TMEM16A as a potential treatment target for head and neck cancer

Kohei Okuyama[1,2,3]* and Souichi Yanamoto[4]

Abstract

Transmembrane protein 16A (TMEM16A) forms a plasma membrane-localized Ca^{2+}-activated Cl- channel. Its gene has been mapped to an area on chromosome 11q13, which is amplified in head and neck squamous cell carcinoma (HNSCC). In HNSCC, TMEM16A overexpression is associated with not only high tumor grade, metastasis, low survival, and poor prognosis, but also deterioration of clinical outcomes following platinum-based chemotherapy. Recent study revealed the interaction between TMEM16A and transforming growth factor-β (TGF-β) has an indirect crosstalk in clarifying the mechanism of TMEM16A-induced epithelial-mesenchymal transition. Moreover, human papillomavirus (HPV) infection can modulate TMEM16A expression along with epidermal growth factor receptor (EGFR), whose phosphorylation has been reported as a potential co-biomarker of HPV-positive cancers. Considering that EGFR forms a functional complex with TMEM16A and is a co-biomarker of HPV, there may be crosstalk between TMEM16A expression and HPV-induced HNSCC. EGFR activation can induce programmed death ligand 1 (PD-L1) synthesis via activation of the nuclear factor kappa B pathway and JAK/STAT3 pathway. Here, we describe an interplay among EGFR, PD-L1, and TMEM16A. Combination therapy using TMEM16A and PD-L1 inhibitors may improve the survival rate of HNSCC patients, especially those resistant to anti-EGFR inhibitor treatment. To the best of our knowledge, this is the first review to propose a biological validation that combines immune checkpoint inhibition with TMEM16A inhibition.

Keywords: TMEM16A, Ca^{2+}-activated Cl^- channel, TGF-β, EGFR, PD-L1, NF-kB, STAT3, HPV, Proliferation, Metastasis, Head and neck squamous cell carcinoma

Introduction

Calcium-activated chloride channels (CaCCs) are abundant in nearly every cell type, where they perform various functions [1]. It is now well-accepted that transmembrane protein 16A (TMEM16A), also known as Anoctamin-1, DOG1, ORAOV2, and TAOS2, forms the localized plasma membrane of the CaCC [2–4]. TMEM16A has been mapped to an area on chromosome 11 (11q13), which is often magnified in cancer cells [5]. Apart from glands, CaCCs have long been known to be present pr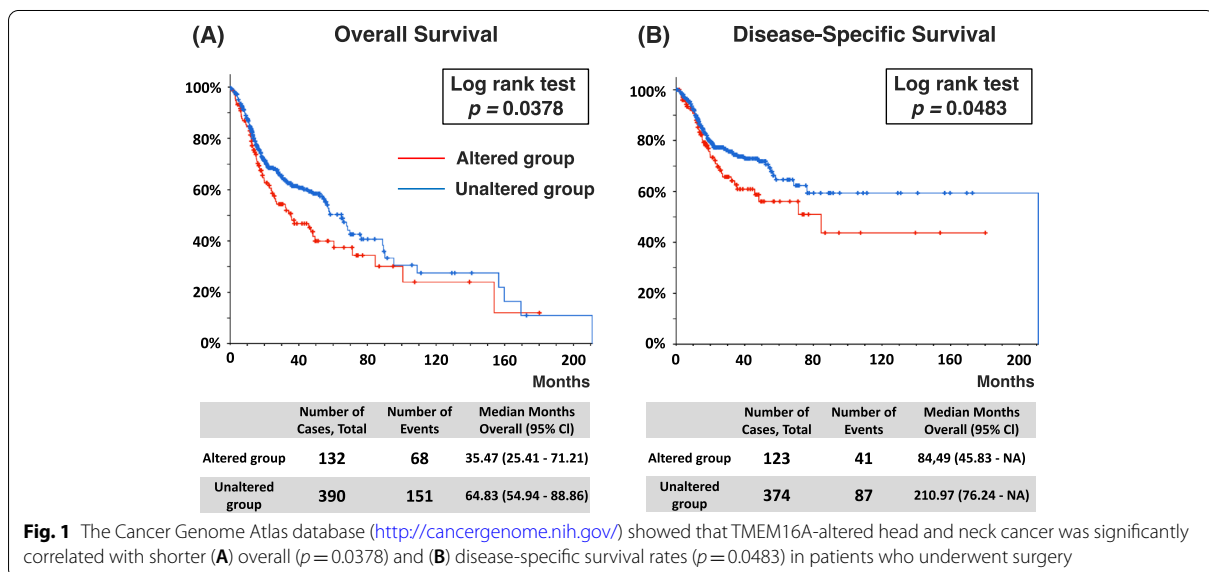imarily in proliferating cells in culture and various types of cancer cells [1, 6, 7]. TMEM16A is a proven significant and reliable tumor marker, particularly for gastrointestinal stromal tumors (GISTs) and head and neck squamous cell carcinoma (HNSCC) [8, 9]. Duvvuri et al. reported that TMEM16A is substantially amplified and highly expressed in 85% of HNSCC patients and correlates with decreased patient survival [10]. According to the data obtained from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/), the overall and disease-specific survival rates of the TMEM16A-altered HNSCC patient group were significantly lower than those of the unaltered group (Fig. 1). Thus, TMEM16A has tumor-specific functions and supports cell proliferation and possible development of malignancy in any cancer cell type [11, 12].

*Correspondence: okuyamak.0429@gmail.com
[1] Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI 48109, USA
Full list of author information is available at the end of the article

Fig. 1 The Cancer Genome Atlas database (http://cancergenome.nih.gov/) showed that TMEM16A-altered head and neck cancer was significantly correlated with shorter (**A**) overall ($p = 0.0378$) and (**B**) disease-specific survival rates ($p = 0.0483$) in patients who underwent surgery

Based on the nature of TMEM16A, many biological experiments have been conducted, and TMEM16A is now considered an excellent biomarker for certain cancer types. On the other hand, Qu et al. insisted that TMEM16A functions in cell growth as an oncogene; its overexpression is not the final step of tumorigenesis as it is involved in the initiation of tumorigenesis, whereas its overexpression is not associated with its amplification [13]. The role of TMEM16A as an oncogene has been well-investigated, however, how TMEM16A interacts with the other biomarkers and related pathways is not fully understood.

HNSCC is an aggressive malignancy with high morbidity and mortality rates. Worldwide, HNSCCs are responsible for over 550,000 new cases and over 380,000 deaths annually [14]. The main risk factors of HNSCCs are long-term tobacco use, alcohol consumption, and infection with high-risk types of human papillomavirus (HPV) [15]. Despite advances in diagnosis and improvements in imaging modalities in recent decades, the survival of patients with HNSCC has remained unchanged owing to the high recurrence rate and high risk of cervical lymph node or distant metastasis [16, 17]. The current standard treatment for HNSCC in most patients is surgery, and postoperative concurrent chemoradiotherapy using platinum-based agents is a widely accepted standard of treatment for patients with a high risk of recurrence, as determined by surgical pathological findings [18, 19]. However, treatments such as radio- and chemotherapy can cause toxicity to other organs that can lead to a reduction in the quality of life. Moreover, therapeutic strategies, particularly for patients with locoregional recurrent or distant metastatic HNSCC, remain limited. Therefore, there is a constant need for improved therapeutic strategies. The most promising option remains targeted therapy [20, 21]. Many CaCC inhibitors have been reported to date. Each substance has been identified with a target organ/structure; however, their practicality for daily clinical use is far from realistic [22].

HPV infection is now one of the prognostic factors in HNSCC, indicating a favorable prognosis [23, 24]. Moreover, this infection can contribute to several functional, genetic, and metabolic changes [25–27], including immuno-escape of HNSCC [28], and can be considered a potential effector for cancer cell metabolism. Some literatures reported the biological and cellular metabolic relationship between the expression of TMEM16A and HPV infection.

A surprising range of biological and molecular functions associated with TMEM16A have emerged from the literature, suggesting that TMEM16A plays a multifaceted role in cancer. In this review, we discuss the current understanding of the molecular dynamics of TMEM16A in cancer, summarise evidence of the biological and molecular functions of TMEM16A, and discuss a potential combination treatment for HNSCC based on TMEM16A inhibition.

Is TMEM16A really a promising biomarker?

Overexpression of TMEM16A is widely thought to promote cancer cell proliferation and migration. Duvvuri et al. reported that TMEM16A overexpression in HNSCC significantly promoted anchorage-independent growth in vitro, and the loss of TMEM16A resulted in

the inhibition of tumor growth both in vitro and in vivo [10]. Ayoub et al. found TMEM16A amplification and expression in HNSCC, accompanied by a high propensity for distant metastasis [29]. In esophageal SCC (ESCC), Shi et al. reported that TMEM16A mRNA expression and protein overexpression were associated with lymph node metastasis (LNM) and advanced clinical stage [30]. LNM is also more common in HNSCC patients with TMEM16A overexpression than in those without [9]. The role of TMEM16A as a prognosticator has been characterized in other organ cancers. Bae et al. found that TMEM16A overexpression was an independent indicator of poor prognosis of shorter overall survival and relapse-free survival of breast cancer patients [31]. A detailed study on the reaction of tamoxifen for breast cancer treatment reported that high TMEM16A expression correlated with decreased survival in patients not treated with tamoxifen and with increased survival in tamoxifen-treated patients [32]. Wu et al. also reported that TMEM16A overexpression is associated with good prognosis in progesterone-positive or HER2-negative breast cancer patients following tamoxifen treatment, especially in patients with low Ki67 expression [33]. Therefore, TMEM16A can serve as a biomarker for clinical outcomes and breast cancer therapeutic responses to tamoxifen [5]. In colorectal cancer (CRC), Li et al. reported that TMEM16A mRNA expression could be a novel predictive marker of LNM, and TMEM16A expression may be an important regulator of tumor proliferation and metastasis [34]. Moreover, Liu et al. (2015) found that high expression levels of TMEM16A were associated with LNM, higher disease grade, and poor prognosis of gastric cancer (GC) [35], whereas Liu et al. (2012) confirmed a positive correlation between TMEM16A expression and tumor grade in prostate cancer [36]. Atala et al. reported that TMEM16A knockdown decreased the proliferation of prostate cancer cells and suppressed xenograft tumor growth in vivo [37]. Britschgi et al. obtained similar results showing that TMEM16A is critical for cell survival and proliferation in 11q13-amplified breast cancer, HNSCC, and ESCC cells [38]. The above data show that overexpression of TMEM16A correlates with high tumor grade, low survival, and poor prognosis. On the other hand, mutations such as S741T increased TMEM16A calcium sensitivity and channel activity, and rendered TMEM16A calcium gating voltage-independent, whereas mutants like L759Q diminished TMEM16A-dependent chloride conductance without affecting TMEM16A expression level or localization on the membrane. One of the key findings is that the S741T mutation could be functional even at very low intracellular calcium levels suggesting that TMEM16A has the capacity to function biochemically in the absence of high calcium in the cytoplasm [39, 40]. It could be that in a tumor cell setting TMEM16A can function in the absence of calcium to drive downstream signaling, with or without chloride channel activity. These dynamics of TMEM16A no longer show a simple ion-channel but a tumor driver, suggesting its potential use as a promising tumor biomarker.

