REVIEW

Molecular Mechanisms of Apoptosis and Roles in Cancer Development and Treatment

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Abstract

   Programmed cell death (PCD) or apoptosis is a mechanism which is crucial for all multicellular organisms to control cell proliferation and maintain tissue homeostasis as well as eliminate harmful or unnecessary cells from an organism. Defects in the physiological mechanisms of apoptosis may contribute to different human diseases like cancer. Identification of the mechanisms of apoptosis and its effector proteins as well as the genes responsible for apoptosis has provided a new opportunity to discover and develop novel agents that can increase the sensitivity of cancer cells to undergo apoptosis or reset their apoptotic threshold. These novel targeted therapies include those targeting anti-apoptotic Bcl-2 family members, p53, the extrinsic pathway, FLICE-inhibitory protein (c-FLIP), inhibitor of apoptosis (IAP) proteins, and the caspases. In recent years a number of these novel agents have been assessed in preclinical and clinical trials. In this review, we introduce some of the key regulatory molecules that control the apoptotic pathways, extrinsic and intrinsic death receptors, discuss how defects in apoptotic pathways contribute to cancer, and list several agents being developed to target apoptosis.

Keywords: Apoptosis - apoptotic pathways - cancer - treatment target

Background

   The term 'apoptosis' has long been utilized as a synonym for programmed cell death (PCD) since the seminal article of Kerr et al., published in 1972 (Kerr et al., 1972). Apoptosis, or type 1 cell death, is a significant terminal pathway for cells of multicellular organisms, and causes a diversity of biological events such as proliferation/homeostasis, differentiation, development and elimination of harmful cells as a protective strategy to remove infected cells (Elmore, 2007). Apoptosis is fundamentally distinct from other types of cell death based on its incidence, morphology, and biochemistry. Particular morphological alterations of apoptotic cells include nuclear condensation and fragmentation, cell shrinkage, dynamic membrane blebbing, and loss of adhesion to extracellular matrices or to neighbors. Biochemical alterations consist of chromosomal DNA cleavage into internucleosomal fragments, phosphatidylserine externalization, and activation of a family of proteases recognized as the caspases (Ouyang et al., 2012). A minor dysfunction of PCD results in various pathological disorders in humans such as various cancers (Sun and Peng, 2009). Apoptosis is a cellular procedure that is regulated by different groups of executioner and regulatory molecules (Mukhopadhyay et al., 2014) and their aberrant function is fundamental to the growth of tumors and the development of anticancer

drug resistance. Therefore, apoptosis has become one of the prime molecular targets for drug discovery and development, particularly for diseases like cancer.

Mechanisms of Apoptosis

   Apoptosis is recognized as the most important form of cell death and its molecular signaling pathway is well known. Identification of apoptotic mechanisms is critical and facilitates the understanding of the pathogenesis of diseases as a result of dysfunctional apoptosis. This, in turn, may assist in developing new drugs that target specific apoptotic pathways or genes. In mammals, there are two central apoptotic pathways: the extrinsic pathway (death receptor mediated pathway) and the intrinsic pathway (mitochondrial mediated pathway) (Figure 1) (Hassen et al., 2012). Moreover, besides these two pathways, there are additional pathways of caspase activation that are less well known, including an initiator role of caspase-12 or caspase-2 in apoptosis activated by endoplasmic reticulum stress (ER) (Szegezdi et al., 2006). The perforin/granzyme pathway is another pathway that involves T-cell mediated cytotoxicity. This pathway can induce apoptosis via either granzyme A or granzyme B. All these apoptotic pathways (extrinsic, intrinsic, and granzyme B pathways) converge on the same terminal or execution pathway (Jin and El-Deiry, 2005). The key regulatory proteins in both

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intrinsic and extrinsic pathways are the caspases (Li and Yuan, 2008). Three groups of mammalian caspases exist on the basis of specific functions in different pathways, including developmental, inflammatory, and apoptotic pathways (Weyhenmeyer et al., 2012). Thus, some caspases, such as caspase-1, do not have a role in apoptosis execution, while several caspases have dual roles both in nonapoptotic and apoptotic signaling. In addition, caspases are classified as initiator (caspase-8 and -9) and effector or executioner caspases (caspase-3, -6 and -7), based on their position in apoptotic signaling cascades (Hu et al., 2013). The initiator caspases, which are activated by autocleavage, sequentially cleave and activate the downstream "executioner" caspases. Subsequently, the executioner caspases cleave essential cellular components to orchestrate the proteolytic dismantling of the cell (Green and Evan, 2002).

The extrinsic death receptor pathway

Apoptotic signaling through the extrinsic pathway begins with the attachment of extracellular ligands, for example, tumor necrosis factor (TNF), Fas ligand (Fas-L), and TNF-related apoptosis-inducing ligand (TRAIL) to the extracellular domain of transmembrane receptors [death receptors, i.e. the type 1 TNF receptor (TNFR1), Fas (also called CD95/Apo-1), TRAIL receptors] (Jin and El-Deiry, 2005; Guicciardi and Gores, 2009). Upon binding of ligand to receptor, the death receptors bind via their intracellular death domain with a corresponding protein motif in adapter proteins such as Fas-associated death domain (FADD) and TNF receptor-associated death domain (TRADD). These adaptor proteins also have another protein interaction domain, called the Death Effector Domain (DED). Pro-caspase-8 also contains DED, which interacts with the DED of FADD (Khosravi-Far and Esposti, 2004).At this time, a death-inducing signaling complex (DISC) is developed, leading to auto-catalytic activation of procaspase-8 (Boatright et al., 2003). Active caspase-8 activates effector/executioner caspases, which cause cell death by damage or destruction of the nucleus and other intracellular structures (Jin and El-Deiry, 2005).

The intrinsic mitochondrial apoptosis pathway

A number of internal stimuli cause an increase in mitochondrial membrane permeability (Kroemer et al., 1998). These different stressors are recognized by several intracellular proteins that send the signal to the mitochondria, ending in Mitochondrial Outer Membrane Permeabilization (MOMP) (Chipuk et al., 2006). MOMP is most commonly mediated via a variety of protein-membrane and protein-protein interactions of the B-cell lymphoma 2 protein (BCL-2) family (Chipuk et al., 2004). The Bcl-2 family of proteins comprise 25 pro- and anti-apoptotic members; cell health relies on the balance among these pro-apoptotic and anti-apoptotic Bcl-2 proteins. Bcl-2-family members contain one or more Bcl-2 homology (BH) domains, those that have four BCL-2 homology domains BCL-2, MCL-1, A1/Bfl-1, Bcl-B/Bcl2L10, and BCL-xL (BCL extralarge) are anti-apoptotic, which block apoptosis by inhibiting their pro-apoptotic counterparts via protein-protein interactions (Czabotar et al., 2014). The pro-apoptotic family members are classified into two further subgroups, according to whether they have multiple BH domains (effector proteins) including: BAX, BAK, and BOK (BCL-2 related ovarian killer) or whether they contain only the BH3 domain, to include BID, BIM, PUMA, NOXA, BIK, BAD, HRK, and BMF (Chipuk et al., 2006; Czabotar et al., 2014).

