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PII: S0304-419X(22)00116-0

DOI: https://doi.org/10.1016/j.bbcan.2022.188791

Reference: BBACAN 188791

To appear in: *BBA - Reviews on Cancer*

Received date: 8 June 2022

Revised date: 29 August 2022

Accepted date: 29 August 2022

Please cite this article as: N. Rosa, F. Speelman-Rooms, J.B. Parys, et al., Modulation of Ca2+ signaling by antiapoptotic Bcl-2 versus Bcl-xL: From molecular mechanisms to relevance for cancer cell survival, *BBA - Reviews on Cancer* (2022), https://doi.org/10.1016/j.bbcan.2022.188791

Modulation of Ca^{2+} signaling by antiapoptotic Bcl-2 *versus* Bcl-xL: from molecular mechanisms to relevance for cancer cell survival

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Abstract

Members of the Bcl-2-protein family are key controllers of apoptotic cell death. The family is divided into antiapoptotic (including Bcl-2 itself, Bcl-xL, Mcl-1, etc.) and proapoptotic members (Bax, Bak, Bim, Bim, Puma, Noxa, Bad, etc.). These proteins are well known for their canonical role in the mitochondria, where they control mitochondrial outer membrane permeabilization and subsequent apoptosis. However, several proteins are recognized as modulators of intracellular Ca^{2+} signals that originate from the endoplasmic reticulum (ER), the major intracellular Ca^{2+}-storage organelle. More than 25 years ago, Bcl-2, the founding member of the family, was reported to control apoptosis through Ca^{2+} signaling. Further work elucidated that Bcl-2 directly targets and inhibits inositol 1,4,5-trisphosphate receptors (IP_3Rs), thereby suppressing proapoptotic Ca^{2+} signaling. In addition to Bcl-2, Bcl-xL was also shown to impact cell survival by sensitizing IP_3R function, thereby promoting pro-survival oscillatory Ca^{2+} release. However, new work challenges this model and demonstrates that Bcl-2 and Bcl-xL can both function as inhibitors of IP_3Rs. This suggests that, depending on the cell context, Bcl-xL could support very distinct Ca^{2+} patterns. This not only raises several questions but also opens new possibilities for the treatment of Bcl-xL-dependent cancers. In this review, we will discuss the similarities and divergences between Bcl-2 and Bcl-xL regarding Ca^{2+} homeostasis and IP_3R modulation from both a molecular and a functional point of view, with particular emphasis on cancer cell death resistance mechanisms.

Keywords:

Bcl-2 proteins; Bcl-xL, IP_3 receptor, Ca^{2+} signaling, cell death, cancer

1 Introduction

The B cell lymphoma 2 (Bcl-2)-protein family exerts control over mitochondrial dynamics and apoptosis [1, 2]. The family is named after its founding member, the proto-oncogene *BCL2*, which was identified in the 1980s at the chromosomal breakpoint of the t(14;18) translocation in human follicular B cell lymphomas, the most common blood cancer in humans [3]. The gene was basically named *B-cell lymphoma 2* because it was the second oncogene identified at a breakpoint of translocations in B-cell lymphomas [4]. In the next decades, more Bcl-2-related proteins were discovered, and the family currently includes approximately 30 members [5]. Most of the characterized Bcl-2-family members are known to interact with each other at the mitochondrial membranes to promote or inhibit apoptosis according to the cellular context [2, 6, 7].

1.1 The proapoptotic Bcl-2-family proteins and their role in apoptosis

The Bcl-2 family of proteins is classified according to their structure and function as antiapoptotic and proapoptotic proteins (Fig. 1). They are characterized by the presence of one to four Bcl-2 homology (BH) motifs, labeled BH1 to BH4 [2]. Proapoptotic Bcl-2-family members are divided into multidomain effectors and BH3-only proteins. On the one hand, multidomain effectors (Bak & Bax) possess four BH domains and directly execute mitochondrial outer membrane permeabilization (MOMP). On the other hand, BH3-only proteins contain one single BH3 domain and permit Bax/Bak activation. The multidomain proapoptotic proteins Bak and Bax and most of the antiapoptotic Bcl-2-family members, such as Bcl-2 itself, are characterized by the presence of four BH domains and one transmembrane domain (TMD). The proteins are organized from the *N*- to C-terminus as follows: BH4 domain, BH3 domain, BH1 domain, BH2 domain and TMD (Fig. 1). The function of these proteins critically depends on their organization, as BH3-BH1-BH2 domains form a hydrophobic cleft, sometimes referred to as the binding groove, that can interact with the BH3 domain of other Bcl-2-family members [2, 6].

Bak and Bax are proteins that directly initiate apoptosis. Inactive in healthy cells, Bak and Bax are activated by BH3-only proteins that are highly expressed upon cell stress. BH3-only proteins are classically divided into two groups: BH3-only activators (Bim & Bid) and sensitizers (Bik, Puma, Noxa, etc.). The former are direct activators of Bax/Bak, while the latter sensitize Bax/Bak activation by inhibiting antiapoptotic Bcl-2-family members (Fig. 2a) [2, 6]. Inactive Bax proteins reside in the cytosol due to continuous retro-translocation by B-cell lymphoma-extra large (Bcl-xL) [8], while Bak constitutively resides at the outer mitochondrial membrane (OMM), where it is inhibited by the voltage-dependent anion channel (VDAC) 2 [9]. Bax and Bak are directly activated by BH3-only activators Bim and tBid (Fig. 2a). These two proteins may have distinct roles since Bim preferentially activates Bax, whereas tBid preferentially activates Bak [10]. This preferential activation has important consequences for therapeutic treatments since various cancer cell types may rely specifically on the Bax/Bim or on the Bak/Bid duo [10]. Activation of Bax by BH3-only activators modifies its conformation and releases its C-terminal α9 helix from its own hydrophobic pocket. This helix therefore anchors Bax in the OMM [11] via a process that likely involves Bak and VDAC2 [12]. Instead, activation of Bak triggers its escape from VDAC2 [9]. Activated Bax/Bak undergoes dimerization by mutual interactions between their BH3 domain and the hydrophobic groove of another Bax/Bak protein [13]. Eventually, Bax/Bak dimers assemble into

larger oligomeric complexes that cause MOMP and the release of cytochrome *c* and SMAC/Diablo in the cytosol. These proapoptotic factors thereby trigger the formation of the apoptosome and the activation of the caspases that execute the apoptotic program [2, 6, 7]. Of note, ongoing and unrestricted Bax/Bak oligomerization not only results in mitochondrial OMM rupture but also in permeabilization of the inner mitochondrial membrane (IMM), thereby causing the release of mitochondrial DNA and leading to subsequent proapoptotic STING/cGAS signaling [14, 15]. In contrast, limited Bax/Bak activation can result in MOMP in a subset of mitochondria that is sufficient to elicit caspase activity but not sufficient to set off apoptosis. As a consequence, cell survival in the presence of caspase activity results in cellular stress and genomic alterations due to DNA damage. This so-called 'minority MOMP' promotes cell transformation and triggers the formation of malignant cells [16]. As such, MOMP should not be considered the point-of-no-return in apoptosis, as this clearly depends on the cellular context and the extent of MOMP occurring among the mitochondrial population in cells [7]. Moreover, very recent work directly compared Bax/Bak-cluster formation [17]. In cells exposed to apoptotic triggers, Bak protein clusters and pores very rapidly arise, though they are smaller than clusters and pores formed by Bax proteins, which are much more slowly assembled. Furthermore, Bax and Bak also exert mutual impacts on each other. Bak accelerates Bax pore formation by forming foci for the mitochondrial recruitment of Bax, while Bax decelerates Bak pore formation, thereby promoting the formation of larger Bak pores [17].

In the canonical model of apoptosis induction described above, multidomain effectors (Bax & Bak) are activated by BH3-only activator proteins (Bim & Bid). In healthy cells, these activators are scaffolded and inhibited by antiapoptotic family members (Bcl-2, Bcl-xL, Mcl-1, etc.) to prevent cell death. However, BH3-only sensitizers (Bik, Puma, Noxa, etc.) can repress the antiapoptotic Bcl-2-family proteins without direct activation of Bax/Bak (Fig. 2a). In the classical model, the binding of BH3-only sensitizers to antiapoptotic Bcl-2-family proteins displaces and releases BH3-only activators from the antiapoptotic proteins, enabling them to directly activate Bax/Bak. In addition, allosteric mechanisms have emerged, indicating that the binding of Bad to the Bid/Bcl-xL complex changes its conformation, allowing Bid to activate Bax, although Bid is still attached to Bcl-xL [18]. In any case, both BH3-only proapoptotic activators and sensitizers are usually highly expressed upon cell stress to trigger apoptosis. The different roles of the two categories of BH3-only proteins induce cell death by both activation of Bak/Bax and inhibition of antiapoptotic Bcl-2 proteins [2, 6, 7].

However, this canonical model may require further consideration as the classification in BH3-only activators and pro-sensitizers becomes increasingly more difficult to maintain [19, 20]. In one alternative model (Fig. 2b), BH3-only proteins have been proposed to activate Bax/Bak indirectly by acting exclusively as BH3-only sensitizers [21, 22]. This model is based on evidence indicating that Bax and Bak could be activated in the absence of interaction with BH3-only proteins [22]. Furthermore, the BH3-only activators Bim and Bid could be able to inhibit the antiapoptotic proteins Bcl-xL and Mcl-1 while being unable to stimulate Bak and Bax in the absence of these antiapoptotic proteins [21]. Hence, Bax/Bak would be spontaneously active upon their release from antiapoptotic Bcl-2-family proteins. In another alternative model (Fig. 2b), BH3-only proteins would not have any direct activator function, as Bax/Bak would undergo activation at the OMM by mitochondrial lipids. Similar to the previous model, the Bax/Bak activation process is prevented by antiapoptotic Bcl-2-family members that need to be

counteracted by BH3-only proteins to initiate Bax/Bak activation [20, 23]. Finally, BH3-only proteins may also directly execute MOMP without the need for Bax and Bak to be present. Indeed, a recent study demonstrated that helix α6 of truncated Bid can perform MOMP by acting as a pore-forming region [24], a feature that can also be counteracted by antiapoptotic Bcl-2-family members.

In conclusion, the Bcl-2 family encompasses several pro- and antiapoptotic proteins, classified in different categories according to their structure and function. These proteins are inserted in the OMM where they interact with each other, regulating Bax/Bak activity and ultimately MOMP. The expression of proapoptotic BH3-only and of antiapoptotic family members is highly dynamic and depends on the presence of pro- or antiapoptotic signaling [2]. Overall, it is clear that tight regulation of all Bcl-2-family proteins is necessary to balance cell death and survival.

1.2 The antiapoptotic role of Bcl-2 and Bcl-xL in cancer

Bcl-2-family proteins have been extensively studied in the context of cancer, a disease characterized by dysregulated cell death pathways. Upregulated *BCL2* expression was originally described and investigated in hematopoietic cancers such as diffuse large B cell lymphoma. Moreover, other genetic and nongenetic alterations (such as modified transcription/translation due to other oncogenic factors, posttranslational modifications, and miRNA-mediated regulation) of other Bcl-2-family members have also been reported in various cancer types. While downregulation of proapoptotic Bcl-2-family proteins has rarely been observed, upregulation of antiapoptotic family members has been significantly documented in both blood and solid cancers [2, 19]. In a widespread study of over 3000 samples representing 26 tumor types, *MCL1* (Mcl-1) and *BCL2L1* (Bcl-xL) were highlighted among the most frequently amplified genes, especially in breast and lung cancers [25]. Such genetic alterations of antiapoptotic Bcl-2-family members are rarely tumorigenic *per se*. In fact, overexpression experiments have shown that the tumorigenic antiapoptotic effect of antiapoptotic Bcl-2-family proteins is usually compensated by an anti-proliferative effect. Bcl-2 and Bcl-xL, in particular, have been shown to promote the resting G0 phase and delay the G0 to S transition [26-28]. This mechanism is likely related to the antiapoptotic functions of the proteins since quiescent cells are commonly more resistant to cell death. The paradoxical consequence is that overexpressed Bcl-2 can act as a tumor suppressor in some cancers, especially during the early stages [28-31]. Dysregulated antiapoptotic Bcl-2-family proteins become oncogenic when they encounter another amplified oncogene, usually *MYC* [32]. Myc is also quite paradoxical in the context of cancer since it combines hyperproliferative and proapoptotic functions. Although the pro- and antitumorigenic effects of Myc and Bcl-2 vary according to the context, the combination of the two generally synergistically amplifies their oncogenic capacities. Hence, overexpressed or overactivated Myc with Bcl-2/Bcl-xL is recognized as a powerful tumorigenic combination [19, 28].

Interfering with the interaction between the hydrophobic cleft of antiapoptotic proteins and the BH3 domain of proapoptotic proteins has emerged as a promising approach to antagonize the activity of antiapoptotic Bcl-2-family proteins in cancer. A new class of drug, termed BH3 mimetics, able to specifically and selectively target the hydrophobic groove of Bcl-2, Bcl-xL and/or Mcl-1 has been

developed [2]. These small molecules display great specificity and efficiency both *in vitro* and *in vivo*, and several are presently the subject of clinical trials (Table 1). The effectiveness of these molecules is based on the fact that the targeted cancers have a specific feature termed “primed for death”. Those cancers usually express high levels of proapoptotic Bcl-2-family proteins due to ongoing oncogenic stress and thus can only survive by the virtue of concomitant upregulation of antiapoptotic Bcl-2-family members. BH3 mimetics that target the hydrophobic cleft of antiapoptotic members and thus release proapoptotic BH3-only proteins are therefore highly effective in killing cancer cells while sparing healthy, nonmalignant cells [2, 33, 34]. Thus, new compounds are being developed, as well as innovative techniques to validate their efficacy and specificity *in vitro* and *in vivo* [35]. Many of these drugs are currently under clinical trial and are starting to fulfill their promises (Table 1). The selective Bcl-2 inhibitor venetoclax/ABT-199 became the first BH3 mimetic approved for the treatment of human cancer, namely, relapsed chronic lymphocytic leukemia (CLL), in 2016. Thus, Bcl-2 inhibitors have been explored in advanced clinical trials for other hematological cancer types. On the other hand, BH3 mimetics selectively targeting Bcl-xL and Mcl-1 have proven very promising in the context of solid cancers [2, 34, 36]. For this reason, dynamic BH3 profiling of patient tumors has been proposed to be systematically applied [33]. This approach consists of collecting tumor biopsies, culturing cancer cells and assessing their sensitivity to proapoptotic Bcl-2-family members in combination with classical chemotherapeutics. Hence, this would permit the prediction of responses to treatment, the anticipation of potential resistance and the determination of the best drug combination to propose individually adapted treatments for patients [33].

Despite the strong efficiency and selectivity of BH3 mimetics, the clinical use of such drugs to target Bcl-xL has been quite challenging compared to those targeting other antiapoptotic Bcl-2-family proteins. BH3 mimetics inhibiting Bcl-xL (including Bcl-2/Bcl-xL & Bcl-2/Bcl-xL/Mcl-1 inhibitors) are indeed very effective *in vitro* and *in vivo* [37-39]. However, the application of such compounds in the human clinic is still limited due to the crucial role of Bcl-xL in the survival of platelets [40]. Considering that the high sensitivity of these BH3 mimetics may reduce the concentrations used in humans, the BH3 mimetic ABT-263 targeting both Bcl-xL and Bcl-2 has been clinically evaluated. However, the first trials have unfortunately been marked by the frequent occurrence of deleterious side effects, particularly thrombocytopenia [41, 42]. Of note, no specific Bcl-xL inhibitor has been selected for clinical trials [36]. Considering the limitations of these compounds regarding the highly Bcl-xL-dependent platelets, a new class of compounds has been recently developed based on the modifications of BH3-mimetic Bcl-xL antagonists. Proteolysis-targeting chimeras (PROTACs) are molecules that degrade a specific protein through recruitment of an E3 ligase that ubiquitinates the protein and sends it to the proteasome [43]. The interest of this system is that it permits the recruitment of particular E3 ligases, including tissue-specific ligases. Indeed, this approach is of particular interest in the context of Bcl-xL. The first PROTAC molecule validated to target and degrade Bcl-xL was named DT2216. This compound encompasses an ABT-263-like backbone (to target Bcl-xL) combined (though a linker sequence) to a molecular structure recruiting the Von Hippel-Lindau (VHL) E3 ligase, an E3 ligase poorly expressed in platelets. Fascinatingly, the application of DT2216 *in vitro* and *in vivo* has been extremely successful in killing cancer cells and showed no toxicity to platelets [44]. This outcome is even more surprising since DT2216 is based on the molecular structure of ABT-263, which is known to impair platelet survival. In fact, the high efficiency

and specificity of the compound provides an effective antitumor effect at substoichiometric concentrations, thereby sparing the platelets. Hence, other PROTAC-targeting Bcl-xL – with another BH3 mimetic backbone or another E3 ligase-addressing signal – have been developed and have been effective in a wide variety of cancer models [43]. These PROTAC molecules might become the most notable achievement of the last decade for Bcl-xL cancer therapies.

The dysregulation of the expression of Bcl-2-family proteins is therefore a hallmark of cancer. Tumor cells are often characterized by a dependency to one of or more antiapoptotic Bcl-2-family members, compensating the high expression levels of proapoptotic members in response to oncogenic signaling. This "primed for death" property has spurred the development of novel precision anti-cancer pharmacological tools that selectively and efficiently kill cancer cell by acting as BH3 mimetics, antagonizing antiapoptotic Bcl-2-family proteins [33]. These compounds have a great potential for cancer care and are currently investigated for use in human clinics.

2 Bcl-2 counteracts proapoptotic Ca^{2+} signaling and apoptosis through inositol 1,4,5-trisphosphate receptor (IP_3R) inhibition

Beyond the well-established role of Bcl-2 and Bcl-xL in the regulation of apoptosis at the mitochondria, these proteins appear to be involved in an increasing number of processes, including autophagy, regulation of transcription and DNA repair [45]. Here, we will focus on apoptosis regulation at the endoplasmic reticulum (ER) and at the mitochondria through modulation of IP_3R-mediated Ca^{2+} signaling by antiapoptotic Bcl-2-family proteins, particularly Bcl-xL.

2.1 IP_3R functions at mitochondria-associated ER membranes (MAMs)

IP_3Rs are intracellular Ca^{2+}-release channels located at the ER membrane. IP_3Rs open upon binding of IP_3, produced through activation of phospholipase C and the subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate in response to an extracellular stimulus. The ensuing Ca^{2+} release from the ER, the main intracellular Ca^{2+} store, to the cytosol can lead to the initiation of a plethora of cellular processes, including neuronal transmission, muscle contraction, gene transcription, cell proliferation and apoptosis [46-48]. Three IP_3R isoforms exist, IP_3R1, IP_3R2 and IP_3R3 (encoded in humans by the genes *ITPR1*, *ITPR2*, and *ITPR3*, respectively), which exhibit strong sequence homology [49, 50]. IP_3Rs are ubiquitously expressed proteins, but the expression levels of each isoform vary according to tissue type, subcellular location and stage of development [51, 52]. Although the three IP_3R isoforms are very similar, they exhibit different affinities for IP_3, as well as for Ca^{2+} and ATP which both are main regulators of the channel [53-55]. Functional channels are made of four IP_3R proteins that together form a large tetrameric complex. All four subunits must bind IP_3 before channel opening can occur [56]. While the majority of IP_3Rs are mobile in the ER membrane and nuclear envelope, recent work has demonstrated that significant numbers of active IP_3Rs are immobile and located close to ER-plasma membrane junctions. These channels are tethered to actin filaments beneath the plasma membrane due to KRas-induced actin-interacting protein (KRAP), forming IP_3R clusters that are "licensed" to respond to IP_3 and generate Ca^{2+} puffs [57, 58]. In addition, a subset of all IP_3R channels present in cells are located in

specialized areas, the MAMs, where ER and mitochondrial membranes reside in close apposition [59]. MAMs serve as functional contact sites that enable signaling microdomains for lipids, reactive oxygen species and Ca^{2+} [60]. At these ER-mitochondrial contact sites, Ca^{2+} released via IP_3Rs is transferred from the ER to the mitochondria in a "quasi-synaptic" manner [61] and transported across the OMM through VDAC [62, 63]. MAMs form Ca^{2+} microdomains [64] where a number of specific proteins are expressed that tightly regulate Ca^{2+} fluxes or physically tether the contact sites [65-67]. As such, IP_3Rs and VDACs are physically coupled via the chaperone glucose-regulated protein 75 (GRP75), forming the base of a large protein complex also involving other actors, such as DJ-1 [68], transglutaminase type 2 [69] and Tespa1 [70]. In addition, other protein partners, such as mitofusin 2 [71], TOM70 [72] and Bok [73], were identified for tethering the MAM while supporting IP_3R-mediated Ca^{2+} fluxes. IP3Rs themselves participate in the establishment of contact sites, independently of their Ca^{2+}-driving activity [74].

MAMs therefore form unique contact sites for ER/mitochondrial Ca^{2+} signaling. These Ca^{2+} transfers are essential for two opposite but essential processes, cell survival and cell death [66, 75]. On the one hand, Ca^{2+} that enters the mitochondria is transported from the intermembrane space to the mitochondrial matrix through the mitochondrial Ca^{2+} uniporter (MCU) expressed in the inner mitochondrial membrane [76, 77]. Since Ca^{2+} is a cofactor of several enzymes of the citric acid cycle (pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase), basal mitochondrial Ca^{2+} dynamics are necessary to sustain cell survival [78-80]. Oscillatory Ca^{2+} release is optimal to promote mitochondrial bioenergetics [81], and constant IP_3R-mediated Ca^{2+} transfer to the mitochondria should be maintained to avoid a high AMP/ATP ratio leading to autophagy [82]. On the other hand, excessive Ca^{2+} uptake in the mitochondria leads to apoptosis induction. Thus, IP_3R activity is directly modulated by cell stress sensors [83]. During the early phases of ER stress, the unfolded protein response regulator GRP78/BiP detach from IP_3R to bind misfolded proteins, thereby allowing ER-resident protein 44 (ERp44) to interact with the channel. The binding of ERp44 to IP_3R suppresses IP_3R-driven Ca^{2+} release [84, 85]. However, upon prolonged stress, the ER oxidase 1α (ERO1α) and the inositol-requiring enzyme 1α (Ire1α) generate proapoptotic Ca^{2+} fluxes by fostering IP_3R expression or activity [86, 87]. In addition, tumor suppressors such as phosphatase and tensin homolog (PTEN) or promyelocytic leukemia protein (PML) are also known to promote IP_3R-mediated cell death [88-90]. Then, the Ca^{2+} released in the MAM is transferred to the mitochondria, and excessive mitochondrial Ca^{2+} triggers the opening of the mitochondrial permeability transition pore (mPTP), eventually leading to the release of proapoptotic factors such as cytochrome *c* into the cytoplasm [91]. This process is mediated through Ca^{2+}, which binds to the β subunit of the F_1F_0-ATP synthase, inducing a conformational change in ATP synthase [92]. The F_1F_0-ATP synthase is believed to turn itself into the mPTP upon binding excessive Ca^{2+} [93, 94]. In addition, Ca^{2+} also targets the diphosphatidylglycerol cardiolipin of the IMM, leading to the disruption of respiratory chain complex II and the generation of excessive reactive oxygen species (ROS) [95]. In turn, proapoptotic factors released from mitochondria, such as cytochrome *c* and ROS, can stimulate IP_3R activity and amplify apoptosis induction [96, 97].

It is also important to note that the three IP_3R isoforms display very distinct characteristics. Although recent data indicate that the three isoforms release similar Ca^{2+} puffs [98], it has been extensively documented that the spatiotemporal Ca^{2+} dynamics strongly differ from one IP_3R isoform to another. It is typically assumed that IP_3R1 and IP_3R2 generate oscillatory Ca^{2+} signaling, while IP_3R3 engenders

monophasic Ca^{2+} transients [55, 99]. These divergences are indeed very significant in the context of cell death and survival and have provided a first explanation for the strong implication of IP_3R3 in Ca^{2+}-dependent cell death [89, 100-102]. Moreover, depending on the cell type, certain isoforms can exhibit a specific localization and function at the ER-mitochondria contact sites. Comparative analysis using cell models with genetic deletion of all three isoforms and reconstituted with one single isoform revealed that while the three IP_3R isoforms can be expressed at the MAMs, the type 2 and type 3 IP_3R isoforms are predominant in these regions [74]. On the other hand, IP_3R1 resides essentially near the plasma membrane. Moreover, the study demonstrated that IP_3Rs have a structural role at the contact sites and support the correct architecture of the MAMs. This work also determined that among the different isoforms in the MAMs, IP_3R2 is the most effective in delivering Ca^{2+} to the mitochondria [74]. Nevertheless, the best described isoform at the ER-mitochondria contact sites is IP_3R3, and this protein is even considered a MAM marker [103]. IP_3R3 is particularly enriched in MAMs [74, 100] and preferentially transmits apoptotic Ca^{2+} signals into mitochondria, even when it is not the predominant isoform in the whole cell [100]. Of note, IP_3R3 does not exclusively mediate proapoptotic Ca^{2+} signaling but can also promote prosurvival Ca^{2+} transfer at MAMs to sustain mitochondrial bioenergetics [72].

By mediating Ca^{2+} flux from the ER to the cytosol, IP_3R channels participate in a plethora of cellular processes, including cell death and survival. Appropriate regulation of IP_3R-mediated Ca^{2+} release is thus essential for cell function. Several mechanisms underlie such tight control, including a still expanding list of IP_3R-accessory proteins. Among these interacting proteins, several antiapoptotic members of the Bcl-2 family have emerged as critical modulators of IP_3Rs Ca^{2+}-flux properties. We will now discuss how Bcl-2 and Bcl-xL can control cell death and survival through regulation of Ca^{2+} signaling.

2.2 The regulation of IP_3R by Bcl-2

Beyond their canonical role in scaffolding proapoptotic Bcl-2 proteins, antiapoptotic Bcl-2-family proteins have also emerged as modulators of intracellular Ca^{2+} homeostasis and dynamics. Currently, it is clear that antiapoptotic Bcl-2-family members can form complexes with IP_3Rs and VDAC1 channels, thereby impacting Ca^{2+} flux from the ER and Ca^{2+} transfer to the mitochondrial matrix [104].

The first Bcl-2-family protein identified for its capacity to bind IP_3R was Bcl-2 itself [105, 106]. Endogenous Bcl-2 interacts with the three IP_3R isoforms in various cell types [105, 107-109]; though the functional impact of Bcl-2 on distinct IP_3R isoforms has not yet been systematically analyzed. Importantly, Bcl-2 directly inhibits IP_3Rs, thereby suppressing sustained, proapoptotic Ca^{2+} signals that arise from ER Ca^{2+} stores [105, 107, 108, 110, 111]. Accordingly, multiple cancer cell types depend on IP_3R inhibition by Bcl-2 for their survival, especially B-cell lymphomas [109, 112-114].

Additionally, Bcl-2 can also regulate ER Ca^{2+} release indirectly by contributing to a negative feedback mechanism that desensitizes activated IP_3R1s [115]. Bcl-2 scaffolds a protein complex comprising the Ca^{2+}/calmodulin-dependent phosphatase calcineurin, the protein DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) and the protein phosphatase 1 (PP1) in proximity to IP_3R. Depending on which plasma membrane receptors are stimulated, the activation of intracellular signaling pathways results in the generation of IP_3 as well as in the activation of protein kinases such as protein

kinase A (PKA). While IP_3 directly triggers IP_3R, PKA sensitizes IP_3R via its phosphorylation at Ser1755. Sensing the Ca^{2+} rise, calcineurin is activated and initiates negative feedback by dephosphorylating DARPP-32. Dephosphorylated DARPP-32 then releases PP1, withdrawing its inhibition. Finally, PP1 removes the PKA-mediated phosphorylation of IP_3R, leading to a desensitization of the channel [115]. Of note, this interplay between Bcl-2 and PKA has only been deciphered for the IP_3R1 isoform. Hence, the relevance of this mechanism for controlling the IP_3R2 and IP_3R3 isoforms, which lack consensus PKA-phosphorylation sites [52, 116], remains largely unknown. However, forskolin, which evokes PKA activation, not only increases the open probability of IP_3R1 but also of IP_3R2 channels [117]. The stimulation of IP_3R2-mediated Ca^{2+} fluxes by PKA appeared to be dependent on Ser937. Also, IP_3R3 can be phosphorylated by PKA though without major functional impact on agonist-evoked Ca^{2+} signals [118].

