Original Article

Expression and clinical significance of FGFR1 and FGFR2 in laryngeal squamous cell carcinoma

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Background: Fibroblast growth factor receptor 1 (FGFR1) and fibroblast growth factor receptor 2 (FGFR2) may be of significance in the development of laryngeal squamous cell carcinoma (SCC) tissues. Examination of the expression results of these factors may offer new insights into treatment of the disease, such as genetic and histological targeted target therapy.

Methods: We selected tissue from 30 cases of laryngeal SCC, 23 cases of adjacent normal mucosa, and 26 cases of benign laryngeal mucosal tissues from patients who received surgery at the Otolaryngology Department of the Affiliated Hospital of Chengde Medical College between September 2020 and January 2022. The laryngeal cancers included nine cases of supraglottic, 20 glottic (vocal cord), and one case of subglottic cancer, while all benign laryngeal mucosal lesions were obtained from vocal cord polyps. The expression of FGFR1 and FGFR2 was detected in 30 laryngeal cancers, 23 adjacent normal mucosa, and 26 vocal cord polyps by immunohistochemical technology [immunohistochemistry (IHC)], and the correlation analysis of their expression in laryngeal cancer was performed. $P<0.05$ was represented statistically significant.

Results: The expression of FGFR1 and FGFR2 was significantly different in laryngeal SCC and the normal tissue >0.5 cm from the tumor margin ($P<0.05$), and between laryngeal SCC and vocal polyps ($P<0.05$). There was no difference in FGFR1 and FGFR2 expression ($P>0.05$) between normal mucosal margins and vocal cord polyp tissue, and no correlation between FGFR1 and FGFR2 in laryngeal SCC and sex, age, smoking history, alcohol consumption history, tumor diameter, tumor lymph node metastasis, tumor differentiation degree, and Tumor-Node-Metastasis (TNM) stage ($P>0.05$), A moderate positive correlation between FGFR1 expression and FGFR2 expression in laryngeal SCC was seen ($Rs=0.499$, $P<0.01$).

Conclusions: FGFR1 and FGFR2 may participate in the occurrence of SCC of the throat: (I) positive FGFR1 and FGFR2 expressions are not associated with gender, age, smoking history, alcohol consumption history, tumor diameter, lymph node metastasis, degree of differentiation, or TNM stage. (II) FGFR2 increases successively with higher FGFR1 expression and with a positive correlation in laryngeal SCC.

Keywords: Fibroblast growth factor receptor 1 (FGFR1); fibroblast growth factor receptor 2 (FGFR2); laryngeal squamous cell carcinoma

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Introduction

Malignant tumors occurring in the human larynx are commonly seen in otolaryngology head and neck surgery. Of these, 85–95% are classified as squamous cell carcinoma (SCC) (1), with other pathologic types extremely rare. Throat cancer is the second largest respiratory tumor after lung cancer (2), and accounts for approximately 1.5% of all cancers in adults (1). A report shows the incidence in China has gradually decreased in recent years, and is mainly concentrated in men aged 40 years, increasing with age (3). The urban incidence rate is higher and is closely related to environmental pollution (4). Surgery remains the most effective treatment, and the application of CO_2 laser cautery is widely popular at home and abroad because of its advantages of small trauma and quick recovery. This method is not only widely used in the treatment of glottic laryngeal cancer (5), but also in the treatment of supraglottic laryngeal cancer (6). Adjuvant chemoradiotherapy and postoperative rehabilitation treatment are also indispensable, and it is worth mentioning that an increasing number of a study show postoperative psychosocial factors in patients with advanced laryngeal cancer such as family support, communication, language clarity, and work and family relationships all help to improve postoperative survival (7). While targeted drug therapy has become a research boom in recent years, cytoimmunological diagnostic markers for laryngeal cancer have not been found. Therefore, discussion of its basic cellular immunology is extremely important.

Fibroblast growth factor receptor1 (FGFR1) and fibroblast growth factor receptor 2 (FGFR2) are members of the fibroblast growth factor (FGF) receptor family and belong to the transmembrane polypeptide tyrosine kinase, which produce different isoforms due to alternative splicing. When FGFR1 and FGFR2 are dimerized with specific ligand FGF, autophosphorylation will occur to produce a cascade, activating downstream channel conduction and participating in embryonic development, cell differentiation, nerve regeneration, wound healing, and other functions. Abnormal signaling can cause disease and is closely related to multiple tumorigenesis. FGFR1 and FGFR2 are widely studied in breast cancer (8), bladder cancer (9), lung cancer (10), and interstitial sarcoma (11), but rarely in laryngeal and hypopharyngeal cancers. This study involves examined the expression and clinical significance of FGFR1 and FGFR2 in laryngeal SCC, to provide new ideas in its pathogenesis and gene and molecular targeted therapy.

In this experiment, FGFR1 and FGFR2 expression were measured by immunohistochemistry (IHC). The specimens included were the carcinoma tissue of 30 laryngeal SCC patients admitted for parallel surgery for the first time, their corresponding 23 adjacent normal mucosa tissue, and vocal cord polyp tissue from 26 other patients. Correlation between FGFR1 and FGFR2 was explored through correlation analysis. We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1936/rc).

