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AUTHOR'S VIEW

A mitochondrial checkpoint to adaptive anticancer immunity

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ABSTRACT

BCL2 robustly preserves mitochondrial integrity, hence inhibiting innate immune signaling and apoptotic cell death in several cell types. Here, we comment on our recent data demonstrating that BCL2 also limits the ability of dendritic cells to elicit adaptive immune responses, lending support to a universal immunosuppressive function for the mitochondrial immune checkpoint.

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Main text

Mammalian mitochondria contain a number of molecules that, once released in the cytosol or in the extracellular space, mediate prominent immunostimulatory functions.[1] In line with this notion, mitochondrial outer membrane permeabilization (MOMP) as regulated by the balance between pro- and antiapoptotic proteins of the Bcl-2 family[2] has been associated with the cytosolic accumulation of potentially interferogenic mitochondrial DNA (mtDNA) and/or mitochondrial RNA (mtRNA) in a number of cell types.[3,4] However, cytochrome c, somatic (CYCS) release by permeabilized mitochondria generally drives the rapid activation of apoptotic caspases via apoptotic peptidase activating factor 1 (APAF1), resulting in the engagement of numerous immunosuppressive pathways, including (but not limited to) several mechanisms that directly quench mtDNA/mtRNA-driven type I interferon (IFN) signaling.[5–8] Besides suggesting that at least part of the therapeutic effects of the FDA-approved BCL2 apoptosis regulator (BCL2) inhibitor venetoclax[9] might originate from restored anticancer immunosurveillance, these data support the notion that simultaneously boosting MOMP while inhibiting apoptotic caspase activation may establish a metastable cell state in malignant cells associated with superior immunostimulatory effects. Recent data from our team demonstrate that the antiapoptotic BCL2 also inhibits the ability of dendritic cells (DCs) to elicit adaptive immune responses,[10] lending support to a universal immunosuppressive function for the mitochondrial immune checkpoint.

Using an immortalized precursor cell line that can differentiate into immature DCs resembling conventional type 1 DCs (cDC1s), we performed an unbiased screening to uncover genes that limit antigen presentation. BCL2 emerged from this genome-wide approach as *Bcl2* deletion resulted in a gain-of-function effect. Specifically, the loss of *Bcl2* endowed cDC1s with improved antigen-presenting capabilities *in vitro*, an effect that was coupled with increased expression of co-stimulatory molecules and superior cytokine signaling. Notably, a similar phenotype could be observed upon pharmacological BCL2 inhibition with venetoclax, but venetoclax failed to improve the antigen-presenting functions of *Bcl2*$^{-/-}$ cDC1s. Moreover, superior antigen presentation as effected by cDC1s upon genetic or pharmacological inhibition of BCL2 could be reversed by blocking costimulatory DC surface molecules such as CD80 and CD86, as well as type I IFN receptors.[10] Mechanistically, BCL2 inhibition prompted the accumulation of mtDNA in the cytosol of cDC1s, culminating with type I IFN secretion downstream of cyclic GMP-AMP synthase (CGAS) and stimulator of interferon response cGAMP interactor 1 (STING1) signaling, and an autocrine/paracrine transcriptional reprogramming impinging on type I IFN receptors.[10]

Driven by these findings, we wondered whether BCL2 inhibition would elicit a broad immunostimulatory response that could boost cancer immunosurveillance *in vivo*. We found that while venetoclax and navitoclax (another pharmacological BCL2 inhibitor) fail to kill mouse fibrosarcoma MCA205 cells and lung adenocarcinoma TC1 cells *in vitro*, they robustly control the growth of MCA205 and TC1 lesions established subcutaneously in immunocompetent syngeneic hosts, unless the latter lack cDC1s, pointing to an immunological mechanism of action. In line with this notion, venetoclax improved the sensitivity of an endogenous mouse model of hormone receptor (HR)-positive breast cancer with superior translational potential[11] to radiation

