SHORT COMMUNICATION

Immunohistochemical localization of c-myc oncogene protein correlated with malignancy of oral epithelium

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Introduction

The oral mucosa provides one of the most potentially informative systems in which to investigate the process from premalignant conditions to carcinoma¹⁻⁴⁾.

The human c-myc oncogene is a cellular homologue of the avian v-myc gene found in some leukaemogenic retroviruses, and is a protooncogene related to proliferation and differentiation of normal and tumor cells⁵⁻⁷⁾. The product protein of c-myc oncogene commonly locates in the nucleus, and is considered to control entry of cells in the cell cycle although its function is still not well established^{5,8)}. Amplification and enhanced expression of c-myc oncogene have been demonstrated in a variety of pathological states including several premalignant and malignant conditions⁹⁻¹⁶⁾, and is closely related to the biological behavior of normal and tumor cells¹⁷⁾.

In the present study, the intracellular localization of c-myc oncogene product p62^{c-myc} was examined in hyperkeratosis, epithelial dysplasia and squamous cell carcinoma of oral mucosa by using an immunohistochemical approach, and the correlation between staining patterns and the degree of histological malignancy was investigated.

Materials and Methods

Tissues

Tissue blocks were obtained from a total of 54 patients. They consisted of normal oral epithelium (10 cases), hyperkeratosis (15 cases), epithelial dysplasia (10 cases) and squamous cell carcinoma (19 cases) including 7 of well, 8 of moderately and 4 of poorly differentiated types. Tissues of normal epithelium were autopsied at the Department of Pathology, Tohoku University Hospital and the other tissues were biopsied at the Oral Surgery of Dental Hospital, Tohoku University. The specimens were all fixed in 10 % neutral formalin for 1 to a few days and embedded in paraffin wax. Sections of 3 μ m thick were taken and processed for immunohistochemistry.

Immunohistochemistry

The primary antibody was mouse monoclonal antibody raised to a synthetic peptide consisting of residues 171-188 of the human p62^{c-myc} sequence (Myc 1-6 E 10). Peptide synthesis, the immunisation protocol and screening procedures for deriving Myc 1-6 E 10 have been fully described elsewhere^{18,19)}.

The sections were deparaffinized, dehydrated and immersed in methanol with 0.3 % H₂O₂ for 30 min. They were washed in 0.01

Fig. 1 Immunostaining for c-myc oncoprotein. Immunoactivities are observed in nucleus (1), perinuclear (2) and entire (3) cytoplasm, and both nucleus and cytoplasm (4). ($\times 370$)

M PBS and treated with normal rabbit serum for 30 min. After washing, they were incubated with the primary antibody (diluted 1 : 150) at 4 °C overnight.

Histofine SAB-PO kits (Nichirei) were employed to bind the primary antibodies. The sections were immersed in 0.03 % DAB solution with 2mM H_2O_2 for 2 to 4 min and counterstained with 1 % methyl green.

Results

Three patterns of immunostaining with c-myc oncoprotein were shown and classified as nuclear staining, perinuclearly cytoplasmic staining and entirely cytoplasmic staining. All of the cases were positive for anti-c-myc antibody with single or implicated staining patterns (Fig. 1). The results showed a correlation between staining patterns and histological types, and were tested for statistical significance by standard error (Fig. 2).

Normal oral epithelium demonstrated a high percentage of nuclear localization in the basal cell layer, and the reduction in prickle, granular and keratotic cell layers (Fig. 3-1). Hyperkeratosis tended to demonstrate increases of nuclear localization in the prickle cell layer, and staining intensity was stronger than normal epithelium (Fig. 3-2). No statistical differences were revealed between normal epithelium and hyperkeratosis (Fig. 2).

Epithelial dysplasia demonstrated decreases of nuclear localization in the the basal cell layer, and increases of both nuclear and cytoplasmic staining in basal and prickle cell layers (Fig. 3-3). Squamous cell carcinoma demonstrated the reduction of nuclear local-

Fig. 2 Immunoreactivity for c-myc oncoprotein in nucleus alone (N), both nucleus and cytoplasm (N+C), and cytoplasm alone (C).

Upper: Staining patterns of basal cells (BC), prickle cells (PC), granular cells (GC) and keratotic cells (KC) in the normal epithelium and the lesions of hyperkeratosis, epithelial dysplasia and squamous cell carcinoma (SCC). Lower: Correlations between c-myc oncoprotein expression and the differentiation of SCC. (w: well, m: moderately, p: poorly)

The observed differences reached statistical significances by standard error. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

Fig. 3 Immunostaining for c-myc oncoprotein.

- 1: Normal epithelium showing nuclear localization in basal cell layer. ($\times 135$)
- 2: Hyperkeratosis showing nuclear localization in basal and prickle cell layer. ($\times 115$)
- 3: Epithelial dysplasia showing both nuclear and cytoplasmic staining in basal and prickle cell layers. ($\times 170$)
- 4: Squamous cell carcinoma showing both nuclear and cytoplasmic staining. ($\times 135$)

ization, and a higher percentage of both nuclear and cytoplasmic staining (Fig. 3-4). The staining pattern revealed a high tendency of cytoplasmic localization in poorly differentiated carcinoma (Fig. 2). The nucleus of tumor cells in the forefront of proliferating tumor tissue and the chromatin under mitosis were obviously stained. No statistical differences were revealed between epithelial dysplasia and squamous cell carcinoma (Fig. 2).

The results compared with histological patterns are summarized in a schematic diagram (Fig. 4).

Discussion

Expression of c-myc oncogene is considered to be a major component in the regulatory processes associated with normal cell proliferation and differentiation^{6,7}, and the protein product of the oncogene is considered to be a DNA-associated nuclear protein^{5,8}. Basal cells are proliferative on stratified squamous epithelium. The predominant nuclear staining in the basal cell layer of normal oral epithelium suggested the potentiality of proliferation.

In this study, precancerous lesions involved in so-called leukoplakia and carcinoma were

Fig. 4 Schematic diagram of correlation between immunohistochemical localization of c-myc oncogene protein and malignancy of oral epithelium. (1: normal epithelium, 2: hyperkeratosis, 3: epithelial dysplasia, 4: squamous cell carcinoma)

examined by immunohistochemical method, and statistical differences were revealed in the various degrees of the malignancy on oral epithelial lesions. Especially, it was impressive that atypical cells demonstrated cytoplasmic distribution of c-myc oncoprotein. Observations in familial polyposis demonstrated the nuclear staining of normal colonic epithelium and the cytoplasmic staining of the proliferating epithelium of adenomatous polyp²⁰⁾ with the same immunohistochemical procedures as those of this study. It was thought that the c-myc oncoprotein had a relatively weak association with the nuclear matrix and could be reversibly modulated by localized variations²¹⁾. The different staining patterns in this study were considered to be probably caused by the association of DNA and c-myc oncoprotein.

It was reported that the subcellular distribution of c-myc oncogene product p62^{c-myc} depended upon the fixation method²²⁾. Staining patterns of formalin-fixed and paraffin-embedded tissue sections in this study might be modified through the procedures.

Immunohistochemical differences of c-myc oncoprotein were considered to indicate biological differences according to the degree of histological malignancy. The immunohistochemical analysis of c-myc oncoprotein has been considered to be useful for the diagno-

sis of oral epithelial lesions. Moreover, correlations between the immunolocalization of c-myc oncoprotein and the prognostic factors should be required.

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