

The clinical pathological significance of FRAT1 and ROR2 expression in cartilage tumors

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Abstract

Purpose Chondrosarcoma is a malignant bone tumor with poor prognosis. Surgical treatment is the first choice for chondrosarcomas. Chondrosarcoma is not sensitive to chemotherapy and radiotherapy. Identification of biological markers is important for the early diagnosis and targeted treatment of chondrosarcoma. This study investigated the protein expression and clinicopathological significance of ROR2 and FRAT1 in 59 chondrosarcomas and 33 osteochondromas.

Methods ROR2 and FRAT1 protein expression in tissues was measured by immunohistochemistry.

Results The percentage of positive ROR2 and FRAT1 expression was significantly higher in patients with chondrosarcoma than in patients with osteochondroma ($P < 0.01$). The percentage of positive ROR2 and FRAT1 expression was significantly lower in patients with histological grade I, AJCC stage I/II stage, Enneking stage I, non-metastatic and invasive chondrosarcoma than patients with histological grade III, AJCC stage III/IV, Enneking stage II + III, metastatic and invasive chondrosarcoma

($P < 0.05$ or $P < 0.01$). ROR2 expression was positively correlated with FRAT1 expression in chondrosarcoma. Kaplan–Meier survival analysis demonstrated that histological grade, AJCC stage, Enneking stage, metastasis, invasion, and ROR2 and FRAT1 expression significantly correlated with a short mean survival time of patients with chondrosarcoma ($P < 0.05$ or $P < 0.01$). Cox multivariate analysis showed that positive ROR2 and FRAT1 expression was an independent prognostic factor that negatively correlated with postoperative survival and positively correlated with mortality.

Conclusion Positive ROR2 and FRAT1 expression is associated with the progression and poor prognosis of chondrosarcoma.

Keywords ROR2 · FRAT1 · Immunohistochemistry · Chondrosarcoma · Osteochondroma

Introduction

Chondrosarcoma is a malignant bone tumor derived from cartilage cells that accounts for approximately 20 % of all malignant bone tumors [1]. In contrast, osteochondroma is the most common benign tumor of the bone derived from hyaline cartilage in the medullary cavity and periosteum [2]. Currently, surgical treatment is the preferred method for effective treatment of chondrosarcoma and osteochondroma. Chondrosarcoma is not sensitive to radiotherapy and chemotherapy. Therefore, patients with metastatic tumors or tumors that occur in specific locations of the body that prevent surgical resection often have poor prognosis [1]. Unfortunately, molecular therapy of chondrosarcoma has not been effectively established in the clinic. Therefore, identification of biomarkers that can be

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developed into a target for molecular therapy or a predictive factor of prognosis is still important for the treatment of chondrosarcoma [3].

Wnt signaling is essential for cellular proliferation, survival, migration, and adhesion, which are important functions necessary for tumorigenesis and metastasis [4]. Deregulation of Wnt signaling has been observed in a variety of cancers [5]. ROR2 (receptor tyrosine kinase-like orphan receptor 2) is a member of the (RTKs) receptor tyrosine kinase family and the receptor of Wnt5a. ROR2 is involved in the regulation of JAK-STAT3 and Wnt/JNK signaling pathway [4, 6]. Recent studies have found abnormal ROR2 expression in a variety of tumor cells, which is believed to be associated with its pro-oncogenic and tumor suppressive roles [4, 6]. FRAT1 (frequently rearranged in advanced T-cell lymphomas 1) is a proto-oncogene encoding the FRAT1 protein, which is a positive regulatory factor of the Wnt/ β -catenin signaling pathway [7, 8]. Overexpression of FRAT1 may cause aberrant activation of Wnt signaling. Recent studies have observed high FRAT1 expression in numerous cancers and found that FRAT1 is involved in the progression and prognosis of these cancers. However, the expression of ROR2 and FRAT1 in chondrosarcoma and osteochondroma has not been reported.

In this study, the expression of ROR2 and FRAT1 proteins in chondrosarcoma and osteochondroma was investigated using immunohistochemical methods. The clinical and pathological significance of ROR2 and FRAT1 protein as well as their predictive role in the prognosis of patients with chondrosarcoma was analyzed.

