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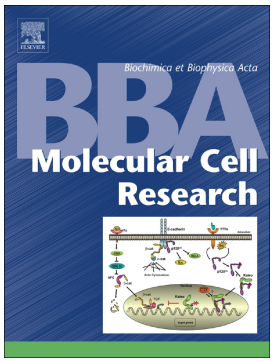

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The role of Bcl-2 proteins in modulating neuronal Ca^{2+} signaling in health and in Alzheimer's disease

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Abstract

The family of B-cell lymphoma-2 (Bcl-2) proteins exerts key functions in cellular health. Bcl-2 primarily acts in mitochondria where it controls the initiation of apoptosis. However, during the last decades, it has become clear that this family of proteins is also involved in controlling Ca^{2+} signaling in cells, a critical process for the function of most cell types, including neurons. Several anti- and pro-apoptotic Bcl-2 family members are expressed in neurons and impact neuronal function. Importantly, expression levels of neuronal Bcl-2 proteins are affected by age. In this review, we focus on the emerging roles of Bcl-2 proteins in neuronal cells. Specifically, we discuss how their dysregulation contributes to the onset, development, and progression of neurodegeneration in the context of Alzheimer's disease (AD). Aberrant Ca^{2+} signaling plays an important role in the pathogenesis of AD, and we propose that dysregulation of the Bcl-2-Ca^{2+} signaling axis may contribute to the progression of AD and that herein, Bcl-2 may constitute a potential therapeutic target for the treatment of AD.

Keywords: Bcl-2, calcium, neurons, mitochondria, apoptosis, Alzheimer disease

1. Neuronal Ca^{2+} signaling: an overview

In this section we provide a brief overview of neuronal Ca^{2+} signaling systems relevant for this review; for a more extensive description a recent review by Bootman and Bultynck is highly recommended [1]. Ca^{2+} is the most important second messenger in neurons and converts incoming signals into activation of effector enzymes that regulate key aspects of neuronal function. The main conductors of Ca^{2+} in neurons include voltage-gated Ca^{2+} channels (VGCC), ligand-operated ion channels and store-operated calcium entry channels (SOCE) (Fig 1). SOCE channels are activated by depletion of the endoplasmic reticulum (ER), the main intracellular Ca^{2+} store, which is detected by STIM1/2, both intraluminal Ca^{2+} sensor proteins (Fig 1). Metabotropic glutamate receptors (mGluR) mobilize Ca^{2+} from the ER into the cytoplasm by activating inositol 1,4,5-trisphosphate receptors (IP_3Rs), the major ER-localized Ca^{2+} release channels (Fig 1). Ca^{2+} can also be released from the ER via ryanodine receptors (RyRs), which are activated by Ca^{2+} itself via Ca^{2+}-induced Ca^{2+} release, allowing to amplify cytosolic Ca^{2+} signals. Besides the ER, other intracellular Ca^{2+} stores include the Golgi apparatus, the nuclear envelope and lysosomes. A low concentration of Ca^{2+} in the cytoplasm of neurons is maintained due to the presence of these intracellular stores in which Ca^{2+} is sequestered via activity of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) [2]. Mitochondria and peroxisomes are involved in Ca^{2+} signaling as well but are not considered to be constitutive Ca^{2+} stores. To control Ca^{2+} levels, neurons utilize different mechanisms including Na^{+}/Ca^{2+} exchanger (NCE), plasma membrane Ca^{2+} pumps and Ca^{2+}- buffering proteins like calbindin–D28, calretinin and parvalbumin in the cytosol and calreticulin and calnexin in the ER [3] (Fig 1).

Mitochondria have a special role in neurons acting not only as the main source of ATP necessary to maintain the electrochemical gradients and membrane excitability, but also to provide additional Ca^{2+} buffering capacity and to participate in many Ca^{2+}-mediated signaling processes [4]. The close proximity of organelles and Ca^{2+} stores allows for direct communication via membrane contact sites (Loncke J et al, *Trends Cell Biol*, 2021, accepted for publication [5]). In general, membrane contact sites are enriched with chaperones that stabilize the close apposition of the two lipid bilayers [6]. The membrane contact sites between ER and mitochondria are termed mitochondria-associated ER membranes (MAMs). MAMs are dynamic structures that provide crosstalk between the ER and mitochondria and are necessary to maintain the bioenergetic balance in cells. It was demonstrated that the voltage-dependent anion channel (VDAC) 1 (VDAC1) on the outer mitochondrial membrane is physically linked with the IP_3R through glucose-regulated protein 75 (GRP75), thus facilitating the transfer of IP_3R-mediated Ca^{2+} signals towards the mitochondria and allowing “quasi-synaptic” Ca^{2+} flux from ER to mitochondria [7]. IP_3R presence and function at the MAMs is sustained by accessory proteins such as IRE1α [8] and translocase of the outer membrane 70 (TOM70) [9], thereby supporting proper mitochondrial metabolism. A recent review of Loncke et al. further illustrates the impact and function of IP_3Rs at MAMs [10]. Impairing IP_3R function limits mitochondrial bioenergetics, thereby augmenting the AMP/ATP ratio. This activates AMP-activated protein kinase and subsequently initiates autophagy [11].

For Ca^{2+} to enter the mitochondrial matrix, it has to pass two membranes. First, the mitochondrial outer membrane where VDAC1 is responsible for Ca^{2+} transport from the cytosol to the intermitochondrial membrane space (Fig 1). Second, the mitochondrial inner membrane where the mitochondrial calcium uniporter (MCU) complex is responsible for Ca^{2+} transport

from the intermitochondrial membrane space to the mitochondrial matrix [12] (Fig 1). The intimate connection between Ca^{2+} signaling and mitochondrial metabolism is due to the close apposition of ER and the mitochondria. Additionally, mitochondria have a high driving force for Ca^{2+} accumulation due to the negative mitochondrial potential (-180 mv).

A key aspect of Ca^{2+} signaling in neurons is its involvement in the mechanisms of synaptic transmission and synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) are a facilitation or attenuation in synaptic transmission between two neurons which persists for a long time after the termination of the stimulus and these are considered to be the cellular mechanisms of learning and memory formation. Both LTP and LTD trigger complex post-synaptic Ca^{2+} signaling pathways enabling continual changes in synaptic strength. Typically, LTP requires initial phosphorylation and subsequent autophosphorylation of CaMKII, while calcineurin initiates dephosphorylation events which often lead to LTD. [13]. Long-lasting changes in the synaptic activity require transcriptional responses, which are also driven by Ca^{2+} signals and specifically propagate to the nucleus in order to maintain the synthesis of the proteins involved in neuroplasticity. The most studied Ca^{2+}-dependent transcription factor is a cAMP-responsive element-binding protein (CREB) that mediates the conversion of short-term memory to long-term memory (Fig 1). Massive Ca^{2+} influx through N-methyl-D-aspartate receptor (NMDAR) during LTP induces activation of Ca^{2+}-dependent kinases like CaMKII and subsequently induces phosphorylation of CREB, which in turn is required for activity-induced Ca^{2+}-dependent gene transcription [14]. Another transcription factor called nuclear factor of activated T cells (NFAT) is also regulated by Ca^{2+} and calcineurin in neurons (Fig 2). NFAT proteins are phosphorylated and reside in the cytoplasm of resting cells. Upon stimulation, they are dephosphorylated by calcineurin, translocate to the nucleus, and become transcriptionally

active, thus providing a direct link between intracellular Ca^{2+} signaling and gene expression in neurons [15].

