Modulation of Ca²⁺ Signaling by Anti-apoptotic B-Cell Lymphoma 2 Proteins at the Endoplasmic Reticulum–Mitochondrial Interface

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Mitochondria are important regulators of cell death and cell survival. Mitochondrial Ca²⁺ levels are critically involved in both of these processes. On the one hand, excessive mitochondrial Ca²⁺ leads to Ca²⁺-induced mitochondrial outer membrane permeabilization and thus apoptosis. On the other hand, mitochondria need Ca²⁺ in order to efficiently fuel the tricarboxylic acid cycle and maintain adequate mitochondrial bioenergetics. For obtaining this Ca²⁺, the mitochondria are largely dependent on close contact sites with the endoplasmic reticulum (ER), the so-called mitochondria-associated ER membranes. There, the inositol 1,4,5-trisphosphate receptors are responsible for the Ca²⁺ release from the ER. It comes as no surprise that this Ca²⁺ release from the ER and the subsequent Ca²⁺ uptake at the mitochondria are finely regulated. Cancer cells often modulate ER-Ca²⁺ transfer to the mitochondria in order to promote cell survival and to inhibit cell death. Important regulators of these Ca²⁺ signals and the onset of cancer are the B-cell lymphoma 2 (Bcl-2) family of proteins. An increasing number of reports highlight the ability of these Bcl-2-protein family members to finely regulate Ca²⁺ transfer from ER to mitochondria both in healthy cells and in cancer. In this review, we focus on recent insights into the dynamic regulation of ER–mitochondrial Ca²⁺ fluxes by Bcl-2 family members and how this impacts cell survival, cell death and mitochondrial energy production.

Keywords: endoplasmic reticulum–mitochondria contact sites, Ca²⁺-transport systems, apoptosis, autophagy, mitochondrial bio energetics, IP₃ receptors, voltage-dependent anion channels, Bcl-2

INTRODUCTION

Ca²⁺ signaling plays important roles in a vast amount of cell physiological processes (1). In cancer cells, Ca²⁺ signaling is altered to promote mitochondrial bioenergetics, cell proliferation, migration, and survival while inhibiting cell death (2–6). The involvement of Ca²⁺ signaling in the development of cancer and consequently the potential of Ca²⁺ signaling as a target for treatment is becoming increasingly apparent (5–11). In cancer cells, proteins involved in Ca²⁺ signaling have been reported to have differential expression profiles compared to healthy cells (12–15). In addition, an increasing number of proto-oncogenes and tumor suppressors impact Ca²⁺-signaling pathways by directly...
modulating intracellular Ca$^{2+}$-transport systems with critical functions in cell survival and cell death (16–19).

An important Ca$^{2+}$-signaling pathway involved in both cell death and cell survival is the transfer of Ca$^{2+}$ from the endoplasmic reticulum (ER) to the mitochondria (20). These Ca$^{2+}$ transfers occur at the so-called mitochondria-associated ER membranes (MAMs), which are close contact sites between the ER and the mitochondria (21). A continuous small Ca$^{2+}$ transfer to the mitochondria is necessary to maintain proper energy production (22). Ca$^{2+}$ is required by several enzymes of the tricarboxylic acid (TCA) cycle (like pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate) to promote NADH and ATP production (23). Besides this, Ca$^{2+}$ also modulates the ATP synthase complex V and the adenine nucleotide translocator (24). In addition to this mitochondrial pathway, pro-survival Ca$^{2+}$ oscillations activate calcineurin, which in turn dephosphorylates the nuclear factor of activated T-cells (NFAT), conferring its translocation into the nucleus (25). Here, NFAT triggers the transcription of genes involved in cell proliferation. In contrast, large Ca$^{2+}$ transfers from the ER to the mitochondria may result in both Ca$^{2+}$-induced mitochondrial outer membrane permeabilization (MOMP) and opening of the mitochondrial permeability transition pore (mPTP), the latter formed by dimers of the F0F1 ATP synthase (29) is an important intracellular Ca$^{2+}$-release channel involved in these Ca$^{2+}$ transfers, whereas at the mitochondria, the voltage-dependent anion channel (VDAC) (at the outer mitochondrial membrane) (30) and the mitochondrial Ca$^{2+}$ uniporter (MCU) (at the inner mitochondrial membrane) (31, 32) are important for transporting Ca$^{2+}$ into the mitochondrial matrix.