TMEM16A downstream pathways and crosslink to other pathways

TMEM16A-induced cancer cell proliferation and tumor growth were accompanied by an increase in the induction of extracellular signal-regulated kinase (ERK)1/2 and cyclin D1 [10]. Sui et al. reported that TMEM16A small-interfering RNA decreased the in vitro proliferation of CRC cells by inhibiting the expression of ERK1/2 [41]. It is now well-known that the rat sarcoma (RAS)- rapidly accelerated fibrosarcoma (RAF)-Mitogen-activated protein kinase (MAPK) signaling pathway can activate ERK1/2. Mutations in Kirsten rat sarcoma virus (KRAS), neuroblastoma-RAS (NRAS), and B-RAF have also been demonstrated to be involved in the initiation and progression of CRC [42]. TMEM16A is also overexpressed in hepatocellular carcinoma, and that the inhibition of TMEM16A suppressed MAPK and tumor growth [43]. In addition, it is generally known that mutant p53 overexpression is related to tumor metastasis, recurrence, and poor prognosis. Multivariate statistical analysis by Sui et al. showed that TMEM16A protein expression positively correlated with KRAS mutation status and negatively correlated with mutant p53 protein expression in CRC. Thus, their data suggest that TMEM16A may play a dual role in tumor formation and metastasis by interacting with mutated KRAS and mutant p53 proteins in CRC tissues [41]. Because HNSCC carries a high mutation rate of p53 [44], studying how its gene mutations interact with TMEM16A expression could be a promising direction for future research.

Epidermal growth factor receptor (EGFR) family members (EGFR1, HER2, EGFR3, and EGFR4) can activate many signaling pathways including phosphatidylinositol 3-kinases (PI3K)/PTEN/AKT/Mammalian/mechanistic Target of Rapamycin (mTORC1)/glycogen synthase kinase-3 (GSK-3), Ras/Raf/MEK/ERK, Janus kinase (JAK)/signal transducer and activator of transcription (STAT), and c-Jun-NH2-terminal kinase (JNK) [45, 46]. TMEM16A has been suggested to regulate EGFR signaling. Bill et al. reported that TMEM16A and EGFR form a functional complex that regulates HNSCC cell proliferation. EGFR signaling increases TMEM16A protein levels, and knockdown of TMEM16A reduces EGFR protein levels without affecting EGFR mRNA levels [47]. Moreover, EGF promotes TMEM16A expression in breast cancer cells through the EGFR-STAT3 pathway [32]. STAT3

signaling also plays an important role in programmed cell death-ligand 1 (PD-L1) upregulation and the antitumor immune response of HNSCC [48]. Recently, Luo et al. reported a novel mechanism underlying TMEM16A-mediated breast cancer metastasis; increased TMEM16A channel activities activate EGFR/STAT3/ROCK1 signaling, and ROCK1 activation by RhoA increased TMEM16A channel activities via moesin phosphorylation at T558. TMEM16A and ROCK1/moesin signaling cooperatively promotes breast cancer metastasis [49]. These data suggest that there is a promising link between TMEM16A and EGFR, which regulates the proliferation and metastasis of cancer cells.

TMEM16A expression may be regulated at the translational level by several microRNAs (miRs), such as miR-9, miR-132, and miR-144, as previously reported [50–52]. MiRs can also regulate TMEM16A function. MiR-132 and miR-381 bind to the 3′ untranslated region of TMEM16A mRNA, resulting in TMEM16A downregulation in patients with CRC [52] and GC [53]. Mokutani et al. revealed that TMEM16A was a direct target of miR-132 and its overexpression was inversely associated with the downregulation of miR-132 in CRC, and correlated with poor clinical outcomes in patients with CRC [52]. TMEM16A protein, then, can interact with other molecular targets including ERK1/2, AKT, calmodulin kinase II (CaMKII), and EGFR at the post-translational level [5, 38, 54]. To investigate the unknown mechanisms underlying TMEM16A-related miRs, regulation of cancer progression and metastasis, are required to clarify how those miRs function as messengers among various pathways.
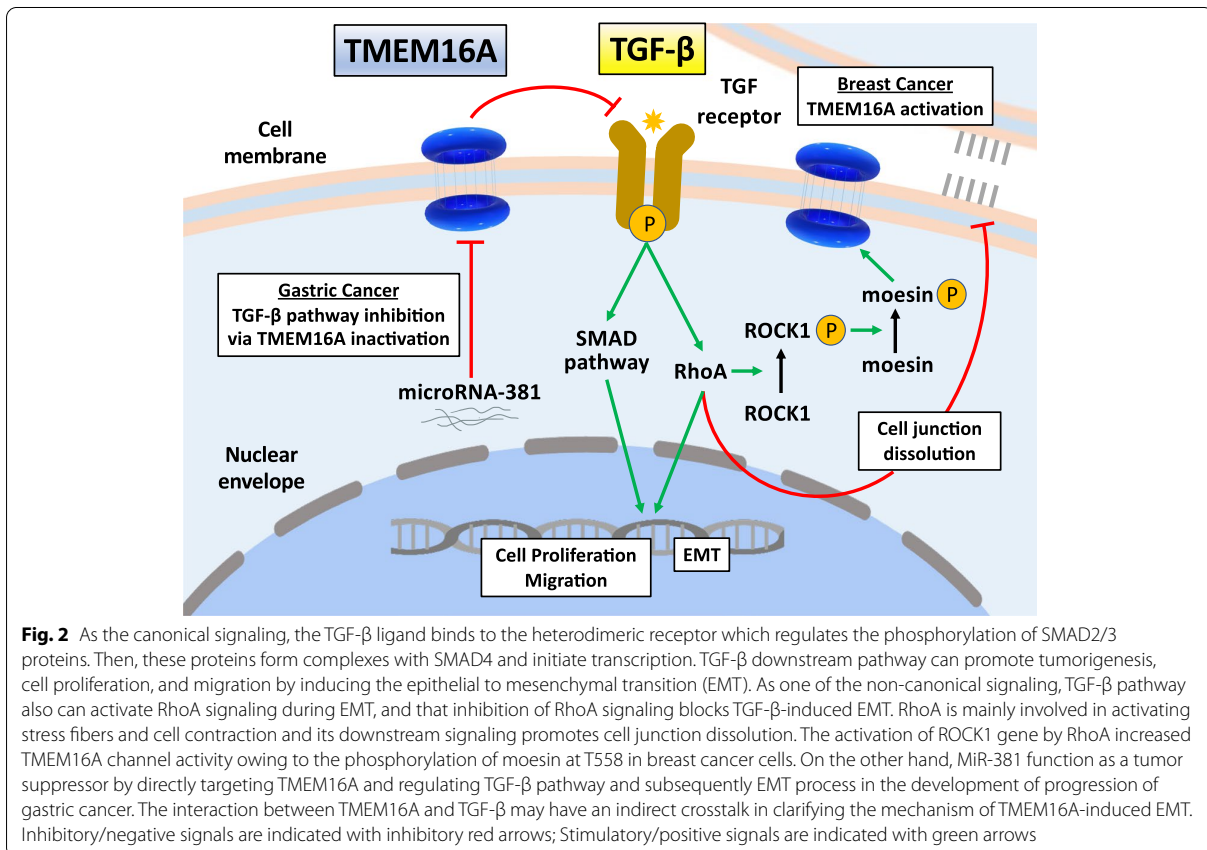
Transforming growth factor-β (TGF-β) plays a key role in controlling embryogenic development, inflammation, and tissue repair, as well as in maintaining adult tissue homeostasis. TGF-β elicits a broad range of context-dependent cellular responses, and consequently, alterations in TGF-β signaling have been implicated in many diseases, including cancer. It is well-known that TGF-β acts as a tumor suppressor during the early phase of cancer progression and as a tumor promotor in advanced stage. During the early stages of tumorigenesis, TGF-β acts as a tumor suppressor by inducing cytostasis and the apoptosis of normal and premalignant cells. However, at advanced stages, when cancer cells have acquired oncogenic mutations and/or have lost tumor suppressor gene function, cells are resistant to TGF-β-induced growth arrest, and TGF-β functions as a tumor promotor by stimulating tumor cells to undergo epithelial-mesenchymal transition (EMT) [55]. Thus, the expression of TGF-β signaling is an important factor that should be considered in the tumor metastasis step. TGF-β is found to be a potent secreted cytokine that drives cancer progression, not only through its immunosuppressive and proangiogenic roles, but also more importantly as a potent inducer of EMT by regulating E-cadherin expression [56]. The activation of TGF-β receptor complex recruit and induce many signaling proteins such as protein and lipid kinases, scaffolding proteins and small GTPases, whereas some of these proteins become directly phosphorylated by the TGF-β receptors [57]. It is now generally accepted that TGF-β and other members of its family control many fundamental aspects of cellular behaviour, including migration, adhesion, differentiation, and modification of the microenvironment [58]. Actually, TGF-β receptor type-I is known as the family of SMAD proteins, which, upon phosphorylation, oligomerize with SMAD4, accumulate in the nucleus and regulate gene transcription by binding to regulatory sequences in the genome together with other transcription factors and chromatin proteins [59, 60].

Regarding the relationship between TGF-β and TMEM16A, Atala et al. reported that the knockdown of TMEM16A decreased $I_{Cl,Ca}$, impaired TGF-β secretion, reduced E-cadherin expression, and inhibited migration and invasion without affecting the proliferation of prostate cancer cells [37]. In addition, Cao et al. discovered that miR-381 could inhibit the TGF-β signaling pathway and partially downregulate EMT phenotype by targeting TMEM16A [53]. These data suggest that TMEM16A participates in the TGF-β signaling pathway to regulate cell proliferation and migration. Moreover, Liu et al. suggested that TGF-βs might serve as intermediators between TMEM16A and oncogenic signaling such as MAPK. The activation of TMEM16A chloride channel depolarized membrane potential, promoted TGF-β secretion, downregulated E-cadherin expression, and facilitated GC migration and invasion [35]. In addition, one of non-canonical TGF-β pathway can activate RhoA signaling during EMT, and that inhibition of RhoA signaling blocks TGF-β-induced EMT [61, 62]. In this regard, Luo et al. reported ROCK1 increased TMEM16A channel activity via moesin phosphorylation and the increase in TMEM16A channel activities promoted cell migration and invasion. They investigated the activation of ROCK1 gene by RhoA increased TMEM16A channel activity owing to the phosphorylation of moesin at T558 in breast cancer cells [49]. Taken together, the interaction between TMEM16A and TGF-β may have an indirect crosstalk in clarifying the mechanism of TMEM16A-induced EMT. We show the representative sherma on TGF-β pathway and its surroundings related to TMEM16A expression in Fig. 2.