The mechanisms of Ca^{2+} regulation are especially important in synapses where the processes of synaptic transmission and synaptic plasticity take place. The postsynaptic structures of the excitatory synapses, called dendritic spines, provide compartmentalization of Ca^{2+} signals to local microdomains. Morphology of the spines is tightly coupled with synaptic transmission and strongly correlates with ongoing neuronal activity. The structure and function of spines are regulated by Ca^{2+} activated proteins such as CAMKII and calcineurin (Fig 1). Recent studies also highlight the importance of intracellular Ca^{2+} stores in formation and maintenance of dendritic spines. Although dendritic spines contain internal Ca^{2+} stores and several plasma membrane located Ca^{2+} channels including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), NMDAR and VGCC, they utilize less potent Ca^{2+}-extrusion mechanisms and have lower endogenous Ca^{2+}-buffer capacity when compared to soma and dendrites [16]. Mitochondria play an important role in synaptic function, providing synapses with ATP for neurotransmitter synthesis and release, as well as buffering Ca^{2+} levels [17]. Synaptic mitochondria are considered to be more vulnerable. It has been shown that non-synaptic mitochondria are capable of accumulating higher amounts of exogenously added Ca^{2+} compared to synaptic mitochondria before undergoing mitochondrial permeability transition pore (mPTP) opening [18].

The role of Ca^{2+} as a second messenger in neurons is difficult to overestimate since the most important functions of neurons, such as control of excitability, synaptic transmission, synaptic plasticity, changes in gene expression and the activation of survival and programmed cell death pathways are regulated by Ca^{2+}. Not surprisingly, Ca^{2+} levels in neurons are tightly

controlled. Even small imbalances in Ca^{2+} handling in neurons can disrupt the delicate mechanisms of Ca^{2+} regulation and ultimately lead to cell death [19]. Importantly, many of these pathways become impaired with age and the relation between brain aging and changes in cellular Ca^{2+} homeostasis is well known [20]. This connection may provide a link with age-related neurodegeneration since similar processes can occur in neuronal cells with the development of neurodegenerative diseases [21–23], as discussed below in more detail for Alzheimer's disease (AD).

2. Structure and function of the Bcl-2 family

Members of the B-cell lymphoma-2 (Bcl-2) protein family critically control cell death and survival processes by regulating mitochondrial outer membrane permeabilization (MOMP) [24–26]. Bcl-2-family members can be classified into three main groups based on their structure and the presence of Bcl-2-homology (BH) domains. The latter being highly conserved α-helical motifs. The group of anti-apoptotic Bcl-2-family members (i.e. Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, Bfl-1, and Bcl-10) is characterized by the presence of four BH domains arranged, from N- to C-terminus, BH4, BH3, BH1, and BH2 (Fig 2). On the other hand, the pro-apoptotic multidomain Bcl-2-family members (i.e. Bax, Bak, and Bok) possess at least three BH domains. Lastly, the BH3-only proteins only contain a single BH3 domain, which is necessary for their pro-apoptotic function [27,28]. BH3-only proteins can be further subdivided into direct activators of Bax and Bak (i.e. Bid and Bim), and sensitizers (i.e. Bad, Bik, Noxa, Puma, Hrk, Bmf). These sensitizers are unable to activate Bax and Bak, but bind to anti-apoptotic Bcl-2 proteins and consequently neutralize them [24,29].

Most forms of apoptosis in vertebrates occur via the intrinsic mitochondrial pathway rather than through receptors of cell death (TNF and Fas receptors) [30]. In this pathway, apoptosis is initiated after the release of apoptogenic proteins from the intermembrane space of mitochondria into the cell cytoplasm. The key event in the mitochondrial apoptosis pathway is an increase in the permeability of the outer mitochondrial membrane [31,32]. The apoptotic Bcl-2 proteins, Bax and Bak, play a significant role in increasing MOMP. The activation and oligomerization of these proteins trigger the onset of apoptosis by forming proteinaceous pores in the mitochondrial outer membrane, resulting in MOMP [33]. This process is responsible for the release of cytochrome c and Smac/Diablo into the cytosol, triggering the formation of the apoptosome and subsequent activation of caspases. While in general MOMP results in apoptotic cell death, low levels of MOMP can actually promote cell transformation and tumorigenesis through cell damage elicited by the sublethal activity of caspases (so-called 'minority MOMP') [25,34]. Under normal circumstances, Bak is associated with the mitochondrial outer membrane, whereas Bax resides mostly in the cytosol and is inserted into the mitochondrial outer membrane upon apoptosis induction [27,35–37]. The relevant interaction between the activator BH3-only proteins and Bax/Bak in cells occurs at and within the intracellular membranes [33]. The anti-apoptotic Bcl-2-family members contain a hydrophobic cleft, formed by their BH3, BH1, and BH2 domains, that allows the scaffolding of the BH3 domain of pro-apoptotic members. The formation of such complexes neutralizes Bax/Bak and BH3-only proteins, thereby preventing their pro-apoptotic functions [27]. Recently, Bax/Bak-inhibiting molecules have been developed such as MSN-125 and MSN-150, which can effectively protect cells against pro-apoptotic stimuli [38]. On the other hand, BH3 mimetics drugs, a promising class of precision anti-cancer medicines, have been

developed to occupy the hydrophobic groove of the anti-apoptotic Bcl-2 proteins, thereby releasing pro-apoptotic Bcl-2-family members and thus stimulating apoptosis induction [28,39] (Fig. 2). More recently, several BH3 mimetic antagonists that selectively antagonize distinct Bcl-2-family members, including Bcl-2, Bcl-X_L and Mcl-1, have emerged [40].

Finally, besides the hydrophobic cleft, Bcl-2's BH4 domain is also critical for its anti-apoptotic function. At the mechanistic level, the BH4 domain of Bcl-2 can directly target Bax, preventing its conformational activation and its pore-formation properties through non-canonical interaction sites [41]. Additionally, Bcl-2 deletion mutants lacking the BH4 domain (Bcl-2ΔBH4) fail to scaffold Bax and thereby even become pro-apoptotic [42,43]. Besides a potential role of Bcl-2's BH4 domain in controlling Bax activity, the BH4 domain of anti-apoptotic Bcl-2 proteins has mainly emerged as a key determinant to control intracellular Ca^{2+} signaling [44,45]. More recently, this BH4 domain also appears an attractive target in anti-cancer strategies, yet on-target small molecule BH4-domain antagonists ought to emerge [46–48].

3. Role of Bcl-2 family members in Ca^{2+} signaling

The first association between Bcl-2 and intracellular Ca^{2+} was described in 1993 by Baffy et al. [49]. Later on, multiple studies revealed a profound impact of Bcl-2-family proteins on intracellular Ca^{2+} signaling [50–52]. We here focus on the importance of Bcl-2 in mediating mitochondrial and ER Ca^{2+} signaling (Fig 3A).