Following apoptotic stimuli, the BH3-only proteins (BAX and BAK) activate and insert into the outer mitochondrial membrane to cause the release of cytochrome c and other mitochondrial proteins, including apoptosis-inducing factor (AIF) and endonuclease G, Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low PI) and the serine protease Omi/HtrA2 (high-temperature requirement protein A2) (Acehan et al., 2002; Chipuk et al., 2006). Subsequently, in the cytosol, cytochrome c interacts with apoptosis protease-activating factor 1 (Apaf-1), and forms a complex recognized as the apoptosome (Zou et al., 1997). The apoptosome, a multi-protein platform comprising a seven-spoke ring-shaped complex (Yuan and Akey, 2013), leads to activation of initiator caspase (usually caspase-9), which in turn activates executioner caspase-3 and initiates a caspase cascade, which eventually leads to demolition of the cell (Jin and El-Deiry, 2005). AIF and endonuclease G can both promote caspase-independent cell death through inducing chromatin condensation and cleavage of nuclear DNA (Li et al., 2001; Sevrioukova, 2011). Besides the fundamental role of AIF in the execution of caspase-independent cell death, AIF has emerged as an important protein for cell survival (Sevrioukova, 2011). Other mitochondrial proteins that released in the cytosol are Smac/DIABLO and Omi/HtrA2, which provides an additional mechanism for caspase activation. Smac/DIABLO and Omi/HtrA2, interact and antagonize Inhibitor of Apoptosis (IAP) proteins and promote caspase activation (Gustafsson and Gottlieb, 2008; Vande Walle et al., 2008).

Role of Apoptosis in Cancer

Appropriate apoptotic signaling is fundamentally important to preserving a healthy balance between cell death and cell survival and in maintaining genome integrity, as highlighted by the establishment of the evasion of apoptosis as a prominent hallmark of cancer (Plati et al., 2011). Apoptotic pathways can be altered by cancer cells transcriptionally, translationally, and post translationally. Significantly, these mechanisms are not exclusive and cancer cells can use a number of diverse strategies to evade apoptosis (Fulda, 2010). Several proteins have been described that apply pro- or anti-apoptotic activity in the cell. The ratio of these pro-and anti-apoptotic proteins plays an important role in the regulation of cell death, and disruption in the balance of pro- and anti-apoptotic proteins has been established to contribute to carcinogenesis by reducing apoptosis in malignant cells. For example, the disequilibrium between pro- and anti-apoptotic BCL-2 proteins can promote cancer cell survival (Kang and Reynolds, 2009). This

can be due to an under-expression or over-expression of the pro-apoptotic proteins and anti-apoptotic proteins, respectively, or a combination of both. In a wide variety of human cancers, both genetic and epigenetic alterations of several pro-apoptotic members of the Bcl-2 family have been reported (Galluzzi et al., 2010). Besides inactivation of pro-apoptotic BCL-2 family proteins, the presence of elevated levels of anti-apoptotic family members is an obvious mechanism of apoptosis dysregulation in cancer (Fulda, 2009). The anti-apoptotic proteins are widely over-expressed in cancer cells to overcome stress signals. Over-expression of anti-apoptotic BCL-2 family proteins is often correlated with recurrence, poor prognosis, and resistance to cancer therapeutics (Wuilleme-Toumi et al., 2005).

In addition to genetic and epigenetic alterations, there is evidence that dysregulation of MicroRNAs (miRNAs) are associated with different human cancers and miRNAs can function as oncogenes and tumor suppressors (Croce, 2009). MiRNAs are a class of non-coding RNAs that regulate post-transcriptional gene expressions and silence target mRNAs. MiRNAs have been established to act as anti-apoptotic or pro-apoptotic regulators by targeting different mRNAs involved in the apoptotic pathways, especially BCL-2 family proteins (Garofalo et al., 2010; Chen et al., 2014). For example, miR-15/16 targets the anti-apoptotic factor (Bcl-2) and the loss or reduced expression of the tumor suppressor of miR-15/16 may elucidate the elevation of Bcl-2 level in some tumors (Cimmino et al., 2005; Allegra et al., 2014). Furthermore, pro-apoptotic miR-34 family members and MiR-125b down-regulate anti-apoptotic proteins and lead to decreased cellular proliferation and increased apoptosis. On the other hand, some miRNAs have oncogenic functions, including miRNA-21, the miRNA-17/92 cluster, miRNA-272/273 and miRNA-221/222, which negatively control apoptotic activity to enhance the rate of cancer cell proliferation and contribute to drug resistance (Garofalo et al., 2008; Xiao et al., 2008; Lima et al., 2011). In different types of cancer, the most constantly up-regulated miRNA is anti-apoptotic miRNA-21. It is believed that miRNA-21 can modulate apoptosis by targeting the programmed cell death 4 gene (PCD 4 gene or PDCD4), tropomyosin 1 (TPM1), and the phosphatase and tensin homologue (PTEN) (Wang and Lee, 2009). Experimental evidence shows that the utilization of anti-microRNAs or miRNA mimics may act as a potent therapeutic strategy to inhibit key molecular pathways that are present in cancer (Garofalo and Croce, 2011). Besides miRNAs, cancer cells often show altered expression patterns of diverse long noncoding RNAs (lncRNAs) in specific kinds of tumors when compared with normal cells and tissues. Therefore, their modulation (overexpression or down-regulation according to the specific lncRNA) in cancer cells often sensitizes cells to apoptotic treatments and consequently induces apoptosis, suggesting that targeting lncRNAs to modulate apoptosis in cancer cells can be utilized for cancer treatment (Rossi and Antonangeli, 2014).

Cellular-FLICE inhibitory protein (c-FLIP) is a critical negative regulator of the apoptotic pathway mediated by the death receptors Fas, TNF-R1, DR4 and DR5 (Micheau,

2003). There are three isoforms of c-FLIP protein that are derived from diverse mRNA splice variants which are transcribed under the same promoter, namely c-FLIPL, c-FLIPS, and c-FLIPR. At high expression levels, all three isoforms function at the DISC level by inhibiting the activation of procaspase-8 and -10, due to its structural homology with the cysteine protease (Subramaniam et al., 2013). Enhanced expression of c-FLIP (long or the short isoform) has been generally found in a wide variety of cancers (Safa and Pollok, 2011). Some reports have also demonstrated that down-regulation of c-FLIP *in vitro* can restore apoptosis mediated by TRAIL and CD95L. Thus, c-FLIP can be utilized as a promising target for cancer therapy, particularly if combined with other treatments, for example, TRAIL or conventional chemotherapy (Sung et al., 2010; Shirley and Micheau, 2013). In contrast to the anti-apoptotic role of c-FLIP at high levels of expression, c-FLIPL has been demonstrated to induce apoptosis at low and more physiologically relevant expression levels by recruiting at the DISC to increase caspase-8 activation (Chang et al., 2002). In spite of the known dual function of c-FLIPL as a pro- or anti-apoptotic factor in normal tissues, c-FLIPL has usually been identified as taking action as a fundamental negative regulator of apoptosis in human cancer cells (Hassan et al., 2014). C-FLIP is a transcriptional target of a number of transcription factors. Among these transcription factors, AP-1 (c-Fos and c-Jun), CREB, SP1, and NF-kB induce c-FLIP expression. Additionally, other transcription factor binding sites have been reported that are associated with the down-regulation of c-FLIP transcription (Subramaniam et al., 2013). Activation of NF-kB by CD40 ligand or TNFα results in overexpression of c-FLIP and the prohibition of TNFR1, Fas- and TRAIL receptors induce apoptosis (Micheau et al., 2001; Travert et al., 2008). Similarly, growth factor stimulation or activation of several pathways, such as mitogen-activated protein kinase (MAPK), the phosphatidylinositol-3 kinase (PI3K)/Akt, can promote the expression of c-FLIP and inhibit apoptosis induced by death receptors (Micheau, 2003).