A capacity of Bcl-2 to lower the Ca^{2+}-store content was originally proposed to account for the decrease in IP_3R-driven Ca^{2+} release [119, 120]. In fact, several different mechanisms by which Bcl-2 can directly or indirectly mediate ER Ca^{2+} leakage have been described [121], including the inhibition of the sarco/endoplasmic reticulum Ca-ATPase (SERCA) [122], increased Ca^{2+} leakage through sensitized IP_3R channels [123] hyperphosphorylated by PKA [124] or directly by creating cation-selective channels in the ER membrane [125]. Nevertheless, further investigations clearly demonstrated that Bcl-2 can suppress Ca^{2+}-driven apoptosis without altering the ER Ca^{2+} store content [105, 107, 108, 110, 126]. To date, the varying impact of Bcl-2 on steady-state $[Ca^{2+}]_{ER}$ among different cell models and research groups remains poorly understood. One obvious reason could be that the effect of Bcl-2 on steady-state ER Ca^{2+} levels depends on its protein levels. Another reason could be that Bcl-2 affects ER Ca^{2+} levels indirectly through accessory proteins such Bax Inhibitor-1 (BI-1) or CISD2/Naf-1 [104, 127-130]. Hence, the effect of Bcl-2 on basal $[Ca^{2+}]_{ER}$ would depend on the presence or absence of these other proteins. Similarly, regulation of ER stores by Bcl-2 could be dependent on its posttranslational modifications since these alterations modulate the function of the protein [131]. Likewise, occurrence of these modifications would also depend on the presence or absence of other proteins. Moreover, the capacity of Bcl-2 to regulate Ca^{2+} homeostasis is strongly dependent on other members of the Bcl-2 family of proteins, whose expression and activity vary according to cell stress. In fact, most pro- and antiapoptotic Bcl-2-family members are expressed at the ER membranes, and many of them, such as Bcl-xL, Mcl-1, Bax, Bak, and Bok, modulate Ca^{2+} homeostasis and/or directly regulate IP_3Rs [104, 132-134]. As such, cells in which the balance of antiapoptotic over proapoptotic proteins is high, such as Bax/Bak double knockout cells, were initially reported to display a decreased steady-state $[Ca^{2+}]_{ER}$ [135]. Indeed, Bax and Bak have been particularly studied in this context since their depletion or overexpression have major consequences on basal $[Ca^{2+}]_{ER}$. Although it is still not clear whether these proteins prevent [124, 135, 136] or favor [137, 138] ER Ca^{2+} store depletion, they always exert the opposite effect of Bcl-2 on $[Ca^{2+}]_{ER}$. It is thus assumed that Bax and Bak present at the ER are targeted by Bcl-2 and diminish the pool of free Bcl-2 available in ER membranes. Thus, they compete with IP_3R for Bcl-2 binding, ultimately governing the capacity of Bcl-2 to regulate IP_3R-mediated Ca^{2+} release [124, 138]. Interestingly, the activity of Bax/Bak at the ER relies, like at the mitochondria, on BH3-only proteins such as Bid, Bim, Bik or Bad [139-142]. Overall, these reports suggest that pro- and antiapoptotic Bcl-2-family members can interact together and neutralize/activate each other at the ER membranes in a similar fashion as they do at the mitochondrial membranes. Furthermore, the possible interplay between Bcl-2 expressed at the ER membranes and mitochondrial

Bax [141] suggests that the pro- and antiapoptotic pathways of the Bcl-2 family of proteins at the ER and at the mitochondria could be tightly connected. We therefore propose that in the narrow environment of the MAMs, Bcl-2-family proteins present in those membranes mutually regulate ER Ca^{2+} homeostasis/IP_3R activity on the one hand and mitochondrial permeability on the other hand, eventually supporting or preventing apoptosis according to the cellular context.

Irrespective of the diverging mechanisms, Bcl-2 is thus acknowledged as an important IP_3R modulator. More interestingly, modulation of IP_3R-driven Ca^{2+} signaling by Bcl-2 has emerged as a promising target for cancer therapies.

2.3 Molecular determinants underlying IP_3R/Bcl-2 complex formation

As stated above, IP_3R channels are tetrameric. Each monomer is organized into three large functional regions: the N-terminal ligand-binding domain (LBD) (amino acids 1-604 for mouse IP_3R1), the central, modulatory region (a.a. 605-2216), and the C-terminal region (a.a. 2217-2749) (Fig. 3). The LBD consists of the IP_3-binding core (IBC) and the suppressor domain (SD). The C-terminal region consists of six TMDs, whereby the 5th and 6th TMDs form the channel pore and a cytosolic tail [143]. This overall organization is similar among the three IP_3R isoforms. Recently, more insights into the structure of the IP3R channel were obtained from cryo-EM analyses of the full-length tetrameric IP_3R1 and IP_3R3 channels [144-148]. Recent insights provided by the publication of these structures to our understanding of the channel function were recently discussed [149].

Furthermore, beyond the functional domains of the channel, it is well established that the IP_3R1 can be experimentally divided into five reproducible proteolytic fragments, generated upon controlled trypsinization of the channel (Fig. 3). These five fragments (labeled Fragment 1 to Fragment 5) [150] have proven to be an excellent tool for deciphering the binding sites of different IP_3R partners to the channel [107, 151, 152]. This strategy has been applied with success for Bcl-2-family proteins [107, 110, 151, 153]. First, the Fragment 3, which corresponds to the middle part of the central, modulatory region of IP_3R1, was identified to bind Bcl-2 (a.a. 923-1581) [107]. Moreover, a conserved segment of 20 amino acids in the Fragment 3 (dubbed Fragment 3a1; a.a. 1389-1408) has been identified as a critical determinant of the interaction of IP3R1 with Bcl-2 [107]. This amino acid stretch is present in all three IP_3R isoforms, enabling the binding of Bcl-2 to the central, modulatory regions of all three isoforms [110]. These amino acids are also highly conserved among IP_3R orthologues, including the IP_3R from the unicellular organism *Capsaspora owczarzaki*, which is capable to bind human Bcl-2 [154]. Moreover, a peptide based on the Fragment 3a1 of IP_3R1 (IDP, for IP_3R-derived peptide) is capable of disrupting Bcl-2/IP_3R complexes and reversing Bcl-2-mediated inhibition of IP_3R by binding to Bcl-2's BH4 domain [110, 155]. Interestingly, since this sequence is conserved among the three IP_3R isoforms, the IDP is not only able to prevent the binding of Bcl-2 to the IP_3R1 but also to the other isoforms [109]. Nevertheless, it later appeared that the interaction between Bcl-2 and IP_3Rs is more complex and relies in fact on multidomain binding determinants. Hence, Bcl-2 binds to the Fragment 5 (a.a. 1932-2749), especially to a segment consisting of the 6th TMD and the C-terminal tail (a.a. 2512-2749) [151]. Finally, by discovering that IP_3R1 channels lacking a.a. 1389-1408 could still be inhibited by Bcl-2, and our group recently identified that Bcl-2 could also interact with the LBD [111]. Interestingly, Bcl-2 and IP_3 displayed mutual

competition to bind the LBD of IP_3R. Consequently, in the presence of a high concentration of IP_3, Bcl-2 binding to the LBD was impaired, while Bcl-2 failed to inhibit IP_3Rs opened by high [IP_3]. Conversely, Bcl-2 was more efficient in inhibiting IP_3Rs at low [IP_3] [111]. Consistently, overexpression of Bcl-2 only suppressed Ca^{2+} signals elicited by low/medium [agonist] but not those elicited by high [agonist]. An interesting consequence of the competition between IP_3 and Bcl-2 towards binding to the LBD is that one Bcl-2 molecule per channel could be theoretically sufficient to inhibit IP_3R, as all four subunits have to be occupied by IP_3 to open the channel [56].

At the level of Bcl-2, the interaction with IP_3R is also quite complex since multiple domains of Bcl-2 interact with the channel. Basically, the N-terminal BH4 domain of Bcl-2 binds to the LBD [111] as well as to the Fragment 3 [110, 155] of IP_3R, whereas the C-terminal TMD of Bcl-2 binds to the Fragment 5 [126]. Both the BH4 domain and the TMD of Bcl-2 are necessary for the proper interaction and modulation of IP_3R. However, among these two domains, the BH4 domain clearly appears to be the most prominent [110, 111, 126]. In fact, peptides representing the BH4 domain of Bcl-2 are able to bind to IP_3Rs (and the purified Fragment 3 of IP_3R) and are sufficient to completely inhibit IP_3R channel opening [110]. This mechanism appeared to be strongly conserved during evolution since BH4 domains of Bcl-2 orthologues from different classes of vertebrates can also act as inhibitors of mammalian IP_3Rs [156]. In addition, the TMD is also significant for the inhibition of IP_3Rs by Bcl-2 [126]. In living cells, the TMD is necessary for Bcl-2 to bind and completely inhibit the channel. Peptides representing the TMD are also able to inhibit the channel by themselves in permeabilized cells but to a lower extent compared to BH4 peptides [126]. The inhibitory capacity of the TMD is actually lower than that of the BH4 domain. This is supported by the fact that, in patch-clamp experiments, a purified Bcl-2 protein deleted from its TMD is as efficient as full-length Bcl-2 in abrogating IP_3R single-channel openings [111, 153]. In conclusion, we have proposed a model in which the TMD anchors Bcl-2 in the membrane of the ER, where it serves to "concentrate" Bcl-2 in proximity to IP_3R. Hence, the TMD moderately inhibits IP_3R but facilitates the positioning of the BH4 domain in the close proximity of its binding sites on the channel [126]. Interestingly, the hydrophobic cleft (made of the BH1, BH2 and BH3 domains) located between the BH4 domain and the TMD is apparently not involved in the regulation of IP_3Rs [157]. Consequently, BH3 mimetics such as venetoclax/ABT-199, a selective Bcl-2 inhibitor that targets the hydrophobic cleft, do not have a major impact on Bcl-2's ability to inhibit IP_3Rs [157, 158]. However, BH3 mimetics, including ABT-737 and venetoclax, appear to stabilize the Bcl-2 protein [159, 160]. Detailed analysis of IP_3R channel gating suggests that allosteric modulation of the BH4 domain through Bcl-2's hydrophobic cleft might exist, thereby subtly impacting the efficiency by which Bcl-2 inhibits low-level IP_3R single channel activity [160].

The capacity of Bcl-2 to bind and inhibit the activity of IP_3Rs was extensively examined and exploited in the context of cancer [161, 162]. Indeed, many cell cancer models, in particular B cell lymphomas, are addicted to Bcl-2, which prevents proapoptotic Ca^{2+} signaling. These cancers are also considered "primed for death" since inhibition of Bcl-2 directly triggers cell death. To study this mechanism, a stabilized, proteolysis-resistant, and cell-permeable version of the IDP, dubbed BIRD-2 (Bcl-2/IP_3 receptor disruptor-2), was developed [112]. This molecule disrupts the Bcl-2/IP_3 interaction and provokes cell death by itself in a variety of Bcl-2-dependent cancer cell models, including diffuse large B-cell lymphoma (DLBCL) and chronic lymphocytic leukemia [109, 112, 114, 163, 164], follicular lymphoma and multiple myeloma [165]. It also enhances cisplatin-induced Ca^{2+} signaling and cell death in ovarian cancers [166] and

synergistically kills small-cell lung cancer cells in combination with the BH3 mimetic ABT-263 [167]. BIRD-2-induced cell death is dependent on IP_3R isoform expression levels, whereby BIRD-2 sensitivity correlated with IP_3R2 protein levels among different DLBCL cell models [109, 113, 114]. Furthermore, lowering IP_3R2 levels using siRNA in a BIRD-2-sensitive cell model decreased the sensitivity to BIRD-2, while increasing IP_3R levels in a BIRD-2-resistant cell model augmented its sensitivity to BIRD-2 [109]. Interestingly, IP_3R2 is recognized as the most efficient IP_3R isoform in establishing ER-mitochondrial contact sites and mediating ER-mitochondrial Ca^{2+} transfers [74]. Ultimately, disrupting the IP_3R/Bcl-2 interaction in such cancer cells triggers excessive IP_3R-mediated Ca^{2+} release, resulting in Bax/Bak-independent mitochondrial Ca^{2+} overload, rapidly followed by mPTP opening, release of cytochrome *c* and induction of apoptosis [164].

Furthermore, there is an interesting reciprocal sensitivity of B-cell lymphomas to BIRD-2 *versus* BH3 mimetics (such as venetoclax, navitoclax or HA14-1, etc.) [114, 163, 165]. This suggests that some cancer cells may require Bcl-2 at the mitochondria, preventing Bax/Bak activation via the hydrophobic cleft, while others require Bcl-2 at the ER, impeding cytotoxic Ca^{2+}-signaling events via their BH4 domain. This indicates some kind of separation of the domains of Bcl-2 according to their functions and interactions, with Bak/Bax on the one hand and IP_3Rs on the other hand. Nevertheless, this simplistic division may not be completely exact since noncanonical interaction mechanisms between Bax and Bcl-2's BH4 domain are also possible [168]. In any case, combining drugs targeting both the BH4 domain and the hydrophobic groove could be an approach to potentialize the effect of these molecules and potentially prevent Bcl-2-dependent cancer from escaping cell death [114, 163, 165]. In fact, the combination of BIRD-2 and BH3 mimetics at low concentrations resulted in synergistic cell death. Consequently, in addition to the new BH3 mimetics under development, new drugs targeting Bcl-2's BH4 domain are being investigated. For instance, BDA-366, a small molecule efficient against lung cancer, was identified as a putative selective Bcl-2 inhibitor targeting the BH4 domain [169]. This drug was originally described to conformationally change Bcl-2 into a proapoptotic protein with an exposed BH3 domain that triggers Bax activation. However, our group recently demonstrated that BDA-366 induced apoptotic cell death in CLL and DLBCL, though independently of Bcl-2-protein levels [159, 170]. Additionally, BDA-366-based alternative molecules were identified by molecular modeling approaches [171], yet these molecules failed to provoke cell death in Bcl-2-dependent cancer cell models [172]. Recently, another screening endeavor using computer-assisted and biophysical approaches resulted in the identification of DC-B01 as a novel Bcl-2 inhibitor that directly targets Bcl-2's BH4 domain [173]. DC-B01 induced mitochondria-mediated apoptosis in a Bcl-2-dependent manner and suppressed *in vivo* tumor growth of lung cancer cell models. At the mechanistic level, DC-B01 perturbed the Bcl-2/cMyc complex and abrogated pro-oncogenic cMyc-driven transcriptional processes. However, it remains to be established whether DC-B01 can also disrupt IP_3R/Bcl-2 complexes and thus elicit apoptosis through Ca^{2+}-driven pathways. In any case, BIRD-2 has proven its efficacy *in vitro* and *in vivo* [165], supporting the development of BH4-domain antagonists as promising anticancer therapeutic agents [161, 162, 172].

From the above it appears that the interaction of Bcl-2 with IP_3 is complex as it involves distinct domains/regions of both proteins. Further work is therefore needed to elucidate the full-length IP_3R/Bcl-2 complex to exactly understand its binding characteristics and stoichiometry. In any case, analyzing the molecular determinants underlying the IP_3R/Bcl-2 complex has permitted the development of peptide

tools such as BIRD-2 that can efficiently kill Bcl-2-dependent cancer cells by intracellular Ca^{2+} overload as a consequence of disrupting IP_3R/Bcl-2 complexes. As such, BH4-domain-antagonizing compounds may form promising anti-cancer tools eliciting Ca^{2+}-driven apoptosis.

3 The role of Bcl-xL in ER/mitochondria Ca^{2+} signaling

Bcl-xL is probably the best described antiapoptotic Bcl-2-family protein after Bcl-2 itself. The protein was discovered nearly a decade after Bcl-2 and identified as another cell death regulator [174]. Bcl-xL was designated "extra large" because its gene (*BCL2L1*) also encodes a smaller isoform named Bcl-xS (B-cell lymphoma-extra small) [174]. The protein Bcl-xL shares approximately 65% similarity with Bcl-2 and has a comparable molecular weight (26 kDa). It also has a similar structure with the same organization of the functional BH domains [19] (Fig. 1). As a member of the Bcl-2 family of proteins, Bcl-xL has been widely investigated for its antiapoptotic role in cancer. However, other evidence has demonstrated that this protein possesses a large number of noncanonical functions, including in noncancerous cells, such as neuronal function and development [175].

3.1 Bcl-xL regulates IP_3Rs to promote cell survival

In line with the role of Bcl-2 as a regulator of IP_3Rs, Bcl-xL emerged as a putative IP_3R modulator. Interestingly, the first study that investigated the relationship between these two proteins reported that overexpression of Bcl-xL reduced the expression of IP_3R1 [176]. This effect was explained by the inhibition of mitochondrial oxidative phosphorylation upon Bcl-xL overexpression, resulting in the reduced binding of the transcription factor NFAT to the IP_3R1 promoter. However, such an effect of Bcl-xL on IP_3R expression was not observed in subsequent studies by other groups, regardless of the cell model considered [177-179], and Bcl-xL was demonstrated to stimulate oxidative phosphorylation (see section 4.1).

Until recently, Bcl-xL was, in contrast to Bcl-2, predominantly seen as a sensitizer of the IP_3R [177]. In patch-clamp assays, recombinant Bcl-xL increased the open probability of single IP_3R1 channels gated by low concentrations of IP_3 but did not exhibit any effect at high $[Ca^{2+}]$. Moreover, in DT40 cells, overexpression of Bcl-xL did not affect agonist-induced IP_3R-mediated Ca^{2+} release but did promote spontaneous Ca^{2+} oscillations. Accordingly, this oscillatory pattern – usually considered prosurvival – resulted in enhanced mitochondrial energetics and augmented cell viability [177]. Equivalent findings were later obtained in distinct DT40 cell lines expressing only one of the three IP_3 isoforms [180]. The authors later provided more insights by exploring IP_3R modulation by Bcl-xL in a comprehensive electrophysiology study [181]. They demonstrated that Bcl-xL has in fact a dual role, activating the channel at low concentrations (with a maximal potentiating effect at 1 µM) but inhibiting it at higher concentrations (10 µM or more). This bell-shaped regulation of IP_3Rs suggested that Bcl-xL could execute various cellular functions according to its levels of expression.

Furthermore, the conclusions of the functional experiments were strengthened by detailed molecular interaction studies. First, coimmunoprecipitation (co-IP) studies from DT40-3KO cells (i.e. depleted from

the three endogenous IP_3R isoforms) reconstituted with a single specific IP_3R isoform and overexpressing Bcl-xL allowed to determine that Bcl-xL can interact with the three isoforms. It was also established using co-IP that endogenous Bcl-xL interacts with endogenous IP_3R3 in COS cells [177]. Next, using recombinant purified IP_3R fragments (Fig. 3), the authors showed through pull-down assays that Bcl-xL can bind the C-terminal Fragment 5 [177, 181] and, to a lesser extent, the Fragment 3a1 [181] – which is essential for Bcl-2/IP_3R interaction [107] – of IP_3R, but not the N-terminal LBD [177]. Using similar pull-down assays, we also measured that, contrary to Bcl-2, Bcl-xL had a noticeably stronger binding to the C-terminal Fragment 5 (more precisely to the Fragment 5b+ but not to the Fragment 5b, indicating the involvement of the 6^{th} TMD) than to the Fragment 3 of IP_3R [151]. Fascinatingly, two BH3-like domains in the Fragment 5 of the channel (dubbed H1 and H4, for helixes 1 and 4) were identified as two major binding sites for Bcl-xL [181]. A BH3 mimetic (ABT-737) was able to disrupt the interaction between Bcl-xL and those helixes, revealing that Bcl-xL binds the BH3-like domains in IP_3R through its hydrophobic cleft, apparently in a similar way as it does with the BH3 domains of proapoptotic Bcl-2-family members. In addition, the authors demonstrated that the bell-shaped regulation of the channel is dictated by the various binding sites targeted by Bcl-xL on the channel. Explicitly, Bcl-xL stimulates channel activity by interacting with the two BH3-like domains in the C-terminus, whereas it switches to its inhibitory function when binding to one of the BH3-like domains and to the Fragment 3a1. Finally, the authors were able to verify that disturbing the Bcl-xL/IP_3R interaction by specifically targeting the BH3-like helixes strongly impeded Bcl-xL's antiapoptotic function [181], convincingly linking the molecular interaction studies with Bcl-xL's functional regulation of the channel.

Furthermore, in line with the role of the hydrophobic cleft of Bcl-xL described for its interaction with the IP_3R channel, we determined that the BH4 domain of Bcl-xL was likely not involved in this interaction [110]. Our group scrutinized in detail the role of the BH4 domains of Bcl-2 and Bcl-xL in their interaction with IP_3Rs [110, 182-184]. In the BH4 domain of Bcl-2, a number of residues critical for the inhibitory effect of the protein were identified. Among them, lysine 17 (K17) is of particular interest [110]. This residue is especially well conserved in Bcl-2 orthologs but not in other antiapoptotic Bcl-2-family proteins. In Bcl-xL and Bcl-w orthologs, this positively charged lysine residue is replaced by a conserved negatively charged aspartate [154], namely, D11 in Bcl-xL. Fascinatingly, mutating K17 into an aspartate in Bcl-2's BH4 domain renders the protein unable to bind and modulate IP_3Rs, therefore impairing protection against IP_3R-mediated cell death. Inversely, mutating D11 into a lysine in Bcl-xL's BH4 domain (which displays poor IP_3R-binding properties by itself) made it as effective as Bcl-2's BH4 domain to bind and regulate IP_3Rs [110]. These molecular divergences provide a rationale for the distinct actions of Bcl-2 and Bcl-xL towards IP_3R.

Thus, until recently, functional and structural evidences pointed to major differences in the regulation of IP_3R by Bcl-2 *versus* Bcl-xL. Although both have a clear antiapoptotic effect, the former has been shown to diminish proapoptotic Ca^{2+} fluxes while the latter has been reported to stimulate prosurvival Ca^{2+} signaling. However, more recent findings indicate that also Bcl-xL is capable of inhibiting IP_3R activity thereby preventing Ca^{2+}-driven apoptosis. This novel feature of Bcl-xL will be developed in the next section.

3.2: Bcl-xL is an inhibitor of IP_3R activity, thereby preventing Ca^{2+}-mediated apoptosis

The first evidence suggesting an inhibitory role of Bcl-xL on IP_3Rs originated from the exhaustive characterization of Ca^{2+} signaling and apoptosis in Bcl-xL-knockout MEFs [185, 186]. The authors observed that the loss of Bcl-xL resulted in an increase in IP_3R-driven Ca^{2+} release, pointing to an inhibitory effect of Bcl-xL. However, they also measured an augmented [Ca^{2+}] in the ER of Bcl-$xL^{-/-}$ MEFs. They therefore concluded that the reduced IP_3R-mediated Ca^{2+} response in WT MEFs compared to Bcl-$xL^{-/-}$ MEFs is the indirect consequence of the lower amount of Ca^{2+} in the ER of WT MEFs [185].

New clues pointing to a direct inhibitory effect of Bcl-xL later arose from the work of our group concerning the ryanodine receptors (RyR) [187-192], a class of Ca^{2+}-release channels structurally and evolutionarily related to IP_3Rs [149, 193]. We first established that Bcl-2 interacts with and inhibits RyRs [187, 190], as it does with IP_3Rs. Moreover, we also demonstrated that Bcl-xL binds RyRs to dampen Ca^{2+} release [189]. In this study, we identified a critical lysine residue (K87) in the BH3 domain of Bcl-xL. *In silico* structure superposition of Bcl-2 and Bcl-xL revealed that K87 is spatially constrained in a similar position as K17 of Bcl-2 (Fig. 4). Mutating this K87 into an aspartate (K87D) abrogated the capacity of Bck-xL to bind and regulate RyRs [191].

In our recent work, we focused on the characterization of IP_3R regulation by Bcl-xL. First, we demonstrated using multiple techniques that Bcl-xL is able to bind to the LBD of IP3R [178], similar to Bcl-2 [111]. Next, we used our insights obtained from the regulation of RyRs by Bcl-xL to demonstrate that the K87 of Bcl-xL is also critical for the interaction of Bcl-xL with IP_3R. The K87D mutation severely impeded the binding of Bcl-xL to IP_3R, including to the LBD and Fragment 3 [178]. Interestingly, K87 is located on the opposite side of Bcl-xL's hydrophobic cleft, not in the binding pocket itself (Fig. 4). This suggests that the interaction of Bcl-xL with IP_3R may not exclusively involve binding through the hydrophobic cleft. Interestingly, a recent study identified a "double bolt" interaction between Bcl-xL and Bim, namely, via Bcl-xL's hydrophobic cleft and lysine 185 [194], validating the idea that multiple regions of Bcl-xL can be required for binding to one single partner.

At the functional level, we demonstrated that Bcl-xL does not systematically sensitize IP_3Rs but can also have a major inhibitory role. Overexpression of Bcl-xL in a variety of cell lines resulted in diminished agonist-induced IP_3R-driven Ca^{2+} release. Likewise, the application of purified Bcl-xL proteins in patch-clamp experiments reduced the open probability (P_O) of single IP_3R channels directly elicited by physiological concentrations of IP_3. Interestingly, we also observed in these electrophysiology experiments that high [IP_3] abolished Bcl-xL's inhibitory effect [178]. This outcome is noteworthy because it is similar to what we previously observed in the context of IP_3R regulation by Bcl-2. In that case, this effect was attributed to competition between Bcl-2 and IP_3 for binding the LBD of IP_3R [111]. As we have shown that Bcl-xL also binds to the LBD, we propose that a similar mechanism may occur in the context of the Bcl-xL/IP_3R interaction. In addition, consistent with our interaction studies, we also determined that the Bcl-xL^{K87D} mutant is severely impaired in its capacity to regulate IP_3R activity, either upon overexpression in cells or when purified for electrophysiology experiments, confirming the critical role of this residue. Another significant molecular determinant we identified is the TMD of Bcl-xL. While a purified Bcl-2 protein depleted of its TMD (Bcl-$2^{\Delta TMD}$) is absolutely capable of efficiently diminishing the P_O of IP_3R, the purified Bcl-$xL^{\Delta TMD}$ fails to inhibit IP_3Rs despite proper α-helical folding [178]. This indicates

that, contrary to Bcl-2's TMD, Bcl-xL's TMD might be an important and unappreciated molecular determinant for the interaction of Bcl-xL with IP_3Rs and not only serve to anchor the protein in the ER membrane. Until now, Bcl-xL's TMD has been essentially known for its role in the oligomerization of the protein [195]. Bcl-xL usually exists in both a membrane-bound state and a soluble cytosolic state. Upon apoptosis induction, the cytosolic form shifts from conformation and relocates to the mitochondrial membrane, where it can exert its antiapoptotic function [196]. Bcl-xL dimers are soluble in the cytosol since the TMDs are sequestered in the protein complex, while monomers are anchored in membranes through the TMD [195]. Although the membrane-inserted form of Bcl-xL is very likely the state required for interaction with the IP_3R (as it is for interaction with mitochondrial proteins), a mutated Bcl-xL protein unable to dimerize appears severely hampered in its capacity to modulate mitochondrial Ca^{2+} uptake [197], suggesting that Bcl-xL homodimers may also be involved in the regulation of Ca^{2+} channels. Overall, our data highlighting Bcl-xL's inhibitory function of IP_3R [178] challenge Bcl-xL's sensitizing effect described in earlier publications [177, 181]. However, our observations may be consistent with an inhibitory effect reported at high [Bcl-xL] [181]. It is nonetheless surprising that we did not observe any sensitization using purified Bcl-xL proteins in patch-clamp experiments. In our hands, both Bcl-2 and Bcl-xL diminish the P_O of the channel in a similar fashion. Furthermore, either at low [IP_3] or at low [Bcl-xL] – likely the best conditions to visualize a sensitization of the channel – we did not measure any augmentation of the P_O. In any case, we postulate that Bcl-xL may mediate various types of modulation of the IP_3R, either sensitizing prosurvival Ca^{2+} signaling or inhibiting proapoptotic Ca^{2+} signaling, potentially dependent on its expression levels and/or other yet poorly defined parameters such as post-translational modifications, accessory proteins, etc.

