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High Frequency of Allelic Loss in Dysplastic Lichenoid Lesions

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SUMMARY: Oral lichen planus (OLP) is a common mucosal condition that is considered premalignant by some, whereas others argue that only lichenoid lesions with epithelial dysplasia are at risk of progressing into oral carcinoma. A recent study from this laboratory used microsatellite analysis to evaluate OLP for loss of heterozygosity (LOH) at loci on three chromosomal arms (3p, 9p, and 17p) (Am J Path 1997;Vol151:Page323-Page327). Loss on these arms is a common event in oral epithelial dysplasia and has been associated with risk of progression of oral leukoplakia to cancer. The data showed that, although dysplastic epithelium demonstrated a high frequency of LOH (40% for mild dysplasia), a significantly lower frequency of LOH was noted in OLP (6%), which is even lower than that in hyperplasia (14%). Such results do not support OLP as a lesion at risk for malignant transformation. As a second step of the research, we determined LOH frequencies in 61 dysplastic lichenoid lesions (mild 35; moderate 19; severe 7) using the same microsatellite markers and compared these results with data obtained from the first study and from 13 normal mucosal specimens. Dysplastic lichenoid lesions showed a high frequency of loss (54% for lichenoid lesions with mild dysplasia), but values did not differ significantly from those observed in dysplasia of similar degree without lichenoid appearance. None of the normal mucosa demonstrated LOH. Epithelial dysplasia is a sign of malignant risk, independent of lichenoid changes. Such results suggest that pathologists should search for dysplasia carefully in lesions that otherwise qualify as OLP and that caution should be used when discounting dysplasia as being merely a reactive condition in lichenoid lesions. (*Lab Invest 2000, 80:233–237*).

Oral lichen planus (OLP) is one of the most common oral mucosa diseases, occurring in approximately 1% of the general population (Pindborg et al, 1972; Scully and el-Kom, 1985). Unlike cutaneous lichen planus, OLP tends to be chronic. It has been suggested that complete remission of OLP is either nonexistent or infrequent (see review in Eisen 1993). Histologically, OLP is characterized by a dense band-like lymphohistiocytic infiltrate in the immediate subepithelial region, with basal epithelial cell destruction.

There have been heated debates as to whether OLP per se is precancerous or whether only OLP-like lesions demonstrating epithelial dysplasia are potentially at risk of developing into cancer (Eisenberg and Krutchkoff, 1992; Holmstrup 1992; Krutchkoff and Eisenberg, 1985; Lovas et al, 1989). After retrospective analysis of original photomicrographs from a number of published cases of alleged malignant transformation of OLP, Krutchkoff and Eisenberg concluded that many reported cases of oral carcinomas arising from OLP may have developed from lichenoid lesions with epithelial dysplasia (Eisenberg and Krutchkoff, 1992). As a result, the authors recommended that more strict criteria be applied in the diagnosis of OLP and that dysplastic lesions not be called OLP.

Are many lichenoid lesions with epithelial dysplasia called OLP? Data suggest that this may be the case. Dysplasia was reported in 11% of OLP ($n = 100$) by Urbizo-Velez et al (1990), and 25% ($n = 100$) by De Jone et al (1984).

The presence and degree of epithelial dysplasia has been the histologic hallmark or gold standard for judging the malignant potential of preinvasive lesions. Why has it been widely ignored in these lichenoid lesions? There are two obvious reasons. First, the biopsies were not carefully examined for the presence of dysplasia because of the striking lichenoid features. An alternative explanation is that dysplasia was recognized but discounted as being due to changes in response to lymphohistiocytic infiltrate.

Inflammation is known to cause atypical epithelial changes resembling dysplasia and the atypia is believed to be reactive and not regarded as an indication

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of malignant potential. Such reactive changes are usually seen in the case of intense acute inflammation or mixed acute and chronic inflammation, typically near an ulcer. It is less clear whether such reactive changes readily occur in specific lichenoid dermatoses such as lichen planus and discoid lupus. Naturally, there is uncertainty about the significance of dysplasia when it is present in a lesion with striking lichenoid features that otherwise qualifies for the diagnosis of OLP. As a result, a lichenoid lesion with epithelial dysplasia may be signed out as dysplasia by one pathologist (who believes dysplasia is dysplasia regardless of lichenoid infiltrate) or as OLP by another pathologist (who overlooks the dysplasia or discounts the dysplasia as an inflammatory response).

Two important questions arise from these debates and disagreements: (a) Is OLP without dysplasia premalignant? (b) Is dysplasia still a sign of malignant potential in a lesion with striking lichenoid features? One way to answer these questions is to determine whether the genetic changes that commonly occur in early oral carcinogenesis (oral dysplasia) are found in OLP and dysplastic lichenoid lesions.

