosis, and inhibits growth of different tumor types.
	-Doxorubicin loaded into multivalent aptamer-siRNA conjugates containing Bcl2-specific siRNA activated caspase-3/7 and decreased cell viability.
	· HDAC inhibitors
	-Sodium butyratedowregulatesBcl-X_L protein in mesothelioma cells. It stimulates hepatocellular carcinoma cells into their normal phenotype and upregulate of Bcl-2 and Mcl-1/EAT
	-Depsipeptidedecreases the expression of Bcl-2, Bcl-X_L, and Mcl-1 in multiple myeloma cells and activates Bim to initiate apoptosis.
	-Fenretinide down-regulates Bcl-2, Bcl-X_L, and Mcl-1 in leukemia
	-Flavopiridol reduces Mcl-1 levels in lung cancer cells.
IAPs	· XIAP and cIAPs
	-Birinapant (TL32711), a second-generation bivalent antagonist of both XIAP and cIAP1. Birinapant stabilized the cIAP1-BUCR (BIR3-UBA-CARD-RING) dimer and promoted auto-ubiquitylation of cIAP1 in vitro. Improved analogs were also obtained from the development of bivalent SMAC mimics that targeted more than one BIR domain region on XIAP, increasing the rate of apoptosis.
	-Cyclo-peptidic SMAC mimetics, 2 and 3 are endogenous second mitochondria-derived activators of caspase/direct IAP binding protein (SMAC/DIABLO) protein. Endogenous SMAC/DIABLO binding to BIR domain of XIAP, competitively preventing its binding with effector caspases-9, 3 and 7, thus blocking their inhibition resulting in active caspases.
	-The non-peptidic SM-164, an IAP inhibitor strongly enhances TRAIL activity by concomitantly targeting XIAP and cIAP1.
	· Survivin
	-Ardisianone, a natural benzoquinone that demonstrated a time-dependent degradation of Survivin in human refractory prostate cancer cell lines. This molecule hinders cell proliferation and induces both caspase-dependent and caspase-independent apoptosis via down-regulation of Bcl-2 proteins.
	-YM-155 suppresses transactivation and expression of Survivin. It effective in in vivo models of prostate, pancreatic, and lung cancer.
	-GDP366 potently and selectively inhibited the expression of Survivin–FL1188 Survivin inhibitor has superior antitumor efficacy in human tumor xenograft models versus to standard anti-cancer drugs.
	-Survivin derived peptides prime CTLs in vivo in murine model of melanoma.
	-Survivin specific CTLs that can lyse Human Leucocyte Antigens (HLA) matched tumor target cells have been observed in cancer patients.
	- Dendritic cell vaccines, DNA vaccines peptide vaccines or VLP based vaccine for Survivin have also been assessed in preclinical or clinical investigations.

	-SiRNA approach targeting Survivin diminished radio resistance in pancreatic cancer cells and non-small cell lung cancer cells.
	-siRNA aimed at blocking Survivin-hsp90 connection have also shown anti-cancer effects in androgen independent prostate cancer models.
	-Disrupting BRIC5 expression with lentiviral CRISPR/Cas9 nickase vector significantly reduced cell growth and invasion and induced cell apoptosis in ovarian cancer cells.
Caspases	-Justicidin A stimulates apoptosis in hepatocellular carcinoma through activation of caspase-8,
	-Saussurealappa herbal medicine suggested for anticancer effects in neuroblastoma,lung cancer,hepatocellular carcinoma gastric cancer,and prostate cancer. It suppresses the expression of pro-caspases-8/9/3 and induce apoptosis in prostate cancer cells.
	-Combined natural medicine berberine and evodiamine have synergetic effects against human breast cancer MCF-7 cells in vitro and in vivo induced apoptosis and activate of caspase-7, and caspase-9.
	-Synthetic drugs carbamate and indolone compounds promote Apaf-1 oligomerization and apoptosomeformation with activation of caspase-3 and -9.
	-Biotinylated platinum(IV) complexes (1-3) exhibited effective cytotoxicity against the tested cancer cell lines. Complex 1 activated caspase-3.
	-PETCM speed up apoptosome formation by interacting with the inhibitor prothymosin-α.
	-Caspase-1 and caspase- 3 replication-deficient adenoviral vectors transduced into prostate tumors decreased tumor growth.
	-Prostate-specific promoter, ARR_2PB, drives the expression of inducible caspase 9 in $ADV.ARR_2PB$-iCasp9 adenoviral vector and the expression of inducible caspase-9 yielded in substantial apoptosis in prostate cancer xenografts.
	- 5-aza-2′ deoxycytidine (decitabine) increases caspase-8 promoter availability, allowing for the binding of SP1 and ETS-like transcription factors. The decitabine-mediated restoration of caspase-8 occurs through genome-wide demethylation which up-regulates the expression of transactivators in brain cancer.
	-Suitable acetylation and transcriptional availability of the caspase-3 promoter, histone deacetylase blockers activated apoptosis and elevated sensitization to TRAIL-, radiation- and chemotherapeutic-stimulated apoptosis in prostate, lung, Ewing's sarcoma and medulloblastoma.
	-HDAC8 knockdown induced apoptotic cell death through caspases activation in oral squamous cell carcinoma.