Materials and methods

Specimens and clinical data

Resected tumor specimens of 59 patients with chondrosarcoma were collected from January 2001 to June 2011 at the Second Xiangya Hospital and Third Xiangya Hospital, Central South University. The diagnosis was confirmed by histopathology. The clinicopathological characteristics of 59 patients with chondrosarcoma are presented in Table 1. Survival information of 59 patients with chondrosarcoma was obtained with the longest follow-up being 134 months; 13 patients died during the follow-up period (22.0 %), while 46 patients survived (i.e. censored cases) (78.0 %). Osteochondroma specimens were collected from 33 patients including 27 males (81.8 %) and 6 females (18.2 %). Of the 33 patients, 29 patients were aged 45 years or younger (87.9 %), while 4 patients were older than 45 (12.1 %); 28 patients had a tumor mass with a maximal diameter ≤ 5 cm (84.8 %) and 5 patients had a tumor with a diameter > 5 cm (15.2 %).

Table 1 The clinicopathological characteristics of chondrosarcoma patients

Clinicopathological characteristics	Case number	Percentage
Age (years)		
≤ 45	24	40.7
> 45	35	59.3
Gender		
Male	30	50.9
Female	29	49.1
Differentiation		
Well	24	40.7
Moderate	22	37.3
Poor	13	22.0
Maximal tumor size		
≤ 5 cm	24	40.7
6–10 cm	27	45.8
> 10 cm	8	13.5
AJCC stages		
I	10	16.9
II	34	57.6
III	5	8.5
IV	10	16.9
Enneking stages		
I	38	64.4
II	11	18.6
III	10	17.0
Metastasis		
No	45	17.0
Yes	14	23.7
Invasion		
No	8	13.6
Yes	51	86.4

Immunohistochemistry

Rabbit anti-human ROR2 and FRAT1 polyclonal antibodies were purchased from Abgent Company (California, CA, USA). EnVisionTM Detection kit was purchased from Dako Laboratories (California, CA, USA). The positive controls were the positive breast cancer sections provided by Abgent Company. EnVision immunohistochemical staining was performed according to the manufacturer's protocol. Briefly, 4 μ m-thick sections were cut from routinely paraffin-embedded tissues. The sections were deparaffinized and then incubated with peroxidase inhibitor (3 % H_2O_2) in the dark for 15 min. Next, the sections were incubated with rabbit anti-human ROR2 and FRAT1 primary antibody for 60 min. After being soaked with PBS for 3 \times 5 min, Solution A was added to the sections for 30 min followed by DAB staining and haematoxylin counter-staining. The slides were then dehydrated with

Fig. 1 Immunohistochemical staining of ROR2 expression. Original magnification $\times 200$. Positive reaction was mainly localized in the cytoplasm.
a Positive ROR2 expression in chondrosarcoma tissues.
b Negative ROR2 expression in chondrosarcoma tissues.
c Positive ROR2 expression in osteochondroma tissues.
d Negative ROR2 expression in osteochondroma tissues

