Role of TGF-β/Smad Signaling Pathway-Related Proteins in Clinicopathological Features and Prognosis and Survival of Patients with Nasopharyngeal Carcinoma

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Abstract: Nasopharyngeal carcinoma (NPC) is the most common cancer originating in the nasopharynx with high incidence of nasopharyngeal carcinoma. This study aims to identify possible prognostic factors of related proteins in the TGF-β/Smad signaling pathway in NPC. The expression of TGF-β1, TGF-βRI, TGF-βRII, TGF-β2, Smad4, Smad7 and RUNX3 in NPC tissues and nasopharyngitis tissues was detected. Besides, the association of TGF-β/Smad signaling pathway-related protein expressions and prognosis of NPC was analyzed. Initially, the NPC tissues showed higher expression of TGF-β1 and Smad7 and lower expression of Smad4, TGF-βRII, TGF-β2 and RUNX3. Meanwhile, the nonkeratinizing differentiated squamous cell carcinoma showed higher positive expression of TGF-βRI, patients in stage III-IV presented higher positive expression of TGF-β1 and Smad7, and patients with lymph node metastasis showed higher positive expression of TGF-β1 and Smad7. Furthermore, TGF-β1, TGF-β2 and Smad4 were independent factors for the prognosis of NPC. Our study suggests that NPC presents with up-regulated expression of positive expression rates of TGF-β1 and Smad7 and down-regulated TGF-β2, TGF-βRII, Smad4 and RUNX3, and additionally, TGF-β1, TGF-β2 and Smad4 are independent factors for the prognosis of NPC, which could be regarded as the novel target for the treatment of NPC.

Keywords: nasopharyngeal carcinoma; TGF-β; Smad; TGF-β/Smad signaling pathway; clinicopathological features; prognosis

1. Introduction

Nasopharyngeal carcinoma (NPC) ranks the 11th common cancer with high incidence in China, with the rate of approximately 2.8/100,000 among men and about 1.9/100,000 among women [1]. NPC has obvious geographical distribution characteristics, mostly occurred in the southern area of China [2]. Generally, NPC is caused by the risk factors of Epstein-Barr virus (EBV), genetic predisposition, smoking and alcohol [3]. Radiotherapy is the basic clinical treatment for patients with early NPC, and intensive modulated radiation therapy (IMRT) involved with a variety of systemic therapy has demonstrated a significant effect in recent years [4]. Although the local control rate of IMRT for NPC can reach up to 90%, the prognosis of NPC reduced considerably [5]. With further study of the regulation mechanism of NPC, a growing number of signaling pathways, such as the epidermal growth factor receptor (EGFR) signaling pathway [6] and oncprotein 18/stathmin signaling pathway [7] were disclosed, which would affect the development process of NPC and play a key regulatory role in NPC. Therefore, the signaling pathway for the regulation of NPC caused extensive concern.

Transforming growth factor β (TGF-β) is first found to induce phenotypic transformation in fibroblasts, and one study found that TGF-β exerts a key regulatory function on a variety of tumor cells and non-tumor cells [8]. TGF-β can inhibit the replication of normal epithelial cells and regulate epithelial cells for migration, differentiation and apoptosis; meanwhile, TGF-β can promote proliferation and growth of tumor cells [9]. The number of amino acids for Smad proteins is generally 400 to 500 with molecular weight of about 42 ~ 60 ku, and Smad proteins are the substrate for the extracellular kinase for TGF-β receptor yet discovered. Among which, smad4 in Smads family is a newly discovered tumor suppressor gene, and smad7 play a role in inhibition of TGF-β signal pathway [10]. A previous study showed that, some abnormalities in TGF-β signaling pathway, such as downstream abnormal signaling proteins Smads due to gene mutations or loss, were also proved to be an important mechanism related to carcinogenesis [11]. Therefore, this article intends to further investigate the role of TGF-β/Smad signaling pathway in clinicopathological features and prognosis of patients with NPC.
2. Material and methods

2.1. Ethical Statement

This study was carried out in conformity to medical ethical standards and was approved by Ethics Committee of Jilin Provincial People’s Hospital. Written informed consents were obtained from eligible patients.

