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ARTICLES

p53 Expression in the Carcinogenesis in the Oral Mucosa

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Abstract Hyperplastic lesions of the oral mucosa such as leukoplakia and oral lichen planus can eventually develop into squamous cell carcinomas. In the clinical treatment of these lesions it would be very important to be able to predict the biological behaviour of an individual lesion. In 64 hyperplastic lesions and 85 squamous cell carcinomas of the oral mucosa, the expression of the mutant tumor suppressor gene p53 was investigated. A positive correlation was seen between the expression of the mutant tumor suppressor gene p53 and the grade of dysplasia of the lesions.

Key words: carcinogenesis, oral mucosa, p53, hyperplastic lesions, squamous cell carcinomas

For benign, hyperplastic lesions of the oral mucosa lesions with an increased risk of malignant development, the term *preneoplastic lesions* is widely used [1]. Examples of such lesions are oral leukoplakia and oral lichen planus. In up to 43%, oral leukoplakia develops into squamous cell carcinoma [2–5]. In oral lichen planus the frequency of carcinomatous changes varies between 1% and 10% [6–9]. It is impossible, though, to assess in an individual patient the biological behaviour of the premalignant lesion. Histological evaluation of the lesions for presence or absence of dysplasia still seems to be the most reliable indicator for future carcinomatous development [5]. It is therefore necessary to understand the molecular changes in the carcinogenesis of the oral mucosa.

Oncogenes and tumor suppressor genes are known to play an important role in the development of the malignant phenotype. Mutations in the p53 tumor suppressor gene are the most common genetic alterations in human cancer [10,11]. Increased levels of p53 protein are often found in malignant tumors but rarely in benign tumors and normal tissue [12,13]. The purpose of this study was to determine whether p53 plays a role in the carcinogenesis of the oral mucosa.

MATERIALS AND METHODS

Eighty-five paraffin embedded tissue sections of squamous cell carcinomas (SCC) of the oral mucosa (58 primary SCC and 27 recurrences) and 64 tissue sections of hyperplastic lesions (leukoplakia and lichen planus) were immunohistochemically stained with a monoclonal antibody for the presence of p53 (MAb Do 7, DaKo). The antibody recognizes an epitope between residues 35 and 45 of wild type and mutant p53.

Cell lines from SCC of the oropharnyx (SCC 4, 9, 15, 25; ATCC, Rockville, MD) were grown in tissue culture and after fixing with formaldehyde and embedding in paraffin were also immunohistochemically stained for the presence of p53 (MAb Do 7, DaKo, Capenteria, CA).

Immunohistochemistry was performed using the APAAP technique. The tissue section and embedded cells were first dewaxed with xylene (30 min), dehydrated with ethanol, and rehydrated gradually with ethanol and water. The cells and tissue sections were then incubated with TBS (pH 7.4) in the microwave (650 W) twice for 5 min to resolve the protein fixation. The TBS-treated sections were then incubated with goat serum (1:10, DaKo X 907) for 10 min, and then incubation with the p53 antibody (MAb Do 7, DaKo, 1:25) for 12 h at 4°C followed. After washing twice with TBS, the sections were incu-

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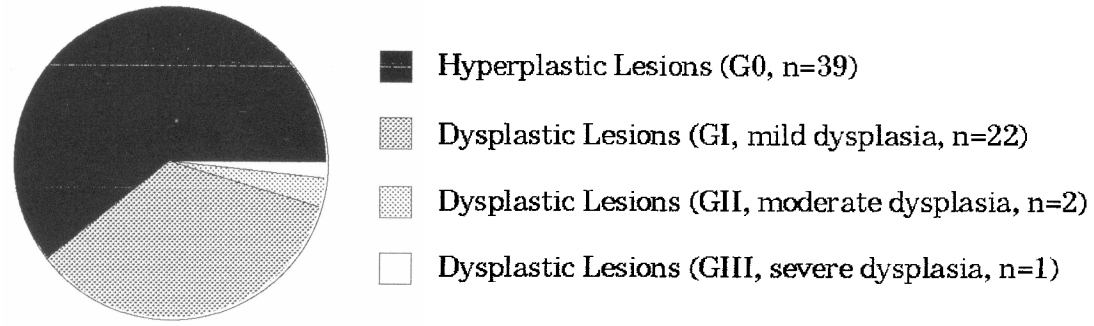

Fig. 1. Histological dysplasia grade in preneoplastic lesions in the oral mucosa (n = 64).

