Journal of Advanced Research 33 (2021) 53–68

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Review

The diverse roles of TMEM16A Ca^{2+}-activated Cl^- channels in inflammation

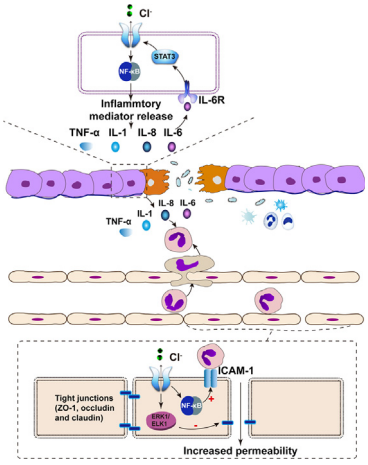
Weiliang Bai [a], Mei Liu [b], Qinghuan Xiao [b,*]

[a] Department of Otolaryngology Head and Neck Surgery, Shengjing Hospital of China Medical University, Shenyang 110004, China
[b] Department of Ion Channel Pharmacology, School of Pharmacy, China Medical University, Shenyang 110122, China

HIGHLIGHTS

- TMEM16A Ca^{2+}-activated Cl^- channel involves in many inflammatory diseases.
- Inflammatory mediators and pathogens promote TMEM16A expression and activity.
- TMEM16A activates diverse inflammatory signaling pathways in inflammatory diseases.
- TMEM16A plays multiple roles in inflammatory processes and pain.
- Cell type-dependent mechanisms underlie the roles of TMEM16A in inflammation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 October 2020
Revised 17 December 2020
Accepted 24 January 2021
Available online 4 February 2021

Keywords:
TMEM16A
Ca^{2+}-activated Cl^- channels
Inflammation
Signaling
Pain

ABSTRACT

Background: Transmembrane protein 16A (TMEM16A) Ca^{2+}-activated Cl^- channels have diverse physiological functions, such as epithelial secretion of Cl^- and fluid and sensation of pain. Recent studies have demonstrated that TMEM16A contributes to the pathogenesis of infectious and non-infectious inflammatory diseases. However, the role of TMEM16A in inflammation has not been clearly elucidated.

Aim of review: In this review, we aimed to provide comprehensive information regarding the roles of TMEM16A in inflammation by summarizing the mechanisms underlying TMEM16A expression and activation under inflammatory conditions, in addition to exploring the diverse inflammatory signaling pathways activated by TMEM16A. This review attempts to develop the idea that TMEM16A plays a diverse role in inflammatory processes and contributes to inflammatory diseases in a cellular environment-dependent manner.

Key scientific concepts of review: Multiple inflammatory mediators, including cytokines (e.g., interleukin (IL)-4, IL-13, IL-6), histamine, bradykinin, and ATP/UTP, as well as bacterial and viral infections, promote TMEM16A expression and/or activity under inflammatory conditions. In addition, TMEM16A activates diverse inflammatory signaling pathways, including the IP_3R-mediated Ca^{2+} signaling pathway, the NF-κB signaling pathway, and the ERK signaling pathway, and contributes to the pathogenesis of many

Peer review under responsibility of Cairo University.

* Corresponding author at: Department of Ion Channel Pharmacology, School of Pharmacy, China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang 110122, China.
E-mail address: qinghuanxiao12345@163.com (Q. Xiao).

https://doi.org/10.1016/j.jare.2021.01.013
2090-1232/

inflammatory diseases. These diseases include airway inflammatory diseases, lipopolysaccharide-induced intestinal epithelial barrier dysfunction, acute pancreatitis, and steatohepatitis. TMEM16A also plays multiple roles in inflammatory processes by increasing vascular permeability and leukocyte adhesion, promoting inflammatory cytokine release, and sensing inflammation-induced pain. Furthermore, TMEM16A plays its diverse pathological roles in different inflammatory diseases depending on the disease severity, proliferating status of the cells, and its interacting partners. We herein propose cellular environment-dependent mechanisms that explain the diverse roles of TMEM16A in inflammation.

Introduction

Transmembrane protein 16A (TMEM16A) belongs to the 10-member TMEM16 family (including TMEM16A-K), which functions as a Ca^{2+}-activated Cl^- channel (TMEM16A and TMEM16B), a Ca^{2+}-activated cation channel (TMEM16F), or a lipid scramblase (TMEM16C-K) [1]. TMEM16A was first discovered to be a novel Ca^{2+}-activated Cl^- channel in 2008 [2–4]; it was found to mediate Ca^{2+}-activated Cl^- currents in many cells, including airway and intestinal epithelial cells [5–9], acinar cells of exocrine glands such as salivary and pancreatic glands [4,10,11], smooth muscle cells of the airway [12–14], endothelial cells [15,16], interstitial cells of Cajal (ICC) [12,17], and dorsal root ganglion (DRG) neurons [18–20]. Considering its wide cellular distribution, it is predictable that TMEM16A has many physiological roles, including fluid secretion of epithelial cells, contraction of smooth muscle, transmission of nociceptive signals, and gastrointestinal mobility (reviewed in [1,21–26]).

As a Ca^{2+}-activated Cl^- channel, TMEM16A is mainly expressed in the plasma membrane, where TMEM16A plays its physiological and pathological roles through Cl^- transport. The expression levels of TMEM16A differ greatly among distinct cells. For example, in airways, TMEM16A is mainly expressed in mucin-secreting goblet cells, but is less expressed in ciliated epithelial cells, especially under inflammatory conditions [6,13,27]. In addition, the cellular localization of TMEM16A varies between different cell types. For example, TMEM16A is mainly expressed in the apical plasma membrane of acinar cells of the salivary gland and exocrine pancreas [12,28] and airway epithelial cells [12], but in the basolateral membrane of colonic epithelial cells [29]. The cell type-dependent expression of TMEM16A indicates that TMEM16A may exert different cellular functions in different cells, and thus may play a cell type-dependent role in different disease conditions.

In the past decade, increasing evidence has revealed that TMEM16A dysfunction contributes to a wide range of diseases, including various cancers [30,31], pain [32–34], cardiovascular diseases such as hypertension [16] and pulmonary hypertension [35], respiratory diseases such as cystic fibrosis (CF) [27,36], asthma [13,37,38], chronic rhinosinusitis [39], and gastrointestinal diseases such as eosinophilic esophagitis [40], steatohepatitis [41], and acute pancreatitis [10]. Inflammation plays a dominant pathological role in many of these TMEM16A-associated diseases, such as CF, asthma, acute pancreatitis, steatohepatitis, and eosinophilic esophagitis. In addition, several studies have reported that TMEM16A upregulation contributes to the pathogenesis of infectious diseases such as rotaviral diarrhea [42–44], and lipopolysaccharide (LPS)-induced sepsis [45]. Therefore, TMEM16A appears to underlie the pathogenesis of infectious and non-infectious inflammation-associated diseases.

In this article, we review recent findings involving TMEM16A, focusing on its role in inflammation. This review summarizes the mechanisms underlying the upregulation of TMEM16A expression and functions in inflammation and discusses the diverse inflammatory signaling pathways activated by TMEM16A. TMEM16A plays important roles in inflammatory processes by increasing vascular permeability and leukocyte adhesion in endothelial cells [15], promoting inflammatory cytokine release from tissue cells [6,10,41], and sensing inflammation-induced nociceptive stimuli in DRG neurons [19,20,46,47]. In this review, we highlight the diverse roles of TMEM16A in inflammation and propose cellular environment-dependent mechanisms underlying these roles in inflammatory diseases.

Structure of TMEM16A

Several studies have investigated the topology of TMEM16A channels using site-directed mutagenesis in combination with cysteine-scanning accessibility, and topology models with eight transmembrane helices have been proposed [4,48]. The TMEM16A topology with ten transmembrane helices (TM1-10) was first identified in 2014 based on the crystal structure of the fungal TMEM16A scramblase nhTMEM16 [49], and was later confirmed by the cryo-electron microscopy (EM) structures of the mTMEM16A Ca^{2+}-activated Cl^- channel [50,51]. The cryo-EM structures of the TMEM16A Cl^- channel have revealed a homodimeric architecture, in which each monomer contains one independent ion-conducting pore and two Ca^{2+}-binding sites (Fig. 1A). The pore, which is surrounded by TM3-7, forms an hourglass shape with a wide intracellular vestibule and a smaller extracellular vestibule that is connected by a restricted neck region [51]. Ten pore-lining residues on TM3-7 were identified to be important for the anion selectivity of TMEM16A channels [50]. The Ca^{2+}binding sites are formed by five conservative acidic amino acids (E654, E702, E705, E734, and D738) on TM6-8 [50,51] (Fig. 1B), consistent with earlier electrophysiology and mutagenesis data [48,52]. The Ca^{2+}-binding sites are located in proximity to the cytosolic end of the pore within the membrane.

The cryo-EM structures of the TMEM16A channel in the apo- and Ca^{2+}-bound forms reveal the mechanisms underlying Ca^{2+}-dependent gating of TMEM16A channels. The apo- and Ca^{2+}-bound structures show that Ca^{2+} binding to the TMEM16A channel mainly induces a conformational rearrangement of TM6 [50,51]. In the closed state without Ca^{2+} binding, the cytosolic end of TM6 bends around the glycine hinge residue (G644), resulting in narrowing of the pore [50,51]. When Ca^{2+} binding occurs, the cytosolic end of TM6 straightens to dilate the pore, thus rendering the channel in the open state [50,51,53] (Fig. 1C).