IAPs are a family of proteins that prevent both intrinsic and extrinsic apoptosis and increase cell survival during cellular stresses such as ER stress (Hamanaka et al., 2009) and loss of attachment from the extracellular matrix (Liu et al., 2006). Furthermore, IAP expression is induced by potent oncogenes, including E6 (Wu et al., 2010) and Ras (Liu et al., 2005); prosurvival signaling such as nuclear factor (NF)-ϰB can also induce IAPs (Chu et al., 1997). In particular, well known members of this family include XIAP (X-linked IAP), surviving (BIRC5), cIAP1 (BIRC2), cIAP2 (BIRC3), Livin/MLIAP (BIRC7), Apollon (BRUCE, BIRC6), IAP-like protein 2(BIRC8), and NAIP (BIRC1) (Hawkins et al., 2001; Eckelman et al., 2006). All IAP proteins are characterized by the presence of one to three baculovirus IAP repeat (BIR) domains that mediate protein-protein interactions (Hinds et al., 1999). Inhibition of caspase activity by these proteins occurs via interaction of IAPs conserved BIR domains with active sites of caspases, which keeps the caspases away from their substrates, promoting degradation of active caspases (Wei et al., 2008). Deregulation of IAPs may

lead to neurodegenerative disorders and cancer (Saleem et al., 2013). Direct genetic evidence has confirmed that cIAPs are proto oncogenes (Wright and Duckett, 2005), and enhanced levels of diverse members of the IAP family have been observed in many malignancies (Yang et al., 2003). Besides the oncogenic role of the IAPs, recent studies have demonstrated a tumor-suppressing role for the cIAPs in restricting NF-χB activity. A number of studies have shown that mutations or translocations of cIAPs are in charge of constitutive activation of NF-χB signaling in numerous cancers (Lau and Pratt, 2012). These studies reveal that both over-expression and loss of cIAPs can promote metastasis and tumor invasion (Lau and Pratt, 2012; Tchoghandjian et al., 2013).

Due to the importance of caspases in the initiation and execution of apoptosis, it is logical to consider that impairment in caspase function or low levels of caspases may result in a reduction of apoptosis and carcinogenesis. Down-regulation of different caspases has been observed in diverse cancers; for example, down-regulation of caspase-9 can leads to colorectal cancer and correlates with poor clinical outcomes. In addition, Devarajan et al. observed decreased levels of caspase 3 in ovarian, breast, and cervical cancers and it is established that restoring caspase-3 expression in breast cancer cells (MCF7) leads to increased sensitivity of these cells to undergo apoptosis in response to anticancer drugs or other apoptotic stimuli of (Devarajan et al., 2002). Notably, there is some evidence that has shown the down-regulation of more than one caspase, which contributes to tumor development (Fong et al., 2006).

Targeting Apoptosis in Cancer Treatment

In recent years, a lot of attention has been paid to developing diverse promising experimental anti-cancer drugs that can modulate apoptotic pathways. Due to apoptosis being a double-edged sword, researchers target every defect or abnormality along the apoptotic pathways to restore the apoptotic signaling pathways and eliminate cancer cells (Table 1).

Targeting anti-apoptotic Bcl-2 family members

As mentioned above, over-expression of prosurvival BCL-2 family members are associated with aggressive cancer and/or chemoresistance which mark these proteins as highly promising therapeutic targets to develop pharmacological manipulation of cell death through down-regulating anti-apoptotic BCL-2 family members. Recent studies showed the inhibition of prosurvival BCL-2 family members by small interfering RNAs (siRNAs) could result in the induction of cells apoptosis and subsequently a reduction in tumor growth. For example, Specific down-regulation of Mcl-1 by siRNA induced significant apoptosis in leukemia cells *in vitro* (Karami et al., 2014). Furthermore, several microRNAs have been identified that regulate Bcl-2 expression. For instance, Sing et al. demonstrated that miR-195, miR-24-2, and miR-365-2 act as negative regulators of Bcl-2 via direct binding to the 3'-UTR of the *BCL2* gene, which shows the therapeutic potential of these miRNAs (Singh and Saini, 2012). In addition, miRNA-15a and miRNA-16-1 have been reported to play an important role in the targeting

Table 1. Preclinical and Clinical Trials of Some Novel Therapeutic Agents that Target Apoptosis in Cancer Cells

Agents	Target/ mechanism	Phase	Target disease	References/ClinicalTrials.gov Identifier & sponsor
I) Targeting Anti-apoptotic Bcl-2 Family Members using Bcl-2 antisense				
Oblimersen Sodium (G3139, Genasense (trial name))	Inhibits bcl-2	Phase I	-Acute Myeloid Leukemia (combined with Cytarabine, and Daunorubicin)	NTC00039117* / (NCI**)
		Phase I/II	-Advanced Colorectal Cancer (combined with Chemotherapy)	NTC00055822/ (NTC)
		Phase I/II	-Advanced Esophageal, Gastro-Esophageal Junction and Gastric Cancer(combined with Cisplatin and Fluorouracil)	NTC00064259/ (NTC)
		Phase I/II	-Advanced Breast Cancer (combined with Doxorubicin and Docetaxel)	NCT00063934/ (NTC)
		Phase II	-Merkel Cell Carcinoma	NCT00079131/ (NCT)
		Phase II	-Chronic Myelogenous Leukemia(combined with Imatinib Mesylate)	NCT00049192/ (NCT)
		Phase II	-B-cell-Hodgkin lymphoma (combined with Rituximab)	NCT00054639/ (NCT)
		Phase II	-Chronic Myelogenous Leukemia (combined with Imatinib Mesylate)	NCT00049192/ (NTC)
		Phase II	-Small cell Lung Cancer (combined with carbo-platin/etoposide)	NCT00042978/ (NTC)
		Phase III	-Multiple Myeloma and plasma cell Neoplasm (with or without dexamethasone)	NCT00017602/ (Genta Incorporated)
		Phase III	-Chronic lymphocytic leukemia (CLL) (combined with Fludarabine and Cyclophosphamide)	NCT00024440/ (Genta Incorporated)
		Phase III	-Myeloma(plus dacarbazine)	NCT00016263 &NCT00518895/ (Genta Incorporated)

Table 1 (cont). Preclinical and Clinical Trials of Therapeutic Agents that Target Apoptosis in Cancer Cells