Methods

Object of study

General clinical data

Thirty cases of throat SCC tissue and its corresponding 23 cases of adjacent normal mucosa tissue were obtained from patients operated on in the Otolaryngology Department of the Affiliated Hospital of Chengde Medical College between September 2020 to January 2022. There were nine cases of supraglottic portion, 20 of glottic portion, and one of infraglottic portion, and the corresponding adjacent normal tissues were >0.5 cm away from the tumor margin. Another 26 cases with benign laryngeal mucosa were used as control, all of which were vocal cord polyps. The general clinical characteristics are shown in *Table 1*. Tumor-Node-Metastasis (TNM) staging according to the eighth edition of laryngeal cancer staging criteria was used. The experiment was approved by the Ethics Committee of the Affiliated Hospital of Chengde Medical College (ethical No. CYFYLL2022163), and all patients signed the informed consent form. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Inclusion criteria

(I) Laryngeal cancer tissues were confirmed as SCC by pathological histology at the Affiliated Hospital of Chengde Medical College, and adjacent normal tissues and benign laryngeal mucosa lesions were diagnosed as normal laryngeal mucosa or inflammatory lesions by the pathology department;
(II) None of the included cancer patients had a preoperative history of radiation, chemical, or other related treatment;
(III) No patients included in the study had a history of other malignancies;
(IV) Data on the medical records of all patients were intact

Table 1 General clinical data of patients

Clinical characteristics	Cases (n=30), n (%)
Gender	
Male	29 (96.67)
Female	1 (3.33)
Age (years)	
≥60	19 (63.33)
<60	11 (36.67)
Smoking	
Yes	28 (93.33)
No	2 (6.67)
Smoking quantity (year × No./d)	
≥400	24 (80.00)
<400	6 (20.00)
Drinking	
Yes	22 (73.33)
No	8 (26.67)
Tumor diameter (cm)	
≥3	10 (33.33)
<3	20 (66.67)
Lymphatic metastasis	
Yes	4 (13.33)
No	26 (86.67)
Degree of differentiation	
High and medium	28 (93.33)
Low	2 (6.67)
TNM staging	
I, II	23 (76.67)
III, IV	7 (23.33)

TNM, Tumor-Node-Metastasis.

during admission.

Exclusion criteria

(I) The pathological and histological diagnosis results of the patient were unknown;
(II) The pathological specimens of the patients had two or more histological credit types;
(III) Tissue was shed during the staining process or the tissue materials were incomplete.

Table 2 Experimental instruments

Company	Experimental instrument
Shanghai Kehuai	YD-12P intelligent environmental-friendly biological tissue dehydrator
Jinhua Kodi, Zhejiang	KD-BMII computer biological tissue embedding
	KD-BLIV cryogenic table of embedding machine
	KD-1508A rotary slicing machine
Hubei Taikang	TKY-TPA pathological tissue drift sheet instrument
	TKY-KPC pathological tissue oven instrument
NIKON	Biological microscope
Thermo	Transfer liquid gun
Sitai Laboratory Equipment	Glass slide and cover slide

Research method

Experimental instruments

In the whole process of the experiment, related professional experimental instruments and equipment are involved, information on the main instruments and their production companies is shown in *Table 2*. Except for the glass slides and cover slides purchased by the author, the other experimental instruments were provided by the central laboratory of the South Branch area of the Affiliated Hospital of Chengde Medical College.

Experimental reagents

The information of experimental reagents applied during the experiment is shown in *Table 3*, mainly including primary antibodies and 5 reaction reagents. Among them, all the primary antibodies used in the experiments were the rabbit anti-human polyclonal antibodies.

Experimental methods and steps

IHC staining was performed using the SP method. The expression of FGFR1 and FGFR2 in laryngeal SCC, adjacent normal mucosa margin, and vocal cord polyp tissue was detected, and the FGFR1 antibody was diluted at a concentration of 1:250 and FGFR2 antibody at 1:450. Five individual tissue sections were taken, and each section was stained at the same antibody dilution concentration, and the final staining results were averaged across the five sections. Tissue sections of positive laryngeal SCC diagnosed by a high seniority pathologist were selected for positive control

Table 3 Experimental reagents

Company	Reagent
BOSTER	Primary antibody: rabbit against human polyclonal FGFR1 antibody
	Primary antibody: rabbit against human polyclonal FGFR2 antibody
	Antibody dilution
Zhongshanjinqiao	Endogenous peroxidase blocker
	Normal goat serum working fluid for blocking
	Biotin-labeled goat anti-rabbit IgG polymer
	Horseradish Peroxidase-conjugated Streptavidin
	DAB color show kit

FGFR, fibroblast growth factor receptor.

and phosphate buffered saline (PBS) instead of primary antibody for negative control.

