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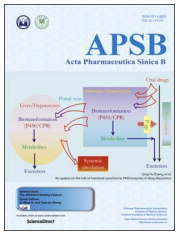

REVIEW

How to unleash mitochondrial apoptotic blockades to kill cancers?

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KEY WORDS

Apoptosis;
BCL-2 family;
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Abstract Apoptosis, especially the intrinsic mitochondrial cell death pathway, is regulated by the BCL-2 family of proteins. Defects in apoptotic machinery are one of the main mechanisms that cells employ to evade cell death and become cancerous. Targeting the apoptotic defects, either by direct inhibition of BCL-2 family proteins or through modulation of regulatory pathways, can restore cell sensitivity to cell death. This review will focus on the aspects of BCL-2 family proteins, their interactions with kinase pathways, and how novel targeted agents can help overcome the apoptotic blockades. Furthermore, functional assays, such as BH3 profiling, may help in predicting responses to chemotherapies and aid in the selection of combination therapies by determining the mitochondrial threshold for initiating cell death.

Abbreviations: ASH, American Society of Hematology; ATAP, amphipathic tail-anchoring peptide; BAD, BCL-2-associated death promoter protein; BAK, BCL-2 homologous antagonist killer; BAX, BCL-2-associated X protein; BCL-2, B-cell lymphoma 2; BCL-w (BCL2L2), BCL-2-like protein 2; BCL-xL, B-cell lymphoma X long; BFL-1 (BCL2A1), BCL-2-related protein A1; BCR, B-cell receptor; BH3, BCL-2 homology 3; BID, BH3 interacting domain death agonist; BIK, BCL-2-interacting killer; BIM, BCL-2-interacting mediator of cell death; BOK, BCL-2 related ovarian killer; BTK, Bruton's tyrosine kinase; CDK, cyclin-dependent kinase; CLL, chronic lymphocytic leukemia; CHOP, cyclophosphamide, hydroxydaunorubicin, oncovin-vincristine and prednisone; CML, chronic myelogenous leukemia; CR, complete response;EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FDA, U. S. Food and Drug Administration; GSK-3, glycogen synthase kinase-3; ITK, interleukin-2-inducible T-cell kinase; MCL, myeloid cell leukemia; MOMP, mitochondrial outer membrane permeabilization; NHL, non-Hodgkin lymphoma; NIH, National Institutes of Health; NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol-3-kinase; PUMA, p53 up-regulated modulator of apoptosis; SLL, small lymphocytic lymphoma; T-ALL, T-acute lymphocytic leukemia

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1. Targeted therapies fueled by the discovery of cancer genome and signaling webs

In 2003, the human genome project was pronounced completed, after thirteen years of U. S. National Institutes of Health (NIH) leading multi-national research efforts. The "finish" period has continued since the final version of sequences was published in the journal Nature in 2004[1], and more detailed information for the human genome has yet to be revealed. With the evolutionary development of sequencing and computing technology, which lead to a drastic decrease in costs for sequencing (from 100 million to 1000 dollars per genome[2]), and possible interpretation of the results, the focus of current DNA sequencing is naturally oriented towards human diseases. And cancer is one of them; the discovery of important gene variants/mutations in cancer genome has changed treatment regimens and benefits many cancer patients. The most exciting examples are mutations uncovered in epidermal growth factor receptor (EGFR) and BRAF in lung cancer[3] and melanoma[4], respectively.

In parallel, proteomics is expanding its web to the most of the signaling networks governing our physiological functions. New nodes and loops continue to be discovered, and novel interventions are being tested and exploited in disease treatments. In 2015, the U. S. Food and Drug Administration (FDA) approved 45 novel drugs, including four used to treat multiple myeloma[5]. About a decade ago, most chemotherapies were limited to conventional pan-cytotoxic drugs that targeted DNA or microtubules such as the CHOP regimen (cyclophosphamide, hydroxydaunorubicin, oncovin-vincristine and prednisone), which cured millions of people of otherwise fatal diseases, but also were accompanied by severe side effects due to damage to healthy tissues. At present, targeted agents, newly approved or in clinical trials, are more precise and bring the overall response rates to much higher levels than before. This is true even for subgroups with adverse prognostic features and those who respond poorly to conventional therapies, especially in hematological cancers demonstrated at the annual meeting of American Society of Hematology—ASH 2015 (Orlando, Florida, USA). In April, 2016, following promising activity in its clinical trials[6,7], the selective BCL-2 inhibitor venetoclax (AbbVie) was approved by the FDA[8] for chronic lymphocytic leukemia (CLL) with chromosomal abnormalities, and it becomes the first-in-class direct inhibitor targeting BCL-2 family proteins that has got approved. With so many novel agents and biomarkers emerging, there is no doubt that cancer research and therapies have entered a new era, in which hopefully more cancers will become manageable in the near future.

