Chapter

The Program Cell Death (Apoptosis) and the Therapy of Cancer

Hilal Kalkan

Abstract

Apoptosis plays many vital roles in maintaining organ homeostasis and represents type I programmed cell death. Programmed cell death happens when the DNA damage is irremediable and has two important pathways, the intrinsic death pathway also known as the mitochondrial pathway, and the extrinsic programmed cell death pathway. Any defects in the regulation of these crucial pathways have been associated with many disorders, most importantly cancer. Therefore, understanding the molecular basis of apoptosis is essential for the treatment of incurable cancer. To date, several anti-cancer drugs have been developed by targeting anti-apoptotic proteins, which are upregulated in many cancers. Nonetheless, a disease progression often time warranted due to the deregulation of several anti or pro-apoptotic proteins which also contribute to drug resistance. Hence, it is important to understand the maintenance and counteraction of apoptosis and improve successful new pharmacological applications of cell death mechanisms for future therapies. This chapter discusses the mechanism of apoptosis and emerging principles of drug resistance in cancer.

Keywords: Apoptosis, Caspases, Cell death pathways, Drug resistance, Cancer

1. Introduction

Apoptosis is a type of programmed cell death that is a genetically controlled process of self-destruction. Upon appropriate internal and external stimulus, apoptosis is activated to eliminate the unwanted cells. Hence, damaged cells undergo DNA condensation, membrane blebbing, and cell shrinkage which is the result of activated caspases and proteases [1]. Programmed cell death (PCD) or apoptosis plays an important role to maintain tissue homeostasis. The major aspect of the PCD mechanism is the removal of infected, damaged, or mutation carrying cells (that cause cell-cycle defects). Apoptosis or type I programmed cell death is a well-defined regulated mechanism for the elimination of unwanted cells in the later-period of life. Apoptosis happens in a silent-manner, without causing any damaging effect on the neighboring cells, tissues, and so extinguish stimulation of immunological response [2]. Although, unregulated or deficit form of apoptosis can lead to disease conditions such as cancer and autoimmune diseases [3]. Therefore, understanding the physiological control of apoptosis is crucial for the development of novel approaches to target various diseases. In this chapter, we will provide

a brief introduction of apoptosis, the program cell death pathways, the role of caspases, the role of PCD in cancer, and anti-cancer therapy.

2. Apoptosis: type 1 program cell death

Apoptosis (will be referred to as PCD) as the name suggest is the targeted killing of cells that occurs as a part of homeostasis, development, and pathogenic processes throughout life. PCD is well-operated in the physiological processes in an orchestrated manner to eliminate any defect in cells or tissues [2, 4]. The three well-recognized apoptotic cell disassembly are (i) cell shrinkage, (ii) membrane blebbing, and (iii) the formation of membrane protrusions and fragmentation [2]. Apoptosis can be triggered via two ways; i) the extrinsic pathway where the cell death receptors such as Fas, TNFαR, and Death receptors (DR3, DR4, and DR5) bind to their ligands ii) the intrinsic pathway when Bcl-2 family pro-apoptotic proteins lead to permeabilization of the mitochondrial outer membrane. Both extrinsic and intrinsic pathways are converged on the activation of the caspase family, which is responsible for the removal of the damaged cell [5, 6]. The stimulation of apoptosis via the extrinsic pathway requires classic ligand-cell-surface receptor interaction. Death-receptors-induced extrinsic cell death is mainly critical for the function of the immune system [7]. The intrinsic apoptotic cell death starts in a cell-autonomous manner. Cellular defects and stresses, such as DNA damage (due to toxic agents or defect s in the cell-cycle process) or endoplasmic reticulum (ER) stress (caused by accumulated unfolded proteins), leads to apoptosis when the damage is further than repair [8].

2.1 The activation of caspases, functions, and regulation

The initiation of apoptosis requires the activation of caspases, which then promote a cascade of events culminating in the death of a cell. Caspases are a family of cysteine proteases, very well conserved proteins playing many vital roles. Caspases can be divided into two categories (i) involved in immunity and (ii) the ones facilitating apoptotic cell death. These enzymes are expressed inactively inside the cell and activated to cleave for specific target substrates [9]. Pro-apoptotic caspases cleave a much broader subset of intracellular proteins to intercede cell death via apoptosis, on contrary, the caspases those involved in immunity can remove the cells via other non-apoptotic mechanisms. Pro-apoptotic caspases are fundamental for the development, and maintenance of tissue homeostasis [10]. Although many different caspases are found in mammals, here attention will be given to those which are very well characterized. During program cell death, the activation of caspases leads to biochemical and structural changes such as condensation of chromatin, mitochondria permeabilization, and membrane blebbing which are characteristic of apoptotic cell bodies. These essential processes display two important functions, firstly, the promotion of cell death and secondly, the elimination of damaged cells. Caspase-dependent program cell death generally ends with cell death [11]. On the contrary, the failure of apoptosis or non-complete process cause many disorders, most importantly, cancer. Nonetheless, cells have many other regulators that positively or negatively maintaining the activation and/or function of caspases [12]. The expression of caspases differs with stimuli type and expression level. While some caspases are expressed following a death trigger, the others are expressed constantly but being in an inactive state [13, 14]. Once the cell senses a death signal and/or being damaged the initiator caspases are being activated and recruited to the plasma membrane or in the cytoplasm. Upon the cleavage of