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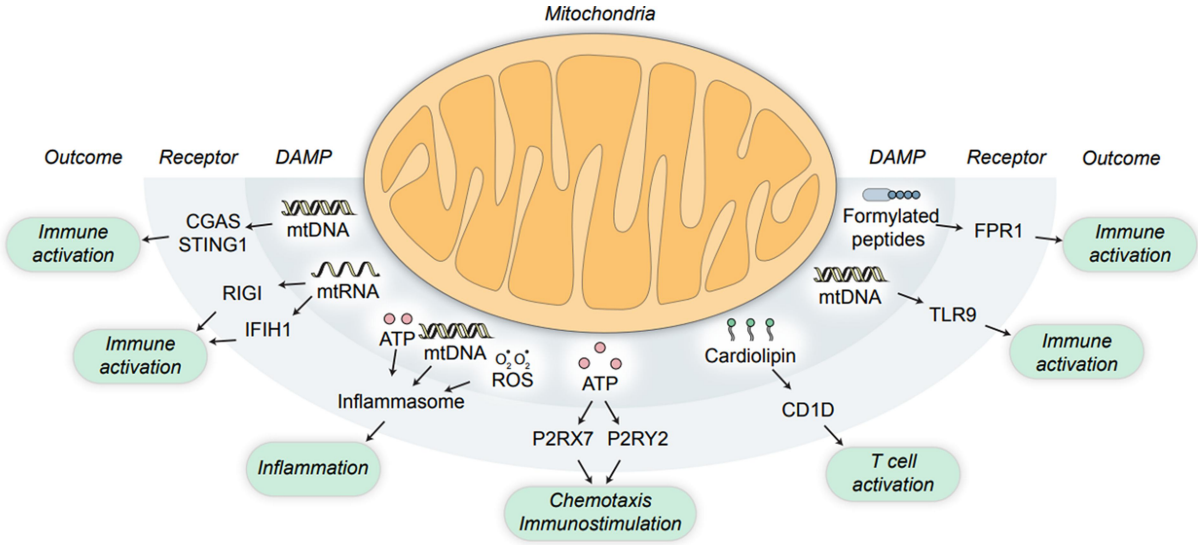

Figure 1. The mitochondrial immune checkpoint. Mitochondrial integrity as promoted by antiapoptotic members of the Bbcl-2 protein family, such as BCL2 apoptosis regulator (BCL2) itself, not only prevents the activation of apoptotic caspases downstream of cytochrome c, somatic (CYCS) accumulation in the cytosol, but also limits the spillage of damage-associated molecular patterns (DAMPs) that (either in the cytosol or in the extracellular space) mediate potent immunostimulatory effects. These molecules include (but are not limited to) ATP, mitochondrial nucleic acids, formylated peptides, reactive oxygen species (ROS) and cardiolipin. Importantly, the mitochondrial immune checkpoint is operational in a number of cell types including immune cells, *de facto* controlling both innate and adaptive immune responses. CGAS, cyclic GMP-AMP synthase; IFIH1, interferon induced with helicase C domain 1; FPR1, formyl peptide receptor 1; P2RX7, purinergic receptor P2X 7; P2RY2, purinergic receptor P2Y2; RIGI, RNA sensor RIG-I; mtDNA, mitochondrial DNA; mtRNA, mitochondrial RNA; STING1, stimulator of interferon response cGAMP interactor 1; TLR9, toll-like receptor 9.

therapy (RT), a therapeutic interaction that was entirely abrogated by type I IFN receptor blockage.[10] Moreover, venetoclax and navitoclax administered intravenously to mice were found to activate cDC1s *in vivo*, as indicated by increased expression of costimulatory factors, MHC Class II molecules and chemokine receptors. High-dimensional immunofluorescence cytometry of circulating cDC1s from patients with acute myeloid leukemia (AML) receiving venetoclax as part of their disease management confirmed the upregulation of same markers. Similar findings were obtained when venetoclax was applied to peripheral blood mononuclear cells (PBMCs) from healthy donors *ex vivo*.[10] In summary, these mouse and human data convergently demonstrate that pharmacological BCL2 inhibition activates cDC1s.

