Ion Channels as Therapeutic Targets for the Immune, Inflammatory, and Metabolic Disorders

Review

Ca²⁺-Activated K⁺ Channel K_{Ca}3.1 as a Therapeutic Target for Immune Disorders

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In lymphoid and myeloid cells, membrane hyperpolarization by the opening of K⁺ channels increases the activity of Ca²⁺ release-activated Ca²⁺ (CRAC) channels and transient receptor potential (TRP) Ca²⁺ channels. The intermediate-conductance Ca²⁺-activated K⁺ channel K_{Ca}3.1 plays an important role in cell proliferation, differentiation, migration, and cytokine production in innate and adaptive immune systems. K_{Ca}3.1 is therefore an attractive therapeutic target for allergic, inflammatory, and autoimmune disorders. In the past several years, studies have provided new insights into 1) K_{Ca}3.1 pharmacology and its auxiliary regulators; 2) post-transcriptional and proteasomal regulation of K_{Ca}3.1; 3) K_{Ca}3.1 as a regulator of immune cell migration, cytokine production, and phenotypic polarization; 4) the role of K_{Ca}3.1 in the phosphorylation and nuclear translocation of Smad2/3; and 5) K_{Ca}3.1 as a therapeutic target for cancer immunotherapy. In this review, we have assembled a comprehensive overview of current research on the physiological and pathophysiological significance of K_{Ca}3.1 in the immune system.

Key words Ca²⁺-activated K⁺ channel; K_{Ca}3.1; extracellular K⁺ concentration; cytokine production; immune disorder; cancer immunotherapy

1. INTRODUCTION

More than 70 different K⁺ channel genes have been identified in the mammalian genome. These are classified as i) voltage-gated, ii) inward-rectifier, iii) Ca²⁺-activated, and iv) two-pore domain superfamilies. In non-excitable cells, including immune cells, the activation of K⁺ channels promotes Ca²⁺ signaling and transcriptional regulation by increasing the electrical driving force for Ca²⁺ entry from Ca²⁺ release-activated Ca²⁺ (CRAC) and transient receptor potential (TRP) Ca²⁺ channels. K⁺ channels therefore regulate proliferation, differentiation, migration, and cytokine/chemokine/chemical mediator expression in immune cells.^{1,2)} The auxiliary subunits of K⁺ channels influence gating kinetics, membrane trafficking, and the Ca²⁺ sensitivity of pore-forming α subunits.^{3,4)} A recent study has shown that the elevation of extracellular K⁺ concentration prevents T cell function and signaling, without changing membrane potential or Ca²⁺ entry; conversely, the reduction of intracellular K⁺ concentration by K⁺ channel activators improves T cell dysfunction.⁵⁾

The intermediate-conductance Ca²⁺-activated K⁺ channel K_{Ca}3.1 contributes to the control of Ca²⁺ signaling in the immune system.⁶⁾ Pharmacological blockade of K_{Ca}3.1 reduces the expression and secretion of pro-inflammatory cytokines and chemokines.^{6–8)} Therefore, K_{Ca}3.1 is an attractive therapeutic target for multiple sclerosis (MS), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), asthma, atherosclerosis, allergic rhinitis, and various tissue fibrosis.¹⁾ In addition, auxiliary subunits that positively or negatively control K_{Ca}3.1 activity, as well as transcriptional and post-

transcriptional regulators that control K_{Ca}3.1 gene expression, have been identified: i) phosphoinositide-3-kinase, class 2, β polypeptide (PI3K-C2B), nucleoside diphosphate kinase-B (NDPK-B), phosphohistidine phosphatase 1 (PHPT-1), myotubularin related protein 6 (MTMR6), tripartite motif containing 27 (TRIM-27), and phosphoglycerate mutase family member 5 (PGAM5) as auxiliary subunits,^{9–13)} and ii) activating protein-1 (AP-1), repressor element 1-silencing transcription factor (REST/NRSF), histone deacetylase 2/3 (HDAC2/3), and microRNA-497-5p (miR-497-5p) as transcriptional and post-transcriptional regulators.^{14–18)} Here, we review current topics on the pathophysiological significance of K_{Ca}3.1 and its regulators in the immune system.