initiator caspases, they activate the destructor caspases. The activation of destructor caspases in turn cleave important cellular substrates during program cell death. The initiator caspases (caspase −8, −9, and − 10) and destructor caspases (caspase −3, −6, and − 7) prefer different cleavage sites [15]. Many of the destructor caspases include a cleavage site that is consistent with the peptide substrate specificity. Even though, caspase −3 and − 7 contain identical peptide substrate preferences they cleave different substrates. Such as Bid, Caspase −6 and − 9, Bid, XIAP, and gelsolin are cleaved by caspase −3, while p23 is cleaved by caspase-7 [16]. The destructor caspases can cleave more than thousands of substrates to enable program cell death. Also, the initiator caspases have many additional important mediators for program cell death. The activation of initiator caspase-8 is through the extrinsic program cell death pathway which is mediated via death receptors [17]. The other initiator caspase-9 is activated by the intrinsic program cell death pathway which is regulated by the apoptosome complex. The active initiator caspases (both −8, and − 9) can cleave downstream destructor caspases (caspase −3, −6, and − 7) [18]. The activation of initiator caspases can be via several cell-death signaling complexes. These different signaling complexes respond to many intra- and extracellular events, which induce the recruitment of initiator caspases for the activation process [19, 20]. It is important to mention that the interactions of caspase-activation platforms and those caspases indicate a death-fold domain in the proteins. These sorts of forms are existing in caspases and adaptor proteins: the caspase-recruitment domain (CARD) and death effector domain (DED). There are also different other death domains, such as the death domain (DD), and pyrin domain (PYR), which are included in some of the activations of caspases but absent on caspases [21]. Indeed, death fold domains generally intervene in interactions between proteins via homotypic interaction. For example, some extracellular ligands are tumor-necrosis factor (TNF) family, TNF-related apoptosis-inducing ligand (TRAIL), and FasL. These specific ligands bind to different TNF receptor (TNFR) family, so, upon their binding stimulating conformational changes. The specific signaling complex that is linked with Fas is DISC (death-inducing signaling complex) and contains FADD (Fas-associated death domain), which is a small adaptor protein. TNFR induces the activation of several cell-death signaling complexes [22]. Complex IIa involves FADD and TRADD, and the other complex IIb involves FADD, and RIPK1, RIPK3 (receptor-interacting serine–threonine kinases). Both complexes can activate caspase-8 and program cell death [23]. Once caspase-9 is activated, the cell death stimuli induce Bcl-2 family proteins Bax or Bak and lead to mitochondrial outer membrane permeabilization (MOMP) [24]. Later, MOMP is freeing cytochrome-c (Cyt-c) and other pro-death factors from the mitochondrial membrane to the cytosol [25]. The electrons are carried by Cyt-c in the respiratory chain of healthy cells, which function as a co-factor of Apaf-1 in the apoptotic cell's cytosol. The Cyt-c and ATP bind to Apaf-1 to form the apoptosome complex, which results in the recruitment and activation of caspase-9 [26]. The active caspase-9 cleaves, and in turn, induces the activation of caspase-3. Importantly, this intrinsic program cell death pathway is related to a death-receptor pathway through Bcl-2 family member, Bid. The cleavage of Bid through death-receptor activated caspase-8 leads to truncated Bid, which in turn causes the activation of Bax/Bak-induced MOMP [27]. Hence, result in the activation of caspase-9. Caspase-2 activation induced by the PIDDosome protein complex involves the Rip-associated protein with a death domain (RAIDD) and p53-induced protein with a death domain (PIDD) [28].
The activation of caspase-1 in turn cleaves pro-IL-1b to cause inflammation [23]. Different inflammasomes form particular responses to different stimuli such as Nod-like receptors (NLRs) including NLRP1, NLRP3, and NLRC4 [29–31]. The NLR inflammasome protein contains an adapter binding domain and adaptor

apoptosis-associated speck-like protein (adaptor ASC) containing CARD. Once ASC oligomerizes, it, in turn, leads to the recruitment of caspase-1 and activates caspase-1 [23]. The activation process of caspases has many violent consequences. Thus, the regulation is tightly managed. The cFLIP (cellular FLICE inhibitory protein), which is caspase-8's inactive mimic, binds to signaling complexes that are associated with death receptors to repress caspase-8 [32]. XIAP (X-linked inhibitor of apoptosis) is the only active site inhibitor of caspases and contains three BIR (baculovirus IAP repeat) motifs. The region BIR2 inhibits the activation of destructor caspases, caspase-3 and -7 via two different mechanisms. The first one, by binding the catalytic side of caspase-3 and -7, and the second one is by binding the newly cleaved protein terminus [33]. Also, the BIR3 motif inhibits the caspase-9 and blocks the dimerization of caspase-9. Of note, the regulation of caspases can be post-transcriptionally by phosphorylation. Several studies reported to inhibit the activity of caspase-2, −8, and − 9 [34]. Besides, caspases can play roles in diverse signaling pathways by their constant activation, for example, some of the specific morphological features of apoptotic cells are due to caspase-intervened activation of some actin cytoskeleton modulators such as p21-activated kinase 2 (PAK2), Rho-associated kinase I (ROCKI) and gelsolin [35–37] (**Figure 1**).