II) Small molecule inhibitors of BCL-2 proteins				
a) Molecules that affect gene or protein expression:				
Drugs that modulate the activity of retinoic acid receptors (RAR), retinoid X receptors (RXR), PPAR, vitamin D receptors (VDR):				
Troglitazone Δ2-TG	Inhibit the anti-apoptotic functions of Bcl-xL and Bcl-2	Phase II	-Advanced prostate cancer	(Grillier-Vuissoz and Isabelle, 2012)
Histon deacetylase inhibitors:				
Sodium butyrate	Downregulate the expression of Bcl-2	Preclinical Preclinical preclinical	-Lymphoma -Prostate cancer -gastric cancer cells	(Duan et al., 2005) (Qiu et al., 2012) (Shin et al., 2012a)
Synthetic cytotoxic retinoid:				
Fenretinide	Caspase 9 activation , Bcl-2 and Mcl-1 down-regulation and not effect on Bax expression	Preclinical Preclinical	-Rhabdomysarcoma -Ovarian carcinoma	(Herrero Martin et al., 2013) (Tiberio et al., 2010)
Cyclin-dependent kinase inhibitor:				
Flavopiridol (Alvoidib)	Down-regulation of Mcl-1 and Bcl-xL, Bid cleavage, and mitochondrial translocation of Bax	Preclinical Preclinical Preclinical Phase I/II Phase II Phase II Phase II Phase II	-B-cell chronic lymphocytic -Myeloma (combined with HA14-1) -Acute myelogenous leukemia -Small cell lung cancer (combination with Topotecan) -Metastatic, or Primary Adrenocortical cancer -Glioblastoma Multiforme -Non-Small Cell Lung Cancer (combined with Docetaxel) -Advanced Laryngeal Cancer (combined with Chemotherapy)	(Kitada et al., 2000) (Pei et al., 2004) (Bose and Grant, 2013) NCT00397293 (Ascenta therapeutics) NCT00848016 (NCI) NCT00540722 (NCI) NCT00544960 (Ascenta therapeutics) NCT01633541 Francis(Frank)Worden
b) Molecules acting on proteins themselves				
BH-3 domain Inhibitors				
Gossypol/ AT-101	Inhibitor of Bcl-2 family proteins including Bcl-2, Bcl-xL, Mcl-1and Bcl-w	Phase II Phase II	-Small Cell Lung Cancer -Non-Small Cell Lung Cancer	NCT00773955 NCT00544960 (Ascenta Therapeutics)
Obatoclax Mesylate (GX15-070MS)	antagonizes Bcl-2, Bcl-XL, Bcl-w and Mcl-1(pan-BCL-2 antagonist)	Phase I Phase I Phase I Phase I Phase I/II Phase II Phase II	-Chronic Lymphocytic Leukemia (CLL) -Non-Hodgkin Lymphoma (combined with Bortezomib) -Hematological Malignancies -Solid Tumors, Lymphoma, or Leukemia (combined with Vincristine Sulfate, Doxorubicin Hydrochloride, and Dexrazoxane Hydrochloride) -Non-Small Cell Lung Cancer (combined with Docetaxel) -Acute Myeloid Leukemia (AML) -Hodgkin's Lymphoma	NCT00600964 (Gemin X) NCT00538187 (NCI) NCT00438178 (Gemin X) NCT00933985 (NCI) NCT00405951 (Gemin X) NCT00684918 (Gemin X) NCT00359892 (Gemin X)
ABT-737	Inhibits Bcl-2, Bcl-xL, and Bcl-W	preclinical Phase I/II Phase I/II Phase II	-Leukemia lymphoma, myeloma, glioma and small cell lung cancer cells -Metastatic breast cancer -Castrate-resistant prostate cancer(CRPC) -Ovarian Tumors (association of platinum)	(Oltersdorf et al., 2005; Chauhan et al., 2007; Trudel et al., 2007; Tagscherer et al., 2008) (Van Poznak et al., 2001) (Liu et al., 2009) NCT01440504 (Centre Francois Baclesse)

Table 1 (cont). Preclinical and Clinical Trials of Therapeutic Agents that Target Apoptosis in Cancer Cells

ABT-263 or navitoclax	Inhibits Bcl-2, Bcl-XL, and Bcl-w but not for MCL-1 or A1	Phase I PhaseI Phase II Phase II	-Solid Tumors (combined with Paclitaxel) -Solid Tumors (combined with Gemzar (Gemcitabine)) -Chronic Lymphocytic Leukemia (CLL) -Small Cell Lung Cancer	NCT00891605 (Abbott) NCT00887757 (Abbott) NCT01557777 AbbVie (prior sponsor, Abbott)(Rudin et al., 2012)
ABT-199/ GDC-0199	Selective inhibitor of Bcl-2 proteins	Phase I Phase I	-Human multiple myeloma -Relapsed or Refractory Chronic Lymphocytic Leukemia (CLL) and Non-Hodgkin's Lymphoma	(Touzeau et al., 2014) (Roberts; Matthew S. Davids, 2013) NCT01328626 AbbVie (prior sponsor, Abbott)
		Preclinical	-Estrogen receptor (ER)-positive breast cancer	(Vaillant et al., 2013)
VDAC1-based cell-penetrating peptides	Targeted HK[3], Bcl-2 and Bcl-xL to ihibit the interaction of VDAC1 and the anti-apoptotic proteins	Phase I	-B-cell chronic lymphocytic leukemia (CLL)	(Prezma et al., 2013)

of prosurvival Bcl-2 proteins in chronic lymphocytic leukemia (CLL) (Calin et al., 2008).

A number of drugs directed at inhibiting anti-apoptotic BCL-2 family proteins have been developed, with some showing great promise in clinical settings (Weyhenmeyer et al., 2012; Roy et al., 2014). Two strategies are recommended to block Bcl-2 and Bcl-xL, including I) application of Bcl-2 antisense, and II) application of small molecule inhibitors of the Bcl-2 family of proteins.

Bcl2 antisense: Bcl2 antisense down-regulates Bcl-2 proteins by target the corresponding mRNA. These include Oblimersen sodium (G3139, Genasense), an 18-antisense oligonucleotide that is the first agent targeting Bcl-2 mRNA to enter clinical trials. The apoptotic effect of this compound happens through an increase in Bax and PARP, releasing cytochrome c and Smac/DIABLO from mitochondria, which can activate caspases (Emi et al., 2005). Reduction of Bcl-2 expression by Oblimersen sodium in preclinical studies had cytotoxic and cytostatic effects on breast (Emi et al., 2005), ovarian (Bartholomeusz et al., 2005), prostate (Chi et al., 2001; Leonetti et al., 2007), melanoma (De Cesare et al., 2005), NSCLC (Hu et al., 2004), bladder (Duggan et al., 2001), and gastric cancers cells (Wacheck et al., 2001). This compound is entering phase I to III human clinical trials (O'Brien, 2009).Also, most clinical trials combine the antisense agent with cytotoxic anticancer drugs for in order to promote the drug sensitivity of different cancer cells (Table 1).

