Modulation of Ca^{2+}-signaling by anti-apoptotic Bcl-2 proteins at the ER-mitochondrial interface

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Abstract

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

Introduction

Ca^{2+} signaling plays important roles in a vast amount of cell physiological processes [1]. In cancer cells, Ca^{2+} signaling is altered in order to promote mitochondrial bioenergetics, cell proliferation, migration and survival whilst inhibiting cell death [2-6]. The involvement of Ca^{2+} signaling in the development of cancer and consequently the potential of Ca^{2+} signaling as a target for treatment, is becoming increasingly apparent [5-11]. In cancer cells, proteins involved in Ca^{2+} 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 Ca^{2+}-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 (MAM), which are close contact sites between the ER and the mitochondria [21]. A continuous small Ca^{2+} transfer to the mitochondria is necessary in order to maintain proper energy production [22]. This Ca^{2+} is required by several enzymes (like pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate) of the tricarboxylic acid (TCA), promoting NADH and ATP production [23]. Besides this, Ca2+ also modulated 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 is formed by dimers of the F_0F_1 ATP synthase [4,26,27]. In this process, Ca^{2+} overload in the mitochondria triggers cardiolipin oxidation, resulting in the disassembly of the respiratory chain complex 2 (also known as succinate Dehydrogenase) subsequently leading to excessive reactive oxygen species (ROS) production [28]. Mitochondrial produced ROS can open the mPTP, ultimately leading to MOMP. At the level of the ER, the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) [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 implicated in oncogenesis, but also represent an "Achilles heel" in cancer cells that can be exploited by the use of Bcl-2 inhibitors [37-39]. Anti-apoptotic Bcl-2 proteins (like Bcl-2, Bcl-X_L and Mcl-1) have been extensively described to inhibit apoptosis by binding to the Bcl-2 homology (BH) 3 domains of the pro-apoptotic Bcl-2-family members (like Bax, Bak, Bim, Bid…) via their hydrophobic cleft formed by the BH1, BH2 and BH3 domains, thereby inhibiting cell death [40]. A recently developed class of compounds, so-called BH3-mimetic drugs, [40-42] are able to compete with pro-apoptotic Bcl-2-family members for the hydrophobic cleft of the anti-apoptotic Bcl-2-family members. Hence, BH3-mimetics alleviate the inhibition of Bax and Bak by the anti-

apoptotic Bcl-2-family members, effectively killing cancer cells that are dependent on anti-apoptotic Bcl-2 proteins for their survival. In addition to this, the BH4 domain of Bcl-2 also contributes to the interaction with Bax via a site that is distinct from Bax's BH3-domain [43]. Moreover, the isolated BH4 domain, delivered as a stapled peptide, neutralized the pro-apoptotic activity of Bim-derived BH3 pptides by restricting Bax conformational change[44].

Besides this, 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-X_L, are present in the MAMs [45,46] (Fig. 1). At the ER, anti-apoptotic Bcl-2, Bcl-X_L and Mcl-1 promote pro-survival IP_3R-mediated Ca^{2+} oscillations, enhancing cell proliferation and mitochondrial energy production [47-49]. Bcl-2 (and Bcl-X_L at high concentrations) also inhibits excessive pro-apoptotic IP_3R-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-X_L 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_3Rs and VDAC, anti-apoptotic Bcl-2-family members also regulate other members of the Ca^{2+} toolkit at different locations in the cell (extensively reviewed in [33]). Mcl-1 located at the inner mitochondrial membranes was also shown to be crucial for normal mitochondrial bioenergetics by regulating the assembly of the F_1F_0-ATP synthase oligomers [58]. Finally, the F_0F_1 ATP synthase also emerged as a target for anti-apoptotic Bcl-X_L allowing the direct regulation of ATP production via Bcl-2-family members [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 (Fig. 2); three important aspects of cancer development.

ER side of the MAMs

ER Ca^{2+} release is an important determinant for both cell survival by regulating mitochondrial bioenergetics and for cell death via promoting mPTP opening. In most cells, including cancer cells, the IP_3R is an important intracellular Ca^{2+}-release channel responsible for this Ca^{2+} release from the ER. Cancer cells have developed several ways to modulate IP_3R-mediated Ca^{2+} release, of which Bcl-2-dependent regulation is one.

