

DISTRIBUTION OF P53 ONCOGENE PROTEINS IN ORAL MALIGNANT LESIONS

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Abstract—Immunohistochemical determination of oncogene proteins in human tumors is becoming increasingly important for obtaining a better understanding of process of tumorigenesis. The nuclear p53 protein has been detected in a variety of tumors and has been implicated as a diagnostic marker for malignancy detection. In current study, the distribution of p53 was determined in paraffin-embedded sections obtained from oral squamous cell carcinoma (SCC) and oral malignant melanoma (MM). Normal oral mucosa, hyperplastic/dysplastic oral lesions and benign pigmented lesions were included for comparison. Expression of p53 was detected by means of immunohistochemistry method using anti-p53 monoclonal antibody, which was specific to all known forms of human p53. Results showed that only 1/15 normal oral mucosa had a few nuclear p53+ cells scattered in the basal cell layer in all section. Positive cells were not found in any of the benign hyperplastic lesions but 8/11 dysplastic lesions had a few p53+ cells (approximately 5% to 10%) spread in the dysplastic region of the sections. Nuclear p53 staining was found in 19/22 oral SCC samples examined. Numerous malignant cells (approximately 90% to 95%) were strongly positive for p53 oncogene, in these sections. In pigmented lesions, p53+ cells were present in the majority of benign pigmented lesions. Each section had over 95% melanocytes stained positively for the antigen. Malignant melanomas showed strong immunoreactivity in all the samples examined. Approximately up to 90% to 95% of malignant melanocytes were p53 positive. It is concluded that expression of p53 oncogene was more enhanced in dysplastic oral lesions than was expressed in benign and normal oral mucosa. The highest level of p53 expression was found in oral SCC sections. Accordingly, p53 expression corresponded to the aggressiveness of tumor cells in oral mucosal lesions. Secondly, p53 expression in malignant melanoma was not associated with increasing malignancy and provided inadequate indication of malignancy development in pigmented lesions. Finally, archives of paraffin embedded lesions provided useful sources for detection of specific tumor markers, in a variety of pathological conditions. Acta Medica Iranica 33 (3&4): 74-78; 1995

Key words: p53; immunohistochemistry; oral malignant lesions

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INTRODUCTION

The nuclear tumor antigen p53 is expressed by a gene localized on the p-arm of human chromosome 17 (1). P53 (so termed because it is a protein with a molecular weight of 53 kilo daltons) (2) is the product of tumor suppressor genes. These genes may play an important role in the development of neoplasia (3). During tumor development, tumor suppressor genes often become mutate or deleted and lose their normal function which is to negatively regulate cell growth (1,3).

P53 protein has its site of action in the nucleus and is thought to be involved in regulating the replication of DNA (4). In normal cells p53 is present in minute concentrations and is almost undetectable. The protein however, is over expressed in a high percentage of tumors including those of breast cancer (5,6), carcinoma of lung (7), and colon carcinoma (8). The wild type p53 protein has a short half-life while mutant p53 proteins are more stable thus making it amenable to detection by immunohistochemistry (9).

In recent studies on distribution of p53 the oncogene protein was expressed by malignant cell-lines derived from head and neck carcinoma (10) and was present in frozen section taken from oral cancer lesions (11). Also, positive p53 expression was reported in the parotid gland carcinomas indicating that over expression of this protein is a reliable marker of malignancy (12).

Additional observations of p53 expression are related to the studies on cutaneous moles and malignant melanomas. Results of these studies however, are inconsistent regarding the value of p53 expression in pigmented lesions of the skin (13,14,15,16,17). Some studies have shown that p53 may have a functional role in development of malignant melanomas (13,14,15,16) and some indicated otherwise by showing that p53 immunoreactivity did not discriminate between benign and melanotic proliferation (17). Information regarding the characteristics of p53 expression in oral mucosal pigmented lesions is scarce.

Current study was designed to investigate two specific aims: 1) To understand whether distribution and

occurrences of p53 immunoreactivity in formalin-fixed paraffin-embedded sections of oral malignant tissues was similar to those of cryostat specimens reported in the literature and 2) To determine whether p53 distribution pattern in benign and malignant pigmented oral lesions was similar or dissimilar to that of cutaneous pigmented lesions.

