Predictive Value of Transforming Growth Factor-α and Ki-67 for the Prognosis of Skull Base Chordoma

Shuheng Zhang[1,2], Jiwei Bai[1,3], Mingxuan Li[1], Yixuan Zhai[4], Shuai Wang[1], Qian Liu[1], Chuzhong Li[1], Songbai Gui[3], Yazhuo Zhang[1-4]

■ OBJECTIVE: **We aimed to characterize the expression of transforming growth factor-α (TGF-α) and Ki-67 and to assess the relationship between TGF-α and Ki-67 expression and prognostic factors in skull base chordoma.**

■ METHODS: **We retrospectively analyzed the data from 46 patients with skull base chordoma. The follow-up duration ranged from 1 to 168 months (mean, 74.1). The survival data were statistically analyzed using the Kaplan-Meier method and multivariate Cox regression analysis. The expression of TGF-α and Ki-67 were detected by immunohistochemical staining of paraffin-embedded patient tissue specimens.**

■ RESULTS: **The total resection (TR) group had longer overall survival compared with the non-TR group ($P = 0.042$). The TR group also had longer progression-free survival (PFS) than did the non-TR group ($P = 0.046$). The group with a high Ki-67 labeling index (Ki-67LI) had shorter overall survival than did the group with a low Ki-67LI ($P = 0.039$). Also, the group with a high Ki-67LI had significantly shorter PFS than did the group with a low Ki-67LI ($P = 0.016$). Moreover, the group with high TGF-α expression had significantly shorter PFS compared with the group with low TGF-α expression ($P = 0.005$).**

■ CONCLUSIONS: **Our results have shown that high levels of TGF-α and Ki-67 are associated with shorter PFS in patients with chordoma. We have confirmed the role of Ki-67 as a functional molecular marker of poor prognosis. We also identified TGF-α as a potential novel biomarker for predicting prognosis for patients with skull base chordoma.**

INTRODUCTION

Chordoma is a rare, primary malignant tumor that arises in the axis of bones, with an incidence rate of ~0.1/100,000 persons annually.[1,2] It accounts for 1%–4% of all primary bone tumors. The most common site of chordoma is the sacrococcygeal region, followed by the skull base.[3] Although its pathogenesis has remained unclear, chordoma is thought to originate from remnants of the notochord.[4] Chordoma is a tumor with slow growth, low malignant potential, and a certain degree of invasiveness. Unlike some tumors, chordoma has been largely resistant to conventional radiotherapy and chemotherapy.[5] Clinically, surgical removal has remained the primary treatment; however, owing to the complexity of the skull base anatomy and frequent invasion into peripheral critical nerves and blood vessels, it can be difficult to completely excise the tumor. Even after complete surgical resection, the local recurrence rate of chordoma has remained very high.[6] Although some progress has occurred, it is urgent to explore the pathogenesis and molecular biological characteristics of chordomas to find new therapeutic targets.

Transforming growth factor-α (TGF-α) was originally discovered in rodents and humans as an acid- and heat-stable 50-amino

Key words
- Ki-67
- Skull base chordoma
- Survival analysis
- TGF-α

Abbreviations and Acronyms
EGF: Epidermal growth factor
EGFR: Epidermal growth factor receptor
H-score: Histochemistry score
Ki-67LI: Ki-67 labeling index
NTR: Non–total resection
OS: Overall survival
PFS: Progression-free survival
TGF-α: Transforming growth factor-α
TR: Total resection

From the [1]Beijing Neurosurgical Institute, Capital Medical University, Beijing; [2]Department of Neurosurgery, Anshan Central Hospital, Anshan; [3]Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University, Beijing; and [4]Department of Neurosurgery, First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

To whom correspondence should be addressed: Yazhuo Zhang, Ph.D., M.D. [E-mail: zyz2004520@yeah.net]