The B-cell lymphoma 2 (Bcl-2)-protein family, consisting of both anti- and pro-apoptotic members, is critically involved in regulating cell death and survival (33–36). Dysregulated expression and function of Bcl-2 proteins have been not only implicated in oncogenesis but also represent an “Achilles’ heel” in cancer cells. A recent study showed that Bcl-2 proteins have been not only implicated in oncogenesis but also represent an “Achilles’ heel” in cancer cells.

Anti-apoptotic Bcl-2 proteins are also known to regulate ER to mitochondrial Ca$^{2+}$ signaling at both organelles, and several Bcl-2-family members, including Bcl-2 and Bcl-XL, are present in the MAMs (45, 46) (Figure 1). At the ER, anti-apoptotic Bcl-2, Bcl-XL and Mcl-1 promote pro-survival IP$_3$-mediated Ca$^{2+}$ oscillations, enhancing cell proliferation and mitochondrial energy production (47–49). Bcl-2 (and Bcl-XL at high concentrations) also inhibits excessive pro-apoptotic IP$_3$-mediated Ca$^{2+}$ release (50–53), thereby preventing Ca$^{2+}$-induced MOMP. At the mitochondrial side of the MAMs, anti-apoptotic Bcl-2 and Bcl-XL proteins inhibit VDAC1-mediated Ca$^{2+}$ uptake in the mitochondria (45, 54, 55). However, also stimulatory roles of Bcl-2-family members on VDAC1-mediated mitochondrial Ca$^{2+}$ transfer have been described, thereby maintaining adequate mitochondrial Ca$^{2+}$ levels that promote survival and mitochondrial bioenergetics (56, 57). Besides IP$_3$,Rs and VDAC, anti-apoptotic Bcl-2-family members on VDAC1-mediated mitochondrial Ca$^{2+}$ transfer have been described, thereby maintaining adequate mitochondrial Ca$^{2+}$ levels that promote survival and mitochondrial bioenergetics.
members also regulate other members of the Ca^{2+} toolkit at different locations in the cell [extensively reviewed in Ref. (33)]. Mcl-1, located at the inner mitochondrial membrane, was also shown to be crucial for normal mitochondrial bioenergetics by regulating the assembly of the F_{0}F_{1} ATP synthase oligomers (58). Finally, the F_{0}F_{1} ATP synthase emerged as a target for anti-apoptotic Bcl-X_{l}, allowing the direct regulation of ATP production (59, 60). In this review, we will focus on recent insights into the dynamic regulation of ER–mitochondrial Ca^{2+} fluxes, the involvement of anti-apoptotic Bcl-2-family members and how this impacts cell survival, cell death, and mitochondrial energy production (Figure 2), three important aspects of cancer development.

**ER SIDE OF THE MAMs**

ER Ca^{2+} release is an important determinant for cell survival by regulating mitochondrial bioenergetics and for cell death via promoting mPTP opening. In most cells, including cancer cells, the IP_{3}-R is an important intracellular Ca^{2+}-release channel responsible for Ca^{2+} release from the ER. Cancer cells have developed several ways to modulate IP_{3}-R-mediated Ca^{2+} release, among which Bcl-2-dependent regulation.