2.2. Subjects

The archived paraffin blocks of biopsy specimens from 142 patients with pathologically diagnosed NPC in Jilin Provincial People’s Hospital between March 2009 and March 2011 were collected. There were 112 males and 30 females, aged from 19 to 78 (median age of 44.6) years old. Patients were enrolled into this study if they met the following criteria: 1, diagnosed with NPC through clinical symptoms, signs, computed tomography (CT) or magnetic resonance imaging (MRI), and histopathology; 2, with complete imaging data; 3, aged between 18 to 70 years old; 4, Karnofsky score not less than 70 points. Correspondingly, these patients were excluded: 1, patients receiving chemotherapy as well as radiotherapy sensitizers and radiation protective agent; 2, patients experienced surgery or had received radiotherapy or chemotherapy; 3, patients had a history of other tumors. Specimens were fixed in 10% formalin, embedded in paraffin and cut into 4 μm serial sections. According to the International histological classification criteria of World Health Organization (WHO) [12], specimens were classified as follows: the keratinizing squamous cell carcinoma (n = 20), non-differentiated keratinizing squamous cell carcinoma (n = 29) and non-keratinizing undifferentiated squamous cell carcinoma (n = 93). All cases had not received radiotherapy or chemotherapy before surgery. The clinical staging of NPC was based on American Joint Committee on Cancer (AJCC) staging system edited in 2002 [13] and the cases were classified as follows: stage I (n = 4), stage II (n = 30), stage III (n = 56) and stage IV (n = 52). A total of 54 cases with nasopharyngitis confirmed by biopsy over the same period were randomly selected for control group. Among which there were 40 males and 14 females, aged between 30 ~ 70 years old, with mean age of (44.2 ± 9.1) years old and the median age of 48 years old. There was no significant difference of gender and age between two groups (both p > 0.05).

2.3. Immunohistochemical Staining

Immunohistochemical staining (streptavidin-peroxidase (SP) method) was applied to detect the expression of TGF-β1, TGF-βRI, TGF-βRII, TGF-β2, Smad4, Smad7 and RUNX3 in nasopharyngitis tissues and NPC tissues. Antigen retrieval and SP staining were performed under instructions of an immunohistochemistry kit (purchased from Fuzhou Maixin Biotech. Co., Ltd, Fuzhou, China). The known NPC slice was used as a positive control, with phosphate buffer saline (PBS) as a negative control instead of primary antibody.

The determination of results: brown particles occurred inside the nucleus and (or) the cytoplasm for Smad4 and TGF-BRI as positive, brown coloration occurred for TGF-β1, TGF-βRII, TGF-β2, Smad7, and RUNX3 inside membrane and (or) cytoplasm as positive. Staining results were determined based on each slice with five high-power fields, where positive cells were calculated within 500 cells, marked (-) for number of positive cells < 20% and (+) for ≥ 20%. The results determined by two independent pathologists, or decided by senior pathologist in case of dispute.

2.4. Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Tissues or peripheral blood cells in 1 mL of Trizol homogenizing tube, were placed on ice, with ethylene diamine tetraacetic acid (EDTA) used as anticoagulant. Total RNA isolation was performed with RNA extraction tool according to the instructions of reagent (Shanghai Huashun Biotechnology Company, Shanghai, China). RNA was reversely transcribed at 42°C for 60 min using 20 units of avian myeloblastosis virus (AMV) reverse transcriptase (BBI), 0.5 ng oligosaccharides (dT) 12–18 primer, 0.5 mM nucleotides and 20 units of ribonuclease (20 μL). The sample was heated for 10 min and then the reaction was terminated when the temperature was up to 70°C. After reverse transcription, the reaction system (20 μL) consisted of 1 × PCR reaction buffer, 0.2 mM nucleotides, and 0.5 μM primers (Table 1) and 1 unit of DNA polymerase. The reaction conditions for TGF-βRII, Smad4 and Smad7 were as follows: at 95°C for 30 s, cooling to 55°C for another 30 s, and finally warmed to 72°C for 45 s. The reaction cycle for TGF-βRII was set to 30, and for Smad4 and Smad7 were 28. The reaction conditions for TGF-β1 and RUNX3 were as follows: pre-denaturation at 95°C for 30 s, 45 cycles of cooling at 95°C for another 5 s, and finally warmed to 60°C for 30 s. The reaction conditions for TGF-β2 and TGF-βRI were as follows: pre-denaturation at 95°C for 3 min, 45 cycles of denaturation at 94°C for 50 s, annealing at 60°C for 50 s, and extension at 72°C for 50 s. Finally, all the proteins was extended at 72°C for 10 min. PCR products were visualized by 2% agarose gel electrophoresis containing ethidium bromide as fluorescent dye.
Table 1. The primer sequences for RT-qPCR.