2. K_{Ca}3.1 PHARMACOLOGY AND ITS AUXILIARY REGULATORS

Recent advances regarding the therapeutic potential of K_{Ca}3.1 blockers have been reviewed.^{1,18)} Gene silencing and the pharmacological blockade of K_{Ca}3.1 exhibit significant efficacy in treating RA, asthma, IBD, allergic rhinitis, Sickle cell disease, delayed type hypersensitivity, and immunoglobulin E (IgE)-mediated anaphylaxis.^{1,6–8,18)} Recently, Nguyen *et al.* visualized the mechanism of a small molecule K_{Ca}3.1 blocker (TRAM-34, PF-05416266, and NS6180) at the atomistic level using Rosetta ligand computational molecular modeling software.¹⁹⁾ This Rosetta K_{Ca}3.1 pore model enables the structure-based drug design of new K_{Ca}3.1 blockers belonging to different structural classes. Several studies on post-translational modifications of K_{Ca}3.1 have recently been reported.

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Fig. 1. Positive and Negative Auxiliary Regulators of $K_{Ca3.1}$

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Oliván-Viguera *et al.* showed that $\omega 3$ -fatty acids such as α -linolenic acid and docosahexaenoic acid inhibited $K_{Ca3.1}$ by negatively modulating its expression in fibroblasts.²⁰⁾ These $\omega 3$ -fatty acids decrease the disease activity of autoimmune diseases such as MS, RA, and IBD,²¹⁾ suggesting that they may down-regulate increased $K_{Ca3.1}$ expression in inflammatory helper T type 1 (Th1) and Th17 cells, in addition to suppressing pro-inflammatory cytokine production. The angiotensin II receptor type 1 (AT1) antagonist, telmisartan (TLM), also exerts an anti-inflammatory effect. Zhang *et al.* showed the inhibitory effect of TLM on $K_{Ca3.1}$ activity, suggesting that the TLM-induced reduction of pro-inflammatory cytokine production may be due, at least in part, to an inhibitory effect on $K_{Ca3.1}$ activity.²²⁾ Furthermore, the activation of protein kinase A reduced $K_{Ca3.1}$ gating and trafficking to the plasma membrane by phosphorylation of Ser332/334 in the calmodulin-binding C-terminus,²³⁾ and also led to the inhibition of $K_{Ca3.1}$ synthesis and down-regulation of PI3K-C2B.²⁴⁾

The following have been shown to be potent $K_{Ca3.1}$ activators: 1-ethylbenzimidazolin-2-one (1-EBIO), 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one (DCEBIO), 6,7-dichloro-1H-indole-2,3-dione 3-oxime (NS309), and naphtho[1,2-d]thiazol-2-ylamine (SKA-31), and they exhibit similar potency for small-conductance $K_{Ca2.x}$. The selective $K_{Ca3.1}$ activator SKA-121, a derivative of SKA-31, was developed using Rosetta modeling.²⁵⁾ Eil *et al.* showed that $K_{v1.3}$ and $K_{Ca3.1}$ activators are possible candidates for cancer immunotherapy,⁵⁾ suggesting that SKA-121 may have an effective therapeutic profile for cancer immunotherapy. In addition, $K_{Ca3.1}$ plays an important role in the fine-tuning of microglia activation and migration. In microglial cells, $K_{Ca3.1}$ was activated through protein kinase G (PKG)-dependent pathways,²⁶⁾ and was up-regulated by interleukin-4 (IL-4) receptor activation through Janus kinase 3 (JAK3) and Ras/MEK/extracellular signal-regulated kinase (ERK) signal pathways.²⁷⁾

