REVIEW ARTICLE

Molecular markers in oral lichen planus: A systematic review

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ABSTRACT

Oral lichen planus (OLP) is a chronic inflammatory mucosal disease that is usually detected in 0.5–2.2% of the human population. Among these, only 0.5–2.9% of the lesions progress to carcinoma. However, there are no prognostic markers available presently to recognize the increased risk in malignant transformation of the lesions. Selected markers for cell proliferation, adhesion, apoptosis and lymphocytic infiltration were analyzed by immunohistochemistry in addition to static cytometry for DNA content. The concept linking OLP and oral squamous cell carcinoma states that chronic inflammation results in crucial DNA damage, which further progresses to development of carcinoma. Even though in the past decade, enormous information has been accumulated on malignant potential of OLP, its transformation still remains unclear. Hence, the purpose of this article was to review cellular and molecular markers to understand the pathogenesis of OLP and its progression toward malignancy.

Key words: Desmocollin-1, DNA content, E-cadherin, Ki-67, oral lichen planus

INTRODUCTION

The oral cavity is lined by a mucous membrane that forms a barrier between the environment and the body. The oral mucosa is classified into keratinized and nonkeratinized oral mucosa. Oral mucosa consists of two distinct layers: stratified squamous epithelium and connective tissue termed lamina propria. The lamina propria is a fibrous connective tissue layer that consists of fibroblasts which are responsible for the production of the fibers as well as the extracellular matrix. Submucosa is a deep, dense layer of the lamina propria which contains a layer of loose fatty or glandular connective tissue containing the major blood vessels and nerves. This tissue separates the oral mucosa from the underlying bone or muscle.[1,2]

Oral lichen planus (OLP) is a chronic inflammatory disease of oral mucosa. The World Health Organization (WHO) has defined OLP as a potentially malignant disorder, representing a generalized state associated with a significantly increased risk of cancer.[3,4]

OLP is the most common noninfectious oral mucosal disease in adult patients referred to oral pathology clinics.[5] It affects 0.5–2.2% of the population and is more often seen in women than men.[6,7] Even though the typical age of presentation is 30–60 years, it is usually spotted in middle-aged women and younger-aged men. In children, OLP is uncommon and it usually appears together with cutaneous disease.[8] Only 17% of the affected patients recover totally from OLP,[6] but remission in 39% of the OLP lesions has also been reported.[9]

Patients with OLP may develop extra OLP lesions that affect the skin or other mucosal sites. About 15% of patients

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with OLP have or will develop cutaneous lesions. The most frequent site of the lesions is the flexor surface of the forearm, but they also commonly appear on the legs, back and chest. Cutaneous lesions present as erythematosus to violaceous, flat-topped, polygonal papules that are covered by a network of fine lines (Wickham's striae) and they are seen typically within several months after the appearance of oral lesions.[10] The main symptom of cutaneous LP is itching and patients complain of pruritus. LP is also seen on the scalp as lichen planopilaris which results in scarring alopecia[11] and also rarely affects the nails.[12]

OLP, in association with genital lesions, is known as vulvovaginal gingival syndrome and was first described by Pelisse.[13] It affects 20% of the women with OLP and patients with genital lesions are always identified with gingival lesions.[14,15] Vaginal lesions are mainly of an erosive type, presenting with burning, pain, vaginal discharge and dyspareunia although asymptomatic reticular lesions are seen. Esophageal LP has been described in OLP patients and it is obviously much more common than what has been reported because of its asymptomatic nature.[10]

The histology of OLP is characterized by a band-like lymphocytic infiltrate in juxta-epithelial lamina propria. In addition, there is hyperkeratinization, acanthosis, liquefaction degeneration of the basal cells, colloid bodies, saw-tooth appearance of rete pegs and distribution of the epithelial basement membrane (BM). Despite these well-characterized histological features of OLP, inter- and intra-observer reproducibility to diagnose OLP are modest, however. The etiology of OLP is still unknown. Cell-mediated mechanisms are involved in the initiation and progression of the disease. Further, localized autoimmunity has been suggested as playing a role in the pathogenesis of OLP. As there is no known causative factor for OLP, its cure remains unspecific. Despite the WHO definition of OLP as a precancerous condition, the premalignant potential of OLP is still debatable. Malignant transformation has been estimated to occur in 0.5-2.9% of the OLP patients. Currently, there are no prognostic markers to identify which chronic OLP lesions are at a higher risk for progression. Thus, every OLP patient should be monitored carefully to detect early cancer development. To understand the etiopathogenesis of OLP, it is important to recognize the key molecules in it.