Experimental operation procedure:

(I) Isolated surgical specimens were soaked in 10% formaldehyde solution for 24–72 h;

(II) Specimens were washed in normal saline and placed in a dehydrator for dehydration. We then used 75% ethanol for 1 h, 85% ethanol for 50 min, 90% ethanol for 40 min, 95% ethanol for 40 min, absolute ethyl alcohol I and II for 40min each, xylene solution I, II and III for 20 min each, and paraffin solution I and II for 1 h each (65 ℃);

(III) Tissue was embedded in paraffin and frozen in a freezing table (–4 ℃) for 20 min;

(IV) Tissue was cut into 3-μm thick pieces, spread out and placed on glass-adhesive slides, baked in a constant temperature baking machine (63 ℃) for 1 h, and placed into the carrier rack;

(V) The glass-adhesive slides were placed into xylene I and II and dewaxed for 10 min each, absolute ethanol I and II for 10 min each, 95% ethanol, 90% ethanol, 85% ethanol, and 75% ethanol for 5 min each. They were then placed in tap water once for 3 min, pure water three times for 3 min each time, and PBS buffer three times, 3 min each time;

(VI) Antigen repair solution was placed in the microwave oven, 100 fire for 4 min boiling. A slide was then placed into it and 50 fire microwaved for 5 min, 30 fire microwaved for 5 min, then cooled to room temperature. After cooling, slides were soaked in pure water for three times, 3 min each time, and finally, soaked in PBS buffer for three times, 3 min each time;

(VII) After wiping the excess liquid off the slides, the endogenous peroxidase (reagent 1) was added, and the slide incubated with a temperature box for 10 min (37 ℃) before soaking in PBS buffer for three times, 3 min each time;

(VIII) After excess liquid was wiped, slides were sealed with normal goat serum working fluid (reagent 2), then incubated in a temperature box for 25 min (37 ℃);

(IX) Excess reagent 2 was gently shaken off, then each slide was dropped the rabbit against human polyclonal FGFR1 antibody and rabbit against human polyclonal FGFR2 antibody, before being placed into the moisturizing box, then placed in the refrigerator overnight (4 ℃);

(X) Slides were incubated in a temperature box for 1 h (37 ℃) then soaked in PBS buffer for three times, 3 min each time;

(XI) After wiping the excess liquid off each slide, biotin-labeled goat anti-rabbit IgG polymer (reagent 3) was added, then incubated for 30 min (37 ℃) and soaked in PBS buffer three times, 3 min each time;

(XII) After wiping the excess liquid off each slide, horseradish peroxidase-conjugated streptavidin (reagent 4) was added, before incubation for 30 min (37 ℃), then soaking in PBS buffer three times, 3 min each time;

(XIII) After dropping DAB color development solution onto each slide to show the color in the light avoidance environment for 5 min, the slides were soaked in pure water three times to terminate the color rendering reaction, 5 min each time;

(XIV) Slides were then redyed with hematoxylin solution for 5 min, tap water to rinse for 5 min, 0.9% hydrochloric acid alcohol differentiated for 5 s, tap water to rinse for 5 min, soaked in lithium carbonate reverse blue solution for 1 min, and a tap water rinse for 5 min;

(XV) Dehydration in gradient alcohol (75% ethanol, 85% ethanol, 90% ethanol, 95% ethanol, absolute ethanol for 2 min each) was then performed, before soaking in xylene solution I,

II for 5 min each;

(XVI) Slides were then sealed with neutral resin and placed into a draught cupboard until fully dry (24–72 h);

(XVII) Photographs were taken with a light microscope after observation under a biological microscope.

Staining results

Under low magnification, the positive expression of FGFR1 and FGFR2 appeared brown and yellow, with the most concentrated coloring in the cytoplasm and very few colored in the cell nucleus. A semi-quantitative scoring method according to different staining degrees was used with 0—uncoloured, 1—light yellow, 2—claybank, 3—brown or granular composure, and according to different color areas: 0, 0–5%; 1, 6–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%. The final two score values were multiplied, with 0–1 as negative (–), 2–4 as weak positive (+), 5–7 as positive (++), and 8–12 as strong positive (+++). In the double-blind method, five high-fold visual fields were randomly selected to read the results successively, and the average of five scores was used as the final score.

Statistical analysis

SPSS 23.0 software was used for data collation, analysis, and statistics. The value of the count data was recorded in N%, and the relationship between the positive expression of FGFR1 and FGFR2 and clinical characteristics was determined with the four-grid table Pearson χ^2 test, continuous corrected χ^2 test, or the Fisher exact probability method. The relationship between FGFR1 and FGFR2 expression in laryngeal cancer tissues was analyzed by Spearman rank correlation analysis. If $P<0.05$, the difference was significant.

Results

Histopathological morphology observation

Paraffin sections of laryngeal SCC, its corresponding normal mucosal margin, and vocal cord polyp tissue were stained with hematoxylin and eosin (HE) and viewed with a biological microscope under high magnification (400×). The epithelium of adjacent normal tissues was stratified squamous epithelium, in which the stratified pavement epithelium was in the outer layer and the compound columnar epithelium was in the inner layer. The squamous epithelium was neatly arranged, with normal morphology and no nucleosis, with an even ratio between cytoplasm and nucleus and no proliferation of basal cells. Dysplastic cells could be seen in cancer tissue, with diverse morphology and different sizes, mostly round, ovoid, and polygonal, an enlarged nucleus, deep dye, an increased nucleoplasm ratio, and the nucleus was oval or spindle-shaped and in a dividing state. The cell arrangement was extremely disordered and had broken through the basal cell layer, showing a nest-like and invasive growth. Keratinized beads and intercellular bridges were visible in the center of highly differentiated squamous carcinoma nests, with red and rich cytoplasm. Vocal cord polyp tissue could be seen with overlying squamous epithelium, the epithelial cells were regularly arranged and unspecific, the stroma was loose and edema-like, and the blood vessels were dilated (*Figure 1*).

