钙激活氯通道TMEM16A在肿瘤发生发展及治疗前景的研究进展

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摘要: 跨膜蛋白16A (transmembrane protein 16A, TMEM16A) 作为一种Ca^{2+}激活Cl^-通道, 在许多恶性肿瘤中高表达。位于染色体11q13区域的*TMEM16A*基因扩增是导致其过表达的主要原因之一。另外, 转录调控、表观遗传调控及microRNAs (miRNAs) 在许多肿瘤细胞中促进TMEM16A的表达。TMEM16A不仅激活多种促进肿瘤细胞增殖、迁移及侵袭的信号通路, 还在抑制肿瘤细胞凋亡方面发挥了重要作用。因此, 靶向TMEM16A的抗肿瘤药物研发具有重要的临床意义。本文综述了TMEM16A在恶性肿瘤中的作用机制, 深入总结了TMEM16A促进肿瘤发生发展的信号通路, 并分析了TMEM16A作为潜在生物标志物在肿瘤发生发展中的关键作用。TMEM16A抑制剂可能成为治疗高表达TMEM16A肿瘤的新方案。

关键词: 跨膜蛋白16A; Ca^{2+}激活Cl^-通道; 肿瘤; 生物标记物; 信号通路; 靶向药物

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Research progress of Ca^{2+}-activated Cl^- channel TMEM16A for the occurrence and development and therapeutic potentials in cancer

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Abstract: Transmembrane protein 16A (TMEM16A) is a Ca^{2+}-activated Cl^- channel that is overexpressed in various malignant tumors. The gene is located in chromosome 11q13 region, and its amplification is one of the main reasons for the high expression of this protein. In addition, transcriptional regulation, epigenetic regulation and microRNAs (miRNAs) can promote TMEM16A expression in many cancer cells. TMEM16A not only activates a variety of signaling pathways that promote tumor cell proliferation, migration and invasion, but also plays an important role in inhibiting tumor cell apoptosis. Therefore, the development of anticancer drugs targeting TMEM16A is of great clinical significance. This article reviews the mechanism of TMEM16A in malignant tumors, summarizes the signaling pathways of TMEM16A in promoting tumor development, and analyzes the key role of TMEM16A as a potential biomarker in tumor development. TMEM16A inhibitors may become a new treatment for cancers with high expression of TMEM16A.

Key words: transmembrane protein 16A; Ca^{2+}-activated Cl^- channel; cancer; biomarker; signaling pathway; targeted drug

恶性肿瘤目前仍是全球第二大死亡原因。肿瘤细胞在人体内的迅速增殖和转移, 是导致恶性肿瘤高死亡率的重要因素之一。此外, 肿瘤细胞中关键基因的异常表达导致细胞周期改变和信号转导失控, 增加了恶性肿瘤诊断和治疗难度[1]。因此, 发现促进肿瘤生物学行为的生物标记物并给予靶向治疗有望成为肿瘤治疗领域的重要突破。

离子通道在调节细胞功能和维持机体稳态等方面发挥关键作用。近年来, 多项研究表明离子通道可通过调节肿瘤细胞内的多种信号通路影响肿瘤的发生和

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进展[2]。氯离子通道广泛分布于多种组织细胞, 通过膜电压、胞内 Ca^{2+} 触发和与配体或第二信使作用等多种机制被激活, 参与了包括静息电位调节、细胞容积调节、胞内 pH 稳态维持和信号通路调控在内的众多生理过程[3]。多项针对肿瘤细胞的研究表明, 氯离子通道的表达与多种恶性肿瘤的发生和进展相关, 如细胞内氯离子通道 1 (chloride intracellular channel 1, CLIC1) 在多种恶性肿瘤中过表达, 与肿瘤的侵袭性和不良预后相关, 有望成为诊断和治疗恶性肿瘤的分子标记物[4]。2008 年, 跨膜蛋白 16A (transmembrane protein 16A, TMEM16A, 又名 anoctamin 1) 被多个研究团队证实为钙离子激活氯通道 (Ca^{2+}-activated Cl^- channel, CaCC)[5-7], 该跨膜蛋白在许多组织细胞中广泛表达, 包括分泌上皮细胞、气道和血管平滑肌细胞等[8-24], 并参与众多生理过程, 如分泌上皮的黏液分泌、平滑肌收缩、血压调节等[8,9,17,19,23]。此外, TMEM16A 功能障碍可导致炎症、囊性纤维化、哮喘、高血压等多种疾病的发生[8,9,17,19,24-27]。由此可见, TMEM16A 在正常的生理条件及病理条件下发挥着至关重要的作用。

近期, 许多研究表明TMEM16A在多种恶性肿瘤中过表达, 包括乳腺癌、结直肠癌 (colorectal cancer, CRC)、食管鳞状细胞癌 (esophageal squamous cell carcinoma, ESCC)、头颈部鳞状细胞癌 (head and neck squamous cell carcinoma, HNSCC) 等 (表 1[28-53]), 且与肿瘤的不良预后相关。TMEM16A 可通过调控肿瘤细胞的增殖、迁移、侵袭、凋亡等生物学功能, 进而影响肿瘤的预后、分期和淋巴结转移等临床结果。由此可见, TMEM16A 在恶性肿瘤的发生和进展中发挥着非常关键的作用。尽管已有研究揭示了 TMEM16A 在肿瘤发展中的多重角色, 但其作为生物标记物和治疗靶点的潜力尚未充分开发。因此, 本综述总结近年 TMEM16A 在肿瘤中表达的相关文献, 着重关注了 TMEM16A 在多种恶性肿瘤中过表达的作用机制及对肿瘤细胞增殖、迁移、侵袭、凋亡的影响。并从基因扩增、转录调控、表观遗传调控、信号通路调控、抑制肿瘤细胞凋亡的角度阐述 TMEM16A 作为肿瘤的生物标记物对肿瘤发生和进展的作用, 揭示钙离子激活氯通道 TMEM16A 在肿瘤中表达的调控机制, 并总结了具有抗肿瘤作用的靶向 TMEM16A 抑制剂, 为 TMEM16A 的肿瘤药理学研究和靶向 TMEM16A 抗癌药物的开发提供新思路。

Table 1 TMEM16A's role in the pathogenesis of various malignant tumors. TMEM16A: Transmembrane protein 16A; P: Proliferation; M: Migration; I: Invasion; A: Apoptosis; +: Increased; -: Inhibited; NR: Not reported; NE: No effect; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor 2; STAT3: Signal transducer and activator of transcription 3; ER: Estrogen receptor; ROCK1: Rho-associated protein kinase 1; CRC: Colorectal cancer; MAPK: Mitogen-activated protein kinase; ESCC: Esophageal squamous cell carcinoma; TGF-*β*: Transforming growth factor-*β*; Sp1: Specificity protein 1; H3K4: Histone H3 lysine 4; NF-*κ*B: Nuclear factor-*κ*B; HCC: Hepatocellular carcinoma; HNSCC: Head and neck squamous cell carcinoma

Cancer type	Tumor cell	Mechanism	TMEM16A overexpression				TMEM16A knockdown				Clinical outcome
			P	M	I	A	P	M	I	A	
Breast cancer	SKBR3[28]	EGFR signaling	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is correlated with decreased survival in HER2-amplified breast cancer
	MCF-7, T47D[29]	EGFR/STAT3 signaling	+	NR	NR	NR	-	NR	NR	NR	Overexpression of TMEM16A is correlated with shorter overall survival in ER-positive breast cancer
	MCF-7, T47D[30]	EGFR/STAT3/ROCK1 signaling	NR	+	+	NR	NR	-	-	NR	Overexpression of TMEM16A is correlated with lymph node metastasis and shorter survival
CRC	DLD-1, HCT116[31]	Epigenetic regulation *via* miR-132 down-regulation	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is correlated with poor prognosis
	HCT116[32]	Epigenetic regulation *via* miR-9 down-regulation, MAPK signaling	NR	NR	NR	NR	-	-	-	+	Overexpression of TMEM16A is correlated with advanced tumor stage and lymph node metastasis
	SW480[33]	Epigenetic regulation *via* miR-144 down-regulation, MAPK signaling	NR	NR	NR	NR	NR	-	-	NR	Overexpression of TMEM16A is correlated with poor histology differentiation and lymph node metastasis
	SW480, SW620, HCT116, HT-29[34]	Wnt/*β*-catenin signaling	NR	NR	NR	NR	-	NR	NR	+	Overexpression of TMEM16A is correlated with advanced tumor stage, histological type and pathological differentiation
ESCC	KYSE30lm3, KYSE 450lm2, KYSE30 luc, KYSE450luc[35]	EGFR signaling	NR	+	+	NR	NR	-	-	NR	Overexpression of TMEM16A is correlated with lymph node metastasis