IP_3Rs

A continuous Ca^{2+} flow from the ER to the mitochondria is necessary in order to maintain normal energy production. At the ER, the IP_3R is responsible for this Ca^{2+} release and is therefore present at the MAMs (Fig. 1). Inhibition of the IP_3R and thus of this 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_3R-driven Ca^{2+} transfer to the mitochondria [61,62]. Similarly to normal/non-tumorigenic cells, cancer cells increase their autophagic flux upon IP_3R 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, this increase in 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_3R. 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_3R activation [25,53], have been described. As such, it was reported that in T-cell models, Bcl-2 can suppress IP_3R-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_3R [52,64]. Peptides derived from this amino acid stretch were able to disrupt IP_3R/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].

In contrast, Bcl-2, Bcl-X_L and Mcl-1 also sensitize the IP_3R to low levels of IP_3 in order to promote pro-survival Ca^{2+} oscillations, thereby feeding Ca^{2+} into the mitochondria to maintain normal mitochondrial bioenergetics [47-49]. The C-terminus of the IP_3R and the hydrophobic cleft of the Bcl-2 proteins have been proposed as important molecular determinants for generating these Ca^{2+} oscillations [67]. Recently, this was further explored and elucidated for Bcl-X_L [53]. Indeed, two BH3-like domains were identified in the C-terminal region of the IP_3R. Bcl-X_L bound to these BH3-like domains via its hydrophobic cleft, apparently with different affinities. Functionally, using nuclear patch clamp approaches, simultaneous binding of Bcl-X_L proteins to both BH3-like domains was shown to increase the open probability of the IP_3R in response to low levels of IP_3. Similarly to Bcl-2, high Bcl-X_L concentrations were also able to inhibit IP_3R-mediated Ca^{2+} release in response to strong IP_3R stimulation. Important for this inhibition were the interaction with both the BH3-like domain conferring the highest affinity towards Bcl-X_L as well as the region in the coupling domain of the IP_3R targeted by Bcl-2's BH4 domain. Binding of Bcl-X_L to the coupling domain appeared with much lower affinity than the binding to the C-terminal tail. Also, compared to Bcl-2, Bcl-X_L binding to this domain was much less prominent, which is consistent with previous findings from our group [48]. This may indicate that moderate levels of Bcl-X_L will most likely operate in IP_3R-sensitizing modus and thus will promote Ca^{2+} oscillations, while high levels of Bcl-X_L will be needed to operate in IP_3R-inhibiting modus. Finally, binding of Bcl-X_L proteins 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_3R by Bcl-X_L [67], 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_3R-mediated Ca^{2+} release in order to promote mitochondrial bioenergetics was recently further highlighted [68]. The authors showed that Bcl-X_L interacts with IP_3R3 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 depend 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_0F_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 is located at the outer mitochondrial membranes [30]. At the MAMs, VDAC is physically linked to the IP_3R via molecular tethers like the chaperone protein glucose-regulated protein 75, allowing efficient Ca^{2+} transfer from the ER into the mitochondria [69]. 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 [45,54,55,70]. For the BH4 domain of Bcl-X_L, but not Bcl-2, was sufficient to bind to VDAC1 and to directly inhibit VDAC1 single-channel activity [45]. Although different regions of Bcl-2, Bcl-X_L as well VDAC-1 seem to be involved in this interaction, both anti-apoptotic proteins have been reported to 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 protection against apoptosis, illustrating that VDAC1 could be a target for anti-cancer drugs [54,70-72]. However, at the level of the BH4 domains, the N-terminal peptide of VDAC1 could only counteract the inhibitory action of Bcl-X_L, but not that of Bcl-2's BH4 domain. The BH4 domain of Bcl-2 also suppressed agonist-induced mitochondrial Ca^{2+} uptake and staurosporin-induced cell death, but acted through inhibition of IP_3Rs, since IP_3R-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 may be dichotomous. Besides inhibiting VDAC1 [45,54], Bcl-X_L has been reported to enhance VDAC1 activity. Bcl-X_L knockout MEF cells displayed a reduced VDAC1-mediated Ca^{2+} uptake in the mitochondria compared to the wild-type MEF cells [56]. Similarly, N-terminal peptides derived from VDAC1 that disrupt Bcl-X_L binding to VDAC1 could also antagonize mitochondrial Ca^{2+} uptake in wild-type MEF cells, while these peptides lacked any effect in Bcl-X_L-deficient MEF cells. While differences in experimental conditions may underlie the seemingly contrasting observations, these results may indicate that Bcl-X_L can 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-X_L 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-X_L on VDAC1 remains poorly understood.

