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Original Article

Epigallocatechin gallate stimulated histamine production and downregulated histamine H1 receptor in oral cancer cell lines expressing histidine decarboxylase

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ABSTRACT

Objectives: Increased histamine production and the overexpression of receptors (H1R~H4R) has been reported in several tumors. The effects of TGFβ1 and epigallocatechin gallate (EGCG) on histamine synthesizing enzymes (HDCs), and the histamine transporter systems and receptors were investigated in this study.

Methods: Four oral cancer cell lines (HSC2, HSC3, HSC4, and SAS) were treated with or without TGFβ1 or EGCG for 24 h. The expression levels of HDC, SLC22A3, H1R~H4R, and TAS2R14 were investigated by Western blotting. Histamine concentrations were determined using the enzyme immune assay. Bitter taste receptor (TAS2R14 and TAS2R39) mRNAs were investigated by RT-PCR.

Results: Varying expression levels of HDC, SLC22A3, H1R~H4R, and TAS2R14 were observed in the four cell lines, where histamine concentrations were found to be ~500 fmol/ml in cell culture media and induced 2–2.5 times higher amounts of histamine following EGCG treatment. TGFβ1 increased HDC expression in three cell lines, SLC22A3 expression in three cell lines, H1R expression in two cell lines, H2R expression in three cell lines, H3R expression in three cell lines, and H4R expression in three cell lines. EGCG decreased HDC expression in all four cell lines, SLC22A3 expression in three expression, H1R expression in all four cell lines, H2R expression in two cell lines, H3R expression in three cell lines, and H4R expression in two cell lines.

Conclusions: EGCG upregulated histamine production and decreased the expression level of H1R in the oral cancer cell lines. It might prove useful for cancer therapy during histamine regulation.

1. Introduction

Squamous epithelial cell carcinoma is the most frequent type of oral cancer with high metastatic and invasive potentials: the prognosis of this disease has not shown any significant improvement over the years [1]. Hence, novel molecular targets that inhibit the proliferation, migration, and drug resistance of the tumor cells are warranted.

Histamine is a pleiotropic biogenic amine with a broad range of activities involved in the regulation of both physiological and pathological processes, including cell growth regulation, differentiation, neurotransmitter, and chemical mediator by binding to G-protein coupled type of histamine receptors (H1R, H2R, H3R and H4R) [2]. It is synthesized from L-histidine by the catalytic enzyme L-histidine decarboxylase (HDC) and is mainly produced largely in two types of cells: professional histamine producing cells and non-professional histamine producing cells [3]. The quantities of histamine synthesized and released by non-professional histamine producing cells about 1000-fold less than that produced by

Abbreviations: EGCG, epigallocatechin gallate; SLC22A3, solute carrier 22A3; HDC, L-histidine decarboxylase; CAFs, cancer-associated fibroblasts; aFMH, alpha-fluoromethylhistidine; TME, tumor microenvironment; TAS2Rs, bitter taste receptors (type II taste receptors); RT-PCR, reverse-transcription polymerase chain reaction.

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SLC22A3 (organic cation transporter-3) in a concentration dependent manner [4–6]. Recent studies have shown that some cancer cells, such as melanomas, cholangiocarcinoma, and small cell lung carcinomas in humans, express HDC and release histamine from non-professional histamine producing cells [7–10]. Additionally, they are known to express histamine receptors, although differences in the types and levels of histamine receptors have been observed [11]. H2R blockage [12] or upregulation of SLC22A3 [13] was found to improved overall survival in patients with head and neck squamous cell carcinoma. Histamine released from cancer cells activates the histamine receptors expressed in tumor cells, immune cells and cancer-associated fibroblasts (CAFs) located within the tumor microenvironment (TME) [14]. CAFs express a high level of TGFβ1, which induces the epithelial to mesenchymal transition (EMT) [15].

(−)-Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea and is a strong inhibitor of HDC in vitro [16,17]. Additionally, it is reported to act as an inhibitory compound in several cancer cell lines, including oral cancer cells [18]. However, its inhibitory effect on HDC activity with EGCG treatment in vivo has not been clarified so far. EGCG elicits a strong bitterness in human. Bitter compounds are detected by bitter taste receptors (type II taste receptors: TAS2Rs), which are primarily expressed in the tongue; they were recently reported to be expressed in extraoral tissues and cancer cells and are known to exist as 25 subtypes in humans [19]. Furthermore, they increase the intracellular Ca^{2+} concentration through G-protein-coupled receptor signaling [20]. EGCG was reported to activate TAS2R14 and TAS2R39, which was confirmed by the increase in the concentration of intracellular Ca^{2+} in HEK293T cells that genetically overexpressed both TAS2Rs and Ga16gut44 [21]. However, the effects of EGCG on the production and release of histamine and its receptors are not been well understood.

The aim of this study was to investigate the expression levels of HDC, histamine receptors, and SLC22A3 to evaluate the extent of histamine production following the stimulation of oral cancer cell lines with TGFβ1 or EGCG.

2. Material and methods

2.1. Reagents

Dulbecco's Modified Eagle Medium (DMEM) was obtained from Wako Pure Chemical, Japan. Fetal bovine serum (FBS) and penicillin/streptomycin/amphotericin B solution were purchased from Sigma-Aldrich (St Louis, MO, USA). The oral cancer cell lines, HSC4, HSC3, HSC2 and SAS were obtained from Japan cell repository bank in Japan. Antibodies against histidine decarboxylase, SLC22A3 (also called organic cation transporter 3, histamine transporter), human bitter taste receptor 14 (TAS2R14), H1R, H2R, H3R, H4R, and β-actin were obtained from Abcam (Cambridge, UK). HRP conjugated goat-anti-rabbit immunoglobulin (Ig)G secondary antibody was obtained from Cell Signaling Technology, USA. TGFβ1 was purchased from R&D Systems (Minneapolis MN, USA). Epigallocatechin gallate hydrate was obtained from Tokyo Chemical Industry, Japan. aFMH was obtained from TRC Toronto Research Chemicals INC (Toronto, Canada).