Continued

Cancer type	Tumor cell	Mechanism	TMEM16A overexpression				TMEM16A knockdown				Clinical outcome
			P	M	I	A	P	M	I	A	
	KYSE30, KYSE410[36]	TGF-β signaling	NR	NR	NR	NR	-	-	-	NE	Overexpression of TMEM16A is correlated advanced tumor stage and lymph node metastasis
Gastric cancer	AGS, SGC7901[37]	Transcriptional regulation *via* Sp1, epigenetic regulation *via* H3K4 trimethylation	NR	NR	NR	NR	NR	-	-	NR	Overexpression of TMEM16A is correlated with advanced tumor stage and tumor metastasis
	AGS, BGC823[38]	Epigenetic regulation *via* miR-381 down-regulation, TGF-β signaling	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is correlated with poor prognosis and tumor metastasis
	AGS, BGC-823[39]	TGF-β signaling	NR	NR	NR	NR	NE	-	-	NR	Overexpression of TMEM16A is correlated with advanced tumor stage and lymph node metastasis
Glioma	U87MG[40]	NF-κB signaling	NR	NR	NR	NR	-	-	-	NR	Overexpression of TMEM16A is correlated with pathological stage
HCC	SMMC-7721[41]	MAPK signaling	NR	NR	NR	NR	-	-	-	NE	NR
	HepG2, SMMC-7721[42]	MAPK signaling	+	+	+	-	-	-	-	+	Overexpression of TMEM16A is correlated with tumor grade, tumor size, distant metastasis and poor differentiation
HNSCC	Patient tissue[43]	TMEM16A gene amplification	NR	NR	NR	NR	NR	NR	NR	NR	Dysplasia with amplification of the TMEM16A gene carries a higher risk of malignant transformation
	Patient tissue[44]	TMEM16A gene amplification	NR	NR	NR	NR	NR	NR	NR	NR	Amplification of TMEM16A gene and overexpression of its protein are associated with poor prognosis in HPV-negative HNSCC tumors
	Patient tissue[45]	TMEM16A gene amplification	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is associated with lymph node metastasis and pathological stage in HPV-negative HNSCC tumors
	PE/CA-PJ34, Fa-Du[46]	Epigenetic regulation *via* promoter hypermethylation	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is correlated with decreased survival in HPV-negative HNSCC tumors
	Patient tissue[47]	Epigenetic regulation *via* promoter hypermethylation	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is correlated with decreased survival
Lung cancer	Patient tissue[48]	TMEM16A gene amplification, EGFR overexpression	NR	NR	NR	NR	NR	NR	NR	NR	Overexpression of TMEM16A is correlated with advanced tumor stage and high recurrence rate
	A549, H1650[49]	Epigenetic regulation *via* H3K4 trimethylation	+	NR	NR	NR	NR	NR	NR	NR	NR
	H1299[50]	EGFR signaling, MAPK signaling	NR	+	+	NR	-	-	-	NR	NR
Melanoma	A-375[51]	MAPK signaling	NR	NR	NR	NR	-	NR	NR	NR	Overexpression of TMEM16A is correlated with low survival rate
Prostate cancer	PC-3[52]	MAPK signaling, TNF-α signaling	+	NR	NR	-	-	NR	NR	+	NR
Pancreatic cancer	AsPC-1[53]	EGFR-Ca^{2+} signaling	NR	NR	NR	NR	NR	-	NR	NR	Overexpression of TMEM16A is correlated with decreased survival

1 TMEM16A简介

1.1 TMEM16家族 TMEM16A作为具有10个成员的TMEM16家族的首位成员, 与TMEM16B一样, 是一种钙离子激活氯通道, 主要参与调节跨上皮液体运输、平滑肌收缩和神经元兴奋性[54-56]。该家族其他成员 (TMEM16C、16D、16F、16G和16J) 多数被证实为Ca^{2+}依赖的磷脂翻转酶, 它们介导的磷脂酰丝氨酸外化在凝血、骨骼发育和细胞融合等多种生物学过程中发挥重要作用[56]。

1.2 TMEM16A结构 作为CaCC, TMEM16A通过胞内Ca^{2+}直接结合[57,58]而被激活。TMEM16A的激活展现出显著的电压依赖性和Ca^{2+}浓度敏感性: 在低Ca^{2+}浓度条件下, 其电流表现出依赖电压的激活特性、显著的外向整流现象和失活的尾电流; 而在高Ca^{2+}浓度条件下, TMEM16A则表现出非电压依赖性的激活模式和电流与电压的线性关系[59]。近期的冷冻电镜 (cryo-electron microscopy, cryo-EM) 结构研究深化了研究者对TMEM16A结构的理解。2014年, 真菌*Nectria*

haematococca 磷脂翻转酶 (nhTMEM16) 的晶体结构首次提供了对 TMEM16 家族的结构分析, 显示了 nhTMEM16 是一个具有 10 个跨膜螺旋 (TM1～10) 同源二聚体, 每个亚基具有两个 Ca^{2+} 结合位点[60]。2017 年, 基于小鼠 TMEM16A (mTMEM16A) 的 cryo-EM 结构分析进一步证实了其同源二聚体的本质, 每个亚基由 10 个跨膜螺旋 (TM1～10) 构成, 配备两个 Ca^{2+} 结合位点和一条独立的离子传导通道[57,58]。特别是, 跨膜螺旋 TM3～7 构成了一个沙漏形状的阴离子传导通道, 其中的 10 个关键残基对 TMEM16A 的离子选择性和阴离子的渗透性起着至关重要的作用[57,58,61] (图 1A、B)。此外, 位于 TM6～8 上的带负电的酸性氨基酸残基 (N650、N651、E654、E702、E705、E734 和 D738) 构成了 Ca^{2+} 结合位点 (图 1C), Ca^{2+} 的结合诱导 TM6 的构象变化, 导致通道开启[57]。在没有 Ca^{2+} 结合的情况下, TM6 的胞质末端围绕甘氨酸残基 (G644) 发生弯曲, 从而使通道变窄; Ca^{2+} 结合后, TM6 胞质末端伸直, 使通道开放[57,62] (图 1D)。值得注意的是, TMEM16A 的两个离子传导孔道能够独立激活, 这一特性为通道功能的多样性和复杂性提供了结构基础[63]。

2 TMEM16A 在肿瘤发生发展中的作用

多项研究证实, TMEM16A 在多种恶性肿瘤中高表达, 并且与肿瘤的临床分期[32,34,37,39,40,42,45,48]、淋巴结转移[30,32-35,39,45]、分化程度[33-35,42]等临床病理因素紧密相关, 提示其在恶性肿瘤的发生和进展中的潜在作用 (表 1)。具体而言, 在结直肠癌、肝癌和胃癌等恶性肿瘤细胞中, TMEM16A 的过表达与淋巴结转移、肿瘤分级及分化程度呈现显著相关性[31-34,37,39,42]。TMEM16A 参与调控多种肿瘤细胞的增殖[29,32,34,36,40-42,49-52]、迁移[30,32,33,35-37,39-42,50,53]、侵袭[30,32,33,35-37,39-42,50] 和凋亡[32,34,42,52] (表 1)。对于大多数肿瘤来说, TMEM16A 的过表达已被证实能够促进细胞的增殖、迁移和侵袭, 并抑制肿瘤细胞凋亡, 而敲低 TMEM16A 的表达产生相反作用 (表 1)。有研究报道, TMEM16A 通过上调细胞周期蛋白 (cyclin) 如 cyclin D1、cyclin A2、cyclin E 以及细胞周期依赖性激酶 (cyclin-dependent kinases, CDK) 如 CDK1 和 CDK2 的表达水平促进细胞增殖[40,41,64], 并且 TMEM16A 能够激活与细胞增殖相关的转录因子如信号转导及转录激活蛋白 3 (signal transducer and activator of transcription 3, STAT3)、c-myc 和核因子 κB (nuclear

