TMEM16A在上皮细胞中的表达和调控

杨雯静 于波*

(辽宁师范大学生命科学学院, 辽宁省生物技术与分子药物研发重点实验室, 大连 116081)

摘要 TMEM16A(tranmenbrane protein 16A)作为一种已知的钙激活氯离子通道(calcium-activated chloride channel, CaCC)在机体中广泛表达, 并介导多种重要的生理功能。在上皮细胞中, TMEM16A可以通过多级反应介导细胞的膜电位变化和液体分泌。此外, 在多种炎症相关的上皮组织疾病如囊性纤维化、哮喘和急性胰腺炎中均发现TMEM16A表达上调的现象, 调节TMEM16A的表达和通道活性可能是炎症治疗的一种潜在策略。研究TMEM16A在上皮细胞中的表达和调控机制, 对阐明TMEM16A的生理病理功能具有重要意义。该文就上皮细胞中TMEM16A的研究进展进行综述。

关键词 TMEM16A; 上皮细胞; 表达; 调控

Expression and Regulation of TMEM16A in Epithelial Cells

YANG Wenjing, YU Bo*

(*Liaoning Provincial Key Laboratory of Biotechnology and Drug Discovery, School of Life Sciences, Liaoning Normal University, Dalian 116081, China*)

Abstract TMEM16A (tranmenbrane protein 16A) is widely expressed in the body as a known CACC (calcium-activated chloride channel), which mediates many important physiological functions. In epithelial cells, TMEM16A can mediate membrane potential changes and fluid secretion through multiple reactions. In addition, TMEM16A expression is up-regulated in a variety of inflammation-related epithelial diseases, such as cystic fibrosis, asthma and acute pancreatitis; regulating TMEM16A expression and channel activity may be a potential strategy for the treatment of inflammation. Studying the expression and regulation mechanism of TMEM16A in epithelial cells is of great significance for elucidating the physiological and pathological functions of TMEM16A. Here, this artical reviewed the latest research progress of TMEM16A in epithelial cells.

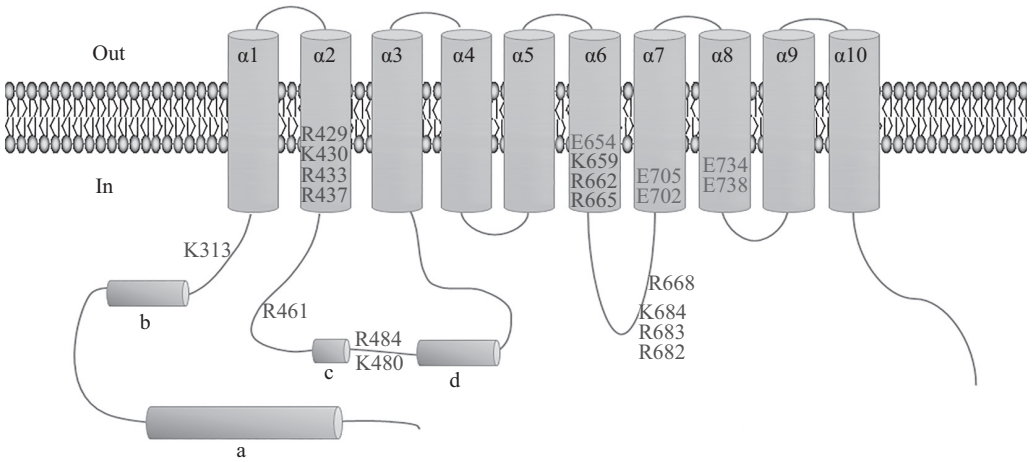
Keywords TMEM16A; epithelial cells; expression; regulation

TMEM16A(tranmenbrane protein 16A)(也被称为ANO1)是一种钙激活的氯离子通道(calcium-activated chloride channel, CaCC)[1-3], 在机体内参与液体分泌、感觉神经信号的传导、平滑肌收缩、细胞增殖和发育等多种生理过程[4]。TMEM16A在多种上皮细胞, 如视网膜细胞[5]、嗅觉上皮细胞[6]、肠道上皮细胞[7]、胰腺导管上皮细胞[8]、胆管上皮细胞[9]中表达, 并与这些细胞中Ca^{2+}激活Cl^-电流的产生有关。研究TMEM16A的表达和作用机制对揭示其生理功能以及与多种疾病的相关性具有十分重要的意义。

1 TMEM16A的结构

TMEM16A含有10个跨膜结构域[10], 是TMEM16蛋白家族的10个成员之一。TMEM16家族成员

收稿日期: 2021-01-15 接受日期: 2021-04-19
辽宁省科学技术计划项目(批准号: 2019-BS-155)资助的课题
*通讯作者。Tel: 0411-85827085, E-mail: yubo821208@163.com
Received: January 15, 2021 Accepted: April 19, 2021
This work was supported by the Natural Science Foundation Project of Liaoning Province (Grant No.2019-BS-155)
*Corresponding author. Tel: +86-411-85827085, E-mail: yubo821208@163.com



TMEM16A的4个可变剪切片段分别用字母a、b、c、d标记。

The four alternative splicing fragments of TMEM16A is labeled with a, b, c and d, respectively.

图1 TMEM16A拓扑结构示意图(根据参考文献[19-22]修改)

Fig.1 TMEM16A topology diagram (modified from references [19-22])

具有相似的拓扑结构, 并且序列具有高度的保守性。TMEM16A具有4个可变剪接片段: a(1~116)、b(268~289)、c(470~473)和d(498~523)(图1)。其中, a段的N-端缩短会影响TMEM16A的表达和功能[11]; b段和c段与TMEM16A的Ca^{2+}敏感性有关[12]; c段可以调节离子通道的电压依赖性[13]; 而尚未发现d段的缺失对TMEM16A通道的活性有明显的影响[13]。

目前已经发现四种不同状态的TMEM16A结构, 包括2个Ca^{2+}结合态(5OYB、6BGI)、1个Ca^{2+}结合态6BGJ、1个Ca^{2+}自由态5OYG[14-16]。其中, 5OYB的晶体结构最完整且分辨率最高。Ca^{2+}结合对TMEM16A中Ca^{2+}依赖性门控起着关键作用[17-18]。在TMEM16A中存在多个酸性残基(E654、E702、E705、E734和E738), 它们分布在TMEM16A的第6~8跨膜α螺旋中, 形成Ca^{2+}结合部位[19-21]。除上述5个关键的酸性残基之外, N650、N651和N730也辅助Ca^{2+}的结合[15]。

除Ca^{2+}结合调控机制外, TMEM16A的激活也受4,5-二磷酸磷脂酰肌醇[$PI(4,5)P_2$]的调控[16]。在TMEM16A中至少存在3个$PI(4,5)P_2$结合位点[22], 分别由5个(R433、K430、R429、R437、K313)、7个(K659、R662、R665、R668、R682、R683、K684)和3个(R461、K480、R484)碱基组成, 这些位点均匀分布在静电势较高的细胞膜附近(图1)。有研究推测, 细胞内$PI(4,5)P_2$的减少会导致TMEM16A介导的电流下降[21], $PI(4,5)P_2$可能通过改变离子的信号传导通路或影响Ca^{2+}的结合对TMEM16A起调控作用[22]。