**IP_{3}-R**

A continuous Ca^{2+} flux from the ER to the mitochondria is necessary in order to maintain normal energy production. At the ER, the IP_{3}-R is responsible for the Ca^{2+} release and is present at the MAMs (Figure 1). Inhibition of the IP_{3}-R and thus of the continuous Ca^{2+} transfer to the mitochondria was already shown to result in the induction of autophagy, thereby managing the decrease in mitochondrial energy production (22). New findings emerged, showing that cancer cells are addicted to constitutive IP_{3}-R-driven Ca^{2+} transfer to the mitochondria (61, 62). Similar to normal/non-tumorigenic cells, cancer cells increase their autophagic flux upon IP_{3}-R inhibition in order to cope with the loss of Ca^{2+} influx into the mitochondria and subsequent reduction in energy production. However, in normal cells, the increased autophagy is accompanied by a decrease in the proliferation rate at the G1/S checkpoint (63), addressing the decreased availability of mitochondrial substrates for biosynthetic pathways of nucleosides and other cellular building blocks. In this way, cells may survive until normal Ca^{2+} transfer to the mitochondria is restored. In cancer cells, this increase in autophagy is not accompanied by a reduction in cell proliferation, likely due to a loss of the link between the monitoring of the mitochondrial health...
and the G1/S checkpoint. As such, these malignant cells proceed through the cell cycle without the necessary pool of nucleosides, resulting in a mitotic catastrophe and necrotic cell death.

Anti-apoptotic Bcl-2-family members have been shown to regulate the IP$_3$R. Both inhibitory (50–52) and stimulatory (47, 49) effects, largely dependent on the Bcl-2-family member involved (64, 65) and the strength of IP$_3$R activation (25, 53), have been described. As such, it was reported that in T-cell models, Bcl-2 suppresses IP$_3$R-mediated Ca$^{2+}$ release generated by strong T-cell receptor stimulation, thereby preventing excessive Ca$^{2+}$ transfer into the mitochondria. This interaction occurs via Bcl-2’s BH4 domain and a stretch of 20 amino acids in the central coupling domain of the IP$_3$R (52, 64). Peptides derived from this amino acid stretch were able to disrupt IP$_3$R/Bcl-2 complexes in several cell types and models, thereby augmenting cell death in response to apoptotic triggers that act through Ca$^{2+}$ signaling (25, 66). The efficient IP$_3$R inhibition by anti-apoptotic Bcl-2 critically depended on the presence of Bcl-2’s C-terminal transmembrane domain, which interacted with the C-terminal domain of the IP$_3$R channel (67). Bcl-2 lacking its transmembrane domain failed to inhibit IP$_3$R-mediated Ca$^{2+}$ release and to suppress Ca$^{2+}$-dependent apoptosis in an in cellulo context. In contrast, the hydrophobic cleft of Bcl-2, responsible for scaffolding pro-apoptotic family members, was dispensable for IP$_3$R binding and inhibition.

Bcl-2, Bcl-X$_L$, and Mcl-1 were reported to sensitize the IP$_3$R to low levels of IP$_3$ in order to promote pro-survival Ca$^{2+}$ oscillations, thereby feeding Ca$^{2+}$ into the mitochondria to maintain adequate mitochondrial bioenergetics (47–49). The interaction of Bcl-2-family members with the C-terminus of the IP$_3$R has been proposed as the underlying molecular mechanism for generating these Ca$^{2+}$ oscillations (68). Recently, the mechanism underlying IP$_3$R sensitization by Bcl-X$_L$ has been identified with a prominent role for its hydrophobic cleft (53). Two BH3-like domains were identified in the C-terminus of IP$_3$Rs. Indeed, in contrast to Bcl-2, for which its hydrophobic cleft was shown to be dispensable for IP$_3$R modulation, Bcl-X$_L$ via its hydrophobic cleft could target, with different affinities, both BH3-like domains present in the C-terminal region of the IP$_3$R. At low concentrations, Bcl-X$_L$ increased the open probability of the IP$_3$R in response to low levels of IP$_3$ by simultaneous binding to both BH3-like domains. Similar to Bcl-2, high Bcl-X$_L$ concentrations were able to inhibit IP$_3$R-mediated Ca$^{2+}$ release in response to strong IP$_3$R stimulation. The "dual" interaction with the BH3-like domain which conferred the highest affinity toward Bcl-X$_L$ as well as the region in the coupling domain of the IP$_3$R targeted by Bcl-2’s BH4 domain, was important for IP$_3$R inhibition by Bcl-X$_L$. Binding of Bcl-X$_L$ to the coupling domain appeared with much lower affinity than the binding to the C-terminal tail, which is in line with our previous study that focused on the binding efficiency of Bcl-2 versus Bcl-X$_L$ for both IP$_3$R domains (48). This may indicate that moderate levels of Bcl-X$_L$ will most likely operate in IP$_3$R-sensitizing modus and thus will promote Ca$^{2+}$ oscillations, whereas high levels of Bcl-X$_L$ will be needed to operate in IP$_3$R-inhibiting modus. Finally, binding of Bcl-X$_L$ to both BH3-like domains is involved in maintaining cell viability and in protecting cells from stress inducers. These molecular results substantiate the previously observed sensitization of the IP$_3$R by Bcl-X$_L$ (68), resulting in pro-survival Ca$^{2+}$ oscillations, and underscore the importance of this interaction for cell viability.

