We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,500
Open access books available

136,000
International authors and editors

170M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

The Role of Apoptosis as a Double-Edge Sword in Cancer

Reyhaneh Farghadani and Rakesh Naidu

Abstract

The pathogenesis of many diseases is most closely related to inappropriate apoptosis (either too little or too much) and cancer is one of the situations where too little apoptosis happens, leading to malignant cells that highly proliferate. Defects at any points along apoptotic pathways may lead to malignant transformation of the affected cells, tumor metastasis, and resistance to anti-cancer drugs. Several major molecular mechanisms are involved in the evasion of apoptosis in cancer initiation and progression. Bcl-2 family of proteins and caspases are the central players in the apoptotic mechanism and regulate cell death. Their imperfections cause to the deficient apoptotic signaling and thereby the inadequate apoptosis in cancer cells and eventually carcinogenesis. Strategies targeting these master regulators in carcinoma cells has been a major focus of interest in cancer studies. Therefore, despite being the cause of problem, apoptosis can be targeted in cancer therapy. This chapter provides a comprehensive review of apoptotic cell death and how deficiencies in apoptotic master regulators, caspases and Bcl-2 family proteins, influence carcinogenesis and can be targeted in cancer treatment.

Keywords: apoptosis, cancer, Bcl-2, caspase, regulation, dysfunction, intrinsic pathway, extrinsic pathway, carcinogenesis

1. Introduction

Cancer as a complicated and heterogeneous disorder is the major threat to human beings and is still the significant leading cause of mortality around the world. According to the world health organization report, cancer is the second leading cause of death around the world with 9.6 million deaths in 2018.That is nearly 1 in 6 of all global deaths [1, 2]. The incidence of cancer is expected to rise approximately 70% within the next two decades around the world, from 14 million new cases in 2012 to 25 million new cases a year [3–5]. Cancer development comprises of a multiple steps happening progressively and beginning with initial mutations that promote tumorigenesis and, eventually, metastasis. The genetic alterations ultimately cause to a disturbance in the balance between cell proliferation and programmed cell death or apoptosis [6].

Apoptosis is a process of the cell's natural mechanism for death which occurred in multicellular organisms to maintain tissue homeostasis and act as a defensive strategy to remove infected, damaged or mutated cells. Apoptosis can be triggered through two major pathways, either mitochondrial- or death receptor-mediated pathways resulting from the intracellular (e.g. stress, DNA damage) and extracellular signals (death-inducing signals by other cells), respectively. This machinery

mainly depends on caspases cascades for executing cell death that eventually cause proteolytic cleavage of thousands of target proteins within the cells that are essential for normal cellular function such as cytoskeletal and nuclear proteins. Consequently, the apoptotic cells undergo a series of morphological and biochemical alterations leading to recognition by macrophages and cell phagocytosis. Moreover, B-cell lymphoma-2 (Bcl-2) family of proteins has long been identified for their significant involvement in regulating the cellular program of apoptosis through mitochondrial outer membrane permeabilization, as the critical decision-point at which cells commit to death, representing their vital role in protecting against cancer [7–9].

Deficiencies at any point along apoptotic pathways and dysfunction of the controlling mechanisms may result in impaired apoptosis that cause to carcinogenesis, allowing cancer cells to survive over intended lifespans and eventually uncontrolled cell proliferation, tumor development and progression. Tumor cells evade apoptosis through a variety of mechanisms. Understanding these molecular mechanisms not only provide insight into the cancer pathogenesis, but also provide clues on cancer treatment [7, 10]. Besides, genomic instability, nutrient deficiency, cellular hypoxia and oncogenic stress may cause to continuous stress within cancer cells which make them more sensitive to apoptotic stimulation. Hence, the ability to target the molecular components of this machinery and restore an apoptotic pathway has long been considered as an intriguing approach in cancer drug discovery. Consequently, being as a double-edged sword, apoptosis plays a critical role in both tumorigenesis and cancer therapy [6, 11, 12]. Therefore, as evasion of apoptosis is well known as the hallmark of all types of cancers, this chapter will be mainly emphasizing the role of apoptosis in cancer, from pathogenesis and cancer development to cancer therapy and treatment with primarily focus on two key mediators of apoptosis, caspases and Bcl-2 family of proteins, which have been receiving great attention in targeted cancer therapies.

2. The role of apoptosis in pathogenesis and treatment of cancer

2.1 Overview of apoptosis

The term “apoptosis” is derived from Greek, meaning “dropping off” or “falling off” as leaves from a tree, was first used in 1972 to describe a morphologically distinct form of cell death. Apoptosis also known as programmed cell death is a highly regulated energy-dependent process that occurs normally during development and aging. It plays an important role as a homeostatic mechanism to maintain cell populations in the tissue of multicellular organisms. In addition to its importance in biological process, defects in apoptosis mechanism has been implicated in the pathophysiology of diseases including cancer [13, 14]. There are many factors, mostly proteins, involved in the activation and regulation of apoptotic mechanism. This highly complicated and regulated process involves an energy-dependent cascade of molecular events and includes the mitochondria-dependent (intrinsic) and death receptor-dependent (extrinsic) pathway. Caspases play a vital role in initiation and execution of both intrinsic and extrinsic pathways which is mediated through the cleavage of hundreds of proteins essential for normal cellular function. [15].

2.1.1 Caspases: key apoptotic proteins

Caspases are a family of cysteine protease enzymes that are able to selectively cleave proteins at aspartic acid residues using the sulfur atom of cysteine in their

catalytic site, hence, named as cysteine-aspartic proteases or caspases. They play an essential role in maintaining homeostasis through regulating cell death and inflammation. Caspases have been generally categorized by their known functions in apoptosis (caspase-2, -3, -6, -7, - 8, -9 and -10) and in inflammation (caspase-1, -4, -5 and -12) in human. Caspases involved in apoptotic cell death have been then subgrouped based on their position and mechanism of action in apoptotic signaling cascades and are either initiator caspases (caspase-8, -9 and -10) or executioner caspases (caspase-3, -6, and -7) in apoptotic pathway. Therefore, caspases as a conserved family of cysteine proteases, which are essential in initiation and execution of intrinsic and extrinsic pathways, are the main emphasis of apoptosis studies [16–18].

Caspases are initially synthesized as an inactive monomeric proenzyme, named zymogens or procaspases, containing a large subunit, small subunit, and prodomain that is only activated through proteolytic cleavage and dimerization following an appropriate stimulus. Therefore, this post-translational level of control provides rapid and tight regulation of the caspase enzyme activities [19, 20]. Initiator caspases have prodomains containing one of the two specific protein–protein interaction domain including caspase recruitment domain (CARD) and death effector domain (DED) that promote caspase dimerization through binding to adapter proteins. Two examples of activating multiprotein complexes include death-inducing signaling complex (DISC) and the apoptosome, which are formed during extrinsic and intrinsic pathway of apoptosis, respectively [19].

Once properly assembled into dimers, pro-caspases undergo cleavage by autocatalysis resulting in the removal of pro-domain and cleavage at the linker region between the large and small subunit resulting in the heterotetramer formation and provides the active-site loops to get a proper conformation for enzymatic activity [17, 19].

Although, initiator caspases are capable of autocatalytic cleavage and activation, effector caspases are cleaved by initiator caspases resulting in the formation of active heterotetramer. Each active caspase is a tetramer consists of two identical big subunits and two identical small subunits. Accordingly, activation of apoptotic caspases leads to the inactivation or activation of substrates, and therefore initiation of a protease cascade events in the apoptotic signaling pathway resulting in rapid cell death. Activated caspases trigger cytoplasmic endonuclease, cleave many vital cellular proteins and break up the nuclear scaffold and cytoskeleton as well as activate DNase, which further degrade nuclear DNA into 180 to 200 base pair. Collectively, caspase activity results in various morphological and biochemical changes in apoptotic cells [19, 21, 22].

2.1.2 Morphological changes in apoptosis

Apoptotic cells are differentiated from healthy and necrotic cells based on certain cellular morphological changes. Characteristic features of apoptosis in the nucleus are chromatin condensation and nuclear fragmentation which are accompanied by cell shrinkage, membrane blebbing and formation of apoptotic bodies in the final stage of apoptosis which are rapidly engulfed by phagocytosis that avoids an inflammatory response in surrounding tissues [23–25]. The shrinkage of the cell is one of the most common morphological changes in apoptotic cell death resulted from the extreme alteration in intracellular water. Intracellular water plays a critical role in apoptotic and necrotic cell death. Although necrotic cells absorb the water resulting in enlarging the size and finally burst, apoptotic cells lose water leads to reduction in cellular volume. Therefore, the apoptotic cells become smaller in size, the cytoplasm is dense and the organelles are more tightly packed. Consequently, due to the occurrence of cell shrinkage, the cell will lose its contact

with neighboring cells, or the extracellular matrix and acquire more rounded morphology. Although the plasma membrane is intact during the entire process, at later stage of apoptosis, loss of membrane integrity and formation of the blebs at the cell surface due to the separation of the plasma membrane from cytoskeleton occur in apoptotic cells [26–28].

