The Role of BFl-1 in Cancer Unravels Inhibition Mechanism

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Abstract: The release of cytochrome c into the cytoplasm via the mitochondrial membrane represent a significant step in programmed cell death. Bcl-2 family proteins play a crucial role as they regulate mitochondrial membrane permeability to cytochrome c. Bfl-1 belongs to the antiapoptotic subfamily of Bcl-2 protein which forms inhibitory heterodimeric complex with Bid thereby preventing the role of Bid in the disruption of Bax/Bcl-X_L and Bak/Bcl-X_L heterodimeric assembly critical for Bax and Bak dimerization in membrane permeability to cytochrome c. This Bfl-1 intracellular attitude connotes one major mechanism of cellular immortality in cancer cells. Other role played by Bfl-1 includes regulation of immunity, neutrophil development, maturation of B-cells and in post allergic mast cell. Cancer cells overexpress Bfl-1. Here, we hypothesize that cancer cells also overexpress Bid due to accumulated mutation but not to a threshold commensurate with the overexpressed Bfl-1. Therefore, using inhibitors of Bfl-1 and the mimetics of Bid/BH3-only domain may represent one way to subvert apoptotic resistance in cancer. Sadly, Bfl-1 selective inhibitors remained undrugged. However, synergistic inhibition of Bfl-1 and other co-dependent proteins may also represent a major breakthrough in overcoming apoptotic resistance.
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Introduction

Apoptosis, usually induced by the p53 tumor suppressor protein, is an integral process in cell proliferation and turnover. As cells are being constantly exposed to toxic environmental conditions, which leads to their dysfunction and consequent death, new cells are being formed to compensate for the lost ones. Cells express certain proteins that regulate the rate of their proliferation and ensure that a balance is strucked between the rate of cell death (apoptosis) and cell survival. Among the mostly expressed proteins are the anti-apoptotic Bcl-2 family of proteins such as Bcl-2, Bcl-X_L, Mcl-1, Bfl-1/A1(commonly referred to as Bfl-1) that play a very pivotal regulatory roles in mitochondrial apoptotic pathway (Leverson, 2016). This family of proteins are over-expressed in cancer cells and have been reported to confer immortality on those cells by inhibiting the permeabilization of the mitochondrial outer membrane in the intrinsic apoptotic pathway rather than by increasing cancer cell proliferation (Letai, 2008).

During stress or disease state, pro-death (pro-apoptotic) proteins Bax and Bak play an obligatory critical role in cell death by oligomerizing into pores in the mitochondrial outer membrane thereby triggering the rapid release of mitochondrial cytochrome c, activating caspase 3/7 and inducing apoptosis (Guerra et al., 2017). The pro-apoptotic proteins have a critical α-helical Bcl-2 homology3 (BH3) domains which is crucial to their roles as pro-death proteins. These proteins only oligomerize when activated by Bid and Bim, members of the activator BH3-only subclass of Bcl-2 family proteins. Bfl-1 and its paralogs Bcl-2, Bcl-xL, Bcl-w, and Mcl-1 are structurally homologous and promote the survival of cancer cells by binding and sequestering activator proteins Bim and Bid, thereby preventing the activation of Bax and Bak. Yecies and co-workers showed in their work that Bcl-2 ensures continuous survival of cancer cells by sequestrating BIM rather than Bax in resistant cells. They argued that despite the cell sensitivity to Bcl-2 antagonist ABT-737, there was no detection of Bax sequestration by Bcl-2 (Yecies, 2010). However, Bcl-2 family proteins can also bind and sequester the monomeric forms of pro-death counterparts Bax, Bak and Noxa proteins (Willis et al., 2005, Frappier et al., 2019). These pro-survival proteins have large hydrophobic surface grooves where they accommodate, bind with high affinity and trap the α-helix corresponding to the Bcl-2 homology 3 (BH3) motifs found in pro-apoptotic proteins (Willis et al., 2007, Rezaei Araghi et al., 2018).

Of the Bcl-2 family proteins, Bfl-1 is proving to be the most important because of its roles in the

pathogenesis and chemoresistance of melanomas, lymphomas and other malignancies. It has been shown to be over-expressed in those forms of cancer and provide resistance against small-molecules that inhibit other anti-apoptotic proteins (Hiraki et al., 2018). There was a dramatic up-regulation in the level of Bfl-1 protein and mRNA transcript cells that showed acquired resistance against ABT-737, a small-molecule antagonist of Bcl-2 (Yecies, 2010). Similarly, elevated level of bfl-1 mRNA has been reported in Epstein Barr virus (EBV)-immortalized B-cell lines and Burkitt's lymphoma cell lines expressing the full spectrum of EBV latent proteins (D'Souza et al., 2000). D'Souza and co. demonstrated that the tetracycline-induced expression of EBV latent membrane protein 1 (LMP1) raised the level of bfl-1 mRNA in the cell, effectively pointing to a link between the oncoprotein LMP1 and its ability to transform cellular growth. In a another study, the regulatory genes that were responsible for the survival of the leukemic cells and for the development of a chemotherapy resistant phenotype in B-cell chronic lymphocytic leukemia (B-CLL) and their data suggested Bfl-1 as important player in apoptosis suppression and therefore a future potential therapeutic target (Morales et al., 2005).

