Histamine H_4 receptor signalling in tongue cancer and its potential role in oral carcinogenesis

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Abstract

Purpose

Recent reports indicate that histamine and its novel, high-affinity histamine H_4 receptor (H_4R) play a role in carcinogenesis, and thus H_4R signalling has become a focus of increasing interest in the pathogenesis of many cancers. The roles of H_4R in oral epithelial dysplasia (OED), and oral tongue squamous cell carcinomas (OTSCC) are unknown. The purpose of this study was to assess H_4R expression in OTSCC patients and in cancer cell lines.

Methods

Biopsies taken from OED, OTSCC, and healthy oral mucosa were studied by immunostaining. Primary human oral keratinocytes (HOKs) and two cancer cell lines (HSC-3 and SCC-25) were used for the in vitro studies. Quantitative

real-time polymerase chain reaction was used to measure oncogene expression in the stimulated HOKs.

Results

H_4R-immunoreactivity was significantly reduced in OED and OTSCC samples, especially in the samples that had higher histopathological grades, which were also associated with noticeably increased mast cell counts. The presence of H_4R in HSC-3 cells had clearly waned, in contrast to the HOKs. Gene expression data indicated that histamine-relevant inflammatory and environmental elements may participate in the regulation of some studied oncogenes.

Conclusions

The results suggest a possible association between H_4R and oral carcinogenesis. Furthermore, our findings raise a potential implication of histamine-mediated factors in the regulation of oncogenes, possibly via mast cells, as crucial components of tumor microenvironment. The identification of new elements that govern oral cancer development is highly important for the development of novel therapeutic approaches in OTSCC.

Keywords

Histamine H_4 receptor
Histamine
Mast cells
Oral epithelial dysplasia
Oral tongue squamous cell carcinoma
Oral cancer

1. Introduction

Cancer is a major cause of mortality that accounted for more than 500,000 deaths in the United States in 2015 [1]. Oral cancer is the most common form of head and neck malignancy and the 11th most common cancer in the world with a high mortality rate due to late diagnosis and early metastases [2 3]. Among oral cancers, oral tongue squamous cell carcinoma (OTSCC) is the most frequent type and it has high metastasis and invasion propensities [4 5]. Uncontrolled cell proliferation is one of the major molecular process in tumour development, and therefore the identification of molecular targets that could inhibit, halt, or arrest the proliferation would be advantageous [3 4 5].

Histamine [2-(4-imidazolyl)-ethylamine] is a pleiotropic biogenic amine that has been shown to mediate different pathophysiological mechanisms, including proliferation of various normal and malignant cells [6 7 8 9 10]. It is noteworthy that many cancer cell lines and human neoplasia exhibit deranged levels of histamine and its synthesizing enzyme, L-histidine decarboxylase (HDC) [6 10 11 12]. Recently, we reported that oral keratinocytes can synthesize and release histamine, in a biphasic manner, to regulate diverse responses in the oral mucosa [13]. We also showed that histamine metabolism is deranged in oral lichen planus (OLP), which is regarded as a potentially malignant lesion. Histamine exerts its physiological and pathological effects by binding to four distinct G protein-coupled histamine receptors (H_1R, H_2R, H_3R and H_4R). Of these subtypes, H_1R and H_2R have been frequently studied in a variety of tumour cells and tissues [10 14]. However, the expression and distribution of the latest discovered family member, H_4R, and its role in carcinogenesis of OTSCC is still largely unknown.

The discovery of H_4R at the turn of the millennium, and its varying levels of expression within a wide range of tissues, including tumours, have paved the way for novel perspectives in histamine research [15 16]. In fact, H_4R is about 10,000-fold more sensitive to histamine effect compared with the classical H_1R and H_2R, and it has been reported as a crucial player in cancer [17 18 19]. In addition to the hematopoietic and immune cells, H_4R is also expressed in oral epithelial cells where it was found to exhibit a uniform staining pattern [20]. Interestingly, H_4R and its ligand, histamine, modulated the proliferation of cells of a breast cancer cell line [17]. Furthermore, H_4R has been implicated in lung, pancreatic, ovarian and colon cancers, which signify H_4R as a potential target in cancer therapy [21 22]. Owing to its immunomodulatory characteristics and involvement in the growth and progression of many neoplasias, H_4R seems to be one of the most promising histamine receptor-subtypes that might be used as a molecular therapeutic target in cancer management [23]. In fact, mast cells are important players in the tumour microenvironment, and they have been

suggested – being a rich source of histamine – as potent regulators of H_4R [20 24]. In addition to histamine, mast cells also release inflammatory cytokines such as IL-17A, which has been implicated in the progression of OTSCC [25].