2.2. Cell culture and treatment of cells

Oral cancer cell lines (HSC4, HSC3, HSC2, and SAS) were cultured in DMEM supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), amphotericin B (250 ng/ml), and heat-inactivated FBS in a humidified atmosphere of 5% CO^2 at 37 °C. The cells were exposed to TGFβ1 (10 ng/ml) for 24 h using an equivalent volume of PBS for the controls. The cells were then exposed to EGCG (100 μM) for 24 h and an identical volume of DMSO for the controls because EGCG was dissolved in DMSO.

2.3. Western blotting

The cell pellets (1X10 [6] cells) obtained from oral cancer cell lines were lysed in RIPA buffer (Thermo Scientific/pierce, USA) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, MO, USA) and phosphatase inhibitors (Sigma-Aldrich, MO, USA). After 10 min on ice, the soluble lysates were collected following centrifugation at 14,000g for 20 min at 4 °C, and boiled for 10 min in Laemmli sample buffer. The total soluble proteins (30 μg) were separated via 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using β-mercaptoethanol as a reducing agent. Following electrophoresis, the proteins were transferred from the gels onto a PVDF membrane (Whatman™, GE Healthcare Life Sciences, NJ, USA) with blotting buffer and blocked in 5% milk powder/phosphate-buffer saline (PBS)-T (PBS-0.05% Triton X-100) for 3h at room temperature. The membranes were incubated with primary antibodies against HDC (1:1000), SLC22A3 (1:1000), H1R (1:1000), H2R (1:1000), H3R (1:1000), H4R (1:1000), TAS2R14 (1:1000) and β-actin (1:2000, which was used as a loading control), followed by the secondary antibodies. Immunological detection was performed using Chemi-Lumi One Ultra (Nacalai, Japan). Images were obtained using ImageQuant LAS500 (GE Healthcare, Bioscience AB, Uppsala, Sweden). Densitometric evaluations of the signal intensities were performed using the Image J software. The ratios of the densities of the proteins to that of β-actin reflected their relative abundance in each lysate.

2.4. Measurements of histamine concentration in cell culture medium

The oral cancer cell lines were cultured in a 6-well chamber. After achieving 80% confluency, EGCG (100 μM) or DMSO (equivalent volumes) was added to the culture medium and the cells were incubated for 24h. The cell culture media were collected and analyzed. The concentration of histamine in the cell culture medium was analyzed using a histamine test kit (Bertin Pharma, France).

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the cells using the ISOGEN (Nippon Gene, Japan) according to manufacturer's instruction. Total RNA samples were subjected to recombinant DNase I (TaKaRaBio, Japan) digestion to avoid the contamination of the genomic DNA. Total RNA was reverse transcribed using the PrimeScriptRT Master Mix (TaKaRaBio Technology), and PCR was performed using Premix Taq (Takara Taq Ver.2.0, TaKaRaBio Technology). The specific PCR primers are listed in Table 1.

Table 1
PCR primers for RT-PCR.

Primer names	Sequence	Amplicon size
TAS2R14-Forward	5′-TTCCCAGCTTTATTTGCCACTGA-3′	98bp
TAS2R14-Reverse	5′-CCGAGGCCTGTAGCCTAACCAGA-3′	
TAS2R39-Forward	5′-TGAGATCAATGTGGTCGGTCTG-3′	141bp
TAS2R39-Reverse	5′-GACCCTGTGGCATTGCTTC-3′	
GAPDH-Forward	5′-GCACCGTCAAGGCTGAGAAC-3′	138bp
GAPDH-Reverse	5′-TGGTGAAGACGCCAGTGGA-3′	

bp, base pairs.