Mcl-1 has 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 [73]. In this study, increasing the 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 the 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 towards more pro-apoptotic levels in comparison to the long pro-survival Mcl-1 isoform.

F_0F_1 ATP synthase

In cultured hippocampal neurons, Bcl-X_L was shown to be present at the inner mitochondrial membranes, where it directly targets the β-subunit of the F_0F_1 ATP synthase [59,60]. This interaction stabilized the mitochondrial membrane potential via the closure of a membrane leak pathway. This increased the enzymatic activity of the F_0F_1 ATP synthase, thereby promoting ATP production during neural activity. In addition, the interaction seems to occur via Bcl-X_L's hydrophobic cleft, since ABT-737 could reverse the effects of Bcl-X_L on the F_0F_1 ATP synthase. Recently, this process was further explored and was shown to be important for neuronal survival [74]. In response to excitotoxic stimuli, cyclin B1 and cyclin dependent kinase 1 (CdK1) accumulated in the mitochondria. There, the cyclin B1-Cdk1 complex phosphorylates Bcl-X_L, leading to its dissociation from the ATP-synthase. This leads to decreased ATP synthesis and the 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 non-specific side effect of the drug, but in many cases it contributes to obtain maximal therapeutic effects [75]. Moreover, recent studies have unraveled the molecular mechanisms underlying the impact of chemotherapeutics and photodynamic therapy on intracellular Ca^{2+} homeostasis [18,76,77]. These anti-cancer regimens caused the accumulation of the tumor suppressor p53 at the ER membranes, where it enhanced SERCA2b activity. These effects were independent of the transcriptional roles of p53. Recruitment of p53 at the ER augmented 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 modulating intracellular Ca^{2+} signaling, more specifically the Ca^{2+} transfer from ER to mitochondria, in cancer is nicely illustrated by recent work of the group of Dr. Foskett [9,61]. They showed that inhibition of ER to mitochondrial Ca^{2+} transfer via IP_3Rs in cancer cells, like in normal cells, results in the induction of autophagy. However, when this increase in autophagy is not accompanied by a halt in proliferation, these 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 here will be to specifically target the Ca^{2+} transfer into the mitochondria.

Antagonizing anti-apoptotic Bcl-2 proteins

A lot of effort is being made in developing BH3-mimetic drugs targeting 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-X_L, resulting in severe side effects related to thrombocytopenia due to the dependence of thrombocytes on Bcl-X_L for their survival [41,78]. 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 [79]. 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 studies reported this more recently. With the identification of the two BH3-like domains at the C-terminus of the IP_3R, the ABT-737 compound was shown to disrupt the binding of Bcl-X_L to the C-terminus of the IP_3R thereby abolishing both the stimulatory and inhibitory effects of Bcl-X_L on IP_3R-mediated Ca^{2+} release [53]. However, the contribution of Ca^{2+} 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 [Ca^{2+}] [80].

Besides a direct impact on IP_3R/Bcl-X_L 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 [81]. 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 Ca^{2+}. Besides increased ER-mitochondrial contact sites, ABT737, when co-applied with cisplatin in cholangiocarcinoma cells, has been shown to induce mitochondrial fragmentation and mitophagy, resulting in cell death, while cisplatin alone induced mitochondrial hyperfusion, potentially underlying cell death resistance [82]. The combined ABT737/cisplatin treatment led to a decrease in Mcl-1 and an increase in Bax. Interestingly, Mcl-1 has recently been shown to be implicated in controlling mitochondrial dynamics [73].