Small molecule inhibitors of BCL-2 proteins: Small molecule inhibitors of BCL-2 proteins are divided in two groups, 1) molecules that affect gene or protein expression, and 2) molecules acting on proteins themselves (Table 1).

i) Molecules that affect gene or protein expression: There are some components that represent potentially "druggable" modulators of Bcl-2 and Bcl-xL genes, such

as some members of the steroid/retinoid superfamily of ligand-activated transcription factors (SRTFs). Some members of the SRTF superfamily that can down-regulate the expression of Bcl-2 or Bcl-xL are some small molecule drugs that modulate the activity of retinoic acid receptors (RAR), retinoid X receptors (RXR), PPAR, and vitamin D receptors (VDR) in specific types of cancer and leukemia cells (Hassan et al., 2014). For instance, Troglitazoneor Δ2-TG, a potent PPAR agonist, can inhibit the anti-apoptotic functions of Bcl-xL and Bcl-2 and cause caspase-dependent apoptosis via caspase-9 activation (Shiau et al., 2005; Grillier-Vuissoz and Isabelle, 2012). In addition, there is evidence that pretreatment of PC-3 cells with the pan-caspase inhibitor Z-VAD-FMK protects cells from troglitazone- and Δ2-TG-activated apoptosis, which verifies the involvement of caspase activation in apoptotic death (Shiau et al., 2005). Furthermore, histone deacetylase inhibitors (e.g., sodium butyrate, trichostatin A) (Duan et al., 2005), synthetic cytotoxic retinoids (e.g., Fenretinide) in a ROS-independent manner(Tiberio et al., 2010; Herrero Martin et al., 2013), cyclin-dependent kinase inhibitors (e.g., Flavopiridol) (Kitada et al., 2000; Pei et al., 2004; Bose and Grant, 2013), Deubiquitinase inhibitors (e.g., WP1130) (Sun et al., 2011) are several other molecules that affect gene or protein expression by down-regulating RNA expression in various cancer cells.

ii) Molecules acting on proteins themselves: These molecules directly bind with anti-apoptotic Bcl-2 proteins at their BH3-binding groove and mimic the action of BH3 proteins (de Almagro and Vucic, 2012). These small molecules, all less than 750 daltons (Azmi and Mohammad, 2009), demonstrate targeting of the different anti-apoptotic members of the Bcl-2- family, such as Bcl-2, Bcl-xL, Bcl-W, Mcl-1, Bcl-B, and Bfl-1, in terms of balancing antitumor efficacy. The most prominent examples of these agents include gossypol, Obatoclax (GX15-070), ABT-737, and ABT-263 or navitoclax (Oltersdorf et al., 2005; Kang and Reynolds,

Table 1 (cont). Preclinical and Clinical Trials of Therapeutic Agents that Target Apoptosis in Cancer Cells

III) Strategies that target p53 to activate apoptosis				
a) Small-molecule HDM2 inhibitors:				
R7112(Nultlin-3, analogues of cis imidazoline)	Preventing MDM2-P53 interaction	Phase I	-Advanced solid Tumors	NCT00559533 (Hoffmann-La Roche)
JNJ-26854165 (a tryptamine derivative)	Prevention of MDM2-P53 inter-action	Phase I	-Advanced Stage or Refractory Solid Tumors	NCT00676910 (Johnson & Johnson Pharmaceutical Research & Development, L.L.C)
b) Small molecules which restore the mutated p53 to wild-type p53:				
SCH529074	Reactivates mutant P53 and inhibits ubiquitination of P53 by HDM2	Preclinical	-WiDr, DLD-1, KLE, H322, MCF7, SJSA, WS-1, AND MB-468 Cancer cells	(Demma et al., 2010)
MIRA-3	Reactivates mutant p53	Preclinical	-Tumor cells	(Bykov et al., 2005a)
PK083	Reactivation of Y220C mutant p53	Preclinical	-Tumor cells	(Boeckler et al.,2008)
PRIMA-1	Reactivates mutant p53	Preclinical	-Tumor cells (combined treatment with cisplatin)	(Bykov et al., 2005b)
APR-246	Reactivates mutant p53	Phase I	-Malignancies or hormone-refractory prostate cancer	(Lehmann et al., 2012)
c) p53 vaccines:				
Peptide-based vaccines Dendritic-cell based vaccines Recombinant Virus	Facilitate the induction of antitumor immune responses, particularly antitumor CTL	Phase I/II Phase II Phase II Phase I/II Phase I/II Phase I/II Phase I	-Colorectal cancer -Ovarian cancer -Advanced (Breast, Cervical, Colorectal, Lung, Ovarian, Pancreatic) -Breast Cancer -Colorectal Cancer -Small cell lung cancer -Brain and Central Nervous System Tumors	(Speetjens et al., 2009) (Leffers et al., 2009) NCT00019084 (Svane et al., 2004; 2007) (Menon et al., 2003) (Atencio et al., 2006) (Antonia et al., 2006) NCT00004080 (NCI)
IV) Antibodies which target TRAIL-receptors				
Dulanermin	Targets TRAIL-R1 and TRAIL-R2	Phase I/II	-Non-Hodgkin's Lymphoma (Combined with Rituximab)	NCT00400764 (Genentech, Inc)
Mapatumumab (HGS ETR1,TRM-1)	Targets TRAIL-R1	Phase II	-Multiple Myeloma (combined with Bortezomib)	NCT00315757 (Human Genome Sciences Inc.)
Lexatumumab (HGS-ETR2)	Targets TRAIL-R2	Phase I	-Metastatic Tumors	NCT00428272 (NCI)
Conatumumab (AMG 655)	Targets TRAIL-R2	Phase I/II	-Colon Cancer Colorectal Cancer Rectal Cancer Metastatic Colorectal Cancer Oncology (combined with panitumumab)	NCT00630786 (Agmen)
Drozitumab	Targets TRAIL-R2	Phase Ib	-Metastatic colorectal cancer (mCRC) (combined with Cetuximab + irinotecan or FOLFIRI ± bevacizumab)	(Baron, 2011)
Tigatuzumab	Targets TRAIL-R2	Phase II	-Breast cancer (combined with Abraxane)	NCT01307891 (University of Alabama at Birmingham)
LBY135	Targets TRAIL-R2	Phase I/II	-Solid tumor (with or without capecitabine)	(Sharma et al., 2014)

*[1]http://clinicaltrials.gov/; [2]National Cancer Institute; [3]hexokinase

2009; Albershardt et al., 2011). ABT-737 is a pan-BCL-2 inhibitor which mimics the BH3 domain of BAD, binds selectively to BCL-2, BCL-XL and BCL-W, and exhibits remarkable antitumor activity in human cancers (Oltersdorf et al., 2005). ABT-199/GDC-0199, a BH3-mimetic, is a selective inhibitor of BCL-2 proteins through restoring the communication system that tells cancer cells to self-destruct. This compound is currently in clinical trials for the treatment of CLL; Non-Hodgkin's Lymphoma (NHL), and multiple myeloma, breast cancer (Roberts; Matthew S. Davids, 2013; Vaillant et al., 2013; Touzeau et al., 2014). However, the safety and efficacy of ABT-199/GDC-0199 have not been established.

Voltage-dependent anion channel (VDAC, also known as porin) which is located in the mitochondrial outer membrane, directly contributes to MOMP and intercedes with cytochrome c release, either by oligomer formation (Shoshan-Barmatz et al., 2010) or by the formation of a large pore comprising VDAC and Bax/Bak (Martinez-Caballero et al., 2009). Tajeddine N. et al. reported that knockdown of the VDAC1 was able to inhibit cell death induced by cisplatin (Tajeddine et al., 2008). In addition, Arbel N. et al have demonstrated that direct interaction of anti-apoptotic Bcl-2 and Bcl-xL with VDAC results in decreased channel conductance and causes anti-apoptotic actions (Arbel et al., 2012).Thus, searching for compounds that block the binding of VDAC1 and prosurvival/anti-apoptotic hexokinase (HK) and Bcl-2 family proteins may be promising targets for anticancer drug research (Shoshan-Barmatz and Mizrachi, 2012). Recently, Prezma T. et al. have designed VDAC1-based cell-penetrating peptides which target HK, Bcl-2 and Bcl-xL to inhibit the interaction of VDAC1 and the anti-apoptotic proteins in B-cell CLL (Prezma et al., 2013).