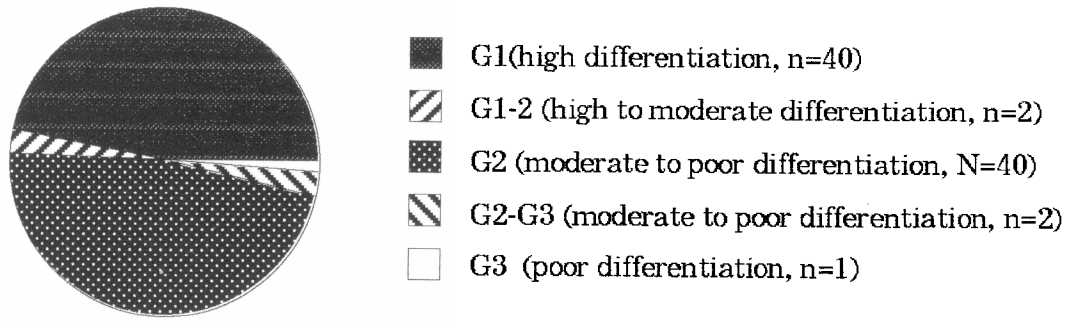

Fig. 2. Histological differentiation grade of SCC and recurrent SCC of the oral mucosa (n = 85).

bated with pig serum (1:20, DaKo X 901) for 10 min followed by incubation with the bridging antibody (DaKo Z 259) for 60 min. After further washing with TBS the sections were incubated with goat serum (1:10, 10 min) and the APAAP complex (1:50, 60 min, DaKo D 651). The steps starting with the pig serum, incubation with the bridging antibody, washing, and incubation with goat serum and the APAAP complex were repeated once. After further washing with TBS, the reaction product was stained with Fast Red (Sigma F-1500) and counterstained with hematoxilin. Levamisole (1 mM) was added to block endogenous alkaline phosphatase activity. All incubations were carried out at room temperature if not indicated otherwise. Each set of experiments included positive (mammary carcinoma) and negative controls (normal mucosa).

The hyperplastic lesions (n = 64) were classified according to the grade of dyplasia they showed (Fig. 1). For the classification of dysplasia, the nomenclature of the CIN (cervical intraepithelial neoplasia) classification was used (GI–GIII as compared to CIN I–CIN III—that is, mild, moderate, and severe dysplasia) [14]. The squamous cell carcinomas were also classified according to the loss of differentiation the lesions showed (UICC classification; G1–G3 for differentiated, moderately differentiated, and poorly differentiated SCC) (Fig. 2). Tissue samples and cells lines were counted as positive for p53 expression when a single positive cell of presumably epithelial origin could be detected in the specimen. In the p53 positive tissue samples, cell counts of the positive cells in ten different areas of the slide, in defined size and standarized location, were performed to quantify the p53 expression.

RESULTS

As in mutant p53 the protein is stabilized and the half-life is extended, it becomes detectable by immunohistogical staining [10]. Detection of p53 by immunohistology can therefore either be caused by stabilization of the protein due to the presence of mutation or result from promotion-driven mechanisms that lead to increased p53 at the steady state in a cell cycle checkpoint response mechanism [11].

Of 64 tissue samples of hyperplastic lesions of the oral mucosa (leukoplakia and lichen planus), 39 samples (61%) did not show any dyplasia (G0) (Figs. 1, 4). Twenty-two samples (34%) showed a low grade dysplasia (GI), and 2 samples (3%) showed moderate dysplasia (GII) (Fig. 5). One tissue sample was classified as severe dyspla-