FGFR1 expression in laryngeal SCC, its corresponding adjacent normal mucosa margin, and vocal cord polyp tissue

Paraffin sections of laryngeal SCC, its corresponding normal mucosal margin, and vocal cord polyp tissue were stained with IHC and viewed with a biological microscope under high magnification (×400). FGFR1 positive expression was brown, most concentrated in the cytoplasm, and on the very few colored nuclei, the total positive expression was 24 (80.00%), including 6 weakly positive (+) (20.00%), 3 positive (++) (10.00%), and 15 strongly positive (+++) (50.00%) (*Figure 2*). The total positive expression of 23 cases was 5 (21.74%), and only weakly positive, while the total positive expression of 26 vocal cord polyps was 11 (42.31%), all of which were weakly positive (*Figure 3*). In the squamous epithelium of normal adjacent mucosa and vocal cord polyp tissue, FGFR1 was often colorless in the outer flat epithelium, while a few pale-yellow colors appeared in the inner columnar epithelium, which may be related to the slight enrichment of columnar epithelial cytoplasm. According to the Pearson χ^2 test, FGFR1 expression was significantly different in laryngeal SCC and normal tissues >0.5 cm away from the adjacent tumor margin, and this difference was statistically significant ($\chi^2=17.835$, $P<0.001$). FGFR1 expression was significantly different in cancerous tissue and vocal cord polyp tissue, and was also statistically significant ($\chi^2=8.443$, $P=0.004<0.05$). There was no difference in the expression of adjacent normal margins and vocal cord polyps ($\chi^2=2.348$, $P=0.125>0.05$) (*Table 4*).

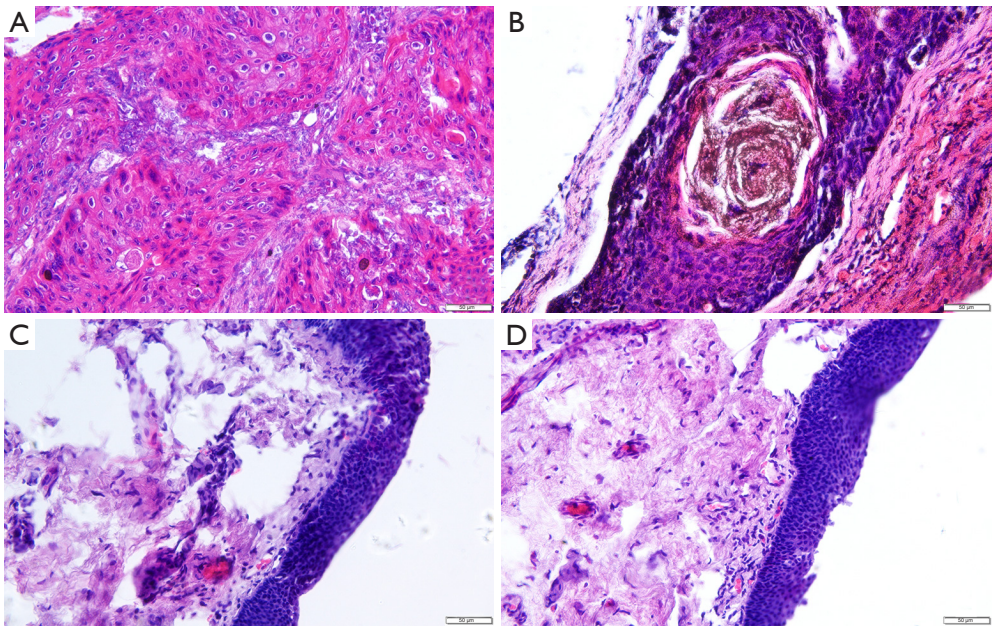

Figure 1 Histopathological observation (HE, ×400). (A) Squamous carcinoma tissue 1, nest-like and invasive growth. (B) Squamous carcinoma tissue 2, nest-like and invasive growth. (C) Normal throat mucosa beside the cancer, neat and normal morphology. (D) Tissue of vocal cords polyps, cell interstitial loosening and rich blood vessels. HE, hematoxylin and eosin.