2 TMEM16A在上皮细胞中的表达、功能和调控机制

大量上皮细胞以单层、分层、假分层和移行等组织形式与少量细胞间质共同构成上皮组织。尽管上皮细胞的功能根据所处位置存在差异, 但也具有相似的共性, 例如: 保护黏膜上皮、吸收离子和辅助液体分泌等。TMEM16A的门控机制和Cl^-分泌特性与多种上皮组织的功能相匹配, 是上皮组织发挥特定生理功能的重要调节组分(表1)。

2.1 TMEM16A的表达及功能

视网膜主要由色素上皮细胞、感光细胞、双极细胞和神经节细胞构成[23], 可以将光信号转换为神经信号并传递至中枢神经系统。TMEM16A在小鼠的视网膜色素上皮(retinal pigment epithelium, RPE)的质膜、感光细胞和双极细胞的终端表达, 并且广泛定位于光感受器的突触末端[23]。TMEM16A在杆状和锥状光感受器上表达并呈点状分布[24], 在各种视网膜神经元的突触前、终、末端均可以观察到TMEM16A的蛋白表达[25]。在脊椎动物的杆状双极细胞中可检测到Ca^{2+}激活的Cl^-电流($I_{Cl(Ca)}$), 该电流的产生与Ca^{2+}通道的活性密切相关。Ca^{2+}通道阻滞剂Co^{2+}、L型Ca^{2+}通道阻滞剂硝苯地平、Cl^-通道阻滞剂5-硝基-2-(3-苯基丙基氨基)苯甲酸(NPPB)、TMEM16A抑制剂$T16A_{inh}$-A01和TMEM16A抗体可以抑制杆状双极细胞中$I_{Cl(Ca)}$的产生[25]。$I_{Cl(Ca)}$通过稳

表1 TMEM16A在上皮细胞上的表达和功能

Table 1 Expression and function of TMEM16A in epithelial cells

上皮组织 Epithelium	细胞类型 Cell type	表达部位 Expression pattern	TMEM16A的功能 The function of TMEM16A	参考文献 References
Retina	RPE (retinal pigment epithelium)	Cell parietal membrane	Promoting the absorption of water and electrolytes in the subretinal space	[23]
	Rod bipolar cells	Cell terminals	Involved in the regulation of synaptic transmission at the ends of photoreceptors, it is related to the activity of Ca^{2+} channel	[25]
	Photoreceptor	Presynaptic terminal	An intrinsic regulator of presynaptic membrane potential. Modulating synaptic transmission at the ends of photoreceptors	[24-25]
Olfactory epithelium	Supporting cells	Cell parietal membrane, high expression in the ventral zone and low expression in the dorsal zone	Controlling Cl^- homeostasis and dynamics in the mucus covering the olfactory epithelium Maintaining proper endocytic trafficking Affecting the TMEM16B-mediated current, modifying the odorant response of the olfactory sensory neurons	[26-30]
Airway epithelium	Ciliated epithelial cells	Cell parietal membrane	Controlling the mucus production/secretion. Facilitating effective compartmentalized Ca^{2+} signaling. Leading to fusion of mucus containing granules, exocytosis, and release of mucus	[35-39]
	ASM (airway smooth muscle) cells	Cell parietal membrane	It is related to the development of airway smooth muscle. Controlling paracrine release of inflammatory mediators. Regulating contraction of ASM	[31-35]
Intestinal tract	Intestinal epithelial cells	Low expression in ileum; high expression in proximal and distal colon	Regulation of calcium signaling required for basolateral mucocele; associated with diarrhea caused by rotavirus toxin NSP4; playing a role in Cl^- secretion in intestines; forming the basis for future studies of the expression and function of TMEM16A in normal and inflammatory intestinal diseases *in vivo*	[41-44]
Pancreas	Acinous cells	Low expression in normal acinar cells High expression in ductal-like epithelial cells of pancreatic cancer tissue	Related to HCO_3^- transport. The driving force for the secretion of fluid by acinar cells TMEM16A inhibitors for the treatment of acute pancreatitis. Associated with the glucose-induced membrane fluctuation in β cell that is necessary for insulin secretion	[46-51]
Biliary duct	Bile duct epithelial cells	Cell parietal membrane	The driving force for cholangiocyte secretion. Promoting alkalization of bile duct fluid. Associated with changes in the composition of bile. A potential target to modulate bile formation in the treatment of cholestatic liver disorders	[55-56]

定膜电位和Ca^{2+}通道的活性来调节光感受器末端的突触传递, 证明TMEM16A作为一种突触前膜电位的内在调节因子参与突触传递过程。

嗅上皮是位于鼻腔最上部的黏膜, 是由嗅觉感觉神经元、鲍曼氏腺、支持细胞、基底细胞和微绒毛细胞等组成的假复层上皮, 鲍曼氏腺(嗅腺)和支持细胞所分泌的水、离子和蛋白质组成的保护性黏液层覆盖在嗅上皮表面, 形成天然防护屏障[26-27]。在小鼠的胚胎发育过程中, TMEM16A在嗅上皮呈现带状、动态、不均匀的表达[28]。MAURYA等[28-29]将野生型(wild type, WT)和*TMEM16A*敲除(knock-out, KO)小鼠进行交配, E12.5天TMEM16A在嗅觉上皮腹侧区(ventral zones)表达, 从E16.5天开始在过渡区(transition zones)高表达, 而在背侧区(dorsal zones)低表达, 这一结果证明TMEM16A在嗅上皮中表达的动态特性。HENRIQUES等[27]通过免疫荧光检测方法, 分别测定了成熟嗅神经元标记蛋白(olfactory marker protein, OMP)、神经元纤毛标记物(乙酰化微管蛋白)以及TMEM16A在嗅上皮的表达定位, 结果显示TMEM16A表达于嗅上皮的顶层, 但不与乙酰化微管蛋白重叠, 证实了TMEM16A的表达位点是支持细胞的顶端部分, 而不是嗅神经元。在支持细胞中, 存在多种异种生物代谢酶, 对异种生物的吞噬是其解毒的必要步骤。HE等[30]发现, 上皮细胞中的TMEM16A所调控的细胞质中Cl^-浓度稳态对适当的内吞运输是必要的。

在气道中, TMEM16A在纤毛上皮细胞和气道平滑肌(airway smooth muscle, ASM)中均有表达, 是气

道上皮细胞Cl^-分泌的主要通道[31-32]。研究显示, 在TMEM16A突变小鼠中, 横跨气管背侧的气管肌发育异常, 证明TMEM16A与气道平滑肌的发育有关。有研究表明, TMEM16A受Th2型细胞因子IL-4和IL-13调控, 从而调节气道上皮的液体分泌[33-35]。在IL-4和IL-13的刺激下, TMEM16A和$Na^+/K^+/2Cl^-$共转运体表达显著上调, 导致Cl^-的分泌增加, 而Cl^-的分泌伴随着水的分泌[36-37], 可诱导气道黏膜的水合作用, 有利于纤毛摆动, 形成防止微生物感染的保护机制。在哮喘患者中, TMEM16A表达于黏蛋白5AC(MUC5AC)阳性细胞中, 并调节黏蛋白的分泌[35]。TMEM16A通过诱发黏蛋白的分泌, 保持气道黏液层的厚度, 通过纤毛摆动、喷嚏等方式将异物排出呼吸道, 从而抵御外界有害物质的入侵[38]。有研究表明, TMEM16A在黏液颗粒与顶膜融合以及胞吐过程中起着重要的作用。在缺乏TMEM16A表达时, ATP诱导的黏液分泌受到强烈抑制, 杯状细胞内Ca^{2+}浓度减弱, 导致黏液颗粒的融合、胞吐和黏液的释放受到抑制[39]。