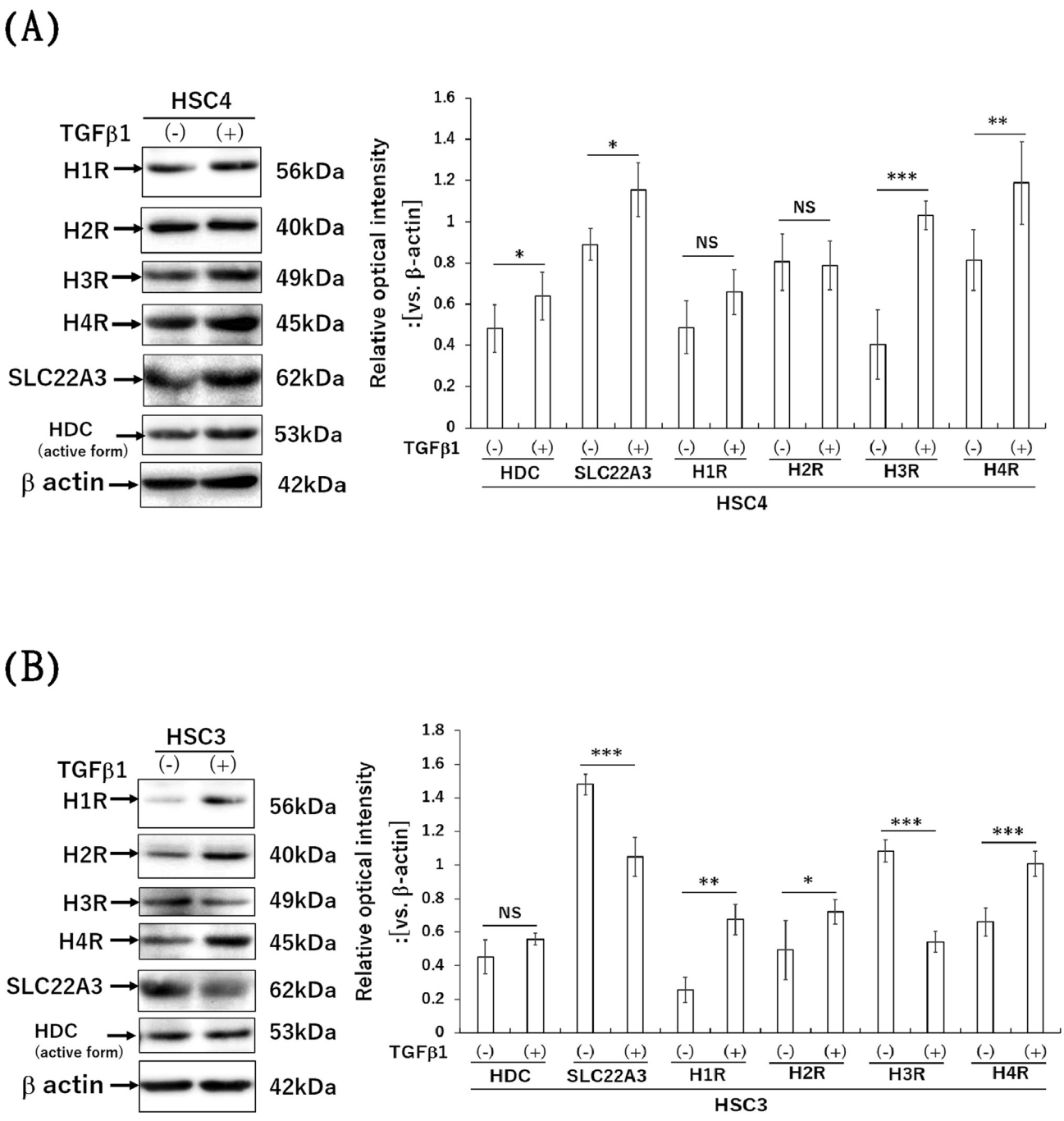

Fig. 1. Expression of human histidine decarboxylase (HDC), SLC22A3, and histamine H1~H4 receptors in oral cancer cell lines with or without TGFβ1 (10 ng/ml) determined by Western blot analysis. Oral cancer cell lines (HSC4, HSC3, HSC2, and SAS) were cultured in DMEM in the presence or absence of TGFβ1 for 24 h. Total cell extracts were prepared with RIPA buffer containing protease inhibitors and phosphatase inhibitors. (A) HSC4 cells were stimulated with TGFβ1 (10 ng/ml) for 24 h. (B) HSC3 cells were stimulated with TGFβ1 (10 ng/ml) for 24 h. (C) HSC2 cells were stimulated with TGFβ1 (10 ng/ml) for 24 h. (D) SAS cells were stimulated with TGFβ1 (10 ng/ml) for 24 h. (A)~(D) indicates representative Western blots performed using the different antibodies. β-actin was used as an internal control. Data were statistically analyzed using independent *t-test* and bars represent means ± SEM (n = 4). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

2.6. Statistical analyses

All experiments were conducted at least four times. Bars present the means ± standard error of the means (SEM). Differences between two groups were analyzed using the student's unpaired *t*-test. For more than two groups, analysis of variance was performed using Tukey's multiple comparison test with SPSS Statistics 24 software (IBM: Armonk, NY, USA). The significance was set at $p < 0.05$.

3. Results

3.1. The expression of HDC, SLC22A3, and histamine receptors is regulated by TGFβ1 in oral cancer cell lines

HDC was detected by Western blotting in all the oral cancer cell lines. HSC4 and SAS cells showed significant induction of the active form of HDC (53 kDa), whereas no significant difference in between the absence and the presence of TGFβ1 in the HSC3 and HSC2 cells