Targeting p53

One of the most essential roles of p53 is its ability to activate apoptosis through both transcription-dependent and transcription-independent ways (Figure 1) (Chi, 2014). The disturbance of this process can cause tumor progression and chemoresistance. There are different strategies that target p53 to activate apoptosis, such as
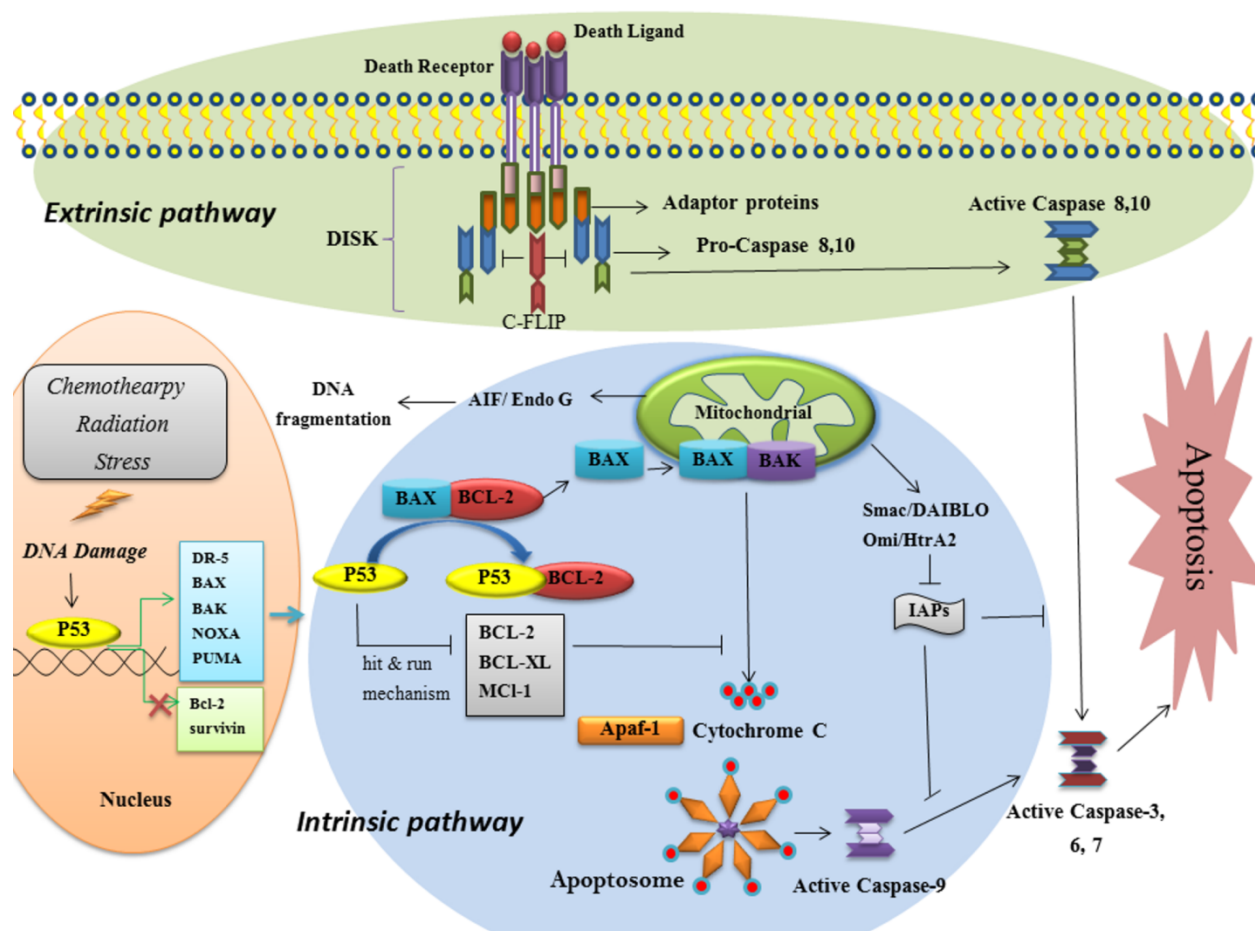

Figure 1. Schematic Diagram of the Intrinsic, Extrinsic Pathways of Apoptosis. Apoptosis is executed via two central apoptotic pathways, the extrinsic and the intrinsic pathways. Both apoptotic pathways converge on the same terminal or execution pathway. The extrinsic pathway begins with the attachment of extracellular ligands to the extracellular domain of transmembrane receptors, whereas mitochondrial apoptosis is initiated by several intracellular stimuli. Initiator caspase of both pathways (caspase 8 and 9, 10) can catalyse the proteolytic maturation of effector caspases (such as caspase 3, 6, 7), which lead to initiation of a caspase cascade and finally result in demolition of the cell. DNA damage triggers apoptosis by activation of the tumor-suppressor protein p53, which induces the transcription of DR-5, BAX, BAK, NOXA, PUMA and also can inhibits the transcription of anti-apoptotic genes such as Bcl-2 and survivin. The cytoplasmic interaction of p53 with the anti-apoptotic Bcl-2 family proteins in the mitochondria induces the release of apoptogenic factors such as cytochrome c from the mitochondrial outer membrane. In addition, p53 can interact directly with Bak and/or Bax and activate these through a "hit and run" mechanism that prompts the permeabilization of the outer mitochondrial membrane

targeting p53 family proteins, inhibition of p53-MDM2 interaction, restoring mutated p53 back to their wild-type functions, eliminating mutant p53, creating p53-based vaccines, and gene therapy to restore p53 function (Table 1) (Hong et al., 2014). The function of p53 is negatively regulated by oncoproteins such as MDM2 (known as HDM2 in humans) and MDMX through an interaction with the p53 transactivation domain (p53TAD) (Oliner et al., 1993). Therefore, one of the main targets for cancer therapy is inhibition of the p53TAD-MDM2/MDMX interaction via the small molecule MDM2 antagonist that stabilizes p53 by blocking its interaction with MDM2 and selectively induces senescence in cancer cells(Iancu-Rubin et al., 2014). Some of these small molecule MDM2 inhibitors such as R7112 (Nutlin-3, analogues of cis-imidazoline) and JNJ-26854165 (a tryptamine derivative) are currently under clinical assessment in phase I studies (Tabernero et al., 2011; Shaomeng Wang et al., 2012; Sosin et al., 2012). In addition, Jae-sun Shin et al. have shown that a p53TAD-mimetic MDM2 antagonist, R7112, can directly interact with BH3 peptide-binding grooves of diverse anti-apoptotic Bcl-2 family proteins such as Bcl-2, Bcl-xL, Mcl-1 and Bcl-w in an analogous mode (Shin et al., 2012b; Shin et al., 2014).

Another class of drugs restores mutated p53 to wild-type p53 through binding to and stabilization of mutant p53, such as SCH529074, MIRA-3, PK083 (Y220C mutant p53), PRIMA-1 and its analog APR-246 ((PRIMA-1MET) (Bykov and Wiman, 2014). PK083 (the carbazole derivative) interacts with a small cleft in the destabilized p53 mutant and raises the melting temperature of Y220C mutant p53 and restores its function (Boeckler et al., 2008).