Multiple mechanisms for upregulated TMEM16A expression in inflammation

TMEM16A expression is upregulated by various inflammatory mediators such as interleukin (IL)-4, IL-13, and IL-6 in inflammatory diseases such as asthma [13,37], eosinophilic esophagitis [40], chronic rhinosinusitis [39,54], and acute pancreatitis [10]. It is also upregulated by pathogenic virulence factors such as bacterial pyocyanin [55] and lipopolysaccharide (LPS) [56–58]. Here,

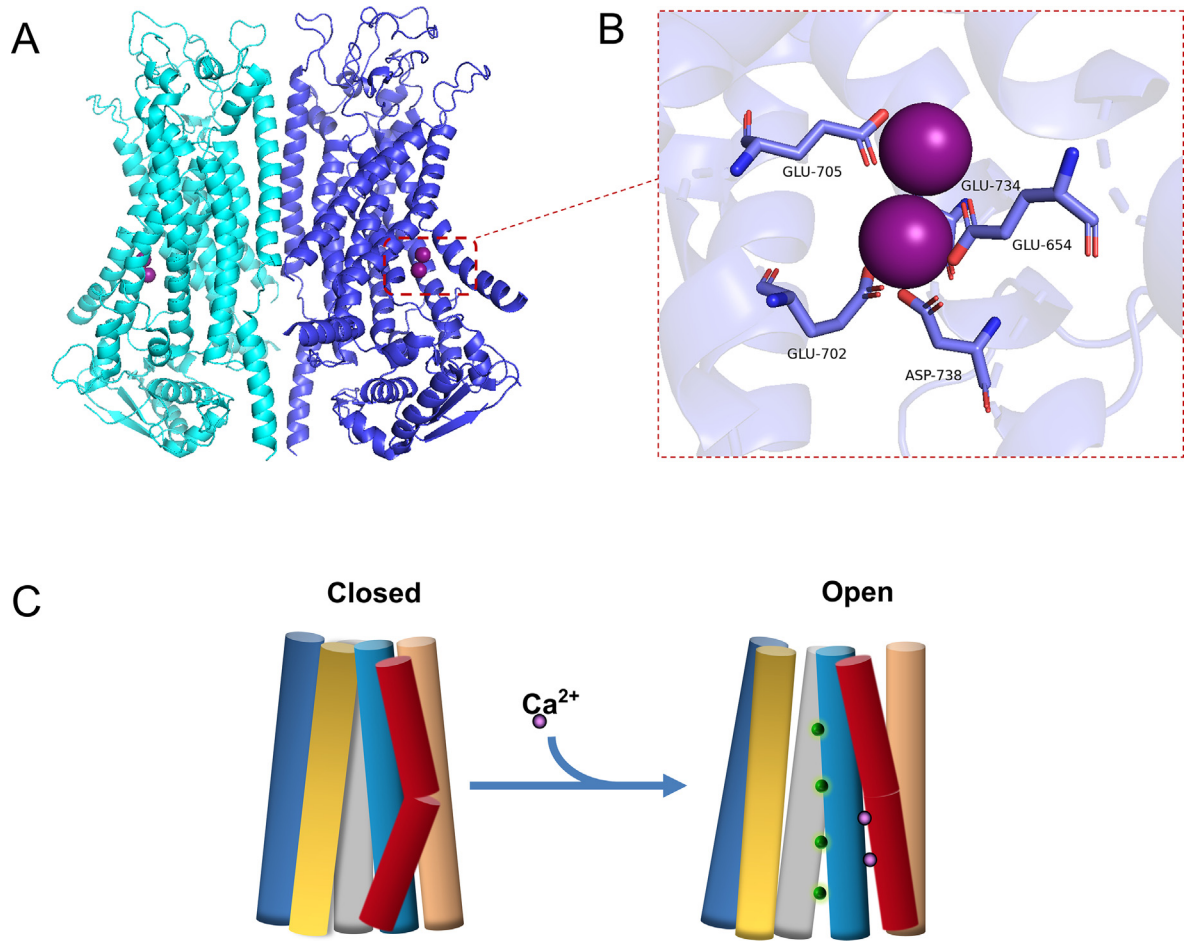


Fig. 1. Cryo-EM structure of TMEM16A and Ca^{2+}-dependent gating of TMEM16A channels. A. The homodieric structure of Ca^{2+}-bound TMEM16A with ten transmembrane helices (PDB: 5OYB). B. The structure of the Ca^{2+}-binding site, which is formed by five conservative amino acids (E654, E702, E705, E734, and D738) on TM6-8. Purple spheres: Ca^{2+} ions. The figures were generated using PyMol (https://pymol.org/2/). C. Schematic illustration showing Ca^{2+}-dependent gating of TMEM16A channels. Closed state (no Ca^{2+} binding): TM6 bends around the glycine hinge residue (G644), resulting in a narrowing of the pore. Open state: Ca^{2+} binding straightens the cytosolic end of TM6 to dilate the pore.

we summarize multiple mechanisms for regulating TMEM16A expression in inflammation (Fig. 2).

The IL-4/IL-13/STAT6 signaling pathway

The identification of TMEM16A as a Ca^{2+}-activated Cl^- channel by Caputo et al. [2] is based on a previous finding that the Th2 cytokine IL-4 promotes the Ca^{2+}-activated Cl^- current in human bronchial epithelial cells [59]. Caputo et al. found that TMEM16A mediates the Ca^{2+}-activated Cl^- current in epithelial cells treated with IL-4 [2]. This was the first evidence showing that TMEM16A expression is upregulated under inflammatory conditions. Subsequently, several studies have confirmed that IL-4 promotes TMEM16A expression in airway epithelial cells [60–62], biliary epithelial cells [63], and cholangiocytes [64]. Mazzone et al. found that IL-4 promoted TMEM16A expression via STAT6, which binds to the STAT6 binding site on the promoter region of the TMEM16A gene [61]. Similar to IL-4, the Th2 cytokine IL-13 also promotes TMEM16A expression via STAT6 in human airway epithelial cells [65,66], cholangiocytes [64], and esophageal epithelial cells [40]. Therefore, IL-4 and IL-13 upregulated TMEM16A expression in epithelial cells by increasing the transcription of the TMEM16A gene via STAT6.

Asthma is a Th2-mediated inflammatory airway disease characterized by goblet cell hyperplasia and mucus hypersecretion. TMEM16A overexpression induced by IL-4 or IL-13 predominantly occurs in mucin-secreting goblet cells, where TMEM16A plays a critical role in goblet cell hyperplasia and mucus secretion [6,13,62]. Huang et al. found that TMEM16A expression was upregulated in goblet cells in Th2-high asthmatic patients, in animal models of asthma induced by ovalbumin, or in transgenic mice overexpressing IL-13 [13]. Increased TMEM16A expression level was also observed in a guinea pig asthma model induced by IL-13 and ovalbumin challenge [37]. Therefore, TMEM16A expression is upregulated by IL-13 in asthma.

Chronic rhinosinusitis with nasal polyps (CRSwNP) is also a Th2-mediated inflammatory disorder characterized by persistent high levels of IL-4 and IL-13 [67]. TMEM16A expression was upregulated in the sinonasal mucosa tissues of patients with CRSwNP, and IL-13 promoted TMEM16A expression in human sinonasal epithelial cells [39]. Eosinophilic esophagitis is another Th2-mediated inflammatory disease with a characteristic feature of intraepithelial eosinophil infiltration and basal zone hyperplasia (BZH). TMEM16A expression was enhanced in the basal layer of the esophagus in patients with eosinophilic esophagitis, and IL-13 upregulated TMEM16A expression in esophageal cells via STAT6 [40]. TMEM16A knockdown reduced IL-13-induced proliferation of esophageal cells, suggesting that TMEM16A overexpression induced by IL-13 contributes to esophageal epithelial proliferation and BZH in eosinophilic esophagitis [40].

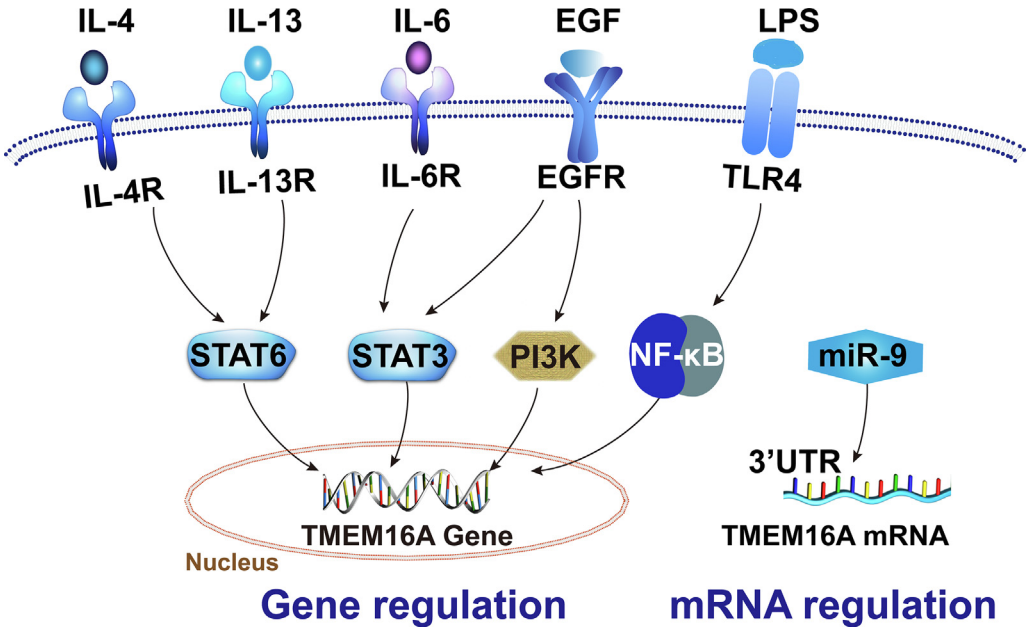


Fig. 2. Multiple mechanisms for regulating TMEM16A expression in inflammation. TMEM16A expression is upregulated in epithelial cells by Th2 cytokine IL-4/IL-13/STAT6 signaling activation in many inflammatory airway diseases, such as asthma, CRSwNP, eosinophilic esophagitis. In acute pancreatitis, the proinflammatory cytokine IL-6 increases TMEM16A expression level in pancreatic acinar cells via the IL-6R/STAT3 signaling pathway. EGF promotes TMEM16A upregulation in airway epithelial cells via the PI3K signaling pathway. LPS induced TMEM16A expression in intestinal epithelial cells via the NF-κB signaling pathway. In addition, TMEM16A expression is regulated by miR-9 in many inflammatory diseases such as CF, IPF, and LPS-induced sepsis.