2.1.3 Biochemical changes in apoptosis

Apart from structural alterations, several biochemical changes also play key events in apoptosis. Apoptotic cells generally display major types of biochemical modifications such as caspase activation, protein and DNA cleavage, and plasma membrane alterations, which lead to phagocytic recognition [13]. Disruption of plasma membrane asymmetry is a common feature of apoptotic cells, independent of the form of apoptotic stimulus. The maintenance of lipid asymmetry of plasma membrane is regulated through transporters named flippases and floppases. In addition, the activated scramblase enzymes have an important role in the loss of lipid asymmetry and enhanced phosphatidylserine (PS) exposure to the outer leaflet of plasma membrane [13, 29].

Therefore, in a healthy cell, PS is limited to the inner layer of the plasma membrane. However, during apoptosis, effector caspases cleave and activate scramblase, as well as cleave and inactivate flippase, responsible for transmitting PS from the outer to the inner leaflet that lead to externalization of PS. Therefore, phosphatidylserine, which is normally localized in the inner membrane layer of cells is flipped out and externalized on the outer layer of the plasma membrane. This PS externalization not only is the indicator of loss of membrane asymmetry during apoptosis, but also allows early recognition by phagocytes and prevents the release of proinflammatory cellular components [29–31].

2.1.4 Pathway of apoptosis

As mentioned earlier, the mechanism of apoptosis involves an energy-dependent cascade of molecular events. Apoptotic cell death machinery includes the mitochondria-dependent (intrinsic) pathway and death receptor-dependent (extrinsic) pathway. The intrinsic pathway arises from intracellular signals like cellular stress and DNA damage and relies on the release of proteins from the intermembrane space of mitochondria. However, the extrinsic pathway is activated through the binding of extracellular ligands to death receptors at the cell surface that trigger the multiprotein complex formation known as death-inducing signaling complex (DISC). These two mitochondria- and death receptor-mediated pathways are interconnected and the molecules in one pathway can affect another pathway [32, 33].

2.1.5 The intrinsic mitochondrial pathway

As its name implies, the intrinsic pathway is activated in response to internal stimuli such as hypoxia, severe DNA damage and oxidative stress and mitochondria play a critical role throughout this apoptosis signaling pathway [34, 35]. The intrinsic pathway is mainly controlled by the members of Bcl-2 family proteins, which regulate the permeabilization of mitochondrial outer membrane (MOM) and are structurally and functionally classified into three groups. BH3-only proteins, like Bim and Bik, that sense cellular stress and directly or indirectly activate the executioner proteins, like Bax, Bak, Bid, that are able to oligomerize in

and permeabilize the MOM. The oligomerization of these pro-apoptotic proteins leads to component release form the intermembrane space to the cytoplasm and activation of effector caspases of apoptosis. The first two groups are known as the pro-apoptotic proteins of Bcl-2 family. The third group is the anti-apoptotic proteins, like Bcl-2 and Bcl-xL that hinder the overall process by inhibiting pro-apoptotic proteins. However, not the absolute quantity but rather the relative levels and balance between the pro- and anti-apoptotic proteins regulates whether the apoptosis event would be initiated. Although the excess of pro-apoptotic proteins makes the cells sensitive to apoptosis, excess of anti-apoptotic proteins makes the cells resistant and prevents the occurrence of apoptosis [36–38]. However, in the presence of apoptotic stimuli, the death signal is sensed initially by the BH3-only protein, which then interacts with the downstream mediators of apoptosis such as Bax. As the intrinsic mitochondrial pathway is initiated, Bax is translocated from cytosol to the outer mitochondrial membrane. The assembly of Bax proteins in mitochondrial outer membrane results in protein-lined channels or pore formation and intensely increase its permeability that cause a dramatic loss of electrical potential in mitochondria and cytochrome c release to cytoplasm. Subsequently, released cytochrome c binds to APAF-1 to facilitate the formation of the apoptosome, a wheel shaped heptametrical complex, which can then recruit and activate pro-caspase-9. Consequently, caspase-9 activates effector caspases (caspase-3/-7) that eventually lead to apoptosis (**Figure 1**) [39–41].

2.1.6 The extrinsic death receptor pathway

The extrinsic pathway is activated through the interactions between the transmembrane death receptors of the tumor necrosis factor (TNF) superfamily and their related ligands. The TNF receptor family has common cysteine-rich extracellular domains and cytoplasmic death domains that involve in transmitting the death signal from the cell surface to the intracellular signaling pathways. Ligation of death receptors with death ligands causes conformational change in death domain and consequently recruits apoptosis-related adaptor proteins that associate with procaspase-8/-10. At this point, a death-inducing signaling complex (DISC) consisting of the death receptor, an adaptor molecule, and pro-caspase-8/−10 is formed, resulting in the auto-catalytic activation of procaspases (**Figure 1**). The activated form of the caspase-8/-10 enzyme, as an initiator caspase, subsequently cleaves and activates other downstream or executioner caspases [42, 43]. Finally, both apoptotic pathways result in the activation of effector caspases (caspase-3/-7) causing the cleavage of key cellular macromolecules which are required for normal cellular function. They cleave the structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes and activate degradative enzymes such as DNases, which together contribute to the typical morphological changes and promote cell death [44, 45].

2.2 Dysregulation of apoptosis in carcinogenesis

The pathogenesis of many diseases is most closely related to inappropriate apoptosis (either too little or too much) and cancer is one of the situations where too little apoptosis happens, leading to malignant cells that highly proliferate. Defects at any points along apoptotic pathways may lead to malignant transformation of the affected cells, tumor metastasis, and resistance to anti-cancer drugs [12, 46]. Defects in Bcl-2 family of proteins and caspases are well-known chief factors to be involved in the evasion of apoptosis by tumor cells.

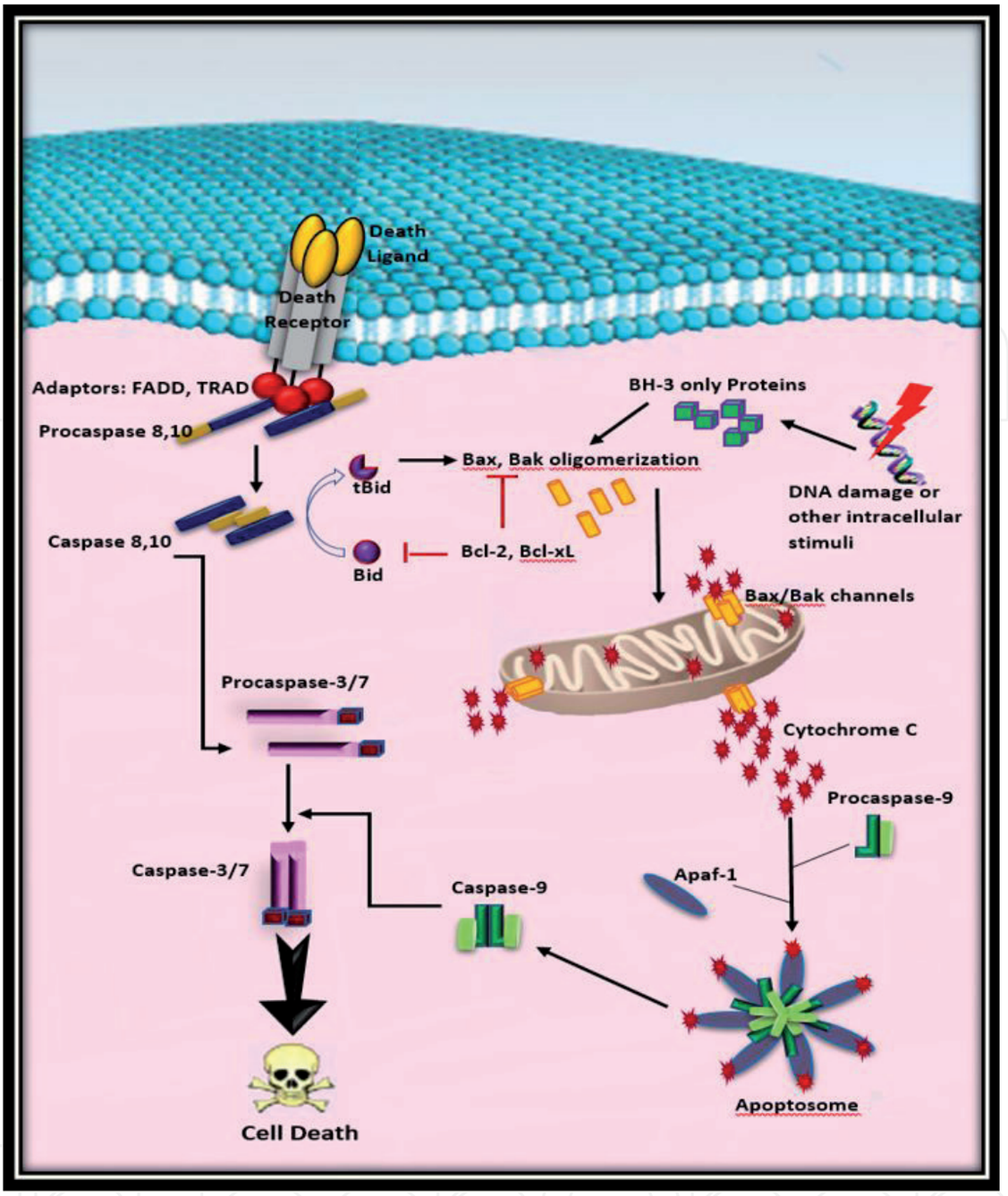

Figure 1.
Apoptosis signaling pathways. Abbreviations: TRADD, TNF receptor-associated death domain protein; FADD, Fas-associated death domain protein; Bid, BH3 interacting-domain death agonist; Bak, Bcl-2 homologous antagonist/killer; tBid, truncated BID; Bax, Bcl-2 associated X protein; APAF-1, apoptotic protease activating factor-1; Bcl-2, B-cell lymphoma 2, Bcl-xL; B-cell lymphoma-extra large.