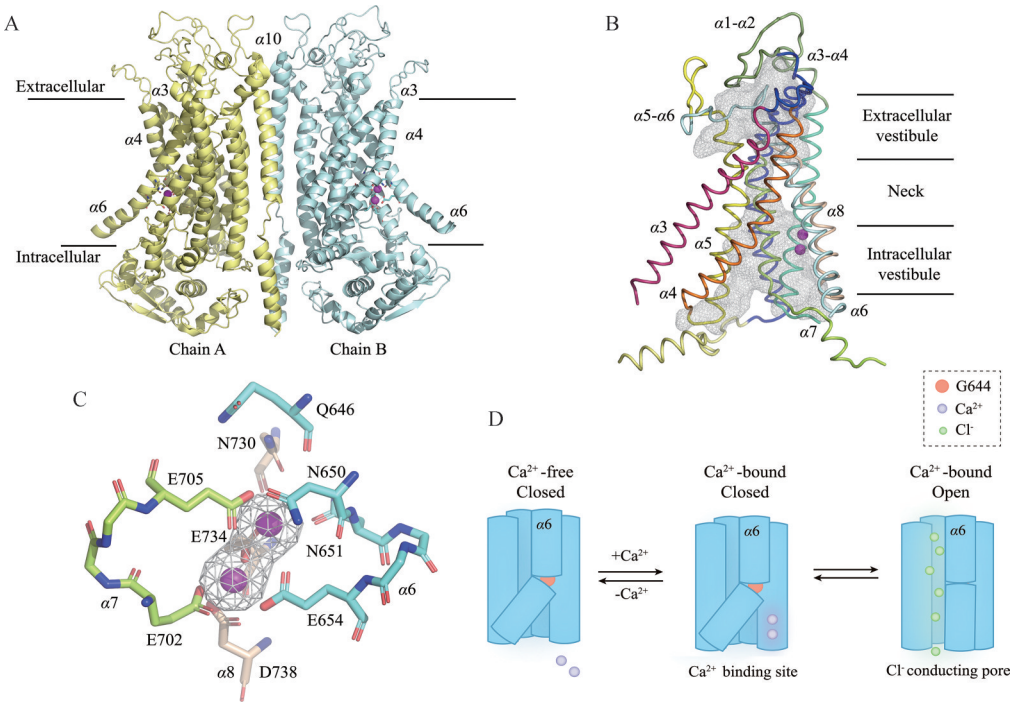


Figure 1 The structure of TMEM16A and activation mechanism. A: Cryo-EM structure of mTMEM16A (PDB ID: 5OYB). The homodimer structure of Ca^{2+}-bound TMEM16A is presented. Each subunit comprises TM1−10, two Ca^{2+}-binding sites, and an independent ion conduction pore. Purple spheres represent Ca^{2+} ions; B: Ion conduction pore of mTMEM16A. The grey mesh represents the pore surface, and purple spheres denote Ca^{2+} ions; C: Ca^{2+} binding site of mTMEM16A. This site is formed by seven conserved acidic amino acid residues (N650, N651, E654, E702, E705, E734, D738) on T6−8. Sticks represent the side chains of selected residues, and purple spheres indicate Ca^{2+} ions. The figures A−C were generated by PyMOL (https://pymol.org/2/); D: Schematic depiction of Ca^{2+}-dependent gating mechanism of TMEM16A. Ca^{2+} binding to the TMEM16A channel primarily induces a conformational rearrangement of TM6. The image displays an intracellular perspective of the membrane structure

factor-κB, NF-κB) [29,30,40]。此外, TMEM16A能够促进抗凋亡蛋白如B细胞淋巴瘤因子2 (B-cell lymphoma 2, Bcl-2)和髓细胞白血病因子1 (myeloid cell leukemia 1, MCL-1)的表达, 而抑制促凋亡蛋白如含半胱氨酸的天冬氨酸蛋白水解酶3 (cysteinyl aspartate specific proteinase 3, caspase-3)、caspase-7、caspase-8、Fas相关死亡结构域蛋白 (Fas-associated death domain protein, FADD)及肿瘤坏死因子α (tumor necrosis factor α, TNF-α)的表达[52,65,66]。另外, TMEM16A通过调节肿瘤细胞内的多种信号通路, 如表皮生长因子受体 (epidermal growth factor receptor, EGFR)信号通路、丝裂原激活蛋白激酶 (mitogen-activated protein kinase, MAPK)信号通路等, 促进了肿瘤细胞的增殖、迁移和侵袭[28-30,32,33,35,41,42,50-53]。由此可见, TMEM16A在肿瘤细胞的生物学行为中发挥着关键作用, 有望成为一个重要的肿瘤生物标记物, 并可能成为开发针对恶性肿瘤的新治疗策略的靶点。

3 TMEM16A在肿瘤中高表达的调控机制

目前研究已经发现, 在肿瘤中TMEM16A高表达受多种机制调控。这里总结了基因扩增、转录调控、表观遗传调控及miRNAs对肿瘤细胞中TMEM16A表达水平的影响 (图2、表1)。

3.1 *TMEM16A*基因扩增促进肿瘤的增殖 *TMEM16A*基因定位于染色体11q13区域, 这一区域的基因扩增在多种恶性肿瘤中较为常见, 如HNSCC和非小细胞肺癌 (non-small cell lung cancer, NSCLC)等[43-45,48]。多项研究表明, *TMEM16A*基因扩增与肿瘤细胞中TMEM16A过表达存在密切联系。有报道HNSCC癌患者手术切除的肿瘤组织和癌前病变组织中均出现*TMEM16A*基因扩增, 且*TMEM16A*基因扩增的患者具有更高的恶变风险[43], 表明*TMEM16A*基因扩增与患者的不良预后相关[44]。类似地, He等[48]在11q13扩增的NSCLC肿瘤组织中也观察到了*TMEM16A*的基因扩增与过表达, 并发现TMEM16A的过表达与肿瘤的分期进展相关。此外, 与人乳头瘤病毒 (human papillomavirus, HPV)阳性的HNSCC相比, *TMEM16A*基因扩增与HPV阴性的HNSCC的淋巴结转移和病理分期呈现显著相关性[45]。这些研究结果表明, 位于染色体11q13区域的*TMEM16A*基因扩增在肿瘤诊断和预后评估中具有重要的临床应用价值。

3.2 *TMEM16A*转录调控对肿瘤细胞的恶性生物学行为的影响 生物信息学分析揭示了*TMEM16A*基因启动子区域的复杂性, 该区域缺乏经典的核心序列TATA盒, 但富含多种转录调控元件, 包括起始子 (initiator, INR)、STAT6结合位点、特异性蛋白1 (specificity protein 1, Sp1)结合位点等, 这些元件参与了*TMEM16A*基因的转录调控[67]。STAT6作为转录因子, 在白细胞介素4 (interleukin-4, IL-4)和IL-13的诱

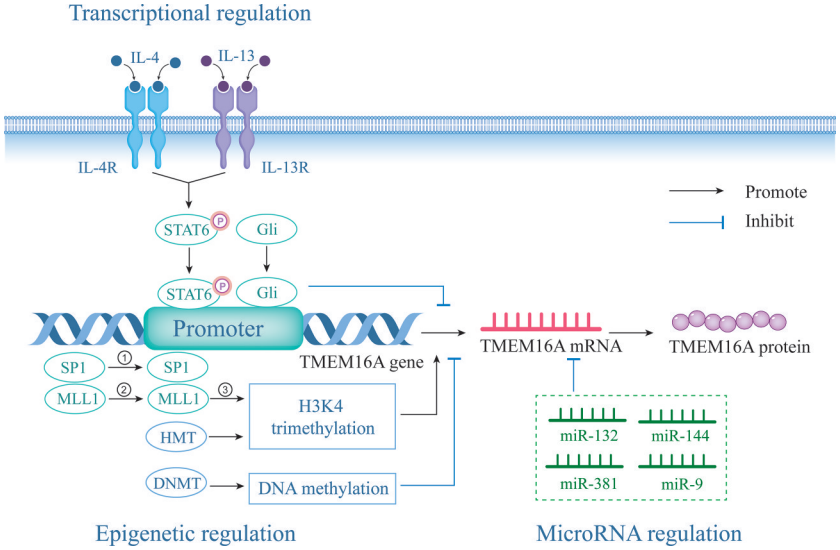


Figure 2 TMEM16A overexpression in cancer *via* transcriptional and epigenetic regulation as well as microRNA regulation. ① Transcriptional regulation: IL-4 and IL-13-induced TMEM16A overexpression: IL-4 and IL-13 promote the transcription of TMEM16A by activation of STAT6. Gil inhibits gene transcription by targeting the TMEM16A promoter. ② Epigenetic regulation: DNMTs induce CpG islands hypermethylation to inhibit gene transcription. Conversely, HMTs induce histone H3K4 trimethylation to promote gene transcription. Sp1 binds to TMEM16A promoter and recruits MLL1, which facilitates histone H3K4 trimethylation to promote gene transcription. ③ MicroRNA regulation: miR-132, miR-144, miR-381, and miR-9 bind to the 3′-UTR of TMEM16A, leading to mRNA degradation or post-transcriptional repression

导下促进 *TMEM16A* 基因的转录。截短或定点突变 *STAT6* 结合位点和使用 *STAT6* 小干扰 RNA (small interfering RNA, siRNA) 处理能够抑制 IL-4 介导的 *TMEM16A* 转录上调[67]。此外, TMEM16A 抑制剂 $T16A_{inh}$-A01或TMEM16A-siRNA处理可显著抑制IL-13介导的ERK磷酸化[68]。研究人员发现经IL-13处理后的食管上皮细胞中 *TMEM16A* mRNA的表达显著上调, 染色质免疫共沉淀测序 (chromatin immunoprecipitation sequencing, CHIP-seq) 结果显示 STAT6 与 *TMEM16A* 基因转录起始点 (transcriptional start site, TSS) 上游的基因组区域存在相互作用[69], 这些结果表明, IL-4 和 IL-13 诱导 TMEM16A 的表达可能涉及 STAT6 的调控作用。尽管 IL-4 和 IL-13 在 *TMEM16A* 转录激活中的作用已被广泛研究[69,70], 但它们通过 STAT6 介导的 TMEM16A 表达上调在肿瘤增殖、迁移和侵袭中的具体作用尚需进一步研究。