Although literature is replete with the use of stapled peptide inhibitors as a covalent target against Bcl-2 family proteins, there has been little or no success in efforts to synthesize therapeutic inhibitors against Bfl-1 proteins. Recently, small molecules, peptides and mini-proteins are being touted as a promising leads in the development of drug candidates (Rezaei Araghi et al., 2018) because, unlike other Bcl-2 proteins, the BH3-binding groove of BFL-1 has a cysteine residues that can be selectively and covalently targeted by these BH3-based stapled peptide inhibitors (Harvey et al., 2018). In this review, we considered the intracellular involvement of the Bcl-2 families in apoptosis. We then point out some of the inhibitors of this protein and the mechanism of inhibition after hypothesizing that imbalance between the expression of Bfl-1 and Bid is critical to cellular immortality in cancer. We finally delved into some other physiological roles played by Bfl-1.

BFl-1 is a Member of the Anti-apoptotic Bcl-2 Family

The Bfl-1/A1 protein was cloned initially from the bone marrow as a granulocyte macrophage colony stimulating factor-inducible Bcl-2-related gene (Lin et al., 1993). Although the human paralogue has been reportedly cloned, but its production seems to be limited to bone marrow, lymphoid organs, leukocytes and lungs (Choi et al., 1995; Karsan et al., 1996; Kenny et al., 1997; Zong et al., 1999). Bfl-1 protein is a member of the six characterized antiapoptotic Bcl-2 family including Bcl-2, Bcl-X_L, Mcl-1, Bcl-W, Bfl-1, and Bcl-B (Wang et al., 2000, Reed., 1998). Bfl-1 is a direct transcriptional target of nuclear factor-kappa B (NF-kB) (Wang et al., 1999; Zong et al., 1999), Researchers (Cheng et al., 2000, Kitada et al., 2003) have shown that the anti-apoptotic Bcl-2 family proteins including Bfl-1 are over expressed in many cancers. This over expression is responsible for tumor progression and resistance to chemotherapeutic drugs (Morales et al., 2005). Drugs or compounds that can inhibit Bfl-1 can therefore compromise tumorigenesis.

The Bcl-2 family is the delicate controller of apoptosis by critically mediating the release of mitochondrial cytochrome c (Desagher and Martinou, 2000). Importantly, The Bcl-2 family has both pro- and anti- apoptotic function and this is usually dictated by the conserved α-helical domain (s) peculiar to each protein subfamily. This domain is called the **B**cl-2-**H**omology (BH) domain. There are four domains (BH1, BH2, BH3 and BH4) concertedly conserved in the anti-apoptotic Bcl-2 family. However, there are two classes of the pro-apoptotic protein: the multidomain pro-apoptotic protein contains three conserved domains (BH1, BH2 and BH3) and the second; BH3-only which contain only the BH3 conserved α-helical domain (Table 1). It has been long known that the pro-apoptotic protein lacks the BH4 domain. Nevertheless, it appears that almost all the Bcl-2 family members contain a transmembrane (TM) domain (Gross et al., 1999; Scorrano and Korsmeyer, 2003).

S/N	Protein Family	Sub-family	Members	Conserved Domains
1.	Anti-apoptotic	-	Bcl-x_L, Bcl-W, Mcl-1, A1/Bfl-1, Boo/Diva, Nr-13	BH1, BH2, BH3, BH4, TM
2.	Pro-apoptotic	Multidomain	Bax, Bak, Bok/Mtd	BH1, BH2, BH3, TM
3.	Pro-apoptotic	BH3-Only	Bid, Bad, Bik/Nbk, Blk, Hrk, Bim/Bod, Bnip3, Nix, Noxa	BH3

Table 1 is showing the classification of the Bcl-2 family proteins. The anti-apoptotic Bcl-2 protein family contains four conserved BH domains (BH1, BH2, BH3 and BH4) and a transmembrane (TM) domain. The multidomain subfamily of the pro-apoptotic Bcl-2 family contain three consensus BH domains (BH1, BH2 and BH3) and a transmembrane (TM) domain while the 'BH3-only' subfamily domain of the proapoptotic Bcl-2 family contains only a conserved BH3 domain. However, some members of the BH3-only subfamily contain a transmembrane (TM) domain (Gross *et al*., 1999; Scorrano and

Korsmeyer, 2003). The BH3 domain is uniquely conserved in all the Bcl-2 protein family members.

The release of cytochrome c from the mitochondria is the hallmark of apoptotic potentiation. Multiple studies have demonstrated the critical involvement of Bax and Bak (a multidomain subfamily of the Bcl-2) proapoptotic protein in the release of cytochrome c. The efficient participation of Bid and Bad in the permeabilization of the mitochondrial inner membrane has been elucidated (Chang et al., 1999; Kelekar et al., 1997; O'Connor et al., 1998; Puthalakath et al., 2001; Vanderheiden et al., 1999). It is now known that the exit of cytochrome c from the mitochondria into the cytoplasm activates the caspase-9 cascade by forming an oligomeric complex with the apoptome initially formed by Apaf-1 (Apoptotic protease-activating factor 1) and caspase-9. Activated caspase-9 activates its downstream effectors; caspase-3 and caspase-7 culminating into the apoptotic pathway. Studies on cells lacking cytochrome c, Apaf-1 and caspase-9 showed compromise in apoptosis thereby reinforcing their involvement in apoptotic pathway (Kuida et al., 1998; Li et al., 2000; Yoshida et al., 2000; Zong et al., 2001).