The intravenous adoptive transfer of tumor antigen-primed cDC1s mediated some antitumor activity *in vivo* in immunocompetent mice bearing established syngeneic neoplasms, an immunotherapeutic effect that could be enhanced by (1) harnessing *Bcl2*$^{-/-}$ cDC1s rather than their wild-type counterparts or (2) pretreating cDC1s with venetoclax or navitoclax.[10] This effect was particularly striking in *Batf3*$^{-/-}$ mice, which naturally lack cDC1s and hence exhibit compromised cancer immunosurveillance, but was lost in mice devoid of mature T cells. Notably, *Bcl2*$^{-/-}$ cDC1s displayed a transcriptional profile of migratory cDC1s, and actually infiltrated pulmonary neoplasms more efficiently than wild-type cDC1s.[10] BCL2 inhibition thus conferred functional advantages to cDC1s, *de facto* enhancing their capacity to stimulate immune responses against tumor-associated antigens.

The anticancer effects of BCL2 inhibitors were further amplified by immune checkpoint blockade with a programmed cell death 1 (PDCD1, best known as PD-1) inhibitor. Conversely, BCL2 inhibitors failed to mediate anticancer activity in immunosuppressed hosts, including *Batf3*$^{-/-}$ mice, mice subjected to cDC1 depletion upon repeated recombinant CYCS injections, as well as mice receiving type I IFN receptor-blocking or CD4^{+} and CD8^{+} T cell-depleting antibodies. Interestingly, venetoclax promoted the upregulation of PD-1 and other co-inhibitory receptors on CD8^{+} T cells *in vivo*, an effect that also depended on the presence of cDC1s.[10] In summary, these findings suggest that the impact of BCL2 inhibition on BCL2-independent cancers, which do not undergo apoptosis upon venetoclax administration, is largely mediated via the immune system, notably through cDC1 activation.

Taken together, these findings demonstrate that the mitochondrial immune checkpoint (Figure 1) is also operational in immune cells, hence directly impacting the activation of adaptive immune responses.[10] It is therefore tempting to speculate that BCL2 inhibitors such as venetoclax may aggravate conditions mechanistically linked with dysregulated type I IFN signaling downstream of mitochondrial instability, such as systemic lupus erythematosus.[12] This possibility, however, has not yet been formally explored. Irrespective of this and other incognita, mitochondria stand out as critical regulators of numerous mammalian (cell) functions, spanning from bioenergetic and anabolic metabolism to cell death and immunity, likely reflecting their ancient evolutionary origin as endosymbionts of early proto-eukaryotic cells.

Author contributions

GK and LG conceived the article. LG wrote the first version of the manuscript with constructive input from OK, PL and GK. OK designed display

items under supervision from GK and LG. All authors approved the submitted version of the article.

Disclosure statement

Outside of this work, OK is a scientific co-founder of Samsara Therapeutics. GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, and Vascage. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of EverImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio. GK is in the scientific advisory boards of Hevolution, Institut Servier and Longevity Vision Funds. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. GK's wife, Laurence Zitvogel, has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Pilege, Merus, Transgene, 9 m, Tusk and Roche, is on the Board of Directors of Transgene, is a cofounder of everImmune and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. LG is/has been holding research contracts with Lytix Biopharma, Promontory and Onxeo, has received consulting/advisory honoraria from Boehringer Ingelheim, AstraZeneca, OmniSEQ, Onxeo, The Longevity Labs, Inzen, Imvax, Sotio, Promontory, Noxopharm, EduCom, and the Luke Heller TECPR2 Foundation, and holds Promontory stock options.