The IL-6/STAT3 signaling pathway

The proinflammatory cytokine IL-6 contributes to the pathogenesis of acute pancreatitis [68]. In acute pancreatitis, inflammatory mediators such as IL-6 are released from pancreatic acinar cells following activation of inflammatory pathways (e.g., the nuclear factor-κB (NFκB) signaling pathway), subsequently causing local pancreatic injury and inducing a systemic inflammatory response [69]. TMEM16A is expressed in many pancreatic tissues, including pancreatic acinar cells [10,12,28,70], and functions as a Cl^- channel and/or HCO_3^- transporter [5,71]. As an HCO_3^- transporter, TMEM16A is implicated in a cell model of acute pancreatitis induced by a supramaximal concentration of cholecystokinin, in which TMEM16A inhibition contributes to luminal acidosis [71]. TMEM16A expression has been found to be upregulated in pancreatic acinar cells in mouse and cell models of cerulein-induced acute pancreatitis, accompanied by increased serum and pancreatic levels of IL-6 [10]. IL-6 treatment increases TMEM16A expression levels in pancreatic acinar cells via the IL-6R/STAT3 signaling pathway [10]. The STAT3-dependent mechanisms underlying TMEM16A overexpression are consistent with our previous findings showing TMEM16A upregulation via EGFR/STAT3 signaling activation in breast cancer cells [72]. Since TMEM16A overexpression also activates NF-κB signaling to increase IL-6 release from pancreatic acinar cells, TMEM16A expression and NFκB/IL-6 may form a positive feedback loop, resulting in a sustained inflammatory response and high TMEM16A expression level in acute pancreatitis.

The EGFR signaling pathway

Upregulation of TMEM16A expression by EGFR signaling activation has been reported in cancer cells [72,73]. High TMEM16A expression correlated with high EGFR expression in breast cancer [72] or non-small cell lung cancer [74]. EGFR signaling activation promotes TMEM16A expression in head and neck squamous cell carcinoma (HNSCC) cells overexpressing EGFR [73], and in breast cancer cells via EGFR/STAT3 signaling activation [72]. Furthermore, EGF increases TMEM16A expression in non-cancer cells, including intestinal epithelial cells [8] and human nasal epithelial cells [54]. A recent study has shown that EGF promoted TMEM16A upregulation via the PI3K signaling pathway. TMEM16A also mediated EGF-induced mucin secretion in human nasal epithelial cells from patients with CRSwNP [54], suggesting that EGFR-mediated increases in TMEM16A expression is important for mucin secretion in airway inflammatory diseases. Since abnormal EGF/EGFR signaling is associated with many inflammatory airway diseases such as asthma, CF, and chronic obstructive pulmonary disease (COPD) [75], EGFR signaling activation may contribute to TMEM16A overexpression in these inflammatory diseases.

The LPS/NF-κB signaling pathway

Bacterial infection is one of the main causes of many inflammatory diseases (e.g., acute lung injury and inflammatory bowel disease) involving airway and intestinal epithelial cells, wherein TMEM16A is important for fluid and mucus secretion [5–7,13]. TMEM16A expression is upregulated in airway epithelial cells, intestinal epithelial cells, and RAW264.7 macrophages following LPS treatment [56–58]. Mechanistically, LPS activates NF-κB signaling by binding to the TLR4 receptor [76]. Recently, Sui et al. reported that NF-κB knockdown inhibited LPS-induced upregulation of TMEM16A expression in intestinal epithelial cells [58], suggesting that LPS upregulates TMEM16A expression via the NF-κB signaling pathway.

microRNAs

miRNAs are small non-coding RNAs that repress gene expression by targeting the 3′ untranslated region (UTR) of the target gene. TMEM16A overexpression due to downregulation of microRNAs has been observed in many cancers. For example, TMEM16A

overexpression is caused by miR-132, miR-9, and miR-144 downregulation in colorectal cancer [77–79], and by miR-381 downregulation in gastric cancer [80]. Regulation of TMEM16A by miR-9 has been reported in many inflammatory conditions such as idiopathic pulmonary fibrosis (IPF) [81] and CF [82], as well as in LPS-induced sepsis [45]. Bioinformatics and luciferase reporter assays have demonstrated that miR-9 directly binds to the 3′ UTR of TMEM16A mRNA and inhibits TMEM16A expression [81,82]. A target site blocker that inhibits the binding of miR-9 to the 3′ UTR region of TMEM16A mRNA increased TMEM16A-mediated Cl^- currents, promoted mucus clearance, and increased airway epithelial cell migration in CF [82]. In addition, miR-9-mediated TMEM16A downregulation promoted inflammation, increased proliferation, and inhibited apoptosis in fibroblasts in mice with IPF [81]. Furthermore, miR-9 knockdown ameliorated LPS-induced sepsis by inhibiting TGF-β signaling by targeting TMEM16A [45]. Therefore, miR-9 is an important regulatory mechanism for TMEM16A expression in many inflammatory diseases, such as CF, IPF, and LPS-induced sepsis.

TMEM16A activation by various inflammatory inducers and mediators via Ca^{2+}

The inflammatory response is initiated by many exogenous inducers, such as bacterial and viral pathogens [83]. In addition, a wide range of inflammatory mediators such as histamine, leukotrienes (LT), and bradykinin are produced by inflammatory cells or secreted by tissue cells during inflammation [83]. It is known that bacterial or viral pathogens can cause an increase in $[Ca^{2+}]_i$ [84], and many inflammatory mediators promote IP_3R-mediated Ca^{2+} release from the endoplasmic reticulum (ER) by activating their corresponding Gq protein-coupled receptors (GqPCRs) [85,86]. Recent studies have demonstrated that TMEM16A can be activated by $[Ca^{2+}]_i$ elevation induced by bacterial or viral pathogens, or by inflammatory mediators via IP_3R-mediated Ca^{2+} release following GqPCR activation (Fig. 3).

TMEM16A activation following bacterial or viral infection

Several studies have shown that TMEM16A Cl^- channel activity is increased in infectious diseases such as rotaviral diarrhea [42–44]. TMEM16A has been reported to mediate Cl^- secretion in colonic epithelial cells [5]. Ousingsawat et al. reported that the rotavirus toxin NSP4, which is known to activate PLC to increase $[Ca^{2+}]_i$ [87], activated TMME16A Cl^- channels in colonic epithelial cells and TMEM16A-overexpressing HEK293 cells [43]. TMEM16A inhibition by many compounds such as red wine extract [88], trans-delta-viniferin [89], and shikonin [42] relieved rotaviral diarrhea. Therefore, rotaviral endotoxin induces excessive fluid secretion by activating TMEM16A Cl^- channels in colonic epithelial cells.

Notably, Lee et al. reported that cAMP-induced Cl^- currents were significantly decreased in the colonic epithelial cells of mice with TMEM16A gene deletion independent of CFTR, suggesting that TMEM16A mediates cAMP-activated Cl^- currents in colonocytes [9]. Furthermore, fluid secretion induced by cholera toxin, which stimulates an increase in intracellular cAMP, was reduced in TMEM16A-deficient colonocytes [9], suggesting that TMEM16A mediates cholera toxin-induced fluid secretion. Since cAMP can promote Cl^- secretion in intestinal cells by elevating $[Ca^{2+}]_i$ [90], it is possible that cAMP activates TMEM16A channels via increasing $[Ca^{2+}]_i$. Therefore, cholera toxin activates TMEM16A Ca^{2+}-activated Cl^- channels via cAMP-induced increases in $[Ca^{2+}]_i$.

Bacterial infection commonly occurs in CF, and *Pseudomonas aeruginosa* is the most prevalent bacterium that deteriorates lung function in CF [91]. TMEM16A expression is upregulated in bronchial epithelial cells treated with pyocyanin, the virulence factor of *P. aeruginosa* [55]. Furthermore, pyocyanin can induce an increase in $[Ca^{2+}]_i$ and promote TMEM16A-mediated Cl^- secretion in bronchial epithelial cells [55]. Thus, pyocyanin can activate TMEM16A Cl^- channels by elevating $[Ca^{2+}]_i$.

TMEM16A activation by inflammatory mediators via GqPCRs

Histamine is a major proinflammatory mediator that is mainly released from mast cells and basophiles under inflammatory conditions, such as allergic rhinitis and asthma [92]. TMEM16A expression level was increased in patients with allergic rhinitis, and histamine promoted TMEM16A-mediated Cl^- secretion in airway epithelial cells, especially upon IL-4 treatment [60]. Histamine-stimulated TMEM16A activation is likely mediated by the GqPCR H_1R, because H_2R or H_3R agonists do not induce fluid secretion [60]. In addition, histamine activates TMEM16A channels in airway smooth muscle cells [38]. Therefore, TMEM16A can be activated by histamine under inflammatory conditions.

The inflammatory mediators 5-HT, thromboxane A2, and LTD4 induce an increase in $[Ca^{2+}]_i$ by activating their corresponding GqPCRs, In addition to histamine, Wang et al. reported that these mediators induced airway smooth muscle contraction by activating TMEM16A Cl^- channels [38]. In addition, Jin et al. found that bradykinin activated TMEM16A Cl^- channels via the G-protein coupled B2 receptor in DRG neurons [93]. Lee et al. reported that inflammatory soups containing a mixture of bradykinin, histamine, prostaglandin E2, and serotonin, which function as agonists of their corresponding GqPCRs, induced membrane depolarization in DRG neurons. This effect was absent in TMEM16A-knockout DRG neurons [19], suggesting that these inflammatory mediators induced Cl^- efflux by activating TMEM16A channels. Therefore, TMEM16A can be activated by many inflammatory mediators via GqPCRs.

Intracellular ATP can be passively released from damaged cells under inflammatory conditions [94]. ATP can increase $[Ca^{2+}]_i$ by activating Gq-protein-coupled P2Y receptors. Activation of TMEM16A Cl^- channels by ATP or UTP via P2Y receptors has been demonstrated in oocytes with heterologous expression of TMEM16A and P2Y1 receptors [95] or in TMEM16A-overexpressing HEK293 cells [96]. TMEM16A activation by ATP or UTP has been observed in many epithelial cells, including airway epithelial cells [13,37,97–99], colonic epithelial cells [100], cholangiocytes [64], and supporting cells of the mouse olfactory epithelium [101], and mediates ATP/UTP-induced Cl^- secretion in these epithelial cells.