| Gene      | Primer sequence (5'-3') |
|-----------|-------------------------|
| TGF-β1    | F: TGG CGA TAC CTC AGC AAC |
|           | R: CTC GTG GAT CCA CTG AGT CC |
| TGF-βRI   | F: ACC TTC TGA TCC ATC CCT T |
|           | R: CCC AAA GCT GTC AGC CTAG |
| TGF-βRII  | F: AGC AAC TGC AGC ATC ACC TC |
|           | R: TGA TGT CTG AGA AGA TGT CC |
| TGF-β2    | F: CTG CAT TTG CAA GAC TTT AC |
| Smad4     | F: GCA TCG ACA GAG ACA TAC AG |
|           | R: CAA CAG TAA CAA TAG GGC AG |
| Smad7     | F: ACC GCA GCA GTG ACC CCA TCT T |
|           | R: GCC TAC CGG CTG TTG AA |
| RUNX3     | F: TTA CGA GGG GCG GTC GTA GCA GGG |
| β-actin   | F: ACC ACA GTC CAT GCC ATC AC |
|           | R: TCC ACC ACC CTG TTG CTG TA |

Notes: RT-qPCR, reverse transcription quantitative polymerase chain reaction; TGF-β, transforming growth factor β; F, forward; R, reverse.

2.5. Follow-up

Follow-up was started when the treatment completed. The follow-up, performed every 3 months in the first two years of follow-up, included checking, mail and telephone interview. By February 31, 2016, no patients were lost to follow-up. Records of survival condition of each patient were recorded during the follow-up period. All patients had completed clinical data and five-year follow-up records.

2.6. Statistical Analysis

SPSS 21.0 software package (IBM Corp., Armonk, New York, USA) was used for statistical analysis of experimental data. The χ2 test and Fisher's exact probability in 2 × 2 tables were used for the differences of TGF-β/Smad signaling pathway-related proteins expression. Spearman rank correlation analysis was used for correlation analysis with \( p < 0.05 \) indicated a significant difference. For univariate analysis, Kaplan-Meier survival curve was used to analyze the relationship between TGF-β/Smad signaling pathway-related proteins expression and prognosis of NPC, with log-rank test for the difference of survival curves. COX proportional hazard model was also used for multivariate survival analysis.

3. Results

3.1. The Positive Protein Expression of TGF-β1 and Smad7 was Increased while that of Smad4, TGF-βRII, TGF-β2 and RUNX3 was Decreased in NPC tissues

Immunohistochemical staining was conducted to examine the protein expression of TGF-β/Smad signaling pathway-related proteins in tissues. The results showed that, TGF-βRII expressed mainly in the cytoplasm and (or) nucleus, TGF-βRII expressed mainly in the membrane and (or) cytoplasm, Smad4 protein expressed in the cytoplasm and (or) within the nucleus, TGF-β2, Smad7 and RUNX3 expressed in the cytoplasm with positive signals localized in the cytoplasm. The difference of positive expression rates of TGF-βRII between NPC and nasopharyngitis tissues were not statistically significant (\( p > 0.05 \)). However, the positive expression rates of TGF-β1 and Smad7 in the NPC tissues were higher than that in the nasopharyngitis tissues, and the positive expression rate of Smad4, TGF-βRII, TGF-β2 and RUNX3 in the NPC tissues were significantly lower than those in the nasopharyngitis tissues (all \( p < 0.05 \)) (Table 2 and Figure 1). The results above revealed that higher positive protein expression of TGF-β1, Smad7 and lower that of Smad4, TGF-βRII, TGF-β2 and RUNX3 were found in NPC tissues.
Figure 1. NPC tissues show higher positive protein expression of TGF-β1 and Smad7 and lower Smad4, TGF-βRII, TGF-β2 and RUNX3 (SP × 400). Notes: Panel A, positive expression of TGF-β1; Panel B, positive expression of Smad7; Panel C, negative expression of TGF-β2; Panel D, negative expression of RUNX3; Panel E, negative expression of TGF-βRI; Panel F, negative expression of TGF-βRII; Panel G, negative expression of Smad4. NPC, nasopharyngeal carcinoma; TGF-β, transforming growth factor β.