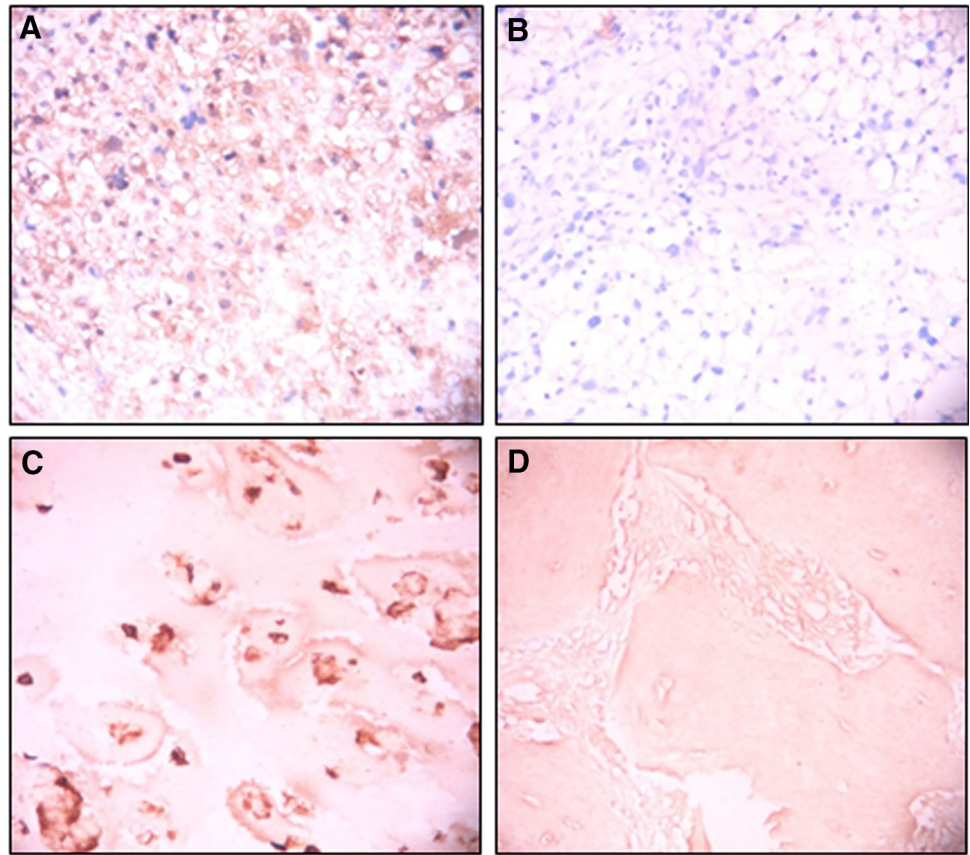


Fig. 2 Immunohistochemical staining of FRAT1 expression. Original magnification $\times 200$. Positive reaction was mainly localized in the cytoplasm.
a Positive FRAT1 expression in chondrosarcoma tissues.
b Negative FRAT1 expression in chondrosarcoma tissues.
c Positive FRAT1 expression in osteochondroma tissues.
d Negative FRAT1 expression in osteochondroma tissues

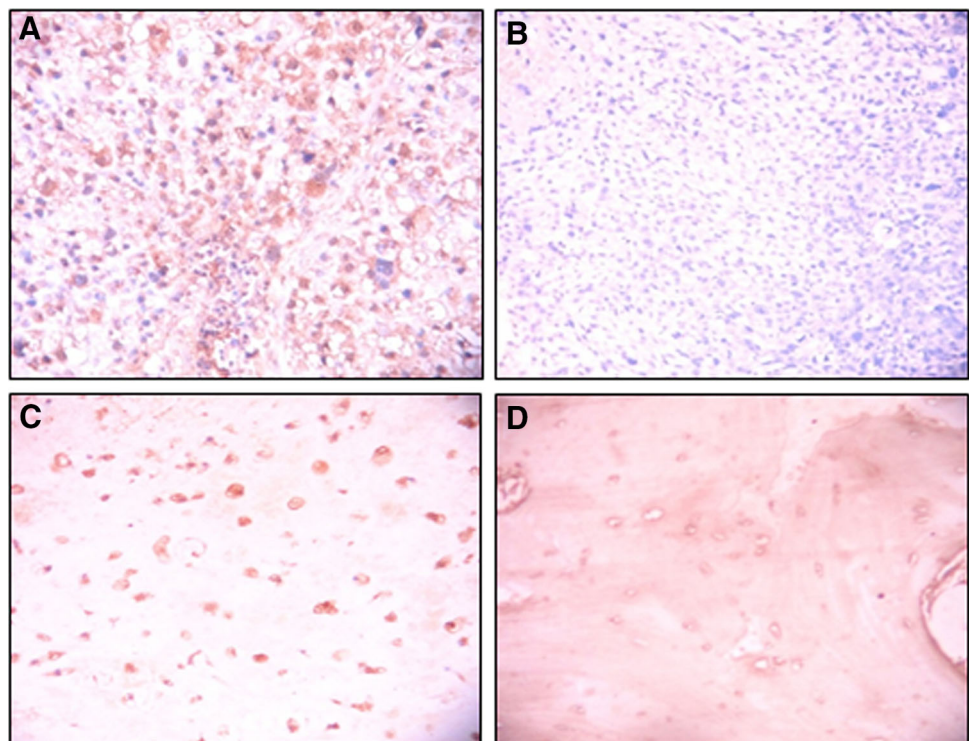


Table 2 The association of ROR2 and FRAT1 expression with the clinicopathological characteristics of chondrosarcoma