2.2.1 Dysfunction of the Bcl-2 family of proteins in apoptosis

The Bcl-2 family of proteins consist of pro-apoptotic and anti-apoptotic proteins that act as a master regulator of initiation of apoptosis through intrinsic pathway and function chiefly at the mitochondrial level. The first protein of this family, B-cell lymphoma 2 (Bcl-2), was recognized almost 30 years ago. Currently 25 members of the Bcl-2 family have been determined and based on the presence of conserved Bcl-2 homology (BH) domains and their role in mitochondrial-mediated apoptosis, they are categorized into the following three subfamilies [47, 48]. Anti-apoptotic subgroup consisting of Bcl-2, Bcl-w, Bcl-xL, A1/Bfl-1, Mcl-1and Bcl-B/Bcl2L10 proteins contain four BH domains designated as 1–4 and inhibit the

apoptosis occurrence so named pro-survival proteins. However, second group, known as apoptosis effectors, belongs to pro-apoptotic members of this family containing BH 1–3 and missing the BH4 domain. Some example of this group are Bak, Bax, and Bok/Mtd. The last group that can be considered as subdivision of pro-apoptotic proteins including Bik, Bid, Bim, Bmf, Puma, Bad, Hrk and Noxa are named "BH3-only"' proteins as they contain only the BH3 domain. The members of this group function as initiators of apoptosis and the major mediators of the interaction with anti-apoptotic proteins [47, 49, 50]. Structural studies have determined that BH1, BH2 and BH3 areas together form a hydrophobic pocket that can be filled by the amphipathic a-helical BH3 domain of pro-apoptotic Bcl-2 proteins. Consequently, Bcl-2 family interactions regulate mitochondrial outer membrane (MOM) integrity and function and eventually onset of mitochondrial-mediate apoptosis [37, 51].

The balance disturbance of anti-apoptotic and pro-apoptotic proteins cause to dysregulated apoptosis in the affected cells. Altered expression of these proteins frequently occurs in cancers. Overexpression of anti-apoptotic proteins such as Bcl-2 or Bcl-xL occurs in a huge number of human cancers [52–55]. In one study, targeted proteomic analysis have revealed the contribution of Bcl-2 overexpression to cell survival of laryngeal carcinoma (LC) though its interaction with Hsp90β and formation of Bcl-2 Hsp90β complex involving in the anti-apoptotic progression of LC [56]. In cervical cancer SiHa cells, overexpressing Bcl-2 gene, the suppression of down-regulation of HPV16 E7 and c-myc gene expression may inhibit the induction of apoptosis [57]. Besides, high levels of Bcl-2 have been reported in hematological malignancies. Various mechanisms such as gene amplification, chromosomal translocations and dysregulation of miRNAs involved in Bcl-2 RNA degradation may cause to Bcl-2 upregulation [58–60]. Furthermore, there have been a number of studies reporting the involvement of Bcl-xL anti-apoptotic protein in tumorigenesis. The increased level of Bcl-xL gene expression determined in human cancers such as colorectal cancer, breast cancer, gastric adenomas and carcinomas, hepatocellular carcinoma and prostate cancer promotes cancer cell survival [61–65]. In addition, several attempts have revealed the association of enhanced levels of Bcl-xL and MCL1 with the malfunction of miRNAs that usually diminish their expression such as miR-29, miR-125b, miR-193 [66–68]. Furthermore, overexpression of anti-apoptotic Bcl-2 and its close relatives have been recognized as chief components of chemoresistance [69–72].

Deficiency in pro-apoptotic members of the Bcl-2 family has also been extensively studied in tumorigenesis and cancers. Pro-apoptotic gene Bim is frequently silenced in human Burkitt's lymphoma [73, 74]. Homozygous deletion and the loss of mRNA and protein expression have also been determined in mantle cell lymphoma and renal cell carcinoma. Hence, blocking Bim expression caused by gene deletion or epigenetic silencing is mainly contributed to pathogenesis of these tumors [75, 76]. Furthermore, a number of researchers have reported that down-regulation and mutation of Bax plays a significant role in tumor resistance to apoptosis. Reduced Bax expression was reported to be correlated with acquiring resistance to 5-FU in colorectal cancer cell line and cisplatin in ovarian carcinoma [77, 78]. Sensitivity of non-small cell lung cancer to Zoledronic was also found to be Bax dependent [79]. Suppressed Bax activity is one of the major reasons of TRAIL resistance in melanoma [80–82]. Besides, inactivated mutation in gene Bax such as frameshift mutations, loss of function mutations and point mutations has been reported in colon cancers, certain hematopoietic malignancies and acquired resistance to antineoplastic drugs [83–85]. Additionally, cells lacking both Bax and Bak have confirmed to be completely resistant to truncated Bid (t-Bid)-induced cytochrome c release and apoptosis [86]. Therefore, all these abnormalities regarding

Bcl-2 family protein members affect the ratio and equilibrium of pro-apoptotic to anti-apoptotic proteins which result in apoptosis dysfunction and resistance to cell death.

2.2.2 Dysfunction of caspases in apoptosis

Caspases are a family of cysteine proteases that play crucial role in initiation and execution of apoptosis signaling pathway. During tumorigenesis, altered caspase activity or deficiency in their functions may lead to impairing apoptosis induction resulting in intense misbalance in the growth dynamics that eventually cause to decreased apoptosis, irregular growth of cancer cells and carcinogenesis [17, 87]. Human cancer cells dysregulate caspase activity through a different mechanism such as inactivated mutation, down-regulation and epigenetic alteration blocking their apoptotic activity [88–90].

Caspase-3/-7 is a critical executioner molecule in apoptotic mechanism through cleaving a variety of key cellular proteins. Many studies have demonstrated the close association of altered caspase-3 expression and various cancers such as cervical adenocarcinoma, colon cancer, glioma and breast cancer [91–97]. However, the role of caspase-3 in breast cancer patients has been an area of controversy. Meta-analysis study of 3091 cases have revealed that enhanced expression of caspase 3 is related to poor overall survival in patients [98].

As mentioned earlier, the activation of executioner caspases involves their proteolytic cleavage through mature and functioning initiator caspases. Therefore, deficiency in initiator caspases activity has been determined in cancer development and progression [99, 100]. Caspase-9 plays a critical role in the initiation phase of the intrinsic apoptosis pathway. Decreased levels of caspase-9 was reported in patients with stage II colorectal cancer associated with poor clinical outcome [90, 101]. Inhibition of caspase 9 activity has been reported to be involved in acquired cisplatin resistance in head and neck squamous cell carcinoma cells [102]. Several functional polymorphism of caspase-9 has also been determined which may influence its expression or activity and therefore alter susceptibility to cancer [103–106].

Since extrinsic signaling of apoptosis mechanism after external stimulation of the death receptors is mediated through initiators caspase-8 and caspase-10, their deregulated expression or function can block death receptor signaling pathway contributing to cancer development. Expression of caspase-10 was found to be reduced in rectal cancer [107]. The cDNA array analysis has also detected the reduced co-expression of initiator caspases of extrinsic pathway, caspase 8 and 10, that might contribute to the pathogenesis of choriocarcinoma [108]. In previous investigations, expression analysis of caspase-8 has shown its down regulation in breast cancer cell lines and tumor tissues of breast cancer and revealed significant association between altered caspase-8 expression and status of HR in breast cancer patients [109]. Some studies also revealed that loss of caspase-8 expression not only cause to reduced apoptosis, but also involved in enhanced cell migration, tumor microenvironment and amplification of MYCN oncogene which highlight its contribution in carcinogenesis. The lack of caspase-8 expression happens very commonly in neuroendocrine cancers such as glioblastoma, medulloblastoma, neuroblastoma [110–112]. Furthermore, the correlation between caspase-8 with cancer prognosis, cancer stage and therapy resistance has been reported [109, 110]. Loss of initiator caspase-8 protein expression has been shown to be related with undesirable survival outcome in medulloblastoma and gynecological tumors such as ovarian and breast cancers and stage of head and neck squamous cell carcinoma (HNSCC) [113, 114].