Environmental factors can also affect histamine metabolism. We have already shown that oral microbiota, via bacterial lipopolysaccharides (LPS), can affect HDC levels and stimulate the release of histamine from oral epithelial cells [13]. The present study was undertaken to characterize H_4R in oral epithelial dysplasia (OED), OTSCC tissue samples and in two OTSCC cell lines. In addition, the effects of mast cell derived mediators, such as histamine, IL-17A, mast cell releasate (MCR), and also bacterial LPS, on the H_4R expression in OTSCC were studied.

2. Materials and methods

2.1. Patients

Paraffin-embedded oral mucosal biopsies were taken from patients whom had been diagnosed with OED (n = 17) or OTSCC (n = 30) in addition to oral biopsies from healthy donors (n = 17) were obtained from Oulu University hospital. OED samples were diagnosed as moderate dysplasia (dysplasia moderata). OTSCC samples were histopathologically graded according to a three-tier grading scheme as follows: 1) grade I, or low-grade tumour, where the majority of cells are well-differentiated; 2) grade II, or intermediate-grade, where tissues are dominated by moderately-differentiated cells; 3) grade III, or high-grade tumour, which is characterized by poorly-differentiated cancer cells. The use of patient samples was approved by the ethics committee of Oulu university hospital. The clinical and pathological characteristics of the patients are listed in Table 1.

Table 1

The clinical and pathological characteristics of patients with oral epithelial dysplasia and oral tongue squamous cell carcinoma

Characteristic	Cases	%
Oral tongue squamous cell carcinoma (n = 30)		
Age		
≤ 60 years old	6	80
> 60 years old	24	20
Gender		
Male	14	46.6
Female	16	53.3
Grade		
Grade I	10	33.3
Grade II	10	33.3
Grade III	10	33.3
Oral epithelial dysplasia (n = 17)		
Age		
≤ 60 years old	3	17.6
> 60 years old	14	82.3
Gender		
Male	7	41.1
Female	10	58.8

2.2. Immunohistochemical staining

Tissue sections (5 μm) that had been obtained from OED and OTSCC patients and from healthy controls, were treated as described in our recent study [26]. The slides were incubated overnight in: 1) 1 μg/ml rabbit anti-human polyclonal H_4R antibody (LifeSpan Biosciences Inc., Seattle, WA, USA) and 1 μg/ml rabbit anti-human monoclonal mast cell tryptase antibody (Abcam, Cambridge, UK) at +4 °C; 2) biotin-conjugated secondary antibodies (Vector Laboratories;

1:200) for 1 h; 3) avidin–biotin–peroxidase complex (Vector Laboratories; 1:200) for 1 h; 4) diaminobenzidine tetrahydrochloride for 10 min. Counterstaining of the slides was performed using hematoxylin and mounted in Mountex (HistoLab, Gothenburg, Sweden).

2.3. Immunofluorescence staining of cultured cells

The HOKs and HSC-3 cell line cells were cultured on coverslips, washed in PBS and fixed with 4% paraformaldehyde for 30 min. The cells were then permeabilized with 0.5% Triton X-100 in PBS for 10 min. The background was blocked with 1% bovine serum albumin (BSA)-in-PBS. The cells were incubated at +4 °C overnight with 1 μg/ml rabbit polyclonal antibodies that had been raised against human H_4R (LifeSpan Biosciences Inc., Seattle, WA, USA). The cells were then incubated with goat anti-rabbit secondary antibodies conjugated with Alexa Fluor 488 (1:200, Invitrogen, Carlsbad, CA, USA) for 1 h, diamidino-2-phenylindole (DAPI; Sigma, Steinheim, Germany) and Alexa Fluor-568 Phalloidin for 10 min at RT. The cells were then mounted with ProLong® Gold Antifade Mountant (Thermo Fisher Scientific, MA, USA).

