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High proliferative activity and chromosomal instability in oral lichen planus

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Abstract. The study aimed to assess the proliferative activity and karyotype in Oral Lichen Planus (OLP) lesions. G-banding chromosomal analysis of short-term primary cultures, and immunohistochemical expression of Ki67 and p53 were applied in 30 consecutive OLP patients divided into two groups according to clinical presentation of the lesions, and in nine subjects as negative controls. Mean values of Ki67 and p53 expression differed significantly ($P < .01$) between controls and patients groups with reticular or atrophic-erosive forms of OLP, whereas there was no significant difference between the two groups of patients with reticular or atrophic-erosive lesions. Six OLP patients showed clonal chromosome alterations, four of them associated with p53 overexpression.

In conclusion, OLP is characterized by a high cellular turnover in most patients irrespective of clinical disease presentation. The genetic instability found in some patients should be interpreted as a consequence of the enhanced epithelial turnover, although we cannot rule out the possibility that some of the cytogenetic non-random anomalies observed represent early steps in cancer development.

Key words: oral lichen planus; keratinocytes; Ki67; p53; cytogenetic.

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Oral lichen planus (OLP) is a chronic inflammatory autoimmune disease involving T lymphocytes with cytotoxic activity against the epithelial cells affecting skin and mucosa in a prevalence estimated at 1–2% in the population[17].

In addition to leukoplakic lesions where dysplastic changes are evident which are the ones with potential malignant change, WHO has included OLP among the conditions at risk of malignant transformation[6] following many clinical investigations that showed that oral cancer had developed in lesions previously diagnosed as OLP[23]. Whether OLP itself can progress to oral cancer is still the subject of controversy. In addition, the frequency of malignant transformation varies between 0.4 to 5% over periods of observation from 6 months to 20 years[13,17].

This controversy probably results from the different criteria applied for the diagnosis of OLP in the different published studies. Hence, it is possible that lesions with impaired primary epithelial maturation, often presenting dysplasia, associated with an immune reaction were frequently misdiagnosed as OLP[5].

On the contrary, some studies have clearly documented that oral cancer can develop in patients with classic OLP[9].

During the last 20 years many studies have shed more light on the genetic basis of tumourigenesis in oral squamous cell carcinoma. It is now widely accepted that oral squamous cell carcinoma is characterized by a chromosomal instability consisting in multiple numeric and structural alterations, and that chromosomal

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aberrations successively accumulate with tumour progression[1,4].

The importance of genetic alterations in potentially malignant oral lesions such as leukoplakias has been emphasized, and the presence of gross genomic aberrations and other chromosomal anomalies has been shown to be discriminatory for progression to oral squamous cell cancer[15,3,20,24].

Few cytogenetic studies have been conducted on OLP to date. Two investigated specific chromosome alterations, and another aimed to detect gross genomic damage[7,14,25]. In 1997, ZHANG et al.[25] showed that a loss of heterozygosity (LOH) on chromosomes arms 3p, 9p and 17p is found in 6% of OLP patients. In addition, chromosome 9 monosomy was demonstrated to play a critical role in progress to malignancy in two cases of lichenoid dysplasia[14]. More recently, FEMIANO et al.[7] found 5% DNA aneuploidy in patients with OLP. All these data suggest that OLP might present genomic instability.

To obtain more cytogenetic information on oral mucosa from OLP patients, we applied a classic G-banding chromosome analysis to short-term primary cultures. This analysis provided useful information on structural and numerical karyotype alterations, and has been successfully applied to the study of oral squamous cell carcinoma[2,21].

The present study aimed to evaluate the presence of chromosomal aberrations in OLP patients and investigate a possible correlation with clinical presentation and degree of epithelial proliferation activity assessed by immunohistochemical expression of Ki67 and p53.

Material and methods

Consecutive patients with oral lesions clinically and histologically consistent with classic OLP were enrolled. In all cases the diagnosis met currently accepted criteria.

Clinical criteria included the following:

- presence of bilateral lesions and
- presence of reticular lesions elsewhere in the oral mucosa.