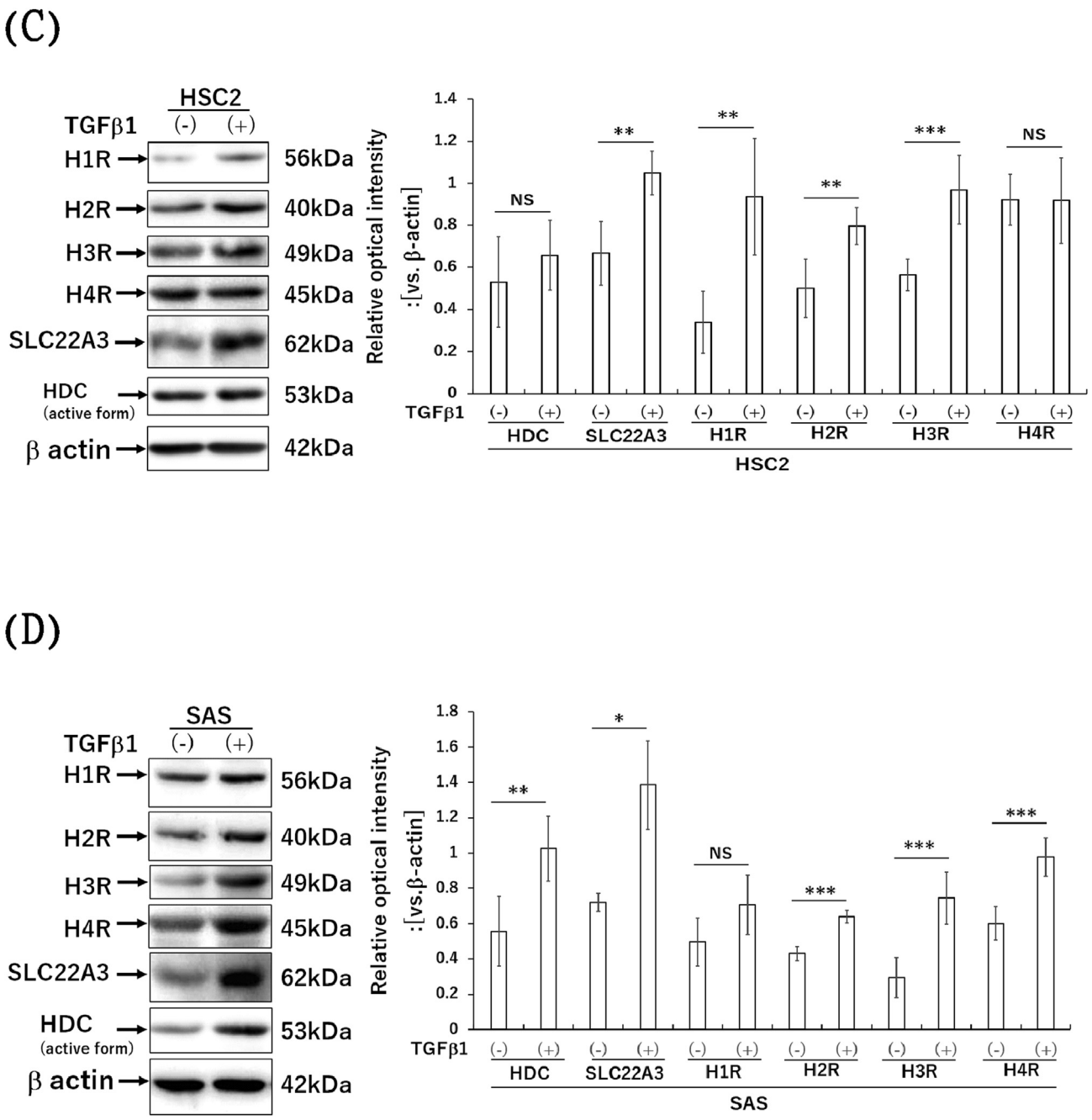

Fig. 1. (*continued*).

exhibited (Fig. 1A–D). All four cell lines expressed SLC22A3. HSC4, HSC2, and SAS cells showed significant induction of SLC22A3 in response to TGFβ1, whereas HSC3 cells demonstrated a significant reduction in SLC22A3 expression (Fig. 1A–D). HSC3, HSC2, and SAS cells presented significant increase in the expression of H1R in response to TGFβ1 (10 ng/ml), whereas HSC4 cells expressed comparable quantities in the absence and presence of TGFβ1. Alternatively, the HSC3, HSC2, and SAS cells expressed significant decreases of H2R in response to TGFβ1, whereas HSC4 cells indicated comparable quantities of H2R protein. HSC4, HSC2, and SAS cell lines presented with a significant induction of H3R in response to TGFβ1, whereas a significant decrease in H3R was observed in the HSC3 cells. Furthermore, a significant increase in the expression level of H4R was observed in the HSC4, HSC3, and SAS cells in response to TGFβ1; alternatively, the HSC2 cells presented with comparable quantities of the protein (Fig. 1A–D).

3.2. EGCG downregulated the expression levels of histamine H1R and SLC22A3 in the oral cancer cell lines

HSC4 cells showed a significant decrease in the expression levels of H1R, H3R, and SLC22A3 following EGCG treatment (Fig. 2A). Likewise, the HSC3 cells showed significant decreases in the expression levels of H1R, H2R, H3R, and SLC22A3 following EGCG treatment (Fig. 2B). Significant decreases in the levels of H1R~H4R were observed in the HSC2 cells (Fig. 2C), whereas significant decreases in the levels of H1R, H4R, and SLC22A3 were observed in the SAS cell following EGCG treatment (Fig. 2D). EGCG negatively

