

## Review Article

# Functions of BCL-X<sub>L</sub> at the Interface between Cell Death and Metabolism

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The BCL-2 homolog BCL-X<sub>L</sub>, one of the two protein products of *BCL2L1*, has originally been characterized for its prominent prosurvival functions. Similar to BCL-2, BCL-X<sub>L</sub> binds to its multidomain proapoptotic counterparts BAX and BAK, hence preventing the formation of lethal pores in the mitochondrial outer membrane, as well as to multiple BH3-only proteins, thus interrupting apical proapoptotic signals. In addition, BCL-X<sub>L</sub> has been suggested to exert cytoprotective functions by sequestering a cytosolic pool of the pro-apoptotic transcription factor p53 and by binding to the voltage-dependent anion channel 1 (VDAC1), thereby inhibiting the so-called mitochondrial permeability transition (MPT). Thus, BCL-X<sub>L</sub> appears to play a prominent role in the regulation of multiple distinct types of cell death, including apoptosis and regulated necrosis. More recently, great attention has been given to the cell death-unrelated functions of BCL-2-like proteins. In particular, BCL-X<sub>L</sub> has been shown to modulate a number of pathophysiological processes, including—but not limited to—mitochondrial ATP synthesis, protein acetylation, autophagy and mitosis. In this short review article, we will discuss the functions of BCL-X<sub>L</sub> at the interface between cell death and metabolism.

## 1. Introduction

According to current models, cell death most often proceeds via either of two relatively independent subroutines, apoptosis, and necrosis [1, 2]. For a long time, apoptotic and necrotic instances of cell death have exclusively been identified based on morphological criteria [2]. In addition, while apoptosis was believed to constitute the sole regulated (i.e., genetically encoded, and hence susceptible to pharmacological modulation) modality of cell death, necrosis was viewed as a purely accidental process [3]. Recently, a functional classification of cell death mechanisms, based on measurable biochemical features, has been proposed [1], and the concept of regulated necrosis has gained large consensus [4]. In this scenario, the true relevance of additional processes that were

previously catalogued as *bona fide* cell death subroutines is being reevaluated. In particular, while macroautophagy (hereafter referred to as autophagy) turned out to constitute a prominent homeostatic and cytoprotective mechanism [5, 6], autophagic cell death (a lethal subroutine that is mediated, rather than merely accompanied, by autophagy) has been shown to occur in a limited number of, mostly developmental, scenarios [1, 7]. Along similar lines, mitotic catastrophe, a signaling cascade elicited in mitosis-incompetent cells that was initially viewed as a particular case of apoptosis [8], has recently been proposed to constitute an oncosuppressive mechanism with multiple functional outcomes, including cell senescence as well as apoptotic and necrotic cell death [9].

Apoptotic stimuli can be propagated via two distinct, but not entirely disjointed, molecular cascades: extrinsic

apoptosis, transducing lethal signals that originate in the extracellular microenvironment, and intrinsic (also known as mitochondrial) apoptosis, responding to perturbations of intracellular homeostasis [10]. Extrinsic apoptosis can be initiated either by the ligand-induced activation of plasma membrane death receptors (e.g., FAS/CD95, tumor necrosis factor receptor 1 (TNFR1)) or by so-called dependence receptors (e.g., deleted in colorectal carcinoma (DCC)), when the concentration of their ligands falls below a specific threshold [1]. Death receptors promote the activation of caspases (a class of cysteine proteases that play a central role in multiple instances of apoptosis) [11] via the formation of a multiprotein complex that includes—among other components—receptor-interacting protein kinase 1 (RIPK1), FAS-associated protein with death domain (FADD), cellular inhibitor of apoptosis proteins (cIAPs), and multiple isoforms of cellular FLICE-inhibitory protein (c-FLIP). Such a death-inducing signaling complex (DISC) allows for the proximity-induced autoactivation of caspase-8, in turn catalyzing the proteolytic maturation of caspase-3, the central effector of most cases of apoptosis [10]. The mechanisms whereby dependence receptors are connected to the execution of apoptosis have only recently begun to emerge and appear to involve caspase-9, a caspase that was long believed to exclusively regulate mitochondrial apoptosis [12].

Intrinsic apoptosis can be triggered by a plethora of perturbations in intracellular homeostasis, including—among others—DNA damage, oxidative stress, and cytosolic  $\text{Ca}^{2+}$  overload [10]. Independent of the initiating stimulus, the signaling cascades that mediate intrinsic apoptosis as well as the prosurvival signals that are generated alongside (to facilitate the reestablishment of homeostasis) are opposed to each other at the level of mitochondria [13–15]. If lethal signals prevail, the majority of mitochondria become permeabilized, an event that *de facto* seals the cell fate. Indeed, upon mitochondrial outer membrane permeabilization (MOMP), (i) the mitochondrial transmembrane potential ( $\Delta\psi_m$ ), that is, the electrochemical gradient driving ATP synthesis as well as many other mitochondrial functions, is rapidly dissipated; and (ii) cytotoxic proteins that are normally sequestered within the mitochondrial intermembrane space (e.g., cytochrome *c*; apoptosis-inducing factor (AIF), endonuclease G (ENDOG)) are released into the cytosol, where they promote the activation of caspases as well as of caspase-independent cell death executioner mechanisms. The former relies on the cytochrome *c*-elicited, dATP-, and apoptotic peptidase activating factor 1 (APAF1)-dependent assembly of the so-called apoptosome, a molecular platform for the activation of the caspase-9  $\rightarrow$  caspase-3 cascade. The latter involves the caspase-independent endonuclease activity of AIF and ENDOG as well as the bioenergetic and redox crisis that ensues  $\Delta\psi_m$  dissipation [13, 14]. Of note, extrinsic and intrinsic apoptosis are not entirely disjointed. Indeed, while in some cell types (e.g., lymphocytes) the caspase-8  $\rightarrow$  caspase-3 cascade is sufficient to mediate death receptor-dependent apoptosis, in others (e.g., hepatocytes), this process requires the caspase-8-mediated cleavage of the BH3-only protein BID, generating a mitochondrion-permeabilizing fragment (see below) [16, 17].

Given its position at the frontier between cell life and death, it is not surprising that MOMP constitutes a highly regulated phenomenon. So far, two models have been put forward to explain MOMP in molecular terms [14, 18]. On one hand, MOMP has been suggested to originate at the mitochondrial outer membrane (OM), thanks to the pore-forming activity of multidomain proapoptotic members of the BCL-2 protein family, namely, BAX and BAK [14, 19]. On the other hand, it has been proposed that—in response to specific triggers—MOMP would stem from the so-called mitochondrial permeability transition (MPT), an abrupt increase in the permeability to small solutes of the mitochondrial inner membrane (IM). In this latter scenario, a critical role has been ascribed to the permeability transition pore complex (PTPC), a large molecular entity assembled at the junctions between the OM and the IM by several proteins, including (though presumably not limited to) voltage-dependent anion channels (VDACs), adenine nucleotide translocase (ANTs), and cyclophilin D (CYPD) [13, 18].

Importantly, antiapoptotic multidomain members of the BCL-2 protein family, including BCL-2 itself, BCL- $X_L$ , and MCL-1, not only counteract the pore-forming activity of BAX and BAK by engaging in direct inhibitory interactions, but also (i) intercept upstream proapoptotic signals such as those mediated by BH3 only proteins like BAD, BID, BIM, and BBCR3 (best known as p53-upregulated modulator of apoptosis (PUMA)) [20, 21], (ii) bind to, hence regulating, several components of the PTPC, including VDAC1 and ANT [22–24], and (iii) prevent the generation of proapoptotic cytosolic  $\text{Ca}^{2+}$  waves, either by interacting with inositol 1,4,5-trisphosphate (IP3)-gated  $\text{Ca}^{2+}$  channels on the endoplasmic reticulum (ER) or by limiting the capacity of ER  $\text{Ca}^{2+}$  stores [25–27]. In addition, both pro- and antiapoptotic BCL-2-like proteins have recently been shown to modulate multiple processes that are not directly connected to the execution of cell death, including—among others—bioenergetic metabolism, mitochondrial functions, mitosis, and autophagy [28, 29].