Another point that will need to be clarified to elucidate the dual role of Bcl-xL towards IP_3Rs is the capacity of Bcl-xL to regulate Ca^{2+} homeostasis. As mentioned above, the work of Li and White clearly demonstrates the essential role of Bcl-xL in the maintenance of ER Ca^{2+} stores [185], likely by promoting enhanced basal IP_3R activity in resting cells [177, 180]. The ability of Bcl-xL to lower ER Ca^{2+} stores has been observed in several studies [177, 180, 185, 198, 199] but not systematically [178, 180, 189]. This discrepancy is very interesting because a similar discrepancy was also reported for Bcl-2 (see section 2.1). Moreover, Bcl-2-mediated reduction of $[Ca^{2+}]_{ER}$ has been related to a sensitization of IP_3Rs. While it is evident that Bcl-2 suppresses large IP_3R-driven proapoptotic Ca^{2+} fluxes [105, 110, 111, 155], Bcl-2 was also shown to amplify IP_3R-mediated prosurvival oscillatory Ca^{2+} release [123, 198, 200]. This ability of Bcl-2 to favor such oscillatory Ca^{2+} signaling was not consistently observed and appeared limited to the context of reduced steady-state $[Ca^{2+}]_{ER}$ [123, 198]. The authors of these studies therefore established that Bcl-2 depleted the Ca^{2+} stores via this enhanced IP_3R activity. Hence, these observations strongly converge to the findings reported for Bcl-xL [177, 180]. It is striking that comparable dual mechanisms – sensitization versus inhibition of IP_3R – were reported for both Bcl-2 and Bcl-xL as well as the relationship between sensitization and altered Ca^{2+} stores. This suggests an important functional similarity of the two proteins in their regulation of IP_3R activity and Ca^{2+} homeostasis. Furthermore, most of the mechanisms proposed in section 2.1 to account for the Bcl-2-mediated reduction of Ca^{2+} stores are also valid for Bcl-xL, including sensitization of IP_3R at steady-state [IP_3] [177], formation of ion channels by Bcl-xL itself [201], interaction of Bcl-xL at the ER with proteins involved in the regulation of $[Ca^{2+}]_{ER}$ such as BI-1 [128], CISD2 [127] or other Bcl-2-family proteins [142]. Similar to Bcl-2, the nonsystematic observation of an effect of Bcl-xL on Ca^{2+} stores indicates that such mechanisms might depend on the level of expression of Bcl-xL or vary depending on the presence/absence of the abovementioned partners. While most Bcl-2

and Bcl-xL partners mentioned above are shared between both proteins, interaction and/or regulation by some other partners are rather specific to either Bcl-2 or Bcl-xL. This is the case with the proto-oncoprotein K-Ras [202]. This protein can bind to both Bcl-2 and Bcl-xL, but only the protein kinase C-phosphorylated K-Ras can block Bcl-xL sensitization of IP_3Rs, limiting cell survival [202, 203]. Another example is the ER pseudoproteases iRhom1 and iRhom2 [204]. These proteins critically support IP_3R-mediated cell death induced by ER stress. iRhoms bind to both IP_3Rs and Bcl-2 and in this way prevent the inhibition of IP_3R-driven Ca^{2+} flux by Bcl-2 upon ER stress. However, iRhoms neither interact nor regulate Bcl-xL [204].

Regarding the consequences of the inhibition of IP_3R by Bcl-xL on cell death and survival, our recent data demonstrate that Bcl-xL can prevent proapoptotic Ca^{2+} signaling. To study this mechanism, we used staurosporine (STS), which has been previously recognized for provoking Ca^{2+}-driven apoptosis [91, 205]. STS provokes transient mitochondrial depolarizations and flickering mPTP openings, therefore generating microdomain oxidative bursts and subsequent oxidization of IP_3Rs at the MAMs [206]. Thus, the oxidation of IP_3Rs elicits proapoptotic IP_3R-driven Ca^{2+} release to the mitochondria. Interestingly, STS-induced cell death relies on Bax and Bak, but they are only activated by excessive mitochondrial Ca^{2+} uptake, not by flickering mitochondrial depolarizations or mPTP openings [206]. However, we validated that STS-induced apoptosis depends on IP_3R, which is absent in HeLa-3KO cells. We subsequently demonstrated that overexpressing Bcl-xL dampened STS-induced Ca^{2+} release and STS-induced apoptosis in wild-type HeLa cells but not in HeLa-3KO cells. This confirmed that Bcl-xL, like Bcl-2, can suppress proapoptotic Ca^{2+} flux (Fig. 5a). Interestingly, the Bcl-xL^{K87D} mutant was not able to prevent such IP_3R-mediated proapoptotic mechanisms, suggesting that K87 is essential for Bcl-xL binding to IP_3R [178]. These results can appear at odds with the work of White and Li. Using Bcl-$xL^{-/-}$ MEFs reconstituted with either ER-targeted or mitochondria-targeted Bcl-xL, these authors have shown that Bcl-xL expressed at the ER does not provide any antiapoptotic protection in response to various cell death inducers, including the IP_3R-mediated apoptosis induced STS [185]. However, it should be kept in mind that the distinction between ER and mitochondrial membranes can be challenging in the context of Ca^{2+} transfer at the MAMs. In these areas, ER and mitochondrial proteins are in close proximity, with IP_3R and VDAC physically tethered [207]. Hence, it is not impossible that one pool of Bcl-xL protein could relocate from one membrane to another upon various stimuli and synergistically control IP_3R and VDAC channels (see section 3.3).

In any case, we hypothesized, as proposed above, that Bcl-xL-mediated inhibition of IP_3R-driven apoptotic Ca^{2+} fluxes could rather be specific for cancer cells. This claim is supported by our findings in MDA-MB-231 cells, triple-negative breast cancer cells that express high levels of Bcl-xL and were identified to be a highly Bcl-xL-dependent cell line [208]. Knockdown of Bcl-xL resulted in increased IP_3R-driven Ca^{2+} release, thereby revealing that IP_3R inhibition occurs by endogenous Bcl-xL, at least in MDA-MB-231 cells. Moreover, MDA-MB-231 cells are resistant to STS-induced apoptosis though decreasing Bcl-xL expression renders them sensitive to STS, thereby provoking cell death [178]. Thus, these results established for the first time that endogenous Bcl-xL can dampen IP_3R-mediated Ca^{2+} signaling and protect cells against IP_3R-dependent apoptosis. This work also raises questions regarding the role of IP_3R-driven Ca^{2+} release and Bcl-xL in cancer. Indeed, ER-mitochondrial Ca^{2+} signaling has emerged as a fascinating prospect for cancer therapies [65, 209, 210]. Many chemotherapeutics, such as cisplatin, arsenic trioxide, and resveratrol, directly or indirectly affect Ca^{2+} flux, leading to cytotoxic mitochondrial Ca^{2+} overload [209]. It would be interesting to determine whether the BH3 mimetics, by targeting Bcl-xL,

beyond acting via the canonical Bcl-2-family pathway, also stimulate proapoptotic Ca^{2+} fluxes to kill cancer cells.

In conclusion, we propose that Bcl-xL may support either a sensitizing or an inhibitory effect of IP_3R-mediated Ca^{2+} release according to its level of expression and/or to the cellular context. In healthy cells, low protein levels of Bcl-xL would sensitize IP_3R-driven prosurvival oscillatory Ca^{2+} signaling and mitochondrial metabolism, while upon ER stress or in cancer cells, high protein levels of Bcl-xL would inhibit IP_3Rs and proapoptotic Ca^{2+} fluxes. Hence, both mechanisms would improve cell survival. Of note, IP_3R regulation by Bcl-xL could be more complex, particularly in cancer cells in which different levels of expression and isoforms of IP_3R, especially IP_3R3, can promote either proapoptotic or prosurvival Ca^{2+} signaling [211, 212]. We therefore do not exclude the possibility that Bcl-xL could also support prosurvival Ca^{2+} signaling in cancer cells.

3.3 Bcl-xL is a regulator of ER/mitochondria Ca^{2+} signaling, thereby promoting cellular metabolism and cell survival

Ca^{2+} release through IP_3Rs is not an isolated process but the trigger of multiple specific cellular functions. The effects mediated by Bcl-xL at the level of IP_3R must be seen in the wider context of ER/mitochondria Ca^{2+} transfer and mitochondrial metabolism (Fig. 5). At the MAMs, Ca^{2+} release from IP_3Rs is directly linked to mitochondrial Ca^{2+} uptake through VDAC, the counterpart channel of IP_3Rs in the OMM [59]. Interestingly, VDACs are also linked to RyR in particular cell contexts, such as cardiomyocytes [213]. In vertebrates, there are three VDAC isoforms – VDAC1 to VDAC3 – coded by three different genes. VDAC1 and VDAC2 are the best documented isoforms and among the most abundant proteins expressed in the OMM [214]. Among the three VDAC isoforms, VDAC1 is the only isoform that forms complexes with IP_3R at MAMs and transfers proapoptotic Ca^{2+} signals from the ER to mitochondria [102].

VDAC is a nonspecific voltage-dependent channel known for its crucial role in mitochondrial metabolite transport. Originally known as a mitochondrial porin due to its β barrel structure, it was renamed after the discovery of its capacity to transport anions under specific voltages. Hence, its closed and open states are defined in terms of flux of anions [215, 216]. The electrical characteristics of VDACs are mostly based on the VDAC1 isoform [62, 216-218]. VDAC2 displays similar properties [219, 220], while VDAC3 is poorly voltage-dependent [220]. The characterization of the channel has been mainly performed in planar lipid membranes [62, 216-218, 220-222]. VDAC exhibits a bell-shaped voltage-dependence with gating at resting, moderate potentials (between −30 to +30 mV). In this open state, the channel has a high conductance for negatively-charged ions, mitochondrial metabolites and respiratory substrates. At higher (> 40 mV) and lower (< -40 mV) applied voltages, VDAC becomes less permeable to anions. In this closed state, the channel is about 50% less permeable to anions and totally impermeable to negatively-charged molecules [214, 217, 218, 220, 223-225]. It is important to note that the channel adopts different closed states, dependent on the applied voltages, with a so-called “negatively closed” state for voltages < -40 mV and “positively closed” state for voltages > 40 mV [215, 216]. Concerning Ca^{2+} fluxes through VDAC, the mechanism is rather paradoxical. In fact, the channel exhibits a higher permeability for cations in its closed states, particularly in the “negatively closed” conformation. In this latter state,

Ca^{2+} permeability is increased about four times compared to the open state [216]. It has been proposed that these different states of VDAC have different structural conformation, particularly at the level of the ion selectivity filter [215, 225]. Nevertheless, the physiological relevance of this process has been debated due to the difficulty to demonstrate the existence of a membrane potential across the OMM [214, 225]. Moreover, some studies suggest that voltage-dependent gating of VDAC is negligible in physiological conditions compared to its regulation by other factors such as accessory proteins [224, 225]. Indeed, a large number of molecules able to interact with the channel and to modulate its gating properties have been identified [224], including NADH [226], hexokinases [222], tubulin [227], α-synuclein [228] and Ca^{2+} itself [229]. Since the channel is usually open in steady-state conditions, it is not surprising that most VDAC regulators do close the channel.

Due to the importance of metabolites and Ca^{2+} fluxes between the cytosol and the mitochondria, VDACs have been involved in numerous biological processes, including regulation of cell death and survival. Accordingly, several Bcl-2-family proteins interact with VDAC, thereby regulating its gating [104, 224, 230]. However, whether VDAC opening or closure contributes to apoptosis has been strongly debated [223, 230]. Nowadays, opened VDAC is generally accepted as the prosurvival state of the channel. The current model is that gated VDAC promotes a large flux of metabolites while sustaining the limited Ca^{2+} uptake necessary to maintain mitochondrial bioenergetics (Fig. 5a). Contrariwise, VDAC in its closed state stimulates cell death by impeding metabolic fluxes and supporting wide cytotoxic Ca^{2+} entry into the mitochondria (Fig. 5b) [214, 216].

Among all Bcl-2-family members, the best-investigated protein interacting with VDAC is Bcl-xL. Since VDAC and Bcl-xL are predominantly expressed in the same organelle, the interaction between these two proteins at the mitochondria was discovered long before the interaction involving IP_3R at the ER [231, 232]. Nevertheless, the role of Bcl-xL in opening *versus* closure of VDAC has been intensely discussed [230]. In accordance with the model depicted above, Bcl-xL has been described to promote the open configuration of the channel, therefore facilitating prosurvival metabolite exchanges [232, 233]. On the other hand, Bcl-xL has also been implicated in the closure of the channel [221, 231], thereby preventing the proapoptotic release of cytochrome *c* [231, 234]. The concept of cytochrome *c* release through VDAC pores has been highly controversial since the diameter of the channel pore is not sufficient for the passage of proteins [235, 236]. Furthermore, the channel initially appeared dispensable for mitochondrial cell death since fibroblasts depleted for the three VDAC isoforms exhibited equivalent viability and cytochrome *c* release than WT cells [237]. Afterwards, the capacity of VDAC to homo- or hetero-oligomerize (with other proteins such as Bax) was revealed. Furthermore, these VDAC oligomers display unique structural conformations and noticeable larger pores that can account for the passage of large molecules, including cytochrome *c* [223, 235, 238, 239]. The assemblage of these oligomers is usually a consequence of amplified VDAC expression and is strongly associated with apoptosis induction. Overexpression and oligomerization of VDAC has been observed in many diseases, such as cancer, and is responsible for aberrant and pathological Ca^{2+} signaling [104, 224, 240]. Proapoptotic drugs commonly used in research are also known to trigger such VDAC oligomerizations [224]. In addition, recent data demonstrated that oxidative stress promotes the release of small mitochondrial DNA (mtDNA) through pores formed by VDAC oligomers, with this mtDNA amplifying such oligomerizations [241]. Interestingly, Bcl-2 and Bcl-xL appear to dampen such VDAC oligomerization [239, 242], thereby validating the role of

Bcl-xL in inhibiting cytochrome *c* release through VDAC [231]. Overall, the different oligomeric states of VDAC likely explain the discrepancies regarding Bcl-xL-mediated closure or opening of the channel. Bcl-xL would increase channel gating to stimulate prosurvival metabolic transfers, whereas it would diminish channel opening/oligomerization upon high VDAC expression to prevent the release of proapoptotic mediators [230].

Since before VDAC was recognized as the major Ca^{2+} transporter in the OMM [62], contradictory reports were published regarding the prosurvival *vs*. proapoptotic role of Ca^{2+} influx into mitochondria [230]. The discrepancy could easily be elucidated by the opposite roles of Ca^{2+} depending on its concentration and spatiotemporal dynamics. Indeed, basal mitochondrial Ca^{2+} levels are required to support mitochondrial bioenergetics [80] but excessive mitochondrial Ca^{2+} levels trigger apoptosis in the mitochondria [95, 243]. Similar conflicting data emerged concerning the ability of anti-apoptotic Bcl-2-family proteins to modulate VDAC gating and prevent apoptosis, either by facilitating or impeding mitochondrial Ca^{2+} uptake [184, 186, 230]. Hence, Bcl-xL was described to either promote [197, 244, 245] or prevent mitochondrial Ca^{2+} uptake [184, 199, 246]. In fact, both schemes can account for the antiapoptotic effect of Bcl-xL. From our own data, we believe that Bcl-xL can switch the channel in an open conformation, thereby preventing proapoptotic Ca^{2+} signaling and favoring metabolite exchanges (Fig. 5a). On the other hand, studies showing that Bcl-xL stimulates mitochondrial Ca^{2+} entry also reported that this Ca^{2+} fosters mitochondrial metabolism [177, 244]. Nevertheless, in that context, the global underlying mechanism remains difficult to conceptualize since increased Ca^{2+} uptake should imply a closure of VDAC [216]. Then, how can a situation leading to an inhibition of metabolite exchanges account for improved mitochondrial bioenergetics? Again, an answer may be found in the distinct properties and regulation of the VDAC monomers *versus* oligomers. It is still an open question whether VDAC oligomers are present in MAMs in contact with IP_3R-driven proapoptotic Ca^{2+} fluxes. Nevertheless, several evidences suggest that VDAC regulation inside and outside the MAM might be different [225]. Another explanation is that Bcl-xL could trigger prosurvival, oscillatory Ca^{2+} release by the IP_3R while keeping VDAC in a prosurvival (open) state. This means that VDAC in its low Ca^{2+} permeability state could still allow the passage of the IP_3R-mediated Ca^{2+} flux, when the Ca^{2+} release is stimulated by Bcl-xL. This is not impossible since VDAC is massively expressed in the OMM: prosurvival Ca^{2+} released by one IP_3R channel could thus be transferred into the mitochondria through many VDAC channels in their low Ca^{2+} permeability state. Another hypothesis is that Bcl-xL controls VDAC gating in a subtler way than just regulating opening and closure. One could hypothesize that Bcl-xL could convert the Ca^{2+} signal released through VDAC to a prosurvival pattern. However, analyzing such modulation remains challenging due to the difficulty to measure Ca^{2+} signaling through VDAC without being influenced by other ER/mitochondrial Ca^{2+}-transport systems.

Globally, increased or decreased mitochondrial Ca^{2+} uptake can rely on various mechanisms beyond VDAC itself. This comprises the upstream modulation of IP_3R-dependent Ca^{2+} release or the downstream regulation of MCU opening. Of note, MCU, in contrast to VDAC, is highly selective for Ca^{2+} [77]. To validate that the effects mediated by Bcl-xL directly target VDAC, peptides derived from VDAC's N-terminal region that represent the Bcl-xL-binding part of the channel have been largely used. These peptides are able to compete with Bcl-xL and specifically disrupt the VDAC/Bcl-xL interaction, thereby impeding Bcl-xL's function towards VDAC [104, 184, 186, 245], in the same way that the BIRD-2 peptide

disrupts IP_3R/Bcl-2 binding. At the level of Bcl-xL, the region that accounts for the interaction with VDAC is the BH4 domain [184, 234]. Similar to what was observed with Bcl-2's BH4 domain and IP_3R [110], the BH4 domain of Bcl-xL is able to bind and inhibit VDAC activity by itself [184, 234]. Interestingly, Bcl-2's BH4 domain is not able to modulate VDAC [184], suggesting that the conserved aspartate residue present in Bcl-xL (D11) but not in Bcl-2's BH4 domain (see section 3.1) is critical for the interaction with VDAC. These observations indicate that the BH4 domains of each protein have specific targets, although both are involved in modulating ER/mitochondria Ca^{2+} signaling. In addition, peptides derived from the BH4 domain of Bcl-xL were also used in cancer cell models exhibiting high expression levels of Bcl-xL to disrupt the endogenous VDAC/Bcl-xL interaction [246].

Overall, it is striking to realize that one single protein, Bcl-xL, is able to regulate two completely different channels that are sequentially involved in the same pathway (Fig. 5). It is also curious that the capacity of Bcl-xL to open or close the channel has been highly discussed for both IP_3R and VDAC. Considering the global scheme, one obvious question arising is whether Bcl-xL can also directly regulate MCU. Although Bcl-xL has also been found in the IMM [247], no other evidence has pointed to this direction for now.

4 Relevance of Bcl-xL-mediated regulation of Ca^{2+} signaling for Bcl-xL-dependent cancer cells

Bcl-xL was established long ago as a major antiapoptotic protein with a malignant role in cancer [248-251]. However, the recent evidences of the aptitude of the protein to regulate IP_3R and Ca^{2+} signaling open new avenues to develop anti-cancer strategies.

4.1 The tumorigenic functions of Bcl-xL

As indicated in section 1.2, Bcl-2 has a paradoxical role in cancer. Although it has been clearly established as a tumorigenic protein, it is sometimes recognized as a favorable prognostic marker. In fact, high Bcl-2 expression is mostly associated with poor prognosis in leukemias [252-254] while it is rather a good prognostic marker in solid cancers [30, 31, 255, 256]. This is not the case with Bcl-xL, which has an established and recognized malignant function in many kinds of tumors. The difference between Bcl-2 and Bcl-xL can be explained by two main reasons. First, Bcl-xL-dependent cancers are characterized by their powerful metastatic features [257-260]. Although both Bcl-2 and Bcl-xL support tumor invasiveness, Bcl-xL is likely able to promote metastasis by itself (See section 4.2) while Bcl-2's invasive role appears more complex and may depend on other protein partners [261]. Second, Bcl-xL by itself has a more potent antiapoptotic effect than Bcl-2 [262]. Bcl-xL, when overexpressed, provides more efficient protection against a wide range of various apoptotic stimuli than Bcl-2 overexpressed at the same level. In a breast cancer cell line, for example, it has been calculated that Bcl-xL is ten times more effective than Bcl-2 in protecting cells from apoptosis. This effect has been attributed to the capacity of Bcl-xL to prevent apoptosis originating from different organelles, contrary to Bcl-2 [262], and even more to the capacity of Bcl-xL to inhibit both Bax and Bak, while Bcl-2 only targets Bax [263]. Hence, high Bcl-xL expression has been recognized as a marker of poor prognosis in a large number of cancers, including breast, urothelial, hepatocellular, colorectal, bladder, gastric, esophageal, tongue and pancreatic

carcinoma [246, 257, 264-274], melanoma [275], osteosarcoma [276], follicular lymphoma [277, 278] and acute myeloid leukemia [279].

Several studies have pointed out that the antiapoptotic and invasive features provided by Bcl-xL in cancer cells are two completely independent mechanisms [179, 259, 280]. In fact, the metastatic function provided by Bcl-xL appears to be the most significant. Accordingly, the genetic ablation of Bcl-xL in pancreatic neuroendocrine cancer attenuates tumor invasiveness without affecting cancer survival and growth [281]. Conversely, when overexpressed in nonmalignant glioma cells, Bcl-xL was shown to strongly enhance metastatic potential but not primary tumor growth [259]. These observations suggest that other oncogenic proteins may protect cancers from cell death, while the metastatic function specifically relies on Bcl-xL. Furthermore, there is also an interesting time-dependent dissociation between the antiapoptotic and invasive functions of Bcl-xL. When overexpressed in malignant glioma cells, Bcl-xL provides protection against apoptosis upon its expression, whereas it only induced an invasive phenotype after a prolonged time [179]. The distinction between these two tumorigenic effects has been explained by the different sites of action of Bcl-xL. Bcl-xL prevents apoptosis when present at the mitochondria, while it exerts metastatic function through epigenetic modification of genes involved in epithelial–mesenchymal transition (EMT) [280]. However, many studies have demonstrated that Bcl-xL can also control cell migration at the mitochondrial level (see section 4.2), suggesting that the mechanisms used by Bcl-xL to promote tumor invasiveness are multiple and involve more than one cellular localization. Overall, although the antiapoptotic and metastatic roles of Bcl-xL can be separated into two independent mechanisms, they remain quite connected in their final purpose. Thus, promoting invasiveness through EMT induction is also a method employed by Bcl-xL to aid cancer cells in avoiding apoptosis [282]. This Bcl-xL-mediated survival/invasiveness relationship has been validated in a wide systematic analysis of Bcl-2-protein dependencies for their survival in an extensive number of cancer lines [208]. In this study, a gene set enrichment analysis revealed that the top four pathways enriched in Bcl-xL/Mcl-1-dependent cell lines are related to EMT. Furthermore, upon transition to an invasive mesenchymal state, this dual dependence is lost and shifted to a dependency on Bcl-xL only [208].

The antiapoptotic role of Bcl-xL becomes particularly prominent in the context of anticancer therapies. Overexpression of Bcl-xL has been clearly documented as a mechanism employed by tumors to avoid cell death induced by chemotherapy [248, 283, 284], radiotherapy [285] or targeted therapies such as tyrosine kinase inhibitors [286]. Activation of the NF-κB pathway has been observed in leukemia and solid cancer to account for this upregulation of Bcl-xL [287-289]. Eventually, overexpressed Bcl-xL inhibits classical apoptosis at the mitochondria, thereby protecting cells against anticancer treatments [290]. Overexpression of Bcl-xL has also been observed in the context of resistance to BH3 mimetics, namely, in the case of Bcl-2-dependent B cell lymphomas that have acquired resistance to venetoclax [291, 292]. Several mechanisms have been involved in this upregulation, including the activation of the phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathways [292] and of the eIF4F translation initiation complex [293] as well as the downregulation of miRNA targeting Bcl-xL's mRNA [294]. Here, overexpressed Bcl-xL binds and inhibits proapoptotic Bim proteins released in the presence of venetoclax, thereby preventing apoptosis [292]. In addition, our recent data demonstrate that inhibition of IP_3R-mediated Ca^{2+} signaling can also be a method employed by Bcl-xL to provide resistance to cell death stimuli, at least to STS [178], which has been shown to provoke intracellular Ca^{2+} fluctuations in

breast cancer cells [295] and IP_3R-mediated Ca^{2+}-dependent apoptosis [178]. In any case, specifically targeting Bcl-xL in such resistant cancer cells effectively reduces their tumorigenic characteristics and sensitizes them to die upon chemotherapy or radiotherapy treatment *in vitro* [296-298] as well as in *in vivo* xenograft models [37, 282, 286, 299]. The combination of classical anticancer therapy and Bcl-xL inhibitors might thus be a novel promising approach to treat Bcl-xL-expressing cancers and avoid resistance mechanisms [300].

4.2 Bcl-xL promotes metastasis invasion

Beyond its antiapoptotic role in cancer, Bcl-xL also impacts cell migration and metastasis invasion. In oncology, Bcl-xL is very well known as a highly prometastatic protein and, consequently, as a marker of poor prognosis [264-266, 268, 274, 277]. Mechanistically, the methods used by Bcl-xL to promote invasiveness are as diverse as the classes of cancer. Bcl-xL translocates to the nucleus, where it regulates the expression of genes involved in EMT [280]. Accordingly, long-term expression of Bcl-xL promotes the expression of matrix metalloproteinase (MMP)-2 and transforming growth factor (TGF)-β, which are both involved in cell migration [179]. However, Bcl-xL is mostly present in the mitochondria, where it also exerts its metastatic activity, mostly through the regulation of mitochondrial metabolism. In addition, Bcl-xL has been reported to stimulate mitochondrial fission and fusion dynamics [301], a mechanism that supports the migration and invasion of breast cancer cells [302].

Mitochondrial metabolism in cancer cells is significantly different from that in nonmalignant cells. One of the most frequent metabolic alterations, known as the Warburg effect, is a switch from oxidative phosphorylation to glycolysis, even in conditions when oxygen is not limiting [303]. This enhanced aerobic glycolysis was found to be directly involved in metastatic development [261, 304-306] and has been recognized as a poor prognosis marker in many tumors [307-309]. However, some recent data have demonstrated that cancers can in reality switch from one bioenergetic pathway to another [310, 311]. This metabolic plasticity has been proposed to allow the tumors to adjust their bioenergetics to the different stages of their development and to adapt to restrictions in nutrients and oxygen [261, 310]. Moreover, recent insights have pointed out the importance of oxidative phosphorylation in metastasis [261, 312, 313]. Indeed, Bcl-xL is very active at the mitochondria and substantially fosters bioenergetics. In addition to the regulation of metabolite and Ca^{2+} transport through VDAC mentioned above, Bcl-xL also acts by regulating mitochondrial homeostasis [314], stabilizing membrane potential [314, 315], increasing metabolic fitness [316], facilitating adenine nucleotide translocation [317] and improving the activity of the F_1F_0-ATP synthase [247, 315]. This latter mechanism is interesting because it has been observed in neurons [247, 315] but is absent in breast cancer cells [316], suggesting that the mechanisms of action of Bcl-xL beyond Bax/Bak regulation could be specific to tissues, or even specific to tumorigenic *versus* non-tumorigenic cell context. In any case, many of these actions participate in amplifying oxidative phosphorylation. The extra ATP generated through this process is believed to fulfill the metabolic requirements necessary for the structural changes of the cells undergoing metastasis [318]. However, this is not the ATP generated by the stimulation of oxidative phosphorylation that is most crucial for cancer invasion but produces ROS [261, 312, 319-321]. ROS are normally and continuously produced by the electron transport chain during mitochondrial respiration. They are intrinsically

cytotoxic, but cancer cells have managed to cope with high levels of ROS and to use them for their own benefit [313]. ROS are highly diffusible molecules, although only at short range, that are able to inactivate protein tyrosine phosphatases by oxidizing their cysteine residues [312, 322, 323]. The best-known example is the phosphatase PTEN, a notable tumor suppressor [322]. Therefore, ROS are involved in the modulation of various protein tyrosine phosphatase-dependent signaling pathways, including the PI3K/Akt/MMP-2, mitogen-activated protein kinase (MAPK), NF-kB, focal adhesion kinase (FAK), and TGF-β pathways [261, 312, 321-324]. Modulation of these pathways stimulates the malignant features of the tumor cells in general and metastatic invasion in particular [261, 313]. Indeed, Bcl-xL was reported to stimulate ROS production in cancer cells [246, 325, 326] while also protecting the cells from ROS-associated toxicity [325, 327]. Notably, in addition to stimulating mitochondrial respiration, Bcl-xL also generates ROS indirectly by inhibiting Bak and Bax, which are known to target the electron transport chain to limit ROS production [199, 326].

