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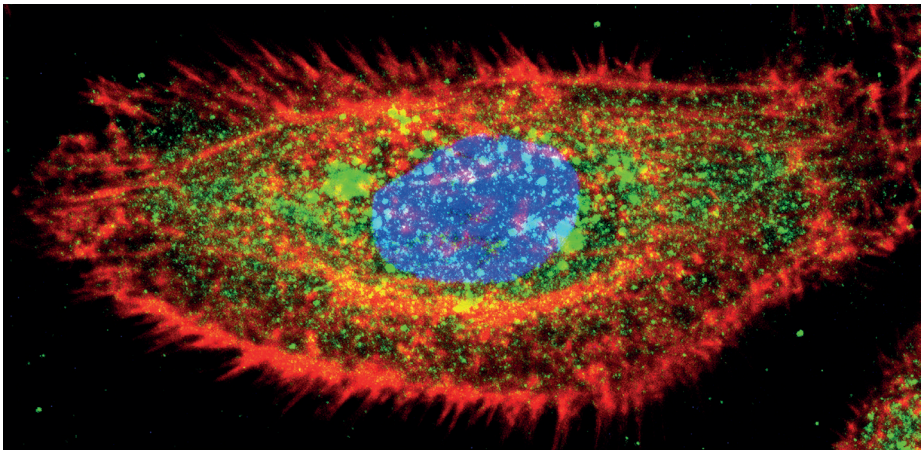
ABDELHAKIM SALEM

Histamine H4 Receptor: A Potential Novel Therapeutic Target in Oral Lichen Planus and Oral Tongue Cancer



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Cover figure:

Human oral epithelial cell expressing Organic Cation Transporter 3 (OCT3). Photo by Author, 2016.

Histamine H4 Receptor: A Potential Novel Therapeutic Target in Oral Lichen Planus and Oral Tongue Cancer

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ACADEMIC DISSERTATION

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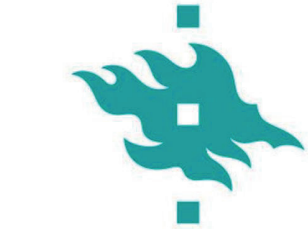

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1. LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis is based on the following original studies:

I. **Salem A**, Al-Samadi A, Stegajev V, Stark H, Häyrinen-Immonen R, Ainola M, Hietanen J, Konttinen YT. Histamine H4 receptor in oral lichen planus. *Oral Dis*. 2015; 21(3):378-385.

II. **Salem A**, Rozov S, Al-Samadi A, Stegajev V, Listyarifah D, Kouri VP, Han X, Nordström D, Hagström J, Eklund KK. Histamine metabolism and transport are deranged in human keratinocytes in oral lichen planus. *Br J Dermatol*. 2017; 176(5):1213-1223.

III. **Salem A**, Mustafa R, Listyarifah D, Al-Samadi A, Barreto G, Nordström D, Eklund KK. Altered Expression of Toll-like Receptors in Human Oral Epithelium in Oral Lichenoid Reactions. *Am J Dermatopathol*. 2017; 39:811-818.

IV. **Salem A**, Almahmoudi R, Listyarifah D, Siponen M, Maaninka K, Al-Samadi A, Salo T, Eklund KK. Histamine H4 receptor signalling in tongue cancer and its potential role in oral carcinogenesis. *Cell Oncol*. 2017; 40(6):621-630.

V. Stegajev V, Kouri VP, **Salem A**, Rozov S, Stark H, Nordström DC, Konttinen YT. Activation of histamine H4 receptor inhibits TNFα/IMD-0354-induced apoptosis in human salivary NS-SV-AC cells. *Apoptosis*. 2014; 19(12):1702-1711.

VI. **Salem A**, Almahmoudi R, Hagström J, Stark H, Nordström D, Salo T, Eklund KK. Histamine Regulates Human β-Defensin 2 Expression in Oral Epithelium: Potential Therapeutic Targets in Oral Lichen Planus (submitted).

2. ABBREVIATIONS

AD	Atopic dermatitis
AOC1	Amine Oxidase, Copper Containing 1
BAX	Bcl-2-associated X protein
Bcl-XL	B cell lymphoma-extra-large protein
BM	Basement membrane
CCA	Cholangiocarcinoma
cDNA	Complementary DNA
CMV	Cytomegalovirus
cTNM	Clinical tumour–node–metastasis
DAMP	Damage-associated molecular pattern
DAO	Diamine oxidase
ddPCR	Droplet digital polymerase chain reaction
EBV	Epstein-Barr virus
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
FFPE	Formalin-fixed, paraffin embedded
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H1R	Histamine H1 receptor
H2R	Histamine H2 receptor
H3R	Histamine H3 receptor
H4R	Histamine H4 receptor
HBD-2	Human Beta-defensin 2
HCV	Hepatitis C virus
HDC	L-histidine decarboxylase
HNMT	Histamine N-methyl transferase
HOKs	Human oral keratinocytes
HPLC	High-performance liquid chromatography
HPV	Human papillomavirus
HSV-1	Herpes simplex virus 1
HSV-6	Human herpesvirus 6
IFN-γ	Interferon gamma
IL-10	Interleukin 10
IL-13	Interleukin 13
IL-17A	Interleukin 17A
IL-17F	Interleukin 17F
IL-6	Interleukin 6
ITGA6	Integrin subunit alpha 6
ITGB4	Integrin subunit beta 4
kDa	Kilodalton

LP	Lichen planus
LPS	Lipopolysaccharides
LPT	Laser phototherapy
MC	Mast cell
MCR	Mast cell releasate
MrgX2	Mas-related gene X2
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NS-SV-AC	Normal submandibular salivary gland-derived acinar cells
OCT1	Organic cation transporter-1
OCT2	Organic cation transporter-2
OCT-3	Organic cation transporter-3
OED	Oral epithelial dysplasia
OKM	Oral keratinocyte medium
OLLs	Oral lichenoid lesions
OLP	Oral lichen planus
OPMDs	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
OTSCC	Oral tongue squamous cell carcinoma
PAMP	Pathogen- associated molecular pattern
PARP	Poly-ADP-ribose polymerase
PRR	Pattern-recognition receptors
PVDF	Polyvinylidene fluoride
PVL	Proliferative verrucous leukoplakia
qRT-PCR	Quantitative real-time polymerase chain reaction
RPLP0	Ribosomal Protein Lateral Stalk Subunit P0
RT	Room temperature
SCC	Squamous cell carcinoma
STAT1	Signal transducer and activator of transcription 1
TAMs	Tumour-associated macrophages
TLRs	Toll-like receptors
TME	Tumour microenvironment
TNF-α	Tumour necrosis factor alpha
TNM	Tumor–node–metastasis
VEGF	Vascular endothelial growth factor
VMAT	Vesicular monoamine transporter
WHO	World Health Organization

Gene symbols are *italicized* in the text in accordance with the Human Genome Organization nomenclature committee (HGNC) guidelines. Gene names used in this book can be found at: http://www.genenames.org/.

3. ABSTRACT

Background and objective: Oral lichen planus (OLP) is a common immune disorder of the oral mucosa, which is categorized as an oral potentially malignant disorder (OPMD) to highlight its potential progression to oral cancer. Oral cancer commonly affects the mobile tongue as oral tongue squamous cell carcinoma (OTSCC), which has dismal prognosis. Histamine signals via four G protein-coupled histamine receptors (H1R-H4R). Classical H1R and H2R medications are ineffective in treating OLP or OTSCC patients. The discovery of H4R has paved the way for novel perspectives in histamine research by modulating immune responses. We therefore hypothesized that H4R is involved in the pathogenesis of OLP and may contribute to oral carcinogenesis.

Materials and methods: Tissue samples from OLP, oral epithelial dysplasia (OED) and OTSCC patients, and from healthy control participants were utilized. The *in vitro* experiments were performed on normal human oral keratinocytes (HOKs), two OTSCC-derived cell lines (HSC-3 and SCC-25), normal salivary gland cells, in addition to supernatants from activated human mast cells (MCs). For *in vitro* internalization and functional assays, two specific H4R ligands (agonist HST-10, and inverse agonist ST-1007) were used. Protein expression of histamine receptors, transporters and metabolizing enzymes, and other antigens were assessed in tissue samples and cell lines by immunohistochemistry and immunofluorescence staining. The expression levels of mRNA were quantified by qRT-PCR and the highly-sensitive droplet-digital PCR technology. Western blotting assays were performed to assess apoptotic markers following H4R stimulation, while flow cytometry was used to study Annexin-V and PI labelling of dead cells. Histamine levels were analysed using high-performance liquid chromatography.

Results: Briefly, H4R is expressed in healthy oral epithelial cells on mRNA and protein levels, and they were able to fully internalize H4R-ligands in a time-dependent manner. In contrast, samples from OLP, OED and OTSCC patients

exhibited lower H4R level, which was negatively correlated with MC-count and OTSCC-grade. We also reported that normal HOKs are histamine-producing cells—fully equipped with histamine synthesizing, transporting and degrading molecules. Interestingly, OLP samples exhibit high levels of the histamine synthesizing and transporting molecules, whereas histamine degrading enzyme was strongly inhibited. HOKs showed a dose-dependent Lipopolysaccharides (LPS)-driven release of histamine, while high histamine levels inhibited epithelial adhesion molecules. We next showed that toll-like receptors (TLRs) are essential players in OLP. TLRs were upregulated in OLP lesions, particularly for TLR4, which is necessary for LPS signalling. Importantly, LPS and MC-mediators regulated several oral oncogenes, while H4R-stimulated cells revealed a marked resistance to apoptosis. Furthermore, LPS and histamine influenced human beta defensin 2 (hBD-2) expression, which was highly induced in OLP. Unexpectedly, hBD-2 protein was subsided in OTSCC tissues with a marked downregulation of its transcript in cancer cells. Histamine synergistically induced TNF-α- and IFN-γ-mediated hBD-2 production in HOKs. Interestingly, targeting H4R seems to regulate TNFα- and LPS-mediated expression of hBD-2.

Conclusions: Briefly, human oral epithelial cells are "non-professional" histamine producing cells—capable to synthesize, release, and degrade low levels of endogenic histamine. High levels of histamine may downregulate H4R as well as key integrity molecules in HOKs and may enhance subsequent bacterial invasion in OLP. In this regard, our findings suggest a potential role of TLRs in OLP pathogenesis, by mediating LPS signalling and enhancing further immune response and histamine production. In addition, our results indicate that histamine/H4R crosstalk signalling with LPS and MCs could in part be involved in OLP and the potential inflammation-driven tumorigenesis. This was further supported by the ability of H4R to regulate cell apoptosis and modulate antibacterial response in HOKs. Further functional and preclinical studies are therefore warranted.

4. INTRODUCTION

Oral lichen planus (OLP) is common immune-mediated oral inflammatory disease that affects about 0.5–2.6% of general population (Kanemitsu 2014; Varghese et al., 2016). Clinically, OLP may present with various white-and-red morphological changes that can wax and wane over an extended period of time (Müller 2011). The most common type of OLP is the reticular form. Other forms of OLP include papular, plaque-like, atrophic, erosive and bullous types. Typically, OLP is found in middle-aged or elderly patients (Kanemitsu 2014; Cheng et al., 2016). In fact, one of the most important concerns regarding OLP lesions is their potential progression to oral cancer (Kanemitsu 2014). Hence, in 1978, the World Health Organization has categorized OLP, as well as several other lesions, under the term oral potentially malignant disorders (OPMDs) to highlight their possible tendency for carcinogenesis in about 1% of cases (Ismail et al., 2007; Speight 2007; Tovaru et al., 2013).

Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumours in the world with more than 354,864 newly diagnosed cases in 2018 (GLOBOCAN 2018; Bray et al., 2018; Warnakulasuriya 2009; Garavello et al., 2007). In fact, the lateral borders of the tongue are considered as the most intraoral OSCC-prone locations (Moore et al., 2000; Torre et al., 2015). Pooling of various oral carcinogens in these channels has been suggested as a potential explanation for such location-dependent higher incidence of OSCC (Johnson and Warnakulasuriya 1993; Moore et al., 2000). In spite of the recent advances in cancer management, oral tongue squamous cell carcinoma (OTSCC) remains to have a relatively poor prognosis and low survival rate due to its high potential for swift invasion and occult metastasis (Sano and Myers, 2007; Brinkman and Wong, 2006).

The transformation of normal epithelium to cancer is a complex multistep process that consists of intricate interactions among environmental and inflammatory mediators (Gonda et al., 2009). In this regard, chronic inflammation has been considered as an important contributor to carcinogenesis (Okada 2014). It is hence

sensible that OPMDs, such as OLP and lesions exhibiting oral epithelial dysplasia (OED), predispose patients to an increased risk of oral cancer (Gillenwater et al., 2006; Speight 2007; Halonen et al. 2018; Yardimci et al., 2014). Therefore, a better understanding of the molecular mechanisms involved in such gradual tumorigenic process could pave the way for developing more effective drugs for OTSCC prevention and treatment. Importantly, oral cavity is easy to examine and risk factors for oral cancer are known; therefore there is a considerable opportunity to improve the patient prognosis and survival rate through better diagnostic and therapeutic approaches of OPMDs and early-stage OTSCC (Gillenwater et al., 2006).

Histamine [2-(4-imidazolyl)-ethylamine] is a pleiotropic biogenic amine found in most tissues of the body, and it is highly elevated in chronic inflammatory lesions and autoimmune diseases (Smuda and Bryce 2011). It mediates its pathophysiological effects via four G-protein-coupled receptors (H1R through H4R), which are expressed in different cell types (Dy and Schneider, 2004). Histamine is synthesized in a wide variety of cell types, however, mast cells (MCs) represent the most relevant source of histamine in the immune system, where it is stored in large intracellular granules (Riley and West, 1952; Maintz and Novak, 2007; Borriello et al., 2017). Once exposed to various immunologic stimuli, MCs release histamine in large amounts into extracellular milieu. These stimuli can be immunologic like allergens and pathogens, or non-immunologic stimuli, such as neuropeptides, lipoproteins, adenosine, superoxidases and physical traumas (Vlieg-Boerstra et al., 2005; Maintz and Novak, 2007; Smuda and Bryce 2011).

Of note, most cancer cell lines and tumour models exhibit the histamine-synthesizing enzyme, L-histidine decarboxylase (HDC), and associate with high levels of endogenous histamine, which showed the potential to regulate tumour growth (Bartholeyns and Fozard, 1985; Garcia-Caballero et al., 1994; Medina and Rivera, 2010). In spite of the successful history of histamine-derived medications in treating many diseases, clinical trials proved, however, ineffectiveness of targeting

classical H1R and H2R to treat OPMDs or OTSCC patients (Day et al., 2003; Gillenwater et al., 2006; Lodi et al., 2012; Wolff et al., 2012). The discovery of histamine H4 receptor (H4R) and its varying expression within a wide range of tissues, including tumours, have paved the way for novel perspectives in histamine research (Zampeli and Tiligada, 2009). In fact, H4R exhibits about 10,000-fold more affinity for histamine compared with other conventional histamine receptors, such as H1R (Thurmond et al., 2008). As expected, H4R antagonists showed marked clinical effects in atopic dermatitis (AD) patients as well as in preclinical models of asthma (Ohsawa et al., 2014; Thurmond, 2015). Moreover, targeting H4R suppresses the proliferation and invasion of esophageal squamous cell carcinoma via different signalling pathways (He et al., 2018). Furthermore, H4R modulated the proliferation of breast, lung, pancreatic, ovarian and colon cancer cell lines (Massari et al., 2018).

Overall, the identification of H4R, along with the enormous pharmaceutical success of drugs targeting the H1R and H2R, has generated immediate interest in this novel receptor, which has shortly paved the way for new era of studies on the immunomodulatory histamine/H4R-mediated effects (Hough, 2001; Thurmond, 2015). Consequently, efforts are still ongoing to understand the function of this new receptor and to determine if it can represent a viable drug target. The aim of this study was, therefore, to explore the role of H4R in OLP, as a model of OPMDs, and its potential contribution to development of OTSCC.

5. LITERATURE REVIEW

5.1. Human oral mucosa

The oral cavity is separated from the outer environment by a mucous membrane barrier that is called oral mucosa. Oral mucosa is categorized in general into keratinized and non-keratinized mucosa. The keratinized mucosa helps to resist hard mechanical forces induced by mastication, and it involves the mucosae of hard palate and marginal gingiva. These tissues undergo terminal differentiation resulting in dead (cornified) squames lacking nuclei or vital organelles and containing keratin, and thus impart protection to such areas (Presland and Jurevic, 2002). Non-keratinized mucosa helps to facilitate speech and swallowing, and it is primarily found in the soft palate, floor of the mouth, buccal mucosa, alveolar mucosa, and lips. In spite of the histology and thickness variations, each type of oral mucosa imparts protection against mechanical damage and preserves the underlying structures according to the functional demand of its location (Dale et al., 1990; Squier and Kremer, 2001; Presland and Jurevic, 2002).

Oral mucosa is composed of two distinct parts: 1) the stratified squamous cell epithelium layers; and 2) the lamina propria (**Figure 1**). The oral epithelium is composed of stratified squamous cells and separated from the lamina propria by the basement membrane (BM). The oral epithelium consists of several layers depending on the type of mucosa whether keratinized or non-keratinized. In keratinized mucosa, the epithelium consists of four layers: stratum basale or basal layer, stratum spinosum or prickle layer, stratum granulosum or granular layer, and stratum corneum or the keratinized layer. In non-keratinized mucosa, it consists of basal cell layer, prickle cell layer, intermediate and superficial cell layers. The basal cell layer is the dividing layer—it separates the epithelium from the underlying lamina propria and maintains the epithelial cell flow.

In addition to keratinocytes, oral epithelium contains also some different cell types, including melanocytes, Langerhans' cells, and Merkel cells (Squier and Hill, 1989; Squier and Kremer, 2001; Moharamzadeh et al., 2012). The lamina propria consists of loose connective tissue that supplies the oral mucosa with blood vessels, nutrients and nerves, and separates the oral mucosa from the underlying tissue, bone, fat or muscles. Many cell types are found in the lamina propria, including endothelial cells, fibroblasts, MCs, macrophages, as well as lymphocytes and plasma cells.

Epithelial adhesion to the extracellular matrix is vital to maintain tissue integrity and for the maintenance of systemic and oral health. Pathogens will first encounter the host at epithelial-mucosal interface, and therefore it is not surprising that oral epithelium takes a central part in defending underlying tissues from pathogenic onslaught. Such protective mechanisms comprise development of a tightly sealed physical barrier, releasing of direct antibacterial peptides, and organizing both innate and adaptive immune responses (Groeger and Meyle, 2015). Furthermore, following tissue physical injury or wounding, epithelial cells offer immediate protective responses by initiating the epithelial cover and restore the deranged barrier against pathogenic infections (Presland and Jurevic, 2002).

In oral epithelium, cytokeratins are the most abundant proteins and main component of keratinized epithelium, which are attached with desmosomes to form extensive cadherin-mediated cytoskeletal architectures (Presland and Jurevic, 2002). Desmosomes play crucial role in maintaining the epithelial intercellular adhesion while hemidesmosomes promoting the adhesion of epithelial cells to the underlying BM (Borradori and Sonnenberg, 1999). Additionally, epithelial cells tightly connect with each other by gap and tight junctions, which promote cell signalling and regulate selective movement of solutes across the epithelium, respectively (Bazzoni and Dejana, 2002).

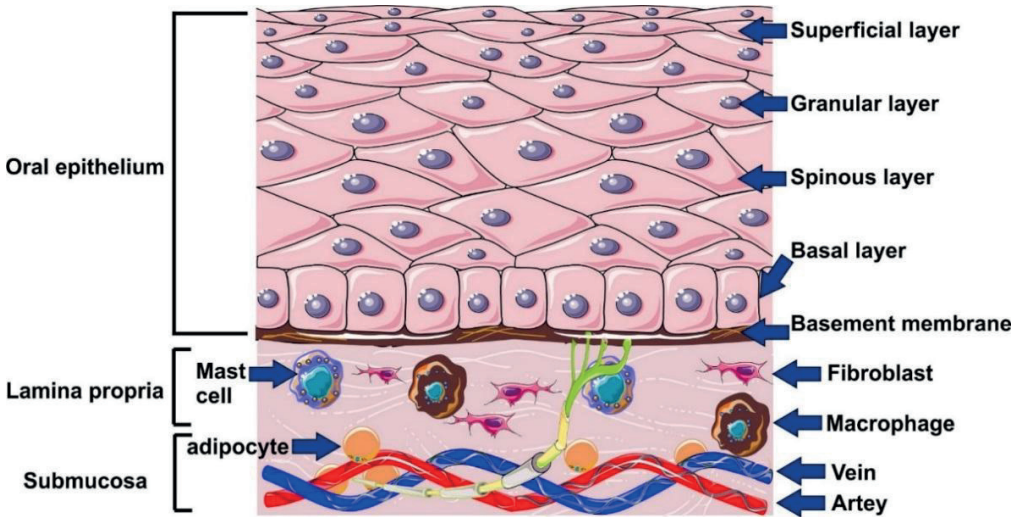

Figure 1. Histological features of human oral mucosa.

5.2. Oral lichen planus: definition and incidence

Oral lichen planus (OLP), which was first described by Dr. Wilson in 1869, is a common chronic inflammatory disease that affects oral mucosa with yet unknown etiology and unclear pathogenesis (Cheng et al., 2016). While cutaneous lesions of lichen planus, in most of cases, are self-limiting, OLP lesions are chronic, less likely to undergo spontaneous remission, and exhibit high potential to develop significant complications (Edwards and Kelsch, 2002; Eisen 2002). In the same context, the World Health Organization (WHO) has regarded OLP as a potentially malignant lesion that is associated with an increased risk of oral cancer (Kramer et al., 1978; van der Waal 2009; El-Naggar et al., 2017). Approximately 60% of cutaneous LP patients exhibit oral manifestations; while only 15% of OLP patients develop cutaneous lesions (Crincoli et al., 2011; De Rossi and Ciarrocca, 2014; Cheng et al., 2016).

Epidemiologically, OLP is one of the most common non-infectious oral mucosal diseases in adults affecting between 0.5 to 2.6% of population, although the percentage was up to 5% in some reports (Thorn et al. 1988; Sugerman et al. 2000; Eisen 2002; Gorouhi et al., 2014). OLP has a higher incidence (over than 60% of

cases) in women than in men (Eisen 2002, Xue et al., 2006, Bermejo-Fenoll et al. 2010). The disease may arise at any age but it is most commonly diagnosed in persons between 30 to 80 years of age (Thorn et al. 1988; Eisen 2002; Bermejo-Fenoll et al. 2010; Regezi et al. 2017). However, OLP incidence in younger patients and children have also been reported (Kanwar and De, 2010).

5.3. Clinical features

OLP patients commonly experience lesions with bouts of exacerbations and remission, and varying degrees of intensity (Wang and van der Waal, 2015). The active lesions have distinctive clinical features and characteristic bilateral distribution commonly on buccal, tongue and gingival mucosa (Eisen et al., 2005; Carbone et al., 2009). The most common area of OLP lesions is in the buccal mucosa, and the second most common site is in the tongue, followed by gingiva (Eisen 2002; Fernández-González et al., 2011). It is, however, less common to find OLP lesions on other mouth regions like palate, floor of the mouth or lip vermilion (Thorn et al. 1988; Edwards and Kelsch 2002; Eisen 2002). There are six clinical variants for OLP lesions, namely: reticular, atrophic, ulcerative, papular, bullous, and plaque-type lesions (Andreasen 1968; Thorn et al. 1988; Alrashdan et al., 2016;

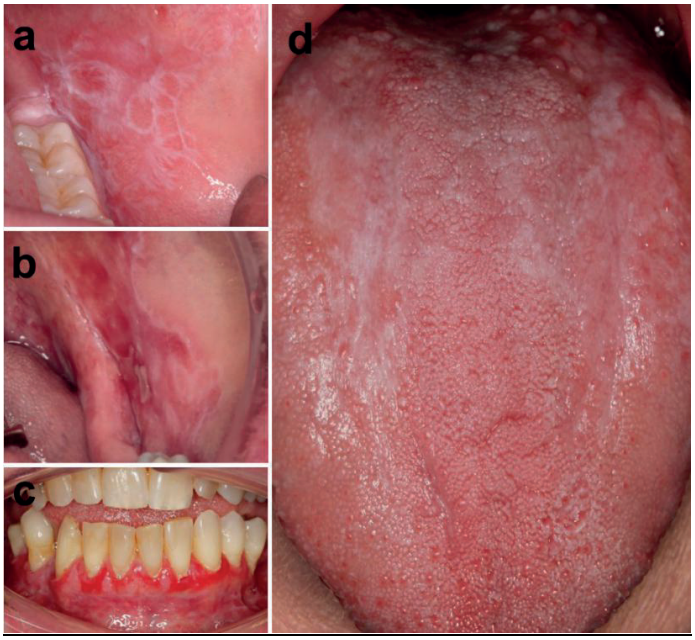

Figure 2. Common clinical forms of OLP: a) reticular; b) combination of reticular, atrophic and ulcerative; c) atrophic; d) plaque-like form. Figures were kindly provided by Dr. Siponen M.

