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COX-2, MMP-7 expression in oral lichen planus and oral squamous cell carcinoma

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ABSTRACT

Objective: To observe cyclooxygenase (COX)-2 expression in normal oral mucosa (NOM), oral lichen planus (OLP) and oral squamous cell carcinoma (OSCC) and explore its significance in the incidence of oral cancer. **Methods:** The immunohistochemical method and RT-PCR method were applied to detect the expression of COX-2 and MMP-7 in 10 cases with NOM, 33 cases of with OLP and 38 cases with OSCC. **Results:** The expression of COX-2 mRNA in OSCC tissues (68.4%, 26/38) was significantly higher than in the OLP (24.2%, 8/33) and NOM (0.0%, 0/10) (P<0.01). The expression of MMP-7 mRNA in OSCC tissues (65.8%, 25/38) was significantly higher than in the OLP (30.3%, 10/33) and NOM (0.0%, 0/10) (P<0.01). The expression of MMP-7 in OLP was significantly higher than in the NOM (P<0.05). There was no significant expression of COX-2 protein in NOM, and the positive rate was 42.4% (14/33) and 89.5% (34/38) in OLP and OSCC group, respectively. The COX-2 expression in cancer tissues was significantly higher than in NOM and OLP (P<0.05). The MMP-7 protein expression in cancer tissues (84.2%, 32/38) was significantly higher than in NOM (10.0%, 1/10) and in OLP (42.4%, 14/33), and the positive rate in OLP was significantly higher than in NOM (P< 0.01). The COX-2 expression was associated with clinical stage (P<0.05), the MMP-7 expression was associated with clinical stage and lymph node metastasis (P<0.05). The expressions of COX-2 and MMP-7 mRNA were positively correlated with OSCC. **Conclusions:** The abnormal expressions of COX-2 and MMP-7 are closely related to the biological behavior of OSCC, the MMP-7 may be induced by COX-2, and further lead to the invasion and metastasis of OSCC.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumors of the oral and maxillofacial, surgery combined with radiotherapy and chemotherapy is still its main treatment[1]. Early detection of malignancy has a positive significance for the prognosis of patients, some studies showed that as an oral mucosa chronic non-specific inflammation, oral lichen planus (OLP) has a cancerous potential. Now we detect the expression of cyclooxygenase (COX)-2, MMP-7 mRNA and protein in the normal oral mucosa (NOM), OLP and OSCC by immunohistochemical SP method and RT-PCR, and to explore their role in the occurrence and development of the OSCC.

2. Materials and methods

2.1. Materials

All specimens were selected from patients who received dentistry treatment in our hospital between 2009 and 2011 and then confirmed by pathological analysis. There were

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33 cases with OLP (18 males and 15 females, age from 20-63 years old), 38 cases with OSCC (21 males and 17 females, age from 25-72 years old), which included 16 cases without lymph node metastasis and 22 cases with lymph node metastasis. According to 5th edition of oral histopathological squamous cell carcinoma diagnostic criteria, there were 6 cases with class Ⅰ squamous cell carcinoma, 14 cases with class Ⅱ squamous cell carcinoma, 18 cases with class Ⅲ squamous cell carcinoma, 8 cases with well-differentiation, 12 cases with moderately differentiation,18 cases with poorly differentiation. And there were 10 cases in normal control group. Samples were obtained from oral cleft lip and palate plastic surgery.

2.2. Reagents

COX-2 and MMP-7 were both rabbit anti-human polyclonal antibody, SP immunohistochemistry kit and DAB color kit were purchased from Wuhan Boster Biological Engineering Co., Ltd. COX-2 (304 bp) primer sequences was as follows: upstream: 5′-TTC AAA TGA GAT TGT GGG AAA ATT GCT-3′, downstream: 5′ - GAT CAT CTC TGC CTG AFT ATC TT-3′; MMP-7 (418 bp) primer sequences was as follows: upstream: 5′-ATG TTA AAC TCC GCG TCA TA-3′, downstream: 5′-CAG CAT ACA GGA AGT TAA TCC-3′; Internal reference sequences was GADPH (298 bp) upstream: 5′-GCG-GGC TCT CCA GAA CAT CAT-3′, downstream: 5′-CCA GCC CCA GCG TCA AAG GTG-3′. They were all synthesized by Shanghai Sangon Biological Engineering Co.

2.3. Experimental methods

All specimens were divided into two groups after surgical resection, specimens for RT-PCR were immediately placed in EP tube treated by diethylprocarbonate (diethyl pyrocarbonate, DEPC), then prepared in low temperature refrigerator at -80 ℃. All specimens used for immunohistochemistry were fixed in 10% formalinfand paraffin-embedded, and were measured by COX-2 and MMP-7 immunohistochemical staining.