Furthermore, an unactivated Bax and Bak is usually in an inhibitory assembly with the anti-apoptotic Bcl-2 proteins which therefore prevent their overall activity in mitochondrial membrane permeability to cytochrome c. Molecular dynamic simulations revealed that Bfl-1 contains stable helical segment in its N-terminal region which have high affinity for Bid BH3 peptides (Modi et al., 2013). This coupled with an immunoprecipitaion procedure that utilized anti-Bid serum and also showed efficient complex formation between Bfl-1 and Bid (Arlette et al., 2002) suggest that, Bfl-1 anti-apoptotic activity is fundamental to its ability to selectively bind BH3-only domain subfamily protein Bid, as oppose other Bcl-2 antiapoptotic proteins. The same immunoprecipitation procedure showed that Bfl-1 neither form complex with ant-Bax nor anti-Bak sera. This may be due to the fact that Bfl-1 proteins do not contain a C-terminal hydrophobic anchor akin to other antiapoptotic Bcl-2 family (Kelekar and Thompson, 1998). Although this sequence has been shown to be crucial for membrane localization, however, more work is needed to know whether the localization of Bfl-1/Bid heterodimer to the membrane is critical for cellular survival.

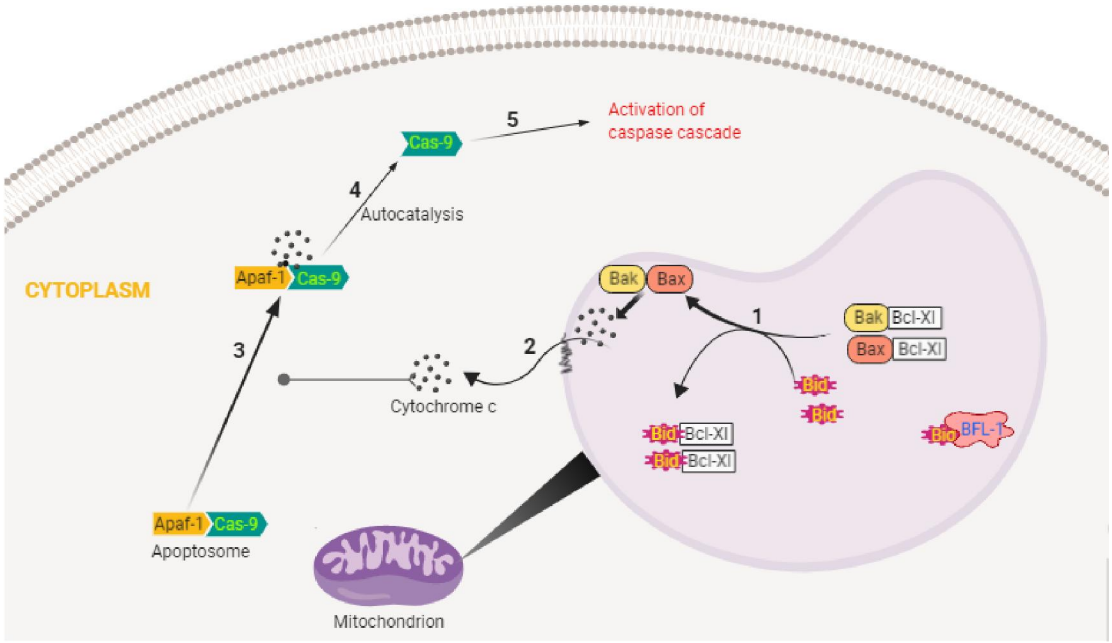

Figure 1 shows the intracellular perspective of apoptotic process and the role of Bfl-1. This protein sequesters Bid and inhibit its participatory role in programmed cell death. (1) free Bid displaces Bak and Bax from the inhibitory heterodimeric assembly of Bak/Bcl-X_L and Bax/Bcl-X_L leading to dimerization of Bax and Bak as well as formation of new inert heterodimeric assembly of Bid/Bcl-X_L. The dimerization of Bax and Bak is critical to mitochondrial membrane alteration and permeability. (2) the permeabilization of mitochondrial membrane allows the exit of cytochrome c into the cytoplasm. (3) furthermore, cytochrome c participate in an oligomeric association with Apaf-1/caspase-9 assembly (apoptosome). (4) this culminate to the autocatalysis of caspase-9 from the assembly. (5) activated caspase-9 activate its downstream effectors in a caspase cascade that lead to apoptosis (Image was created by Biorender Application).

Although the displacement of Bax and Bak from Bcl-X_L heterodimeric assembly is not the only model proposed for the involvement of Bid, however, we focused on this model because of the inhibitory association of Bfl-1 with Bid. We hypothesize that, there is a balance between Bid and Bfl-1 expression in normal cell especially during growth and development. On the contrary, disease conditions or when the DNA is damaged beyond repair, there may be upregulation of Bid so that the balance between Bid and Bfl-1 is upset in favour of Bid. It may be the outnumbered amount of overexpressed Bid that participates in apoptosis. Again, we hypothesize that, overexpression of Bfl-1 in cancer cell also shift the balance between Bfl-1 and Bid. In cancer cells, there may also be overexpression of Bid due to accumulated mutation, but we propose that the overexpression of Bid in cancer cell is not commensurate with the sustained upregulation of Bfl-1 so that Bid is continually arrested by Bfl-1. It is from this point of view that we propose a probable intracellular/mechanistic effect of Bfl-1 inhibitors in cancer cells. Therefore, potent Bfl-1 inhibitors will avail adequate Bid that can ensure persistent apoptosis of cancer cell. Also, we suggest that, peptide mimicks of bid may help to arrest the proliferation of cancer cell. Firstly by binding to the upregulated Bfl-1 and secondly by disrupting the assembly of Bak/Bcl-X_L and Bax/Bcl-X_L.

