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MINIREVIEW

Intermediate-conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ and its related molecules in T-lymphocytes

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The intermediate-conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ (also called IK_{Ca}, IK1 and KCNN4) plays an essential role for the positive-feedback mechanism required for the enhancement of Ca^{2+} signaling in activated T-lymphocytes, and regulates the T cell activation, proliferation and differentiation. Recent reports have suggested that T-lymphocyte $K_{Ca}3.1$ K^+ channel is an attractive target for the therapeutic strategies of inflammatory bowel disease (IBD). In addition, the potential $K_{Ca}3.1$ regulators also play critical roles in the T cell functions: phosphoinositide-3-kinase, class 2, beta polypeptide (PI3K-C2B), nucleoside diphosphate kinase B (NDPK-B), phosphohistidine phosphatase 1 (PHPT-1) and myotubularin related protein 6 (MTMR-6). We recently described that the up-regulation of $K_{Ca}3.1$ and NDPK-B might constitute an initiation step in $CD4^+$ T-lymphocyte proliferation in acute IBD and might be one of important mechanisms underlying the pathogenesis of IBD (Ohya *et al*., *Am J Physiol Gastrointest Liver Physiol.* 306:G873-G885). $K_{Ca}3.1$ K^+ channel and its regulators may be potential therapeutic targets for inflammatory diseases such as IBD.

Keywords: Ca^{2+}-activated K^+ channel; inflammatory bowel disease; T-lymphocyte

Abbreviations: IBD, inflammatory bowel disease; PI3K-C2B, phosphoinositide-3-kinase, class 2, NDPK-B, nucleoside diphosphate kinase B; PHPT-1, phosphohistidine phosphatase 1; MTMR-6, myotubularin related protein 6; TCR,T cell receptor ; CRAC, Ca2+-release activated Ca2+; Th, helper T; DIS, dominant-inhibitory segment; AP-1, activator protein-1; REST, repressor element-1 silencing transcription factor; CLT, clotrimazole; HLMC, Human lung mast cells; LAR, late asthmatic response; DTH, delayed type hypersensitivity; SNP, Single Nucleotide Polymorphism; CD, Crohn's disease; TRIM27, tripartite motif containing protein 27; RING, Really Interesting New Gene; IS, immunological synapse; APC, antigen-presenting cell; ICAP1A, integrin cytoplasmic domain-associated protein A; p-SMAC, peripheral supramolecular activating complex; c-SMAC, central supramolecular activating complex

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Introduction

In the immune cells such as lymphocytes, mast cells and macrophages, Ca^{2+}-activated K^+ channel is characterized by an intermediate-conductance $K_{Ca}3.1$ channel [1]. In T-lymphocytes, the activation of $K_{Ca}3.1$ channels by the intracellular Ca^{2+}rise during T cell receptor (TCR)-evoked Ca^{2+} signaling hyperpolarizes the membrane potential, and thereby promotes Ca^{2+} influx via Ca^{2+}-release activated Ca^{2+} (CRAC) channels composed of the complex of Orai and STIM families[2-4]. $K_{Ca}3.1$ channel plays an essential role for the positive-feedback mechanism required for the enhancement of Ca^{2+} signaling in activated T-lymphocyte. $K_{Ca}3.1$ expression is relatively low in quiescent human naive (T_N), central memory (T_{CM}), and effector memory (T_{EM}) T cells. Upon T cell activation, $K_{Ca}3.1$ channel is up-

regulated in T_N and T_{CM} cells, whereas voltage-gated K^+ channel, $K_V1.3$ instead of $K_{Ca}3.1$ is up-regulated in T_{EM} cells [5, 6]. In addition, $K_{Ca}3.1$ currents are significant higher in helper T (Th1) cells compared with that in Th2 cells, corresponding to the larger Ca^{2+} signaling in Th1 cells [7]. Similar to the case with T cells, quiescent naive and early memory B cells express low levels of $K_{Ca}3.1$ channels, and effector cells derived from naive memory B cells express high levels of them[8]. $K_{Ca}3.1$ channel is a potential molecular target for pharmacological intervention in a variety of diseases, e.g. autoimmune diseases such as multiple sclerosis and rheumatoid arthritis, transplant rejection, sickle cell anemia, asthma, atherosclerosis, fibrosis, and traumatic brain injury [9-11].