The fact that Bcl-xL promotes cancer invasion is also interesting since this process relies strongly on Ca^{2+} signaling [328, 329]. We have already extensively described in the previous sections how Bcl-xL controls Ca^{2+} signaling and cell death. On the other hand, regulation of Ca^{2+} signaling by Bcl-xL also influences invasiveness [318]. Metastasis is based on cell migration, a process that is highly supported by Ca^{2+} [330, 331]. This process depends on the spatiotemporally regulated action of multiple Ca^{2+}-dependent effectors triggered following the activation of various Ca^{2+} channels (voltage-gated Ca^{2+} channels, ORAI1 channels, transient receptor potential channels, IP_3Rs, etc.). Ca^{2+}-dependent effectors (Ca^{2+}/calmodulin-dependent protein kinase II, myosin light chain kinase, calpain, etc.) mediate several cellular functions, such as the reorganization of the cytoskeleton, focal adhesions and extracellular matrix, that are necessary for the movements of the cell [318, 330]. Beyond these wide-ranging Ca^{2+}-dependent effects, tumor invasion also relies on ER/mitochondria Ca^{2+} signaling and mitochondrial metabolism [318, 329]. It was proposed that Bcl-xL exerts its antiapoptotic function by sensitizing IP_3Rs to elicit prosurvival Ca^{2+} oscillations from the ER to the mitochondria, therefore fostering mitochondrial bioenergetics [177]. This work is in line with studies that reported that Bcl-xL promotes Ca^{2+} flux through VDAC [186] and may account for the capacity of Bcl-xL to stimulate oxidative phosphorylation [244]. Therefore, Bcl-xL-stimulated IP_3R/VDAC-driven Ca^{2+} signaling can theoretically promote the generation of ATP and ROS to favor cell migration. Accordingly, Ca^{2+} uptake in the mitochondrial matrix through the MCU strongly correlates with cancer invasiveness [332]. However, our group and others have reported that Bcl-xL inhibits IP_3R/VDAC-mediated Ca^{2+} flux [178, 184, 246]. Recently, another group demonstrated that Bcl-xL inhibition of mitochondrial Ca^{2+} uptake in breast cancer cells can also lead to an improvement of respiration and ROS production and thus cell migration. This effect appears to rely specifically on the inhibition of VDAC by Bcl-xL since disruption of the Bcl-xL/VDAC complexes prevented breast cancer cell mobility [246]. These findings support the model whereby Bcl-xL prevents Ca^{2+} flux though VDAC while promoting metabolite exchanges to support bioenergetics (see section 3.3). Hence, the role of Bcl-xL at the ER-mitochondrial interface in controlling Ca^{2+} flux from the ER to mitochondria by targeting IP_3Rs and/or VDAC1 channels requires further study. This role of Bcl-xL at the MAMs may account for increased invasive features as well as increased cell death resistance of cancer cells with upregulated Bcl-xL protein levels.

Overall, Bcl-xL is obviously a critical regulator of cell death and survival in cancer cells but the functions of this protein extend further, including stimulation of invasion/migration. Therefore, Bcl-xL has a large interactome and multiple mechanisms of action to foster tumorigenesis. However, we assume that these mechanisms may be specific to well-defined tumor types or to certain stage of tumor development (i.e. some tumors become invasive at later stages). Yet, the fact that Bcl-xL-mediated control of Ca^{2+} signaling may regulate apoptosis as well as invasiveness suggests that some of these mechanisms of action could be interconnected.

Concluding remarks

In the last three decades, the molecular mechanisms underlying the cellular functions of Bcl-xL and how these contribute to the neoplastic behavior of cancer cells have extensively increased. Nevertheless, treating Bcl-xL-dependent cancers remains a challenging matter. Despite the development of new kinds of drugs, such as BH3 mimetics that target Bcl-xL very selectively and efficiently, the application of such tools in human therapies remains challenging. Thus, the development of new innovative approaches to target Bcl-xL in cancers is crucial. One interesting example is the recently developed PROTAC molecules targeting Bcl-xL. Moreover, peptides or other molecules targeting ER/mitochondrial Ca^{2+} signaling, especially the regulation of IP_3R and VDAC by Bcl-xL, may be promising. Indeed, more research will be necessary to improve our understanding of the modulation of these two channels by Bcl-xL.

Conflict of Interest

The authors report that no conflict of interest exists.

Acknowledgements

Research in the authors' laboratory was funded by grants from the Research Foundation – Flanders (G090118N, G0E7520N, G081821N and G094522N to GB) and from the Research Council – KU Leuven (C14/19/099 and AKUL/19/34 to GB). GB and JBP are part of the FWO Scientific Research Network CaSign (W0.019.17N and W0.014.22N).

Author contributions

GB and NR designed the review and were involved in primary writing. NR and FSR prepared figures. GB and JBP further edited and revised the manuscript.

References

[1] A. Aouacheria, S. Baghdiguian, H.M. Lamb, J.D. Huska, F.J. Pineda, J.M. Hardwick, Connecting mitochondrial dynamics and life-or-death events via Bcl-2 family proteins, Neurochem Int 109 (2017) 141-161. https://doi.org/10.1016/j.neuint.2017.04.009.
[2] R. Singh, A. Letai, K. Sarosiek, Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins, Nature reviews Molecular cell biology 20 (2019) 175-193. https://doi.org/10.1038/s41580-018-0089-8.
[3] Y. Tsujimoto, L.R. Finger, J. Yunis, P.C. Nowell, C.M. Croce, Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation, Science 226 (1984) 1097-1099. https://doi.org/10.1126/science.6093263.
[4] D. Vaux, Early work on the function of Bcl-2, an interview with David Vaux, Cell Death Differ 11 Suppl 1 (2004) S28-32. https://doi.org/10.1038/sj.cdd.4401439.
[5] F. Pentimalli, BCL2: a 30-year tale of life, death and much more to come, Cell Death Differ 25 (2018) 7-9. https://doi.org/10.1038/cdd.2017.189.
[6] J. Kale, E.J. Osterlund, D.W. Andrews, BCL-2 family proteins: changing partners in the dance towards death, Cell Death Differ 25 (2018) 65-80. https://doi.org/10.1038/cdd.2017.186.
[7] F.J. Bock, S.W.G. Tait, Mitochondria as multifaceted regulators of cell death, Nature reviews Molecular cell biology 21 (2020) 85-100. https://doi.org/10.1038/s41580-019-0173-8.
[8] F. Edlich, S. Banerjee, M. Suzuki, M.M. Cleland, D. Arnoult, C. Wang, A. Neutzner, N. Tjandra, R.J. Youle, Bcl-x_L retrotranslocates Bax from the mitochondria into the cytosol, Cell 145 (2011) 104-116. https://doi.org/10.1016/j.cell.2011.02.034.
[9] E.H. Cheng, T.V. Sheiko, J.K. Fisher, W.J. Craigen, S.J. Korsmeyer, VDAC2 inhibits BAK activation and mitochondrial apoptosis, Science 301 (2003) 513-517. https://doi.org/10.1126/science.1083995.
[10] K.A. Sarosiek, X. Chi, J.A. Bachman, J.J. Sims, J. Montero, L. Patel, A. Flanagan, D.W. Andrews, P. Sorger, A. Letai, BID preferentially activates BAK while BIM preferentially activates BAX, affecting chemotherapy response, Mol Cell 51 (2013) 751-765. https://doi.org/10.1016/j.molcel.2013.08.048.
[11] M. Suzuki, R.J. Youle, N. Tjandra, Structure of Bax: coregulation of dimer formation and intracellular localization, Cell 103 (2000) 645-654. https://doi.org/10.1016/s0092-8674(00)00167-7.
[12] D. Chandra, G. Choy, P.T. Daniel, D.G. Tang, Bax-dependent regulation of Bak by voltage-dependent anion channel 2, J Biol Chem 280 (2005) 19051-19061. https://doi.org/10.1074/jbc.M501391200.
[13] G. Dewson, R.M. Kluck, Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis, J Cell Sci 122 (2009) 2801-2808. https://doi.org/10.1242/jcs.038166.
[14] K. McArthur, L.W. Whitehead, J.M. Heddleston, L. Li, B.S. Padman, V. Oorschot, N.D. Geoghegan, S. Chappaz, S. Davidson, H. San Chin, R.M. Lane, M. Dramicanin, T.L. Saunders, C. Sugiana, R. Lessene, L.D. Osellame, T.L. Chew, G. Dewson, M. Lazarou, G. Ramm, G. Lessene, M.T. Ryan, K.L. Rogers, M.F. van Delft, B.T. Kile, BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis, Science 359 (2018) eaao6047. https://doi.org/10.1126/science.aao6047.
[15] J.S. Riley, G. Quarato, C. Cloix, J. Lopez, J. O'Prey, M. Pearson, J. Chapman, H. Sesaki, L.M. Carlin, J.F. Passos, A.P. Wheeler, A. Oberst, K.M. Ryan, S.W. Tait, Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis, EMBO J 37 (2018) e99238. https://doi.org/10.15252/embj.201899238.
[16] G. Ichim, J. Lopez, S.U. Ahmed, N. Muthalagu, E. Giampazolias, M.E. Delgado, M. Haller, J.S. Riley, S.M. Mason, D. Athineos, M.J. Parsons, B. van de Kooij, L. Bouchier-Hayes, A.J. Chalmers, R.W. Rooswinkel, A. Oberst, K. Blyth, M. Rehm, D.J. Murphy, S.W.G. Tait, Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death, Mol Cell 57 (2015) 860-872. https://doi.org/10.1016/j.molcel.2015.01.018.

[17] K. Cosentino, V. Hertlein, A. Jenner, T. Dellmann, M. Gojkovic, A. Pena-Blanco, S. Dadsena, N. Wajngarten, J.S.H. Danial, J.V. Thevathasan, M. Mund, J. Ries, A.J. Garcia-Saez, The interplay between BAX and BAK tunes apoptotic pore growth to control mitochondrial-DNA-mediated inflammation, Mol Cell 82 (2022) 933-949 e939. https://doi.org/10.1016/j.molcel.2022.01.008.
[18] C. Bogner, J. Kale, J. Pogmore, X. Chi, A. Shamas-Din, C. Fradin, B. Leber, D.W. Andrews, Allosteric Regulation of BH3 Proteins in Bcl-xL Complexes Enables Switch-like Activation of Bax, Mol Cell 77 (2020) 901-912 e909. https://doi.org/10.1016/j.molcel.2019.12.025.
[19] K.J. Campbell, S.W.G. Tait, Targeting BCL-2 regulated apoptosis in cancer, Open Biol 8 (2018) 180002. https://doi.org/10.1098/rsob.180002.
[20] X. Luo, K.L. O'Neill, K. Huang, The third model of Bax/Bak activation: a Bcl-2 family feud finally resolved?, F1000Res 9 (2020) 935. https://doi.org/10.12688/f1000research.25607.1.
[21] K. Huang, K.L. O'Neill, J. Li, W. Zhou, N. Han, X. Pang, W. Wu, L. Struble, G. Borgstahl, Z. Liu, L. Zhang, X. Luo, BH3-only proteins target BCL-xL/MCL-1, not BAX/BAK, to initiate apoptosis, Cell Res 29 (2019) 942-952. https://doi.org/10.1038/s41422-019-0231-y.
[22] S.N. Willis, J.I. Fletcher, T. Kaufmann, M.F. van Delft, L. Chen, P.E. Czabotar, H. Ierino, E.F. Lee, W.D. Fairlie, P. Bouillet, A. Strasser, R.M. Kluck, J.M. Adams, D.C. Huang, Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak, Science 315 (2007) 856-859. https://doi.org/10.1126/science.1133289.
[23] K.L. O'Neill, K. Huang, J. Zhang, Y. Chen, X. Luo, Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane, Genes & development 30 (2016) 973-988. https://doi.org/10.1101/gad.276725.115.
[24] H. Flores-Romero, L. Hohorst, M. John, M.C. Albert, L.E. King, L. Beckmann, T. Szabo, V. Hertlein, X. Luo, A. Villunger, L.P. Frenzel, H. Kashkar, A.J. Garcia-Saez, BCL-2-family protein tBID can act as a BAX-like effector of apoptosis, EMBO J 41 (2022) e108690. https://doi.org/10.15252/embj.2021108690.
[25] R. Beroukhim, C.H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J.S. Boehm, J. Dobson, M. Urashima, K.T. Mc Henry, R.M. Pinchback, A.H. Ligon, Y.J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M.S. Lawrence, B.A. Weir, K.E. Tanaka, D.Y. Chiang, A.J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F.J. Kaye, H. Sasaki, J.E. Tepper, J.A. Fletcher, J. Tabernero, J. Baselga, M.S. Tsao, F. Demichelis, M.A. Rubin, P.A. Janne, M.J. Daly, C. Nucera, R.L. Levine, B.L. Ebert, S. Gabriel, A.K. Rustgi, C.R. Antonescu, M. Ladanyi, A. Letai, L.A. Garraway, M. Loda, D.G. Beer, L.D. True, A. Okamoto, S.L. Pomeroy, S. Singer, T.R. Golub, E.S. Lander, G. Getz, W.R. Sellers, M. Meyerson, The landscape of somatic copy-number alteration across human cancers, Nature 463 (2010) 899-905. https://doi.org/10.1038/nature08822.
[26] L.A. O'Reilly, D.C. Huang, A. Strasser, The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry, EMBO J 15 (1996) 6979-6990. https://doi.org/10.1002/j.1460-2075.1996.tb01090.x.
[27] Y.M. Janumyan, C.G. Sansam, A. Chattopadhyay, N. Cheng, E.L. Soucie, L.Z. Penn, D. Andrews, C.M. Knudson, E. Yang, Bcl-xL/Bcl-2 coordinately regulates apoptosis, cell cycle arrest and cell cycle entry, EMBO J 22 (2003) 5459-5470. https://doi.org/10.1093/emboj/cdg533.
[28] S. Zinkel, A. Gross, E. Yang, BCL2 family in DNA damage and cell cycle control, Cell Death Differ 13 (2006) 1351-1359. https://doi.org/10.1038/sj.cdd.4401987.
[29] S. Buglioni, I. D'Agnano, M. Cosimelli, S. Vasselli, C. D'Angelo, M. Tedesco, G. Zupi, M. Mottolese, Evaluation of multiple bio-pathological factors in colorectal adenocarcinomas: independent prognostic role of p53 and bcl-2, Int J Cancer 84 (1999) 545-552. https://doi.org/10.1002/(sici)1097-0215(19991222)84:6<545::aid-ijc1>3.0.co;2-2.
[30] K.T. Hwang, J.W. Woo, H.C. Shin, H.S. Kim, S.K. Ahn, H.G. Moon, W. Han, I.A. Park, D.Y. Noh, Prognostic influence of BCL2 expression in breast cancer, Int J Cancer 131 (2012) E1109-1119. https://doi.org/10.1002/ijc.27539.

[31] R.H. Pierce, M.E. Vail, L. Ralph, J.S. Campbell, N. Fausto, Bcl-2 expression inhibits liver carcinogenesis and delays the development of proliferating foci, Am J Pathol 160 (2002) 1555-1560. https://doi.org/10.1016/S0002-9440(10)61101-7.
[32] D.L. Vaux, S. Cory, J.M. Adams, Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells, Nature 335 (1988) 440-442. https://doi.org/10.1038/335440a0.
[33] J. Montero, A. Letai, Why do BCL-2 inhibitors work and where should we use them in the clinic?, Cell Death Differ 25 (2018) 56-64. https://doi.org/10.1038/cdd.2017.183.
[34] S. Kehr, M. Vogler, It's time to die: BH3 mimetics in solid tumors, Biochim Biophys Acta Mol Cell Res 1868 (2021) 118987. https://doi.org/10.1016/j.bbamcr.2021.118987.
[35] M. Villalobos-Ortiz, J. Ryan, T.N. Mashaka, J.T. Opferman, A. Letai, BH3 profiling discriminates on-target small molecule BH3 mimetics from putative mimetics, Cell Death Differ 27 (2020) 999-1007. https://doi.org/10.1038/s41418-019-0391-9.
[36] S. D'Aguanno, D. Del Bufalo, Inhibition of Anti-Apoptotic Bcl-2 Proteins in Preclinical and Clinical Studies: Current Overview in Cancer, Cells 9 (2020) 1287. https://doi.org/10.3390/cells9051287.
[37] T. Oltersdorf, S.W. Elmore, A.R. Shoemaker, R.C. Armstrong, D.J. Augeri, B.A. Belli, M. Bruncko, T.L. Deckwerth, J. Dinges, P.J. Hajduk, M.K. Joseph, S. Kitada, S.J. Korsmeyer, A.R. Kunzer, A. Letai, C. Li, M.J. Mitten, D.G. Nettesheim, S. Ng, P.M. Nimmer, J.M. O'Connor, A. Oleksijew, A.M. Petros, J.C. Reed, W. Shen, S.K. Tahir, C.B. Thompson, K.J. Tomaselli, B. Wang, M.D. Wendt, H. Zhang, S.W. Fesik, S.H. Rosenberg, An inhibitor of Bcl-2 family proteins induces regression of solid tumours, Nature 435 (2005) 677-681. https://doi.org/10.1038/nature03579.
[38] J.D. Leverson, D.C. Phillips, M.J. Mitten, E.R. Boghaert, D. Diaz, S.K. Tahir, L.D. Belmont, P. Nimmer, Y. Xiao, X.M. Ma, K.N. Lowes, P. Kovar, J. Chen, S. Jin, M. Smith, J. Xue, H. Zhang, A. Oleksijew, T.J. Magoc, K.S. Vaidya, D.H. Albert, J.M. Tarrant, N. La, L. Wang, Z.F. Tao, M.D. Wendt, D. Sampath, S.H. Rosenberg, C. Tse, D.C. Huang, W.J. Fairbrother, S.W. Elmore, A.J. Souers, Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy, Sci Transl Med 7 (2015) 279ra240. https://doi.org/10.1126/scitranslmed.aaa4642.
[39] C. Tse, A.R. Shoemaker, J. Adickes, M.G. Anderson, J. Chen, S. Jin, E.F. Johnson, K.C. Marsh, M.J. Mitten, P. Nimmer, L. Roberts, S.K. Tahir, Y. Xiao, X. Yang, H. Zhang, S. Fesik, S.H. Rosenberg, S.W. Elmore, ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor, Cancer Res 68 (2008) 3421-3428. https://doi.org/10.1158/0008-5472.CAN-07-5836.
[40] H. Zhang, P.M. Nimmer, S.K. Tahir, J. Chen, R.M. Fryer, K.R. Hahn, L.A. Iciek, S.J. Morgan, M.C. Nasarre, R. Nelson, L.C. Preusser, C.A. Reinhart, M.L. Smith, S.H. Rosenberg, S.W. Elmore, C. Tse, Bcl-2 family proteins are essential for platelet survival, Cell Death Differ 14 (2007) 943-951. https://doi.org/10.1038/sj.cdd.4402081.
[41] L. Gandhi, D.R. Camidge, M. Ribeiro de Oliveira, P. Bonomi, D. Gandara, D. Khaira, C.L. Hann, E.M. McKeegan, E. Litvinovich, P.M. Hemken, C. Dive, S.H. Enschede, C. Nolan, Y.L. Chiu, T. Busman, H. Xiong, A.P. Krivoshik, R. Humerickhouse, G.I. Shapiro, C.M. Rudin, Phase I study of Navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors, J Clin Oncol 29 (2011) 909-916. https://doi.org/10.1200/JCO.2010.31.6208.
[42] C.M. Rudin, C.L. Hann, E.B. Garon, M. Ribeiro de Oliveira, P.D. Bonomi, D.R. Camidge, Q. Chu, G. Giaccone, D. Khaira, S.S. Ramalingam, M.R. Ranson, C. Dive, E.M. McKeegan, B.J. Chyla, B.L. Dowell, A. Chakravartty, C.E. Nolan, N. Rudersdorf, T.A. Busman, M.H. Mabry, A.P. Krivoshik, R.A. Humerickhouse, G.I. Shapiro, L. Gandhi, Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer, Clin Cancer Res 18 (2012) 3163-3169. https://doi.org/10.1158/1078-0432.CCR-11-3090.
[43] P. Zhang, X. Zhang, X. Liu, S. Khan, D. Zhou, G. Zheng, PROTACs are effective in addressing the platelet toxicity associated with BCL-XL inhibitors, Explor Target Antitumor Ther 1 (2020) 259-272. https://doi.org/10.37349/etat.2020.00017.

[44] S. Khan, X. Zhang, D. Lv, Q. Zhang, Y. He, P. Zhang, X. Liu, D. Thummuri, Y. Yuan, J.S. Wiegand, J. Pei, W. Zhang, A. Sharma, C.R. McCurdy, V.M. Kuruvilla, N. Baran, A.A. Ferrando, Y.M. Kim, A. Rogojina, P.J. Houghton, G. Huang, R. Hromas, M. Konopleva, G. Zheng, D. Zhou, A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity, Nat Med 25 (2019) 1938-1947. https://doi.org/10.1038/s41591-019-0668-z.
[45] C.F.A. Warren, M.W. Wong-Brown, N.A. Bowden, BCL-2 family isoforms in apoptosis and cancer, Cell Death Dis 10 (2019) 177. https://doi.org/10.1038/s41419-019-1407-6.
[46] M.D. Bootman, G. Bultynck, Fundamentals of Cellular Calcium Signaling: A Primer, Cold Spring Harb Perspect Biol 12 (2020) a038802. https://doi.org/10.1101/cshperspect.a038802.
[47] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nature reviews Molecular cell biology 1 (2000) 11-21. https://doi.org/10.1038/35036035.
[48] K. Hamada, K. Mikoshiba, IP_3 Receptor Plasticity Underlying Diverse Functions, Annu Rev Physiol 82 (2020) 151-176. https://doi.org/10.1146/annurev-physiol-021119-034425.
[49] J.K. Foskett, C. White, K.H. Cheung, D.O. Mak, Inositol trisphosphate receptor Ca^{2+} release channels, Physiol Rev 87 (2007) 593-658. https://doi.org/10.1152/physrev.00035.2006.
[50] M.J. Berridge, Inositol trisphosphate and calcium signalling mechanisms, Biochim Biophys Acta 1793 (2009) 933-940. https://doi.org/10.1016/j.bbamcr.2008.10.005.
[51] E. Vermassen, J.B. Parys, J.P. Mauger, Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants, Biol Cell 96 (2004) 3-17. https://doi.org/10.1016/j.biolcel.2003.11.004.
[52] H. Ivanova, T. Vervliet, L. Missiaen, J.B. Parys, H. De Smedt, G. Bultynck, Inositol 1,4,5-trisphosphate receptor-isoform diversity in cell death and survival, Biochim Biophys Acta 1843 (2014) 2164-2183. https://doi.org/10.1016/j.bbamcr.2014.03.007.
[53] S. Patel, S.K. Joseph, A.P. Thomas, Molecular properties of inositol 1,4,5-trisphosphate receptors, Cell Calcium 25 (1999) 247-264. https://doi.org/10.1054/ceca.1999.0021.
[54] L.E. Wagner, 2nd, D.I. Yule, Differential regulation of the $InsP_3$ receptor type-1 and -2 single channel properties by $InsP_3$, Ca^{2+} and ATP, J Physiol 590 (2012) 3245-3259. https://doi.org/10.1113/jphysiol.2012.228320.
[55] M. Hattori, A.Z. Suzuki, T. Higo, M. Miyauchi, T. Michikawa, T. Nakamura, T. Inoue, K. Mikoshiba, Distinct roles of inositol 1,4,5-trisphosphate receptor types 1 and 3 in Ca^{2+} signaling, J Biol Chem 279 (2004) 11967-11975. https://doi.org/10.1074/jbc.M311456200.
[56] K.J. Alzayady, L. Wang, P. Chandrasekhar, L.E. Wagner, 2nd, F. Van Petegem, D.I. Yule, Defining the stoichiometry of inositol 1,4,5-trisphosphate binding required to initiate Ca^{2+} release, Sci Signal 9 (2016) ra35. https://doi.org/10.1126/scisignal.aad6281.
[57] N.B. Thillaiappan, A.P. Chavda, S.C. Tovey, D.L. Prole, C.W. Taylor, Ca^{2+} signals initiate at immobile IP_3 receptors adjacent to ER-plasma membrane junctions, Nat Commun 8 (2017) 1505. https://doi.org/10.1038/s41467-017-01644-8.
[58] N.B. Thillaiappan, H.A. Smith, P. Atakpa-Adaji, C.W. Taylor, KRAP tethers IP_3 receptors to actin and licenses them to evoke cytosolic Ca^{2+} signals, Nat Commun 12 (2021) 4514. https://doi.org/10.1038/s41467-021-24739-9.
[59] L. Scorrano, M.A. De Matteis, S. Emr, F. Giordano, G. Hajnoczky, B. Kornmann, L.L. Lackner, T.P. Levine, L. Pellegrini, K. Reinisch, R. Rizzuto, T. Simmen, H. Stenmark, C. Ungermann, M. Schuldiner, Coming together to define membrane contact sites, Nat Commun 10 (2019) 1287. https://doi.org/10.1038/s41467-019-09253-3.
[60] G. Csordas, D. Weaver, G. Hajnoczky, Endoplasmic Reticulum-Mitochondrial Contactology: Structure and Signaling Functions, Trends Cell Biol 28 (2018) 523-540. https://doi.org/10.1016/j.tcb.2018.02.009.

[61] G. Csordas, A.P. Thomas, G. Hajnoczky, Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria, EMBO J 18 (1999) 96-108. https://doi.org/10.1093/emboj/18.1.96.
[62] D. Gincel, H. Zaid, V. Shoshan-Barmatz, Calcium binding and translocation by the voltage-dependent anion channel: a possible regulatory mechanism in mitochondrial function, Biochem J 358 (2001) 147-155. https://doi.org/10.1042/0264-6021:3580147.
[63] E. Rapizzi, P. Pinton, G. Szabadkai, M.R. Wieckowski, G. Vandecasteele, G. Baird, R.A. Tuft, K.E. Fogarty, R. Rizzuto, Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria, J Cell Biol 159 (2002) 613-624. https://doi.org/10.1083/jcb.200205091.
[64] R. Rizzuto, M. Brini, M. Murgia, T. Pozzan, Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria, Science 262 (1993) 744-747. https://doi.org/10.1126/science.8235595.
[65] G. Morciano, S. Marchi, C. Morganti, L. Sbano, M. Bittremieux, M. Kerkhofs, M. Corricelli, A. Danese, A. Karkucinska-Wieckowska, M.R. Wieckowski, G. Bultynck, C. Giorgi, P. Pinton, Role of Mitochondria-Associated ER Membranes in Calcium Regulation in Cancer-Specific Settings, Neoplasia 20 (2018) 510-523. https://doi.org/10.1016/j.neo.2018.03.005.
[66] J. Loncke, A. Kaasik, I. Bezprozvanny, J.B. Parys, M. Kerkhofs, G. Bultynck, Balancing ER-Mitochondrial Ca^{2+} Fluxes in Health and Disease, Trends Cell Biol 31 (2021) 598-612. https://doi.org/10.1016/j.tcb.2021.02.003.
[67] G. Csordas, C. Renken, P. Varnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A. Mannella, G. Hajnoczky, Structural and functional features and significance of the physical linkage between ER and mitochondria, J Cell Biol 174 (2006) 915-921. https://doi.org/10.1083/jcb.200604016.
[68] Y. Liu, X. Ma, H. Fujioka, J. Liu, S. Chen, X. Zhu, DJ-1 regulates the integrity and function of ER-mitochondria association through interaction with IP_3R3-Grp75-VDAC1, Proc Natl Acad Sci U S A 116 (2019) 25322-25328. https://doi.org/10.1073/pnas.1906565116.
[69] M. D'Eletto, F. Rossin, L. Occhigrossi, M.G. Farrace, D. Faccenda, R. Desai, S. Marchi, G. Refolo, L. Falasca, M. Antonioli, F. Ciccosanti, G.M. Fimia, P. Pinton, M. Campanella, M. Piacentini, Transglutaminase Type 2 Regulates ER-Mitochondria Contact Sites by Interacting with GRP75, Cell Rep 25 (2018) 3573-3581 e3574. https://doi.org/10.1016/j.celrep.2018.11.094.
[70] H. Matsuzaki, T. Fujimoto, M. Tanaka, S. Shirasawa, Tespa1 is a novel component of mitochondria-associated endoplasmic reticulum membranes and affects mitochondrial calcium flux, Biochem Biophys Res Commun 433 (2013) 322-326. https://doi.org/10.1016/j.bbrc.2013.02.099.
[71] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, Nature 456 (2008) 605-610. https://doi.org/10.1038/nature07534.
[72] R. Filadi, N.S. Leal, B. Schreiner, A. Rossi, G. Dentoni, C.M. Pinho, B. Wiehager, D. Cieri, T. Cali, P. Pizzo, M. Ankarcrona, TOM70 Sustains Cell Bioenergetics by Promoting IP_3R3-Mediated ER to Mitochondria Ca^{2+} Transfer, Curr Biol 28 (2018) 369-382 e366. https://doi.org/10.1016/j.cub.2017.12.047.
[73] M.A. Carpio, R.E. Means, A.L. Brill, A. Sainz, B.E. Ehrlich, S.G. Katz, BOK controls apoptosis by Ca^{2+} transfer through ER-mitochondrial contact sites, Cell Rep 34 (2021) 108827. https://doi.org/10.1016/j.celrep.2021.108827.
[74] A. Bartok, D. Weaver, T. Golenar, Z. Nichtova, M. Katona, S. Bansaghi, K.J. Alzayady, V.K. Thomas, H. Ando, K. Mikoshiba, S.K. Joseph, D.I. Yule, G. Csordas, G. Hajnoczky, IP_3 receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer, Nat Commun 10 (2019) 3726. https://doi.org/10.1038/s41467-019-11646-3.