The auxiliary regulators of $K_{Ca3.1}$ play a crucial role in T cell activation and quiescence, and are potential therapeutic targets in the treatment of $K_{Ca3.1}$ -related immune disorders (Fig. 1). NDPK-B directly activates $K_{Ca3.1}$ by phosphorylating His358 in the C terminus,²⁸⁾ and PHPT1 directly inhibits $K_{Ca3.1}$ by dephosphorylation. PI3K-C2B acts upstream of

NDPK-B to activate $K_{Ca3.1}$, and TRIM27 inhibits $K_{Ca3.1}$ activity by ubiquitinating PI3K-C2B. MTMR6 inactivates NDPK-B by dephosphorylating phosphatidylinositol 3-phosphate, which acts upstream of NDPK-B. Recently, a new $K_{Ca3.1}$ regulator, PGAM5, was identified as a negative regulator of CD4⁺ T cells.¹³⁾ PGAM5 negatively regulates NDPK-B to inactivate $K_{Ca3.1}$. It has also been shown that a reduction of $K_{Ca3.1}$ activity by gene silencing of PI3K-C2B decreased Fc ϵ RI-stimulated Ca^{2+} entry, cytokine production, and degranulation in mast cells.²⁹⁾ These positive and negative $K_{Ca3.1}$ regulators are both expected to be new pharmacologic targets for treating immune disorders, although their potent and selective inhibitors or activators have not yet been discovered.

3. POST-TRANSCRIPTIONAL AND PROTEASOMAL REGULATION OF $K_{Ca3.1}$

Our previous review summarized the transcriptional, epigenetic, spliceosomal, and proteasomal regulation of $K_{Ca3.1}$.³⁰⁾ The recycling endosome Rab8 contributes to the trafficking of $K_{Ca3.1}$ from the endoplasmic reticulum (ER) Golgi to the plasma membrane. Recent studies have identified the Rab8-dependent recycling pathway as an intracellular route for protein delivery to an immunological synapse (IS)^{31,32)} (Fig. 2). $K_{Ca3.1}$ is compartmentalized in the IS of T lymphocytes,³³⁾ suggesting that Rab8 may be an essential contributor to the localization of $K_{Ca3.1}$ and to the efficient coupling between $K_{Ca3.1}$ and CRAC channels in the IS. However, specific and potent promoting compounds of $K_{Ca3.1}$ degradation have not yet been developed.

Epigenetic mechanisms such as DNA methylation, histone modification, and RNA interference modulate gene expression. Histone deacetylase inhibitors are expected to be potentially therapeutic against autoimmune diseases such as type I diabetes, MS, pancreatitis, and IBD.^{34–37)} For instance, HDAC isoforms such as HDAC2, HDAC3, and HDAC6 are involved in chronic intestinal inflammation, and pan-HDAC inhibitors (HDACis) suppress pro-inflammatory cytokine production such as IL-6, tumor necrosis factor α (TNF- α), and interferon γ (IFN- γ).³⁷⁾ In $K_{Ca3.1}$ -expressing breast and prostate cancer cells, pharmacological and small interfering RNA (siRNA)-

Fig. 2. Transcriptional, Post-Transcriptional, and Post-Translational Regulation of $K_{Ca}3.1$

(Color figure can be accessed in the online version.)

mediated inhibition of HDAC2 and HDAC3 resulted in the down-regulation of $K_{Ca}3.1$.¹⁷⁾ In addition, $K_{Ca}3.1$ activity and expression were significantly increased in $CD4^+$ T cells of IBD model mice.⁸⁾ These suggest that $K_{Ca}3.1$ amplification in $CD4^+$ T cells in IBD model mice may be induced by the up-regulation of HDAC2 and/or HDAC3, whereas the inhibition of $K_{Ca}3.1$ may, at least in part, be involved in HDACis-induced improvement in the pathogenesis of IBD (Fig. 2).