2.3 Targeting cellular apoptosis machinery in cancer treatment

Since inhibition of apoptosis lies at the heart of all abnormal malignant growth, metastasis and conferring therapeutic failure, targeting the apoptosis mechanism players is of vital importance in cancer therapy. In this regard, Bcl-2 family of proteins as gate-keepers of intrinsic apoptotic pathway mediating the pro- and anti-apoptotic function at the mitochondrial level and caspases as the central player in the initiation and execution of apoptotic cell death have been the center of attraction for drug discovery studies and development of anticancer agents [10, 115, 116]. Here, various therapeutic strategies designed to target them have been reviewed.

2.3.1 Therapeutic opportunities based on Bcl-2 family proteins modulation

In view of the critical role of Bcl-2 proteins in regulation of mitochondrial pathway of apoptosis, targeting various members of this family have been considered amongst the most promising therapeutic strategies in cancer, a well-known dysfunctional apoptosis disorder [117]. Numerous attempts have been carried out to target the modifications in Bim expression and therefore regulate tumor cell response to apoptosis. Histone deacetylase inhibitors have been shown not only cause to up regulation of Bim in transformed cells, but also they are able to reverse silencing of Bim in cancer cells and consequently restored their sensitivity to various anticancer-agents reported in leukemia and Burkitt's lymphoma cells [118]. The proteasome inhibitors are also recognized to promote accumulation of Bim and enhance the lethality of cancer cells [119, 120]. Another approach is through diminishing its degradation by blocking its phosphorylation. Ras/Raf/MEK/ERK pathway have a key role in regulating the expression and function of Bim through its phosphorylation and triggering its proteasomal degradation. MEK1/2 Inhibitors has been applied to disrupt this process leading to accumulation of Bim and consequently apoptosis. MEK1/2 Inhibitors are also able to modify the interaction between BIM and other Bcl-2 family members contributing to cell death [118, 121, 122].

Furthermore, structure-based drug design can be applied to discover anticancer agents which are able to effectively activate a pro-apoptotic Bcl-2 protein through changing its conformation promoting cell death. Bax as a unique entry point for intrinsic apoptotic signaling is another major pro-apoptotic member of the Bcl-2 family proteins which has been greatly getting attention to be targeted in order to control apoptosis. Recent studies have revealed that direct binding and activation of Bax can be a promising approach for cancer treatment. Discovery of small-molecule functioning as a Bax activators may result in selective induction of tumor cell apoptosis and overcome chemoresistance which has been proved through invitro and invivo studies [117, 123]. Besides, some studies targeting a regulatory site in Ser184 of Bax protein have determined that its agonists SMBA1–SMBA3 can effectively bind to and trigger its oligomerization through the suppression of its phosphorylation that eventually lead to cyrochrome c release and induction of apoptosis in mouse lung cancer xenografts [124] . Similar results were also reported with other Bax agonists as promising Bax direct activators in breast cancer, glioblastoma and acute myeloid leukemia cells. These drug candidates demonstrated noteworthy in vivo efficiency inhibiting xenograft tumor growth though induction of apoptotic cell death [125–127].

The next emerging strategy in cancer drug discovery was the BH3 mimetics which are able to antagonize the function of Bcl-2 and selectively kill cancer cells. In this approach, BH3 mimetics are antagonists of the anti-apoptotic Bcl-2 proteins. These small molecules acting as the competitive inhibitors induce apoptosis though binding to their hydrophobic cleft and therefore affect the interactions between

anti- and pro-apoptotic proteins [128]. Various BH3 mimetics with different level of specificity and efficiency have been reported. For instance, TW-37 derived from phenolic aldehyde gossypol has been showing high affinity to bind MCL-1, Bcl-2 and Bcl-xL anti-apoptotic proteins and induce apoptosis in B-cell lymphomas and pancreatic cell lines along with decreasing tumor size in xenograft models [129–131]. As ABT-737 mimicked the BH3 domain of Bad protein, it was able to bind selectively to Bcl-2, Bcl-xL and Bcl-W. It also demonstrated poor affinity to other member of ani-apoptic proteins including MCL-1 and BFL-1. ABT-737 has shown efficacy in the killing of several cancer cell lines including leukemia, lymphoma, multiple myeloma, glioma and small cell lung cancer cell lines as well as primary samples. Also, these two inhibitors of Bcl-2 families are currently in clinical trials [132–134].

Another approach to antagonize the function of Bcl-2 anti-apoptotic proteins is focusing on the protein interaction among members of Bcl-2 family through their essential death domain. In this regard, peptide-based inhibitors have been significant achievements in targeting and regulating intracellular protein–protein interaction. Stapled peptides are synthetic, bioactive α-helical peptides locked into their bioactive structure that have brought new hope to target drug discovery [135, 136]. For instance, stabilized alpha-helix of Bcl-2 domains, SAHBs, is the peptide having the ability to penetrate leukaemia cells and trigger induction of apoptosis through its binding to the Bcl-xL which its function has been further confirmed though invivo mouse xenograft models of leukaemia [137]. Another research study has also revealed that exclusive MCL-1 stapled peptide inhibitor (MCL-1 SAHBD) can effectively resensitize cancer cells to caspase-mediated apoptosis through directly targeting of MCL-1 and suppress its inhibitory interaction with Bak protein [138].

2.3.2 Therapeutic opportunities based on caspase modulation

Given the vital role of caspases in the regulation of apoptosis, it is not surprising that numerous therapeutic opportunities targeting caspase activity demonstrate great promise for the cancer treatment. Different strategies have been investigated to upregulate caspase-8 expression to restore its function in tumors. As hypermethylation of its promotor has been recognized as the main mechanism of silencing, one approach for its reactivation is using demethylation agents. Azacytidine, decitabine and nucleoside analogs promoting the demethylation of caspase-8 promotor have been successfully applied in neuroblastoma, medulloblastoma, breast cancer and lung carcinoma [139, 140]. Another interesting strategy is designing the small molecules that selectively and directly target and trigger caspase-8 activation. These small molecules has been reported to potentiate TRAIL-induced cell death [141]. Proteosomal inhibitors such as bortezomib has been also reported to increase total cellular caspase-8 levels apparently by blocking its degradation [111, 142]. Some studies have also reported that the use of interferons can elevate the caspase-8 expression through modification at transcriptional level. This strategy targeting interferon-sensitive response elements within the caspase-8 promoter leading to sensitize cancer cell to apoptotic cell death in cancer chemotherapy or irradiation therapy [139, 143, 144].

Besides, developing molecules that are able to directly activates caspase 3 have been of research interests as well. For this purpose, particular sequence of inactive procaspase-3 consisting of the triplet of aspartic acid residues has been targeted. In vitro studies have exhibited that PETCM, gambonic acid and its derivatives have the potential to effectively activate caspase 3 leading to apoptotic cell death in cancer cell lines [145–147]. Furthermore, procaspase-activating compound1 (PAC-1) has been shown to induce anticancer activity through promoting the procaspase-3 activation. PAC-1 exerted its effect by chelation of inhibitory labile zinc ions and currently is in phase I clinical trial for cancer treatment [148].

In order to sensitize tumor cells to apoptotic stimuli, caspase –9 can be also regarded as a potential target in cancer therapy. There are a wide range of molecules such as protein kinase, microRNAs and heat shock protein that have been identified to modulate caspase-9 expression and hence have been getting interest as candidates for new drug development though regulating intrinsic apoptosis in cancer cells [149, 150]. Targeting caspase-9 have been also initiated in clinical trials (phase I) against several cancer including Chronic Myeloid Leukemia, non-Hodgkin's lymphoma, Acute Lymphoblastic Leukemia, [151, 152].

In addition, several attempts have also been conducted on cancer gene therapy focusing on apoptotic caspases. Gene transfer technologies may restore caspase gene expression resulting in selectively induction of apoptosis in various tumor types [153–155]. In this regard, caspase-9 and caspase-3 has been suggested for being used in gene therapy strategies. A main benefit of involving these caspases is that they start apoptosis at the downstream of the mitochondrial outer membrane potential and they will not be affected with the enhanced expression of anti-apoptotic of Bcl-2 proteins. The researchers conducted on inducible version of these caspases have shown encouraging results related to remarkable reduction in size of lung and gastric tumors, respectively [156–158].

Other than directly targeting of caspases, another area of research has focused on discovery of anticancer agents that trigger the caspases activity indirectly. In this approach, certain members of the inhibitors of apoptosis proteins (IAP) are targeted. IAPs are functioning as the endogenous caspase inhibitors and prevent apoptosis event by binding and inhibiting caspases through the degradation of active caspases or keeping them away from their substrate. In this regard, numerous researches have investigated various IAP inhibiting agents, accomplishing a breakthrough in cancer treatment [159, 160]. Some of these agents are acting as the IAP antagonist and exert their effect via suppression of their activity, while others are analogs of the endogenous IAP inhibitor Smac. Several Smac mimetics such as LCL161 and birinapant IAP inhibitors have currently being tested in phaseI/II in clinical trials, with promising outcomes [161–164]. Besides, IAP inhibitors have been reported to exert the synergistic effect in combination chemotherapy and sensitize the cancer cells to radiotherapy which is of particular interest in malignant gliomas [165–167].