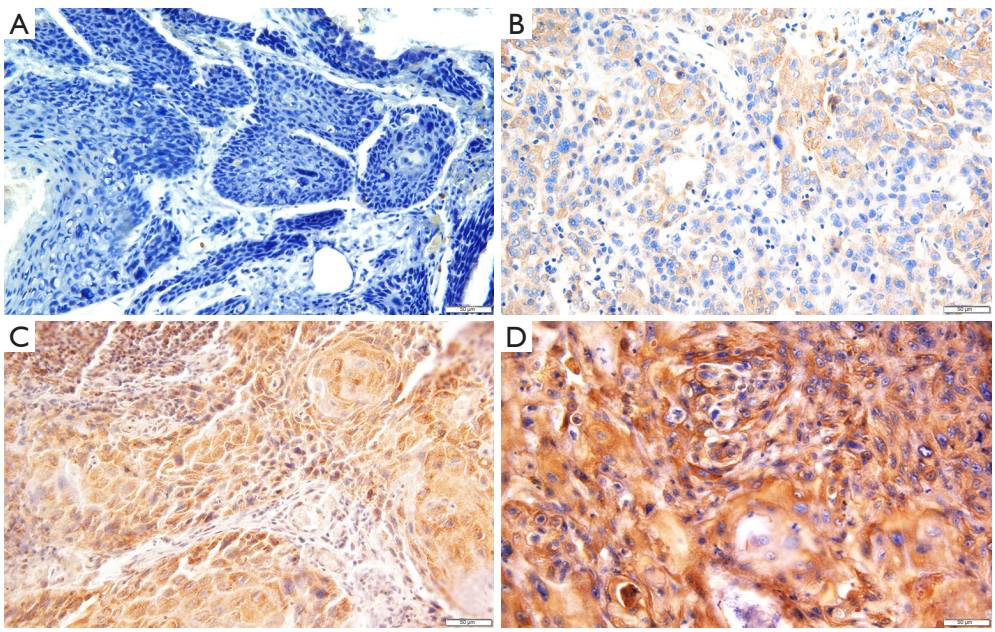

Figure 2 FGFR1 expression in throat scale cancer tissue (IHC, ×400). (A) Negative (–), blue—no coloring. (B) Weak positive (+), light yellow—partially colored. (C) Positive (++), yellow tan—clearly colored. (D) Strong positive (+++), tan with granular pigmentation—significant colored. FGFR, fibroblast growth factor receptor; IHC, immunochemistry.

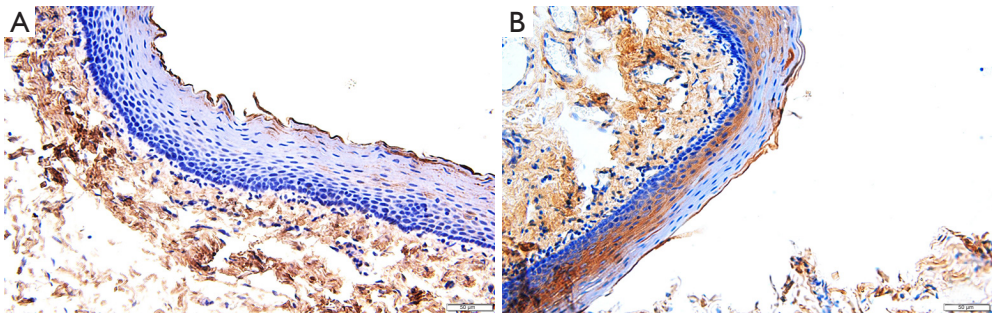

Figure 3 Expression of FGFR1 in the adjacent normal laryngeal mucosa and vocal cord polyps (IHC, ×400). (A) Negative (–), blue—no coloring. (B) Weak positive (+), claybank—partially colored. FGFR, fibroblast growth factor receptor; IHC, immunochemistry.

Table 4 Comparison of FGFR1 expression in throat scale cancer, adjacent normal tissues, and vocal cord polyps

Tissue	Positive cases, n (%)	Negative cases, n (%)	χ^2	P
Cancer *vs.* normal			17.835	<0.001
Cancer	24 (0.80)	6 (0.20)		
Normal	5 (0.22)	18 (0.78)		
Cancer *vs.* polyp of vocal cord			8.443	0.004
Cancer	24 (0.80)	6 (0.20)		
Polyp of vocal cord	11 (0.42)	15 (0.58)		
Normal *vs.* polyp of vocal cord			2.348	0.125
Normal	5 (0.22)	18 (0.78)		
Polyp of vocal cord	11 (0.42)	15 (0.58)		

FGFR, fibroblast growth factor receptor.

FGFR2 expression in laryngeal SCC, its corresponding adjacent normal mucosa margin, and vocal cord polyp tissue

Paraffin sections of laryngeal SCC, its corresponding normal mucosal margin, and vocal cord polyp tissue were stained with IHC and viewed in the biological microscope under high magnification (400×). FGFR2 positive expression was brown and colored in the cytoplasm, with a total positive expression of 25 (83.33%), including 11 weakly positive (+) (36.67%), four positive (++) (13.33%), and 10 strongly positive (+++) (33.33%) (*Figure 4*). In the 23 cases of adjacent normal margins, total positive expression was observed in two cases (8.70%), while of the 26 vocal cord polyps, total positive expression was observed in three cases (11.54%). According to the four-grid table Pearson χ^2 test or Serial corrected χ^2 test, FGFR2 expression was significantly different in laryngeal SCC and normal tissues >0.5 cm away from the adjacent tumor margin, and this difference was statistically significant (χ^2=29.020, P<0.001). FGFR2 expression was significantly different in cancer tissue and vocal cord polyp tissue, and this difference was statistically significant (χ^2=28.718, P<0.001), while there was no difference in the expression of adjacent normal margins and vocal cord polyps (χ^2=0.000, P=1.000 >0.05) (*Table 5*).