To answer the first question, a recent paper from this laboratory studied the molecular changes in OLP by microsatellite analysis (Zhang et al, 1997; editorial on the study in Allen, 1998). We chose this technique because of its sensitivity. Oral premalignant lesions are generally small with minute amounts of DNA. Microsatellite analysis requires only 5 ng of DNA per reaction, but yields valuable data on a biologically significant event, the loss of chromosomal regions (loss of heterozygosity, LOH) that contain putative tumor suppressor genes (Rosin et al, in press; Califano et al, 1996). The study compared LOH at 3p, 9p, and 17p in OLP (without dysplasia) with LOH in oral epithelial dysplasia and benign hyperplasia. Although dysplastic epithelium demonstrated a high frequency of LOH (40% for mild dysplasia), a significantly lower frequency of LOH was noted in OLP (6%), which is even lower than that in hyperplasia (14%). Such results seem to indicate that OLP (without dysplasia) has no apparent malignant risk.

As a second step of the investigation, this study tested the hypothesis that epithelial dysplasia is a sign of malignant risk even when the lesion demonstrates striking lichenoid features. LOH at 3p, 9p, and 17p was analyzed in dysplastic lichenoid lesions. The results were compared with data obtained from the first study and from 13 normal mucosal specimens.

Results

Table 1 presents the mean age and smoking habits of individuals in the two study groups, together with similar data for the OLP and dysplasia cases from our previous study. A significantly higher number of smokers were found in patients with dysplasia (87.5%) and dysplastic lichenoid lesions (77%) compared with those patients with OLP (25%) and normal epithelium (25%) (p value varies from $p = 0.0018$ to $p < 0.0001$).

Table 2 presents LOH frequencies for the 61 dysplastic lichenoid lesions and the concurrently run samples of normal oral epithelium ($n = 13$). The data are presented as the percentage of cases in each group with LOH for loci on 3p, 9p, or 17p. In addition, a determination was made of the percentage of cases showing any allelic loss (3p, 9p, or 17p), or with loss on more than one of these arms. Figure 1 represents a typical LOH analysis.

None of the samples with normal oral epithelium showed LOH. In contrast, a significant percentage of dysplastic lichenoid lesions demonstrated such loss (Table 2). LOH frequencies tended to increase with the severity of dysplasia in the lesions. High-grade dysplastic lichenoid lesions (those with severe dysplasia or carcinoma in situ, CIS) contained higher LOH frequencies than low-grade dysplastic lichenoid lesions (those with mild and moderate dysplasia) (χ^2 test for trend, $p = 0.0489$).

Of the 61 patients with dysplastic lichenoid lesions, seven patients had biopsies from lesions from two geographic locations in the oral cavity. All of these biopsies showed intense submucosal lymphocytic infiltrate. Six of seven patients had dysplasia in both biopsied lichenoid lesions and one patient had one dysplastic lichenoid lesion and one OLP. Information on smoking habits was available for five of the seven patients; of these five patients, four were smokers.

Discussion

Although OLP is considered a premalignant lesion by the World Health Organization (see review in Holmstrup, 1992), it is also argued that OLP per se is not precancerous and that only OLP-like lesions demonstrating dysplasia are potentially at risk of developing into cancer (Eisenberg and Krutchkoff, 1992; Krutchkoff & Eisenberg, 1985; Lovas et al, 1989). A recent study from this laboratory has shown that OLP (without dysplasia) lacked the characteristic genetic alter-

Table 1. Smoking Habit and Age of Patients with Oral Lesions

Diagnosis	Number of cases	Mean age (years)	Number of patients with known habit (%)	
			Smoker (present or past)	Non-smoker
Oral licheu planus	33	47	8 (25%)	24 (75%)
Dysplasia	37	60	21 (87.5%)	3 (12.5%)
Dysplastic lichenoid lesion	61	53	30 (77%)	9 (23%)
Normal	13	42	3 (25%)	9 (75%)

Table 2. Loss of Heterozygosity in Normal Epithelium and Lichenoid Lesions with Dysplasia[a]

Diagnosis	Number of cases	Total allelic loss/informative cases (%)				
		3p	9p	17p	Any loss	>1 loss
Normal	13	0	0	0	0	0
Lichenoid lesions with mild dysplasia	35	9/33 (27)	14/35 (40)	4/33 (12)	19/35 (54)	6/33 (18)
Lichenoid lesions with moderate dysplasia	19	5/17 (29)	6/18 (33)	5/19 (26)	10/19 (53)	5/19 (26)
Lichenoid lesions with severe dysplasia or CIS	7	3/7 (43)	6/7 (86)	3/7 (43)	6/7 (86)	5/7 (71)

[a] Values given as number of samples showing loss/total number of informative cases (% of cases in parentheses).
CIS, carcinoma in situ.