3. Conclusion

It is well established that the apoptosis dysfunction promotes the malignant transformation and renders the cancer cell resistant to treatment. Targeting apoptotic pathways in tumor cells has been a main clinical interest as the evasion of apoptosis is a hallmark of all cancers regardless of their causes or types. There are numerous defects found in apoptotic mechanism contributing to inhibition of cancer cell death. As demonstrated in this chapter, impaired activation of caspases and disturbance in the balance between anti-apoptotic and pro-apoptotic members of Bcl-2 family proteins are remarkably involved in tumorgenesis. The enhanced knowledge about their critical roles in apoptosis and cell fate in recent years has eventually made them promising therapeutic targets. This also has facilitated the generation of more specific anticancer agents and led to shifting in anticancer therapy form typical cytotoxic approaches to the designing and development of apoptosis-inducing drugs that particularly target the cancer cells. An exciting development in successful eradication of cancer cells involves structure-based drug design of small molecules such as BH3 mimetics, specifically targeting Bcl-2 proteins, that is currently being tested in clinical trials with promising effects of

selective induction of tumor cell apoptosis and overcoming chemoresistance as well. These inhibitor molecules are in continuous development and a great deal of effort is required to discover the most efficient ones having more specificity for individual Bcl-2 proteins and offer maximal clinical efficacy. Besides, new therapeutic applications targeting apoptotic caspases including gene therapy approaches and small molecules suppressing inhibitors of caspases are beginning to show some promise through selectively and directly targeting of individual caspases and eventually triggering their activity. Caspase-targeted approaches, epigenetic modulators and their combinations with established therapies may have the potential to overcome the limitation of previous strategies through exerting synergistic pro-apoptotic activity and may enhance the effectiveness of conventional cancer therapy, worthy of further investigation in preclinical advanced models and clinical trial. Apoptosis-targeted therapies are now remarkably advancing and remain a promising approaches in future clinical practice of oncology.

Acknowledgements

I would like to thank Prof. Mahmood Ameen Abdulla and Dr. Jayakumar Rajarajeswaran for their valuable guidance.

Conflict of interest

There is no conflict of interest.

Author details

Reyhaneh Farghadani* and Rakesh Naidu*
Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway, Selangor Darul Ehsan, Malaysia

*Address all correspondence to: r_farghadani@yahoo.com; reyhaneh.farghadani@monash.edu and rakesh.naidu@monash.edu

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Hejmadi M. Introduction to cancer biology. London: Bookboon; 2009.

[2] World Health Organization. Cancer 2018 [Available from: http://www.who.int/news-room/fact-sheets/detail/cancer.

[3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

[4] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-E86.

[5] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5-29.

[6] Fernald K, Kurokawa M. Evading apoptosis in cancer. Trends Cell Biol. 2013;23(12):620-33.

[7] Pfeffer C, Singh A. Apoptosis: a target for anticancer therapy. Int J Mol Sci. 2018;19(2):448.

[8] Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nature reviews Clinical oncology. 2020;17(7):395-417.

[9] Campbell KJ, Tait SW. Targeting BCL-2 regulated apoptosis in cancer. Open biology. 2018;8(5):180002.

[10] Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY). 2016;8(4):603.

[11] Koff JL, Ramachandiran S, Bernal-Mizrachi L. A time to kill: targeting apoptosis in cancer. Int J Mol Sci. 2015;16(2):2942-55.

[12] Wong RS. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res. 2011;30(1):87.

[13] Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495-516.

[14] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wideranging implications in tissue kinetics. Br J Cancer. 1972;26(4):239.

[15] Pfeffer CM, Singh AT. Apoptosis: a target for anticancer therapy. Int J Mol Sci. 2018;19(2):448.

[16] Goodsell DS. The molecular perspective: caspases. Stem Cells. 2000;18(6):457-8.

[17] McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol. 2015;7(4).

[18] Parrish AB, Freel CD, Kornbluth S. Cellular mechanisms controlling caspase activation and function. Cold Spring Harb Perspect Biol. 2013;5(6):a008672.

[19] Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene. 2008;27(48):6194.

[20] Shi Y. Caspase activation: revisiting the induced proximity model. Cell. 2004;117(7):855-8.

[21] Cohen GM. Caspases: the executioners of apoptosis. Biochem J. 1997;326(1):1-16.

[22] Kitazumi I, Tsukahara M. Regulation of DNA fragmentation: the

role of caspases and phosphorylation. The FEBS journal. 2011;278(3):427-41.

[23] Doonan F, Cotter TG. Morphological assessment of apoptosis. Methods. 2008;44(3):200-4.

[24] Farghadani R, Rajarajeswaran J, Hashim NBM, Abdulla MA, Muniandy S. A novel β-diiminato manganese III complex as the promising anticancer agent induces G 0/G 1 cell cycle arrest and triggers apoptosis via mitochondrial-dependent pathways in MCF-7 and MDA-MB-231 human breast cancer cells. RSC Advances. 2017;7(39):24387-98.

[25] Caruso S, Atkin-Smith GK, Baxter AA, Tixeira R, Jiang L, Ozkocak DC, et al. Defining the role of cytoskeletal components in the formation of apoptopodia and apoptotic bodies during apoptosis. Apoptosis. 2019;24(11-12):862-77.

[26] Model MA, Schonbrun E. Optical determination of intracellular water in apoptotic cells. The Journal of Physiology. 2013;591(23):5843-9.

[27] Gómez-Angelats M, Cidlowski JA. Invited review: cell volume control and signal transduction in apoptosis. Toxicol Pathol. 2002;30(5):541-51.

[28] Orlov SN, Platonova AA, Hamet P, Grygorczyk R. Cell volume and monovalent ion transporters: their role in cell death machinery triggering and progression. American Journal of Physiology-Cell Physiology. 2013.

[29] Julian L, Olson MF. Apoptotic membrane dynamics in health and disease. Cell Health and Cytoskeleton. 2015;2015(7):133-42.

[30] Segawa K, Nagata S. An Apoptotic 'Eat Me' Signal: Phosphatidylserine Exposure. Trends Cell Biol. 2015;25(11):639-50.

[31] Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL. Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. J Biol Chem. 2000.

[32] Green DR, Llambi F. Cell death signaling. Cold Spring Harb Perspect Biol. 2015;7(12):a006080.

[33] Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nature Reviews Cancer. 2002;2(4):277.

[34] Li MX, Dewson G. Mitochondria and apoptosis: emerging concepts. F1000prime reports. 2015;7.

[35] Wu C-C, Bratton SB. Regulation of the intrinsic apoptosis pathway by reactive oxygen species. Antioxidants & redox signaling. 2013;19(6):546-58.

[36] Montero J, Letai A. Why do BCL-2 inhibitors work and where should we use them in the clinic? Cell Death Differ. 2018;25(1):56.

[37] Shamas-Din A, Kale J, Leber B, Andrews DW. Mechanisms of action of Bcl-2 family proteins. Cold Spring Harb Perspect Biol. 2013;5(4):a008714.

[38] Siddiqui WA, Ahad A, Ahsan H. The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update. Arch Toxicol. 2015;89(3):289-317.

[39] Chipuk JE, Green DR. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends Cell Biol. 2008;18(4):157-64.

[40] Tait SW, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol. 2010;11(9):621-32.

[41] Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD,

et al. Ordering the cytochrome c–initiated caspase cascade: hierarchical activation of caspases-2,-3,-6,-7,-8, and-10 in a caspase-9–dependent manner. The Journal of cell biology. 1999;144(2):281-92.

[42] Nair P, Lu M, Petersen S, Ashkenazi A. Apoptosis initiation through the cell-extrinsic pathway. Methods Enzymol. 544: Elsevier; 2014. p. 99-128.

[43] Ricci MS, El-Deiry WS. The extrinsic pathway of apoptosis. Apoptosis, Senescence, and Cancer: Springer; 2007. p. 31-54.

[44] Behzadi P, Ranjbar R. Caspases and apoptosis. Cell. 2015;4(7):11-3.

[45] Jänicke RU, Sprengart ML, Wati MR, Porter AG. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem. 1998;273(16):9357-60.

[46] Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. Cell Death Differ. 2005;12(S1):942.

[47] Hatok J, Racay P. Bcl-2 family proteins: master regulators of cell survival. Biomolecular Concepts. 2016;7(4):259-70.

[48] Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev. 1999;13(15):1899-911.

[49] Shamas-Din A, Brahmbhatt H, Leber B, Andrews DW. BH3-only proteins: Orchestrators of apoptosis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2011;1813(4):508-20.