Here, we discuss the multifaceted role of BCL- $X_L$ , a prototypic antiapoptotic member of the BCL-2 family, at the hub between cell death and metabolism.

## 2. BCL- $X_L$ and Cell Death

In humans, BCL- $X_L$  is encoded by *BCL2L1*, a *BCL2*-related gene mapping to chromosome 20q11.21 [30]. *BCL2L1* was shown from the beginning to code for two distinct protein products, owing to the alternative splicing of *BCL2L1* mRNA: a cytoprotective factor of 233 residues (BCL- $X_L$ ) and a smaller polypeptide (170 residues) that exerts BCL- $X_L$ -antagonizing functions (BCL- $X_S$ ) [30]. Similar to BCL-2, BCL- $X_L$  contains four distinct BCL-2 homology (BH) domains (BH1–BH4) as well as a transmembrane region, through which it localizes—at least in part—to several membranous compartments, including the OM, the ER, and the nuclear envelope [30–32]. The same does not apply to BCL- $X_S$ , which lacks both the BH1 and BH2 domains [30, 33]. Of note, in most physiological settings the BCL- $X_L$ -coding mRNA is expressed to higher levels than its BCL- $X_S$ -coding counterpart [34]. Conversely, BCL- $X_S$  often predominates in

situations of developmental and pharmacological cell death [35, 36]. Importantly, a caspase-generated cleavage product of BCL-X<sub>L</sub> (lacking an N-terminal fragment) has recently been shown to mediate neuronal cell death in rodent models of ischemic brain injury [37], suggesting that chemical inhibitors of BCL-2-like proteins might also be employed (at least in selected circumstances) as cytoprotective agents.

BCL-2 and BCL-X<sub>L</sub> were soon recognized as critical antiapoptotic factors, although this function was initially attributed to their ability to mediate antioxidant effects [38, 39]. This notion has quickly been abandoned in favor of the so-called “rheostat” model, proposing that BCL-2 and BCL-X<sub>L</sub> would physically sequester their proapoptotic counterparts BAX and BAK in inhibitory interactions [40–42]. In the following decade, along with the discovery of several other members of the BCL-2 protein family, this model has been progressively refined to include the concepts of “activating” and “derepressing” BH3-only proteins [21]. According to current viewpoints, the former would promote MOMP by engaging in direct activatory liaisons with BAX and BAK, while the latter would do so by competitively displacing BAX and BAK from inhibitory interactions with BCL-2, BCL-X<sub>L</sub>, and MCL-1 [21]. Of note, the core concept of the rheostat model as first theorized by Stanley Korsmeyer in 1993 [43], that is, that cell death is governed by the balance between pro- and antiapoptotic BCL-2 family members, has remained remarkably unmodified since its original formulation.

Nevertheless, during the last two decades, antiapoptotic BCL-2 family members, including BCL-X<sub>L</sub>, have been shown to exert cytoprotective functions via a myriad of mechanisms that do not necessarily rely on their capacity to block the pore-forming activity of BAX and BAK although—at least in some instances—they do involve a BAX-/BAK-antagonizing effect. Similar to BCL-2, BCL-X<sub>L</sub> prevents the generation of proapoptotic cytosolic Ca<sup>2+</sup> waves by reducing capacity of ER Ca<sup>2+</sup> stores, an effect that is antagonized by BAX and BAK [25–27]. Moreover, BCL-X<sub>L</sub> has been shown to critically regulate the opening status of VDAC1, and hence of the PTPC, thus influencing MPT-dependent apoptotic cell death [23, 24, 44].

Of note, while some authors proposed that the MPT would stem from an unselectively open conformation of the PTPC [23], others concluded that the MPT would originate from the closed state of the pore [24, 44]. Irrespective of this controversy, which has not yet been fully resolved, recent data have confirmed a critical role for the interaction between VDAC1 and BCL-X<sub>L</sub> in the antiapoptotic properties of the latter [45]. Interestingly, many other BCL-2 family members such as BAX, BAK, BID, and BCL-2 appear to interact with (and hence modulate the activity of) PTPC components (i.e., VDAC1, VDAC2, and ANT) [22, 23, 46, 47], suggesting that the crosstalk between these two systems might constitute a particularly important point of functional regulation. However, the actual relevance of the PTPC for the cellular demise in physiological settings remains matter of debate. Indeed, mice lacking one or more of the most critical PTPC components (including all VDAC and ANT isoforms known thus far) [48–50], with the single exception of *Ppif*<sup>-/-</sup>

animals (lacking CYPD) [51–53], fail to exhibit remarkable cell death defects in response to ischemic, traumatic, and pharmacological challenges. Hence, it seems that—at least in physiological settings—the complex crosstalk between BCL-2 family members and the PTPC mainly modulates cell-death unrelated cellular functions and impacts on the cellular demise only via indirect circuitries (see below).

One of the most central regulators of apoptosis as triggered by perturbations of intracellular homeostasis such as DNA damaging conditions, imbalances in redox homeostasis and oncogenic stress is p53 [54]. Besides operating as a stress-responsive transcription factor that regulates the synthesis of both pro- and antiapoptotic proteins, including a large panel of BCL-2 family members (i.e., BAX, BAK, BAD, BID, PUMA, BCL-2, and BCL-X<sub>L</sub>) [55], p53 can also exert apoptotic functions in a transcription-independent fashion [56, 57]. In particular, p53 has been shown to operate similar to BH3-only proteins, that is, to promote MOMP either by engaging in activatory (though labile) interactions with BAX [58] or by displacing BAX and BAK from inhibitory liaisons with BCL-2 and BCL-X<sub>L</sub> [59]. In this setting, the mitochondrial pools of BCL-2 and BCL-X<sub>L</sub> constitute the main target for the “derepressor” activity of p53 [59]. In addition, a cytoplasmic pool of BCL-X<sub>L</sub> appears to work as a PUMA-sensitive inhibitor of p53, *de facto* operating at the interface between p53 transcriptional and transcription-independent functions [60]. Thus, BCL-X<sub>L</sub> exerts cytoprotective effects not only as it antagonizes its proapoptotic counterparts but also as it counteracts the activity of p53. In addition, BCL-X<sub>L</sub> has recently been reported to interact with the mitochondrial phosphatase phosphoglycerate mutase family member 5 (PGAM5) [61], a central effector of regulated necrosis [62, 63]. Hence, although Niture et al. did not address this question in a direct fashion, BCL-X<sub>L</sub> may soon be discovered to regulate necrotic instances of cell death.

### 3. BCL-X<sub>L</sub> and Metabolism

The hypothesis that BCL-2 family members, notably BAX, BAD, BCL-2, and BCL-X<sub>L</sub>, would influence bioenergetic and intermediate metabolism began to gain consensus along with the discoveries that (i) these proteins interact with components of the PTPCs that, in physiological circumstances, regulate various facets of mitochondrial functions (e.g., ANT, VDAC, and glucokinase) [22–24, 44, 64], (ii) these proteins modulate Ca<sup>2+</sup> homeostasis at the ER [25–27], and (iii) p53 not only operates as a potent proapoptotic factor in response to stress but also exerts an homeostatic control over metabolism [54]. In particular, BCL-X<sub>L</sub> (i) reportedly preserves the physiological conformation of VDAC, hence promoting the exchange of metabolites, including ADP, across the OM [44]; (ii) functionally antagonizes BAD [42], which has been found to exert prominent metabolic functions by regulating a mitochondrial multiprotein complex that involves (among other enzymes) glucokinase, protein kinase A, and protein phosphatase 1 [64]; (iii) has been shown to lower the concentrations of Ca<sup>2+</sup> ions within the ER, hence quenching the bioenergetic burst that normally results from

the opening of IP<sub>3</sub>-gated Ca<sup>2+</sup> channels [25–27]; and (iv) binds to cytoplasmic p53 in steady-state conditions [60], thus—at least theoretically—modulating its functions related to bioenergetic and redox metabolism [54, 65, 66].