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acid protein. TGF-α is produced by macrophages, epidermal cells, and brain cells and can induce the development of epithelial cells.[7] TGF-α is expressed in a variety of tissues, including hematopoietic tissue, epithelial tissue, brain tissue, and smooth muscle. It plays a physiological role in wound healing, angiogenesis, and cell proliferation. TGF-α inhibits cartilage formation and promotes osteoclast development to stimulate bone resorption, thereby playing a role in bone remodeling.[8,9] TGF-α has been reported to be upregulated in many tumors, including ovarian cancer,[10,11] kidney cancer,[12] pancreatic cancer,[13] colon cancer,[14] hepatocellular carcinoma,[15] breast cancer,[16,17] and esophageal and gastric carcinoma.[18] In these tumors, TGF-α has been shown to drive tumor growth through autocrine pathways, thereby inducing and sustaining tumor invasion and metastasis. However, whether TGF-α is expressed in skull base chordoma and what role it might play in the development and progression of chordoma have not been extensively studied. Because the expression of the Ki-67 protein can reflect the number of dividing cells in tumors, Ki-67 has been used as an important marker for assessing the degree of malignancy in tumors.[19-22] Nevertheless, the role of Ki-67 in skull base chordoma is still controversial.

In the present study, we evaluated the expression of TGF-α and Ki-67 in skull base chordoma and analyzed the clinical factors and patient prognosis.

METHODS

Patients

In the present study, the data from 46 patients with skull base tumors who had undergone surgical resection and with a pathological diagnosis of chordoma at Beijing Tiantan Hospital, Capital Medical University, were retrospectively collected from February 2008 to January 2012. The follow-up interval for the present study ranged from 1 to 168 months (mean, 74.1; median, 79). The ethics committee of Beijing Tiantan Hospital, Capital Medical University, approved the present study.

Definition of Degree of Surgical Resection

From the patients' surgical records, we compared the postoperative magnetic resonance imaging (MRI) studies of the patients with preoperative MRI studies available. The tumor volume reduction was used as a criterion for assessing the extent of resection. We defined the criteria as follows: total resection (TR; removal of visible tumor, with no residual tumor seen on the postoperative MRI studies); and non–total resection (NTR; postoperative MRI studies showing residual tumor, regardless of the extent).[23]

Immunohistochemistry and Evaluation

Immunohistochemical staining was performed using the BOND III automated system (Leica Biosystems, Wetzlar, Germany). In brief, slides with paraffin-embedded tissue slices were baked at 65°C for 0.5 hour. The slides were successively washed in xylene, hydrated with an ethanol gradient (100% and 95%), and subjected to antigen retrieval. The slides were then treated with 3% hydrogen peroxide to block endogenous hydrogen peroxide. Subsequently, the sections were incubated with primary antibodies (anti-TGFα [D-6] antibody; sc-374433 [Santa Cruz Biotechnology, Santa Cruz, California, USA], 1:100 dilution; and anti–Ki-67 antibody; ZM-0166 [Zhongshan Jinqiao Biotechnology Co. Ltd., Beijing, China]; 1:150 dilution). The Bond Polymer Precision Detection Kit (Leica Biosystems) was used to treat the slides according to the manufacturer's protocol. Diaminobenzidine was used as a chromogenic substrate for immunohistochemical staining. Finally, we dehydrated, cleaned, and mounted the slides. We used phosphate-buffered saline instead of primary antibody for the negative control.

The Aperio AT2 scanner (Leica) and ImageScope, version 12.3.0.5056 (Leica), were used to scan and evaluate the immunostained slides using an automated algorithm, respectively. The staining intensity was graded as follows: none, 0; weak, 1+; moderate, 2+; and strong, 3+. The Ki-67 labeling index (Ki-67LI) was calculated as a percentage of the nuclear cells with positive staining, which was the sum of the proportions of cells with a staining grade of 1+–3+. The histochemistry score (H-score) was calculated as follows: conventional formula: H-score = 1 × (percentage of cells with 1+) + 2 × (percentage of cells with 2+) + 3 × (percentage of cells with 3+).[24] The H-score ranged from 0 to 300. The assessment of each slice was independently completed by 2 pathologists who were unaware of the patient's clinical pathologic features.