[50] Dewson G, Kluck RM. Bcl-2 family-regulated apoptosis in health and disease. Cell Health and Cytoskeleton. 2010;2:9-22.

[51] Petros AM, Olejniczak ET, Fesik SW. Structural biology of the Bcl-2 family of proteins. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2004;1644(2-3):83-94.

[52] Amundson SA, Myers TG, Scudiero D, Kitada S, Reed JC, Fornace AJ. An informatics approach identifying markers of chemosensitivity in human cancer cell lines. Cancer Res. 2000;60(21):6101-10.

[53] Reed JC. Bcl-2–family proteins and hematologic malignancies: history and future prospects. Blood, The Journal of the American Society of Hematology. 2008;111(7):3322-30.

[54] Trisciuoglio D, Tupone MG, Desideri M, Di Martile M, Gabellini C, Buglioni S, et al. BCL-X L overexpression promotes tumor progression-associated properties. Cell Death Dis. 2017;8(12):1-15.

[55] D'Aguanno S, Del Bufalo D. Inhibition of anti-apoptotic Bcl-2 proteins in preclinical and clinical studies: current overview in cancer. Cells. 2020;9(5):1287.

[56] Li S, Li J, Hu T, Zhang C, Lv X, He S, et al. Bcl-2 overexpression contributes to laryngeal carcinoma cell survival by forming a complex with Hsp90β. Oncol Rep. 2017;37(2):849-56.

[57] Deng Y, Lin C, Zheng J, Fu M, Liang X, Chen J, et al. Overexpression of Bcl-2 partly inhibits apoptosis of human cervical cancer SiHa cells induced by arsenic trioxide. Chin Med J. 2000;113(1):84-8.

[58] Perini GF, Ribeiro GN, Neto JVP, Campos LT, Hamerschlak N. BCL-2 as therapeutic target for hematological malignancies. J Hematol Oncol. 2018;11(1):65.

[59] Huang J, Fairbrother W, Reed JC. Therapeutic targeting of Bcl-2 family

for treatment of B-cell malignancies. Expert Rev Hematol. 2015;8(3):283-97.

[60] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proceedings of the National Academy of Sciences. 2005;102(39):13944-9.

[61] Schott A, Apel I, Nunez G, Clarke M. Bcl-XL protects cancer cells from p53-mediated apoptosis. Oncogene. 1995;11(7):1389-94.

[62] Jin-Song Y, Zhao-Xia W, Cheng-Yu L, Xiao-Di L, Ming S, Yuan-Yuan G, et al. Prognostic significance of Bcl-xL gene expression in human colorectal cancer. Acta Histochem. 2011;113(8):810-4.

[63] Scherr A-L, Gdynia G, Salou M, Radhakrishnan P, Duglova K, Heller A, et al. Bcl-x L is an oncogenic driver in colorectal cancer. Cell Death Dis. 2016;7(8):e2342-e.

[64] Watanabe J, Kushihata F, Honda K, Mominoki K, Matsuda S, Kobayashi N. Bcl-xL overexpression in human hepatocellular carcinoma. Int J Oncol. 2002;21(3):515-9.

[65] Castilla C, Congregado Bn, Chinchón D, Torrubia FJ, Japón MA, Sáez C. Bcl-xL is overexpressed in hormone-resistant prostate cancer and promotes survival of LNCaP cells via interaction with proapoptotic Bak. Endocrinology. 2006;147(10):4960-7.

[66] Chen J, Zhang X, Lentz C, Abi-Daoud M, Paré GC, Yang X, et al. miR-193b regulates Mcl-1 in melanoma. The American journal of pathology. 2011;179(5):2162-8.

[67] Gong J, Zhang J, Li B, Zeng C, You K, Chen M, et al. MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. Oncogene. 2013;32(25):3071-9.

[68] Shimizu S, Takehara T, Hikita H, Kodama T, Miyagi T, Hosui A, et al. The let-7 family of microRNAs inhibits Bcl-xL expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma. J Hepatol. 2010;52(5):698-704.

[69] Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26(9):1324-37.

[70] Konopleva M, Zhao S, Hu W, Jiang S, Snell V, Weidner D, et al. The anti-apoptotic genes Bcl-X(L) and Bcl-2 are over-expressed and contribute to chemoresistance of non-proliferating leukaemic CD34+ cells. Br J Haematol. 2002;118(2):521-34.

[71] de Melo Silva AJ. Bcl-2 Family Overexpression and Chemoresistance in Acute Myeloid Leukemia. Serbian Journal of Experimental and Clinical Research. 2018;19(4):299-309.

[72] Maji S, Panda S, Samal SK, Shriwas O, Rath R, Pellecchia M, et al. Bcl-2 antiapoptotic family proteins and chemoresistance in cancer. Adv Cancer Res. 137: Elsevier; 2018. p. 37-75.

[73] Anderton E, Yee J, Smith P, Crook T, White R, Allday M. Two Epstein–Barr virus (EBV) oncoproteins cooperate to repress expression of the proapoptotic tumour-suppressor Bim: clues to the pathogenesis of Burkitt's lymphoma. Oncogene. 2008;27(4):421-33.

[74] Richter-Larrea JA, Robles EF, Fresquet V, Beltran E, Rullan AJ, Agirre X, et al. Reversion of epigenetically mediated BIM silencing overcomes chemoresistance in Burkitt lymphoma. Blood, The Journal of the American Society of Hematology. 2010;116(14):2531-42.

[75] Tagawa H, Karnan S, Suzuki R, Matsuo K, Zhang X, Ota A, et al. Genome-wide array-based CGH for

mantle cell lymphoma: identification of homozygous deletions of the proapoptotic gene BIM. Oncogene. 2005;24(8):1348-58.

[76] Zantl N, Weirich G, Zall H, Seiffert BM, Fischer SF, Kirschnek S, et al. Frequent loss of expression of the pro-apoptotic protein Bim in renal cell carcinoma: evidence for contribution to apoptosis resistance. Oncogene. 2007;26(49):7038-48.

[77] Manoochehri M, Karbasi A, Bandehpour M, Kazemi B. Down-regulation of BAX gene during carcinogenesis and acquisition of resistance to 5-FU in colorectal cancer. Pathology & Oncology Research. 2014;20(2):301-7.

[78] Perego P, Giarola M, Righetti SC, Supino R, Caserini C, Delia D, et al. Association between cisplatin resistance and mutation of p53 gene and reduced bax expression in ovarian carcinoma cell systems. Cancer Res. 1996;56(3):556-62.

[79] Aoyagi T, Morii T, Ohtsuka K, Ohnishi H, Tajima T, Yoshiyama A, et al. Lung cancer cell line sensitivity to zoledronic acid is BAX-dependent. Anticancer Res. 2013;33(12):5357-63.

[80] Ravi R, Bedi A. Requirement of BAX for TRAIL/Apo2L-induced apoptosis of colorectal cancers: synergism with sulindac-mediated inhibition of Bcl-xL. Cancer Res. 2002;62(6):1583-7.

[81] Eberle J. Countering trail resistance in melanoma. Cancers (Basel). 2019;11(5):656.

[82] Deng Y, Lin Y, Wu X. TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. Genes Dev. 2002;16(1):33-45.

[83] Miquel C, Borrini F, Grandjouan S, Aupérin A, Viguier J, Velasco V, et al. Role of bax mutations in apoptosis in colorectal cancers with microsatellite instability. Am J Clin Pathol. 2005;123(4):562-70.

[84] Fresquet V, Rieger M, Carolis C, García-Barchino MJ, Martinez-Climent JA. Acquired mutations in BCL2 family proteins conferring resistance to the BH3 mimetic ABT-199 in lymphoma. Blood. 2014;123(26):4111-9.

[85] Kitada S, Pedersen IM, Schimmer AD, Reed JC. Dysregulation of apoptosis genes in hematopoietic malignancies. Oncogene. 2002;21(21):3459-74.

[86] Wei MC, Zong W-X, Cheng EH-Y, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science. 2001;292(5517):727-30.

[87] Fiandalo M, Kyprianou N. Caspase control: protagonists of cancer cell apoptosis. Exp Oncol. 2012;34(3):165.

[88] Ando M, Kawazu M, Ueno T, Fukumura K, Yamato A, Soda M, et al. Cancer-associated missense mutations of caspase-8 activate nuclear factor-κB signaling. Cancer Sci. 2013;104(8):1002-8.

[89] Teng Y, Dong YC, Liu Z, Zou Y, Xie H, Zhao Y, et al. DNA methylation-mediated caspase-8 downregulation is associated with anti-apoptotic activity and human malignant glioma grade. Int J Mol Med. 2017;39(3):725-33.

[90] Shen XG, Wang C, Li Y, Wang L, Zhou B, Xu B, et al. Downregulation of caspase-9 is a frequent event in patients with stage II colorectal cancer and correlates with poor clinical outcome. Colorectal Dis. 2010;12(12):1213-8.