[75] J. Loncke, M. Kerkhofs, A. Kaasik, I. Bezprozvanny, G. Bultynck, Recent advances in understanding IP_3R function with focus on ER-mitochondrial Ca^{2+} transfers, Current Opinion in Physiology 17 (2020) 80-88. https://doi.org/10.1016/j.cophys.2020.07.011.
[76] D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336-340. https://doi.org/10.1038/nature10230.
[77] Y. Kirichok, G. Krapivinsky, D.E. Clapham, The mitochondrial calcium uniporter is a highly selective ion channel, Nature 427 (2004) 360-364. https://doi.org/10.1038/nature02246.
[78] B. Glancy, R.S. Balaban, Role of mitochondrial Ca^{2+} in the regulation of cellular energetics, Biochemistry 51 (2012) 2959-2973. https://doi.org/10.1021/bi2018909.
[79] R. Rizzuto, D. De Stefani, A. Raffaello, C. Mammucari, Mitochondria as sensors and regulators of calcium signalling, Nature reviews Molecular cell biology 13 (2012) 566-578. https://doi.org/10.1038/nrm3412.
[80] A. Rossi, P. Pizzo, R. Filadi, Calcium, mitochondria and cell metabolism: A functional triangle in bioenergetics, Biochim Biophys Acta Mol Cell Res 1866 (2019) 1068-1078. https://doi.org/10.1016/j.bbamcr.2018.10.016.
[81] G. Hajnoczky, L.D. Robb-Gaspers, M.B. Seitz, A.P. Thomas, Decoding of cytosolic calcium oscillations in the mitochondria, Cell 82 (1995) 415-424. https://doi.org/10.1016/0092-8674(95)90430-1.
[82] C. Cardenas, R.A. Miller, I. Smith, T. Bui, J. Molgo, M. Muller, H. Vais, K.H. Cheung, J. Yang, I. Parker, C.B. Thompson, M.J. Birnbaum, K.R. Hallows, J.K. Foskett, Essential regulation of cell bioenergetics by constitutive $InsP_3$ receptor Ca^{2+} transfer to mitochondria, Cell 142 (2010) 270-283. https://doi.org/10.1016/j.cell.2010.06.007.
[83] S. Kiviluoto, T. Vervliet, H. Ivanova, J.P. Decuypere, H. De Smedt, L. Missiaen, G. Bultynck, J.B. Parys, Regulation of inositol 1,4,5-trisphosphate receptors during endoplasmic reticulum stress, Biochim Biophys Acta 1833 (2013) 1612-1624. https://doi.org/10.1016/j.bbamcr.2013.01.026.
[84] T. Higo, K. Hamada, C. Hisatsune, N. Nukina, T. Hashikawa, M. Hattori, T. Nakamura, K. Mikoshiba, Mechanism of ER stress-induced brain damage by IP_3 receptor, Neuron 68 (2010) 865-878. https://doi.org/10.1016/j.neuron.2010.11.010.
[85] T. Higo, M. Hattori, T. Nakamura, T. Natsume, T. Michikawa, K. Mikoshiba, Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44, Cell 120 (2005) 85-98. https://doi.org/10.1016/j.cell.2004.11.048.
[86] G. Li, M. Mongillo, K.T. Chin, H. Harding, D. Ron, A.R. Marks, I. Tabas, Role of ERO1-alpha-mediated stimulation of inositol 1,4,5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis, J Cell Biol 186 (2009) 783-792. https://doi.org/10.1083/jcb.200904060.
[87] A. Carreras-Sureda, F. Pihan, H. Urra, S. Durand, D.E. Mortenson, A. Sagredo, G. Bustos, Y. Hazari, E. Ramos-Fernandez, M.L. Sassano, P. Pihan, A.R. van Vliet, M. Gonzalez-Quiroz, A.K. Torres, C. Tapia-Rojas, M. Kerkhofs, R. Vicente, R.J. Kaufman, N.C. Inestrosa, C. Gonzalez-Billault, R.L. Wiseman, P. Agostinis, G. Bultynck, F.A. Court, G. Kroemer, J.C. Cardenas, C. Hetz, Non-canonical function of IRE1alpha determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics, Nat Cell Biol 21 (2019) 755-767. https://doi.org/10.1038/s41556-019-0329-y.
[88] M. Bittremieux, J.B. Parys, P. Pinton, G. Bultynck, ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca^{2+} signaling, Biochim Biophys Acta 1863 (2016) 1364-1378. https://doi.org/10.1016/j.bbamcr.2016.01.002.
[89] S. Kuchay, C. Giorgi, D. Simoneschi, J. Pagan, S. Missiroli, A. Saraf, L. Florens, M.P. Washburn, A. Collazo-Lorduy, M. Castillo-Martin, C. Cordon-Cardo, S.M. Sebti, P. Pinton, M. Pagano, PTEN counteracts FBXL2 to promote IP_3R3- and Ca^{2+}-mediated apoptosis limiting tumour growth, Nature 546 (2017) 554-558. https://doi.org/10.1038/nature22965.

[90] C. Giorgi, K. Ito, H.K. Lin, C. Santangelo, M.R. Wieckowski, M. Lebiedzinska, A. Bononi, M. Bonora, J. Duszynski, R. Bernardi, R. Rizzuto, C. Tacchetti, P. Pinton, P.P. Pandolfi, PML regulates apoptosis at endoplasmic reticulum by modulating calcium release, Science 330 (2010) 1247-1251. https://doi.org/10.1126/science.1189157.
[91] G. Szalai, R. Krishnamurthy, G. Hajnoczky, Apoptosis driven by IP_3-linked mitochondrial calcium signals, EMBO J 18 (1999) 6349-6361. https://doi.org/10.1093/emboj/18.22.6349.
[92] V. Giorgio, V. Burchell, M. Schiavone, C. Bassot, G. Minervini, V. Petronilli, F. Argenton, M. Forte, S. Tosatto, G. Lippe, P. Bernardi, Ca^{2+} binding to F-ATP synthase beta subunit triggers the mitochondrial permeability transition, EMBO Rep 18 (2017) 1065-1076. https://doi.org/10.15252/embr.201643354.
[93] P. Bernardi, M. Carraro, G. Lippe, The mitochondrial permeability transition: Recent progress and open questions, FEBS J Online ahead of print (2021) https://doi.org/10.1111/febs.16254.
[94] V. Giorgio, L. Guo, C. Bassot, V. Petronilli, P. Bernardi, Calcium and regulation of the mitochondrial permeability transition, Cell Calcium 70 (2018) 56-63. https://doi.org/10.1016/j.ceca.2017.05.004.
[95] M.S. Hwang, C.T. Schwall, E. Pazarentzos, C. Datler, N.N. Alder, S. Grimm, Mitochondrial Ca^{2+} influx targets cardiolipin to disintegrate respiratory chain complex II for cell death induction, Cell Death Differ 21 (2014) 1733-1745. https://doi.org/10.1038/cdd.2014.84.
[96] D. Boehning, R.L. Patterson, L. Sedaghat, N.O. Glebova, T. Kurosaki, S.H. Snyder, Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis, Nat Cell Biol 5 (2003) 1051-1061. https://doi.org/10.1038/ncb1063.
[97] I. Kruman, Q. Guo, M.P. Mattson, Calcium and reactive oxygen species mediate staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells, J Neurosci Res 51 (1998) 293-308. https://doi.org/10.1002/(SICI)1097-4547(19980201)51:3<293::AID-JNR3>3.0.CO;2-B.
[98] J.T. Lock, K.J. Alzayady, D.I. Yule, I. Parker, All three IP_3 receptor isoforms generate Ca^{2+} puffs that display similar characteristics, Sci Signal 11 (2018) eaau0344. https://doi.org/10.1126/scisignal.aau0344.
[99] T. Miyakawa, A. Maeda, T. Yamazawa, K. Hirose, T. Kurosaki, M. Iino, Encoding of Ca^{2+} signals by differential expression of IP_3 receptor subtypes. EMBO J 18 (1999) 1303-1308. https://doi.org/10.1093/emboj/18.5.1303.
[100] C.C. Mendes, D.A. Gomes, M. Thompson, N.C. Souto, T.S. Goes, A.M. Goes, M.A. Rodrigues, M.V. Gomez, M.H. Nathanson, M.F. Leite, The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca^{2+} signals into mitochondria, J Biol Chem 280 (2005) 40892-40900. https://doi.org/10.1074/jbc.M506623200.
[101] A.A. Khan, M.J. Soloski, A.H. Sharp, G. Schilling, D.M. Sabatini, S.H. Li, C.A. Ross, S.H. Snyder, Lymphocyte apoptosis: mediation by increased type 3 inositol 1,4,5-trisphosphate receptor, Science 273 (1996) 503-507. https://doi.org/10.1126/science.273.5274.503.
[102] D. De Stefani, A. Bononi, A. Romagnoli, A. Messina, V. De Pinto, P. Pinton, R. Rizzuto, VDAC1 selectively transfers apoptotic Ca^{2+} signals to mitochondria, Cell Death Differ 19 (2012) 267-273. https://doi.org/10.1038/cdd.2011.92.
[103] M.R. Wieckowski, C. Giorgi, M. Lebiedzinska, J. Duszynski, P. Pinton, Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells, Nat Protoc 4 (2009) 1582-1590. https://doi.org/10.1038/nprot.2009.151.
[104] H. Ivanova, T. Vervliet, G. Monaco, L.E. Terry, N. Rosa, M.R. Baker, J.B. Parys, Serysheva, II, D.I. Yule, G. Bultynck, Bcl-2-Protein Family as Modulators of IP_3 Receptors and Other Organellar Ca^{2+} Channels, Cold Spring Harb Perspect Biol 12 (2020) a035089. https://doi.org/10.1101/cshperspect.a035089.
[105] R. Chen, I. Valencia, F. Zhong, K.S. McColl, H.L. Roderick, M.D. Bootman, M.J. Berridge, S.J. Conway, A.B. Holmes, G.A. Mignery, P. Velez, C.W. Distelhorst, Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate, J Cell Biol 166 (2004) 193-203. https://doi.org/10.1083/jcb.200309146.

[106] M. Lam, G. Dubyak, L. Chen, G. Nunez, R.L. Miesfeld, C.W. Distelhorst, Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes, Proc Natl Acad Sci U S A 91 (1994) 6569-6573. https://doi.org/10.1073/pnas.91.14.6569.
[107] Y.P. Rong, A.S. Aromolaran, G. Bultynck, F. Zhong, X. Li, K. McColl, S. Matsuyama, S. Herlitze, H.L. Roderick, M.D. Bootman, G.A. Mignery, J.B. Parys, H. De Smedt, C.W. Distelhorst, Targeting Bcl-2-IP_3 receptor interaction to reverse Bcl-2's inhibition of apoptotic calcium signals, Mol Cell 31 (2008) 255-265. https://doi.org/10.1016/j.molcel.2008.06.014.
[108] C.J. Hanson, M.D. Bootman, C.W. Distelhorst, R.J. Wojcikiewicz, H.L. Roderick, Bcl-2 suppresses Ca^{2+} release through inositol 1,4,5-trisphosphate receptors and inhibits Ca^{2+} uptake by mitochondria without affecting ER calcium store content, Cell Calcium 44 (2008) 324-338. https://doi.org/10.1016/j.ceca.2008.01.003.
[109] H. Akl, G. Monaco, R. La Rovere, K. Welkenhuyzen, S. Kiviluoto, T. Vervliet, J. Molgo, C.W. Distelhorst, L. Missiaen, K. Mikoshiba, J.B. Parys, H. De Smedt, G. Bultynck, IP_3R2 levels dictate the apoptotic sensitivity of diffuse large B-cell lymphoma cells to an IP_3R-derived peptide targeting the BH4 domain of Bcl-2, Cell Death Dis 4 (2013) e632. https://doi.org/10.1038/cddis.2013.140.
[110] G. Monaco, E. Decrock, H. Akl, R. Ponsaerts, T. Vervliet, T. Luyten, M. De Maeyer, L. Missiaen, C.W. Distelhorst, H. De Smedt, J.B. Parys, L. Leybaert, G. Bultynck, Selective regulation of IP_3-receptor-mediated Ca^{2+} signaling and apoptosis by the BH4 domain of Bcl-2 versus Bcl-Xl, Cell Death Differ 19 (2012) 295-309. https://doi.org/10.1038/cdd.2011.97.
[111] H. Ivanova, L.E. Wagner, 2nd, A. Tanimura, E. Vandermarliere, T. Luyten, K. Welkenhuyzen, K.J. Alzayady, L. Wang, K. Hamada, K. Mikoshiba, H. De Smedt, L. Martens, D.I. Yule, J.B. Parys, G. Bultynck, Bcl-2 and IP_3 compete for the ligand-binding domain of IP3Rs modulating Ca^{2+} signaling output, Cell Mol Life Sci 76 (2019) 3843-3859. https://doi.org/10.1007/s00018-019-03091-8.
[112] F. Zhong, M.W. Harr, G. Bultynck, G. Monaco, J.B. Parys, H. De Smedt, Y.P. Rong, J.K. Molitoris, M. Lam, C. Ryder, S. Matsuyama, C.W. Distelhorst, Induction of Ca^{2+}-driven apoptosis in chronic lymphocytic leukemia cells by peptide-mediated disruption of Bcl-2-IP_3 receptor interaction, Blood 117 (2011) 2924-2934. https://doi.org/10.1182/blood-2010-08-307405.
[113] M. Bittremieux, R.M. La Rovere, H. Akl, C. Martines, K. Welkenhuyzen, K. Dubron, M. Baes, A. Janssens, P. Vandenberghe, L. Laurenti, K. Rietdorf, G. Morciano, P. Pinton, K. Mikoshiba, M.D. Bootman, D.G. Efremov, H. De Smedt, J.B. Parys, G. Bultynck, Constitutive IP_3 signaling underlies the sensitivity of B-cell cancers to the Bcl-2/IP_3 receptor disruptor BIRD-2, Cell Death Differ 26 (2019) 531-547. https://doi.org/10.1038/s41418-018-0142-3.
[114] T. Vervloessem, H. Akl, T. Tousseyn, H. De Smedt, J.B. Parys, G. Bultynck, Reciprocal sensitivity of diffuse large B-cell lymphoma cells to Bcl-2 inhibitors BIRD-2 versus venetoclax, Oncotarget 8 (2017) 111656-111671. https://doi.org/10.18632/oncotarget.22898.
[115] M.J. Chang, F. Zhong, A.R. Lavik, J.B. Parys, M.J. Berridge, C.W. Distelhorst, Feedback regulation mediated by Bcl-2 and DARPP-32 regulates inositol 1,4,5-trisphosphate receptor phosphorylation and promotes cell survival, Proc Natl Acad Sci U S A 111 (2014) 1186-1191. https://doi.org/10.1073/pnas.1323098111.
[116] V. Arige, D.I. Yule, Spatial and temporal crosstalk between the cAMP and Ca^{2+} signaling systems, Biochim Biophys Acta Mol Cell Res 1869 (2022) 119293. https://doi.org/10.1016/j.bbamcr.2022.119293.
[117] M.J. Betzenhauser, J.L. Fike, L.E. Wagner, 2nd, D.I. Yule, Protein kinase A increases type-2 inositol 1,4,5-trisphosphate receptor activity by phosphorylation of serine 937, J Biol Chem 284 (2009) 25116-25125. https://doi.org/10.1074/jbc.M109.010132.
[118] M.D. Soulsby, R.J. Wojcikiewicz, The type III inositol 1,4,5-trisphosphate receptor is phosphorylated by cAMP-dependent protein kinase at three sites, Biochem J 392 (2005) 493-497. https://doi.org/10.1042/BJ20051325.

[119] R. Foyouzi-Youssefi, S. Arnaudeau, C. Borner, W.L. Kelley, J. Tschopp, D.P. Lew, N. Demaurex, K.H. Krause, Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum, Proc Natl Acad Sci U S A 97 (2000) 5723-5728. https://doi.org/10.1073/pnas.97.11.5723.
[120] P. Pinton, D. Ferrari, P. Magalhaes, K. Schulze-Osthoff, F. Di Virgilio, T. Pozzan, R. Rizzuto, Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells, J Cell Biol 148 (2000) 857-862. https://doi.org/10.1083/jcb.148.5.857.
[121] F.O. Lemos, G. Bultynck, J.B. Parys, A comprehensive overview of the complex world of the endo- and sarcoplasmic reticulum Ca^{2+}-leak channels, Biochim Biophys Acta Mol Cell Res 1868 (2021) 119020. https://doi.org/10.1016/j.bbamcr.2021.119020.
[122] E.S. Dremina, V.S. Sharov, K. Kumar, A. Zaidi, E.K. Michaelis, C. Schoneich, Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), Biochem J 383 (2004) 361-370. https://doi.org/10.1042/BJ20040187.
[123] A.E. Palmer, C. Jin, J.C. Reed, R.Y. Tsien, Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor, Proc Natl Acad Sci U S A 101 (2004) 17404-17409. https://doi.org/10.1073/pnas.0408030101.
[124] S.A. Oakes, L. Scorrano, J.T. Opferman, M.C. Bassik, M. Nishino, T. Pozzan, S.J. Korsmeyer, Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum, Proc Natl Acad Sci U S A 102 (2005) 105-110. https://doi.org/10.1073/pnas.0408352102.
[125] S.L. Schendel, Z. Xie, M.O. Montal, S. Matsuyama, M. Montal, J.C. Reed, Channel formation by antiapoptotic protein Bcl-2, Proc Natl Acad Sci U S A 94 (1997) 5113-5118. https://doi.org/10.1073/pnas.94.10.5113.
[126] H. Ivanova, A. Ritaine, L. Wagner, T. Luyten, S. Shapovalov, K. Welkenhuyzen, B. Seitaj, G. Monaco, H. De Smedt, N. Prevarskaya, D.I. Yule, J.B. Parys, G. Bultynck, The trans-membrane domain of Bcl-2alpha, but not its hydrophobic cleft, is a critical determinant for efficient IP_3 receptor inhibition, Oncotarget 7 (2016) 55704-55720. https://doi.org/10.18632/oncotarget.11005.
[127] N.C. Chang, M. Nguyen, M. Germain, G.C. Shore, Antagonism of Beclin 1-dependent autophagy by BCL-2 at the endoplasmic reticulum requires NAF-1, EMBO J 29 (2010) 606-618. https://doi.org/10.1038/emboj.2009.369.
[128] C. Xu, W. Xu, A.E. Palmer, J.C. Reed, BI-1 regulates endoplasmic reticulum Ca^{2+} homeostasis downstream of Bcl-2 family proteins, J Biol Chem 283 (2008) 11477-11484. https://doi.org/10.1074/jbc.M708285200.
[129] S. Kiviluoto, L. Schneider, T. Luyten, T. Vervliet, L. Missiaen, H. De Smedt, J.B. Parys, A. Methner, G. Bultynck, Bax inhibitor-1 is a novel IP_3 receptor-interacting and -sensitizing protein, Cell Death Dis 3 (2012) e367. https://doi.org/10.1038/cddis.2012.103.
[130] Z.Q. Shen, Y.L. Huang, Y.C. Teng, T.W. Wang, C.H. Kao, C.H. Yeh, T.F. Tsai, CISD2 maintains cellular homeostasis, Biochim Biophys Acta Mol Cell Res 1868 (2021) 118954. https://doi.org/10.1016/j.bbamcr.2021.118954.
[131] O. Kutuk, A. Letai, Regulation of Bcl-2 family proteins by posttranslational modifications, Curr Mol Med 8 (2008) 102-118. https://doi.org/10.2174/156652408783769599.
[132] I. de Ridder, N. Rosa, M. Kerkhofs, G. Bultynck, Bok joining the "Ca^{2+} club", Cell Calcium 98 (2021) 102438. https://doi.org/10.1016/j.ceca.2021.102438.
[133] J. Loncke, T. Vervliet, J.B. Parys, A. Kaasik, G. Bultynck, Uniting the divergent Wolfram syndrome-linked proteins WFS1 and CISD2 as modulators of Ca^{2+} signaling, Sci Signal 14 (2021) eabc6165. https://doi.org/10.1126/scisignal.abc6165.
[134] N. Popgeorgiev, L. Jabbour, G. Gillet, Subcellular Localization and Dynamics of the Bcl-2 Family of Proteins, Front Cell Dev Biol 6 (2018) 13. https://doi.org/10.3389/fcell.2018.00013.

[135] L. Scorrano, S.A. Oakes, J.T. Opferman, E.H. Cheng, M.D. Sorcinelli, T. Pozzan, S.J. Korsmeyer, BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis, Science 300 (2003) 135-139. https://doi.org/10.1126/science.1081208.
[136] Z. Pan, M.B. Bhat, A.L. Nieminen, J. Ma, Synergistic movements of Ca^{2+} and Bax in cells undergoing apoptosis, J Biol Chem 276 (2001) 32257-32263. https://doi.org/10.1074/jbc.M100178200.
[137] W.X. Zong, C. Li, G. Hatzivassiliou, T. Lindsten, Q.C. Yu, J. Yuan, C.B. Thompson, Bax and Bak can localize to the endoplasmic reticulum to initiate apoptosis, J Cell Biol 162 (2003) 59-69. https://doi.org/10.1083/jcb.200302084.
[138] M. Chami, A. Prandini, M. Campanella, P. Pinton, G. Szabadkai, J.C. Reed, R. Rizzuto, Bcl-2 and Bax exert opposing effects on Ca^{2+} signaling, which do not depend on their putative pore-forming region, J Biol Chem 279 (2004) 54581-54589. https://doi.org/10.1074/jbc.M409663200.
[139] M. Klee, K. Pallauf, S. Alcala, A. Fleischer, F.X. Pimentel-Muinos, Mitochondrial apoptosis induced by BH3-only molecules in the exclusive presence of endoplasmic reticular Bak, EMBO J 28 (2009) 1757-1768. https://doi.org/10.1038/emboj.2009.90.
[140] J.P. Mathai, M. Germain, G.C. Shore, BH3-only BIK regulates BAX, BAK-dependent release of Ca^{2+} from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death, J Biol Chem 280 (2005) 23829-23836. https://doi.org/10.1074/jbc.M500839200.
[141] M.J. Thomenius, N.S. Wang, E.Z. Reineks, Z. Wang, C.W. Distelhorst, Bcl-2 on the endoplasmic reticulum regulates Bax activity by binding to BH3-only proteins, J Biol Chem 278 (2003) 6243-6250. https://doi.org/10.1074/jbc.M208878200.
[142] X. Wang, K.E. Olberding, C. White, C. Li, Bcl-2 proteins regulate ER membrane permeability to luminal proteins during ER stress-induced apoptosis, Cell Death Differ 18 (2011) 38-47. https://doi.org/10.1038/cdd.2010.68.
[143] D.L. Prole, C.W. Taylor, Structure and Function of IP_3 Receptors, Cold Spring Harb Perspect Biol 11 (2019) a035063. https://doi.org/10.1101/cshperspect.a035063.
[144] G. Fan, M.L. Baker, Z. Wang, M.R. Baker, P.A. Sinyagovskiy, W. Chiu, S.J. Ludtke, Serysheva, II, Gating machinery of $InsP_3R$ channels revealed by electron cryomicroscopy, Nature 527 (2015) 336-341. https://doi.org/10.1038/nature15249.
[145] G. Fan, M.R. Baker, Z. Wang, A.F. Seryshev, S.J. Ludtke, M.L. Baker, Serysheva, II, Cryo-EM reveals ligand induced allostery underlying $InsP_3R$ channel gating, Cell Res 28 (2018) 1158-1170. https://doi.org/10.1038/s41422-018-0108-5.
[146] C.M. Azumaya, E.A. Linton, J. Risener, T. Nakagawa, E. Karakas, Cryo-EM structure of human type-3 inositol triphosphate receptor reveals the presence of a self-binding peptide that acts as an antagonist, J Biol Chem 295 (2020) 1743-1753. https://doi.org/10.1074/jbc.RA119.011570.
[147] M.R. Baker, G. Fan, A.F. Seryshev, M.A. Agosto, M.L. Baker, Serysheva, II, Cryo-EM structure of type 1 IP_3R channel in a lipid bilayer, Commun Biol 4 (2021) 625. https://doi.org/10.1038/s42003-021-02156-4.
[148] E.A. Schmitz, H. Takahashi, E. Karakas, Structural basis for activation and gating of IP_3 receptors, Nat Commun 13 (2022) 1408. https://doi.org/10.1038/s41467-022-29073-2.
[149] K.A. Woll, F. Van Petegem, Calcium-release channels: structure and function of IP_3 receptors and ryanodine receptors, Physiol Rev 102 (2022) 209-268. https://doi.org/10.1152/physrev.00033.2020.
[150] F. Yoshikawa, H. Iwasaki, T. Michikawa, T. Furuichi, K. Mikoshiba, Trypsinized cerebellar inositol 1,4,5-trisphosphate receptor. Structural and functional coupling of cleaved ligand binding and channel domains, J Biol Chem 274 (1999) 316-327. https://doi.org/10.1074/jbc.274.1.316.
[151] G. Monaco, M. Beckers, H. Ivanova, L. Missiaen, J.B. Parys, H. De Smedt, G. Bultynck, Profiling of the Bcl-2/Bcl-X_L-binding sites on type 1 IP_3 receptor, Biochem Biophys Res Commun 428 (2012) 31-35. https://doi.org/10.1016/j.bbrc.2012.10.002.