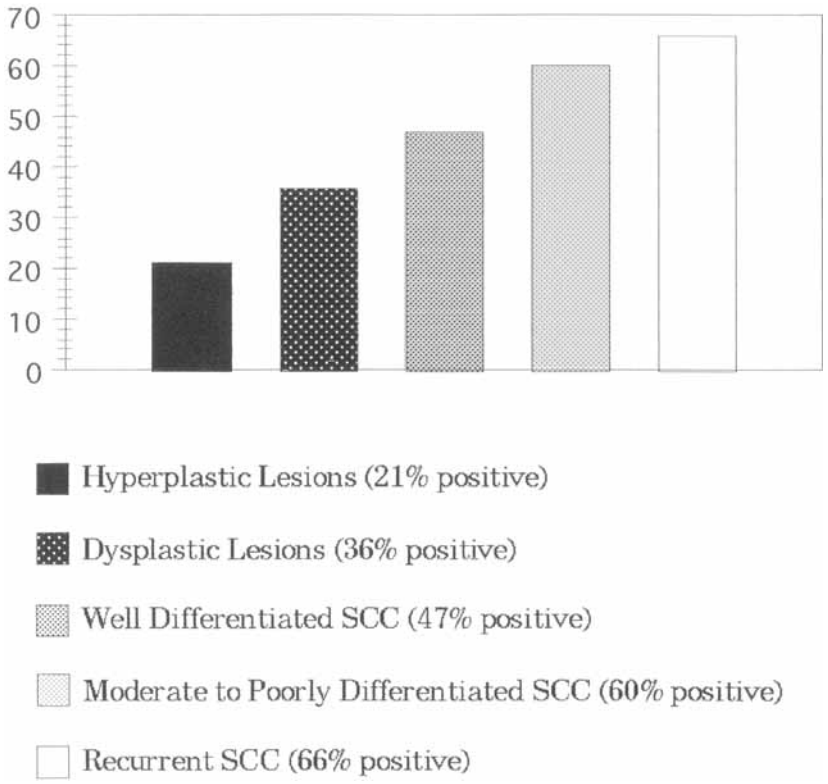

Fig. 3. Positive p53 expression in the lesions in the oral mucosa (in percent).

sia (GIII) [13]. The SCC of the oral mucosa (n = 85) were classified according to the loss of differentiation (G1–G3) (Fig. 2). Forty samples (47%) showed high differentiation (Fig. 2). Two tumors were highly to moderately differentiated. Another 40 tissue samples (47%) were classified as moderately differentiated. Two tumors showed a moderate to low differentiation (Fig. 6). Only one tumor showed a low differentiation. In the group of hyperplastic lesions without dysplasia (n = 39) 31 lesions (79%) did not show any p53 expression. A detectable level of p53 could only be found in 8 (21%) of the G0 samples (Fig. 3). In the hyperplastic lesions with dysplasia (GI–III) (n = 25) nine samples (36%) showed p53 expression at a detectable level (Fig. 5). Notably, the only GIII lesion was positive for p53, but only one of 2 GII lesions showed p53 expression (Fig. 3).

Forty-six (54%) of all SCC showed a detectable level of p53 expression. In the GI group (high differentiation) (n = 40), 19 tumors (47%) were positive. Among the SCC that showed only moderate to low differentiation (n = 45), 27 tumors (60%) were positive for p53.

In 27 recurrent SCC of the oral mucosa, 18 tissue samples (66%) showed a detectable level

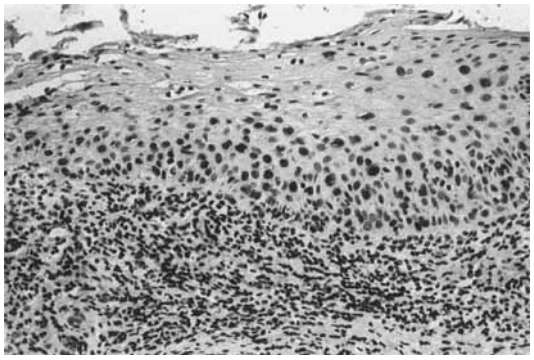

Fig. 4. p53 expression in hyperplasia of the oral mucosa without dysplasia (lichen planus). ×375.

of p53 by immunohistology. The majority of the recurrent SCC showed a moderate to poor level of differentiation (G1: n = 5; G2–3: n = 13). In four SCC cell lines (SCC 4, 9, 15, 25) a detectable level of p53 was only found in SCC 4. None of the other cell lines investigated showed a single p53 positive cell.

The quantification of p53 expression in the positive tissue samples did not show any correlation with histological parameters such as dysplasia and loss of differentiation. p53 positive cells

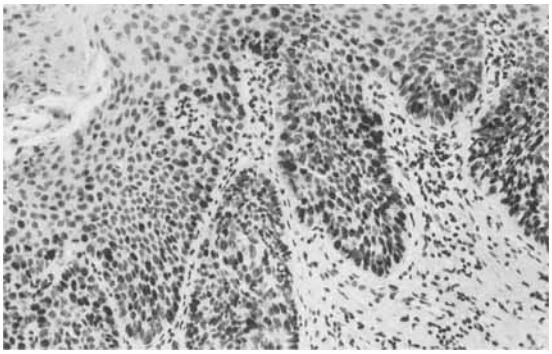

Fig. 5. p53 expression in hyperplasia of the oral mucosa with dysplasia (leukoplakia). ×375.

