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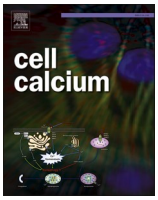

Commentary

The LRRC8C-STING-p53 axis in T cells: A Ca^{2+} affair

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ABSTRACT

Up to now, no role has been associated with VRAC channels in T cells. In a recent paper published in Nature Immunology, LRRC8C has been described as an essential component of VRAC in T cells. These data raise the intriguing possibility that the LRRC8C-STING-p53 signaling axis may represent a new inhibitory pathway in T cells that controls their function and adaptive immunity.

Up to now, no role has been associated with VRAC channels in T cells. In a recent paper published in *Nature Immunology*, LRRC8C has been described as an essential component of VRAC in T cells. These data raise the intriguing possibility that the LRRC8C-STING-p53 signaling axis may represent a new inhibitory pathway in T cells that controls their function and adaptive immunity.

1. Main text

In mammalian cells, the volume-regulated anion channel (VRAC) plays a central role in the volume regulation, mediating Cl^-secretion and facilitating the regulatory volume decrease (RVD) after hypotonic cell swelling [1]. The molecular identity of VRAC was unveiled in 2014 when it was demonstrated that VRAC was formed by heteromers of the leucine-rich repeat-containing protein 8 (LRRC8) family, consisting of five members LRRC8A-LRRC8E, with LRRC8A being the only essential subunit [2,3]. The function of the other paralogs LRRC8B-E is much less understood. Beyond RVD, VRAC is involved in cell proliferation and migration, cancer drug resistance, transport of metabolites, and second messengers.

Recent data published in *Nature Immunology* raise the intriguing possibility that LRRC8C, as an essential component of VRAC in T cells, could represent a target for modulating T cell function and adaptive immunity [4]. Concepcion et al. used a gene-expression-based screening approach to identify LRRC8C as a selective and highly expressed marker in $CD4^+$T cells, after T cell receptor (TCR) stimulation or treatment with interleukin (IL)−2 or IL-15, demonstrating that T cell-specific expression of *Lrrc8c* is controlled by IL-2/STAT5 signaling and STAT5 tetramerization. Abolished LRRC8C protein expression results in RVD impairment compared to the wild-type (WT) counterpart or after STAT5 inhibition, indicating that LRRC8C is an essential component of VRAC in T cells [4].

To understand whether LRRC8C plays a role in T cells function, the authors investigated the effect of *Lrrc8c* deletion on gene expression after TCR stimulation under isotonic conditions, funding that several signaling pathways are dysregulated in the absence of LRRC8C. Expression of total and phosphorylated p53 protein was reduced, indicating that the p53 signaling pathway was significantly downregulated in the absence of LRRC8C. Moreover, $Lrrc8c^{-/-}$ cells showed enhanced cell cycle progression and proliferation.

Based on these considerations, increasing p53 levels (pharmacologically or by genetic manipulation) in $Lrrc8c^{-/-}$ results in the suppression of enhanced survival and proliferation compared to the untreated counterpart, suggesting that the enhanced activation of $Lrrc8c^{-/-}$ T cells is due to decreased p53 expression. However, how LRRC8C regulates p53 signaling in $CD4^+$T cells remains unclear. The key missing mechanistic links seem to be the transport of cyclic dinucleotides (CDNs) such as 2′3′cGAMP and the activation of STING. Intriguingly, LRRC8C mediates CDN transport in T cells and STING activation, as observed upon treatment of $Lrrc8c^{-/-}$ cells with a hypotonic buffer to activate VRAC, resulting in no uptake of 2′3′cGAMP, a significant reduction of STING phosphorylation, the downregulation of NF-kB dependent gene expression, and p53 downregulation [4]. Akin to Lrrc8c deletion, STING inhibition also increased T cell proliferation and decreased p53 expression; a direct activation of p53 can reverse these effects, suggesting that p53 signaling is downstream of STING. These observations suggest that in T cells, LRRC8C mediates the uptake of CDN, including 2′3′cGAMP, to activate STING and the p53 signaling pathway, thus regulating T cell proliferation and survival. Loss of LRRC8C is also associated with higher production of calcium (Ca^{2+})-dependent cytokines (IL-2 and IFN-g), which impacts the T cell-dependent in vivo