[152] M.W. Harr, Y. Rong, M.D. Bootman, H.L. Roderick, C.W. Distelhorst, Glucocorticoid-mediated inhibition of Lck modulates the pattern of T cell receptor-induced calcium signals by down-regulating inositol 1,4,5-trisphosphate receptors, J Biol Chem 284 (2009) 31860-31871. https://doi.org/10.1074/jbc.M109.005579.
[153] N. Rosa, H. Ivanova, L.E. Wagner, 2nd, J. Kale, R. La Rovere, K. Welkenhuyzen, N. Louros, S. Karamanou, V. Shabardina, I. Lemmens, E. Vandermarliere, K. Hamada, H. Ando, F. Rousseau, J. Schymkowitz, J. Tavernier, K. Mikoshiba, A. Economou, D.W. Andrews, J.B. Parys, D.I. Yule, G. Bultynck, Bcl-xL acts as an inhibitor of IP_3R channels, thereby antagonizing Ca^{2+}-driven apoptosis, Cell Death Differ 29 (2022) 788-805. https://doi.org/10.1038/s41418-021-00894-w.
[154] N. Rosa, V. Shabardina, H. Ivanova, A. Sebe-Pedros, D.I. Yule, G. Bultynck, Tracing the evolutionary history of Ca^{2+}-signaling modulation by human Bcl-2: Insights from the Capsaspora owczarzaki IP_3 receptor ortholog, Biochim Biophys Acta Mol Cell Res 1868 (2021) 119121. https://doi.org/10.1016/j.bbamcr.2021.119121.
[155] Y.P. Rong, G. Bultynck, A.S. Aromolaran, F. Zhong, J.B. Parys, H. De Smedt, G.A. Mignery, H.L. Roderick, M.D. Bootman, C.W. Distelhorst, The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP_3 receptor, Proc Natl Acad Sci U S A 106 (2009) 14397-14402. https://doi.org/10.1073/pnas.0907555106.
[156] H. Ivanova, T. Luyten, E. Decrock, T. Vervliet, L. Leybaert, J.B. Parys, G. Bultynck, The BH4 domain of Bcl-2 orthologues from different classes of vertebrates can act as an evolutionary conserved inhibitor of IP_3 receptor channels, Cell Calcium 62 (2017) 41-46. https://doi.org/10.1016/j.ceca.2017.01.010.
[157] T. Vervloessem, H. Ivanova, T. Luyten, J.B. Parys, G. Bultynck, The selective Bcl-2 inhibitor venetoclax, a BH3 mimetic, does not dysregulate intracellular Ca^{2+} signaling, Biochim Biophys Acta Mol Cell Res 1864 (2017) 968-976. https://doi.org/10.1016/j.bbamcr.2016.11.024.
[158] M.A. Jakubowska, M. Kerkhofs, C. Martines, D.G. Efremov, J.V. Gerasimenko, O.V. Gerasimenko, O.H. Petersen, G. Bultynck, T. Vervliet, P.E. Ferdek, ABT-199 (Venetoclax), a BH3-mimetic Bcl-2 inhibitor, does not cause Ca^{2+} -signalling dysregulation or toxicity in pancreatic acinar cells, Br J Pharmacol 176 (2018) 4402–4415. https://doi.org/10.1111/bph.14505.
[159] T. Vervloessem, B.K. Sasi, E. Xerxa, S. Karamanou, J. Kale, R.M. La Rovere, S. Chakraborty, F. Sneyers, M. Vogler, A. Economou, L. Laurenti, D.W. Andrews, D.G. Efremov, G. Bultynck, BDA-366, a putative Bcl-2 BH4 domain antagonist, induces apoptosis independently of Bcl-2 in a variety of cancer cell models, Cell Death Dis 11 (2020) 769. https://doi.org/10.1038/s41419-020-02944-6.
[160] G. Shapovalov, A. Ritaine, N.C. Essonghe, I. de Ridder, H. Ivanova, S. Karamanou, A. Economou, G. Bultynck, R. Skryma, N. Prevarskaya, Allosteric cross-talk between the hydrophobic cleft and the BH4 domain of Bcl-2 in control of inositol 1,4,5-trisphosphate receptor activity, Exploration of Targeted Anti-tumor Therapy 3 (2022) 375-391. https://doi.org/10.37349/etat.2022.00088.
[161] M. Kerkhofs, G. Bultynck, T. Vervliet, G. Monaco, Therapeutic implications of novel peptides targeting ER-mitochondria Ca^{2+}-flux systems, Drug Discov Today 24 (2019) 1092-1103. https://doi.org/10.1016/j.drudis.2019.03.020.
[162] C.W. Distelhorst, M.D. Bootman, Creating a New Cancer Therapeutic Agent by Targeting the Interaction between Bcl-2 and IP_3 Receptors, Cold Spring Harb Perspect Biol 11 (2019) a035196. https://doi.org/10.1101/cshperspect.a035196.
[163] H. Akl, R.M. La Rovere, A. Janssens, P. Vandenberghe, J.B. Parys, G. Bultynck, HA14-1 potentiates apoptosis in B-cell cancer cells sensitive to a peptide disrupting IP_3 receptor / Bcl-2 complexes, Int J Dev Biol 59 (2015) 391-398. https://doi.org/10.1387/ijdb.150213gb.
[164] M. Kerkhofs, R. La Rovere, K. Welkenhuysen, A. Janssens, P. Vandenberghe, M. Madesh, J.B. Parys, G. Bultynck, BIRD-2, a BH4-domain-targeting peptide of Bcl-2, provokes Bax/Bak-independent cell death in B-cell cancers through mitochondrial Ca^{2+}-dependent mPTP opening, Cell Calcium 94 (2021) 102333. https://doi.org/10.1016/j.ceca.2020.102333.

[165] A.R. Lavik, F. Zhong, M.J. Chang, E. Greenberg, Y. Choudhary, M.R. Smith, K.S. McColl, J. Pink, F.J. Reu, S. Matsuyama, C.W. Distelhorst, A synthetic peptide targeting the BH4 domain of Bcl-2 induces apoptosis in multiple myeloma and follicular lymphoma cells alone or in combination with agents targeting the BH3-binding pocket of Bcl-2, Oncotarget 6 (2015) 27388-27402. https://doi.org/10.18632/oncotarget.4489.
[166] Q. Xie, Y. Xu, W. Gao, Y. Zhang, J. Su, Y. Liu, Y. Guo, M. Dou, K. Hu, L. Sun, TAT-fused IP_3R-derived peptide enhances cisplatin sensitivity of ovarian cancer cells by increasing ER Ca^{2+} release, Int J Mol Med 41 (2018) 809-817. https://doi.org/10.3892/ijmm.2017.3260.
[167] E.F. Greenberg, K.S. McColl, F. Zhong, G. Wildey, A. Dowlati, C.W. Distelhorst, Synergistic killing of human small cell lung cancer cells by the Bcl-2-inositol 1,4,5-trisphosphate receptor disruptor BIRD-2 and the BH3-mimetic ABT-263, Cell Death Dis 6 (2015) e2034. https://doi.org/10.1038/cddis.2015.355.
[168] L.A. Barclay, T.E. Wales, T.P. Garner, F. Wachter, S. Lee, R.M. Guerra, M.L. Stewart, C.R. Braun, G.H. Bird, E. Gavathiotis, J.R. Engen, L.D. Walensky, Inhibition of Pro-apoptotic BAX by a noncanonical interaction mechanism, Mol Cell 57 (2015) 873-886. https://doi.org/10.1016/j.molcel.2015.01.014.
[169] B. Han, D. Park, R. Li, M. Xie, T.K. Owonikoko, G. Zhang, G.L. Sica, C. Ding, J. Zhou, A.T. Magis, Z.G. Chen, D.M. Shin, S.S. Ramalingam, F.R. Khuri, W.J. Curran, X. Deng, Small-Molecule Bcl2 BH4 Antagonist for Lung Cancer Therapy, Cancer Cell 27 (2015) 852-863. https://doi.org/10.1016/j.ccell.2015.04.010.
[170] R.W. Birkinshaw, Challenges in small-molecule target identification: a commentary on "BDA-366, a putative Bcl-2 BH4 domain antagonist, induces apoptosis independently of Bcl-2 in a variety of cancer cell models", Cell Death Differ 28 (2021) 1130-1132. https://doi.org/10.1038/s41418-020-00717-4.
[171] I.Y. Joel, T.O. Adigun, A.O. Ajibola, O.O. Bankole, O.O. Ozojiofor, I.A. Remi-Esan, L.A. Sulaimon, Virtual Screening and Elucidation of Putative Binding Mode for Small Molecule Antagonist of BCL2 BH4 Domain, BioRxiv Preprint (2020) https://doi.org/10.1101/2020.07.29.226308.
[172] I. de Ridder, M. Kerkhofs, S.P. Veettil, W. Dehaen, G. Bultynck, Cancer cell death strategies by targeting Bcl-2's BH4 domain, Biochim Biophys Acta Mol Cell Res 1868 (2021) 118983. https://doi.org/10.1016/j.bbamcr.2021.118983.
[173] J.Y. Zhou, R.R. Yang, J. Chang, J. Song, Z.S. Fan, Y.H. Zhang, C.H. Lu, H.L. Jiang, M.Y. Zheng, S.L. Zhang, Discovery and identification of a novel small molecule BCL-2 inhibitor that binds to the BH4 domain, Acta Pharmacol Sin (2022) https://doi.org/10.1038/s41401-022-00936-0.
[174] L.H. Boise, M. Gonzalez-Garcia, C.E. Postema, L. Ding, T. Lindsten, L.A. Turka, X. Mao, G. Nunez, C.B. Thompson, bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death, Cell 74 (1993) 597-608. https://doi.org/10.1016/0092-8674(93)90508-n.
[175] J. Bas, T. Nguyen, G. Gillet, Involvement of Bcl-xL in Neuronal Function and Development, Int J Mol Sci 22 (2021) 3202. https://doi.org/10.3390/ijms22063202.
[176] C. Li, C.J. Fox, S.R. Master, V.P. Bindokas, L.A. Chodosh, C.B. Thompson, Bcl-X_L affects Ca^{2+} homeostasis by altering expression of inositol 1,4,5-trisphosphate receptors, Proc Natl Acad Sci U S A 99 (2002) 9830-9835. https://doi.org/10.1073/pnas.152571899.
[177] C. White, C. Li, J. Yang, N.B. Petrenko, M. Madesh, C.B. Thompson, J.K. Foskett, The endoplasmic reticulum gateway to apoptosis by Bcl-X_L modulation of the $InsP_3R$, Nat Cell Biol 7 (2005) 1021-1028. https://doi.org/10.1038/ncb1302.
[178] N. Rosa, H. Ivanova, L.E. Wagner, 2nd, J. Kale, R. La Rovere, K. Welkenhuyzen, N. Louros, S. Karamanou, V. Shabardina, I. Lemmens, E. Vandermarliere, K. Hamada, H. Ando, F. Rousseau, J. Schymkowitz, J. Tavernier, K. Mikoshiba, A. Economou, D.W. Andrews, J.B. Parys, D.I. Yule, G. Bultynck, Bcl-xL acts as an inhibitor of IP_3R channels, thereby antagonizing Ca^{2+}-driven apoptosis, Cell Death Differ Online ahead of print (2021) https://doi.org/10.1038/s41418-021-00894-w.
[179] M. Weiler, O. Bahr, U. Hohlweg, U. Naumann, J. Rieger, H. Huang, G. Tabatabai, H.W. Krell, H. Ohgaki, M. Weller, W. Wick, BCL-xL: time-dependent dissociation between modulation of apoptosis and

invasiveness in human malignant glioma cells, Cell Death Differ 13 (2006) 1156-1169. https://doi.org/10.1038/sj.cdd.4401786.
[180] C. Li, X. Wang, H. Vais, C.B. Thompson, J.K. Foskett, C. White, Apoptosis regulation by Bcl-x_L modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating, Proc Natl Acad Sci U S A 104 (2007) 12565-12570. https://doi.org/10.1073/pnas.0702489104.
[181] J. Yang, H. Vais, W. Gu, J.K. Foskett, Biphasic regulation of $InsP_3$ receptor gating by dual Ca^{2+} release channel BH3-like domains mediates Bcl-xL control of cell viability, Proc Natl Acad Sci U S A 113 (2016) E1953-E1962. https://doi.org/10.1073/pnas.1517935113.
[182] G. Monaco, E. Decrock, K. Nuyts, L.E. Wagner, 2nd, T. Luyten, S.V. Strelkov, L. Missiaen, W.M. De Borggraeve, L. Leybaert, D.I. Yule, H. De Smedt, J.B. Parys, G. Bultynck, Alpha-helical destabilization of the Bcl-2-BH4-domain peptide abolishes its ability to inhibit the IP_3 receptor, PLoS One 8 (2013) e73386. https://doi.org/10.1371/journal.pone.0073386.
[183] G. Monaco, R. La Rovere, S. Karamanou, K. Welkenhuyzen, H. Ivanova, E. Vandermarliere, M. Di Martile, D. Del Bufalo, H. De Smedt, J.B. Parys, A. Economou, G. Bultynck, A double point mutation at residues Ile14 and Val15 of Bcl-2 uncovers a role for the BH4 domain in both protein stability and function, FEBS J 285 (2018) 127-145. https://doi.org/10.1111/febs.14324.
[184] G. Monaco, E. Decrock, N. Arbel, A.R. van Vliet, R.M. La Rovere, H. De Smedt, J.B. Parys, P. Agostinis, L. Leybaert, V. Shoshan-Barmatz, G. Bultynck, The BH4 domain of anti-apoptotic Bcl-XL, but not that of the related Bcl-2, limits the voltage-dependent anion channel 1 (VDAC1)-mediated transfer of pro-apoptotic Ca^{2+} signals to mitochondria, J Biol Chem 290 (2015) 9150-9161. https://doi.org/10.1074/jbc.M114.622514.
[185] C.O. Eno, E.F. Eckenrode, K.E. Olberding, G. Zhao, C. White, C. Li, Distinct roles of mitochondria- and ER-localized Bcl-xL in apoptosis resistance and Ca^{2+} homeostasis, Mol Biol Cell 23 (2012) 2605-2618. https://doi.org/10.1091/mbc.E12-02-0090.
[186] H. Huang, X. Hu, C.O. Eno, G. Zhao, C. Li, C. White, An interaction between Bcl-xL and the voltage-dependent anion channel (VDAC) promotes mitochondrial Ca^{2+} uptake, J Biol Chem 288 (2013) 19870-19881. https://doi.org/10.1074/jbc.M113.478230.
[187] T. Vervliet, E. Decrock, J. Molgo, V. Sorrentino, L. Missiaen, L. Leybaert, H. De Smedt, N.N. Kasri, J.B. Parys, G. Bultynck, Bcl-2 binds to and inhibits ryanodine receptors, J Cell Sci 127 (2014) 2782-2792. https://doi.org/10.1242/jcs.150011.
[188] T. Vervliet, J.V. Gerasimenko, P.E. Ferdek, M.A. Jakubowska, O.H. Petersen, O.V. Gerasimenko, G. Bultynck, BH4 domain peptides derived from Bcl-2/Bcl-XL as novel tools against acute pancreatitis, Cell Death Discov 4 (2018) 58. https://doi.org/10.1038/s41420-018-0054-5.
[189] T. Vervliet, I. Lemmens, E. Vandermarliere, E. Decrock, H. Ivanova, G. Monaco, V. Sorrentino, N. Nadif Kasri, L. Missiaen, L. Martens, H. De Smedt, L. Leybaert, J.B. Parys, J. Tavernier, G. Bultynck, Ryanodine receptors are targeted by anti-apoptotic Bcl-XL involving its BH4 domain and Lys87 from its BH3 domain, Sci Rep 5 (2015) 9641. https://doi.org/10.1038/srep09641.
[190] T. Vervliet, I. Lemmens, K. Welkenhuyzen, J. Tavernier, J.B. Parys, G. Bultynck, Regulation of the ryanodine receptor by anti-apoptotic Bcl-2 is independent of its BH3-domain-binding properties, Biochem Biophys Res Commun 463 (2015) 174-179. https://doi.org/10.1016/j.bbrc.2015.04.131.
[191] T. Vervliet, J.B. Parys, G. Bultynck, Bcl-2 and FKBP12 bind to IP_3 and ryanodine receptors at overlapping sites: the complexity of protein-protein interactions for channel regulation, Biochem Soc Trans 43 (2015) 396-404. https://doi.org/10.1042/BST20140298.
[192] T. Vervliet, I. Pintelon, K. Welkenhuyzen, M.D. Bootman, H. Bannai, K. Mikoshiba, W. Martinet, N. Nadif Kasri, J.B. Parys, G. Bultynck, Basal ryanodine receptor activity suppresses autophagic flux, Biochem Pharmacol 132 (2017) 133-142. https://doi.org/10.1016/j.bcp.2017.03.011.
[193] K.J. Alzayady, A. Sebe-Pedros, R. Chandrasekhar, L. Wang, I. Ruiz-Trillo, D.I. Yule, Tracing the Evolutionary History of Inositol, 1, 4, 5-Trisphosphate Receptor: Insights from Analyses of Capsaspora

owczarzaki Ca2+ Release Channel Orthologs, Mol Biol Evol 32 (2015) 2236-2253. https://doi.org/10.1093/molbev/msv098.
[194] Q. Liu, E.J. Osterlund, X. Chi, J. Pogmore, B. Leber, D.W. Andrews, Bim escapes displacement by BH3-mimetic anti-cancer drugs by double-bolt locking both Bcl-XL and Bcl-2, Elife 8 (2019) e37689. https://doi.org/10.7554/eLife.37689.
[195] S.Y. Jeong, B. Gaume, Y.J. Lee, Y.T. Hsu, S.W. Ryu, S.H. Yoon, R.J. Youle, Bcl-x_L sequesters its C-terminal membrane anchor in soluble, cytosolic homodimers, EMBO J 23 (2004) 2146-2155. https://doi.org/10.1038/sj.emboj.7600225.
[196] Y.T. Hsu, K.G. Wolter, R.J. Youle, Cytosol-to-membrane redistribution of Bax and Bcl-X_L during apoptosis, Proc Natl Acad Sci U S A 94 (1997) 3668-3672. https://doi.org/10.1073/pnas.94.8.3668.
[197] S. Rajan, M. Choi, Q.T. Nguyen, H. Ye, W. Liu, H.T. Toh, C. Kang, N. Kamariah, C. Li, H. Huang, C. White, K. Baek, G. Gruber, H.S. Yoon, Structural transition in Bcl-xL and its potential association with mitochondrial calcium ion transport, Sci Rep 5 (2015) 10609. https://doi.org/10.1038/srep10609.
[198] E.F. Eckenrode, J. Yang, G.V. Velmurugan, J.K. Foskett, C. White, Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling, J Biol Chem 285 (2010) 13678-13684. https://doi.org/10.1074/jbc.M109.096040.
[199] A.V. Teles, R.P. Ureshino, D.J. Dorta, G.S. Lopes, Y.T. Hsu, S.S. Smaili, Bcl-x_L inhibits Bax-induced alterations in mitochondrial respiration and calcium release, Neurosci Lett 442 (2008) 96-99. https://doi.org/10.1016/j.neulet.2008.06.073.
[200] F. Zhong, M.C. Davis, K.S. McColl, C.W. Distelhorst, Bcl-2 differentially regulates Ca^{2+} signals according to the strength of T cell receptor activation, J Cell Biol 172 (2006) 127-137. https://doi.org/10.1083/jcb.200506189.
[201] A.J. Minn, P. Velez, S.L. Schendel, H. Liang, S.W. Muchmore, S.W. Fesik, M. Fill, C.B. Thompson, Bcl-x_L forms an ion channel in synthetic lipid membranes, Nature 385 (1997) 353-357. https://doi.org/10.1038/385353a0.
[202] P.J. Sung, F.D. Tsai, H. Vais, H. Court, J. Yang, N. Fehrenbacher, J.K. Foskett, M.R. Philips, Phosphorylated K-Ras limits cell survival by blocking Bcl-xL sensitization of inositol trisphosphate receptors, Proc Natl Acad Sci U S A 110 (2013) 20593-20598. https://doi.org/10.1073/pnas.1306431110.
[203] T.G. Bivona, S.E. Quatela, B.O. Bodemann, I.M. Ahearn, M.J. Soskis, A. Mor, J. Miura, H.H. Wiener, L. Wright, S.G. Saba, D. Yim, A. Fein, I. Perez de Castro, C. Li, C.B. Thompson, A.D. Cox, M.R. Philips, PKC regulates a farnesyl-electrostatic switch on K-Ras that promotes its association with Bcl-XL on mitochondria and induces apoptosis, Mol Cell 21 (2006) 481-493. https://doi.org/10.1016/j.molcel.2006.01.012.
[204] I. Dulloo, P. Atakpa-Adaji, Y.C. Yeh, C. Levet, S. Muliyil, F. Lu, C.W. Taylor, M. Freeman, iRhom pseudoproteases regulate ER stress-induced cell death through IP_3 receptors and BCL-2, Nat Commun 13 (2022) 1257. https://doi.org/10.1038/s41467-022-28930-4.
[205] Z. Assefa, G. Bultynck, K. Szlufcik, N. Nadif Kasri, E. Vermassen, J. Goris, L. Missiaen, G. Callewaert, J.B. Parys, H. De Smedt, Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis, J Biol Chem 279 (2004) 43227-43236. https://doi.org/10.1074/jbc.M403872200.
[206] D.M. Booth, P. Varnai, S.K. Joseph, G. Hajnoczky, Oxidative bursts of single mitochondria mediate retrograde signaling toward the ER, Mol Cell 81 (2021) 3866-3876 e3862. https://doi.org/10.1016/j.molcel.2021.07.014.
[207] G. Szabadkai, K. Bianchi, P. Varnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I. Nagy, T. Balla, R. Rizzuto, Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels, J Cell Biol 175 (2006) 901-911. https://doi.org/10.1083/jcb.200608073.
[208] R.S. Soderquist, L. Crawford, E. Liu, M. Lu, A. Agarwal, G.R. Anderson, K.H. Lin, P.S. Winter, M. Cakir, K.C. Wood, Systematic mapping of BCL-2 gene dependencies in cancer reveals molecular

determinants of BH3 mimetic sensitivity, Nat Commun 9 (2018) 3513. https://doi.org/10.1038/s41467-018-05815-z.
[209] M. Kerkhofs, M. Bittremieux, G. Morciano, C. Giorgi, P. Pinton, J.B. Parys, G. Bultynck, Emerging molecular mechanisms in chemotherapy: Ca^{2+} signaling at the mitochondria-associated endoplasmic reticulum membranes, Cell Death Dis 9 (2018) 334. https://doi.org/10.1038/s41419-017-0179-0.
[210] A. Danese, S. Leo, A. Rimessi, M.R. Wieckowski, F. Fiorica, C. Giorgi, P. Pinton, Cell death as a result of calcium signaling modulation: A cancer-centric prospective, Biochim Biophys Acta Mol Cell Res 1868 (2021) 119061. https://doi.org/10.1016/j.bbamcr.2021.119061.
[211] N. Rosa, F. Sneyers, J.B. Parys, G. Bultynck, Type 3 IP_3 receptors: The chameleon in cancer, Int Rev Cell Mol Biol 351 (2020) 101-148. https://doi.org/10.1016/bs.ircmb.2020.02.003.
[212] J.B. Parys, G. Bultynck, T. Vervliet, IP_3 Receptor Biology and Endoplasmic Reticulum Calcium Dynamics in Cancer, Prog Mol Subcell Biol 59 (2021) 215-237. https://doi.org/10.1007/978-3-030-67696-4_11.
[213] C.K. Min, D.R. Yeom, K.E. Lee, H.K. Kwon, M. Kang, Y.S. Kim, Z.Y. Park, I. Jeon, D.H. Kim, Coupling of ryanodine receptor 2 and voltage-dependent anion channel 2 is essential for Ca^{2+} transfer from the sarcoplasmic reticulum to the mitochondria in the heart, Biochem J 447 (2012) 371-379. https://doi.org/10.1042/BJ20120705.
[214] W.M. Rosencrans, M. Rajendran, S.M. Bezrukov, T.K. Rostovtseva, VDAC regulation of mitochondrial calcium flux: From channel biophysics to disease, Cell Calcium 94 (2021) 102356. https://doi.org/10.1016/j.ceca.2021.102356.
[215] M. Colombini, VDAC structure, selectivity, and dynamics, Biochim Biophys Acta 1818 (2012) 1457-1465. https://doi.org/10.1016/j.bbamem.2011.12.026.
[216] W. Tan, M. Colombini, VDAC closure increases calcium ion flux, Biochim Biophys Acta 1768 (2007) 2510-2515. https://doi.org/10.1016/j.bbamem.2007.06.002.
[217] T. Rostovtseva, M. Colombini, VDAC channels mediate and gate the flow of ATP: implications for the regulation of mitochondrial function, Biophys J 72 (1997) 1954-1962. https://doi.org/10.1016/S0006-3495(97)78841-6.
[218] M. Colombini, Structure and mode of action of a voltage dependent anion-selective channel (VDAC) located in the outer mitochondrial membrane, Ann N Y Acad Sci 341 (1980) 552-563. https://doi.org/10.1111/j.1749-6632.1980.tb47198.x.
[219] V.A. Menzel, M.C. Cassara, R. Benz, V. de Pinto, A. Messina, V. Cunsolo, R. Saletti, K.D. Hinsch, E. Hinsch, Molecular and functional characterization of VDAC2 purified from mammal spermatozoa, Biosci Rep 29 (2009) 351-362. https://doi.org/10.1042/BSR20080123.
[220] X. Xu, W. Decker, M.J. Sampson, W.J. Craigen, M. Colombini, Mouse VDAC isoforms expressed in yeast: channel properties and their roles in mitochondrial outer membrane permeability, J Membr Biol 170 (1999) 89-102. https://doi.org/10.1007/s002329900540.
[221] N. Arbel, D. Ben-Hail, V. Shoshan-Barmatz, Mediation of the antiapoptotic activity of Bcl-xL protein upon interaction with VDAC1 protein, J Biol Chem 287 (2012) 23152-23161. https://doi.org/10.1074/jbc.M112.345918.
[222] H. Azoulay-Zohar, A. Israelson, S. Abu-Hamad, V. Shoshan-Barmatz, In self-defence: hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death, Biochem J 377 (2004) 347-355. https://doi.org/10.1042/BJ20031465.
[223] A.K.S. Camara, Y. Zhou, P.C. Wen, E. Tajkhorshid, W.M. Kwok, Mitochondrial VDAC1: A Key Gatekeeper as Potential Therapeutic Target, Front Physiol 8 (2017) 460. https://doi.org/10.3389/fphys.2017.00460.
[224] V. Shoshan-Barmatz, Y. Krelin, A. Shteinfer-Kuzmine, VDAC1 functions in Ca^{2+} homeostasis and cell life and death in health and disease, Cell Calcium 69 (2018) 81-100. https://doi.org/10.1016/j.ceca.2017.06.007.

[225] P. Sander, T. Gudermann, J. Schredelseker, A Calcium Guard in the Outer Membrane: Is VDAC a Regulated Gatekeeper of Mitochondrial Calcium Uptake?, Int J Mol Sci 22 (2021) 946. https://doi.org/10.3390/ijms22020946.
[226] M. Zizi, M. Forte, E. Blachly-Dyson, M. Colombini, NADH regulates the gating of VDAC, the mitochondrial outer membrane channel, J Biol Chem 269 (1994) 1614-1616.
[227] T.K. Rostovtseva, K.L. Sheldon, E. Hassanzadeh, C. Monge, V. Saks, S.M. Bezrukov, D.L. Sackett, Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration, Proc Natl Acad Sci U S A 105 (2008) 18746-18751. https://doi.org/10.1073/pnas.0806303105.
[228] T.K. Rostovtseva, P.A. Gurnev, O. Protchenko, D.P. Hoogerheide, T.L. Yap, C.C. Philpott, J.C. Lee, S.M. Bezrukov, alpha-Synuclein Shows High Affinity Interaction with Voltage-dependent Anion Channel, Suggesting Mechanisms of Mitochondrial Regulation and Toxicity in Parkinson Disease, J Biol Chem 290 (2015) 18467-18477. https://doi.org/10.1074/jbc.M115.641746.
[229] G. Báthori, G. Csordas, C. Garcia-Perez, E. Davies, G. Hajnoczky, Ca^{2+}-dependent control of the permeability properties of the mitochondrial outer membrane and voltage-dependent anion-selective channel (VDAC), J Biol Chem 281 (2006) 17347-17358. https://doi.org/10.1074/jbc.M600906200.
[230] S.J.F. Chong, S. Marchi, G. Petroni, G. Kroemer, L. Galluzzi, S. Pervaiz, Noncanonical Cell Fate Regulation by Bcl-2 Proteins, Trends Cell Biol 30 (2020) 537-555. https://doi.org/10.1016/j.tcb.2020.03.004.
[231] S. Shimizu, M. Narita, Y. Tsujimoto, Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC, Nature 399 (1999) 483-487. https://doi.org/10.1038/20959.
[232] M.G. Vander Heiden, N.S. Chandel, P.T. Schumacker, C.B. Thompson, Bcl-xL prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange, Mol Cell 3 (1999) 159-167. https://doi.org/10.1016/s1097-2765(00)80307-x.
[233] M.G. Vander Heiden, X.X. Li, E. Gottleib, R.B. Hill, C.B. Thompson, M. Colombini, Bcl-xL promotes the open configuration of the voltage-dependent anion channel and metabolite passage through the outer mitochondrial membrane, J Biol Chem 276 (2001) 19414-19419. https://doi.org/10.1074/jbc.M101590200.
[234] S. Shimizu, A. Konishi, T. Kodama, Y. Tsujimoto, BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death, Proc Natl Acad Sci U S A 97 (2000) 3100-3105. https://doi.org/10.1073/pnas.97.7.3100.
[235] V. Shoshan-Barmatz, A. Israelson, D. Brdiczka, S.S. Sheu, The voltage-dependent anion channel (VDAC): function in intracellular signalling, cell life and cell death, Curr Pharm Des 12 (2006) 2249-2270. https://doi.org/10.2174/138161206777585111.
[236] K.S. McCommis, C.P. Baines, The role of VDAC in cell death: friend or foe?, Biochim Biophys Acta 1818 (2012) 1444-1450. https://doi.org/10.1016/j.bbamem.2011.10.025.
[237] C.P. Baines, R.A. Kaiser, T. Sheiko, W.J. Craigen, J.D. Molkentin, Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death, Nat Cell Biol 9 (2007) 550-555. https://doi.org/10.1038/ncb1575.
[238] S. Shimizu, T. Ide, T. Yanagida, Y. Tsujimoto, Electrophysiological study of a novel large pore formed by Bax and the voltage-dependent anion channel that is permeable to cytochrome c, J Biol Chem 275 (2000) 12321-12325. https://doi.org/10.1074/jbc.275.16.12321.
[239] Y. Zheng, Y. Shi, C. Tian, C. Jiang, H. Jin, J. Chen, A. Almasan, H. Tang, Q. Chen, Essential role of the voltage-dependent anion channel (VDAC) in mitochondrial permeability transition pore opening and cytochrome c release induced by arsenic trioxide, Oncogene 23 (2004) 1239-1247. https://doi.org/10.1038/sj.onc.1207205.

