Published in final edited form as:

Med Hypotheses. 2010 December ; 75(6): 492–494. doi:10.1016/j.mehy.2010.07.002.

Oral lichen planus is a unique disease model for studying chronic inflammation and oral cancer

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Summary

Oral lichen planus (OLP) is a chronic inflammatory disease, which has been defined by the World Health Organization as a potential precancerous condition, representing a generalized state associated with a significantly increased risk of oral cancer. We would like to put forward a hypothesis that inflammatory mediators such as cytokines and chemokines released from infiltrating T lymphocytes induce fundamental changes of proteins in oral epithelial cells, leading to the progression of OLP to oral squamous cell carcinoma (OSCC). These altered proteins can act as the key risk factors associated with the local microenvironment and development of OSCC. Identification of these proteins would add to our understanding of the connection between chronic inflammation and OSCC.

Introduction

The association of inflammation with cancer was first made in the 19th century, on the basis of observations that tumors often arose at sites of chronic inflammation and that inflammatory cells were present in tumor tissues. Since then, numerous epidemiological or molecular biology studies have further highlighted the connections between inflammation and cancer, leading to a general acceptance that inflammation is a strong risk factor for cancer development. For instance, chronic inflammation has been causally associated with the cancer development in inflammatory diseases such as ulcerative colitis, atrophic gastritis and Barrett's esophagus [1-4].

OLP is a T cell-mediated mucocutaneous inflammatory disease of unknown etiology [5-7]. Most T cells in the affected epithelium of OLP and adjacent to the damaged basal keratinocytes are activated CD⁸⁺ lymphocytes, which are known to trigger apoptosis of keratinocytes. Although the World Health Organization (WHO) classifies OLP as a

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Conflict of interest statement: There are no potential conflicts of interest that need to be declared.

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precancerous disease, there is considerable controversy regarding the malignant potential of OLP because a wide range of malignant transformation rates (0-12.5%) was observed in patients with OLP [8, 9]. Some of the controversy can be attributed to the factors such as different clinical criteria used for diagnosis of OLP or various time periods of follow up studies. Nevertheless, most studies indicated that patients with OLP develop oral cancer at an increased rate (~ 10 times higher risk) when compared to the general population [9]. A new meta-analysis reported that, from a total of 11,225 cases of OLP in the last 20 years (1988-2008), 183 (1.63%) developed malignant transformation approximately 6 years after the initial diagnosis. The computed mean rate was 0.27% per year [10].

Proteomic analysis of OLP and associated oral cancer is important to understand the connection between OLP and oral carcinogenesis. The generally accepted statement lies in that both malignant and host cells have been shown to produce cytokine networks and induce DNA damage, cell growth and immortalization, angiogenesis, invasion and metastasis [11]. Although numerous investigations have begun to identify genetic and protein aberrations within OLP, few of them were focused on the progression of OLP to oral cancer [12, 13]. Since only a few percent of the patients with OLP progress to oral squamous cell carcinoma (OSCC), we do not know whether these molecular alterations within OLP have significant role in malignant transformation. A proteomic analysis of OLP and OLP-associated OSCC will help determine the malignant potential of OLP and understand the molecular mechanism of malignant transformation of OLP to OSCC.

Formalin-fixed paraffin-embedded (FFPE) tissue can be used for proteomic analysis of OLP and related OSCC. Although fresh tissue specimens are desirable, the use of fresh tissue has difficulties including collection of sufficient human tissue samples for proposed studies and the need to design the collection of tissues in a prospective manner. In contrast, FFPE tissue blocks are routinely preserved and stored after pathological diagnosis, and therefore represent an extremely valuable resource for cancer proteomics [14]. A major problem of using FFPE specimens for proteomic analysis lies in that formaldehyde-induced intermolecular and intramolecular cross-linking hinders the solubility of proteins and complicates the extraction of intact proteins from the samples [15]. Recently, improved methods for isolating proteins from FFPE tissues have allowed the MS identification of trace amounts of proteins present in complex samples [16-20].

Hypothesis

We hypothesize that inflammatory mediators such as cytokines and chemokines released from infiltrating T cells induce fundamental changes of proteins in oral epithelial cells, leading to the progression of OLP to OSCC. The proteomic alteration in epithelial cells may in turn cause the release of cytokines/chemokines, attracting more T cells into the epithelium. The protein determinants in epithelial cells underlying the progression of OLP to OSCC can be identified through a proteomic analysis of OLP and OLP-associated OSCC based on MS and database searching. Subsequent functional analysis of these proteins in OSCC will lead to potential target proteins that may be used for therapeutic treatment or early cancer detection.

