Mitochondrial control of IFNγ signaling

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VDAC2 enforces a mitochondrial checkpoint to cancer immunity

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Mitochondrial integrity controls cellular and immunological homeostasis in various pathophysiological settings. Recent findings suggest that VDAC2 antagonizes the ability of IFNγ to drive mitochondrial permeabilization in malignant cells by inhibiting BAK, thus promoting cell survival and immunoevasion.

Mitochondrial integrity is paramount for the survival of mammalian cells, not only because mitochondria have an irreplaceable role in bioenergetic and anabolic metabolism, but also because the irreversible permeabilization of mitochondrial membranes actively promotes intrinsic apoptosis[1]. Moreover, mitochondria contain several molecules that mediate powerful pro-inflammatory functions once released in the cytosol or the extracellular milieu (such as mitochondrial DNA (mtDNA)), suggesting that the structural breakdown of these organelles can have important inflammatory consequences, unless compensatory mechanisms are activated[2]. Accordingly, malignant cells often acquire defects in the molecular mechanisms that subvert such a mitochondrial checkpoint to cell death and immunity as a way to evade cell-intrinsic and immunological barriers to tumor progression[3]. In this issue of *Nature Immunology*, data from Yuan et al.[4] demonstrate that VDAC2, an endogenous inhibitor of mitochondrial outer membrane permeabilization (MOMP) as promoted by BAK1 (best known as BAK), is particularly relevant for cancer cells to evade immunosurveillance by resisting both the pro-apoptotic and immunostimulatory effects of IFNγ.

To identify factors involved in immune evasion, Yuan et al.[4] performed an in vivo CRISPR–Cas9 screen targeting 3,017 metabolism-associated genes in mouse B16F10 melanoma cells expressing the model antigen OVA (B16-OVA) under immunological pressure by $CD8^+$ T cells. Among several other hits, VDAC2 emerged as a previously unrecognized mediator of immune evasion. Accordingly, *Vdac2* deletion in B16-OVA cells or OVA-expressing mouse colorectal cancer MC38 (MC38-OVA) cells rendered them more sensitive to $CD8^+$ T cell cytotoxicity, both in vitro and in vivo. VDAC2 deficiency also improved tumor sensitivity to immune checkpoint inhibition, even when malignant cells were unresponsive to TNF. The lack of *Vdac2* did not affect tumor control in immunodeficient *Rag1*$^{-/-}$ mice, further suggesting that VDAC2 supports the evasion of adaptive anticancer immunity. In line with these findings, the protection afforded to tumor cells by VDAC2 was mechanistically linked to IFNγ, as its blockade limited the cytotoxic effects of $CD8^+$ T cells against *Vdac2*$^{-/-}$ B16-OVA cells. Furthermore, VDAC2 deficiency rendered B16-OVA cells more sensitive to IFNγ-induced, caspase- and GSDME-dependent inflammatory cell death[4].

To assess the immunological consequences elicited by the loss of *Vdac2* in malignant cells, the authors analyzed the tumor microenvironment of VDAC2-proficient versus deficient tumors. Both B16-OVA and MC38-OVA tumors lacking *Vdac2* exhibited increased infiltration by $CD8^+$ T cells expressing effector molecules such as IFNγ, TNF and GZMB, as well as an increased $CD8^+$ T cell/$FOXP3^+$ T regulatory (T_{reg}) cell ratio compared with their VDAC2-competent counterparts, globally resembling the immunological alterations in the tumor microenvironment elicited by PD-1 blockage. Blocking IFNγ or specifically deleting *Ifng* in $CD8^+$ T cells efficiently restored the in vivo growth of *Vdac2*$^{-/-}$ B16-OVA melanomas, highlighting the crucial role of $CD8^+$ T cell-derived IFNγ in the suppression of VDAC2-deficient cancer cells[4].

Next, Yuan et al.[4] harnessed transcriptomic and chromatin accessibility analyses to reveal that VDAC2 deficiency enhances IFNγ-induced type I IFN responses via cGAS signaling through STING1 (best known as STING). Immunoblotting experiments confirmed that STING expression increases in response to IFNγ in both VDAC-proficient and deficient B16-OVA cells. However, the cGAS-elicited phosphorylation of STING and its signal transducers TBK1 and IRF3 was exacerbated by the absence of *Vdac2*, which suggests that VDAC2 restrains IFNγ-driven STING activation. In line with the notion that VDAC2 is localized to the outer mitochondrial membrane[5] and that mtDNA is an established cGAS activator[6], *Vdac2*$^{-/-}$ B16-OVA cells demonstrated superior accumulation of cytosolic double-stranded DNA after IFNγ treatment, which could be effectively prevented by mtDNA depletion[4]. These results suggest that VDAC2 modulates IFNγ-driven STING signaling by maintaining mitochondrial stability.