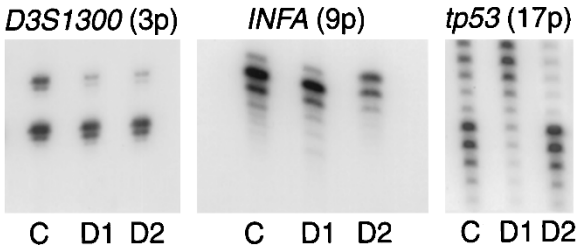

Figure 1.
Microsatellite analysis of 2 mildly dysplastic lichenoid lesions (*D1* and *D2*) identified in concurrent biopsies from different oral sites in one patient. DNA was isolated from cells microdissected from the stroma (*C*), as a source of normal DNA, and from each dysplastic lichenoid lesion. Microsatellite markers and the chromosomal arm being assayed are indicated above each block. Both lesions (*D1* and *D2*) showed loss of the upper allele at *D3S1300* on 3p. In contrast, biopsy D2 showed a loss of the lower allele at *INFA* on 9p and biopsy *D1* showed retention of both alleles at this locus. Furthermore, one of the dysplastic lichenoid lesions (*D1*) showed loss of the lower allele at *TP53* on 17p, whereas the other (*D2*) had a loss of the upper allele.

ations (loss of tumor suppressor genes) commonly seen in oral premalignant lesions (Zhang et al, 1997). In contrast to these results, the present study has shown that dysplastic lichenoid lesions contain a high frequency of LOH, with values that did not differ significantly from those observed among dysplastic lesions of similar degree without lichenoid appearance: LOH at any arm was noted in 54% mildly dysplastic lichenoid lesions compared with 40% of mild dysplasia ($p = 0.4908$); 53% moderately dysplastic lichenoid lesions compared with 46% of moderate dysplasia ($p = 1$); and 86% of lichenoid lesions with severe dysplasia or CIS compared with 81% in severe dysplasia/CIS ($p = 1$) (Zhang et al, 1997). Similar to dysplasia (without lichenoid mucosites), dysplastic lichenoid lesions showed a significantly higher frequency of LOH when compared with hyperplastic lesions, even when the comparison was made between mildly dysplastic lichenoid lesions and hyperplasias (any LOH: 54% versus 14%, $p = 0.0014$; > 1 loss: 18% versus 0%, $p = 0.0257$) (Table 2; Zhang et al, 1997).

Such high frequencies of loss in chromosome regions containing presumptive tumor suppressor genes would suggest that the presence of epithelial dysplasia is a sign of malignant risk, independent of lichenoid changes. The results suggest that atypical epithelial changes in the presence of lichenoid mucosites is more likely to represent true dysplastic changes and should not be discounted as reactive change, which is more commonly seen in the case of intense acute or mixed acute and chronic inflammation typically near an ulcer. In view of the fact that there is some tendency for pathologists to miss dysplasia in a lesion otherwise qualifying for the diagnosis of OLP, or to discount dysplasia as reactive in such lesions, we would echo the caution from Krutchkoff and Eisenberg that more strict criteria should be used in diagnosing OLP and more attention should be paid to examining lichenoid lesions for dysplasia (Eisenberg and Krutchkoff, 1992).

Because dysplastic lichenoid lesions resemble both OLP and dysplasia, one would question whether there is any relationship between OLP and dysplasia. There may be two scenarios. In many cases, the dysplastic lichenoid lesion may represent a dysplasia de novo and the lesion may have no relation to OLP. This is supported by the fact that oral epithelial dysplasia with varying degrees of submucosal lymphocytic infiltrate is a common phenomenon, although a striking lichenoid infiltrate suggestive of OLP (cases used in this study) is less frequent. Such clinically white lesions are more likely leukoplakias rather than OLP, especially when they present as single plaque-like lesions.

On the other hand, a relationship may indeed exist between OLP and dysplasia in some cases and the dysplastic lichenoid lesions may actually represent OLP that have undergone dysplastic changes. In the previous study, we have shown that one patient (a smoker) with both OLP and a dysplastic lichenoid lesion demonstrated LOH only in the dysplastic lichenoid lesion. We hypothesized that this patient originally had only OLP (multiple lesions at different sites), and subsequently, one lesion, possibly due to tobacco exposure, underwent dysplastic changes and LOH. In this study of 61 patients with dysplastic lichenoid lesions, 7 had multiple biopsies available for histology and LOH analysis. The presence of multiple white lesions clinically, the presence of dense submucosal lymphohistiocytic infiltrates in these multiple lesions histologically, and the presence of OLP in one of the patients would suggest an OLP process. The majority (4 of 5) of these patients with known habits were smokers. Again one could hypothesize that these dysplastic lichenoid lesions may have been originally OLP which, under the influence of oral carcinogens, underwent dysplastic changes. Of interest, in 5 of the 7 cases that had multiple biopsies, a different pattern of loss was observed in the different lesion sites (see