在肠道中, TMEM16A在小鼠肠上皮细胞和杯状细胞中均有表达, 调节基底外侧黏液胞吐所需的Ca^{2+}信号传导[40]。蛋白质组分析和免疫印记检测显示, TMEM16A在十二指肠和空肠上无表达, 在回肠少量表达, 在近端及远端结肠中大量表达[41-42]。有研究表明, 在敲除*TMEM16A*基因的小鼠肠上皮细胞中, 结肠Ca^{2+}依赖的Cl^-分泌受到了抑制[42], 而表皮生长因子(epidermal growth factor, EGF)可上调Ca^{2+}依赖的Cl^-分泌, 并提高结肠上皮细胞的TMEM16A表达[43]。一种轮状病毒毒素(NSP4)可引起婴儿腹泻, 也会引起Ca^{2+}依赖的Cl^-分泌。利用NSP4处理转染TMEM16A的HEK293细胞后, 可诱导细胞产生Ca^{2+}依赖的Cl^-分泌[44]。由此可见, TMEM16A是轮状病毒感染腹泻中Cl^-过度分泌的主要通道。尽管TMEM16A具有调节Cl^-分泌的生理特性[42], 在肠上皮中囊性纤维化跨膜电导调节因子(cystic fibrosis transmembrane regulator, CFTR)看似是介导Cl^-持续分泌的主要途径, 而CFTR的功能在某种程度上依赖于TMEM16A[45], 敲除*TMEM16A*会减弱结肠上皮细胞中cAMP和Ca^{2+}激活的Cl^-电流[43]。

在胰腺中, TMEM16A在腺泡细胞[46-47]、胰岛β细胞[48]、胰腺导管细胞[49]以及胰腺癌细胞[8]中都有表达。在正常胰腺腺泡内TMEM16A表达量较低, 而在胰腺癌组织导管样上皮细胞中高表达。TMEM16A的过表达会增强腺泡细胞的Cl^-分泌, 成为液体分泌的主要驱动力[46], 同时TMEM16A介导的HCO_3^-转运可调节腔内pH稳态[50]。此外, TMEM16A表达和功能下调可抑制NF-κB的激活, 能够有效改善急性胰腺炎(acute pancreatitis, AP)模型小鼠的胰腺损伤[50]。在胰岛β细胞中, TMEM16A抑制剂$T16A_{inh}$-A01可消除葡萄糖诱导的胰岛素分泌以及膜电位的振荡[51], TMEM16A表达缺失也会影响小鼠正常的胰岛素分泌[52]。因此, TMEM16A是胰岛β细胞中调节胰岛素分泌的重要分子。

在胆管中, 胆汁的形成主要借助于细胞的合成和肝胆管的运输。研究显示, 在人Mz-Cha-1细胞[53]、正常大鼠胆管细胞(normal rat cholangiocytes, NRCs)、小鼠大胆管细胞(large mouse cholangiocytes, MLCs)和小鼠小胆管细胞(small mouse cholangiocytes, MSCs)中均可检测到TMEM16A的表达[54]。大鼠和小鼠肝脏免疫染色显示, TMEM16A在肝细胞中表达较弱, 而在胆管细胞中的表达较强[55]。有研究证明, TMEM16A在胆管上皮细胞(biliary epithelial cells, BECs)顶膜表达, ATP可刺激BECs产生$I_{Cl(Ca)}$, 利用siRNA干扰TMEM16A表达或在无Ca^{2+}的条件下该电流显著降低, 证明TMEM16A是BECs中主要的CaCCs, 并为胆汁的分泌提供驱动力[55]。TMEM16A作为胆管Cl^-外排的通道, 可能成为胆汁淤积性肝病中调节胆汁流量的潜在靶点[56]。

2.2 TMEM16A的调控机制

2.2.1 TMEM16A在视网膜中的调控机制　研究显示, ATP在RPE细胞的顶膜侧可以诱导$I_{Cl(Ca)}$的产生。RPE中TMEM16A激活的机制和生理意义尚不清楚, 推测可能涉及P_2Y受体的激活所引起的Ca^{2+}释放所导致的通道开放, 进而从视网膜下间隙吸收水和电解质[57]。

视网膜中电压门控Ca^{2+}通道(voltage-dependent calcium channel, VGCC)由α1、α2δ、β1-4和γ四种不同的亚基组成[23], 其中α2δ亚基分为α2δ1(CACNA2D1)、α2δ2(CACNA2D2)、α2δ3(CACNA2D3)和α2δ4(CACNA2D4)四种不同的亚型, 介导突触末端的Ca^{2+}内流, 促使神经递质的释放。TMEM16A通道与Ca^{2+}通道的α1亚单位相连, 形成复合物VGCC/TMEM16A, 有利于光感受器突触末端的功能发挥, 与Ca^{2+}内流结构域的紧密结合可以对突触活动产生最佳的反馈控制[23]。在CACNA2D4突变小鼠视网膜中,

VGCC的α2δ4亚单位会因无义突变, 使光感受器突触末端的结构和功能遭到破坏[58], TMEM16A通道失去其特有的定位, 导致光感受器突触紊乱[59]。突触蛋白从突触末端向外核层(outer nuclear laye, ONL)转移, 导致杆状光感受器向下游传递信号的能力严重受损[23]。

2.2.2 TMEM16A在嗅上皮细胞中的调控机制 ATP通过激活支持细胞G蛋白偶联的P_2Y受体促使细胞内储存的Ca^{2+}释放, 引起细胞内Ca^{2+}的瞬时增加, 激活细胞中的TMEM16A依赖性电流[60]。Ca^{2+}在支持细胞的胞质侧促使TMEM16A Cl^-通道开放, 导致Cl^-外排, 从而控制Cl^-在嗅觉上皮黏液中的动态平衡。嗅上皮可以受到三叉神经各种分支的广泛神经支配, 这些纤维可以通过轴突反射释放ATP[61]。TMEM16A可以与内质网膜上的三磷酸肌醇受体(inositol 1,4,5-trisphosphate receptor, IP_3R)相互作用来放大ATP介导的Ca^{2+}信号, 参与嗅觉上皮细胞ATP转导通路的级联反应。另外, 有研究表明一些气味分子会与位于嗅感觉神经元(olfactory sensory neurons, OSNs)纤毛上的气味受体结合, 进而导致G蛋白偶联的反应, 激活腺苷酸环化酶III, 使环核苷酸门控离子通道(cyclic nucleotide-gated channel, CNG)和Ca^{2+}激活的Cl^-通道TMEM16B大量表达[62-65]。此外, 乙酰胆碱通过激活M3毒蕈碱受体, 使细胞内Ca^{2+}浓度升高, 进而激活TMEM16A介导的电流[66-67]。

2.2.3 TMEM16A在气道上皮细胞中的调控机制 气道上皮中的TMEM16A与P_2Y受体、CFTR共定位于细胞顶膜[68], P_2Y受体被激活后可引起内质网Ca^{2+}释放, Ca^{2+}不仅激活TMEM16A, 还刺激腺苷酸环化酶1(adenylate cyclase type 1, ADCY1)的酶活性, 促使cAMP水平升高, cAMP刺激蛋白激酶A(protein kinase A, PKA)的活性, 进而激活CFTR, 促进Cl^-分泌[68]。组胺作为一种促分泌素存在于气道组织中, 引起气道上皮中Ca^{2+}浓度短暂升高, 促使黏蛋白分泌。在气道炎症反应过程中, Th2细胞可以释放多种促炎性因子[35]。其中, IL-4在过敏性炎症机制中起着重要的调节作用, 包括免疫球蛋白(immunoglobulin E, IgE)的产生以及嗜酸性粒细胞、嗜碱性粒细胞和肥大细胞的活化[69]。在过敏性炎症期间, IL-4可以提高TMEM16A的表达, 在促进液体分泌中起着重要作用[69-70]。靶向IL-4或TMEM16A的治疗剂可有效缓解变应性鼻炎中液体的高分泌。