BCL-X<sub>L</sub> has also been demonstrated to regulate distinct facets of intermediate metabolism in a direct fashion. In neurons, a pool of BCL-X<sub>L</sub> localized to the IM appears to physically interact with the β subunit of the F<sub>1</sub>F<sub>0</sub> ATP synthase, hence increasing its enzymatic efficiency, stabilizing the Δψ<sub>m</sub> and—consequently—maximizing mitochondrial ATP generation [67, 68]. Similar functions have been attributed to a truncated variant of MCL-1 that localizes to the mitochondrial matrix [69]. In addition, the transfection-enforced overexpression of BCL-2 has been associated with increased oxygen consumption and higher rates of mitochondrial respiration [70, 71]. Taken together, these observations suggest a conserved role for BCL-2 proteins in the regulation of ATP synthesis. Of note, antiapoptotic members of the BCL-2 family have been suggested to exert prooxidant functions, at least under selected circumstances [70, 71]. Such a (slight) prooxidant state, presumably reflecting the ability of BCL-2 and BCL-X<sub>L</sub> to stimulate mitochondrial respiration [67, 68, 70], appears to be linked to the interaction of BCL-2-like proteins with small GTPases of the RAC family [72, 73] and to exert cytoprotective effects by contributing to the maintenance of baseline energetic homeostasis [71].

Recent data have indicated that BCL-X<sub>L</sub> operates (in a BAX- and BAK-independent manner) to limit the intracellular levels of acetyl-CoA [74]. Acetyl-CoA is not only a critical intermediate of the Krebs cycle, but also required for protein acetylation, including N-α-acetylation, that is, the posttranslational modification that consists in the addition of an acetyl moiety (provided by acetyl-CoA) to the N-terminus of nascent polypeptides [74]. Hence, high expression levels of BCL-X<sub>L</sub> exert cytoprotective effects along with the establishment of a state characterized by decreased levels of virtually all the metabolites involved in the Krebs cycle (but not of glycolytic substrates) as well as by reduced extents of N-α-acetylation [74]. Although Yi and colleagues ascribed such an antiapoptotic state (which could be reversed by the exogenous supply of citrate and acetate) only to the inhibition of N-α-acetylation [74], reduced levels of reactive oxygen species (ROS), which constitute a normal byproduct of mitochondrial respiration, may equally well underpin (at least part of) the cytoprotective effects that originate from BCL-X<sub>L</sub> metabolic functions [75]. In support to this notion, (i) the overactivation of several metabolic circuitries (including glycogenolysis and glutaminolysis) and the overgeneration of ROS have been linked to both apoptotic and necrotic cell death [13, 76]; and (ii) a predominantly glycolytic metabolism, as observed in cancer cells even in the presence of normal oxygen levels (i.e., the so-called Warburg effect), reportedly exerts cytoprotective effects as it increases the amounts of reduced glutathione (a potent antioxidant) [75]. Of note, the pyruvate kinase M2, a glycolytic enzyme variant that is known to sustain the Warburg effect [77], has been shown to stimulate the expression of BCL-X<sub>L</sub> at the transcriptional level [78]. Although the cytoprotective transcription factor NF-κB may play a role in this setting [78], the precise

molecular mechanisms underlying this phenomenon remain to be identified. In addition, protein acetylation has recently been involved in the regulation of autophagy (see below) [79–82], suggesting that BCL-X<sub>L</sub> might exert a broad control over multiple cellular functions.

#### 4. BCL-X<sub>L</sub> and Autophagy

Autophagy is a catabolic pathway driving the lysosomal degradation of cellular constituents such as portions of the cytoplasm, protein aggregates, and dysfunctional/supernumerary organelles [83]. Under physiological conditions, autophagy plays a prominent role in the maintenance of intracellular homeostasis [5, 84, 85]. In addition, the autophagic flux is dramatically upregulated in response to a large panel of stress conditions, including (but not limited to) glucose and amino acid deprivation, hypoxia, intracellular pathogens, and cytotoxic xenobiotics [83, 86]. Although in some, mostly developmental, scenarios an autophagic program *de facto* mediates cell death [1, 7], stress-elicited autophagy near to invariably exerts prominent cytoprotective functions [87]. In line with this notion, both pharmacological and genetic maneuvers that block autophagy most often exacerbate, rather than limit, cell death as triggered by several distinct stimuli [88].

The stress-elicited upregulation of autophagy is a tightly regulated phenomenon, involving distinct molecular sensors and signal transduction cascades that impinge at various levels on the autophagic machinery. A detailed description of the proteins and factors that are involved in the regulation and execution of autophagy largely exceeds the scope of this paper and can be found elsewhere [83, 84, 87, 89]. Nevertheless, it is important to note that—in most (but not all) settings—autophagy critically relies on a class III phosphoinositide-3-kinase (PI3K) enzymatic activity [90]. In human cells, this function is mediated by phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3, best known as hVPS34), which operates under the control of a multiprotein complex involving—among other interactors—the haploinsufficient oncosuppressor Beclin 1 [91, 92]. Importantly, by virtue of a *bona fide* BH3 domain [93], Beclin 1 can physically interact with antiapoptotic members of the BCL-2 protein family, including BCL-2 itself and BCL-X<sub>L</sub> [94–96].

By interacting with Beclin 1, BCL-2 and BCL-X<sub>L</sub> *de facto* prevent the stress-induced activation of autophagy [94]. In line with this notion, both “derepressor” BH3-only proteins (e.g., BAD) and chemical inhibitors of BCL-2-like proteins (e.g., ABT-737) have been shown to activate autophagy as they displace Beclin 1 from inhibitory liaisons with BCL-2 and BCL-X<sub>L</sub> [94, 97]. Interestingly, some BH3-only proteins like BNIP3 have been shown to be critical for the execution of specific autophagic programs, such as the selective removal of damaged mitochondria (mitophagy) [5, 98], while their relevance in cell death regulation seems rather limited [99]. The binding of Beclin 1 to antiapoptotic BCL-2 family members can also be resolved by the phosphorylation of either binding partner [100–102]. Conversely, it seems that BCL-2 and BCL-X<sub>L</sub> do not affect the steady-state levels of the autophagic flux in a direct fashion [100]. Of note, Tian et al. have recently

suggested that BCL-2/BCL-X<sub>L</sub>-targeting compounds might activate a Beclin 1- and PIK3C3-independent autophagic program leading to cell death [103]. However, the authors failed to provide robust data to mechanistically explain their findings.

Besides a direct autophagy-modulatory function stemming from its interactions with Beclin 1 and other BCL-2-like proteins [94, 96], BCL-X<sub>L</sub> is expected to regulate autophagy via less direct metabolic circuitries, notably as it (i) controls the efficiency of mitochondrial ATP production [67, 68], (ii) influences the exchange of critical bioenergetic metabolites (e.g., ATP and ADP) by PTPC components [24, 44], (iii) reduces the intracellular levels of acetyl-CoA [74], and (iv) interacts with the cytoplasmic pool of p53 [60, 65, 66]. Hence, the expression levels of BCL-X<sub>L</sub> might also influence the autophagic flux in steady-state, as opposed to adaptive, conditions. This aspect of the crosstalk between BCL-X<sub>L</sub> and autophagy warrants further investigation.