Molecular markers offer the possibility to identify patients with potentially malignant lesions which are in progression toward cancer before malignant cells are detectable histologically, at the primary site.[16] Numerous potential markers have been identified and their associations with early detection, progression and prognosis of oral squamous cell carcinoma (OSCC) have also been discussed. Tumor markers can be allocated in four groups according to their function: (1) Enhancement of tumor growth: cell cycle accelerations and proliferations, (2) tumor suppression and antitumor defense: immune responses and apoptosis, (3) angiogenesis, (4) tumor invasion and metastatic potential: Adhesion molecules and matrix degradations.[17] Currently, there are no specific markers to identify the risk lesions from stable disease. The general purpose of this review was to identify certain molecular markers to understand the molecular mechanism involved in etiopathogenesis of OLP and to predict the progression of the lesion toward malignancy.

MATERIALS AND METHODS

A literature search was conducted with keywords such as oral lichen planus, DNA content, Ki-67, E-cadherin, desmocollin-1 and cluster of differentiation for peer reviewed articles published in English for OLP from January 1, 1900 to June 30, 2011 in Medline (PubMed), Ovid, Cochrane search and Google Scholar. Articles retrieved from the electronic search were manually searched for relative references and cross references. The available articles were then reviewed and assessed for suitability for the stated purpose.

Cell cycle markers

Abnormal nuclear DNA content (aneuploidy) is an indicator of chromosomal aberrations and is associated with malignant and premalignant lesions.[18] In oral precancer studies, DNA index measurement is thought to be more suitable for risk assessment of an identified precancerous field and of less value in early diagnoses despite the higher analytical sensitivity.[19] Aneuploid dysplastic lesions are shown to develop SCC in a shorter period than diploid; thus, measurement of DNA index might be valuable to determine the time to cancer progression.[20]

Based on the studies, 2.5c exceeding rate (ER) and the proliferation index of DNA content are shown to be useful parameters in predicting malignant transformation.[21,22] With more strict criteria defined by Auer *et al*., aneuploidy is classified as 2.5cER more than 35% and 5cER valued over 0%.[23] These results are compatible with the figures on potential risk of cancer development in OLP (0.46.25%).[24] In nuclear DNA content studies, many cellular parameters can be detected with static cytometry analysis. Former studies showed that in prostate and cervical carcinomas, the G2/M phase is a strong prognostic marker in cancer development.[25,26]

A few additional reports exist on epithelial DNA content measurements including OLP biopsies and cytology.[27-30] The results are conflicting and DNA content varies from diploid to aneuploid DNA content in OLP. The most important difference among these studies seems to be the method used in DNA content measurement. In certain studies, cell separation technique was used for the image cytometry measurement.[27-31] In another study, exfoliative cytology samples from OLP lesions were used.[28] In static cytometry, both the morphology and exact location of the measured cells can be assessed simultaneously. Another distinct difference is that most of the authors used the reticular form of OLP which

is the most unlikely form for malignant transformation.[32,33] However, there is one previous study where few erosive OLP lesions were classified as aneuploid; hence aneuploid changes and DNA cytometry can be suitable screening methods for OLP to detect the high-risk lesions.[27,34]

Role of p53

Cell cycle arrest helps in maintaining tissue integrity and facilitating DNA repair mechanisms; however, at the same time, entry into senescence could favor malignant transformation. Inactivation of p53 is a frequent phenomenon in OSCC. This is caused by mutations, presence of HPV virus and other molecular alteration occurring in the p53 pathway.