Bfl-1 Inhibitors and Inhibition Mechanism

G Brien and his co-researchers (G Brien et al., 2007) for the first time demonstrated that Bfl-1 is an essential protein for survival of malignant B cells where they showed that Bfl-1 silencing in a drug resistant lymphoblastoid B-cell line potently induces their apoptosis by chemotherapeutic drugs such as doxorubicin and cisplatin. This suggests that Bfl-1 may represent a potential target for future drug development. Therefore, inhibition of the activities of Bfl-1 represents a promising strategy for designing new classes of chemotherapeutic drug that can target the resistance of cancer cells towards currently available chemotherapeutics (Azmi et al., 2011). This part of this review will focus on various inhibitors of Bfl-1 protein and the mechanism of inhibition.

A strategy for the inhibition of Bfl-1 and other antiapoptotic Bcl-2 family proteins is based on mimicking the actions of endogenous inhibitors that binds them and pro-apoptotic BH3 helices. (Oltersdorf et al., 2005; Reed 2002). X-ray crystallographic study and nuclear magnetic resonance (NMR) spectroscopy used to study the structure of the Bcl-2 family protein elucidated that a hydrophobic crevice/hollow is present on their surfaces which binds the BH3 domain of pro-apoptotic family members. (Muchmore et al., 1996). Reed (2000) showed that the BH3 domain is a protein motif consisting of 16-25 amino-acid amphipathic alpha helix. Thus, molecules that mimic the BH3 domain or other peptides corresponding to the BH3 domain may be effective in inhibiting Bfl-1 and other antiapoptotic Bcl-2 proteins (Sattler et al., 1997).

Several compounds that compete with BH3 peptides preventing their binding to the apoptotic Bcl-2 family proteins have been identified. An example is Venetoclax, which have been approved by the Drug Administration (FDA) as a potent inhibitor of BCL-2 family proteins. It has been demonstrated to be clinically efficient in triggering apoptosis in BCL-2-dependent cancers cells (Roberts et al., 2016). A promising compound discovered at the Abbott Laboratories, ABT-737, a BH3 mimetic, inhibits Bcl-2 family (van Delft et al., 2006), this compound was discovered by using structure–activity relationship aided by nuclear magnetic resonance strategy (Oltersdorf et al., 2005). However, ABT-737 binds poorly with Bfl-1 as it is fairly specific to Bcl-2, Bcl-X_L and Bcl-w (Oltersdorf et al., 2005). Few compounds which have Bcl-2 family protein's inhibitory potential have been identified to inhibit Bfl-1 protein.

Earlier researchers have reported the inhibitory potential of Gossypol on Bcl-2 proteins through its BH3 mimicking property (Kitada et al., 2003, Mohammad et al., 2005, Wang et al., 2006). Gossypol is a natural compound isolated from the seeds and roots of cotton (Wang et al., 2009). It is known to be the first compound to reach clinical trials (Bushunow et al., 1999; Meng et al., 2008). Although, a more potent compound derived from gossypol, Apogossypol, has been designed (Wei et al., 2009). And this derived compound was found to have superior efficacy compared to Gossypol and also possessing markedly reduced toxicity (Kitada et al., 2008). However, Zhai and his colleagues in 2006 found that Gossypol and Apogossypol displayed little binding affinity for Bfl-1 protein when treated against the BH3 peptide using fluorescence polarization assays (FPAs) (Zhai et al., 2006).

In a compound screening for inhibitors of Bfl-1 protein, Gambonic acid was found as a lead compound. (Zhai et al.,2008). Gambonic Acid is a natural compound which was derived from gamboges resin of the Garcinia hanburyi tree. Interests on the compound developed when it was shown to have cytotoxic activity against tumor cell lines in culture (Zhang et al., 2004). Similar study used Bfl-1 as a target for screening the inhibitory activities of Gambonic acid and other natural products on the Bcl-2 family proteins (Zhai et al., 2008). They found that Gambonic Acid displaced BH3 peptides from Bfl-1 in

a fluorescence polarization assay. Further analysis showed that it inhibits all Bcl-2 family proteins.

Recently, using competitive binding of peptides comprising the BH3 domain to anti-apoptotic Bfl-1 in a high throughput screening of 66,000 compounds, N-aryl maleimides have been identified as potential Bfl-1 inhibitors (Cashman et al., 2010). Similarly, in 2011, sulfonylpyrimidine series compounds together with N-aryl maleimides were identified as Bfl-1-specific inhibitors using fluorescence polarization assay (FPA) and a secondary assay based on time-resolved fluorescence resonance energy transfer (TR-FRET) (Zhai et al., 2011).

Again, of all chemical inhibitors of Bcl-2 family proteins described in the literature thus far, only few natural or synthetic compounds that have biologically relevant affinity to selectively bind and inhibit Bfl-1 have been reported (Konopleva et al., 2006). A presumed difference in the pocket on Bfl-1 that binds BH3 peptides compared to other Bcl-2 proteins may be responsible for its limited inhibitors reported in literature. Hence, developing more selective inhibitors of BFL-1 has become high priority goal for researchers (Haq et al., 2013; Yecies et al., 2010). Precision targeting of Bfl-1 and ATM-codependency revealed that, synergestic inhibition of Bfl-1 and ATM subverted apoptotic resistance in cancer cell (Guerra *et al*., 2018).