[91] Jing Z, Heng W, Xia L, Ning W, Yafei Q, Yao Z, et al. Downregulation of

phosphoglycerate dehydrogenase inhibits proliferation and enhances cisplatin sensitivity in cervical adenocarcinoma cells by regulating Bcl-2 and caspase-3. Cancer Biol Ther. 2015;16(4):541-8.

[92] Zheng Ba, Chai R, Yu X. Downregulation of NIT2 inhibits colon cancer cell proliferation and induces cell cycle arrest through the caspase-3 and PARP pathways. Int J Mol Med. 2015;35(5):1317-22.

[93] Jia G, Wang Q, Wang R, Deng D, Xue L, Shao N, et al. Tubeimoside-1 induces glioma apoptosis through regulation of Bax/Bcl-2 and the ROS/Cytochrome C/Caspase-3 pathway. Onco Targets Ther. 2015;8:303.

[94] Zhou L, Luo Y, Li K, Tian L, Wang M, Li C, et al. Molecular markers of therapeutic resistance in breast cancer. Hum Pathol. 2013;44(7):1421-8.

[95] Zhou M, Liu X, Li Z, Huang Q, Li F, Li CY. Caspase-3 regulates the migration, invasion and metastasis of colon cancer cells. Int J Cancer. 2018;143(4):921-30.

[96] Pu X, Storr SJ, Zhang Y, Rakha EA, Green AR, Ellis IO, et al. Caspase-3 and caspase-8 expression in breast cancer: caspase-3 is associated with survival. Apoptosis. 2017;22(3):357-68.

[97] Palmerini F, Devilard E, Jarry A, Birg F, Xerri L. Caspase 7 downregulation as an immunohistochemical marker of colonic carcinoma. Hum Pathol. 2001;32(5):461-7.

[98] Yang X, Zhong D-N, Qin H, Wu P-R, Wei K-L, Chen G, et al. Caspase-3 over-expression is associated with poor overall survival and clinicopathological parameters in breast cancer: a meta-analysis of 3091 cases. Oncotarget. 2018;9(9):8629.

[99] Zhao R, Kaakati R, Lee AK, Liu X, Li F, Li C-Y. Novel roles of apoptotic caspases in tumor repopulation, epigenetic reprogramming, carcinogenesis, and beyond. Cancer Metastasis Rev. 2018;37(2-3):227-36.

[100] Kumar S. Caspase function in programmed cell death. Cell Death Differ. 2007;14(1):32-43.

[101] Asadi M, Shanehbandi D, Kermani TA, Sanaat Z, Zafari V, Hashemzadeh S. Expression level of caspase genes in colorectal cancer. Asian Pacific journal of cancer prevention: APJCP. 2018;19(5):1277.

[102] Kuwahara D, Tsutsumi K, Oyake D, Ohta T, Nishikawa H, Koizuka I. Inhibition of caspase-9 activity and Apaf-1 expression in cisplatin-resistant head and neck squamous cell carcinoma cells. Auris Nasus Larynx. 2003;30:85-8.

[103] Liamarkopoulos E, Gazouli M, Aravantinos G, Tzanakis N, Theodoropoulos G, Rizos S, et al. Caspase 8 and caspase 9 gene polymorphisms and susceptibility to gastric cancer. Gastric Cancer. 2011;14(4):317-21.

[104] Li P, Zhou L, Zhao T, Liu X, Zhang P, Liu Y, et al. Caspase-9: structure, mechanisms and clinical application. Oncotarget. 2017;8(14):23996.

[105] Yilmaz SG, Yencilek F, Yildirim A, Yencilek E, Isbir T. Effects of caspase 9 gene polymorphism in patients with prostate cancer. In Vivo. 2017;31(2):205-8.

[106] Ercan S, Arinc S, Yilmaz SG, Altunok C, Yaman F, Isbir T. Investigation of Caspase 9 Gene Polymorphism in Patients With Non-small Cell Lung Cancer. Anticancer Res. 2019;39(5):2437-41.

[107] Xu B, Zhou Z-G, Li Y, Wang L, Yang L, Zhou B, et al.

Clinicopathological significance of caspase-8 and caspase-10 expression in rectal cancer. Oncology. 2008;74(3-4):229-36.

[108] Fong P, Xue W, Ngan H, Chiu P, Chan K, Tsao S, et al. Caspase activity is downregulated in choriocarcinoma: a cDNA array differential expression study. J Clin Pathol. 2006;59(2):179-83.

[109] Aghababazadeh M, Dorraki N, Javan FA, Fattahi AS, Gharib M, Pasdar A. Downregulation of Caspase 8 in a group of Iranian breast cancer patients–A pilot study. J Egypt Natl Canc Inst. 2017;29(4):191-5.

[110] Kostova I, Mandal R, Becker S, Strebhardt K. The role of caspase-8 in the tumor microenvironment of ovarian cancer. Cancer Metastasis Rev. 2020:1-16.

[111] Stupack DG. Caspase-8 as a therapeutic target in cancer. Cancer Lett. 2013;332(2):133-40.

[112] Graf RP, Keller N, Barbero S, Stupack D. Caspase-8 as a regulator of tumor cell motility. Curr Mol Med. 2014;14(2):246-54.

[113] Pingoud-Meier C, Lang D, Janss AJ, Rorke LB, Phillips PC, Shalaby T, et al. Loss of caspase-8 protein expression correlates with unfavorable survival outcome in childhood medulloblastoma. Clin Cancer Res. 2003;9(17):6401-9.

[114] Elrod HA, Fan S, Muller S, Chen GZ, Pan L, Tighiouart M, et al. Analysis of death receptor 5 and caspase-8 expression in primary and metastatic head and neck squamous cell carcinoma and their prognostic impact. PLoS One. 2010;5(8):e12178.

[115] Baig S, Seevasant I, Mohamad J, Mukheem A, Huri H, Kamarul T. Potential of apoptotic pathway-targeted cancer therapeutic research: Where do we stand? Cell Death Dis. 2016;7(1):e2058-e.

[116] Boice A, Bouchier-Hayes L. Targeting apoptotic caspases in cancer. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2020;1867(6):118688.

[117] Liu Z, Ding Y, Ye N, Wild C, Chen H, Zhou J. Direct activation of bax protein for cancer therapy. Med Res Rev. 2016;36(2):313-41.

[118] Harada H, Grant S. Targeting the regulatory machinery of BIM for cancer therapy. Critical Reviews™ in Eukaryotic Gene Expression. 2012;22(2).

[119] Pigneux A, Mahon F-X, Moreau-Gaudry F, Uhalde M, De Verneuil H, Lacombe F, et al. Proteasome inhibition specifically sensitizes leukemic cells to anthracyclin-induced apoptosis through the accumulation of Bim and Bax pro-apoptotic proteins. Cancer Biol Ther. 2007;6(4):603-11.

[120] Dai Y, Chen S, Wang L, Pei X-Y, Kramer LB, Dent P, et al. Bortezomib interacts synergistically with belinostat in human aml and all cells in association with perturbations in NF-κB and BIM. Br J Haematol. 2011;153(2):222.

[121] Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ. Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. J Biol Chem. 2003;278(21):18811-6.

[122] Ewings KE, Wiggins CM, Cook SJ. Bim and the pro-survival Bcl-2 proteins: opposites attract, ERK repels. Cell cycle. 2007;6(18):2236-40.

[123] Zhao G, Zhu Y, Eno CO, Liu Y, DeLeeuw L, Burlison JA, et al. Activation of the proapoptotic Bcl-2 protein Bax by a small molecule induces tumor cell apoptosis. Mol Cell Biol. 2014;34(7):1198-207.

[124] Xin M, Li R, Xie M, Park D, Owonikoko TK, Sica GL, et al. Small-molecule Bax agonists for cancer therapy. Nature communications. 2014;5(1):1-11.

[125] Liu G, Kim H, Ding C, Yu Z, Wang H, Chen H, et al. Abstract 3: Discovery and optimization of small molecule Bax activators for cancer therapy. Cancer Res. 2019;79(13 Supplement):3-.

[126] Daniele S, Pietrobono D, Costa B, Giustiniano M, La Pietra V, Giacomelli C, et al. Bax activation blocks self-renewal and induces apoptosis of human glioblastoma stem cells. ACS Chem Neurosci. 2018;9(1):85-99.

[127] Reyna DE, Garner TP, Lopez A, Kopp F, Choudhary GS, Sridharan A, et al. Direct activation of BAX by BTSA1 overcomes apoptosis resistance in acute myeloid leukemia. Cancer Cell. 2017;32(4):490-505. e10.

[128] Chonghaile TN, Letai A. Mimicking the BH3 domain to kill cancer cells. Oncogene. 2008;27(1):S149-S57.

[129] Verhaegen M, Bauer JA, de la Vega CM, Wang G, Wolter KG, Brenner JC, et al. A novel BH3 mimetic reveals a mitogen-activated protein kinase–dependent mechanism of melanoma cell death controlled by p53 and reactive oxygen species. Cancer Res. 2006;66(23):11348-59.