Figure 2). It is not uncommon for the same patient to exhibit several variants of OLP lesions occurring simultaneously (**Figure 2b**; Edwards and Kelsch, 2002; Alrashdan et al., 2016).

The most common pattern of OLP is the reticular form, which is often asymptomatic and exhibits multiple interweaved papules with lacy and elevated whitish lesions known as Wickham's striae (Eisen 2002; Eisen et al., 2005; Alrashdan et al., 2016). Atrophic and erosive variants of OLP are also common, and they are often associated with considerable pain and discomfort (Eisen 2002; Gorouhi et al., 2014). Erosive OLP lesions may involve local erythematous patches, induced by inflammatory responses, and ulcerative lesions, enclosed by thinly radiating keratotic striae (Gorouhi et al., 2014; Edwards and Kelsch, 2002; Eisen 2002). When the atrophic/erosive forms of OLP affect the attached gingivae, the condition may be termed desquamative gingivitis (Rogers et al., 1982; Edwards and Kelsch 2002). The papular and bullous lesions appear as tiny whitish papules or soft mucous extensions. These lesions are less commonly encountered forms of OLP and usually seen in the buccal mucosa and lateral border of tongue (Thorn et al. 1988). Plaque-like OLP lesions are more frequent in smokers and they are usually affecting buccal mucosa and tongue (Thorn et al. 1988).

5.4. Microscopic features

The classical histopathological triad of OLP includes: 1) dense and confluent band-like inflammatory cell infiltrate (consisting mainly of T-cells) in the lamina propria; 2) degenerated BM; and 3) apoptotic basal keratinocytes (**Figure 3**; Kramer et al., 1978; Epstein et al., 2003; Rad et al., 2009). The degenerated basal keratinocytes (i.e. apoptotic cells and cells with hydropic changes) tend to form structures in the lower epidermis known as "Civatte bodies" or eosinophilic colloid bodies, which contain DNA fragments and immunoglobulins from the apoptotic basal cells (Burgdorf and Plewig, 2014). Moreover, the basal cell layer manifests signs of

degeneration such as elongated cytoplasmic processes, and widening in intercellular spaces (Paul and Shetty, 2013). Furthermore, degeneration of BM is usually observed as breaks, irregular branches and patch-like disruptions (Jungell P et al., 1987). It is also common to find inflammatory cells within the epithelial layers in OLP tissues (Bloor et al., 1999; Neppelberg et al., 2001; Sugerman et al. 2002; Fernández-González et al., 2011).

Other less-specific histopathological features of OLP include hyperkeratosis (ortho- or parakeratosis), shortened/pointed (saw-teeth) rete pegs that can be also lost, acanthosis, epithelial atrophy, and alteration of the epithelial anchoring elements (Sugerman et al. 2002; Fernández-González et al., 2011; Haapalainen et al., 1995). As a result of epithelial degeneration and apoptotic basal cells, the epithelial barrier in OLP lesions, in general, is thinner compared with normal oral mucosa, and hence it offers less protection against physical and chemical trauma (Karatsaidis et al., 2003).

The inflammatory cell infiltrate consists predominantly of activated cytotoxic ($CD8^+$) and helper ($CD4^+$) T-lymphocytes. Additionally, there are increased counts of local "degranulated" MCs, dendritic cells (Langerhans cells), natural killer cells, and macrophages (Iijima et al., 2003; Santoro et al., 2005; Salem et al., 2015; Choi et al., 2016). When making a diagnosis of a seemingly OLP lesion, it is important to assess both clinical and histopathological features to rule out any other similar conditions and malignant diseases (Cheng et al., 2016).

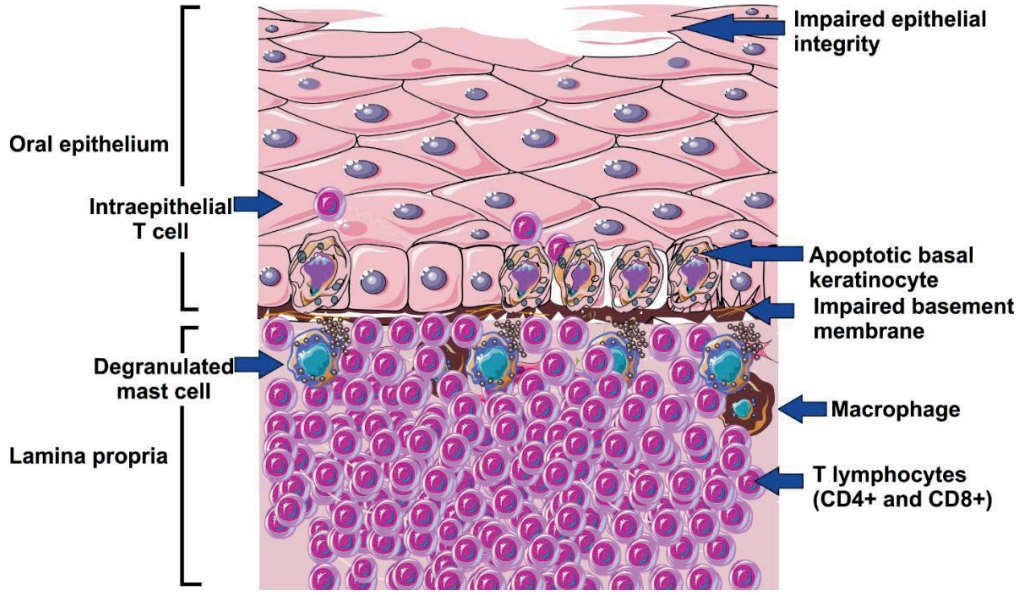

Figure 3. Main histopathological features of oral lichen planus.

5.5. Etiology

Although OLP is considered one of the most extensively studied oral lesion for over than a century, the actual etiology of the disease remains unknown. However, some predisposing factors have been attributed to its pathogenesis, including genetic factors, mechanical trauma, autoimmunity, metal allergy and smoking (Gorouhi et al., 2014; Cheng et al., 2016).

5.6. Genetic factors

Some recent reports support the close association between genetic predisposition and OLP pathogenesis. For instance, Wu et al. examined the association between mitochondrial DNA haplogroups and susceptibility to OLP in 242 Chinese patients, and they found that a certain haplogroup (i.e. B4) had a significantly higher incidence in controls than in OLP patients, implying this haplogroup may play a protective role in OLP susceptibility (Wu et al., 2014).

OLP lesions were also determined in a recent twin study to determine the genetic burden of the disease (Wei et al., 2018). In the same context, a former report of family-related OLP patients indicated that there may be some genetic predisposition toward OLP (Bermejo-Fenoll and López-Jornet, 2006). Furthermore, several studies highlighted the potential role of genetic background in OLP pathogenesis, including gene polymorphisms of TNF-α and IL-10 (Xavier et al., 2007; Bai et al., 2009), IFN-γ (Kimkong et al., 2012; Al-Mohaya et al., 2016). However, a recent meta-analysis study failed to prove any statistical associations between the polymorphisms of IL-6 and IL-10 and susceptibility to OLP, which necessitates the need to more well-designed studies with larger sample sizes (Shi et al., 2017).

5.7. Systemic diseases

Many research studies that have been carried out over the last few decades have shed light on the relationship between OLP and several systemic diseases. Such potential causative relationship is summarised in **Table 1.**

5.8. Differential diagnosis

There are several oral mucosal lesions that exhibit a multifocal bilateral presentation as in OLP, and hence they should be considered when making a clinical differential diagnosis (Dissemond 2004; Lopes et al., 2015; Regezi et al., 2017). Such lesions may include oral lichenoid lesions (OLLs), proliferative verrucous leukoplakia (PVL), lupus erythematosus, graft-versus-host disease, and candidiasis. Therefore, in addition to the clinical and histopathological features (i.e. clinicopathologic correlation), precise diagnosis should also take into consideration the course history of the disease (Alrashdan et al., 2016; Regezi et al., 2017). Overall, a definitive diagnosis of OLP is highly crucial due to the implications for therapeutic management (Müller 2011; Cheng et al., 2016; Müller 2017).

Table 1. The possible association between OLP and certain systemic diseases

Disease	Association aspects	References
Viral	• HCV seropositivity was increased in OLP patients compared with controls. • HCV-specific T-cells were found in the oral mucosa of OLP/HCV patients. • EBV genome and specific serum antibodies was found in OLP patients. • Some literatures indicated a higher incidence of HPV, HSV-1, HSV-6 and CMV in OLP patients.	Lodi et al., 2010; Pilli et al., 2002; Pedersen 1996; Shariati et al., 2018; Ma et al., 2016; Mattila et al., 2012; Yildirim et al., 2011; Ding et al., 2017.
Autoimmune	• Autoimmunity has been suggested to play a key role in OLP pathogenesis. • OLP patients have higher levels of circulating antibodies (e.g. antinuclear, autoantibodies). • Certain autoimmune diseases have been linked with OLP, such as Sjögren's syndrome, systemic lupus erythematosus, Hashimoto's thyroiditis, and ulcerative colitis.	Chung et al., 2015; Chang et al., 2009; Kobayashi et al., 2008; Likar-Manookin et al., 2013; Lo et al., 2013; Sardana et al., 2002; Li et al., 2017.
Psychological	• Some observational studies and case reports showed an association between anxiety, stress and OLP incidence. However, there is no strong evidence to support such observations so far. • Discomfort from OLP lesions or the associated anxiety about its malignant potential could lead to a deteriorated psychological state, and thus the causal association between stress and OLP remains speculative.	Rojo-Moreno et al., 1998; Le Cleach and Chosidow, 2012; Pippi et al., 2016;

HCV: Hepatitis C virus; EBV: Epstein–Barr virus; HPV: Human papillomavirus; HSV-1: Herpes simplex virus 1; HSV-6: Human herpesvirus 6; CMV: Cytomegalovirus.

5.9. Management

Currently, there is no specific treatment for OLP, and thus management is primarily aimed to relieve the patient symptoms (Carbone et al., 2003; Eisen et al. 2005; Scully and Carrozzo 2008; Hodgson and Chaudhry, 2010; Lodi et al., 2012;

Regezi et al., 2017). Moreover, due to the fluctuation in severity and activity during the disease course, finding a single and definitive treatment for OLP remains challenging (Thongprasom and Dhanuthai, 2008; Gupta et al., 2017). Nevertheless, some anti-inflammatory and immunosuppressive medications can provide satisfactory control of the lesion. Topical corticosteroids, such as clobetasol propionate, fluocinonide, triamcinolone acetonide, and betamethasone sodium phosphate are commonly considered as the first-line treatment for OLP (Gupta et al., 2017). However, the long-term use of topical steroids has been associated with increased incidence of oral candidiasis (Gonzalez-Moles, 2010). Systemic steroids, such as cyclosporine, tacrolimus, or prednisolone are indicated in severe OLP lesions and in patients unresponsive to topical steroids (Gupta et al., 2017). Intralesional injections of corticosteroids, such as dexamethasone, have also been indicated for extensive lesions, but they are not consistently effective, and may cause localized side-effects such as mucosal atrophy and pain (Thongprasom and Dhanuthai, 2008). Overall, topical steroids, such as ointments, pastes, gels, or mouthwashes, are generally recommended over other modes of treatment. In Finland, the available topical corticosteroids include: 1) mid-potent class such as 0.1% triamcinolone acetonide, beclomethasone dipropionate or beclomethasone dipropionate monohydrate, and fluticasonepropionate; 2) potent class such as 0.1% betamethasone 17-valerate; 3) highly potent preparations such as 0.05% clobetasolpropionate (Siponen 2017). Methylprednisolone is available for intralesional injections, while prednisolone is aimed for systemic treatment of OLP (Siponen 2017).

Immunosuppressants, including calcineurin inhibitors (e.g. cyclosporine, tacrolimus, and pimecrolimus) have also been recommended for OLP management due to their anti-inflammatory effects (McCaughey et al, 2011). Although topical cyclosporine has been popular choice for treating OLP, Harpenau et al. concluded that it is not superior to steroids due to its frequent relapse rates, burning sensation

and higher cost (Harpenau et al, 1995; Gupta et al., 2017). The synthetic derivatives of vitamin A, retinoids, have been suggested as an effective therapeutic alternative/adjuvant to corticosteroids (Gupta et al., 2017). In this context, mouthwashes containing a combination of vitamin and triamcinolone produced more favourable effects in OLP patients compared to use of steroid alone (Dalirsani et al, 2010).

Laser phototherapy (LPT) has been employed for the management of OLP lesions. By using diode- or CO2-laser, the diseased epithelial layers together with the underlying inflammatory component are destroyed via protein denaturation (Derikvand et al., 2017; Gupta et al., 2017). Dillenburg et al. concluded that LPT was more effective than topical (0.05%) clobetasol for the treatment of atrophic and erosive OLP; however, LPT treatment was inferior to other topical steroids such as dexamethasone and triamcinolone (Dillenburg et al, 2014; Gupta et al., 2017). Based on these reports, LPT can be used as an adjunct therapy with topical steroids in refractory cases.

It is also important in the management course of OLP patients to maintain excellent oral hygiene, eliminate any local irritating factors, removing calculi, and treating any concurring periodontal lesions (Regezi et al., 2017).

5.10. Prognosis and cancer progression

The potential malignant transformation of OLP into squamous cell carcinoma (SCC) has been an unresolved controversial issue for many decades. It has been reported in a recent cohort study of 13,100 Finnish female patients that OLP diagnosis is significantly correlated with an increased risk of cancer of lip, tongue, and oral cavity (Halonen et al., 2018). However, the rate of malignant transformation in OLP lesions varies considerably between different reports. In this context, Landini et al. found that such reported transformation rates generally fall between 0% and 10% among an accumulating 24097 cases of OLP regardless of the follow up time and diagnostic

criteria (Landini et al., 2014). Nevertheless, a general estimate of the overall malignant transformation rate in OLP was estimated as 1.09-2% (Fitzpatrick et al., 2014; Aghbari et al., 2017; Giuliani et al., 2018). Therefore, it was concluded by WHO experts that OLP is a potentially precancerous lesion that exhibit an increased risk of cancer, and hence it was later categorized as a potentially malignant disorder (van der Waal 2009; Mortazavi et al., 2014).

5.11. Oral potentially malignant disorders

The older term "oral premalignant lesion" is not currently advocated as it implies that a lesion will inevitably progress to cancer, and hence the alternative term "oral potentially malignant disorders" or (OPMDs) has been adopted and has become widely used (Warnakulasuriya et al., 2007; Reibel et al., 2017; Speight et al., 2018). Such new term, OPMDs, suggests that a progression of the lesion to cancer is only a potential risk (i.e. more statistically probable).

The category of OPMDs comprises numerous disorders with an elevated risk of being transformed to SCC including, inter alia, leukoplakia, proliferative, PVL, erythroplakia, OLP/OLLs, lupus erythematosus, actinic cheilitis, and oral submucous fibrosis (Mortazavi et al., 2014; Speight et al., 2018). Several factors may influence the progression of OPMDs to malignant lesions, such as the anatomical site of the lesions, its clinical pattern, and presence of OED (Speight et al., 2018). In fact, several OPMDs can share both clinical and histological resemblance and thus both biopsy and microscopic analysis are necessary to minimize misdiagnosis, and provide better specific management. In this regard, the initial clinical presentation of PVL, which has a high tendency to recur after treatment, has close similarities to OLP, particularly in early stages, such as the presence of multifocal white lesions and dominance in adult female patients (Lopes et al., 2015). Therefore, it has recently been suggested that many malignantly transformed cases may represent an under-

recognized spectrum of PVL, which is characterized by a lichenoid pattern, and hence could lead to misdiagnosis (Fernandes et al., 2017)

The distribution of OPMDs in oral cavity can reflect existing habitual risk factors, such as betel quid chewing and reverse smoking prevalent in parts of India, Philippines, and South America. Accordingly, buccal mucosa is the most commonly affected site in betel quid and Khat (or Catha edulis) chewers (Kassie et al., 2001; El-Zaemey et al., 2015). On the other hand, OPMDs in palate, for example, is most the commonly affected site in reverse and pipe smokers (Naveen-Kumar et al., 2016; Speight et al., 2018). It was reported in a retrospective analysis of OPMDs that 40% of dysplastic or malignant lesions develop in tongue and floor of mouth (Dos et al., 2013). Furthermore, Jaber et al. found that most dysplastic lesions that associated with oral carcinogenesis were found in the lateral and ventral borders of tongue, and the floor of mouth (Jaber et al., 2003). Buccal mucosa also is considered as a common site to develop some OPMDs such as leukoplakia, however, it was reported that buccal leukoplakia is associated with less tendency to develop oral cancer (Warnakulasuriya and Ariyawardana, 2016). Overall, the most common sites of malignant transformation of OPMDs were observed in tongue, floor of mouth, palate and buccal mucosa (Speight et al., 2018; Regezi et al. 2017).

The clinical appearance of OPMDs varies considerably, which may occasionally render their diagnosis complex or confusing (Speight et al., 2018). Nevertheless, there is a general agreement in the literature that nonhomogeneous oral lesions pose a higher risk of malignant transformation as compared with homogeneous lesions (van der Waal 2009; Napier and Speight, 2008; Warnakulasuriya et al., 2008; Speight et al., 2018). Additionally, some other patterns of OPMDs, such as erosive, ulcerative, and atrophic forms were also reported to correlate with an increased incidence of OSCC, such as in OLP lesions (Ismail et al., 2007; Tovaru et al., 2013; Fitzpatrick et al., 2014).

5.12. Oral tongue squamous cell carcinoma

Oral tongue (AKA mobile tongue) squamous cell carcinoma (OTSCC) is a subtype of oral cavity cancer, which is regarded as one of the most common malignancies in head and neck region, with an increased incidence in recent years (Moore et al., 2000; Marur and Forastiere, 2016; Ferlay et al., 2015; Siegel et al., 2017). In spite of the noticeable advances in cancer therapy, OTSCC prognosis and survival rate remain among the poorest of oral malignancies, due to the relatively swift metastasis to regional lymph nodes and high cancer-related mortality (Sano and Myers, 2004; Brinkman and Wong, 2006).

Risk factors for OTSCC development are numerous and their combination may enhance OTSCC development in susceptible individuals. Various forms of tobacco, betel quid and Khat chewing, excessive alcohol consumption, and existence of OPMDs are regarded among the most prominent risk factors for OTSCC (Agnihotri and Gaur, 2014; Scully 2011; Casparis et al., 2015). In this regard, early diagnosis of OPMDs and malignant lesions of tongue is of a prime importance, as it corresponds to increased 5-year survival rates and reduced comorbidity, compared with the advanced stages of OTSCC (Sciubba 2001). Importantly, there is a significant association between the delay in diagnosis and increased comorbidity rate (Teppo and Alho 2009). Despite the fact that oral cavity is easily accessible for clinical evaluation, yet many OTSCC cases are diagnosed at advanced stages (van der Waal et al., 2011).

Histopathologically, OTSCC is classified by WHO according to its resemblance to the normal tissue (Thompson 2006), into well differentiated (few mitotic changes, higher resemblance to normal findings), moderately-differentiated (less keratinized cancer cells, more atypical mitotic changes and nuclear pleomorphism), and poorly-differentiated cancer cells (very few or no keratinized

cells, more frequent atypical mitotic changes, with higher degree of nuclear pleomorphism).

Clinical staging (i.e. tumor–node–metastasis or cTNM system) of OSCC has long been used as the main reference for risk evaluation and treatment planning (Akhter et al., 2011). It is based on assessing tumour diameter (T), lymph node status (N), and distant metastases (M) (Brierley et al., 2016; Amin et al., 2017). In spite of its popularity in clinical setting, cTNM staging system has been criticized for not encompassing the biological behaviour of tumour cells, which remains crucial in deciding the future outcomes of cancers (Anneroth et al., 1987; Bryne et al., 1992; Akhter et al., 2011). Furthermore, the prognostic value of cTNM staging system, particularly in early stages of OTSCC, has been questionable (Yanamoto et al. 2013). Hence, the introduction of new prognostic parameters that taking into account the biological activity of tumour cells is vital for more precise therapeutic strategies.

Surgical resection and radiation are the mainstay treatment for early-stage OTSCC patients, while elective neck dissection without or with radiotherapy, chemotherapy, or both, could be indicated for the advanced cases (Ord and Blanchaert 2001; Langendijk et al., 2010). In spite of the radical changes in oral cancer management in the recent decades, it should be taken into consideration that advanced OTSCC patients are more prone to develop distant metastases and second primary cancers (Ord and Blanchaert 2001). This may explain the fact that OTSCC prognosis has not significantly improved, and the 5-year overall survival rate remains relatively low (Rusthoven et al., 2008; Jadhav and Gupta 2013). Therefore, it is important to enhance the early diagnosis and follow-up of the OPMDs. Additionally, investigating various signalling pathways that perpetuate the chronic inflammation in such OPMDs could improve the diagnostic and preventive strategies, which may translate to better clinical outcomes in most patients (Sciubba 2001; Ord and Blanchaert 2001; Bhatia and Burtness 2017).

5.13. Inflammation and carcinogenesis

The causal relationship between the chronic inflammation and carcinogenesis is not a new concept but dates back to 1863, when the pathologist Rudolf Virchow reported that cancer was developed at sites of chronic inflammation and dominated by "lymphoreticular infiltrate" (Balkwill and Mantovani 2001). Indeed, the recent extensive research in this field has supported Virchow's hypothesis, and the causal relationship between chronic inflammation, immune response and carcinogenesis is now more widely accepted (Balkwill and Mantovani 2001; Coussens and Werb 2001). However, a considerable part of the intricate molecular and cellular signalling that mediate such relationship remain unresolved (Coussens and Werb 2001).

Several mechanisms were suggested as potential contributors to the inflammation-mediated tumorigenesis, including the ability of many inflammatory mediators (e.g. Mediators of nuclear factor-κB (NF-κB) activation, growth factors and matrix-proteases) to induce the survival and proliferation rate of affected cells (Karin and Greten 2005; Grivennikov and Karin 2010). Additionally, *in vivo* studies of inflammation-mediated cancer showed that activation of NF-κB pathway can inhibit cancer cell apoptosis by inducing crucial anti-apoptotic genes, such as B cell lymphoma-extra-large (*Bcl-XL*) and others (Greten et al., 2004). Moreover, tumour-associated inflammatory response can downregulate the anti-tumorigenic immune response and enhance the pro-tumorigenic and angiogenic effector functions in immune cells, as reported in tumour-associated macrophages (TAMs) and MCs (Sica et al., 2008; Grivennikov et al., 2010; Varricchi et al., 2017). Activation of PI3k pathway, as reported in OLP, and its downstream events has been also suggested to play a role in the inflammation-mediated carcinogenesis and subsequent cell proliferation, motility and invasion (Giacomelli and Covani, 2010; Sonis et al., 2016).

The malignant transformation of normal epithelium to OTSCC is a multistep progressive process that consists of dynamic and complex interactions between various environmental and inflammatory mediators (Gonda et al., 2009; Whitmore and Lamont, 2014; Sun et al., 2016; Ryan and Faupel-Badger, 2016). As discussed, persistent inflammatory mediators have the potential to facilitate cancer development through enhancing immune cell infiltration and remodelling of stromal tissue that seem compatible with oral carcinogenesis (Hanahan and Coussens 2012). It is therefore sensible that OPMDs, such as OLP, can increase the risks to develop OTSCC (Yardimci et al., 2014; Casparis et al., 2015; Halonen et al. 2018). Mediators associated with OPMDs, such as inflammatory cytokines and reactive oxygen species, can facilitate epigenetic alterations in such lesions and downregulate tumour suppressor genes (Grivennikov and Karin, 2010). Therefore, a better understanding of the molecular mechanisms associated with such tumorigenic process will pave the way for identifying novel and more viable drug targets for OTSCC.

Importantly, tumour microenvironment (TME), such as in OTSCC, is highly miscellaneous and contains diverse immune cells (including TAMs, lymphocytes, MCs, etc.) that communicate with tumour cells and shape cancer behaviour (de Visser et al., 2006). In this regard, it was reported that higher numbers of TAMs in TME is proportionally correlated with treatment shortcomings and can promote cancer angiogenesis, invasion, and metastasis (Condeelis and Pollard, 2006; Murdoch et al., 2008). Furthermore, T lymphocytes can have dual effects by inducing either antitumorigenic or protumorigenic effects, depending on their effector functions (Kohrt et al., 2005; Grivennikov et al., 2010). For instance, increased T cell numbers, specifically high $CD4^+/CD8^+$ ratios was suggested as indicator of poor prognosis in breast cancer (Kohrt et al., 2005).

MCs have long been suspected as potential players in cancer pathogenesis. Recently, these cells were also revealed as potent and versatile effector components

of TME, where they play far more complex roles than their role in allergy, and thus representing promising therapeutic targets in cancer (Maciel et al., 2015; Aponte-López et al., 2018).

5.14. The role of MCs in cancer

MCs can exert both tumor-suppressive and -promoting effects mediated by their potent, and versatile, granular constituents (Maciel et al., 2015; Varricchi et al., 2017; Ribatti et al., 2018). Almahmoudi et al. showed in a recent study that MC-derived IL-17F correlates with better disease-specific survival in OTSCC patients (Almahmoudi et al., 2018). Likewise, the abundance of stromal MCs in invasive breast cancer patients was correlated with a favourable prognosis (Dabiri et al., 2004). In another study, colorectal cancer patients with a low MC count had significantly deeper depth of invasion and lower rates of metastasis compared with patients with higher MC count (Tan et al., 2005).