Other Physiological Role of Bfl-1

Compelling evidences about the role of Bfl-1 is coalescing in literature including their roles in post allergic mast cells, cellular survival, maturity of B-cell and regulation of immunity (Karsan et al., 1996; Tomayo and Cancro, 1998; Youle and Strasser, 2008). The role of Bfl-1 in inflammation has been elucidated albeit; there are limited evidences about the exact mechanism involved. In a particular study, constitutive expression of A1(Bfl-1) in murine vascular endothelial cell – anchored by human intercellular adhesion molecule 2 promoter – provided survival stamina against cell death caused by the proinflammatory TNF-α. However, this study further demonstrated transplant tolerance and cellular survival in Bfl-1-constitutively expressing mice who received a transplanted heart allograft. The improved survival has been tentatively correlated to the inflammatory-modification role played by the anti-apoptotic molecule (Bfl-1) in the murine's endothelial vasculature (Smyth et al., 2017). In another study, pro-inflammatory stimulation followed by in vitro Hoxb8 system of expression analysis and regulation of A1/Bfl-1 in progenitors and differentiating neutrophils demonstrated upregulation of A1/Bfl-1evidenced by the presence of low constitutive mRNA and A1/Bfl-1 protein. This shows that, A1/Bfl-1 is pivotal to the thriving and homeostatic regulation of neutrophils and neutrophil progenitors (Vier et al., 2016). Other researchers have also correlated persistent A1/Bfl-1 expression to neutorphils in a physiological meeting with pathogens (Ge et al., 2006; Sarkar et al., 2013; Schwartz et al., 2013). Gene knockout approach revealed the significance of A1/Bfl-1 in neutrophil homeostasis; gene knockout of murine A1/Bfl-1 culminated to commensurate neutrophil apoptosis (Hamasaki et al., 1998) further confirming its role in the development and regulation of neutrophil functions. Most evidences in literature supported the regulatory role of A1/Bfl-1 in normal cell, it appears that this regulatory role allows balance between cell proliferation through mitosis and cell death via apoptosis, but the exact anti-inflammatory mechanism is not well known. However, the C-terminal domain of the anti-apoptotic Bfl-1 protein has been shown to be responsible for its anti-inflammatory function in human endothelial cells (Guedes et al., 2013).

The role of Bfl-1 in mast cells has been reported. Ekoff et al (2011) reported that the antiapoptotic Bfl-1 protein is the major effector in activation-induced human mast cell survival. The research group utilized cord blood-induced mast cells and the mast cell lines were activated by FcεRI-mediated crosslinking in the presence and absence of chemical inhibitors of Bfl-1 expression and activity. Real time PCR analysis of the group without inhibitors showed FcεRI-mediated crosslinking, enhanced activation-induced survival of human mast cell and this is due to upregulation of Bfl-1. However, the use of roscovitine inhibitor diminished the viability of human mast cells although with a sustained activation. The diminished viability is due to the inhibition of Bfl-1 activity and expression in the mast cell (Ekoff et al., 2011). Constitutive knockdown experiment through RNA interference has unveiled the pivotal significance of A1/Bfl-1 protection against mast cell-mediated allergic responses. Knockdown of A1/Bfl-1 reportedly disturbed the IgE-induced inert systemic and cutaneous anaphylaxis, Bfl-1 has been essentially linked to the homeostatic regulation of connective tissue mast cell (Ottina et al., 2015). Although the overall function of the anti-apoptotic bfl-1 protein is poorly understood, expression analysis has suggested its prominent role in leukocyte development (Ottina et al., 2012). Recently, there are consistent reports of the paramount role of Bfl-1 in the various component of the immune system. One suggestive mechanism may be its ability to provide survival stamina to immunological cells.

Conclusion

The intracellular revelation of the role of Bfl-1 in apoptotic resistance seems to be Bfl-1-favoured tilt in balance between Bid and Bfl-1. Therefore as we call

on many researchers to work more on the intracellular role on Bfl-1, we also recommend that more research should be done to detect potent selective inhibitors of Bfl-1 and its codependent proteins as this may attenuate apoptotic resistance in cancer.