However, although STING deletion prevented type I IFN responses elicited by IFNγ in *Vdac2*$^{-/-}$ B16-OVA cells, it did not suppress IFNγ cytotoxicity. Yuan et al.[4] performed a genetic interaction screen and identified BAK as a key effector of IFNγ-driven mitochondrial destabilization and cell death in the absence of *Vdac2*. Accordingly, *Bak1* deletion prevented mtDNA release, cGAS activation and type I IFN expression in *Vdac2*$^{-/-}$ B16-OVA cells. Interestingly, similar observations could not be made after deleting *Bax*, which encodes a pro-apoptotic protein that in most settings cooperates with (and can effectively compensate for the loss of) BAK at MOMP induction[7]. This is even more surprising given that IFNγ caused IRF1- and STAT1-dependent upregulation of BAK as well as both BAK- and BAX-activating proteins including BID and BCL2L11 (also known as BIM). Finally, blocking apoptotic caspases further enhanced type I IFN responses as driven by IFNγ in *Vdac2*$^{-/-}$ B16-OVA cells[4], which is in line with the ability of CASP3 to potently suppress cGAS signaling[8].

In summary, the study by Yuan et al.[4] delineates a new VDAC2-dependent molecular mechanism through which malignant cells evade the cytotoxic and immunostimulatory effects of BAK activation as elicited by IFNγ signaling[4] (Fig. 1). These findings suggest VDAC2 may constitute a target for the development of new therapeutic interventions aimed at restoring cancer immunosurveillance by limiting the

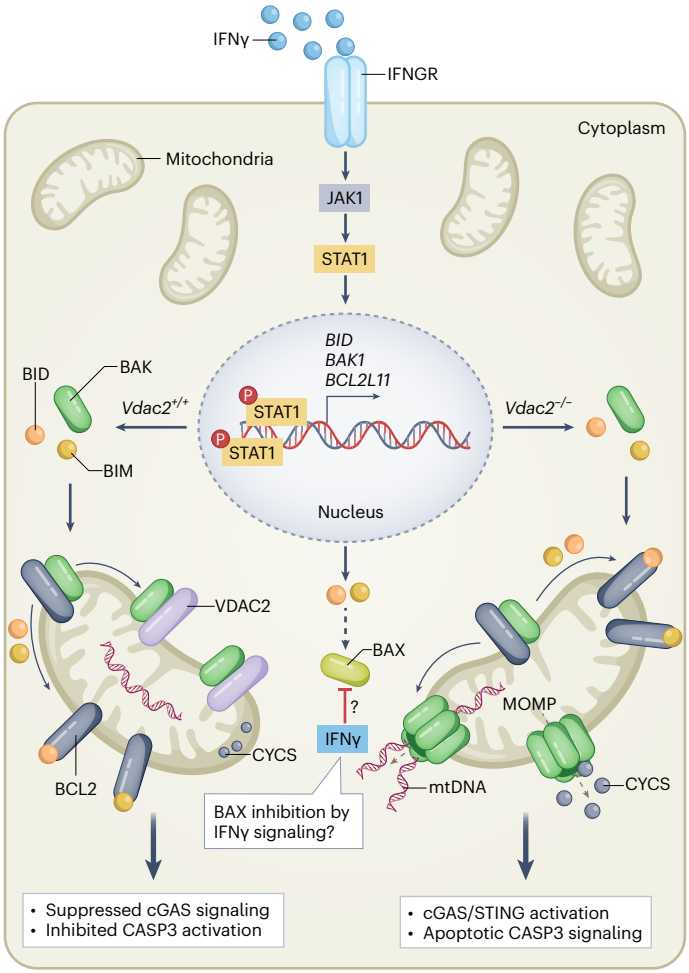

Fig. 1 | VDAC2 actively suppresses the BAK-dependent pro-apoptotic and immunostimulatory effects of IFNγ in cancer cells. Malignant cells exposed to IFNγ undergo a transcriptional rearrangement involving the upregulation of several genes encoding inducers of mitochondrial outer membrane permeabilization (MOMP), including *BAK1*, *BID* and *BCL2L11*. Normally, such a transcriptional program fails to effectively drive apoptosis and inflammation downstream of MOMP as malignant cells express high levels of the endogenous BAK inhibitor VDAC2, despite normal expression of (at least theoretically activatable) BAX. Conversely, in the absence of VDAC2, IFNγ results in rapid BAK-dependent (but BAX-independent) MOMP coupled with: (1) effective cyclic cGAS signaling downstream of cytosolic mitochondrial DNA (mtDNA) accumulation, and (2) apoptotic cell death as elicited by the CYCS-dependent activation of CASP3. These observations point to the intriguing possibility that IFNγ may simultaneously promote BAK activation and BAX inactivation to generate a temporal window for mtDNA-driven cGAS signaling before cGAS degradation by apoptotic caspases.

resistance of malignant cells to the BAK-dependent pro-apoptotic and immunostimulatory effects of IFNγ[4].

The molecular mechanisms through which BID, BIM and BAK upregulation by IFNγ in malignant cells would generate a MOMP-inducing signal in the absence of a sizeable contribution from BAX, however, remain to be determined. Cells expressing increased relative levels of BAK (over BAX) have previously been shown to release mtDNA downstream of MOMP according to an accelerated kinetic, which may generate a temporal window for pro-inflammatory cGAS signaling before effective CASP3 activation (and hence cGAS degradation)[9]. That said, only the absence of both BAX and BAK effectively interrupts MOMP, thus mediating bona fide cytoprotective effects, in most cellular systems[7]. It is therefore tempting to speculate that IFNγ signaling might simultaneously drive BAK activation and BAX inhibition, thus setting the stage for superior cGAS signaling downstream of MOMP despite canonical CASP3 activation. Additional work is required to formally assess this possibility.

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Competing interests

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