On contrary, it has been documented that MCs are able to induce angiogenesis in cancer models, where MC-deficient mice exhibited decreased angiogenesis and metastatic capacities during tumour induction (Ribatti et al., 2001). This can be explained by the ability of MCs to synthesize and release pro-angiogenic mediators (e.g. VEGF and IL-8) and proteases (e.g. Matrix metallopeptidase 9, chymase, tryptase) that can degrade extracellular matrix and potentiate invasion (Ribatti et al., 2018). Malfettone et al. reported that higher density of tryptase^{+} MCs at invasive front of human colon adenocarcinomas was associated with cancer cell motility and dismal prognostic outcomes (Malfettone et al., 2013). Furthermore, elevated levels of MCs within TME has been correlated with poor prognosis, enhanced metastasis, and reduced overall survival in oropharyngeal carcinoma, melanoma, and prostate cancer (Elpek et al., 2001; Ribatti et al., 2003; Nonomura et al., 2007).

Beside angiogenesis and matrix degradation, the contribution of MCs to carcinogenesis can also include protumorigenic immunoregulation (Kalesnikoff and Galli 2011; Ribatti et al., 2018). Supporting this role, Gounaris et al. showed that MCs are essential components for the development of the premalignant adenomatous colon polyps, and they further demonstrated that MCs can promote tumour development by inducing immunosuppressive and anti-inflammatory phenotypic changes in tumour-associated regulatory T cells (Gounaris et al., 2007; Gounaris et al., 2009). In addition, MCs produce IL-13, which plays a crucial role in TME by activating TAMs and myeloid-derived suppressor cells (McLeod et al., 2015; Suzuki et al., 2015). Histamine, the most known MC mediators, has also been linked to the MC-mediated immunosuppression and was thereby able to increase the risk for cancer development in animal models (Hart et al., 2001).

In fact, histamine can induce tumour proliferation and progression via signalling with certain receptor subtypes, while in contrast, targeting histamine with specific inhibitors has yielded promising antitumorigenic effects (Medina and Rivera 2010; Fernández-Nogueira et al., 2018). Indeed, this places histamine, as one of the most exploited substances in medicine, in promising therapeutic approaches for OPMDs and cancer (Martinel Lamas DJ et al., 2013).

5.15. Histamine synthesis, release, and degradation

Histamine [2-(4-imidazolyl)-ethylamine] is a short-acting yet potent endogenous amine. It derives its name from the Greek word (*histos*, meaning tissue), which implies its wide distribution throughout body tissues and involvement in many physiological and pathological conditions (Smolinska et al., 2014). Histamine is synthesized only by HDC enzyme, which converts the amino acid L-histidine to histamine (Maintz and Novak, 2007). HDC is widely expressed in different cell types and tissues such as central nervous system (CNS), neurons, gastric mucosal tissue, MCs, basophils, and enterochromaffin-like cells (Thangam et al., 2018; Walter and

Stark, 2012). There are two isoforms of HDC: 1) low activity full-length 74-kDa HDC, and 2) high activity cleaved 53-kDa HDC. Both HDC isoforms are found in the endoplasmic reticulum of mammalian cells, but only the 53-kDa isoform undergoes internalization by vesicular monoamine transporter-2 (VMAT-2) to facilitate storage of the newly produced histamine (Stegaev et al., 2013).

Histamine-producing cells vary considerably in origin and functions. In this context, our group has coined a new term to differentiate cells based on their histamine-production characteristics, as either "professional" or "non-professional" histamine-producing cells (Konttinen et al., 2013). "Professional" histamine-producing cells (e.g. MCs, basophils and enterochromaffin-like cells) use the low-molecular weight, post-translationally cleaved, 53-kDa HDC to produce extensive amounts of histamine, store it in special granules and response to stimulants (eg. Allergens) by releasing histamine in "burst-release" exocytosis manner. On the other hand, "non-professional" histamine-producing cells (e.g. epithelial cells and lymphocytes) utilize the high-molecular weight, full-length, 74-kDa HDC which produces as low histamine amounts as 100-1000-fold lower rate than MCs. Moreover, "non-professional" histamine-producing cells do not store histamine in granules but it is passively transferred along its concentration gradient via histamine channels such as organic cation transporters or OCTs (Konttinen et al., 2013; Panula et al., 2015).

Histamine degradation is controlled by two enzymes: histamine N-methyl transferase (HNMT) and diamine oxidase (DAO), which facilitate ring methylation and oxidative deamination, respectively (Smolinska et al., 2014). HNMT is a cytosolic enzyme, and thus it degrades histamine only intracellularly, whereas DAO is mainly responsible for scavenging histamine from extracellular spaces (Klocker et al., 2005). Experimental studies showed that HNMT deficiency enhanced brain histamine concentrations and regulated sleep-wake cycle (Naganuma et al., 2017).

5.16. Histamine receptors

Histamine-mediated effects occur via four known G-coupled protein receptors (GPCR) of histamine (histamine H1 through H4 receptor). Firstly, it was noted that some effects produced by antihistamine compounds were compatible with antagonizing effects at a receptor that is now called the histamine H1 receptor (H1R; Panula et al., 2015). The H1R was the first discovered receptor subtype of histamine, and it was the first to be harnessed as treatment for allergic inflammation (Massari et al., 2018; Panula et al., 2015). The first generation of antihistamines paved the way for very successful medications, which many are still in use. However, some histamine-mediated effects were not blocked by such H1- antihistamines and a second receptor was suggested to exist. This has led to the pharmacological characterization of H2R, the second histamine receptor subtype to be discovered, which was intended clinically to block the histamine-mediated secretion of gastric acid (Panula et al., 2015). Later, scientists characterized the third receptor of histamine, H3R, which showed very promising clinical effects and has been involved in studies targeting CNS disorders (Massari et al., 2018). Finally, the H4R was discovered at the turn of the millennium, and it represents the latest characterized receptor subtype of histamine family (Nakamura et al., 2000; Oda et al., 2000; Simons and Simons, 2011; Ferre et al., 2014; Panula et al., 2015).

Histamine receptors consist of three main parts or domains: intracellular, transmembranous, and extracellular domains. The ligand-binding sites of the receptor are located on the extracellular domain, while different G-proteins are bound to the intracellular domain Therefore, G-proteins play crucial role in the signal conduction through histamine receptors via downstream activation/inhibition of signalling cascades. Activation of histamine receptors will eventually result in various subsequent events including swift release of calcium ions, protein phosphorylation, and gene transcription (Konttinen et al., 2013; Tiligada and Ennis 2018).

In fact, different concentrations of histamine are required to stimulate the receptor subtypes. For instance, H1R and H2R have relatively low affinity for histamine, and thus require higher histamine concentrations for activation, compared with H3R and H4R. Therefore, histamine responses are largely based on its local concentration and what kind of receptor subtype exists in certain tissue. The increased pace in histamine research area has improved our understanding of histamine receptor signalling, which was associated with developments in histamine receptor pharmacology (Ferre et al., 2014; Panula et al., 2015; Tiligada and Ennis 2018). Summary of some features of histamine receptor subtypes are listed in **Table 2**.

In spite of the fact that histamine has been exploited in medicine for over 80 years, H1R and H2R ligands remain largely ineffective in the management of several immune-associated diseases particularly those with elevated MC and histamine levels, such as autoimmune diseases and cancer (Konttinen et al., 2013; Massari et al., 2018; Panula et al., 2015). This has been attributed to the variation of histamine levels in various body tissues, as it could be very low to activate the classical low affinity H1R ($pK_i = 4.2$) and H2R ($pK_i = 4.3$), but high enough to stimulate the high-affinity H4R ($pK_i = 8.3$) that is expressed on immune cells and tumour tissues (Thurmond et al., 2008; Konttinen et al., 2013; Massari et al., 2018). Therefore, the latter novel receptor has generated enormous interests due to the fact that histamine can exert immunomodulatory effects on different cell types via H4R signalling and hence it may provide a promising therapeutic target in various diseases (Thurmond et al., 2008; Konttinen et al., 2013).

5.17. Histamine H4 Receptor

The first evidence of H4R existence was shown when nanomolar concentrations of histamine increased cytosolic Ca^{2+} in eosinophils about 7.5-fold more compared with specific H3R agonists (Raible et al. 1994). The discovery of

H3R gene has encouraged enthusiasm to search for additional related sequences, which resulted in a receptor that carries ~35% homology to H3R and exhibits a high affinity for histamine (**Table 2**).

Table 2. Summary of certain key features of histamine receptor subtypes.

Features	**H1R**	**H2R**	**H3R**	**H4R**
Receptor cloning	1993	1991	1999	2000
Histamine affinity (pK_i)	4.2	4.3	7.8	8.3
Protein length	487 amino acids	359 amino acids	445 amino acids	390 amino acids
G-protein type	$G\alpha_{q/11}$	$G\alpha_{s}$	$G_{i/o}$	$G_{i/o}$
Distribution	Smooth muscle cells of airways, endothelial cells, central nervous system, chondrocytes, myeloid cells, T- and B-lymphocytes, Gastrointestinal tract, heart, genitourinary system and adrenal medulla.	Gastric mucosa, neutrophils, eosinophils, macrophages, dendritic cells, lymphocytes, epithelial cells, endothelial cells, bronchioles and bowel.	Central nervous system; basal ganglia, cortex, hippocampus and striatal area.	Hematopoietic cells, eosinophils, MCs, neutrophils, dendritic cells, T-lymphocytes and basophils.
General effects	Allergic responses, pain, vasodilation, itching, flushing, headache, tachycardia, Broncho-constriction	Increased gastric acid secretion, Vascular perm., hypotension, flushing, headache, tachycardia, bronchodilation, mucus production.	Modulate the release of neurotransmitter, counteracts bronchoconstriction; mediates pruritus	Differentiation of cells, Increased Ca^{2+} flux; Mediate chemotaxis; increases secretion of IL-16

Several groups have later cloned the H4R protein, which has revived the scientific interest in histamine research as a hot topic for potential therapeutic drug approaches (Nakamura et al. 2000; Oda et al. 2000; Konttinen et al., 2013). Interestingly, H4R has been characterized in hematopoietic cells such as eosinophils, neutrophils, basophils, MCs, NK-cells, dendritic cells and T-lymphocytes (Damaj et al. 2007), suggesting its crucial role in inflammation and immune responses. Moreover, H4R activation resulted in increased secretion of IL-16 (from T-lymphocytes), neutrophils recruitment, chemotactic activity in MCs and eosinophils, and decreased monocytes secretion of CCL2 chemokine (Gantner et al. 2002; Takeshita et al. 2003; Buckland et al. 2003; Hofstra et al. 2003; Ling et al. 2004; Dijkstra et al. 2007).

H4R has also been characterized in non-immune cells, such as the neuronal cells of nasal mucosa and the grey matter of the spine (Nakaya et al. 2004; Kajihara et al. 2010; Lethbridge and Chazot 2010). Additionally, H4R receptors were found to be expressed in the human cortex, and mouse thalamus (Breunig et al. 2007) and other regions of CNS tissues of both mice and humans (Connelly et al. 2009; Strakhova et al. 2009). Other cell types that were reported to express H4R include dermal fibroblasts, dermal keratinocytes, osteoclasts, synovial cells and chondrocytes (Biosse-Duplan et al. 2009; Yamaura et al. 2013). In fact, such expression of H4R in a wide variety of tissues and cells suggests a crucial biological role of this receptor.

5.18. Histamine and H4R in cancer

The role of histamine and histamine receptors in regulating cell growth and proliferation has been widely studied, and its association with carcinogenesis remains an attractive area in cancer research (Cianchi et al., 2008; Median and Rivera, 2010; Massari et al., 2018). Indeed, histamine can regulate various biological processes associated with tumour growth with different responses based on its local

concentration and the type of receptor subtype. Such processes including cell proliferation, migration, differentiation and apoptosis (Faustino-Rocha et al., 2017). Moreover, MC-derived histamine can affect angiogenesis and the expression of vascular endothelial growth factor (VEGF), and thus it can regulate tumour growth, invasion and distant metastasis (Johnson et al., 2016).

Experimental research reports indicate that histamine, through its receptor subtypes, imparts a regulatory function on mammary cell growth (Median and Rivera, 2010). Recent *in vivo* studies demonstrated that xenograft tumours of the highly invasive human breast cancer cell line MDA-MB-231 were significantly decreased in growth when stimulated with H4R agonists. Furthermore, administration of histamine has significantly increased median survival in mice and tumoural apoptosis (Martinel Lamas et al., 2013). On the other hand, the proliferation rate of MDA-MB-231 cell line was increased moderately through the H3R pathway; which signifies the role of histamine concentration and receptor subtype in determining the outcome (Medina et al., 2006; Medina et al., 2008). However, no effect on proliferation is observed in the non-tumorigenic HBL-100 cells (Medina et al., 2006; Medina et al., 2008).

In melanoma, histamine was shown to induce melanogenesis via H2R and growth-differentiation factor-15 in melanoma cell lines (Lee et al., 2012). In contrast, the *in vitro* activation of H4R showed inhibitory effects on melanoma cell proliferation, while the *in vivo* administration of H4R agonist (JNJ28610244) exhibited a significant antitumorigenic effect and reduced metastatic potential in experimental mice (Massari et al., 2017). Furthermore, H3R and H4R are involved in pancreatic carcinoma cell growth. Targeting H3R in human pancreatic cancer cells induced their proliferative capacity, whereas such effect was reversed via H4R agonists (Cricco et al. 2008).

Meng et al. found that Clobenpropit, a potent H3R antagonist/H4R agonist, is able to reduce cholangiocarcinoma (CCA) growth and metastases *in vivo*. Downregulation of H3R expression or overexpression of H4R significantly decreased CCA proliferation (Meng et al., 2011). Recent reports indicated that histamine and H4R are involved in esophageal carcinogenesis (He et al., 2018). Importantly, H4R exhibited antitumorigenic properties where stimulation of the receptor significantly blocked cancer cell proliferation and inhibited invasive properties. Moreover, xenograft-bearing mice treated with H4R agonists were associated with an increased survival period (He et al., 2018).

5.19. Constitutive or basal H4R activity

A two-state model of receptor activation that describes ligand-receptor interactions for H4R and other G-protein coupled receptors has been widely accepted (Leff 1995). It is suggested that H4R exists in two states: resting or ground receptor state (R, GDP-bound), which indicates the absence of a ligand; and active receptor state (R*, GTP-bound) that characterizes the binding of a ligand to its receptor (signalling state).

Some G-protein coupled receptors spontaneously attain R* conformation, or state, and promote G-protein signalling even in ligand absence, which is known as constitutive or basal activity. H4R exhibits high constitutive activity, compared to H1R and H2R, which indicates sustained and extended histamine interactions under physiological and inflammatory conditions (Konttinen et al. 2013). The constitutive or intrinsic H4R activity is described as the ratio between R* and R, and this intrinsic activity is considered to be about 50% of H4R total activity (i.e. half-maximally active), which makes H4R responsive to very low, nanomolar, histamine concentrations (Thurmond et al. 2008; Jablonowski et al. 2003; Akdis and Simons, 2002).

Histamine produced by "non-professional" histamine-producing cells is too low to stimulate the conventional low-affinity H1R and H2R, but sufficiently high to stimulate H4R. In this regard, the low-affinity H1R and H2R become maximally activated during allergy and helminthic infections, leading to acute and transient responses in the host tissue. In contrast, the high-affinity H4R, is believed to maintain and regulate mechanisms responsible for homeostasis and immunity, and thus it is also expressed in non-professional histamine-producing cells including various cells such as dendritic cells, T-cells, and monocytes/macrophages (Kubo and Nakano 1999; Szeberenyi et al. 2001; Konttinen et al. 2013). Additionally, non-immune cells, such as epithelial cells, mammary gland cell lines, skeletal muscle cells, and sperm cells produce considerably lower concentrations of histamine (Tanaka and Ichikawa 2011; Niijima-Yaoita et al. 2012).

Another important aspect of such constitutive activity is that receptor ligands can act as agonists, neutral antagonists or inverse agonists. When agonists bind the receptor they promote the active (R*) conformation and induce signal transduction. On the other hand, binding of a neutral antagonist to a constitutively-active receptor does not promote either R or R* states alone, but it competes with an agonist for receptor binding, and therefore inhibits agonist-mediated effects. Importantly, binding of inverse agonists (formerly were also called antagonists) promotes the inactive receptor conformation (R-state), which thus inhibits the constitutive receptor activity (Konttinen et al., 2013).

Indeed, such new findings added extra layer of complexity to our understanding of histamine signalling, and revived interest in histamine, and its novel H4R, as viable therapeutic targets in inflammatory disorders and cancer (Dib et al., 2014; Thurmond 2015).

6. AIMS OF THE STUDY

The hypothesis of our study was that "histamine and its latest discovered receptor, H4R, are involved in the pathogenesis of OLP and may contribute to inflammation-mediated oral carcinogenesis". To assess this hypothesis, the following objectives were set out:

1) To investigate H4R expression and internalization in human oral epithelial cells, and to determine its cellular sources in OLP;

2) To assess the capacity of oral epithelial cells to synthesize and release histamine, and to study the role of bacterial/inflammatory mediators in regulating such processes;

3) To assess the potential outcomes of derangement of histamine metabolism in oral epithelial cells and its possible contribution to oral mucosal integrity and subsequent bacterial invasion;

4) To elucidate the role of pattern-recognition receptors (PRRs) in promoting H4R response by mapping the toll-like receptors in OLP lesions;

5) To investigate the expression of H4R in cancer cell lines and in OTSCC patients. Additionally, we aimed to study the effects of bacterial components and MC mediators on oncogene expression in oral epithelial cells;

6) To assess the ability of H4R agonist to modulate the proliferation/apoptosis of oral epithelial cells;

7) To assess the ability of H4R agonist and inverse agonist to modulate epithelial-mediated antimicrobial response, and to further envision a potential pro-cancerous, vicious inflammatory circle in OLP lesions.

7. MATERIALS AND METHODS

7.1. Ethical Permissions

Studies utilized OLP patient samples (I-IV, VI) were approved by the Hospital District of Helsinki and Uusimaa (Ethical permission number 42/13/03/01/2013). The usage of OED (study IV) and OTSCC (studies IV, VI) patient samples was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Finland (49/2010, 56/2010) and the Finnish National Supervisory Authority for Welfare and Health (6865/05.01.00.06/2010). Patient involvement in this study was voluntary; written and signed informed consent was obtained from all participants (patients and controls).

7.2. Patient Samples

In study **I**, paraffin-embedded oral mucosal biopsies taken from eighteen OLP patients and ten healthy controls were included. In study **II**, twenty-five OLP and seventeen healthy control samples were used. In study **III**, twenty-five OLP and fifteen healthy control samples were used. In study **IV**, seventeen OED (*dysplasia moderata*) patients, thirty OTSCC patients and 17 healthy controls. Histopathologically, OTSCC specimens were graded according to a three-tier grading scheme (Thompson 2006) as follows: a) grade I, or low-grade tumour, where the majority of cells are well-differentiated; b) grade II, or intermediate-grade, tumour where tissues are dominated by moderately-differentiated cells; c) grade III, or high-grade tumour, which is characterized by poorly-differentiated cancer cells. In study **V**, no patient samples were used. In study **VI**, we used samples from fourteen OLP patients, forty OTSCC patients, and fourteen healthy controls.

OLP patients were clinically and histologically diagnosed by specialized oral pathologists. The inclusion criteria of patients enrolled in this study were as follows: (a) clinical and histological diagnosis of OLP lesions according to the WHO recommendation and its 2009 modification (Kramer et al., 1978; Rad et al., 2009);

(b) patients are not diagnosed with any systemic diseases at the time of sampling; (c) patients have not yet received treatment for OLP or have been off corticosteroids for at least twelve months at the time of biopsy; and (d) lesions do not represent any non-characteristic features of OLP (Rad et al., 2009). OLP samples were obtained from the department of pathology at Helsinki University hospital. OTSCC samples were obtained from Oulu University hospital. The OLP biopsies were taken from active lesion areas, mostly from buccal mucosa. Control samples were obtained from healthy oral mucosa from volunteers during wisdom tooth extraction. The surgical procedures were performed under local anaesthesia (xylocaine-adrenaline, 20 mg/ml 12.5 μg/ml) by specialised oral surgeons. Samples were preserved in 10% formalin and then embedded in paraffin blocks. Formalin-fixed, paraffin embedded (FFPE) samples are stored at room temperature (RT) until further use.

7.3. Cell Culture and Stimulation (studies I, II, IV-VI)

Normal human oral keratinocytes (HOKs)

Primary HOKs and oral keratinocyte culture medium (OKM) were purchased from Science Cell Research Laboratory (ScienCell, Uppsala, Sweden). Oral keratinocyte growth supplements and penicillin/streptomycin solution were added to OKM at concentration of 500 unit per ml. Cells were cultured in a 75 T-flask until 80-90% confluent was reached. Culturing procedure was performed as described in the manufacturer's instruction sheet. For stimulation assays, cells were collected by adding trypsin-ethylenediaminetetraacetic (EDTA) acid, and cells were then cultured in a multi-well plates (BD Falcon, Lawrence, KS, USA) and exposed to various stimulants **(Table 3)**. HOKs were used within cell passages 2-7.

Table 3. Details of different cell-culture stimulants used in this study.

Protein	Company/Provider	Concentration	Time
Study I			
HST-10	Prof. Holger Stark[1]	50 nM	10, 20, 30, 60 min
Histamine	Sigma-Aldrich, MO, USA	50 µM	24 hours
TNF-α	R&D Systems, MN, USA	10 ng/ml	24 hours
IFN-γ	R&D Systems, MN, USA	10 ng/ml	24 hours
Study II			
HST-10	Prof. Holger Stark[1]	100 nM 5, 25, 50, 100 nM	5, 30, 60, 180 min 8 hours
Clathrin inhibitor methyl-β cyclodextrin	Sigma, Steinheim, DE	10 mg/ml	1 hour
TNF-α	R&D Systems, MN, USA	100 ng/ml	36 hours
NF-κB inhibitor IMD-0354	R&D Systems, MN, USA	125 nM	36 hours
Staurosporine	Merk KGaA, DE	100 nM	36 hours
Study III			
Ultrapure LPS	Invivogen, CA, USA	0.05, 0.1,1, 5 µg/ml	24 hours 36 hours
Histamine	Sigma-Aldrich, MO, USA	50 µM	24 hours
TNF-α	R&D Systems, MN, USA	100 ng/ml	24 hours
IFN-γ	R&D Systems, MN, USA	100 ng/ml	24 hours
Study V			
MC-releasate[2]	Katariina Maaninka[3]	90 µg/ml	24 hours
IL-17A	R&D Systems, MN, USA	100 ng/ml	24 hours
Histamine	Sigma-Aldrich, MO, USA	50 µM	24 hours
LPS-binding protein	R&D Systems, MN, USA	100 ng/ml	24 hours
Ultrapure LPS	Invivogen, CA, USA	100 ng/ml	24 hours
Study VI			
LPS-binding protein	R&D Systems, MN, USA	50 ng/ml	8 hours
Ultrapure LPS	Invivogen, CA, USA	5 µg/ml	24 hours
TNF-α	R&D Systems, MN, USA	100 ng/ml	8 hours
IFN-γ	R&D Systems, MN, USA	100 ng/ml	8 hours
Histamine	Sigma-Aldrich, MO, USA	100 µM	8 hours
HST-10	Prof. Holger Stark[1]	1 µM	8 hours
ST-1007	Prof. Holger Stark[1]	1 µM	8 hours

[1]Institut für Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universität Düsseldorf, Germany; [2]MC-releasate was prepared as described in this report (Lappalainen et al., 2007); [3]Wihuri Research Institute, Biomedicum Helsinki, Finland.

Human OTSCC cell lines

Two OTSCC cell lines were used in this research project: the highly-aggressive OTSCC cell line (HSC-3; JCRB Cell Bank; Osaka National Institute of Health Sciences, Osaka, Japan), in addition to the less-aggressive OTSCC cell line (SCC-25; ATCC® CRL-1628™, Rockville MD, USA). Both OTSCC cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM)-12 (Gibco, Payisley, UK), 10% foetal bovine serum, 1000 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.1% hydrocortisone (Life Technologies, Grand Island, NY, USA). Confluent cells were detached, counted and plated in cell culture well-plates for further stimulations **(Table 3)**.

7.4. Purification of Total RNA (studies I, II, IV-VI)

The total RNA samples from healthy and patient FFPE specimens were purified using Maxwell® 16 Low Elution Volume RNA-FFPE Kit and AS2000 Maxwell® 16 Instrument (firmware 4.97; Promega Corp, Madison, WI, USA) according to the recommended isolation program. Total cellular RNA from cultured HOKs and OTSCC cell lines were purified using RNeasy Mini-Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions. The total amount of RNA was measured using the NanoDrop 1000 spectrophotometer (Thermo scientific, Waltham, MA, USA).