Disclosure Statement

No Conflicts of Interest Exist among Authors

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References

1. Alejo AM, Anna O, Fredrik C, Anders O, Mikael J, and Lyda M. Osorio. High expression of Bfl-1 contributes to the apoptosis resistant phenotype in B-cellchronic lymphocytic leukemia. Int. J. Cancer: 2005; 113: 730–737.
2. Arlette BW, Evert de Vries, Stephen GT, Ilja B, Jannie B**.** Bcl-2 Family Member Bfl-1/A1 Sequesters Truncated Bid to Inhibit Its Collaboration with Pro-apoptotic Bak or Bax. The Journal of Biological Chemistry. 2002; 277: 22781-22788.
3. Azmi AS, Zhiwei W, Philip AP, Ramzi MM, Fazlul HS. Emerging Bcl-2 inhibitors for the treatment of cancer. Expert Opin Emerg Drugs. 2011;16(1):59–70.
4. Brendan D, Martin R, Dermot W. The bfl-1 Gene Is Transcriptionally Upregulated by the Epstein-Barr Virus LMP1, and Its Expression Promotes the Survival of a Burkitt's Lymphoma Cell Line. Journal of Virology. 2000; 74(14): 6652–6658.
5. Bushunow P, Reidenberg MM, Wasenko J, et al. Gossypol treatment of recurrent adult malignant gliomas. J Neurooncol. 1999; 43:79–86.
6. Cashman JR, MacDonald M, Ghirmai S, Okolotowicz KJ, Sergienko E, Brown B, Garcia X, Zhai D, Dahl R, Reed JC. Inhibition of Bfl-1 with N-aryl Maleimides. Bioorg. Med. Chem. Lett. 2010; 20(22):6560–6564.
7. Cheng Q, Lee HH, Li Y, Parks TP, Cheng G. Upregulation of Bcl-x and Bfl-1 as a potential mechanism of chemoresistance, which can be overcome by NF-kappa B inhibition. Oncogene. 2000; 19: 4936–4940.
8. Choi SS, Park I-S, Yun JW, Sung YC, Hong S-I, Shin H-S. A novel Bcl-2 related gene, Bfl-1, is overexpressed in stomach cancer and preferentially expressed in bone marrow. Oncogene. 1995; 11: 1693–1698.
9. Derek Y, Nicole EC, Jing D, Anthony L. Acquired resistance to ABT-737 in lymphoma cells that up-regulate MCL-1 and BFL-1. Blood. 2010; 115(16).
10. Desagher S, Martinou JC. Mitochondria as the central control point of apoptosis**.** Trends Cell Biol. 2000; 10:369–377.
11. Edward PH, Hyuk-SooSeo, Rachel MG, Gregory HB, Sirano D, Loren DW. Structural basis for selective therapeutic targeting of BFL-1 in cancer by a covalent stapled peptide inhibitor [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2018; 2018 Apr 14-18; Chicago, IL. Philadelphia (PA): AACR; Cancer Res. 2018; 78(13 Suppl): Abstract nr 1901.
12. Ekoff M., Nilsson G. Mast cell apoptosis and survival. Adv. Exp. Med. Biol. 2011; 716: 47–60.
13. Frappier V, Jenson JM., Zhou J, Grigoryan G, Keating AE. Tertiary Structural Motif Sequence Statistics Enable Facile Prediction and Design of Peptides that Bind Anti-apoptotic Bfl-1 and Mcl-1. Structure. 2019.
14. Ge Y, Rikihisa Y. Anaplasma phagocytophilum delays spontaneous human neutrophil apoptosis by modulation of multiple apoptotic pathways. Cell Microbiol 2006; 8: 1406–1416.
15. Gross A, Yin XM, Wang K, Wei MC, Jockel J, Milliman C, Erdjument BH, Tempst P, Korsmeyer SJ. Caspase Cleaved BID Targets Mitochondria and Is Required for Cytochrome c Release, while BCL-X_L Prevents This Release but Not Tumor Necrosis Factor-R1/Fas Death. J. Biol. Chem. 1999; 274:1156-1163.
16. Guedes RP, Rocha E, Mahiou J, Moll HP, Arvelo MB, Taube JM, Peterson CR, Kaczmarek E, Longo CR, da Silva CG, Ferran C. The C-terminal domain of A1/Bfl-1 regulates its anti-inflammatory function in human endothelial cells. Biochim Biophys Acta. 2013; 833(6):1553-61.
17. Hamasaki A, Sendo F, Nakayama K, Ishida N, Negishi I, Nakayama K et al. Accelerated neutrophil apoptosis in mice lacking A1-a, a subtype of the bcl-2-related A1 gene. J Exp Med. 1998; 188: 1985–1992.
18. Haq R, Yokoyama S, Hawryluk EB, Jo¨ nsson GB, Frederick DT, McHenry K, Porter D, Tran TN, Love KT, Langer R et al. BCL2A1 is a lineage-specific antiapoptotic melanoma oncogene that confers resistance to BRAF inhibition. Proc. Natl. Acad. Sci. USA. 2013; 110: 4321–4326.