CP characteristic	Total no.	ROR2			FRAT1		
		Pos no. (%)	χ^2	<i>P</i>	Pos no. (%)	χ^2	<i>P</i>
Age							
≤45 years	24	17 (70.8)	0.404	0.525	14 (58.3)	0.008	0.928
>45 years	35	22 (62.9)			20 (57.1)		
Gender							
Male	30	17 (56.7)	2.425	0.119	16 (53.3)	0.461	0.497
Female	29	22 (75.9)			18 (62.4)		
Pathological type							
Conventional	53	36 (66.0)	3.003	0.432	31 (58.5)	2.207	0.508
Clear cell	1	0 (0.0)			1 (100.0)		
Mucoid	1	1 (100.0)			0 (0.0)		
Dedifferentiated	4	2 (50.0)			2 (50.0)		
Histological grade							
I	24	12 (50.0)	6.803	0.035	7 (29.2)	15.369	0.000
II	22	15 (68.2)			15 (58.2)		
III	13	12 (92.3)			12 (92.3)		
Tumor size							
≤5 cm	24	16 (66.7)	1.149	0.563	15 (62.5)	1.590	0.452
6–10 cm	27	19 (70.4)			16 (59.3)		
>10 cm	8	4 (50.0)			3 (37.5)		
AJCC stage							
I	10	4 (40.0)	8.885	0.038	4 (40.0)	7.398	0.057
II	34	21 (61.8)			17 (50.0)		
III	5	4 (80.0)			4 (80.0)		
IV	10	10 (100.0)			9 (90.0)		
Enneking stage							
I	38	22 (57.9)	3.907	0.0142	15 (39.5)	14.410	0.001
II	11	8 (72.7)			10 (90.9)		
III	10	9 (90.0)			9 (90.0)		
Metastasis							
No	45	26 (57.8)	5.864	0.017	21 (46.7)	9.330	0.002
Yes	14	13 (92.9)			13 (92.9)		
Invasion							
No	8	2 (25.0)	6.978	0.008	2 (25.0)	4.035	0.042
Yes	51	37 (70.6)			32 (62.8)		

alcohol, soaked in xylene, and mounted with neutral balsam. 400 cells in 10 random fields were examined per section. Patients with positive cells ≥25 % was considered positive whereas everything else was considered negative.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences version 18.0 (SPSS 18.0). The inter-relationship of ROR2 and FRAT1 expression with histological or clinical factors was analyzed using χ^2 or Fisher’s exact test. Kaplan–Meier and log-rank tests were used for

univariate survival analysis. Cox proportional hazards model was used for multivariate analysis and determining the 95 % confidence interval. A *P* < 0.05 was considered statistically significant.

Results

ROR2 and FRAT1 protein expression in chondrosarcoma and osteochondroma tissues

Positive ROR2 and FRAT1 staining was mainly located in the cytoplasm, and less at the nucleus of tumor cells

(Figs. 1, 2). Of the 59 chondrosarcomas, positive ROR2 staining was observed in 39 tissues (66.1 %), and positive FRAT1 staining was observed in 34 tissues (57.6 %). Of the 33 osteochondromas, positive ROR2 staining was observed in 3 patients (9.1 %), and positive FRAT1 was observed in 3 patients (9.1 %). The percentage of positive ROR2 and FRAT1 expression in patients with chondrosarcoma was significantly higher than that in patients with osteochondroma ($P = 0.000$).