Statistical Analysis

All statistical analyses were performed using SPSS, version 22.0, software (IBM Corp., Armonk, New York, USA). The χ^2 test, 2-independent samples t test, and Wilcoxon Mann-Whitney U test were used to determine the differences between groups. The Kaplan-Meier method with the log-rank test for univariate survival analysis and multivariate Cox regression analysis were used for statistical analysis of the survival data. All tests were 2-sided, and $P < 0.05$ was regarded as statistically significant. Statistical graphs were created using GraphPad Prism, version 8.0 (GraphPad Software, San Diego, California, USA).

RESULTS

Clinical Features

A total of 46 patients with skull base chordoma, aged 15 to 69 years (mean age, 40.9), were enrolled in the present study. Of the 46 patients, 28 were male and 18 were female, with a male/female ratio of 1.56:1. Of the cases of chordoma, 35 were primary and 11 were recurrent. Of the 46 patients, 21 underwent transsphenoidal surgery and 25, craniotomy. TR was performed in 13 patients (28.26%) and non-TR in 33 patients. By the last follow-up examination, 12 patients (26.08%) had died. Of the 46 patients, 25 had had cranial nerve involvement before surgery and 22 had had brain stem or pituitary compression. The details regarding the preoperative clinical status (Karnofsky performance status) are presented in Table 1. Of the 46 patients, 18 had undergone postoperative radiotherapy, including conventional radiotherapy, gamma knife treatment, and proton therapy. Five patients experienced serious complications, including 2 cases of intracranial hemorrhage and 3 cases of cerebrospinal fluid leakage with intracranial infection; 1 of the patients died of surgery.

Table 1. Association Between Expression of Transforming Growth Factor-α, Expression of Ki-67, and Clinical Factors of Patients with Skull Base Chordoma

		TGF-α Expression				Ki-67 Expression		
Clinical Factor	**Patients (n)**	**High**	**Low**	***P* Value**	**Patients (n)**	**High**	**Low**	***P* Value**
Age (years)				1.000				0.459
≤50	37	19	18		37	17	20	
>50	9	4	5		9	6	3	
Sex				1.000				0.763
Male	28	14	14		28	15	13	
Female	18	9	9		18	8	10	
KPS score		NA	NA	NA	NA	NA	NA	NA
90	4							
80	31							
70	10							
50	1							
Tumor size (mm^3)				0.376				0.786
≤30	22	13	9		22	10	12	
>30	24	10	14		24	13	11	
Surgical approach				0.554				0.075
TRA	21	12	9		21	14	7	
CRA	25	11	14		25	9	16	
Resection extent				1.000				
TR	13	7	6		13	5	8	0.514
NTR	33	16	17		33	18	15	
Operative history				0.491				1.000
Yes	35	16	19		35	17	18	
No	11	7	4		11	6	5	
Blood supply				0.554				
Poor	21	12	9		21	11	10	1.000
Rich	25	11	14		25	12	13	
Tumor texture				0.514				
Soft	33	18	15		33	16	17	1.000
Hard	13	5	8		13	7	6	
Radiotherapy				0.763				0.130
Yes	18	10	8		18	6	12	
No	28	13	15		28	17	11	

TGF-α, transforming growth factor-α; KPS, Karnofsky performance scale; NA, not applicable; TRA, transsphenoidal approach; CRA, craniotomy approach; TR, total resection; NTR, non—total resection.