TMEM16A expression and anti-tumor drug sensitivity

TMEM16A overexpression correlates with improved clinical outcomes following chemotherapy [34]. Vyas

Fig. 2 As the canonical signaling, the TGF-β ligand binds to the heterodimeric receptor which regulates the phosphorylation of SMAD2/3 proteins. Then, these proteins form complexes with SMAD4 and initiate transcription. TGF-β downstream pathway can promote tumorigenesis, cell proliferation, and migration by inducing the epithelial to mesenchymal transition (EMT). As one of the non-canonical signaling, TGF-β pathway also can activate RhoA signaling during EMT, and that inhibition of RhoA signaling blocks TGF-β-induced EMT. RhoA is mainly involved in activating stress fibers and cell contraction and its downstream signaling promotes cell junction dissolution. The activation of ROCK1 gene by RhoA increased TMEM16A channel activity owing to the phosphorylation of moesin at T558 in breast cancer cells. On the other hand, MiR-381 function as a tumor suppressor by directly targeting TMEM16A and regulating TGF-β pathway and subsequently EMT process in the development of progression of gastric cancer. The interaction between TMEM16A and TGF-β may have an indirect crosstalk in clarifying the mechanism of TMEM16A-induced EMT. Inhibitory/negative signals are indicated with inhibitory red arrows; Stimulatory/positive signals are indicated with green arrows

et al. reported a strong positive correlation between TMEM16A and ATP7B expressions, which balances the copper level in the body by excreting excess copper into bile and plasma in HNSCC. TMEM16A overexpression and depletion in HNSCC cell lines caused parallel changes in ATP7B expression. A previous study showed that increased oxidative stress in cells overexpressing TMEM16A liberated the chelated copper in the cytoplasm, leading to the transcriptional activation of ATP7B expression, which in turn decreased the efficacy of platinum compounds by promoting their vesicular sequestration [63]. TMEM16A also promotes the sensitivity of other antitumor agents [47, 64], and TMEM16A overexpression could correlate with the deterioration of clinical outcomes by chemotherapy [34]. Platinum-based chemotherapy is the cornerstone treatment for a variety of human malignancies, including HNSCC. Clinically, resistance to cisplatin therapy is a major problem contributing to poor patient outcomes [65]. Godse et al. provided strong evidence that in a portion of cisplatin-resistant malignancies, TMEM16A overexpression may lead to inhibition of apoptosis and subsequent cisplatin resistance. Furthermore, they concluded that this

raises the possibility that pharmacologic inhibition of TMEM16A or its relevant downstream effectors, such as ERK1/2, could be strategically used to overcome cisplatin-resistant malignancies in TMEM16A expressing malignancies [66]. Thus, there is a need to construct a platinum-based combination therapy that includes a TMEM16A inhibitor. Moreover, the c-KIT negative GIST that expresses TMEM16A could be imatinib resistant population that could benefit from a TMEM16A inhibitor. There is a need to define the role of TMEM16A in GIST using both c-KIT positive and negative cell lines and xenografts [67].

TMEM16A expression and HPV infection on HNSCC
HPV + HNSCC is significantly different from HPV − HNSCC in many aspects, such as DNA mutation and mRNA and protein expression. In contrast, HPV − HNSCC is activated by several risk factors and is highly related to TP53 mutations. To date, there have been some reports on the interrelationship between TMEM16A and HPV in HNSCC. Dixit et al. found that TMEM16A was preferentially overexpressed in HPV − HNSCC compared to HPV + HNSCC, and that

this overexpression was associated with decreased patient survival. They revealed that promoter hypomethylation contributes to TMEM16A overexpression in HPV − HNSCC [68]. HPV + and HPV − HNSCC are considered two entirely different types of cancer, in part due to their unique molecular landscapes [69]. HPV proteins E6 and E7 downregulate tumor suppressor proteins p53 and Rb, respectively, although their true interactome encompasses a wide variety of cellular targets [70]. Recent work has shown that the E6 and E7 proteins act synergistically to induce HNSCC in a genomic engineered mouse model and that additional targets besides Rb are targeted by E7 [71–73]. E6 and E7 also activate oncogenic signaling pathways, including EGFR and PI3K [74, 75]. This coordinated perturbation of the critical tumor suppressor pathway results in uncontrolled growth and proliferation, although with a mutational landscape that is significantly restricted when compared with HPV − HNSCC [76, 77].

EGFR has been reported as a potential co-biomarker of HPV infection [74, 78]. EGFR is associated with HPV-related prognosis in oropharyngeal cancer [79]. EGFR expression in HPV + cancer may be regulated by multiple factors, including existing complex mechanisms and HPV viral proteins. EGFR signaling can also be affected by the Hippo/YAP pathway, which eventually influences cancer progression in HPV + cervical cancer [80]. Qiu et al. reported that E7 protein downregulates EGFR, which downregulates the phosphorylation of EGFR and inhibits EGFR downstream pathway that in turn induces a better prognosis [81]. Moreover, Cai et al. identified a mechanism of immunoregulation mediated via the STING-TBK1- nuclear factor-kappa B (NF-κB) pathway in HPV + cervical cancer [82]. Taken together, considering that EGFR can establish a functional complex with TMEM16A, there may be an indirect relationship between the expression of TMEM16A and HPV + cancer. Further research could reveal the therapeutic implications of HPV + HNSCC and develop a novel treatment strategy such as establishment of HPV vaccination and proteolysis targeting chimera (PROTAC) for E7 combined with TMEM16A inhibition.

TMEM16A behavior in HNSCC metastatic regions
Cervical lymph node metastasis
Importantly, TMEM16A expression is modulated by tumor progression. Shiwarski et al. reported that TMEM16A overexpression exerts an inhibitory effect on the migration, invasion, and metastasis of HNSCC cells. They showed that primary tumors exhibit a high level of TMEM16A, whereas LNM exhibits a low expression of TMEM16A. This is because promoter hypermethylation results in decreased TMEM16A expression in LNM tissues [83]. In general, TMEM16A expression level can be

changed due to cancer heterogeneity, and various subtypes can be observed owing to the role of TMEM16A in cancer cells.

Roepman et al. reported that the gene alteration pattern of LNM in HNSCC was maintained, which emphasizes the importance of the primary tumor gene expression profile for the development and treatment of metastasis [84]. In addition, Sugahara et al. reported a comprehensive physical map of the 11q13 amplification region and identified two core regions separated by a breakpoint. Copy number amplification of CTTN (core 2) and/or TPCN2/MYEOV (core 1) in the 11q13 region is strongly associated with LNM in patients with HNSCC. They concluded that the detailed assessment of selected loci, followed by real-time quantitative PCR, appears to be a valid strategy for identifying genetic markers in regions with complicated alterations, as is the case with 11q13. However, despite TMEM16A belonging to 11q13, no significant expression of TMEM16A as a single marker for cervical LNM was detected [85]. Therefore, TMEM16A expression alone may not be a promising marker for predicting LNM in HNSCC but evaluating core-combination gene status may improve the prediction accuracy.

Bone metastasis
According to a previous study, the reported frequency of the bone metastasis of HNSCC is approximately 15–39% of distant metastasis depending on the study population and other complicated factors [86]. Unlike in LNM, gene expression in the tumor microenvironment and functional heterogeneity in the bone metastatic region can be different from their primary form because bone resorption occurs due to the activity of osteoclasts, multinucleated cells derived from the haematopoietic colony forming a unit-granulocyte macrophage/monocyte-macrophage family [87]. When cancer cells arrest in the bone, the bone becomes a storehouse of a variety of cytokines and growth factors, such as TGF-β, and thus provides a fertile environment for cancer cell growth. In breast cancer cells, cell-to-cell interaction between vascular cell adhesion molecule 1-positive bone-tropic cells and very late antigen 4-positive osteoclast precursors activate the survival signal through the PI3K/AKT pathway; osteoclast precursors differentiate into osteoclasts through the receptor activator of NF-κB ligand (RANKL)-RANK signaling to promote osteolysis. When osteolysis due to bone metastasis of cancer is advanced, TGF-β is released from the bone matrix that promotes cell growth [88]. As mentioned, TGF-β signaling pathway is a promising treatment target inhibiting TMEM16A-mediated tumor progression and EMT phenotype [32]. At this time, we could not find a manuscript that demonstrated a difference in the expression patterns of the

TMEM16 family members in the bone metastatic region. Since TMEM16A functions as a CaCC, the authors expect that the dynamics of the TMEM16 family in the bone metastatic site can be largely different from the primary cancer or other soft tissue metastatic cancers and extracellular Ca^{2+} population may be considerably high in the bone metastatic microenvironment. We believe that there may be a crosstalk between these factors and TMEM16A in the bone metastatic region to drive tumor progression in the bone metastatic microenvironment.

Inhibition of TMEM16A

There are at least two ways to explain the relationship between TMEM16A overexpression and tumorigenesis. First, TMEM16A overexpression is a causal factor for tumorigenesis. If TMEM16A functions as an oncogene, it participates in tumorigenesis initiation. Second, the overexpression of TMEM16A may be an end step in tumorigenesis. In either case, inhibition of this channel could interrupt tumorigenesis. In particular, if the factors upstream of TMEM16A transcription could be revealed, inhibition of these factors may prevent the overexpression of TMEM16A and help stop the tumorigenic process that includes multiple progressive pathways, such as the EGFR downstream pathway [89]. From this viewpoint, ROCK1 gene, which was revealed by Luo et al., is an indirect TMEM16A driver that might be a reasonable inhibitor for TMEM16A overexpression [49].

To date, including natural products, quite a few TMEM16A inhibitors have been developed. T16Ainh-A01 reduced TMEM16A expression in SKBR3 breast cancer cells [64], and in AR42J cells [90]. Bill, et al. reported that CaCCinh-A01 promoted loss of TMEM16A proteins via endoplasmic reticulum-associated proteasomal degradation of TMEM16A in HNSCC cell lines [91]. Arctigenin and dehydroandrographolide also decreased the protein expression of TMEM16A in lung adenocarcinoma cells [92] and colorectal cancer cells [93], respectively. Moreover, Wang, et al. found that simvastatin, a widely used cholesterol-lowering drug, suppressed TMEM16A channel activities, and inhibited cell proliferation in OSCC cells via TMEM16A in a mevalonate acid-independent way [94]. In addition, luteolin, a common dietary flavonoid, has various biological effects including anticancer activity against multiple types of human cancer cell lines [95]. Seo, et al. reported that luteolin inhibited cell proliferation and migration of PC-3 cells expressing high levels of TMEM16A more potently than that of TMEM16A-deficient PC-3 cells. Notably, luteolin not only inhibited TMEM16A channel activity, but also strongly decreased protein expression levels of TMEM16A [96]. Miner et al. identified niclosamide as a potent inhibitor of TMEM16A [97]. Niclosamide

is an anthelminthic drug that was also shown to inhibit NOTCH signaling [98], a pathway that is well known to participate in tumorigenesis [99]. Niclosamide was also shown to inhibit NF-κB, Wnt/ß-catenin signaling, the IL-6-JAK1-STAT3 pathway, GSK-3 and more [100–108]. Moreover, homoharringtonine was reported as a novel natural product inhibitor of TMEM16A by Guo, et al. [109]. Homoharringtonine inhibited TMEM16A activity in a concentration-dependent manner and cancer cell proliferation by inhibiting protein and DNA syntheses [110]. In breast cancer, homoharringtonine suppresses cell growth and promotes apoptosis by regulating the miR-18a-3p-AKT-mTOR signaling pathway [111]. Zhang X, et al. reported cepharanthine as selective TMEM16A inhibitors with potential for lung adenocarcinoma therapy [112]. In addition, they found some avermectins have limited effects on the proliferation, migration, and apoptosis of LA795 cells. Avermectins could also dramatically inhibited the growth of xenograft tumors in mice [113]. Zhang G, et al. identified benzophenanthridine alkaloids inhibited proliferation, migration, and induced apoptosis of lung adenocarcinoma cells [114]. Shi, et al. reported theaflavin effectively inhibited the proliferation and migration of TMEM16A high-expressing lung adenocarcinoma cells, displaying antitumor potential [115]. Guo, et al. reported silibinin as a novel TMEM16A inhibitor for lung adenocarcinoma which induced apoptosis and downregulation of cyclin D1 [116]. Shi, et al. identified zafirlukast as a novel TMEM16A channel inhibitor with excellent anticancer activity for lung adenocarcinoma LA795 cells and in vivo study [117]. Duo, et al. also reported matrine is an effective and safe TMEM16A inhibitor in vitro and in vivo [118]. Bai, et al. reported administration of nuciferine, a novel TMEM16A inhibitor, significantly enhanced the cancer therapy effect of cisplatin and counteracted the toxicity of high concentrations of cisplatin [119]. Ani9 is reported to inhibit the proliferation of TMEM16A overexpressed malignant glioma cell [120]. Since these inhibitors are relatively novel, and in some of them, a mechanism of TMEM16A inhibition is unclear, more basic experiments will be needed (Table 1). Other, there are also lots of TMEM16A inhibitors which are not still fully investigated the antitumor effect; MONNA [121], AACT [122], digallic acid [123], evodiamine, rutecarpine [124], purpactin A [125], nimodipine [126], and more. Actually, blocking TMEM16A may be difficult when clarifying the intended target because inhibition of TMEM16A can suppress multiple pathways. Therefore, to find a combined antitumor agent with a TMEM16A inhibitor, more studies are needed before clinical trials are conducted.