2.4. Cell cultures

Primary normal HOKs (ScienCell, Uppsala, Sweden) and two oral cancer cell lines were used in this study. The HOKs were cultured in oral keratinocyte medium (ScienCell, Uppsala, Sweden). The oral cancer cell lines were prepared thus: the highly-invasive human tongue squamous cell carcinoma HSC-3 cell line (JCRB Cell Bank; Osaka National Institute of Health Sciences, Osaka, Japan) and the relatively less-invasive SCC-25 cell line (ATCC, Rockville, MD, USA) were cultured in Dulbecco's Modified Eagle with Nutrient Mixture F-12 Medium, 10% foetal bovine serum, penicillin-streptomycin, and 0.1% hydrocortisone (Life Technologies, Grand Island, NY, USA). Confluent cells were detached, counted and plated in cell culture well-plates (BD Falcon, Lawrence, KS, USA).

2.5. Stimulation of cultured HOK cells

To explore the putative role of mast cell derived mediators and LPS in oral carcinogenesis, the HOKs were incubated at a density of ~100×10^3 cells per ml for 24 h with: 1) 90 μg/ml MCR (supernatants from activated mast cells). Mast cells were obtained as described in [27]. The MCR was prepared as follows: At week 9 of culture, MCs at a concentration of 2 × 106 MCs/ml were activated by 1 μM calcium ionophore A21387 (Sigma-Aldrich) in DPBS. After 30 min of incubation, the MCs were sedimented and the MCR was collected and stored at −80 °C until further analysis; 2) 100 ng/ml IL-17A (R&D Systems, MN, USA); 3) 50 μM histamine (Sigma-Aldrich, MO, USA); 4) 100 ng/ml recombinant human Lipopolysaccharide-binding protein (rhLBP; R&D Systems, Inc., MN, USA) combined with 100 ng/ml *E.coli* ultrapure-lipopolysaccharide (LPS-EB Ultrapure; Invivogen, CA, USA). Pilot experiments were conducted to determine the optimal time slots and concentrations.

2.6. RNA isolation and quantitative real-time polymerase chain reaction [qRT-PCR]

Total RNA was purified from controls and stimulated HOKs, HSC-3 and SCC-25 cells, by using RNeasy Mini-Kit (Qiagen GmbH, Düsseldorf, Germany). QRT-PCR was performed by utilizing SuperScript® First-Strand Synthesis System: 2 μl of cDNA from purified RNA and 250 nM primers in iQ SYBR® Green supermix or in SsoAdvanced Universal SYBR® Green Supermix (Bio-Rad Laboratories Inc.). Primers were synthesized in the core facilities of the University of Helsinki. Sequences of the used primers are listed in Table 2.

Table 2

Human primers used for quantitative real-time PCR

Gene	Forward	Reverse
HRH4	5′-TGG AAG CGT GAT CAT CTC AG-3’	5′-ATA TGG AGC CCA GCA AAC AG-3’
HDC	5′-TTG ATT GCC CTG CTG GCA GC-3′	5′-TGC ACA GAC AAA GAC GGG CAC C-3′
EGF	5′-ATG CGG TTG TTC CTC ACC CG-3′	5′-GCT GGC TGA GCA GAG TTC CA-3′
EGFR	5′-CTC TTC GGG GAG CAG CGA T-3’	5′-AAA GTG CCC AAC TGC GTG AG-3′
Bcl-2	5′-CGC GAC TCC TGA TTC ATT GG-3’	5′-TGC ATA AGG CAA CGA TCC CA-3′
Bcl-xL	5′-GAT CCC CAT GGC AGC AGT AAA GCA AG-3′	5′-CCC CAT CCC GGA AGA GTT CAT TCA CT-3′
GAPDH	5′-AAG GTC ATC CCT GAG CTG AA-3′	5′-TGC TGT AGC CAA ATT CGT TG-3′

RPLP0	5′-GGC GAC CTG GAA GTC CAA CT-3′	5′-CCA TCA GCA CCA CAG CCT TC-3′

HRH4, human histamine H_4 receptor; *HDC*, histidine decarboxylase; *EGF*, epidermal growth factor; *EGFR*, epidermal growth factor receptor;

Bcl-2, B-cell lymphoma 2; *Bcl-xL*, B-cell lymphoma extra-large; *RPLP0*, Ribosomal protein, large, P0; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase

2.7. Imaging and image analysis

A Leica DM6000 microscope (Leica Microsystems, Wetzlar, Germany) was used for imaging the immunostained samples. Representative figures of each immunostaining were selected and presented in the results. Image analysis was performed by ImageJ software (version 1.47; National Institute of Health, Bethesda, MD, USA), which measured the relative intensity of antigen expression in healthy, OED, OTSCC samples.