MATERIALS AND METHODS

Non-pigmented lesions

Fifteen surgical specimens of normal oral mucosa, 9 benign hyperplastic and 11 dysplastic mucosal lesions were examined. Malignant mucosal lesions were 22 samples of primary oral squamous cell carcinoma (SCC) with different stages of differentiations.

Pigmented lesions

Benign pigmented lesions were 2 nevi and 7 focal melanosis. Three focal melanosis with dysplasia were also included. A range of oral malignant melanomas (n=18) from different stages of tumor progression were examined and 5 cutaneous melanomas were included for comparison.

Immunohistochemistry method

Serial sections (5 μ m) taken from paraffin-embedded samples were first deparaffinized in 2 changes of xylene, 10 minutes each. Rehydration was obtained in a sequence of ethanol changes with decreasing concentrations. To bleach melanin pigmentations, all section taken from pigmented lesions were treated with 0.5% potassium permanganate for 15 minutes, washed in distilled water and then incubated with 0.1% oxalic acid for 1-2 minutes. After washing in distilled water all sections employed in the study were incubated with 0.1% H₂O₂ for 30 minutes to block the endogenous peroxidase activity. Sections were then washed in distilled water and incubated 30 minutes with 0.05% saponin (Sigma). Prior to application of the primary antibody, a protein blocking agent (10% normal goat serum) was used followed by washing in 2 changes of phosphate buffered solution (PBS, PH: 7-2). The primary monoclonal used was 1:10 dilution of anti-p53 monoclonal Ab-6 (obtained from Oncogene Science Inc., San Diego, CA, U.S.A). Incubation with the primary antibody was terminated after an over-night period and was followed by application of multilink biotinylated secondary antibodies. The next step was incubation of sections with peroxidase conjugated streptavidin label (Bio Genex Super Sensitive Multilink Kit, San Ramon, CA). Incubation for each layer was 30 minutes in room temperature and was followed

with 10 minute washing in 2 changes of PBS, between each layers. The color reaction was developed with AEC chromogen (from the kit) and counter staining was with Mayer's hematoxylin for up to 5 minutes.

Positive and negative control sections were processed with each batch of tissues. Sections of breast cancer which are known to express p53 (5,6) were used as positive controls. Non-immune sera and PBS were used in place of primary monoclonal and served as negative controls. Staining intensity in each section was assessed as very weak (\pm), weak (+), moderate (+2), strong (+3) and very strong (+4) reaction. Using an eyepiece graticule (\times 400) positive cell population was counted in five randomly selected regions of each section.

RESULTS

Normal oral mucosa

The p53 nuclear staining was detected in only 1/15 section of normal oral mucosa (Fig. 1). An interesting finding in all normal sections was a uniform and widely-spread cell membrane staining expressed exclusively by the epithelial cells located above the basal cell layer (Fig. 2).

Benign and dysplastic mucosal lesions

There were no p53 positive cells detected in any section of the benign hyperplastic lesions examined in this study. The majority of mucosal samples (8/11) with moderate to severe dysplasia had a variable number of positive cells (5% to 10%) stained for p53. Most of the positive cells were initially located in the distinct regions of the tissues which all at least showed a light to moderate level of dysplasia (Fig. 3). Staining intensity in dysplastic region of tissues varied from moderate (+) in 4 samples and increased to relatively high level (+2) in 5 specimens.

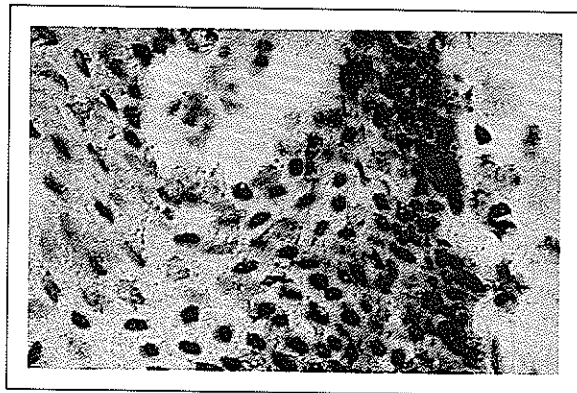


Fig. 1. Normal oral mucosa had only few nuclear positive staining in basal cell layer (\times 200).