2.2.4 TMEM16A在肠上皮细胞中的调控机制 目前, 关于肠道上皮中TMEM16A的调控机制的报道较少, 影响其表达和活性的因子和细胞信号通路尚需要更多的实验证据[71]。现有的研究表明, 在低剂量和高剂量脂多糖诱导的上皮屏障功能障碍中, TMEM16A起双重作用。TMEM16A会通过激活ERK1/MLCK信号通路, 加重低剂量脂多糖诱导的细胞屏障功能障碍; 相反, 通过ERK/Bcl-2/Bax信号通路可以保护高剂量脂多糖诱导的细胞屏障功能[72]。免疫荧光结果显示, 脂多糖可以显著刺激TMEM16A的表达, 而这种作用会被NF-κB显著抑制[72]。TMEM16A的复杂调控机制和靶向性为肠上皮屏障损伤提供潜在的治疗策略, 并为研究TMEM16A在正常的肠道细胞和炎症性肠道疾病中的表达和功能奠定基础[71-73]。

2.2.5 TMEM16A在腺泡细胞中的表达机制 TMEM16A在腺泡细胞中的表达往往与胰腺组织和血清中IL-6相关。研究表明, 在AR42J细胞(大鼠胰腺肿瘤细胞)中, IL-6通过IL-6受体(IL-6R)/信号转导子和转录激活子3(signal transducer and activator of transcription 3, STAT3)信号通路, 促进腺泡细胞中TMEM16A的表达, 引起TMEM16A表达上调[50]。TMEM16A过表达会激活腺泡细胞中IP_3R/Ca^{2+}/NF-κB信号通路, 并被IP_3R介导的Ca^{2+}释放所激活, 细胞内Ca^{2+}升高激活NF-κB信号通路, 导致腺泡细胞分泌的IL-6增加[50,74]。因此, TMEM16A与IP_3R/Ca^{2+}/NF-κB/IL-6通路之间的正向激活回路对Ca^{2+}升高、NF-κB激活和IL-6释放至关重要。

2.2.6 TMEM16A在胆管上皮细胞中的调控机制 在小鼠和人胆管细胞中, 胆汁酸可以刺激TMEM16A介导$I_{Cl(Ca)}$产生, 该过程依赖于PKCα, 涉及细胞外ATP和P_2Y及IP_3受体的激活[75]。肝细胞和胆管细胞释放ATP到胆汁中[76], 与胆管细胞的P_2Y受体结合, 进而激活IP_3受体, 导致Ca^{2+}浓度升高和Cl^-通道活性增加, 促进TMEM16A介导的Cl^-分泌[77]。管腔中Cl^-浓度的增加促使Cl^-和HCO_3^-通过AE2(阴离子交换蛋白)进行交换, 水分子由水通道蛋白4(aquaporin 4, AQP4)流出, 最终使胆管内液碱化[78]。胆管细胞接受胆汁中主要成分UDCA或TUDCA的刺激后, 细胞的胞吐水平和ATP释放速率进一步增加, UDCA刺激ATP释放到胆汁中, 激活与TMEM16A共定位的CFTR, 进一步促进ATP以自分泌或旁分泌的方式释

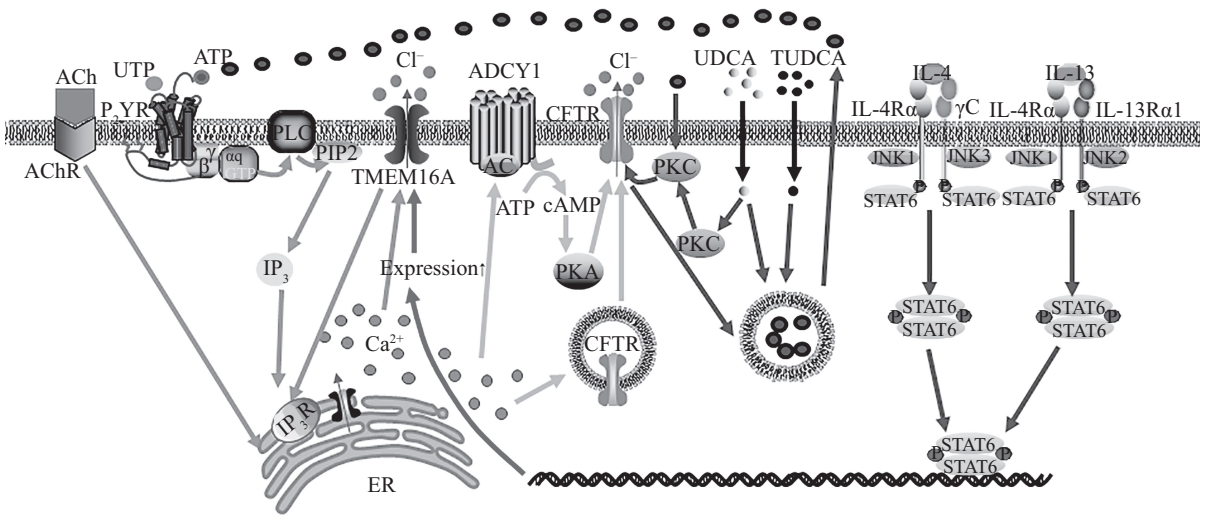


图2 TMEM16A在上皮细胞中的调控机制(根据参考文献[50,57,60,68,74,79]修改)

Fig.2 The regulatory mechanism of TMEM16A in epithelial cells (modified from references [50,57,60,68,74,79])

放并与P_2Y受体结合, 通过IP_3受体介导的细胞内钙库释放Ca^{2+}, 导致细胞内Ca^{2+}增加, 激活TMEM16A通道[79]。因此, TMEM16A是ATP刺激的Cl^-流出的"下游"通道, CFTR是参与胆汁酸刺激的ATP释放的"上游"靶点[79-80]。此外, PKCα在UDCA的作用下被转运到质膜上, 并与ATP刺激的TMEM16A激活耦联[80]。因此, 胆汁酸可能通过次级信使或其他信号分子参与TMEM16A的调节[81]。

正常大鼠胆管细胞[54]、人胆管癌细胞[53]和小鼠肝脏组织分离的胆管细胞[82]中均表达IL-4Rα/IL-13Rα1受体复合物。经IL-13或IL-4处理后的胆管细胞TMEM16A蛋白表达增加, ATP刺激产生的$I_{Cl(Ca)}$显著增强[82]。IL-4和IL-13介导的TMEM16A表达增加与STAT6磷酸化相关, 特异性抑制STAT6可逆转TMEM16A表达的增加和ATP刺激的Cl^-分泌。这些研究表明, IL-13和IL-4通过涉及STAT6的信号通路调控胆道TMEM16A通道的表达和功能[82]。目前已知的TMEM16A表达调控机制总结于图2。

