Expression of Cyclooxygenase-2 and Basic Fibroblast Growth Factor in Nasopharyngeal Carcinoma Tissues and Their Correlation with Radiosensitivity: Postprint

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Abstract

Objective: To investigate the expression of cyclooxygenase-2 (COX-2) and basic fibroblast growth factor (bFGF) in nasopharyngeal carcinoma tissues and their correlation with radiosensitivity.

Methods: Immunohistochemistry was employed to detect the expression of COX-2 and bFGF in pre-radiotherapy biopsy tissues from 97 patients with nasopharyngeal carcinoma, and the relationship between these two markers and radiosensitivity was analyzed.

Results: The positive expression rates of COX-2 and bFGF in the 97 nasopharyngeal carcinoma tissues were 71.1% (69/97) and 64.9% (63/97), respectively. The positive expression of COX-2 was significantly correlated with T stage and N stage of nasopharyngeal carcinoma ($P<0.05$), while the positive expression of bFGF was significantly correlated with N stage ($P<0.05$). The positive expression rates of COX-2 in the radiosensitive group and the non-sensitive group were 62.8% and 92.6%, respectively, and those of bFGF were 57.1% and 85.2%, respectively. Spearman correlation analysis in nasopharyngeal carcinoma tissues revealed that COX-2 expression was significantly positively correlated with bFGF expression (correlation coefficient $r=0.486$, $P<0.05$). Based on the expression status of COX-2 and bFGF, patients were divided into four groups: COX-2(-) and bFGF(-), COX-2(-) and bFGF(+), COX-2(+) and bFGF(-), and COX-2(+) and bFGF(+). Combined analysis of COX-2 and bFGF expression demonstrated that different subgroups were significantly associated with radiosensitivity of nasopharyngeal carcinoma ($P<0.05$).

Conclusion: The expression of COX-2 and bFGF is elevated in nasopharyngeal carcinoma tissues, and both are significantly correlated with tumor radiosensi-

tivity, which may serve as important indicators for predicting radiosensitivity in nasopharyngeal carcinoma.

Full Text

Preamble

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Abstract

Objective: To detect the expressions of cyclooxygenase-2 (COX-2) and basic fibroblast growth factor (bFGF) and evaluate their value in predicting radiotherapy sensitivity in nasopharyngeal carcinoma (NPC).

Methods: The expressions of COX-2 and bFGF were detected immunohistochemically in biopsy samples from 97 NPC patients before radiotherapy, and their relationship with radiotherapy sensitivity was analyzed.

Results: The positive expression rates of COX-2 and bFGF in 97 NPC tissues were 71.1% (69/97) and 64.9% (63/97), respectively. COX-2 positivity was significantly correlated with T stage and N stage ($P<0.05$), while bFGF positivity was significantly correlated with N stage ($P<0.05$). The positive rates of COX-2 in radiotherapy-sensitive and radiotherapy-insensitive groups were 62.8% and 92.6%, respectively; the corresponding rates for bFGF were 57.1% and 85.2%. Spearman correlation analysis revealed a significant positive correlation between COX-2 and bFGF expression ($r=0.486$, $P<0.05$). Patients were divided into four subgroups based on COX-2 and bFGF expression: COX-2(-) and bFGF(-), COX-2(-) and bFGF(+), COX-2(+) and bFGF(-), and COX-2(+) and bFGF(+). Combined analysis showed that different subgroups were significantly associated with radiotherapy sensitivity ($P<0.05$).

Conclusion: COX-2 and bFGF are overexpressed in NPC tissues and both are significantly associated with tumor radiotherapy sensitivity, suggesting they may serve as important biomarkers for predicting radiotherapy sensitivity in NPC.

Keywords: nasopharyngeal carcinoma; COX-2; basic fibroblast growth factor; radiotherapy sensitivity

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Introduction

Nasopharyngeal carcinoma (NPC) is a common malignant tumor in Southern China with high malignancy and early lymph node metastasis [1-2]. Radiotherapy is the primary treatment modality for NPC. With advances in imaging and radiotherapy techniques, local and regional control rates have improved significantly [3-4]. However, a major clinical challenge is the lack of individualized radiotherapy dosing. The NCCN guidelines [5] recommend a uniform radiation dose of 68-70 Gy to the nasopharyngeal tumor for all stage II-VIb NPC patients receiving concurrent chemoradiotherapy. Nevertheless, different tumors exhibit varying radiosensitivity, and even within the same tumor type, individual radiosensitivity differs. Therefore, identifying radiotherapy-sensitive NPC patients is crucial for precision therapy [6-7].