Activation of diverse inflammatory signaling pathways by TMEM16A

TMEM16A can activate diverse signaling pathways in distinct cells [30,31]. In cancer cells, TMEM16A activates mitogen-activated protein kinase (MAPK) signaling in HNSCC [102], NF-κB signaling in glioma [103], EGFR/Ca^{2+} signaling in pancreatic cancer [104], and EGFR/STAT3 signaling in breast cancer [72]. In non-cancer cells, TMEM16A promotes reactive oxygen species (ROS) generation in endothelial cells by directly binding to Nox2 NADPH oxidase [16], activates the p38/JNK signaling pathway in podocytes [105], and regulates Ca^{2+} signaling via receptor- (ROCE) and store- (SOCE) operated Ca^{2+} entry in pulmonary arterial smooth muscle cells [106]. TMEM16A can also activate signaling pathways in many inflammatory diseases. For example, TMEM16A increases $[Ca^{2+}]_i$ via IP_3R-mediated Ca^{2+} release, and promotes excessive mucus secretion from goblet cells in airway inflammatory diseases [6,27]. TMEM16A also activates NF-κB signaling and promotes the

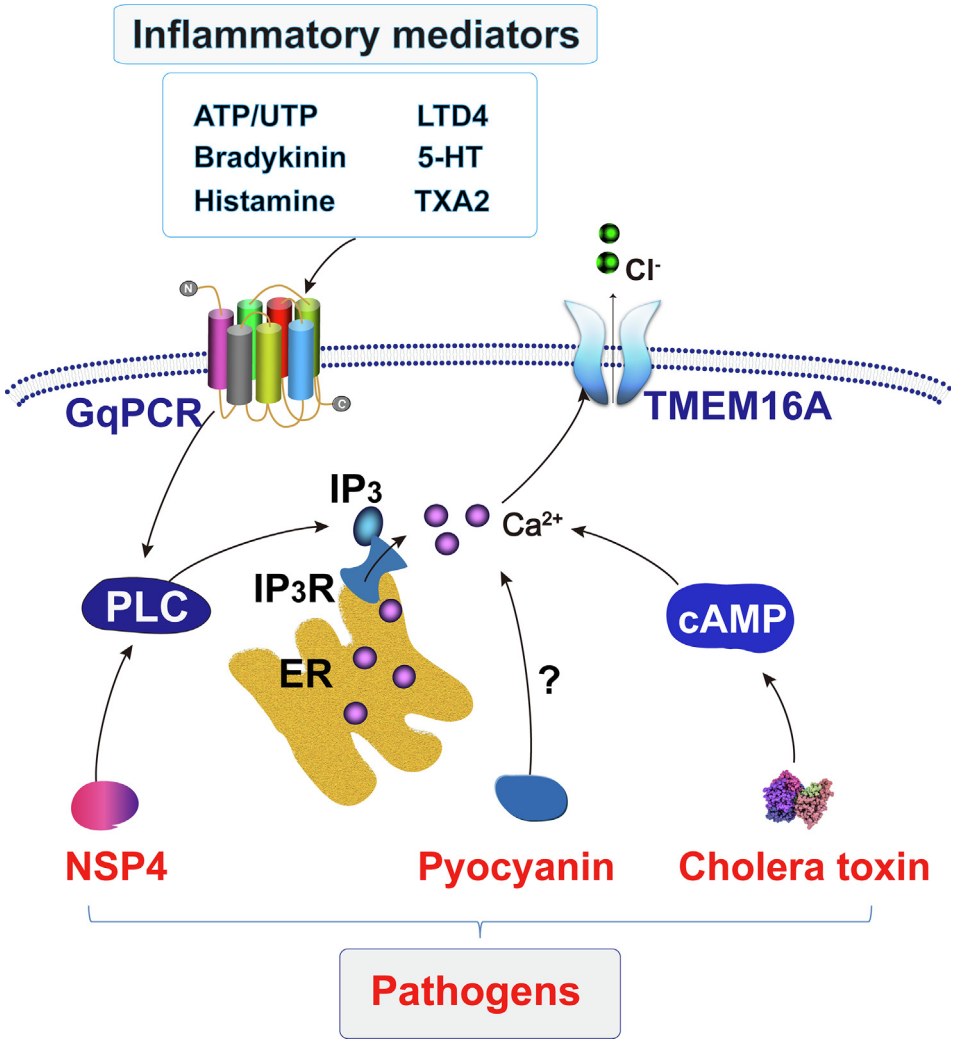


Fig. 3. TMEM16A activation by various inflammatory inducer and mediators. TMEM16A is activated by rotavirus toxin NSP4 via PLC-mediated Ca^{2+} release from the ER, by cholera toxin via cAMP-mediated increases in $[Ca^{2+}]_i$, and by pyocyanin via increasing $[Ca^{2+}]_i$. In addition, many inflammatory mediators including histamine, 5-HT, thromboxane A2 (TXA2), LTD4, ATP/UTP, and bradykinin activate TMEM16A via increasing IP_3R-mediated Ca^{2+} release by acting on their corresponding GqPCRs.

pathogenesis of acute pancreatitis [10] and steatohepatitis [41]. TMEM16A promotes mucus hypersecretion in inflammatory airway diseases [65] and aggravates LPS-induced epithelial barrier dysfunction [58] by activating the ERK1/2 signaling pathway. In this review, we summarize the diverse inflammatory signaling pathways activated by TMEM16A (Fig. 4).

IP_3R-mediated Ca^{2+} signaling

TMEM16A is localized to the ER-plasma membrane contact sites, where many Ca^{2+} channels (e.g., IP_3R, TRP channels, STIM1 and Orai channels) and receptors (e.g., GPCRs, EGFR) are located to regulate Ca^{2+} signaling [107,108]. Jin et al. first found that TMEM16A forms a complex with IP_3R and GPCR (including B2R and PAR-2) in DRG cells, and is activated by IP_3R-mediated Ca^{2+} release following GqPCR activation [93]. Similarly, the activation of TMEM16A by IP_3R-mediated Ca^{2+} release has been observed in HeLa [109] and AR42J cells [10]. The importance of TMEM16A activation by IP_3R-medicated Ca^{2+} release was further demonstrated by a recent study showing that IP_3R mediates TMEM16A activation via TRPV1 [110].

The direct interaction of TMEM16A with IP_3R suggests that TMEM16A also controls IP_3R-mediated Ca^{2+} signaling elicited by GqPCR activation [111]. Increasing evidence has shown that TMEM16A promotes $[Ca^{2+}]_i$ via IP_3R-mediated Ca^{2+} release. For example, Schreiber et al. reported that increases in $[Ca^{2+}]_i$ induced by the muscarinic agonist carbachol was inhibited in isolated colonic crypt cells from TMEM16A-knockout mice [29]. Conditional knockout or pharmacological inhibition of TMEM16A impaired Ca^{2+} transients in ICC [17,112], and TMEM16A knockdown inhibited IP_3R-mediated Ca^{2+} release from the ER in HeLa cells [109], human bronchial epithelial cells [98], and M1 cells [113]. Wang et al. recently found that TMEM16A inhibition by its inhibitor T16Ainh-A01 blocked IP_3R-induced Ca^{2+} release in AR42J cells treated with the cholesystokinin receptor agonist cerulein [10].

Increases in TMEM16A expression and function contribute to the development of inflammatory diseases by promoting IP_3R-mediated Ca^{2+} signaling. For example, TMEM16A expression is upregulated in mucin-secreting goblet cells in asthma [13,27]. Increases in TMEM16A expression level and function facilitate IP_3R-mediated Ca^{2+} release elicited by ATP, and thus lead to subsequent exocytosis of mucus-containing granules [6,27]. Therefore, TMEM16A may promote excessive mucus secretion from goblet cells in many inflammatory airway diseases such as asthma, CF, and COPD. Our recent findings demonstrating that TMEM16A promotes acute pancreatitis represent a second example showing the role of TMEM16A in inflammatory diseases, which it achieves by facilitating IP_3R-mediated Ca^{2+} release [10]. TMEM16A expression

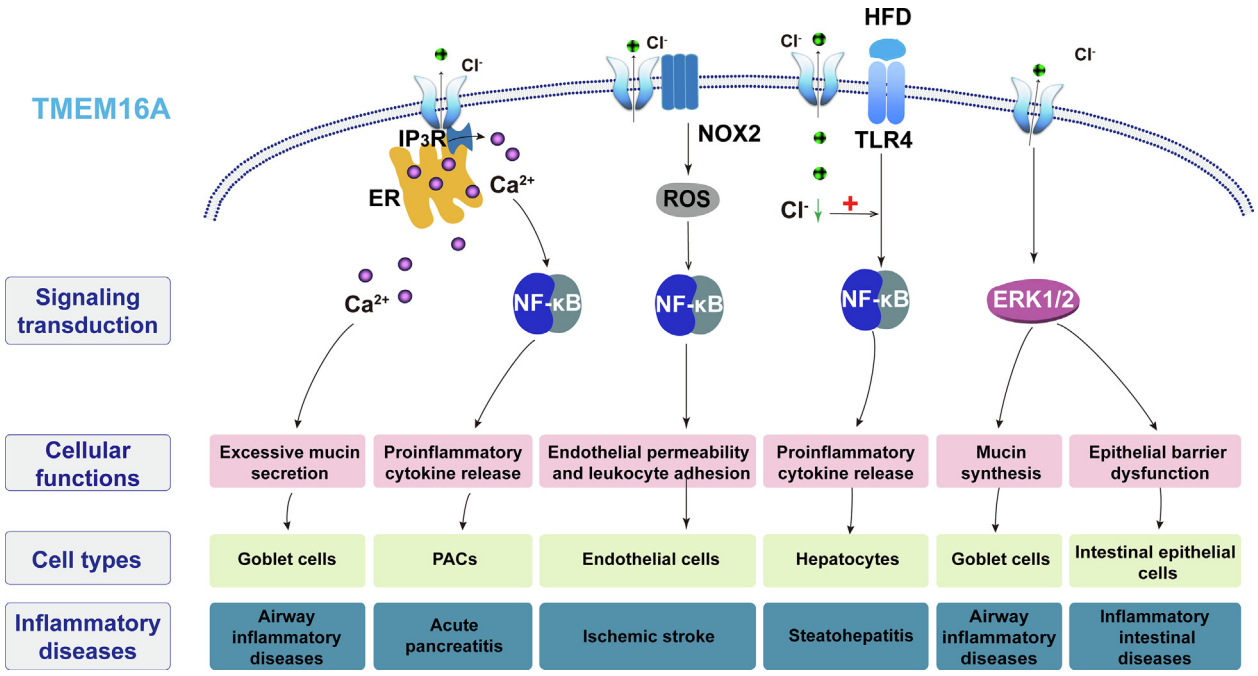


Fig. 4. TMEM16A-mediated activation of diverse inflammatory signaling pathways in distinct cells in many inflammatory diseases. TMEM16A directly interacts with IP_3R and increases $[Ca^{2+}]_i$ via IP_3R-mediated Ca^{2+} release, thus promoting excessive mucus secretion from goblet cells in airway inflammatory diseases such as asthma, COPD, and CF. TMEM16A-mediated Ca^{2+} elevation activates NF-κB signaling in pancreatic acinar cells (PACs) and promotes the pathogenesis of acute pancreatitis. TMEM16A activates the NF-κB signaling pathway in endothelial cells by increasing ROS generation in ischemic stroke. TMEM16A-mediated Cl^- efflux facilitates high fat diet (HFD)/TLR4-induced NF-κB activation in hepatocytes by reducing intracellular Cl^- concentration, and thus contributes to the pathogenesis of steatohepatitis. TMEM16A activates the ERK1/2 signaling to promote mucus synthesis and release in goblet cells and thus is important for mucus hypersecretion in inflammatory airway diseases such as asthma. TMEM16A aggravated LPS-induced epithelial barrier dysfunction in intestinal epithelial cells by activating the ERK1/2 signaling pathway.

level is increased in cell and mouse models of cerulein-induced acute pancreatitis [10]. TMEM16A knockdown or inhibitors blocked IP_3R-mediated Ca^{2+} release and subsequent activation of NF-κB signaling in pancreatic acinar cells, and reduced pancreatic tissue damage *in vivo* [10]. Therefore, TMEM16A promotes acute pancreatitis by maintaining sustained Ca^{2+} elevation and NF-κB activation [10], which are key contributors to the pathogenesis of acute pancreatitis [114].