Mazzone 等[71]进一步揭示了胶质瘤相关癌基因 (glioma-associated oncogene, Gli) 蛋白在TMEM16A表达调控中的作用。Gli蛋白的异常表达与肿瘤发生和不良预后相关[72], 并且通过CHIP检测发现HEK293细胞内源表达的Gli能够直接与 *TMEM16A* 启动子结合, 抑制其转录[71]。但该效果被GANT61抑制Gli蛋白与DNA结合后所逆转, 导致 *TMEM16A* 的mRNA和蛋白表达水平上升, 表明Gli蛋白可能通过抑制 *TMEM16A* 的转录来调控肿瘤细胞的增殖和迁移[71]。

由此可见, *TMEM16A* 的转录调控是一个多层次、多因素参与的过程, 涉及多种转录因子和信号通路。未来的研究需要深入探索这些调控元件和转录因子如何相互作用, 以及它们在不同肿瘤类型中的特异性作用。

3.3 表观遗传修饰调控 *TMEM16A* 基因转录参与肿瘤的进展 表观遗传学是指在不改变DNA序列的情况下, 通过可遗传的方式调控基因表达的机制。这些机制在肿瘤的发生和发展中起着至关重要的作用。表观遗传调控主要包括DNA甲基化和组蛋白修饰。

DNA甲基化是由DNA甲基转移酶 (DNA methyltransferase, DNMT) 催化的, 主要发生在CpG岛上的胞嘧啶碳原子上。在 *TMEM16A* 基因的启动子区域, CpG岛的存在已被确认, 并且其甲基化状态与 *TMEM16A* 基因的表达密切相关。具体而言, CpG岛上的高甲基化状态被报道抑制了 *TMEM16A* 基因的表达, 而低甲基化状态则促进了其表达[46,67,73]。与HPV阴性的HNSCC细胞相比, HPV阳性的HNSCC细胞中TMEM16A的表达水平较高, 这与其启动子区域的低甲基化程度相关, 提示TMEM16A的表达可能受启动子甲基化状态的调控[46]。然而, 在口咽鳞状细胞癌 (oropharyngeal squamous cell carcinoma, OPSCC) 细胞中, 研究人员发现5'远端调节元件 (5' distal regulatory element, 5'-DRE) 附近的CpG岛高甲基化以及TSS附近的CpG岛低甲基化可能共同上调TMEM16A的表达, 表明DNA甲基化对TMEM16A在肿瘤中表达的调控可能存在区域差异[47]。

组蛋白修饰是调控基因表达的另一种重要机制。TMEM16A被认为是组蛋白H3第4位赖氨酸 (histone H3 lysine 4, H3K4) 甲基化的潜在靶点, 因此组蛋白甲基转移酶 (histone methyltransferase, HMT) 可以通过调控H3K4的甲基化水平来影响基因转录[37,49]。CHIP实验显示Sp1能够结合 *TMEM16A* 启动子区, 并募集混合谱系白血病蛋白1 (mixed-lineage leukemia 1, MLL1) 导致H3K4三甲基化, 从而上调TMEM16A的表达, 促进胃癌细胞的生长和转移[37]。类似地, 组蛋白甲基转移酶SET和MYND结构域蛋白3 (histone methyltransferase SET and MYND domain-containing 3, SMYD3) 通过结合 *TMEM16A* 启动子并诱导H3K4三甲基化, 促进了 *TMEM16A* 的转录, 进而推动NSCLC细胞的异常增殖[49]。这些研究结果表明, 组蛋白甲基转移酶介导的H3K4三甲基化可能通过促进 *TMEM16A* 基因转录进而诱导肿瘤细胞的增殖, 尽管如此, 组蛋白甲基化在 *TMEM16A* 转录调控中的具体机制仍需进一步阐明。

3.4 miRNAs靶向 *TMEM16A* mRNA抑制肿瘤的发生和进展 miRNAs是一类由内源基因编码的、长度约为22个核苷酸的单链非编码小型RNA分子。它们通过与靶mRNA的3'-非翻译区 (3' untranslated regions, 3'-UTR) 碱基配对, 导致mRNA的降解或抑制其翻译, 从而在表观遗传调控中发挥重要作用。miRNAs在调控关键原癌基因和抑癌基因的表达方面起着至关重要的作用, 进而影响肿瘤细胞的增殖、凋亡和侵袭等生物学过程[31-33,38]。在TMEM16A的调控中, 特定的miRNAs已被证实可直接靶向其3'-UTR, 从而调控其表达。Mokutani等[31]研究表明, miR-132通过直接靶向 *TMEM16A* 的3'-UTR, 下调其mRNA的表达, 进而抑制CRC细胞的生长和侵袭, 且miR-132的下调与CRC患者的不良预后相关, 表明miR-132过表达抑制TMEM16A的表达, 可能抑制肿瘤的发生和进展。Cao等[38]进一步揭示了miR-381在胃癌中的作用, 发现miR-381的表达与TMEM16A的表达呈负相关, miR-381通过调控转化生长因子β (transforming growth factor-β, TGF-β) 通路发挥抑癌作用, 表明miR-381高表达诱导的TMEM16A低表达通过抑制TGF-β通路进而阻碍胃癌细胞的迁移和侵袭。此外, 荧光素酶报告基因检测

证实了miR-9和miR-144也能通过靶向*TMEM16A* mRNA的3'-UTR来抑制其表达, 进而抑制CRC细胞的增殖、迁移和侵袭[32,33]。这些研究结果均表明, miRNAs可以通过靶向*TMEM16A* mRNA的3'-UTR来调控其表达, 进而影响肿瘤细胞的生物学行为。特别是, miR-132、miR-381、miR-9和miR-144的过表达能够抑制TMEM16A的表达, 从而可能抑制肿瘤细胞的增殖、迁移和侵袭。这些发现为理解TMEM16A在肿瘤发展中的作用提供了新的视角, 并为肿瘤治疗提供了潜在的治疗靶点。

4 TMEM16A参与信号通路调控肿瘤细胞生物学过程

TMEM16A激活多种信号通路促进肿瘤细胞增殖、迁移、侵袭、凋亡等生物学过程。本文总结了TMEM16A促进肿瘤细胞增殖、迁移、侵袭、凋亡等生物学过程相关的信号通路机制 (图3、表1)。

EGFR是一种酪氨酸激酶受体, 其在多种肿瘤细胞中的磷酸化激活与肿瘤细胞的增殖、迁移和侵袭等过程密切相关[29,30,35,50,53]。TMEM16A已被多项研究证实与EGFR信号通路存在交叉作用。Kulkarni等[28]研究发现, TMEM16A被敲低可降低EGFR和人表皮生长因子受体2 (human epidermal growth factor receptor 2, HER2)的表达水平和活性, 且该抑制作用与HER2扩增的乳腺癌细胞的存活率降低相关, TMEM16A抑制剂和靶向EGFR单克隆抗体西妥昔单抗联合处理使HNSCC细胞的活力明显丧失, 表明EGFR的过表达在TMEM16A介导的肿瘤细胞生长和生存中发挥重要作用。本课题组的前期研究揭示了EGF通过EGFR/STAT3信号通路促进TMEM16A表达, 从而相互激活并促进乳腺癌细胞的增殖[29]。此外, TMEM16A过表达可以通过EGFR/STAT3信号通路促进Rho相关蛋白激酶1 (Rho-associated protein kinase 1, ROCK1)的表达, 进而促进乳腺癌细胞的迁移和侵袭[30]。在肺癌H1299细胞系中, TMEM16A的高表达促进EGFR和细胞外调节蛋白激酶1/2 (extracellular regulated kinase 1/2, ERK1/2)的磷酸化, 而$T16A_{inh}$-A01处理可以显著抑制这些蛋白磷酸化, 表明TMEM16A通过EGFR/ERK1/2信号通路促进肺癌细胞的迁移和侵袭[50]。Crottès等[53]研究也表明, EGF通过激活TMEM16A和TMEM16A依赖的钙库操纵性Ca^{2+}内流 (store-operated calcium entry, SOCE)促进胰腺癌细胞的迁移, 并证明TMEM16A通过参与EGFR/Ca^{2+}信号通路影响胰腺癌的预后。这些研究结果表明, TMEM16A过表达可能通过促进EGFR磷酸化和EGF分泌进而激活EGFR信