In contrast to Bcl-X_L, the hydrophobic cleft of Bcl-2 appeared dispensable for IP_3R modulation [83]. Hydrophobic cleft Bcl-2 mutants that failed to bind Bax/Bak remained capable to interact with and inhibit the IP_3R. In contrast, efficient IP_3R inhibition by anti-apoptotic Bcl-2 critically depended on the presence of Bcl-2's transmembrane domain. Bcl-2 lacking its C-terminal transmembrane domain failed to inhibit IP_3R-mediated Ca^{2+} release and to suppress Ca^{2+}-dependent apoptosis in an *in cellulo* context. Consistent with this, IP_3R/Bcl-2-protein complexes and IP_3R inhibition by Bcl-2 were resistant to ABT199/venetoclax treatment. Also, acute addition of ABT199/venetoclax to a variety of permeabilized and intact cell systems did neither trigger Ca^{2+} release by itself nor directly affected ER-located Ca^{2+}-uptake and –release systems. Related to this, ABT199/venetoclax-induced apoptosis in Bcl-2-dependent cancer cells appeared to occur independently of intracellular Ca^{2+} overload [83,84] although, in same instances, inhibition of Bcl-2 by BH3 mimetics resulted in a rapid impairment of mitochondrial oxidative phosphorylation [85]. Notably, over the years, it has also 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 via the BH4 domain using Bcl-2/IP_3 receptor Disrupter-2 (BIRD-2), a stabilized TAT-linked peptide containing the 20 amino acids that represent the Bcl-2 interaction motif of IP_3Rs, triggers intracellular Ca^{2+} overload and apoptotic cell death in a variety of cancer cell models, including chronic lymphocytic leukemia [80], diffuse large B-cell lymphoma [89], multiple myeloma, follicular lymphoma [90] and small cell lung carcinoma [91]. This cell death could be suppressed by buffering intracellular Ca^{2+} and by inhibiting IP_3R activity [80,89]. Very recently, a small molecule (BDA-366) that targets the BH4 domain of Bcl-2, which was effective in lung cancers and multiple myeloma, has been developed [92,93]. The mechanism involved a conformational switch in Bcl-2 that turned it from a pro-survival protein 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, too impaired IP_3R/Bcl-2 complex formation and raised cytosolic Ca^{2+} levels, although further work is needed to determine the contribution of Ca^{2+} 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, a 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 counteracting metastasis. However, at this point, it is not clear whether these Mcl-1 inhibitors can disrupt VDAC1/Mcl-1 complex formation.

Conclusions

Ca^{2+} transfer from ER to mitochondria is important for maintaining proper energy production, cell survival and cell death. The anti-apoptotic Bcl-2-family members regulate these Ca^{2+} transfers at the level of the ER as well as of the mitochondria by directly targeting ER and mitochondrially located Ca^{2+}-transport systems. Moreover, the molecular determinants underlying complex formation with these systems start to emerge, allowing to develop

strategies and tools to interfere with the Ca^{2+}-signaling control functions of these Bcl-2 proteins. 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 Ca^{2+}-modulating abilities of these proteins may offer novel anti-cancer strategies. In addition to this, Ca^{2+} signaling might contribute to the cell death properties of recently developed Bcl-2 inhibitors, including BH3 mimetics and BH4-domain antagonists.

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Figure legend

Figure. 1. Bcl-2 and Bcl-X_L and their targets in Ca^{2+} signaling, IP_3R and VDAC1, are present in the MAMs. Representative immunoblots showing the presence of VDAC1, IP_3Rs, Bcl-2, and Bcl-X_L in the MAMs of MEFs. Calnexin (CNX) and cytochrome c (Cyt c) served as specific MAMs and mitochondrial markers, respectively. This research was originally published in Journal of Biological Chemistry with following reference: Monaco G, Decrock E, Arbel N, van Vliet AR, La Rovere RM, De Smedt H, Parys JB, Agostinis P, Leybaert L, Shoshan-Barmatz V, Bultynck G. The BH4 domain of anti-apoptotic Bcl-X_L, 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. Journal of Biological Chemistry; 290(14):9150-61. © the American Society for Biochemistry and Molecular Biology. The original results have been produced by Dr. Alex van Vliet in the laboratory of Prof. Patrizia Agostinis (KU Leuven, Belgium).