The NF-κB signaling pathway

The NF-κB signaling pathway regulates the expression of various proinflammatory genes and plays a key role in many inflammatory diseases [115]. NF-κB signaling activation by TMEM16A was first reported in glioma cells [103]. Subsequently, TMEM16A has been found to activate the NF-κB signaling pathway in many different cells. For example, TMEM16A activates NF-κB signaling to promote mucus production in human airway epithelial cells [66]. TMEM16A knockdown inhibits oxygen-glucose deprivation/reoxygenation (OGDR)-induced NF-κB activation in brain endothelial cells [15], suggesting that TMEM16A is important for OGDR-induced NF-κB activation. Furthermore, Guo et al. found that high fat diet (HFD)-induced NF-κB signaling activation was increased by hepatocyte-specific transgenic overexpression of TMEM16A [41], suggesting that TMEM16A promotes NF-κB signaling activation in steatohepatitis. Wang et al. have previously found that TMEM16A activates the NF-κB signaling pathway in pancreatic acinar cells in mice with acute pancreatitis [10].

The mechanisms underlying NF-κB activation by TMEM16A have been investigated in acute pancreatitis [10]. Sustained Ca^{2+} elevation is an important initiator of acute pancreatitis, and Ca^{2+} is required for NF-κB activation in pancreatic acinar cells [114]. TMEM16A directly interacts with IP_3R [10,93,109], and promotes IP_3R-mediated Ca^{2+} release [10,109]. TMEM16A inhibition by T16Ainh-A01 blocked IP_3R-mediated Ca^{2+} release in AR42J cells [10], suggesting that TMEM16A facilitates IP_3R-mediated Ca^{2+} in AR42J cells. Furthermore, TMEM16A activated NF-κB signaling in pancreatic acinar cells during cerulein-induced acute pancreatitis; this effect was blocked by the Ca^{2+} chelator BAPTA-AM, suggesting that TMEM16A activates NF-κB signaling by increasing $[Ca^{2+}]_i$ via IP_3R [10].

NF-κB activation by TMEM16A overexpression has also been observed in human brain microvascular endothelial cells, since TMEM16A knockdown reduced NF-κB activation induced by OGDR [15]. However, the mechanisms underlying NF-κB activation by TMEM16A in endothelial cells remain unclear. Since TMEM16A is not co-immunoprecipitated with p65/NF-κB [15], TMEM16A may not activate NF-κB signaling via direct interaction. OGDR can induce IP_3R-mediated Ca^{2+} release [116], which is controlled by TMME16A [10,109]. Thus, TMEM16A may mediate OGDR-induced NF-κB signaling activation in endothelial cells by promoting IP_3R-mediated Ca^{2+} release, similar to the mechanism in AR42J cells. In agreement with this idea, Ca^{2+} has been found to activate NF-κB signaling in human cerebral microvascular endothelial cells [117]. In addition, Ma et al. found that TMEM16A promoted ROS generation via Nox2-containing NADPH oxidase in vascular endothelial cells [16]. ROS can activate NF-κB signaling in many cells, including endothelial cells [118,119]. Therefore, TMEM16A may activate the NF-κB signaling pathway via ROS.

NF-κB activation by TMEM16A also occurs in hepatocytes [41]. Guo et al. reported that hepatocyte-specific TMEM16A overexpression promoted HFD-induced TLR4/NF-κB signaling activation, suggesting that TMEM16A enhances NF-κB signaling activation to promote inflammation in hepatocytes [41]. However, the mechanisms underlying this effect are not known. Since decreases in intracellular Cl^- concentration promote NF-κB signaling activation [120], it is hypothesized that TMEM16A-mediated Cl^- efflux facilitates NF-κB activation by reducing the

intracellular Cl^- concentration [41]. Therefore, TMEM16A may activate NF-κB signaling in hepatocytes by reducing intracellular Cl^- concentration, thus contributing to the pathogenesis of steatohepatitis.

The extracellular signal-regulated kinase (ERK) signaling pathway

ERK 1/2 belongs to the MAPK family that regulates many cellular processes, such as proliferation, migration, and apoptosis, and is an important oncogenic driver of human cancers [121,122]. TMEM16A is preferentially coupled to the ERK/MAPK signaling cascade in cancers [31], and several studies have reported that TMEM16A overexpression activates ERK1/2 signaling in breast cancer [123], HNSCC [102], colorectal cancer [124], and hepatoma [125]. Recent studies have also shown that TMEM16A activates ERK1/2 signaling in inflammatory diseases [58,65]. For example, Qin et al. reported that TMEM16A inhibition by genetic silencing or pharmacological inhibitors decreased ERK1/2 phosphorylation in airway epithelial cells, and TMEM16A-mediated ERK1/2 signaling activation is important for mucus hypersecretion in inflammatory airway diseases such as asthma [65]. In addition, Sui et al. reported that TMEM16A aggravated LPS-induced epithelial barrier dysfunction in intestinal epithelial cells via ERK1/2 signaling activation [58], suggesting that TMEM16A-mediated activation of the ERK signaling pathway contributes to inflammatory intestinal diseases.

Multifunctional roles of TMEM16A in inflammatory processes

Inflammation is an adaptive response that is triggered by harmful exogenous stimuli such as pathogens, toxic compounds, allergens, and irritants, or by endogenous molecules released from damaged cells and tissues [83]. Acute inflammation consists of multiple biological processes, including increased vascular permeability, local exudation formation, leukocyte infiltration and accumulation, and inflammatory mediator release from immune and tissue cells [126]. TMEM16A overexpression contributes to increased vascular permeability and leukocyte recruitment in endothelial cells [15], and activates many inflammatory signaling pathways (e.g., Ca^{2+} signaling and NF-κB signaling) to promote the release of inflammatory cytokines from epithelial cells (Fig. 4). We developed the idea that TMEM16A is involved in inflammatory processes by increasing vascular permeability and leukocyte adhesion, and by promoting the release of proinflammatory cytokines from tissue cells (Fig. 5).

TMEM16A promotes endothelial permeability and leukocyte adhesion in inflammation

The vascular endothelium, which constitutes the innermost layer of the blood vessels, controls the transport of fluid, ions, nutrients, and macromolecules across the vessel wall. During inflammation, the permeability of endothelial cells is increased to allow extravasation of blood components (e.g., plasma proteins, fluids, and leukocytes), resulting in excessive accumulation of fluid and inflammatory cells surrounding the tissue. Tight junctions, which are composed of claudins and occludins, mediate cell–cell interactions and are important for controlling the permeability of endothelial cells [127]. Recently, Liu et al. reported that TMEM16A was upregulated in brain endothelial cells, and the TMEM16A inhibitor CaCCinh-A01 reduced cerebral infarct size and improved neurological function in a mouse model of ischemic stroke [15]. Downregulation of TMEM16A expression reduced transendothelial permeability in *in vivo* and *in vitro* models of ischemia/perfusion injury, accompanied by an increase in the expression levels of zonula occludens (ZO)-1, occludin, and claudin-5 [15]. Thus, TMEM16A overexpression contributes to increased endothelial cell permeability by disrupting tight junctions during ischemic stroke. However, the mechanisms underlying TMEM16A-mediated tight junction dysfunction in endothelial cells remain unclear. Sui et al. found that TMEM16A overexpression disrupted tight junctions by activating the ERK1/ELK1 signaling pathway in LPS-treated colonic epithelial cells [58]. It remains to be determined whether TMEM16A promotes endothelial permeability by activating the ERK1/ELK1 signaling pathway.

NF-κB signaling activation promotes the expression of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), which is important for regulating endothelial adhesion, transendothelial migration, and extravasation of circulating leukocytes during inflammation [128]. TMEM16A overexpression has been reported to activate NF-κB signaling and subsequently promote ICAM-1 expression in brain endothelial cells [15]. Furthermore, TMEM16A knockdown reduced OGDR-induced adhesion of leukocytes to endothelial cells by decreasing ICAM-1 expression [15], suggesting that TMEM16A promotes extravasation of circulating leukocytes via NF-κB/ICAM-1 signaling activation.

TMEM16A promotes inflammatory response by increasing proinflammatory cytokine release

TMEM16A activates the NF-κB signaling pathway, which regulates the expression of proinflammatory cytokines in inflammatory diseases [115]. TMEM16A expression was upregulated in the hepatocytes of mice fed a HFD, and hepatocyte-specific TMEM16A overexpression promoted the expression of inflammatory cytokines such as IL-6, IL-1β, and TNF-α in HFD-treated mice [41]. Since conditional TMEM16A knockout reduced NF-κB signaling activation via a reduction in proinflammatory cytokines, TMEM16A in hepatocytes may aggravate the inflammatory response by promoting the production and release of proinflammatory cytokines via NF-κB signaling in steatohepatitis [41].

Wang et al. have previously reported that TMEM16A overexpression induces IL-6 secretion in pancreatic acinar cells by activating the NF-κB signaling pathway [10]. TMEM16A inhibition by genetic silencing or its inhibitor T16Ainh-A01 inhibited NF-κB activation and reduced IL-6 secretion in *in vitro* and *in vivo* models of cerulein-induced acute pancreatitis [10], suggesting that TMEM16A aggravates acute pancreatitis by activating NF-κB signaling and promoting proinflammatory cytokine release.