Table 2. The protein expression of TGF-β/Smad signaling pathway-related proteins between NPC tissues and nasopharyngitis tissues.

| Factor   | Result | NPC tissues (n = 142) | Nasopharyngitis tissues (n = 54) | χ²  | p    |
|----------|--------|-----------------------|----------------------------------|-----|------|
| TGF-β1   | Positive | 90                    | 12                               | 26.550 | <0.001 |
|          | Negative | 52                    | 42                               | 26.550 | <0.001 |
| TGF-βRI  | Positive | 101                   | 32                               | 2.526  | 0.112 |
|          | Negative | 41                    | 22                               | 2.526  | 0.112 |
| TGF-βRII | Positive | 92                    | 43                               | 4.020  | 0.045 |
|          | Negative | 50                    | 11                               | 18.08  | 0.045 |
| TGF-β2   | Positive | 100                   | 50                               | 10.710 | 0.001 |
|          | Negative | 42                    | 4                                | 18.08  | 0.001 |
| Smad4    | Positive | 98                    | 46                               | 5.249  | 0.022 |
|          | Negative | 44                    | 8                                | 18.08  | <0.05 |
| Smad7    | Positive | 54                    | 6                                | 13.340 | <0.001 |
|          | Negative | 88                    | 48                               | 18.08  | <0.05 |
| RUNX3    | Positive | 80                    | 49                               | 20.580 | <0.001 |
|          | Negative | 62                    | 5                                | 18.08  | <0.05 |

Notes: TGF-β, transforming growth factor β; NPC, nasopharyngeal carcinoma.

3.2. NPC Tissues Show Higher mRNA Expression of TGF-β1 and Smad7, and Lower that of Smad4, TGF-βRII, TGF-β2 and RUNX3

Next, RT-qPCR was conducted to examine the mRNA expression of TGF-β/Smad signaling pathway-related proteins in tissues. The mRNA expression of TGF-β1 and Smad7 in the NPC tissues was significantly higher than those in the nasopharyngitis tissues (p < 0.01). The mRNA expression of TGF-βRI between the NPC and nasopharyngitis tissues was not statistically significant (p > 0.05). The mRNA expression of TGF-βRII, TGF-β2, Smad4 and RUNX3 in the NPC tissues was lower than that in the nasopharyngitis tissues (all p < 0.05) (Figure 2). Therefore, the higher mRNA expression of TGF-β1 and Smad7 and lower that of Smad4, TGF-βRII, TGF-β2 and RUNX3 were found in the NPC tissues.
Figure 2. NPC tissues show higher mRNA expression of TGF-β1 and Smad7, and lower Smad4, TGF-βRII, TGF-β2 and RUNX3. Note: NPC, nasopharyngeal carcinoma; TGF-β, transforming growth factor β.

3.3. NPC is Relevant to the Histological Classification, the Clinical Staging and Lymph Node Metastasis

Subsequently, results of examining the relationship between TGF-β/Smad signaling pathway and clinicopathological features of NPC showed that no significant difference was found in the positive expression rates of TGF-β1, TGF-βRI, TGF-βRII, TGF-β2, Smad4, Smad7 and RUNX3 in NPC patients with different gender and age (all p > 0.05). The positive expression rate of TGF-βRI in non-keratinizing differentiated squamous cell carcinoma was significantly lower than that in non-keratinizing undifferentiated squamous cell carcinoma (p < 0.05). The positive expression rates of TGF-β1 and Smad7 in patients in stage III-IV were significantly higher than patients in stage I-II (both p < 0.05). The positive expression rate of Smad4 in patients without lymph node metastasis was significantly higher than those with lymph node metastasis (p < 0.05). The positive expression rates of TGF-β1 and Smad7 in patients with lymph node metastasis were higher than those without lymph node metastasis (all p < 0.05) (Table 3). All these results suggested that NPC was related to the histological classification, the clinical staging and lymph node metastasis.