As p53 expression has been identified as a response to DNA damage, the identification of p53 in OLP tissue is interpreted as an indication of precancerous potential by some researchers.[28] In support to this concept, Chaiyarit *et al*. showed an inducible nitric oxide synthase-dependent DNA damage and p53-elevated expression in OLP patients. Another concept is that the high expression of p53 in OLP is a result of the higher cellular proliferation. To prove that p53 expression in OLP is not just a result of the inflammatory process, Safadi *et al*. compared the immunohistochemical expression of p53 and its downstream effector p21WAF1 between OLP and other inflammatory oral conditions and found significantly higher expression in OLP.[29,30]

Cell proliferation markers

The previous studies showed that topo IIα is a confidential marker of cell proliferation in breast and vulva tumors and also in head and neck precancerous and cancerous lesions.[35-38] Studies on oral mucosa indicate that topo IIα expression has increased as the disease progressed from intraepithelial dysplasia to SCC. Similarly, an increased expression has been found in well or moderately differentiated SCC than in highly differentiated SCC.[36-38]

In the previous studies, topo IIα is considered a better proliferation marker than Ki-67. This is stated by its shorter expression time during the cell cycle and thus giving a better estimation of the number of actively cycling cells than Ki-67 does.[39] Differences in expression of Ki-67 and topo IIα can also be explained by repairing or apoptotic role of topo IIα. During cell proliferation, topo IIα not only repairs DNA damage but also plays a role in apoptosis by inducing apoptotic cell death.[40,41] The susceptibility of topo IIα to trigger apoptosis is found to be independent of the enzyme's DNA cleavage activity but yet required nuclear localization of topo IIα. Consequently, inappropriate expression of topo IIα confuses the temporal order of events that topo IIα mediates during cell division.[40] Hence, failure in DNA repair due to increased expression of topo IIα might be one key event leading to apoptosis of basal and parabasal cells. It is also shown that protein kinase C delta, which has an essential role in the genotoxic stress response, activates topo IIα to induce apoptotic cell death in response to DNA damage.[41] Thus, based on these findings, it might be that increased expression of topo IIα in OLP is a marker for not only proliferation but also for unsuccessful DNA repair which finally will result in apoptosis. Ki-67 accumulates during the S-phase and it rapidly disappears from postmitotic cells.[42] The rapid disappearance and short half-life of Ki-67 during the G2/M phase could be some explanations for the negativestaining pattern of Ki-67. As a cell proliferation marker, however, studies on Ki-67 were in line with previous studies showing greater increased proliferation in OLP than in normal oral mucosa.[43,44]

Ruutu *et al*. previously showed that increased mRNA levels of cdk-1 and Rad-51 in cell lines established from oral cancers originally developed from OLP with microarray analysis.[45] In tumor cells, overexpression of Rad-51 may protect the altered cells from apoptosis by increasing the resistance to DNA damage.[46] In addition, elevated levels of Rad-51 provide chromosomal instability, which is associated with tumor progression. Moreover, deregulation of cdk-1 phosphatase activity is one of the common events in cancer and is associated with a poor prognosis. Inactivation of cdk-1 is also shown to increase the level of apoptosis.[47]

Intensified expression of proliferative antigens such as proliferating cell nuclear antigen and Ki-67 can be found in numerous malignant and premalignant lesions. Diffuse and continuous reactions were observed in parabasal and basal layer cells of epithelium and spinous cell layer showed mostly focal positive reaction. The reaction was intense in lymphatic infiltrate of lamina propria and highly intense in stromal macrophages.

Ki-67 immunohistochemically showed discontinued mosaic type reaction in basal layer cells of epithelium and was negative in other epithelium layers such as oral leukoplakia. Further reaction was positive in lamina propria with diffusely thick lymphoid infiltrate.[48]

Cell adhesion molecules

Oral epithelia have a disposition to transmit the expression type and pattern of several adhesion-related gene products during cancer development. In malignant transformation, interactions with neighboring cells and extracellular cytoskeleton are altered. Normal nonkeratinized oral epithelia do express CK-19; however, in the normal keratinized addition and hyperplastic proliferation of keratinizing oral mucosa, no CK-19 expression is found in any cell layer.[49] The detection of CK-19 expression in OLPs located on the tongue is suggested as high-risk lesions by others.[49,50]

E-cadherin is expressed in normal oral mucosa but the expression is lost in oral SCC.[51] However, there was no relation to E-cadherin expression and malignant

transformation or other histological parameters or localization of OLP lesions. Bánkfalvi *et al*. showed that in the early stages of oral carcinogenesis, there is a general transient increase of E-cadherin expression, which finally turns into a loss of expression as the tumor acquires an invasive phenotype with significantly shortened survival.[52] Thus, E-cadherin might inhibit the epidermal growth factor receptor to enhance the progression of oral cancer. Other studies showed focal loss of E-cadherin expression in the epithelium of OLP, but the downregulation could not be related to malignant transformation.[33,53]

In keratinized normal oral mucosa, the level of desmocollin-1 is low which could reflect weakened adhesion in the epithelium. It is speculated that this allows oral keratinocytes to undergo a faster transition through the living layers of the epithelium.[54] Thus, the degree of keratinization as such cannot explain the desmocollin-1 expression.