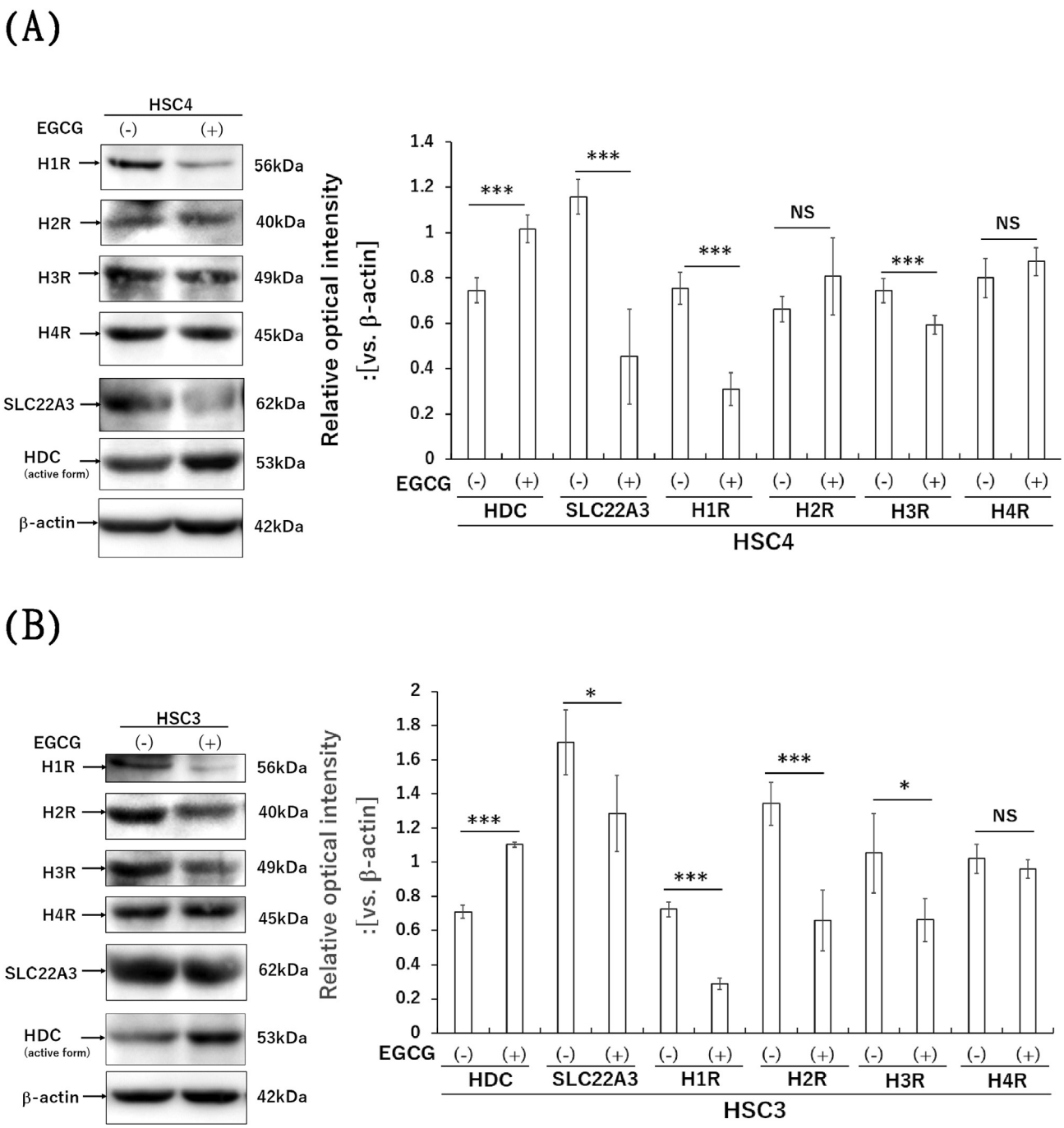

Fig. 2. Expression of HDC, SLC22A3, and histamine receptors in oral cancer cell lines with or without EGCG (100 μM) as determined by Western blot analysis. The oral cancer cell lines (HSC4, HSC3, HSC2, and SAS) were cultured in DMEM in the presence or absence of EGCG (100 μM) for 24 h. Total cell extracts were prepared with RIPA buffer containing protease inhibitors and phosphatase inhibitors. (A) HSC4 cells were stimulated with EGCG (100 μM) for 24 h. (B) HSC3 cells were stimulated with EGCG (100 μM) for 24 h. (C) HSC2 cells were stimulated with EGCG (100 μM) for 24 h. (D) SAS cells were stimulated with EGCG (100 μM) for 24 h. (A)~(D) indicates representative Western blots performed using the different antibodies. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

regulated the protein expression of H1R in the four cell lines; there were differences of regulation in H2R, H3R, H4R, and SLC22A3 protein expression among HSC4, HSC3, HSC2, and SAS cells.

3.3. EGCG induced the expression of HDC and increased histamine production

Cancer cells secrete most of the histamine into the medium after production due to the lack of an apparent organelle for histamine storage. The concentrations of histamine were found to be ~500 femto-mol/ml, indicating release of histamine as a non-professional histamine producing cells when compared to that in professional histamine producing cells (Fig. 3). Histamine was not detected in cultured media that did not contain cancer cells. To verify the production of histamine in the cells, alpha-fluoromethylhistidine (aFMH; 10 μM), which was reported as a strong inhibitor of HDC [16], was used in vitro experiments. The HSC4 and SAS cells, but not the HSC3 and HSC2 cells, presented

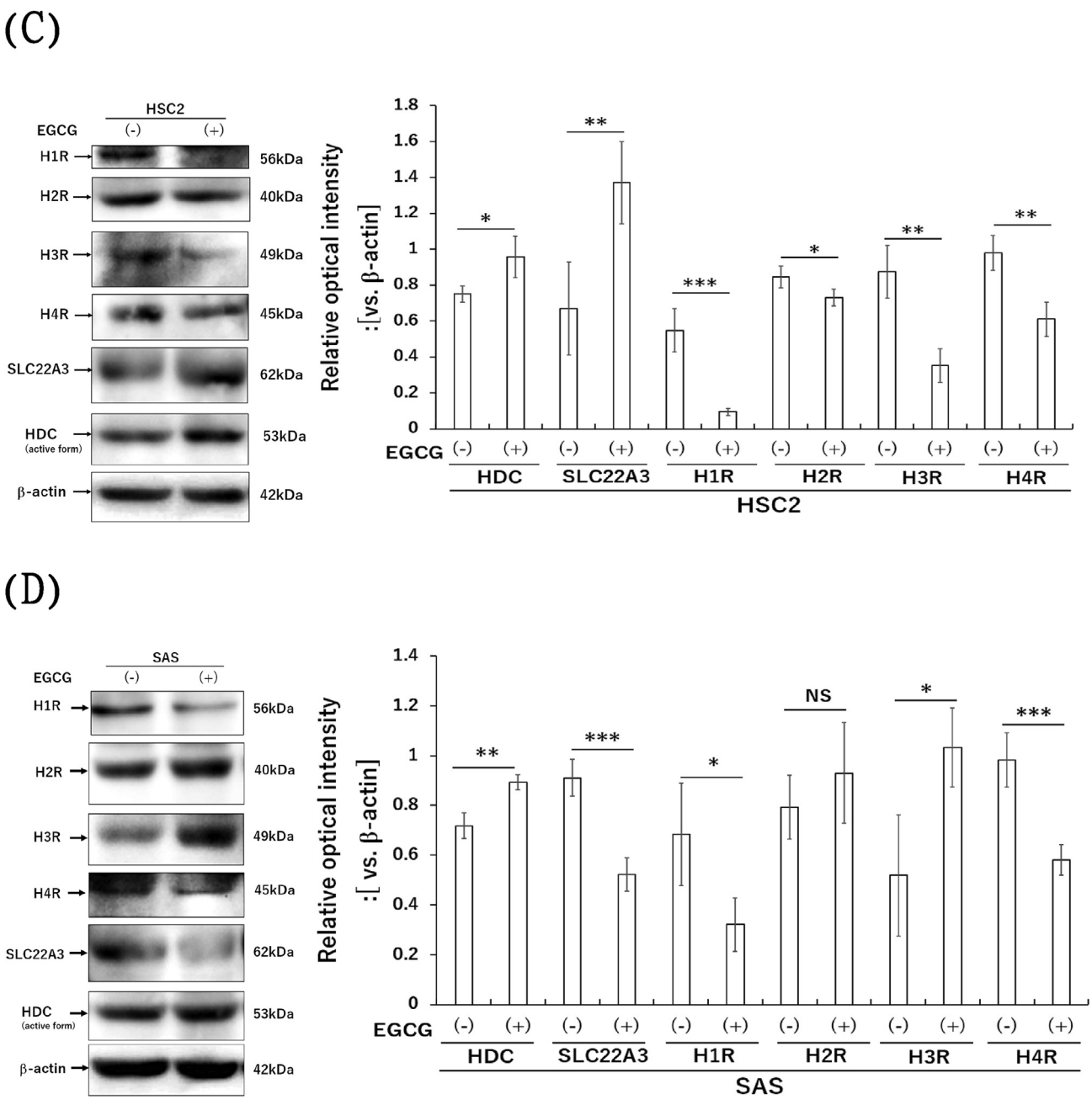

Fig. 2. (*continued*).

with a significant reduction in histamine (Fig. 3). However, the cells stimulated with EGCG were found to produce 2–2.5 times higher amounts of histamine in the HSC4, HSC3, HSC2, and SAS cells (Fig. 3), although EGCG is reported to act as a strong inhibitor of the recombinant human HDC protein [17,18]. Therefore, we analyzed the expression of HDC after exposure to EGCG (100 μM) for 24h. EGCG significantly induced HDC expression in the four oral cancer cell lines, indicating that it could not inhibit HDC activity (Fig. 2), which is consistent with the report that EGCG exhibits difficulty in absorbing into cells [19], EGCG could target other molecules in signaling pathways for HDC production.