2.2 The mitochondrial pathway of apoptosis (the intrinsic pathway)

The intrinsic pathway contains the let off of cytochrome c from the mitochondria to the cytoplasm in response to cellular stress [26]. The cellular stress by the variety of factors such as DNA damage, deprivation of growth factors, defective ER, and several developmental factors activate the intrinsic pathway. In the mitochondrial pathway, the cleavage and activation of caspases begin with caspase-9, which leads to apoptosome machinery activation [19, 20]. The apoptosis protease-activating factor1 (APAF1) is a key molecule in the intrinsic pathway of apoptosis, besides, an important target for its pro-survival member, and scaffolding the assembled apoptosome [38]. In the Intrinsic apoptosis pathway, firstly, cytochrome c is discharged from mitochondria and enters into the cytosol, by ending with its

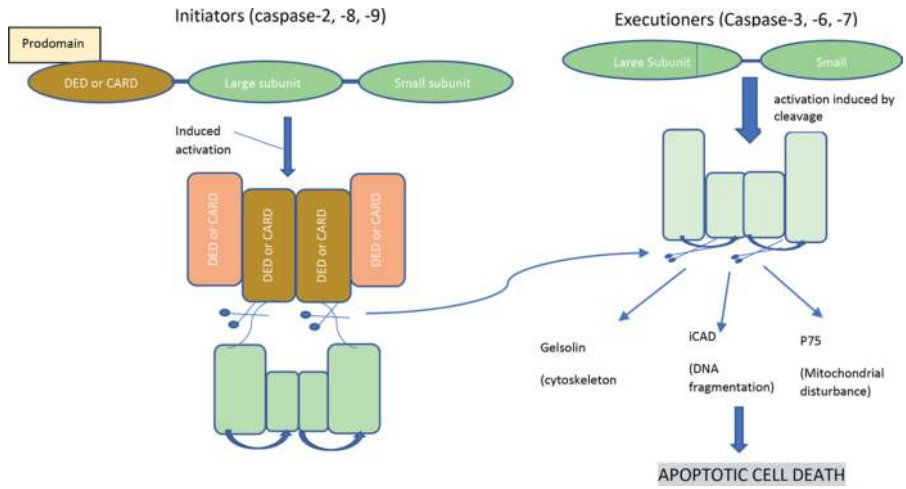

Figure 1.
The caspase family proteases and apoptosis. The initial signal on the cell membrane or from the mitochondria activates the initiator caspases (caspase-2, caspase-8, and caspase-9), which act on execution caspases. The initiator caspases are produced inactively and composed of a pro-domain (containing a CARD or a death effector domain [DED]) and, a large and small subunit. On contrary, executioner caspases (caspase-3, caspase-6, and caspase-7) are activated by cleavage of the initiator caspases. Thus, dependent on initiator signaling. The active caspases including two small and large subunits.

interaction to APAF1 [39, 40]. The cytochrome c, together with APAF1 forms the apoptosome, which leads to inactive caspase-9 monomers into active auto-process [26]. The active caspase-9, in turn, leads to activation of downstream effector caspases. Further, the mitochondria let off pro-apoptotic proteins includes apoptosis-inducing factor (AIF) and endonuclease-G [41]. Cytochrome c is only present in the mitochondrial intermembrane in healthy cells, to interact with APAF1, the apoptotic stimuli trigger the mitochondrial outer membrane (MOM) permeabilization (MOMP) [27]. Hereby, the MOMP conduce to discharge of the entire soluble proteins from mitochondrial intermembrane space to the cytosol. Additionally, rather than cytochrome c, two important pro-apoptotic proteins which are Smac and Omi, were also released meanwhile the above-mentioned procedure. These two proteins are crucial for increasing the potential of apoptosome via antagonizing the caspase inhibitor called X-linked inhibitor of apoptosis (XIAP) [26, 42]. In the lack of Smac and/or Omi, XIAP binding to caspase-9, and inhibits its catalytic activity in which also affects the caspase-3 and caspase-7 [42]. The intrinsic apoptotic pathway is regulated by the Bcl-2 family of proteins [5, 43]. The regulation of MOMP is tightly controlled by the Bcl-2 family proteins, they share BH region homology with their sequence, function, and structure. The Bcl-2 family can be sub-grouped into three categories: the pro-apoptotic proteins, Bax and Bak, play a key role for MOMP regulation; the anti-apoptotic proteins, Bcl-2, Bcl-xL, and Mcl-1, these are the blockers of MOMP; and the BH3-only proteins, Bid, Bim, Bad and Noxa, which are activators of pro-apoptotic proteins and neutralizers for Bcl2 anti-apoptotic proteins. Anti-apoptotic Bcl2 proteins control many other cellular processes such as autophagy and mitochondrial fusion [24, 44]. Bax and Bak are in charge of the loss of mitochondrial outer membrane integrity. Over their activation, they form broad oligomers, and by disturbing MOM enduing their entry into MOM. The disturbing process still unknown, but this lets off the release of entire intermembrane space proteins [45, 46]. Bax and Bak are acting on MOMP involuntarily but importantly at least one of them is needed for re-permeabilization of mitochondria. These proteins are generally being at an inactive state, and gets activated upon upstream stimuli [47–49]. The BH3-only proteins, at least two of them, Bim and active Bid activate Bax and Bak via their transient interaction [25, 50–52]. The antagonization of MOMP accrued by the Bcl2 anti-apoptotic proteins in which they bind and inhibit both Bax and Bak, also BH3-only proteins via interacting with their BH3-only domains [53]. The Bcl2 proteins are regulated both transcriptionally and post-transcriptionally. Especially, via the ubiquitin-proteasome system, the degradation of Mcl-1 involve in apoptosis due to many cellular stresses. Over DNA damage, Mcl1 ubiquitin ligase E3 (MULE) binds to Mcl1 and initiates it catalyzes [54]. Mcl1 is phosphorylated via mitogen-activated protein kinase (MAPK) upon cellular stress conditions [55]. Also, Mcl1 is degraded with growth factors stimuli such as IL3 and the deficiency of phosphoinositide 3-kinase (PI3K)-AKT signaling. This important process eases glycogen synthase kinase 3 (GSK3) from AKT inhibition. Then, GSK3 leads to phosphorylation of Mcl1 and its subsequent degradation [55, 56]. Intrinsic pathway signaling with pro-and anti-apoptotic proteins maintain many signaling processes in the important point of life and death decision. BH3-only proteins are essential upstream sensors of the mitochondrial apoptotic pathway. The regulation of several signaling pathways converge on the BH3-only proteins, and therefore, control and regulate their expression levels and activity [57, 58]. Such as the activation of Bid over cleavage by caspase-8 follow up DR ligation [59, 60]. Hereby, the Bid mediates the cross-regulation of both extrinsic and intrinsic apoptotic pathways. Also, the response to genotoxic stress can be given with Puma and Noxa, which are the direct target of p53 [61–64]. Also, the primary apoptotic factor Bim similarly can be up-regulated via forkhead transcription factor FOXO3A over