Apoptotic markers

Apoptosis of basal keratinocytes, caused by the activity of cytotoxic T-cells, could be a possible explanation for one of the histopathologic hallmarks of OLP that is the vacuolar degeneration of basal membrane. This is also supported by several molecular studies demonstrating the presence of apoptotic signals in OLP.[5]

An increased rate of apoptosis in the epithelium in OLP as compared to normal mucosa is shown in previous studies, but a wide variation of apoptotic cells in different OLP lesions exists.[55,56]

There are no earlier reports on caspase cascade pathways of apoptosis in OLP. Caspase-2 activation by cytotoxic stress, such as DNA damage, is required for the permeabilization of mitochondria, which, release proteins that promote cell death.[57] Caspase-2 is also required in cell death receptor-mediated apoptosis by contributing to caspase-8 activation.[58]

Like caspase-2, caspase-12 activation is linked to ER stress, resulting in increased cell death by intrinsic apoptotic pathway. It has been reported that caspase-12 is one of the microsomal components required in ER stress-induced apoptosis independent from mitochondria-dependent apoptosis activation.[59] With other ER stress-induced molecules, caspase-12 is needed to activate caspase-9 during the process. In OLP lesions, high expression of caspase-12 may implicate intracellular disorders occurring in ER such as protein synthesis and $Ca2^{+}$ homeostasis. Furthermore, caspase-12 is a member of the inflammatory caspase family which is an essential protease for processing and maturation of the inflammatory cytokines, such as interleukin 1 (IL-1) and IL-18. Caspase-12 appears to inhibit caspase-1, another member of the inflammatory caspase family, resulting in the reduction of proinflammatory mediators IL-1 and IL-18 formation and release. Thus, caspase-12 plays a role in decreasing the macrophage-elicited Th-1 and Th-2 cytokine responses.[60] These findings may suggest that caspase-12 not only has a role in apoptosis, it might also have an important part in inflammatory process in OLP lesions.

Downstream cascade, caspase-3 expression in OLP has been studied previously.[61,62] Caspase-3 cleavage activation means no returning of cell death. As most of the caspase-3 expressing cells are located in the basal cell area of epithelium, wherein also the cell proliferation occurs, it may be assumed that dividing cells could be focused for elimination in OLP. Despite this, no correlation with apoptosis and cell proliferation in OLP has been reported earlier as Bcl-2 and Bax were used as apoptosis marker and MIB-1 as proliferation marker.[56]

Lymphocytic markers (CD5, CD20, CD27 and CD38)

Autoimmune disease has been defined as a clinical syndrome caused by the activation of T-cells or B-cells, or both, in the absence of an ongoing infection or other discernible cause.[63,64] Evidence of autoimmunity in OLP has been described indicating the simultaneous occurrence of a known autoimmune disease such as systemic lupus erythematosus (SLE), primary biliary cirrhosis, Sjögren's syndrome and OLP in the same incident. Thus, it could be hypothesized that at least, a part of the OLP lesions may be related to autoimmunity. In addition, one of the possible contributions of autoreactivity to OLP has been provided by studies of CD4 + helper-induced T-cell subset in the peripheral blood and lesions of patients affected with OLP.[65,66]

Likewise, the CD38+ expression in B-cells is evidently connected with autoimmune diseases, such as type II diabetes mellitus, systemic lupus erythematosus (SLE) and Sjögren's syndrome.[66,67] In intestinal mucosa, CD38+ targets at the mucosal addressin cell adhesion molecule-1 (Mad/CAM-1), but oral mucosa and tonsils lack this expression.[66] Thus, the target of CD38+ in oral mucosa is also still unknown.