The role of Bcl-X$_L$ in modulating IP$_3$R-mediated Ca$^{2+}$ release in order to promote mitochondrial bioenergetics was recently further highlighted (69). The authors showed that Bcl-X$_L$ interacts with IP$_3$R3 at the MAMs, where it increased Ca$^{2+}$ transfer into the mitochondria, thereby enhancing TCA cycling. Upon ER-stress induction, Bcl-X$_L$ translocated more to the MAMs, where the subsequent facilitation of Ca$^{2+}$ transfer to the mitochondria and thus increased energy production helped the cells cope with the induced ER stress. This further highlights that Bcl-X$_L$ exerts its protective effects against stress inducers in large part via modulating Ca$^{2+}$ signaling.

**MITOCHONDRIAL SIDE OF THE MAMs**

Cancer cells are highly dependent on the mitochondria for their energy production. For sustaining this energy production, adequate control of mitochondrial Ca$^{2+}$ levels is important. Anti-apoptotic Bcl-2 proteins are known regulators of this mitochondrial Ca$^{2+}$ influx, thereby regulating mitochondrial bioenergetics. In addition, the F$_{0}$F$_{1}$ ATP synthase has also been identified as a target for anti-apoptotic Bcl-2-family members, thereby directly linking them to the production of ATP (58–60).

**VDAC**

The large conductance channel VDAC, of which three isoforms are known to exist, is located at the outer mitochondrial membranes (30). At the MAMs, VDAC is physically linked to the IP$_3$R via molecular tethers like the chaperone protein, glucose-regulated protein 75, allowing efficient Ca$^{2+}$ transfer from the ER into the mitochondria (70). Close regulation of mitochondrial Ca$^{2+}$ uptake via VDAC is critical for maintaining mitochondrial energy production. Anti-apoptotic Bcl-2-family members are known to modulate this mitochondrial Ca$^{2+}$ transfer through interactions with VDAC. Both Bcl-2 and Bcl-X$_L$ have been reported to inhibit VDAC1-mediated Ca$^{2+}$ uptake into the mitochondria, thereby protecting cells from Ca$^{2+}$-induced MOMP (45, 54, 55, 71). The BH4 domain of Bcl-X$_L$, but not the one of Bcl-2, was sufficient to bind to VDAC1 and to directly inhibit VDAC1 single-channel activity (45). Although different regions of Bcl-2 and Bcl-X$_L$ seem to be involved in this interaction, both anti-apoptotic proteins target the N-terminus of VDAC1. Introducing VDAC1’s N-terminal into cells was shown to inhibit both Bcl-2’s and Bcl-X$_L$’s anti-apoptotic function, illustrating that VDAC1 could be a target for anti-cancer drugs (54, 71–73). However, at the level of the BH4 domains, the N-terminal peptide of VDAC1 could only counteract the inhibitory action of Bcl-X$_L$’s, but not that of Bcl-2’s BH4 domain. The BH4 domain of Bcl-2 also suppressed agonist-induced mitochondrial Ca$^{2+}$ uptake and staurosporine-induced cell death, but acted through inhibition of IP$_3$Rs, since IP$_3$R-derived peptides were able to alleviate the inhibitory effects of Bcl-2’s, but not those of Bcl-X$_L$’s BH4 domain (45).