and development. These investigators found a co-downregulation of both capase-8 and -10 in a cDNA array hybridization and postulated that it may contribute to the pathogenesis of choriocarcinomas [63].

Collectively, these data show that abnormal changes in the extrinsic and intrinsic signaling pathway proteins promote apoptosis dysfunction and incidence of cancer.

The use of the extrinsic and the intrinsic pathways for cancer therapeutics

The recognition that aberrant apoptosis was a major clinical hurdle to overcome in the treatment of cancer led to the creation of a variety of strategies aimed at exploiting the extrinsic and the intrinsic pathways in cancer therapeutics as discussed in the subsequent sections and in Table 3.

Agents targeting the extrinsic pathways

Death receptors and their ligands specifically, Fas/FasL and Apo2L/TRAIL have extensive anti-tumor activity and subsequently have been pursued as potential targets for cancer therapy [64]. The main agents' characteristics are summarized below.

Agents against Fas/FasL: Several synthetic drugs have been created aiming at increasing Fas pathway cancer therapeutic molecules. One promising synthetic agent is the ABT-510 that mimics the anti-angiogenic activity of the endogenous protein thrombospondin-1 (TSP1, Abbott Laboratories) and competes with it for endothelial cell binding acting as an angiogenesis inhibitor with 1000-fold greater antiangiogenic activity. Similarly to TSP-1, ABT-510 cause's endothelial cell apoptosis via activation of pathways, including Fas/FasL ultimately causing apoptosis [65,66]. Data from a Phase II clinical trial in patients with advanced renal cell carcinoma indicated that ABT-510 slightly improved the toxicity profile of other treatments, but exhibited insufficient clinical activity as a single agent. Therefore, ABT-510 was recommended for the use in combined therapies. Some other drugs that upregulate Fas include doxorubicin in tumor cells [67], 5-fluorouracil (5-FU) in melanoma [68] and colon cancer cells [69], phosphaplatinsin breast cancer cells [70] and ovarian cancer cells [71,72] and cisplatin-resistant cancers [73]. Besides these chemotherapeutic drugs, two monoclonal antibodies, anti-APO-1 and anti-Fasinduceapoptosis in different types of cancer cells by binding to a receptor on the surface of the cells they kill [74]. Other drugs such as HDIs not only show selective cytotoxicity against cancer cells, they also enhance the cytotoxic effects of radiation and synergistically stimulate the effects of chemotherapy [75]. HDIs act through the upregulation ofthe expression of Fas and FasL, cytochrome c release and activation of caspase-9 and caspase-3 [76, 77]. Apicidin is anexample of HDI agent that stimulates apoptosis through Fas/FasL expression in human acute promyelocytic leukemia cells [77].