Surprisingly, TMEM16A is also known to be activated by noxious heat over 44 °C [127]. Further research that

Table 1 Representative TMEM16A inhibitors for cancer

Name	Target	Cancer types	Reported year	References
T16Ainh-A01	NF-kB, EGFR signaling	Breast, HNSCC, Pancrea	2014-	[64] [90]
CaCCinh-A01	ER-associated proteasomal degradation	HNSCC	2014	[91]
Arctigenin	MAPK pathway	Lung adenocarcinoma	2020	[92]
Dehydroandrographolide	Directly inhibit TMEM16A	Colorectal	2015	[93]
Simvastatin	Mevalonate acid-dependent pathway	OSCC	2021	[94]
Luteolin	PI3K/AKT, NF-kB, XIPA, p53	Prostate	2008-	[95] [96]
Niclosamide	NOTCH signaling, NF-kB, Wnt/beta-Catenin signaling, IL-6-JAK1-STAT3 pathway, GSK-3, other	Leukemia, OSCC, Ovarian, Hepatocellular carcinoma	2009-	[97–108]
Homoharrimgtonine	MiR-18a-3p-AKT-mTOR pathway	Lung, Breast, Leukemia	2019-	[109–111]
Cepharanthine	Detailes unknown	Lung adenocarcinoma	2021	[112]
Avermectin	Endogenous TMEM16A-mediated currents	Lung adenocarcinoma	2020	[113]
Benzophenanthridine alkaloid	Endogenous TMEM16A currents	Lung adenocarcinoma	2020	[114]
Theaflavin	Directly inhibit TMEM16A	Lung adenocarcinoma	2021	[115]
Silibinin	Apoptosis, Cyclin D1	Lung adenocarcinoma	2021	[116]
Zafirlukast	Directly inhibit TMEM16A	Lung adenocarcinoma	2022	[117]
Matrine	Directly inhibit TMEM16A and its currents	Lung adenocarcinoma	2018	[118]
Nuciferine	Enhance anti-cancer effect of cisplatin	Lung adenocarcinoma	2022	[119]
Ani9	Directly inhibit TMEM16A	Malignant glioma	2016	[120]

focuses on the relationship between heat and TMEM16 family diversity, including the relationship with the release of heat shock proteins that are associated with tumor-specific antigens from heat-stressed or dying tumor cells that are phagocytised by antigen-presenting cells are needed [128]. High temperature-focused research may reveal another viewpoint of hyperthermia treatment combined with TMEM16A inhibitor.

Radiotherapy combined with TMEM16A inhibitor
Radiotherapy is one of a promising treatment strategy for cancer. In HNSCC, combination of high-dose cisplatin administration with radiotherapy is recommended as a standard of care for the postoperative HNSCC patients who are at intermediate/high risk of recurrence [129]. In various cancer, the development of effective radiosensitizers can improve the survival rates. At this time, we cannot detect papers which analyzed TMEM16A as a potential radiosensitizer. It is well-known that TGF-β promotes radioresistance by inducing EMT, cancer stem cells and cancer-associated fibroblasts, suppresses the immune system and facilitates cancer resistance [130]. Moreover, as mentioned, miR-381 functions as a tumor suppressor by directly targeting TMEM16A and regulating TGF-β pathway and subsequently EMT process in the progression of GC [53]. Thus, the inhibition of TMEM16A may gain radiosensitivity via inhibition of TGF-β downstream pathway. On the other hand, hypoxia also enhances the radioresistance, and most advanced solid cancers are hypoxic [131, 132]. There is also no

report about the relationship between hypoxia and TMEM16A expression in cancer, but in normal epithelia, such as in cultured sinonasal epithelial layers, long-term hypoxia can enhance TMEM16A expression [133]. Taken together, TMEM16A inhibitor is a candidate for the potential radiosensitizer. The analysis for the relationship between hypoxia and TMEM16A in cancer and the development of reoxygenation methods will be needed in the future experiments.

TMEM16A and Immune-checkpoint inhibition
Several clinical trials using immune-checkpoint inhibitors have been conducted, and their results have led to the development of pembrolizumab as a first-line treatment for recurrent/metastatic HNSCC [134, 135]. However, PD-L1 expression alone did not completely capture the sensitivity of HNSCCs to PD-1 inhibitors. Furthermore, most patients who initially respond to PD-1 inhibitors eventually develop acquired resistance to immunotherapy through mechanisms that have not yet been completely understood [136].

To date, there are no reports highlighting the use of combination therapy with TMEM16A inhibitors and immune-checkpoint inhibitors. Several studies reported that high PD-L1 expression was associated with the presence of EGFR mutations in non-small cell lung cancer and was an independent negative prognostic factor for this disease [137–139]. Moreover, Xu et al. reported that HPV + HNSCC patients display improved outcomes with PD-1/PD-L1 axis blockade compared to HPV − HNSCC

patients. These improved outcomes are likely driven to a greater extent by anti-PD-L1 inhibitors [140]. Sirianant et al. indicated that HPV infections and cancer caused by mutations in TMC8 are related to upregulated Zn^{2+}/ Ca^{2+} signaling and activation of TMEM16A in HNSCC [141]. Moreover, according to Finegersh et al., three CpG islands near the TMEM16A transcriptional start site correlate with TMEM16A expression, including two that positively correlate with TMEM16A expression. They found that positively correlated CpGs drive TMEM16A expression, revealing this to likely be a fundamental mechanism for regulating TMEM16A in HNSCC. Moreover, because HPV E7-, but not E6-transfected cancer cells led to hypermethylation of a positively correlated CpG island without a change in TMEM16A expression, they concluded that this hypermethylation caused only by E7 was not sufficient to increase TMEM16A mRNA levels that showed the clinical relevance of both negatively and positively correlated CpG expression levels [142]. Taken together, HPV infection can indirectly stimulate TMEM16A expression and function.

Overall, there can be an indirect relationship among the expression of TMEM16A, HPV infection, and PD-L1 and EGFR expression. Here, we show the downstream pathways involved in PD-L1 expression. Both HPV infection and EGFR downstream can affect the NF-κB pathway, a central player in inflammation and immunity, which then forms the p65 and p50 complex. Its transcript COPS5 inhibits PD-L1 ubiquitination; thus, more PD-L1 can be synthesized. Moreover, Antonangeli et al. reported that the upregulation of PD-L1 in cancer cells is controlled via NF-κB downstream of several signals, including oncogene- and cell stress-induced pathways, inflammatory cytokines, and chemotherapeutic drugs [143]. NF-κB has emerged as a key positive regulator of PD-L1 expression. Moreover, some groups have reported an indirect link between EGFR and PD-L1 expression via NF-κB in non-small cell lung cancer [144, 145]. In fact, even in HNSCC, PD-L1 is significantly elevated in cancer models of acquired cetuximab resistance [146]. In addition, Chen et al. reported that STAT3 also has been shown to be key transcriptional regulators of PD-L1 in cancer [147].

TMEM16A can also promote cancer progression by activating EGFR and CAMK signaling. Knockdown of TMEM16A contributes to a reduction in AKT, ERK1/2, and v-Src sarcoma viral oncogene homologue (SRC) phosphorylation levels due to decreased autocrine EGFR ligand secretion, reduced EGFR signaling, and decreased cell viability in breast [38] and ovarian cancer cells [148]. Furthermore, TMEM16A inhibition of its chloride-channel activity reduced CaMKII signaling, which subsequently attenuated AKT, SRC, and ERK1/2
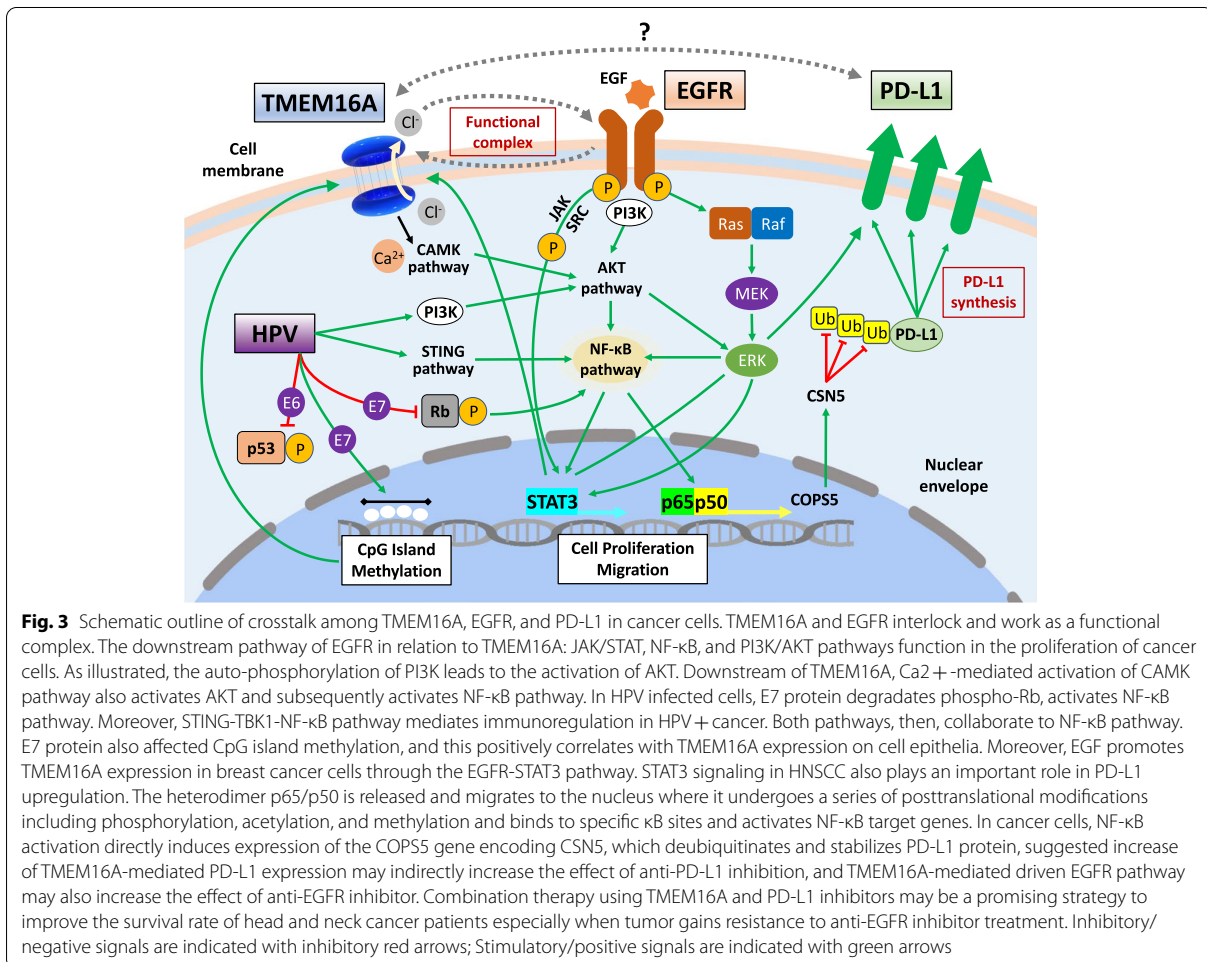
activation in breast cancer [38]. Bill et al. also discovered that TMEM16A and EGFR form a functional complex that jointly regulates HNSCC cell proliferation: EGFR signaling increases TMEM16A protein levels, whereas knockdown of TMEM16A reduces EGFR protein levels [47]. MAPK signaling pathway is an evolutionarily conserved kinase module that controls fundamental cellular processes such as growth, proliferation, differentiation, migration, and apoptosis [149]. Almaça et al. reported that TMEM16A was activated by ATP through an increase in intracellular Ca^{2+} and a Ca^{2+}-independent mechanism involving ERK1/2 [150]. These data indicated that the expression of TMEM16A is also functionally linked to the MAPK pathway.