3.1 Bcl-2-family members and mitochondrial Ca^{2+} handling

VDAC is located in the mitochondrial outer membrane and is permeable to mitochondrial metabolites and ions, including Ca^{2+} [53]. Up until now, three isoforms of VDAC have been

identified in higher eukaryotes: VDAC1, VDAC2, and VDAC3, with VDAC1 being the most abundant isoform [54]. Several studies have highlighted the pivotal role of VDAC1 in mitochondria-mediated apoptosis, as silencing of VDAC1 prevented apoptosis [55,56], whereas overexpression induced apoptotic cell death [53,57–60]. VDAC1 is responsible for the transfer of pro-apoptotic Ca^{2+} signals towards the mitochondria, in part due to its ability to form complexes with IP_3Rs located at the MAMs [61]. Moreover, VDAC1 serves as a mitochondrial permeation path that mediates the cross-talk between mitochondria and the rest of the cell, e.g. as an exit pathway for ATP from the intermitochondrial membrane space and the cytosol [62]. VDAC1 channels interact with several pro-survival proteins including hexokinase-I and Bcl-2-family members, thereby promoting cell survival and preventing apoptosis [63]. Particularly, the N-terminal region of VDAC1 is an interaction hub for these proteins [64,65]. These insights have been exploited to elicit cell death in different cancer types using a variety of VDAC1-derived peptides [66]. Also, different anti-apoptotic Bcl-2-family members, Bcl-2, Bcl-X_L, and Mcl-1, interact with VDAC1 impacting its channel conductance [53,67]. Bcl-2 and Bcl-X_L have been implicated in inhibiting VDAC1 by targeting its N-terminal region, thereby rendering cells more resistant to Ca^{2+}-driven apoptosis. The inhibition of VDAC1 by Bcl-X_L involved its BH4 domain, which by itself is sufficient to inhibit VDAC1 single-channel activity and VDAC1-mediated mitochondrial Ca^{2+} uptake [64]. However, besides an inhibitory effect, Bcl-X_L (and also Mcl-1) have been reported to augment VDAC1 activity, thereby boosting mitochondrial metabolism and cancer cell proliferation [68,69]. The binding of Bax to VDAC1 on the other hand evokes cytochrome c release [67,70]. In addition to this, VDAC2 has emerged as an important partner for the mitochondrial import of Bak [71,72] and Bax during apoptosis induction [73]. Besides its interaction with VDAC, the Bcl-2-family proteins display

other effects at the mitochondrial level. For example, overexpression of Bcl-2 in mice revealed a significant reduction in the activity of the mitochondrial Na^+/Ca^{2+} exchanger [74]. Additionally, different Bcl-2-family members (i.e. Bak, Bax, Bcl-X_L, and Bok) are able to modulate mitochondrial morphology and dynamics by targeting different proteins responsible for mitochondrial fission and fusion [75–78]. Bcl-X_L is able to control the mitochondrial respiratory capacity and ATP production, and Bcl-X_L knockout (KO) results in increased oxidative stress [79]. Finally, Bcl-X_L has been shown to interact directly with the β-subunit of the F_1F_0 ATP synthase causing an increased transport of H^+ during F_1F_0 ATPase activity [80].

3.2 Bcl-2-family members and ER Ca^{2+} handling

Bcl-2-family members do not only affect the mitochondrial Ca^{2+} handling but also control intracellular Ca^{2+} dynamics at the level of the ER. Bcl-2 directly binds to and inhibits the IP_3R, the major Ca^{2+} release channel and an important player in the crosstalk between the ER and mitochondria. This Bcl-2-IP_3R interaction seems crucial for the inhibition of the IP_3R-mediated Ca^{2+} release from the ER by Bcl-2, and thereby Ca^{2+}-mediated apoptosis is prevented [46,81]. The domain responsible for this interaction is the BH4 domain of Bcl-2, which binds to two regions in the IP_3R: i. a sequence of 20 amino acids in the central modulatory domain [81]; and ii. the ligand-binding region near the N-terminal part of the IP_3R channel [82]. Moreover, the BH4-domain by itself is sufficient to bind to and inhibit IP_3Rs [26,44,83]. However, relatively high concentrations of BH4-domain peptides are needed to impact the IP_3R function [83]. *In cellulo*, the relatively low affinity of the BH4 domain for IP_3Rs is alleviated by recruiting Bcl-2 in close proximity of IP_3Rs through Bcl-2's transmembrane domain that targets the C-terminal region of the channel [84]. Interestingly, Bcl-2's binding to IP_3Rs appears to occur

independently of its hydrophobic cleft that is responsible for scaffolding pro-apoptotic Bcl-2-family members [84].

Other Bcl-2-family members containing a BH4 domain motif too, may interact with these IP_3Rs. This has been shown for Nrz/NrH, another anti-apoptotic Bcl-2-family member, as well as for Bcl-w [85–87]. However, the BH4 domain of Bcl-X_L, which closely resembles Bcl-2, appears not to be able to target and control IP_3R function. At the molecular level, Lys17 in Bcl-2's BH4 domain was identified as a residue that was critical for its binding to IP_3Rs and inhibition of the channel. In the BH4-domain of Bcl-X_L, this Lys residue was not present and was replaced by an Asp residue. This may underlie the reported differences between Bcl-2 and Bcl-X_L concerning the ability of the BH4 domain of Bcl-2 *versus* the BH4 domain of Bcl-X_L to inhibit IP_3Rs [88]. Besides the IP_3R, there is another major Ca^{2+} release channel located in the ER, namely the RyR [89]. The central domain of the RyR contains a stretch of amino acids that displays a strong similarity with the binding site of Bcl-2 in the central domain of the IP_3R. Bcl-2 via its BH4 domain can bind to this central domain of RyR channels, thereby enabling RyR/Bcl-2-complex formation and suppressing RyR-mediated Ca^{2+} release. However, the Lys17 residue of the BH4-domain was not critical for RyR binding and modulation by Bcl-2. Consistent with this, both Bcl-2 and Bcl-X_L proteins bind to RyRs and inhibit RyR-mediated Ca^{2+} release [90].

3.3 Subcellular localization of Bcl-2-family members

It is clear that many of the Bcl-2-family members are localized at the mitochondria where these proteins have several functions such as regulating MOMP [24], mitochondrial Ca^{2+} uptake [63] and energy production [80]. In addition, several anti-apoptotic Bcl-2 family members also

localize to the ER where they modulate ER Ca^{2+} release (Bcl-2, Bcl-X_L and Mcl-1) [91–93] and the structural organization of the ER (Mcl-1) [94]. Pro-apoptotic Bcl-2-family members have also been shown to localize to the ER, thereby regulating ER Ca^{2+} levels (Bax/Bak) [52] or the stability of the IP_3R (Bok) [95]. Besides the ER and mitochondria, Bcl-2-protein family members can also localize in the cytosol or at other compartments such as the nuclear outer membrane, the nucleus, peroxisomes and the Golgi apparatus. As an in-dept discussion of this is beyond the scope of this review, we would like to direct the reader to a recent review of Popgeorgiev et al. dedicated to this topic [96].

4. Functions of Bcl-2-family members in neurons

A large number of Bcl-2-family members, both pro- and anti-apoptotic, are expressed in the central nervous system (CNS) [97]. The pro-apoptotic protein Bok is expressed in the cerebral cortex and hippocampus, whereas Bax is more widely expressed in the brain [98,99]. The anti-apoptotic Bcl-2-family members, Bcl-X_L and Bcl-w are present in mature neurons, whereas Bcl-2 is mostly expressed in the developing brain [100–102]. In the following part, we will briefly discuss the known functions of pro-and anti-apoptotic Bcl-2 family members in neurons and refer the reader to Pemberton et al. for an extensive discussion [103].