3 TMEM16A与疾病

3.1 TMEM16A与囊性纤维化

囊性纤维化(cystic fibrosis, CF)是一种由CFTR功能失调引起气道黏膜水合不足所引发的气道炎症性疾病, 该病临床表现主要为黏液分泌过多、气道阻塞和支气管收缩[83]。在干燥的上皮环境下, 黏液更倾向于黏附在气道上皮, 进而导致纤毛的清除能力受损[84], 增加细菌感染风险。但也有研究表明, 除气道黏膜水化不足外, CF还可能与CFTR介导的碳酸氢盐分泌障碍有关。目前CF的治疗策略主要是试图直接恢复或补偿CFTR的功能, 一些研究者认为, TMEM16A可以作为增强CF气道黏液清除能力的候选靶点, 通过刺激气道ATP/UTP的释放, 急性增加TMEM16A介导的离子分泌, 从而改善气道黏液水化状况并增加黏液纤毛清除能力。化合物ETX001(一种TMEM16A增强剂)已被证明可增强CF患者上皮中的$I_{Cl(Ca)}$[85], Cl^-分泌增强驱动更多的液体进入气道黏膜, 该通路具有加速黏液清除的能力, 可以作为临床开发的候选治疗药物。

3.2 TMEM16A与哮喘

在气道中, TMEM16A的过表达会引起ASM的收缩, 可导致临床相关的支气管痉挛, 而TMEM16A拮抗剂可以阻断TMEM16A, 进而诱导支气管舒张[86]。由于TMEM16A在多种上皮细胞中均有表达, 所以全身性地使用TMEM16A拮抗剂来治疗支气管痉挛极可能导致非靶效应, 因此直接雾化吸入给药可以更好地避免副作用[87]。与纤毛上皮细胞相比, TMEM16A在黏液分泌细胞中表达更丰富, 在哮喘模型的气道上皮细胞中表达也会增加。TMEM16A拮抗剂可能对哮喘患者的ASM的张力和黏液分泌具有双重治疗作用[87]。

3.3 TMEM16A与急性胰腺炎

急性胰腺炎(acute pancreatitis, AP)是一种胰腺的急性炎症过程, 目前尚缺乏有效的针对AP的治疗药物。AP的发病机制主要是由于腺泡细胞中Ca^{2+}

持续升高, 进而激活胰蛋白酶原, 导致线粒体功能障碍和NF-κB的过度激活。$T16A_{inh}$-A01、Ca^{2+}螯合剂(BAPTA-AM)、抗IL-6受体(anti-IL-6R)抗体、STAT3抑制剂JSI-124可以抑制TMEM16A在AR42J细胞中过表达, 抑制Ca^{2+}释放, 这可能是治疗AP的一种新策略[50]。

3.4 TMEM16A与癌症

TMEM16A在头颈部鳞状细胞癌[88]、胃癌[89]、胰腺导管腺癌[90]、结肠癌[91]等癌细胞中表达异常上调。TMEM16A过表达后通过ras-raf-MEK-ERK通路和cyclin D1激活信号调节激酶ERK, 但不激活AKT[92], 从而诱导体内肿瘤生长和细胞增殖, ERK或MEK/ERK的特异性抑制剂可阻断TMEM16A介导的细胞增殖。抑制TMEM16A可降低癌细胞的增殖、迁移和侵袭能力, 提高化疗治疗的效果[93-94]。因此, TMEM16A是一种十分重要的癌症标志物和有潜力的癌症治疗靶点。

肿瘤中*TMEM16A*基因表达水平与肿瘤患者生存率有显著的相关性[95-96], 与肿瘤分级[97]、细胞迁移增加[98]、肿瘤生长或转移[99]呈正相关。一些已知的TMEM16A抑制剂并不能抑制细胞增殖, 而促进TMEM16A降解的$CaCC_{inh}$-A01(CaCCs的抑制剂)却能有效抑制细胞增殖[100]。这些结果表明, 在TMEM16A诱导的细胞增殖的过程中, TMEM16A蛋白水平比TMEM16A通道活性更重要。

4 结语和展望

TMEM16A作为一种典型的CaCCs, 在多种上皮细胞中表达并发挥与组织结构相对应的生理功能。在视网膜中, TMEM16A在Ca^{2+}介导的哺乳动物视网膜的兴奋和突触传递中起着重要的作用。在嗅觉上皮中, TMEM16A的表达除了与上皮组织的黏液分泌有关, 也可能参与细胞因子、活性因子的膜泡转运。在气道上皮中, TMEM16A的活性增强是CF和其他以黏液阻塞为特征的疾病的共同特点。在胆管上皮中, 胆汁酸可能通过直接或间接途径调节TMEM16A的活性, 进而影响胆汁的组成和分泌。

TMEM16A在上皮细胞中的表达定位和功能研究尚未完成, 这可能与TMEM16A并未像CFTR突变一样导致严重的系统性病变有关。目前, 对TMEM16A的生理活性的研究多以细胞株或分离的肿瘤细胞为实验材料, 并发现其表达和通道活性变化是影响肿瘤细胞增殖、迁移和浸润的重要因素。然而在生理条件下, 特别是在机体组织上, TMEM16A是否也具有类似的活性, 仍需要进一步确定。一方面, 随着基因条件敲除技术的不断发展, 多种上皮细胞中*TMEM16A*被条件性敲除, 这避免了全身敲除*TMEM16A*而导致的早死现象, 为研究特定细胞类型中TMEM16A的表达和功能提供了实验基础。另一方面, 目前*TMEM16A*条件性敲除动物并未表现出明显的病理变化, 但在疾病动物模型中, TMEM16A的表达异常却与哮喘、慢阻肺、糖尿病、炎症性肠病、胆汁淤积性肝病等疾病的发生和发展密切相关, 系统研究疾病模型中TMEM16A的表达、定位和功能将为病理学的发展提供新的见解, 也为疾病的治疗提供新的策略。

参考文献 (References)

[1] CAPUTO A, CACI E, FERRERA L, et al. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity [J]. Science, 2008, 322(5901): 590-4.

[2] SCHROEDER B C, CHENG T, JAN Y N, et al. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit [J]. Cell, 2008, 134(6): 1019-29.

[3] YANG Y D, CHO H, KOO J Y, et al. TMEM16A confers receptor-activated calcium-dependent chloride conductance [J]. Nature, 2008, 455(7217): 1210-5.

[4] CHA J Y, WEE J, JUNG J, et al. Anoctamin 1 (TMEM16A) is essential for testosterone-induced prostate hyperplasia [J]. Proc Natl Acad Sci USA, 2015, 112(31): 9722-7.

[5] DAUNER K, MOBUS C, FRINGS S, et al. Targeted expression of anoctamin calcium-activated chloride channels in rod photoreceptor terminals of the rodent retina [J]. Invest Ophthalmol Vis Sci, 2013, 54(5): 3126-36.

[6] MENCO B P, FARBMAN A I. Ultrastructural evidence for multiple mucous domains in frog olfactory epithelium [J]. Cell Tissue Res, 1992, 270(1): 47-56.

[7] MALYSZ J, GIBBONS S J, SARAVANAPERUMAL S A, et al. Conditional genetic deletion of TMEM16A in interstitial cells of Cajal impairs Ca^{2+} transients and slow waves in adult mouse small intestine [J]. Am J Physiol Gastrointest Liver Physiol, 2017, 312(3): G228-45.

[8] NOVAK I, HAANES K A, WANG J. Acid-base transport in pancreas-new challenges [J]. Front Physiol, 2013, 4: 380.

