Int J Clin Exp Med 2017;10(1):1145-1150
www.ijcem.com /ISSN:1940-5901/IJCEM0040071

Original Article

HJURP overexpression indicates unfavorable prognosis in osteosarcoma

Haitao Li[1], Hongya Liu[2], Zhaohong Zhang[3], Xiaodong Xu[1], Jinqing Kan[1], Shiyou Dai[4], Yubao Yang[2], Yuan Li[2]

[1]Department of Joint Surgery, Linyi People's Hospital, Linyi, Shandong, China; [2]Department of Joint Surgery, Affiliated Hospital of Shandong Medical College, Shandong, China; [3]Department of Orthopedics, Affiliated Hospital of Shandong Medical College, Shandong, China; [4]Department of Orthopedics, Eastern District of Qingdao Municipal Hospital, Shandong, China

Received September 15, 2016; Accepted November 7, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: Aim: To elucidate the expression and prognostic significance of HJURP in osteosarcoma (OS). Materials and methods: A total of 61 samples of OS were collected and used for the detection of HJURP by immunohistochemistry (IHC). The cohort was divided into HJURP negative expression and positive expression according to the IHC score. We analyzed the correlation between HJURP expression and clinicopathologic factors with Chi-square test, and further evaluated the prognostic value of HJURP with univariate and multivariate analysis. Results: The positive staining of HJURP was mainly observed in cell nucleus. The percentage of HJURP positive expression was 32.7% (20/61). Positive HJURP expression (P=0.006), advanced Enneking stage (P=0.026), tumor histopathology (P=0.002), positive metastasis (P=0.041) and poor response to chemotherapy (P=0.033) were proved to be significantly associated with unfavourable prognosis with univariate analysis. Moreover, positive HJURP expression was identified as an independent prognostic factor of OS (HR=34.1, P=0.005) with multivariate analysis. Conclusions: In our study, positive HJURP expression was identified as an independent prognostic biomarker and could predict poorer prognosis in patients with OS, indicating HJURP as a potential therapeutic drug target of OS treatment.

Keywords: HJURP, biomarker, osteosarcoma, prognosis

Introduction

Osteosarcoma (OS) is the most frequent solid malignancy of bone [1]. The morbidity of OS is 0.2-3/100,000 in the general population worldwide [2], but the number increased to 0.8-11/100,000 in adolescence. OS is the third leading cause of cancer-related death in children and adolescents, and about 53% OS occurs in ages 0 to 24 years old [3]. OS is characterized by early metastasis and poor prognosis without treatment. It is reported that approximately 80% patients with OS have metastatic or micro-metastatic disease at diagnosis [4], making chemotherapy an important approach of OS treatment. The survival rate of OS patients improved remarkably along with the development of adjuvant therapy and surgical method. Identifying new biomarker and chemo-target for OS is still a promising way to find the effective drug target and improve prognosis of OS patients.

Holliday junction recognition protein (HJURP) is a kind of centromeric protein that plays a central role in the maintenance, assembling and recycling of histone H3-like centromeric protein A (CENPA) at centromeres [5], by which regulating cell mitosis and proliferation. Dysfunction of chromatin regulators is demonstrated to be involved in tumorigenesis and progression, including histone, histone chaperones and histone-modifying enzymes [6, 7]. Among the chromatin regulators, HJURP overexpression was observed in several kinds of cancers, including lung cancer, gliomas and breast cancer, etc [8, 9]. As the regulated protein of HJURP, CENP-A was proved to be unregulated in OS [10], but the clinical significance of HJURP is not elucidated in OS.

In our study, we investigated the expression of HJURP in OS samples and divided the cohort into HJURP positive expression and negative expression group. With univariate and multivari-