Histopathological criteria included the following:

- presence of well-defined bandlike zones of inflammatory infiltration confined to the superficial part of the connective tissue, consisting mainly of mature lymphocytes,
- vacuolar alteration of the basal layer of the epithelium and
- absence of epithelial dysplasia.

History-taking failed to disclose smoking, irradiation or alcohol abuse. All patients consented to the research, approved by the Local Ethics Committee, and underwent haematological and immunological analysis to rule out any identifiable causes such as a hypersensitivity to dental restorative materials or drugs.

The patients were divided into two groups according to the clinical presentation of the oral lesions, and compared with a third group of nine healthy controls from whom a specimen of oral mucosa was obtained during a dental extraction.

Fresh biopsies from all subjects were divided into two specimens: one was formalin fixed for histological and immunohistochemical analysis, while the other was submitted for cytogenetic investigation.

Immunohistochemistry

Immunostaining was performed on 2-μm thick sections serially cut from the selected blocks and placed for 30 min at 60 °C. The following antibodies were employed: monoclonal anti-Ki67 (Dako, Denmark, clone MIB-1, diluted 1:200) and monoclonal anti-p53 (Dako, clone p53, diluted 1:50). The processing was performed in an automatic stainer (Autostainer, Ventana, USA). Counting the percentage of positive nuclei in 400 consecutive epithelial cells of selected areas representative of the lesion gave a semi-quantitative evaluation of the immunohistochemical results[8]. In addition the site of positive cells (basal-parabasal, intermediate and superficial) was also evaluated.

Cytogenetic analysis

Cell suspensions, obtained after enzymatic digestion with collagenase (2000 U/ml; GIBCO, Invitrogen Corporation) at 37 °C for 16–18 h, were seeded and cultured for 3 to 10 days in DMEM-F12 (GIBCO, Milan, Italy) supplemented with 10% foetal bovine serum (Hy-Clone Laboratories, Celbio, Milan, Italy) and antibiotics/fungizone. Short-term cultures were processed to minimize fibroblast contamination[12], following the method currently applied for cultures on solid tumours[2]. Cytogenetic analysis was carried out according to standard procedures on *in situ* G-banding metaphases taken from primary short-term cultured cells. After culture and overnight incubation with Colcemid (0.03 μg/mL), the cells were harvested using hypotonic solution with 0.8% sodium citrate preheated to 37 °C at room temperature for 35 min. The metaphases were fixed four times with methanol and glacial acetic acid (3:1). Chromosomes were G-banded using HCL and Wright stain. Twenty G-banded metaphases, when present in sufficient number, were evaluated on three slides to exclude a clonal growth of the same cell. The karyotypes were described according to the ISCN 1995 Guidelines for Cancer.

Only Clonal Alterations (CA) were considered, defined as follows: gains of chromosomes (trisomy) and structural alterations when present in a minimum of two metaphases and/or in composite karyotypes (cp); monosomy when present in a minimum of three metaphases and/or in composite karyotypes (cp).

Statistical analysis

Since the standardized Skewness and Kurtosis values were within the range expected for data from a normal distribution, one-way ANOVA was used to compare the mean values of Ki67 and p53 between the different groups of subjects. Tukey's honestly significant difference (HSD) procedure was used to discriminate among the means.

A cross-tabulation was also performed to construct a two-way table showing the frequency of occurrence of unique pairs of values for Ki67 or p53 and clinical aspects of oral lesions; the Chi square test was used to determine whether the two variables in the cross-tabulation were independent of each other.

A linear regression was applied to evaluate the presence of a relationship between Ki67 and p53 values.

Results

Cytogenetic analysis was informative in 30 OLP patients and in nine controls.

The OLP patients included 13 men aged 35 to 71 years (mean 51.8 ± 11.7), and 17 women aged 33 to 75 years (mean 55.1 ± 10.5). Twenty patients (10 men) aged 33 to 75 years (mean 53.6 ± 11.6) had exclusively reticular lesions (white lesions) characterized by a lacelike network of slightly raised grey-white lines, whereas ten patients (3 men) aged 35 to 71 years (mean 53.9 ± 10.4) had mainly atrophic-erosive lesions (red lesions) with associated reticular lesions.

The control group included 9 subjects (5 women) aged 32 to 71 years (mean 53 ± 12.5).