Molecular determinants of $K_{Ca}3.1$ channel and its transcriptional factors

$K_{Ca}3.1$/KCNN4 orthologs and their spliced isoforms have been molecularly identified from various species and tissues [12, 13]. Alternative splicing isoforms can function as the dominant-negative by incorporation into tetrameric K^+ channel α subunits. In small-conductance $K_{Ca}2.x$ channels, two different spliced isoforms, $K_{Ca}2.3$-1B and $K_{Ca}2.3$-1C lacking first transmembrane segment, have caused dominant-negative suppression of $K_{Ca}2.x$ by trapping channel proteins intracellularly [14, 15]. Both $K_{Ca}2.x$ and $K_{Ca}3.1$ have the dominant-inhibitory segment (DIS) in the C-terminus with the sequence similarity to tetramerization-coiled-coiled domains, and DIS plays an essential role in $K_{Ca}2.x$ and $K_{Ca}3.1$ tetrameric interactions [15]. We have recently identified the N-terminus-lacking spliced isoforms of $K_{Ca}3.1$ ($K_{Ca}3.1$-ΔN), from mammalian lymphoid tissues. $K_{Ca}3.1$-ΔN isoforms have shown dominant-negative effects on membrane trafficking and the channel activity of functional $K_{Ca}3.1$, $K_{Ca}3.1$-wt [13]. Additionally, up-regulation and over-expression of $K_{Ca}3.1$-ΔN in mice thymocytes differentiated $CD4^+CD8^+$ double positive phenotype into $CD4^-CD8^-$ double negative one, and suppressed concanavalinA-stimulated thymocyte growth by down-regulation of IL-2 expression[13].

As transcriptional factors of $K_{Ca}3.1$, activator protein-1 (AP-1) (Fos/Jun heterodimers) and repressor element-1 silencing transcription factor (REST) have been identified [16, 17]. $K_{Ca}3.1$ gene expression is positively and negatively regulated by AP-1 and REST, respectively. Disease-associated alternative splicing of $K_{Ca}3.1$ has not been identified yet, however, the increase in $K_{Ca}3.1$ channel activity by the down-regulation of $K_{Ca}3.1$-ΔN may have a critical role in the pathogenesis of *inflammatory diseases.*

Potential therapeutic uses of $K_{Ca}3.1$ channel blockers

In 1990's, it has been reported that pharmacological blockade of $K_{Ca}3.1$ by clotrimazole (CLT) prevents sickle cell dehydration [18, 19]. However, the imidazole ring of CLT which is essential for the inhibition of hepatic cytochrome P450 enzyme activity has caused abnormal hepatic function [19]. To prevent cytochrome P450 inhibition by CLT, the imidazole ring has been chemically modified, and selective $K_{Ca}3.1$ blockers, TRAM-34 (1-[2-chlorophenyl] diphenylmethyl)-1H -pyrazole) (IC_{50}=20 nM) [18] and ICA -17043(4-fluoro-α-phenylbenzaneacetamide) (Senicapoc) (Icagen Inc.) (IC_{50}=11 nM) have been developed [20]. Also, novel chemical compounds as $K_{Ca}3.1$ blockers are developed by Brugnara *et al.*: 11-phenyl-dibenzazepine (IC_{50}=90 nM) and diphenylindanone 12 (IC_{50}=189 nM) (US patent No. US6992079, US7342038).