cytokine deficiency or due to ER stress to unbind the accumulated proteins [65–67]. To this end, the activity of Bad regulated via several kinases' phosphorylation including AKT, which leads to its sequestration through 14–3-3- proteins [68, 69]. Also, when Bad is released, it acts against Bcl2 anti-apoptotic proteins over growth factor deficiency and/or lacking the AKT signaling [68, 70]. It is noteworthy that MOMP is obliged the cell to death even though the caspase activation is hindered which might be the conclusion of the loss of mitochondrial function, which causes the failure of energy [26, 71]. Some cells can survive under these conditions though [26]. Nevertheless, the MOMP activation through its downstream is an important point in the regulation of caspases and might be settled. Finally, mitochondrial apoptosis can be harassed through the phosphorylation and inhibition of caspase-9 by acting downstream of MOMP [72].

2.3 The extrinsic pathway of apoptosis

The extrinsic pathway of apoptosis can be initiated through the binding of ligands to the death receptors such as tumor necrosis factor receptors (TNFRs) and Fas receptor (FasR) on the cell surface [7, 17, 73]. These receptors possess death domains at the cytosolic side, which helps in recruiting adaptor proteins such as FAS associated via death domain (FADD), and also, for some receptors, TNFRSF1A is associated via death domain (TRADD) [74, 75]. The recruitment of these adaptor proteins occurs through the interaction of death domains present in both receptors and adaptors. The death effector domains of these adaptor proteins have different upstream procaspases such as caspase-8 and caspase-10 to form a death-inducing signaling complex (DISC), which then promote activation of these caspases [17, 76]. The downstream executioner caspases such as caspase-3, caspase-6, and caspase-7 activated through their upstream caspases to execute cell death [77]. The main feature of apoptosis is the lack of systemic damage to neighboring cells during its initiation and clearance of the dead cells. This feature of apoptosis is provided by the attentive wrapping of the cell membrane around the fragmented cell by forming apoptotic bodies. The release of ATP (adenosine 5′-triphosphate) and UTP (uridine- 5′-triphosphate) from apoptotic bodies display a signal for macrophages, leads to the migration of macrophages to the apoptotic site [78]. Besides, apoptotic cells also expose the eat me signal most likely phosphatidylserine (PtdSer) on their surface, which initiates phagocytes to engulf the must-remove cells [79].

3. Apoptosis and cancer

Cancer is a multi-complex disease arising from mutation of tumor suppressor or oncogenes, which can be due to impairment of several signaling pathways and associated with apoptosis [80]. The evasion of apoptosis is an important sign of cancer, which mostly results in a very high level of resistance to many chemo-therapeutic agents. Therefore, attention has been given to apoptotic proteins and became the most attractive anti-cancer target for effective treatment. The resistance against apoptosis is seen in many types of cancers, and required much higher doses of drugs to overcome the resistance, which increases the risk of "off-target" negative effects [81]. Apoptosis is also a very complex system with its regulation and/or function via several proteins, kinases, and links with several pathways might provide important therapeutic benefit for novel approaches to treat cancer. A better understanding of apoptotic pathways and their alteration in cancer cells had the attention of the development of pro-apoptotic agents. In cancer cells, cell death has been reported for a long time and associated with cancer therapy

due to the designation of killing malignant cells by chemo- and/or radiotherapy. Unfortunately, this also leads to the death of normal cells along with cancer cells [80]. Kerr et al. reported that radiation increases the rate of apoptosis in cancer cells. Therefore, apoptosis-associated directly with the development of cancer cells in humans. Most importantly, it can also promote resistance to the therapies which aim to kill cancer cell by targeting apoptosis [2]. In healthy cells, the recognition of damage leads to activation of program cell death, which ending up with their elimination by killing themselves. Such as the activation of tumor suppressor p53 can activate apoptosis inducers including Puma and Noxa, in normal cells [61, 62, 64]. Thus, the un-endowed cells which are not able to eliminate themselves may cause an accumulation of genetic damage. The oncogenes (e.g. Myc) induced apoptosis is a very crucial protective mechanism against cancer development, controlled by a p53-dependent pathway, activated in response to abnormal mitogenic signals. Such as the cells overexpressing the apoptosis inhibitor Bcl2 or have damage in the p53 gene are incapable of this removal process including cancer cells. The loss of function mutations in the p53 tumor suppressor gene causes defection in the apoptotic mechanism and also impacts its tumor-suppressive functions, which involving DNA repairment, cell-cycle arrest, and cell senescence [82, 83]. Also, the incapable cells with an additional mutation can lead to atypical cell proliferation. Such as chromosome translocation which ends up with over-expression of c-Myc, then will lead to quick malignant cell growth. Of note, this explains that the defects in apoptotic mechanism such as over-expression of apoptosis inhibitor Bcl-2 and unregulated cell proliferation due to c-Myc overexpression in the development of lymphoma [82, 84, 85].