## 5. BCL-X<sub>L</sub> and Other Cellular Functions

The implication of BCL-2 family members, including BCL-X<sub>L</sub>, in cell death-unrelated processes is not limited to the aspects of cell biology discussed above [28, 29]. For instance, it has recently been shown that BCL-X<sub>L</sub> is phosphorylated at multiple serine residues (including S49 and S62) in a cell cycle-dependent fashion [104, 105]. The mitotic kinase Polo-like kinase 3 (PLK3) appears to be responsible for the cell cycle-dependent phosphorylation of BCL-X<sub>L</sub> at S49 (starting at the S phase and abruptly falling at the onset of mitosis) [104], whereas PLK1 and mitogen-activated protein kinase 9 (MAPK9) have been suggested to catalyze BCL-X<sub>L</sub> phosphorylation at S62 in response to DNA-damaging agents, hence stabilizing a cell cycle arrest at the G<sub>2</sub> checkpoint [105]. Hence, similar to BCL-2 [106, 107], BCL-X<sub>L</sub> plays a role in both physiological cell cycle progression and DNA damage-induced cell cycle checkpoints [104, 105].

A few years ago, rather unspecific inhibitors of BCL-2-like proteins such as ABT-737 and ABT-263 have generated an intense wave of enthusiasm and quickly entered clinical trials as part of antineoplastic regimens for the treatment of—mostly hematological—malignancies [108]. One of the most prominent on-target side effects of ABT-737 and ABT-263 turned out to be a dose-limiting thrombocytopenia [109, 110], linked to the fact that BCL-X<sub>L</sub> is critical for the survival of platelets [111]. More recently, BCL-X<sub>L</sub> has also been involved in the adhesive function of platelets [112, 113]. Hence, ABT-737 and ABT-263 appear to impair aggregation not only as they trigger the demise of a consistent fraction of circulating platelets but also as they exert consistent thrombocytopathic effects among residual platelets [113].

The implication of the BCL-2 protein family in mitochondrial dynamics as well as the actual relevance of mitochondrial fission/fusion events in apoptosis have been and still are the subject of a vivid debate [114–116]. While a detailed discussion of this topic largely exceeds the scope of this paper, it is worth noting that BCL-X<sub>L</sub> has recently been shown to interact with (and stimulate the GTPase activity of) dynamin-related protein 1 (DRP1), a central component of the

mitochondrial fission machinery [117]. In doing so, BCL-X<sub>L</sub> appears to alter the mitochondrial function of neurons in a manner that stimulates the formation of synapses [117]. As DRP1 also participates in the execution of regulated necrosis [63], mitochondrial dynamics may constitute yet another point of control of cell death-related and -unrelated processes by BCL-X<sub>L</sub>.

BCL-2 and BCL-X<sub>L</sub> have been reported to negatively regulate the NLRP1 inflammasome, a supramolecular platform that is required for the full-blown activation of caspase-1—and hence the production of interleukin (IL)-1 $\beta$  and IL-18—in response to proinflammatory stimuli [118, 119]. In particular, the flexible loop domain of both BCL-2 and BCL-X<sub>L</sub> (which is located between the 1st and 2nd  $\alpha$  helices of the proteins) appears to engage in physical interactions with NLRP1, thereby blocking the capacity of the latter to bind ATP and oligomerize [118, 120]. Besides playing a critical role in innate immunity, inflammasomes are crucial for the translation of immunogenic cell death (a functionally peculiar form of apoptosis) into a robust adaptive immune response [121]. It is therefore tempting to speculate, yet remains to be formally proved, that the interaction between antiapoptotic BCL-2 family members and inflammasomes may constitute a promising therapeutic target for enhancing the immunogenicity of (cancer) cell death [121].

## 6. Concluding Remarks

Following an initial wave of interest on the role of BCL-2-like proteins in the regulation of apoptosis, several laboratories have refocused their attention on distinct aspects of the biology of pro- and antiapoptotic members of the BCL-2 family [28, 29]. During the last decade, this intense investigational effort has led to the identification of several processes that are modulated by BCL-2 family proteins independent of (or at least not directly impacting on) their cell death-regulatory functions [28, 29]. As discussed in this paper, BCL-X<sub>L</sub> has been shown to exert a consistent degree of control on various aspects of bioenergetic metabolism, including mitochondrial ATP production, Ca<sup>2+</sup> fluxes, autophagy, and protein acetylation, as well as on several other cellular and organismal processes such as mitosis, platelet aggregation, and synaptic efficiency (Table 1). Hence, similar to other BCL-2 family members, BCL-X<sub>L</sub> appears to operate at critical hubs to coordinately control multiple cellular functions including the three-step switch between homeostatic metabolisms, adaptive responses to stress, and cell death [122]. In this scenario, it is tempting to speculate, yet remains to be formally demonstrated, that BCL-2-like proteins may have originated as regulators of non-apoptotic functions and only later in evolution may have acquired the capacity of control cell death. Irrespective of this unresolved issue, we expect that—similar to what happened (and is still happening) for p53 [54]—the number of cellular functions that are regulated by BCL-2 family members, including BCL-X<sub>L</sub>, will grow further.

## Abbreviations

AIF: Apoptosis-inducing factor

TABLE 1: Functions of BCL-X<sub>L</sub> at the interface between cell death regulation and other aspects of the cell biology.

Interactor	Main localization	Notes(s)	Reference
BAD	Cytosol mitochondria	By antagonizing BAD, BCL-X <sub>L</sub> modulates the metabolic functions of a mitochondrial multiprotein complex involving glucokinase, PKA, and PPI	[64]
Beclin 1	Cytosol Golgi network	BCL-X <sub>L</sub> binds to Beclin 1, thus inhibiting stress-induced, but not baseline, autophagy	[94, 97, 100]
DRP1	Mitochondria	BCL-X <sub>L</sub> interacts with DRP1, altering the mitochondrial function of neurons to stimulate the formation of synapses	[117]
F <sub>1</sub> F <sub>0</sub> ATP synthase	Mitochondria	BCL-X <sub>L</sub> increases the enzymatic activity of the F <sub>1</sub> F <sub>0</sub> ATP synthase, hence, stabilizing the $\Delta\psi_m$ and maximizing mitochondrial ATP synthesis	[67, 68]
IP <sub>3</sub> R	ER	BCL-X <sub>L</sub> reduces Ca <sup>2+</sup> concentration in the ER	[25–27]
Krebs's cycle	Mitochondria	BCL-X <sub>L</sub> overexpression reduces the levels of virtually all TCA (but not glycolytic) intermediates, modulating both N- $\alpha$ -acetylation and autophagy	[74, 79–82]
MAPK9 PLK1	Cytosol nucleus	BCL-X <sub>L</sub> is phosphorylated at S49 by PLK1 and MAPK9 in a cell cycle-dependent fashion	[104]
NLRP1 inflammasome	Cytosol	BCL-X <sub>L</sub> inhibits the NLRP1 inflammasome, interfering with the secretion of IL-1 $\beta$ and IL-18	[118, 120]
p53	Cytosol mitochondria	BCL-X <sub>L</sub> binds to p53, hence, inhibiting both its pro-apoptotic and metabolic functions	[54, 60, 66]
PKM2	Cytosol mitochondria	PKM2 stimulates the expression of BCL-X <sub>L</sub> at the transcriptional level	[78]
PLK3	Cytosol nucleus	PLK3 phosphorylates BCL-X <sub>L</sub> at S62 in response to DNA-damaging agents, favoring a cell cycle arrest at the G <sub>2</sub> checkpoint	[105]
RAC2	Cytosol plasma membrane	In some settings, antiapoptotic BCL-2 family members exert prooxidant functions, perhaps linked to their interaction with RAC2	[70–72]
VDAC1	Mitochondria	BCL-X <sub>L</sub> promotes the exchange of metabolites between the cytosol and the mitochondrial matrix	[24, 44]

$\Delta\psi_m$ : mitochondrial transmembrane potential; DRP: dynamin-related protein 1; ER: endoplasmic reticulum; IL: interleukin; IP<sub>3</sub>R: inositol 1,4,5-triphosphate receptor; MAPK9: mitogen-activated protein kinase 9; PKA: protein kinase A; PKM2: pyruvate kinase M2; PLK: polo-like kinase 1; PPI: protein phosphatase 1; VDAC1: voltage-dependent anion channel 1.