Senicapoc has been evaluated in clinical trials for sickle cell anemia and asthma, however, phase II and III studies of Senicapoc for patients of these diseases have been recently terminated [21, 22]. Nevertheless, Senicapoc is recognized as a safe and well-tolerated drug because long-term treatment with Senicapoc has shown no serious toxic and adverse effects in preclinical and clinical trials. Human lung mast cells (HLMC), which are implicated in the pathology of asthma, express high levels of $K_{Ca}3.1$ channels, and pharmacological blockade of $K_{Ca}3.1$ channels attenuates HLMC proliferation and migration [23]. Senicapoc has indicated the significant efficacy for the late asthmatic response (LAR) (www.pfizer.com). In addition to allergic asthma, $K_{Ca}3.1$ is a potential therapeutic target for inflammatory and autoimmune diseases. Genetic disruption and/or pharmacological blockade of $K_{Ca}3.1$ have indicated the significant efficacy for IgE-mediated anaphylaxis and DTH (delayed type hypersensitivity) [24, 25], and also developed less severe colitis in two different IBD model mice [26].In $K_{Ca}3.1$ knock-out mice, Th1 and Th2 cells show smaller Ca^{2+} influx and cytokine production during TCR activation, whereas Th17 and regulatory T cells possess normal function. Moreover, agenome-wide association study has identified a certain $K_{Ca}3.1$ SNP (Single Nucleotide Polymorphism) (rs2306801) as a potential susceptibility factor in ileal Crohn's disease (CD) in the Australian and New Zealand populations [27].Clinical trials to evaluate the efficacy of Senicapoc and the other $K_{Ca}3.1$ blockers for inflammatory diseases will be conducted in near future.

Functional regulators of $K_{Ca}3.1$ channel

In $CD4^+$ T-lymphocytes, $K_{Ca}3.1$ on histidine 358 (His358) in the C-terminus is phosphorylated by NDPK-B, a positive $K_{Ca}3.1$ regulator, and dephosphorylated by PHPT-1, a negative $K_{Ca}3.1$ regulator [28, 29]. Genetic disruption of NDPK-B negatively regulates $CD4^+$ T cells, and suppresses cytokine production in both T helper 1 (Th1) and Th2 cells [30]. In contrast, genetic disruption of PHPT-1 positively regulates $CD4^+$ T cells. Apart from these molecules, a certain phosphatidylinositol 3 kinase, PI3K-