Agents against TRAIL: TRAIL-induced apoptosis is an attractive way to combat cancer becauseTRAIL takes advantage of the patient's immune cells [17] whileit canminimize cytotoxicity to normal, healthy tissue [17]. In fact, exogenous TRAIL was shown to selectively destroy tumor cells without negatively affecting normal cells, making TRAIL and TRAIL-receptor agonists attractive anticancer therapeutics [78,79]. Notably, TRAIL ismore efficient in the induction of tumor cell death in combined therapies [80]. For instance, TRAIL acts in synergy with the chemotherapeutic drug 5-Fluorouracil to yield increased apoptosis in mutant V-Ki-ras2 Kirsten rat sarcoma (KRAS) non-small cell lung carcinoma cells [81]. TRAIL suppresses the growth of colon and breast xenografts without the side effects observed with TNF and FasL [78] with synergistic antitumor effects observed when TRAIL was combined with chemotherapy or radiation [82,83]. Similarly, TRAIL tested in combination with agents such as doxorubicin resulted in significant inhibition of tumor growth in a prostate cancer *in vivo* model [84]. Multiple clinically relevant agents upregulate the expression of TRAIL death receptors and cooperate with TRAIL as well as DR4 and DR5-specific agonistic antibodies to exhibit tumor cell death [85]. HGS-ETR1 was the first fully human agonistic TRAIL-R1 monoclonal antibody [86] developed as a specific and potent antitumor agent with favorable pharmacokinetic characteristics. This antibody has the potential to provide therapeutic benefit for a broad range of human malignancies. TRAIL-R2-specific antibodies and recombinant TRAIL can synergize to kill cancer cells as well [87]. It is important to note that the development of TRAIL as

an anticancer drug suffered some setback when Jo et al. [88] reported that human primary hepatocytes were also sensitive to TRAIL-induced apoptosis. Studying *in vitro* normal hepatocytes derived from 20 individuals, the authors observed that in striking contrast with mice and nonhuman primates, >60% of the human hepatocytes underwent apoptosis after exposure to TRAIL [88]. Furthermore, TRAIL was found to induce apoptosis in a human brain cells *in vitro* but in preclinical studies TRAIL failed to induce apoptosis in the brain of laboratory animals [82,83].

Altogether, the promising findings on TRAIL *in vitro* and preclinical studies on animals are limited in their ability to predict toxicity of TRAIL agents in humans. More detailed investigations assessing the different TRAIL drug preparations as a potential cause of toxicity in humans are therefore still required.

Agents targeting the intrinsic pathways.

Inhibitors of the Bcl-2 family

Following the molecular cloning of Bcl-2 by Korsmeyer et al. [89] atonable progress in identifying Bcl-2 family members as targets for cancer drug development has been made with several categories of Bcl-2 family inhibitors created as described in the subsequent sections.

BH3 mimetics: The polyphenol gossypol is a BH3 mimetic derived from the cotton seed plant. It was the first natural compound, which inhibited anti-apoptotic Bcl-2, Bcl-xL and Mcl-1 proteins [90]. In preclinical studies, gossypolhas shown potent pro-apoptotic activity [91]. Unfortunately, the results of a Phase II clinical trial using levo gossypol (AT-101, Ascenta),an oral inhibitor of the anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-XL, Bcl-W, and Mcl-1) and an inducer of the pro-apoptotic proteins NOXA and PUMA tested in patients with recurrent chemosensitive extensive-stage small cell lung cancer were unsatisfactory [92]. A gossypol analog, apogossypol (Burnham Institute) seems to better target Bcl-2 and Mcl-1. It also displays single-agent activity in Bcl-2–transgenic mice and has superior efficacy with less toxicity compared with gossypol, implying that further development of this agent for cancer therapy may be reasonable [93].