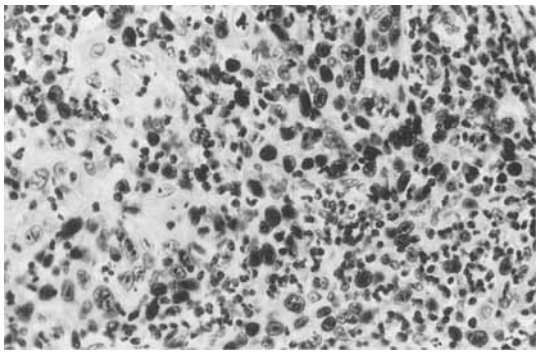

Fig. 6. p53 expression in SCC of the oral mucosa (G2–3). ×600.

were commonly found in the basal cell layer (Figs. 4, 5).

DISCUSSION

Clinically it is well known that oral leukoplakia and lichen planus can develop into squamous cell carcinoma [1–9]. In their retrospective study of 200 patients with oral leukoplakia and a clinical follow up of more than 5 years, Maerker and Burkhardt found a correlation between the development of carcinomas and the grade of dysplasia of the primary lesions [15,16]. Other investigators found that the grade of dysplasia seems to be unreliable as the only diagnostic parameter in predicting cancer development [5]. This illustrates the need for further investigations concerning parameters that could help predict the development of cancer in oral leukoplakia and lichen planus.

p53 mutation is a common genetic change in human oral cancer. Most cell lines of SCC of the oropharnx show mutations of the p53 tumor suppression gene [13,17,18]. Using different monoclonal antibodies, Langdon and Partridge found detectable levels of p53 in up to 80% of 15 SCC lesions they studied [12], whereas no detectable level of p53 could be found in normal mucosa. Thus p53 mutation seemed to be an interesting gene to investigate in carcinogenesis in the oral mucosa.

Sixty-four hyperplastic lesions of the oral mucosa were stained with p53 MAb. Seventeen of the lesions showed detectable levels of p53, which is assumed to be synonymous with the presence of p53 mutation. Interestingly, the grade of dysplasia of the lesion was positively correlated to the degree of expression of detectable levels of the phosphoprotein p53. In 39 hyperplastic lesions of the oral mucosa without dysplasia, only 21% showed detectable p53 expression. In contrast, among the dysplastic lesions, 36% were positive for p53 expression (Fig. 3). It was also noted that seven p53 positive hyperplastic lesions without dysplasia were diagnosed as lichen planus. Since only a small percentage of lichen planus lesions develop into SCC [6,7,8,9], it would be interesting to investigate whether p53 mutation in oral lichen planus is related to an increased risk of malignant transformation.

In 85 squamous cell carcinomas of the oral mucosa, the loss of differentiation among these tumors was correlated with the detectable expression of p53. In 40 highly differentiated tumors, p53 expression was detectable in 47% of the tissue samples. In 45 tumors with a low grade of differentiation, 60% showed p53 expression. The number of p53 positive tumors was highest in the group of recurrent SCC. In the recurrent SCC, 66% were positive for p53.

The presented data suggest that p53 gene mutation is positively correlated to increasing dysplasia and loss of differentiation in carcinogenesis in the oral mucosa.

It was also attempted to quantify p53 expression among the lesions by counting the positive cells. No correlation could be found between the number of positive cells and the grade of dysplasia of the lesions. This could be due to the heterogenicity of the polyclonal tumors and hyperplastic lesions as well as to the fact that increased levels of p53 protein are expressed in growing cells [19]. In addition, with morphology being the least sensitive technique, dysplasia may indicate an already advanced state of atypia. Demonstration of p53 mutation may suggest atypia which is not yet overt by light microscopy [20]. Follow-up studies should be done to further elucidate this phenomenon.

The results of this immunohistological investigation imply that mutation of the p53 tumor suppressor gene plays an important role in carcinogenesis in the oral mucosa as it also has been confirmed at the molecular level [21]. Further investigations are needed, though, to determine whether detection of p53 is caused by the presence of mutation or due to promotion-driven mechanisms that lead to increased p53 at the steady state in a cell cycle checkpoint response mechanism.

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