2.8. Statistical analyses

Statistical analyses were performed using SPSS software programme version 21.0 (IBM SPSS Statistics, SPSS Inc., Chicago, IL, USA). The Student's t test and the One-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison post-hoc test were used. Data are presented as means ± standard deviation. *P* values <0.05 were considered to be statistically significant.

3. Results and discussion

To the best of our knowledge, this is the first study that sheds light on H_4R expression and distribution in OTSCC in relation to its microenvironment. The expression and distribution of H_4R in human oral epithelium have recently been established, and H_4R was found to exhibit a uniform pattern in all epithelial layers, except for the actively-dividing H_4R^- basal cell layer [20 28]. First, we showed that H_4R was deranged and shifted up towards the spinous [prickle] and granular cell layers in OED (Fig. 1a). Such a finding is consistent with our previous report, which showed that H_4R-immunoexpression was downregulated in an oral premalignant lesion – OLP [20].

Fig. 1

Deranged H_4R staining patterns are observed in oral epithelial dysplasia (OED) and oral tongue squamous cell carcinoma (OTSCC) **a.** Immunostained sections from patients with OED show the H_4R^- area at the basal and adjoining suprabasal epithelial cell layers. The upper spinous and granular cell layers are moderately-stained compared with the strongly-positive epithelium in healthy controls **b.** H_4R is inversely associated with the histopathological grade of OTSCC. In grade I samples, the H_4R^- zone is seen at the basal, suprabasal cells and parts of the spinous layers, under faint and irregularly-stained upper epithelial layers ($p = 0.002$). In grade II samples, the H_4R protein is markedly weakened in all epithelial layers and it presents as somewhat scattered spots ($p = 0.005$), until they are difficult to discern in the grade III samples ($p < 0.001$) **c.** H_4R staining intensity was analysed by ImageJ software. All *p*-values are derived from the Student's t test and One-way Analysis of Variance followed by Tukey's post-hoc test (for OTSCC, $n = 30$; for OED, $n = 17$; for healthy controls, $n = 17$)

Interestingly, the H_4R protein staining was intensely reduced in OTSCC in a seemingly grade-dependent manner (Fig. 1b and c). Indeed, compared with the strong and uniform pattern observed for the normal oral epithelium, the H_4R expression in all the pathological grades of OTSCC was considerably weaker. The staining intensity for H_4R clearly decreased with increasing cancer grade. We found that grade I samples showed a H_4R^- zone at the basal, suprabasal and parts of the spinous layers, below weak and irregularly-stained upper epithelial cells. In grade II samples, H_4R staining was markedly weaker in all the epithelial layers and was seen as a few scattered spots, which were difficult to detect in grade III samples. The prognostic value of histopathological grading remains controversial in many tumour types. However, histopathological grading of cancer samples when properly performed, as in breast cancer, could be considered as a simple, affordable, and reliable method to interpret tumour behaviour and patient prognosis [29]. In this regard, it is interesting that the low expression of H_4R was also reported in colorectal carcinomas [22 30], breast cancer cell lines [31] and in gastric carcinomas, where H_4R was negatively associated with higher tumor grades [32].

Noteworthy, H_4R activation induces cell cycle arrest in several cell lines. For example, H_4R-agonist induced cell growth

arrest at G0/G1 phase followed by apoptosis in MDA-MB-231 cancer cells, colon cancer cells, and human hematopoietic progenitor cells [10 22 33]. In this context, the immunostaining of HOKs revealed positive expression of the receptor in most cells, compared with very little or no H_4R-immunoreactivity in HSC-3 cells (Fig. 2a). The SCC-25 cells showed inconsistent staining pattern (not shown). This finding was also supported by the low H_4R and HDC mRNA in cancer cell lines (Fig. 2b). Such a low H_4R profile in cancer cells could in part be related to its "pro-apoptotic" effect as it interferes with their unlimited proliferative capacity, and thus they could "skip" growth inhibition and subsequent apoptosis. This assumption is also supported by the attenuated expression of the H_4R protein and also of the *HRH4* gene in the highly invasive, poorly-differentiated, cancer cell line HSC-3. It is therefore conceivable that H_4R expression in cancer tissue is perhaps related to the degree of cancer cell differentiation and tumour progression [22 31].