Although the interaction of Bcl-X$_L$ with VDAC1 is well established, the impact of Bcl-X$_L$ on VDAC1’s functional properties

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may be dichotomous. Besides inhibiting VDAC1 (45, 54), Bcl-Xi has been reported to enhance VDAC1 activity. Bcl-Xi knockout MEF cells displayed a reduced VDAC1-mediated Ca\(^{2+}\) uptake in the mitochondria compared with the wild-type MEF cells (56). Similarly, N-terminal peptides derived from VDAC1 that disrupt Bcl-Xi binding to VDAC1 could also antagonize mitochondrial Ca\(^{2+}\) uptake in wild-type MEF cells, while these peptides lacked any effect in Bcl-Xi-deficient MEF cells. While differences in experimental conditions may underlie the seemingly contrasting observations, these results indicate that Bcl-Xi might have a dual impact on VDAC1’s Ca\(^{2+}\)-flux properties dependent on VDAC1’s function as a pro-survival or pro-death protein. Hence, Bcl-Xi could stimulate basal pro-survival and inhibit excessive pro-apoptotic VDAC1-mediated mitochondrial Ca\(^{2+}\) transfer, thereby fine-tuning mitochondrial Ca\(^{2+}\) handling according to cellular needs, with respect to cell fate decisions. The molecular basis for these opposite effects of Bcl-Xi on VDAC1 remains poorly understood.

Mcl-1 has also been shown to positively regulate VDAC in non-small cell lung carcinoma cells (57). In these cancer cells, Mcl-1 interacted with VDAC, with a pronounced role for its N-terminus, thereby increasing mitochondrial Ca\(^{2+}\) uptake, resulting in increased ROS production and cell migration. Disrupting the Mcl-1/VDAC interaction utilizing N-terminal VDAC-derived peptides could inhibit ROS production and cell migration. The importance of Mcl-1 at the mitochondria was further underscored by a recent study concerning different Mcl-1 splice variants (74). In this study, the increased expression of the short pro-apoptotic Mcl-1 isoform resulted in increased mitochondrial fusion via a reduced Mcl-1-dependent recruitment of dynamin-related protein 1 to the mitochondria. This was accompanied by hyperpolarization of the mitochondrial potential and increased mitochondrial Ca\(^{2+}\) uptake, thereby increasing susceptibility to apoptotic stimuli. Whether this increase in mitochondrial Ca\(^{2+}\) uptake was also mediated through the interaction with VDAC was not evaluated. Nevertheless, it would be interesting to assess whether the short pro-apoptotic Mcl-1 isoform would shift VDAC-mediated mitochondrial Ca\(^{2+}\) uptake toward more pro-apoptotic levels in comparison to the long pro-survival Mcl-1 isoform.

**F\(_{0}\)F\(_{1}\) ATP Synthase**

In cultured hippocampal neurons, Bcl-Xi was shown to be present at the inner mitochondrial membranes, where it directly targets the \(\beta\)-subunit of the F\(_{0}\)F\(_{1}\) ATP synthase (59, 60). The interaction stabilized the mitochondrial membrane potential via the closure of a membrane leak pathway. This increased the enzymatic activity of the F\(_{0}\)F\(_{1}\) ATP synthase, thereby promoting ATP production during neural activity. In addition, the interaction seems to occur via Bcl-Xi’s hydrophobic cleft, since ABT-737 could reverse the effects of Bcl-Xi on the F\(_{0}\)F\(_{1}\) ATP synthase. Recently, this process was further explored and was shown to be important for neuronal survival (75). In response to excitotoxic stimuli, cyclin B1 and cyclin-dependent kinase 1 (Cdk1) accumulated in the mitochondria. There, the cyclin B1-Cdk1 complex phosphorylated Bcl-Xi, leading to its dissociation from the ATP-synthase. This led to decreased ATP synthesis and production of ROS species, resulting in the inhibition of respiratory chain complex I, mitochondrial dysfunction, and potentially neuronal death.