Understanding of extrinsic apoptotic pathway on death receptors especially on TRIAL signaling, including TRAIL-1 and -2, shown to lower toxicity compared to TNF or FAS signaling were targeted. The lowering expression levels of TRAIL receptors via recombinant human TRAIL-R1 and -R2 opened up an important therapeutic window. The tumor cells pre-dominantly signal via TRAIL-R1 and -R2, and the expression of these receptors displayed that they do not behave as an indicator of favored receptor signaling [86]. The higher expression levels of these receptors have been found on brain tissue and hepatocytes [87, 88]. However, these findings opened up several windows about the use of TRIAL in the clinic which has been reported notable apoptotic cell death in primary hepatocytes. These findings underlining the importance of selective models to evaluate appropriate drug combinations and their potential on- and/or off-target adverse effects. A study by Von Karstedt et al. in KRAS mutant cancers, known to have an elevated level of TRAIL-R2 expression shown that triggering TRAIL signaling is worsen the development of cancer, and metastasis [89, 90]. Several other studies in different cancers such as pancreatic cancer also shown that TRIAL-R2 signaling interacts with the miRNA processing complex and leads to the inhibition of the maturation of mRNA, called let-7. The result of TRAIL R2-miRNA interaction displayed an up-regulated level of let-7 transcriptional target genes which also prompt cancer development [91]. The sublethal concentrations of TRAIL were shown to induce the activation of caspase-8 dependent apoptotic nucleases, which leads to increased mutations caused by DNA damage in surviving cells [92].

The IAP proteins which are downstream of DRs and Bcl2 family proteins are excessively expressed in cancer cells, therefore associated with cancer development and as well as resistance to therapies. The involvement of cIAPs has also been associated with the regulation of the NF-KB pathway. The lack of cIAPs leads to an increase of inflammatory cytokines including TNF family and IL6, IL8, IL10, and Mcl1, which are effectively involved in systemic inflammation [93]. Nevertheless,

these cytokines have also been reported to be increased in cancer progression due to their link with inflammation which causes to include immune cells to the cancer site and prompt the tumor development (**Figure 2**).

3.1 p53 phosphorylation: is it a driver to cell death?

DNA damage comprises different causes that involve cell stress, mutations, toxic agents, or genomic lesions. Upon damage, the confrontation of cells to damage lead to their potential responses to repair the damage or eliminate the harmed cells by apoptosis. Usually, the response of the damaged cell fate differs from strength to the