Figure. 2. Modulation of ER to mitochondrial Ca^{2+} transfers by anti-apoptotic Bcl-2 proteins. ER to mitochondrial Ca^{2+} transfers are critical for the regulation of cell death and cell survival decisions. In order to fuel the TCA cycle, a continuous influx of Ca^{2+} into the mitochondria is required (green arrow), thereby promoting cell survival. Excessive mitochondrial Ca^{2+} uptake leads to Ca^{2+}-induced MOMP and cell death (red arrow). The anti-apoptotic side of the Bcl-2-protein family regulates these Ca^{2+} transfers at both organelles. During pro-survival Ca^{2+} signaling at the ER, Bcl-2, Bcl-X_L and Mcl-1 modulate IP_3R-mediated Ca^{2+} release to generate Ca^{2+} oscillations. At the mitochondria Bcl-X_L and Mcl-1 can increase VDAC1-mediated Ca^{2+} uptake. Combining the effects at the two organelles results in an efficient and finely regulated Ca^{2+} uptake at the mitochondria which increases mitochondrial bioenergetics and promotes cell survival. In addition, Mcl-1 and Bcl-X_L target the F_0F_1 ATP synthase, thereby regulating ATP-production. During pro-death signaling, Bcl-2 and Bcl-X_L can inhibit both pro-apoptotic Ca^{2+} release from the IP_3R and the Ca^{2+} uptake into the mitochondria via VDAC. Finally, abolishing ER to mitochondrial Ca^{2+} transfers by either blocking IP_3Rs or knocking down the MCU induces autophagy. When this is coupled to decreased cell proliferation (healthy cells) this increase in autophagy may rescue the cell. However, when proliferation is not halted (cancer cells) this results in cell death.

