

Expression and Correlation of MMP-2, TIMP-2 in Oral Squamous Cell Cancer

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Abstract: Oral Squamous Cell Cancer is the most common malignant tumour in head and neck area being the top of Oral Carcinoma. The malignancy is high, invasion is strong, and metastasis is fast. This report will use the immunohistochemical method to test the expression of MMP-2 and TIMP-2 in normal oral mucosa, dysplastic oral mucosa and oral squamous tissue, in order to investigate the correlativity and the effect to the biological behaviour of Oral Squamous Cell Cancer. In the process of invasion and metastasis of carcinoma, imbalance of MMP and TIMP expression plays an important role. Detecting MMP and TIMP the protein expression may be of great significance to estimating the biological behavior of carcinoma and evaluating the prognosis of patients.

Keywords: Gene expression, Matrix Metalloproteinase- 2 (MMP-2), Tissue inhibitors of metalloproteinase-2(TIMP-2), Immunohistochemistry

Introduction

The invasion and metastasis of tumor cell can be separated into three steps: attachment; matrix dissolution; migration. Only when the three processes join in a line, can direct invasion come true. Many studies has demonstrated that the increased expression of MMP-2 is closely related to the invasion and metastasis of oral squamous cell cancer. However, the conclusions are incongruous. The research about the correlation of MMP-2 and TIMP-2 with its role in the invasion and metastasis of oral squamous cell cancer is rare. The aim of the present study is to test the expression of MMP-2, TIMP-2 in oral squamous cell cancer, using the immunohistochemical method, in order to investigate the correlativity and the effect to the biological behaviour of oral squamous cell cancer.

Materials and Methods

1. Clinical data

1.1 Source of tissue

30 random samples were selected from paraffin blocks of primary oral squamous cell cancer tissue which were removed from patients of Da Lian oral Hospital from January 2002 to October 2004. 10 random samples were selected from dysplastic oral mucosa and normal oral mucosa respectively as a control reaction, review the clinicopathological features of all the oral squamous cell cancer cases.

2. Methods

2.1 Main reagents

Mouse anti MMP-2 and TIMP-2 monoclonal antibody, SP immunohistochemical reagents (provided by Shanghai Biological Science and Technique Ltd).

2.2 Staining method of immunohistochemistry

For the double immunostainings, two successive labeling reactions for MMP-2 and TIMP-2 were done, sequentially as follows:

- 1) Paraffin-embedded tissues were dewaxed in xylene and rehydrated through graded alcohol.
- 2) According to the first antibody, the tissue antigen was renovated correspondingly.
- 3) The sections was treated with methanol-hydrogen peroxide complex solution for 30 minutes so that endogenous dioxygenase was lost.
- 4) Washed with PBS Solution (2min × 3).
- 5) The section was incubated with first antibody for 1hr at room

temperature in a moist chamber.

- 6) Washed with PBS Solution
- 7) Incubated with the second antibody for 30min at room temperature discontinuously.
- 8) Washed by PBS Solution.
- 9) Added DAB Solution and observed the color reaction under microscope, stopped the color reaction timely with water, the reaction continued not more than 10min.
- 10) Counter stained nucleus with haematoxylin or methyl green for 10 sec.
- 11) Dehydrated with gradient alcohol, made it transparent with xylene, fixed with neutral gum.

Results and Conclusion

1. The expression of MMP-2, TIMP-2 in normal oral mucosa, dysplastic oral mucosa and oral squamous cell cancer tissue.

1.1 Expression of MMP-2

From normal oral mucosa epithelium, dysplastic oral mucosa epithelium to oral squamous cell cancer, the expression of MMP-2 increased. The difference of oral squamous cell cancer and normal oral mucosa epithelium, dysplastic oral mucosa epithelium and normal oral mucosa epithelium was significant ($p < 0.01$). The difference of oral squamous cell cancer and dysplastic oral mucosa epithelium was not significant ($p > 0.05$).

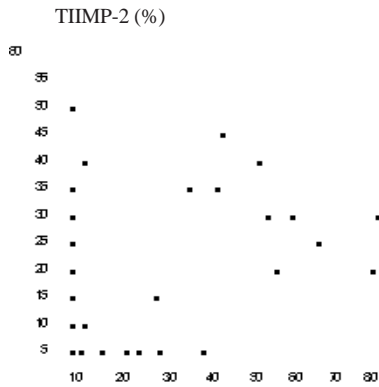
1.2 Expression of TIMP

The positive expression of TIMP was also light brown granules in the cytoplasm. The expression of TIMP in normal oral mucosa epithelium was negative or low positive, the expression in dysplastic oral mucosa epithelium varied from low positive to high positive, the expression in oral squamous cancer varied from low positive to high positive.

2. The correlation between the expression of MMP-2 and the expression of TIMP-2 in oral squamous cell cancer (table-1).

Twenty of the thirty oral squamous cell cancer cases displayed positive expression of MMP-2, eighteen of the thirty cases displayed positive expression of TIMP-2, fourteen cases displayed positive expression of both MMP-2 and TIMP-2, four cases displayed negative expression of both MMP-2 and TIMP-2. According to statistical analysis, there was inverse correlation between the MMP-2 expression and TIMP-2 expression in oral squamous cell cancer tissues ($r = -3.89$, $P < 0.01$). In the process of invasion and metastasis of carcinoma, imbalance of MMP and TIMP expression plays an important role. Detecting MMP and

Table 1 The correlation between the expression of MMP-2 and the expression of TIMP-2 in oral squamous cell cancer



MMP-2(%)

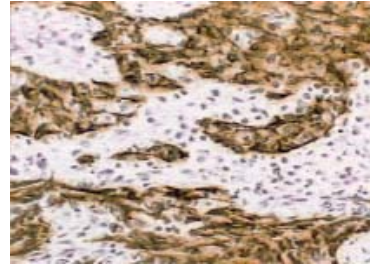


Fig 1 the expression of MMP-2 in poor differentiation oral squamous cell cancer (+++) (x 200)

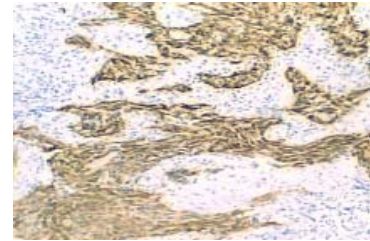


Fig2 the expression of TIMP-2in poor differentiation oral squamous cell cancer (++) (x100)

TIMP the protein expression may be of great significance to estimating the biological behavior of carcinoma and evaluating the prognosis of patients.

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