3.4. The Prognostic Survival is Related to TGF-β1, TGF-β2, Smad4 and Smad7

Follow-up was performed to observe the association of TGF-β/Smad expression and prognosis of NPC patients. The total five-year survival rate of patients was 47.8%. There were significant differences for the five-year survival rate in positive cases of TGF-β1, TGF-β2, Smad4 and Smad7 when compared with those negative cases (all p < 0.05). However, no significant difference was found for the five-year survival rate between the positive cases of TGF-βRI, TGF-βRII and RUNX3 and those negative cases (all p > 0.05) (Figure 3). Therefore, TGF-β1, TGF-β2, Smad4 and Smad7 were related to the prognosis of patients in NPC.

Figure 3. The prognostic survival of NPC is related to TGF-β1, TGF-β2, Smad4 and Smad7. Notes: Panel A, curve for TGF-β1; Panel B, curve for TGF-βRI; Panel C, curve for TGF-βRII; Panel D, curve for TGF-β2; Panel E, curve for Smad4; Panel F, curve for Smad7; Panel G, curve for RUNX3. NPC, nasopharyngeal carcinoma; TGF-β, transforming growth factor β.
Table 3. The relationship between TGF-β/Smad expressions and clinicopathological features in NPC tissues.

| Features                              | TGF-β1 | TGF-βRI | TGF-βRII | TGF-β2 | Smad4 | Smad7 | RUNX3 |
|---------------------------------------|--------|---------|----------|--------|-------|-------|-------|
|                                       | +     | -       | +        | -      | +     | -     | +     | -     | +     | -     | +     | -     | +     | -     | P     |
| Gender                                |       |         |          |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
| Male                                  | 0.995 | 0.544   | 0.808    | 0.955  | 0.754 | 0.802 | 0.229 |
| Female                                | 0.779 | 0.931   | 0.147    | 0.512  | 0.754 | 0.922 | 0.897 |
| Gender (years)                        |       |         |          |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
| < 45                                  | 0.052 | 0.028   | 0.580    | 0.978  | 0.505 | 0.415 | 0.698 |
| ≥45                                   | 0.779 | 0.931   | 0.147    | 0.512  | 0.754 | 0.922 | 0.897 |
| Pathological type                     |       |         |          |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
| Nonkeratinizing Squamous Cell Carcinoma | 0.960 | 0.420   | 0.225    | 0.217  | 0.698 | 0.165 | 0.166 |
| Nonkeratinizing undifferentiated squamous cell carcinoma | 0.024 | 0.937   | 0.991    | 0.981  | 0.054 | 0.046 | 0.046 |
| Nonkeratinizing differentiated squamous cell carcinoma | 0.034 | 0.530   | 0.266    | 0.516  | 0.001 | 0.001 | 0.151 |
| Skull base encroachment               |       |         |          |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
| No                                    | 0.960 | 0.420   | 0.225    | 0.217  | 0.698 | 0.165 | 0.166 |
| Yes                                   | 0.024 | 0.937   | 0.991    | 0.981  | 0.054 | 0.046 | 0.046 |
| Clinical stage                        |       |         |          |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
| I+II                                  | 0.034 | 0.530   | 0.266    | 0.516  | 0.001 | 0.001 | 0.151 |
| III+IV                                | 0.960 | 0.420   | 0.225    | 0.217  | 0.698 | 0.165 | 0.166 |
| Lymph node metastasis                 |       |         |          |        |       |       |       |       |       |       |       |       |       |       |       |       |       |
| No                                    | 0.024 | 0.937   | 0.991    | 0.981  | 0.054 | 0.046 | 0.046 |
| Yes                                   | 0.034 | 0.530   | 0.266    | 0.516  | 0.001 | 0.001 | 0.151 |