Regardless of their categorization as conventional or targeted therapies, most of chemotherapies kill cancer cells *via* the apoptotic cell death pathway. In this review, the focus will be on research progress made on this intrinsic mitochondrial cell death pathway regulated by B-cell lymphoma 2 (BCL-2) family proteins; on targeted therapies that have been developed to intervene in its dysfunction in cancers; and how we can use BH3 profiling, a functional assay measuring mitochondrial priming, to predict patient responses and provide guiding information for potential combination therapies.

2. Apoptosis is regulated by BCL-2 family proteins

Apoptosis, which includes both extrinsic and intrinsic pathways, is one of the most important forms of cell death in multicellular organisms. The intrinsic cell death pathway is regulated mostly by BCL-2 family proteins residing in or recruited to the mitochondria after death insults imposed on cells[9,10]. The BCL-2 family comprises both anti- and pro-apoptotic proteins. Anti-apoptotic proteins include at least BCL-2, BCL-xL, MCL-1, BCL-w and BFL-1. High expression of anti-apoptotic proteins, especially BCL-2[11–14], BCL-xL[15] and MCL-1[16–20], has been shown in various types of cancers, and they play important roles in tumorigenesis in different tumor models [9,21–25]. Pro-apoptotic proteins can be further divided into two subgroups, including multi-domain proteins, like the death effectors/executioners BAX and BAK; and BH3-only proteins, like activators BIM, BID and PUMA, or sensitizers including BAD, NOXA, HRK and BMF. Recently, BOK, a non-canonical BCL-2 family effector of apoptosis, has been shown to mediate cell death triggered by endoplasmic reticulum (ER)-associated degradation independent of BAX and BAK, or when BAX/BAK are absent and cells are overwhelmed by unfolded proteins[26].

The interactions within the BCL-2 family members are complex, and the interplay of anti- and pro-apoptotic proteins determines cell fate (see Fig. 1). The activation of BAX, BAK or BOK (in some circumstances)[26] can lead to their oligomerization, which forms pores in the mitochondrial outer membrane and resulting the release of cytochrome *c*[27,28]. Thus mitochondrial outer membrane permeabilization (MOMP) is generally considered a point-of-no-return, and triggers downstream caspase activation, proteolysis and DNA fragmentation. Different apoptotic blockades, resulting from BCL-2 family protein interactions, in which pro-death signals were sequestered or counteracted by anti-apoptotic proteins, have been observed in cancer cells as a means for cells to evade apoptosis[29].

Apoptosis is initiated at mitochondria; however, the regulation of BCL-2 family proteins is tightly controlled by upstream signaling networks, from receptors on cell surface to transcription factors residing in the nuclei. There are usually two sets of regulations when cells face death insults. One is to upregulate pro-death signals and the other is to downregulate anti-death factors. For example, pro-apoptotic protein BIM-EL is kept low through phosphorylation (by extracellular signal-regulated kinase—ERK[30]) mediated degradation when cells are stimulated with growth signals, but its protein level can be stabilized and increased when the ERK pathway is inhibited. BIM can be induced transcriptionally by transcription factor FOXO3A[31,32] when it is translocated to nuclei after PI3K/AKT inhibition. AKT can phosphorylate BAD[33,34] and BAX[35,36], and regulate their pro-apoptotic functions. *BID*[37], *PUMA*[38], *NOXA*[39] and *BAX*[40] are targets of p53 transcription factor, and their induction by p53 in response to DNA damage or other death insults keep cells in balance between cell cycle arrest and cell death. Besides p53, PUMA can also be transcriptionally regulated by FOXO3A[41]. On the other hand, for anti-apoptotic proteins, *BCL-2*[42], *BCL-xL*[43] and *BFL-1*[44] are target genes of NF-κB signaling, which is consistent with the pro-survival function of the NF-κB pathway. The anti-apoptotic function of these proteins also can be modulated by phosphorylation[45–47]. Nevertheless, the best example of phosphorylation and its sub-sequential effects on apoptosis lies in MCL-1. This short half-life protein can be rapidly degraded *via* the proteasome pathway after phosphorylation by glycogen synthase kinase-3 (GSK-3) in the AKT pathway[48,49]. The mutation of E3 ligase FBW7 and resulting stabilization of MCL-1 protein is critical in tumorigenesis of T- acute lymphocytic leukemia

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