7.5. Quantitative Real-Time Polymerase Chain Reaction (qPCR; studies I-VI)

Following total RNA isolation, qRT-PCR was carried out using two µl of first-strand cDNA and 250 nmol/L primers in iQ SYBR® Green supermix, or in SsoAdvanced Universal SYBR® Green Supermix (BioRad Laboratories Inc., Hercules, CA, USA), which is based on Ss7d Fusion Enzyme Technology. Human primer sequences are listed in **Table 4**.

7.6. Droplet Digital Polymerase Chain Reaction (ddPCR; study VI)

The ddPCR is a highly-sensitive technology that can detect a few positive molecules and rare target mutations (van Ginkel et al., 2017). The ddPCR was performed using 20 µl reaction mix that contained 10 µl of QX200™ EvaGreen® ddPCR™ Supermix (Bio-Rad Laboratories), 2 µl of cDNA, 1 µl of gen primers. Samples were pipetted into droplet-generator cartridge, then 70 microliters of Droplet-Generation Oil for EvaGreen was pipetted into the cartridge, followed by droplet generation (QX200™ Droplet-Generator). Forty µl of droplets were then loaded into the columns of a 96-well PCR plate, which was sealed with a supplied foil in PX1-PCR Plate Sealer instrument (Bio-Rad). The plate was then loaded into a T100 Thermal Cycler (Bio-Rad). The sealed plate was then transferred into the droplet reader for detection of completed PCR reactions in droplets. The data were analysed using and QX200™ Droplet Digital™ PCR Systems (Bio-Rad Laboratories) according to the manufacturer's instructions. The Quanta-Soft version 1.7.4.0917 software (Bio-Rad Laboratories) was used for data analysis.

Table 4. Details of human primer sequences used in this study.

Primer	Primer sequences	Study
H4R	Forward: 5′-TGGAAGCGTGATCATCTCAG-3′ Reverse: 5′-ATATGGAGCCCAGCAAACAG-3′	I, V
Bcl-XL	Forward:5′-GATCCCCATGGCAGCAGTAAAGCAAG-3′ Reverse: 5′-CCCCATCCCGGAAGAGTTCATTCACT-3′	II, V
BAX	Forward: 5′-CGGGTTGTCGCCCTTTTCTA-3′ Reverse: 5′-GTCCAATGTCCAGCCCATGA-3′	II, V
HDC	Forward: 5′-TTGATTGCCCTGCTGGCAGC-3′ Reverse: 5′-TGCACAGACAAAGACGGGCACC-3′	III, V
OCT3	Forward: 5′-CGGGTTGTCGCCCTTTTCTA-3′ Reverse: 5′-GTCCAATGTCCAGCCCATGA-3′	III
HNMT	Forward: 5′-TGGCATCTTCCATGAGGAGCTT-3′ Reverse: 5′-AAAATCCCAAAGCAGGTCTCCAT-3′	III
AOC1	Forward: 5′-GCTACGTCCACGCCACCTTCTA-3′ Reverse: 5′-CCCAGGCCACCAGGTCCTCA-3′	III
ITGA6	Forward: 5′-GGAGACCCCGGGAGCCTCTTC-3′ Reverse: 5′-AGCCCTCCCGTTCTGTTGGCT-3′	III
ITGB4	Forward: 5′-CTACACGGTGAAGGCGCGCAA-3′ Reverse: 5′-CCAGCAGGGGCTCGAACTTCC-3′	III
TLR1	Forward: 5′-CGGAGGCAATGCTGCTGTTCAG-3′ Reverse: 5′-TGTAGGGGTGCCCAATATGCCT-3′	IV
TLR2	Forward: 5′-GCTGCTCGGCGTTCTCTCAGG-3′ Reverse: 5′-TGTCCAGTGCTTCAACCCACAACT-3′	IV

TLR3	Forward: 5′-TGCCGTCTATTTGCCACACACTTC-3′ Reverse: 5′-GTGCACTTGGTGGTGGAGGATGC-3′	IV
TLR4	Forward: 5′-CCTGCGTGGAGGTGGTTCCTA-3′ Reverse: 5′-CCAGAAAAGGCTCCCAGGGCTA-3′	IV
TLR5	Forward: 5′-TGTTGGCGCTGTCCGAACCT-3′ Reverse: 5′-AGGTGGTCTCCCATGATCCTCG-3′	IV
TLR6	Forward: 5′-AAGAGATCTTGAATTTGGACTCATATC-3′ Reverse: 5′-TGAAGCTCAGCGATGTAGTTC-3′	IV
TLR7	Forward: 5′-TCTTGGCACCTCTCATGCTCTGC-3′ Reverse: 5′-GTGAGGTTCGTGGTGTTCGTGGG-3′	IV
TLR8	Forward: 5′-CTGCGCTGCTGCAAGTTACGGA-3′ Reverse: 5′-TTGCCCACCGTTTGGGGAACTTC-3′	IV
TLR9	Forward: 5′-CCCAGCATGGGTTTCTGC-3′ Reverse: 5′-ACTTCAGGAACAGCCAGTTG-3′	IV
TLR10	Forward: 5′-CTCCCAACTTTGTCCAGAAT-3′ Reverse: 5′-TGGTGGGAATGCAATAGAAT-3′	IV
EGF	Forward: 5′-ATGCGGTTGTTCCTCACCCG-3′ Reverse: 5′-GCTGGCTGAGCAGAGTTCCA-3′	V
EGFR	Forward: 5′-CTCTTCGGGGAGCAGCGAT-3′ Reverse: 5′-AAAGTGCCCAACTGCGTGAG-3′	V
hBD-2	Forward: 5′-ATCAGCCATGAGGGTCTTGT-3′ Reverse: 5′-GAGACCACAGGTGCCAATTT-3′	VI
TNF-α	Forward: 5′-CTTTGGAGTGATCGGCCCC-3′ Reverse: 5′-GGTTATCTCTCAGCTCCACGC-3′	VI
NF-κB	Forward: 5′-CCAGACCAACAACAACCCCT-3′ Reverse: 5′-TCACTCGGCAGATCTTGAGC-3′	VI
STAT1	Forward: 5′-AGTCTGGCGGCTGAATTTCG-3′ Reverse: 5′-GATCACCACAACGGGCAGAG-3′	VI
GAPDH	Forward: 5′-AAGGTCATCCCTGAGCTG-3′ Reverse: 5′-TGCTGTAGCCAAATTCGTTG-3′	I-VI
RPLP0	Forward: 5′-GGCGACCTGGAAGTCCAACT-3′ Reverse: 5′-CCATCAGCACCACAGCCTTC-3′	I-VI

7.5. Immunostaining (studies I-VI)

Immunohistochemistry (IHC)

Formalin fixed and paraffin embedded tissue samples were cut to 5-µm sections and incubated overnight at 37°C. The slides were first deparaffinised, and the antigen retrieval was performed in 10 mM sodium citrate buffer, pH 6.0, in Micromed T/T Mega Microwave processing Lab station for Histology (HACKER Instruments & Industries Inc., Winnsboro, SC, USA) for 25 min at +95°C, followed by cooling at RT for 20 min. Endogenous peroxide was blocked using 3% H_2O_2 in phosphate-

buffered saline (PBS) for 10 min, then washed 3x5 min in PBS. The nonspecific staining was blocked by incubation in 10% normal serum for 1 hour at RT. Slides were then incubated with the primary antibodies (**Table 5**) overnight at +4°C and washed 3x5 min in PBS. Appropriate biotin-conjugated secondary antibodies against IgG (Vector Laboratories; 1:200 in 1.25% bovine serum albumin (BSA)-PBS) were added to the slides for 1 hour and then washed 3x5 min in PBS. Slides were then followed by incubation with ABC complex (1:200 in H_2O, Vector Laboratories) for 1 hour at RT and washed 3x5 min in PBS. To develop the colour, slides were then incubated in 0.006% H_2O_2 and 3,3´-diaminobenzidine (DAB) chromogen for 10 min at RT and washed 3x5 min in dH_2O. Cell nuclei were stained with hematoxylin, dehydrated and mounted in Mountex (HistoLab, Gothenburg, Sweden). For negative controls, slides were incubated with non-immune IgG at the same concentration, the same incubation time, and in the same environment as the primary antibodies. Antibody concentration was optimized through pilot experiments with controls.

Immunofluorescence (IF)

Cultured cells on coverslips and paraffin-embedded tissue sections were first permeabilized with 0.5% Triton X-100 for 10 min at RT, and washed 3x5 min in PBS. Non-specific staining was blocked by incubation in a 10% normal serum for 1 hour at RT. The coverslips and slides were incubated with primary antibodies (**Table 5**) overnight at +4°C and washed 3x5 min in PBS. Then, they were incubated with fluorescein-conjugated secondary antibodies for 1 hour at RT, and washed 3x5 min in PBS. Cells nuclei were stained using 4'6-Diamidino-2-phenylindole (DAPI) for 10 min at RT and washed 3x5 min in PBS. Samples were mounted in Vectashield® (Vector Laboratories, Burlingame, CA) and kept at +4°C in a dark place for overnight incubation to ensure an optimum curing time of the reagent. For the negative staining of controls, slides were incubated with a non-immune IgG at the

same concentration, duration, and in the same environment as the primary antigen-specific antibodies. Positive tissue controls were also used where applicable.

Table 5. Primary antibodies used in this study.

Antibody	Source and clonality	Manufacturer or provider	Concentration or dilution	Method
		Study I		
H4R	Rabbit, p	MBL International, MA, USA	1 µg/ml	IHC-IF
Tryptase	Mouse, m	AbD Serotec, Oxford, UK	0.05 µg/ml	IHC
		Study II		
H4R	Rabbit, p	MBL International, MA, USA	1 µg/ml	IHC-IF
cPARP	Rabbit, m	Abcam, Cambridge, UK	50 mM	WB
ERK1/2	Mouse, m	Millipore, Temecula, CA	0.25 µg/ml	WB
pERK1/2	Mouse, m	Millipore, Temecula, CA	0.1 µg/ml	WB
pJNK	Rabbit, p	Millipore, Temecula, CA	1 µg/ml	WB
Bcl-XL	Rabbit, p	Abcam, Cambridge, UK	0.128 µg/ml	WB
BAX	Rabbit, m	Abcam, Cambridge, UK	0.114 µg/ml	WB
		Study III		
HDC	Mouse, m	Dr. Francisca Sanchez Jimenez[1]	1:350	IHC
DAO	Mouse, m	Dr. Hubert Schwelberger[2]	1:400	IHC
HNMT	Rabbit, p	Abcam, Cambridge, UK	2.5 µg/ml	IHC
OCT1, 2	Rabbit, p	Dr. Anne T. Nies[3]	1:100	IF
OCT3	Goat, p	Santa Cruz Biotech., CA, USA	1 µg/ml	IF
		Study IV		
TLR1 to 10*	Rabbit, p	Santa Cruz Biotech., CA, USA	(1;2;1.8;1.5;1;1;1;1;1;2) µg/ml	IHC
		Study V		
H4R	Rabbit, p	LifeSpan Biosciences, WA, USA	1 µg/ml	IHC-IF
MC-tryptase	Rabbit, m	Abcam, Cambridge, UK	1 µg/ml	IHC
		Study VI		
β-defensin-2	Rabbit, p	Bioss, Woburn, MA, USA	5 µg/ml	IHC-IF
H1R; H2R; H3R; H4R	Rabbit, p	LifeSpan Biosciences,WA, USA	1 µg/ml	IHC
CD4	Mouse, m	Dako, Glostrup, Denmark	3 µg/ml	IF
CD8	Mouse, m	Dako, Glostrup, Denmark	1.5 µg/ml	IF
CD163	Mouse, m	Leica Biosystems, Newcastle, UK	10 µg/ml	IF
MC-chymase	Mouse, m	Thermo Fisher Scientific, MA, USA	5 µg/ml	IF

[1]University of Málaga, Málaga, Spain; [2]Medical University Innsbruck, Innsbruck, Austria; [3]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany; *respectively. m= monoclonal; p= polyclonal.

7.6. High Performance Liquid Chromatography (HPLC; studies II, V)

HPLC combined with fluorescence detection was used to detect histamine level in supernatants from bacterial lipopolysaccharides-stimulated and non-stimulated HOKs. The HPLC system comprised of 4 pumps, autosampler (SIL-20AC), fluorescence detector (RF-10Axl) and controller. The software (LC-Solution 1.21) was employed for system control and data collection and processing. The lower limit of detection of histamine with signal-to-noise ratio 3:1 was approximately 5 fmol. We used Yamatodani's method used for histamine analysis (Yamatodani et al., 1985).

7.7. Western Blotting (WB; study V)

Following induction of apoptosis, NS-SV-AC cells were collected at different time points for analysis of cleaved poly-ADP-ribose polymerase (PARP), Bcl-XL and Bcl-2-associated X (BAX) proteins. After brief centrifugation, cells were lysed and sonicated the centrifuged again at 14.000 g for 10 min at +4°C. Supernatants were collected and total protein concentration was measured using Pierce BCA Protein Assay Kit (Thermo Scientific, IL). Total protein amount of the samples was between 9-22 µg per lane. The sample proteins were denatured by heating up at +95°C for 10 minutes, then followed by electrophoresis and blotting samples to methanol-activated polyvinylidene fluoride (PVDF) membranes. PVDF membranes were then washed in TBS buffer, blocked in BSA buffer for 1 hour at RT, and incubated overnight at +4°C in primary antibodies (**Table 5)**. PVDF membranes were then washed 3x10 minutes and secondary antibodies were applied for 1 hour at RT. The membranes were washed 4x10 minutes in TTBS and detection was performed by chemiluminescent reaction using Bio-Rad Immun-Star™ WesternC™ Kit and Bio-Rad ChemiDoc™ MP Imaging system. Band intensity was analysed by Bio-Rad Image Lab software.

7.8. Microscopy and Image Analysis (studies I-VI)

ZEISS Primo Vert microscope (Carl Zeiss Microscopy, Thornwood, NY, USA) was used to monitor culture and examine cell appearance and growth. Fully-automated Leica DM6000 microscope equipped with Leica DFC365-FX digital camera (Leica Microsystems, Wetzlar, Germany) was used to image immunostained sections and cells. For immunolocalization (study III), cells were photographed using Zeiss Confocal Laser Scanning microscope (CLSM 880; Zeiss Corp, Oberkochen, Germany). The staining intensity was measured using ImageJ program (version 1.47; National Institute of Mental Health, Bethesda, MD, USA). For staining intensity analysis, three different areas per tissue section of OLP samples and controls were analysed at a field magnification of 40x. A stop filter type was used with a hue value of between 0 and 161. The relative staining intensity of each antigen was determined by applying the inverse mean grey value.

7.9. Statistical Analysis

Study results are presented as means ± standard deviation, and data were obtained from at least three independent experiments. Data analysis were performed using SPSS software programme version 21.0 (studies I-V; IBM SPSS Statistics, SPSS Inc., Chicago, IL, USA) or GraphPad Prism version 6 (GraphPad Software, La Jolla, CA) for study VI. In study I, two-tailed Student's t-test was used, and linear regression analysis was used to assess the correlation between H_4R staining intensity and MC count. In studies II-VI, two-tailed Student's t-test or one-way Analysis of variance (ANOVA) followed by Bonferroni or Tukey's multiple comparison post-hoc tests were used to estimate the statistical significance between two-grouped and multiple-grouped data, respectively. Mann–Whitney U test, or Kruskal-Wallis followed by Dunn's multiple comparisons test were applied where appropriate. Statistical significance was set at P values ≤ 0.05. Significance was indicated as *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, and ****P ≤ 0.0001.

8. RESULTS

8.1. Characterization of H4R in OPMDs, OTSCC, and oral epithelial cell lines (studies I, IV)

Healthy oral epithelium expressed high levels of constitutive H4R at both protein and mRNA levels, which exhibited a uniform pattern of staining in healthy epithelial controls (**Figure 4a**). On contrary, H4R protein staining was noticeably reduced in specimens obtained from OLP, OED, and OTSCC patients.

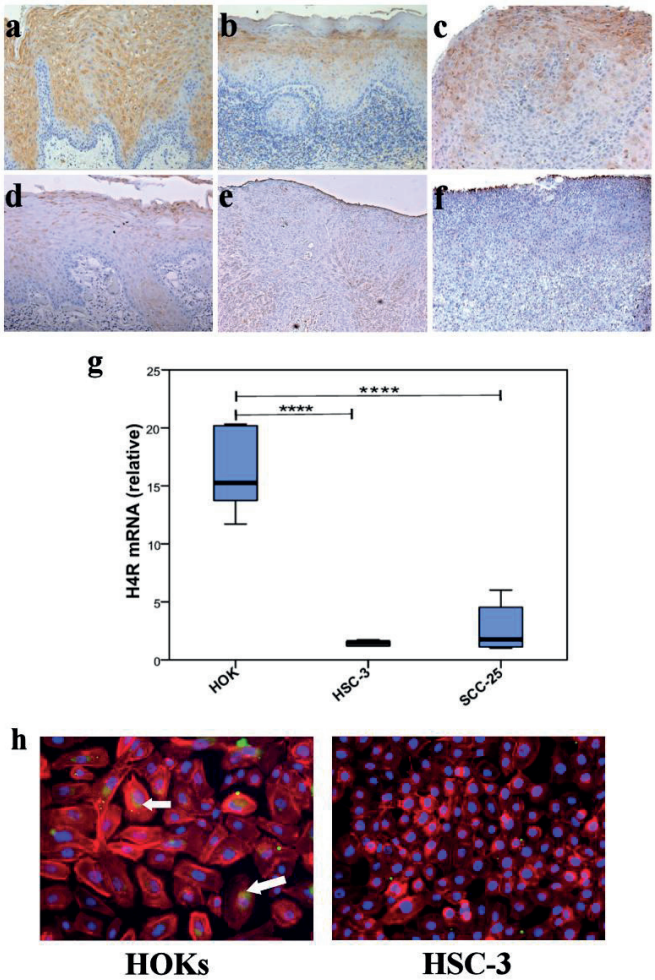

Figure 4. Expression of H4R in: a) healthy oral epithelium, b) OLP, c) OED, d) grade II OTSCC, e) grade II OTSCC, f) grade III OTSCC; g) *H4R* mRNA in HOKs and cancer cells; h) H4R in HOKs and HSC-3.

In OLP and OED tissues, H4R immunoreactivity was less intense and not detectable in basal and suprabasal epithelial layers, while H4R-expressing cells were found in the middle spinous cell layer and with faint and irregular staining of the granular cell layer (**Figure 4b, c**). In OTSCC tissues, H4R protein immunopositivity was decreased in all cases and, interestingly, exhibited a clear grade-dependent manner, with least detectable H4R-immunoreactivity in grade III samples (**Figure 4d-f**). These findings were also supported by low *H4R* expression in HSC-3 and SCC-25 cells (**Figure 4g**). Immunocytochemistry results revealed high expression of H4R in normal HOKs compared with very faint and inconsistent immunostaining in OTSCC cells (**Figure 4h)**.

8.2. High histamine concentration and IFN-γ downregulate H4R expression in HOKs (study I)

Quantitative RT-PCR results showed that the mRNA expression of *H4R* was significantly downregulated in oral epithelial cells after 24 hour exposure to 50 μM histamine and 10 ng/ml IFN-γ. The effect of TNF-α was statistically not significant (**Figure 5a**).

8.3. Negative correlation between MCs and H4R in OPMDs and OTSCC lesions (studies I, IV)

MC-tryptase staining revealed few MCs in healthy control samples compared with higher cell count in OLP and OTSCC tissues. Moreover, we found a clear negative correlation between degranulated MCs and the staining intensity of H4R in oral epithelium of OLP lesions (**Figure 5b**).We assessed MC distribution in tissue biopsies of OLP, OED and OTSCC. MCs were markedly increased in OLP and OTSCC tissues mainly in the lamina propria and subepithelial connective tissue regions, while a moderate rise in MC count was seen in OED (**Figure 5c**). Of note, MC count was highest in grade III OTSCC samples.

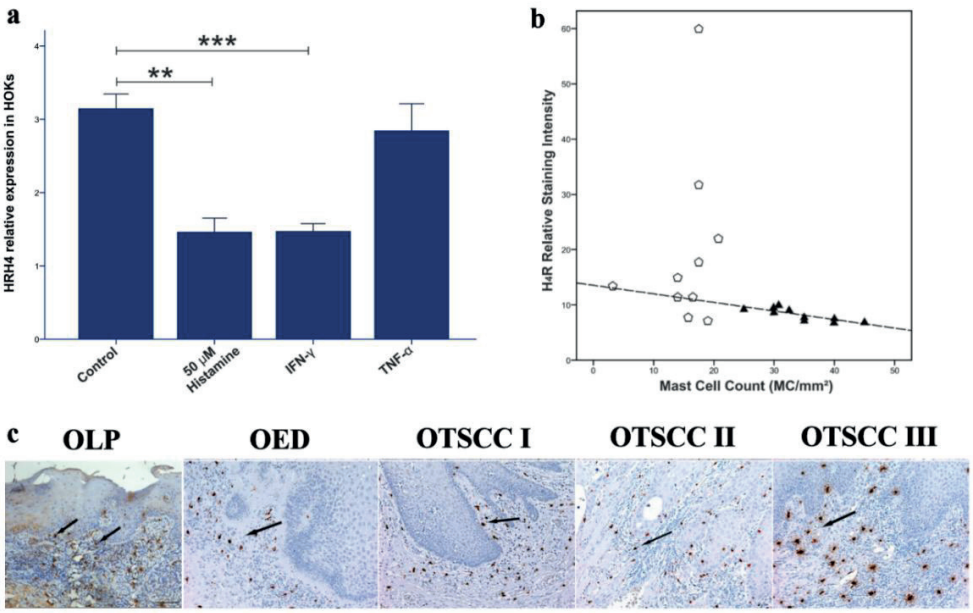

Figure 5. a) MC mediators affect *H4R* expression; b) MCs correlate negatively with H4R staining intensity, triangles represent healthy samples, pentagons represent OLP; c) MC Tryptase staining in different lesions.

8.4. Rapid internalization of H4R in human oral epithelial cell lines (studies I, V)

Although we showed that human oral epithelial cells express H4Rs, it was necessary to test if such cells can bind and internalize the specific ligands of H4R. To avoid potential cross-binding of histamine with the other receptors, cells were stimulated with H_4R-specific agonist HST-10 [N-(3-(1H-imidazol-4-yl) propyl)-2-cyclohexylacetamide], which has been suggested as the most selective agonist to human H4R (Kottke et al., 2011). Cells were stimulated for different times at +37°C (**Table 3**). For HOKs and NS-SV-AC cells, non-stimulated, zero time-point controls showed weak or no labelling signal. However, an increased staining intensity of H4R was observed at 5, 10 and 20 min time points, which then turned into cytoplasmic and becomes somewhat weaker at 30 min, until it disappears after 60 min stimulation with HST-10 (**Figure 6a**).

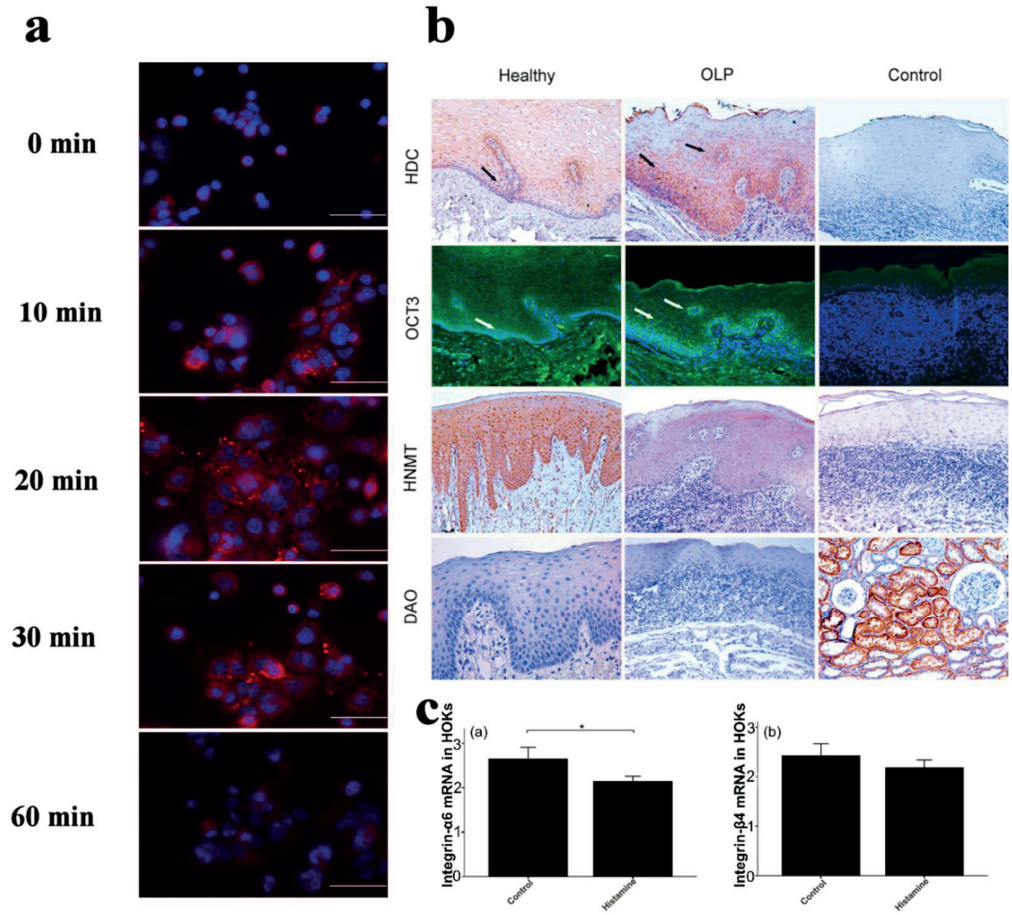

Figure 6. a) Internalization of H4R ligand (HST-10) in cultured HOKs; b) Histamine metabolizing molecules are deranged in OLP lesions; c) High histamine levels affect mRNA expression of integrins in HOKs.