Fig. 2

a. H_4R immunoexpression in cultured normal and cancer cell lines. H_4R is positively expressed in most of the normal human oral keratinocytes (HOKs). In contrast, the highly invasive tongue cancer cell line, HSC-3, shows very little or no H_4R-immunoreactivity. **b.** *HRH4* and L-histidine decarboxylase (*HDC*) gene transcripts are downregulated in cancer cell lines. *HRH4* and *HDC* gene transcripts were higher in the HOKs ($p < 0.001$). In contrast, *HRH4* and *HDC* were downregulated in HSC-3 and SCC-25 ($p = 0.005$ and $p < 0.001$, respectively). All *p*-values are derived from One-way Analysis of Variance followed by Tukey's post-hoc test

The microenvironment of the tumour is ~~comprises~~comprised of a wide variety of cells, signalling molecules and extracellular matrix, which are closely related and persistently interact and influence tumour initiation and perpetuation. Among cells surrounding the tumours, mast cells have been implicated in the immune responses to cancer and disease progression [24]. Mast cells are distinguished by their secretory granules that contain a plethora of potent inflammatory constituents, such as histamine. Micromolar histamine has been shown to downregulate the *HRH4* gene expression in oral epithelial cells [20]. This raises the hypothesis that such low expressions of H_4R in OTSCC might have resulted from an inhibitory feedback effect of "burst-released" histamine from local mast cells. Therefore, we assessed the distribution of mast cells in tissue samples of OED and OTSCC by tryptase staining. Only a few mast cells were detected in the healthy controls, but a marked rise in mast cell numbers was observed in the lamina propria and subepithelial connective tissue in OTSCC samples, whereas a moderate increase in mast cell numbers was found in OED (Fig. 3a). Granular staining by tryptase, at the close peri-cellular vicinity, was also noticeable in advanced grades of OTSCC samples (Fig. 3a, grade III).

Fig. 3

a. Mast cell numbers are increased in the vicinity of OTSCC. Mast cell tryptase staining reveals a marked rise in the mast cell count in the lamina propria and the subepithelial connective tissue in OED and OTSCC in marked contrast to only a few positive cells observed in the healthy controls. Advanced grades of OTSCC were associated with mast cell tryptase staining at the peri-cellular areas (grade III) **b.** Mast cell releasate (MCR) downregulated *HRH4* and L-histidine decarboxylase (*HDC*) genes in the human oral keratinocytes (HOKs; $p = 0.030$; 0.010, respectively). The HOKs were incubated with MCR and IL-17A for 24 h to explore the effects of tumorigenic inflammatory milieu on *HRH4* and *HDC* gene expression**.** The *HRH4* and *HDC* genes were downregulated by MCR. All *p*-values are derived from One-way Analysis of Variance followed by Tukey's post-hoc test; for OTSCC, $n = 30$; for OED, $n = 17$; for healthy controls, $n = 17$

The mast cells number was increased in tissues surrounding OTSCC, therefore we explored the effects of mast cell derived mediators on *HRH4* and *HDC* gene expression. The HOKs were incubated with mast cell derived mediators. Our findings showed that MCR downregulated *HRH4* and *HDC* gene transcripts in the HOKs, while IL-17A did not have an effect on the transcriptional profile of either gene (Fig. 3b). However, the HDC protein is expressed in the HOKs in addition to most other studied cancer cell lines in which histamine is released via organic cation transporters in autocrine and paracrine modes [10 13].