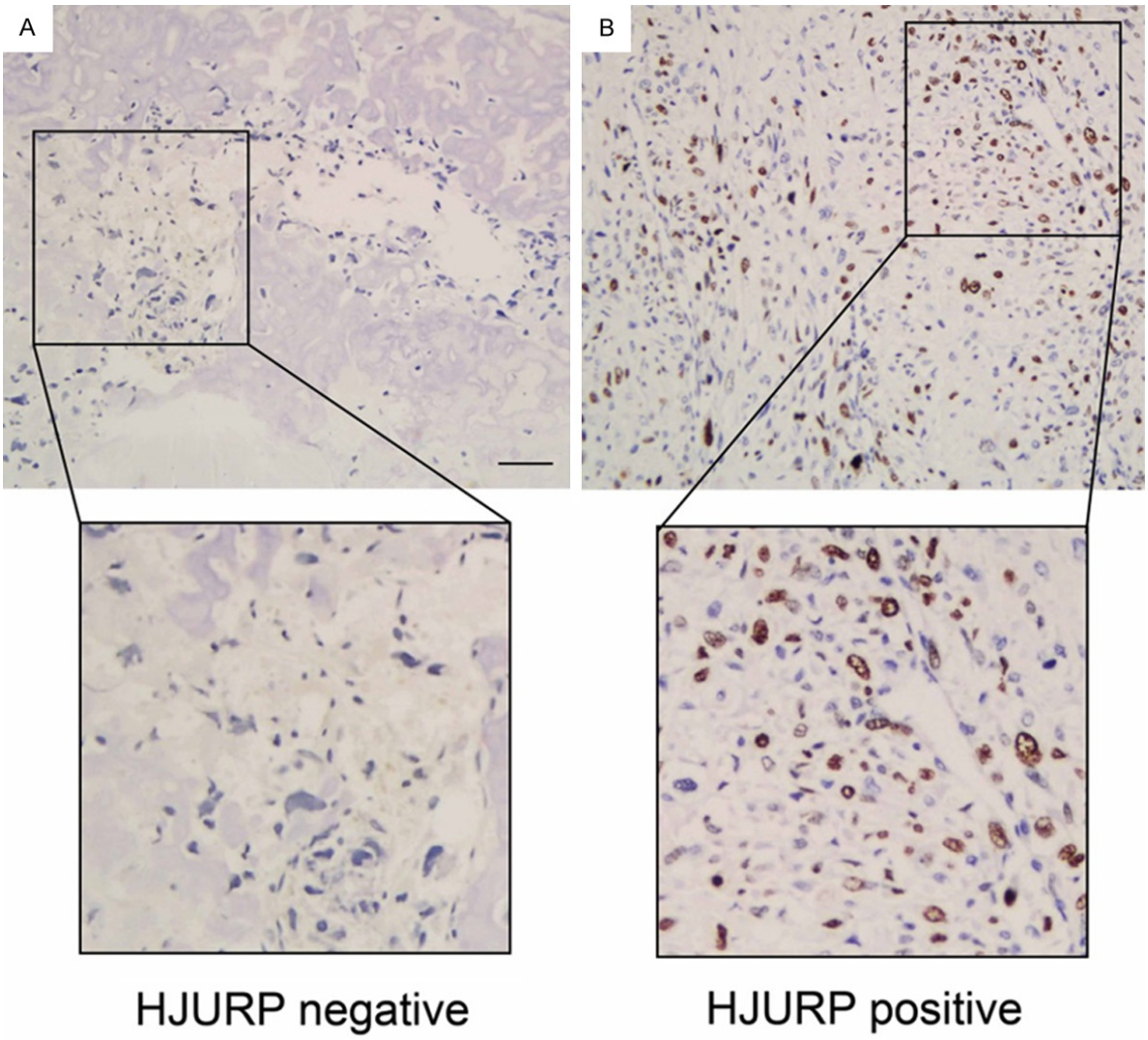

Figure 1. Representative images of HJURP negative and positive staining with immunohistochemistry. A: Representative HJURP negative immunohistochemistry staining and its magnified image. B: Representative HJURP positive immunohistochemistry staining and its magnified image. Scale bar: 50 μm.

ate analysis, we further analyzed the prognostic value of HJURP expression in OS.

Materials and methods

Patients and tissue samples

Osteosarcoma specimens were collected from the Affiliated Hospital of Shandong Medical College and Linyi People's Hospital from 2003 to 2010. The primary cohort consisted of 174 patients of osteosarcoma who underwent curative tumor resection in above hospitals. The validation cohort was selected from primary cohort according to the following criteria: (1) patients received neoadjuvant chemotherapy with high-dose methotrexate, adriamycin and cisplatin; (2) available biopsy tissue specimen before chemotherapy for IHC; (3) follow ups more than 5 months. The experiment was approved by the Ethics Broad of involved hospitals, and the samples were obtained with the prior consent of the patients. Tumor response to the neoadjuvant chemotherapy was judged according to the system of Salzer-Kuntschik (S-K) histologic 6-graded scale [11, 12]. Clinical stage of OS was referred to the criteria made by Enneking *et al* [13].