In addition, numerous clinical trials have been performed using different kinds of p53 vaccines including I) short and long peptide-based vaccines, II) dendritic cell-based vaccines, and III) recombinant replication-defective adenoviral vectors with human wild-type p53 (Kuball et al., 2002; Svane et al., 2004; Vermeij et al., 2011).

One attractive p53 gene-based strategy is the utilization of a genetically engineered oncolytic adenovirus. One p53-expressing adenovirus which is used for the treatment of tumors lacking p53 function is ONYX-015(dl1520), in which the E1B-55 kDa gene has been removed. ONYX-015 selectively replicates and lyses tumor cells deficient in p53 and leaves normal cells unaffected (Nemunaitis et al., 2000). Therefore it can be used for the treatment of tumors lacking p53 function (Nemunaitis et al., 2000; Cohen and Rudin, 2001).Over-expression of the wild-type p53 gene has been demonstrated to sensitize tumor cells of colorectal cancers, prostate cancers, glioma, and head and neck to ionizing radiation(P:, 2001). Moreover, Mirza A. et al. have shown that the expression of wild-type p53 was associated with strong repression of the survivin expression at both mRNA and protein levels in various cell types (Mirza et al., 2002).

Targeting the extrinsic pathway
Besides disequilibrium between pro- and anti-apoptotic BCL-2 proteins which leads to the evasion of the intrinsic pathway of apoptosis, some abnormalities in the death signaling pathways have been reported to have an important place in the evasion of the extrinsic pathway of apoptosis. For example, changes in death ligand and death receptor expression and abnormal expression of decoy receptors have been described in various cancers (Reesink-Peters et al., 2005). Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in cancer cell lines. TRAIL-receptor 1 (TRAIL-R1 or death receptor 4) and TRAIL-receptor 2(TRAIL-R2 or death receptor 5) are the main agonistic receptors for TRAIL. TRAIL appears to have minimal activity in cancer cells against most normal cell types but TRAIL-R1 and TRAIL-R2 expression increases in a variety of solid tumors (Spierings et al., 2004). There is some evidence that has shown pro-apoptotic receptor agonists (PARAs), such as antibodies against TRAIL-receptors and recombinant human (rh) TRAIL antibodies, can induce apoptosis in a variety of tumor types and can be utilized as an effective anti-cancer strategy (Ashkenazi et al., 1999; Ichikawa et al., 2001).

Some of these antibodies which target TRAIL-receptors include Dulanermin (a recombinant human TRAIL antibody targeting both TRAIL-R1 and TRAIL-R2), Mapatumumab (a monoclonal agonistic antibody targeting TRAIL-R1), Lexatumumab, Conatumumab, Drozitumab, tigatuzumab, and LBY135 (a monoclonal agonistic antibody targeting TRAIL-R2) (Table 1) (Khan et al., 2014). Mapatumumab (TRM-1, HGS-ETR1) is a fully human immunoglobulin G1 lambda (IgG1 λ) monoclonal antibody (mAb) agonist of TRAIL-R which has shown a tolerable toxicity profile during phase1 development (Tolcher et al., 2007). Researchers have observed promising preclinical activity with mapatumumab in patient with advanced solid cancers (Hotte et al., 2008). Currently, mapatumumab in combination with bortezomib in patients with refractory myeloma (Kapoor et al., 2012) and cisplatin and radiotherapyin patients with cervical cancer is in phase II clinical trials (Vici et al., 2014).

Lexatumumab (HGS-ETR2) is another fully human agonistic mAb that targets TRAIL-R2 and activates death receptor mediated pathway and has been evaluated in phase I/II settings (Sikic BI et al., 2007; Wakelee et al., 2010).

Targeting c-FLIP
C-FLIP is an important target for the development of siRNA that, in particular, inhibit the expression of c-FLIP in various human tumor cell lines and sensitize cancer cells to TRAIL, FASL, and chemotherapeutic agents (Day and Safa, 2009). However, there are many limitations for siRNA *in vivo* and the extensive utilization of targeting FLIP by siRNAs in clinical settings relies on the safe delivery of siRNA (Whitehead et al., 2009). Besides siRNA mediated down-regulation of c-FLIP, there are some small molecules that cause degradation of c-FLIP as well as down-regulating mRNA and protein levels of c-FLIPL and c-FLIPS splice variants (Safa and Pollok, 2011). It should be noted that due to the structural similarity of c-FLIP to caspase-8, small molecules which have the ability to inhibit c-FLIP's recruitment to the DISC could also block the recruitment of caspase-8, and,

consequently, inhibit apoptosis. This resemblance makes the c-FLIP protein a very complex target for drugs to block c-FLIP function; thus, in order to inhibit c-FLIP expression, small molecules should be developed which inhibit c-FLIP without blocking caspase-8 and -10 (Safa and Pollok, 2011).

Several agents have been established that down-regulate levels of c-FLIP through affecting c-FLIP transcription, degrading c-FLIP through the ubiquitin proteasome system, or decreasing c-FLIP translation (Safa et al., 2008; Safa and Pollok, 2011). Some of these agents include DNA damaging agents and chemotherapeutic drugs (such as cisplatin and Oxaliplatin), topoisomerase I inhibitors (camptothecin, 9-nitrocamptothecin (9NC), irinotecan) (Kinoshita et al., 2000; Chatterjee et al., 2001; Song et al., 2003; Galligan et al., 2005; Longley et al., 2006; Abedini et al., 2008), or Histone deacetylase (HDAC) inhibitors (such as suberoylanilidehydroxamic acid (SAHA, vorinostat), Depsipeptide, Trichostatin A, Valproic acid, MS-275 (entinostat)) (Pathil et al., 2006; Park et al., 2009; Rao-Bindal et al., 2013; Riley et al., 2013; Feng et al., 2014). One of the most promising HDAC inhibitors is SAHA, which causes degradation of c-FLIP variants via an ubiquitin/proteasome-dependent Itch/AIP4-independent mechanism (Yerbes and Lopez-Rivas, 2012). Depsipeptide is another member of HDACi that leads to caspase-dependent apoptosis in chronic lymphocytic leukemia and osteosarcoma cells through selectively involving TNF-R (extrinsic pathway) and down-regulation of c-FLIP without evidence of Fas (CD95) up-regulation (Aron et al., 2003; Watanabe et al., 2005). Additionally, MS-275 has been reported to block c-FLIP expression in CLL, osteosarcoma and melanoma cells (Lucas et al., 2004; Rao-Bindal et al., 2013; Venza et al., 2014). Recently, a small molecule inhibitor of c-FLIP, 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide (CMH), has been recognized as using a high-throughput chemical library screen (Schimmer et al., 2006). 4-(4-Chloro-2-methylphenoxy)-N-hydroxybutanamide (CMH) (5809354) down-regulates c-FLIP mRNA and protein levels, and causes degradation of PARP and reduces cell survival, as well as inducing apoptosis in cancer cells (Mawji et al., 2007b; Bijangi-Vishehsaraei et al., 2010). Moreover, an antipsychotic agent, Thioridazine, is known to have an anticancer activity due to the ability of thioridazine to sensitize TRAIL-mediated apoptosis and down-regulate c-FLIP(L) and Mcl-1 expression at the post-translational level (Min et al., 2014). Kerr et al. reported the Ku70 /FLIP interaction affects FLIP protein stability by preventing its polyubiquitination. In addition, they showed that SAHA disrupts the FLIP/Ku70 complex via increasing the acetylation of Ku70, which consequently triggers FLIP polyubiquitination and degradation by the proteasome (Kerr et al., 2012). C-FLIP has been demonstrated to be down-regulated by several compounds such as actinomycin D (Hernandez et al., 2001), cycloheximide (Kaminskyy et al., 2013), and anisomycin (Mawji et al., 2007a); the first is an RNA synthesis inhibitor, and the second and third are protein synthesis inhibitors. Moreover, protease inhibitors such as Bortezomib (PS-34) have been extensively studied and

shown to have the ability to reduce c-FLIP in diverse cell lines (Koschny et al., 2007; Perez et al., 2010).