TGF-α and Ki-67 Expression

TGF-α was positively expressed in the cytoplasm and nucleus of the cells in the skull base chordoma specimens. The H-score ranged from 38.92 to 229.66, with a median of 140.33 ± 50.17 (Figure 1A, B). Ki-67 was expressed in the nucleus of the chordoma cells, and the Ki-67LI ranged from 1.25% to 27.62% (median, 5.08% ± 5.47%; Figure 1C, D). Using the median H-score for TGF-α (140.33) or the median Ki-67LI (5.08%) as the cutoff point, the patients were equally divided into 2 groups. We did not find a significant correlation between

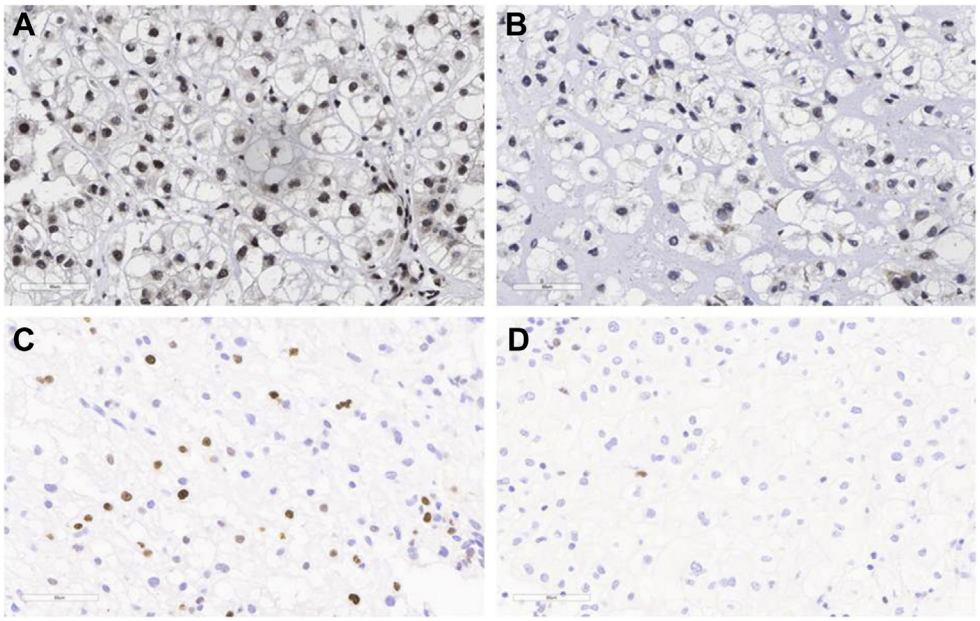

Figure 1. (**A**) High expression of transforming growth factor-α (TGF-α). (**B**) Low expression of TGF-α. (**C**) High expression of Ki-67. (**D**) Low expression of Ki-67 (original magnification ×400).