Immunohistochemical staining and score

Formalin-fixed, paraffin-embedded OS tissues were used for immunohistochemistry. Samples were first incubated in xylene and graded alco-

Table 1. Correlation between HJURP and clinicopathologic factors

Characters	Number	HJURP		P*
		Low	High	
Gender				
Female	15	10	5	0.959
Male	46	31	15	
Age				
<20	47	34	13	0.126
≥20	14	7	7	
Tumor size (cm)				
<8	37	27	10	0.273
≥8	24	14	10	
Enneking stage				
I	6	6	0	0.180
II	35	23	12	
III	20	12	8	
Site				
Femur	29	21	8	0.499
Tibia	13	10	3	
Humerus	10	6	4	
Fibula	5	2	3	
Others	4	2	2	
Histopathology				
Osteoblastic	23	15	8	0.817
Fibroblastic	14	11	3	
Chondroblastic	8	5	3	
Telangiectatic	7	5	2	
Others	9	5	4	
Metastasis				
No	41	29	12	0.562
Yes	20	12	8	
Response to chemotherapy				
Good	32	19	13	0.187
Poor	29	22	7	

*means calculated by Chi-square test. Abbreviation: HJURP for holliday junction recognition protein.

hol for deparaffinization and rehydration, and then soaked in 3% hydrogen peroxide for endogenous peroxidase inactivation. Citrate buffer (pH=6.0) was used to achieve better antigen retrieval of OS. After blockage with 5% bovine serum albumin for 1 hour, slides were incubated in primary antibody of HJURP (Abcam Ltd., Cambridge, UK) overnight at 4°C. Samples were then rinsed with phosphate buffer saline and incubated in secondary antibody for 2 hours at 37°C. Then secondary antibodies and streptavidin peroxidase complex reagent were added. Finally, 3,3'-diaminobenzidine solution was applied for visual staining and hematoxylin was used for counterstaining.

Each slide was evaluated by two pathologists blindly who were unaware of the clinical data of patients. Sections without consensus were re-evaluated by a third doctor. The IHC score was defined as the multiplied product of staining intensity and positive cells percentage. Scores of staining intensity included: 0, negative staining; 1, weak staining; 2, median staining; 3, strong staining. The scores of positive cells percentage included: 1, 0-25% positive cells; 2, 25%-50% positive cells; 3, above 50% positive cells. So the final score of IHC ranged from 0 to 9. The cut-off of IHC score was generated from ROC curve and it was set as the point with the highest specificity and sensitivity.

Statistical analysis

All statistical analyses were carried out with SPSS17.0 software (IBM Corporation). The relationship between the HJURP expression and clinicopathologic parameters was analyzed by Chi-square test without special instruction. Overall patient survival curves were analyzed by the Kaplan-Meier method, and the difference of survival rate of HURP negative or positive group was calculated with log-rank test. Independent prognostic riskers were defined with multivariate analysis by Cox proportional hazards. *P* value less than 0.05 was considered as statistically significant.

Results

Expression of HJURP in OS

The expression and location of HJURP were investigated by IHC in samples of OS (**Figure 1**). As a centromeric protein, HJURP was mainly observed in nucleus. The cohort was divided into HJURP positive and HJURP negative group according to the IHC score detailed explained in *Patients and Materials*. HJURP positive group accounted for 32.7% (20/61), while HJURP negative group took up about 67.3% (41/61).

Table 2. Univariate analysis of clinicopathologic factors

Characters	5-year survival rate	P*
Gender		
Female	77.1	0.513
Male	51.5	
Age		
<20	67.9	0.388
≥20	43.2	
Tumor size (cm)		
<8	66.6	0.821
≥8	47.4	
Enneking stage		
I+II	58.3	0.026
III	56.1	
Site		
Femur	69.5	0.087
Tibia	90	
Humerus	33.8	
Fibula	0	
Others	0	
Histopathology		
Osteoblastic	54.2	0.002
Fibroblastic	50	
Chondroblastic	0	
Telangiectatic	85.7	
Others	87.5	
Metastasis		
No	56.8	0.041
Yes	56.1	
Response to chemotherapy		
Good	73.9	0.033
Poor	37.6	
HJURP		
Low	80.4	0.006
High	21.1	

*means calculated by Log-rank test. Abbreviation: HJURP for holliday junction recognition protein.

Correlation between HJURP expression and clinicopathologic factors

The correlation between HJURP expression and clinicopathologic factors including patients' age, gender, Enneking stage, tumor size was analyzed by Chi-square test to select the relevant factors with HJURP (**Table 1**). In our study, all the enrolled clinicopathologic factors had no significant correlation with HJURP expression.

Prognostic value of HJURP

In previous study, CENPA, the target protein of HJURP, was demonstrated to be associated with unfavorable prognosis in OS, but the prognostic significance of HJURP in OS is still blank. In our experiment, we evaluated the prognostic significance of HJURP with univariate analysis and multivariate analysis.