Relationship between FGFR1 and FGFR2 expression and clinical features

According to the Fisher's exact probability method, there was no correlation between positive FGFR1 expression and other clinical characteristics, including gender (χ^2=1.000), age (χ^2=0.641), smoking history (χ^2=0.366), smoking quantity (χ^2=0.750), drinking history (χ^2=0.645), tumor diameter (χ^2=1.000), lymph node metastasis (χ^2=0.557), degree of differentiation (χ^2=1.000), and TNM staging

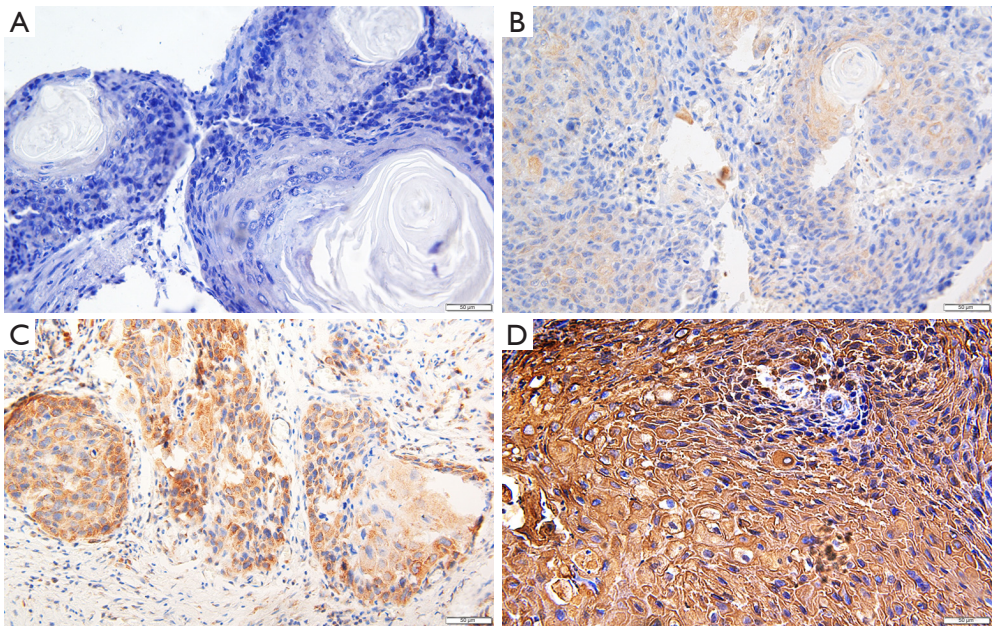

Figure 4 FGFR2 expression in throat scale cancer tissue (IHC, ×400). (A) Negative (–), blue—no coloring. (B) Weak positive (+), light yellow—partially colored. (C) Positive (++), yellow tan—clearly colored. (D) Strong positive (+++), tan with granular pigmentation-significant colored. FGFR, fibroblast growth factor receptor; IHC, immunochemistry.

Table 5 Comparison of FGFR2 expression in throat scale cancer, adjacent normal tissues and vocal cord polyps

Tissue	Positive cases, n (%)	Negative cases, n (%)	χ^2	P
Cancer *vs.* normal			29.020	<0.001
Cancer	25 (0.83)	5 (0.17)		
Normal	2 (0.09)	21 (0.91)		
Cancer *vs.* polyp of vocal cord			28.718	<0.001
Cancer	25 (0.83)	5 (0.17)		
Polyp of vocal cord	3 (0.12)	23 (0.88)		
Normal *vs.* polyp of vocal cord			0.000	1.000
Normal	2 (0.09)	21 (0.91)		
Polyp of vocal cord	3 (0.12)	23 (0.88)		

FGFR, fibroblast growth factor receptor.

(χ^2=1.000), and the differences were statistically insignificant (P>0.05) (*Table 6*). There was also no correlation between positive FGFR2 expression and these clinical characteristics, with gender (χ^2=1.000), age (χ^2=0.327), smoking history (χ^2=0.310), smoking quantity (χ^2=0.254), drinking history (χ^2=0.589), tumor diameter (χ^2=0.300), lymph node metastasis (χ^2=1.000), degree of differentiation (χ^2=1.000), and TNM staging (χ^2=0.304), The difference were also statistically insignificant (P>0.05) (*Table 7*).

Correlation between FGFR1 and FGFR2 expression in laryngeal SCC

The expression of FGFR1 and FGFR2 was significantly higher in throat SCC tissue than in adjacent normal mucosal margins and benign laryngeal lesions (vocal cord

Table 6 Relationship between FGFR1 expression and clinical characteristics

Clinical characteristics	Cases	FGFR1		P
		Positive	Negative	
Gender				1.000
Male	29	23	6	
Female	1	1	0	
Age (years)				0.641
≥60	19	16	3	
<60	11	8	3	
Smoking				0.366
Yes	28	23	5	
No	2	1	1	
Smoking quantity (year × No./d)				0.750
≥400	24	21	3	
<400	6	3	3	
Drinking				0.645
Yes	22	18	4	
No	8	6	2	
Tumor diameter (cm)				1.000
≥3	10	8	2	
<3	20	16	4	
Lymphatic metastasis				0.557
Yes	4	4	0	
No	26	20	6	
Degree of differentiation				1.000
High and medium	28	22	6	
Low	2	2	0	
TNM staging				1.000
I, II	23	18	5	
III, IV	7	6	1	

FGFR, fibroblast growth factor receptor; TNM, Tumor-Node-Metastasis.