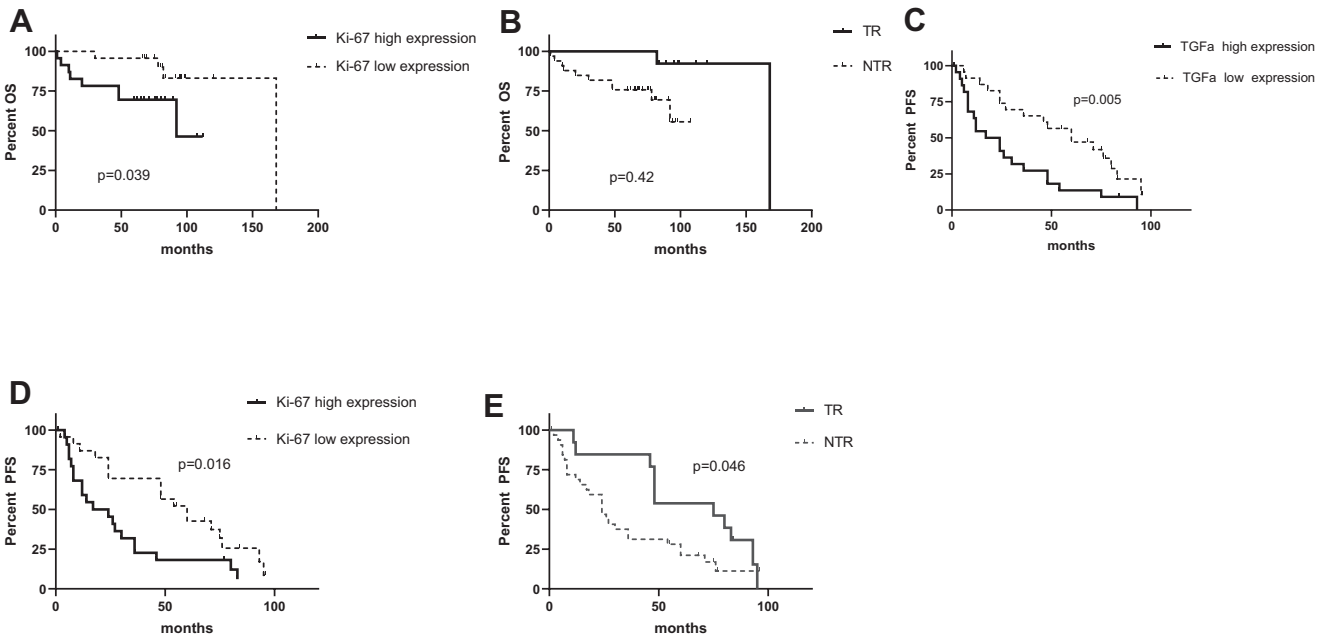

Figure 2. Analysis of overall survival (OS) and progression-free survival (PFS) using Kaplan-Meier survival curves. (**A**) OS and expression of Ki-67. (**B**) OS and extent of resection. (**C**) PFS and expression of transforming growth factor-α. (**D**) PFS and expression of Ki-67. (**E**) PFS and extent of resection.

Table 2. Univariate Kaplan-Meier Analysis of Clinical Features Affecting Progression-Free Survival and Overall Survival

	PFS			OS		
Clinical Factor	**HR**	**95% CI**	***P* Value**	**HR**	**95% CI**	***P* Value**
Age (≤50 vs. >50 years)	NA	NA	0.353	NA	NA	0.943
Sex (male vs. female)	NA	NA	0.553	NA	NA	0.370
Tumor size (≤30 vs. >30 mm^3)	NA	NA	0.585	NA	NA	0.977
Surgical approach (TRA vs. CRA)	NA	NA	0.114	NA	NA	0.528
Extent of resection (NTR vs. TR)	0.516	0.272—0.979	0.046	0.265	0.085—0.831	0.042
Operative history (no vs. yes)	NA	NA	0.160	NA	NA	0.175
Blood supply (poor vs. rich)	NA	NA	0.763	NA	NA	0.172
Tumor texture (soft vs. hard)	NA	NA	0.359	NA	NA	0.292
Radiotherapy (no vs. yes)	NA	NA	0.618	NA	NA	0.559
TGF-α (low vs. high)	2.355	1.206—4.599	0.005	NA	NA	0.091
Ki-67 (low vs. high)	2.076	1.065—4.045	0.016	3.144	0.98—10.044	0.039

PFS, progression-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; NA, not applicable; TRA, transsphenoidal approach; CRA, craniotomy approach; NTR, non—total resection; TR, total resection; TGF-α, transforming growth factor-α.

TGF-α or Ki-67 expression and the clinical features of age, sex, tumor size, surgical approach, extent of resection, history of operation, blood supply, or tumor texture (Table 1).

Relationship Between Overall Survival and TGF-α Expression, Ki-67 Expression, and Clinical Features

For the survival data, the interval between the first diagnosis and death or the last follow-up examination was defined as overall survival (OS). In the present study, the OS ranged from 1 to 168 months (median, 79 ± 31.05).

The group with high TGF-α expression had a shorter mean OS (88.0 months) compared with the group with low TGF-α expression (mean, 141.8 months). High TGF-α expression correlated negatively with OS; however, the difference was not statistically significant (P = 0.091).