Targeting IAPs

Aberrantly high expression of IAP proteins has been reported to increase resistance to apoptotic stimuli in many cancer types (Wright and Duckett, 2005); based on the idea that IAPs contribute to blockage of apoptosis, this can provide new opportunities for cancer treatment through the development of drugs that can inhibit IAPs (Dubrez et al., 2013) . Presently, there are two approaches for inhibiting IAPs that include *(i)* inhibiting IAPs by mimicking Smac, and *(ii)* anti-sense mediated interference of XIAP and survivin mRNA and protein expression.

Smac mimetic compounds (SMCS): Recently, several peptidic and non-peptidic small molecule pharmacologic inhibitors of IAP proteins that mimic the binding domain of Smac (SMAC mimetics) to IAP proteins have been developed (Fulda, 2014). Cyclopeptidic SMAC mimetics bind to the BIR2 or BIR3 domain of cIAP-1/2 and XIAP and restore the activity of caspases which have been blocked by IAPs (Sun et al., 2010). Alternatively, a non-peptidic IAP inhibitor(SM-164) was described to increase TRAIL activity at the same time as targeting cIAP1and XIAP (Lu et al., 2011).

Numerous studies report the therapeutic effect of SMAC mimetics when used in combination with conventional chemotherapeutic drugs, radiation therapy or death receptor agonist. For example, Dai et al. (2009) have shown that the SMAC mimetic SH122 sensitizes human prostate cancer cells to TRAIL-induced apoptosis by inhibiting both IAPs and NF- κB. Moreover, they reported that another small-molecule inhibitor of IAPs, SH130, can increase ionizing-radiation-induced apoptosis in prostate cancer models (Dai et al., 2008). Varfolomeev E. et al. demonstrated that a SMAC mimetic which binds to cIAPs results in c-IAP auto-ubiquitination activity and proteasomal degradation (Varfolomeev et al., 2007).

Several SMAC mimetic compounds that have shown activity in cancer cell lines, include AZD5582, which sensitizes CLL cells to killing by TRAIL (Zhuang et al.), and JP1201 in NSCLC (Greer et al., 2011),HT-29 colorectal cancer(Huerta et al., 2010) cell lines, and multiple animal models of pancreatic cancer (Dineen et al., 2010). Interestingly, cIAPs target NF- κB-inducing kinase (NIK), therefore acting as negative regulators of NF-κB activation, and inducing loss of cIAPs by SMAC mimetics leads to NIK stabilization and consequent NF-κB activation (Baud and Karin, 2009). YM155 (sepantronium bromide) and EM1421which are inhibitors of Birc5 transcription can antagonize survivin expression and induce apoptosis in human cancer cell lines (Nakahara et al., 2007). YM155, a selective small-molecule survivin suppressant, is currently in phase 2 trials in non-small cell lung carcinoma, solid tumors, prostate cancer and melanoma (Giaccone et al., 2009; Tolcher et al., 2012; Kudchadkar et al., 2014).

Antisense oligonucleotides and small siRNA molecules: Besides targeting IAPs through small molecule inhibitors and peptidomimetics, antisense oligonucleotides and short interfering RNA (siRNA) molecules have also

been exploited to target survivin and XIAP in cancers (Dai et al., 2005; Karami et al., 2013). Several antisense have been described as down-regulating XIAP and surviving expression, such as AEG35156 (down-regulates XIAP expression) (Carter et al., 2011) and LY2181308 (binds to survivin mRNA and inhibits its expression) (Tanioka et al., 2011). Nevertheless, partial positive responses have been seen in clinical trials of existing IAPs using targeting strategies .YM155 monobromide is a small molecule survivin gene suppressant through binding to the Sp1 rich region of the promoter of survivin, and blocks the transcription of survivin in different cancer cell lines (Nakahara et al., 2007; Cheng et al., 2012). Croci DO et al. have shown that after siRNA transfection against survivin and down-regulation of survivin, translocation of the AIF from the cytoplasm to the nucleus occurs, which is a molecular indicator of the caspase-independent apoptosis of cells (Croci et al., 2008). Combined use of cisplatin, fluorouracil, paclitaxel, etoposide, and XIAP siRNA have been shown to result in better chemotherapeutic activity and to efficiently decrease XIAP expression and make it sensitive to cancer treatments (Zhang et al., 2007; Wei et al., 2008).

Targeting caspases

A number of drugs have been developed to synthetically activate caspases. For instance, Apoptin is a caspase-inducing agent which exerts a tumor-preferential apoptotic activity and selectively induces apoptosis in cancer but not normal cells (Rohn and Noteborn, 2004). Another class of drugs which are activators of caspases is the small molecule caspase activators. Small molecule caspase activators are characterized by presence of the arginine-glycine-aspartate motif. These agents have the potential to decrease the activation threshold of caspase and to induce auto-activation of procaspase-3, and, finally, can sensitize cancer cells to chemotherapeutic agents (Philchenkov et al., 2004). Thus, targeting caspase-8 expression may lead to a significant therapeutic effect, particularly in tumors suffering from a gene dosage effect or hypermethylation of caspase-8 promoters. In this context, in tumors suffering from hypermethylation of caspase-8 promoters, researchers are using 5-aza-2′deoxycytidine (decitabine), a cytosine nucleoside analog which inhibits DNA methyltransferase covalent binding and promotes demethylation. Additionally, there is evidence that shows 5-aza-2′ deoxycytidine (decitabine) boosts *caspase-8* promoter availability, and provides a situation for binding of SP1 and ETS-like transcription factors (Liedtke et al., 2005; Fulda and Debatin, 2006).

Besides caspase-based drug therapy, caspase-based gene therapy has been tried. It has been shown that caspase-3 gene therapy, in addition to etoposide treatment, can induce widespread apoptosis and leads to reduction of tumor volume in an AH130 liver tumor model (Yamabe et al., 1999).

Conclusion

In conclusion, apoptosis is a cellular procedure that is closely regulated by diverse groups of regulatory molecules and is characterized by specific morphological and biochemical features in which activation of caspase plays a crucial role. One of the fundamental features of human cancers is evasion of apoptosis, which contributes to both tumor progression and resistance to cancer treatments. An enhanced understanding of the apoptotic signaling mechanisms, the key proteins involved in apoptosis, and the mechanisms by which cancer cells evade apoptotic death has led to effective therapeutic strategies to inhibit cancer cell proliferation, which will significantly benefit cancer patients. Some drugs that selectively target different components of apoptosis are in clinical trials, and the combination of these novel agents with traditional chemotherapy seems to be well tolerated.

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