Several synthetic BH3 mimetics such as antagonist ABT-737 [94] exhibits cytotoxicity in lymphoma, small cell lung carcinoma cell line and primary patient-derived cells, and caused regression of established tumors with a high percentage of cure in mice [94] ABT-737 blocks Bcl-2, Bcl-XL, and Bcl-W proteins andbinds the anti-apoptotic Bcl-2 family with affinities in the nanomolar magnitude (Ki ≤ 1 nmol/L for Bcl-2, Bcl-XL, and Bcl-w; Ki ≅ 0.46 nmol/L for Bcl-B, Mcl-1, Bfl1/A-1). As ABT-737 does not block the pro-survival proteins e.g. Mcl-1, Bcl-B, Bfl-1 (A1); tumors that over express these Bcl-2 family proteins are likely to be resistant to ABT-737.Another Bcl-2 inhibitor, GX15-070 (obatoclax, Gemin X) is a small molecule indole bipyrrole compound that blocks most anti-apoptotic Bcl-2 family of proteins. Compared with ABT-737, GX15-070 has a relatively low affinity for Bcl-2, Bcl-XL, Bcl-w, and Mcl-1 (Ki =220 nmol/L for Bcl-2 and ~0.5 μmol/L for Bcl-XL, Bcl-w, and Mcl-1[95]. *In vitro* experiments revealed that GX15-070 produce cooperative growth-inhibitory effects in chronic lymphocytic leukemia cells when combined with PI3-kinase/mTOR-inhibitor BEZ235 [96]. An additional interesting compound is the A-1210477, a potent and selective Mcl-1 inhibitor with Ki and IC50 of 0.454 nM and 26.2 nM, respectively, >100-fold selectivity over other Bcl-2 family members. A-1210477 induces the hallmarks of intrinsic apoptosis and demonstrates single agent killing of multiple myeloma and non-small cell lung cancer cell lines. Owing to their potent anti-tumorigenic activity many BH3 mimetics such as ABT-737, ABT-263, obatoclax, and A-1210477 have been developed and entered in clinical trials [97].

In silico screening for compounds that bound the hydrophobic groove of Bcl-2 identified the HA14-1 (Ethyl 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate) resulted in a small organic compound, [98] which inhibits the binding of Bcl-2 and Bcl-XL to Bax and Bak [99] and induce apoptosis in a wide variety of cancer cells, including glioma cells [99] and colon cancer cells [100]. Notably, the binding affinity of this compound for Bcl-2 is quite high and is significantly higher than affinities of other inhibitors for Bcl-2 [98].

Silencing the Bcl2 anti-apoptotic proteins/genes: Anti-apoptotic Bcl-2 specific siRNA inhibits the expression of target gene *in vitro* and *in vivo* with anti-proliferative and pro-apoptotic effects [101]. The first drug for the Bcl-2 family members that pharmacologically silencinggenes coding for Bcl-2 family memberswasG3139 (Oblimersen sodium, Genasense, Genta Inc., Berkeley Heights, NJ) [102]. This antisense oligonucleotide was designed to specifically bind to the first 6 codons of the human Bcl-2 mRNA sequence to decrease in Bcl-2 protein translation [102]. Unfortunately, this agent did not obtain US Food and Drug Administration approvalsince it did produce survival differences in a melanoma trial [103]. Wu et al. [104] demonstrated that silencing proto-oncogene Bmi-1 in MCF breast cancer cells downregulated the expression of Bcl-2, rendering these cells more sensitive to doxorubicin as evidenced by an increase in apoptotic cells *in vitro* and *in vivo*. Because Bcl-XL shares high sequence homology regions with Bcl-2, they can be co-expressed in many tumors, but they exhibit different biological roles. Hence, antisense Bcl-XLolignucleotide and bispecific antisense bcl-2/bcl-xL oligonucleotide, targeting the mRNA homology region of both bcl-2 and bcl-xL, simultaneously down regulates Bcl-2 and Bcl-XL expression, induces apoptosis, and inhibits growth of different tumor types *in vitro* and *in vivo* [105]. Doxorubicin loaded into multivalent aptamer-siRNA conjugates containing Bcl2-specific siRNA exert promising anticancer effects by activating caspase-3/7 and decrease of cell viability, on multidrug-resistant cancer cells due to their high intracellular uptake efficiency [106].