Fig. 1, for example). The presence of independent mutations at separate lichenoid regions suggests these inflamed sites may be susceptible to genetic changes. However, the only convincing way of proving that OLP develop into dysplastic lichenoid lesions would be to undertake prospective studies and to demonstrate that clinically and histologically confirmed OLP cases become dysplastic. Similarly the only convincing way of proving that lichenoid lesions in a smoker have a higher chance of malignant transformation than the rest of similarly smoking-exposed oral mucosa would again be through prospective studies.

In summary, the results of the study suggest that lichenoid lesions should be carefully examined for the presence of dysplastic changes, because such alterations often contain genetic changes associated with malignant risk. Furthermore, the presence of epithelial dysplastic changes in lichenoid lesions should not be readily discounted as reactive, as in the case of intense acute inflammation and ulceration. Finally, the study results suggest that patients with OLP should all be under periodic observation by qualified personnel, especially if they are smokers, to ensure that clinical and histologic evidence of premalignant and malignant changes are detected.

Materials and Methods

Tissues and DNA Extraction

Most of the specimens were selected from archival paraffin blocks obtained from the Division of Oral Pathology at Vancouver Hospital and Health Sciences Centre, Vancouver, Canada. Some specimens came from oral pathology biopsy services in other North American Institutes. Two groups of lesions were used: dysplastic lichenoid lesions and specimens with normal oral epithelium. The first group consisted of lesions with epithelial dysplasia as well as striking lichenoid features. The criteria used for the diagnosis of dysplastic lichenoid lesions were those described by Krutchkoff and Eisenberg (1985). The latter group consisted of biopsies with unremarkable histology, amalgam tattoo, melanotic macules, and vascular lesions (varicose vein and hemangioma). Histologic diagnoses of the specimens were reconfirmed by two of the authors (LZ and RP), both oral pathologists.

All lesions were microdissected by LZ. Connective tissue from each specimen was submitted as a source of normal DNA. The dissected tissues were placed in sodium dodecyl sulfate/proteinase K at 48° C and spiked twice a day for 72 hours with fresh proteinase K. Genomic DNA was extracted with phenol-chloroform and precipitated with ethanol as previously described (Zhang et al, 1997).

LOH Assay

DNA was analyzed for LOH by using microsatellite markers (Research Genetics, Huntsville, Alabama) that mapped to the following regions: 3p14.2 (*D3S1234*, *D3S1300*), 3p25.3–25.1 (*D3S1110*), 9p21 (*IFNA*, *D9S171*, *D9S1751*, *D9S1748*), 17p13.1 (*TP53*), and 17p11.1–12 (*CHRNB1*). These markers are localized in regions previously shown to be frequently lost in head and neck tumors. The 9p21 locus has been linked to a putative tumor suppressor $p16^{INK4A}$, a gene that codes for a cyclin-dependent kinase inhibitor involved in regulation of the cell cycle (Kamb et al, 1994). Three regions of loss have been identified for 3p (3p14, 3p21.3, and 3p24) (Maestro et al, 1993). We chose to focus primarily on the 3p14 locus because LOH at this region has not only been reported high in head and neck squamous cell carcinoma (SCC) (Mao et al, 1996a; 1996b; Virgilio et al, 1996), but also has been recently shown to be associated with the risk of progression of oral premalignant lesions to SCC (Mao et al, 1996a; 1996b). We did, however, include one marker in the more telomeric region of loss for comparison (*D3S1110*). Finally, the study included a marker located within the p53 gene (*TP53*), as well as one at 17p11.1-p12 (*CHRNB1*) because this locus is sometimes lost in the absence of LOH at TP53 (Adamson et al, 1994). Although the putative suppressor gene in this region is believed to be p53, there is some suggestion that a second tumor suppressor gene could be present in this region (Adamson et al, 1994).

The protocol used for LOH analysis is described in Zhang et al (1997). After PCR amplification, PCR products were separated on 7% urea-formamide-polyacrylamide gels and visualized by autoradiography. Samples were coded and LOH was scored without knowledge of diagnosis. For informative cases, allelic loss was inferred when the signal intensity of one allele was at least 50% decreased in the DNA sample from a lesion, compared with the corresponding allele in the matching connective tissue DNA. All samples showing allelic loss were subjected to repeat analysis after a second independent amplification.

Statistical Analysis

Groups were compared with the Fisher exact test (two-tailed) and χ^2 test for trend. All p values were two-sided. A p value of 0.05 or less was considered significant.

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