Considering the above-mentioned crosstalk between EGFR and TMEM16A, we can describe the indirect interplay among the expressions of EGFR, PD-L1, and TMEM16A (Fig. 3). Combination treatment using a TMEM16A inhibitor and an anti-PD-L1 inhibitor may be a promising strategy. Considering that the co-inhibition of EGFR and TMEM16A had an additive effect on HNSCC cell proliferation, adding PD-L1 inhibition may have a synergistic effect. Moreover, co-targeting of TMEM16A and EGFR could enhance the clinical potential of EGFR-targeted therapy and might delay or prevent resistance to single anti-EGFR inhibitor treatment [47]. Taken together, the authors believe that a combination therapy using anti-TMEM16A and anti-PD-L1 inhibitors can be a promising strategy to improve the survival rate of HNSCC, particularly when the tumor gains resistance to anti-EGFR inhibitor treatment.

This hypothesis may prove useful in the near future. In fact, Katsurahara, et al. reported that ANO9, one of the TMEM family members, promotes cancer growth via STAT3 pathway and regulates PD-L2 expression via interferon (IFN)-related genes in GC. They also found that the knockdown of ANO9 attenuated the PD-L2 expression when IFNα was added in an in vitro analysis. Conversely, PD-L1 increased when IFNα was added [151]. In the case of ANO9, IFNα may play a crucial role for the control of PD-L1 and PD-L2 expression. This study evidently showed the possibility of a crosstalk between the expressions of TMEM family members and PD-L1.

Prospective

Further research is required to elucidate how the disparity between the intra- and extracellular ion distribution modulates EGFR, PD-L1, and TMEM16A expressions and how it affects the formation and function of the PD-1/PD-L1 axis. Moreover, investigation of the expression levels of EGFR, PD-L1, and TMEM16A in the early-stage HNSCC is necessary to confirm whether

Fig. 3 Schematic outline of crosstalk among TMEM16A, EGFR, and PD-L1 in cancer cells. TMEM16A and EGFR interlock and work as a functional complex. The downstream pathway of EGFR in relation to TMEM16A: JAK/STAT, NF-κB, and PI3K/AKT pathways function in the proliferation of cancer cells. As illustrated, the auto-phosphorylation of PI3K leads to the activation of AKT. Downstream of TMEM16A, Ca2 + -mediated activation of CAMK pathway also activates AKT and subsequently activates NF-κB pathway. In HPV infected cells, E7 protein degradates phospho-Rb, activates NF-κB pathway. Moreover, STING-TBK1-NF-κB pathway mediates immunoregulation in HPV + cancer. Both pathways, then, collaborate to NF-κB pathway. E7 protein also affected CpG island methylation, and this positively correlates with TMEM16A expression on cell epithelia. Moreover, EGF promotes TMEM16A expression in breast cancer cells through the EGFR-STAT3 pathway. STAT3 signaling in HNSCC also plays an important role in PD-L1 upregulation. The heterodimer p65/p50 is released and migrates to the nucleus where it undergoes a series of posttranslational modifications including phosphorylation, acetylation, and methylation and binds to specific κB sites and activates NF-κB target genes. In cancer cells, NF-κB activation directly induces expression of the COPS5 gene encoding CSN5, which deubiquitinates and stabilizes PD-L1 protein, suggested increase of TMEM16A-mediated PD-L1 expression may indirectly increase the effect of anti-PD-L1 inhibition, and TMEM16A-mediated driven EGFR pathway may also increase the effect of anti-EGFR inhibitor. Combination therapy using TMEM16A and PD-L1 inhibitors may be a promising strategy to improve the survival rate of head and neck cancer patients especially when tumor gains resistance to anti-EGFR inhibitor treatment. Inhibitory/ negative signals are indicated with inhibitory red arrows; Stimulatory/positive signals are indicated with green arrows

the target therapy we suggested in this review can be adopted in such an early disease. In addition, our previous study revealed that prolonged cetuximab treatment induced the inhibition of HNSCC cell migration, resulting in high cell density-related cell stress and persistent cell cycle arrest at G1 phase culminating in autophagy [152]. Hence, we cannot ignore how activated autophagy modulates the intracellular crosstalk among TMEM16A-EGFR-PD-L1-related proteins. Alternatively, targeted protein degradation using a bifunctional PROTAC consisting of a TMEM16A-binding molecule coupled to a degradation inducing moiety could present a promising strategy to target TMEM16A in cancer [40]. In innate immune sensing, further investigations should also focus on how TMEM16A overexpression modulates intratumoral lymphocyte populations and surrounding T lymphocyte recruitment and their functions. Dr Laura Conforti mentioned that Ca^{2+} is in fact a key regulator of the activity of important transcription factors that

control transcriptional activity in T lymphocytes. The overall T lymphocyte activation process resides in ion channel proteins controling the Ca^{2+} signaling triggered by antigen presentation that is necessary for T lymphocyte proliferation and cytokine release [153]. Moreover, intratumoral hypoxia and immunity have been correlated with patient outcome in various tumor settings [154], and with EGFR expression [155]. Investigating the relationship between these factors involving treatment resistance and TMEM16A expression is necessary in the future.

Conclusion
In the present review, we have elaborated on TMEM16A as a promising target in the treatment of HNSCC because it can modulate cell metabolism through interactions with EGFR and PD-L1. This indicates the possibility of combining synergistic therapy with immune-checkpoint inhibitors. To our knowledge, this is the first review to present a validation and

mechanism that combines immune-checkpoint inhibitor with TMEM16A inhibition. Considering that the overexpression of TMEM16A is a common feature observed in multiple cancers, suggesting a conserved mechanism for the promotion of carcinogenesis, tumor proliferation, and migration, a crosstalk may exist among these hallmarks in HNSCC.

Abbreviations
CaCC: Ca^{2+}-activated Cl^- channels; TMEM16A: Transmembrane protein 16A; GISTs: Gastrointestinal stromal tumors; HNSCC: Head and neck squamous cell carcinoma; TCGA: The Cancer Genome Atlas; HPV: Human papillomavirus; LNM: Lymph node metastasis; ESCC: Esophageal squamous cell carcinoma; CRC: Colorectal cancer; GC: Gastric cancer; ERK: Extracellular signal-regulated kinase; MAPK: Mitogen-activated protein kinase; RAS: Rat sarcoma; RAF: Rapidly accelerated fibrosarcoma; EGFR: Epidermal growth factor receptor; PI3K: Phosphatidylinositol 3-kinases; mTORC1: Mammalian/mechanistic target of rapamycin complex-1; GSK-3: Glycogen synthase kinase-3; PD-L1: Programmed cell death-ligand 1; miRs: MicroRNAs; CaMKII: Calmodulin kinase II; TGF-β: Transforming growth factor-β; EMT: Epithelial-mesenchymal transition; PROTAC: Proteolysis targeting chimera; NF-κB: Nuclear factor-kappa B; CpG: Capsid protein gene; STING: Stimulator of interferon genes; STAT: Signal transducer and activator of transcription; JAK: Janus kinase; JNK: C-Jun-NH2-terminal kinase; RANK: Receptor activator of nuclear factor-kappa B; RANKL: Receptor activator of nuclear factor-kappa B ligand; IFN: Interferon.

Acknowledgements
Not applicable.

Authors' contributions
KO obtained research funding, wrote the original manuscript, and made figures/table. SY provided ideas for the research design and supervised the study. All authors reviewed and revised the manuscript and approved the final version of the manuscript.

Funding
This work was supported in part by Grant-in-Aid for Young Scientists (#21K16942) from the Japan Society for the Promotion of Science.

Availability of data and material
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The consent for publication has been given by all authors.

Competing interests
The authors declare no competing interests.

Author details
¹Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, Ann Arbor, MI 48109, USA. ²University of Michigan Rogel Cancer Center, Ann Arbor, MI 48109, USA. ³Department of Oral and Maxillofacial Surgery, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo 113-8510, Japan. ⁴Department of Oral Oncology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima 734-8553, Japan.