19. Hiraki M, Maeda T, Mehrotra N, Jin C, Alam M, Bouillez A, Hata T, Tagde A, Keating, A, Kharbanda S et al. Targeting MUC1-C suppresses BCL2A1 in triple-negative breast cancer. Signal Transduct. Target. Ther. 2018; 3: 13.
20. Karsan A, Yee E, Kaushansky K, Harlan JM. Cloning of human Bcl-2 homologue: inflammatory cytokines induce human A1 in cultured endothelial cells. Blood 1996; 87(8):3089–96.
21. Kelekar A, Chang BS, Harris MH, Harlan JE, Fesik SW, Thompson CB. The BH3 domain of Bcl-xS is required for the inhibition of the anti-apoptotic function of Bcl-xL. Mol. Cell. Biol. 1999; 19: 6673-6681.
22. Kelekar A., Thompson C. B. Bcl-2 family proteins: the role of the BH3 domain in apoptosis. Trends Cell Biol. 1998; 8:324–330.
23. Kenny JJ, Knobloch TJ, Augustus M, Carter KC, Rosen CA, Lang JC. (1997). GRS, a novel member of the Bcl-2 gene family, is highly expressed in multiple cancer cell lines and in normal leukocytes. Oncogene. 1997; 14: 997–1001.
24. Kitada S, Kress CL, Krajewska M, Jia L, Pellecchia M, Reed JC. Bcl-2 antagonist apogossypol (NSC736630) displays single agent activity in Bcl-2-transgenic mice and has superior efficacy with less toxicity compared with gossypol (NSC19048). Blood. 2008; 111: 3211–3219.
25. Kitada S, Leone M, Sareth S, Zhai D, Reed JC, Pellecchia M. Discovery, characterization, and structure activity relationships studies of proapoptotic polyphenols targeting B-cell lymphocyte/leukemia2 proteins. J. Med. Chem. 2003; 46: 4259–4264.
26. Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi YX, Sneed T et al. Mechanisms of Apoptosis Sensitivity and Resistance to the BH3 Mimetic ABT-737 in Acute Myeloid Leukemia. Cancer Cell. 2006; 10(5): 375–388.
27. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, P. Rakic P, Flavell RA. Reduced apoptosis and cytochrome C-mediated caspase activation in mice lacking Caspase 9. Cell, 1998; 94: 325-337.
28. L. O'Connor A, Strasser LA, O'Reilly G, Hausmann JM, Adams S, Cory DC, Huang. Bim: a novel member of the Bcl‐2 family that promotes apoptosis. EMBO J. 1998; 17: 384-395.
29. Letai AG. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. Nat Rev Cancer. 2008; 8(2): 121-132.
30. Leverson A. New Staple: Peptide-Targeted Covalent Inhibitors, Cell Chemical Biology. chembiol. 2016.
31. Li K, Li Y, Shelton JM, Richardson JA, Spencer E, Chen ZJ, Wang X, Williams RS. Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis.
32. Cell. 2000; 101: 389-399.
33. Lin EY, Orlowsky A, Berger MS, Prystowsky MB. Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to bcl-2. J. Immunol. 1993; 151: 1979–1988.
34. Meng Y, Tang W, Dai Y, Wu X, Liu M, Ji Q, Ji M, Pienta K, Lawrence T, Xu L. Natural BH3 mimetic (-)-gossypol chemosensitizes human prostate cancer via Bcl-xL inhibition accompanied by increase of Puma and Noxa. Mol. Cancer Ther. 2008; 7: 2192–2202.
35. Modi V, Lama D, Sankararamakrishnan R. Relationship between helix stability and binding affinities: molecular dynamics simulations of Bfl-1/A1-binding pro-apoptotic BH3 peptide helices in explicit solvent. J Biomol Struct Dyn. 2013; 31(1): 65-77.
36. Mohammad RM, Wang S, Aboukameel A, Chen B, Wu X, Chen J, Al-Katib A. Preclinical studies of a nonpeptidic small-molecule inhibitor of Bcl-2 and Bcl-X (L) [(-)-gossypol] against diffuse large cell lymphoma. Mol. Cancer Ther. 2005; 4: 13–21.
37. Morales AA, Olsson A, Celsing F, Osterborg A, Jondal M, Osorio LM. High expression of bfl-1 contributes to the apoptosis resistant phenotype in B-cell chronic lymphocytic leukemia. Int J Cancer 2005; 113: 730–737.
38. Muchmore, SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong SL, Ng SL, Fesik SW. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature. 1996; 381: 335–341.
39. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005; 435: 677–681.
40. Ottina E, Lyberg K, Sochalska M., Villunger A, Nilsson PM. Knockdown of the antiapoptotic Bcl-2 family member A1/Bf-1 protects mice from anaphylaxis. J Immunol. 2015; 194(3): 1316 – 1322.