Benedetto et al. reported that TMME16A knockdown reduced IL-8 release in Calu3 epithelial cells, indicating that TMEM16A may be important for IL-8 release in inflammatory diseases [6]. Since IL-8 is a chemokine that can induce leukocyte migration [129], decreased IL-8 release from airway epithelial cells may explain the observed reduction in leukocyte infiltration in TMEM16A-knockout mice with ovalbumin challenge [6]. These findings suggest that TMEM16A promotes an inflammatory response by increasing the release of proinflammatory cytokines.

In summary, expression level and function of TMEM16A are increased in response to many inflammatory mediators, including histamine, IL-6, and IL-4, as well as pathogens such as bacteria and viruses (Figs. 2, 3). In endothelial cells, TMEM16A overexpression increases endothelial permeability by disrupting tight junctions and promoting leukocyte adhesion and extravasation. Thus, TMEM16A is important for increased vascular permeability and recruitment of leukocytes into the inflammatory site. In epithelial cells, TMEM16A overexpression promotes the release of many inflammatory cytokines by activating the NF-κB signaling pathway. Of these cytokines, IL-6 is important for maintaining high TMEM16A expression by activating IL-6R/STAT3 signaling, and IL-8 promotes leukocyte migration into the inflammatory sites

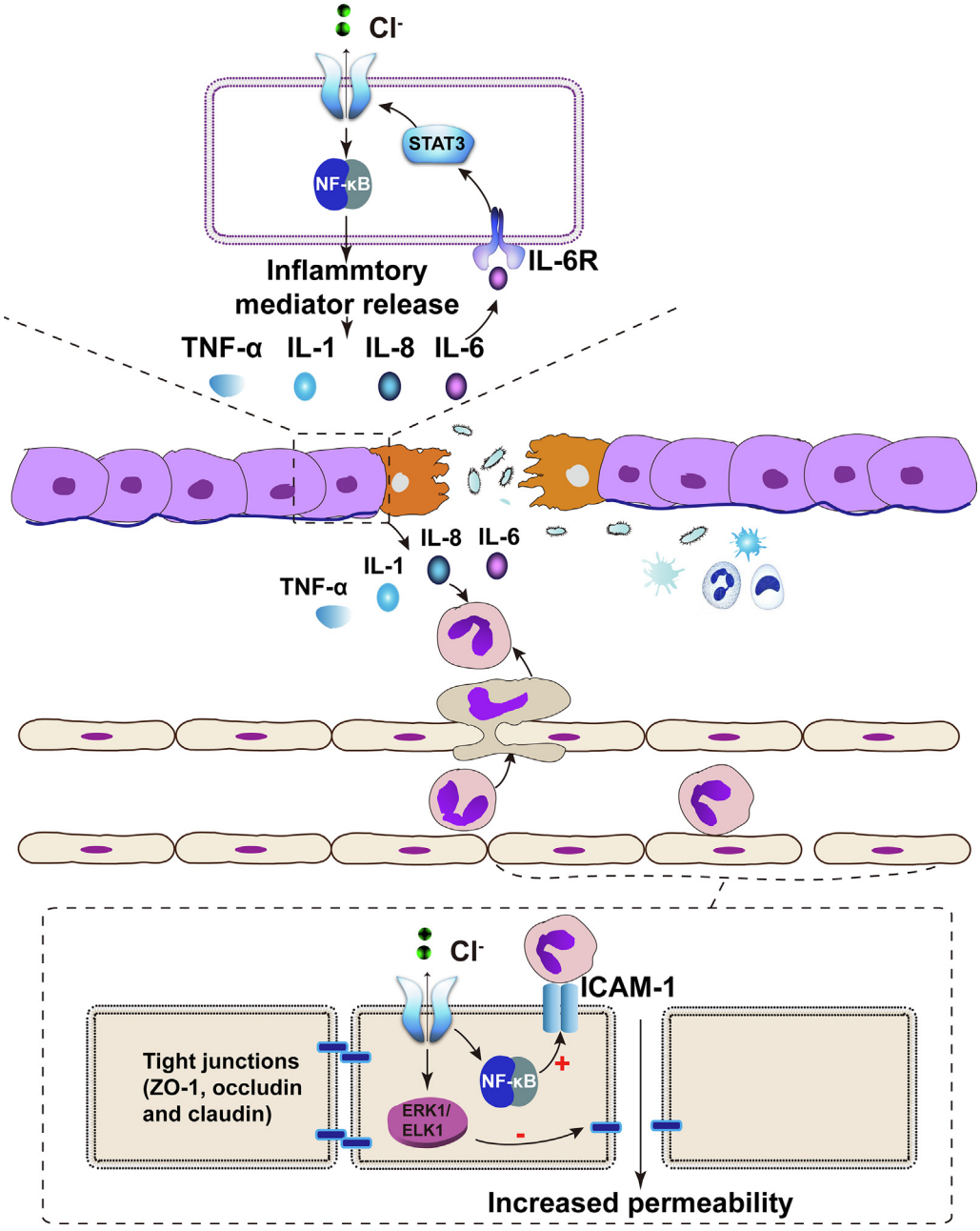


Fig. 5. TMEM16A mediates inflammatory processes by increasing vascular permeability and leukocyte adhesion and promoting release of proinflammatory cytokines from tissue cells. The inflammatory process is regulated by multiple cells including tissue cells, immune cells, and vascular cells. Immune cells such as macrophages or mast cells initially recognize noxious stimuli (tissue injury or pathogens) and release proinflammatory cytokines such as histamine, IL-6, and IL-4. These cytokines promote TMEM16A expression and function. Endothelial TMEM16A overexpression disrupts tight junctions, possibly by activating the ERK1/ELK1 signaling pathway, and thus increases vascular permeability. Endothelial TMEM16A also increases ICAM-1 expression level by activating the NF-κB signaling pathway, and subsequently promotes adhesion and extravasation of circulating leukocytes. TMEM16A overexpression in epithelial cells activates NF-κB signaling and promotes release of proinflammatory cytokines such as IL-6, IL-8, IL-1β, and TNF-α. IL-6 further promotes TMEM16A expression by activating IL-6R/STAT3 signaling pathway. IL-8 promotes leukocyte infiltration into the inflammatory site.

(Fig. 5). Therefore, TMEM16A is involved in multiple steps of inflammatory processes, including vascular permeability, leukocyte infiltration, and inflammatory mediator release.

TMEM16A contributes to inflammation-induced pain

Pain is one of the cardinal features of inflammation, and is triggered by inflammatory mediators released from inflammatory cells or damaged tissues. DRG neurons extend their peripheral axons to the target tissues, where the plasma membrane receptors, particularly GPCRs and ion channels, sense and transduce nociceptive stimuli such as temperature, mechanical pressure, and chemical stimuli [130]. TMEM16A is expressed in DRG neurons, especially in a subpopulation of small DRG neurons that are responsible for nociceptive sensation [4,18]. Since DRG neurons maintain a high intracellular Cl^- concentration, Cl^- efflux as a result of TMEM16A activation leads to membrane depolarization of nociceptive neurons [18,32]. Cho et al. reported that TMEM16A was directly activated by noxious heat (temperature > 44 °C) in the absence of Ca^{2+}; furthermore, heat-activated Cl^- currents were reduced in

nociceptive DRG neurons isolated from conditional TMEM16A-knockout mice, which are insensitive to noxious heat-induced pain [18]. Thus, TMEM16A is important for heat-induced pain sensation.

The role of TMEM16A in inflammation-induced pain was first demonstrated in a study by Liu et al. [20], showing that bradykinin, an inflammatory mediator that causes pain sensation, activates TMEM16A channels to induce membrane depolarization and action potential firing in DRG neurons. Bradykinin activates TMEM16A Cl^- channels by IP_3R-mediated Ca^{2+} release via the Gq-protein coupled B2 receptor, which directly interacts with IP_3R and TMEM16A in DRG neurons [93]. TMEM16A inhibitors or ablation of the TMEM16A gene in DRG neurons inhibited nocifensive behaviors in mice treated with bradykinin or formalin [19,20,46,47], suggesting that TMEM16A contributes to inflammation-induced pain sensation (Fig. 6). In addition, TMEM16A is also mediated by hyperalgesia and allodynia responses under inflammatory and nerve-injury conditions [19,131].

It is also known that the G-protein coupled receptor PAR2 (protease-activated receptor 2) is involved in pain and inflammation [132]. TMEM16A and PAR2 are reportedly co-expressed in DRG neurons, and TMEM16A and PAR2 expression is upregulated in rats with neuropathic pain induced by chronic constriction injury [133]. In addition, TMEM16A is co-expressed with PAR2 in the caveolin-rich microdomain in small DRG neurons. TMEM16A can be activated by local Ca^{2+} release induced by PAR2 stimulation [93], suggesting that TMEM16A activation is important for PAR2-mediated pain sensation (Fig. 6).

In DRG neurons, TMEM16A is also co-expressed with TPRV1, a Ca^{2+} permeant cation channel that is well known for its role in pain sensation [18]. Takayama et al. reported that TMEM16A and TRPV1 interact, and that TMEM16A Cl^- channels are activated by Ca^{2+} influx via TRPV1 in DRG neurons [33]. TMEM16A inhibition by T16Ainh-A01 inhibited capsaicin-induced currents in DRG neurons and reduced capsaicin-induced pain-related behaviors in mice, suggesting that the TMEM16A-TRPV1 interaction is critical for pain sensation [33]. In addition, Shah et al. reported that TMEM16A, TRPV1, and IP_3R1 are in close proximity to the ER-plasma membrane contact site of DRG neurons, where TRPV1 activates TMEM16A by promoting IP_3R-mediated Ca^{2+} release from the ER, presumably through Ca^{2+}-dependent PLC activation [110]. Thus, the mechanisms underlying TMEM16A activation by TRPV1 may include direct TMEM16A activation by Ca^{2+} influx through TRPV1 and indirect activation by IP_3R-mediated Ca^{2+} release following PLC activation by TRPV1 (Fig. 6).