References
1. Hartzell C, Putzier I, Arreola J. Calcium-activated chloride channels. Annu Rev Physiol. 2005;67:719–58.
2. Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, et al. TMEM16A confers receptor-activated calcium- dependent chloride conductance. Nature. 2008;455(7217):1210–5.
3. Schroeder BC, Cheng T, Jan YN, Jan LY. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell. 2008;134(6):1019–29.
4. Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, et al. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science. 2008;322(5901):590–4.
5. Wang H, Zou L, Ma K, Yu J, Wu H, Wei M, et al. Cell-specific mechanisms of TMEM16A Ca2+-activated chloride channel in cancer. Mol Cancer. 2017;16(1):152.
6. Schreiber R. Ca2+ signaling, intracellular pH and cell volume in cell proliferation. J Membr Biol. 2005;205(3):129–37.
7. Kunzelmann K. Ion channels and cancer. J Membr Biol. 2005;205(3):159–73.
8. Espinosa I, Lee CH, Kim MK, Rouse BT, Subramanian S, Montgomery K, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. Am J Surg Pathol. 2008;32(2):210–8.
9. Ruiz C, Martins JR, Rudin F, Schneider S, Dietsche T, Fischer CA, et al. Enhanced Expression of ANO1 in Head and Neck Squamous Cell Carcinoma Causes Cell Migration and Correlates with Poor Prognosis. PLoS ONE. 2012;7(8): e43265.
10. Duvvuri U, Shiwarski DJ, Xiao D, Bertrand C, Huang X, Edinger RS, et al. TMEM16A induces MAPK and contributes directly to tumorigenesis and cancer progression. Cancer Res. 2012;72(13):3270–81.
11. Kunzelmann K, Ousingsawat J, Benedetto R, Cabrita I, Schreiber R. Contribution of Anoctamins to Cell Survival and Cell Death. Cancers (Basel). 2019;11(3):382.
12. Kunzelmann K, Schreiber R, Kmit A, Jantarajit W, Martins JR, Faria D, et al. Expression and function of epithelial anoctamins. Exp Physiol. 2012;97(2):184–92.
13. Qu Z, Yao W, Yao R, Liu X, Yu K, Hartzell C. The Ca (2+) -activated Cl(-) channel, ANO1 (TMEM16A), is a double-edged sword in cell proliferation and tumorigenesis. Cancer Med. 2014;3(3):453–61.
14. McDermott JD, Bowles DW. Epidemiology of Head and Neck Squamous Cell Carcinomas: Impact on Staging and Prevention Strategies. Curr Treat Options Oncol. 2019;20(5):43.
15. Koteluk O, Bielicka A, Lemańska Ż, Jóźwiak K, Klawiter W, Mackiewicz A, et al. The Landscape of Transmembrane Protein Family Members in Head and Neck Cancers: Their Biological Role and Diagnostic Utility. Cancers (Basel). 2021;13(19):4737.
16. Haddad RI, Shin DM. Recent advances in head and neck cancer. N Engl J Med. 2008;359(11):1143–54.
17. Döbrossy L. Epidemiology of head and neck cancer: Magnitude of the problem. Cancer Metastasis Rev. 2005;24(1):9–17.
18. Bernier J, Domenge C, Ozsahin M, Matuszewska K, Lefèbvre JL, Greiner RH, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med. 2004;350(19):1945–52.
19. Cooper JS, Pajak TF, Forastiere AA, Jacobs J, Campbell BH, Saxman SB, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med. 2004;350(19):1937–44.
20. Marur S, Forastiere AA. Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment. Mayo Clin Proc. 2016;91(3):386–96.
21. Alsahafi E, Begg K, Amelio I, Raulf N, Lucarelli P, Sauter T, et al. Clinical update on head and neck cancer: Molecular biology and ongoing challenges. Cell Death Dis. 2019;10(8):540.
22. Ji Q, Guo S, Wang X, Pang C, Zhan Y, Chen Y, et al. Recent advances in TMEM16A: Structure, function, and disease. J Cell Physiol. 2019;234(6):7856–73.
23. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363(1):24–35.

24. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011;29(32):4294–301.

25. Fleming JC, Woo J, Moutasim K, Mellone M, Frampton SJ, Mead A, et al. HPV, tumour metabolism and novel target identification in head and neck squamous cell carcinoma. Br J Cancer. 2019;120(3):356–67.

26. Oh JE, Kim JO, Shin JY, Zhang XH, Won HS, Chun SH, et al. Molecular genetic characterization of p53 mutated oropharyngeal squamous cell carcinoma cells transformed with human papillomavirus E6 and E7 oncogenes. Int J Oncol. 2013;43(2):383–93.

27. Lechner M, Fenton TR. The Genomics, Epigenomics, and Transcriptomics of HPV-Associated Oropharyngeal Cancer-Understanding the Basis of a Rapidly Evolving Disease. Adv Genet. 2016;93:1–56.

28. Luo X, Donnelly CR, Gong W, Heath BR, Hao Y, Donnelly LA, et al. HPV16 drives cancer immune escape via NLRX1-mediated degradation of STING. J Clin Investig. 2020;130(4):1635–52.

29. Ayoub C, Wasylyk C, Li Y, Thomas E, Marisa L, Robé A, et al. ANO1 amplification and expression in HNSCC with a high propensity for future distant metastasis and its functions in HNSCC cell lines. Br J Cancer. 2010;103(5):715–26.

30. Shi ZZ, Shang L, Jiang YY, Hao JJ, Zhang Y, Zhang TT, et al. Consistent and differential genetic aberrations between esophageal dysplasia and squamous cell carcinoma detected by array comparative genomic hybridization. Clin Cancer Res. 2013;19(21):5867–78.

31. Bae JS, Park JY, Park SH, Ha SH, An AR, Noh SJ, et al. Expression of ANO1/DOG1 is associated with shorter survival and progression of breast carcinomas. Oncotarget. 2017;9(1):607–21.

32. Wang H, Yao F, Luo S, Ma K, Liu M, Bai L, et al. A mutual activation loop between the Ca2+-activated chloride channel TMEM16A and EGFR/STAT3 signaling promotes breast cancer tumorigenesis. Cancer Lett. 2019;455:48–59.

33. Wu H, Guan S, Sun M, Yu Z, Zhao L, He M, et al. Ano1/TMEM16A overexpression is associated with good prognosis in PR-positive or HER2-negative breast cancer patients following Tamoxifen treatment. PLoS ONE. 2015;10(5): e0126128.

34. Li H, Yang Q, Huo S, Du Z, Wu F, Zhao H, et al. Expression of TMEM16A in Colorectal Cancer and Its Correlation With Clinical and Pathological Parameters. Front Oncol. 2021;11: 652262.

35. Liu F, Cao QH, Lu DJ, Luo B, Lu XF, Luo RC, et al. TMEM16A overexpression contributes to tumor invasion and poor prognosis of human gastric cancer through TGF-beta signaling. Oncotarget. 2015;6(13):11585–99.

36. Liu W, Lu M, Liu B, Huang Y, Wang K. Inhibition of Ca (2+)- activated Cl(−) channel ANO1/TMEM16A expression suppresses tumor growth and invasiveness in human prostate carcinoma. Cancer Lett. 2012;326(1):41–51.

37. Atala A. Re: Inhibition of Ca2+-activated Cl- channel ANO1/TMEM16A expression suppresses tumor growth and invasiveness in human prostate carcinoma. J Urol. 2013;189(6):2393.

38. Britschgi A, Bill A, Brinkhaus H, Rothwell C, Clay I, Duss S, et al. Calcium-activated chloride channel ANO1 promotes breast cancer progression by activating EGFR and CAMK signaling. Proc Natl Acad Sci USA. 2013;110(11):E1026–34.

39. Bill A, Popa MO, van Diepen MT, Gutierrez A, Lilley S, Velkova M, et al. Variomics screen identifies the re-entrant loop of the calcium-activated chloride channel ANO1 that facilitates channel activation. J Biol Chem. 2015;290(2):889–903.

40. Bill A, Gaither LA. The Mechanistic Role of the Calcium-Activated Chloride Channel ANO1 in Tumor Growth and Signaling. Adv Exp Med Biol. 2017;966:1–14.

41. Sui Y, Sun M, Wu F, Yang L, Di W, Zhang G, et al. Inhibition of TMEM16A expression suppresses growth and invasion in human colorectal cancer cells. PLoS ONE. 2014;9(12): e115443.

42. Yang Q, Huo S, Sui Y, Du Z, Zhao H, Liu Y, et al. Mutation status and immunohistochemical correlation of KRAS, NRAS, and BRAF in 260 Chinese colorectal and gastric cancers. Front Oncol. 2018;8:487.

43. Deng I, Yang J, Chen H, Ma B, Pan K, Su C, et al. Knockdown of TMeM16a suppressed MaPK and inhibited cell proliferation and migration in hepatocellular carcinoma. Onco Targets Ther. 2016;9:325–33.

44. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. N Engl J Med. 2007;357(25):2552–61.

45. McCubrey JA, Abrams SL, Fitzgerald TL, Cocco L, Martelli AM, Montalto G, et al. Roles of signaling pathways in drug resistance, cancer initiating cells and cancer progression and metastasis. Adv Biol Regul. 2015;57:75–101.

46. Davis NM, Sokolosky M, Stadelman K, Abrams SL, Libra M, Candido S, et al. Deregulation of the EGFR/ PI3K/PTEN/Akt/mTORC1 pathway in breast cancer: Possibilities for therapeutic intervention. Oncotarget. 2014;5(13):4603–50.

47. Bill A, Gutierrez A, Kulkarni S, Kemp C, Bonenfant D, Voshol H, et al. ANO1/TMEM16A interacts with EGFR and correlates with sensitivity to EGFR-targeting therapy in head and neck cancer. Oncotarget. 2015;6(11):9173–88.

48. Bu LL, Yu GT, Wu L, Mao L, Deng WW, Liu JF, et al. STAT3 Induces Immunosuppression by Upregulating PD-1/PD-L1 in HNSCC. J Dent Res. 2017;96(9):1027–34.

49. Luo S, Wang H, Bai L, Chen Y, Chen S, Gao K, et al. Activation of TMEM16A Ca 2+-activated Cl-channels by ROCK1/moesin promotes breast cancer metastasis. J Adv Res. 2021;33:253–64.

50. Jiang Y, Cai Y, Shao W, Li F, Guan Z, Zhou Y, et al. MicroRNA144 suppresses aggressive phenotypes of tumor cells by targeting ANO1 in colorectal cancer. Oncol Rep. 2019;41(4):2361–70.

51. Park YR, Lee ST, Kim SL, Zhu SM, Lee MR, Kim SH, et al. Down- regulation of miR-9 promotes epithelial mesenchymal transition via regulating anoctamin-1. (ANO1) in CRC cells. Cancer Genet. 2019;231–232:22–31.

52. Mokutani Y, Uemura M, Munakata K, Okuzaki D, Haraguchi N, Takahashi H, et al. Down-regulation of microRNA-132 is associated with poor prognosis of colorectal cancer. Ann Surg Oncol. 2016;23(Suppl 5):599–608.

53. Cao Q, Liu F, Ji K, Liu N, He Y, Zhang W, et al. MicroRNA-381 inhibits the metastasis of gastric cancer by targeting TMEM16A expression. J Exp Clin Cancer Res. 2017;36(1):29.

54. Dulin NO. Calcium-activated chloride channel ANO1/TMEM16A: regulation of expression and signaling. Front Physiol. 2020;11: 590262.

55. Hao Y, Baker D, Ten Dijke P. TGF-β-Mediated Epithelial-Mesenchymal Transition and Cancer Metastasis. Int J Mol Sci. 2019;20(11):2767.

56. Katsuno Y, Lamouille S, Derynck R. TGF-beta signaling and epithelial-mesenchymal transition in cancer progression. Curr Opin Oncol. 2013;25(1):76–84.

57. Rodrigues-Junior DM, Tsirigoti C, Lim SK, Heldin CH, Moustakas A. Extracellular Vesicles and Transforming Growth Factor β Signaling in Cancer. Front Cell Dev Biol. 2022;10: 849938.