Association of ROR2 and FRAT1 expression with the clinicopathological features of chondrosarcoma

As shown in Table 2, the percentage of positive ROR2 and FRAT1 expression in patients with histological grade I, AJCC stage I + II, Enneking stage I, non-metastatic and invasive chondrosarcomas was significantly lower than that in patients with histological grade III, AJCC stage III + IV, Enneking stage II + III, metastatic and invasive chondrosarcomas ($P < 0.05$ or $P < 0.01$). No significant association between ROR2 and FRAT1 expression and the age, gender, histological type, or maximum tumor diameter was observed in patients with chondrosarcoma ($P > 0.05$). Of the 39 chondrosarcomas with positive ROR2 expression, 29 patients were FRAT1 positive. Of the 20 patients with negative ROR2 expression, 15 were FRAT1 negative. A significantly positive correlation between the expression of ROR2 and FRAT1 was observed ($P = 0.000$).

Correlations between clinicopathological parameters, ROR2 and FRAT1 expression, and the mean survival of patients with chondrosarcoma were observed

Survival information of 59 cases with chondrosarcoma was collected via letter or telephone interviews. 13 cases (22.0 %) died during the follow-up of 134 months. Kaplan–Meier survival analysis showed that the gender, histological grade, AJCC stage, Enneking stage, metastasis, and invasion were significantly associated with the mean survival time of patients with chondrosarcoma (Table 3. $P < 0.05$ or $P < 0.01$). Patients with positive ROR2 and FRAT1 expression survived significantly shorter than patients with negative ROR2 and FRAT1 expression (Table 3 & Fig. 3. $P < 0.05$).

Cox multivariate analysis showed that histological grade III, AJCC stage III or IV, Enneking stage III, tumor metastasis, invasion, and positive ROR2 or FRAT1 expression negatively correlated with postoperative survival rate and positively correlated with mortality (Table 3). Positive ROR2 and FRAT1 expression was an independent prognostic factor in patients with chondrosarcoma (Table 4).

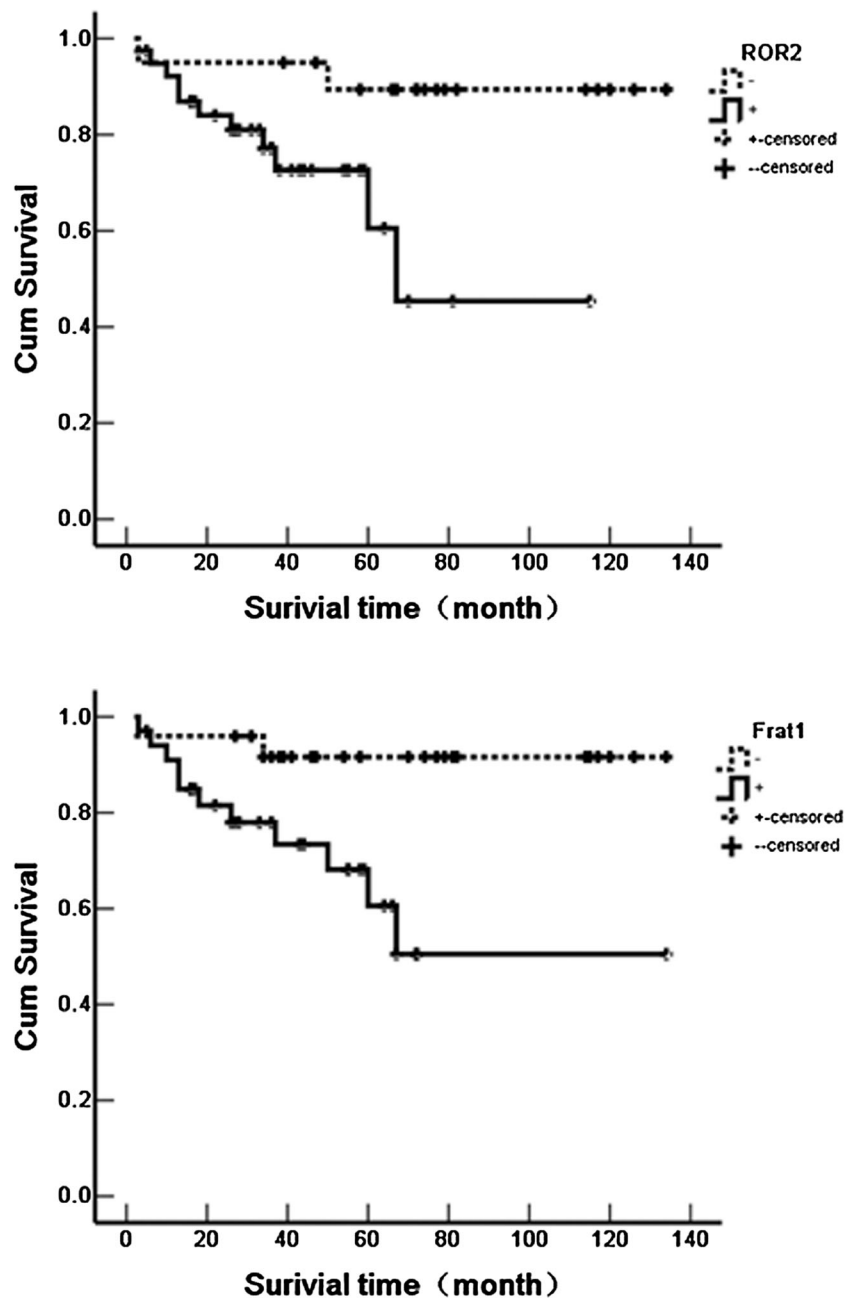
Table 3 Relationship between clinical, pathological characteristics, ROR2 and FRAT1 expression and survival of chondrosarcoma patients