4.1 Functions of pro-apoptotic Bcl-2-family members in neurons

Bax is one of the major proteins promoting cell death in the developing CNS. More than half of the neurons in the developing CNS die via the apoptotic pathway regulated by Bax [104]. The main proteins responsible for Bax activation in neurons are the BH3-only activators Bid, Bim, and Puma. For instance, single deletion of Puma prevents both neuronal apoptosis [103,105,106] and axon degeneration [107] in *vitro* and in a variety of neuronal cell types from

both the central and peripheral nervous system. This suggests that BH3-only activators play a significant role in regulating neuronal MOMP [103]. The central role of Bax in neuronal apoptosis is illustrated by the observation that Bax KO mice do not exhibit developmental programmed cell death of dorsal root ganglion sensory neurons, superior cervical ganglion sympathetic neurons, or motoneurons (MNs) [108–110]. Multiple studies have indicated that the deletion of the *Bax* gene protects neurons against excitotoxic apoptosis [97,111,112]. Moreover, the Ca^{2+} transients during NMDA excitation were reduced in Bax-deficient neurons, and this effect seemed independent of the role of Bax in apoptosis [113]. Besides regulating neuronal cell death, Bax suppresses neurogenesis in the hippocampus and the cerebellum of adult brains [104,114].

Neuronal death in Bak KO mice was found to be more complex as it was either inhibited or enhanced depending on the developmental stage, death stimulus or neuronal subtype [115]. Only Bax/Bak double KO mice demonstrated an increase in neuroprogenitor cells in the periventricular zone of the brain, whereas single KO of Bax (or Bak), did not contribute to an increase in the survival of neuroprogenitor cells in the brain of mice. This suggests a redundancy for Bax and Bak in these progenitor cells, which is not the case in postmitotic differentiating neurons which only require Bax for apoptosis induction [116,117].

Thus, Bak and Bax can contribute to both survival and death of neurons. How Bak and Bax participate in these processes strongly depends on the stage of development, stress stimuli, and the neuronal type. However, it should be noted that despite the almost canonical belief that apoptosis is necessary to ensure proper development, mice lacking both Bax and Bak can successfully develop [116]. Moreover, early stages of embryogenesis occur without any defects despite the loss of both key apoptotic molecules [116]. These data suggest that the

induction of Bax/Bak-dependent apoptosis may not be critical for successful embryogenesis to occur.

4.2 Functions of anti-apoptotic Bcl-2-family members in neurons

Similar data were obtained when studying the effects of some anti-apoptotic members of the Bcl-2 family on the process of embryogenesis. Bcl-2-deficient mice embryos did not exhibit any significant neuronal development disturbances or abnormal neuronal apoptosis [118,119]. Nevertheless, severe defects of Bcl-2 loss are observed in postnatal animals, which may indicate a more important role in post-natal development than during early neurogenesis [119,120]. High levels of Bcl-2 expression were detected during early neurulation, which emphasizes its role in preventing apoptosis at this stage. Bcl-2 expression decreases in the neurons of CNS after neural tube formation, while it remains highly expressed in the peripheral nervous cells [102,121].

In the mature brain, Bcl-2 is principally retained in the granule cells of the cerebellum and dentate gyrus of the hippocampus, as well as in sensory and sympathetic adult neurons [102]. Chen et al. reported that Bcl-2 plays a major role in promoting growth and axon regeneration in retinal neurons. Bcl-2 seemed essential but not sufficient for the regeneration of retinal axons in the CNS [122]. Altered Bcl-2 expression is deemed to impair cellular plasticity and resilience in neuropsychiatric patients [123,124]. The mechanisms by which Bcl-2 promotes axonal growth are suggested to be by enhancing the intracellular Ca^{2+} signaling and by activating CREB and extracellular-regulated kinase (ERK). These latter two proteins are known to induce gene expression essential for neurite growth and plasticity. Bcl-2 reduces the ER Ca^{2+} uptake in neurons, which leads to increased Ca^{2+} influx over the plasma membrane. This

consequently causes the activation of CREB and ERK transcriptional programs that regulate neurite extension [125]. A recent review of Pemberton et al. further focuses on the regulation of axon degeneration and neuronal cell death by Bcl-2 proteins [103]. Additionally, overexpression of Bcl-2 protects motor neuron cell bodies from apoptosis in a progressive motor neuropathy mouse model. However, the life span of these mice was not affected as overexpression of Bcl-2 did not prevent the degeneration of myelinated motor fibers [126]. Bcl-2 can also influence neuronal Ca^{2+} signaling through its interaction with RyRs [90]. Co-immunoprecipitation assays on lysates of rat hippocampi proved the presence of RyR-Bcl-2 complexes in rat neurons. The BH4 domain of Bcl-2 was sufficient to inhibit the RyR-mediated Ca^{2+} release in these neurons. This further underpins an important function of Bcl-2 in the brain.

Bcl-2 expression appears to protect neuroepithelial and hippocampal cells against glutamate-mediated excitotoxicity [127]. It was shown that overexpression of Bcl-2 may improve cortical neuron survival by blocking the translocation of apoptosis-inducing factor (AIF) from mitochondria to the nucleus following focal cerebral ischemia [128]. Instead, mice with Bcl-2 deficiency demonstrate enhanced oxidative stress and alterations in antioxidants levels in the brain [129]. Transgenic mice overexpressing Bcl-2 in neurons display nervous system hypertrophy caused by decreased neuronal cell death, but they also show a 50% reduction in cerebral infarction volume compared to wild-type mice after permanent middle cerebral artery occlusion (MCAO)-induced ischemia [130]. Moreover, transplantation of embryonic stem cells overexpressing Bcl-2 into the brain cavity of adult rats after MCAO led to neuronal differentiation and improved functional recovery [131]. Up-regulation of Bcl-2 may also aid DNA repair following oxidative stress damage [132]. In the model of MCAO, Bcl-2 expression

inhibits apoptosis of neonatal neurons in the brains of adult rats [133]. It should be noted that changes in Ca^{2+} homeostasis of the ER have been shown to induce apoptosis in neurons [134].

As Bcl-2 expression decreases after neurulation, Bcl-X_L expression increases and remains elevated throughout neuronal ontogeny with the highest levels in differentiating cells [102,121]. It was demonstrated that Bcl-X_L may regulate programmed cell death via supporting the viability of immature cells during the development of the nervous and hematopoietic systems. Strikingly, Bcl-X_L-deficient mice die around 13^{th} embryonic day showing extensive apoptotic cell death in postmitotic immature neurons of the developing brain, spinal cord, and dorsal root ganglia [100]. However, the mechanisms and signals regulating Bcl-X_L expression in the brain are still not clear. Postnatally, in mature neuronal cells Bcl-X_L has been identified as regulator of synaptic plasticity and neurite growth [131]. Injection of Bcl-X_L in the presynaptic terminal of squids led to potentiation of the synaptic neurotransmitter release, both in healthy synapses as in synapses in which the transmission had run down. Additionally, Bcl-X_L reportedly improved recovery after synaptic depression [135].

Bcl-w, another anti-apoptotic Bcl-2-family member is considered to play an important role in neurons and may be potentially therapeutically relevant target in neurodegenerative diseases [87]. Indeed, Bcl-w contributes to the maintenance of axons [136] and is involved in promoting cell survival after cerebral ischemia. Cell death, and more specifically apoptosis, is implicated in brain injury following cerebral ischemia. Bcl-w is deemed to have a neuroprotective effect given its increased expression in the brain, and mainly in the surviving cells, after the ischemic insult [137,138]. The role of Bcl-w as an endogenous neuroprotector is not limited to cerebral ischemia but is also observed in the β-amyloid-induced cell death in AD [139]. Bcl-w is also

implicated in the maintenance of axon integrity in sensory neurons, and a lack of this anti-apoptotic protein can lead to small-fiber sensory neuropathy [140]. Bcl-w can protect against axon degeneration via interaction of its BH4 domain with the IP_3R1, the isoform predominantly expressed in the brain [141].