**POTENTIAL THERAPEUTIC OPPORTUNITIES**

**Promoting ER–Mitochondrial Ca\(^{2+}\) Transfer**

Many chemotherapeutics trigger intracellular Ca\(^{2+}\) release from the ER, causing, or at least contributing to, mitochondrial Ca\(^{2+}\) overload. This Ca\(^{2+}\) release is often considered as a nonspecific side effect of the drug, but in many cases, it contributes to obtain maximal therapeutic effects (76). Moreover, recent studies have unraveled the molecular mechanisms underlying the impact of chemotherapeutics and photodynamic therapy on intracellular Ca\(^{2+}\) homeostasis (18, 77, 78). These anti-cancer regimens caused the accumulation of the tumor suppressor p53 at the ER membranes, where it enhanced sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) 2b activity. The effects were independent of the transcriptional roles of p53. Recruitment of p53 at the ER increased the Ca\(^{2+}\) filling state of the ER stores, increasing the susceptibility to apoptotic stimuli and the likelihood for mitochondrial Ca\(^{2+}\) overload. Cells deficient in p53 did not display this effect and were resistant to chemotherapy. This resistance could be overcome by SERCA and/or MCU overexpression.

**Inhibiting ER–Mitochondrial Ca\(^{2+}\) Transfer**

The therapeutic potential of dampening Ca\(^{2+}\) transfer from ER to mitochondria has recently been proposed as an anti-cancer strategy (9, 61). It was shown that dysregulation of the ER to mitochondrial Ca\(^{2+}\) transfer via inhibition of IP\(_3\)Rs results in the induction of autophagy in both cancer and normal cells. However, when this increase in autophagy is not accompanied by a halt in proliferation, the cancer cells will die mainly through necrosis. This could prove to be a very specific way of eliminating cancer cells by effectively turning the increased proliferative capacity of cancer cells against themselves, whereas healthy cells can cope with this loss of Ca\(^{2+}\) transfer to the mitochondria. A major challenge will be to selectively target the Ca\(^{2+}\) transfer into the mitochondria without affecting global Ca\(^{2+}\) signaling and to mainly limit the effect of IP\(_3\)-inhibiting drugs to the malignant cells.

**Antagonizing Anti-apoptotic Bcl-2 Proteins**

Major efforts have been dedicated towards the development of BH3-mimetic drugs, which target the hydrophobic cleft of anti-apoptotic Bcl-2-family members. The first generation of BH3 mimetics (ABT-737 and ABT-263) inhibited both Bcl-2 and Bcl-Xi, resulting in severe side effects related to thrombocytopenia due to the dependence of thrombocytes on Bcl-Xi for their survival (41, 79). More recently, a Bcl-2-selective BH3-mimetic inhibitor was developed, namely ABT-199/venetoclax, which is a very promising anti-cancer drug that has been approved for the treatment of chronic lymphocytic leukemia (80). Whether these BH3-mimetic drugs also influence the ability of anti-apoptotic Bcl-2-family members to modulate intracellular Ca\(^{2+}\) release is less well understood, although some recent studies aimed to...
address this. With the identification of the two BH3-like domains at the C-terminus of the IP3R, the ABT-737 compound was shown to disrupt the binding of Bcl-XL to the C-terminus of the IP3R, thereby abolishing both the stimulatory and inhibitory effects of Bcl-XL on IP3R-mediated Ca2+ release (53). However, the contribution of Ca2+ signaling to ABT-737-induced cell death requires further investigation, since ABT-737 could cause cell death in primary chronic lymphocytic leukemia cells without inducing elevations in intracellular [Ca2+](81).

Besides a direct impact on IP3R/Bcl-XL complexes, ABT-737 has also been proposed to modulate the sensitivity of cancer cells to chemotherapy via a mechanism that involves remodeling of ER–mitochondrial contact sites (82). As such, cisplatin-resistant ovarian cancer cells could be re-sensitized to cisplatin by ABT-737. This drug increased ER–mitochondrial contact sites, thereby increasing cisplatin-induced elevations in mitochondrial Ca2+. When co-applied with cisplatin in cholangiocarcinoma cells, ABT-737 has been shown to induce mitochondrial fragmentation and mitophagy, resulting in cell death, whereas cisplatin alone induced mitochondrial hyperfusion, potentially underlying cell-death resistance (83). The combined ABT-737/cisplatin treatment led to a decreased Mcl-1 and an increased Bax expression. Interestingly, Mcl-1 has recently been shown to be implicated in controlling mitochondrial dynamics (74).