[130] Wang Z, Azmi AS, Ahmad A, Banerjee S, Wang S, Sarkar FH, et al. TW-37, a small-molecule inhibitor of Bcl-2, inhibits cell growth and induces apoptosis in pancreatic cancer: involvement of Notch-1 signaling pathway. Cancer Res. 2009;69(7):2757-65.

[131] Mohammad RM, Goustin AS, Aboukameel A, Chen B, Banerjee S, Wang G, et al. Preclinical studies of TW-37, a new nonpeptidic small-molecule inhibitor of Bcl-2, in diffuse large cell lymphoma xenograft model reveal drug action on both Bcl-2 and Mcl-1. Clin Cancer Res. 2007;13(7):2226-35.

[132] Chauhan D, Velankar M, Brahmandam M, Hideshima T, Podar K, Richardson P, et al. A novel Bcl-2/Bcl-X L/Bcl-w inhibitor ABT-737 as therapy in multiple myeloma. Oncogene. 2007;26(16):2374-80.

[133] Tagscherer KE, Fassl A, Campos B, Farhadi M, Kraemer A, Böck B, et al. Apoptosis-based treatment of glioblastomas with ABT-737, a novel small molecule inhibitor of Bcl-2 family proteins. Oncogene. 2008;27(52):6646-56.

[134] Xia J, Bai H, Yan B, Li R, Shao M, Xiong L, et al. Mimicking the BIM BH3 domain overcomes resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer. Oncotarget. 2017;8(65):108522.

[135] Ali AM, Atmaj J, Van Oosterwijk N, Groves MR, Dömling A. Stapled peptides inhibitors: a new window for target drug discovery. Computational and structural biotechnology journal. 2019;17:263-81.

[136] McDougall L, Jamieson AG. Stapled Peptides as Potential Therapeutics. eLS. 2019:1-9.

[137] Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, et al. Activation of apoptosis in vivo by a hydrocarbon-stapled BH3 helix. Science. 2004;305(5689):1466-70.

[138] Stewart ML, Fire E, Keating AE, Walensky LD. The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. Nat Chem Biol. 2010;6(8):595-601.

[139] Fulda S, editor Therapeutic opportunities based on caspase modulation. Semin Cell Dev Biol; 2018: Elsevier.

[140] Hensley P, Mishra M, Kyprianou N. Targeting caspases in cancer therapeutics. Biol Chem. 2013;394(7):831-43.

[141] Bucur O, Gaidos G, Yatawara A, Pennarun B, Rupasinghe C, Roux J, et al. A novel caspase 8 selective small molecule potentiates TRAIL-induced cell death. Sci Rep. 2015;5:9893.

[142] Thorpe JA, Christian PA, Schwarze SR. Proteasome inhibition blocks caspase-8 degradation and sensitizes prostate cancer cells to death receptor-mediated apoptosis. The Prostate. 2008;68(2):200-9.

[143] Häcker S, Dittrich A, Mohr A, Schweitzer T, Rutkowski S, Krauss J, et al. Histone deacetylase inhibitors cooperate with IFN-γ to restore caspase-8 expression and overcome TRAIL resistance in cancers with silencing of caspase-8. Oncogene. 2009;28(35):3097.

[144] Ruiz-Ruiz C, de Almodóvar CR, Rodríguez A, Ortiz-Ferrón G, Redondo JM, López-Rivas A. The up-regulation of human caspase-8 by interferon-γ in breast tumor cells requires the induction and action of the transcription factor interferon regulatory factor-1. J Biol Chem. 2004;279(19):19712-20.

[145] Zhang H-Z, Kasibhatla S, Wang Y, Herich J, Guastella J, Tseng B, et al. Discovery, characterization and SAR of gambogic acid as a potent apoptosis inducer by a HTS assay. Biorg Med Chem. 2004;12(2):309-17.

[146] Roy S, Bayly CI, Gareau Y, Houtzager VM, Kargman S, Keen SL, et al. Maintenance of caspase-3 proenzyme dormancy by an intrinsic "safety catch" regulatory tripeptide. Proceedings of the National Academy of Sciences. 2001;98(11):6132-7.

[147] McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol. 2013;5(4):a008656.

[148] S Roth H, J Hergenrother P. Derivatives of procaspase-activating compound 1 (PAC-1) and their anticancer activities. Curr Med Chem. 2016;23(3):201-41.

[149] Kim B, Srivastava SK, Kim S-H. Caspase-9 as a therapeutic target for treating cancer. Expert Opin Ther Targets. 2015;19(1):113-27.

[150] Serrano BP, Hardy JA. Phosphorylation by protein kinase A disassembles the caspase-9 core. Cell Death Differ. 2018;25(6):1025-39.

[151] Zhou X, Dotti G, Krance RA, Martinez CA, Naik S, Kamble RT, et al. Inducible caspase-9 suicide gene controls adverse effects from alloreplete T cells after haploidentical stem cell transplantation. Blood. 2015;125(26):4103-13.

[152] Administration of Donor T Cells With the Caspase-9 Suicide Gene (DOTTI) [Internet]. 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT01494103.

[153] Cam L, Boucquey A, Coulomb-L'hermine A, Weber A, Horellou P. Gene transfer of constitutively active caspase-3 induces apoptosis in a human hepatoma cell line. The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications. 2005;7(1):30-8.

[154] Chen X, Godbey W. 638. Expression-Targeted Cancer Gene Therapy Using pran to Drive Caspase 3 Expression. Mol Ther. 2015;23:S254.

[155] Jia L-T, Chen S-Y, Yang A-G. Cancer gene therapy targeting cellular apoptosis machinery. Cancer Treat Rev. 2012;38(7):868-76.

[156] Zhang D-X, Zhao P-T, Xia L, Liu L-L, Liang J, Zhai H-H, et al. Potent inhibition of human gastric cancer by HER2-directed induction of apoptosis with anti-HER2 antibody and caspase-3 fusion protein. Gut. 2010;59(3):292-9.

[157] Ando M, Hoyos V, Yagyu S, Tao W, Ramos CA, Dotti G, et al. Bortezomib sensitizes non-small cell lung cancer to mesenchymal stromal cell-delivered inducible caspase-9-mediated cytotoxicity. Cancer Gene Ther. 2014;21(11):472-82.

[158] Hoyos V, Del Bufalo F, Yagyu S, Ando M, Dotti G, Suzuki M, et al. Mesenchymal stromal cells for linked delivery of oncolytic and apoptotic adenoviruses to non-small-cell lung cancers. Mol Ther. 2015;23(9):1497-506.

[159] Finlay D, Teriete P, Vamos M, Cosford N, Vuori K. Inducing death in tumor cells: roles of the inhibitor of apoptosis proteins [version 1; referees: 3 approved]. F1000Research. 2017;6(587).

[160] Cong H, Xu L, Wu Y, Qu Z, Bian T, Zhang W, et al. Inhibitor of apoptosis protein (IAP) antagonists in anticancer agent discovery: Current status and perspectives. J Med Chem. 2019;62(12):5750-72.

[161] Infante JR, Dees EC, Olszanski AJ, Dhuria SV, Sen S, Cameron S, et al. Phase I dose-escalation study of LCL161, an oral inhibitor of apoptosis proteins inhibitor, in patients with advanced solid tumors. J Clin Oncol. 2014;32(28):3103-10.

[162] Bardia A, Parton M, Kümmel S, Estévez LG, Huang C-S, Cortés J, et al. Paclitaxel with inhibitor of apoptosis antagonist, LCL161, for localized triple-negative breast cancer, prospectively stratified by gene signature in a biomarker-driven neoadjuvant trial. J Clin Oncol. 2018;36(31):3126-33.

[163] Noonan AM, Bunch KP, Chen JQ, Herrmann MA, Lee JM, Kohn EC, et al. Pharmacodynamic markers and clinical results from the phase 2 study of the SMAC mimetic birinapant in women with relapsed platinum-resistant or-refractory epithelial ovarian cancer. Cancer. 2016;122(4):588-97.

[164] Amaravadi RK, Schilder RJ, Martin LP, Levin M, Graham MA, Weng DE, et al. A phase I study of the SMAC-mimetic birinapant in adults with refractory solid tumors or lymphoma. Mol Cancer Ther. 2015;14(11):2569-75.

[165] Mohamed MS, Bishr MK, Almutairi FM, Ali AG. Inhibitors of apoptosis: clinical implications in cancer. Apoptosis. 2017;22(12):1487-509.

[166] Najem S, Langemann D, Appl B, Trochimiuk M, Hundsdoerfer P, Reinshagen K, et al. Smac mimetic LCL161 supports neuroblastoma chemotherapy in a drug class-dependent manner and synergistically interacts with ALK inhibitor TAE684 in cells with ALK mutation F1174L. Oncotarget. 2016;7(45):72634.

[167] Ziegler DS, Keating J, Kesari S, Fast EM, Zawel L, Ramakrishna N, et al. A small-molecule IAP inhibitor overcomes resistance to cytotoxic therapies in malignant gliomas in vitro and in vivo. Neuro Oncol. 2011;13(8):820-9.