Prognostic factors were first screened out with Kaplan-Meier method by univariate analysis (**Table 2**). All the investigated factors were enrolled. In our study, HJURP positive expression was identified as a significant factor indicating poorer prognosis (P=0.006) (**Figure 2**). Additionally, advanced Enneking stage (P= 0.026), tumor histopathology (P=0.002), positive metastasis (P=0.041) and poor response to chemotherapy (P=0.033) were significantly associated with unfavourable prognosis of OS.

To further confirm the results of univariate analysis and identify independent prognostic factors, we performed multivariate analysis with Cox-regression model (**Table 3**). All the prognostic factors demonstrated in univariate analysis were enrolled into the Cox-regression model except Enneking stage because of its interaction with metastasis. In the metastasis, HJURP positive expression was identified as an high-risk factor of poor prognosis in OS (HR= 34.1, P=0.005), which indicated that HJURP positive expression could predict unfavorable prognosis of OS. Moreover, positive metastasis (HR=47.9, P=0.004) and poor response (HR=6.6, P=0.019) to chemotherapy were also defined as independent prognostic factors of OS.

Discussion

Lots of evidence indicated that CENP-A is overexpressed in more aggressive carcinomas [8, 14-16], such as hepatocellular carcinoma, ovarian cancer, lung cancer, which are featured with early lymphatic invasion or metastasis. Primary study identified CENP-A as an independent poor prognostic factor for osteosarcoma with 123 cases of osteosarcoma. Additionally, CENP-A overexpression was significantly correlated with tumor size, poor response to neoadjuvant chemotherapy, local recurrence/lung metastasis, high Ki-67 index, and P53 positivi-

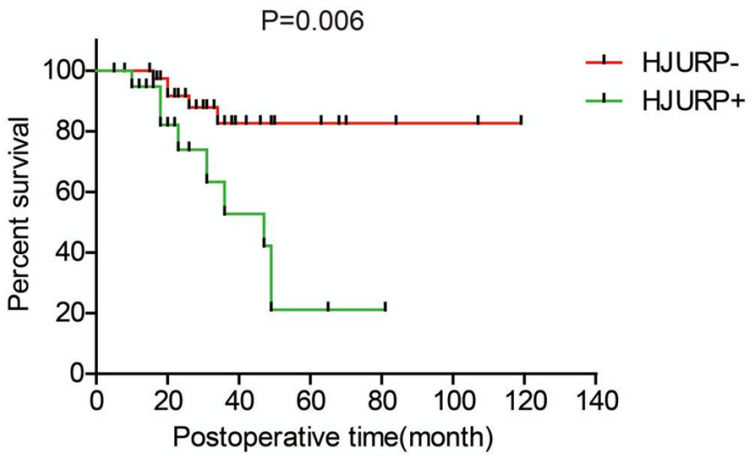

Figure 2. The overall survival curves of HJURP negative group and HJURP positive group are displayed by Kaplan-Meier analysis. The statistical difference is analyzed by log-rank test.

Table 3. Multivariate analysis of OS

Characters	HR	95% CI	P
Enneking stage			
I+II	-		
III	-	-	-
Site			
Femur	1		
Tibia	1.18	0.13-10.9	0.880
Humerus	-	-	0.959
Fibula	0.924	0.78-109	0.078
Others	0.758	0.047-12.2	0.845
Histopathology			
Osteoblastic	1		
Fibroblastic	0.075	0.003-01.89	0.115
Chondroblastic	0.105	0.004-3.05	0.190
Telangiectatic	2.74	0.21-36.5	0.446
Others	0.026	0.001-1.62	0.083
Metastasis			
No	1		
Yes	47.9	3.48-660	0.004
Response to chemotherapy			
Good	1		
Poor	6.6	1.36-32.2	0.019
HJURP			
Low	1		
High	34.1	2.81-409	0.005

ty [10]. However, there is no hypothesis or demonstration for the underlying mechanisms. As the most important regulator of CENP-A, HJURP should be considered as a candidate biomarker for OS.

As a relatively recognized new protein, the function of HJURP for depositing and assembling CENP-A was just acknowledged in recent years [5, 17]. The study of other HJURP is still in mist although it is attracting the focus of scientists more and more. In our study, we demonstrated HJURP overexpression as a biomarker of poorer prognosis in OS with very remarkable significance (P=0.006). However, our experiments did not involve the underlying mechanism of HJURP affecting OS prognosis. In consideration of that HJURP may be a potential drug target of OS treatment; new fundamental experiments should be performed *in vitro* and *in vivo* to reveal the function of HJURP in OS oncogenesis and progression.