$K_{Ca}3.1$ is one of the putative target genes of the tumor suppressor miR-497-5p: miR-497-5p down-regulates $K_{Ca}3.1$ expression and contributes to the inhibition of angiosarcoma malignancy development.³⁸⁾ Additionally, miR-497-5p is up-regulated during myofibroblast differentiation in pulmonary fibrosis,³⁹⁾ and $K_{Ca}3.1$ has been associated with the induction of pulmonary fibrosis myofibroblasts.⁴⁰⁾ It remains to be determined whether immune disorder-induced changes in miR-497-5p are associated with the up-regulation of $K_{Ca}3.1$ in inflammatory T cells and macrophages (Fig. 2).

4. $K_{Ca}3.1$ AS A REGULATOR OF IMMUNE CELL MIGRATION, CYTOKINE PRODUCTION, AND PHENOTYPIC POLARIZATION

$K_{Ca}3.1$ is related to immune cell migration. In activated T cells, $K_{Ca}3.1$ accumulates in the uropod rear portion, but not at the leading edge (predominant in $K_v1.3^{high}$ T cells); together with TRPM7 Ca^{2+} channels it regulates T cell migration of IL-7 $R\alpha^{low}$ effector memory $CD8^+$ cells^{41,42)} (Fig. 3). $\beta 1$ integrins play important roles in the adhesion, migration, proliferation, and differentiation of T cells, and contribute to the efficient and functional assembly of ion channels and their regulators in lipid rafts. Recent studies have shown that the $\beta 1$ integrin contributes to swelling activated $K_{Ca}3.1$ and its cellular localization in the rear.^{43,44)} The roles of ion channels in chemokine-induced migration are largely unknown. The blockade of $K_{Ca}3.1$ decreased chemokine-induced dendritic cell migration and monocyte chemotaxis by the down-regulation of migration markers (CCR5 and CCR7) and chemotaxis related factor CCL7, respectively.^{45–47)} In neutrophils, $K_{Ca}3.1$ is

Fig. 3. Role of $K_{Ca}3.1$ in Immune Cell Migration

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a key factor in the chemotactic response and migration capacity, without affecting Ca^{2+} entry.⁴⁸⁾

Our previous study showed that pharmacological blockade of $K_{Ca}3.1$ reduced the expression of IFN- γ in $CD4^+$ T cells and of IL-6 in inflammatory tissues from IBD model mice.⁸⁾ In mast cells and RA synovial fibroblasts, $K_{Ca}3.1$ blockade reduced the expression and secretion of pro-inflammatory cytokines IL-6 and IL-8.^{7,49)} Similarly, in activated brain microglia, $K_{Ca}3.1$ blockade suppressed pro-inflammatory cytokines and chemical mediators.^{50,51)} Inflammatory responses were regulated by the AKT/mammalian target of rapamycin (mTOR) signaling pathway, and the blockade of $K_{Ca}3.1$ inhibited the activation of the PI3K/AKT signaling pathway in immune cells.^{50,52)} A recent study showed that increased intracellular K^+ by K^+ channel blockade may reduce pro-inflammatory cytokine expression by inhibiting the AKT/mTOR signaling pathway.⁵⁾

$K_{Ca}3.1$ -mediated activation of the AKT/mTOR signaling pathway is also responsible for an increase in tumor-infiltrating microglia/macrophages.⁵³⁾ Furthermore, $K_{Ca}3.1$ -mediated activation of the signal transducer and activator of transcrip-

tion 1 (STAT1) signaling pathway is responsible for macrophage polarization toward the M1 phenotype.⁵⁴⁾