HDAC inhibitors: Several HDIs including sodium butyrate, depsipetide, fenretinide and flavipirodol can target Bcl2 family in cancer cells. Sodium butyratedowregulates Bcl-XL protein levels by decreasing its RNA expression in mesothelioma cells [107]. This agent can stimulate hepatocellular carcinoma cells into their normal phenotype with an upregulation of anti-apoptotic Bcl-2 and Mcl-1/EAT as well [108]. Similarly, Bak is upregulated by butyrate through increased binding of Sp3 [109]. The depsipeptide is a peptide, which decreases the expression of Bcl-2, Bcl-XL, and Mcl-1 in multiple myeloma cells [110] and activates Bim to initiate apoptosis by Acetylation of FoxO1 [111]. The fenretinide, a synthetic cytotoxic retinoid, down-regulates Bcl-2, Bcl-XL, and Mcl-1 in leukemia cells [112,113]. The flavopiridol, a cyclin-dependent kinase inhibitor, reduces Mcl-1 levels in lung cancer cells [114]. The specificity for the anti-apoptotic Bcl-2 family members for fenretinide, or flavopiridol is lower than for agents that are designed to directly target anti-apoptotic Bcl-2 family members and the ability of such agents to modulate drug resistance mechanisms via the Bcl-2 family proteins

offers opportunities for perhaps synergistic drug.

Collectively, the Bcl-2 family of proteins plays an important role in the response of cancers to both classic chemotherapies and targeted therapies.

Targeting the IAPs

Therapies to target IAPs in cancer has gained substantial scientific interest nowadays. Various drugs that have been developed against IAPs are discussed below.

XIAP and cIAPs: The discovery of SMAC mimetics or IAP antagonists was unveiled following the elucidation of the crystal structure of the interaction between SMAC and IAPs1, 2. SMAC binding to IAPs is facilitated by the interaction of its 4 N-terminal residues (alanine–valine–phenylalanine–isoleucine) with BIR domains on XIAP [115]. Monomeric class of SMAC inhibitors exhibit strong binding affinities with XIAP and cIAP1/2 at nanomolar ranges [116]. These include Birinapant (TL32711), a second-generation bivalent antagonist of both XIAP and cIAP1 with Kd values of 45 nM and <1 nM, respectively (Kd is the equilibrium constant involved in the dissociation of a compound into two or more compounds; the lower the Kd value the higher the affinity of the compound with the IAPs) [117]. A range of assays that evaluated cIAP1 stability and oligomeric state demonstrated that Birinapant stabilized the cIAP1-BUCR (BIR3-UBA-CARD-RING) dimer and promoted auto-ubiquitylation of cIAP1 *in vitro*. This enhanced tolerability has allowed Birinapant to proceed into clinical studies [118]. Improved analogs were also obtained from the development of bivalent SMAC mimics that targeted more than one BIR domain region on XIAP, increasing the rate of cell death by apoptosis [119]. Other IAP antagonists consist of peptidic and non-peptidic small molecules. Two cyclo-peptidic SMAC mimetics, 2 and 3 are endogenous second mitochondria-derived activators of caspase/direct IAP binding protein (SMAC/DIABLO) protein [23,120]. Endogenous SMAC/DIABLO exerts its inhibitory effect on IAPs by binding to BIR domain of XIAP, [115] competitively preventing its binding with effector caspases-9, 3 and 7, resulting in active caspases. The non-peptide SM-164, an IAP inhibitor strongly enhances TRAIL activity by concomitantly targeting XIAP and cIAP1 [121].

The finding that many cancer cell lines and tumors have increased IAP gene copy number, upregulated IAP protein levels, or translocation of specific IAP genes reinforced the premise that deregulated IAP activity might result in tumor initiation, progression, and/or resistance to anticancer treatment through aberrant caspase inhibition [122, 123]. Consistent with this hypothesis, selective elimination of XIAP by genetic depletion or Silencing RNA (SiRNA) treatment would make tumor cells sensitive to a variety of conventional chemotherapeutic drugs. Indeed, using antisense strategies and siRNA XIAP molecules resulted in an improved *in vivo* tumor control by radiotherapy [124]. Ohnishi et al. [125] reported that siRNA targeting of XIAP increased radiation sensitivity of human cancer cells. Also, when used together with anticancer drugs, XIAP antisense oligonucleotide exhibit enhanced chemotherapeutic activity in lung cancer cells *in vitro* and *in vivo* [124].Yamaguchi et al. [126] showed that targeting XIAP by siRNAs sensitize hepatoma cells to death receptor- and chemotherapeutic agent-induced cell death.