Subgroup	Case number (n)	Mean survival (month)	Chi square	P value
Gender				
Male	30	81.63 (2–126)	6.112	0.013
Female	29	121.10 (5–134)		
Age				
≤45	24	97.36 (2–126)	0.021	0.886
>45	35	108.51 (3–134)		
Histological grade				
I	24	123.59 (16–134)	12.975	0.002
II	22	91.74 (6–115)		
III	13	40.05 (2–64)		
Tumor size				
≤5 cm	24	97.16 (2–134)	1.157	0.561
6–10 cm	27	107.33 (10–134)		
>10 cm	8	63.75 (3–10)		
AJCC stage				
I	10	124.75 (4–134)	12.544	0.006
II	34	115.75 (2–134)		
III	5	50.50 (16–67)		
IV	10	37.99 (3–64)		
Enneking stage				
I	38	119.99 (10–134)	8.650	0.013
II	11	67.38 (3–114)		
III	10	49.04 (2–67)		
Metastasis				
No	45	114.72 (2–134)	5.716	0.017
Yes	14	53.35 (5–67)		
Invasion				
No	8	16.68 (16–134)	55.535	0.000
Yes	51	6.98 (2–134)		
ROR2				
–	20	122.76 (3–134)	5.351	0.021
+	39	75.03 (2–115)		
FRAT1				
–	25	124.36 (2–134)	6.117	0.013
+	34	86.17 (3–134)		

Discussion

Current therapy of chondrosarcoma includes surgical resection and chemotherapy. However, patients with metastatic tumors or resistance to initial chemotherapy often have poor prognosis [9]. In addition, conventional chemotherapy is highly toxic. Therefore, various new therapeutic approaches have been developed in the past decade to target tumor cells by interfering with signaling pathways involved in cell proliferation, survival, or chemoresistance

Fig. 3 ROR2 and FRAT1 expression and survival in patients with chondrosarcoma. **a** Kaplan–Meier plots of mean survival in patients with chondrosarcoma and with ROR2 positive and negative expression. **b** Kaplan–Meier plots of mean survival in patients with chondrosarcoma and with FRAT1 positive and negative expression



[4, 5]. However, effective targeting therapy against tumor cells of chondrosarcoma has not yet been well established in the clinic. In this study, we identified high expression of ROR2 and FRAT1 protein in chondrosarcoma. Both positive ROR2 and FRAT1 expression are associated with malignancy of chondrosarcoma and poor prognosis of patients with chondrosarcoma. Low expression of ROR2 and FRAT1 was observed in benign tumors of the bone, which implicates the value of ROR2 and FRAT1 as a target for targeted therapy of chondrosarcoma.