5. Bcl-2 in Alzheimer's disease

5.1 Deranged Ca^{2+} signaling in Alzheimer's disease

AD is the most frequently occurring form of dementia worldwide with an enormous impact on the quality of life of patients and their family. The disease leads to an irreversible loss of neurons and is clinically represented by impaired memory formation, disorientation, troubled judgment, and behavioral changes [142,143]. The main histopathological features of AD are an accumulation of amyloid-beta (Aβ) that aggregates in senile plaques and hyperphosphorylated tau protein which is a major constituent neurofibrillary tangles typically observed in AD patients. Rare familial forms of Alzheimer's disease (FAD) are linked to mutations in the genes encoding the amyloid precursor protein (APP), presenilin1 (PSEN1), and presenilin 2 (PSEN2). The latter constitute the catalytic subunit of γ-secretase [142,144] that liberates Aβ peptides from their precursor, the APP C-terminal fragment. Based on the genetic nature of these familial forms of AD, an amyloid cascade hypothesis was formulated that linked the Aβ accumulation with degenerative changes in the brain leading to the death of neuronal cells and the development of cognitive impairment [145]. However, the cause of sporadic forms of AD (SAD), still constituting over 95% of all cases, remains elusive. Besides, some controversy exists concerning the Aβ- hypothesis, given that Aβ-deposits are also found in healthy, cognitively well-functioning individuals [146]. Whereas anti-amyloid therapy strategies have so

far not been successful in patients [147], the more recently developed Aducanumab bears more promise [148]. Memantine, an NMDAR antagonist, is currently the only disease-modifying drug approved for AD therapy.

The Ca^{2+} hypothesis of AD was formulated by Zaven S. Khachaturian and is based on the similarity of the processes occurring in neurons during aging and AD [149]. Indeed, many pathological processes observed in neurons during AD progressions such as oxidative and metabolic stress, a decrease in ATP production, and Ca^{2+} dysregulation are also observed with senescence. Increasing evidence points to an important role for altered Ca^{2+} signaling in AD, as changes in intracellular Ca^{2+} signaling occur before the major neuronal loss in AD [150–152]. Among these changes, synaptic dysfunction is especially important since synapses are units of high energy consumption and extremely vulnerable to changes in Ca^{2+} balance. The elimination of synapses is observed early in the AD development and better correlates with cognitive impairments than other histopathological signs [153,154].

Many physiological processes mediated by Ca^{2+} can turn into a pathological cascade with aging and AD. An example of such a process is the excitotoxic effect of glutamate. Glutamate is the main excitatory neurotransmitter in the brain, and one of its key effects is the induction of LTP necessary for short-term memory formation. However, the constant massive Ca^{2+} influx in case of NMDAR overstimulation can become detrimental if neurons are unable to rapidly remove of Ca^{2+} from the cytosol. The decrease in Ca^{2+} buffer proteins observed in AD [155] may contribute to the development of excitotoxicity since the accumulation of Ca^{2+} in the cytoplasm leads to the activation of Ca^{2+}-dependent calpain proteases, which induce neuronal apoptosis. Considering the physiological/pathophysiological effects of glutamate, it is necessary to carefully consider the strategy for correcting Ca^{2+} imbalance in neurons. Perhaps

this is a key feature of memantine which at therapeutic concentrations blocks the excitotoxicity mediated by extrasynaptic NMDARs but does not affect the physiological processes of neuroplasticity in synapses [156].

Evidence that supports the Ca^{2+} hypothesis comes from studies indicating dysfunctional ER-mediated Ca^{2+} signaling in FAD [157]. A specific interest in the ER Ca^{2+} homeostasis related to the AD pathology arises as certain mutations that cause FAD also interfere with ER Ca^{2+} signaling. An important finding indicating the close relationship between FAD and impaired Ca^{2+} signaling was the discovery of the role of PSEN1 as potential Ca^{2+} leak channels [158]. Mutations in PSEN1 that affect this function, disrupt steady-state resting ER Ca^{2+} levels and promote excessive Ca^{2+} accumulation in the ER [159]. Alternatively, other groups showed an enhancement of IP_3R-mediated Ca^{2+} signaling in fibroblasts [160] and B lymphoblast's [161] from AD patients carrying mutations in PSEN1. These changes in Ca^{2+} signaling were already present before the patients became clinically symptomatic [162]. Similar results have been shown in Xenopus oocyte expression system experiments with AD-linked mutation in PSEN [163] and in mouse cortical neurons of the mutant PSEN1, PS1-M146V knock-in (PS1-M146V-KI), model of AD [164].

Several groups also indicated the role of RyRs in dysregulated Ca^{2+} signaling as neuronal RyR expression was increased in different AD mouse models and cell lines [165–170]. Dantrolene treatment, a RyRs inhibitor, has been reported to normalize the dysregulated ER Ca^{2+} signaling and to reduce the Aβ deposition [167]. Despite some uncertainty remaining in proposed mechanisms, the main concept of excessive Ca^{2+} release from the ER via IP_3R1 and RyR caused by FAD-associated mutations in PSEN is gaining in popularity [171].

Another pathological aspect of overfilling of the ER Ca^{2+} content is decreased activity of neuronal SOCE (nSOCE). As a compensatory response to elevated ER Ca^{2+} level, a reduction in STIM2 protein has been observed in PSEN1-M146V-KI [172] and APP-KI mouse models [173]. TRPC6-mediated nSOCE activity is necessary for maintaining the persistent activity of CAMKII and stabilization of mature mushroom spines [174]. Downregulation of STIM2 expressions reduces the constant activity of nSOCE and the expression of the active (phosphorylated) form of CAMKII leading to destabilization of mature spines. This provides an important connection between deranged Ca^{2+} signaling in the ER and the earliest signs of neurodegenerative processes observed in AD. It was also demonstrated that application of Aβ oligomers (Aβo) promotes the loss of nSOCE in aged rat hippocampal cultures. Aβo also exacerbate the increased resting cytosolic Ca^{2+} concentration and Ca^{2+} store content. In young neuronal cultures Aβo promoted ER to mitochondrial Ca^{2+} transfer without detrimental effects whereas, in aged cultured neurons, Aβo suppressed Ca^{2+} transfer from ER to mitochondria. In these aged cultures Aβo also decreased mitochondrial potential, enhanced reactive oxygen species (ROS) generation and promoted apoptosis [175].

While deranged Ca^{2+} signaling is mainly considered as a downstream effect of AD-linked mutations, some studies indicate that nSOCE downregulation selectively elevates Aβ42 generation suggesting that reduced Ca^{2+} entry might be an early cellular event associated with PSEN mutations [176]. It was also shown that exposure of cultured neurons to Ca^{2+} ionophores increases their production of Aβ [177]. Moreover, a physiological Ca^{2+} stimulus increases α-secretase cleavage of APP and may thereby decrease Aβ production [178,179].

Impaired Ca^{2+} balance in the ER leads to dysfunction of mitochondria since its additional buffering capacity is needed to reduce high cytosolic Ca^{2+} concentration in neurons.