Consistent with the lack of contribution of Bcl-2’s hydrophobic cleft to the interaction with and regulation of IP3R, IP3R/Bcl-2-protein complexes and IP3R inhibition by Bcl-2 were resistant to ABT199/venetoclax treatment (67). Acute addition of ABT199/venetoclax to a variety of permeabilized and intact cell systems did neither trigger Ca2+ release by itself nor directly affected ER-located Ca2+ -uptake and -release systems. Related to this, ABT199/venetoclax-induced apoptosis in Bcl-2-dependent cancer cells appeared to occur independently of intracellular Ca2+ overload (67, 84). However, the inhibition of Bcl-2 by BH3-mimetics has been reported to result in a rapid impairment of mitochondrial oxidative phosphorylation (85). This may underlie the increased sensitivity of Bcl-2-dependent cancer cells to ABT199/venetoclax in the presence of the intracellular Ca2+ buffer, BA-PAM (67, 84).

Over the years, it has become clear that Bcl-2 inhibition via targeting its BH4 domain has potential as an effective anti-cancer treatment (38, 86–88). Targeting Bcl-2/IP3R complex with Bcl-2/IP3 receptor disrupter-2 (BIRD-2), a stabilized TAT-linked peptide containing the 20 amino acids that represent the Bcl-2 interaction motif of IP3Rs, triggers intracellular Ca2+ overload and apoptotic cell death in a variety of cancer cell models, including chronic lymphocytic leukemia (81), diffuse large B-cell lymphoma (89), multiple myeloma, follicular lymphoma (90), and small-cell lung carcinoma (91). The cell death could be suppressed by buffering intracellular Ca2+ and by inhibiting IP3R activity (81, 89). Very recently, a small molecule (BDA-366) that targets the BH4 domain of Bcl-2 has been developed and shown to be effective in lung cancers and multiple myeloma (92, 93). The mechanism involved a conformational switch in Bcl-2 that turned it from a pro-survival to a pro-death protein by exposing its BH3 domain. A decrease in Bcl-2 phosphorylation may contribute to this pro-apoptotic switch induced by BDA-366. BDA-366 also impaired IP3R/Bcl-2 complex formation and raised cytosolic Ca2+ levels, although further work is needed to determine the contribution of Ca2+ signaling to BDA-366-induced cell death in cancer cells.

Mcl-1 gene amplifications are frequently found in many types of cancer (94). Very recently, an Mcl-1 inhibitor (S63845) targeting Mcl-1’s hydrophobic cleft has been developed (95). This compound was shown to be very specific for Mcl-1, well-tolerated by animal models and efficient at triggering cell death in Mcl-1-dependent tumor cells. As the regulation of VDAC1 by Mcl-1 also stimulates cancer cell migration (57), Mcl-1 inhibitors may not only be useful to eliminate Mcl-1-dependent cancers by provoking cell death but also by countering metastasis. However, at this point, it is not clear whether these Mcl-1 inhibitors can disrupt VDAC1/Mcl-1 complex formation.

**CONCLUSIONS**

Ca2+ transfer from ER to mitochondria is important for maintaining proper energy production and balance between cell survival and cell death. The anti-apoptotic Bcl-2-family members regulate these Ca2+ transfers at the level of the ER as well as of the mitochondria by directly targeting Ca2+-transport systems located at the ER and mitochondria. Moreover, the molecular determinants underlying the complex formation between the Bcl-2 proteins and these systems are emerging as a hot topic, which allows the development of strategies and tools to interfere with the Bcl-2 protein-mediated control of Ca2+-signaling. These mechanisms also appear to be exploited by cancer cells to promote survival and mitochondrial bioenergetics, to contribute to cell-death resistance and control metastasis. Thus, targeting the Ca2+-modulating abilities of Bcl-2 proteins may offer novel anti-cancer strategies. In addition to this, Ca2+ signaling might contribute to the cell-death properties of recently developed Bcl-2 inhibitors, including BH3-mimetics and BH4-domain antagonists.

**AUTHOR CONTRIBUTIONS**

TV and GB drafted the manuscript. All authors critically read, amended, and/or corrected the manuscript. TV made the figures.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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