58. Siegel PM, Massagué J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer. 2003;3(11):807–21.

59. Morikawa M, Koinuma D, Miyazono K, Heldin CH. Genome-wide Mechanisms of Smad Binding. Oncogene. 2013;32:1609–15.

60. Tzavlaki K, Moustakas A. TGF-β Signaling Biomolecules. 2020;10:487.

61. Bhowmick NA, Ghiassi M, Bakin A, Aakre M, Lundquist CA, Engel ME, et al. Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA- dependent mechanism. Mol Biol Cell. 2001;12(1):27–36.

62. Vardouli L, Moustakas A, Stournaras C. LIM-kinase 2 and cofilin phosphorylation mediate actin cytoskeleton reorganization induced by transforming growth factor-beta. J Biol Chem. 2005;280(12):11448–57.

63. Vyas A, Duvvuri U, Kiselyov K. Copper-dependent ATP7B upregulation drives the resistance of TMEM16A-overexpressing head-and-neck cancer models to platinum toxicity. Biochem J. 2019;476(24):3705–19.

64. Kulkarni S, Bill A, Godse NR, Khan NI, Kass JI, Steehler K, et al. TMEM16A/ANO1 suppression improves response to antibody-mediated targeted therapy of EGFR and HER2/ERBB2. Genes Chromosomes Cancer. 2017;56(6):460–71.

65 Ozols RF. Ovarian cancer: new clinical approaches. Cancer Treat Rev. 1991;18(Suppl A):77–83.

66. Godse NR, Khan N, Yochum ZA, Gomez-Casal R, Kemp C, Shiwarski DJ, et al. TMEM16A/ANO1 Inhibits Apoptosis Via Downregulation of Bim Expression. Clin Cancer Res. 2017;23(23):7324–32.

67. Berglund E, Akcakaya P, Berglund D, Karlsson F, Vukojevic V, Lee L, et al. Functional role of the Ca-activated Cl channel DOG1/

TMEM16A in gastrointestinal stromal tumor cells. Exp Cell Res. 2014;326(2):315–25.

68. Dixit R, Kemp C, Kulich S, Seethala R, Chiosea S, Ling S, et al. TMEM16A/ANO1 is differentially expressed in HPV-negative versus HPV positive head and neck squamous cell carcinoma through promoter methylation. Sci Rep. 2015;5:16657.

69. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018;18(5):269–82.

70. Wise-Draper TM, Wells SI. Papillomavirus E6 and E7 proteins and their cellular targets. Front Biosci. 2008;13:1003–17.

71. Shin MK, Pitot HC, Lambert PF. Pocket proteins suppress head and neck cancer. Cancer Res. 2012;72(5):1280–9.

72. Jabbar S, Strati K, Shin MK, Pitot HC, Lambert PF. Human papillomavirus type 16 E6 and E7 oncoproteins act synergistically to cause head and neck cancer in mice. Virology. 2010;407(1):60–7.

73. Strati K, Lambert PF. Role of Rb-dependent and Rb-independent functions of papillomavirus E7 oncogene in head and neck cancer. Cancer Res. 2007;67(24):11585–93.

74. Devaraja K, Aggarwal S, Verma SS, Gupta SC. Clinico-pathological peculiarities of human papilloma virus driven head and neck squamous cell carcinoma: A comprehensive update. Life Sci. 2020;245: 117383.

75. Network CGA. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature. 2015;517(7536):576–82.

76. Nichols AC, Yoo J, Palma DA, Fung K, Franklin JH, Koropatnick J, et al. Frequent mutations in TP53 and CDKN2A found by next-generation sequencing of head and neck cancer cell lines. Arch Otolaryngol Head Neck Surg. 2012;138(8):732–9.

77. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, et al. The mutational landscape of head and neck squamous cell carcinoma. Science. 2011;333(6046):1157–60.

78. Pectasides E, Rampias T, Sasaki C, Perisanidis C, Kouloulias V, Burtness B, et al. Markers of Epithelial to Mesenchymal Transition in Association With Survival in Head and Neck Squamous Cell Carcinoma (HNSCC). PLoS ONE. 2014;9(4): e94293.

79. Taberna M, Torres M, Alejo M, Mena M, Tous S, Marquez S, et al. The Use of HPV16-E5, EGFR, and pEGFR as Prognostic Biomarkers for Oropharyngeal Cancer Patients. Front Oncol. 2018;8:589.

80. He C, Mao D, Hua G, Lv X, Chen X, Angeletti PC, et al. The Hippo/ YAP Pathway Interacts With EGFR Signaling and HPV Oncoproteins to Regulate Cervical Cancer Progression. EMBO Mol Med. 2015;7(11):1426–49.

81. Qiu J, Hu F, Shao T, Guo Y, Dai Z, Nie H, et al. Blocking of EGFR Signaling Is a Latent Strategy for the Improvement of Prognosis of HPV-Induced Cancer. Front Oncol. 2021;27: 633794.

82. Cai H, Yan L, Liu N, Xu M, Cai H. IFI16 promotes cervical cancer progression by upregulating PD-L1 in immunomicroenvironment through STING-TBK1-NF-kB pathway. Biomed Pharmacother. 2020;123: 109790.

83. Shiwarski DJ, Shao C, Bill A, Kim J, Xiao D, Bertrand CA, et al. To "Grow" or "Go": TMEM16A expression as a switch between tumor growth and metastasis in SCCHN. Clin Cancer Res. 2014;20(17):4673–88.

84. Roepman P, de Jager A, Groot Koerkamp MJA, Kummer JA, Slootweg PJ, Holstege FC. Maintenance of head and neck tumor gene expression profiles upon lymph node metastasis. Cancer Res. 2006;66(23):11110–4.

85. Sugahara K, Michikawa Y, Ishikawa K, Shoji Y, Iwakawa M, Shibahara T, et al. Combination effects of distinct cores in 11q13 amplification region on cervical lymph node metastasis of oral squamous cell carcinoma. Int J Oncol. 2011;39(4):761–9.

86. Takes RP, Rinaldo A, Silver CE, Haigentz M Jr, Woolgar JA, Triantafyllou A, et al. Distant metastases from head and neck squamous cell carcinoma. Part I Basic aspects Oral Oncol. 2012;48(9):775–9.

87. Roodman GD. Advances in bone biology: the osteoclast. Endocr Rev. 1996;17(4):308–32.

88. Lu X, Mu E, Wei Y, Riethdorf S, Yang Q, Yuan M. VCAM-1 Promotes Osteolytic Expansion of Indolent Bone Micrometastasis of Breast Cancer by Engaging α4β1-Positive Osteoclast Progenitors. Cancer Cell. 2011;20(6):701–14.

89. Qu Z, Yao W, Yao R, Liu X, Yu K, Hartzell C. The Ca2+-activated Cl— channel, ANO1 (TMEM16A), is a double-edged sword in cell proliferation and tumorigenesis. Cancer Med. 2014;3(3):453–61.

90. Wang Q, Bai L, Luo S, Wang T, Yang F, Xia J, et al. TMEM16A Ca (2+)-activated Cl(-) channel inhibition ameliorates acute pancreatitis via the IP (3) R/Ca (2+)/NFκB/ IL-6 signaling pathway. J Adv Res. 2020;23:25–35.

91. Bill A, Hall ML, Borawski J, Hodgson C, Jenkins J, Piechon P, et al. Small molecule-facilitated degradation of ANO1 protein: a new targeting approach for anticancer therapeutics. J Biol Chem. 2014;289:11029–41.

92. Guo S, Chen Y, Shi S, Wang X, Zhang H, Zhan Y, et al. Arctigenin, a novel TMEM16A inhibitor for lung adenocarcinoma therapy. Pharmacol Res. 2020;155: 104721.

93. Sui Y, Wu F, Lv J, Li H, Li X, Du Z, et al. Identification of the Novel TMEM16A Inhibitor Dehydroandrographolide and Its Anticancer Activity on SW620 Cells. PLoS ONE. 2015;10(12): e0144715.

94. Wang H, Wang T, Zhang Z, Fan Y, Zhang Y, Gao K, et al. Simvastatin inhibits oral squamous cell carcinoma by targeting TMEM16A Ca2+-activated chloride channel. J Cancer Res Clin Oncol. 2021;147:1699–711.

95. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. Curr Cancer Drug Targets. 2008;8(7):634–46.

96. Seo Y, Ryu K, Park J, Jeon DK, Jo S, Lee HK, et al. Inhibition of ANO1 by luteolin and its cytotoxicity in human prostate cancer PC-3 cells. PLoS ONE. 2017;12(3): e0174935.

97. Miner K, Labitzke K, Liu B, Elliot R, Wang P, Henckels K, et al. Drug Repurposing: The Anthelmintics Niclosamide and Nitazoxanide Are Potent TMEM16A Antagonists That Fully Bronchodilate Airways. Front Pharmacol. 2019;10:51.

98. Wang AM, Ku HH, Liang YC, Chen YC, Hwu YM, Yeh TS. The autonomous notch signal pathway is activated by baicalin and baicalein but is suppressed by niclosamide in K562 cells. J Cell Biochem. 2009;106:682–92.

99. Meurette O, Mehlen P. Notch Signaling in the Tumor Microenvironment. Cancer Cell. 2018;34:536–48.

100. Kim SY, Kang JW, Song X, Kim BK, Yoo YD, Kwon YT, et al. Role of the IL-6-JAK1-STAT3-Oct-4 pathway in the conversion of non-stem cancer cells into cancer stem-like cells. Cell Signal. 2013;25:961–9.

101. Jin Y, Lu Z, Ding K, Li J, Du X, Chen C, et al. Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: Inactivation of the NF-kappaB pathway and generation of reactive oxygen species. Cancer Res. 2010;70:2516–27.

102. Ren X, Duan L, He Q, Zhang Z, Zhou Y, Wu D, et al. Identification of Niclosamide as a New Small-Molecule Inhibitor of the STAT3 Signaling Pathway. ACS Med Chem Lett. 2010;1:454–9.

103. Osada T, Chen M, Yang XY, Spasojevic I, Vandeusen JB, Hsu D, et al. Anti-helminth compound niclosamide downregulates Wnt signaling and elicits antitumor responses in tumors with activating APC mutations. Cancer Res. 2011;71:4172–82.

104. Wang LH, Xu M, Fu LQ, Chen XY, Yang F. The Antihelminthic Niclosamide Inhibits Cancer Stemness, Extracellular Matrix Remodeling, and Metastasis through Dysregulation of the Nuclear beta-catenin/c-Myc axis in OSCC. Sci Rep. 2018;8:12776.

105. Arend RC, Londono-Joshi AI, Gangrade A, Katre AA, Kurpad C, Li Y, Samant RS, et al. Niclosamide and its analogs are potent inhibitors of Wnt/beta-catenin, mTOR and STAT3 signaling in ovarian cancer. Oncotarget. 2016;7:86803–15.

106. Ahn SY, Yang JH, Kim NH, Lee K, Cha YH, Yun JS, et al. Anti-helminthic niclosamide inhibits Ras-driven oncogenic transformation via activation of GSK-3. Oncotarget. 2017;8:31856–63.

107. Chen B, Wei W, Ma L, Yang B, Gill RM, Chua MS, et al. Computational Discovery of Niclosamide Ethanolamine, a Repurposed Drug Candidate That Reduces Growth of Hepatocellular Carcinoma Cells In Vitro and in Mice by Inhibiting Cell Division Cycle Signaling. Gastroenterology. 2017;152:2022–36.

