Biochimica et Biophysica Acta 1843 (2014) 2240–2252

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

A dual role for the anti-apoptotic Bcl-2 protein in cancer: Mitochondria *versus* endoplasmic reticulum ☆

Haidar Akl *, Tamara Vervloessem, Santeri Kiviluoto, Mart Bittremieux, Jan B. Parys, Humbert De Smedt, Geert Bultynck *

KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Molecular and Cellular Medicine, Campus Gasthuisberg, O/N-I, Bus 802, Herestraat 49, BE-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 28 January 2014
Received in revised form 4 April 2014
Accepted 5 April 2014
Available online 21 April 2014

Keywords:
Bcl-2
Inositol 1,4,5-trisphosphate receptor
Apoptosis
Cell cycle
Ca^{2+}
Cancer B-cell

ABSTRACT

Anti-apoptotic Bcl-2 contributes to cancer formation and progression by promoting the survival of altered cells. Hence, it is a prime target for novel specific anti-cancer therapeutics. In addition to its canonical anti-apoptotic role, Bcl-2 has an inhibitory effect on cell-cycle progression. Bcl-2 acts at two different intracellular compartments, the mitochondria and the endoplasmic reticulum (ER). At the mitochondria, Bcl-2 via its hydrophobic cleft scaffolds the Bcl-2-homology (BH) domain 3 (BH3) of pro-apoptotic Bcl-2-family members. Small molecules (like BH3 mimetics) can disrupt this interaction, resulting in apoptotic cell death in cancer cells. At the ER, Bcl-2 modulates Ca^{2+} signaling, thereby promoting proliferation while increasing resistance to apoptosis. Bcl-2 at the ER acts via its N-terminal BH4 domain, which directly binds and inhibits the inositol 1,4,5-trisphosphate receptor (IP_3R), the main intracellular Ca^{2+}-release channel. Tools targeting the BH4 domain of Bcl-2 reverse Bcl-2's inhibitory action on IP_3Rs and trigger pro-apoptotic Ca^{2+} signaling in cancer B-cells, including chronic lymphocytic leukemia (CLL) cells and diffuse large B-cell lymphoma (DLBCL) cells. The sensitivity of DLBCL cells to BH4-domain targeting tools strongly correlated with the expression levels of the IP_3R2 channel, the IP_3R isoform with the highest affinity for IP_3. Interestingly, bio-informatic analysis of a database of primary CLL patient cells also revealed a transcriptional upregulation of IP_3R2. Finally, this review proposes a model, in which cancer cell survival depends on Bcl-2 at the mitochondria and/or the ER. This dependence likely will have an impact on their responses to BH3-mimetic drugs and BH4-domain targeting tools. This article is part of a Special Issue entitled: Calcium signaling in health and disease. Guest Editors: Geert Bultynck, Jacques Haiech, Claus W. Heizmann, Joachim Krebs, and Marc Moreau.

1. Introduction

Bcl-2, the founding member of the Bcl-2 family, derives its name from *B-cell lymphoma 2*. It was initially described in 14;18 chromosome translocations, which are a characteristic of follicular lymphoma, the most common lymphoma in humans [1,2]. The Bcl-2-protein family regulates cell death and proliferation [3,4], two processes dysregulated during oncogenic transformation. Thus, it is not surprising to find an upregulation of Bcl-2 expression in many cancers, including chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL) [5,6].

Intracellular Ca^{2+} signals originating from the endoplasmic reticulum (ER), the main intracellular Ca^{2+}-storage organelle, also control cell death and proliferation [7]. In particular, the close proximity of the ER and the mitochondria allows a swift and prominent accumulation of Ca^{2+} into the mitochondria, not only in response to physiological agonists but also in response to apoptotic stimuli that promote Ca^{2+} release from the ER [8–10]. Moreover, intracellular Ca^{2+} elevation induces calcineurin (CN)-dependent dephosphorylation and activation of Bad, a pro-apoptotic Bcl-2 protein, leading to Bcl-2 antagonism and mitochondrial outer membrane permeabilization (MOMP) [11]. In

Abbreviations: ATP, adenosine triphosphate; BI-1, Bax inhibitor-1; BH, Bcl-2-homology; CN, calcineurin; CaM, calmodulin; CLL, chronic lymphocytic leukemia; JNK1, c-Jun N-terminal kinase 1; DLBCL, diffuse large B-cell lymphoma; DARPP-32, dopamine- and cAMP-regulated phosphoprotein of 32 kDa; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinases; GM-CSF, granulocyte–macrophage colony-stimulating factor; IP_3R, inositol 1,4,5-trisphosphate receptor; IRE1α, inositol-requiring enzyme-1 alpha; IL-2 and IL-3, interleukin (IL)-2 and -3; IICR, IP_3-induced Ca^{2+} release; MAMs, mitochondria-associated ER membranes; MOMP, mitochondrial outer membrane permeabilization; NFAT, nuclear factor of activated T-cells; PTEN, phosphatase and tensin homolog; PI3K, phosphatidylinositol 4,5-bisphosphate 3-kinase; Akt/PKB, phosphoinositide-dependent serine–threonine protein kinase/protein kinase B; PKA, protein kinase A; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; TAT-IDP^S, stabilized TAT-IP_3R-derived peptide; Orai1, STIM1-mediated Ca^{2+}-release-activated Ca^{2+}-channel protein 1; STIM1, stromal interaction molecule 1; UPR, unfolded protein response; VDAC, voltage-dependent anion channel