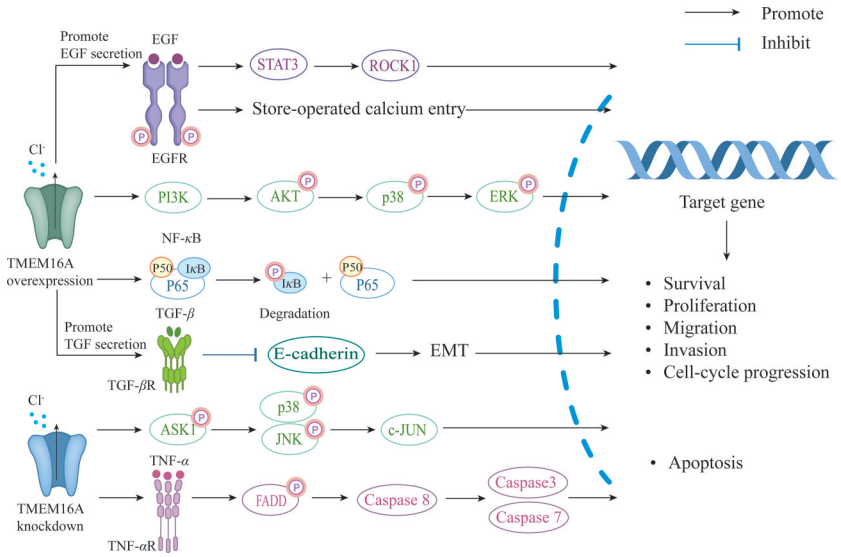


Figure 3 TMEM16A-associated signaling pathways in tumorigenesis. TMEM16A plays a crucial role in tumor development through its involvement in multiple signaling pathways. TMEM16A overexpression can enhance EGFR phosphorylation and promote EGF secretion, thereby activating STAT3/ROCK1 signaling pathways and SOCE. This activation promotes tumor cell proliferation and migration. TMEM16A induces ERK activation through the PI3K/AKT signaling pathway, which promotes tumor cell-cycle progression. TMEM16A activates NF-κB signaling pathway, contributing to tumor cell proliferation, migration and invasion. Overexpression of TMEM16A can activate the TGF-β signaling pathway, promoting TGF-β secretion, downregulating E-cadherin expression, and facilitating EMT, thereby enhancing tumor cell migration and invasion. Knockdown of TMEM16A promotes tumor cell apoptosis by activating the ASK1/p38/JNK/JUN signaling pathway. Knockdown of TMEM16A activates TNF-α signaling pathway, leading to FADD phosphorylation and caspase activation, thereby promoting tumor cell apoptosis

号通路, 调控肿瘤细胞的生物学过程。

MAPK信号通路在肿瘤细胞的增殖、迁移、分化、运动和凋亡等多个细胞进程中起着关键作用[41,42,51,52,74]。Song等[52]发现敲低*TMEM16A*通过显著增高凋亡信号调节激酶1 (apoptosis signal regulating kinase 1, ASK1)、p38、细胞内蛋白Jun氨基末端激酶(c-Jun N-terminal kinase, JNK) 和c-Jun的磷酸化水平进而促进TNF-α介导的前列腺癌细胞凋亡, 表明TMEM16A对MAPK信号通路的激活具有调控作用。此外, TMEM16A-siRNA处理可通过激活丝/苏氨酸蛋白激酶 (serine and threonine kinase, AKT)/哺乳动物雷帕霉素靶蛋白 (mammalian target of rapamycin, mTOR)信号通路抑制乳腺癌细胞中HER2的转录, 说明TMEM16A抑制剂可为对HER2靶向治疗产生耐药性的乳腺癌患者提供潜在的治疗策略[74]。在肝癌SMMC-7721细胞中, *TMEM16A*被敲低导致磷脂酰肌醇-3-激酶 (phosphatidylinositol-3 kinase, PI3K)、p-AKT、p-p38和p-ERK的表达水平降低, 同时cyclin D1的表达受到抑制, 这些结果表明TMEM16A可能通过PI3K/AKT/p38/ERK信号通路调控肝癌细胞的生长和凋亡[41,42]。最新的研究结果显示, 在B-Raf原癌基因(*BRAF*) 突变的恶性黑色素瘤细胞中, 敲低*TMEM16A*可以显著抑制AKT、丝裂原活化蛋白激酶激酶(mitogen-activated protein kinase kinase, MEK) 和ERK的磷酸化激活。这一效果与BRAF抑制剂维莫非尼降低MEK和ERK的磷酸化水平具有协同作用, 并且能够拮抗其诱导的AKT磷酸化激活, 表明靶向TMEM16A可能有助于改善黑色素瘤对BRAF抑制剂的耐药性[51]。因此, 在不同肿瘤细胞中, TMEM16A可能通过激活不同MAPK信号通路, 影响肿瘤细胞的增殖、凋亡和耐药性。

此外, TMEM16A还参与了多种其他信号通路的调控。Liu等[40]发现在TMEM16A过表达的胶质瘤细胞中, NF-κB p65在细胞核内累积, 其靶基因包括参与细胞增殖的cyclin D1、cyclin E、c-myc, 以及与迁移和侵袭相关的基质金属蛋白酶2 (matrix metalloproteinase 2, MMP-2) 和MMP-9的表达水平显著上调, 进而促进胶质瘤细胞的增殖、迁移和侵袭。TMEM16A过表达可通过TGF-β信号通路促进胃癌细胞的迁移和侵袭, 而miR-381通过靶向TMEM16A抑制TGF-β信号通路, 进而抑制上皮−间充质转化 (epithelial-mesenchymal transition, EMT)[38,39]。此外, TMEM16A小发夹RNA (small hairpin RNA, shRNA) 处理不仅下调TGF-β的表达水平, 还降低下游信号蛋白Smad2和Smad3的磷酸化水平, 进而抑制ESCC细胞的迁移和侵袭[36]。Song等[52]发现抑制TMEM16A可以激活TNF-α信号通路, 导致FADD的磷酸化及caspase家族成员的激活, 进而促进前列腺癌细胞凋亡。Yan等[34]研究则揭示了TMEM16A通过Wnt/β-catenin信号通路调控结直肠癌细胞的增殖和凋亡。

综上所述, TMEM16A在不同肿瘤细胞中通过参与多种信号通路的调控, 影响肿瘤细胞的生物学过程。这些发现进一步证明TMEM16A在肿瘤发展中的多功能性, 并为开发针对TMEM16A的靶向治疗策略提供了重要的分子机制信息。

5 TMEM16A抑制肿瘤细胞凋亡

肿瘤细胞常常通过上调抗凋亡基因或下调促凋亡基因的表达来逃避凋亡, 从而获得生存优势[42,52,65,66]。TMEM16A已被多项研究证实在肿瘤细胞凋亡的调节中发挥重要作用。在乳腺癌细胞中, TMEM16A的过表达被发现可以促进抗凋亡蛋白Bcl-2和Mcl-1的表达, 同时抑制促凋亡蛋白caspase-3和caspase-9的活性, 从而抑制细胞凋亡[65]。这一发现表明TMEM16A可能通过调节凋亡相关蛋白的表达来影响乳腺癌细胞的命运。Bim是一种促凋亡的Bcl-2家族蛋白, 其下调有助于肿瘤细胞逃避凋亡。在HNSCC FaDu细胞中, TMEM16A通过下调Bim的表达来抑制细胞凋亡[66]。此外, 在肝细胞癌 (hepatocellular carcinoma, HCC) HepG2和SMMC7721细胞中, *TMEM16A*被敲低后导致细胞凋亡率显著升高, 进一步证实了TMEM16A在肿瘤细胞凋亡中的抑制作用[42]。在前列腺癌PC-3细胞中, *TMEM16A*被敲低后发现可以激活TNF-α下游信号通路, 包括FADD的磷酸化和caspase家族成员(caspase-3、caspase-7和caspase-8) 的激活及聚腺苷二磷酸核糖聚合酶 (poly ADP-ribose polymerase, PARP)的降解, 最终促进细胞凋亡, 表明TMEM16A可能通过调节TNF-α信号通路来影响前列腺癌细胞的凋亡[52]。

因此, TMEM16A在不同类型肿瘤细胞中通过多种机制调节细胞凋亡, 包括调节抗凋亡和促凋亡蛋白的表达, 以及影响TNF-α信号通路的激活。这些发现为理解TMEM16A在肿瘤发展中的作用提供了新的视角, 并为开发针对TMEM16A的抗癌治疗策略提供了潜在的靶点。

6 TMEM16A与肿瘤干性的关系

肿瘤干细胞 (cancer stem cells, CSC) 是一类具有自我更新和多向分化能力的耐药细胞亚群[75,76], 其静止特性和抗凋亡特性与肿瘤的发生、进展和复发密切相关[77,78]。多项研究表明, CSC广泛存在于多种肿瘤类型中, 并在治疗抵抗和患者预后不良中发挥关键作用[79]。因此, 靶向CSC被认为是一种潜在的新颖的肿

瘤治疗策略, 有望实现降低复发风险、减少耐药性及提高患者生存率。

Ca^{2+}对于包括干细胞在内的多种细胞的增殖、存活、分化和基因表达等正常细胞功能至关重要[80]。调控细胞内钙激活的离子通道与包括肿瘤在内的许多病理过程的发展密切相关[81]。研究发现, 钙释放通道IP_3R和SOCE在CSC中异常表达, 并与维持干细胞特性相关, 通过促进细胞增殖、迁移和侵袭及上调干性相关基因、转录因子和标记物的表达水平增强肿瘤干性[79]。值得注意的是, TMEM16A过表达已被证实通过激活IP_3R和SOCE促进肿瘤的恶性进展[42,53], 因此TMEM16A在调控CSC生物学行为中的作用不容忽视, 尽管具体的调控机制仍需进一步研究, 以揭示其在肿瘤发展和治疗中的全面作用。