Mitochondria's dysfunction in AD is well described and includes mitochondrial oxidative stress, impaired bioenergetics and biogenesis and formation of mPTP, inducing neuronal apoptosis [180,181]. Recent studies also point to the pivotal role of MAMs in AD pathogenesis. It was shown that PSENs may localize in MAMs [182] where also APP cleavage has been suggested to take place [183]. A significant increase in the contact sites between ER and mitochondria was also demonstrated in the case of FAD and SAD, indicating an imbalance in the functioning of MAMs [184]. Taking into account that Ca^{2+} transfer from ER into mitochondria occurs predominantly through MAMs [185], it cannot be excluded that the increase in contact sites between the two organelles may result in an increased Ca^{2+} uptake into mitochondria, eventually leading to mPTP opening and neuronal apoptosis. More recently, *in vivo* evidence based on an APP/PS1 mouse model emerged that mitochondrial Ca^{2+} overload occurs prior to neuronal death [186,187]. This further underpins the key role of Ca^{2+} -signaling dysregulation as an early event in AD [181].

5.2 Bcl-2-family members in AD

Much work has been performed on the potential roles of Bcl-2 in AD as we will describe in the next parts. However, also other Bcl-2-family members have been associated with the disease progression. In general, the balance between pro-and anti-apoptotic Bcl-2 family members shifts towards the pro-apoptotic side during the disease thereby increasing the potential occurrence of neuronal cell death. It is suggested that Aβ-deposits alter the balance of the Bcl-2-family proteins favoring the expression of pro-apoptotic family members. This is illustrated by the observation that Bim is upregulated while Bcl-2 is downregulated after injection of mice brain with oligomeric Aβ [188]. Additionally, increased activation of Bax was observed and *bax*$^{-/-}$ neurons seemed resistant against Aβ-induced cell death [188]. A similar increase in Bax and

downregulation of Bcl-2 in human neuronal cultures derived from fetal brain following Aβ treatment was also observed by other researchers [189]. Finally, by studying senile plaques and neurons from AD patients with neurofibrillary degeneration, a strong immunoreactivity has been observed for Bax as well [190,191]. However, this could not be reproduced by others (Tortosa et al.) [192].

Concerning the anti-apoptotic Bcl-2-family members, Bcl-X_L was shown to be expressed in microglia of patients with AD that co-localized with Aβ-deposits and activated astrocytes which may increase the cell survival of these microglia in disease hot spots [193]. Mcl-1 was shown to interact with cyclin dependent kinase 5 (Cdk5) [194], which is involved in neuronal cell death in AD [195]. The interaction between Mcl-1 and Cdk5 induced phosphorylation of Mcl-1 at T91 thereby triggering Mcl-1 ubiquitylation and its subsequent degradation. This renders the neurons more sensitive to cell death. Besides this, selective Mcl-1 antagonism using UMI-77, a BH3 mimetic, induces mitophagy, a mitochondrial quality control process that is inhibited in AD patients. Through this mechanism, UMI-77 could significantly improve the cognitive impairment in mice lacking APP and PSEN1 [196].

5.3 Bcl-2 is downregulated in AD

The expression of members of the Bcl-2-protein family, such as Bcl-X_L, Bak, and Bad, is altered in AD [197] (Fig 3B). The alterations in Bcl-2 in healthy brain aging are dissimilar from those observed in brain of AD models [198]. Multiple studies using post-mortem samples of AD patients have indicated a striking immunoreactivity of Bcl-2 in astrocytes surrounding senile Aβ-plaques. This suggests that Bcl-2 is involved in astroglial survival [199–201]. Moreover, it

was shown that the immunoreactivity for Bcl-2 in neurons of patients with AD was increased relative to controls in most neurons of the entorhinal cortex, subiculum, CA1, CA2, CA3, hilus, and dentate gyrus [201]. Relative Bcl-2 staining increased in parallel with increasing disease severity. In contrast, Bcl-2 immunoreactivity in neurons from patients with AD with confirmed neurofibrillary degeneration is decreased, indicating the downregulation of Bcl-2 in these degenerating neurons [200–202].

Several mechanisms may contribute to the Bcl-2 downregulation observed in AD. For instance, Aβ-deposits are considered to be able to regulate the expression of certain micro RNAs (miRNAs) that can impact neuronal cell death observed in animal models of AD [203–206]. Interestingly, miR-16-5p, a microRNA that targets Bcl-2 mRNA and thus reduces Bcl-2-protein levels, was found to be upregulated in neurons from a FAD mouse model surrounding Aβ-deposits, thereby promoting apoptosis in the neurons affected by Aβ-plaques [206]. Bhatnagar et al. showed increased levels of miR-34a and miR-34c in blood samples of AD patients [207,208]. Four key target genes are silenced by these miRNAs of which Bcl-2 is one of them, leading to a significantly reduced abundance of Bcl-2 in AD plasma samples [209]. Thus, this implicates Bcl-2 as a potential biomarker for neurodegeneration.

Another possible contributing mechanism was unveiled by bio-informatic approaches identifying Ovarian-Carcinoma-Immunoreactive-Antigen-Domain-Containing-1 (OCIAD1) as a neurodegeneration-associated factor for AD in a FAD mouse model [210]. OCIAD1 is upregulated in neurons of both AD mice and sporadic AD patients, and higher OCIAD1 levels are correlated with disease severity. Different pathways are affected by this protein including mitochondrial functionality through interaction with Bcl-2. The OCIAD1-Bcl-2 interaction was suggested to interfere with Bcl-2/Bax-complex formation [210].

A third mechanism that may play a role is a single-nucleotide polymorphism (SNP). The Bcl-2 gene is subject to SNP rs956572, which significantly alters protein and mRNA expression levels as detected in AD patients and patients suffering from bipolar disorder [211–213]. The AA-genotype is associated with reduced expression of Bcl-2, whereas the G-allele is associated with higher Bcl-2 levels. The Bcl-2 rs956572 polymorphism influences age-related volume reductions of the grey matter, mainly in the cerebellum. More specifically, the Bcl-2 G homozygosity has been found to protect against this age-related grey matter volume reduction [123]. In addition, Chang et al. examined the association between the Bcl-2 rs956572 polymorphism and the structural covariance network in AD patient samples and reported a greater covariance strength in the A homozygotes [211]. Therefore, this polymorphism might also be of importance when examining the role of Bcl-2 in AD.

Another mechanism that has been proposed is the interference of ROS. In AD patients, ROS is increased which subsequently causes a decrease in Bcl-2 levels [214,215]. This may then be associated with the Ca^{2+} dysregulation in AD. Finally, nuclear factor erythroid 2-related factor (Nrf2), which maintains the level of redox buffer glutathione, is suggested to be an important player in the prevention of AD [216]. Interestingly, Nrf2 Is known to regulate Bcl-2 levels and besides the downregulation of Bcl-2, Nrf2 levels are also significantly reduced in the brains of AD patients [214,217].

5.4 Bcl-2 as therapeutic target for AD

In this section, we focus on the potential of Bcl-2 as a therapeutic target for AD. As discussed above, Ca^{2+} signaling is dysregulated in AD. Bcl-2 plays a role in the control of intracellular Ca^{2+} signaling and may potentially be used to normalize Ca^{2+} signals in AD neurons (Fig 3B).