8.5. Histamine metabolism capacity is deranged in OLP lesions (study II)

We showed in the second study that normal HOKs are equipped with histamine metabolizing capacities, including HDC, OCT3 and HNMT molecules. In OLP lesions, HDC and OCT3 levels were increased while HNMT was decreased compared with healthy controls (**Figure 6b**). OCT1/OCT2 showed weak and inconsistent staining patterns, whereas DAO was negative in HOKs (not shown). Next, we investigated the potential consequences of deranged histamine metabolism in oral mucosa, and whether surplus histamine can affect the integrity of oral

epithelium. Our data disclosed a significant downregulation of *integrin-α6* and *integrin-β4* transcripts in HOKs when exposed to 50 μM histamine for 24 h (**Figure 6c**). These genes were also downregulated in samples from OLP patients (data not shown).

8.6. Bacterial lipopolysaccharides (LPS) induces histamine synthesis and release by normal HOKs (study II)

Next, after having confirmed the presence of histamine metabolizing molecules in normal cultured HOKs, we studied the potential inflammatory and environmental mediators that govern histamine metabolism in oral epithelium. Therefore, HOKs were stimulated by bacterial LPS and MC-derived mediators (INF-γ, histamine and TNF-α). After 24 hours of stimulation, *HDC* mRNA was upregulated with the highest expression seen at 5, 1 and 0.05 μg/ml ($P = 0.003$; 0.01; 0.02 respectively; **Figure 7a**). Similarly, stimulation with IFN-γ (100 ng/mL) significantly upregulated *HDC*, *OCT3* and *HNMT* transcripts in HOKs ($P = 0.02$; 0.03; 0.03, respectively), while 50 μM histamine and 100 ng/mL TNF-α showed inhibitory effects (**Figure 7b-d**). Furthermore, when HOKs were exposed to LPS, HPLC analysis of the supernatants showed that LPS-stimulated cells produced low (picomolar) concentrations of histamine that were detectable at 36 hours. The highest histamine level was detected in response to 0.05 μg/mL LPS (2.2 pmol/ml; $P = 0.02$; **Figure 7e**).

8.7. Altered expression of TLR family in OLP lesions (study III)

After having confirmed the involvement of bacterial LPS in histamine synthesis and metabolism in human oral epithelium, we next mapped the main innate sensors of such invading bacterial components—TLRs.

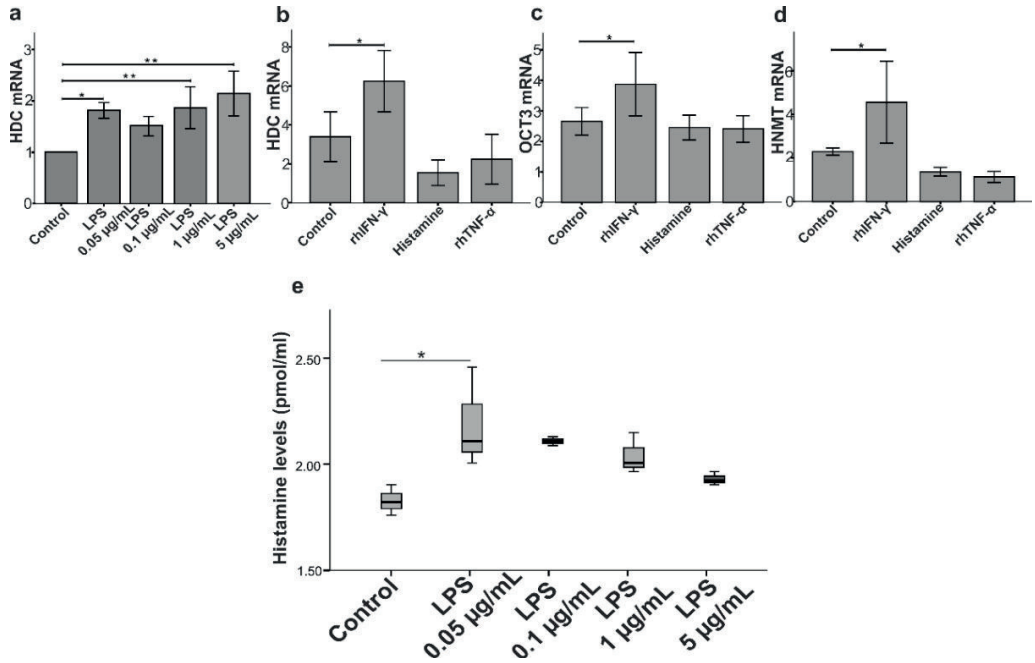

Figure 7. a-d) LPS and MC mediators regulate histamine synthesis in human oral epithelial cells; e) LPS induces histamine synthesis and release from cultured normal HOKs in dose-dependent manner.

The TLR family is a crucial group of PRRs that mediate the recognition of invasive pathogens (e.g. LPS) by immune cells. Once activated, TLRs induce production of proinflammatory cytokines that can initiate and perpetuate the inflammatory lesions. Interestingly, the immunoreactivity for all TLRs was increased in OLP, except for TLR5, which was noticeably decreased. On gene level, *TLR1*, *TLR2*, *TLR4*, *TLR7*, *TLR8*, and *TLR9* transcripts were upregulated in patients compared with controls. On contrary, *TLR3*, *TLR5*, and *TLR6* transcripts were negatively regulated in OLRs. *TLR10* remained unchanged in both study groups. Our findings are summarized in **Table 6**.

The intensity of TLR staining was scored in the basal/suprabasal, intermediate and upper cell layers of the oral epithelium. The intensity of TLR immunoreactivity in

healthy control and OLP patient samples is expressed as follows: (-) to indicate a negative TLR-staining; (+) weakly positive TLR-staining; (++) moderately positive TLR-staining; and (+++) for a strongly positive TLR-staining. Scoring was performed by three independent researchers, who were blinded to the information of the study samples and antibodies.

Table 6. Intensity of TLR-immunoreactivity in different epithelial layers of OLP samples.

	Basal/suprabasal layers		**Intermediate layers**		**Superficial layers**	
	Control	*OLP*	*Control*	*OLP*	*Control*	*OLP*
TLR1	+++	+++	+	++	-	++
TLR2	+++	+++	+	+++	-	+++
TLR3	+++	+++	+	+++	+	++
TLR4	+++	+++	+	+++	-	+++
TLR5	+++	+	++	-	+	+
TLR6	+++	++	+	++	-	+++
TLR7	+++	+++	+	+++	-	+++
TLR8	+++	+++	-	++	-	++
TLR9	+++	+++	+	+++	-	+++
TLR10	+	+	-	+	-	-

8.8. MC-derived mediators and LPS regulate oral oncogene expression in HOKs (study IV)

We performed gene analysis assays to ascertain whether MC-derived mediators or bacterial LPS can influence the expression of certain genes associated with oral carcinogenesis. Hence HOKs were stimulated with IL-17, MCR, histamine and LPS for 24 hours, as detailed in **Table 3**. Both IL-17A and MCR upregulated the expression of two oncogenes: *EGF* ($P = 0.002$; 0.009, respectively) and *EGFR* ($P = 0.030$; 0.010 respectively). In contrast, MCR downregulated *Bcl-2* ($P > 0.05$) and *Bcl-xL* ($P = 0.007$) genes, while histamine downregulated *Bcl-2* ($P = 0.005$) expression. LPS significantly upregulated *EGFR* expression ($P = 0.002$). *EGF* also tends to be induced by LPS; however, the difference was not statistically significant (**Figure 8a**).

8.9. Activation of H4R modulates oral cell proliferation in a dose-dependent manner (study V)

TNF-α combined with NF-κB inhibitor (IMD-0354) induced apoptotic signals in normal oral (NS-SV-AC) cells. Interestingly, H4R agonist (HST-10) seems to exhibit a dual effect based on its concentration. Lower "nanomolar" concentrations of HST-10 inhibited cell apoptosis in a dose-dependent manner. Phase contrast microscopy disclosed that 100 nM of HST-10 was able to preserve cell structure and prevented signs of cell apoptosis (**Figure 8b**). QRT-PCR showed up-regulation of anti-apoptotic *Bcl-XL* mRNA and protein in H4R-stimulated samples, however, no effect was observed on BAX expression (**Figure 8c**). Furthermore, H4R-agonist diminished cleavage of the early apoptotic marker PARP (**Figure 8d)** and flow cytometry showed significant dose-dependent inhibitory effect of H4R stimulation on apoptosis (not shown).

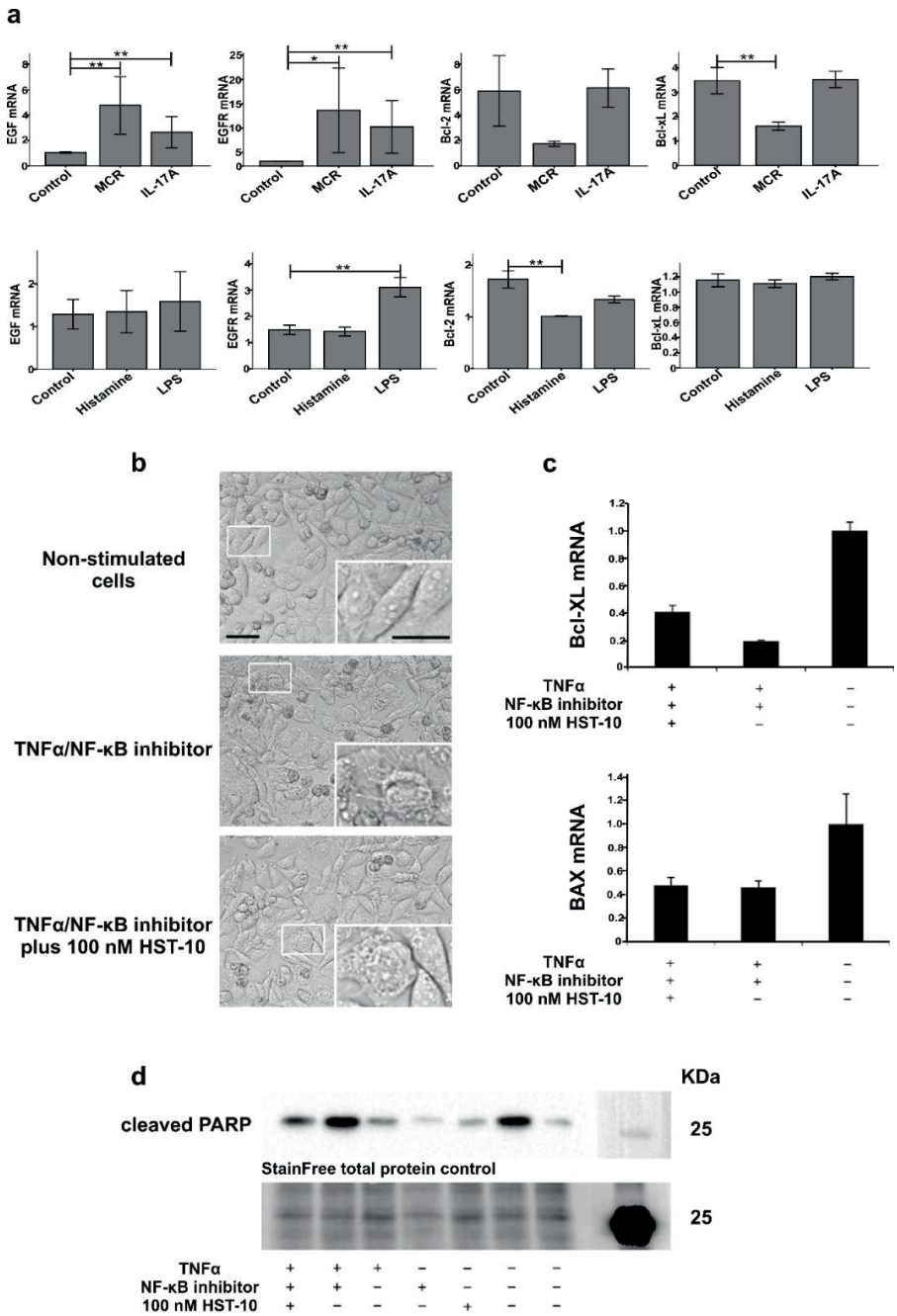

Figure 8. a) MC-derived mediators and LPS regulate the expression of several oncogenes in HOKs; b) Figures from phase-contrast microscopy shows the effect of H4R-agonist (HST-10) on NS-SV-AC cell apoptosis; c) up-regulation of anti-apoptotic *Bcl-xL* mRNA upon HST-10 stimulation while pro-apoptotic *BAX* gene remains unchanged; d) HST-10 inhibited the early apoptotic marker PARP in NS-SV-AC cells.

8.10. Induced expression of hBD-2 in OLP (study VI)

In addition to their role in OLP pathogenesis and histamine signalling, LPS and MCs are considered the main inducers of epithelial hBD-2, which has also been implicated in OSCC pathogenesis and progression (Verma et al., 2007; Shi et al., 2014). To investigate the potential relationship between LPS, histamine and hBD-2 signalling in oral carcinogenesis, immunohistochemical analysis was performed on patient and control tissue samples. HBD-2 expression was either negative or very faint in healthy controls compared with high hBD-2-immunoreactivity in OLP lesions (**Figure 9a**). Interestingly, hBD-2 protein was also induced in OTSCC specimens as compared with controls, but the expression was in general less intense in cancer tissue than in OLP. This finding was also consistent with ddPCR results obtained from healthy and OLP samples, which revealed upregulated *hBD-2* transcript in OLP patients (**Figure 9b**). *HBD-2* was expressed in normal HOKs, while it was not detected in OTSCC cells (**Figure 9c**).

8.11. LPS and inflammatory mediators stimulate hBD-2 expression in HOKs (study VI)

Based on the previous findings that histamine, cytokines and LPS are involved in OLP and OTSCC pathogenesis, we therefore investigated the potential effect of such mediators on hBD-2 expression in HOKs. As expected, LPS and IFN-γ significantly (P=0.03; 0.02, respectively) induced *hBD-2* mRNA in cultured normal HOKs compared with the non-stimulated control cells. TNF-α alone slightly induced *hBD-2* mRNA (**Figure 9d**).

8.12. Subepithelial MCs and macrophages produce hBD-2 in OLP (study VI)

Immunostaining revealed that hBD-2 was expressed in OLP samples in epithelial cells and lamina propria alike, and hence we investigated the plausible "subepithelial" sources of hBD-2 in OLP lesions. Double-label immunofluorescence

staining revealed a very faint hBD-2 in CD4+ and CD8+ T-lymphocytes. CD163+ macrophages were positive for hBD-2. Subepithelial MCs (expressing chymase) revealed strong expression of hBD-2+ (**Figure 9e**).

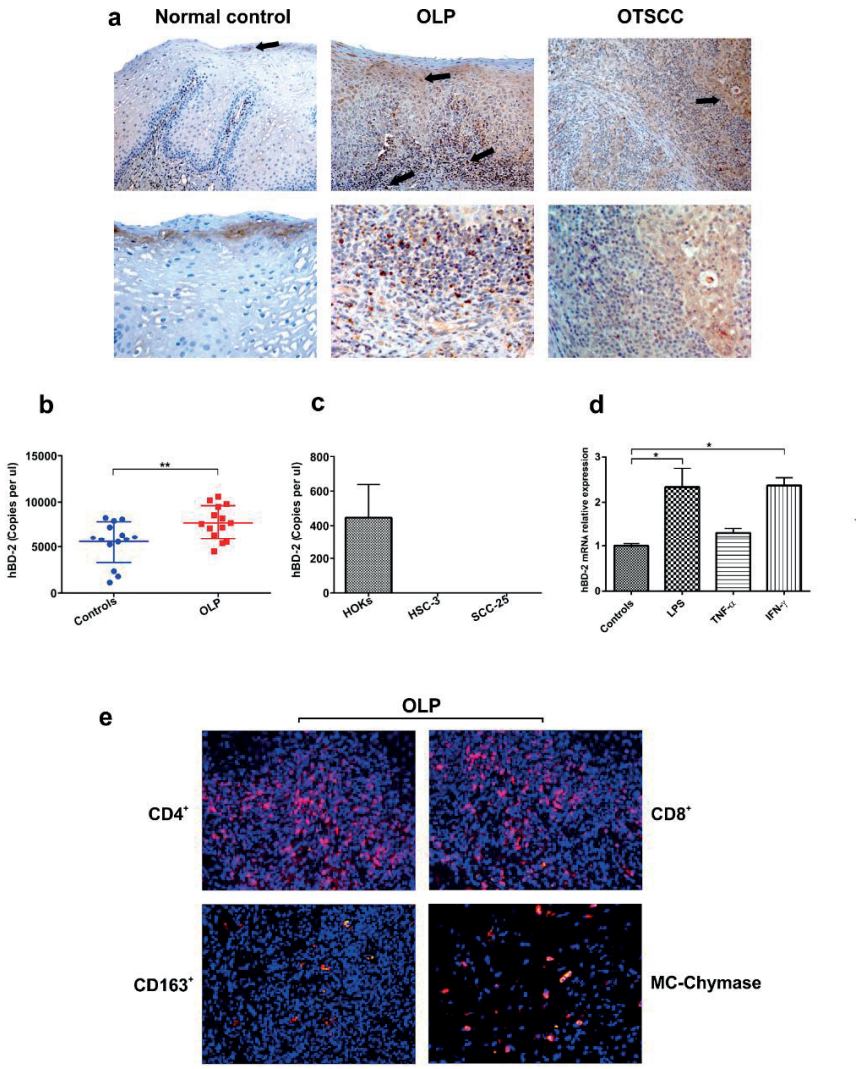

Figure 9. a) Faint staining of hBD-2 in normal oral epithelium which is more confined in the uppermost superficial layers (arrow). OLP lesions exhibited strong epithelial and subepithelial hBD-2 expression; b) *hBD-2* transcript was highly induced in OLP patients; c) *hBD-2* mRNA was expressed in normal HOKs but not detected in OTSCC cell lines (HSC-3 and SCC-25; d) LPS and IFN-γ induced *hBD-2* mRNA in HOKs; e) hBD-2 staining was very weak in subepithelial CD4+ and CD8+ lymphocytes in OLP. CD163+ macrophages were more intense for hBD-2. Subepithelial MCs revealed strong expression of hBD-2.

8.13. Histamine effect on hBD-2 production in HOKs (study VI)

Next we investigated the role of histamine and LPS on hBD-2 production in HOKs. Histamine alone did not have an effect on the *hBD-2* mRNA level, but it enhanced TNF-α-mediated upregulation of *hBD-2* mRNA (**Figure 10a**). Furthermore, histamine regulated the transcriptional promoter region of hBD-2. Histamine mediated the TNF-α-induced expression of NF-κB p65 and the IFN-γ-mediated STAT1 expression (P = 0.006) (**Figure 10b-c**). This was further confirmed by immunofluorescence staining, which revealed intense expression of activated NF-κB p65 and p-STAT1 in OLP patients compared with healthy controls (**Figure 10d**).

8.14. Activation of H4R modulates TNFα- and LPS-mediated hBD-2 pathways (study VI)

After reporting that activation of H4R enhanced prosurvival signals in oral NS-SV-AC cell lines, which protected cells from apoptosis (**study V**), we further show that H4R targeting can regulate hBD-2 expression in HOKs. Cells were initially pre-treated with 1 μM of HST-10 or ST-1007 for 8 hours, and then stimulated with inflammatory mediators (8 hours; **Table 3**) or 5 μg/ml LPS (24 hours). Interestingly, histamine/TNF-α-induced hBD-2 expression was upregulated in ST1007-challanged HOKs (P = 0.04). In contrast, the same pathway was downregulated in HOKs with HST-10 (**Figure 10e**). Similarly, *hBD-2* was induced in HOKs stimulated with ST-1007 and LPS (P = 0.06), while HST-10 diminished such effect in a similarity to the histamine/TNF-α-mediated pathway (**Figure 10f**).

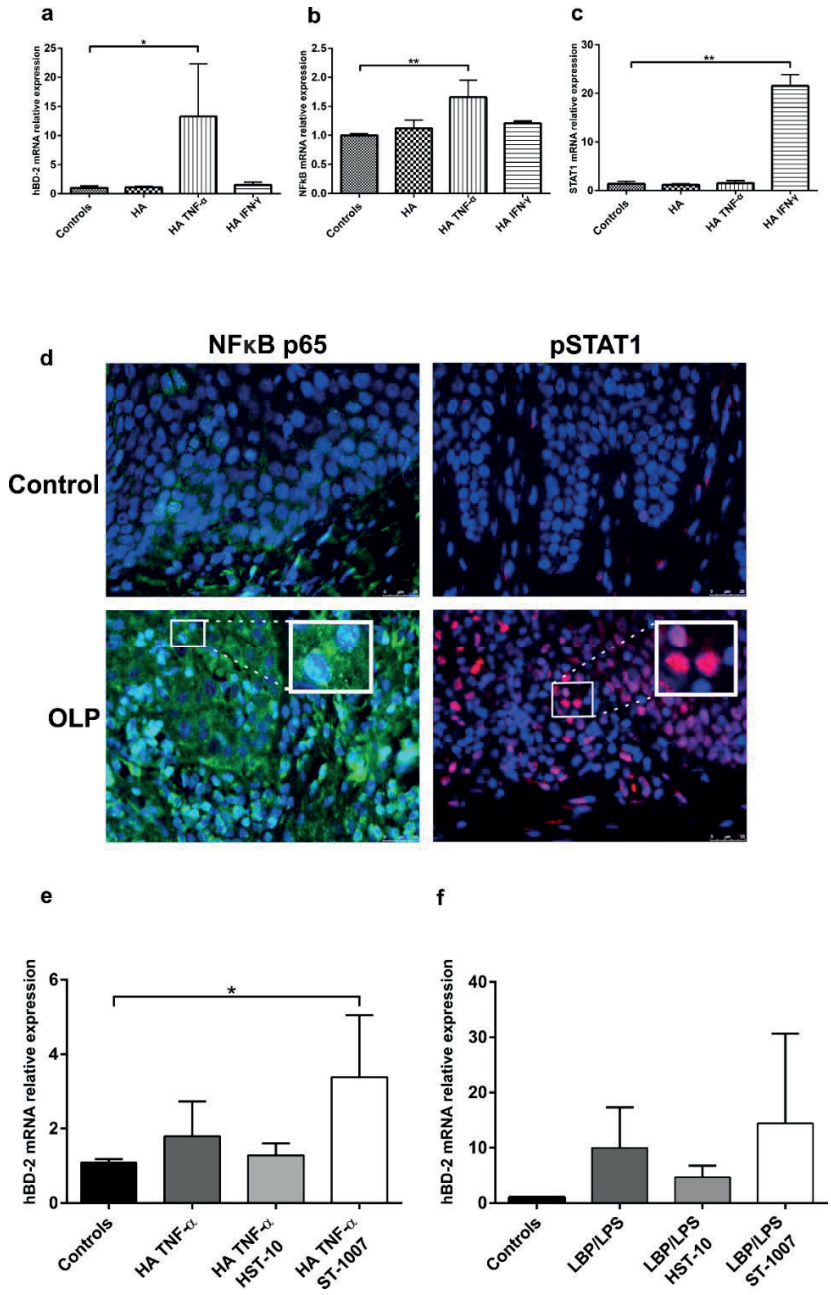

Figure 10. a) Histamine (HA) together with TNF-α, but not alone, induced *hBD-2* mRNA in HOKs; b and c) Histamine synergistically influenced the promoter region (NF-κB p65 and STAT1) of *hBD-2* transcript via both TNF-α- and IFN-γ-mediated pathways; d) Induced expression of activated NF-κB p65 and p-STAT1 in OLP samples compared with healthy controls; e and f) Targeting of H4R modulated hBD-2 expression in HOKs via both histamine and bacterial LPS.

9. DISCUSSION

The most recently discovered member of histamine receptor family, H4R, is essentially expressed by cells of the haematopoietic lineage, immune system, and also on other cell types including cancer cell lines (Thurmond et al., 2008). Such a wide distribution of H4R reflects the crucial immunomodulatory role of this novel receptor, not only in innate and adaptive immune responses, but also in carcinogenesis (Zampeli and Tiligada 2009; Medina and Rivera 2010; Thangam et al., 2018). Accordingly, recent reports indicate the involvement of H4R in mediating the pathogenesis of several disorders, such as autoimmune diseases, bronchial asthma, pruritus and cancer (Thurmond et al., 2008; Ohsawa and Hirasawa 2012; Neumann 2017; Sterle et al., 2018). Indeed, these findings signify the therapeutic potential of H4R ligands, which have already been translated into clinical settings for the treatment of chronic pruritus and AD (Thurmond et al., 2015).