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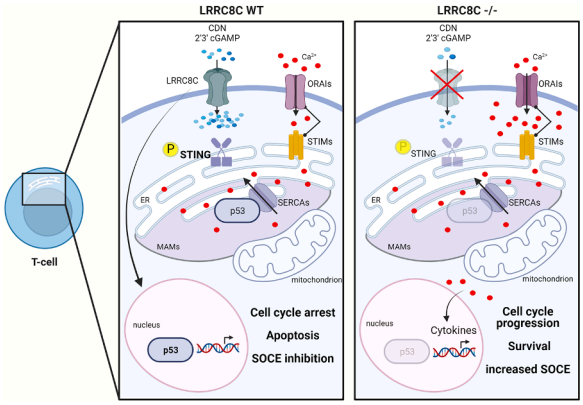

Fig. 1. The volume-regulated anion channel (VRAC) LRRC8C abolishes T cell function by mediating the transport of cGAMP and activating the STING-p53 axis. T cells cope with perturbations of homeostasis by activating a response centered on LRRC8C, an essential component of VRAC. LRRC8C mediates CDN uptake, including 2′3′cGAMP, activating the ER-associated STING and the tumor suppressor p53 signaling pathway. This response inhibits SOCE, hence arresting the cell cycle and inducing apoptosis. Conversely, abolished LRRC8C protein expression results in no uptake of 2′3′cGAMP, a significant reduction of STING phosphorylation, and p53 downregulation. In this scenario, the increased SOCE and cytokines production are related to cell cycle progression and survival. SOCE increment might be explained, at least in part, by SERCA inhibition due to p53 downregulation at the ER-MAMs. CDN: cyclic dinucleotide; ER: endoplasmic reticulum; MAMs: mitochondria-associated membranes; SOCE: Store-operated Ca2+ entry; STING: stimulator of IFN genes. Created with BioRender.com

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immune response. In the experimental autoimmune encephalomyelitis (EAE), deletion of Lrrc8c results in aggravated disease severity associated with an increased presence of myelin-specific $CD4^+$T cells in the spinal cord and elevated production of pro-inflammatory cytokines. At odds with these data, during an antiviral immune response to influenza A virus, Lrrc8c deletion enhances the function of $CD4^+$ and $CD8^+$ T cells by downregulating p53 signaling. This intricate molecular scenario is purely coordinated by Ca^{2+} ions, which operate, as is often the case, as bridging factors between different signaling pathways [5,6]. Indeed, store-operated Ca^{2+} entry (SOCE) could control cytokines production in T cells [7], and accordingly, Concepcion et al. showed that LRRC8C suppresses Ca^{2+} influx. This effect is not linked to an altered expression of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel-related genes or changes in the membrane potential. Rather it is mediated by the CDN transport and its effects on the STING-dependent activation of p53. Although STING regulates SOCE by directly controlling the Ca^{2+} sensor Stromal interaction molecule 1 (STIM1) functions in Jurkat cells [8], STING inhibition is unable to affect Ca^{2+} influx upon pharmacological activation of p53 [4], indicating that SOCE suppression by LRRC8C-STING signaling in T cells is mainly p53-dependent, rather than relies on the effects of STING on capacitative Ca^{2+} entry machinery. In this context, the ability of p53 in controlling Ca^{2+} fluxes at the Endoplasmic Reticulum (ER)-mitochondria interface could be highly relevant. We reported that p53 localized at both the ER and specialized contact domains between the ER and mitochondria called mitochondria-associated membranes (MAMs), where it interacts with Sarco/ER Ca^{2+}-ATPase (SERCA) pumps, modulates ER-mitochondria crosstalk and in turn, Ca^{2+}-dependent apoptosis [9]. Therefore, considering the negative role of SERCA on Ca^{2+} influx [10], the increased SOCE observed upon *Lrrc8c* depletion might be explained, at least in part, by SERCA inhibition, as a result of p53 downregulation (Fig. 1). Nonetheless, SOCE inhibition could be an adjunct mechanism governed by p53 to suppress cytokines production.

Altogether, these observations establish the LRRC8C-STING-p53 signaling axis as a new inhibitory signaling pathway in T cells and adaptative immunity. Further investigations are required to provide mechanistic insight into LRRC8C as a target to modulate T cell function. Moreover, many other issues could be explored, including whether a specific intracellular localization of p53 is critical for SOCE regulation or if p53 mutations may affect Ca^{2+} influx and be involved in the pathogenesis of some autoimmune diseases.

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