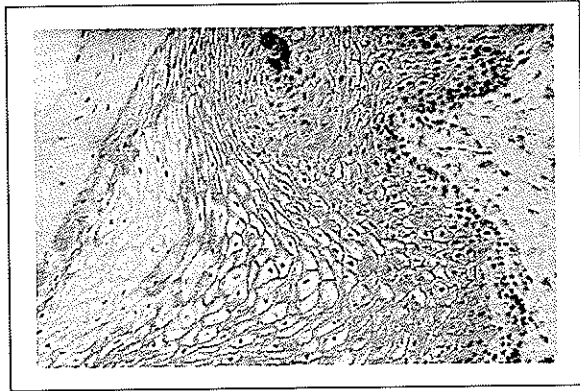


Fig. 2. Exclusive cell membrane staining of suprabasal cells corresponding to the distribution of involucrin molecules in the region ($\times 100$).

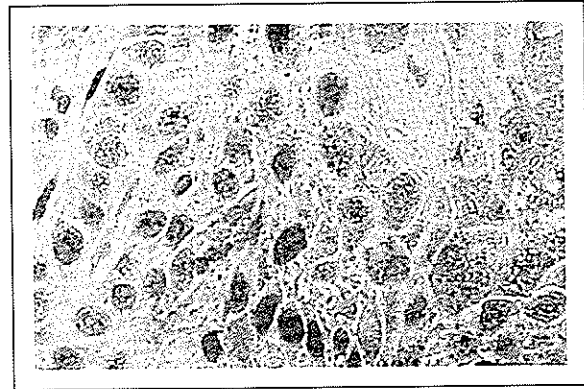


Fig. 4. Strong p53 staining is evident in an oral SCC sample ($\times 400$).

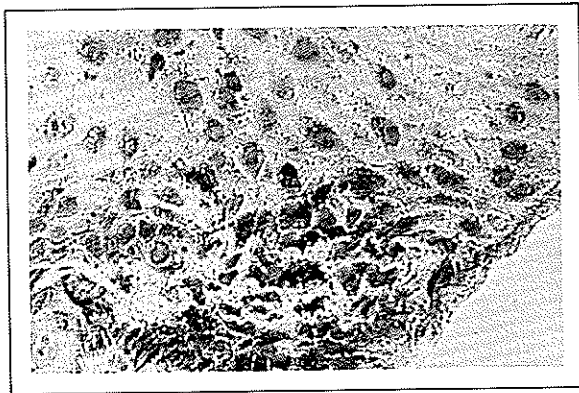


Fig. 3. Dysplastic oral lesions had few p53 positive cells scattered within the tissue ($\times 200$).

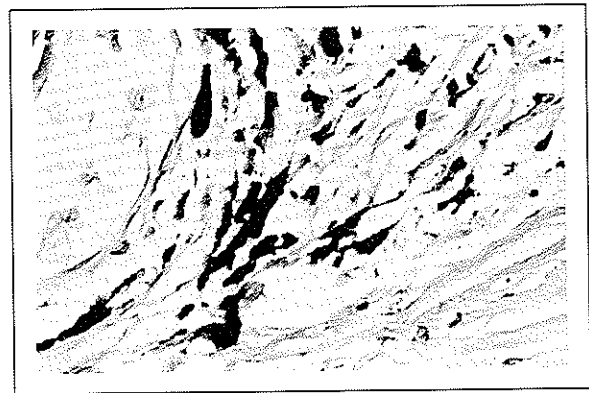


Fig. 5. Routine H and E staining of a benign nevus ($\times 400$).

Oral Squamous cell Carcinoma

Positive p53 immunoreactivity was found in 19/22 samples of oral SCC examined. Many sections (17/22) had the majority (90% to 95%) of malignant cells reacted positively for expression of p53. The positive cells were either present throughout the tissues or were scattered among small numbers of negative cells (Fig. 4). The intensity of nuclear staining varied from moderate (+2) to strong (+3) and very strong (+4) in a given lesion. Staining distribution in some sections (5/22) was focal and had at least up to 35% to 50% cell positively distributed across the tumor specimens. Immunoreactivity for p53 was exclusively nuclear in some sections and was both cytoplasmic and nuclear in some other SCC sections examined.