RT-PCR was operated as follows: total RNA were extracted from tissues by TRIzol (Invitrogen Company), cDNA was synthesized by reverse transcription according to the instructions. The cDNA synthesized by reverse transcription was as a template for PCR amplification, and GADPH was as an internal reference. COX-2 reaction conditions were as follows: after 95 ℃ for 5 min, 95 ℃ for 30 s→62 ℃ for 30 s→72 ℃ for 30 s, 35 cycles; 72 ℃ for 7 min. MMP-7 reaction conditions were as follows: after 95 ℃ for 5 min, 95 ℃ for 30 s→53 ℃ for 30 s→72 ℃ for 30 s, 35 cycles; 72 ℃ for 7 min. The amplified products were performed by 1.5% cleavage agarose gel eletrophoresis and EB staining. The immunohistochemistry was operated as follows: matured by immunohistochemical SP method and coloured by DAB. All specimens were under high temperature and high pressure to repair antigen, and staining in accordance with the reagent instructions. The positive section was used as a positive control, PBS instead of primary antibody was used as a negative control.

2.4. RT-PCR

Positive bands were compared with Marker. Positive bands at 304 bp, 418 bp and 298 bp, respectively were regarded as positive expression of COX-2, MMP-7 and GADPH mRNA. If there was no positive strip band at the corresponding area, the result was negative.

2.5. Immunohistochemical staining criteria

The semi-quantitative method to used to determine the results. Ten typical high power fields (× 400) were selected in each slice, at least 1 000 cells were counted, and then it was calculated by the percentage of positive cells. COX-2, MMP-7 protein cytoplasm and/or nuclei in yellow-brown staining were considered as positive cells. Positive degree of tumor cells was as follows: 10% -25% were (+), 26%-50% were (+ +), more than 51% were (+ + +); Slice positive cells rate ≥ 10% were regarded as positive, <10% as negative (-).

2.6. Statistical analysis

All of the data were analyzed by t test and χ^2 test. Fisher's exact test and Spearman rank correlation analysis was used to analyze the correlation, $P<0.05$ was considered as statistical significance.

3. Results

3.1. COX-2, MMP-7 mRNA levels

The positive expression rate of COX-2 mRNA in NOM, OLP and OSCC were 0% (0/10), 24.2% (8/33) and 68.4% (26/38), respectively. The expression of COX-2 in OSCC tissues was significantly higher than in OLP and NOM ($P<0.01$). The expression of COX-2 in OLP was slightly higher than in NOM ($P>0.05$). The positive expression rate of MMP-7 mRNA in NOM, OLP and OSCC were 0% (0/10), 30.3% (10/33) and 65.8% (25/38), respectively, the expression of MMP-7 in OSCC tissues was significantly higher than in OLP and NOM ($P<0.01$), the expression of MMP-7 in OLP was significantly higher than in NOM ($P<0.05$).

3.2. COX-2, MMP-7 protein expression

Immunohistochemical results showed that COX-2 has no significant expression in NOM, and the positive rate was 42.4% (14/33) and 89.5% (34/38) in OLP and OSCC group, respectively. The COX-2 expression in cancer tissues was

significantly higher than in NOM and OLP ($P<0.05$). There was no significant difference in the positive expression between OLP and NOM. Positive particles of COX-2 protein was brown, mainly distributed in the cytoplasm and cell membrane. The positive expression rates of MMP-7 were 10.0% (1/10) in NOM, 42.4% (14/33) in OLP, 84.2% (32/38) in OSCC, respectively. MMP-7 expression in cancer tissues was significantly higher than in NOM and OLP, and the expression in OLP was significantly higher than in NOM ($P<0.01$). The positive granules of MMP-7 were brow, and mainly distributed in the cytoplasm (Table 1).

Table 1

Expressions of COX-2 and MMP-7 protein in NOM, OLP and OSCC (*n*).

Groups	COX-2				MMP-7			
	−	+	++	+++	−	+	++	+++
NOM	10	0	0	0	10	0	0	0
OLP	19	4	5	5	19	10	3	1
OSCC	4	6	12	16	6	14	11	7

3.3. Relationship of COX-2 and MMP-7 expressions with clinical pathological features

Out of 38 cases with OSCC, aging from 25 to 72 years, 13 cases were older than 60 years old, and 25 cases were less than 60 years old. In the ⩾ 60-year old group, 8 cases had the expression of COX-2 mRNA and MMP-7 mRNA, respectively. In the <60-year old group, 18 and 17 cases had the expression of COX-2 mRNA and MMP-7mRNA, respectively. There were 21 males and 17 females in OSCC group. A total of 15 male cases and 16 male cases had the expression of COX-2 mRNA and MMP-7 mRNA, respectively; while 11 female cases and 9 female cases had the expression of COX-2 mRNA and MMP-7 mRNA, respectively.

There were 8 well-differentiated cases, 12 moderately differentiated cases, 18 poorly differentiated cases in the OSCC group. There were 4 cases, 9 cases and 13 cases respectively of COX-2 mRNA expression in the well differentiated group,moderately differentiated group and the poorly differentiated group. While there were 5 cases, 8 cases and 12 cases respectively of MMP-7mRNA expression in the well differentiated group,moderately differentiated group and the poorly differentiated group.