108. Li Y, Li PK, Roberts MJ, Arend RC, Samant RS, Buchsbaum DJ. Multi-targeted therapy of cancer by niclosamide: A new application for an old drug. Cancer Lett. 2014;349:8–14.

109. Guo S, Bai X, Shi S, Deng Y, Kang X, An H. TMEM16A, a Homoharringtonine Receptor, as a Potential Endogenic Target for Lung Cancer Treatment. Int J Mol Sci. 2021;22(20):10930.

110. Tan M, Zhang Q, Yuan X, Chen Y, Wu Y. Synergistic killing effects of homoharringtonine and arsenic trioxide on acute myeloid leukemia stem cells and the underlying mechanisms. J Exp Clin Cancer Res. 2019;38:308.

111. Wang LB, Wang DN, Wu LG, Cao J, Tian JH, Liu R, et al. Homoharringtonine inhibited breast cancer cells growth via miR-18a-3p/AKT/mTOR signaling pathway. Int J Biol Sci. 2021;17:995–1009.

112. Zhang X, Zhang G, Zhao Z, Xiu R, Jia J, Chen P, et al. Cepharanthine, a novel selective ANO1 inhibitor with potential for lung adenocarcinoma therapy. Biochim Biophys Acta Mol Cell Res. 2021;1868(12): 119132.

113. Zhang X, Zhang G, Zhai W, Zhao Z, Wang S, Yi J. Inhibition of TMEM16A Ca2+-activated Cl- channels by avermectins is essential for their anticancer effects. Pharmacol Res. 2020;156: 104763.

114. Zhang G, Zhu L, Xue Y, Zhao Z, Li H, Niu Z, et al. Benzophenanthridine alkaloids suppress lung adenocarcinoma by blocking TMEM16A Ca2+-activated Cl- channels. Pflugers Arch. 2020;472(10):1457–67.

115. Shi S, Ma B, Sun F, Qu C, An H. Theaflavin binds to a druggable pocket of TMEM16A channel and inhibits lung adenocarcinoma cell viability. J Biol Chem. 2021;297(3): 101016.

116. Guo S, Bai X, Liu Y, Shi S, Wang X, Zhan Y, et al. Inhibition of TMEM16A by Natural Product Silibinin: Potential Lead Compounds for Treatment of Lung Adenocarcinoma. Front Pharmacol. 2021;12: 643489.

117. Shi S, Ma B, Sun F, Qu C, Li G, Shi D, et al. Zafirlukast inhibits the growth of lung adenocarcinoma via inhibiting TMEM16A channel activity. J Biol Chem. 2022;298(3): 101731.

118. Guo S, Chen Y, Pang C, Wang X, Shi S, Zhang H, et al. Matrine is a novel inhibitor of the TMEM16A chloride channel with antilung adenocarcinoma effects. J Cell Physiol. 2019;234(6):8698–708.

119. Bai X, Liu X, Li S, An H, Kang X, Guo S. Nuciferine Inhibits TMEM16A in Dietary Adjuvant Therapy for Lung Cancer. J Agric Food Chem. 2022;70(12):3687–96.

120. Seo Y, Lee HK, Park J, Jeon DK, Jo S, Jo M, et al. Ani9, A Novel Potent Small-Molecule ANO1 Inhibitor with Negligible Effect on ANO2. PLoS One. 2016;11(5):e0155771.

121. Oh SJ, Hwang SJ, Jung J, Yu K, Kim J, Choi JY, et al. MONNA, a potent and selective blocker for transmembrane protein with unknown function 16/Anoctamin-1. Mol Pharmacol. 2013;84:726e35.

122. Truong EC, Phuan PW, Reggi AL, Ferrera L, Galietta LJV, Levy SE, et al. Substituted 2-Acylaminocycloalkylthiophene-3-carboxylic Acid Arylamides as Inhibitors of the Calcium-Activated Chloride Channel Transmembrane Protein 16A (TMEM16A). J Med Chem. 2017;60(11):4626–35.

123. Namkung W, Thiagarajah JR, Phuan PW, Verkman AS. Inhibition of Ca2þ-activated Cl– channels by gallotannins as a possible molecular basis for health benefits of red wine and green tea. FASEB J. 2010;24:4178e86.

124. Zhao Z, Xue Y, Zhang G, Jia J, Xiu R, Jia Y, et al. Identification of evodiamine and rutecarpine as novel TMEM16A inhibitors and their inhibitory effects on peristalsis in isolated Guinea-pig ileum. Eur J Pharmacol. 2021;908: 174340.

125. Yimnual C, Satitsri S, Ningsih BNS, Rukachaisirikul V, Muanprasat C. A fungus-derived purpactin A as an inhibitor of TMEM16A chloride channels and mucin secretion in airway epithelial cells. Biomed Pharmacother. 2021;139: 111583.

126. Wang H, Ma D, Zhu X, Liu P, Li S, Yu B, et al. Nimodipine inhibits intestinal and aortic smooth muscle contraction by regulating Ca 2+-activated Cl – channels. Toxicol Appl Pharmacol. 2021;421: 115543.

127. Cho H, Yang YD, Lee J, Lee B, Kim T, Jang Y, et al. The calcium- activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. Nature Neurosci. 2012;15(7):1015–21.

128. Binder RJ, Han DK, Srivastava PK. CD91: a receptor for heat shock protein gp96. Nat Immunol. 2000;1(2):151–5.

129. Caudell JJ, Gillison ML, Maghami E, Spencer S, Pfister DG, Adkins D, et al. NCCN Guidelines® Insights: Head and Neck Cancers, Version 1.2022. J Natl Compr Canc Netw. 2022;20(3):224–34.

130. Wang J, Xu Z, Wang Z, Du G, Lun L. TGF-beta signaling in cancer radiotherapy. Cytokine. 2021;148: 155709.

131. Begg K, Tavassoli M. Inside the hypoxic tumour: reprogramming of the DDR and radioresistance. Cell Death Discov. 2020;18(6):77.

132. Sørensen BS, Horsman MR. Tumor Hypoxia: Impact on Radiation Therapy and Molecular Pathways. Front Oncol. 2020;10:562.

133. Blount A, Zhang S, Chestnut M, Hixon B, Skinner D, Sorscher EJ, Woodworth BA. Transepithelial Ion Transport Is Suppressed in Hypoxic Sinonasal Epithelium. Laryngoscope. 2011;121:1929–34.

134. Cohen EEW, Soulières D, Le Tourneau C, Dinis J, Licitra L, Ahn MJ, et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study. Lancet. 2019;393(10167):156–67.

135. Burtness B, Harrington KJ, Greil R, Soulières D, Tahara M, de Castro G, Jr, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet. 2019;394(10212):1915–28.

136. Dos Santos LV, Abrahão CM, William WN Jr. Overcoming Resistance to Immune Checkpoint Inhibitors in Head and Neck Squamous Cell Carcinomas. Front Oncol. 2021;11: 596290.

137. Azuma K, Ota K, Kawahara A, Hoshino T, Nakanishi Y, Okamoto I. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected non-small cell lung cancer. Ann Oncol. 2014;25(10):1935–40.

138. Zhang N, Zeng Y, Du W, Zhu J, Shen D, Liu Z, et al. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. Int J Oncol. 2016;49(4):1360–8.

139. Xu Y, Zhu G, Maroun CA, Wu IXY, Huang D, Seiwert TY, et al. Programmed Death-1/Programmed Death-Ligand 1-Axis Blockade in Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma Stratified by Human Papillomavirus Status: A Systematic Review and Meta-Analysis. Front Immunol. 2021;12: 645170.

140. Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. Cancer Res. 2013;73(6):1733–41.

141. Sirianant L, Ousingsawat J, Tian Y, Schreiber R, Kunzelmann K. TMC8 (EVER2) attenuates intracellular signaling by Zn2+ and Ca2+ and suppresses activation of Cl- currents. Cell Signal. 2014;26(12):2826–33.

142. Finegersh A, Kulich S, Guo T, Favorov AV, Fertig EJ, Danilova LV, et al. DNA methylation regulates TMEM16A/ANO1 expression through multiple CpG islands in head and neck squamous cell carcinoma. Sci Rep. 2017;7(1):15173.

143. Antonangeli F, Natalini A, Garassino MC, Sica A, Santoni A, Di Rosa F. Regulation of PD-L1 Expression by NF-κB in Cancer. Front Immunol. 2020;11: 584626.

144. Lin K, Cheng J, Yang T, Li Y, Zhu B. EGFR-TKI down-regulates PD-L1 in EGFR mutant NSCLC through inhibiting NF-kappaB. Biochem Biophys Res Commun. 2015;463(1–2):95–101.

145. Guo R, Li Y, Wang Z, Bai H, Duan J, Wang S, et al. Hypoxia-inducible factor-1alpha and nuclear factor-kappaB play important roles in regulating programmed cell death ligand 1 expression by epidermal growth factor receptor mutants in non-small-cell lung cancer cells. Cancer Sci. 2019;110(5):1665–75.

146. Bhola NE, Njatcha C, Hu L, Lee ED, Shiah JV, Kim MO, et al. PD-L1 is upregulated via BRD2 in head and neck squamous cell carcinoma models of acquired cetuximab resistance. Head Neck. 2021;43(11):3364–73.

147. Chen J, Jiang CC, Jin L, Zhang XD. Regulation of PD-L1: a novel role of pro-survival signalling in cancer. Ann Oncol. 2016;27(3):409–16.

148. Liu Z, Zhang S, Hou F, Zhang C, Gao J, Wang K. Inhibition of Ca 2+ -activated chloride channel ANO1 suppresses ovarian cancer through inactivating PI3K/Akt signaling. Int J Cancer. 2019;144(9):2215–26.

149. Dhillion AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. Oncogene. 2007;26(22):3279–90.

150. Almaça J, Tian Y, Aldehni F, Ousingsawat J, Kongsuphol P, Rock JR, et al. TMEM16 proteins produce volume-regulated chloride currents that are reduced in mice lacking TMEM16A. J Biol Chem. 2009;284(42):28571–8.

151. Katsurahara K, Shiozaki A, Kosuga T, Shimizu H, Kudou M, Arita T, et al. ANO9 regulates PD-L2 expression and binding ability to PD-1 in gastric cancer. Cancer Sci. 2021;112(3):1026–37.

152. Okuyama K, Suzuki K, Naruse T, Tsuchihashi H, Yanamoto S, Kaida A, et al. Prolonged cetuximab treatment promotes p27Kip1-mediated G1 arrest and autophagy in head and neck squamous cell carcinoma. Sci Rep. 2021;11(1):5259.

153. Conforti L. The ion channel network in T lymphocytes, a target for immunotherapy. Clin Immunol. 2012;142(2):105–6.
154. Brooks JM, Menezes AN, Ibrahim M, Archer L, Lal N, Bagnall CJ, et al. Development and Validation of a Combined Hypoxia and Immune Prognostic Classifier for Head and Neck Cancer. Clin Cancer Res. 2019;25(17):5315–28.
155. Zahnreich S, Gebrekidan S, Multhoff G, Vaupel P, Schmidberger H, Mayer A. Oxygen Deprivation Modulates EGFR and PD-L1 in Squamous Cell Carcinomas of the Head and Neck. Front Oncol. 2021;11: 623964.

Publisher's Note