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Original article

RSF1 functions as an oncogene in osteosarcoma and is regulated by XIST/miR-193a-3p axis

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ABSTRACT

RSF1 (HBXAP), is a member of ATP-dependent chromatin remodeling factor. Dysregulated RSF1 has been reported to be related to tumor progression. However, the function of RSF1 in osteosarcoma (OS) remains unclear. In this study, we showed that RSF1 expression was upregulated in OS cells. RSF1 inhibition suppressed OS cell proliferation and invasion. We further showed that MAPK/Erk signaling pathway was inactivated by RSF1 suppression. In addition, RSF1 was identified as a direct target of miR-193a-3p. Clinically, RSF1 was increased and associated with advanced clinical features and poor overall survival of OS patients. MiR-193a-3p expression was decreased and associated with advanced clinical features and poor overall survival of OS patients. In addition, we found that miR-193a-3p was negatively correlated with RSF1 expression in OS tissues. Moreover, our data showed that XIST could function as competing endogenous RNA to repress miR-193a-3p, which regulated its downstream target RSF1. In conclusion, our findings demonstrated that the XIST/miR-193a-3p/RSF1 axis might contribute to the progression and act as a therapeutic target of OS patients.

1. Introduction

Osteosarcoma (OS) is the most frequent primary human sarcoma of the bone and a leading cause of cancer death, mainly in children and adolescents [1]. Despite the development of cancer treatment over the past few decades, the prognosis of advanced OS remains poor [2,3]. Thus, it is necessary to further explore factors that control OS initiation, progression, and metastasis and to develop novel therapeutic strategies to increase the long-term survival of patients.

RSF1, also known as hepatitis B X-antigen-associated protein (HBXAP), is a member of ATP-dependent chromatin remodeling factors [4]. Recent studies showed that RSF1 is commonly increased in diverse cancers and involved in various biological processes [5–7]. However, the biological effect of RSF1 on OS remains unclear.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs of 20–22 nucleotides that play important regulatory roles in biological processes through targeting mRNAs for cleavage and translational repression [8]. During carcinogenesis, miRNAs have been functionally classified as oncogenes or tumor suppressor genes and contributed to tumor progression by modulating target genes [9,10]. For example, Zhao et al. showed that miR-630 was upregulated in renal cancer and promoted renal cancer cells progression [11]. Xiang et al. showed that miR-25 was up-regulated in non-small cell lung cancer and promoted cell proliferation and motility by targeting FBXW7 [12]. Yang et al. showed that miR-506 was down-regulated in clear cell renal cell carcinoma and inhibited cell growth and metastasis via targeting FLOT1 [13].

It has been widely accepted that MAPK/Erk signaling pathway play important roles in tumor progression [14]. For example, Lin et al. showed that activation of MAPK/Erk leading to cell proliferation in non-small cell lung cancer [15]. Ruan et al. showed that knockdown of MAPK/Erk pathway inhibited breast cancer cell migration, invasion and adhesion capacity [16]. Wang et al. suggested that tumor suppressors miR-497 inhibited human ovarian cancer progression by targeting MAPK/Erk [17].

In the present study, our data showed that RSF1 inhibition suppressed OS cell proliferation, invasion and the downstream MAPK/Erk pathway. RSF1 was a direct and functional target of miR-193a-3p, which was affected by lncRNA XIST. Thus, we revealed that the XIST/miR-193a-3p/RSF1 axis was involved in OS progression.

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Table 1
Correlation between RSF1/miR-193a-3p expression and clinicopathological features of OS patients.

Clinico features	Group	Total	RSF1 expression		*P value*	miR-193a-3p expression		*P value*
			Low	High		Low	High	
Gender	Male	25	13	12	0.606	10	15	0.160
	Female	16	7	9		10	6	
Age (years)	< 25	28	15	13	0.368	14	14	0.819
	≥25	13	5	8		6	7	
Tumor size (cm)	< 8 cm	23	13	10	0.262	10	13	0.443
	≥8 cm	18	7	11		10	8	
Anatomic location	Tibia/femur	32	16	16	0.768	15	17	0.645
	Elsewhere	9	4	5		**5**	**4**	
Clinical stage	I/II	20	14	6	0.008	15	5	0.001
	III	21	6	15		5	16	
Metastasis	Absence	30	18	12	0.018	19	11	0.002
	Presence	11	2	9		1	10	

2. Materials and methods

2.1. Clinical samples

A total of 41 primary OS tissues and adjacent non-tumor tissues were surgically obtained from patients who underwent surgery at the The First Affiliated Hospital of Xinxiang Medical University and First Affiliated Hospital of Zhengzhou University from 2010 to 2012. This protocol was approved by the Ethics Committee of the Institutional Review Board at Xinxiang Medical University and Zhengzhou University, and written informed consent was collected before surgery. All the patients were pathologically confirmed and all of the samples were snap-frozen in liquid nitrogen and stored at −80 °C. The clinical parameters of OS patients in this study are presented in Table 1.