[240] N. Keinan, D. Tyomkin, V. Shoshan-Barmatz, Oligomerization of the mitochondrial protein voltage-dependent anion channel is coupled to the induction of apoptosis, Mol Cell Biol 30 (2010) 5698-5709. https://doi.org/10.1128/MCB.00165-10.
[241] J. Kim, R. Gupta, L.P. Blanco, S. Yang, A. Shteinfer-Kuzmine, K. Wang, J. Zhu, H.E. Yoon, X. Wang, M. Kerkhofs, H. Kang, A.L. Brown, S.J. Park, X. Xu, E. Zandee van Rilland, M.K. Kim, J.I. Cohen, M.J. Kaplan, V. Shoshan-Barmatz, J.H. Chung, VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease, Science 366 (2019) 1531-1536. https://doi.org/10.1126/science.aav4011.
[242] T.J. Malia, G. Wagner, NMR structural investigation of the mitochondrial outer membrane protein VDAC and its interaction with antiapoptotic Bcl-xL, Biochemistry 46 (2007) 514-525. https://doi.org/10.1021/bi061577h.
[243] K.N. Alavian, G. Beutner, E. Lazrove, S. Sacchetti, H.A. Park, P. Licznerski, H. Li, P. Nabili, K. Hockensmith, M. Graham, G.A. Porter, Jr., E.A. Jonas, An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore, Proc Natl Acad Sci U S A 111 (2014) 10580-10585. https://doi.org/10.1073/pnas.1401591111.
[244] A. Williams, T. Hayashi, D. Wolozny, B. Yin, T.C. Su, M.J. Betenbaugh, T.P. Su, The non-apoptotic action of Bcl-xL: regulating Ca^{2+} signaling and bioenergetics at the ER-mitochondrion interface, J Bioenerg Biomembr 48 (2016) 211-225. https://doi.org/10.1007/s10863-016-9664-x.
[245] A. Fouqué, E. Lepvrier, L. Debure, Y. Gouriou, M. Malleter, V. Delcroix, M. Ovize, T. Ducret, C. Li, M. Hammadi, P. Vacher, P. Legembre, The apoptotic members CD95, BclxL, and Bcl-2 cooperate to promote cell migration by inducing Ca^{2+} flux from the endoplasmic reticulum to mitochondria, Cell Death Differ 23 (2016) 1702-1716. https://doi.org/10.1038/cdd.2016.61
[246] M. Bessou, J. Lopez, R. Gadet, M. Deygas, N. Popgeorgiev, D. Poncet, A. Nougarede, P. Billard, I. Mikaelian, P. Gonzalo, R. Rimokh, G. Gillet, The apoptosis inhibitor Bcl-xL controls breast cancer cell migration through mitochondria-dependent reactive oxygen species production, Oncogene 39 (2020) 3056-3074. https://doi.org/10.1038/s41388-020-1212-9.
[247] K.N. Alavian, H. Li, L. Collis, L. Bonanni, L. Zeng, S. Sacchetti, E. Lazrove, P. Nabili, B. Flaherty, M. Graham, Y. Chen, S.M. Messerli, M.A. Mariggio, C. Rahner, E. McNay, G.C. Shore, P.J. Smith, J.M. Hardwick, E.A. Jonas, Bcl-xL regulates metabolic efficiency of neurons through interaction with the mitochondrial F1FO ATP synthase, Nat Cell Biol 13 (2011) 1224-1233. https://doi.org/10.1038/ncb2330.
[248] A.J. Minn, C.M. Rudin, L.H. Boise, C.B. Thompson, Expression of bcl-xL can confer a multidrug resistance phenotype, Blood 86 (1995) 1903-1910. https://doi.org/10.1182/blood.V86.5.1903.bloodjournal8651903.
[249] D. Schlaifer, M. Marcu, S. Krajewski, G. Laurent, J. Pris, G. Delsol, J.C. Reed, P. Brousset, High expression of the bcl-x gene in Reed-Sternberg cells of Hodgkin's disease, Blood 85 (1995) 2671-2674.
[250] A.F. Schott, I.J. Apel, G. Nunez, M.F. Clarke, Bcl-XL protects cancer cells from p53-mediated apoptosis, Oncogene 11 (1995) 1389-1394.
[251] M.G. Dole, R. Jasty, M.J. Cooper, C.B. Thompson, G. Nunez, V.P. Castle, Bcl-xL is expressed in neuroblastoma cells and modulates chemotherapy-induced apoptosis, Cancer Res 55 (1995) 2576-2582.
[252] E. Punnoose, F.V. Peale, E. Szafer-Glusman, G. Lei, R. Bourgon, A.D. Do, E. Kim, L. Zhang, P. Farinha, R.D. Gascoyne, F.J. Munoz, M. Martelli, A. Mottok, G.A. Salles, L.H. Sehn, J.F. Seymour, M. Trneny, M.Z. Oestergaard, K.E. Mundt, U. Vitolo, BCL2 Expression in First-Line Diffuse Large B-Cell Lymphoma Identifies a Patient Population With Poor Prognosis, Clin Lymphoma Myeloma Leuk 21 (2021) 267-278 e210. https://doi.org/10.1016/j.clml.2020.11.004.
[253] J. Roh, H. Cho, H.K. Pak, Y.S. Lee, S.W. Lee, J.S. Ryu, E.J. Chae, K.W. Kim, J. Huh, Y.S. Choi, S.H. Jeong, C. Suh, D.H. Yoon, C.S. Park, BCL2 super-expressor diffuse large B-cell lymphoma: a distinct subgroup associated with poor prognosis, Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 35 (2022) 480-488. https://doi.org/10.1038/s41379-021-00962-z.

[254] K. Makino, H. Nakamura, N. Shinojima, J.I. Kuroda, S. Yano, Y. Mikami, A. Mukasa, BCL2 expression is associated with a poor prognosis independent of cellular origin in primary central nervous system diffuse large B-cell lymphoma, J Neurooncol 140 (2018) 115-121. https://doi.org/10.1007/s11060-018-2940-3.
[255] G. Gasparini, M. Barbareschi, C. Doglioni, P.D. Palma, F.A. Mauri, P. Boracchi, P. Bevilacqua, O. Caffo, L. Morelli, P. Verderio, et al., Expression of bcl-2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer, Clin Cancer Res 1 (1995) 189-198.
[256] S.J. Dawson, N. Makretsov, F.M. Blows, K.E. Driver, E. Provenzano, J. Le Quesne, L. Baglietto, G. Severi, G.G. Giles, C.A. McLean, G. Callagy, A.R. Green, I. Ellis, K. Gelmon, G. Turashvili, S. Leung, S. Aparicio, D. Huntsman, C. Caldas, P. Pharoah, BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received, Br J Cancer 103 (2010) 668-675. https://doi.org/10.1038/sj.bjc.6605736.
[257] O.I. Olopade, M.O. Adeyanju, A.R. Safa, F. Hagos, R. Mick, C.B. Thompson, W.M. Recant, Overexpression of BCL-x protein in primary breast cancer is associated with high tumor grade and nodal metastases, Cancer J Sci Am 3 (1997) 230-237.
[258] Y. Fernández, L. Espana, S. Manas, A. Fabra, A. Sierra, Bcl-xL promotes metastasis of breast cancer cells by induction of cytokines resistance, Cell Death Differ 7 (2000) 350-359. https://doi.org/10.1038/sj.cdd.4400662.
[259] S.S. Martin, A.G. Ridgeway, J. Pinkas, Y. Lu, M.J. Reginato, E.Y. Koh, M. Michelman, G.Q. Daley, J.S. Brugge, P. Leder, A cytoskeleton-based functional genetic screen identifies Bcl-xL as an enhancer of metastasis, but not primary tumor growth, Oncogene 23 (2004) 4641-4645. https://doi.org/10.1038/sj.onc.1207595.
[260] D. Trisciuoglio, M.G. Tupone, M. Desideri, M. Di Martile, C. Gabellini, S. Buglioni, M. Pallocca, G. Alessandrini, S. D'Aguanno, D. Del Bufalo, BCL-XL overexpression promotes tumor progression-associated properties, Cell Death Dis 8 (2017) 3216. https://doi.org/10.1038/s41419-017-0055-y.
[261] T.V. Denisenko, A.S. Gorbunova, B. Zhivotovsky, Mitochondrial Involvement in Migration, Invasion and Metastasis, Front Cell Dev Biol 7 (2019) 355. https://doi.org/10.3389/fcell.2019.00355.
[262] A.A. Fiebig, W. Zhu, C. Hollerbach, B. Leber, D.W. Andrews, Bcl-XL is qualitatively different from and ten times more effective than Bcl-2 when expressed in a breast cancer cell line, BMC Cancer 6 (2006) 213. https://doi.org/10.1186/1471-2407-6-213.
[263] S.N. Willis, L. Chen, G. Dewson, A. Wei, E. Naik, J.I. Fletcher, J.M. Adams, D.C. Huang, Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins, Genes & development 19 (2005) 1294-1305. https://doi.org/10.1101/gad.1304105.
[264] H. Friess, Z. Lu, A. Andren-Sandberg, P. Berberat, A. Zimmermann, G. Adler, R. Schmid, M.W. Buchler, Moderate activation of the apoptosis inhibitor bcl-xL worsens the prognosis in pancreatic cancer, Ann Surg 228 (1998) 780-787. https://doi.org/10.1097/00000658-199812000-00009.
[265] S. Yoshimine, E. Kikuchi, T. Kosaka, S. Mikami, A. Miyajima, Y. Okada, M. Oya, Prognostic significance of Bcl-xL expression and efficacy of Bcl-xL targeting therapy in urothelial carcinoma, Br J Cancer 108 (2013) 2312-2320. https://doi.org/10.1038/bjc.2013.216.
[266] J. Watanabe, F. Kushihata, K. Honda, A. Sugita, N. Tateishi, K. Mominoki, S. Matsuda, N. Kobayashi, Prognostic significance of Bcl-xL in human hepatocellular carcinoma, Surgery 135 (2004) 604-612. https://doi.org/10.1016/j.surg.2003.11.015.
[267] Y.L. Zhang, L.Q. Pang, Y. Wu, X.Y. Wang, C.Q. Wang, Y. Fan, Significance of Bcl-xL in human colon carcinoma, World J Gastroenterol 14 (2008) 3069-3073. https://doi.org/10.3748/wjg.14.3069.
[268] D.A. Hameed, A.M. Abdel Raheem, E. Mosad, H.M. Hammouda, N.A. Kamel, M.A. Abdel Aziz, Bcl-XL and Bcl-2 expression in bilharzial squamous cell carcinoma of the urinary bladder: which protein is prognostic?, Urology 72 (2008) 374-378. https://doi.org/10.1016/j.urology.2007.12.063.

[269] P.D. Dunne, H.G. Coleman, P. Bankhead, M. Alderdice, R.T. Gray, S. McQuaid, V. Bingham, M.B. Loughrey, J.A. James, A.M.B. McCorry, A. Gilmore, C. Holohan, D. Klingbiel, S. Tejpar, P.G. Johnston, D.G. McArt, F. Di Nicolantonio, D.B. Longley, M. Lawler, Bcl-xL as a poor prognostic biomarker and predictor of response to adjuvant chemotherapy specifically in BRAF-mutant stage II and III colon cancer, Oncotarget 9 (2018) 13834-13847. https://doi.org/10.18632/oncotarget.24481.
[270] Y. Jin-Song, W. Zhao-Xia, L. Cheng-Yu, L. Xiao-Di, S. Ming, G. Yuan-Yuan, D. Wei, Prognostic significance of Bcl-xL gene expression in human colorectal cancer, Acta Histochem 113 (2011) 810-814. https://doi.org/10.1016/j.acthis.2011.01.002.
[271] W.H. Zhou, F. Tang, J. Xu, X. Wu, S.B. Yang, Z.Y. Feng, Y.G. Ding, X.B. Wan, Z. Guan, H.G. Li, D.J. Lin, C.K. Shao, Q. Liu, Low expression of Beclin 1, associated with high Bcl-xL, predicts a malignant phenotype and poor prognosis of gastric cancer, Autophagy 8 (2012) 389-400. https://doi.org/10.4161/auto.18641.
[272] M. Okumura, Y. Kajiyama, K. Takeda, K. Okumura, M. Tsurumaru, Correlation between loss of Bcl-XL expression and improved prognosis in advanced esophageal cancer treated by preoperative chemoradiotherapy, Eur Surg Res 41 (2008) 260-266. https://doi.org/10.1159/000141515.
[273] K. Zhang, K. Jiao, Z. Xing, L. Zhang, J. Yang, X. Xie, L. Yang, Bcl-xL overexpression and its association with the progress of tongue carcinoma, Int J Clin Exp Pathol 7 (2014) 7360-7377.
[274] B. Karczmarek-Borowska, A. Filip, J. Wojcierowski, A. Smolen, E. Korobowicz, I. Korszen-Pilecka, M. Zdunek, Estimation of prognostic value of Bcl-xL gene expression in non-small cell lung cancer, Lung Cancer 51 (2006) 61-69. https://doi.org/10.1016/j.lungcan.2005.08.010.
[275] L. Zhuang, C.S. Lee, R.A. Scolyer, S.W. McCarthy, X.D. Zhang, J.F. Thompson, P. Hersey, Mcl-1, Bcl-XL and Stat3 expression are associated with progression of melanoma whereas Bcl-2, AP-2 and MITF levels decrease during progression of melanoma, Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 20 (2007) 416-426. https://doi.org/10.1038/modpathol.3800750.
[276] Z.X. Wang, J.S. Yang, X. Pan, J.R. Wang, J. Li, Y.M. Yin, W. De, Functional and biological analysis of Bcl-xL expression in human osteosarcoma, Bone 47 (2010) 445-454. https://doi.org/10.1016/j.bone.2010.05.027.
[277] W.L. Zhao, M.E. Daneshpouy, N. Mounier, J. Briere, C. Leboeuf, L.F. Plassa, E. Turpin, J.M. Cayuela, J.C. Ameisen, C. Gisselbrecht, A. Janin, Prognostic significance of bcl-xL gene expression and apoptotic cell counts in follicular lymphoma, Blood 103 (2004) 695-697. https://doi.org/10.1182/blood-2003-06-1901.
[278] R. Soltani-Arabshahi, C. Leboeuf, J. Rivet, H. Pisonero, W.L. Zhao, H. Bachelez, J.C. Ameisen, A. Janin, Bcl-xL gene expression correlated with lower apoptotic cell numbers and shorter progression-free survival in PCFCL, J Invest Dermatol 129 (2009) 1703-1709. https://doi.org/10.1038/jid.2008.430.
[279] M. Schaich, T. Illmer, C. Seitz, B. Mohr, U. Schakel, J.F. Beck, G. Ehninger, The prognostic value of Bcl-XL gene expression for remission induction is influenced by cytogenetics in adult acute myeloid leukemia, Haematologica 86 (2001) 470-477.
[280] S. Choi, Z. Chen, L.H. Tang, Y. Fang, S.J. Shin, N.C. Panarelli, Y.T. Chen, Y. Li, X. Jiang, Y.N. Du, Bcl-xL promotes metastasis independent of its anti-apoptotic activity, Nat Commun 7 (2016) 10384. https://doi.org/10.1038/ncomms10384.
[281] J.H. Hager, D.B. Ulanet, L. Hennighausen, D. Hanahan, Genetic ablation of Bcl-x attenuates invasiveness without affecting apoptosis or tumor growth in a mouse model of pancreatic neuroendocrine cancer, PLoS One 4 (2009) e4455. https://doi.org/10.1371/journal.pone.0004455.
[282] U. Keitel, A. Scheel, J. Thomale, R. Halpape, S. Kaulfuss, C. Scheel, M. Dobbelstein, Bcl-xL mediates therapeutic resistance of a mesenchymal breast cancer cell subpopulation, Oncotarget 5 (2014) 11778-11791. https://doi.org/10.18632/oncotarget.2634.
[283] J. Cheng, D. Qian, X. Ding, T. Song, M. Cai, X. Dan, Y. Wang, J. Zhao, Z. Liu, Z. Wu, Q. Pang, L. Zhu, P. Wang, X. Hao, Z. Yuan, High PGAM5 expression induces chemoresistance by enhancing Bcl-xL-mediated

anti-apoptotic signaling and predicts poor prognosis in hepatocellular carcinoma patients, Cell Death Dis 9 (2018) 991. https://doi.org/10.1038/s41419-018-1017-8.
[284] J. Williams, P.C. Lucas, K.A. Griffith, M. Choi, S. Fogoros, Y.Y. Hu, J.R. Liu, Expression of Bcl-xL in ovarian carcinoma is associated with chemoresistance and recurrent disease, Gynecol Oncol 96 (2005) 287-295. https://doi.org/10.1016/j.ygyno.2004.10.026.
[285] Z. Dou, D. Zhao, X. Chen, C. Xu, X. Jin, X. Zhang, Y. Wang, X. Xie, Q. Li, C. Di, H. Zhang, Aberrant Bcl-x splicing in cancer: from molecular mechanism to therapeutic modulation, J Exp Clin Cancer Res 40 (2021) 194. https://doi.org/10.1186/s13046-021-02001-w.
[286] J. Jin, Y. Xiong, B. Cen, Bcl-2 and Bcl-xL mediate resistance to receptor tyrosine kinase-targeted therapy in lung and gastric cancer, Anticancer Drugs 28 (2017) 1141-1149. https://doi.org/10.1097/CAD.0000000000000561.
[287] M. Haselager, R. Thijssen, C. West, L. Young, R. Van Kampen, E. Willmore, S. Mackay, A. Kater, E. Eldering, Regulation of Bcl-XL by non-canonical NF-kappaB in the context of CD40-induced drug resistance in CLL, Cell Death Differ 28 (2021) 1658-1668. https://doi.org/10.1038/s41418-020-00692-w.
[288] X. Xiao, G. Yang, P. Bai, S. Gui, T.M. Nyuyen, I. Mercado-Uribe, M. Yang, J. Zou, Q. Li, J. Xiao, B. Chang, G. Liu, H. Wang, J. Liu, Inhibition of nuclear factor-kappa B enhances the tumor growth of ovarian cancer cell line derived from a low-grade papillary serous carcinoma in p53-independent pathway, BMC Cancer 16 (2016) 582. https://doi.org/10.1186/s12885-016-2617-2.
[289] Q.G. Dong, G.M. Sclabas, S. Fujioka, C. Schmidt, B. Peng, T. Wu, M.S. Tsao, D.B. Evans, J.L. Abbruzzese, T.J. McDonnell, P.J. Chiao, The function of multiple IkappaB : NF-kappaB complexes in the resistance of cancer cells to Taxol-induced apoptosis, Oncogene 21 (2002) 6510-6519. https://doi.org/10.1038/sj.onc.1205848.
[290] C. Alcon, J. Gomez Tejeda Zanudo, R. Albert, N. Vagle, M. Scaltriti, A. Letai, J. Samitier, J. Montero, ER+ Breast Cancer Strongly Depends on MCL-1 and BCL-xL Anti-Apoptotic Proteins, Cells 10 (2021) 1659. https://doi.org/10.3390/cells10071659.
[291] M. Kerkhofs, T. Vervloessem, K.B. Stopa, V.M. Smith, M. Vogler, G. Bultynck, DLBCL Cells with Acquired Resistance to Venetoclax Are Not Sensitized to BIRD-2 But Can Be Resensitized to Venetoclax through Bcl-XL Inhibition, Biomolecules 10 (2020) 1081. https://doi.org/10.3390/biom10071081.
[292] G.S. Choudhary, S. Al-Harbi, S. Mazumder, B.T. Hill, M.R. Smith, J. Bodo, E.D. Hsi, A. Almasan, MCL-1 and BCL-xL-dependent resistance to the BCL-2 inhibitor ABT-199 can be overcome by preventing PI3K/AKT/mTOR activation in lymphoid malignancies, Cell Death Dis 6 (2015) e1593. https://doi.org/10.1038/cddis.2014.525.
[293] L.O. Herzog, B. Walter, R. Buono, J.S. Lee, S. Mallya, A. Fung, H. Chiu, N. Nguyen, B. Li, A.B. Pinkerton, M.R. Jackson, R.J. Schneider, Z.A. Ronai, D.A. Fruman, Targeting eIF4F translation initiation complex with SBI-756 sensitises B lymphoma cells to venetoclax, Br J Cancer 124 (2021) 1098-1109. https://doi.org/10.1038/s41416-020-01205-9.
[294] S. Al-harbi, G.S. Choudhary, J.S. Ebron, B.T. Hill, N. Vivekanathan, A.H. Ting, T. Radivoyevitch, M.R. Smith, G.C. Shukla, A. Almasan, miR-377-dependent BCL-xL regulation drives chemotherapeutic resistance in B-cell lymphoid malignancies, Mol Cancer 14 (2015) 185. https://doi.org/10.1186/s12943-015-0460-8.
[295] J.J. Bassett, M. Robitaille, A.A. Peters, A.H.L. Bong, M.W. Taing, I.A. Wood, F. Sadras, S.J. Roberts-Thomson, G.R. Monteith, ORAI1 regulates sustained cytosolic free calcium fluctuations during breast cancer cell apoptosis and apoptotic resistance via a STIM1 independent pathway, FASEB J 36 (2022) e22108. https://doi.org/10.1096/fj.202002031RR.
[296] J. Bai, J. Sui, A. Demirjian, C.M. Vollmer, Jr., W. Marasco, M.P. Callery, Predominant Bcl-XL knockdown disables antiapoptotic mechanisms: tumor necrosis factor-related apoptosis-inducing ligand-based triple chemotherapy overcomes chemoresistance in pancreatic cancer cells in vitro, Cancer Res 65 (2005) 2344-2352. https://doi.org/10.1158/0008-5472.CAN-04-3502.

[297] J. Yang, M. Sun, A. Zhang, C. Lv, W. De, Z. Wang, Adenovirus-mediated siRNA targeting Bcl-xL inhibits proliferation, reduces invasion and enhances radiosensitivity of human colorectal cancer cells, World J Surg Oncol 9 (2011) 117. https://doi.org/10.1186/1477-7819-9-117.
[298] N. Bah, L. Maillet, J. Ryan, S. Dubreil, F. Gautier, A. Letai, P. Juin, S. Barille-Nion, Bcl-xL controls a switch between cell death modes during mitotic arrest, Cell Death Dis 5 (2014) e1291. https://doi.org/10.1038/cddis.2014.251.
[299] Y. de Jong, D. Monderer, E. Brandinelli, M. Monchanin, B.E. van den Akker, J.G. van Oosterwijk, J.Y. Blay, A. Dutour, J. Bovee, Bcl-xl as the most promising Bcl-2 family member in targeted treatment of chondrosarcoma, Oncogenesis 7 (2018) 74. https://doi.org/10.1038/s41389-018-0084-0.
[300] Y. Xu, H. Ye, Progress in understanding the mechanisms of resistance to BCL-2 inhibitors, Exp Hematol Oncol 11 (2022) 31. https://doi.org/10.1186/s40164-022-00283-0.
[301] S.B. Berman, Y.B. Chen, B. Qi, J.M. McCaffery, E.B. Rucker, 3rd, S. Goebbels, K.A. Nave, B.A. Arnold, E.A. Jonas, F.J. Pineda, J.M. Hardwick, Bcl-x L increases mitochondrial fission, fusion, and biomass in neurons, J Cell Biol 184 (2009) 707-719. https://doi.org/10.1083/jcb.200809060.
[302] J. Zhao, J. Zhang, M. Yu, Y. Xie, Y. Huang, D.W. Wolff, P.W. Abel, Y. Tu, Mitochondrial dynamics regulates migration and invasion of breast cancer cells, Oncogene 32 (2013) 4814-4824. https://doi.org/10.1038/onc.2012.494.
[303] R.J. DeBerardinis, N.S. Chandel, Fundamentals of cancer metabolism, Sci Adv 2 (2016) e1600200. https://doi.org/10.1126/sciadv.1600200.
[304] T. Shiraishi, J.E. Verdone, J. Huang, U.D. Kahlert, J.R. Hernandez, G. Torga, J.C. Zarif, T. Epstein, R. Gatenby, A. McCartney, J.H. Elisseeff, S.M. Mooney, S.S. An, K.J. Pienta, Glycolysis is the primary bioenergetic pathway for cell motility and cytoskeletal remodeling in human prostate and breast cancer cells, Oncotarget 6 (2015) 130-143. https://doi.org/10.18632/oncotarget.2766.
[305] C.F. Zhou, X.B. Li, H. Sun, B. Zhang, Y.S. Han, Y. Jiang, Q.L. Zhuang, J. Fang, G.H. Wu, Pyruvate kinase type M2 is upregulated in colorectal cancer and promotes proliferation and migration of colon cancer cells, IUBMB Life 64 (2012) 775-782. https://doi.org/10.1002/iub.1066.
[306] M. Anderson, R. Marayati, R. Moffitt, J.J. Yeh, Hexokinase 2 promotes tumor growth and metastasis by regulating lactate production in pancreatic cancer, Oncotarget 8 (2017) 56081-56094. https://doi.org/10.18632/oncotarget.9760.
[307] M. Yu, S. Chen, W. Hong, Y. Gu, B. Huang, Y. Lin, Y. Zhou, H. Jin, Y. Deng, L. Tu, B. Hou, Z. Jian, Prognostic role of glycolysis for cancer outcome: evidence from 86 studies, J Cancer Res Clin Oncol 145 (2019) 967-999. https://doi.org/10.1007/s00432-019-02847-w.
[308] S.Y. Peng, P.L. Lai, H.W. Pan, L.P. Hsiao, H.C. Hsu, Aberrant expression of the glycolytic enzymes aldolase B and type II hexokinase in hepatocellular carcinoma are predictive markers for advanced stage, early recurrence and poor prognosis, Oncol Rep 19 (2008) 1045-1053. https://doi.org/10.3892/or.19.4.1045.
[309] H. Ogawa, H. Nagano, M. Konno, H. Eguchi, J. Koseki, K. Kawamoto, N. Nishida, H. Colvin, A. Tomokuni, Y. Tomimaru, N. Hama, H. Wada, S. Marubashi, S. Kobayashi, M. Mori, Y. Doki, H. Ishii, The combination of the expression of hexokinase 2 and pyruvate kinase M2 is a prognostic marker in patients with pancreatic cancer, Mol Clin Oncol 3 (2015) 563-571. https://doi.org/10.3892/mco.2015.490.
[310] D. Jia, M. Lu, K.H. Jung, J.H. Park, L. Yu, J.N. Onuchic, B.A. Kaipparettu, H. Levine, Elucidating cancer metabolic plasticity by coupling gene regulation with metabolic pathways, Proc Natl Acad Sci U S A 116 (2019) 3909-3918. https://doi.org/10.1073/pnas.1816391116.
[311] L. Yu, M. Lu, D. Jia, J. Ma, E. Ben-Jacob, H. Levine, B.A. Kaipparettu, J.N. Onuchic, Modeling the Genetic Regulation of Cancer Metabolism: Interplay between Glycolysis and Oxidative Phosphorylation, Cancer Res 77 (2017) 1564-1574. https://doi.org/10.1158/0008-5472.CAN-16-2074.