Table 7 Relationship between FGFR2 expression and clinical features

Clinical characteristics	Cases	FGFR2		P
		Positive	Negative	
Gender				1.000
Male	29	24	5	
Female	1	1	0	
Age (years)				0.327
≥60	19	17	2	
<60	11	8	3	
Smoking				0.310
Yes	28	24	4	
No	2	1	1	
Smoking quantity (year × No./d)				0.254
≥400	24	21	3	
<400	6	4	2	
Drinking				0.589
Yes	22	19	3	
No	8	6	2	
Tumor diameter (cm)				0.300
≥3	10	7	3	
<3	20	18	2	
Lymphatic metastasis				1.000
Yes	4	4	0	
No	26	21	5	
Degree of differentiation				1.000
High and medium	28	23	5	
Low	2	2	0	
TNM staging				0.304
I, II	23	18	5	
III, IV	7	7	0	

FGFR, fibroblast growth factor receptor; TNM, Tumor-Node-Metastasis.

polyps). Spearman rank correlation analysis was applied to analyze the correlation between their expression in laryngeal SCC, and revealed FGFR1 and FGFR2 expression in laryngeal cancer had a positive correlation, and a moderate correlation, with statistically significant differences (Rs=0.499, P=0.005) (*Table 8*).

Discussion

Most laryngeal cancers are SCCs, and according to

Table 8 Correlation between FGFR1 expression and FGFR2 expression in squamous cell carcinoma of the throat

FGFR1	FGFR2				Rs	P
	–	+	++	+++		
–	4	2	0	0	0.499	0.005
+	1	2	1	2		
++	0	1	0	2		
+++	0	6	3	6		

FGFR, fibroblast growth factor receptor. –, negative; +, weak positive; ++, positive; +++, strong positive.

their different anatomical locations, can be divided into supraglottic cancer, glottic cancer (vocal cord cancer), and subglottic cancer. Glottic cancer is the most common, accounting for 64% of laryngeal cancer, supraglottic cancer is the second, accounting for 30%, while subglottic cancer is the least common, accounting for only 6%. In early laryngeal cancer, hoarseness is the most common presenting complaint, accompanied by throat discomfort, and a feeling of the presence of a foreign body. Later symptoms include sore throat, sputum with blood, and eating and eating cough symptoms. Surgical resection remains the main basic treatment for laryngeal cancer, although according to its anatomical classification and lymph node metastasis, auxiliary radiotherapy and chemotherapy may also produce effective results. While the most significant effect was seen in early vocal cord carcinoma and glottic laryngeal carcinoma without lymph node metastasis using plasma cryogenic ablation and microscopic CO_2 laser cautery, compared with traditional laryngotomy, CO_2 laser burning is popular due to its characteristics of short operation time, reducing the damage of vocal cord tissue, reducing destruction of the laryngeal anatomical frame structure, and preserving laryngeal function (12). For advanced laryngeal cancer, the prognosis is poor, and exploring new diagnosis and treatment methods has become a boom of academic research in recent years. Therefore, research on the histology and genetics of laryngeal SCC is very important and provides new ideas for its diagnosis and treatment.

As with other neoplastic diseases, the exact mechanism of SCC of the larynx is hitherto not well defined, with the rapid development of tumor molecular biology techniques, it has been proved that the occurrence of malignancy is a long-term process and caused by multifactorial. Among them, the activation of oncogene and the inactivation of tumor suppressor gene and the disorder of intracellular signaling are believed to be closely related to the occurrence and metastasis of laryngeal SCC. Therefore, the molecular mechanism of laryngeal cancer and related pathological factors to provide a basis for the later immunotargeted therapy.

The human genes encoding FGFR1 and FGFR2 are on band 1 and 2 of band 1 of the short arm of chromosome 8 (8p11,8p12) (13), and band 6 of region 2 of the long arm of chromosome 10 (10q26). As members of the receptor tyrosine kinase (RTK) family, FGFR1 and FGFR2 bind to FGF, increasing tyrosine kinase activity by 500 to 1,000 times (14). Subsequently, signals along different intracellular conduction pathways, such as the RAS-MAPK pathway, JAK/STAT pathway, and PLC pathway, cause further physiological effects. Problems in any link of the conduction pathway will affect the metabolic activities of the body, play an important physiological role in organ synthesis, tissue remodeling, nervous system regeneration, angiogenesis, and metabolism regulation of organisms, and even participate in the whole process of malignant tumor disease. Thus, we infer FGFR1 and FGFR2 are related to the occurrence of laryngeal cancer and metastasis.