Cyclooxygenase-2 (COX-2) is a key rate-limiting enzyme in prostaglandin (PGs) synthesis that is highly expressed in many tumor tissues. It participates in tumorigenesis and development through mechanisms including promoting cell proliferation, inhibiting apoptosis, and stimulating tumor angiogenesis, and is associated with tumor metastasis and radioresistance [8-10]. Our previous research found that basic fibroblast growth factor monoclonal antibody can enhance tumor chemosensitivity by reversing drug resistance in breast cancer cell lines through P-glycoprotein [11]. However, the combined effect of COX-2 and bFGF on NPC radiotherapy sensitivity has not been reported. This study investigates the expression of COX-2 and bFGF in pre-radiotherapy biopsy tissues from NPC patients and explores their impact on radiotherapy sensitivity to provide a basis for future clinical trials on individualized radiotherapy dosing in NPC.

Methods

1.1 Patient Population

We selected 97 NPC patients who received definitive radiotherapy at the First Affiliated Hospital of Jinan University between January 1995 and December 2010. All patients had pathologically confirmed poorly differentiated squamous cell carcinoma via nasopharyngeal biopsy before treatment. The cohort included 51 males and 46 females, aged 25-72 years with a median age of 49 years. Clinical staging was reassessed according to the 7th edition of the AJCC/UICC TNM staging system for NPC. This study was approved by the hospital ethics committee, and all patients provided informed consent.

1.2 Treatment Protocol

All patients completed full-course conventional radiotherapy using a 6 MV-X linear accelerator with 1.8-2.0 Gy per fraction, once daily, five days per week.

The nasopharyngeal region received a total dose of 70-74 Gy in 35-37 fractions, while metastatic cervical lymph nodes received 70-76 Gy in 35-37 fractions.

1.3 Evaluation of Short-term Efficacy

Three months after radiotherapy completion, nasopharyngoscopy, CT, or MRI examinations were performed to assess tumor regression in the nasopharynx and neck. Treatment response was evaluated according to RECIST criteria as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Patients with CR or PR were classified as the radiotherapy-sensitive group, while those with SD or PD (with persistent primary tumor or new lesions three months post-radiotherapy) were classified as the radiotherapy-insensitive group.

1.4 Reagents

Rabbit anti-human COX-2 monoclonal antibody (catalog no. ab62331) and rabbit anti-human bFGF polyclonal antibody (catalog no. ab16828) were purchased from Abcam (USA). The SP two-step detection kit and DAB chromogenic kit were obtained from Beijing Zhongshan Golden Bridge Biotechnology.

1.5 Immunohistochemical Staining

All paraffin-embedded tissues were sectioned at 4 m thickness. After dewaxing in xylene, rehydration through graded alcohols, and antigen retrieval using high-pressure citrate buffer, immunohistochemical staining was performed using the streptavidin-peroxidase (SP) method according to the manufacturer' s instructions. PBS was used as the negative control for primary antibodies.

1.6 Result Interpretation

COX-2 and bFGF immunostaining was considered positive when brown-yellow granules were observed in the cell membrane or cytoplasm with intensity above background nonspecific staining. Five high-power fields (×400) were selected, and 100 cells per field were counted. The average percentage of positive cells was calculated, and positivity was defined as >5% positive cells.

1.7 Statistical Analysis

Data were analyzed using SPSS 17.0 software. Chi-square test was used to compare rates between two groups. Spearman rank correlation analysis was applied to assess the correlation between COX-2 and bFGF expression. Kruskal-Wallis test was used to compare radiotherapy sensitivity among different subgroups based on combined COX-2 and bFGF expression. $P<0.05$ was considered statistically significant.

Results

2.1 High Expression of COX-2 and bFGF in NPC Tissues

Immunohistochemical results showed negative COX-2 and bFGF expression in normal nasopharyngeal mucosal epithelium. In 97 NPC tissues, the positive expression rates were 71.1% (69/97) for COX-2 and 64.9% (63/97) for bFGF, with 56.7% (55/97) showing positivity for both markers in the same patient specimens. COX-2 staining was primarily membranous and cytoplasmic, appearing as brown-yellow granules in tumor cell cytoplasm or linear distribution along the nuclear membrane. bFGF staining was mainly membranous, localized to tumor cell cytoplasm [Figure 1: see original paper].

2.2 Relationship Between COX-2/bFGF Expression and Clinical Characteristics

The relationship between COX-2 and bFGF expression and clinicopathological features is shown in Table 1 . COX-2 positivity was significantly correlated with T stage and N stage ($P<0.05$), while bFGF positivity was significantly correlated with N stage ($P<0.05$). Spearman correlation analysis revealed a significant positive correlation between COX-2 and bFGF expression ($r=0.486$, $P<0.05$).

2.3 Relationship Between COX-2/bFGF Expression and Radiotherapy Efficacy

Three months after completing radiotherapy, 35 patients achieved CR or PR and were classified as the radiotherapy-sensitive group, while 27 patients with SD or PD were classified as the radiotherapy-insensitive group. The relationship between marker expression and radiotherapy efficacy is shown in Table 2 . The positive rate of COX-2 was 62.8% in the sensitive group versus 92.6% in the insensitive group ($P<0.05$). The corresponding rates for bFGF were 57.1% and 85.2% ($P<0.05$). Combined analysis of COX-2 and bFGF expression revealed significant differences in radiotherapy sensitivity among the four subgroups ($P<0.05$).