[9] DUTTA A K, WOO K, KHIMJI A K, et al. Mechanosensitive Cl^- secretion in biliary epithelium mediated through TMEM16A [J]. Am J Physiol Gastrointest Liver Physiol, 2013, 304(1): G87-98.

[10] WANITCHAKOOL P, WOLF L, KOEHL G E, et al. Role of anoctamins in cancer and apoptosis. Philosophical transactions of the Royal Society of London Series B [J]. FELOS Trans R Soc Lond B Biol Sci, 2014, 369(1638): 20130096.

[11] SONDO E, SCUDIERI P, TOMATI V, et al. Non-canonical translation start sites in the TMEM16A chloride channel [J]. Biochim

Biophys Acta, 2014, 1838(1 Pt B): 89-97.
[12] FERRERA L, CAPUTO A, UBBY I, et al. Regulation of TMEM16A chloride channel properties by alternative splicing [J]. J Biol Chem, 2009, 284(48): 33360-8.
[13] XIAO Q, YU K, PEREZ-CORNEJO P, et al. Voltage- and calcium-dependent gating of TMEM16A/TMEM16A chloride channels are physically coupled by the first intracellular loop [J]. Proc Natl Acad Sci USA, 2011, 108(21): 8891-6.
[14] DANG S, FENG S, TIEN J, et al. Cryo-EM structures of the TMEM16A calcium-activated chloride channel [J]. Nature, 2017, 552(7685): 426-9.
[15] PAULINO C, KALIENKOVA V, LAM A K M, et al. Activation mechanism of the calcium-activated chloride channel TMEM16A revealed by cryo-EM [J]. Nature, 2017, 552(7685): 421-5.
[16] SHI S, PANG C, GUO S, et al. Recent progress in structural studies on TMEM16A channel [J]. Comput Struct Biotechnol J, 2020, 18: 714-22.
[17] BOESE S H, AZIZ O, SIMMONS N L, et al. Kinetics and regulation of a Ca^{2+}-activated Cl^- conductance in mouse renal inner medullary collecting duct cells [J]. Am J Physiol Renal Physiol, 2004, 286(4): F682-92.
[18] QU Z, WEI R W, HARTZELL H C. Characterization of Ca^{2+}-activated Cl^- currents in mouse kidney inner medullary collecting duct cells [J]. Am J Physiol Renal Physiol, 2003, 285(2): F326-35.
[19] BRUNNER J D, LIM N K, SCHENCK S, et al. X-ray structure of a calcium-activated TMEM16 lipid scramblase [J]. Nature, 2014, 516(7530): 207-12.
[20] TIEN J, PETERS C J, WONG X M, et al. A comprehensive search for calcium binding sites critical for TMEM16A calcium-activated chloride channel activity [J]. Elife, 2014, 3: e02772.
[21] CRUZ-RANGEL S, ESPINO-SALDAÑA Á E, MARTÍNEZ-TORRES A, et al. Phosphatidylinositol 4,5-bisphosphate, cholesterol, and fatty acids modulate the calcium-activated chloride channel TMEM16A [J]. Biochim Biophys Acta Mol Cell Biol Lipids, 2018, 1863(3): 299-312.
[22] YU K, JIANG T, CUI Y, et al. A network of phosphatidylinositol 4,5-bisphosphate binding sites regulates gating of the Ca^{2+}-activated Cl^-channel TMEM16A [J]. Proc Natl Acad Sci USA, 2019, 116(40): 19952-62.
[23] CAPUTO A, PIANO I, DEMONTIS G C, et al. TMEM16A is associated with voltage-gated calcium channels in mouse retina and its function is disrupted upon mutation of the auxiliary α2δ4 subunit [J]. Front Cell Neurosci, 2015, 9: 422.
[24] DAUNER K, MOBUS C, FRINGS S, et al. Targeted expression of anoctamin calcium-activated chloride channels in rod photoreceptor terminals of the rodent retina [J]. Invest Ophthalmol Vis Sci, 2013, 54(5): 3126-36.
[25] JEON J H, PAIK S S, CHUN M H, et al. Presynaptic localization and possible function of calcium-activated chloride channel anoctamin 1 in the mammalian retina [J]. PLoS One, 2013, 8(6): e67989.
[26] MENCO B P, FARBMAN A I. Ultrastructural evidence for multiple mucous domains in frog olfactory epithelium [J]. Cell Tissue Res, 1992, 270(1): 47-56.
[27] HENRIQUES T, AGOSTINELLI E, HERNANDEZ-CLAVIJO A, et al. TMEM16A calcium-activated chloride currents in supporting cells of the mouse olfactory epithelium [J]. J Gen Physiol, 2019, 151(7): 954-66.
[28] MAURYA D K, HENRIQUES T, MARINI M, et al. Development of the olfactory epithelium and nasal glands in TMEM16A$^{-/-}$ and TMEM16A$^{+/+}$ mice [J]. PLoS One, 2015, 10(6): e0129171.
[29] MAURYA D K, MENINI A. Developmental expression of the calcium-activated chloride channels TMEM16A and TMEM16B in the mouse olfactory epithelium [J]. Dev Neurobiol, 2014, 74(7): 657-75.
[30] HE M, YE W, WANG W J, et al. Cytoplasmic Cl^- couples membrane remodeling to epithelial morphogenesis [J]. Proc Natl Acad Sci USA, 2017, 114(52): E11161-9.
[31] CLARKE L L, GRUBB B R, YANKASKAS J R, et al. Relationship of a non-cystic fibrosis transmembrane conductance regulator-mediated chloride conductance to organ-level disease in Cftr$^{-/-}$ mice [J]. Proc Natl Acad Sci USA, 1994, 91(2): 479-83.
[32] GRUBB B R, VICK R N, BOUCHER R C. Hyperabsorption of Na^+ and raised Ca^{2+}-mediated Cl^- secretion in nasal epithelia of CF mice [J]. Am J Physiol, 1994, 266(5 Pt 1): C1478-83.
[33] KANG J W, LEE Y H, KANG M J, et al. Synergistic mucus secretion by histamine and IL-4 through TMEM16A in airway epithelium [J]. Am J Physiol Lung Cell Mol Physiol, 2017, 313(3): L466-76.
[34] SCUDIERI P, CACI E, BRUNO S, et al. Association of TMEM16A chloride channel overexpression with airway goblet cell metaplasia [J]. J Physiol, 2012, 590(23): 6141-55.
[35] HUANG F, ZHANG H, WU M, et al. Calcium-activated chloride channel TMEM16A modulates mucin secretion and airway smooth muscle contraction [J]. Proc Natl Acad Sci USA, 2012, 109(40): 16354-9.
[36] GORRIERI G, SCUDIERI P, CACI E, et al. Goblet cell hyperplasia requires high bicarbonate transport to support mucin release [J]. Sci Rep, 2016, 6: 36016.
[37] SCUDIERI P, CACI E, BRUNO S, et al. Association of TMEM16A chloride channel overexpression with airway goblet cell metaplasia [J]. J Physiol, 2012, 590(23): 6141-55.
[38] FISCHER B M, VOYNOW J A. Neutrophil elastase induces MUC5AC gene expression in airway epithelium via a pathway involving reactive oxygen species [J]. Am J Respir Cell Mol Biol, 2002, 26(4): 447-52.
[39] CABRITA I, BENEDETTO R, WANITCHAKOOL P, et al. TMEM16A mediates mucus production in human airway epithelial cells [J]. Am J Respir Cell Mol Biol, 2021, 64(1): 50-8.
[40] KUNZELMANN K, CENTEIO R, WANITCHAKOOL P, et al. Control of ion transport by Tmem16a expressed in murine intestine [J]. Front Physiol, 2019, 10: 1262.
[41] OUSINGSAWAT J, MIRZA M, TIAN Y, et al. Rotavirus toxin NSP4 induces diarrhea by activation of TMEM16A and inhibition of Na^+ absorption [J]. Pflugers Arch, 2011, 461(5): 579-89.
[42] SCHREIBER R, FARIA D, SKRYABIN B V, et al. Anoctamins support calcium-dependent chloride secretion by facilitating calcium signaling in adult mouse intestine [J]. Pflugers Arch, 2015, 467(6): 1203-13.
[43] MROZ M S, KEELY S J. Epidermal growth factor chronically upregulates Ca^{2+}-dependent Cl^- conductance and TMEM16A

expression in intestinal epithelial cells [J]. J Physiol, 2012, 590(8): 1907-20.