Gene analysis assays were performed to ascertain whether mast cell or bacterial derived components influence the expression of oncogenes in the HOKs (Fig. 4; *EGF*, epidermal growth factor; *EGFR*, epidermal growth factor receptor; *Bcl-2*, B-cell lymphoma 2; *Bcl-xL*, B-cell lymphoma extra-large). IL-17 can be found in mast cells, and it has been implicated in the pathogenesis of several tumours, and it has also recently been reported to be a strong promoter of invasion and progression in OTSCC [25]. We found that IL-17A, similarly to MCR, upregulated the expression of *EGF*

and *EGFR* that are associated with oral carcinogenesis [34]. On the other hand, MCR and histamine downregulated *Bcl-2*, whereas *Bcl-xL* gene expression was reduced by MCR only. However, both genes were found to be overexpressed in OTSCC, which suggest that factors, other than those derived from mast cells, are participating in oncogene regulation [34 35]. It is worth remembering that oral cancer develops in a tissue that is continuously exposed to microbiota, thus the bacteria and flora of the oral econiche could participate in the process of carcinogenesis [36]. Kurago et al. showed that LPS, as a model of microbial contamination, has the potential to contribute to oral cancer progression [37]. Moreover, LPS has been suggested to be a regulator of histamine metabolism, and it has been shown to induce histamine production by human keratinocytes [13 38]. Interestingly, the present results show that bacterial LPS induces the expression of the *EGFR* oncogene in HOKs. The mechanisms governing oncogene regulation by LPS in OTSCC should be elucidated in future research.

Fig. 4

Mast cell derived mediators and LPS regulate oncogene expression in the human oral keratinocytes (HOKs) **a** and **b.** MCR and IL-17A induced *EGF* ($p = 0.002$; 0.009, respectively) and *EGFR* ($p = 0.030$; 0.010 respectively) in the HOKs **c** and **d.** In contrast, MCR downregulated *Bcl-2* and *Bcl-xL* ($p = 0.007$) genes. IL-17A did not affect gene expression. **e** and **f.** Bacterial LPS induced both *EGF* and *EGFR* expression in the HOKs ($p = 0.002$, EGFR). Histamine (HA) did not affect the expression of such genes **g** and **h.** Histamine (HA) and LPS downregulated the *Bcl-2* gene ($p = 0.005$, HA) but had a minor effect on *Bcl-xL* gene expression. All *p*-values are derived from One-way Analysis of Variance followed by Tukey's post-hoc test

When taken together, our findings indicate that H_4R expression is altered in OED and it also manifests a grade-dependent decrease in OTSCC. This finding may indicate a possible inverse association between H_4R, tumour development and the prognostic outcome. Furthermore, the results suggest that histamine metabolism plays a role in oral carcinogenesis. However, this study has some limitations including a relatively small sample size and the lack of patient's follow-up data for analysing the possible prognostic value of the H_4R staining. Further studies are needed to understand better the role of H_4R in OTSCC, and whether it could represent a novel biomarker or therapeutic target in oral cancer.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2015. CA Cancer J. Clin (2015). doi: 10.3322/caac.21254

2. R.I. Haddad, D.M. Shin, Recent advances in head and neck cancer. N Engl J Med **359**, 1143–1154 (2008)

3. R. Sankaranarayanan, K. Ramadas, H. Amarasinghe, S. Subramanian, N. Johnson, Oral Cancer: Prevention, Early Detection, and Treatment. In Cancer: Disease Control Priorities, Ed. By H. Gelband, P. Jha, R. Sankaranarayanan, S. Horton (The International Bank for Reconstruction and Development/The World Bank, Washington, DC, 2015) Chapter 5

4. P.W. Yuen, K.Y. Lam, A.C. Chan, W.I. Wei, L.K. Lam, Clinicopathological analysis of local spread of carcinoma of the tongue. Am J Surg **175**, 242–244 (1998)

5. R.S. Patel, J.R. Clark, R. Dirven, R. Wyten, K. Gao, C.J. O'Brien, Prognostic factors in the surgical treatment of patients with oral carcinoma. ANZ J Surg **79**, 19–22 (2009)

6. E.S. Rivera, G.P. Cricco, N.I. Engel, C.P. Fitzimons, G.A. Martin, R.M. Bergoc, Histamine as an autocrine growth factor: An unusual role for a widespread mediator. Semin Cancer Biol **10**, 15–23 (2000)

AQ3

7. Z. Darvas, E. Sakurai, H.G. Schwelberger, H. Hegyesi, E. Rivera, H. Othsu, T. Watanabe, E. Pállinger, A. Falus, Autonomous histamine metabolism in human melanoma cells. Melanoma Res **13**, 239–246 (2003)

8. Z. Pós, H. Hegyesi, E.S. Rivera, *Histamine and Cell Proliferation. In: Histamine: Biology and Medical Aspects, Ed. By a. Falus* (SpringMed Publishing, Budapest, 2004), p. 199