The statistical analysis showed COX-2 and MMP-7 expressions were not related with the age, gender, lymph node metastasis and pathological type ($P>0.05$). A total of 15 cases with lymph node metastasis and 11 cases without lymph node metastasis had COX-2 mRNA expression ($P>0.05$); While 18 cases with lymph node metastasis and 7 cases without lymph node metastasis had MMP-7mRNA expression ($P<0.05$). A total of 17 cases in Ⅰ + Ⅱ stage group and 9 cases in Ⅲ group had COX-2 mRNA expression ($P<0.05$); while there were 9 cases in Ⅰ + Ⅱ stage group and 16 cases in Ⅲ group had MMP-7mRNA expression ($P<0.05$); COX-2 expressions were related to the clinical stage ($P<0.05$), MMP-7 expression were related to the clinical stage and lymph node metastasis ($P<0.05$), others showed no correlation with clinical factors.

3.4.Correlation of COX-2 and MMP-7 expression with OSCC

A total of 26 OSCC cases showed positive COX-2 mRNA expression, 25 cases showed positive MMP-7 mRNA expression, and 18 cases showed positive COX-2 and MMP-7 mRNA expression. Spearman rank correlation analysis showed COX-2 and MMP-7 mRNA expression showed a positive correlation with OSCC ($r=0.548$, $P<0.05$).

4. Discussion

Oral lichen planus is an inflammatory disease of the skin and mucous membranes. There are a variety of clinical manifestations, but the specific etiology and pathogenesis is not clear. It is considered to be a pre-cancerous or precancerous state[2]. Tumor progression and metastasis is usually from normal tissue to precancerous lesions, and then the cancer formed. The principles of treatment for cancer are early detection, diagnosis and treatment[3]. With the development of molecular biology, immunology and other inspection techniques, it is possible to detect the presence of tumor markers in patient's body fluids to assist early detection of cancer[4].

Arachidonic acid (AA) metabolites are closely related tumor. As AA metabolism rate-limiting enzyme, COX plays an important role as the valve in tumorigenesis. There are only COX-1 and COX-2 of the COX gene. Many studies have shown that COX-1 as a housekeeping gene has a stable expression in the tissues, while COX-2 has almost no expression in normal epithelial tissues, but was highly expressed in the hyperplastic tissue, and more highly expressed in gastrointestinal cancer, lung cancer, breast cancer, liver cancer, pancreatic cancer and other diseases[5,6]. Some studies suggest that COX-2 overexpression in precancerous lesions prior to the expression of biomarkers of apoptosis and angiogenesis, and involved in tumorigenesis by promoting the cell proliferation and inhibiting the apoptosis[7,8]. In this study, the mRNA and tissue protein analysis showed no expression of COX-2 in NOM, which is higher in OLP than in NOM, but not significant. The expression was significantly increased in OSCC, which is consistent with the relevant research. Associated with clinical features, we found that COX-2 expression is higher in phase Ⅰ than in phase Ⅱ and phase Ⅲ, which indicating that there was obviously expression of COX-2 in early tumorigenesis and is closely related with the tumorigenesis.

Extracellular matrix and basement membrane are stable

tissue cells involuntary transfer organizational structure,of which the matrix metalloproteinases (MMPs) genes as the important gene for the degradation of the extracellular matrix and basement membrane is extensively studied, and its expression was significantly increased in a variety of tumors ,and can promote tumor growth through the activation of tumor tissue growth factor and inhibit the apoptosis of tumor cells[9-12]. The MMPs have more than 20 members, and MMP-7 has a strong degradation type Ⅳ collagen. It is also the only epithelial-specific product of MMPs family, which is related with apoptosis[13-17].

In this study, the mRNA and tissue protein analysis showed no expression of MMP-7 in NOM, which is higher in OLP than in NOM, but not significant. The expression was significantly increased in OSCC. Associated with clinical features, we found that MMP-7 expression is higher in phase Ⅲ than in phase Ⅰ and phase Ⅱ, and higher in groups with lymph node metastasis than groups without lymph node metastasis, which indicating that there was obviously expression of MMP-7 in the process of tumor development and is closely related with the tumorigenesis, and indicates a poor prognosis. COX-2 and MMP-7 mRNA's expression was positively correlated with oral squamous cell carcinoma, and it showed there is a correlation between the expression of these two genes. This study suggests that oral lichen planus has epithelial hyperplasia, once the proliferation of epithelial cells surpass the normal limits, it will change into abnormal differentiation cells, and cancer will become final results. That confirmed the malignant potential of oral lichen planus once again, the application and testing of COX-2 and MMP-7 in oral precancerous lesions may provide clinical preventive information to blocking further malignant transformation of epithelial cells in the precancerous stage.
In the correlative studies of oral cancer and the expression of COX-2 and MMP-7, we presume that COX-2 and MMP-7 can act on the occurrence and development process of oral cancer, COX-2 and MMP-7 can be used as molecular markers to predict oral cancer and precancerous lesions in clinical application.

Conflict of interest statement

We declare that we have no conflict of interest.

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