5. ROLE OF $K_{Ca}3.1$ IN THE PHOSPHORYLATION AND NUCLEAR TRANSLOCATION OF SMAD2/3

IL-10 is an anti-inflammatory cytokine produced by B cells, mast cells, macrophages, dendritic cells, and several T cell lineages (*i.e.*, Th2 and regulatory T, T_{reg}). IL-10 induces immunosuppression and assists in an escape from tumor immune surveillance.^{41,55)} Smad2 and Smad3 are key regulators of the transforming growth factor- β (TGF- β)-mediated T_{reg} cell conversion of naïve T cells. Phosphorylation of Smad2/3 primarily mediates TGF- β -induced transcriptional regulation, and phosphorylated Smad2/3 translocate into the nucleus to regulate genes. In lung myofibroblasts, $K_{Ca}3.1$ constitutively promotes fibroblast to myofibroblast differentiation, mediation of the Smad2/3 signaling pathway, and the blockade of $K_{Ca}3.1$ prevented myofibroblast differentiation by attenuating the phosphorylation and nuclear translocation of Smad2/3.^{56,57)} Similarly, pharmacological blockade of $K_{Ca}3.1$ significantly inhibited the nuclear translocation of Smad2/3, and suppressed tissue fibrosis and vascular calcification.^{58–60)} Blockade of $K_{Ca}3.1$ led to a reduction of the phosphorylation of Smad2/3, and may be a novel target for therapeutic intervention in patients with tissue fibrosis. A recent study has shown that $K_{Ca}3.1$ blockade inhibits the TGF- β 1 signaling pathway through PI3K/AKT/mTOR signaling pathways.⁶¹⁾ In an aggressive B cell lymphoma, the inhibition of PI3K/AKT/mTOR and STAT3 signaling pathways reduced the release of IL-10.⁶²⁾ Further studies will be needed to clarify the mechanisms underlying $K_{Ca}3.1$ -mediated IL-10 expression in IL-10 producing immune cells.

6. $K_{Ca}3.1$ AS A THERAPEUTIC TARGET OF CANCER IMMUNOTHERAPY

Recent studies have reported that ion channels, including K^+ channels, are potential therapeutic targets for cancer immunotherapy.⁶³⁾ Koshy *et al.* concluded that $K_{Ca}3.1$, but not $K_v1.3$, is a beneficial target for cancer immunotherapy. Their study showed that $K_{Ca}3.1$ is preferentially up-regulated by adherent natural killer A-NK cells, and that a $K_{Ca}3.1$ blocker increases the ability of A-NK cells to reduce tumor growth.⁶⁴⁾ In addition, Eil *et al.* showed that $K_{Ca}3.1$ and $K_v1.3$ activators can maintain tumor-specific effector T cell function.⁵⁾ Within a tumor microenvironment, the elevation of extracellular K^+ concentration suppresses the TCR-mediated Akt/mTOR signaling pathway. The reduction of intracellular K^+ by $K_{Ca}3.1$ activators promotes pro-inflammatory cytokine expression through AKT/mTOR signaling, suggesting that $K_{Ca}3.1$ activators may increase the ability of tumor-specific effector T cells to reduce tumor growth.

7. CONCLUDING REMARKS

K^+ channels play important roles in the regulation of immune and inflammatory processes, and are therefore attractive therapeutic targets for immune disorders.¹⁾ Previous studies have suggested the predominance of $K_v1.3$ on effector memory T (T_{EM}) cells, thus $K_v1.3$ inhibitors were developed to treat

chronic immune disorders. However, there is emerging evidence that $K_{Ca}3.1$ is also a potential therapeutic target for immune and inflammatory disorders. Chiang *et al.* showed that $K_v1.3$ and $K_{Ca}3.1$ cooperatively and compensatorily regulate T_{EM} cell function.⁶⁵⁾ In addition, the physiological and pathophysiological roles of many molecules related to transcriptional, post-transcriptional, and post-translational modifications of $K_{Ca}3.1$ have been studied. Recently, $K_{Ca}3.1$ has attracted attention as a key player in cancer immune surveillance. Based on the recent advances in $K_{Ca}3.1$ research overviewed here, new therapeutic strategies may emerge for such immune disorders as autoimmune diseases, chronic inflammatory diseases and cancers.

Conflict of Interest The authors declare no conflict of interest.

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