The group with high Ki-67 expression had a shorter mean OS (79.4 months) than the group with low Ki-67 expression (151.0 months). High Ki-67 expression was significantly and negatively correlated with OS (P = 0.039; Figure 2A). Of the 23 patients in the high Ki-67 expression group, 8 had died compared with 4 patients in the low Ki-67 expression group, for a survival rate of 65.21% and 82.61%, respectively.

The TR group had a longer mean OS (161.4 months) than the NTR group (mean OS, 82.9 months). TR was significantly and positively correlated with OS (P = 0.042; Figure 2B). Of the 13 patients in the TR group, 2 had died compared with 10 of 33 patients in the NTR group, for a survival rate of 84.62% and 69.70%, respectively.

The group of patients who had undergone radiotherapy had a longer mean OS (139.2 months) compared with the group without radiotherapy (94.3 months); however, the difference was not statistically significant (P = 0.559; Table 2).

No statistically significant correlations were found between the other clinical features and OS in the present study (Table 2).

The results of multivariate Cox regression analysis showed that Ki-67 expression and the extent of resection on OS were not significant (P = 0.054 and P = 0.086; Table 3).

Table 3. Cox Multivariable Analysis of Factors Affecting Progression-Free Survival and Overall Survival

Variable	**HR**	**95% CI**	***P* Value**
PFS			
TGF-α (low vs. high)	3.496	1.717—7.120	0.001
Ki-67 (low vs. high)	2.611	1.281—5.320	0.008
Resection extent (NTR vs. TR)	0.369	0.167—0.816	0.014
OS			
Ki-67 (low vs. high)	NA	NA	0.054
Resection extent (NTR vs. TR)	NA	NA	0.086

HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; TGF-α, transforming growth factor-α; NTR, non—total resection; TR, total resection; OS, overall survival; NA, not applicable.

Relationship Between Progression-Free Survival and TGF-α Expression, Ki-67 Expression, and Clinical Features

Progression-free survival (PFS) was defined as the interval from the initial diagnosis to the date of progression or the last follow-up examination. During the follow-up period, 38 of the 46 patients (82.60%) experienced recurrence. The PFS in the present study ranged from 1 to 90 months (median, 33 ± 30.58).

The group with high TGF-α expression had significantly shorter PFS (mean, 29.3 months) than the group with low TGF-α expression (57.0 months; P = 0.005; Figure 2C). Moreover, the group with high Ki-67 expression had significantly shorter PFS (mean, 29.6 months) compared with the group with low Ki-67 expression (56.0 months; P = 0.016; Figure 2D). The TR group had longer mean PFS (63.6 months) compared with the NTR group (35.4 months; P = 0.046; Figure 2E). The group of patients who had undergone radiotherapy had a longer mean PFS (45.9 months) compared with the group without radiotherapy (41.9 months). However, the difference was not statistically significant (P = 0.618; Table 2). Furthermore, the multivariate Cox regression analysis of PFS showed that the independent prognostic factors for PFS were TGF-α expression, Ki-67 expression, and the extent of resection (P = 0.001, P = 0.008, and P = 0.014, respectively; Table 3).

DISCUSSION

The treatment of skull base chordoma has remained challenging. Previous research has shown that the most effective treatment of skull base chordoma is total surgical resection.[25-27] Consistently, we observed that OS and PFS were significantly longer for the TR group than for the NTR group. In addition, TR was an independent prognostic factor, in particular for PFS. As the surgical techniques improve, more patients with chordoma will benefit from them. However, owing to the high postoperative recurrence rate and resistance to conventional radiotherapy and chemotherapy,[27] it is necessary to explore the mechanism of chordoma tumorigenesis and identify new prognostic factors. These studies will facilitate the discovery of new effective treatments for chordoma of the skull base.