Notes: TGF-β, transforming growth factor β; NPC, nasopharyngeal carcinoma.
3.5. TGF-β1, TGF-β2 and Smad4 are Independent Factors for the Prognosis of NPC

COX proportional hazard model was applied to multivariate survival analysis for the prognosis of NPC patients. The clinicopathological features, including TGF-β1, TGF-βRII, TGF-βRII, TGF-β2, Smad4, Smad7, RUNX3, clinical stage and lymph node metastasis, were included into COX proportional hazard model. The results showed that TGF-β1, TGF-β2 and Smad4 were independent factors for the prognosis of NPC (all $p < 0.05$) (Table 4).

| Factor                | B    | SE   | Wald | Sig.  | Exp (B) | 95% CI |
|-----------------------|------|------|------|-------|---------|--------|
| TGF-β1                | 0.826| 0.347| 5.682| 0.017 | 2.284   | 1.158–4.505 |
| TGF-βRII              | 0.344| 0.284| 1.468| 0.226 | 1.410   | 0.809–2.459 |
| TGF-βRII              | 0.138| 0.264| 0.271| 0.603 | 1.147   | 0.683–1.927 |
| TGF-β2                | −1.071| 0.250| 18.295| 0.001 | 0.343   | 0.210–0.560 |
| Smad4                 | −2.584| 1.186| 4.744| 0.029 | 0.076   | 0.007–0.772 |
| Smad7                 | −0.319| 0.582| 0.301| 0.583 | 0.727   | 0.232–2.273 |
| RUNX3                 | −0.187| 0.242| 0.595| 0.440 | 0.830   | 0.516–1.334 |
| Clinical stage        | 0.371| 0.272| 1.860| 0.173 | 1.449   | 0.850–2.470 |
| Lymph node metastasis | −0.120| 0.335| 0.127| 0.721 | 0.887   | 0.460–1.711 |

Notes: TGF-β, transforming growth factor β; NPC, nasopharyngeal carcinoma; B, regression coefficient; S.E, standard error; OR, odd ratio; CI, confidence interval.

4. Discussion

NPC is a serious threat to human health, and most NPC are poorly differentiated, which prone to easy invasion and metastasis [14]. Currently, IMRT is widely used in the treatment for NPC, which significantly improve life of quality and survival rate of NPC patients. However, researchers found that the relapse and metastasis of NPC after treatment occurred in a certain proportion of patients [15]. Therefore, researchers try to reveal the potential mechanism of NPC on the genetic level, in order to provide new ideas for the treatment of NPC and improve the cure rate of NPC.

The study found that the expression of TGF-β1 and Smad7 have an increase tendency in NPC patients. TGF-β1 is one of the three isoforms from TGF-β superfamily, mainly expressed in the immune system, and the cancer cells may overproduce TGF-β as well as induce TGF-β production of peripheral tumor in microenvironment, which indicated that TGF-β1 expression would be increased in NPC [16]. In addition, TGF-β1 would play a dual role in tumor development process, which not only play a role in the inhibition of tumor cell proliferation, but also induce apoptosis of liver cells, bone marrow cells, and epithelial cells [17]. The study concentrating on the mechanism of TGF-β1 in colorectal cancer showed that, TGF-β1 produced by tumor cells can cause reduced permeability of antigen presenting cell, thereby increasing the degree of malignancy of the tumor and significantly reducing patient survival rate, which is consistent with the results of our study [18]. Smad7 is an inhibitory factor of TGF-β signaling pathway, abnormal expression of which can lead to TGF-β signaling pathway disorder, resulting in a variety of diseases. For example, Smad7 exhibit low expression in normal epithelial tissues, whereas in human pancreatic cancer, Smad7 expression was significantly increased; all of which is consistent with the results of this experiment [19]. The study found that both TGF-β1 and Smad7 expression in NPC have shown up-regulation in tumor cells, which demonstrate their synergistic promotion role in tumor development.