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Data availability statement

All data discussed in this article are referenced to.

References

1. Marchi S, Guilbaud E, Tait SWG, Yamazaki T, Galluzzi L. Mitochondrial control of inflammation. Nat Rev Immunol. 2023;23(3):159–173. doi:10.1038/s41577-022-00760-x.
2. Czabotar PE, Garcia-Saez AJ. Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. Nat Rev Mol Cell Biol. 2023;24(10):732–748. doi:10.1038/s41580-023-00629-4.
3. Tigano M, Vargas DC, Tremblay-Belzile S, Fu Y, Sfeir A. Nuclear sensing of breaks in mitochondrial DNA enhances immune surveillance. Nature. 2021;591(7850):477–481. doi:10.1038/s41586-021-03269-w.
4. Yamazaki T, Kirchmair A, Sato A, Buque A, Rybstein M, Petroni G, Bloy N, Finotello F, Stafford L, Navarro Manzano E, et al. Mitochondrial DNA drives abscopal responses to radiation that are inhibited by autophagy. Nat Immunol. 2020;21(10):1160–1171. doi:10.1038/s41590-020-0751-0.
5. Rodriguez-Ruiz ME, Buque A, Hensler M, Chen J, Bloy N, Petroni G, Sato A, Yamazaki T, Fucikova J, Galluzzi L. Apoptotic caspases inhibit abscopal responses to radiation and identify a new prognostic biomarker for breast cancer patients. Oncoimmunology. 2019;8(11):e1655964. doi:10.1080/2162402X.2019.1655964.
6. Rongvaux A, Jackson R, Harman CC, Li T, West AP, de Zoete MR, Wu Y, Yordy B, Lakhani SA, Kuan CY, et al. Apoptotic caspases prevent the induction of type I interferons by mitochondrial DNA. Cell. 2014;159(7):1563–1577. doi:10.1016/j.cell.2014.11.037.
7. White MJ, McArthur K, Metcalf D, Lane RM, Cambier JC, Herold MJ, van Delft MF, Bedoui S, Lessene G, Ritchie ME, et al. Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell. 2014;159(7):1549–1562. doi:10.1016/j.cell.2014.11.036.
8. Chaintreuil P, Laplane L, Esnault F, Ghesquier V, Savy C, Furstoss N, Arcangeli ML, Cluzeau T, Robert G, Droin N, et al. Reprogramming monocyte-derived macrophages through caspase inhibition. Oncoimmunology. 2022;11(1):2015859. doi:10.1080/2162402X.2021.2015859.
9. Diepstraten ST, Anderson MA, Czabotar PE, Lessene G, Strasser A, Kelly GL. The manipulation of apoptosis for cancer therapy using BH3-mimetic drugs. Nat Rev Cancer. 2022;22(1):45–64. doi:10.1038/s41568-021-00407-4.
10. Zhao L, Liu P, Mao M, Zhang S, Bigenwald C, Dutertre CA, Lehmann CHK, Pan H, Paulhan N, Amon L, et al. BCL2 inhibition reveals a dendritic cell-specific immune checkpoint that controls tumor immunosurveillance. Cancer Discov. 2023. doi:10.1158/2159-8290.CD-22-1338.

11. Buque A, Bloy N, Perez-Lanzon M, Iribarren K, Humeau J, Pol JG, Levesque S, Mondragon L, Yamazaki T, Sato A, et al. Immunoprophylactic and immunotherapeutic control of hormone receptor-positive breast cancer. Nat Commun. 2020;11(1):3819. doi:10.1038/s41467-020-17644-0.

12. Caielli S, Athale S, Domic B, Murat E, Chandra M, Banchereau R, Baisch J, Phelps K, Clayton S, Gong M, et al. Oxidized mitochondrial nucleoids released by neutrophils drive type I interferon production in human lupus. J Exp Med. 2016;213:697–713. doi:10.1084/jem.20151876.