A transgenic AD mouse model with neuronal overexpression of Bcl-2 showed a reduction in caspase 9 and caspase 3 activation, suppression in the formation of plaques and NTFs, and improved memory retention [218]. More specifically, the prevention of caspase activation led to a limited caspase-mediated cleavage of tau and an intracellular accumulation of APP without the formation of Aβ-plaques. These findings indicate a neuroprotective role of Bcl-2 overexpression [218]. This is further supported by other research groups as Karlnoski et al. demonstrated an association between increased Bcl-2 expression in brain regions containing Aβ deposits and neuroprotection in APP transgenic mice [219]. Besides Bcl-2, Bcl-X_L too appears to protect against early-stage and late-stage apoptosis/necrosis following Aβ treatment [220]. Moreover, Bcl-X_L was shown to interact with PSENs, which in turn are known to significantly increase Bax expression. However, overexpression of Bcl-X_L abolished the enhanced Bax-induced apoptosis mediated by PSENs [221]. Besides these direct overexpression approaches, several therapeutic regimens that ameliorate AD outcomes, also enhance Bcl-2-expression levels. For instance, ibudilast, montelukast, and pranlukast, all currently used for the treatment of inflammatory diseases such as asthma, improved Aβ-induced memory impairment [222–224]. Moreover, these drugs all prevented Bcl-2 downregulation. Other researchers found similar results for prosaposin-derived 18-mer peptide, an amelioration of both Aβ-induced neurotoxicity and Bcl-2 downregulation was observed in mice [225].

6. Conclusion

In this review, we have highlighted the emerging link between Bcl-2 family proteins, neuronal Ca^{2+} signaling in health and AD. Ca^{2+} is one of the most versatile secondary messengers required for memory formation and other neuronal-specific processes. Multiple evidence points

to an important role of dysregulated Ca^{2+} signaling in AD. Proteins of the Bcl-2 family are key regulators of cell death and survival, but also regulate intracellular Ca^{2+} signaling at both mitochondrial and ER levels. Moreover, several Bcl-2 proteins are involved in the integrity of axons and neurons. Since expression of Bcl-2 is suppressed in AD and Bcl-2 proteins act as inhibitors of Ca^{2+} channels such as IP_3R and RyR, de-inhibition of these channels might therefore contribute to aberrant neuronal Ca^{2+} signaling in AD. Multiple mechanisms have emerged that contribute to the decreased Bcl-2-protein levels, including the expression of miRNAs regulated by Aβ deposition; interaction with OCIAD1, and single nucleotide polymorphism in the Bcl-2 gene. Overexpression of Bcl-2 has multiple neuroprotective effects in models of AD that may go well beyond its canonical anti-apoptotic effects. Indeed, by targeting intracellular Ca^{2+}-release channels, Bcl-2 via its BH4 domain could inhibit excessive IP_3R/RyR-mediated release of Ca^{2+} from the ER. These results suggest that anti-apoptotic Bcl-2 and derived protein domains such as the BH4 domain may have therapeutic potential to prevent the onset of AD and delay neurodegeneration. Therefore, further work is needed to elucidate whether Bcl-2 deregulation contributes to aberrant Ca^{2+} signaling in AD and whether Bcl-2 or its BH4 domain can help to prevent or delay memory loss and neurodegeneration in AD and possibly other neurodegenerative disorders.

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Figure Legends

Figure 1. Neuronal calcium signaling.

Schematic representation of neuronal Ca^{2+} signaling. Main Ca^{2+} influx sources in plasma membrane (PM) are voltage-gated (VGCC), the ligand–operated (ROC) and the store-operated (SOCE) Ca^{2+} channels. Plasma membrane Ca^{2+} ATPase (PMCA) and the Na^{+}/Ca^{2+} exchanger (NCX) extrude Ca^{2+} from cytosol into the extracellular space. Activation of metabotropic glutamate receptors (mGluR) stimulate Ca^{2+} mobilization from endoplasmic reticulum (ER) via inositol 1,4,5 - trisphosphate receptor (IP_3R). Ca^{2+} also can be mobilized from ER via ryanodine receptors (RyR) amplifying cytoplasmic Ca^{2+} signals. Reuptake Ca^{2+} into the ER is operated by the ATPase SERCA. ER Ca^{2+} depletion is detected by Ca^{2+} sensors STIM1/2. To maintain the balance, Ca^{2+} is released from ER through passive leakage channels presenilins (PSEN1/2). The transmission of Ca^{2+}-dependent signals to the nucleus occurs with the help of transcription factors cAMP response element-binding protein (CREB) and nuclear factor of activated T-cells (NFAT). The mitochondrial (mito) Ca^{2+} handling systems include mitochondrial Ca^{2+} uniporter (MCU), voltage-dependent anion channel type 1 (VDAC1), mitochondrial permeability transition pore (mPTP). Ca^{2+} concentration in cytosolic maintain with Ca^{2+}-binding proteins: calbindin-28 (CaB), parvalbumin (PV) and calretinin (CaR), and inside ER with calreticulin (CRT) and calnexin (CNX). Ca^{2+}-activated proteins include calmodulin (CaM), Ca^{2+}/ calmodulin-dependent protein kinase type II (CAMKII) and Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin (CaN). Figure created with BioRender.com.

Figure 2. Domain structure of Bcl-2 protein family structure.

Schematic overview of the linear representation of Bcl-2 protein family. Bcl-2 contains four Bcl-2-homology (BH) domains. The BH4 domain is known to bind and inhibit inositol 1,4,5-trisphosphate receptor (IP_3R), as well as ryanodine receptor (RyR). The hydrophobic cleft is formed by the BH3, BH1 and BH2 domains and scaffolds pro-apoptotic Bax/Bak and activator BH3-only proteins, thereby neutralizing their pro-apoptotic activities. BH3 mimetics and sensitizer BH3-only proteins can bind to the hydrophobic cleft thereby antagonizing Bcl-2's ability to bind Bax/Bak and activator BH3-only proteins and unleashing their pro-activity activities. Figure created with BioRender.com.

Figure 3. Bcl-2 function in healthy and AD neurons

(A) In healthy neurons, Bcl-2 diminishes Ca^{2+} release from the ER by inhibiting ryanodine receptor (RyR) and 1,4,5-trisphosphate receptor (IP_3R), and reduces activity of voltage-dependent anion channel 1 (VDAC1). (B) In AD neurons Bcl-2 expression is reduced via various mechanisms including the formation of amyloid-β deposits, the upregulation of Ovarian-Carcinoma-Immunoreactive-Antigen-Domain-Containing-1 (OCIAD1), the upregulation of reactive oxygen species (ROS) and the downregulation of nuclear factor erythroid 2-related factor (Nrf2). The Bcl-2 downregulation reduces the inhibition of ER Ca^{2+} release and VDAC1 activity, leading to enhanced intracellular Ca^{2+} release and apoptosis. Figure created with BioRender.com.