Cellular environment-dependent role of TMEM16A in inflammation

The TMEM16A Ca^{2+}-activated Cl^- channel exerts diverse cellular functions in inflammatory diseases. For example, TMEM16A overexpression in airway and intestinal epithelial cells can result in excessive fluid secretion in inflammatory diseases such as allergic rhinitis [60,134,135] and infectious diarrhea [42–44,89]. In addition, increased TMEM16A expression level and activity in goblet cells contribute to goblet cell metastasis and mucus hypersecretion under inflammatory conditions [6,60–62,65,66] and airway inflammatory diseases such as asthma [13]. TMEM16A mediates the contraction of airway smooth muscle cells, and increased TMEM16A activity contributes to airway hyper-responsiveness in asthma [13,38]. Furthermore, TMEM16A overexpression activates IP_3R/Ca^{2+}/NF-κB signaling and promotes IL-6 secretion from pancreatic acinar cells in acute pancreatitis [10]. In hepatocytes, TMEM16A aggravates steatosis and inflammation by interacting with VAMP3, and thus contributes to the development of nonalcoholic fatty liver disease (NAFLD). Since TMEM16A plays distinct roles in different cell types, we developed the idea that TMME16A exerts cell-specific effects in inflammatory diseases. The cell-specific roles of TMEM16A in different inflammatory diseases suggest that distinct disease conditions create a unique cellular environment that determines the specific role of TMEM16A.

TMEM16A expression is upregulated in inflammatory conditions by multiple mechanisms such as the IL-4/STAT6, IL-6/STAT3, EGFR, and NF-κB signaling pathways, all of which also promote cell proliferation. IL-4-induced upregulation of TMEM16A expression is dependent on cell proliferation [136]. In turn,

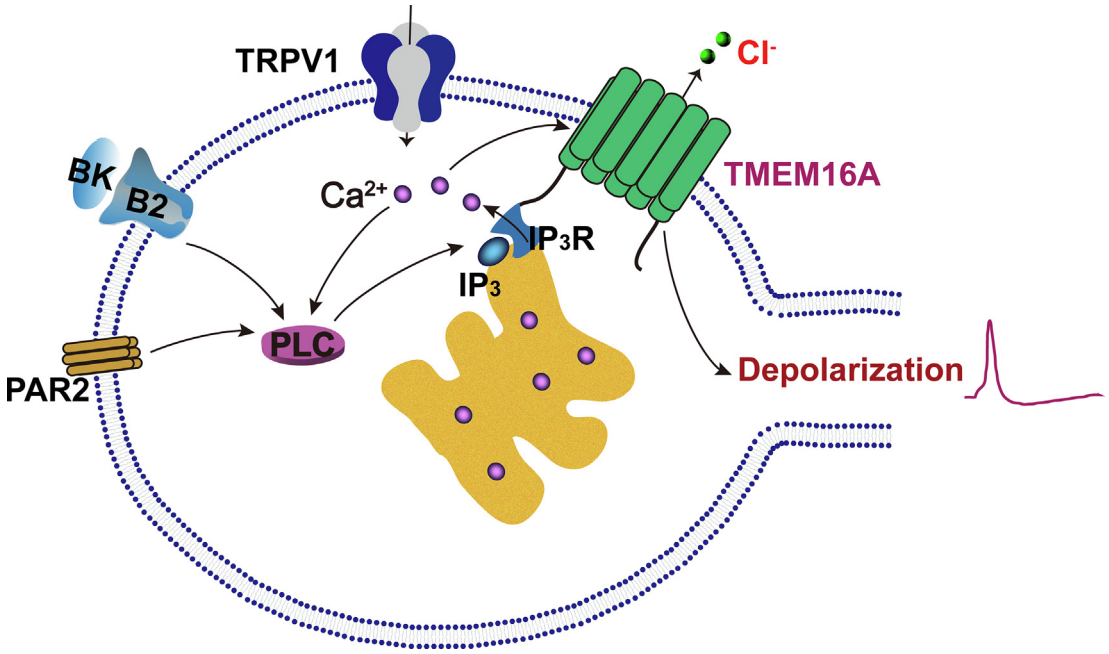


Fig. 6. Role of TMEM16A in inflammation-induced pain. TMEM16A is co-expressed with TRPV1, B2 receptors, and PAR2 receptors in DRG neurons. TMEM16A is activated by increases in $[Ca^{2+}]_i$ via IP_3R following activation of the B2 and PAR2 receptors, the GqPCRs that activate PLC to release IP_3. Ca^{2+} entry via TRPV1, which directly interacts with TMEM16A, can activate TMEM16A. TRPV1 can indirectly activate TMEM16A via IP_3R-mediated Ca^{2+} release following TRPV1/Ca^{2+}-dependent PLC activation. TMEM16A activation results in membrane depolarization due to Cl^- ion efflux. Membrane depolarization initiates and propagate action potentials for pain sensation.

TMEM16A activates multiple proliferation-associated signaling pathways in inflammatory diseases, such as the NF-κB and ERK1/2 signaling pathways. Thus, increased TMEM16A expression level and cell proliferation may be interconnected. Furthermore, TMEM16A promotes mucin synthesis in a cell proliferation-dependent manner [136]. Therefore, TMEM16A may play its pathological role depending on the proliferating state of the cells. Furthermore, since TMEM16A interacts with multiple interacting partners, it can exert diverse pathological effects by regulating its binding partners. Here, we explored the idea that TMEM16A plays a cell-specific role in inflammatory diseases depending on the cellular environment, which is determined by the disease conditions, the proliferating state, and its interacting partner proteins.

Different roles of TMEM16A are dependent on disease severity

The role of TMEM16A in inflammatory diseases may depend on disease severity. For example, TMEM16A promotes or inhibits intestinal epithelial dysfunction depending on the dose of LPS, where TMEM16A aggravates intestinal epithelial damage under low-dose LPS treatment and protects against intestinal epithelial damage under high-dose LPS treatment [58]. Under a mild stage of intestinal inflammatory disease (low-dose LPS), TMEM16A activates the ERK1/MLCK signaling pathways, leading to intestinal barrier dysfunction; meanwhile, under a severe disease stage (high-dose LPS), TMEM16A activates the ERK/Bcl-2/Bax signaling pathway to inhibit inflammation-induced apoptosis [58]. Therefore, it seems that TMEM16A is coupled to distinct signaling pathways in different cellular environments, which are determined by the severity of the disease.

Proliferation-dependent role of TMEM16A

TMEM16A is known to promote proliferation in cancer cells [30,31], as well as in airway epithelial cells [36]. In addition, TMEM16A is critical for goblet cell hyperplasia, since TMEM16A inhibition reportedly reduces IL-13-induced goblet cell hyperplasia [13,62]. Several studies have reported that TMEM16A overexpression promotes mucus production and secretion in airway epithelial cells in response to Th2 cytokine stimulation, and TMEM16A inhibition by small interfering RNAs or TMEM16A inhibitors blocked IL-13-induced mucus production [6,12,62,65,66]. Since overexpression of TMEM16A-containing plasmids increases mucin synthesis in airway epithelial cells in the absence of Th2 cytokines [65,66], TMEM16A alone can drive mucus production. Simoes et al. showed that increased mucin production was mainly caused by cell proliferation [136]. Since TMEM16A overexpression increases mucin production via the ERK1/2 and NF-κB signaling pathways [65,66], which also promotes cell proliferation, it remains unclear whether TMEM16A induces mucus production via its proliferation-promoting effect. Notably, Simoes et al. reported that IL-4-induced TMEM16A upregulation depended on cell proliferation, since cell proliferation arrest by mitomycin C blocked IL-4-induced TMEM16A expression [136]. Since TMEM16A can promote cell proliferation, which in turn promotes TMEM16A expression, it appears that the promoting effect of TMEM16A on mucus production is amplified in airway epithelial cells in a proliferating state.

The argument against the idea that TMEM16A promotes mucus production has been made based on the finding that mucin synthesis can be induced by Notch signaling activation, independent of TMEM16A and cell proliferation [136]. Evidence that TMEM16A is not required for mucus production suggests that TMEM16A-independent mechanisms for mucin synthesis exist in airway epithelial cells, especially when cells are in the non-proliferating state.

In summary, it appears that TMEM16A-dependent and -independent mechanisms for mucus production may exist in a proliferation-dependent manner. In non-proliferating cells, the TMEM16A-independent mechanism (e.g., the Notch signaling pathway) may be responsible for mucin production, whereas in proliferating cells (e.g., under stimulation of IL4 or IL-13), TMEM16A overexpression promotes mucin production by activating the NF-κB and ERK1/2 signaling pathways. Since combined application of NF-κB inhibitors and an ERK1/2 inhibitor did not completely block TMEM16A overexpression-induced MUC5AC expression [65], unknown mechanisms that are responsible for TMEM16A-mediated mucus synthesis may also exist.

Diverse roles of TMEM16A in inflammation via its interacting partners

In addition to its ability to conduct Cl^- ions, TMEM16A, as an integral membrane protein, can form complex protein–protein interaction networks by interacting with a wide range of proteins. When overexpressed in HEK293 cells, TMEM16A binds to various proteins, ranging from membrane-cytoskeleton-associated ezrin-radixin-moesin (ERM) proteins and lipid-interacting proteins to multiple Ca^{2+}-binding proteins and kinases [137]. In addition, several studies have identified TMEM16A-interacting proteins in various cells. For example, TMEM16A interacts with IP_3R in DRG neurons [93], AR42J cells [10], and HeLa cells [109]. Additional TMEM16A-interacting partners include p62 in vascular smooth muscle cells [138], Nox2 NADPH oxidase in endothelial cells [16], EGFR in HNSCC [73], VAMP3 in hepatocytes [41], CFTR in airway epithelial cells [98], TRPV1 in nociceptive neurons [34], and CLCA1 in HEK293T cells [139]. The identification of multiple TMEM16A-interacting proteins in different cells implies that TMEM16A may play distinct pathological roles depending on the function of its interacting partners.

TMEM16A can play its pathological role in inflammatory diseases by enhancing the function of its binding partners. For example, TMEM16A increases $[Ca^{2+}]_i$ via directly interacting with IP_3R in response to ATP treatment, thereby increasing mucus secretion via exocytosis in inflammatory lung diseases [6,27]. Wang et al. reported that TMEM16A increased IP_3R-mediated Ca^{2+} release and promoted the subsequent activation of NF-κB signaling in pancreatic acinar cells, thereby aggravating acute pancreatitis [10].

TMEM16A can also inhibit the functions of its interacting partners under inflammatory conditions. SNARE proteins, including VAMP3 and syntaxin-4, translocate vesicles to the membrane, and these have been discovered in the complex TMEM16A interactome derived from TMEM16A-overexpressing HEK293 cells [137]. It was recently found that VAMP3 directly interacts with TMEM16A in hepatocytes, and the binding of TMEM16A results in VAMP3 degradation via a proteasome-dependent mechanism [41]. VAMP3 degradation in turn impairs GLUT3 translocation to the membrane, leading to glucose metabolic disorder and eventually insulin resistance, steatosis, and inflammatory responses [41]. Therefore, TMEM16A aggravates NAFLD by promoting VAMP3 degradation and subsequently disrupting VAMP3-mediated GLUT3 translocation.

