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Oral lichen planus shows higher expressions of tumor suppressor gene products of p53 and p21 compared to oral mucositis. An immunohistochemical study☆

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ARTICLE INFO

Article history:
Accepted 24 March 2010

Keywords:
Tumor suppressor genes
p53
p21
Oral lichen planus
Malignant transformation
Oral mucositis

ABSTRACT

Objective: Oral lichen planus (OLP) has been speculated to be a chronic inflammatory disease with potential for malignant progression. The aim of this study was to establish a hypothesis on the difference between OLP and oral mucositis (OM) in terms of tumor suppressor gene expression.

Design: Computer based image analysis of immunohistochemical expressions of p53 and p21 was investigated in 18 samples of OLP, 10 normal oral epithelium (NOE), 10 oral squamous cell carcinoma (OSCC), 13 OM, 20 oral focal keratosis (OFK), and 30 samples of oral epithelial dysplasia (OED). Representative fields were digitized and analyzed.

Results: Using independent samples Student's t-test, p53 and p21 the mean percentages of positive nuclei (MPPN) of p53 (40.27%) and of p21 (39.98%) in OLP were significantly higher than that of NOE, OFK and OM (15.06%, 27.87%, 30.08% and 16%, 31.09%, 33.92% respectively, $p < 0.001$). MPPN of p53 in OLP was not different from that of mild OED (40.5%, $p = 0.85$) but lower than of moderate and severe OED, and OSCC (49.78%, 61.36%, 78.16% respectively; $p < 0.001$). MPPN of p21 in OLP was lower than that of moderate and severe OED, and OSCC (47.72%, 57.9%, 85.44% respectively; $p < 0.001$) but slightly higher than that of mild OED (39.86% $p = 0.81$).

Conclusions: As the expression of p53 and p21 in OLP was significantly higher than that of oral mucositis with no significant difference from mild epithelial dysplasia, OLP might need to be followed up and monitored more closely to detect early features of transformation, if any, compared to non-specific oral mucositis which needs no close follow-up.

1. Introduction

Oral lichen planus (OLP) is a relatively common chronic inflammatory disorder of the oral mucosa that affects 0.5–2% of the general population.[1]

The malignant transformation potential of OLP has been the seat of considerable debate. Results of some studies showed that 0.5–12.5% of OLP cases could undergo malignant transformation.[2–5] However, other studies failed to prove the malignant transformation potential of OLP.[6,7]

☆ Grant number 23-2007, Deanship of Scientific Research, Jordan University of Science and Technology, Irbid, Jordan.
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doi:10.1016/j.archoralbio.2010.03.019

p53 and p21 proteins are the products of *p53* and *p21* tumor suppressor genes. p53 plays a role in the maintenance of genomic integrity by controlling the cell cycle, DNA repair, and activation of apoptosis.[8] Impaired function of *p53* gene has been implicated in the development and progression of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC).[9] Therefore, detection of p53 changes may help in the identification of higher risk lesions or lesions with malignant transformation potential[10] and in OLP in specific.[11]

p21 inhibits the activity of cyclin-dependent kinase which controls the transition from G1 to S phase during the cell cycle.[12] It has been found that p21 expression increases as oral epithelial dysplastic lesions progressed into OSCC, which implies that p21 expression might be used as a prognostic marker in the development of oral malignancy.[12]

Reviewing the cited literature, no previous studies have compared cell cycle control proteins expression in oral lichen planus with oral mucositis. The aim of the present study was to compare the quantity and pattern of immunohistochemical expression of tumor suppressor gene products of p53 and p21 in OLP with that of oral mucositis and oral epithelial dysplasia. In addition, we aimed at expanding the knowledge on p21 expression in oral lesions as research on this marker in specific is limited.

2. Materials and methods

2.1. Case selection

An inclusive sample of previously diagnosed specimens of OLP was retrieved between the years 1991 and 2007. Only 24 specimens were found. Control groups retrieved randomly from the same archives included oral mucositis (13 cases), oral focal keratosis (20 cases), oral epithelial dysplasia (10 cases of each of mild, moderate and severe grades), normal oral epithelium (10 cases) and OSCC (10 cases). The diagnoses of these specimens were confirmed by an oral pathologist (RS). Tissues from normal oral epithelium, focal keratosis and for the first time oral mucositis were included in the study to control for the effects of inflammation and hyperkeratosis on the expression of these proteins. The study was approved by the JUST Ethics Committee of the Deanship of Scientific Research.