In fact, human skin keratinocytes were shown to express functional H4Rs, which have been implied in itch and AD pathology (Gutzmer et al., 2011; Yamaura et al., 2013). Here we show that healthy human oral mucosal epithelial cells express H4R protein and mRNA. However, immunostaining assays disclosed a marked alteration in H4R immunoexpression and diminished staining intensity in OLP, OED and OTSCC tissues compared with the healthy oral epithelium. The exact reason for this dramatic loss of H4R-immunoreactivity is not yet understood. However, based on the mechanistic *in vitro* experiments, we suggest that such loss may result from the combination of swift "burst-released" MC-derived histamine (micromolar levels) and slow cytokine-mediated effects. Such explanation is also supported by our findings that negative association exists between local MCs and H4R immunoexpression in the lesional samples, and that MC-releasate downregulated *H4R* in HOKs. MCs were markedly increased in OLP and OTSCC tissues mainly in the lamina propria and connective tissue regions. In fact, MCs play important roles in cancer-associated events (e.g. angiogenesis and matrix degradation). This has

been discussed in the literature review (**section 5.14**). Of note, MCs can induce protumorigenic immunoregulation by inducing immunosuppressive and phenotypic changes in tumour-associated regulatory T cells (Kalesnikoff and Galli 2011; Ribatti et al., 2018).

It was also interesting, in the same context, that H4R immunoexpression was negatively correlated with the respective OTSCC grade. Multiple independent studies have assessed and recognized the prognostic value of tumour histological grade, which reflects the degree of cancer cell differentiation (Henson et al., 1991; Rakha et al., 2008; Rakha et al., 2010). In fact, the histological grade of tumours, when adequately prepared and stained samples, has been considered as a simple, affordable and reliable indicator to interpret tumour behaviour and patient prognosis, as in breast cancer (Rakha et al., 2010). Prognostic evaluation for OTSCC has long been based on cTNM classification, however, this staging system does not take into account the biological activity of tumour cells, and thus it must be supplemented by more precise methods (Akhter et al., 2011). In this regard, and based on our findings, testing the prognostic value of H4R expression in OTSCC patients could be one option. This, however, was not possible in the present study due to the lack of the clinical follow-up data and the limited sample size of OTSCC patients.

The mRNA levels of *H4R* were also investigated in oral cancer cells, which further supported the transcriptionally attenuated *H4R* gene in OTSCC. This is also in agreement with a previous study, in which *H4R* mRNA levels were significantly downregulated in colorectal carcinomas compared with normal enterocytes (Fang et al., 2011). Additionally, authors suggested that such attenuation of H4R level could probably happen gradually during the course of carcinogenesis (Fang et al., 2011). These findings may ultimately unravel the plausible effect of histamine, mediated via H4R, on tumour development and progression.

In spite of the abundance expression of H4Rs in human oral epithelial cells, it was highly important to assess the ability of these cells to bind and internalize the specific ligands of H4R. Consequently, this has been proven in two distinct cell lines: the normal HOKs and NS-SV-AC cells. Indeed, the internalization assays revealed an efficient and fast cellular mechanistic response to low doses of H4R-selective agonist, which starts as soon as 5 min and is completed one hour after stimulation. Furthermore, we show that human oral epithelial cells are equipped with functional molecular machinery necessary for histamine synthesis, transport and degradation. In fact, H4R has high affinity (pKi = 8.3) for histamine that is about 10 000-fold greater than the classical H1R and H2R (Walter and Stark 2012). It is therefore interesting that cultured normal HOKs were able to synthesize and continuously release very low levels of histamine into extracellular supernatants in autocrine and paracrine manners. We suggest, based on these findings, that human oral epithelial cells are "non-professional" histamine producing cells (**section 5.15**). Our finding is congruent with another study, which showed that histamine was released from human skin keratinocytes into the extracellular environment after exposure to ultraviolet light (Malaviya et al., 1996).

Histamine metabolizing molecules exhibit deranged levels in many inflammatory and malignant disorders, such as AD and breast cancer (Gutowska-Owsiak et al., 2014; Medina and Rivera 2010). Here we report that histamine metabolism is also deranged in oral mucosa of OLP patients. Moreover, the high surplus histamine in lesional oral epithelium, which may accumulate due to HNMT attenuation or from locally-degranulated MCs, may contribute significantly to the impaired mucosal integrity in OLP. This is supported by the inhibitory effect of histamine on the expression of integrin-α6 and integrin-β4, which are involved in epithelial cell–cell and epithelial cell–extracellular matrix interactions. Importantly, the loss of such crucial integrins can provoke serious mucosal blistering and

ulcerations, compatible with the pathognomonic symptoms of some forms of OLP (Bouvard et al., 2013).

In fact, the impaired epithelial integrity and ulcerative mucosa in OLP lesions can facilitate bacterial invasion, as well as access of other pathogen- or damage-associated molecular patterns (PAMPs or DAMPs), into deeper epithelial layers (Ismail et al., 2007; Salem et al., 2018). Recently, Choi et al. showed that epithelial layers of OLP lesions were occupied by high levels of intruding bacteria, which were also detected in the subepithelial lymphocytic cell infiltrate (Choi et al., 2016). Of note, gram-negative bacterial species (e.g. *Porphyromonas gingivalis*), with its hallmark LPS, were highly increased in OLP lesions (Choi et al., 2016; Wang et al., 2016). Such bacteria have also been implicated in oral carcinogenesis, and viable bacteria were isolated from OSCC (Sakamoto et al., 1999; Hooper et al., 2006; Abdulkareem et al., 2018). However, the bacterial role in OLP-mediated carcinogenesis should be interpreted with caution as the clinical presentation of OLP patients can be noticeably different from those diagnosed with OTSCC (e.g. gender, age and intraoral distribution). Our explanation is that such variability could in part be attributed to certain key factors that may influence the putative carcinogenesis process including hormones, and male lifestyle habits, such as smoking and alcohol consumption (Petti 2009). Moreover, lateral border of tongue is considered as one of the most common sites for OLP occurrence after buccal mucosa and gingiva (Müller 2017; Cheng et al., 2016). Here, we further assert the role of LPS in OLP pathology by revealing its role in regulating histamine metabolism and stimulating histamine release from cultured HOKs (study **II**). Once invaded the superficial epithelial layers, LPS molecules are recognized by PRRs (e.g. TLRs) that constitutively expressed in oral epithelial cells.

Recognition of PAMPs/DAMPs is indeed a vital element for the initiation of proper immune responses and inflammation. Members of TLR-family can mediate

such recognition process by initiating innate immune responses and orchestrate the adaptive arm of host immunity to fight infections (O'Neill et al., 2013). Bacterial cell wall components are mainly detected by membranous TLRs whereas nucleic acids are detected by intracellular TLRs (Kawai and Akira 2011). This could explain our findings that TLR-architecture in OLP patients was, and for most receptors, induced and shifted towards upper epithelial layers to combat the invading pathogens. Namely, TLR1, TLR2, TLR4, TLR7, TLR8, and TLR9 were strongly upregulated in OLL samples at both protein and gene levels. Components of gram-negative as well as gram-positive bacteria can be detected by many of these TLRs, such as TLR2 (lipoproteins, peptidoglycans, and lipoteichoic acid), TLR4 (LPS), TLR7 (streptococci-derived RNA), TLR9 (DNA motifs) and others (Akira et al., 2006; Mancuso et al., 2009). These findings, taking together, suggest a fundamental role of TLR-mediated response to invading pathogens in the initiation and perpetuation of OLP lesions.

Beside its role in histamine metabolism, LPS has the potential to mediate OSCC development and progression, and TME is well suited for bacterial growth and survival (Kurago et al., 2008). Importantly, we show that ultra-pure LPS and MC-derived mediators can significantly induce the expression of certain genes implicated in oral carcinogenesis. The signalling scheme behind this effect is still poorly understood. However, it was suggested that LPS can directly induce HOKs and cancer cells to produce cancer-promoting factors, and activating STAT3/IL-6 pathway (Kurago et al., 2008). Mediators from MCs were also shown to perpetuate inflammation and induce protumorigenic changes in mucosal tissues and predispose patients to colon cancer (Gounaris et al., 2007; Gounaris et al., 2009). Nevertheless, the mechanisms governing precancerous inflammation and oral oncogenesis via MCs and LPS remain to be further elucidated.

The persistent apoptosis of basal keratinocytes is a key process in OLP pathogenesis, which also shapes its potential transformation to OSCC (Zhao et al., 2018; Tampa et al., 2018). Apoptosis causes loss of oral epithelial cells, compromises the epithelial protective barrier, and, if not properly cleared, perpetuates the precancerous inflammatory reaction, and even cancer (Poon et al., 2014; Zhao et al., 2018; Tampa et al., 2018). The handling of such abnormal, and massive, apoptotic bodies likely poses a challenge for system-scavenging apoptotic bodies (Al-Samadi et al., 2015). Therefore, recent research efforts have focused on suppressing such refractory apoptotic process in oral epithelial cells (Wu et al., 2017; Zhao et al., 2018). We explored the role of H4R in oral epithelial cell apoptosis as well as the protective mechanism of HST-10 signalling against TNF-α-mediated cell death. Interestingly, our findings suggest that H4R attenuates cell apoptosis by repressing the expression of B-cell lymphoma protein 2 (BCL-2) family.

Indeed, normal oral epithelium provides the first line of defence against microbial invasion by secreting potent antimicrobial peptides including, inter alia, hBD-2 (Ganz 2003). This defensive barrier is impaired in many OPMDs, including OLP (Darido et al., 2016; Choi et al., 2016; Danielsson et al., 2017). In this study, we report that hBD-2 is highly induced in OLP and OTSCC lesions as compared with normal oral epithelium. Furthermore, we show that *hBD-2* expression was attenuated in cancer tissues and cells compared with OLP lesions, suggesting an association between hBD-2 expression and the initiation and progression of OTSCC. These findings are in agreement with previous studies showing waned levels of *hBD-2* mRNA in samples obtained from OSCC patients (Joly et al., 2009; Abiko et al., 2001).

Supporting the previously discussed LPS- and MC-driven effects in oral epithelium, we also demonstrate that hBD-2 in HOKs was induced, and partly regulated, by LPS and MC-derived mediators. In fact, immunofluorescences staining

results suggest that MCs are involved in hBD-2-mediated pathogenesis of OLP via two pathways: 1) as its major "non-epithelial" source, they release hBD-2 upon activation; 2) they release histamine, which can induce hBD-2 release from oral epithelium (Kanda and Watanabe, 2007). Importantly, hBD-2 can subsequently stimulate MCs to release more histamine, which leads to further production of hBD-2 in a proinflammatory vicious circle (Niyonsaba et al., 2001; Subramanian et al., 2013).

Histamine has synergistic effect on hBD-2 production via TNF-α and IFN-γ. It is probably driven through the upregulation of hBD-2 promoter region, which contains two binding sites for NF-κB and one site for STAT1 (Mineshiba et al., 2005). Interestingly, activation of H4R regulated *hBD-2* mRNA level in HOKs. H4R agonist seems to downregulate the histamine/TNF-α- and LPS-mediated signalling pathways, while the inverse agonist, ST-1007, potentiated their effect. In this regards, H4R shows suppressive effect on both STAT1- and TNF-α-mediated effects (Horr et al., 2006), and thus it is logical to assume that such HST10-mediated effect could also be mediated by interfering with one or both pathways (**Figure 11**). Supporting this assumption, H4R expression is low in OPMDs and cancer, which could partly elucidate the dysregulated hBD-2 response in such lesions.

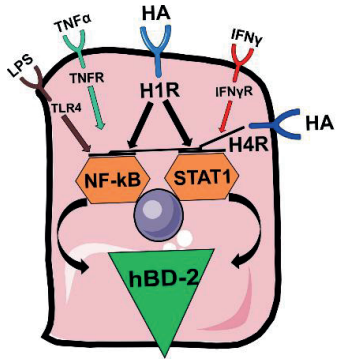

Figure 11. The effect of histamine (HA) on hBD-2 production is likely mediated via NF-κB and, to less extent, via STAT1; hBD-2 promoter region contains two sites for NF-κB and one site for STAT1, which both are induced by HA signalling. Effect of H4R on hBD-2 in HOKs could be mediated via both STAT1 and NF-κB axes.

10. STUDY CONCLUSIONS AND LIMITATIONS

Based on the presented findings, we conclude that human oral epithelial cells are "non-professional" histamine producing cells—capable to synthesize, release, and degrade low levels of endogenic histamine. We also conclude that H4R expression and histamine metabolism are deranged in OLP patients, where high levels of histamine could downregulate key integrity molecules in HOKs, and may enhance subsequent bacterial invasion. In this regard, our findings suggest a potential role of TLRs in OLP pathogenesis, probably by binding bacterial components (i.e. LPS) and enhancing further immune response and histamine production. In addition, our results indicate that histamine/H4R crosstalk signalling with LPS and MCs could partly be involved in OTSCC pathogenesis. This was further supported by the deranged H4R expression and the ability of H4R to regulate cell apoptosis and modulate antibacterial response in HOKs. Therefore, the aims of this study were fulfilled accordingly.

Putting pieces together, we propose the following overall scenario to envision the inflammatory cascade in OLP lesions and the potential oral carcinogenesis (**Figure 12**): a) the impaired epithelial barrier in susceptible patients facilitates bacterial invasion into deeper epithelial layers; b) pathogens (e.g. LPS) bind to TLRs expressed by deeper residing cells and induce antimicrobial response and histamine synthesis; c) epithelial cell-derived hBD-2 (and histamine?) binds Mas-related gene X2 receptor (MrgX2) expressed on MCs and induces their degranulation (Subramanian et al., 2013); d) MC mediators provoke proinflammatory response and recruit more inflammatory cells; e) elevated levels of surplus histamine and hBD-2 may cause further epithelial degeneration and attenuate epithelial H4R, which seems to play crucial immunoregulatory functions by modulating characteristic features of OLP pathology such as epithelial cell apoptosis and antimicrobial response; f) persistent exposure to LPS and MC mediators perpetuates the vicious inflammatory

circle by recruiting more immune cells and driving angiogenesis (angiogenic switch) that could, overtime, promote oral carcinogenesis.

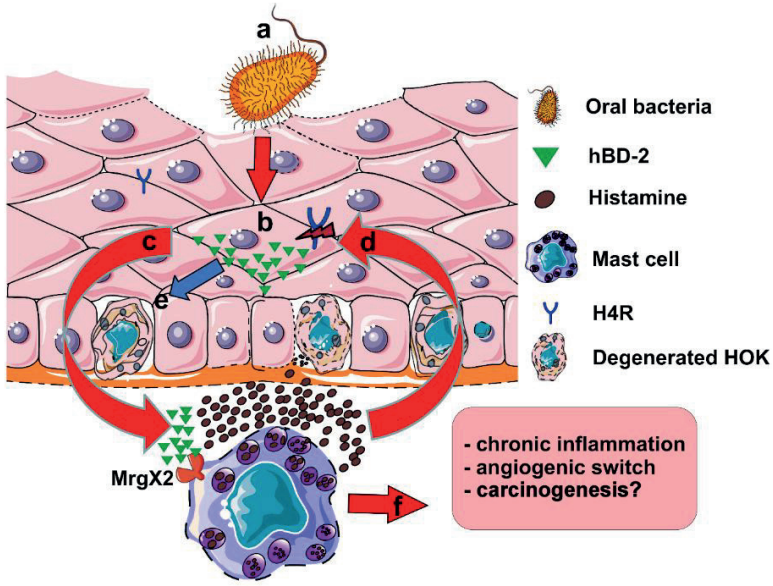

Figure 12. The proposed scenario for the inflammatory cascade in OLP lesions.

Taken together, our findings suggest a possible involvement of H4R in OLP pathogenesis. Additionally, the attenuated expression of H4R in OTSCC and the mechanistic *in vitro* effects of histamine suggest a potential role in oral carcinogenesis. Recently, development of molecules to target specific steps in the progression from premalignant lesions to oral cancer has gained enormous attention (Tanaka and Ishigamori 2011). Therefore, further fictional and preclinical studies of H4R signalling are warranted to assess whether it serves as a promising molecular target for therapeutic interventions in OLP and OTSCC. There are several limitations in this PhD thesis and that should be taken into considerations. We acknowledge that some clinical and follow-up data of the studied OLP/OED patients were not available, and therefore assessing the potential tumorigenic transformation in such lesions was not possible. This also applies to the follow-up data of OTSCC patients and thus prognostic analysis was not achieved. A relatively small sample size and

lack of animal model experiments should also be acknowledged. Hence, further studies to overcome the present limitations and to validate our findings in preclinical *in vivo* models are warranted.

11. ACKNOWLEDGEMENTS

"*Life is nothing more than a book with many chapters*".

Indeed, doing a Ph.D. was one of the most enriching chapters in my life; and it is a time to say "thank you" to those who were crucial to the completion of this journey.

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Hakim

12. REFERENCES

Abdulkareem AA, Shelton RM, Landini G, Cooper PR, Milward MR. Periodontal pathogens promote epithelial-mesenchymal transition in oral squamous carcinoma cells in vitro. Cell Adh Migr. 2018 Mar 4; 12(2):127-137.

Abiko Y, Suraweera AK, Nishimura M, Arakawa T, Takuma T, Mizoguchi I, Kaku T. Differential expression of human beta-defensin 2 in keratinized and non-keratinized oral epithelial lesions; immunohistochemistry and in situ hybridization. Virchows Arch. 2001 Mar; 438(3):248-53.

Aghbari SMH, Abushouk AI, Attia A, Elmaraezy A, Menshawy A, Ahmed MS, Elsaadany BA, Ahmed EM. Malignant transformation of oral lichen planus and oral lichenoid lesions: A meta-analysis of 20095 patient data. Oral Oncol. 2017 May; 68:92-102.

Agnihotri R, Gaur S. Implications of tobacco smoking on the oral health of older adults. Geriatr Gerontol Int. 2014 Jul; 14(3):526-40.

Akdis C, Simons E. Histamine receptors are hot in immunopharmacology. Eur J Pharmacol. 2002 533, 69-76.

Akhter M, Hossain S, Rahman QB, Molla MR. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol. 2011 May; 15(2):168-76.

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006 Feb 24; 124(4):783-801.

Almahmoudi R, Salem A, Sieviläinen M, Sundquist E, Almangush A, Toppila-Salmi S, Paavonen T, Salo T, Al-Samadi A. Extracellular interleukin-17F has a protective effect in oral tongue squamous cell carcinoma. Head Neck. 2018 Oct; 40(10):2155-2165.

Al-Mohaya MA, Al-Otaibi L, Al-Harthi F, Al Bakr E, Arfin M, Al-Asmari A. Association of genetic polymorphisms in interferon-γ, interleukin-6 and transforming growth factor-β1 gene with oral lichen planus susceptibility. BMC Oral Health. 2016 Aug 20; 16(1):76.

Alrashdan MS, Cirillo N, McCullough M. Oral lichen planus: a literature review and update. Arch Dermatol Res. 2016 Oct; 308(8):539-51.

Al-Samadi A, Drozd A, Salem A, Hietanen J, Häyrinen-Immonen R, Konttinen YT. Epithelial Cell Apoptosis in Recurrent Aphthous Ulcers. J Dent Res. 2015 Jul; 94(7):928-35.

Amin MB, Edge SB, Greene FL, et al, eds. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017.

Andreasen JO. Oral lichen planus. 1. A clinical evaluation of 115 cases. Oral Surg Oral Med Oral Pathol. 1968 Jan; 25(1):31-42.

Anneroth G, Batsakis J, Luna M. Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. Scand J Dent Res. 1987 Jun; 95(3):229-49.

Aponte-López A, Fuentes-Pananá EM, Cortes-Muñoz D, Muñoz-Cruz S. Mast Cell, the Neglected Member of the Tumor Microenvironment: Role in Breast Cancer. J Immunol Res. 2018 Feb 5; 2018:2584243.

Azuma M, Tamatani T, Kasai Y, Sato M. Immortalization of normal human salivary gland cells with duct-, myoepithelial-, acinar-, or squamous phenotype by transfection with SV40 ori- mutant deoxyribonucleic acid. Lab Invest. 1993 Jul; 69(1):24-42.

Bai J, Jiang L, Lin M, Zeng X, Wang Z, Chen Q. Association of polymorphisms in the tumor necrosis factor-alpha and interleukin-10 genes with oral lichen planus:

a study in a chinese cohort with Han ethnicity. J Interferon Cytokine Res. 2009 Jul; 29(7):381-8.

Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001 Feb 17; 357(9255):539-45.

Bartholeyns J, Fozard J. Role of histamine in tumor development. Trends Pharmacol Sci. 1985; 6:123–125.

Bazzoni G, Dejana E. Keratinocyte junctions and the epidermal barrier: how to make a skin-tight dress. J Cell Biol. 2002 Mar 18; 156(6):947-9.

Bermejo-Fenoll A, López-Jornet P. Familial oral lichen planus: presentation of six families. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Aug; 102(2):e12-5.

Bermejo-Fenoll A, Sánchez-Siles M, López-Jornet P, Camacho-Alonso F, Salazar-Sánchez N. A retrospective clinicopathological study of 550 patients with oral lichen planus in south-eastern Spain. J Oral Pathol Med. 2010 Jul; 39(6):491-6.

Bhatia A, Burtness B. Novel Molecular Targets for Chemoprevention in Malignancies of the Head and Neck. Cancers (Basel). 2017 Aug 31;9(9). pii: E113.

Biosse-Duplan M, Baroukh B, Dy M, de Vernejoul MC, Saffar JL. Histamine promotes osteoclastogenesis through the differential expression of histamine receptors on osteoclasts and osteoblasts. Am J Pathol. 2009 Apr; 174(4):1426-34.

Bloor BK, Malik FK, Odell EW, Morgan PR. Quantitative assessment of apoptosis in oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999 Aug; 88(2):187-95.

Borradori L, Sonnenberg A. Structure and function of hemidesmosomes: more than simple adhesion complexes. J Invest Dermatol. 1999 Apr; 112(4):411-8.

Borriello F, Iannone R, Marone G. Histamine Release from Mast Cells and Basophils. Handb Exp Pharmacol. 2017; 241:121-139.

Bouvard D, Pouwels J, De Franceschi N, Ivaska J. Integrin inactivators: balancing cellular functions in vitro and in vivo. Nat Rev Mol Cell Biol. 2013 Jul; 14(7):430-42.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov; 68(6):394-424.

Breunig E, Michel K, Zeller F, Seidl S, Weyhern CW, Schemann M. Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. J Physiol. 2007 Sep 1; 583(Pt 2):731-42.

Brierley J, Gospodarowicz M, O'Sullivan B. The principles of cancer staging. Ecancermedicalscience. 2016 Nov 24; 10:ed61.

Brinkman BM, Wong DT. Disease mechanism and biomarkers of oral squamous cell carcinoma. Curr Opin Oncol. 2006 May; 18(3):228-33.

Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. J Pathol. 1992 Apr; 166(4):375-81.

Buckland KF, Williams TJ, Conroy DM. Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. Br J Pharmacol. 2003 Nov; 140(6):1117-27.

Burgdorf WH, Plewig G. Who described Civatte bodies? J Cutan Pathol. 2014 Apr; 41(4):340-6.

Carbone M, Arduino PG, Carrozzo M, Gandolfo S, Argiolas MR, Bertolusso G, Conrotto D, Pentenero M, Broccoletti R. Course of oral lichen planus: a retrospective study of 808 northern Italian patients. Oral Dis. 2009 Apr; 15(3):235-43.

Carbone M, Goss E, Carrozzo M, Castellano S, Conrotto D, Broccoletti R, Gandolfo S. Systemic and topical corticosteroid treatment of oral lichen planus: a comparative study with long-term follow-up. J Oral Pathol Med. 2003 Jul; 32(6):323-9.

Casparis S, Borm JM, Tektas S, Kamarachev J, Locher MC, Damerau G, Grätz KW, Stadlinger B. Oral lichen planus (OLP), oral lichenoid lesions (OLL), oral dysplasia, and oral cancer: retrospective analysis of clinicopathological data from 2002-2011. Oral Maxillofac Surg. 2015 Jun; 19(2):149-56.

Chang JY, Chiang CP, Hsiao CK, Sun A. Significantly higher frequencies of presence of serum autoantibodies in Chinese patients with oral lichen planus. J Oral Pathol Med. 2009; 38: 48–54.

Cheng YS, Gould A, Kurago Z, Fantasia J, Muller S. Diagnosis of oral lichen planus: a position paper of the American Academy of Oral and Maxillofacial Pathology. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016 Sep; 122(3):332-54.

Choi YS, Kim Y, Yoon HJ, Baek KJ, Alam J, Park HK, Choi Y. The presence of bacteria within tissue provides insights into the pathogenesis of oral lichen planus. Sci Rep. 2016 Jul 7; 6:29186.

Chung PI, Hwang CY, Chen YJ, Lin MW, Chen TJ, Hua TC, Wu LC, Chu SY, Chen CC, Lee DD, Chang YT, Liu HN. Autoimmune comorbid diseases associated with lichen planus: a nationwide case-control study. J Eur Acad Dermatol Venereol. 2015 Aug; 29(8):1570-5.