Survivin: Survivin protein is encoded by BIRC5 gene. Various strategies have been envisioned to block the expression or the function of Survivin in tumor cells. Small inhibitors targeting the function of Survivin include Ardisianone, a natural benzoquinone that demonstrated a time-dependent degradation of Survivin in human refractory PC-3 and DU-145prostate cancer cell lines [127]. This molecule hinders cell proliferation and induces both caspase-dependent and caspase-independent apoptosis via down-regulation of Bcl-2 proteins that produces reactive oxygen species, and disturbs the mitochondrial membrane potential [127]. YM-155 suppresses transactivation of Survivin through direct binding to its promoter [128] and selectively preventing its expression. Survivin has also been shown to be effective *in vivo* models of prostate, pancreatic, and lung cancer [128-130]. GDP366 potently and selectively inhibited the expression of Survivin [131]. Another small molecule Survivin inhibitor FL118 exhibited superior antitumor efficacy in human tumor xenografts models in comparison to standard anti-cancer drugs [131].

Various strategies for therapeutic cancer vaccines have revealed good immunogenicity in clinical trials, still clinical translation of antigen specific cancer vaccines has not been as successful as passive immunotherapy [132]. The rationale for cancer vaccine is to focus a specific tumor antigen as vaccine candidate, create robust antigen presentation mostly through Dendritic Cells (DC), and induce Cytotoxic T Cell (CTL) response. Tumor antigens are made into peptides, inserted onto Major Histocompatibility Complex (MHC) molecules. The MHC-peptide complex is then presented by Antigen Presenting Cells (APCs) to T cells for stimulation. Survivin protein promoted CTL response *in vitro* when presented by DC [133]. Survivin derived peptides prime CTLs *in vivo* in murine model of melanoma [134]. Survivin-specific CTLs that can lyse Human Leucocyte Antigens (HLA) matched tumor target cells have been observed in cancer patients [135]. Various vaccine approaches including DC vaccines, DNA vaccines [136], peptide vaccines or VLP based vaccine for Survivin have also been assessed in preclinical or clinical investigations. These immunotherapeutic approaches targeting Survivin are detailed in Garg et al. [132].

Nucleic acid based strategies, which interfere with Survivin gene expression using antisense Survivin transfected into malignant melanoma cells resulted in spontaneous cell apoptosis [137] and sensitized the cells to chemotherapy [138]. siRNA approach targeting Survivin diminished radio resistance in pancreatic cancer cells [139] and non-small cell lung cancer cells [140]. Moreover, it inhibited proliferation and induced apoptosis in human lung adenocarcinoma [141] and ovarian cancer cells [142]. siRNA aimed at blocking Survivin-Hsp90 connection have also shown anti-cancer effects in androgen independent prostate cancer models [143]. Disrupting BRIC5 expression using the lentiviral CRISPR/Cas9 nickase vector significantly reduced cell growth and invasion and induced cell apoptosis in ovarian cancer cells [144].

In sum, various therapeutics strategies targeting of Survivin have entered a number of clinical trials, including vaccination, inhibitors of transcription and immunotherapy reflect on the importance of Survivin in cancer therapy [145,146]. Targeting common proteins of the extrinsic and intrinsic pathways.

Caspase targeting compounds: The natural drug Justicidin A is derived from the plant Justicia procumbens, a traditional herbal remedy for the treatment of cancer [147]. Justicidin A stimulates apoptosis in hepatocellular carcinoma via the activation of both intrinsic and extrinsic apoptosis pathways [147]. Through activation of caspase-8, Justicidin A increases intracellular Bid leading to the

release of cytochrome c; but decreases Bcl-XL. Mitochondrial release of Smac/DIABLO activates caspases-9 and -3 to induce the intrinsic pathway. Saussurea lappa is another traditional herbal medicine suggested for anticancer effects in neuroblastoma [148], lung cancer, [149] hepatocellular carcinoma [150], gastric cancer [151], and prostate cancer [152]. Additionally, Saussurealappa ethanol extract suppresses the expression of pro-caspases-8/9/3 and induce apoptosis in prostate cancer cell [153]. The synergistic effects of combined natural medicine Berberine and Evodiamine against human breast cancer MCF-7 cells *in vitro* and *in vivo* induced apoptosis paralleled by increased expression levels of Bax, reduced expression levels of Bcl-2, and activation of caspase-7, and caspase-9 [154].