7 靶向TMEM16A的抗肿瘤药物研发进展

TMEM16A在多种恶性肿瘤中过表达并促进肿瘤的发生进展, 基于靶向TMEM16A抗肿瘤药物的治疗方法已成为研究热点。目前, TMEM16A抑制剂主要通过以下机制实现靶向抑制: 抑制TMEM16A的通道功能、干扰TMEM16A介导的信号通路激活及促进肿瘤细胞凋亡。这些基于TMEM16A靶向抑制剂的治疗方法有望在恶性肿瘤相关疾病中展现治疗潜力。

抑制离子通道功能是靶向抑制TMEM16A的一种重要机制, 主要包括抑制氯离子电流和阻断离子孔道。在胃肠道间质瘤细胞中, $CaCC_{inh}$-A01的加入可抑制氯离子电流, 导致细胞生存能力下降和细胞周期阻滞[82]。和厚朴酚通过靶向TMEM16A抑制氯离子电流进而抑制CRC细胞的增殖, 其中氨基酸残基R429、K430和N435是和厚朴酚诱导TMEM16A抑制的主要作用靶点, 进一步证实了和厚朴酚对TMEM16A通道功能的抑制作用[83]。此外, 苦参碱以浓度依赖的方式抑制TMEM16A的氯离子电流, 进而抑制肺腺癌细胞的增殖和迁移[84]。另一方面, Shi等[85]研究发现依托泊苷能够与TMEM16A的开放状态结合而抑制TMEM16A, 进而抑制前列腺癌细胞的增殖。类似地, 茶黄素和扎鲁斯特也被证明通过阻断TMEM16A孔道, 进而抑制肺腺癌细胞的增殖和迁移[86,87]。因此, TMEM16A抑制剂可能通过抑制TMEM16A通道活性进而抑制肿瘤细胞的增殖和迁移。

TMEM16A抑制剂已被证实可通过阻断TMEM16A介导的信号通路影响肿瘤细胞的生物学行为。牛蒡子素处理可以显著抑制肺腺癌细胞中TMEM16A和cyclin D1的表达, 以及MEK1/2和ERK1/2的磷酸化水平, 导致肿瘤细胞增殖抑制和细胞周期阻滞, 进而减慢肺腺癌的生长速率[88]。此外, 高三尖杉酯碱和荷叶碱均能通过阻断TMEM16A介导的MAPK信号通路, 抑制肺腺癌细胞的生长[89,90]。

多项研究表明, TMEM16A抑制剂还可通过靶向TMEM16A诱导肿瘤细胞凋亡。Song等[52]发现$CaCC_{inh}$-A01和Ani9以浓度依赖的方式促进TNF-α分泌, 从而诱导前列腺癌细胞凋亡。高三尖杉酯碱能够激活与凋亡相关的信号蛋白如caspase-3和caspase-9, 表明其可通过靶向抑制TMEM16A促进肺腺癌细胞凋亡[89]。最新的研究发现, 血红素能够通过抑制TMEM16A激活caspase-3及降解PARP, 进而促进前列腺癌细胞凋亡[91]。

基于TMEM16A在肿瘤发生发展中的调控机制, TMEM16A抑制剂与其他抗肿瘤药物联合应用有望获得更好的治疗效果。联合应用TMEM16A和程序性死亡配体1 (programmed death ligand 1, PD-L1)抑制剂能够提高HNSCC患者的生存率, 特别是在对EGFR抑制剂产生耐药性的患者中效果显著[92]。类似地, 联合应用靶向TMEM16A单克隆抗体mT16a#5和西妥昔单抗能够协同抑制ESCC的生长和转移, 说明TMEM16A与EGFR的共靶向为ESCC治疗提供了潜在的靶点[35]。这些结果表明, 基于TMEM16A的多靶点联合治疗可表现出显著的协同效应, 能够增强肿瘤治疗效果并克服耐药性。

8 总结与展望

TMEM16A作为一种钙离子激活的氯通道, 已被广泛研究并证实在多种恶性肿瘤中发挥着关键作用。在分子层面, TMEM16A通过调控肿瘤细胞内多种分子的表达水平促进肿瘤细胞的增殖。在细胞层面, TMEM16A参与调控肿瘤细胞的增殖、迁移、侵袭和凋亡过程, 其表达水平和生物学作用具有细胞异质性。在个体层面, TMEM16A在肿瘤细胞中的过表达与肿瘤的进展和患者的不良预后密切相关。位于染色体11q13区域的*TMEM16A*基因扩增是导致其过表达的主要原因之一。转录调控和表观遗传调控也在此过程中扮演着重要角色。多种转录因子、组蛋白修饰酶和miRNAs通过精细调控TMEM16A的转录和翻译过程, 影响肿瘤细胞的增殖、迁移、侵袭和凋亡。特别是, TMEM16A参与并激活了包括EGFR、MAPK、NF-κB、TGF-β和TNF-α在内的多种信号通路, 这些通路在肿瘤细胞的生物学行为中起着至关重要的作用。此外, TMEM16A在维持肿瘤干性中的作用也引起了研究者的关注, 其可能通过调节肿瘤干细胞的恶性生物学行为和干性标志物的表达水平, 进而影响其干细胞特性。尽管TMEM16A的异常表达或功能障碍参与了肿瘤的发展, 但验证TMEM16A作为肿瘤的治疗靶点仍然是

一个重大挑战。TMEM16A在肿瘤发生中的作用并非仅依赖于离子通道功能, 还需考虑肿瘤类型和细胞特异性。

随着对TMEM16A在肿瘤中作用机制的深入理解, 靶向TMEM16A的抗肿瘤药物研发已成为一个重要的研究方向。TMEM16A在癌症中的表达与转录调控、表观遗传调控和翻译后水平调控均有关。多种TMEM16A抑制剂均显示出抗肿瘤的潜力, 但其有效性、安全性和特异性较低。$CaCC_{inh}$-A01、Ani9、扎鲁斯特、和厚朴酚、苦参碱、依托泊苷、茶黄素、牛蒡子素、高三尖杉酯碱、荷叶碱和血红素等化合物已在体外和体内实验中显示出对TMEM16A的抑制效果, 有望成为治疗恶性肿瘤的新方案。这些抑制剂通过阻断TMEM16A的通道活性或影响其蛋白表达, 干扰TMEM16A介导的信号通路激活, 进而抑制肿瘤细胞生长和侵袭, 促进肿瘤细胞凋亡。

尽管TMEM16A已被多项研究证实在多种恶性肿瘤中呈现过表达现象, 且与肿瘤患者的不良预后紧密相关, 未来的研究仍需进一步探索TMEM16A作为肿瘤生物标记物的潜力, 验证其在肿瘤诊断和预后评估中的临床应用价值。同时, 深入研究TMEM16A的调控机制将有助于开发新的肿瘤治疗策略。随着药物研发的不断进展, TMEM16A与多种抑制因子之间的相互作用已被深入研究发现, 这为研究TMEM16A在肿瘤中的生理功能及开发靶向TMEM16A的抗肿瘤药物提供了可能。TMEM16A靶向抑制剂有望成为肿瘤治疗领域的重要突破, 为患者带来新的希望。总之, TMEM16A在肿瘤生物学中的多功能性及其在肿瘤治疗中的潜在应用, 使其成为一个极具前景的研究领域。

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References

[1] Ahmad E, Ali A, Nimisha, et al. Molecular approaches in cancer [J]. Clin Chim Acta, 2022, 537: 60-73.

[2] Ohya S, Kito H, Hatano N, et al. Recent advances in therapeutic strategies that focus on the regulation of ion channel expression [J]. Pharmacol Ther, 2016, 160: 11-43.

[3] Peretti M, Angelini M, Savalli N, et al. Chloride channels in cancer: focus on chloride intracellular channel 1 and 4 (CLIC1 and CLIC4) proteins in tumor development and as novel therapeutic targets [J]. Biochim Biophys Acta, 2015, 1848: 2523-2531.

[4] Zhang L, Chen XY, Yao S, et al. Intracellular chloride channel 1 and tumor [J]. Am J Cancer Res, 2023, 13: 3300-3314.

[5] Caputo A, Caci E, Ferrera L, et al. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity [J]. Science, 2008, 322: 590-594.

[6] Schroeder BC, Cheng T, Jan YN, et al. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit [J]. Cell, 2008, 134: 1019-1029.

[7] Yang YD, Cho H, Koo JY, et al. TMEM16A confers receptor-activated calcium-dependent chloride conductance [J]. Nature, 2008, 455: 1210-1215.

[8] Wang M, Yang H, Zheng LY, et al. Downregulation of TMEM16A calcium-activated chloride channel contributes to cerebrovascular remodeling during hypertension by promoting basilar smooth muscle cell proliferation [J]. Circulation, 2012, 125: 697-707.

[9] Sun WJ, Guo S, Li YH, et al. Anoctamin 1 controls bone resorption by coupling Cl^- channel activation with RANKL-RANK signaling transduction [J]. Nat Commun, 2022, 13: 2899.

[10] Cil O, Chen XL, Askew Page HR, et al. A small molecule inhibitor of the chloride channel TMEM16A blocks vascular smooth muscle contraction and lowers blood pressure in spontaneously hypertensive rats [J]. Kidney Int, 2021, 100: 311-320.