Cianchi F, Vinci MC, Masini E. Histamine in cancer: the dual faces of the coin. Cancer Biol Ther. 2008 Jan; 7(1):36-7.

Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell. 2006 Jan 27; 124(2):263-6.

Connelly WM, Shenton FC, Lethbridge N, Leurs R, Waldvogel HJ, Faull RL, Lees G, Chazot PL. The histamine H4 receptor is functionally expressed on neurons in the mammalian CNS. Br J Pharmacol. 2009 May; 157(1):55-63.

Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002 Dec 19-26; 420(6917):860-7.

Cricco GP, Mohamad NA, Sambuco LA, Genre F, Croci M, Gutiérrez AS, Medina VA, Bergoc RM, Rivera ES, Martín GA. Histamine regulates pancreatic carcinoma cell growth through H3 and H4 receptors. Inflamm Res. 2008; 57 Suppl 1:S23-4.

Crincoli V, Di Bisceglie MB, Scivetti M, Lucchese A, Tecco S, Festa F. Oral lichen planus: update on etiopathogenesis, diagnosis and treatment. Immunopharmacol Immunotoxicol. 2011 Mar; 33(1):11-20.

Dabiri S, Huntsman D, Makretsov N, Cheang M, Gilks B, Bajdik C, Gelmon K, Chia S, Hayes M. The presence of stromal mast cells identifies a subset of invasive breast cancers with a favorable prognosis. Mod Pathol. 2004 Jun; 17(6):690-5.

Dale BA, Salonen J, Jones AH. New approaches and concepts in the study of differentiation of oral epithelia. Crit Rev Oral Biol Med. 1990; 1(3):167-90.

Dalirsani Z, Taghavi Zenouz A, Mehdipour M, Alavi F, Javadzadeh Y. Comparison of the effect of combination of triamcinolone acetonide and vitamin a mouthwash

with triamcinolone mouthwash alone on oral lichen planus. J Dent Res Dent Clin Dent Prospects. 2010; 4(1):21-4.

Damaj BB, Becerra CB, Esber HJ, Wen Y, Maghazachi AA. Functional expression of H4 histamine receptor in human natural killer cells, monocytes, and dendritic cells. J Immunol. 2007 Dec 1; 179(11):7907-15.

Danielsson K, Ebrahimi M, Nylander E, Wahlin YB, Nylander K. Alterations in Factors Involved in Differentiation and Barrier Function in the Epithelium in Oral and Genital Lichen Planus. Acta Derm Venereol. 2017; 97(2):214-218.

Darido C, Georgy SR, Jane SM. The role of barrier genes in epidermal malignancy. Oncogene. 2016; 35(44):5705-5712.

Day TA, Davis BK, Gillespie MB, Joe JK, Kibbey M, Martin-Harris B, Neville B, Richardson MS, Rosenzweig S, Sharma AK, Smith MM, Stewart S, Stuart RK. Oral cancer treatment. Curr Treat Options Oncol. 2003 Feb; 4(1):27-41.

De Rossi SS, Ciarrocca K. Oral lichen planus and lichenoid mucositis. Dent Clin North Am. 2014 Apr; 58(2):299-313.

de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006 Jan;6(1):24-37.

Derikvand N, Ghasemi SS, Moharami M, Shafiei E, Chiniforush N. Management of Oral Lichen Planus by 980 nm Diode Laser. J Lasers Med Sci. 2017 Summer; 8(3):150-154.

Dib K, Perecko T, Jenei V, McFarlane C, Comer D, Brown V, Katebe M, Scheithauer T, Thurmond RL, Chazot PL, Ennis M. The histamine H4 receptor is a potent inhibitor of adhesion-dependent degranulation in human neutrophils. J Leukoc Biol. 2014 Sep; 96(3):411-8.

Dijkstra D, Leurs R, Chazot P, Shenton FC, Stark H, Werfel T, Gutzmer R. Histamine downregulates monocyte CCL2 production through the histamine H4 receptor. J Allergy Clin Immunol. 2007 Aug; 120(2):300-7.

Dillenburg CS, Martins MA, Munerato MC, Marques MM, Carrard VC, Sant'Ana Filho M, Castilho RM, Martins MD. Efficacy of laser phototherapy in comparison to topical clobetasol for the treatment of oral lichen planus: a randomized controlled trial. J Biomed Opt. 2014 Jun; 19(6):068002.

Ding M, Wang X, Wang C, Liu X, Zen K, Wang W, Zhang CY, Zhang C. Distinct expression profile of HCMV encoded miRNAs in plasma from oral lichen planus patients. J Transl Med. 2017 Jun 7; 15(1):133.

Dissemond J. Oral lichen planus: an overview. J Dermatolog Treat. 2004 15(3):136-140.

Dost F, Lê Cao KA, Ford PJ, Farah CS. A retrospective analysis of clinical features of oral malignant and potentially malignant disorders with and without oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Dec; 116(6):725-33.

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

Edwards PC, Kelsch R. Oral lichen planus: clinical presentation and management. J Can Dent Assoc. 2002 Sep; 68(8):494-9.

Eisen D, Carrozzo M, Bagan Sebastian JV, Thongprasom K. Number V Oral lichen planus: clinical features and management. Oral Dis. 2005 Nov; 11(6):338-49.

Eisen D. The clinical features, malignant potential, and systemic associations of oral lichen planus: a study of 723 patients. J Am Acad Dermatol. 2002 Feb; 46(2):207-14.

El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ. WHO Classification of Head and Neck Tumours (4th edition). IARC: Lyon 2017. Page 113.

Elpek GO, Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, Keleş N. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol. 2001 Dec; 54(12):940-4.

El-Zaemey S, Schüz J, Leon ME. Qat Chewing and Risk of Potentially Malignant and Malignant Oral Disorders: A Systematic Review. Int J Occup Environ Med. 2015 Jul; 6(3):129-43.

Epstein JB, Wan LS, Gorsky M, Zhang L. Oral lichen planus: progress in understanding its malignant potential and the implications for clinical management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Jul; 96(1):32-7.

Fang Z, Yao W, Xiong Y, Li J, Liu L, Shi L, Zhang W, Zhang C, Nie L, Wan J. Attenuated expression of HRH4 in colorectal carcinomas: a potential influence on tumor growth and progression. BMC Cancer. 2011 May 24; 11:195:1-11.

Farhi D, Dupin N. Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: facts and controversies. Clin Dermatol. 2010 Jan-Feb; 28(1):100-8.

Faustino-Rocha AI, Ferreira R, Gama A, Oliveira PA, Ginja M. Antihistamines as promising drugs in cancer therapy. Life Sci. 2017 Mar 1; 172:27-41.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015 Mar 1;136(5):E359-86.

Fernandes DT, Santos-Silva AR, Vargas PA, Lopes MA. Letter to the Editor: Development of proliferative verrucous leukoplakia (PVL) in oral lichen planus: Is it not a clinical spectrum of PVL? Med Oral Patol Oral Cir Bucal. 2017 Mar 1;22(2):e142-e143.

Fernández-González F, Vázquez-Álvarez R, Reboiras-López D, Gándara-Vila P, García-García A, Gándara-Rey JM. Histopathological findings in oral lichen planus and their correlation with the clinical manifestations. Med Oral Patol Oral Cir Bucal. 2011 Aug 1; 16(5):e641-6.

Fernández-Nogueira P, Noguera-Castells A, Fuster G, Recalde-Percaz L, Moragas N, López-Plana A, Enreig E, Jauregui P, Carbó N, Almendro V, Gascón P, Bragado P, Mancino M. Histamine receptor 1 inhibition enhances antitumor therapeutic responses through extracellular signal-regulated kinase (ERK) activation in breast cancer. Cancer Lett. 2018 Jun 28; 424:70-83.

Ferré S, Casadó V, Devi LA, Filizola M, Jockers R, Lohse MJ, Milligan G, Pin JP, Guitart X. G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. Pharmacol Rev. 2014 Feb 10; 66(2):413-34.

Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: a systematic review. J Am Dent Assoc. 2014 Jan; 145(1):45-56.

Gantner F, Sakai K, Tusche MW, Cruikshank WW, Center DM, Bacon KB. Histamine h(4) and h(2) receptors control histamine-induced interleukin-16 release from human CD8(+) T cells. J Pharmacol Exp Ther. 2002 Oct; 303(1):300-7.

Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003 Sep; 3(9):710-20.

Garavello W, Spreafico R, Gaini RM. Oral tongue cancer in young patients: a matched analysis. Oral Oncol. 2007 Oct; 43(9):894-7.

Garcia-Caballero M, Neugebauer E, Rodriguez F, Nuñez de Castro I, Vara-Thorbeck C. Histamine synthesis and content in benign and malignant breast tumours. Its effects on other host tissues. Surg Oncol. 1994 Jun; 3(3):167-73.

Giacomelli L, Covani U. Bioinformatics and data mining studies in oral genomics and proteomics: new trends and challenges. Open Dent J. 2010 Jul 16;4:67-71.

Gillenwater A, Papadimitrakopoulou V, Richards-Kortum R. Oral premalignancy: new methods of detection and treatment. Curr Oncol Rep. 2006 Mar;8(2):146-54.

Giuliani M, Troiano G, Cordaro M, Corsalini M, Gioco G, Lo Muzio L, Pignatelli P, Lajolo C. Rate of malignant transformation of oral lichen planus: A systematic review. Oral Dis. 2018 May 8. doi: 10.1111/odi.12885.

Gonda TA, Tu S, Wang TC. Chronic inflammation, the tumor microenvironment and carcinogenesis. Cell Cycle. 2009 Jul 1; 8(13):2005-13.

González-Moles MA. The use of topical corticoids in oral pathology. Med Oral Patol Oral Cir Bucal. 2010 Nov 1; 15(6):e827-31.

Gorouhi F, Davari P, Fazel N. Cutaneous and mucosal lichen planus: a comprehensive review of clinical subtypes, risk factors, diagnosis, and prognosis. ScientificWorldJournal. 2014 Jan 30; 2014:742826.

Gounaris E, Blatner NR, Dennis K, Magnusson F, Gurish MF, Strom TB, Beckhove P, Gounari F, Khazaie K. T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. Cancer Res. 2009 Jul 1; 69(13):5490-7.

Gounaris E, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, Lee DM, Zhang G, Glickman JN, Shin K, Rao VP, Poutahidis T, Weissleder R, McNagny KM, Khazaie K. Mast cells are an essential hematopoietic component for polyp development. Proc Natl Acad Sci U S A. 2007 Dec 11; 104(50):19977-82.

Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell. 2004 Aug 6; 118(3):285-96.

Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010 Mar 19; 140(6):883-99.

Grivennikov SI, Karin M. Inflammation and oncogenesis: a vicious connection. Curr Opin Genet Dev. 2010 Feb; 20(1):65-71.

Groeger SE, Meyle J. Epithelial barrier and oral bacterial infection. Periodontol 2000. 2015 Oct; 69(1):46-67.

Gupta S, Ghosh S, Gupta S. Interventions for the management of oral lichen planus: a review of the conventional and novel therapies. Oral Dis. 2017 Nov; 23(8):1029-1042.

Gutowska-Owsiak D, Greenwald L, Watson C, Selvakumar TA, Wang X, Ogg GS. The histamine-synthesizing enzyme histidine decarboxylase is upregulated by keratinocytes in atopic skin. Br J Dermatol. 2014 Oct; 171(4):771-8.

Gutzmer R, Gschwandtner M, Rossbach K, Mommert S, Werfel T, Kietzmann M, Baeumer W. Pathogenetic and therapeutic implications of the histamine H4 receptor in inflammatory skin diseases and pruritus. Front Biosci (Schol Ed). 2011 Jun 1; 3:985-94.

Haapalainen T, Oksala O, Kallioinen M, Oikarinen A, Larjava H, Salo T. Destruction of the epithelial anchoring system in lichen planus. J Invest Dermatol. 1995 Jul; 105(1):100-3.

Halonen P, Jakobsson M, Heikinheimo O, Riska A, Gissler M, Pukkala E. Cancer risk of Lichen planus: A cohort study of 13,100 women in Finland. Int J Cancer. 2018 Jan 1; 142(1):18-22.

Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012 Mar 20; 21(3):309-22.

Harpenau LA, Plemons JM, Rees TD. Effectiveness of a low dose of cyclosporine in the management of patients with oral erosive lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995 Aug; 80(2):161-7.

Hart PH, Grimbaldeston MA, Finlay-Jones JJ. Sunlight, immunosuppression and skin cancer: role of histamine and mast cells. Clin Exp Pharmacol Physiol. 2001 Jan-Feb;28(1-2):1-8.

He GH, Ding JQ, Zhang X, Xu WM, Lin XQ, Huang MJ, Feng J, Wang P, Cai WK. Activation of histamine H(4) receptor suppresses the proliferation and invasion of esophageal squamous cell carcinoma via both metabolism and non-metabolism signaling pathways. J Mol Med (Berl). 2018 Sep; 96(9):951-964.

Henson DE, Ries L, Freedman LS, Carriaga M. Relationship among outcome, stage of disease, and histologic grade for 22,616 cases of breast cancer. The basis for a prognostic index. Cancer. 1991 Nov 15; 68(10):2142-9.

Hodgson TA and Chaudhry SI. The management of oral lichen planus: Symptom control at what risk? Oral Dis. 2010; 16(6): 512–513.

Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. J Pharmacol Exp Ther. 2003 Jun; 305(3):1212-21.

Hooper SJ, Crean SJ, Lewis MA, Spratt DA, Wade WG, Wilson MJ. Viable bacteria present within oral squamous cell carcinoma tissue. J Clin Microbiol. 2006 May; 44(5):1719-25.

Horr B, Borck H, Thurmond R, Grösch S, Diel F. STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes. Int Immunopharmacol. 2006; 6(10):1577-1585.

Iijima W, Ohtani H, Nakayama T, Sugawara Y, Sato E, Nagura H, Yoshie O, Sasano T. Infiltrating CD8+ T cells in oral lichen planus predominantly express CCR5 and CXCR3 and carry respective chemokine ligands RANTES/CCL5 and IP-10/CXCL10 in their cytolytic granules: a potential self-recruiting mechanism. Am J Pathol. 2003 Jul; 163(1):261-8.

Ismail SB, Kumar SK, Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis, management and malignant transformation. J Oral Sci. 2007 Jun; 49(2):89-106.

Jablonowski JA, Grice CA, Chai W, Dvorak CA, Venable JD, Kwok AK, Ly KS, Wei J, Baker SM, Desai PJ, Jiang W, Wilson SJ, Thurmond RL, Karlsson L, Edwards JP, Lovenberg TW, Carruthers NI. The first potent and selective non-imidazole human histamine H4 receptor antagonists. J Med Chem. 2003 Sep 11; 46(19):3957-60.

Jadhav KB, Gupta N. Clinicopathological prognostic implicators of oral squamous cell carcinoma: need to understand and revise. N Am J Med Sci. 2013 Dec; 5(12):671-9.

Johnson C, Huynh V, Hargrove L, Kennedy L, Graf-Eaton A, Owens J, Trzeciakowski JP, Hodges K, DeMorrow S, Han Y, Wong L, Alpini G, Francis H. Inhibition of Mast Cell-Derived Histamine Decreases Human Cholangiocarcinoma Growth and Differentiation via c-Kit/Stem Cell Factor-Dependent Signaling. Am J Pathol. 2016 Jan; 186(1):123-33.

Johnson NW, Warnakulasuriya KA. Epidemiology and aetiology of oral cancer in the United Kingdom. Community Dent Health. 1993 Jun;10 Suppl 1:13-29.

Joly S, Compton LM, Pujol C, Kurago ZB, Guthmiller JM. Loss of human beta-defensin 1, 2, and 3 expression in oral squamous cell carcinoma. Oral Microbiol Immunol. 2009; 24(5):353-360.

Jungell P, Malmström M, Wartiovaara J, Konttinen Y, Sane J. Ultrastructure of oral leukoplakia and lichen planus. I. Basal region and inflammatory cells. J Oral Pathol. 1987 Apr; 16(4):170-8.

Kajihara Y, Murakami M, Imagawa T, Otsuguro K, Ito S, Ohta T. Histamine potentiates acid-induced responses mediating transient receptor potential V1 in mouse primary sensory neurons. Neuroscience. 2010 Mar 10; 166(1):292-304.

Kalesnikoff J, Galli SJ. Antiinflammatory and immunosuppressive functions of mast cells. Methods Mol Biol. 2011; 677:207-20.

Kanda N and Watanabe S. Histamine enhances the production of human beta-defensin-2 in human keratinocytes. Am J Physiol Cell Physiol. 2007; 293(6):C1916-1923.

Kanemitsu S. Oral lichen planus: malignant potential and diagnosis. Oral Sci Int. 2014; 11:1–7.

Kanwar AJ, De D. Lichen planus in childhood: report of 100 cases. Clin Exp Dermatol. 2010 Apr; 35(3):257-62.

Karatsaidis A, Schreurs O, Helgeland K, Axéll T, Schenck K. Erythematous and reticular forms of oral lichen planus and oral lichenoid reactions differ in pathological features related to disease activity. J Oral Pathol Med. 2003 May; 32(5):275-81.

Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005 Oct; 5(10):749-59.

Kassie F, Darroudi F, Kundi M, Schulte-Hermann R, Knasmüller S. Khat (Catha edulis) consumption causes genotoxic effects in humans. Int J Cancer. 2001 May 1; 92(3):329-32.

Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011 May 27; 34(5):637-50.

Kimkong I, Nakkuntod J, Sodsai P, Hirankarn N, Kitkumthorn N. Association of interferon-gamma gene polymorphisms with susceptibility to oral lichen planus in the Thai population. Arch Oral Biol. 2012 May; 57(5):491-4.

Klocker J, Mätzler SA, Huetz GN, Drasche A, Kolbitsch C, Schwelberger HG. Expression of histamine degrading enzymes in porcine tissues. Inflamm Res. 2005 Apr; 54 Suppl 1:S54-7.

Kobayashi T, Hatamochi A, Kamada N, Matsue H, Shinkai H. Systemic lupus erythematosus with lichen planus-like eruptions associated with pericarditis. J Dermatol. 2008; 35: 306–307.

Kohrt HE, Nouri N, Nowels K, Johnson D, Holmes S, Lee PP. Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. PLoS Med. 2005 Sep;2(9):e284.

Konttinen Y, Husu H, Han X et al. Non-professional histamine producing cells, immune responses and autoimmunity. In: Histamine H4 Receptor: a Novel Drug

Target in Immunoregulation and Inflammation. (H Stark, ed), Warsaw: Versita Ltd., 2013; 201–38.

Kottke T, Sander K, Weizel L, Schneider EH, Seifert R, Stark H. Receptor-specific functional efficacies of alkyl imidazoles as dual histamine H3/H4 receptor ligands. Eur J Pharmacol. 2011 Mar 11; 654(3):200-8.

Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol. 1978 Oct; 46(4):518-39.

Kubo Y, Nakano K. Regulation of histamine synthesis in mouse CD4+ and CD8+ T lymphocytes. Inflamm Res. 1999 Mar; 48(3):149-53.

Kurago ZB, Lam-ubol A, Stetsenko A, De La Mater C, Chen Y, Dawson DV. Lipopolysaccharide-squamous cell carcinoma-monocyte interactions induce cancer-supporting factors leading to rapid STAT3 activation. Head Neck Pathol. 2008 Mar; 2(1):1-12.

Landini G, Mylonas P, Shah IZ, Hamburger J. The reported rates of transformation of oral lichen planus. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2014; (26):213–220.

Langendijk JA, Ferlito A, Takes RP, Rodrigo JP, Suárez C, Strojan P, Haigentz M Jr, Rinaldo A. Postoperative strategies after primary surgery for squamous cell carcinoma of the head and neck. Oral Oncol. 2010 Aug; 46(8):577-85.

Lappalainen J, Lindstedt KA, Kovanen PT. A protocol for generating high numbers of mature and functional human mast cells from peripheral blood. Clin Exp Allergy. 2007 Sep; 37(9):1404-14.

Le Cleach L, Chosidow O. Clinical practice. Lichen planus. N Engl J Med. 2012 Feb 23;366(8):723-32.

Lee HJ, Park MK, Lee EJ, Kim YL, Kim HJ, Kang JH, Kim HM, Lee AY, Lee CH. Histamine receptor 2-mediated growth-differentiation factor-15 expression is involved in histamine-induced melanogenesis. Int J Biochem Cell Biol. 2012 Dec; 44(12):2124-8.

Leff P. The two-state model of receptor activation. Trends Pharmacol Sci. 1995; 16(3):89-97.

Lethbridge NL, Chazot PL. Immunological identification of the mouse H4 histamine receptor on spinal cord motor neurons using a novel anti-mouse H4R antibody. Inflamm Res. 2010 Mar; 59 Suppl 2:S197-8.

Li D, Li J, Li C, Chen Q, Hua H. The Association of Thyroid Disease and Oral Lichen Planus: A Literature Review and Meta-analysis. Front Endocrinol (Lausanne). 2017 Nov 9; 8:310.

Likar-Manookin K, Stewart C, Al-Hashimi I et al. Prevalence of oral lesions of autoimmune etiology in patients with primary Sjogren's syndrome. Oral Dis. 2013; 19: 598–603.

Ling P, Ngo K, Nguyen S, Thurmond RL, Edwards JP, Karlsson L, Fung-Leung WP. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. Br J Pharmacol. 2004 May; 142(1):161-71.

Lo Muzio L, Santarelli A, Campisi G, Lacaita M, Favia G. Possible link between Hashimoto's thyroiditis and oral lichen planus: a novel association found. Clin Oral Investig. 2013; 17: 333–336.

Lodi G, Carrozzo M, Furness S, Thongprasom K. Interventions for treating oral lichen planus: a systematic review. Br J Dermatol. 2012 May; 166(5):938-47.

Lodi G, Pellicano R, Carrozzo M. Hepatitis C virus infection and lichen planus: a systematic review with meta-analysis. Oral Dis. 2010 Oct; 16(7):601-12.

Lopes MA, Feio P, Santos-Silva AR, Vargas PA. Proliferative verrucous leukoplakia may initially mimic lichenoid reactions. World J Clin Cases. 2015 Oct 16;3 (10):861-3.

Ma J, Zhang J, Zhang Y, Lv T, Liu J. The Magnitude of the Association between Human Papillomavirus and Oral Lichen Planus: A Meta-Analysis. PLoS One. 2016 Aug 29; 11(8):e0161339.

Maciel TT, Moura IC, Hermine O. The role of mast cells in cancers. F1000Prime Rep. 2015 Jan 5;7:09. doi: 10.12703/P7-09.

Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr. 2007 May; 85(5):1185-96.

Malaviya R, Morrison AR, Pentland AP. Histamine in human epidermal cells is induced by ultraviolet light injury. J Invest Dermatol. 1996 Apr; 106(4):785-9.

Malfettone A, Silvestris N, Saponaro C, Ranieri G, Russo A, Caruso S, Popescu O, Simone G, Paradiso A, Mangia A. High density of tryptase-positive mast cells in human colorectal cancer: a poor prognostic factor related to protease-activated receptor 2 expression. J Cell Mol Med. 2013 Aug; 17(8):1025-37.

Mancuso G, Gambuzza M, Midiri A, Biondo C, Papasergi S, Akira S, Teti G, Beninati C. Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells. Nat Immunol. 2009 Jun; 10(6):587-94.

Martinel Lamas DJ, Croci M, Carabajal E, Crescenti EJ, Sambuco L, Massari NA, Bergoc RM, Rivera ES, Medina VA. Therapeutic potential of histamine H_4 receptor agonists in triple-negative human breast cancer experimental model. Br J Pharmacol. 2013 Sep; 170(1):188-99.

Marur S, Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. Mayo Clin Proc. 2016; 91:386-396.

Massari NA, Medina VA, Cricco GP, Martinel Lamas DJ, Sambuco L, Pagotto R, Ventura C, Ciraolo PJ, Pignataro O, Bergoc RM, Rivera ES. Antitumor activity of histamine and clozapine in a mouse experimental model of human melanoma. J Dermatol Sci. 2013 Dec; 72(3):252-62.

Massari NA, Medina VA, Martinel Lamas DJ, Cricco GP, Croci M, Sambuco L, Bergoc RM, Rivera ES. Role of H4 receptor in histamine-mediated responses in human melanoma. Melanoma Res. 2011 Oct; 21(5):395-404.

Massari NA, Nicoud MB, Medina VA. Histamine receptors and cancer pharmacology: an update. Br J Pharmacol. 2018 Nov 10. doi: 10.1111/bph.14535. [Epub ahead of print].

Massari NA, Nicoud MB, Sambuco L, Cricco GP, Martinel Lamas DJ, Herrero Ducloux MV, Blanco H, Rivera ES, Medina VA. Histamine therapeutic efficacy in metastatic melanoma: Role of histamine H4 receptor agonists and opportunity for combination with radiation. Oncotarget. 2017 Apr 18; 8(16):26471-26491.

Mattila R, Rautava J, Syrjänen S. Human papillomavirus in oral atrophic lichen planus lesions. Oral Oncol. 2012 Oct; 48(10):980-984.