☆ This article is part of a Special Issue entitled: Calcium signaling in health and disease. Guest Editors: Geert Bultynck, Jacques Haiech, Claus W. Heizmann, Joachim Krebs, and Marc Moreau.

* Corresponding authors at: KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Molecular and Cellular Medicine, Campus Gasthuisberg, O/N-I, Bus 802, Herestraat 49, BE-3000 Leuven, Belgium. Tel.: +32 16 37 73 94, +32 16 33 02 15.

E-mail addresses: haidar.akl@med.kuleuven.be (H. Akl), geert.bultynck@med.kuleuven.be (G. Bultynck).

http://dx.doi.org/10.1016/j.bbamcr.2014.04.017
0167-4889/

normal conditions, inactive Bad is highly phosphorylated and binds to 14-3-3 scaffold proteins and thus cannot interact with Bcl-2 or Bcl-Xl [12]. The inositol 1,4,5-trisphosphate receptor (IP_3R) is the main intracellular Ca^{2+}-release channel located in the ER. Importantly, a subfraction of the IP_3Rs is located in a microdomain of the ER, namely the mitochondria-associated ER membranes (MAMs) [10,13–15]. The magnitude and the duration of Ca^{2+} signals derived from the IP_3Rs are decisive for the cell fate, for which small Ca^{2+} oscillations promote mitochondrial bioenergetics and cellular proliferation, while large Ca^{2+} transients cause MOMP and subsequent apoptosis [16,17]. It is therefore not surprising that Bcl-2 proteins also act at the level of the ER as critical regulators of the IP_3R channels, thereby assuring Ca^{2+}-dependent cell proliferation while protecting them from apoptosis. In this review, the key determinants of Bcl-2's action at the mitochondria and at the ER are discussed, thereby illustrating two different strategies that cancer cells can use to promote survival.

2. The canonical anti-apoptotic role of Bcl-2 at the mitochondria

The Bcl-2 family of proteins is essentially studied for its role in apoptosis [18–21]. Apoptosis is a programmed cell death morphologically characterized by membrane blebbing, cell shrinkage, chromatin condensation and chromosomal DNA fragmentation [22]. Under physiological conditions, apoptosis is a non-immunogenic cell death, because the produced apoptotic bodies are removed by the phagocytic cells before the contents of the dying cell can induce an inflammatory reaction [23]. There are two distinct signaling pathways that lead to apoptosis: (i) the extrinsic or extracellular pathway induced, for example, by an infection that activates a receptor-mediated process, like the tumor necrosis factor receptor (TNFR) [24] or the Fas receptor (FasR) [25] and (ii) the intrinsic or mitochondria-mediated pathway [26–28]. The intrinsic apoptosis signaling pathway is operated by the Bcl-2-family members, which are generally divided into three categories (anti-apoptotic proteins, pro-apoptotic executioners and pro-apoptotic BH3-only proteins) based on their intracellular function and sequence homology [29]. The anti-apoptotic Bcl-2 proteins like Bcl-2, Bcl-w, Mcl-1, Bfl-1 and Bcl-Xl which contain all four Bcl-2-homology (BH) domains 1–4 (BH1–4), can interact with both pro-apoptotic categories, the multi-domain executioner proteins Bax and Bak and the BH3-only proteins. The latter consist of two groups: the activator BH3-only proteins, which can directly activate the executioners Bax and Bak, like Puma, Bim, and Bid and the sensitizer BH3-only proteins, like Bad, Bik, Noxa and Bmf which de-repress Bcl-2's anti-apoptotic function but cannot directly activate Bax and Bak [28] (Table 1). Mechanistically, anti-apoptotic family members prevent death by binding and sequestering the BH3 domains of activator BH3-only proteins and preventing their interaction with Bax/Bak [30,31]. The sensitizers BH3-only proteins induce Bax/Bak oligomerization indirectly by binding anti-apoptotic proteins and thereby displacing activator BH3-only proteins [31]. Very recently, a selective role for Bid and Bim as activator BH3-only proteins has been discovered: Bid preferentially switches on Bak, while Bim preferentially switches on Bax [32] (Fig. 1).