Discussion

NPC exhibits distinct geographic distribution, with the highest incidence in Southern China, earning it the name "Cantonese cancer." It predominantly affects individuals aged 40-50 years [12]. Radiotherapy remains the primary treatment for NPC. The NCCN guidelines [5, 13] recommend a uniform dose of 68-70 Gy for all stage II-VIb patients receiving concurrent chemoradiotherapy. However, the clinical problem is the lack of individualized dosing. Patients with identical pathological types and clinical stages receiving the same standard treatment and radiation doses show vastly different outcomes, primarily due to

inherent differences in tumor radiosensitivity and the use of single-dose regimens [14-15]. Some NPC patients achieve long-term tumor control with only 40 Gy due to various factors [16-17], while others experience residual disease or recurrence despite receiving curative doses of 70-80 Gy [18]. Therefore, identifying radiotherapy-sensitive patients is essential for precision radiotherapy in NPC.

COX is a key rate-limiting enzyme in prostaglandin synthesis, comprising COX-1 and COX-2 isoforms. COX-1 is constitutively expressed in most normal human tissues, whereas COX-2 is an inducible enzyme not expressed under normal conditions but rapidly induced by external factors or endogenous stimuli (cytokines, endotoxins, interleukins, tumor promoters). COX-2 is highly expressed in many tumor tissues and participates in tumorigenesis and development through promoting cell proliferation, inhibiting apoptosis, and stimulating angiogenesis, thereby enhancing tumor invasion and metastasis [8, 19]. Elevated COX-2 expression in various tumors is associated with metastasis and radioresistance [8-10]. Inoue et al. [20] demonstrated that the COX inhibitor diclofenac reduced radiation-induced COX-2 and Ki-67 expression, enhancing radiosensitivity in prostate adenocarcinoma by inhibiting proliferation and promoting apoptosis. Additionally, COX-2 silencing altered radiobiological parameters in NPC cells, reduced radiation-induced G2/M arrest, and increased tumor suppression rates, thereby improving radiosensitivity [21]. In this study, the overall positive expression rate of COX-2 in NPC was 68%, which was closely associated with T stage and N stage. T stage represents primary tumor size and growth, confirming COX-2' s important role in tumor proliferation. N stage indicates regional lymph node metastasis, suggesting COX-2 may be involved in malignant progression of NPC. No significant correlation was observed between COX-2 expression and M stage or overall clinical stage, possibly due to the relatively small sample size requiring further expansion to reduce bias.

bFGF is a mitogenic polypeptide growth factor that promotes cell division, proliferation, and angiogenesis, playing crucial regulatory roles in embryonic development, differentiation, angiogenesis, and tissue repair. It is a major pro-angiogenic factor, and the bFGF/FGFR signaling pathway promotes tumor proliferation, angiogenesis, and drug resistance through PI3K/AKT, PLC /PKC, and Ras/Raf/MEK/ERK pathways. Studies have shown bFGF is highly expressed in various tumors (breast cancer, lung cancer, melanoma, hepatocellular carcinoma) and promotes tumorigenesis, development, invasion, and metastasis through neovascularization [22-25]. Our previous research found that bFGF monoclonal antibody can enhance chemosensitivity by reversing drug resistance in breast cancer cell lines via P-glycoprotein [11]. This study demonstrates that bFGF is positively expressed in NPC and significantly correlated with N stage. Spearman correlation analysis revealed a significant positive correlation between COX-2 and bFGF expression. This study is the first to demonstrate the close relationship between COX-2 and bFGF in NPC tissues.

Therefore, for NPC patients with positive COX-2 and bFGF expression, if we can predict radiotherapy insensitivity, treatment plans could be modified from

the outset by increasing radiation dose, adopting concurrent chemoradiotherapy, or using radiosensitizers to prevent distant metastasis and improve quality of life. Conversely, for patients with negative COX-2 and bFGF expression who are predicted to be radiosensitive, radiation dose could potentially be reduced after further evaluation, thereby decreasing toxic side effects, avoiding overtreatment, reducing late sequelae, and improving quality of life.

In summary, pre-treatment expression levels of COX-2 and bFGF in NPC patients may serve as predictive biomarkers for radiotherapy sensitivity, providing a reference for individualized clinical treatment of NPC.

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Figure and Table Legends

Figure 1. Representative immunohistochemical images showing the expressions of COX-2 and bFGF in normal nasopharyngeal mucosa and NPC tissues (Original magnification: ×400).

Table 1. Correlation of expressions of COX-2 and bFGF with clinicopathological features in NPC patients.

Table 2. Correlation of expressions of COX-2 and bFGF with the radiation response of NPC.

Note: Figure translations are in progress. See original paper for figures.