Discussion

Chronic inflammation has been causally associated with various types of cancer [4, 21, 22]. Numerous studies have reported that the inflammatory infiltrate can be a strong risk factor for cancer development in chronic, inflammatory conditions. Similarly, malignant transformation of OLP may be related to, or dependent on, a series of molecular stimuli originating in the inflammatory infiltrate. The integrated signals from various mediators and

cellular effectors of inflammation may determine the sensitivity of oral keratinocytes to exogenous mutagens and may enhance tumor growth and metastasis following cancer formation in OLP. Some proteins and small molecules generated by inflammatory cells may even act as mutagenic agents for epithelial cells and influence important cell cycle regulation mechanisms [1, 23-38].

OLP basal cells suffer from the intense lymphocyte attack and the mutagenic effects, but remarkably few apoptotic phenomena are observed in this cell compartment. There is growing support for the idea that the scarcity of epithelial apoptotic phenomena may be a consequence of stimuli from the inflammatory infiltrate itself. It has been shown that the macrophage migration inhibitory factor and the chemokine RANTES (Regulated on Activation, Normal T Expressed and Secreted) released by the infiltrate can exert anti-apoptotic effects on epithelial cells.

Studies on cell proliferation in OLP have demonstrated a marked increase in the proliferation rate of basal epithelial cells, which may be induced by stimuli from the inflammatory infiltrate. This is very likely an important event in the development of cancer in OLP. Analyses of the expression of the p53 protein and interpretation of its function in OLP have yielded conflicting results. Most authors found that p53 was significantly over-expressed in the affected epithelium of OLP *versus* normal oral mucosa. However, there is no consensus on the mechanism that would explain this over-expression. Some researchers consider that the response of epithelial cells to intense T lymphocyte attack in OLP, with scarce apoptosis and increased cell proliferation, is aimed at preservation of the epithelial structure. They hypothesized that anti-apoptotic and proliferative stimuli are generated by the inflammatory infiltrate as a defense against this possibility, activating the p53-related DNA repair system in a high proportion of cells.

OLP inflammatory cells may contribute to an excess of nitric oxide *via* expression of inducible nitric oxide synthetase, which finally induces the formation of both 8-oxo-7, 8-dihydro-2'-doxyguanosine (8-oxodG) and 8-nitroguanine in the nucleus of epithelial cells. Formation of 8-oxodG is a known cause of G-T transversion, which can promote carcinogenesis. In addition, mutation in OLP may derive from the action of cyclooxygenase-2, which is also produced by inflammatory infiltrating cells.

Although existing molecular studies seem to support the premalignant nature of OLP, the malignant potential of OLP remains controversial, and different research groups have proposed distinct approaches and interpretations [29]. However, as evidenced in other chronic inflammatory disorders associated with cancer development, the role of the inflammatory microenvironment is crucial in initiating and promoting malignant transformation of keratinocytes. In this regard, OLP can serve as a unique disease model for studying the role of chronic inflammation in oral carcinogenesis. A comprehensive analysis of OLP and OLP-associated OSCC at proteome and cytokine levels may elucidate the mechanisms underlying the malignant transformation of OLP, such as proliferation/anti-apoptosis signals or involvement of oxidative DNA damage. OSCCs that are not associated with OLP should also be included in the comparative proteomic study because OLP-related OSCC very likely develops along a different molecular pathway from that of non-OLP OSCC.

Prospect

If the hypothesis pans out to have merit, it would add to our understanding of the connection between chronic inflammation and oral cancer. We expect to find out the cytokines/chemokines in the tissue microenvironment of OLP that are directly associated with oral carcinogenesis and to understand the activated intracellular mechanisms contributing to cell

transformation in the oral epithelium. In addition, this study will confirm the premalignant nature of OLP and help establish a molecular model for malignant transformation of OLP.

Acknowledgments

Sources of support in the form of grants: NIH/NCI R21-CA122806, NIH/NIDCR R03-DE017144, the California - TRDRP disease program, and the University of California Cancer Research Coordinating Committee seed grant (to S.H.).

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