The study also found that, RUNX3, TGF-β2, and Smad4 expressions in the NPC tissues were lower than those in the nasopharyngitis tissues. Smad4 is one of the independent prognostic factors of NPC, and low expression of Smad4 is indicated of poor prognosis. TGF-βRII is one of the TGF-β1 receptors on the other hand. The researchers found the fact that the cancer cells become insensitive to TGF-β was related to reduced expression or inactivation of TGF-βRII in tumor cell surface, and the deletion or inactivation of its expression can promote tumor proliferation, invasion and metastasis [20]. Tumor suppressor protein RUNX3 is downstream transcription factor, and RUNX3 protein expression coded by RUNX3 gene was significantly down-regulated in NPC, with a suggestion that RUNX3 may be a protective factor in NPC; additionally, RUNX3 inactivation is a reflection of weakened defense force as well, RUNX3 and TGF-β2 expressions in NPC were positively correlated, which indicated that these two factors may have a synergistic effect on the progress of NPC as a protective factor [21]. Smad gene family located on chromosome 18q21, and Smad proteins are essential regulatory factor in TGFβ signaling pathway [22]. Researchers who also detected low expression of Smad4 in colorectal cancer had proposed low Smad4 expression would inhibit tumor growth pathways by blocking TGF-β, so as to promote the development and progression of colorectal cancer; in addition, low expression of Smad4 was independent of tumor invasion and metastasis promoted by TGF-β, which also suggesting that Smad4 was an independent prognostic factor for NPC [23]. Furthermore, studies about the prognostic value of Smad4 expressions in colorectal cancer showed that cancer with metastasis have lower expression of Smad4, and low expression of Smad4 was the main reason for the
metastasis of colorectal cancer [24,25].

In summary, the findings of this study suggest that TGF-β1 and Smad7 expression were up-regulated and the expressions of TGF-β2, TGF-βRII, Smad4 and RUNX3 were down-regulated in NPC. Besides, TGF-β1, TGF-β2 and Smad4 were independent factors in the prognosis of NPC. Thus, up-regulation of TGF-β2 and Smad4 expressions and down-regulation of TGF-β1 expression could be an effective way for NPC treatment. Yet without adequate experimental evidence, the experimental results for the clinical significance remain to be confirmed.

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**References**

1. Cao SM, Simons MJ, Qian CN. The prevalence and prevention of nasopharyngeal carcinoma in China. *Chinese Journal of Cancer*, 2011, 30: 114–119.

2. Xu ZJ, Zheng RS, Zhang SW, Zou XN, Chen WQ. Nasopharyngeal carcinoma incidence and mortality in China in 2009. *Chinese Journal of Cancer*, 2013, 32: 453–460.

3. Li Z, An L, Li H, Wang S, Zhou Y, et al. Identifying novel genes and chemicals related to nasopharyngeal cancer in a heterogeneous network. *Scientific Reports*, 2016, 6: 25515.

4. Su SF, Han F, Zhao C, Huang Y, Chen CY, et al. Treatment outcomes for different subgroups of nasopharyngeal carcinoma patients treated with intensity-modulated radiation therapy. *Chinese Journal of Cancer*, 2011, 30: 565–573.

5. Sun Y, Tang LL, Chen L, Li WF, Mao YP, et al. Promising treatment outcomes of intensity-modulated radiation therapy for nasopharyngeal carcinoma patients with N0 disease according to the seventh edition of the AJCC staging system. *BMC Cancer*, 2012, 12: 68.

6. Ruan L, Li XH, Wan XX, Yi H, Li C, et al. Analysis of EGFR signaling pathway in nasopharyngeal carcinoma cells by quantitative phosphoproteomics. *Proteome Science*, 2011, 9: 35.

7. Lin X, Tang M, Tao Y, Li L, Liu S, et al. Epstein-Barr virus-encoded LMP1 triggers regulation of the ERK-mediated Op18/STAT3 signaling pathway in association with cell cycle. *Cancer Science*, 2012, 103: 993–999.

8. Principe DR, DeCant B, Staudacher J, Vitello D, Mangan RJ, et al. Loss of TGFβ signaling promotes colon cancer progression and tumor-associated inflammation. *Oncotarget*, 2017, 8: 3826–3839.