ANT: Adenine nucleotide translocase  
 APAF1: Apoptotic peptidase-activating factor 1  
 BH: BCL-2 homology  
 cFLIP: Cellular FLICE-inhibitory protein  
 cIAP: Cellular inhibitor of apoptosis protein  
 CYPD: Cyclophilin D  
 DCC: Deleted in colorectal carcinoma  
 DISC: Death-inducing signaling complex  
 DRP1: Dynamin-related protein 1  
 ENDOG: Endonuclease G  
 ER: Endoplasmic reticulum  
 FADD: FAS-associated protein with death domain  
 IL: Interleukin  
 IM: Mitochondrial inner membrane  
 IP<sub>3</sub>: Inositol 1,4,5-trisphosphate  
 MAPK9: Mitogen-activated protein kinase 9  
 MOMP: Mitochondrial outer membrane permeabilization  
 MPT: Mitochondrial permeability transition  
 OM: Mitochondrial outer membrane  
 PGAM5: Phosphoglycerate mutase family member 5  
 PI3K: Phosphoinositide-3-kinase

PI3KC3: Phosphatidylinositol 3-kinase, catalytic subunit type 3  
 PLK3: Polo-like kinase 3  
 PTPC: Permeability transition pore complex  
 PUMA: p53-upregulated modulator of apoptosis  
 RIPK1: Receptor-interacting protein kinase 1  
 ROS: Reactive oxygen species  
 TNFR1: Tumor necrosis factor receptor 1  
 VDAC: Voltage-dependent anion channel  
 $\Delta\psi_m$ : Mitochondrial transmembrane potential.

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## References

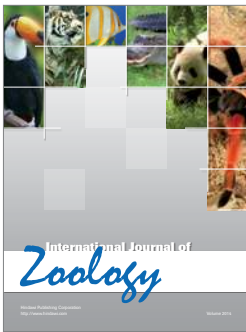
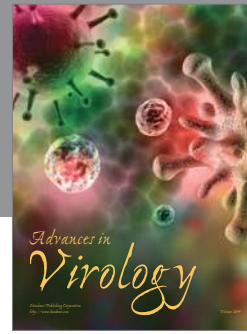
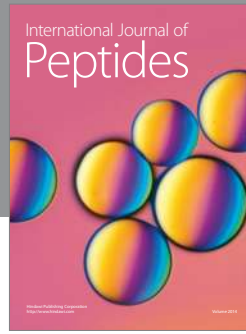
- [1] L. Galluzzi, I. Vitale, J. M. Abrams et al., "Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012," *Cell Death and Differentiation*, vol. 19, pp. 107–120, 2012.
- [2] G. Kroemer, L. Galluzzi, P. Vandenabeele et al., "Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009," *Cell Death and Differentiation*, vol. 16, no. 1, pp. 3–11, 2009.
- [3] L. Galluzzi, M. C. Maiuri, I. Vitale et al., "Cell death modalities: classification and pathophysiological implications," *Cell Death and Differentiation*, vol. 14, no. 7, pp. 1237–1243, 2007.
- [4] P. Vandenabeele, L. Galluzzi, T. Vanden Berghe, and G. Kroemer, "Molecular mechanisms of necroptosis: an ordered cellular explosion," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 10, pp. 700–714, 2010.
- [5] D. R. Green, L. Galluzzi, and G. Kroemer, "Mitochondria and the autophagy-inflammation-cell death axis in organismal aging," *Science*, vol. 333, pp. 1109–1112, 2011.
- [6] G. Kroemer and B. Levine, "Autophagic cell death: the story of a misnomer," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 12, pp. 1004–1010, 2008.
- [7] D. L. Berry and E. H. Baehrecke, "Growth arrest and autophagy are required for salivary gland cell degradation in drosophila," *Cell*, vol. 131, no. 6, pp. 1137–1148, 2007.
- [8] M. Castedo, J. L. Perfettini, T. Roumier, K. Andreau, R. Medema, and G. Kroemer, "Cell death by mitotic catastrophe: a molecular definition," *Oncogene*, vol. 23, no. 16, pp. 2825–2837, 2004.
- [9] I. Vitale, L. Galluzzi, M. Castedo, and G. Kroemer, "Mitotic catastrophe: a mechanism for avoiding genomic instability," *Nature reviews. Molecular cell biology*, vol. 12, no. 6, pp. 385–392, 2011.
- [10] R. C. Taylor, S. P. Cullen, and S. J. Martin, "Apoptosis: controlled demolition at the cellular level," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 3, pp. 231–241, 2008.
- [11] M. Lamkanfi, N. Festjens, W. Declercq, T. V. Berghe, and P. Vandenabeele, "Caspases in cell survival, proliferation and differentiation," *Cell Death and Differentiation*, vol. 14, no. 1, pp. 44–55, 2007.
- [12] F. Mille, C. Thibert, J. Fombonne et al., "The Patched dependence receptor triggers apoptosis through a DRAL-caspase-9 complex," *Nature Cell Biology*, vol. 11, no. 6, pp. 739–746, 2009.
- [13] G. Kroemer, L. Galluzzi, and C. Brenner, "Mitochondrial membrane permeabilization in cell death," *Physiological Reviews*, vol. 87, no. 1, pp. 99–163, 2007.
- [14] S. W. G. Tait and D. R. Green, "Mitochondria and cell death: outer membrane permeabilization and beyond," *Nature Reviews Molecular Cell Biology*, vol. 11, no. 9, pp. 621–632, 2010.
- [15] L. Galluzzi, O. Kepp, and G. Kroemer, "Mitochondria: master regulators of danger signalling," *Nature Reviews Molecular Cell Biology*, vol. 13, pp. 780–788, 2012.
- [16] H. Li, H. Zhu, C. J. Xu, and J. Yuan, "Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis," *Cell*, vol. 94, no. 4, pp. 491–501, 1998.
- [17] X. Luo, I. Budihardjo, H. Zou, C. Slaughter, and X. Wang, "Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors," *Cell*, vol. 94, no. 4, pp. 481–490, 1998.
- [18] C. Brenner and S. Grimm, "The permeability transition pore complex in cancer cell death," *Oncogene*, vol. 25, no. 34, pp. 4744–4756, 2006.
- [19] R. J. Youle and A. Strasser, "The BCL-2 protein family: opposing activities that mediate cell death," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 1, pp. 47–59, 2008.
- [20] J. E. Chipuk, J. C. Fisher, C. P. Dillon, R. W. Kriwacki, T. Kuwana, and D. R. Green, "Mechanism of apoptosis induction by inhibition of the anti-apoptotic BCL-2 proteins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 51, pp. 20327–20332, 2008.
- [21] S. N. Willis and J. M. Adams, "Life in the balance: how BH3-only proteins induce apoptosis," *Current Opinion in Cell Biology*, vol. 17, no. 6, pp. 617–625, 2005.
- [22] C. Brenner, H. Cadiou, H. L. A. Vieira et al., "Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator," *Oncogene*, vol. 19, no. 3, pp. 329–336, 2000.
- [23] S. Shimizu, Y. Shinohara, and Y. Tsujimoto, "Bax and Bcl-x(L) independently regulate apoptotic changes of yeast mitochondria that require VDAC but not adenine nucleotide translocator," *Oncogene*, vol. 19, no. 38, pp. 4309–4318, 2000.
- [24] M. G. Vander Heiden, N. S. Chandel, P. T. Schumacker, and C. B. Thompson, "Bcl-x(L) prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange," *Molecular Cell*, vol. 3, no. 2, pp. 159–167, 1999.
- [25] C. W. Distelhorst and M. D. Bootman, "Bcl-2 interaction with the inositol 1, 4, 5-trisphosphate receptor: role in Ca(2+) signaling and disease," *Cell Calcium*, vol. 50, pp. 234–241, 2011.
- [26] S. A. Oakes, L. Scorrano, J. T. Opferman et al., "Proapoptotic BAX and BAK regulate the type I inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 1, pp. 105–110, 2005.
- [27] Y. Rong and C. W. Distelhorst, "Bcl-2 protein family members: versatile regulators of calcium signaling in cell survival and apoptosis," *Annual Review of Physiology*, vol. 70, pp. 73–91, 2008.
- [28] L. Galluzzi, N. Joza, E. Tasdemir et al., "No death without life: vital functions of apoptotic effectors," *Cell Death and Differentiation*, vol. 15, no. 7, pp. 1113–1123, 2008.
- [29] L. Galluzzi, O. Kepp, C. Trojel-Hansen, and G. Kroemer, "Non-apoptotic functions of apoptosis-regulatory proteins," *The EMBO Reports*, vol. 13, pp. 322–330, 2012.
- [30] L. H. Boise, M. Gonzalez-Garcia, C. E. Postema et al., "bcl-x, A bcl-2-related gene that functions as a dominant regulator of apoptotic cell death," *Cell*, vol. 74, no. 4, pp. 597–608, 1993.
- [31] F. W. H. Ng, M. Nguyen, T. Kwan et al., "p28 Bap31, a Bcl-2/Bcl-X(L)- and procaspase-8-associated protein in the endoplasmic reticulum," *Journal of Cell Biology*, vol. 139, no. 2, pp. 327–338, 1997.
- [32] E. Schmitt, M. Beauchemin, and R. Bertrand, "Nuclear colocalization and interaction between bcl-x<sub>L</sub> and cdk1(cdc2) during G2/M cell-cycle checkpoint," *Oncogene*, vol. 26, no. 40, pp. 5851–5865, 2007.
- [33] W. Fang, J. J. Rivard, D. L. Mueller, and T. W. Behrens, "Cloning and molecular characterization of mouse bcl-x in B and T lymphocytes," *Journal of Immunology*, vol. 153, no. 10, pp. 4388–4398, 1994.