[312] H.D. Um, Bcl-2 family proteins as regulators of cancer cell invasion and metastasis: a review focusing on mitochondrial respiration and reactive oxygen species, Oncotarget 7 (2016) 5193-5203. https://doi.org/10.18632/oncotarget.6405.
[313] L.B. Sullivan, N.S. Chandel, Mitochondrial reactive oxygen species and cancer, Cancer Metab 2 (2014) 17. https://doi.org/10.1186/2049-3002-2-17.
[314] M.G. Vander Heiden, N.S. Chandel, E.K. Williamson, P.T. Schumacker, C.B. Thompson, Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria, Cell 91 (1997) 627-637. https://doi.org/10.1016/s0092-8674(00)80450-x.
[315] Y.B. Chen, M.A. Aon, Y.T. Hsu, L. Soane, X. Teng, J.M. McCaffery, W.C. Cheng, B. Qi, H. Li, K.N. Alavian, M. Dayhoff-Brannigan, S. Zou, F.J. Pineda, B. O'Rourke, Y.H. Ko, P.L. Pedersen, L.K. Kaczmarek, E.A. Jonas, J.M. Hardwick, Bcl-xL regulates mitochondrial energetics by stabilizing the inner membrane potential, J Cell Biol 195 (2011) 263-276. https://doi.org/10.1083/jcb.201108059.
[316] F. Lucantoni, M. Salvucci, H. Dussmann, A.U. Lindner, D. Lambrechts, J.H.M. Prehn, BCL(X)L and BCL2 increase the metabolic fitness of breast cancer cells: a single-cell imaging study, Cell Death Differ 28 (2021) 1512-1531. https://doi.org/10.1038/s41418-020-00683-x.
[317] G. Manfredi, J.Q. Kwong, J.A. Oca-Cossio, M. Woischnik, C.D. Gajewski, K. Martushova, M. D'Aurelio, A.L. Friedlich, C.T. Moraes, BCL-2 improves oxidative phosphorylation and modulates adenine nucleotide translocation in mitochondria of cells harboring mutant mtDNA, J Biol Chem 278 (2003) 5639-5645. https://doi.org/10.1074/jbc.M203080200.
[318] C. White, The Regulation of Tumor Cell Invasion and Metastasis by Endoplasmic Reticulum-to-Mitochondrial Ca^{2+} Transfer, Front Oncol 7 (2017) 171. https://doi.org/10.3389/fonc.2017.00171.
[319] K. Ishikawa, K. Takenaga, M. Akimoto, N. Koshikawa, A. Yamaguchi, H. Imanishi, K. Nakada, Y. Honma, J. Hayashi, ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis, Science 320 (2008) 661-664. https://doi.org/10.1126/science.1156906.
[320] P.E. Porporato, V.L. Payen, J. Perez-Escuredo, C.J. De Saedeleer, P. Danhier, T. Copetti, S. Dhup, M. Tardy, T. Vazeille, C. Bouzin, O. Feron, C. Michiels, B. Gallez, P. Sonveaux, A mitochondrial switch promotes tumor metastasis, Cell Rep 8 (2014) 754-766. https://doi.org/10.1016/j.celrep.2014.06.043.
[321] F. Weinberg, R. Hamanaka, W.W. Wheaton, S. Weinberg, J. Joseph, M. Lopez, B. Kalyanaraman, G.M. Mutlu, G.R. Budinger, N.S. Chandel, Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity, Proc Natl Acad Sci U S A 107 (2010) 8788-8793. https://doi.org/10.1073/pnas.1003428107.
[322] S.R. Lee, K.S. Yang, J. Kwon, C. Lee, W. Jeong, S.G. Rhee, Reversible inactivation of the tumor suppressor PTEN by H2O2, J Biol Chem 277 (2002) 20336-20342. https://doi.org/10.1074/jbc.M111899200.
[323] A. Östman, J. Frijhoff, A. Sandin, F.D. Bohmer, Regulation of protein tyrosine phosphatases by reversible oxidation, J Biochem 150 (2011) 345-356. https://doi.org/10.1093/jb/mvr104.
[324] J.M. Lluis, F. Buricchi, P. Chiarugi, A. Morales, J.C. Fernandez-Checa, Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor-{kappa}B via c-SRC and oxidant-dependent cell death, Cancer Res 67 (2007) 7368-7377. https://doi.org/10.1158/0008-5472.CAN-07-0515.
[325] A.J. Kowaltowski, R.G. Fenton, G. Fiskum, Bcl-2 family proteins regulate mitochondrial reactive oxygen production and protect against oxidative stress, Free Radic Biol Med 37 (2004) 1845-1853. https://doi.org/10.1016/j.freeradbiomed.2004.09.005.
[326] E.M. Kim, J.K. Park, S.G. Hwang, W.J. Kim, Z.G. Liu, S.W. Kang, H.D. Um, Nuclear and cytoplasmic p53 suppress cell invasion by inhibiting respiratory complex-I activity via Bcl-2 family proteins, Oncotarget 5 (2014) 8452-8465. https://doi.org/10.18632/oncotarget.2320.
[327] J.H. Jang, Y.J. Surh, Possible role of NF-kappaB in Bcl-X_L protection against hydrogen peroxide-induced PC12 cell death, Redox Rep 9 (2004) 343-348. https://doi.org/10.1179/135100004225006858.

[328] H. Ivanova, M. Kerkhofs, R.M. La Rovere, G. Bultynck, Endoplasmic Reticulum-Mitochondrial Ca^{2+} Fluxes Underlying Cancer Cell Survival, Front Oncol 7 (2017) 70. https://doi.org/10.3389/fonc.2017.00070.
[329] S. Marchi, C. Giorgi, L. Galluzzi, P. Pinton, Ca^{2+} Fluxes and Cancer, Mol Cell 78 (2020) 1055-1069. https://doi.org/10.1016/j.molcel.2020.04.017.
[330] N. Prevarskaya, R. Skryma, Y. Shuba, Calcium in tumour metastasis: new roles for known actors, Nat Rev Cancer 11 (2011) 609-618. https://doi.org/10.1038/nrc3105.
[331] V. Paupe, J. Prudent, New insights into the role of mitochondrial calcium homeostasis in cell migration, Biochem Biophys Res Commun 500 (2018) 75-86. https://doi.org/10.1016/j.bbrc.2017.05.039.
[332] A. Tosatto, R. Sommaggio, C. Kummerow, R.B. Bentham, T.S. Blacker, T. Berecz, M.R. Duchen, A. Rosato, I. Bogeski, G. Szabadkai, R. Rizzuto, C. Mammucari, The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1alpha, EMBO Mol Med 8 (2016) 569-585. https://doi.org/10.15252/emmm.201606255.

Figure legends

Figure 1: Structure and function of Bcl-2-family proteins

The Bcl-2 family of proteins is classified into antiapoptotic and proapoptotic proteins characterized by the presence of one to four Bcl-2 homology (BH) domains. The antiapoptotic proteins consist exclusively of multidomain effectors, while proapoptotic proteins can be further subdivided into multidomain effectors and BH3-only proteins. The BH3-BH1-BH2 domains of Bcl-2-like proteins form a hydrophobic cleft that targets the BH3 domain of many proapoptotic Bcl-2-family proteins. BH3 mimetics can prevent this effect by acting as a ligand for the hydrophobic cleft. Most Bcl-2-family proteins have a transmembrane domain (TMD) that permits anchoring in the ER and outer mitochondrial membranes (the red asterisk indicates proteins without TMD).

Figure 2: Bax/Bak activation models

a) Canonical direct activation model: BH3-only proteins are divided into activators and sensitizers with different functions. BH3-only activators (such as Bim) can directly activate Bax/Bak, while sensitizers prevent Bax/Bak inhibition by antiapoptotic Bcl-2-family proteins. b) Indirect activation model: All BH3-only proteins act as sensitizers by preventing Bax/Bak inhibition by antiapoptotic Bcl-2-family proteins. Several lines of evidence and hypotheses have been reported to account for the activation of Bax/Bak without direct interaction with BH3-only proteins.

Figure 3: IP_3R structure, regions and domains: interaction sites for Bcl-2 and Bcl-xL

a) Schematic representation of a tetrameric IP_3R channel (two of the four IP_3R monomers are shown for easier representation). IP_3R is organized into three functional regions: the ligand-binding domain, the central, modulatory region and the C-terminal region. The C-terminal region contains transmembrane domains (TMDs) that form the channel pore. b) Linear representation of one IP_3R monomer. In addition to the three functional regions, the channel is divided into five stable proteolytic fragments. The numbers indicate the amino acids of each region/domain/fragment in mouse IP_3R1. The identified interaction sites for Bcl-2 and Bcl-xL are displayed.

Figure 4: Bcl-2/Bcl-xL three-dimensional superposition

In silico representations of Bcl-2 and Bcl-xL three-dimensional structures. The lysine residues involved in the interaction with IP_3Rs are indicated. The superposition of the proteins illustrates that K87 of Bcl-xL is constrained in a similar position as K17 of Bcl-2. Image taken from our previously published work [189]; this work is licensed under a Creative Commons Attribution 4. 0 International License.

Figure 5: Regulation of cell death and survival by Bcl-2-family proteins and Ca^{2+} signaling

Schematic overview highlighting the canonical and noncanonical regulation of cell survival (a) and cell death (b) by Bcl-2-family proteins. a) In healthy conditions, antiapoptotic Bcl-2-family members (Bcl-2, Bcl-xL, etc.) support mitochondrial bioenergetics and tightly control Ca^{2+} transfer at the ER–mitochondria interface through modulation of Ca^{2+} channels. b) Upon cell stress, the expression of proapoptotic Bcl-2-family members switches the balance of Bcl-2-family proteins from predominantly antiapoptotic to predominantly proapoptotic. The latter members trigger canonical apoptotic pathways (Fig. 3). ER stress-triggered mechanisms also sensitize IP_3 receptors and generate proapoptotic Ca^{2+} fluxes. See text for more information. ER: endoplasmic reticulum, MAM: mitochondria-associated membranes, IP_3R: inositol 1,4,5-trisphosphate receptor, VDAC: voltage-dependent anion channel, MCU: mitochondrial Ca^{2+} uniporter, mPTP: mitochondrial permeability transition pore.

Table 1: Overview of BH3 mimetics inhibiting antiapoptotic Bcl-2 family proteins and their use in clinical trials.

Information is up-to-date on March 1st, 2022. More details are available from https://www.clinicaltrials.gov/.

Inhibitor	Target	Clinical trials reported	Context of clinical trials
A-1155463	Bcl-xL	/	/
A-1210477	Mcl-1	/	/
A-1331852	Bcl-xL	/	/
ABT-199 (venetoclax)	Bcl-2	Phase I: 98 trials, phase I/II: 69 trials, phase II: 113 trials, phase II/III: 4 trials, phase III: 34 trials, phase IV: 2 trials	most types of hematologic cancers, breast carcinoma, chondrosarcoma, colorectal cancer, gastric cancer, lung carcinoma, neuroblastoma, pancreatic adenocarcinoma, prostate carcinoma
ABT-263 (navitoclax)	Bcl-2/ Bcl-xL/ Bcl-w	Phase I: 19 trials, phase I/II: 7 trials, phase II: 5 trials, phase III: 2 trials	acute lymphoblastic & myeloid leukemia, chronic lymphocytic leukemia, hepatocellular carcinoma, lung carcinoma, melanoma, myelofibrosis, non-Hodgkin's lymphoma, ovarian cancer, peripheral T-cell lymphoma, prostate cancer
ABT-737	Bcl-2/ Bcl-xL/ Bcl-w	/	/
AMG-176	Mcl-1	Phase I: 3 trials	acute myeloid leukemia, non-Hodgkin lymphoma, multiple myeloma, chronic myelomonocytic leukemia, myelodysplastic syndrome
APG-1252 (palcitoclax)	Bcl-2/ Bcl-xL	Phase I: 1 trials, phase I/II: 3 trials	non-Hodgkin lymphoma, myelofibrosis, neuroendocrine tumors, lung carcinoma
AZD0466	Bcl-2/ Bcl-xL	Phase I: 1 trial, phase I/II: 2 trials	non-hodgkin lymphoma, multiple myeloma, advanced solid tumors
AZD4320	Bcl-2/ Bcl-xL	/	/
AZD5991	Mcl-1	Phase I/II: 2 trials	acute myeloid leukaemia
BCL2-32	Bcl-2/ Bcl-xL	/	/
BM-1197	Bcl-2/ Bcl-xL	/	/
BTSA1	Bax	/	/

S44563	Bcl-2/ Bcl-xL	/	/
S55746	Bcl-2	/	/
S63845	Mcl-1	/	/
S64315 (MIK665)	Mcl-1	Phase I: 4 trials, phase I/II: 1 trial	acute myeloid leukaemia, multiple myeloma, non-Hodgkin lymphoma
S65487	Bcl-2	Phase I: 2 trials, phase I/II: 1 trial	acute myeloid leukaemia, chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin lymphoma
UMI-77	Mcl-1	/	/
WEHI-539	Bcl-xL	/	/

The authors report that no conflict of interests exist.

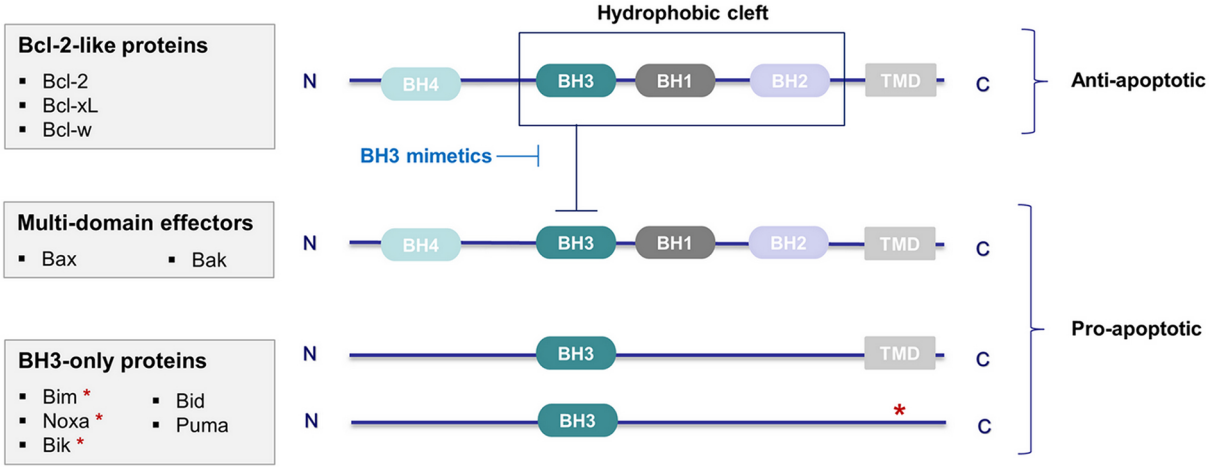
Bcl-2-like proteins
Bcl-2
Bcl-xL
Bcl-w
Multi-domain effectors
Bax
Bak
BH3-only proteins
Bim *
Noxa *
Bik *
Bid
Puma
Hydrophobic cleft
N
BH4
BH3
BH1
BH2
TMD
C
Anti-apoptotic
BH3 mimetics
Pro-apoptotic
*

Figure 1

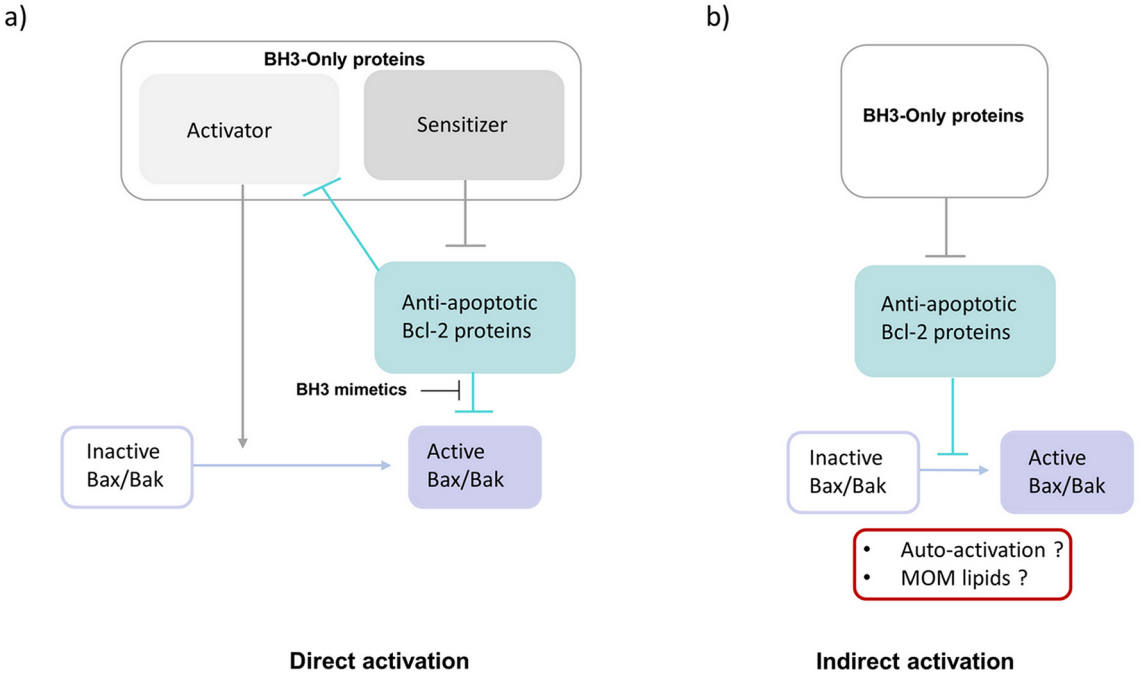
a)
BH3-Only proteins
Activator
Sensitizer
Anti-apoptotic
Bcl-2 proteins
BH3 mimetics
Inactive
Bax/Bak
Active
Bax/Bak
Direct activation
b)
BH3-Only proteins
Anti-apoptotic
Bcl-2 proteins
Inactive
Bax/Bak
Active
Bax/Bak
• Auto-activation ?
• MOM lipids ?
Indirect activation

Figure 2

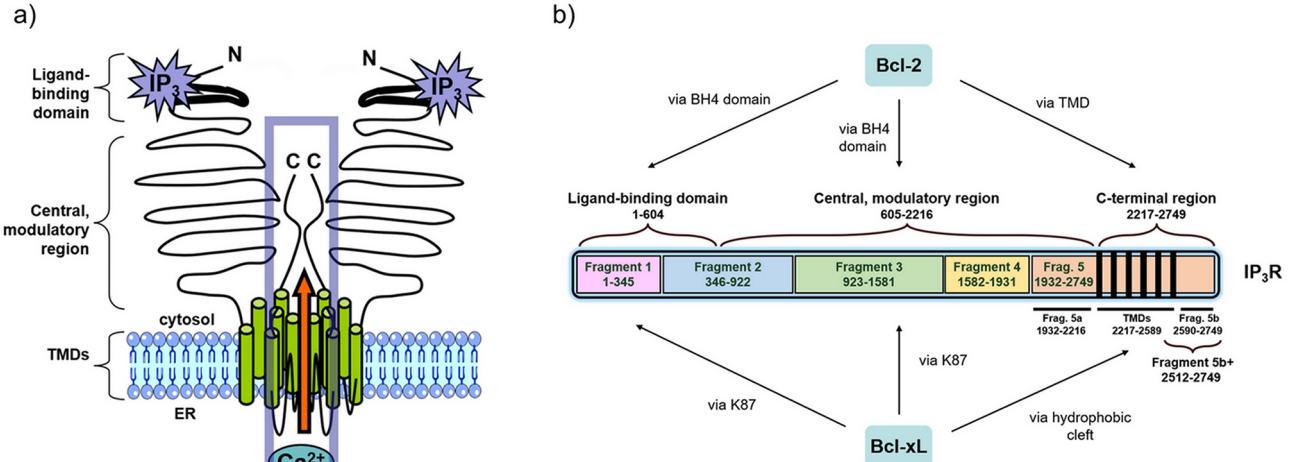
a)
Ligand-binding domain
N
N
IP3
IP3
C C
Central, modulatory region
cytosol
TMDs
ER
Ca2+
C-terminal region
b)
Bcl-2
via BH4 domain
via BH4 domain
via TMD
Ligand-binding domain
1-604
Central, modulatory region
605-2216
C-terminal region
2217-2749
Fragment 1
1-345
Fragment 2
346-922
Fragment 3
923-1581
Fragment 4
1582-1931
Frag. 5
1932-2749
IP3R
Frag. 5a
1932-2216
TMDs
2217-2589
Frag. 5b
2590-2749
Fragment 5b+
2512-2749
via K87
via K87
via hydrophobic cleft
Bcl-xL

Figure 3

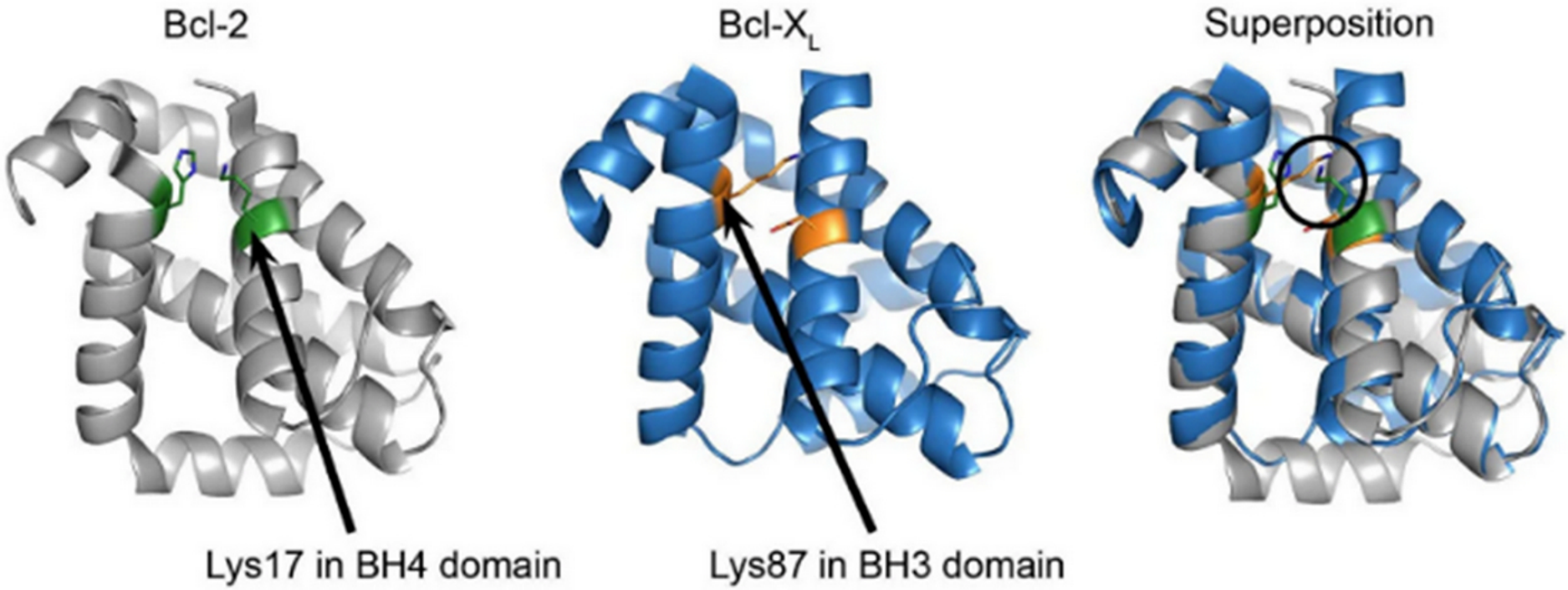
Bcl-2
Bcl-X$_L$
Superposition
Lys17 in BH4 domain
Lys87 in BH3 domain

Figure 4

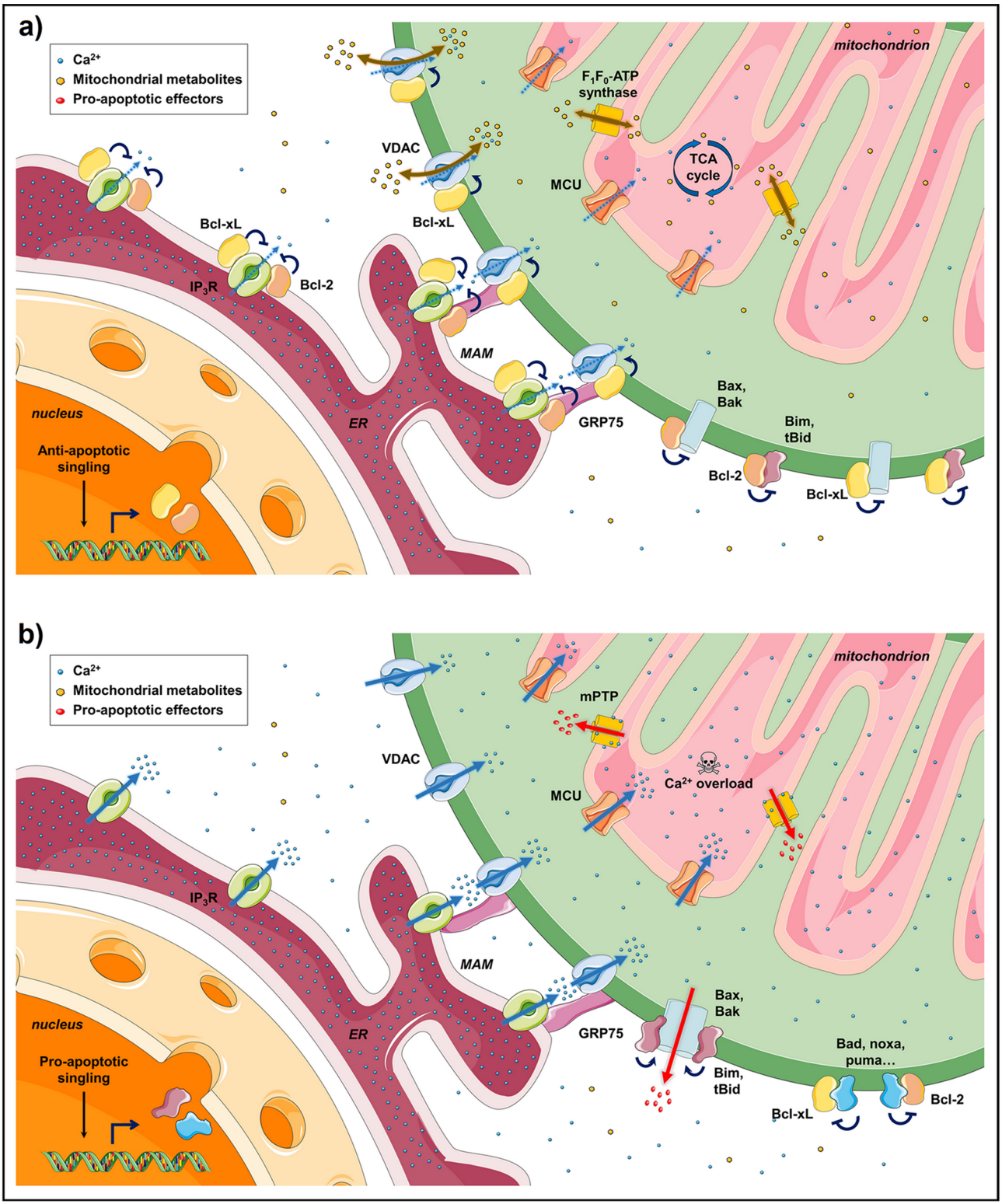
a)
Ca2+
Mitochondrial metabolites
Pro-apoptotic effectors
mitochondrion
F1F0-ATP synthase
VDAC
TCA cycle
MCU
Bcl-xL
Bcl-xL
IP3R
Bcl-2
MAM
nucleus
ER
GRP75
Bax, Bak
Bim, tBid
Bcl-2
Bcl-xL
Anti-apoptotic singling
b)
Ca2+
Mitochondrial metabolites
Pro-apoptotic effectors
mitochondrion
mPTP
VDAC
Ca2+ overload
MCU
IP3R
MAM
nucleus
ER
GRP75
Bax, Bak
Bad, noxa, puma…
Bim, tBid
Bcl-xL
Bcl-2
Pro-apoptotic singling

Figure 5