[44] OUSINGSAWAT J, MIRZA M, TIAN Y, et al. Rotavirus toxin NSP4 induces diarrhea by activation of TMEM16A and inhibition of Na^+ absorption [J]. Pflugers Arch, 2011, 461(5): 579-89.

[45] KUNZELMANN K, MALL M, BRIEL M, et al. The cystic fibrosis transmembrane conductance regulator attenuates the endogenous Ca^{2+} activated Cl^- conductance of Xenopus oocytes [J]. Pflugers Arch, 1997, 435(1): 178-81.

[46] HUANG F, ROCK J R, HARFE B D, et al. Studies on expression and function of the TMEM16A calcium-activated chloride channel [J]. Proc Natl Acad Sci USA, 2009, 106(50): 21413-8.

[47] YOKOYAMA T, TAKEMOTO M, HIRAKAWA M, et al. Different immunohistochemical localization for TMEM16A and CFTR in acinar and ductal cells of rat major salivary glands and exocrine pancreas [J]. Acta Histochem, 2019, 121(1): 50-5.

[48] CRUTZEN R, VIRREIRA M, MARKADIEU N, et al. Anoctamin 1 (TMEM16A) is required for glucose-induced membrane potential oscillations and insulin secretion by murine beta-cells [J]. Pflugers Arch, 2016, 468(4): 573-91.

[49] WANG J, HAANES K A, NOVAK I. Purinergic regulation of CFTR and Ca^{2+}-activated Cl^- channels and K^+ channels in human pancreatic duct epithelium [J]. Am J Physiol Cell Physiol, 2013, 304(7): C673-84.

[50] WANG Q, BAI L, LUO S, et al. TMEM16A Ca^{2+}-activated Cl^- channel inhibition ameliorates acute pancreatitis via the IP_3R/Ca^{2+}/NF-κB/IL-6 signaling pathway [J]. J Adv Res, 2020, 23: 25-35.

[51] CRUTZEN R, VIRREIRA M, MARKADIEU N, et al. Anoctamin 1 (TMEM16A) is required for glucose-induced membrane potential oscillations and insulin secretion by murine β-cells [J]. Pflugers Arch, 2016, 468(4): 573-91.

[52] XU Z, LEFEVRE G M, GAVRILOVA O, et al. Mapping of long-range INS promoter interactions reveals a role for calcium-activated chloride channel TMEM16A in insulin secretion [J]. Proc Natl Acad Sci USA, 2014, 111(47): 16760-5.

[53] KNUTH A, GABBERT H, DIPPOLD W, et al. Biliary adenocarcinoma. Characterisation of three new human tumor cell lines [J]. J Hepatol, 1985, 1(6): 579-96.

[54] WOO K, SATHE M, KRESGE C, et al. Adenosine triphosphate release and purinergic (P2) receptor-mediated secretion in small and large mouse cholangiocytes [J]. Hepatology, 2010, 52(5): 1819-28.

[55] DUTTA A K, KHIMJI A K, KRESGE C, et al. Identification and functional characterization of TMEM16A, a Ca^{2+}-activated Cl^- channel activated by extracellular nucleotides, in biliary epithelium [J]. J Biol Chem, 2011, 286(1): 766-76.

[56] LI Q, DUTTA A, KRESGE C, et al. Bile acids stimulate cholangiocyte fluid secretion by activation of transmembrane member 16A Cl^- channels [J]. Hepatology, 2018, 68(1): 187-99.

[57] SCHREIBER R, KUNZELMANN K. Expression of anoctamins in retinal pigment epithelium (RPE) [J]. Pflugers Arch, 2016, 468(11/12): 1921-9.

[58] WYCISK K A, ZEITZ C, FEIL S, et al. Mutation in the auxiliary calcium-channel subunit CACNA2D4 causes autosomal recessive cone dystrophy [J]. Am J Hum Genet, 2006, 79(5): 973-7.

[59] WYCISK K A, BUDDE B, FEIL S, et al. Structural and functional abnormalities of retinal ribbon synapses due to CACNA2D4 mutation [J]. Invest Ophthalmol Vis Sci, 2006, 47(8): 3523-30.

[60] HASSENKLOVER T, KURTANSKA S, BARTOSZEK I, et al. Nucleotide-induced Ca^{2+} signaling in sustentacular supporting cells of the olfactory epithelium [J]. Glia, 2008, 56(15): 1614-24.

[61] JIA C, ROMAN C, HEGG C C. Nickel sulfate induces location-dependent atrophy of mouse olfactory epithelium: protective and proliferative role of purinergic receptor activation [J]. Toxicol Sci, 2010, 115(2): 547-56.

[62] FERANCHAK A P, ROMAN R M, DOCTOR R B, et al. The lipid products of phosphoinositide 3-kinase contribute to regulation of cholangiocyte ATP and chloride transport [J]. J Biol Chem, 1999, 274(43): 30979-86.

[63] FERANCHAK A P, ROMAN R M, SCHWIEBERT E M, et al. Phosphatidylinositol 3-kinase contributes to cell volume regulation through effects on ATP release [J]. J Biol Chem, 1998, 273(24): 14906-11.

[64] ROMAN R M, FITZ J G. Emerging roles of purinergic signaling in gastrointestinal epithelial secretion and hepatobiliary function [J]. Gastroenterology, 1999, 116(4): 964-79.

[65] BANALES J M, ARENAS F, RODRIGUEZ-ORTIGOSA C M, et al. Bicarbonate-rich choleresis induced by secretin in normal rat is taurocholate-dependent and involves AE2 anion exchanger [J]. Hepatology, 2006, 43(2): 266-75.

[66] FU Z, OGURA T, LUO W, et al. ATP and odor mixture activate trpm5-expressing microvillous cells and potentially induce acetylcholine release to enhance supporting cell endocytosis in mouse main olfactory epithelium [J]. Front Cell Neurosci, 2018, 12: 71.

[67] OGURA T, SZEBENYI S A, KROSNOWSKI K, et al. Cholinergic microvillous cells in the mouse main olfactory epithelium and effect of acetylcholine on olfactory sensory neurons and supporting cells [J]. J Neurophysiol, 2011, 106(3): 1274-87.

[68] BENEDETTO R, OUSINGSAWAT J, WANITCHAKOOL P, et al. Epithelial chloride transport by CFTR requires TMEM16A [J]. Sci Rep, 2017, 7(1): 12397.