- [34] M. Gonzalez-Garcia, R. Perez-Ballester, L. Ding et al., "bcl-x(L) is the major bcl-x mRNA form expressed during murine development and its product localizes to mitochondria," *Development*, vol. 120, no. 10, pp. 3033–3042, 1994.
- [35] K. Heermeier, M. Benedict, M. Li, P. Furth, G. Nuñez, and L. Hennighausen, "Bax and Bcl-xs are induced at the onset of apoptosis in involuting mammary epithelial cells," *Mechanisms of Development*, vol. 56, no. 1-2, pp. 197–207, 1996.
- [36] S. Willimott, T. Merriam, and S. D. Wagner, "Apoptosis induces Bcl-XS and cleaved Bcl-x<sub>L</sub> in chronic lymphocytic leukaemia," *Biochemical and Biophysical Research Communications*, vol. 405, no. 3, pp. 480–485, 2011.
- [37] D. Ofengeim, Y.-B. Chen, T. Miyawaki et al., "N-terminally cleaved Bcl-x<sub>L</sub> mediates ischemia-induced neuronal death," *Nature Neuroscience*, vol. 15, pp. 574–580, 2012.
- [38] D. M. Hockenbery, Z. N. Oltvai, X. M. Yin, C. L. Millman, and S. J. Korsmeyer, "Bcl-2 functions in an antioxidant pathway to prevent apoptosis," *Cell*, vol. 75, no. 2, pp. 241–251, 1993.
- [39] D. J. Kane, T. A. Sarafian, R. Anton et al., "Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species," *Science*, vol. 262, no. 5137, pp. 1274–1277, 1993.
- [40] Z. N. Oltvai, C. L. Millman, and S. J. Korsmeyer, "Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death," *Cell*, vol. 74, no. 4, pp. 609–619, 1993.
- [41] S. Shimizu, Y. Eguchi, H. Kosaka, W. Kamiike, H. Matsuda, and Y. Tsujimoto, "Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-x<sub>L</sub>," *Nature*, vol. 374, no. 6525, pp. 811–813, 1995.
- [42] E. Yang, J. Zha, J. Jockel, L. H. Boise, C. B. Thompson, and S. J. Korsmeyer, "Bad, a heterodimeric partner for Bcl-x(L), and Bcl-2, displaces Bax and promotes cell death," *Cell*, vol. 80, no. 2, pp. 285–291, 1995.
- [43] S. J. Korsmeyer, J. R. Shutter, D. J. Veis, D. E. Merry, and Z. N. Oltvai, "Bcl-2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death," *Seminars in Cancer Biology*, vol. 4, no. 6, pp. 327–332, 1993.
- [44] M. G. Vander Heiden, X. X. Li, E. Gottlieb, R. B. Hill, C. B. Thompson, and M. Colombini, "Bcl-x<sub>L</sub> promotes the open configuration of the voltage-dependent anion channel and metabolite passage through the outer mitochondrial membrane," *The Journal of Biological Chemistry*, vol. 276, no. 22, pp. 19414–19419, 2001.
- [45] N. Arbel, D. Ben-Hail, and V. Shoshan-Barmatz, "Mediation of the antiapoptotic activity of Bcl-x<sub>L</sub> protein upon interaction with VDAC1 protein," *The Journal of Biological Chemistry*, vol. 287, pp. 23152–23161, 2012.
- [46] N. Zamzami, C. E. Hamel, C. Maise et al., "Bid acts on the permeability transition pore complex to induce apoptosis," *Oncogene*, vol. 19, no. 54, pp. 6342–6350, 2000.
- [47] E. H. Y. Cheng, T. V. Sheiko, J. K. Fisher, W. J. Craigen, and S. J. Korsmeyer, "VDAC2 inhibits BAK activation and mitochondrial apoptosis," *Science*, vol. 301, no. 5632, pp. 513–517, 2003.
- [48] C. P. Baines, R. A. Kaiser, T. Sheiko, W. J. Craigen, and J. D. Molkenin, "Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death," *Nature Cell Biology*, vol. 9, no. 5, pp. 550–555, 2007.
- [49] L. Galluzzi and G. Kroemer, "Mitochondrial apoptosis without VDAC," *Nature Cell Biology*, vol. 9, no. 5, pp. 487–489, 2007.
- [50] J. E. Kokoszka, K. G. Waymire, S. E. Levy et al., "The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore," *Nature*, vol. 427, no. 6973, pp. 461–465, 2004.
- [51] C. P. Baines, R. A. Kaiser, N. H. Purcell et al., "Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death," *Nature*, vol. 434, no. 7033, pp. 658–662, 2005.
- [52] E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M. A. Forte, and P. Bernardi, "Properties of the permeability transition pore in mitochondria devoid of cyclophilin D," *The Journal of Biological Chemistry*, vol. 280, no. 19, pp. 18558–18561, 2005.
- [53] A. C. Schinzel, O. Takeuchi, Z. Huang et al., "Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 34, pp. 12005–12010, 2005.
- [54] K. H. Vousden and C. Prives, "Blinded by the light: the growing complexity of p53," *Cell*, vol. 137, no. 3, pp. 413–431, 2009.
- [55] L. Galluzzi, E. Morselli, O. Kepp, I. Vitale, M. Pinti, and G. Kroemer, "Mitochondrial liaisons of p53," *Antioxidants and Redox Signaling*, vol. 15, no. 6, pp. 1691–1714, 2011.
- [56] L. Galluzzi, E. Morselli, O. Kepp, N. Tajeddine, and G. Kroemer, "Targeting p53 to mitochondria for cancer therapy," *Cell Cycle*, vol. 7, no. 13, pp. 1949–1955, 2008.
- [57] E. Morselli, L. Galluzzi, and G. Kroemer, "Mechanisms of p53-mediated mitochondrial membrane permeabilization," *Cell Research*, vol. 18, no. 7, pp. 708–710, 2008.
- [58] J. E. Chipuk, T. Kuwana, L. Bouchier-Hayes et al., "Direct activation of bax by p53 mediates mitochondrial membrane permeabilization and apoptosis," *Science*, vol. 303, no. 5660, pp. 1010–1014, 2004.
- [59] M. Mihara, S. Erster, A. Zaika et al., "p53 has a direct apoptogenic role at the mitochondria," *Molecular Cell*, vol. 11, no. 3, pp. 577–590, 2003.
- [60] J. E. Chipuk, L. Bouchier-Hayes, T. Kuwana, D. D. Newmeyer, and D. R. Green, "Cell biology: PUMA couples the nuclear and cytoplasmic proapoptotic function of p53," *Science*, vol. 309, no. 5741, pp. 1732–1735, 2005.
- [61] S. K. Niture and A. K. Jaiswal, "Inhibitor of Nrf2 (INrf2 or Keap1) protein degrades Bcl-x<sub>L</sub> via phosphoglycerate mutase 5 and controls cellular apoptosis," *The Journal of Biological Chemistry*, vol. 286, pp. 44542–44556, 2011.
- [62] L. Sun, H. Wang, Z. Wang et al., "Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase," *Cell*, vol. 148, pp. 213–227, 2012.
- [63] Z. Wang, H. Jiang, S. Chen et al., "The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways," *Cell*, vol. 148, pp. 228–243, 2012.
- [64] N. N. Danial, C. F. Gramm, L. Scorrano et al., "BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis," *Nature*, vol. 424, no. 6951, pp. 952–956, 2003.
- [65] M. C. Maiuri, L. Galluzzi, E. Morselli, O. Kepp, S. A. Malik, and G. Kroemer, "Autophagy regulation by p53," *Current Opinion in Cell Biology*, vol. 22, no. 2, pp. 181–185, 2010.
- [66] E. Tasdemir, M. C. Maiuri, L. Galluzzi et al., "Regulation of autophagy by cytoplasmic p53," *Nature Cell Biology*, vol. 10, pp. 676–687, 2008.
- [67] Y. B. Chen, M. A. Aon, Y.-T. Hsu et al., "Bcl-x<sub>L</sub> regulates mitochondrial energetics by stabilizing the inner membrane potential," *The Journal of Cell Biology*, vol. 195, pp. 263–276, 2011.
- [68] K. N. Alavian, H. Li, L. Collis et al., "Bcl-x<sub>L</sub> regulates metabolic efficiency of neurons through interaction with the mitochondrial F<sub>1</sub>F<sub>0</sub> ATP synthase," *Nat Cell Biol*, vol. 13, pp. 1224–1233, 2011.