Pigmented lesions

Between two benign nevi examined, one pigmented nevus stained strongly positive (+4) for p53 (Figs. 5 and 6) and one specimen did not stain. Positive immuno-

reactivity was also demonstrated in 5/7 samples of focal melanosis with very strong (+4) staining intensity (Figs. 7 and 8). Two samples in this group did not stain. Focal melanosis with dysplasia ($n=3$) had two samples stained strongly positive (+3) for p53 and one sample with weak staining intensity (+). The number of positive cells demonstrated in the benign nevus was up to 100%. Focal melanosis with or without dysplasia had at least over 95% of cell positivity determined in each section.

Malignant melanomas were assessed for the differential expression of the p53 antigen at all levels within the epithelium and at connective tissues. The results were subsequently matched to the histological grading of the tissues. A pronounced nuclear and cytoplasmic expression of p53 oncogene was detected in all ($n=18$) cases of oral malignant melanomas (Figs. 9 and 10). Some samples had p53 positive cells with uniform staining pattern distributed across the tumor specimen resembling red-cherry branches (Figs. 11 and 12). The staining intensity was strong (+3) and very strong (+4) in

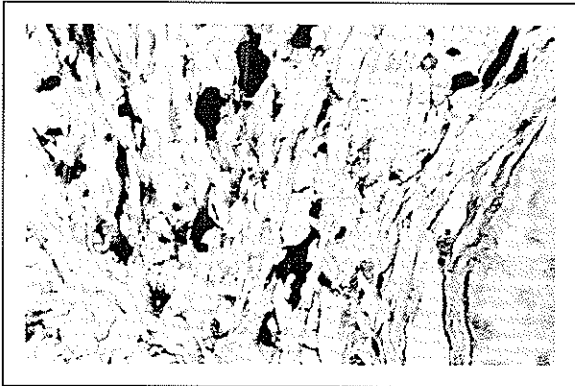


Fig. 6. Pigmented nevus cells seen in Fig. 5 are strongly positive for p53 ($\times 400$).

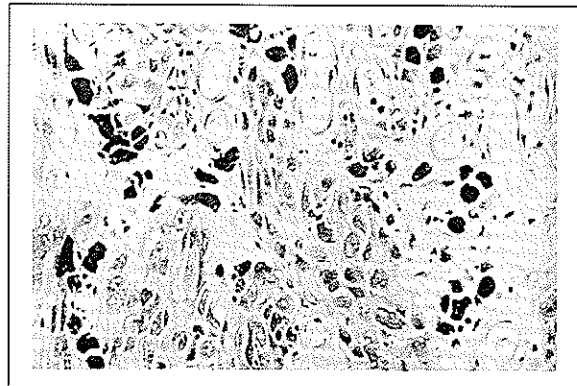


Fig. 9. Routine H and E staining of an oral MM specimen ($\times 200$).

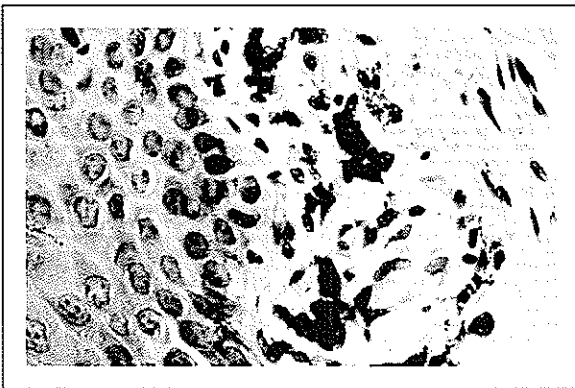


Fig. 7. Routine H and E staining of a focal melanosis ($\times 400$).

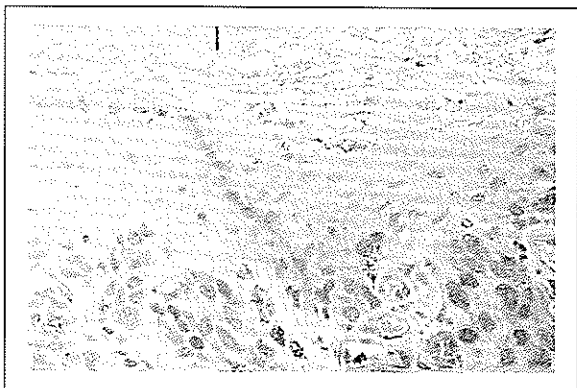


Fig. 10. Strong p53 immunoreactivity is evident in a serial section taken from the same specimen corresponding to the distribution of malignant cells in Fig. 9 ($\times 200$).