[11] Zheng HQ, Li XL, Zeng X, et al. TMEM16A inhibits angiotensin II-induced basilar artery smooth muscle cell migration in a WNK1-dependent manner [J]. Acta Pharm Sin B, 2021, 11: 3994-4007.

[12] Pedemonte N, Galietta LJ. Structure and function of TMEM16 proteins (anoctamins) [J]. Physiol Rev, 2014, 94: 419-459.

[13] Genovese M, Borrelli A, Venturini A, et al. TRPV4 and purinergic receptor signalling pathways are separately linked in airway epithelia to CFTR and TMEM16A chloride channels [J]. J Physiol, 2019, 597: 5859-5878.

[14] Lee B, Hong GS, Lee SH, et al. Anoctamin 1/TMEM16A controls intestinal Cl^- secretion induced by carbachol and cholera toxin [J]. Exp Mol Med, 2019, 51: 1-14.

[15] Ma MM, Gao M, Guo KM, et al. TMEM16A contributes to endothelial dysfunction by facilitating Nox2 NADPH oxidase-derived reactive oxygen species generation in hypertension [J]. Hypertension, 2017, 69: 892-901.

[16] Ma K, Wang H, Yu JK, et al. New insights on the regulation of Ca^{2+}-activated chloride channel TMEM16A [J]. J Cell Physiol, 2017, 232: 707-716.

[17] Hwang SJ, Blair PJ, Britton FC, et al. Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles [J]. J Physiol, 2009, 587: 4887-4904.

[18] Fedigan S, Bradley E, Webb T, et al. Effects of new-generation TMEM16A inhibitors on calcium-activated chloride currents in rabbit urethral interstitial cells of Cajal [J]. Pflugers Arch, 2017,

469: 1443-1455.

[19] Cho H, Yang YD, Lee J, et al. The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons [J]. Nat Neurosci, 2012, 15: 1015-1021.

[20] Takayama Y, Uta D, Furue H, et al. Pain-enhancing mechanism through interaction between TRPV1 and anoctamin 1 in sensory neurons [J]. Proc Natl Acad Sci U S A, 2015, 112: 5213-5218.

[21] Liu SB, Feng J, Luo JL, et al. Eact, a small molecule activator of TMEM16A, activates TRPV1 and elicits pain- and itch-related behaviours [J]. Br J Pharmacol, 2016, 173: 1208-1218.

[22] Sun WJ, Li YH, Li JW, et al. Mechanical stimulation controls osteoclast function through the regulation of Ca^{2+}-activated Cl^- channel anoctamin 1 [J]. Commun Biol, 2023, 6: 407.

[23] Liu YN, Liu ZT, Wang KW. The Ca^{2+}-activated chloride channel ANO1/TMEM16A: an emerging therapeutic target for epithelium-originated diseases?[J]. Acta Pharm Sin B, 2021, 11: 1412-1433.

[24] Cabrita I, Kraus A, Scholz JK, et al. Cyst growth in ADPKD is prevented by pharmacological and genetic inhibition of TMEM16A *in vivo* [J]. Nat Commun, 2020, 11: 4320.

[25] Huang F, Zhang HK, Wu M, et al. Calcium-activated chloride channel TMEM16A modulates mucin secretion and airway smooth muscle contraction [J]. Proc Natl Acad Sci U S A, 2012, 109: 16354-16359.

[26] Papp R, Nagaraj C, Zabini D, et al. Targeting TMEM16A to reverse vasoconstriction and remodelling in idiopathic pulmonary arterial hypertension [J]. Eur Respir J, 2019, 53: 1800965.

[27] Wang QH, Bai LC, Luo SY, 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.

[28] Kulkarni S, Bill A, Godse NR, et al. TMEM16A/ANO1 suppression improves response to antibody-mediated targeted therapy of EGFR and HER2/ERBB2 [J]. Genes Chromosomes Cancer, 2017, 56: 460-471.

[29] Wang H, Yao F, Luo SY, et al. A mutual activation loop between the Ca^{2+}-activated chloride channel TMEM16A and EGFR/STAT3 signaling promotes breast cancer tumorigenesis [J]. Cancer Lett, 2019, 455: 48-59.

[30] Luo SY, Wang H, Bai LC, et al. Activation of TMEM16A Ca^{2+}-activated Cl^- channels by ROCK1/moesin promotes breast cancer metastasis [J]. J Adv Res, 2021, 33: 253-264.

[31] Mokutani Y, Uemura M, Munakata K, et al. Down-regulation of microRNA-132 is associated with poor prognosis of colorectal cancer [J]. Ann Surg Oncol, 2016, 23: 599-608.

[32] Park YR, Lee ST, Kim SL, et al. Down-regulation of miR-9 promotes epithelial mesenchymal transition *via* regulating anoctamin-1 (ANO1) in CRC cells [J]. Cancer Genet, 2019, 231-232: 22-31.

[33] Jiang YS, Cai YH, Shao WW, et al. MicroRNA-144 suppresses aggressive phenotypes of tumor cells by targeting ANO1 in colorectal cancer [J]. Oncol Rep, 2019, 41: 2361-2370.

[34] Yan YF, Ding XY, Han CH, et al. Involvement of TMEM16A/ANO1 upregulation in the oncogenesis of colorectal cancer [J]. Biochim Biophys Acta Mol Basis Dis, 2022, 1868: 166370.

[35] Zheng YT, Meng L, Qu LK, et al. Co-targeting TMEM16A with a novel monoclonal antibody and EGFR with cetuximab inhibits the growth and metastasis of esophageal squamous cell carcinoma [J]. J Transl Med, 2024, 22: 1046.

[36] Yu Y, Cao J, Wu WB, et al. Genome-wide copy number variation analysis identified ANO1 as a novel oncogene and prognostic biomarker in esophageal squamous cell cancer [J]. Carcinogenesis, 2019, 40: 1198-1208.

[37] Zeng XQ, Pan DY, Wu H, et al. Transcriptional activation of ANO1 promotes gastric cancer progression [J]. Biochem Biophys Res Commun, 2019, 512: 131-136.

[38] Cao QH, Liu F, Ji KY, et al. MicroRNA-381 inhibits the metastasis of gastric cancer by targeting TMEM16A expression [J]. J Exp Clin Cancer Res, 2017, 36: 29.

[39] Liu F, Cao QH, Lu DJ, et al. TMEM16A overexpression contributes to tumor invasion and poor prognosis of human gastric cancer through TGF-β signaling [J]. Oncotarget, 2015, 6: 11585-11599.

[40] Liu J, Liu Y, Ren YG, et al. Transmembrane protein with unknown function 16A overexpression promotes glioma formation through the nuclear factor-κB signaling pathway [J]. Mol Med Rep, 2014, 9: 1068-1074.

[41] Deng L, Yang JH, Chen HW, et al. Knockdown of TMEM16A suppressed MAPK and inhibited cell proliferation and migration in hepatocellular carcinoma [J]. Onco Targets Ther, 2016, 9: 325-333.

[42] Zhang CT, Liu JX, Han ZY, et al. Inhibition of TMEM16A suppresses growth and induces apoptosis in hepatocellular carcinoma [J]. Int J Clin Oncol, 2020, 25: 1145-1154.

[43] Rodrigo JP, Menéndez ST, Hermida-Prado F, et al. Clinical significance of anoctamin-1 gene at 11q13 in the development and progression of head and neck squamous cell carcinomas [J]. Sci Rep, 2015, 5: 15698.

[44] Hermida-Prado F, Menéndez ST, Albornoz-Afanasiev P, et al. Distinctive expression and amplification of genes at 11q13 in relation to HPV status with impact on survival in head and neck cancer patients [J]. J Clin Med, 2018, 7: 501.

[45] Pattle SB, Utjesanovic N, Togo A, et al. Copy number gain of 11q13.3 genes associates with pathological stage in hypopharyngeal squamous cell carcinoma [J]. Genes Chromosomes Cancer, 2017, 56: 185-198.

[46] Dixit R, Kemp C, Kulich S, et al. TMEM16A/ANO1 is differentially expressed in HPV-negative *versus* HPV-positive head and neck squamous cell carcinoma through promoter methylation [J]. Sci Rep, 2015, 5: 16657.

[47] Finegersh A, Kulich S, Guo T, et al. DNA methylation regulates

TMEM16A/ANO1 expression through multiple CpG islands in head and neck squamous cell carcinoma [J]. Sci Rep, 2017, 7: 15173.

[48] He Y, Li H, Chen Y, et al. Expression of anoctamin 1 is associated with advanced tumor stage in patients with non-small cell lung cancer and predicts recurrence after surgery [J]. Clin Transl Oncol, 2017, 19: 1091-1098.

[49] Zhong D, Zhan ZQ, Zhang JQ, et al. SMYD3 regulates the abnormal proliferation of non-small-cell lung cancer cells *via* the H3K4me3/ANO1 axis [J]. J Biosci, 2022, 47: 53.