- [69] R. M. Perciavalle, D. P. Stewart, B. Koss et al., "Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration," *Nature Cell Biology*, vol. 14, pp. 575–583, 2012.
- [70] Z. X. Chen and S. Pervaiz, "Bcl-2 induces pro-oxidant state by engaging mitochondrial respiration in tumor cells," *Cell Death and Differentiation*, vol. 14, no. 9, pp. 1617–1627, 2007.
- [71] Z. X. Chen and S. Pervaiz, "Involvement of cytochrome c oxidase subunits Va and Vb in the regulation of cancer cell metabolism by Bcl-2," *Cell Death and Differentiation*, vol. 17, no. 3, pp. 408–420, 2010.
- [72] B. Mizukawa, J. Wei, M. Shrestha et al., "Inhibition of Rac GTPase signaling and downstream prosurvival Bcl-2 proteins as combination targeted therapy in MLL-AF9 leukemia," *Blood*, vol. 118, pp. 5235–5245, 2011.
- [73] R. Velaitan, J. Kang, J. L. Hirpara et al., "The small GTPase Rac1 is a novel binding partner of Bcl-2 and stabilizes its antiapoptotic activity," *Blood*, vol. 117, no. 23, pp. 6214–6226, 2011.
- [74] C. H. Yi et al., "Metabolic regulation of protein N-alpha-acetylation by Bcl-xL promotes cell survival," *Cell*, vol. 146, pp. 607–620, 2011.
- [75] A. E. Vaughn and M. Deshmukh, "Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c," *Nature Cell Biology*, vol. 10, no. 12, pp. 1477–1483, 2008.
- [76] D. W. Zhang, J. Shao, J. Lin et al., "RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis," *Science*, vol. 325, no. 5938, pp. 332–336, 2009.
- [77] H. R. Christofk, M. G. Vander Heiden, M. H. Harris et al., "The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth," *Nature*, vol. 452, no. 7184, pp. 230–233, 2008.
- [78] O. H. Kwon, T. -W. Kanga, J. -H. Kim et al., "Pyruvate kinase M2 promotes the growth of gastric cancer cells via regulation of Bcl-xL expression at transcriptional level," *Biochemical and Biophysical Research Communications*, vol. 423, pp. 38–44, 2012.
- [79] S. Y. Lin et al., "GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy," *Science*, vol. 336, pp. 477–481, 2012.
- [80] E. Morselli, G. Mariño, M. V. Bennetzen et al., "Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome," *Journal of Cell Biology*, vol. 192, no. 4, pp. 615–629, 2011.
- [81] F. Pietrocola, G. Mariño, D. Lissa et al., "Pro-autophagic polyphenols reduce the acetylation of cytoplasmic proteins," *Cell Cycle*, vol. 11, pp. 3851–3860, 2012.
- [82] C. Yi, M. Ma, L. Ran et al., "Function and molecular mechanism of acetylation in autophagy regulation," *Science*, vol. 336, pp. 474–477, 2012.
- [83] L. Galluzzi, J. M. Vicencio, O. Kepp, E. Tasdemir, M. C. Maiuri, and G. Kroemer, "To die or not to die: that is the autophagic question," *Current Molecular Medicine*, vol. 8, no. 2, pp. 78–91, 2008.
- [84] N. Mizushima, B. Levine, A. M. Cuervo, and D. J. Klionsky, "Autophagy fights disease through cellular self-digestion," *Nature*, vol. 451, no. 7182, pp. 1069–1075, 2008.
- [85] N. Mizushima and M. Komatsu, "Autophagy: renovation of cells and tissues," *Cell*, vol. 147, pp. 728–741, 2011.
- [86] B. Levine, N. Mizushima, and H. W. Virgin, "Autophagy in immunity and inflammation," *Nature*, vol. 469, no. 7330, pp. 323–335, 2011.
- [87] G. Kroemer, G. Mariño, and B. Levine, "Autophagy and the integrated stress response," *Molecular Cell*, vol. 40, no. 2, pp. 280–293, 2010.
- [88] P. Boya, R.-A. González-Polo, N. Casares et al., "Inhibition of macroautophagy triggers apoptosis," *Molecular and Cellular Biology*, vol. 25, pp. 1025–1040, 2005.
- [89] D. J. Klionsky, "Autophagy: from phenomenology to molecular understanding in less than a decade," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 11, pp. 931–937, 2007.
- [90] X. Zeng, J. H. Overmeyer, and W. A. Maltse, "Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking," *Journal of Cell Science*, vol. 119, no. 2, pp. 259–270, 2006.
- [91] Z. Yue, S. Jin, C. Yang, A. J. Levine, and N. Heintz, "Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 25, pp. 15077–15082, 2003.
- [92] X. H. Liang, S. Jackson, M. Seaman et al., "Induction of autophagy and inhibition of tumorigenesis by beclin 1," *Nature*, vol. 402, no. 6762, pp. 672–676, 1999.
- [93] W. Feng, S. Huang, H. Wu, and M. Zhang, "Molecular basis of Bcl-xL's target recognition versatility revealed by the structure of Bcl-xL in complex with the BH3 domain of beclin-1," *Journal of Molecular Biology*, vol. 372, no. 1, pp. 223–235, 2007.
- [94] M. C. Maiuri, G. Le Toumelin, A. Criollo et al., "Functional and physical interaction between Bcl-xL and a BH3-like domain in Beclin-1," *The EMBO Journal*, vol. 26, no. 10, pp. 2527–2539, 2007.
- [95] A. Oberstein, P. D. Jeffrey, and Y. Shi, "Crystal structure of the Bcl-xL-beclin 1 peptide complex: beclin 1 is a novel BH3-only protein," *The Journal of Biological Chemistry*, vol. 282, no. 17, pp. 13123–13132, 2007.
- [96] S. Pattingre, A. Tassa, X. Qu et al., "Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy," *Cell*, vol. 122, no. 6, pp. 927–939, 2005.
- [97] M. C. Maiuri, A. Criollo, E. Tasdemir et al., "BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-xL," *Autophagy*, vol. 3, no. 4, pp. 374–376, 2007.
- [98] H. Zhang, M. Bosch-Marce, L. A. Shimoda et al., "Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia," *The Journal of Biological Chemistry*, vol. 283, no. 16, pp. 10892–10903, 2008.
- [99] C. Vande Velde, J. Cizeau, D. Dubik et al., "BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore," *Molecular and Cellular Biology*, vol. 20, no. 15, pp. 5454–5468, 2000.
- [100] C. He, M. C. Bassik, V. Moresi et al., "Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis," *Nature*, vol. 481, pp. 511–515, 2012.
- [101] Y. Wei, S. Pattingre, S. Sinha, M. Bassik, and B. Levine, "JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy," *Molecular Cell*, vol. 30, no. 6, pp. 678–688, 2008.
- [102] E. Zalckvar, H. Berissi, L. Mizrachi et al., "DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-xL and induction of autophagy," *The EMBO Reports*, vol. 10, no. 3, pp. 285–292, 2009.