41. Ottina E, Tischner D, Herold MJ, Villunger A. A1/Bfl-1 in leukocyte development and cell death. Exp Cell Res. 2012; 318(11): 1291-303.
42. Puthalakath H, Villunger A, O'Reilly LA, Beaumont JG, Coultas L, Cheney RE, Huang DC, Strasser A. Bmf: A Proapoptotic BH3-Only Protein Regulated by Interaction with the Myosin V Actin Motor Complex, Activated by Anoikis. Science. 2001; 293: 1829-1832.
43. Rachel MG, Annissa JH, Edward PH, Gregory HB, Loren DW. Reactivating apoptosis in BFL-1/A1 driven cancer with cysteine-reactive stapled peptide inhibitors [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2017; 2017 Apr 1-5; Washington, DC. Philadelphia (PA): AACR; Cancer Res 2017;77(13 Suppl): Abstract nr 2024. doi:10.1158/1538-7445.AM2017-2024
44. Rachel MG, Gregory HB, Edward PH, Neekesh VD, Kyle JK, Michelle SP, Kimberly S, Loren DW. Precision Targeting of BFL-1/A1 and an ATM Co-dependency in Human Cancer. Cell Rep. 2018; 24(13): 3393–3403.
45. Reed JC. Apoptosis-based therapies. Nat. Rev. Drug Discov. 2002; 1: 111–121.
46. Reed JC. Bcl-2 family proteins. Oncogene. 1998; 17: 3225–3236.
47. Rezaei Araghi R, Bird GH, Ryan JA., Jenson JM, Godes M, Pritz, JR, Grant RA, Letai A, Walensky LD, Keating AE. Iterative optimization yields Mcl-1-targeting stapled peptides with selective cytotoxicity to Mcl-1-dependent cancer cells. Proc. Natl. Acad. Sci. U S A. 2018; 115: E886–E895.
48. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, Kipps TJ, Anderson MA, Brown JR, Gressick L et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N. Engl. J. Med. 2016; 374: 311–322.
49. Sarkar A, Aga E, Bussmeyer U, Bhattacharyya A, Moller S, Hellberg L et al. Infection of neutrophil granulocytes with Leishmania major activates ERK 1/2 and modulates multiple apoptotic pathways to inhibit apoptosis. Med Microbiol Immunol 2013; 202: 25–35.
50. Sattler M, Liang H, Nettesheim D, Meadows RP, Harlan JE, Eberstadt M, Yoon HS, Shuker SB, Chang BS, Minn AJ, Thompson CB, Fesik SW. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. Science. 1997; 275: 983–986.
51. Schwartz JT, Bandyopadhyay S, Kobayashi SD, McCracken J, Whitney AR, Deleo FR et al. Francisella tularensis alters human neutrophil gene expression: insights into the molecular basis of delayed neutrophil apoptosis. J Innate Immun 2013; 5: 124–136.
52. Smyth LA, Meader L, Woodward M, Brady HJ, Lechler R, Lombardi G. Constitutive expression of the anti-apoptotic Bcl-2 family member A1 in murine endothelial cells leads to transplant tolerance. Clin Exp Immunol. 2017; 188(2): 219 – 225.
53. Tomayko MM, Cancro MP. Long-lived B cells are distinguished by elevated expression of A1. J Immunol 1998; 160(1): 107–11.
54. Van Delft MF, Wei AH, Mason KD et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell. 2006; 10: 389–99.
55. Vander Heiden M, Chandel NS, Schumacker PT, Thompson CB. Bcl-x (L) prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange.
56. Mol. Cell. 1999; 3: 159-167.
57. Vier J, Groth M, Sochalska M, Kirschnek S. The anti-apoptotic Bcl-2 family protein A1/Bfl-1 regulates neutrophil survival and homeostasis and is controlled via p13k and Jak/Stat signaling. Cell Death Dis. 2016; 7(2); e2013.
58. Wang CY, Guttridge DC, Mayo MW, Baldwin Jr AS. NF-kappa B induces expression of the Bcl-2 homologue A1/ Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. Mol Cell Biol. 1999; 19: 5923–5929.
59. Wang JL, Liu D, Zhang ZJ, Shan S, Han X, Srinivasula SM, Croce CM, Alnemri ES, Huang Z. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. Proc. Natl. Acad. Sci. U.S.A. 2000; 7: 7124–7129.
60. Wang S, Nikolovska-Coleska Z, Yang C.-Y, Chen J. Apogossypolone and the Uses Thereof. PCT Int. Appl, WO 2006050447 A2 20060511. 2006.
61. Wang X, Howell CP, Chen F et al. Gossypol – polyphenolic compound from cotton plant. Adv Food Nutr Res. 2009; 58: 215–63.
62. Wei J, Kitada S, Rega MF, Emdadi A, Yuan H, Cellitti J, Stebbins JL, Zhai D, Sun J, Yang L, Dahl R, Zhang Z, Wu B, Wang S, Reed TA, Lawrence N, Sebti S, Reed JC, Pellecchia M. Apogossypol derivatives as antagonists of antiapoptotic Bcl-2 family proteins. Mol. Cancer Ther. 2009; 8: 904–913.
63. Willis SN, Chen L, Dewson G, et al. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev. 2005; 19(11): 1294-1305.

64. Willis SN, Fletcher JI, Kaufmann T, et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science. 2007; 315(5813): 856-859.

65. Yecies D, Carlson NE, Deng J, Letai A. Acquired resistance to ABT-737 in lymphoma cells that up-regulate MCL-1 and BFL-1. Blood 2010; 115: 3304–3313.

66. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW. Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell, 1998; 94: 739-750.

67. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate celldeath. Nat Rev Mol Cell Biol. 2008; 9(1): 47–59.

68. Zhai D, Jin C, Shiau C, Kitada S, Satterthwait AC, Reed JC. Gambogic acid is an antagonist of antiapoptotic Bcl-2 family proteins. Mol Cancer Ther. 2008; 7: 1639-1646.

69. Zhai D, Jin C, Satterthwait AC, Reed JC. Comparison of chemical inhibitors of antiapoptotic Bcl-2 family proteins. Cell Death Differ. 2006; 13: 1419-21.

70. Zhai D, Godoi P, Sergienko E, Dahl R, Chan X, Brown B, Rascon J, Hurder A, Su Y, Chung D. Y, Jin C, Diaz P, Reed JC. High Throughput Fluorescence Polarization Assay for Chemical Library Screening against Anti- Apoptotic Bcl-2 Family Member Bfl-1. Journal of Biomolecular Screening. 2011; 17(3): 350–360.

71. Zhang HZ, Kasibhatla S, Wang Y, et al. Discovery, characterization and SAR of gambogic acid as a potent apoptosis inducer by a HTS assay. Bioorg Med Chem. 2004; 12: 309 – 17.

72. Zong WX, Edelstein LC, Chen C, Bash J, Gelinas C. The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-kappa B that blocks TNFalpha induced apoptosis. Genes Dev. 1999; 13: 382–387.

73. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. Bmf: A Proapoptotic BH3-Only Protein Regulated by Interaction with the Myosin V Actin Motor Complex, Activated by Anoikis. Genes Dev. 2001; 15: 1481-1486.

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