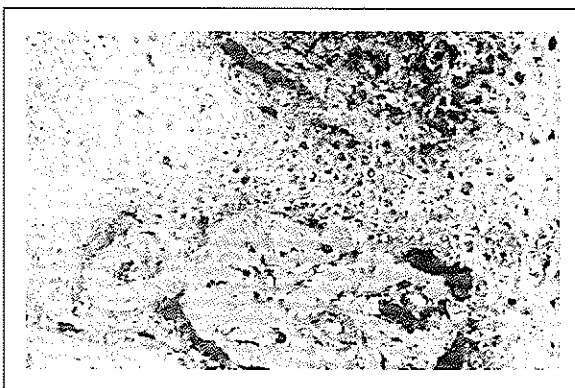


Fig. 8. An intense p53 staining of pigmented cells seen in Fig. 7 is evident in Fig. 8 ($\times 400$).

all samples examined. All cutaneous melanomas ($n=4$) also demonstrated positive p53 immunoreactivity with variable staining intensity ranging from strong (+3) to very strong (+4) reaction.

Controls

Breast cancer sections were all positive for p53 expression. Staining was completely absent in negative control section treated with non-immune sera or PBS in place of the primary monoclonal antibody.

DISCUSSION

Oncogene p53 protein is one of the most important factors the expression of which may be associated with progression from the benign to malignant transformation of oral epithelium (10,11). Results of the current study show that only few basal cells in 1/15 normal oral mucosa were positive for p53 expression (Fig. 1). This may be due to the fact that the p53 levels in normal cells are too low to be detected by immunohistochemistry. A surprising observation in normal tissues was the immunoreactivity of the antibody with a cell-membrane

antigen of suprabasal cells (Fig. 2). The regional variation of reactivity may be related to the differences in the cytokeratin types at different anatomical zones of oral mucosa (18). It is reasonable to assume that the antibody may have cross-reacted exclusively with involucrin keratin molecules as they are the only known keratin expressed by supra-basal cell layers (18).

The function of p53 oncogene proteins in normal cells is not well understood (18). It has been suggested to be a cell cycle protein playing a regulatory role in the control of cell proliferation (18). Thus, the inactivation or mutation of p53 may be a necessary step in the development of malignancy (4).

Results of the current study on dysplastic lesions with light to moderate dysplasia revealed only some focal staining with few positive cells distributed in each section (Fig. 3). This observation may indicate that nuclear expression of p53 is not an early event in the development of malignancy in oral lesions (8). An enhanced staining distribution of p53 was detected in 19/22 oral SCC examined in the current study. Approximately over 95% cell positivity was found in each malignant section examined. Focal areas of p53 expression were also found in some SCC specimens. The explanation for this observation is unclear, however discrete phase of the cell cycle may be associated with elevated levels of p53 (9,10). Enhanced expression of p53 from normal to dysplastic and malignant lesions detected in the current study indicates that p53 over-expression provides a marker for malignant transformation of oral mucosal lesions.

In the group of pigmented lesions, the immunohistochemical positivity was found in a benign nevi, in 5/7 of focal melanosis and in 3/3 of focal melanosis with dysplasia. In these specimens, over 97% of the pigmented cells stained strongly positive indicating that p53 is commonly expressed by benign and dysplastic pigmented oral lesions. All specimens of oral MM and cutaneous melanomas were also strongly positive for p53. The cellular localization of p53 staining in MM sections were variable and included discrete nuclear staining, combined nuclear and cytoplasmic staining and staining of the cytoplasm alone. Since p53 was distributed widely in benign and dysplastic pigmented oral lesions, its association with MM can give little information towards the diagnosis or prognosis of these lesions.

In conclusion, the peculiar association of p53 expression in oral SCC(s) suggests that it may be useful in the detection of neoplastic cell development in mucosal lesions. However, the value of p53 expression in pigmented oral lesions is questionable since the oncogene protein is also associated with non-malignant pigmented oral lesions.

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