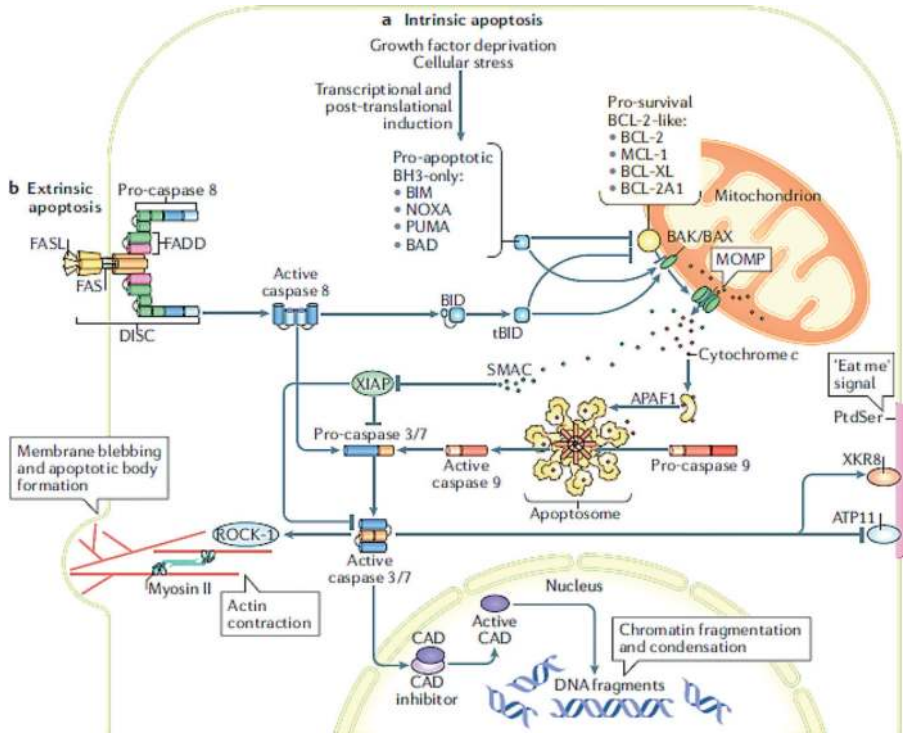

Figure 2.
Molecular mechanisms of intrinsic and extrinsic apoptotic signaling pathways activation. **a.** *the activation of intrinsic apoptotic signaling pathway through cellular stress (ER), and deprivation of growth factor (DNA damage). This alteration promoting the activation of pro-apoptotic members of the BCL-2 family, including BIM, PUMA, BAD, or NOXA. The BH3-only proteins bind and therefore, neutralize BCL-2 pro-survival proteins, involving BCL-2, BCL- XL, MCL-1, and BCL-2- related protein A1. Thus, releasing the important effectors of apoptosis signaling, such as BAK and BAX, which then unite into big complexes and lead to incompliance in the mitochondrial outer membrane. Afterward, this results in the free off cytochrome c and SMAC, which are apoptogenic factors. Some of BH3- only proteins (BIM and PUMA) recently have been reported to bind and promote the activation of MOMP. Also, cytochrome c binding to APAF1 leads to the formation of the apoptosome complex. Caspase-9 is activated in this apoptosome complex, and later proteolytically causes the activation of both caspase- 3 and caspase-7. The caspase inhibitor XIAP activity is blocked by SMAC.* **b.** *Extrinsic apoptotic signaling is stimulated through the activation of DRs, for example, the activation of FAS via FAS ligand (FASL). This cause the pro-caspase 8 recruitment to the death receptor by the adaptor protein FADD, and ending up with the formation of the DISC, which is an important catalyzer for the caspase-8 activation. The activation of caspase 8 promotes cell killing, in two ways: i) via direct activation of caspases 3 and 7, or ii) by indirect activation of the BH3 only protein BID into tBID, thus linking with the intrinsic apoptotic signaling pathway. Effector caspases can be activated in two ways, either by cleavage of hundreds of intracellular proteins to promote the conventional apoptotic morphology and to avoid the efflux of intracellular DAMPs which can result in inflammatory responses. In addition, effector caspases either directly or indirectly can lead to activation of ROCK-1 kinase, which is important for plasma membrane blebbing by actin contraction and caspase-activated DNAse (CAD), which cause inter-nucleosomal DNA cleavage and thus chromatin condensation. These effector caspases also can proteolytically lead to the inactivation of lipid flippases including ATP11, and also can activate the lipid scramblase such as XKR8. Altogether, this results in the exposure of PtdSer on the cell membrane. Therefore, this is called an 'eat me signal' for phagocytic cells and ending up with the engulfment of cells that are undergoing apoptosis [94].*

content of DNA damage, and different signaling pathways are activated. The different cellular decision-making process is guided with specific signaling molecules at the molecular level [95]. The tumor suppressor p53 plays a key role in response to DNA damage and involve in the cellular decision-making process, in turn, found to prompt DNA repair by shutoff the cell cycle to allow time for the repairment and restore genome stability [96]. However, it remains unknown still that how p53 does different cell-fate decisions, activate different target genes, and at a non-transcriptional level have an effect in the cytoplasm of MOMP [97]. The phosphorylation of p53 at Ser15 and Ser20 occurs over immense DNA damage and these are critical sites for stabilization of p53 [98]. Importantly, there is a specific phosphorylation site which is Ser46 and selectively associated with the activity of cell killing caused by genotoxic stress. These crucial different phosphor-sites of p53 might improve the understanding of the function of specific phosphorylation sites. Many studies focused on the phosphorylation of p53 at Ser46 which modulates the target gene transactivation and it has been implicated to potentiate cell death transcriptionally and also, non-transcriptionally [95]. The transactivation of significant pro-apoptotic p53 target genes including BAX, NOXA, p53AIP1, PTEN, and AKT kinase, which are all up-regulated over p53 Ser46 phosphorylation [63, 99–101]. How this different transcriptional effect occurs and which mechanisms are evolved is an open question? The specific role of the p53 Ser46 phosphorylation site in making a cell-fate decision upon cell death leads to the thought of its disturbed regulation might cause cancer cell resistance to therapy. The deregulation of p53 Ser46 kinases in cancer cells could be caused by many factors involving mutations, mis-localization, lower expression level, and disturbed kinase activity. The mutations of p53 at Ser46 have been reported with 1.4–1.6% rates in different human cancer types including breast, colorectal, lung, and stomach. Besides, the down-regulation of several p53 Ser46 kinases are reported such as ATM, DYRK2, HIPK2, and p38α in different tumor types [63, 102–104]. Although, since the regulation of the kinase relies on p53 Ser46 activity, their specific potentials and activities is a challenging task.

3.2 The pro-oncogenic roles of apoptosis

The deregulation of apoptosis is a feature of cancer cells. Apoptosis is a tumor suppressor mechanism in cancer cells. Hence, the dysregulation of apoptosis causing uncontrollable cancer cell-survival [105]. Besides, the tumor-suppressive effects of apoptosis, also, suggested having pro-oncogenic functions. The maintenance of aggressive tumors by apoptosis has been described in the literature. The term tumor aggressiveness defined with increased proliferation, and metastatic features of cancer cells. Such as apoptotic stresses are maybe the reason for aggressive tumor development. Therefore, have pro-oncogenic roles in cancer development and aggressiveness [106, 107]. Importantly, this opens several questions about the beneficial effects of apoptosis in cancer therapy, since strategies aim to trigger apoptosis [108]. Apoptosis-induced proliferation (AIP) is an important compensatory proliferation form, which helps to eliminate damaged cells and maintain tissue homeostasis. Caspase-dependent inflammation and repressed AIP activity might lead to tumor development. One important mediator of AIP is Prostaglandin-E2 (PGE-2), and functioning as a trigger of tumor proliferation in apoptotic cells [109]. The regulation of tumor cells by PGE-2 through caspase-3 dependent manner is an important strategy to treat melanoma. Here, the dying cells trigger the growth of living-cells after cytotoxic therapy [110, 111]. Therefore, targeting PGE-2 to enhance the response to therapy might help to overcome the resistance in cancer cells, when designing cancer therapies [112].