[50] Hu C, Zhang RG, Jiang DP. TMEM16A as a potential biomarker in the diagnosis and prognosis of lung cancer [J]. Arch Iran Med, 2019, 22: 32-38.

[51] Shi MY, Wang JH, Huang XY, et al. Targeting TMEM16A/ANO1 inhibits the progression of BRAF mutant (V600E) melanoma through the MEK/ERK and AKT signaling pathways [J]. Genes Dis, 2024, 11: 101153.

[52] Song Y, Gao J, Guan LZ, et al. Inhibition of ANO1/TMEM16A induces apoptosis in human prostate carcinoma cells by activating TNF-*α* signaling [J]. Cell Death Dis, 2018, 9: 703.

[53] Crottès D, Lin YT, Peters CJ, et al. TMEM16A controls EGF-induced calcium signaling implicated in pancreatic cancer prognosis [J]. Proc Natl Acad Sci U S A, 2019, 116: 13026-13035.

[54] Suzuki J, Fujii T, Imao T, et al. Calcium-dependent phospholipid scramblase activity of TMEM16 protein family members [J]. J Biol Chem, 2013, 288: 13305-13316.

[55] Pifferi S, Boccaccio A. Ca^{2+}-activated chloride channels and phospholipid scramblases [J]. Int J Mol Sci, 2022, 23: 2158.

[56] Le SC, Yang HH. Structure-function of TMEM16 ion channels and lipid scramblases [J]. Adv Exp Med Biol, 2021, 1349: 87-109.

[57] Paulino C, Kalienkova V, Lam AKM, et al. Activation mechanism of the calcium-activated chloride channel TMEM16A revealed by cryo-EM [J]. Nature, 2017, 552: 421-425.

[58] Dang SY, Feng SJ, Tien J, et al. Cryo-EM structures of the TMEM16A calcium-activated chloride channel [J]. Nature, 2017, 552: 426-429.

[59] Xiao QH, Yu KA, Perez-Cornejo P, et al. Voltage- and calcium-dependent gating of TMEM16A/Ano1 chloride channels are physically coupled by the first intracellular loop [J]. Proc Natl Acad Sci U S A, 2011, 108: 8891-8896.

[60] Brunner JD, Lim NK, Schenck S, et al. X-ray structure of a calcium-activated TMEM16 lipid scramblase [J]. Nature, 2014, 516: 207-212.

[61] Peters CJ, Yu HB, Tien J, et al. Four basic residues critical for the ion selectivity and pore blocker sensitivity of TMEM16A calcium-activated chloride channels [J]. Proc Natl Acad Sci U S A, 2015, 112: 3547-3552.

[62] Peters CJ, Gilchrist JM, Tien J, et al. The sixth transmembrane segment is a major gating component of the TMEM16A calcium-activated chloride channel [J]. Neuron, 2018, 97: 1063-1077.e4.

[63] Jeng G, Aggarwal M, Yu WP, et al. Independent activation of distinct pores in dimeric TMEM16A channels [J]. J Gen Physiol, 2016, 148: 393-404.

[64] Guan LZ, Song Y, Gao J, et al. Inhibition of calcium-activated chloride channel ANO1 suppresses proliferation and induces apoptosis of epithelium originated cancer cells [J]. Oncotarget, 2016, 7: 78619-78630.

[65] Britschgi A, Bill A, Brinkhaus H, et al. Calcium-activated chloride channel ANO1 promotes breast cancer progression by activating EGFR and CAMK signaling [J]. Proc Natl Acad Sci U S A, 2013, 110: E1026-E1034.

[66] Godse NR, Khan N, Yochum ZA, et al. TMEM16A/ANO1 inhibits apoptosis *via* downregulation of Bim expression [J]. Clin Cancer Res, 2017, 23: 7324-7332.

[67] Mazzone A, Gibbons SJ, Bernard CE, et al. Identification and characterization of a novel promoter for the human ANO1 gene regulated by the transcription factor signal transducer and activator of transcription 6 (STAT6) [J]. FASEB J, 2015, 29: 152-163.

[68] Qin YL, Jiang YF, Sheikh AS, et al. Interleukin-13 stimulates MUC5AC expression *via* a STAT6-TMEM16A-ERK1/2 pathway in human airway epithelial cells [J]. Int Immunopharmacol, 2016, 40: 106-114.

[69] Vanoni S, Zeng C, Marella S, et al. Identification of anoctamin 1 (ANO1) as a key driver of esophageal epithelial proliferation in eosinophilic esophagitis [J]. J Allergy Clin Immunol, 2020, 145: 239-254.e2.

[70] Dutta AK, Boggs K, Khimji AK, 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: G763-G771.

[71] Mazzone A, Gibbons SJ, Eisenman ST, et al. Direct repression of anoctamin 1 (ANO1) gene transcription by Gli proteins [J]. FASEB J, 2019, 33: 6632-6642.

[72] Wu J, Di DX, Zhao C, et al. Role of glioma-associated GLI1 oncogene in carcinogenesis and cancertargeted therapy [J]. Curr Cancer Drug Targets, 2018, 18: 558-566.

[73] Shin Y, Lee SW, Namkoong E, et al. Epigenetic modification as a regulatory mechanism for spatiotemporal dynamics of ANO1 expression in salivary glands [J]. Int J Mol Sci, 2019, 20: 6298.

[74] Fujimoto M, Kito H, Kajikuri J, et al. Transcriptional repression of human epidermal growth factor receptor 2 by ClC-3 Cl^-/H^+ transporter inhibition in human breast cancer cells [J]. Cancer Sci, 2018, 109: 2781-2791.

[75] Batlle E, Clevers H. Cancer stem cells revisited [J]. Nat Med, 2017, 23: 1124-1134.

[76] Papaccio F, Paino F, Regad T, et al. Concise review: cancer cells, cancer stem cells, and mesenchymal stem cells: influence in cancer development [J]. Stem Cells Transl Med, 2017, 6: 2115-2125.

[77] Han L, Shi S, Gong T, et al. Cancer stem cells: therapeutic implications and perspectives in cancer therapy [J]. Acta Pharm Sin B, 2013, 3: 65-75.

[78] Toledo-Guzmán ME, Bigoni-Ordóñez GD, Ibáñez Hernández M, et al. Cancer stem cell impact on clinical oncology [J]. World J Stem Cells, 2018, 10: 183-195.

[79] O'Reilly D, Buchanan P. Calcium channels and cancer stem cells [J]. Cell Calcium, 2019, 81: 21-28.

[80] Li GR, Deng XL. Functional ion channels in stem cells [J]. World J Stem Cells, 2011, 3: 19-24.

[81] Prevarskaya N, Skryma R, Shuba Y. Ion channels in cancer: are cancer hallmarks oncochannelopathies? [J]. Physiol Rev, 2018, 98: 559-621.

[82] Fröbom R, Sellberg F, Xu C, et al. Biochemical inhibition of DOG1/TMEM16A achieves antitumoral effects in human gastrointestinal stromal tumor cells *in vitro* [J]. Anticancer Res, 2019, 39: 3433-3442.

[83] Wang TY, Wang H, Yang F, et al. Honokiol inhibits proliferation of colorectal cancer cells by targeting anoctamin 1/TMEM16A Ca^{2+}-activated Cl^- channels [J]. Br J Pharmacol, 2021, 178: 4137-4154.

[84] Guo S, Chen Y, Pang C, et al. Matrine is a novel inhibitor of the TMEM16A chloride channel with antilung adenocarcinoma effects [J]. J Cell Physiol, 2019, 234: 8698-8708.

[85] Shi S, Ma B, Ji QS, et al. Identification of a druggable pocket of the calcium-activated chloride channel TMEM16A in its open state [J]. J Biol Chem, 2023, 299: 104780.

[86] Shi S, Ma B, Sun FD, et al. Theaflavin binds to a druggable pocket of TMEM16A channel and inhibits lung adenocarcinoma cell viability [J]. J Biol Chem, 2021, 297: 101016.

[87] Shi S, Ma B, Sun FD, et al. Zafirlukast inhibits the growth of lung adenocarcinoma *via* inhibiting TMEM16A channel activity [J]. J Biol Chem, 2022, 298: 101731.

[88] Guo S, Chen YF, Shi S, et al. Arctigenin, a novel TMEM16A inhibitor for lung adenocarcinoma therapy [J]. Pharmacol Res, 2020, 155: 104721.

[89] Guo S, Bai X, Shi S, et al. TMEM16A, a homoharringtonine receptor, as a potential endogenic target for lung cancer treatment [J]. Int J Mol Sci, 2021, 22: 10930.

[90] Bai X, Liu XY, Li ST, et al. Nuciferine inhibits TMEM16A in dietary adjuvant therapy for lung cancer [J]. J Agric Food Chem, 2022, 70: 3687-3696.

[91] Park SH, Lee Y, Jeon H, et al. Anticancer effect of hemin through ANO1 inhibition in human prostate cancer cells [J]. Int J Mol Sci, 2024, 25: 6032.

[92] Okuyama K, Yanamoto S. TMEM16A as a potential treatment target for head and neck cancer [J]. J Exp Clin Cancer Res, 2022, 41: 196.