3.4. EGCG regulated TAS2R14 protein expression

The mRNA expression of TAS2R14 detected in the four cell lines (Fig. 4A), whereas that of TAS2R39 was not observed (data not shown). Likewise, the protein expression of TAS2R14 was detected in all cell lines; SAS cells exhibited the highest quantities of TAS2R14 protein (Fig. 4A). The protein expression levels of TAS2R14were significantly increased in the HSC4, HSC3, and SAS cells, and significantly reduced in the HSC2 cells following EGCG treatments (Fig. 4B).

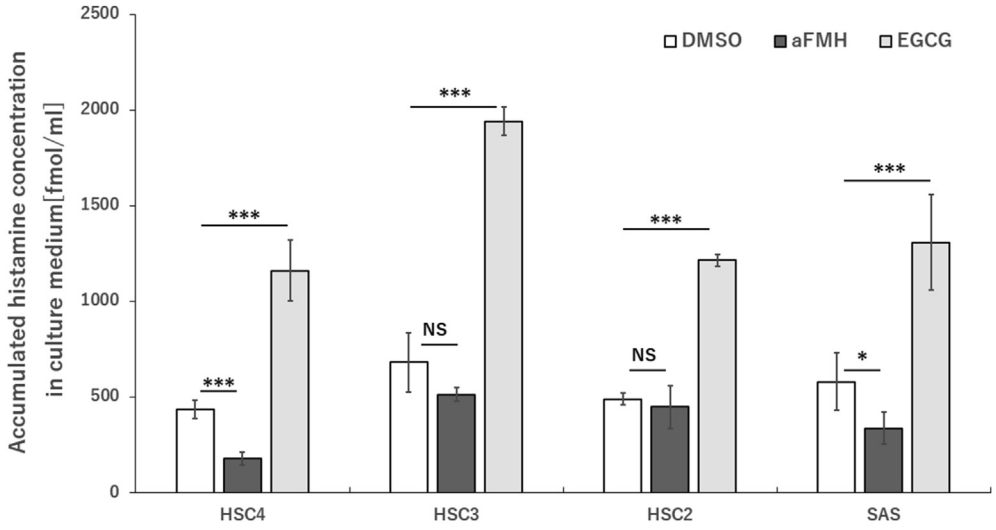

Fig. 3. Effects of aFMH and EGCG on histamine concentrations in the cell culture medium. Oral cancer cell lines were cultured in DMEM. Each cell was exposed to aFMH (10 μM) or EGCG (100 μM) for 24 h. The cell culture medium was collected and the histamine concentrations were analyzed. As a negative control, histamine concentration of medium was determined by enzyme immune assay. Data are statistically analyzed by student's *t*-test and bars represent means ± SEM (n = 4). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

4. Discussion

In the current study, we investigated the effects of TGFβ1 and EGCG on histamine production and the expression levels of the enzyme, transporter, and receptors involved in four oral cancer cell lines. Significant stimulation of HDCs by TGFβ1 (10 ng/ml) was observed in the HSC4 and SAS cells. Histamine production was confirmed by determining the concentration of histamine from the four oral cancer cell lines. The protein expression levels of the histamine transporter SLC22A3 were increased in the HSC4, HSC2, and SAS cells, and decreased in the HSC3 cells, following treatment with TGFβ1. The four cell lines expressed H1R~H4R, although differences in the protein expression levels and responses to TGFβ1 were observed among the cell lines. EGCG increased HDC protein expression in all four cell lines; alternatively, the expression level of SLC22A3 was decreased in all the cell lines, except for HSC2, where it was found to be increased. H1R was significantly decreased by EGCG in the four cell lines. EGCG is an agonist for the bitter taste receptors, TAS2R14 and TAS2R39 [22]; therefore, the mRNA and protein expression levels of these receptors were examined in the four cell lines. The mRNA and proteins of TAS2R14 were detected in all four cell lines, whereas TAS2R39 mRNA was not detected at the mRNA levels. Furthermore, the protein expression level of TAS2R14 was increased in the HSC4, HSC3, and SAS cells, and decreased in the HSC2 cells following EGCG.

To the best of our knowledge, this is the first report on the regulation of histamine biosynthesis and its receptors, including HDC and SLC22A3 transporter, in oral cancer cells following treatment with TGFβ1 and EGCG (Figs. 1 and 2).

The production of histamine has been reported in a variety of non-professional histamine synthesizing cancer cells, including melanoma, breast, cholangiocarcinoma, small lung cell carcinoma and colon cancer cells [7–10]. Histamine is a well-known mediator of immunological reactions and performs a variety of functions via autocrine and paracrine mechanisms, which affect several types of cells such as CAFs, macrophage, monocytes, lymphocytes, and mast cells in the TME [11,14]. Most studies on the role and significance of histamine production focused on cancer cell lines and the pathology involved. Only a few studies conducted an in vivo recapitulated experimental analysis; the detailed pathophysiological roles of histamine production in tumor cells were analyzed in melanoma and cholangiocarcinoma cells and histamine production was reported to increase cell proliferation and invasion due to the inhibition of the usage of the HDC-specific inhibitor both in vitro and in vivo [8,9].