Strict histopathologic diagnostic criteria proposed by Van Der Meij and Van Der Waal[7] were considered while confirming the original diagnoses of OLP specimens. These criteria included: saw-toothed rete ridges, superficial, dense, band like inflammatory infiltrate of lymphocytes, basal cell liquefaction, normal maturation of epithelium, Civatte bodies and hyperkeratosis. Based on these criteria, the number of OLP cases dropped to 18 (12 cases of reticular/plaque-like and 6 of erosive/atrophic form).

The selected cases of OLP included in the study were presented clinically as bilateral buccal mucosal lesions in 14 cases and multifocal oral lesions in four cases. The medical records of patients with OLP in the present study revealed no history of use of medications.

Oral mucositis cases were from specimens diagnosed as focal keratosis with oral mucositis (6/13), fibrous hyperplasia with oral mucositis (5/13), and foreign body reaction with oral mucositis (2/13). Mucositis with candidal infections were excluded from this study after conducting Periodic acid Schiff stain. None of the specimens showed neutrophilic microabscess formation. The area of mucositis selected for analysis exhibited mild to no hyperkeratosis. The inflammatory infiltrate was chronic consisting mainly of lymphocytes and plasma cells which exhibited no characteristic pattern of any specific diagnostic entity. The clinical histories of the patients revealed no medications or radiation.

Cases of normal oral epithelium consisted of reactive fibrous hyperplasias surfaced by apparently normal epithelium. These were re-examined carefully to check for the absence of hyperkeratosis, mucositis, ulceration, or other pathologic changes.

2.2. Immunohistochemical procedure

Ten serial sections of 5 μm thickness were cut from the formalin-fixed, paraffin embedded tissue of each case, using a tissue microtome, and mounted on aminosilane (3-aminopropyltriethoxysilane, Sigma, USA) coated glass microslides. Two sections from each case were stained in separate runs for each of p53 and p21 proteins using monoclonal primary antibodies. Immunohistochemistry was performed by the standard method, using horse radish peroxidase (HRP)–diaminobenzidine (DAB) detection kits (DAB substrate, Biogenex, San Ramon, CA, USA). After deparaffinization and rehydratation of tissue sections, immunoreactivity of the tissue was enhanced by immersion of the sections in antigen retrieval solution (Reveal Decloaker 10×, Biocare Medical, USA) in an autoclave for 7 min, at a temperature of 121 °C and a pressure of 1.5 bar. Sections were allowed to cool down overnight in the antigen retrieval solution. Non-specific stickiness of proteins in the tissue was blocked by non-specific protein block (Protein block, Biogenex, San Ramon, CA, USA), applied for 20 min at room temperature.

p53 protein was detected by incubating the tissue sections with monoclonal antibody Ab-8 for 30 min at room temperature diluted at 1:150 (Antibody diluent, Dako cytomation, Denmark). Ab-8 is a mixture of 2 monoclonal antibodies of different portions of p53 protein (clone DO-7+BP53-12, Lab Vision, UK), which enhances the specificity of detecting p53 protein. p21 protein was detected by incubating tissue sections with a monoclonal antibody $p21^{WAF1}$ Ab3 for 60 min at room temperature (Lab Vision, UK), diluted at 1:40.

The sections were then incubated with biotinylated multilink peroxidase agent (Super sensitive™ link-label immunohistochemical detection system, Biogenex, San Ramon, CA, USA) at room temperature for 20 min. Antigen–antibody reaction was visualized using DAB substrate at room temperature for 10 min (DAB substrate, Biogenex, San Ramon, CA, USA). The sections were then lightly washed in phosphate-buffered saline (PBS), counterstained with hematoxylin for 2 min, washed in water, dehydrated in graded ethanol solutions and cover slipped using Depex (DPX Mountant for histology, Sigma, Germany), according to the standard procedure.

Tissues on the microslides were defined using a wax pen (Biogenex, San Ramon, CA, USA) that enabled better visualization of the tissue and saved the solutions applied on the