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit Author Statement

M.C. - Writing – original draft, Writing – review & editing
N.C. - Writing – original draft, Writing – review & editing
K.D. - Writing – original draft, Writing – review & editing
W.A. - Funding Acquisition , Writing – review & editing,
G.B. – Conceptualization, Supervision, Funding Acquisition, Writing – review & editing
I.B. - – Conceptualization, Supervision, Funding Acquisition, Writing – review & editing
T.V. - – Conceptualization, Supervision, Writing – review & editing

Highlights

1. The function of Bcl-2 protein family in the nervous system is discussed
2. The emerging link between Bcl-2 family proteins, neuronal calcium signaling and Alzheimer's disease is discussed.
3. The role of Bcl-2 as therapeutic target for Alzheimer's disease is discussed

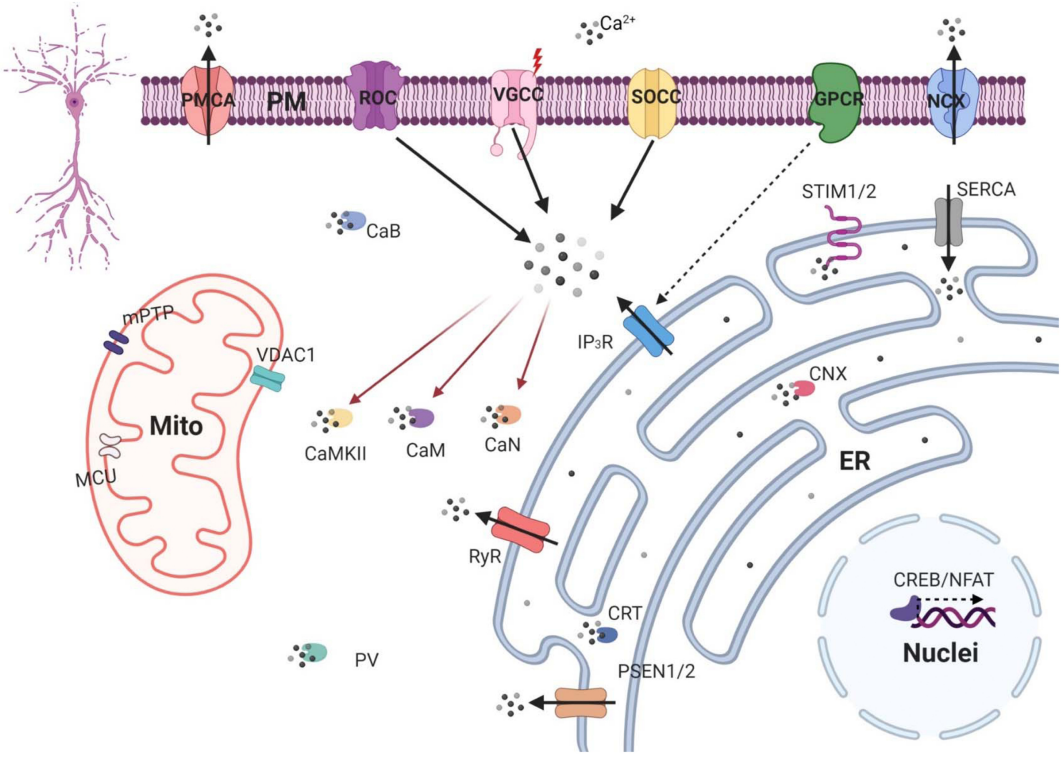
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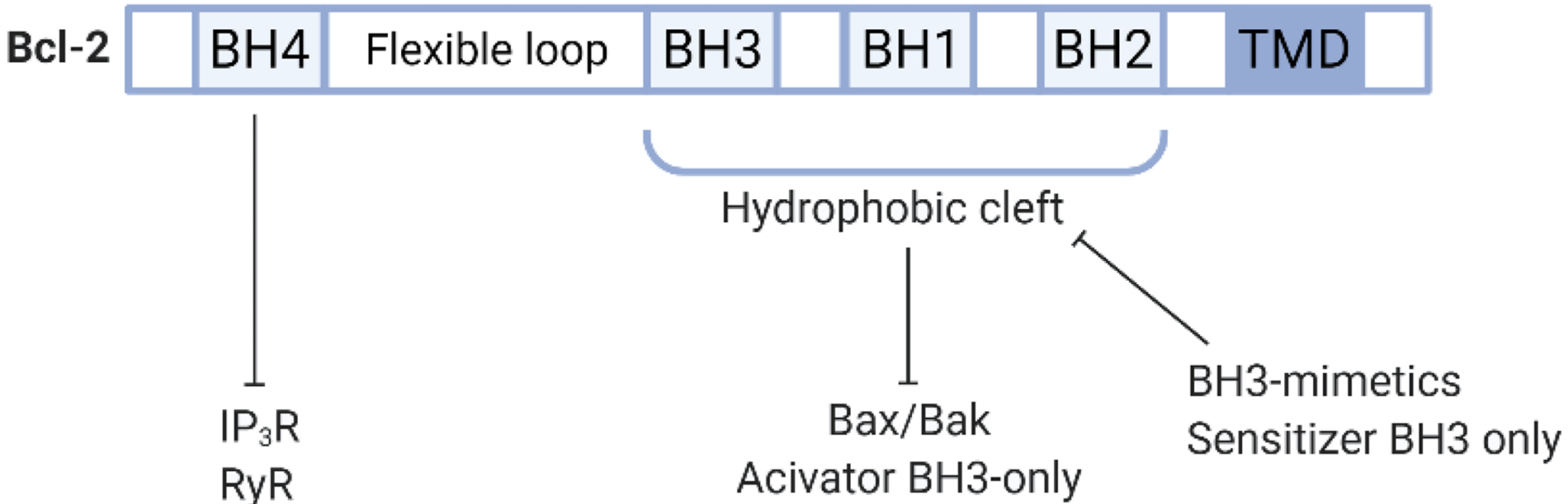
Figure 1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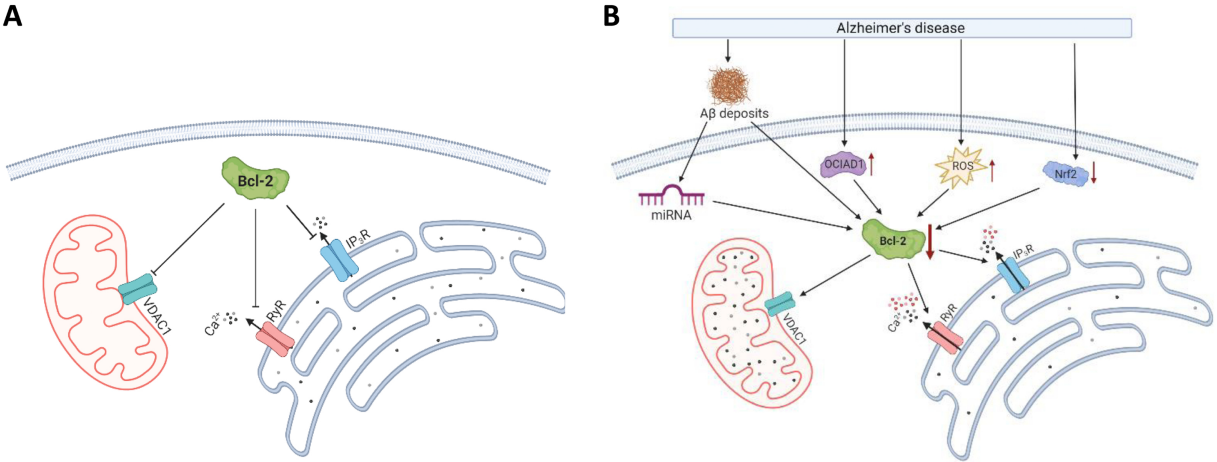
Bax/Bak
Acivator BH3-only
BH3-mimetics
Sensitizer BH3 only

Figure 2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VDAC1
Ca2+
RyR
B
Alzheimer's disease
Aβ deposits
OCIAD1
ROS
Nrf2
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Bcl-2
IP3R
VDAC1
Ca2+
RyR

Figure 3