EGCG increased histamine production via HDC induction in the four cell lines (Fig. 3), although EGCG is known to be a strong inhibitor for HDC, suggesting that EGCG does not act on HDC but interacts with cell surface molecules such as receptors, because EGCG showed resistance to enter the intracellular space [35]. The significance of histamine production in oral cancer cells must be considered from the point of tumor and TME. Histamine acts not only on cancer cells but also on lymphocytes or mast cells in TME. Mast cells as professional histamine producing cells release much higher amounts of histamine and express H1R and H4R, which are activated by histamine followed by the increase of histamine release and migration, and furthermore, Th1 lymphocytes expressing H2R are involved in Th1 hypersensitivity reaction [23]. It should be crucial points to discuss that cancer cells producing histamine activate immunological cells in TME and that activated immunological cells may participate in accelerations of cancer cells proliferation or migration. Additional in vivo studies using animal models would facilitate a more comprehensive understanding.

EGCG is known to play an inhibitory role in cancer cells [19]. The down-regulation of histamine receptors and SLC22A3 by EGCG in the oral cancer cell lines in the present study (Fig. 2) might suggest that EGCG has a direct or indirect interaction with histamine receptors and transport system.

In the present study, the expression levels of the histamine receptors following the treatment of the cancer cells with TGFβ1 and EGCG were evaluated. Our findings might be in accordance with a previous clinical study that the usage of H2R antagonist showed improved prognosis [15]. The effectiveness of H3R antagonists in breast cancer and glioblastoma has been demonstrated in vitro

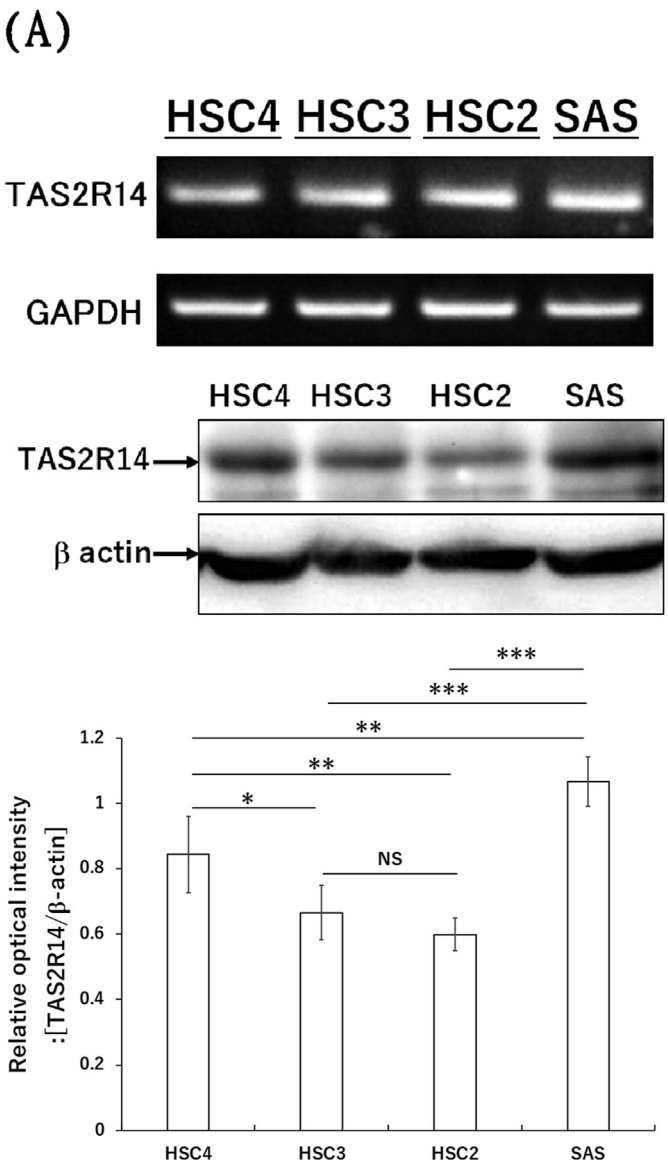

Fig. 4. Bitter taste receptor, TAS2R14 was expressed and regulated by EGCG. (A) TAS2R14 mRNA was detected by RT-PCR. HSC4, HSC3, HSC2, and SAS cells were cultured in DMEM. Total RNA was isolated, followed by DNase I digestion and reverse transcription. PCR was performed using TAS2R14, and glyseraldehyde-3-phosphate dehydrogenase (GAPDH)-specific primers. **(B) TAS2R14 protein expressions were induced by EGCG.** Oral cancer cell lines (HSC4, HSC3, HSC2, and SAS) were cultured in DMEM in the presence or absence of EGCG (100 μM) for 24 h. Total cell extracts were prepared with RIPA buffer containing protease inhibitors and phosphatase inhibitors. The results indicate representative Western blots using the TAS2R14 antibody. β-actin was used as an internal control. Data were analyzed by student's *t*-test and bars represent means ± SEM (n = 4). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

[24,25]. H3R antagonists might be effective in oral cancer growth inhibition based on the significant induction of H3R following TGFβ1 treatment in the three cell lines. The further study on migration or proliferation using H3R antagonist in oral cancer cells are required.

There is an interesting report that histamine demonstrated bivalent behaviors for regulation of tumor growth. In terms of tumor growth, the response at a lower concentration of histamine in the TME is distinct from the response at a higher concentration. For example, in the human pancreatic carcinoma PANC-1 cell line, low concentration (0.01 μM) of histamine increased tumor cell proliferation, whereas, at high concentrations (10 μM), it decreased cell proliferation [26]. In the current study, EGCG increased histamine production and its spontaneous release (1–2 nM) indicated a relatively lower concentration of histamine comparable to the study with a lower concentration of histamine in the pancreatic cancer cell line. Further investigations on cell proliferation and migration are needed to elucidate the histamine concentration dependency, using oral cancer cells with a genetic deletion of HDC or in the presence of an HDC inhibitor. The bivalent functions of histamine might be explained by the different affinities of the histamine receptors. Both H1R and H2R showed ~1000 fold less

affinity for histamine compared to H3R and H4R [3]. The roles of histamine production and the effectiveness of the H2R antagonist could be explained by the histamine concentration and expression levels of histamine receptors; in the presence of the H2R antagonist, higher concentrations of histamine could stimulate H3R and H4R and inhibit tumor proliferation and progression.