Ki-67 is a nuclear protein encoded by the mKi-67 gene and is associated with cell proliferation.[28-31] The expression of Ki-67 in malignant tissues will be significantly greater than that in normal tissues. In addition, the lower the degree of tissue differentiation in tumors, the greater the expression of Ki-67. Therefore, Ki-67 has been used in the evaluation of clinical stage and metastatic potential of some tumors.[32,33] Considering that Ki-67 is involved in cell proliferation,[34-37] we also explored the correlation between Ki-67 expression and the clinicopathological features. The Ki-67 expression in chordoma tissues was low. Kaplan-Meier univariate analysis showed that high Ki-67 expression was associated with shorter survival duration and a shorter time to recurrence. The Cox regression multivariate analysis showed that Ki-67 was an independent prognostic factor affecting PFS (P = 0.008) but not OS (P = 0.054). We, thus, confirmed the role of Ki-67 as a functional molecular marker of a poor prognosis. In line with our findings, Holton et al.[38] reported that higher expression of Ki-67 is indicative of faster growth of the chordoma. However, recent research has suggested that Ki-67 is merely a phenotypic marker reflecting mutation status.[39]

TGF-α has been found to be an important mediator of tumorigenesis and tumor progression.[40] The human TGF-α gene is located on chromosome 2 (2p13) and spans a 138.7-kb region.[41] TGF-α is a member of the epidermal growth factor (EGF) receptor (EGFR) ligand family and competes with EGF to bind to the cell surface of EGFR.[42] In contrast, as an epithelial cell-specific autocrine mitogen, TGF-α increases proliferation, inhibits apoptosis, triggers angiogenesis, and enhances invasion by activating various signaling pathways downstream of EGFR.[43-49] In contrast, TGF-α modulates the tumor microenvironment in a paracrine manner by promoting cross-talk among the tumor, surrounding stroma, and immune system.[49,50]

As previously stated, TGF-α drives tumor growth via autocrine growth factors, and this mechanism has been observed in a variety of tumors.[10-18] However, the role of TGF-α in chordoma is not clear. The results of our study showed that TGF-α was expressed in all chordoma cases. The data from immunohistochemical analyses of skull base chordoma specimens suggested that high levels of TGF-α expression were linked to shorter recurrence intervals. More specifically, the group with high TGF-α expression had significantly shorter PFS (mean, 29.3 months) than that of the group with low TGF-α expression (mean, 57.0 months; P = 0.005).

The results of multivariate Cox regression analysis further confirmed that patients with high TGF-α expression had shorter PFS compared with the other patients (P = 0.001). In the present study, we performed immunohistochemical staining of 46 specimens of skull base chordoma, examined TGF-α expression, and analyzed the relationship between the H-scores of TGF-α expression and survival. To the best of our knowledge, the present study is the first large-scale and long-term follow-up single-center study of TGF-α expression in chordoma. We found that high TGF-α expression was an independent prognostic factor in skull base chordoma. Our data offer a new perspective in the study of chordoma, and further research on the specific mechanisms might contribute to the medical treatment of skull base chordoma.

In some studies, surgical resection combined with adjuvant radiotherapy improved patient survival.[51,52] In the present study, our survival analysis showed that patients who had undergone radiotherapy experienced relatively longer PFS and OS. However, the difference was not statistically significant (P = 0.618 and P = 0.559, respectively). However, this was a group of surgical cases nearly 10 years ago. The method and dosage of radiotherapy were diverse in this group. In recent years, we have routinely recommended patients to receive radiotherapy, especially proton therapy and carbon ion therapy. With the increasing number of patients undergoing proton therapy and carbon ion therapy, the safety and effectiveness of these new methods for the treatment of skull base chordoma can be verified.[53]

Study Limitations

The present study was a retrospective analysis. The method and dosage regimen of radiotherapy were diverse in the present group, which could have limited the evaluation of the therapeutic value of adjuvant radiotherapy for chordoma.

CONCLUSIONS

Our study showed that high expression of TGF-α or Ki-67LI is indicative of a poor prognosis for patients with chordoma. We confirmed the role of Ki-67 as a functional molecular marker of a poor prognosis. We also identified TGF-α as a potential novel biomarker for predicting the prognosis of patients with skull base chordoma.

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