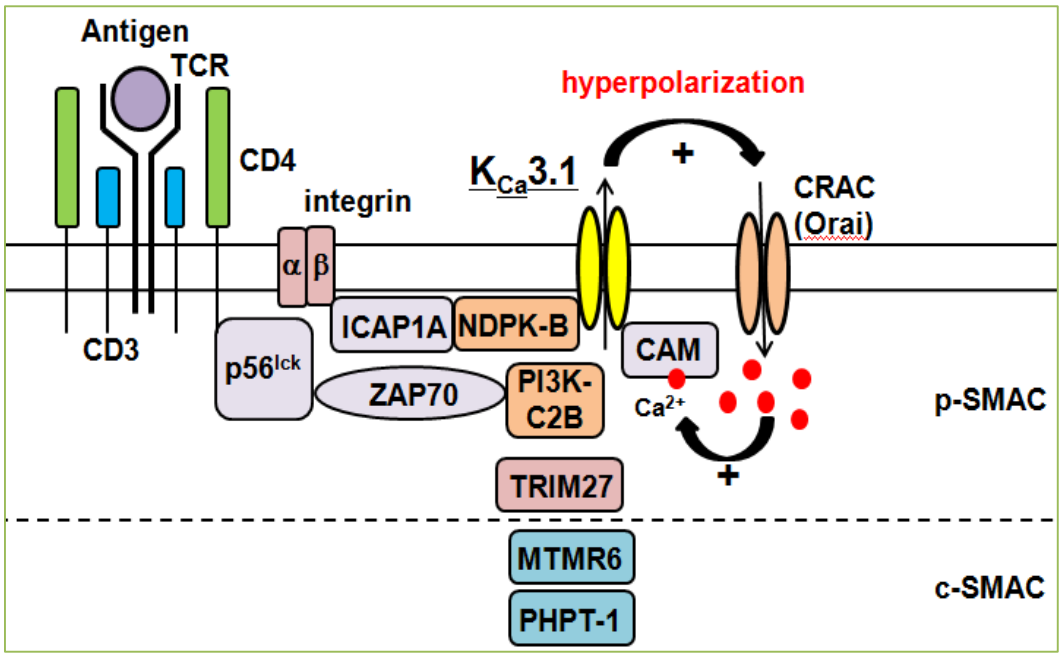

Figure 1. Signal molecules involving in $K_{Ca}3.1$ K^+ channel distribution and Ca^{2+} signal in the immunological synapse. ICAP: integrin cytoplasmic domain-associated protein. $p56^{lck}$: lymphocyte-specific tyrosine protein kinase. ZAP: zeta-chain-associated protein kinase. NDPK: nucleoside diphosphate kinase. PI3K: class II phosphatidylinositol 3 kinase. PHPT: phosphohistidine phosphatase. MTMR: myotubularin-related protein. TRIM: tripartite motif. CAM: calmodulin. CRAC: Ca^{2+} release-activated Ca^{2+} channel. p-SMAC: peripheral-supramolecular adhesion complex. c-SMAC: central-SMAC.

C2Bactivates $K_{Ca}3.1$ channel and positively regulates $CD4^+$ T cells [31], whereas a certain PI3 phosphatase, MTMR6 inhibits $K_{Ca}3.1$ channel and negatively regulates $CD4^+$ T cells [32]. Recently, it has been reported that regulator of PI3K-C2B [33]. By inhibiting PI3K-C2B, TRIM27 suppresses $K_{Ca}3.1$ channel activity, and thereby decreases TCR-stimulated Ca^{2+} influx, proliferation, and cytokine production in T-lymphocytes [33]. Taken together, positive regulators (NDPK-B and PI3K-C2B) and negative regulators (PHPT-1, MTMR6 and TRIM27) of $K_{Ca}3.1$ K^+ channel may provide new strategies for the treatment of inflammatory, autoimmune and allergic diseases.

Localization of $K_{Ca}3.1$ channel and its interacting molecules in immunological synapse

The immunological synapse (IS) is a highly organized structure triggered by sustained TCR engagement on the surface of an antigen-presenting cell (APC), and is characterized by an extensive reorganization of signaling proteins to the T cell-APC contact zone. The IS formation plays an essential role in both the initiation and the maintenance of immune responses [34]. Similar to ion channels contributing to T-lymphocyte Ca^{2+} signaling, such as CRAC and $K_V1.3$ channels, $K_{Ca}3.1$ channels are recruited to the IS upon antigen stimulation, and become part of the signaling complex that facilitates T cell proliferation and cytokine production[35]. However, the tripartite motif containing protein 27 (TRIM27), a member of Really Interesting New Gene (RING) E3 ubiquitin ligases, functions as a negative

distribution of $K_{Ca}3.1$ channels to the IS is not prevented by pharmacological blockade of $K_{Ca}3.1$ channel activity, and the increase in Ca^{2+} influx mediated by $K_{Ca}3.1$ channel activation does not contribute to the initiation and the maintenance of IS formation.