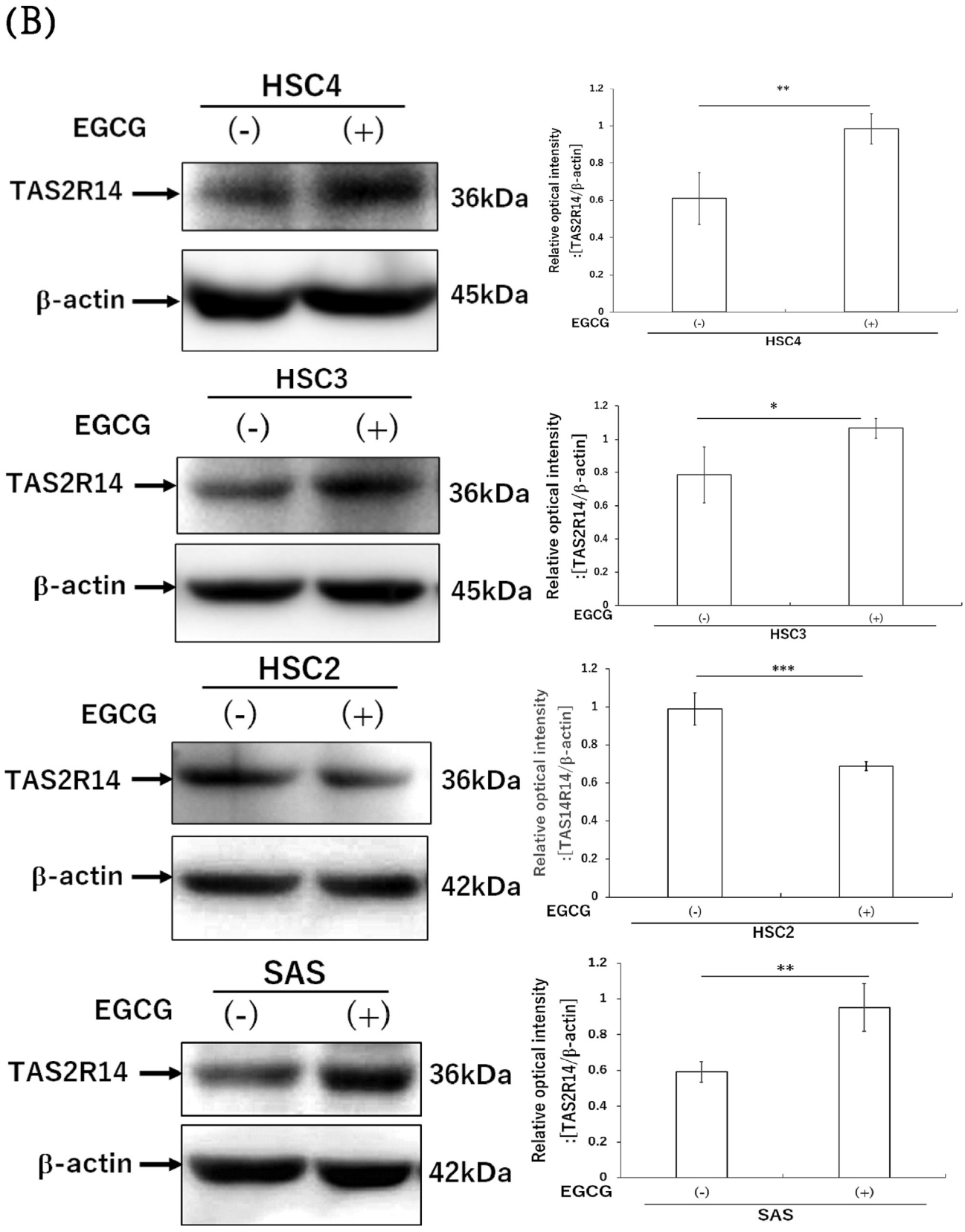

Fig. 4. (*continued*).

SLC22A3, which is ubiquitously expressed in different tissues, is involved in the transportation of histamine and acts as a prognostic surrogate marker of glioblastoma multiforme, human hepatocellular carcinoma, colorectal carcinoma, and higher chemotherapy sensitivity in kidney carcinoma cells [27–31]. SLC22A3 on chromosome 6q26-q27, are physiologically imprinted and under epigenetic regulation. Altered imprinted genes in the region result in decreased mRNA expression in squamous cell carcinoma of the head and neck [32,33]. Furthermore, the SLC22A3 mRNA is subjected to post-transcriptional modification of the RNA nucleotides (RNA editing) in familiar esophageal squamous cell carcinoma [34]. To the best of our knowledge, this is the first study to report the effects of TGFβ1 and EGCG on SLC22A3 expression. The difference in the expression levels among various cell types in this study might reflect the different signaling pathways of TGFβ1 and multiple targets of EGCG in cancer cells.

EGCG showed poor bioavailability and difficulty in entering the cells [35], although it is known to perform a variety of functions in cancer cell lines [19]. The inductions of TAS2R14 protein expression following EGCG treatment in three cell lines suggest EGCG might be involved in transcription/translation and/or protein stability of TAS2R14 directly or indirectly. The increase in HDC expression and H1R down-regulation by EGCG would be explicable by TAS2R14-mediated signaling pathways. The further experiments using specific TAS2R14 inhibitor or siRNA for TAS2R14 are required to confirm TAS2R14-mediated direct action.

5. Conclusions

Oral cancer cell lines produced histamine and expressed HDC, the histamine transporter, SLC22A3, and histamine receptors (H1R~H4R). TGFβ1 and EGCG induced the expression of HDC, whereas EGCG decreased the expression levels of SLC22A3 and histamine receptors in some of the oral cancer cell lines, might prove useful for oral cancer therapy during histamine regulation.

Ethical approval

This research does not include new human data or tissue that require ethical approval and consent.

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CRediT authorship contribution statement

Masashi Kon: Data collection. **Taichi Ishikawa:** Critical assessment of data. **Yu Ohashi:** Research planning. **Hiroyuki Yamada:** Research planning. **Masahito Ogasawara:** Research planning, technical support, and manuscript writing.

Conflict of interest

The authors declare that they have no potential conflicts of interest.

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