9. M. Dy, E. Schneider, Histamine-cytokine connection in immunity and hematopoiesis. Cytokine Growth Factor Rev **15**, 393–410 (2004)

10. V.A. Medina, E.S. Rivera, Histamine receptors and cancer pharmacology. Br J Pharmacol **161**, 755–767 (2010)

11. M. Garcia-Caballero, E. Neugebauer, R. Campos, I. Nuñez de Castro, C. Vara-Thorbeck, Histamine synthesis and content in benign and malignant breast tumors. Surg Oncol **3**, 167–173 (1994)

12. A. Falus, H. Hegyesi, E. Lazar-Molnar, Z. Pos, V. Laszlo, Z. Darvas, Paracrine and autocrine interactions in melanoma: histamine is a relevant player in local regulation. Trends Immunol **22**, 648–652 (2001)

13. A. Salem, S. Rozov, A. Al-Samadi, V. Stegajev, D. Listyarifah, V.P. Kouri, X. Han, D. Nordström, J. Hagström, K. Eklund, Histamine metabolism and transport are deranged in human keratinocytes in oral lichen lanus. Br. J. Dermatol (2016). doi: 10.1111/bjd.14995

14. M. Grimm, M. Krimmel, D. Alexander, A. Munz, S. Kluba, C. Keutel, J. Hoffmann, J. Polligkeit, S. Reinert, S. Hoefert, Prognostic value of histamine H1 receptor expression in oral squamous cell carcinoma. Clin Oral Investig **17**, 949–955 (2013)

15. E. Tiligada, E. Zampeli, K. Sander, H. Stark, Histamine H_3 and H_4 receptors as novel drug targets. Expert Opin Investig Drugs **18**, 1519–1531 (2009)

16. R.L. Thurmond, The histamine H_4 receptor: from orphan to the clinic. Front. Pharmacol (2015). doi: 10.3389/fphar.2015.00065

17. D.J. Martinel Lamas, E.S. Rivera, V.A. Medina, Histamine H_4 receptor: Insights into a potential therapeutic target in breast cancer. Front Biosci (Schol Ed) **7**, 1–9 (2015)

18. M. Walter, H. Stark, Histamine receptor subtypes: A century of rational drug design. Front. Biosci. (Schol. Ed.) **4**, 461–488 (2012)

19. Y. Konttinen, H. Husu, X. Han, M.B. Passani, C. Ballerini, V. Stegaev, T. Sillat, Z. Mackiewicz, Non-professional histamine producing cells, immune responses and autoimmunity, in Histamine H_4 receptor: a novel drug target in immunoregulation and inflammation, ed. By H. Stark (Versita Ltd, 2013) p 201

20. A. Salem, A. Al-Samadi, V. Stegajev, H. Stark, R. Häyrinen-Immonen, M. Ainola, J. Hietanen, Y.T. Konttinen, Histamine H_4 receptor in oral lichen planus. Oral Dis **21**, 378–385 (2015)

21. G.P. Cricco, N.A. Mohamad, L.A. Sambuco, F. Genre, M. Croci, A.S. Gutiérrez, V.A. Medina, R.M. Bergoc, E.S. Rivera, G.A. Martín, Histamine regulates pancreatic carcinoma cell growth through H_3 and H_4 receptors. Inflamm Res **57**(Suppl. 1), S23–S24 (2008)

22. Z. Fang, W. Yao, Y. Xiong, J. Li, L. Liu, L. Shi, W. Zhang, C. Zhang, L. Nie, J. Wan, Attenuated expression of HRH4 in colorectal carcinomas: A potential influence on tumor growth and progression. BMC Cancer **11**, 1–11 (2011)

23. V.A. Medina, G. Coruzzi, D.J. Martinel Lamas, N. Massari, M. Adami, F. Levi-Schaffer, M. Ben-Zimra, H. Schwelberger, E. S. Rivera, Histamine in cancer, in Histamine H_4 receptor: a novel drug target in immunoregulation and inflammation, ed. By H. Stark (Versita Ltd, 2013) p 259

24. K. Khazaie, N.R. Blatner, M.W. Khan, F. Gounari, E. Gounaris, K. Dennis, A. Bonertz, F.N. Tsai, M.J. Strouch, E. Cheon, J.D. Phillips, P. Beckhove, D.J. Bentrem, The significant role of mast cells in cancer. Cancer Metastasis Rev **30**, 45–60 (2011)