Recent studies have found high expression of ROR2 in some malignancies, such as melanoma [10], renal cell

carcinoma [11], prostate carcinoma [12], and stromal tumors [13]. The high expression of ROR2 is associated with poor differentiation, high clinical stage, susceptibility to metastasis and invasion, or poor prognosis of these malignancies. Recently, two groups reported a correlation between high ROR2 expression and disease severity in osteosarcoma [14, 15]. ROR2's oncogenic role is supposed to be associated with Wnt5a-dependent cell proliferation, migration, and invasion [4]. In contrast, the tumor suppressive role of ROR2 has been reported in colon cancer [16], hepatocellular cancer [17], and multiple hematological malignancies [18]. ROR2 expression was significantly

Table 4 Multivariate Cox regression analysis of survival rate in chondrosarcoma patients

Subgroup	Factor	RC	SE	Wald	<i>P</i>	HR	95 % confidence interval	
							Lower	Upper
Histological grade	I/II/III	0.976	0.451	4.690	0.030	2.655	1.097	6.426
Tumor size	<5/6–10 > 10	1.120	0.571	3.852	0.050	3.065	1.002	9.381
AJCC stage	I/II/III/IV	1.057	0.429	6.059	0.014	2.878	1.240	6.679
Enneking stage	I + 1I/III	0.957	0.385	6.192	0.013	2.605	1.225	5.537
Metastasis	No/yes	1.512	0.719	4.418	0.036	4.535	1.107	18.568
Invasion	No/yes	1.191	0.542	4.825	0.028	3.291	1.137	9.525
ROR2	±	1.620	0.713	5.162	0.023	5.025	1.249	20.454
FRAT1	±	1.491	0.637	5.476	0.019	4.440	1.274	15.473

RC Regression coefficients, SE standard error, HR hazards ratio

downregulated or lost in these cancer cells. The tumor suppression of ROR2 is hypothesized to be associated with its ability to inhibit the pro-tumorigenic effects of the canonical Wnt pathway [4]. This study observed high ROR2 expression in chondrosarcoma, which was associated with the disease severity and poor prognosis, suggesting that ROR2 plays oncogenic roles in chondrosarcoma. The role of ROR2 in chondrosarcoma may be associated with its ability in regulating cell proliferation, differentiation, adhesion, migration, and invasion processes [19, 20].

Proto-oncogene FRAT1 encodes a 29kD protein [8, 21] and is first identified as T-cell lymphoma proto-oncogene, which positively regulates Wnt signal transduction pathway [21, 22]. FRAT1 inhibits the role of GSK3 on β -catenin phosphorylation by binding to GSK3 and subsequently elevating the cytoplasmic levels of free β -catenin. β -catenin enters into the nucleus, combines with members of the TCF/LEF family, and then activates the target genes [8, 21, 22]. Previous studies demonstrated that FRAT1 is overexpressed in esophageal cancer [23], lung cancer [24], ovarian cancer [25], Glioma [25], and glioblastoma [26]. The high expression of FRAT1 is associated with high malignancy, disease severity, and/or poor prognosis of these cancers. However, the expression of FRAT1 in both chondrosarcoma and osteochondroma has not been reported. In this study, we demonstrated that FRAT1 was highly expressed in chondrosarcoma, which was associated with tumor metastasis, invasion, high pathological grade and clinical stage, and short postoperative survival.

This study also found that percentage of positive ROR2 and FRAT1 expression was significantly lower in osteochondroma (9.1 %) than that in chondrosarcoma (66.1 and 57.6 %, respectively, $P = 0.000$). Moreover, a significantly positive correlation between the expression of ROR2 and FRAT1 was observed ($P = 0.000$). These findings suggest that both ROR2 and FRAT1 may be involved in the tumorigenesis of bone malignancies, and

these two proteins may collaborate during the development of chondrosarcoma through regulating Wnt signaling. Therefore, ROR2 and/or FRAT1 could be used as biomarkers for the potential for aggressive behavior or metastasis.

In conclusion, positive FRAT1 and ROR2 expression is a biomarker for disease severity and a predictive factor of poor prognosis in patients with chondrosarcoma.

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Conflict of interest All authors declared no any conflict of interest.

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