- [103] S. Tian, J. Lin, J. Zhou et al., "Beclin 1-independent autophagy induced by a Bcl-x<sub>L</sub>/Bcl-2 targeting compound, Z18," *Autophagy*, vol. 6, no. 8, pp. 1032–1041, 2010.
- [104] J. Wang, M. Beauchemin, and R. Bertrand, "Bcl-xL phosphorylation at Ser49 by polo kinase 3 during cell cycle progression and checkpoints," *Cell Signal*, vol. 23, pp. 2030–2038, 2011.
- [105] J. Wang, M. Beauchemin, and R. Bertrand, "Phospho-Bcl-x(L)(Ser62) plays a key role at DNA damage-induced G(2) checkpoint," *Cell Cycle*, vol. 11, pp. 2159–2169, 2012.
- [106] Y. H. Ling, C. Tornos, and R. Perez-Soler, "Phosphorylation of Bcl-2 is a marker of M phase events and not a determinant of apoptosis," *The Journal of Biological Chemistry*, vol. 273, no. 30, pp. 18984–18991, 1998.
- [107] C. D. Scatena, Z. A. Stewart, D. Mays et al., "Mitotic phosphorylation of Bcl-2 during normal cell cycle progression and taxol-induced growth arrest," *The Journal of Biological Chemistry*, vol. 273, no. 46, pp. 30777–30784, 1998.
- [108] M. H. Kang and C. P. Reynolds, "Bcl-2 Inhibitors: targeting mitochondrial apoptotic pathways in cancer therapy," *Clinical Cancer Research*, vol. 15, no. 4, pp. 1126–1132, 2009.
- [109] L. Gandhi, D. R. Camidge, M. R. De Oliveira et al., "Phase I study of navitoclax (ABT-263), a novel bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors," *Journal of Clinical Oncology*, vol. 29, no. 7, pp. 909–916, 2011.
- [110] C. M. Rudin, C. L. Hann, E. B. Garon et al., "Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer," *Clinical Cancer Research*, vol. 18, pp. 3163–3169, 2012.
- [111] K. D. Mason, M. R. Carpinelli, J. I. Fletcher et al., "Programmed anuclear cell death delimits platelet life span," *Cell*, vol. 128, no. 6, pp. 1173–1186, 2007.
- [112] M. T. Harper and A. W. Poole, "Bcl-xL-inhibitory BH3 mimetic ABT-737 depletes platelet calcium stores," *Blood*, vol. 119, pp. 4337–4338, 2012.
- [113] S. M. Schoenwaelder et al., "Bcl-xL-inhibitory BH3 mimetics can induce a transient thrombocytopathy that undermines the hemostatic function of platelets," *Blood*, vol. 118, pp. 1663–1674, 2011.
- [114] P. A. Parone, D. I. James, S. D. Cruz et al., "Inhibiting the mitochondrial fission machinery does not prevent Bax/Bak-dependent apoptosis," *Molecular and Cellular Biology*, vol. 26, no. 20, pp. 7397–7408, 2006.
- [115] R. J. Youle and M. Karbowski, "Mitochondrial fission in apoptosis," *Nature Reviews Molecular Cell Biology*, vol. 6, no. 8, pp. 657–663, 2005.
- [116] R. J. Youle and A. M. van der Bliek, "Mitochondrial fission, fusion, and stress," *Science*, vol. 337, pp. 1062–1065, 2012.
- [117] H. Li, Y. Chen, A. F. Jones et al., "Bcl-x<sub>L</sub> induces Drp1-dependent synapse formation in cultured hippocampal neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 6, pp. 2169–2174, 2008.
- [118] J. M. Bruey, N. Bruey-Sedano, F. Luciano et al., "Bcl-2 and Bcl-x<sub>L</sub> regulate proinflammatory caspase-1 activation by interaction with NALP1," *Cell*, vol. 129, no. 1, pp. 45–56, 2007.
- [119] L. Zitvogel et al., "Inflammasomes in carcinogenesis and anti-cancer immune responses," *Nature Immunology*, vol. 13, pp. 343–351, 2012.
- [120] B. Faustin, Y. Chen, D. Zhai et al., "Mechanism of Bcl-2 and Bcl-x<sub>L</sub> inhibition of NLRP1 inflammasome: loop domain-dependent suppression of ATP binding and oligomerization," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 10, pp. 3935–3940, 2009.
- [121] G. Kroemer, L. Galluzzi, O. Kepp et al., "Immunogenic cell death in cancer therapy," *Annual Reviews Immunology*, 2013.
- [122] S. Luo and D. C. Rubinsztein, "Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-x<sub>L</sub>," *Cell Death and Differentiation*, vol. 17, no. 2, pp. 268–277, 2010.



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