Synthetic drugs associated with caspase activation include carbamate and indolone compounds [154]. They promote Apaf-1 oligomerization and apoptosome formation with the ensuing activation of caspase-3 and -9 [155]. Biotinylated platinum (IV) complexes (1-3) exhibited effective cytotoxicity against the tested cancer cell lines, especially complex 1, which was 2.0-9.6 -fold more potent than cisplatin. The complex 1 activates the expression of Bax, and induced cytochrome c release from the mitochondria, and finally activated caspase-3 [156]. Other potential drugs for selective induction of apoptosis were detected using high-throughput screening of the compounds activating caspase-3 as a vital suicide caspase. Among them PETCM [α-(trichloromethyl)-4-pyridine ethanol [157] uses various pathways; e.g., PETCM speed up apoptosome formation by interacting with the inhibitor prothymosin-α [157].

Caspase-based gene therapy has also been used in several studies. Caspase-1 and caspase- 3 replication-deficient adenoviral vectors transducer into prostate tumors showed focal substantial apoptosis free of cytotoxic damage to surrounding tissue and decrease tumor growth [158]. Xie et al. [159] used a prostate-specific promoter, ARR2PB, to drive the expression of inducible caspase 9 in ADV. ARR2PB-iCasp9 adenoviral vector. This promoter has two androgen response regions located upstream of the rat probasinpromoter. High levels of intra-prostatic androgens drive the expression of inducible caspase-9 yielded in substantial apoptosis in prostate cancer xenografts [159].

Functional loss of caspase-8 by hypermethylationhas been associated with therapeutic resistance of the death-receptor ligands TNFα, TRAIL and Fas [160]. Hence, it was hypothesized that targeting caspase-8 expression may lead to a significant therapeutic effect, especially in tumors experiencing a gene dosage effect or hypermethylation of caspase-8 promoters. In this vein, 5-aza-2′ deoxycytidine (decitabine), a cytosine nucleoside analog, which supports demethylation by preventing DNA methyltransferase covalent binding, increases caspase-8 promoter availability, allowing for the binding of SP1 and ETS-like transcription factors [161]. The decitabine-mediated restoration of caspase-8 occurs through genome-wide demethylation which up-regulates the expression of transactivators [162] in brain cancer [163]. Suitable Acetylation and transcriptional availability of the caspase-3 promoter, histone deacetylase blockers activate apoptosis and elevate sensitization to TRAIL-, radiation- and chemotherapeutic-stimulated apoptosis in prostate, lung, Ewing's sarcoma and medulloblastoma [164-166]. Additionally, HDAC8 knockdown induced apoptotic cell death through caspases activation in Oral Squamous Cell Carcinoma (OSCC) [167] could become a novel therapeutic strategy for OSCC. These data indicate that HDIs may provide an effective mean for the therapeutic manipulation of caspases as well.

Conclusion

The extrinsic and the intrinsic apoptotic signaling pathwaysare pivotal for the normal function of apoptosis in humans. More importantly, they have a crucial role in the development and the progression of carcinogenesis. Understanding of the molecular knowledge acquired on these apoptotic signaling pathways in tumors, resulted in designed anticancer strategies and novel therapeutic avenues as highlighted in this review. Some of these discoveries are in preclinical while others have already entered clinical trials. A search at http://www.clinicaltrials. gov (a registry and results database of federally and privately supported clinical trials conducted in the United States and around the world) returns a wealth of information. Some of the new agents or treatment strategies have been integrated into combination therapy associated with conventional anticancer drugs in several clinical trials to enhance currently available treatment modalities. Whether these treatment strategies can induce resistance in diverse tumorsin the long run, and if they will kill normal cells in overwhelming numbers require immediate further investigations.

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