Figure 1

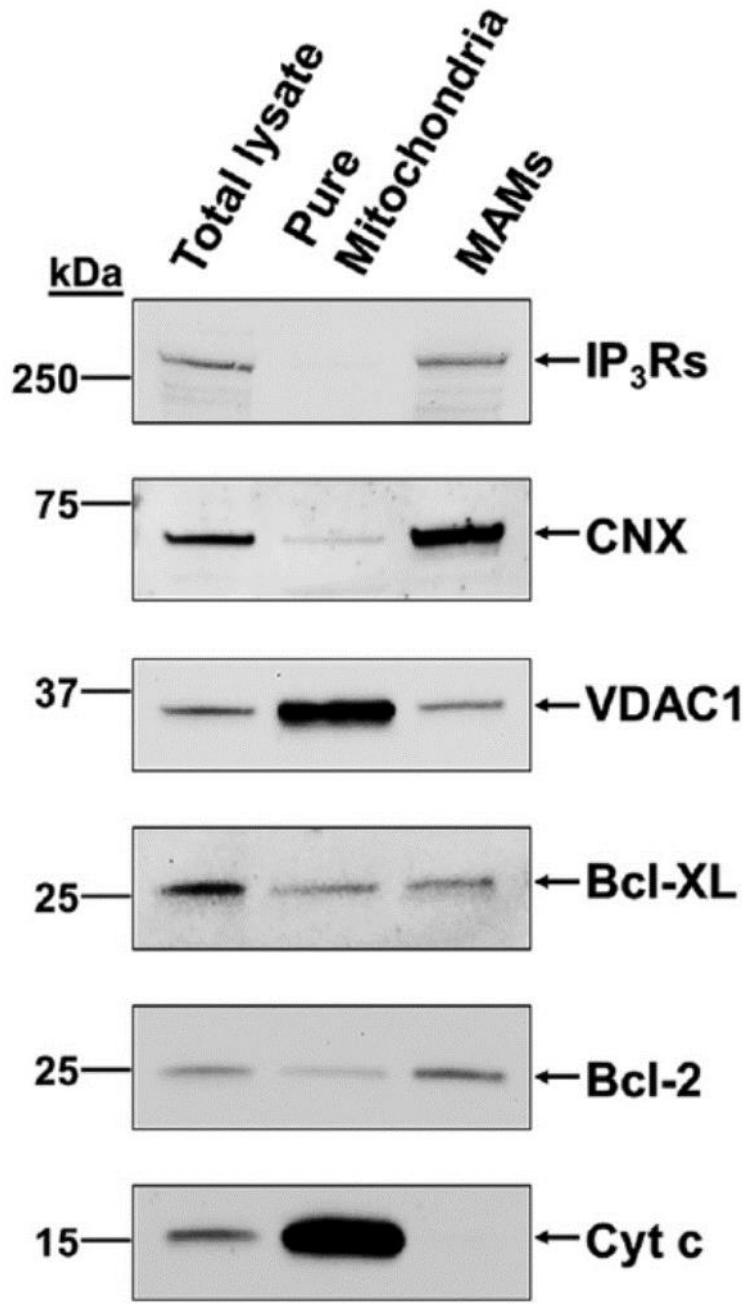
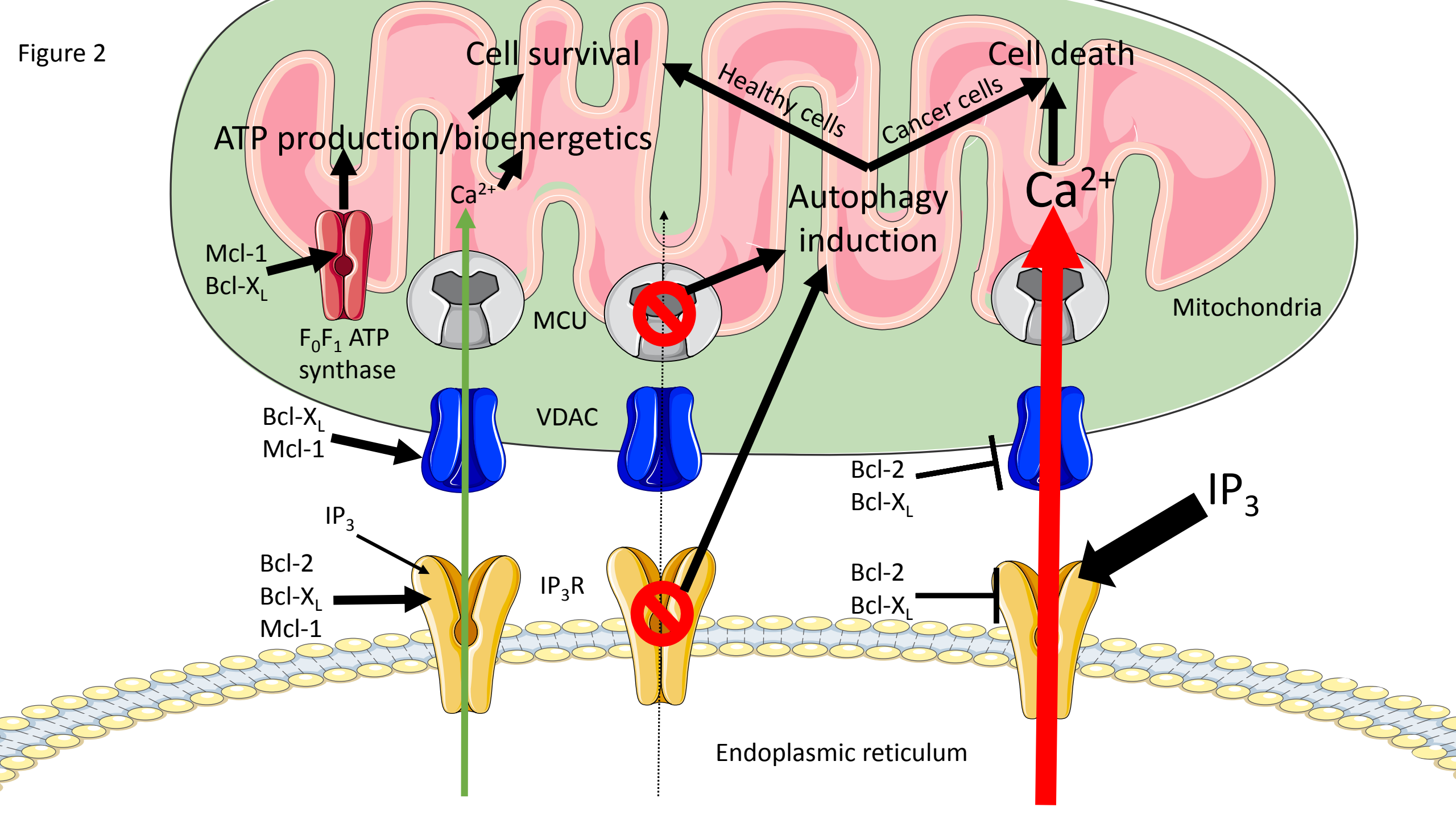

Total lysate
Pure Mitochondria
MAMs
kDa
250
IP3Rs
75
CNX
37
VDAC1
25
Bcl-XL
25
Bcl-2
15
Cyt c

Figure 2


Cell survival
Cell death
Healthy cells
Cancer cells
ATP production/bioenergetics
Ca2+
Autophagy induction
Ca2+
Mcl-1
Bcl-XL
F0F1 ATP synthase
MCU
Mitochondria
Bcl-XL
Mcl-1
VDAC
Bcl-2
Bcl-XL
IP3
IP3
Bcl-2
Bcl-XL
Mcl-1
IP3R
Bcl-2
Bcl-XL
Endoplasmic reticulum