3.3 The oncogenic roles of apoptosis

Apoptotic cells release the "eat-me" signal which is a signal for phagocytosis. These dynamic signals are including fractalkine (FTK), phosphatidylserine (PS), nucleotides ATP, and UTP [113, 114]. These signals are responsible for immune cell activation. Such as the effects of FTK associated with cell migration, and angiogenesis. Also, these signals by apoptotic cells are thought to induce inflammation. Somehow, in the occurrence of normal apoptotic cell death, inflammation is prevented by efferocytosis. The mechanism of efferocytosis is to eliminate the apoptotic cells by immune cells, macrophages. Importantly, it also plays a key role in cancer cells by creating an immunosuppressive effect to organize several signalization events between tumor cells [115]. For example, a study by Ford and colloquies, in 2015, showed that apoptotic cancer cells induce tumor growth and angiogenesis in B-cell lymphoma and also, malignant melanoma [116]. In conclusion, the effects of apoptotic cells in their microenvironment is changed upon their features. The studies that suggest apoptotic cell involvement might play important role in signaling events due to their environment and features. Of note, it remains unknown how they do these roles, therefore, more studies are needed to clarify this point.

4. The therapeutic approaches to cancer

The question what happens when apoptotic machinery is impaired? The answer is impairment of apoptosis results in uncontrolled cell proliferation, tumor development and progression, and resistance to anticancer therapeutics [117, 118]. The deregulation of apoptosis is not only causing the development and progression of the tumor but also lead to resistance to therapeutic approaches [80]. Cancer cells can promote drug resistance to several treatments, which target several different molecular signaling. So many cancers present an internal resistance to chemotherapy, without previous exposure to anti-cancer agents. Therefore, the beginning response to cancer treatment is weak. Furthermore, several tumors might also gain drug resistance because of chemotherapy [119–122]. Among many intrinsic and gained drug resistance are increased drug leakage, defective inflow transporters, changes in the function of drugs and their targets, drug inactivation metabolically, drug compartmentalization, increased activity of DNA repairment [120, 123–126]. To improve therapeutically new approaches, the understanding of these signaling pathways is required to progress cancer therapy and more importantly get over the resistance. Besides, targeting the apoptotic signaling events that are involved in resistance may improve a valid strategy to increase the cell sensitivity of cancer cells to apoptosis and overcome the failure of therapeutic difficulties.

4.1 The role of Bcl2 family proteins in cancer and drug resistance

The Bcl-2 family proteins contains at least one Bcl-2 homology (BH) domain [127–129]. The different BH-domains exist, but especially BH-3 is crucial to form several BCL-2 protein complexes that govern the result between survival and death [130–133]. These interactions between BH3-BH3 are also important to antagonize pro-survival complexes. The increased level of anti-apoptotic Bcl-2 family proteins is associated with carcinogenesis. Such as the transgenic mice model that overexpress Bcl-2 shown to develop tumors spontaneously [134]. However, the high expression of Bcl-2 was also reported to cause a shift in the balance of cancer cells through survival and also lead to resistance to chemotherapy [135]. Overexpression of Bcl-xL, another member of the Bcl2-family is also associated with colorectal

cancer [136]. This unbalanced expression of Bcl-2 family proteins confers failure of apoptosis and resistance to cancer therapies [137]. Especially, overexpression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL is correlated with several cancers including ovarian and breast cancer [138, 139]. On the other side, an increased ratio of Bcl-2/Bax is associated with chronic lymphocytic leukemia (CLL) [140].

4.2 The role of BH3-mimetics as a potential therapeutic option for cancer

Bcl-2 family proteins control the intrinsic apoptosis pathway toward protein–protein interactions, which including all BH-3 domains [141, 142]. Currently, the therapeutic approaches mostly focused on mimicking and target the BH-3 only proteins. BH-3 mimetics are small molecule compounds and bind to anti-apoptotic Bcl-2 proteins. Therefore, inhibit their activity and promotes apoptosis [133, 143]. Of note, BH-3 mimetics can start apoptosis by leading to activation of Bax/Bak or by inhibiting anti-apoptotic Bcl-2 family proteins [142]. Between a variety of BH-3 mimetics that have been generated, ABT-737 and ABT-263 (Navitoclax) showed the most promising antitumor efficacy by engaging anti-apoptotic Bcl-2 family proteins. Both drugs are shown to engage to Bcl-2, Bcl-Xl, and Bcl-w. Somehow, the use of Navitoclax showed to cause thrombocytopenia by inhibition of Bcl-xl, as a major adverse effect [144, 145]. These side effects lead to exclusion of Navitoclax as an effective chemotherapeutic drug and required new findings more selective for Bcl-2 inhibitors, so can induce cell death without causing critical side effects. Indeed, ABT-199 (Navitoclax) was subsequently developed to replace Navitoclax, as a highly selective Bcl-2 inhibitor. Venetoclax binds with high affinity to Bcl-2 and a lesser extent to other Bcl-2 family proteins including Bcl-xl and Bcl-w. Also, Venetoclax lacks off-target effects on BCL-XL [146]. Altogether, the pre-clinical experience with venetoclax and together with combining BCR signaling inhibitors were shown to be very promising with significant efficacy in the patient population Chronic Lymphocytic Leukemia (CLL) and Diffuse Large B-cell Lymphoma (DLBCL). The study by Binu Sasi et al., in 2019, showed that fostamatinib and ibrutinib can sensitize DLBCL cell lines to venetoclax, which marks the importance of combination approaches. In conclusion, the findings provided a rational explanation for testing the use of combination therapy with venetoclax and selective BCR inhibitors in clinical use in DLBCL [147]. The progress of research is underlying the continuous role of Bcl-2 family inhibitors as promising and effective anticancer therapeutic agent. However, another small molecule BDA-366 (BH4 domain inhibitor) is also recently described as a promising approach in anticancer treatment. The potent effects of BDA-366 have been experimentally validated in both in vitro and in vivo, by showing significant anticancer activity [148]. Lastly, a very innovative strategy again comes from Bcl-2, the Bcl-2 gene is known to be rich with GC sequence which is placed in the promoter region, and has the potential to form G-quadruplex structures. Thus, is playing a role as a transcriptional repressor element and these G-quadruplex-specific ligands are capable of regulating Bcl-2 transcriptionally [149, 150].