Functional regulators of $K_{Ca}3.1$ channel, NDPK-B, PHPT-1, PI3K-C2B, and MTMR6 are recruited to the IS, and spatial arrangement in the IS of them is critical to $K_{Ca}3.1$ channel regulation**.** NDPK-B and PHPT-1, which are coupled in the C-terminus of $K_{Ca}3.1$ channel, form the clustering at the IS together with $K_{Ca}3.1$ channel [28, 29] (Fig. 1). At the IS, NDPK-B interacts with the integrin cytoplasmic domain-associated protein A (ICAP1A), linking $K_{Ca}3.1$ to β-integrin [36]. Moreover, PI3K-C2B colocalizes with Zap70 (ζ-Chain-Associated Protein Kinase 80) and $p56^{lck}$ in peripheral micro clusters, and is recruited to the IS during antigen stimulation [31]. Recruitment of NDPK-B and PI3K-C2B with $K_{Ca}3.1$ to the peripheral zone, p-SMAC (peripheral supramolecular activating complex) in the IS is critical for the $K_{Ca}3.1$ channel activation. In contrast, localization of MTMR6 and PHPT-1 to the central supramolecular activating complex (c-SMAC) segregates $K_{Ca}3.1$ from p-SMAC, and could

provide one possible mechanism underlying the negative regulation of $K_{Ca}3.1$ channel activity [31]. Further comprehensive study on the molecules forming Ca^{2+} signal complex sorted to the p-SMAC of the IS may enable the novel drug design for inflammatory, autoimmune and allergic diseases.

Ubiquitin-mediated $K_{Ca}3.1$ channel protein degradation

A balance between the translation and the degradation of ion channels is of importance to regulate its activity. Small-conductance $K_{Ca}2.3$ channel protein is rapidly endocytosed and recycled back to the plasma membrane. Recycling of $K_{Ca}2.3$ channel protein is dependent upon REM1 and Rab35 [37]. In contrast, $K_{Ca}3.1$ channel protein is rapidly (60-90 min after membrane trafficking) endocytosed, however, does not enter the recycling pathway. Subsequent to endocytosis, $K_{Ca}3.1$ channel proteinis targeted to the lysosomes for degradation in Rab7- and ESCRT (Endosomal Sorting Complex Required for Transport)-dependent pathway [38]. Therefore, the inhibition of ubiquitin-activating enzymes results in reduced ubiquitylation and internalization of $K_{Ca}3.1$ channel proteins, and accordingly the ubiquitylase inhibitors attenuate $K_{Ca}3.1$ degradation. Deubiquitylase USP-8 (Ubiquitin Specific Protease 8) interacts with $K_{Ca}3.1$ channel protein, and siRNA-mediated USP-8 knock-out enhances the accumulation of ubquitylated $K_{Ca}3.1$ channel proteins [39]. Interestingly, a high-throughput screening method has been developed to identify small-molecule modulators of $K_{Ca}3.1$ channel endocytosis using fluorescence-tagged $K_{Ca}3.1$. Using this novel method, the ubiquitin-activating enzyme, E1 inhibitor, UBEI-41 has been identified as an inhibitor of $K_{Ca}3.1$ channel endocytosis [40]. The inhibition of deubiquitylases and the activation of ubiquitin-activating enzymes for $K_{Ca}3.1$ channels by pharmacological and/or genetic methods may possibly be novel strategies for drug development of inflammatory, autoimmune and allergic diseases.

Future direction

We recently showed that $K_{Ca}3.1$ inhibitors may be a therapeutic potential for DTH [41] and IBD [42]. In both auricular lymph node $CD4^+$ T-lymphocytes of DTH model and mesenteric lymph node $CD4^+$ T-lymphocytes in IBD model, an increase in $K_{Ca}3.1$ activity concomitant with an up-regulation of $K_{Ca}3.1$ was observed. Pharmacological blockade of $K_{Ca}3.1$ elicited the significant decrease in disease severity. It is of important that the pharmacological blockade of $K_{Ca}3.1$ is associated with the down-regulation of $K_{Ca}3.1$ in lymph node $CD4^+$ T-lymphocytes in both Th1-mediated disease models. These findings may provide novel information about radical treatments for inflammatory, autoimmune and allergic diseases. In addition, we suggest that $K_{Ca}3.1$ regulators such as NDPK-B may be potential therapeutic targets to decrease the risk of the disease development.

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Conflict of interest

The authors declare that there is no conflict of interest.

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