25. T. Wei, X. Cong, X.T. Wang, X.J. Xu, S.N. Min, P. Ye, X. Peng, L.L. Wu, G.Y. Yu, Interleukin-17A promotes tongue squamous cell carcinoma metastasis through activating miR-23b/versican pathway. Oncotarget (2016). doi: 10.18632/oncotarget.14255

26. A. Salem, R. Mustafa, D. Listyarifah, A. Al-Samadi, G. Barreto, D. Nordström, K.K. Eklund, Altered Expression of Toll-like Receptors in Human Oral Epithelium in Oral Lichenoid Reactions. Am. J. Dermatopathol (2016). doi: 10.1097/DAD.0000000000000807

27. J. Lappalainen, K.A. Lindstedt, P.T. Kovanen, A protocol for generating high numbers of mature and functional human mast cells from peripheral blood. Clin Exp Allergy **37**, 1404–1414 (2007)

28. K. Yamaura, A. Shigemori, E. Suwa, K. Ueno, Expression of the histamine H_4 receptor in dermal and articular tissues. Life Sci **92**, 108–113 (2013)

29. E.A. Rakha, J.S. Reis-Filho, F. Baehner, D. Dabbs, T. Decker, V. Eusebi, S.B. Fox, S. Ichihara, J. Jacquemier, S.R. Lakhani, J. Palacios, A.L. Richardson, S.J. Schnitt, F.C. Schmitt, P. Tan, G.M. Tse, S. Badve, I.O. Ellis, Breast cancer prognostic classification in the molecular era: The role of histological grade. Breast Cancer Res **12**, 207 (2010)

30. K. Boer, E. Helinger, A. Helinger, P. Pocza, Z. Pos, P. Demeter, Z. Baranyai, K. Dede, Z. Darvas, A. Falus, Decreased expression of histamine H1 and H4 receptors suggests disturbance of local regulation in human colorectal tumours by histamine. Eur J Cell Biol **87**, 227–236 (2008)

31. V.A. Medina, P.G. Brenzoni, D.J. Lamas, N. Massari, C. Mondillo, M.A. Nunez, O. Pignataro, E.S. Rivera, Role of histamine H_4 receptor in breast cancer cell proliferation. Front Biosci (Elite Ed) **3**, 1042–1060 (2011)

32. C. Zhang, Y. Xiong, J. Li, Y. Yang, L. Liu, W. Wang, L. Wang, M. Li, Z. Fang, Deletion and down-regulation of HRH4 gene in gastric carcinomas: A potential correlation with tumor progression. PLoS One **7**, e31207 (2012). doi: 10.1371/journal.pone.0031207

33. A.F. Petit-Bertron, F. Machavoine, M.P. Defresne, M. Gillard, P. Chatelain, P. Mistry, E. Schneider, M. Dy, H4 histamine receptors mediate cell cycle arrest in growth factor-induced murine and human hematopoietic progenitor cells. PLoS One **4**, e6504 (2009). doi: 10.1371/journal.pone.0006504

34. L.L. Loro, O.K. Vintermyr, A.C. Johannessen, Cell death regulation in oral squamous cell carcinoma: Methodological considerations and clinical significance. J Oral Pathol Med **32**, 125–138 (2003)

35. B. Popović, B. Jekić, I. Novaković, L.J. Luković, Z. Tepavcević, V. Jurisić, M. Vukadinović, J. Milasin, Bcl-2 expression in oral squamous cell carcinoma. Ann N Y Acad Sci **1095**, 19–25 (2007)

36. J.H. Meurman, Oral microbiota and cancer. J. Oral Microbiol **2**, 5195 (2010). doi: 10.3402/jom.v2i0.5195

37. Z.B. Kurago, A. Lam-ubol, A. Stetsenko, C. De La Mater, Y. Chen, D.V. Dawson, Lipopolysaccharide-squamous cell carcinoma-monocyte interactions induce cancer-supporting factors leading to rapid STAT3 activation. Head Neck Pathol **2**, 1–12 (2008)

38. D. Gutowska-Owsiak, L. Greenwald, C. Watson, T.A. Selvakumar, X. Wang, G.S. Ogg, The histamine-