Overall, we detected the expression of HJURP with IHC in 61 cases of OS and demonstrated that HJURP overexpression was significantly correlated with shorter survival time and poorer prognosis with univariate analysis. With multivariate analysis, we further identified HJURP overexpression as an independent prognostic factor, predicting unfavorable prognosis of OS patient. We hope our finding could trigger more interest on HJURP and CENP-A function on OS progression, and help find a new target for OS treatment.

Disclosure of conflict of interest

None.

Address correspondence to: Yubao Yang and Yuan Li, Department of Joint Surgery, Affiliated Hospital of Shandong Medical College, Shandong, China. E-mail: wchly1988@163.com (YBY); easonlinyi@163.com (YL)

References

[1] Ritter J and Bielack SS. Osteosarcoma. Ann Oncol 2010; 21 Suppl 7: vii320-325.

[2] Bielack S, Carrle D and Casali PG. Osteosarcoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2009; 20 Suppl 4: 137-139.

[3] Sborov D and Chen JL. Targeted therapy in sarcomas other than GIST tumors. J Surg Oncol 2015; 111: 632-640.

[4] Moore DD and Luu HH. Osteosarcoma. Cancer Treat Res 2014; 162: 65-92.

[5] Foltz DR, Jansen LE, Bailey AO, Yates JR 3rd, Bassett EA, Wood S, Black BE and Cleveland DW. Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. Cell 2009; 137: 472-484.

[6] Baylin SB and Jones PA. A decade of exploring the cancer epigenome-biological and translational implications. Nat Rev Cancer 2011; 11: 726-734.

[7] Gurard-Levin ZA, Quivy JP and Almouzni G. Histone chaperones: assisting histone traffic and nucleosome dynamics. Annu Rev Biochem 2014; 83: 487-517.

[8] Montes de Oca R, Gurard-Levin ZA, Berger F, Rehman H, Martel E, Corpet A, de Koning L, Vassias I, Wilson LO, Meseure D, Reyal F, Savignoni A, Asselain B, Sastre-Garau X and Almouzni G. The histone chaperone HJURP is a new independent prognostic marker for luminal A breast carcinoma. Mol Oncol 2015; 9: 657-674.

[9] de Tayrac M, Saikali S, Aubry M, Bellaud P, Boniface R, Quillien V and Mosser J. Prognostic significance of EDN/RB, HJURP, p60/CAF-1 and PDLI4, four new markers in high-grade gliomas. PLoS One 2013; 8: e73332.

[10] Gu XM, Fu J, Feng XJ, Huang X, Wang SM, Chen XF, Zhu MH and Zhang SH. Expression and prognostic relevance of centromere protein A in primary osteosarcoma. Pathol Res Pract 2014; 210: 228-233.

[11] Karrer K, Pridun N, Denck H and Sighart H. [Polychemotherapy in patients following radical surgery for bronchial carcinoma]. Osterr Z Onkol 1977; 3: 127-141.

[12] Grabellus F, Kraft C, Sheu-Grabellus SY, Bauer S, Podleska LE, Lauenstein TC, Pottgen C, Konik MJ, Schmid KW and Taeger G. Tumor vascularization and histopathologic regression of soft tissue sarcomas treated with isolated limb perfusion with TNF-alpha and melphalan. J Surg Oncol 2011; 103: 371-379.

[13] Enneking WF. A system of staging musculoskeletal neoplasms. Clin Orthop Relat Res 1986; 9-24.

[14] Lacoste N, Woolfe A, Tachiwana H, Garea AV, Barth T, Cantaloube S, Kurumizaka H, Imhof A and Almouzni G. Mislocalization of the centromeric histone variant CenH3/CENP-A in human cells depends on the chaperone DAXX. Mol Cell 2014; 53: 631-644.

[15] Li Y, Zhu Z, Zhang S, Yu D, Yu H, Liu L, Cao X, Wang L, Gao H and Zhu M. ShRNA-targeted centromere protein A inhibits hepatocellular carcinoma growth. PLoS One 2011; 6: e17794.

[16] Wu Q, Qian YM, Zhao XL, Wang SM, Feng XJ, Chen XF and Zhang SH. Expression and prognostic significance of centromere protein A in human lung adenocarcinoma. Lung Cancer 2012; 77: 407-414.

[17] Dunleavy EM, Roche D, Tagami H, Lacoste N, Ray-Gallet D, Nakamura Y, Daigo Y, Nakatani Y and Almouzni-Pettinotti G. HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. Cell 2009; 137: 485-497.