McCaughey C, Machan M, Bennett R, Zone JJ, Hull CM. Pimecrolimus 1% cream for oral erosive lichen planus: a 6-week randomized, double-blind, vehicle-controlled study with a 6-week open-label extension to assess efficacy and safety. J Eur Acad Dermatol Venereol. 2011 Sep; 25(9):1061-7.

McLeod JJ, Baker B, Ryan JJ. Mast cell production and response to IL-4 and IL-13. Cytokine. 2015 Sep; 75(1):57-61.

Medina V, Cricco G, Nuñez M, Martín G, Mohamad N, Correa-Fiz F, Sanchez-Jimenez F, Bergoc R, Rivera ES. Histamine-mediated signaling processes in human malignant mammary cells. Cancer Biol Ther. 2006 Nov; 5(11):1462-71.

Medina V, Croci M, Crescenti E, Mohamad N, Sanchez-Jiménez F, Massari N, Nuñez M, Cricco G, Martin G, Bergoc R, Rivera E. The role of histamine in human mammary carcinogenesis: H3 and H4 receptors as potential therapeutic targets for breast cancer treatment. Cancer Biol Ther. 2008 Jan; 7(1):28-35.

Medina V, Croci M, Crescenti E, Mohamad N, Sanchez-Jiménez F, Massari N, Nuñez M, Cricco G, Martin G, Bergoc R, Rivera E. The role of histamine in human mammary carcinogenesis: H3 and H4 receptors as potential therapeutic targets for breast cancer treatment. Cancer Biol Ther. 2008 Jan; 7(1):28-35.

Medina VA, Rivera ES. Histamine receptors and cancer pharmacology. Br J Pharmacol. 2010 Oct; 161(4):755-67.

Meng F, Han Y, Staloch D, Francis T, Stokes A, Francis H. The H4 histamine receptor agonist, clobenpropit, suppresses human cholangiocarcinoma progression by disruption of epithelial mesenchymal transition and tumor metastasis. Hepatology. 2011 Nov; 54(5):1718-28.

Mineshiba J, Myokai F, Mineshiba F, Matsuura K, Nishimura F, Takashiba S. Transcriptional regulation of beta-defensin-2 by lipopolysaccharide in cultured human cervical carcinoma (HeLa) cells. FEMS Immunol Med Microbiol. 2005; 45:37–44.

Moharamzadeh K, Colley H, Murdoch C, Hearnden V, Chai WL, Brook IM, Thornhill MH, Macneil S. Tissue-engineered oral mucosa. J Dent Res. 2012 Jul; 91(7):642-50.

Moore SR, Johnson NW, Pierce AM, Wilson DF. The epidemiology of tongue cancer: a review of global incidence. Oral Dis. 2000 Mar; 6(2):75-84.

Mortazavi H, Baharvand M, Mehdipour M. Oral potentially malignant disorders: an overview of more than 20 entities. J Dent Res Dent Clin Dent Prospects. 2014; 8(1):6-14.

Müller S. Oral lichenoid lesions: distinguishing the benign from the deadly. Mod Pathol. 2017 Jan; 30(s1):S54-S67

Müller S. Oral manifestations of dermatologic disease: a focus on lichenoid lesions. Head Neck Pathol. 2011 Mar; 5(1):36-40.

Multhoff G, Molls M, Radons J. Chronic inflammation in cancer development. Front Immunol. 2012 Jan 12; 2:98.

Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer. 2008 Aug; 8(8):618-31.

Naganuma F, Nakamura T, Yoshikawa T, Iida T, Miura Y, Kárpáti A, Matsuzawa T, Yanai A, Mogi A, Mochizuki T, Okamura N, Yanai K. Histamine N-methyltransferase regulates aggression and the sleep-wake cycle. Sci Rep. 2017 Nov 21; 7(1):15899.

Nakamura T, Itadani H, Hidaka Y, Ohta M, Tanaka K. Molecular cloning and characterization of a new human histamine receptor, HH4R. Biochem Biophys Res Commun. 2000 Dec 20; 279(2):615-20.

Nakaya M, Takeuchi N, Kondo K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. Ann Otol Rhinol Laryngol. 2004 Jul; 113(7):552-7.

Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med. 2008 Jan; 37(1):1-10.

Naveen-Kumar B, Tatapudi R, Sudhakara-Reddy R, Alapati S, Pavani K, Sai-Praveen KN. Various forms of tobacco usage and its associated oral mucosal lesions. J Clin Exp Dent. 2016 Apr 1; 8(2):e172-7.

Neppelberg E, Johannessen AC, Jonsson R. Apoptosis in oral lichen planus. Eur J Oral Sci. 2001 Oct; 109(5):361-4.

Neumann D. Role of the Histamine H(4)-Receptor in Bronchial Asthma. Handb Exp Pharmacol. 2017; 241:347-359.

Niijima-Yaoita F, Tsuchiya M, Ohtsu H, Yanai K, Sugawara S, Endo Y, Tadano T. Roles of histamine in exercise-induced fatigue: favouring endurance and protecting against exhaustion. Biol Pharm Bull. 2012; 35(1):91-7.

Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I. Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. Eur J Immunol. 2001; 31(4):1066-1075.

Nonomura N, Takayama H, Nishimura K, Oka D, Nakai Y, Shiba M, Tsujimura A, Nakayama M, Aozasa K, Okuyama A. Decreased number of mast cells infiltrating into needle biopsy specimens leads to a better prognosis of prostate cancer. Br J Cancer. 2007 Oct 8; 97(7):952-6.

Oda T, Morikawa N, Saito Y, Masuho Y, Matsumoto S. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. J Biol Chem. 2000 Nov 24; 275(47):36781-6.

Ohsawa Y, Hirasawa N. The antagonism of histamine H1 and H4 receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. Allergy. 2012 Aug; 67(8):1014-22.

Ohsawa Y, Hirasawa N. The role of histamine H1 and H4 receptors in atopic dermatitis: from basic research to clinical study. Allergol Int. 2014 Dec; 63(4):533-42.

Okada F. Inflammation-related carcinogenesis: current findings in epidemiological trends, causes and mechanisms. Yonago Acta Med. 2014 Jun; 57(2):65-72.

O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors -redefining innate immunity. Nat Rev Immunol. 2013 Jun; 13(6):453-60.

Ord RA, Blanchaert RH Jr. Current management of oral cancer. A multidisciplinary approach. J Am Dent Assoc. 2001 Nov; 132 Suppl:19S-23S.

Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, Liu WL, Stark H, Thurmond RL, Haas HL. International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. Pharmacol Rev. 2015 Jul; 67(3):601-55.

Paul M, Shetty DC. Analysis of the changes in the basal cell region of oral lichen planus: An ultrastructural study. J Oral Maxillofac Pathol. 2013 Jan; 17(1):10-6.

Pedersen A. Abnormal EBV immune status in oral lichen planus. Oral Dis. 1996 Jun; 2(2):125-8.

Petti S. Lifestyle risk factors for oral cancer. Oral Oncol. 2009 Apr-May;45(4-5):340-50.

Pilli M, Penna A, Zerbini A, Vescovi P, Manfredi M, Negro F, Carrozzo M, Mori C, Giuberti T, Ferrari C, Missale G. Oral lichen planus pathogenesis: A role for the HCV-specific cellular immune response. Hepatology. 2002 Dec; 36(6):1446-52.

Pippi R, Romeo U, Santoro M, Del Vecchio A, Scully C, Petti S. Psychological disorders and oral lichen planus: matched case-control study and literature review. Oral Dis. 2016 Apr; 22(3):226-34.

Poon IK, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. Nat Rev Immunol. 2014 Mar; 14(3):166-80.

Presland RB, Jurevic RJ. Making sense of the epithelial barrier: what molecular biology and genetics tell us about the functions of oral mucosal and epidermal tissues. J Dent Educ. 2002 Apr; 66(4):564-74.

Rad M, Hashemipoor MA, Mojtahedi A, Zarei MR, Chamani G, Kakoei S, Izadi N. Correlation between clinical and histopathologic diagnoses of oral lichen planus based on modified WHO diagnostic criteria. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Jun; 107(6):796-800.

Raible DG, Lenahan T, Fayvilevich Y, Kosinski R, Schulman ES. Pharmacologic characterization of a novel histamine receptor on human eosinophils. Am J Respir Crit Care Med. 1994 Jun; 149(6):1506-11.

Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, Blamey RW, Ellis IO. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol. 2008 Jul 1; 26(19):3153-8.

Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt SJ, Schmitt FC, Tan PH, Tse GM, Badve S, Ellis IO. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res. 2010; 12(4):207.

Regezi J, Sciubba J, Jordan R. Oral Pathology: Clinical Pathologic Correlations. 2017; 7th Ed. (3) 80-113; St. Louis MO, Elsevier.

Reibel J, Gale N, Hille J, et al. Oral potentially malignant disorders and oral epithelial dysplasia. In: El-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PPJ, eds.WHO Classification of Head and Neck Tumours. 4th ed. Lyon, France: IARC; 2017: 112-115.

Ribatti D, Ennas MG, Vacca A, Ferreli F, Nico B, Orru S, Sirigu P. Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. Eur J Clin Invest. 2003 May; 33(5):420-5.

Ribatti D, Tamma R, Crivellato E. The dual role of mast cells in tumor fate. Cancer Lett. 2018 Oct 1; 433:252-258.

Ribatti D, Vacca A, Nico B, Crivellato E, Roncali L, Dammacco F. The role of mast cells in tumour angiogenesis. Br J Haematol. 2001 Dec; 115(3):514-21.

Riley JF, West GB. Histamine in tissue mast cells. J Physiol. 1952 Aug; 117(4):72P-73P.

Rogers RS 3rd, Sheridan PJ, Nightingale SH. Desquamative gingivitis: clinical, histopathologic, immunopathologic, and therapeutic observations. J Am Acad Dermatol. 1982 Dec; 7(6):729-35.

Rojo-Moreno JL, Bagán JV, Rojo-Moreno J, Donat JS, Milián MA, Jiménez Y. Psychologic factors and oral lichen planus. A psychometric evaluation of 100 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998 Dec; 86(6):687-91.

Rusthoven K, Ballonoff A, Raben D, Chen C. Poor prognosis in patients with stage I and II oral tongue squamous cell carcinoma. Cancer. 2008 Jan 15; 112(2):345-51.

Ryan BM, Faupel-Badger JM. The hallmarks of premalignant conditions: a molecular basis for cancer prevention. Semin Oncol. 2016 Feb; 43(1):22-35.

Sakamoto H, Naito H, Ohta Y, Tanakna R, Maeda N, Sasaki J, Nord CE. Isolation of bacteria from cervical lymph nodes in patients with oral cancer. Arch Oral Biol. 1999 Oct; 44(10):789-93.

Salem A, Almahmoudi R, Vehviläinen M, Salo T. Role of the high mobility group box 1 signalling axes via the receptor for advanced glycation end-products and toll-like receptor-4 in the immunopathology of oral lichen planus: a potential drug target? Eur J Oral Sci. 2018 Jun; 126(3):244-248.

Salem A, Al-Samadi A, Stegajev V, Stark H, Häyrinen-Immonen R, Ainola M, Hietanen J, Konttinen YT. Histamine H4 receptor in oral lichen planus. Oral Dis. 2015 Apr; 21(3):378-85.

Sano D, Myers JN. Metastasis of squamous cell carcinoma of the oral tongue. Cancer Metastasis Rev. 2007 Dec; 26(3-4):645-62.

Santoro A, Majorana A, Roversi L, Gentili F, Marrelli S, Vermi W, Bardellini E, Sapelli P, Facchetti F. Recruitment of dendritic cells in oral lichen planus. J Pathol. 2005 Mar; 205(4):426-34.

Sardana K, Sharma RC, Koranne RV, Mahajan S. An interesting case of colocalization of segmental lichen planus and vitiligo in a 14-year-old boy. Int J Dermatol. 2002; 41: 508–509.

Sciubba JJ. Oral cancer. The importance of early diagnosis and treatment. Am J Clin Dermatol. 2001; 2(4):239-51.

Scully C, Carrozzo M. Oral mucosal disease: Lichen planus. Br J Oral Maxillofac Surg. 2008; 46(1):15-21.

Scully C. Oral cancer aetiopathogenesis; past, present and future aspects. Med Oral Patol Oral Cir Bucal. 2011 May 1; 16(3):e306-11.

Shariati M, Mokhtari M, Masoudifar A. Association between oral lichen planus and Epstein-Barr virus in Iranian patients. J Res Med Sci. 2018 Mar 27; 23:24.

Shi N, Jin F, Zhang X, Clinton SK, Pan Z, Chen T. Overexpression of human β-defensin 2 promotes growth and invasion during esophageal carcinogenesis. Oncotarget. 2014 Nov 30; 5(22):11333-44.

Shi Q, Zhang T, Huo N, Huang Y, Xu J, Liu H. Association between polymorphisms in interleukins and oral lichen planus: A meta-analysis. Medicine (Baltimore). 2017 Mar; 96(11):e6314.

Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. Cancer Lett. 2008 Aug 28; 267(2):204-15.

Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017 Jan; 67(1):7-30.

Simons FE, Simons KJ. Histamine and H1-antihistamines: celebrating a century of progress. J Allergy Clin Immunol. 2011 Dec; 128(6):1139-1150.e4.

Siponen M. Oral lichen planus – etiopathogenesis and management. Acta Univ. Oul. D 1403, 2017.

Smolinska S, Jutel M, Crameri R, O'Mahony L. Histamine and gut mucosal immune regulation. Allergy. 2014 Mar; 69(3):273-81.

Smuda C, Bryce PJ. New developments in the use of histamine and histamine receptors. Curr Allergy Asthma Rep. 2011 Apr; 11(2):94-100.

Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol. 2006;90:1-50.

Soucek L, Lawlor ER, Soto D, Shchors K, Swigart LB, Evan GI. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. Nat Med. 2007 Oct; 13(10):1211-8.

Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018 Jun; 125(6):612-627.

Speight PM. Update on oral epithelial dysplasia and progression to cancer. Head Neck Pathol. 2007 Sep;1(1):61-6.

Squier CA, Hill MW. Oral mucosa. In: Ten Cate AR, editor. Oral histology: development, structure and function. St. Louis (MO): CV Mosby; 1989. p. 341–81.

Squier CA, Kremer MJ. Biology of oral mucosa and esophagus. J Natl Cancer Inst Monogr. 2001; (29):7-15.

Stegaev V, Nies AT, Porola P, Mieliauskaite D, Sánchez-Jiménez F, Urdiales JL, Sillat T, Schwelberger HG, Chazot PL, Katebe M, Mackiewicz Z, Konttinen YT, Nordström DC. Histamine transport and metabolism are deranged in salivary glands in Sjogren's syndrome. Rheumatology (Oxford). 2013 Sep; 52(9):1599-608.

Sterle HA, Nicoud MB, Massari NA, Táquez Delgado MA, Herrero Ducloux MV, Cremaschi GA, Medina VA. Immunomodulatory role of histamine H4 receptor in breast cancer. Br J Cancer. 2018 Jul 10. doi: 10.1038/s41416-018-0173-z.

Strakhova MI, Nikkel AL, Manelli AM, Hsieh GC, Esbenshade TA, Brioni JD, Bitner RS. Localization of histamine H4 receptors in the central nervous system of human and rat. Brain Res. 2009 Jan 23; 1250:41-8.

Subramanian H, Gupta K, Lee D, Bayir AK, Ahn H, Ali H. β-Defensins activate human mast cells via Mas-related gene X2. J Immunol. 2013; 191(1):345-352.

Subramanian H, Gupta K, Lee D, Bayir AK, Ahn H, Ali H. β-Defensins activate human mast cells via Mas-related gene X2. J Immunol. 2013 Jul 1; 191(1):345-52.

Sugerman PB, Savage NW, Zhou X, Walsh LJ, Bigby M. Oral lichen planus. Clin Dermatol. 2000 Sep-Oct; 18(5):533-9.

Sun Y, Liu N, Guan X, Wu H, Sun Z, Zeng H. Immunosuppression Induced by Chronic Inflammation and the Progression to Oral Squamous Cell Carcinoma. Mediators Inflamm. 2016; 2016:5715719.

Suzuki A, Leland P, Joshi BH, Puri RK. Targeting of IL-4 and IL-13 receptors for cancer therapy. Cytokine. 2015 Sep; 75(1):79-88.

Szeberényi JB, Pállinger E, Zsinkó M, Pós Z, Rothe G, Orsó E, Szeberényi S, Schmitz G, Falus A, László V. Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. Immunol Lett. 2001 Apr 2; 76(3):175-82.

Takeshita K, Sakai K, Bacon KB, Gantner F. Critical role of histamine H4 receptor in leukotriene B4 production and mast cell-dependent neutrophil recruitment induced by zymosan in vivo. J Pharmacol Exp Ther. 2003 Dec; 307(3):1072-8.

Tampa M, Caruntu C, Mitran M, Mitran C, Sarbu I, Rusu LC, Matei C, Constantin C, Neagu M, Georgescu SR. Markers of Oral Lichen Planus Malignant Transformation. Dis Markers. 2018 Feb 26; 2018:1959506.

Tan SY, Fan Y, Luo HS, Shen ZX, Guo Y, Zhao LJ. Prognostic significance of cell infiltrations of immunosurveillance in colorectal cancer. World J Gastroenterol. 2005 Feb 28; 11(8):1210-4.

Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. J Oncol. 2011; 2011:603740.

Tanaka, S, Ichikawa A. Regulation of mammalian histamine synthesis: histidine decarboxylase. In: M. Shahid, N. Khardori, R.A. Khan, & T. Tripathi. (Eds.), Biomedical Aspects of Histamine. Current Perspectives; 2011; 15-30. New York, NY: Springer.

Teppo H, Alho OP. Comorbidity and diagnostic delay in cancer of the larynx, tongue and pharynx. Oral Oncol. 2009 Aug; 45(8):692-5.

Thangam EB, Jemima EA, Singh H, Baig MS, Khan M, Mathias CB, Church MK, Saluja R. The Role of Histamine and Histamine Receptors in Mast Cell-Mediated Allergy and Inflammation: The Hunt for New Therapeutic Targets. Front Immunol. 2018 Aug 13; 9:1873.

Thiagarajan S, Nair S, Nair D, Chaturvedi P, Kane SV, Agarwal JP, D'Cruz AK. Predictors of prognosis for squamous cell carcinoma of oral tongue. J Surg Oncol. 2014 Jun;109(7):639-44.

Thompson L. World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Ear Nose Throat J. 2006 Feb; 85(2):74.

Thongprasom K, Dhanuthai K. Steriods in the treatment of lichen planus: a review. J Oral Sci. 2008 Dec; 50(4):377-85.

Thorn JJ, Holmstrup P, Rindum J, Pindborg JJ. Course of various clinical forms of oral lichen planus. A prospective follow-up study of 611 patients. J Oral Pathol. 1988 May; 17(5):213-8.

Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov. 2008 Jan; 7(1):41-53.

Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov. 2008 Jan; 7(1):41-53.

Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov. 2008 Jan; 7(1):41-53.

Thurmond RL. The histamine H4 receptor: from orphan to the clinic. Front Pharmacol. 2015 Mar 31; 6:65.

Tiligada E, Ennis M. Histamine pharmacology: from Sir Henry Dale to the 21st century. Br J Pharmacol. 2018 Oct 19. doi: 10.1111/bph.14524. [Epub ahead of print]

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015 Mar; 65(2):87-108.

Tovaru S, Parlatescu I, Gheorghe C, Tovaru M, Costache M, Sardella A. Oral lichen planus: a retrospective study of 633 patients from Bucharest, Romania. Med Oral Patol Oral Cir Bucal. 2013 Mar 1;18(2):e201-6.

van der Waal I, de Bree R, Brakenhoff R, Coebergh JW. Early diagnosis in primary oral cancer: is it possible? Med Oral Patol Oral Cir Bucal. 2011 May 1; 16(3):e300-5.

van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009 Apr-May; 45(4-5):317-23.

van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009 Apr-May; 45(4-5):317-23.

van Ginkel JH, Huibers MMH, van Es RJJ, de Bree R, Willems SM. Droplet digital PCR for detection and quantification of circulating tumor DNA in plasma of head and neck cancer patients. BMC Cancer. 2017 Jun 19; 17(1):428.

Varghese SS, George GB, Sarojini SB, Vinod S, Mathew P, Mathew DG, Sebastian J, George A. Epidemiology of Oral Lichen Planus in a Cohort of South Indian Population: A Retrospective Study. J Cancer Prev. 2016 Mar; 21(1):55-9.

Varricchi G, Galdiero MR, Loffredo S, Marone G, Iannone R, Marone G, Granata F. Are Mast Cells MASTers in Cancer? Front Immunol. 2017 Apr 12; 8:424.

Verma C, Seebah S, Low SM, Zhou L, Liu SP, Li J, Beuerman RW. Defensins: antimicrobial peptides for therapeutic development. Biotechnol J. 2007 Nov; 2(11):1353-9.

Vlieg-Boerstra BJ, van der Heide S, Oude Elberink JN, Kluin-Nelemans JC, Dubois AE. Mastocytosis and adverse reactions to biogenic amines and histamine-releasing foods: what is the evidence? Neth J Med. 2005; 63(7):244-9.

Walter M, Stark H. Histamine receptor subtypes: a century of rational drug design. Front Biosci (Schol Ed). 2012 Jan 1; 4:461-88.

Wang J, van der Waal I. Disease scoring systems for oral lichen planus; a critical appraisal. Med Oral Patol Oral Cir Bucal. 2015 Mar 1; 20(2):e199-204.

Wang K, Lu W, Tu Q, Ge Y, He J, Zhou Y, Gou Y, Van Nostrand JD, Qin Y, Li J, Zhou J, Li Y, Xiao L, Zhou X. Preliminary analysis of salivary microbiome and their potential roles in oral lichen planus. Sci Rep. 2016 Mar 10; 6:22943.

Warnakulasuriya S, Ariyawardana A. Malignant transformation of oral leukoplakia: a systematic review of observational studies. J Oral Pathol Med. 2016 Mar; 45(3):155-66.

Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med. 2007; 36:575-580.

Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E. Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. J Oral Pathol Med. 2008 Mar; 37(3):127-33.

Warnakulasuriya S. Causes of oral cancer--an appraisal of controversies. Br Dent J. 2009 Nov 28; 207(10):471-5.

Wei Z, Hou Q, Xu H, Jiang L, Chen Q. Evidence of genetic factors involved in oral lichen planus pathogenesis. Oral Dis. 2018 Jul; 24(5):864-865.

Whitmore SE, Lamont RJ. Oral bacteria and cancer. PLoS Pathog. 2014 Mar 27; 10(3):e1003933.

Wolff KD, Follmann M, Nast A. The diagnosis and treatment of oral cavity cancer. Dtsch Arztebl Int. 2012 Nov; 109(48):829-35.

Wu D, Cheng S, Chen X, Sun M, Wang G, Fu S, Dong G, Wang L, Wu Y. Mitochondrial haplogroup B4 may be a protective factor to oral lichen planus susceptibility in Chinese. Oral Dis. 2014 Jan; 20(1):62-8.

Wu X, Gong Z, Sun L, Ma L, Wang Q. MicroRNA-802 plays a tumour suppressive role in tongue squamous cell carcinoma through directly targeting MAP2K4. Cell Prolif. 2017 Jun;50(3).

Xavier GM, de Sá AR, Guimarães AL, da Silva TA, Gomez RS. Investigation of functional gene polymorphisms interleukin-1beta, interleukin-6, interleukin-10 and tumor necrosis factor in individuals with oral lichen planus. J Oral Pathol Med. 2007 Sep; 36(8):476-81.

Xue JL, Fan MW, Wang SZ, Chen XM, Li Y, Wang L. A clinical study of 674 patients with oral lichen planus in China. J Oral Pathol Med. 2005 Sep; 34(8):467-72.

Yamatodani A, Fukuda H, Wada H, Iwaeda T, Watanabe T. High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. J Chromatogr. 1985 Nov 8; 344:115-23.

Yamaura K, Shigemori A, Suwa E, Ueno K. Expression of the histamine H4 receptor in dermal and articular tissues. Life Sci. 2013 Feb 7; 92(2):108-13.

Yanamoto S, Yamada S, Takahashi H, Kawasaki G, Ikeda H, Shiraishi T, Fujita S, Ikeda T, Asahina I, Umeda M. Predictors of locoregional recurrence in T1-2N0 tongue cancer patients. Pathol Oncol Res. 2013 Oct; 19(4):795-803.

Yardimci G, Kutlubay Z, Engin B, Tuzun Y. Precancerous lesions of oral mucosa. World J Clin Cases. 2014 Dec 16; 2(12):866-72.

Yildirim B, Sengüven B, Demir C. Prevalence of herpes simplex, Epstein Barr and human papilloma viruses in oral lichen planus. Med Oral Patol Oral Cir Bucal. 2011; 16(2):e170-4.

Zampeli E, Tiligada E. The role of histamine H4 receptor in immune and inflammatory disorders. Br J Pharmacol. 2009 May; 157(1):24-33.

Zhao B, Xu N, Li R, Yu F, Zhang F, Yang F, Ge X, Li YC, Du J. Vitamin D/VDR signaling suppresses microRNA-802-induced apoptosis of keratinocytes in oral lichen planus. FASEB J. 2018 Aug 3:fj201801020RRR.

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