After their oligomerization induced by the BH3-only proteins, Bax and Bak directly cause MOMP, a critical step during apoptosis [26,27,33]. Cytochrome c is released after MOMP leading to the activation of caspases and the subsequent progression toward dismantling of the cell [27]. Practically, post-MOMP, mitochondria are impaired in their ability to generate adenosine triphosphate (ATP) and cannot maintain cellular homeostasis [27,34,35]. Therefore, MOMP is considered as the point-of-no-return in mitochondrial apoptosis [21].

The balance between pro-survival and pro-death Bcl-2 proteins is a major factor in determining whether or not cells undergo apoptosis in response to cell stress. Anti-apoptotic Bcl-2 members can via their hydrophobic cleft composed by their BH1, 2 and 3 domains, bind and sequester the pro-apoptotic Bcl-2-family members (Fig. 1). Via this hydrophobic cleft, the anti-apoptotic Bcl-2 proteins can also interact with other apoptosis regulators that are not members of the Bcl-2-protein family, like p53 [28]. Beyond these interactions, Bcl-2 can also interact with several non-Bcl-2-family proteins via its N-terminal BH4 domain. It can e.g. target the serine/threonine protein kinase Raf-1 to mitochondrial membranes, allowing it to phosphorylate Bad [36]. Moreover, Bcl-2 can sequester active CN and subsequently block the nuclear factor of activated T-cells (NFAT)-signaling pathway [37].

Table 1
The Bcl-2 family members are divided into three categories based on their intracellular function and sequence homology.

Anti-apoptotic	Pro-apoptotic		
All four BH domains (BH1–4)	Multi-domain (BH1–3)	BH3-only proteins	
Sequestrate their pro-apoptotic counterparts	Executioners	Activators	Sensitizers
Bcl-2, Bcl-XI, Bcl-w, Bfl-1 and Mcl-1	Bax and Bak	Bim and Bid	Bad, Bik, Noxa and Bmf

3. ER-to-mitochondria Ca^{2+} transfer determines cell fate

3.1. Ca^{2+} is a key factor in mitochondria-based cell fate

Mitochondrial homeostasis is essential for the regulation of bioenergetics, cell proliferation, cell death and autophagy, a survival mechanism that involves cell degradation of unnecessary cellular components in order to produce energy [7,9,17,38–41]. In fact, Ca^{2+} is a key regulator of mitochondrial homeostasis and consequently has a pivotal role in determining cell fate [41]. While moderate Ca^{2+} levels are essential for normal mitochondrial activities, mitochondrial overload of Ca^{2+} is detrimental for the morphology of this energetic organelle. High mitochondrial Ca^{2+} levels cause mitochondrial depolarization, thereby opening the mitochondrial permeability transition pore (mPTP), whose molecular nature has recently been identified as the c subunit of the F_0F_1-ATPase [42-44]. mPTP opening leads to mitochondrial swelling and MOMP, the point-of-no-return for apoptosis induction by triggering the release of cytochrome c [44]. Recently, mitochondrial Ca^{2+} overload has also been implicated in mitophagy, the selective degradation of damaged mitochondria through autophagy (as reviewed in [14]). In contrast, too low mitochondrial Ca^{2+} levels reduce ATP production, thereby leading to the activation of AMP-activated kinase and the induction of autophagy [45].

3.2. The IP_3R is an ER Ca^{2+} channel playing a central role in cell-fate decision

The Ca^{2+} transfer from the ER to the mitochondria involves the IP_3Rs and the voltage-dependent anion channel (VDAC) linked through the 75-kDa glucose-regulated protein (GRP75). This molecular bridge, as well as the presence of other ER–mitochondria tethers like the mitochondria-shaping proteins mitofusin-1 and -2, helps to establish ER/mitochondria contacts at the MAMs [9,10,46,47]. The ER-to-mitochondria Ca^{2+} transfer depends on the filling state of the ER Ca^{2+} stores as well as on the Ca^{2+}-flux properties of the IP_3R [48]. In basal conditions, constitutive low-level IP_3R-mediated Ca^{2+} release is essential to fuel mitochondria with Ca^{2+} necessary for the activity of mitochondrial enzymes like pyruvate-, α-ketoglutarate- and isocitrate-dehydrogenases [45,49,50]. Basal Ca^{2+} oscillations are responsible for the production of ATP and nicotinamide adenine dinucleotide phosphate (NADPH). Ca^{2+} also indirectly fuels bioenergetics by stimulating substrate transporters such as the ARALAR/AGC1-malate aspartate shuttle [51,52]. Previous data have shown that sensitizing the IP_3R to basal IP_3 levels, as via interaction with Bcl-Xl, promotes bioenergetics and cell survival [53,54]. Furthermore, in normal cells, the inhibition of the IP_3R-dependent Ca^{2+} fluxes induces basal autophagy as a pro-survival