4.3 Up-regulation of Bcl-2 (pro-apoptotic) family proteins to overcome resistance in cancer cells

The up-regulated expression level of anti-apoptotic Bcl-2 proteins might be possible be neutralized by increasing the expression of pro-apoptotic Bcl-2 proteins. Puma is an important BH-3 protein, having a key role in mediating pro-apoptotic p53 function and help to release p53 from inactive forms with Bcl-Xl. Hereby, freed cytosolic p53 can activate Bax and Bak, which results in stimulation of apoptotic

signaling through MOMP [44, 151]. p53 increases the expression of pro-apoptotic protein level, called ARTS, and counteracts Bcl-xl to induce apoptosis [152]. Hence, the inhibition of p53 antagonists such as Mdm-2 can cause an increased level of p53, induce pro-apoptotic protein expressions. Additionally, reduction of pro-apoptotic BH-3 only protein levels can also lead to drug resistance. This effect might be due to its interaction with BCL-2 anti-apoptotic proteins in cancer cells [153].

4.4 Therapeutic approach to target death receptor

There are different reasons and ways cause the failure of apoptosis or leading to resistance. Including altered death receptor signaling, an unbalanced level of pro-and anti-apoptotic proteins lowered caspase role and disrupted p53 function. Impairment of the death receptor signaling pathway is associated with many sorts of tumors, showing the importance of the Fas–FasL system and/or the abnormal level of cytosolic components of this pathway such as FADD can improve the tumor conversion [154, 155]. Transcriptionally silencing of Fas is familiar with oncogenic events in the epithelial conversion, the mutation in this gene linked with B-cell germinal center-derived lymphomas [156]. However, the lack of expression level of FADD is a common sign in acute myelogenous leukemia (AML) which leads to chemotherapy resistance [154, 155, 157]. Furthermore, the low and/or lack of caspase-8 expression have been reported in numerous other cancers including neuroblastoma and small cell lung cancer [158–160]. However, the induction of apoptosis by triggering the death receptor pathway might be an important promising approach to overcome the resistance to therapeutic agents. An important therapeutic approach that confers the TRAIL ligand which induces apoptosis in many different cancer cell lines [161]. TRAIL-induced apoptosis, an important mechanistic strategy that has a harmful effect against cancer cells and leads to several different approaches to develop agonistic-featured antibodies against TRAIL death receptors and/or several soluble recombinant derivatives of TRAIL [162, 163]. On the other side, increasing the sensitivity of malignant tumors to TRAIL-induced apoptosis is crucial to design new promising molecules to target and activate caspase-8 [164]. Importantly, the validation of in silico screening some small molecules which promote caspase-8 activation have been reported. In many different cancer cell lines including leukemic cells, the CaspPro molecule is shown to be very promising, and shown to induce not only caspase-8 activation but also caspase 3 activation, and leading to apoptosis in the presence of TRAIL [163–165].

Among other therapeutic approaches, another important strategy is targeting IAPs. Through the use of antisense oligonucleotides and small-molecule inhibitors very potential anticancer therapeutic approach has been developed. In ovarian cancer, the supplementation of antisense agents targeting XIAP through an adenoviral vector was displayed to reduce the expression level of XIAP and underlined its remarkable effect to induce apoptosis [166]. Besides, in lung cancers also its effect has been proven by showing that it increases cell sensitivity to radiation treatment [167]. In this regard, by inhibition of XIAP through the use of specifically designed oligomers also displayed to promote the activation of caspase-3, increasing the apoptotic effect of TRIAL in different cancers such as prostate cancer [168]. Furthermore, the SMAC mimetics are also shown to trigger apoptosis and/or increase the sensitivity of cancer cells to different therapies. These molecules induce caspase activation through the neutralization of XIAP-mediated caspase inhibition [169]. The successful therapeutic approach relies on the capability of the tool to trigger apoptosis mainly by two approaches, i) targeting overexpressed anti-apoptotic Bcl-2 family proteins, or ii) triggering the activation of pro-apoptotic molecules.

5. Conclusion remark

In conclusion, the main hallmark of cancer is the intrinsic or requisite resistance to apoptosis, and the evasion of apoptotic cell death can be part of cellular response to stressful stimuli. Based on our understanding of how the dysregulation of the apoptosis pathway leads to cancer, approaches to target anti-apoptosis Bcl-2 family proteins have proven to be effective in the treatment of cancers, especially lymphatic cancers. However, cancer cells often outsmart these therapeutic interventions by modulating the expression of other molecules of apoptosis. Novel combination approaches are warranted to overcome this drug-induced resistance. More importantly, the new perspective and pieces of evidence are needed for powerful approaches to overcome drug resistance in cancer cells in clinical use. Therefore, the current knowledge needs a further understanding of how different cellular stresses trigger anti-apoptotic mechanisms, and how these effects lead to apoptotic resistance in cancer cells.

Author details

Hilal Kalkan[1,2]

1 Università degli Studi della Campania Luigi Vanvitelli, Naples, Italy

2 Istituto di Chimica Biomolecolare (ICB-CNR), Naples, Italy

*Address all correspondence to: h.kalkan@icb.cnr.it

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