[69] BENSON M, ADNER M, CARDELL L O. Cytokines and cytokine receptors in allergic rhinitis: how do they relate to the Th2 hypothesis in allergy [J]? Clin Exp Allergy, 2001, 31(3): 361-7.

[70] SCUDIERI P, CACI E, BRUNO S, et al. Association of TMEM16A chloride channel overexpression with airway goblet cell metaplasia [J]. J Physiol, 2012, 590(23): 6141-55.

[71] VEGA G, GUEQUÉN A, JOHANSSON M E V, et al. Normal calcium-activated anion secretion in a mouse selectively lacking TMEM16A in intestinal epithelium [J]. Front Physiol, 2019, 10: 694.

[72] SUI J, ZHANG C, FANG X, et al. Dual role of Ca^{2+}-activated Cl^- channel transmembrane member 16A in lipopolysaccharide-induced intestinal epithelial barrier dysfunction *in vitro* [J]. Cell Death Dis, 2020, 11(5): 404.

[73] PORRAS M, MARTIN M T, YANG P C, et al. Correlation between cyclical epithelial barrier dysfunction and bacterial translocation in the relapses of intestinal inflammation [J]. Inflamm Bowel Dis, 2006, 12(9): 843-52.

[74] JAKKAMPUDI A, JANGALA R, REDDY B R, et al. NF-κB in

acute pancreatitis: mechanisms and therapeutic potential [J]. Pancreatology, 2016, 16(4): 477-88.

[75] DUTTA A K, KHIMJI A K, LIU S, et al. PKCα regulates TMEM16A-mediated Cl^- secretion in human biliary cells [J]. Am J Physiol Gastrointest Liver Physiol, 2016, 310(1): G34-42.

[76] FERANCHAK A P, ROMAN R M, DOCTOR R B, et al. The lipid products of phosphoinositide 3-kinase contribute to regulation of cholangiocyte ATP and chloride transport [J]. J Biol Chem, 1999, 274(43): 30979-86.

[77] ROMAN R M, FITZ J G. Emerging roles of purinergic signaling in gastrointestinal epithelial secretion and hepatobiliary function [J]. Gastroenterology, 1999, 116(4): 964-79.

[78] BANALES J M, ARENAS F, RODRIGUEZ-ORTIGOSA C M, et al. Bicarbonate-rich choleresis induced by secretin in normal rat is taurocholate-dependent and involves AE2 anion exchanger [J]. Hepatology, 2006, 43(2): 266-75.

[79] DUTTA A K, WOO K, KHIMJI A K, et al. Mechanosensitive Cl^- secretion in biliary epithelium mediated through TMEM16A [J]. Am J Physiol Gastrointest Liver Physiol, 2013, 304(1): G87-98.

[80] FIOROTTO R, SPIRLI C, FABRIS L, et al. Ursodeoxycholic acid stimulates cholangiocyte fluid secretion in mice via CFTR-dependent ATP secretion [J]. Gastroenterology, 2007, 133(5): 1603-13.

[81] DUTTA A K, KHIMJI A K, LIU S, et al. PKCα regulates TMEM16A-mediated Cl^- secretion in human biliary cells [J]. Am J Physiol Gastrointest Liver Physiol, 2016, 310(1): G34-42.

[82] DUTTA A K, BOGGS K, KHIMJI A K, et al. Signaling through the interleukin-4 and interleukin-13 receptor complexes regulates cholangiocyte TMEM16A expression and biliary secretion [J]. Am J Physiol Gastrointest Liver Physiol, 2020, 318(4): G763-71.

[83] BOUCHER R C. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy [J]. Annu Rev Med, 2007, 58: 157-70.

[84] HENDERSON A G, EHRE C, BUTTON B, et al. Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure [J]. J Clin Invest, 2014, 124(7): 3047-60.

[85] DANAHAY H L, LILLEY S, FOX R, et al. TMEM16A potentiation: a novel therapeutic approach for the treatment of cystic fibrosis [J]. Am J Respir Crit Care Med, 2020, 201(8): 946-54.

[86] RUIZ C, MARTINS J R, RUDIN F, et al. Enhanced expression of TMEM16A in head and neck squamous cell carcinoma causes cell migration and correlates with poor prognosis [J]. PLoS One, 2012, 7(8): e43265.

[87] DANIELSSON J, KUFORIJI A S, YOCUM G T, et al. Agonism of the TMEM16A calcium-activated chloride channel modulates airway smooth muscle tone [J]. Am J Physiol Lung Cell Mol Physiol, 2020, 318(2): L287-95.

[88] ESPINOSA I, LEE C H, KIM M K, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors [J]. Am J Surg Pathol, 2008, 32(2): 210-8.

[89] FARIA D, ROCK J R, ROMAO A M, et al. The calcium-activated chloride channel Anoctamin 1 contributes to the regulation of renal function [J]. Kidney Int, 2014, 85(6): 1369-81.

[90] SCHREIBER R, FARIA D, SKRYABIN B V, et al. Anoctamins support calcium-dependent chloride secretion by facilitating calcium signaling in adult mouse intestine [J]. Pflugers Arch, 2015, 467(6): 1203-13.

[91] OH U, JUNG J. Cellular functions of TMEM16/anoctamin [J]. Pflugers Arch, 2016, 468(3): 443-53.

[92] DUVVURI U, SHIWARSKI D J, XIAO D, et al. TMEM16A induces MAPK and contributes directly to tumorigenesis and cancer progression [J]. Cancer Res, 2012, 72(13): 3270-81.

[93] GALIETTA L J, PAGESY P, FOLLI C, et al. IL-4 is a potent modulator of ion transport in the human bronchial epithelium *in vitro* [J]. J Immunol, 2002, 168(2): 839-45.

[94] HAMA T, YUZA Y, SAITO Y, et al. Prognostic significance of epidermal growth factor receptor phosphorylation and mutation in head and neck squamous cell carcinoma [J]. Oncologist, 2009, 14(9): 900-8.

[95] BRITSCHGI A, BILL A, BRINKHAUS H, et al. Calcium-activated chloride channel TMEM16A promotes breast cancer progression by activating EGFR and CAMK signaling [J]. Proc Natl Acad Sci USA, 2013, 110(11): E1026-34.

[96] DUVVURI U, SHIWARSKI D J, XIAO D, et al. TMEM16A induces MAPK and contributes directly to tumorigenesis and cancer progression [J]. Cancer Res, 2012, 72(13): 3270-81.

[97] LIU Z, ZHANG S, HOU F, et al. Inhibition of Ca^{2+}-activated chloride channel TMEM16A suppresses ovarian cancer through inactivating PI3K/Akt signaling [J]. Int J Cancer, 2019, 144(9): 2215-26.

[98] AYOUB C, WASYLYK C, LI Y, et al. TMEM16A amplification and expression in HNSCC with a high propensity for future distant metastasis and its functions in HNSCC cell lines [J]. Br J Cancer, 2010, 103(5): 715-26.

[99] SHI Z Z, SHANG L, JIANG Y Y, et al. Consistent and differential genetic aberrations between esophageal dysplasia and squamous cell carcinoma detected by array comparative genomic hybridization [J]. Clin Cancer Res, 2013, 19(21): 5867-78.

[100] BILL A, HALL M L, BORAWSKI J, et al. Small molecule-facilitated degradation of TMEM16A protein: a new targeting approach for anticancer therapeutics [J]. J Biol Chem, 2014, 289(16): 11029-41.