Conclusions and perspectives

Multiple inflammatory mediators, including cytokines (IL-4, IL-13, and IL-6), histamine, bradykinin, and ATP/UTP, as well as bacterial and viral infections, promote TMEM16A expression and/or activity (Figs. 2, 3). This suggests that increased TMEM16A function represents a common feature for a wide range of inflammatory diseases. In addition, TMEM16A expression is upregulated by multiple inflammatory signaling pathways, including the IL-4/IL-13/STAT6,

IL-6/STAT3, and NF-κB signaling pathways. In turn, TMEM16A also activates many inflammatory signaling pathways, such as Ca^{2+} signaling, NF-κB signaling, and ERK1/2 signaling pathways. The involvement of TMEM16A in a diverse range of complex inflammatory signaling pathways suggests that TMEM16A is important for the pathogenesis and development of many inflammatory diseases. Furthermore, TMEM16A performs distinct pathological functions in different cells under various disease conditions, implying that TMEM16A regulates a unique cell function in a specific inflammatory disease. Future studies are expected to identify the novel roles of TMEM16A in other inflammatory diseases.

Acute inflammation triggered by tissue injury or infection involves multiple inflammatory processes, including local immune response, vascular response, inflammatory cell infiltration, and inflammatory mediator release from inflammatory and tissue cells. The inflammatory process is cooperatively regulated by many immune cells, such as macrophages and monocytes [126]. Macrophages play a key role in the initiation, maintenance, and resolution of inflammation [140]. TMEM16A expression is upregulated in RAW264.7 macrophages following LPS treatment [58], and TMEM16A mediates miR-9-induced TGF-β/Smad2 signaling in RAW264.7 macrophages [45]. However, the expression and functional role of TMEM16A in other immune cells are largely unknown. Further studies should be performed to investigate whether TMEM16A regulates the function of immune cells during inflammation.

It is known that inflammation is associated with the initiation and development of cancer [141]. TMEM16A activates multiple signaling pathways (such as the NF-κB signaling pathway) that are important for both inflammation and cancer [142]. TMEM16A contributes to cell proliferation under inflammatory conditions. For example, TMEM16A expression is upregulated in mucus-secreting goblet/club cells during goblet cell metaplasia, whereas TMEM16A downregulation by pharmacological inhibitors reduces IL-13-induced goblet cell metaplasia [13,62,143]. TMEM16A promotes esophageal epithelial proliferation in eosinophilic esophagitis [40]. It is well known that TMEM16A overexpression promotes cell proliferation, migration, and invasion in a wide range of cancers [30,31,144]. Hence, it can be hypothesized that enhanced TMEM16A expression in inflammation promotes cancer development. However, Lee et al. reported that colonocyte-specific TMEM16A knockout did not affect colitis-associated colon carcinogenesis in mice treated with azoxymethane and dextran sodium sulfate. Since TMEM16A exhibits a cell-specific role in both inflammatory diseases and cancer [31], future studies are required to examine whether TMEM16A is involved in the transition from inflammation to cancer in a unique cell type.

TMEM16A expression/activity levels reportedly promote proliferation, migration, invasion, tumor growth, and metastasis in many cancers such as breast cancer, HNSCC, and pancreatic cancer. The pro-oncogenic effect of TMEM16A can be blocked by TMEM16A inhibitors [30,31,144]. High TMEM16A expression level has also been reported to be associated with poor clinical prognosis in patients with breast cancer [72,123], HNSCC [145], and pancreatic cancer [104]. We have previously reported that the beneficial effect of the ER modulator tamoxifen may be associated with its pharmacological inhibition of TMEM16A channels [72]. Furthermore, *in vitro* application of TMEM16A inhibitors improves therapeutic responses to EGFR inhibitors such as gefitinib and cetuximab in breast cancer and HNSCC [72,73,146], the HER2 inhibitor trastuzumab in HNSCC [146], and the CK2 inhibitor silmitasertib in HNSCC [147]. Notably, recent studies have found that HNSCC cells overexpressing TMEM16A exhibit platinum resistance via copper-dependent ATP7B upregulation [148]. Therefore, TMEM16A inhibitors may be used to overcome platinum resistance in TMEM16A-overexpressing HNSCC.

Many studies have found that TMEM16A inhibitors are promising for treating TMEM16A-associated inflammatory diseases. However, some issues exist regarding the application of TMEM16A inhibitors for treating inflammatory diseases. First, most TMEM16A inhibitors exhibit non-specific effects on other targets. For example, CaCCinh-A01 inhibits CFTR Cl^- channels [149], bestophin-1 Cl^- channels [150], and Ca^{2+}-activated K^+ channels (KCa3.1) [151]. In addition, many TMEM16A inhibitors alter $[Ca^{2+}]_i$, including T16Ainh-A01, CaCCinh-A01, benzbromarone, niclosamide, and Ai9 [100,152,153]. Due to these non-specific effects, caution should be taken when these inhibitors are used to study the role of TMEM16A in inflammatory diseases. Second, although recent studies have revealed the cryo-EM structure of TMEM16A [50,51], the binding sites of these TMEM16A inhibitors on TMEM16A channels have not been identified. Detection of the binding site for TMEM16A inhibitors is useful for the structure-based development of new TMEM16A inhibitors, in addition to assisting in improving our understanding of the mechanisms underlying TMEM16A regulation by these inhibitors. Third, although TMEM16A inhibitors are being considered to be used for the treatment of TMEM16A overexpression-associated inflammatory diseases, the use of TMEM16A inhibitors or agonists for the treatment of CF is debated [27]. Because the main problem presented by CF is mucus hypersecretion, TMEM16A inhibitors are favored for treating CF over TMEM16A agonists; this is because TMEM16A inhibitors can reduce mucus production and secretion with minimal impairment of TMEM16A-mediated Cl^- secretion [27]. However, a recent study has shown that the TMEM16A potentiator ETX001 improves fluid secretion and accelerates mucus clearance into the airway but does not induce mucus secretion and bronchospasm [154,155]. Future clinical studies should be performed to investigate the efficacy of these inhibitors or activators in the treatment of CF.

In this review, we comprehensively summarize the diverse roles of TMEM16A in inflammation, including the mechanisms underlying TMEM16A expression and activation under inflammatory conditions, diverse inflammatory signaling pathways activated by TMEM16A, and multiple roles in inflammatory processes and pain. Since the roles of TMEM16A vary greatly in different diseases, the cellular environment-dependent mechanisms may explain the diverse roles of TMEM16A in inflammatory diseases. These functions suggest that TMEM16A plays a novel role in certain inflammatory diseases via a cell-specific mechanism. Considering the diversity of inflammatory diseases, future studies should be extensively investigated to reveal the mechanisms of TMEM16A in various inflammatory diseases, thus elucidating its diverse role in inflammatory diseases.

Increased TMEM16A expression level and/or activities contribute to the pathogenesis of many inflammatory diseases, such as airway inflammatory diseases, acute pancreatitis, ischemic stroke, steatohepatitis, and inflammatory intestinal diseases (Fig. 4). Therefore, TMEM16A inhibition represents a novel therapeutic strategy for treating these inflammatory diseases. Recent studies have reported that the approved drugs anthelmintics niclosamide and nitazoxanide, which are well tolerated with mild side effects in the clinic, inhibit TMEM16A channels and are promising for treating airway inflammatory diseases such as asthma, COPD, and CF [96,156]. In addition, many natural compounds, such as shikonin from the dried root of *Lithospermum erythrorhizon* [42], *trans*-δ-viniferin from *Vitis amurensis Rup* [89], and plumbagin from *Plumbago zeylanica L.*[44], inhibit TMEM16A channels, and are used to treat secretory diarrhea in animal models. These compounds are widely used with mild side effects, and thus may be clinically useful for the treatment of secretory diarrhea. Further clinical studies are necessary to investigate the efficacy and safety of these com-

pounds in the treatment of TMEM16A-associated inflammatory diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81572613 and No. 31371145 to Qinghuan Xiao; No. 81802659 to Mei Liu).

Author contributions

W.B., M. L, and Q.X. wrote, revised, and finalized the manuscript for publication.

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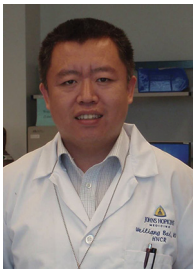
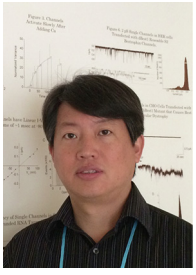


Weiliang Bai is a professor, Department of Otolaryngology Head and Neck Surgery at Shengjing Hospital of China Medical University. He received his M.D./Ph.D. from China Medical University, China. He completed his postdoctoral training at the Department of Otolaryngology Head and Neck Surgery at Johns Hopkins Medical Institute, USA. He has extensive experience in clinical research and basic science in the field of inflammatory diseases. His recent research is on the pathogenesis of inflammatory airway diseases, with a focus on the molecular mechanism of sinusitis and nasal polyps. He has co-authored more than 60 peer-reviewed scientific publications.

Mei Liu has obtained her Ph.D. degree in pharmacology at China Medical University,and is currently working as a post-doctoral fellow at the lab of Professor Qinghuan Xiao at the Department of Ion Channel Pharmacology of China Medical University. Her research interest is on ion channel pharmacology, with a focus on TMEM16A Ca^{2+}-activated Cl^- channels. She recently studies on the pathogenesis of TMEM16A Ca^{2+}-activated Cl^- channels in inflammatory diseases incuding acute pancreatitis. Her study was supported by the grant from the National Natural Science Foundation of China. She has co-authored more than 15 peer-reviewed scientific publications.



Qinghuan Xiao is a professor and chair, Department of Ion Channel Pharmacology at School of Pharmacy of China Medical University. He received his medical degree from China Medical University, and got his Ph.D degree in pharmacology at Northeastern University, Boston, USA. Currently, he is interested in the role of TMEM16A Ca^{2+}-activated Cl^- channels in many diseases, including acute pancreatitis, endothelial dysfunction and cancer. Recently, his study focuses on the pathological role of TMEM16A in inflammatory processes, especially in pancreatitis, and inflammatory airway diseases. He has published more than 40 research and review articles in the internationally recognized peer-reviewed journals. He is a reviewer of many international journals.