CD20 expression is detected in different stages of B-cell maturation from pre-Bcells to immature and mature cells as well as in activated B-cells but not in plasma cells.[68] Although the exact function of the CD20 gene is not clear, it has been the target gene in therapy of B-cell lymphomas and some autoimmune disorders such as rheumatoid arthritis.[69,70] By immunohistochemistry, the activation or immature stage of the B-cells cannot be assessed. Instead, B-cell activating factor (BAFF) appears as a critical factor controlling B-cell homeostasis in survival and maturation, but it does not affect proliferation.[71] In BAFF transgenic mice, it has been shown that CD27+ B-cells translocate from serum to cutaneous tissue and exocrine glands, causing similar changes as found in certain autoimmune diseases, such as rheumatoid arthritis, SLE and Sjögren's syndrome.[72,67] Thus, the finding of CD20+ and CD27+ expressions in OLP lesions may propose the

autoimmune nature of OLP. So far, the expression of BAFF in OLP has not been assessed.

CD5 molecule has been implicated in the proliferative response of activated T-cells and in T-cell helper function. It has been pointed out that autoimmune disorders may result from the disruption of inhibitory receptors, particularly in their conserved intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs) which are sites for alternative phosphorylation, typically by a Src kinase and dephosphorylation, either by tyrosine phosphatase SHP1 or inositol phosphatase SHIP, transducing signals to distinct pathways.[73] Furthermore, it has been noted that CD5 has an ITIM that interacts with SHP1 and opposes activation mediated by the B cell receptor. Spour *et al*. have detected that CD3+CD5-cells represent a discrete small subset of mature T lymphocytes which are cytotoxic in nature.[74]

Heat shock proteins

In the mammalian species, the heat shock protein (HSP) family consists of mitochondrial (mt-HSP-60) and cytosolic HSP 60. HSP 70 family includes constitutive cytosolic HSP70, stress induced cytosolic HSP-70, endoplasmic reticulum Bip (Grp-78) and mitochondrial (mtHSP70).[5] Cellular expression of this antigen may take place under varied conditions as also seen in LP. Both HSP-60 and HSP70 induces the release of cytokines from the lymphocytes and contribute to the pathogenesis of autoimmune disease and chronic inflammation.[75]

Sugerman *et al*. drew comparisons in HSP staining among OLP, dysplastic OLP, normal oral mucosa and nonspecific oral ulceration. They noted a full thickness expression of HSP in 94% of their OLP cases.

According to Bramanti *et al*. and Sugerman *et al*., OLP represents an autoimmune response, directed toward the basal cell antigens or a hypersensitive response to antigens shared by basal cells and a microbial agent. HSPs represent antigenic proteins that may potentially be involved in the initiation or the persistence of the lymphocytic response of LP.[5,64,76]

It is hypothesized that in OLP cases, diverse exogenous agents may cause an upregulated expression of HSP by the basal keratinocytes. A reaction of the cytotoxic lymphocytes against these activated keratinocytes could then result in cell death and tissue destruction, characteristic of OLP lesions.

Once initiated, such a cytotoxic immunological reaction, or cytokines such as gamma interferon and tumor necrosis factor released from the activated T lymphocytes could further upregulate HSP expression by the neighboring keratinocytes, thus propagating lesional chronicity.[64]

Matrix metalloproteinases

The role of matrix metalloproteinases (MMPs) in OLP was initially associated with apoptosis of epithelial cells and grade of inflammation. Basement membrane damage and T-cell migration in OLP may be mediated by MMPs. Transforming growth factor beta (TGF-β) and bone morphogenic protein-4 were suggested as promoting signals for the upregulation of the MMPs. Chen *et al*. studied MMPs, TIMPs and TGF-β in OSCC that developed from the previous OLP and found constant expression with levels comparable to those detected in atrophic OLP, which is the form of OLP reported to have the higher malignant potential. They concluded that their findings are suggestive of the role MMPs have in the malignant transformation in OLP.[77]

SUMMARY

It can be concluded that several molecular alterations implicating pre-neoplastic changes are detectable in OLP. Of all markers included in the present review, desmocollin-1 expression increased the risk of dysplasia and cancer. Hence, it was the only independent predictor of the substitute endpoint of oral cancer emerging from OLP while E-cadherin loss may play roles in different aspects of the pathogenesis of OLP including apoptosis of basal keratinocytes and migration of T-cells into the epithelial compartment. However, further research should be done on finding markers that delimit the patients at risk of OSCC progression. Thus, it is recommended that all OLP patients should be kept under regular observation which can help in early diagnosis and thus treatment.

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Conflicts of interest

There are no conflicts of interest.

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