9. Kamato D, Burch ML, Piva TJ, Rezaei HB, Rostam MA, et al. Transforming growth factor-beta signalling: role and consequences of Smad linker region phosphorylation. *Cellular Signalling*, 2013, 25: 2017–2024.

10. Singh P, Wig JD, Srinivasan R. The Smad family and its role in pancreatic cancer. *Indian Journal of Cancer*, 2011, 48: 351–360.

11. Syed V. TGF-beta Signaling in Cancer. *Journal of Cellular Biochemistry*, 2016, 117: 1279–1287.

12. Gao Y, Zhu Y, Huang X, Wang H, Huang X, et al. Transsudodenal ampullectomy provides a less invasive technique to cure early ampullary cancer. *BMC Surgery*, 2016, 16: 36.

13. Chen P, Li B, Zhu Y, Chen W, Liu X, et al. Establishment and validation of a prognostic nomogram for patients with resectable perihilar cholangiocarcinoma. *Oncotarget*, 2016, 7: 37319–37330.

14. Lo YL, Pan WH, Hsu WL, Chien YC, Chen JY, et al. Partial Least Square Discriminant Analysis Discovered a Dietary Pattern Inversely Associated with Nasopharyngeal Carcinoma Risk. *Plos One*, 2016, 11, e0155892.

15. Wang W, Feng M, Fan Z, Li J, Lang J. Clinical outcomes and prognostic factors of 695 nasopharyngeal carcinoma patients treated with intensity-modulated radiotherapy. *BioMed Research International*, 2014, 2014: 814948.

16. Wang R, Tan Y, Wang X, Ma L, Wang D, et al. Prognoses and long-term outcomes of nasopharyngeal carcinoma in Han and Uyghur patients treated with intensity-modulated radiotherapy in the Xinjiang Autonomous Region of China. *Plos One*, 2014, 9: e111145.

17. Wang H, Li C, Jian Z, Ou Y, Ou J. TGF-beta1 Reduces miR-29a Expression to Promote Tumorigenicity and Metastasis of Cholangiocarcinoma by Targeting HDAC4. *Plos One*, 2015, 10: e0136703.

18. Liu N, Jiao T, Huang Y, Liu W, Li Z, et al. Hepatitis B virus regulates apoptosis and tumorigenesis through the microRNA-15a-Smad7-transforming growth factor beta pathway. *Journal of Virology*, 2015, 89: 2739–2749.

19. Singh P, Wig JD, Srinivasan R, Radotra BD. A comprehensive examination of Smad4, Smad6 and Smad7 mRNA expression in pancreatic ductal adenocarcinoma. *Indian Journal of Cancer*, 2011, 48: 170–174.

20. Javle M, Li Y, Tan D, Dong X, Chang P, et al. Biomarkers of TGF-beta signaling pathway and prognosis of pancreatic cancer. *Plos One*, 2014, 9: e85942.

21. Huang C, Lin P, Wang J, Huang Z. [Differential gene expression profiling for identification of protective transcription factors in different subtypes of nasopharyngeal carcinoma]. *Nan Fang Yi Ke Da Xue Xue Bao. Journal of Southern Medical University,*
22. Bennett J, Cassidy H, Slattery C, Ryan MP, McMorrow T. Tacrolimus Modulates TGF-beta Signaling to Induce Epithelial-Mesenchymal Transition in Human Renal Proximal Tubule Epithelial Cells. *Journal of Clinical Medicine*, 2016, 5: 50.

23. Isaksson-Mettavainio M, Palmqvist R, Forssell J, Stenling R, Oberg A. SMAD4/DPC4 expression and prognosis in human colorectal cancer. *Anticancer Research*, 2006, 26: 507–510.

24. Cheng D, Zhao S, Tang H, Zhang D, Sun H, et al. MicroRNA-20a-5p promotes colorectal cancer invasion and metastasis by downregulating Smad4. *Oncotarget*, 2016, 7: 45199–45213.

25. Wang C, Zhou Y, Ruan R, Zheng M, Han W, et al. High expression of COUP-TF II cooperated with negative Smad4 expression predicts poor prognosis in patients with colorectal cancer. *International Journal of Clinical and Experimental Pathology*, 2015, 8: 7112–7121.