Research of FGFR1 and FGFR2 has increased over the past decade, and the relationship between them and various clinical characteristics has been eagerly sought by studying the detection of their expression in various malignant tumors using FGFR gene screening. Abnormal alteration or overexpression of FGFR1 and FGFR2 proteins has been found in oral SCC (15), esophageal SCC (16,17), lung cancer (18-20), breast cancer (21,22), and pancreatic cancer (23), and a clear association between high expression status and poor prognosis of patients has been observed. The application of FGFR inhibitors in the treatment of cholangiocarcinoma and urothelial malignancies has achieved initial results (24), while FGFR1 overexpression has been found in SCC of the head and neck (HNSCC) (25), and was associated with poor survival. In addition, FGFR1

was associated with poor survival in human papillomavirus (HPV)-negative HNSCC patients, but not with HPV-positive HNSCC patients (26). Twenty years ago, some researchers observed FGFR1 was positively expressed on the nucleus and cytoplasm of tumor cells surrounding the necrotic area, and the nucleus was much stronger than the cytoplasm. Later, some researchers observed the expression of FGFR1 uniformly and significantly in the cytoplasm of tumor cells by IHC staining of tissue sections of 209 laryngeal and hypolaryngeal carcinoma patients, where a weak nuclear expression was occasionally seen and was related to tumor differentiation. Moreover, the overall degree of expression was related to tumor differentiation, with high expression in low differentiation carcinoma and no or low expression in highly differentiated squamous carcinoma (27). Recently, Starska *et al.* tested the expression of FGFR1 mRNA or FGFR1 protein in 137 surgically removed laryngeal SCC patients by PCR and IHC and found high expression levels were associated with increased tumor invasion rate, tumor lymph node metastasis, tumor recurrence, and poor patient prognosis, and that FGFR1 activated tumor cell regeneration and vascular appreciation by activating the downstream PI3K/AKT kinase pathway (28).

A retrospective study of the prognostic factors of laryngeal SCC in China showed that the clinical stage, the surgical margin, and the systemic condition were independent factors influencing the prognosis of laryngeal cancer, the low survival rate of patients with advanced laryngeal cancer, and positive surgical margin or severe comorbidities is low, which indicates the importance of early diagnosis, early treatment, negative surgical margin and good systemic condition of the patient (29). The relationship between FGFR and the prognosis of laryngeal cancer has also gradually become a research hotspot in recent years. By retrieving deleterious genes from 122 HNSCC patients who had undergone primary surgery, Dubot *et al.* observed that FGFR1 could be a prognostic biomarker in HNSCC patients, especially in HPV-negative patients (30). This may be closely related to the involvement of FGFR1 in cell differentiation, and a study has shown that the high expression of FGFR1 is more significant in laryngeal cancer patients with lymphovascular invasion and advanced lymph node metastasis, which also directly affects the clinical stage of tumors and further affects the long-term survival rate (27). However, there are very few studies on the prognostic correlation of FGFR2 and laryngeal cancer, and numerous basic experiments are needed to be analyzed.

In this study, IHC staining of 30 laryngeal SCC tissues showed the expression of FGFR1 and FGFR2 was significantly higher in laryngeal SCC tissue than adjacent normal mucosal margin and vocal cord polyp tissue. However, further research is required to verify whether elevated FGFR1 and FGFR2 are closely related to the occurrence of laryngeal cancer and can be further investigated as oncogenic factors, and whether both are significantly expressed in the cytoplasm. At the same time, correlation analysis of the expression of FGFR1 and FGFR2 in tumor tissues may show whether the two proteins can influence each other and play a role in promoting cancer cell increment and angiogenesis. This experiment involved a small number of specimens, and a larger sample size is required to deepen the genetics, histology, and functional aspects to provide a basis for early diagnosis and screening of laryngeal cancer.

Conclusions

The results of this experimental study showed both FGFR1 and FGFR2 protein had a high expression trend in laryngeal squamous-cell carcinoma patients. However, the expression level of the two proteins was not directly associated with tumor lymph node metastasis, the degree of tumor differentiation, or TNM stage, and there was no correlation with patient age and smoking and drinking history. Since previous studies have related FGFR to multiple clinical features, especially the TNM stage of the tumor, lymph node metastasis, and recurrence rate (27,28,31), further studies are needed.

The results of this experimental study also showed FGFR1 and FGFR2 are partially expressed in the normal laryngeal mucosa margin and vocal cord polyps in laryngeal cancer patients, but the staining intensity was not high, and the results were not statistically significant. These results indicate FGFR1 and FGFR2 can be used as carcinogenic factors for further study in the field of genetic and histological diagnosis and treatment of laryngeal cancer.

Through the analysis of the experimental results, the higher the expression of FGFR1 in laryngeal cancer tissue, the higher the expression of FGFR2. The interaction between the two as independent factors can be studied, providing a new approach for research and application of the diagnosis and treatment of tumor disease. Further studies are needed to investigate whether FGFR1 and FGFR2 is a target of radiosensitization in laryngeal SCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1936/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The experiment was approved by the Ethics Committee of the Affiliated Hospital of Chengde Medical College (ethical No. CYFYLL2022163), and all patients signed the informed consent form

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