2.2. Cell culture

Human OS cell lines U2OS, Saos2, HOS, MG63 and normal osteoblast cell line NHOst were purchased from the Cell Bank of the Institute of Biochemistry and Cell Biology, China Academy of Sciences (Shanghai, China). The cells were maintained in DMEM (Gibco, Carlsbad, USA) containing 10% fetal bovine serum (FBS, Gibco, Carlsbad, USA) and cultured at 37 °C in humidified air containing 5% CO_2.

2.3. RNA extraction and quantitative real-time PCR

Total RNA was extracted from tissues and cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. The reverse transcription was performed by using a PrimeScript RT Reagent kit (Takara). The expression level of miRNAs and mRNAs were measured by qRT-PCR with SYBR Green PCR Kit (Takara) on Applied Biosystems StepOne-Plus Real-Time PCR System. QRT-PCR reactions were performed using the following parameters: 95 °C for 2 min followed by 40 cycles of 95 °C, 15 s, and 60 °C. The U6 or GAPDH was used as an endogenous control. The relative fold expressions were calculated with the $2^{-\Delta\Delta CT}$ method. The following primers were used for the quantitative PCR: RSF1 forward 5′-TCCCAGATGGAGAATGGTTC-3′, reverse 5′-AGTCTGGCTCTTGTGGAGGA-3′; miR-193a-3p forward 5′-GCATAACTGGCCTACAAAGT-3′, reverse 5′-GTGCAGGGTCCGAGGT-3′; GAPDH forward 5′-GAGTCAACGGATTTGGTCGT-3′, reverse 5′-TTGATTTTGGAGGGATCTCG-3′. U6 forward 5′-CTCGCTTCGGCAGCACA-3′, reverse 5′-AACGCTTCACGAATTTGCGT-3′.

2.4. Plasmid construction and transfection

To knockdown the RSF1, we performed the lentivirus infection strategy. Lentivirus containing shRNA targeting RSF1 gene or non-silencing control were constructed by GeneChem (Shanghai, China). Cells were plated at a density of 30% in a well condition before the day of infection. shRNA against lncRNA XIST or scrambled oligonucleotides were synthesized from GenePharma. The miR-193a-3p mimics, inhibitor, and corresponding negative controls were purchased from GenePharma, and transfections were performed using Lipofectamine 2000 (Invitrogen). sh-RSF1: 5′-CACCGGAGCTTTCGTCTACGACTGTTTCAAGAGAACAGTCGTAGACGAAAGCTCCTTTTTTG-3′. sh-XIST: 5′-CACCGCTCTTGAACAGTTAATTTGCTTCAAGAGAGCAAATTAACTGTTCAAGAGCTTTTTG-3′.

2.5. Cell proliferation assay

Cell proliferation was determined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cells (2×10^3) were seeded into 96-well plates and were maintained at 37 °C. MTT (100 μl; 0.5 mg/ml; Sigma) was added to each well at the indicated time points (24, 48, 72 and 96 h). 150 μl of DMSO was used to stop the reaction, followed by measurement of the absorbance at 490 nm.

2.6. Wound healing assay and cell invasion assay

Transfected cells (1.0×10^6) were seeded onto six-well plates and cultured overnight. Wounds were created by scratching cell layer with a sterile plastic pipette tips and washed with culture medium. Cells were further cultured with medium containing 1% FBS in 48 h.

For the invasion assays, a 24-well transwell chamber with the upper chamber coated with Matrigel (BD Bioscience) was used. 1.0×10^5 cells in 100ul serum free DMEM medium were seeded in the top chamber, 500ul medium containing 10% FBS was placed to the lower chamber. After incubation for 48 h, cells on the upper membrane surface were wiped off using a cotton swab and the cells that had traversed the membrane were staining by crystal violet and counted.

2.7. Luciferase activity assay

Both the wild-type (Wt) or mutated (Mut) 3′-UTR of RSF1 mRNA were subcloned into psicheck-2 vector (Promega). The mutant constructs were generated using a QuickChange site-directed mutagenesis kit (Stratagene). For the luciferase reporter assay, HEK293 cells (1×10^5 cells/well) were cultured and co-transfected with 50 nM miR-193a-3p mimics or negative control (miR-NC), 200 ng of Wt or Mut vector, and 2 ng of Renilla luciferase-expressing vector pRL-TK (Promega). 48 h later, cells were harvested and assayed using the Dual-Luciferase Reporter Assay System (Promega).

The fragment from XIST containing the predicted miR-193a-3p binding site was amplified by PCR and cloned into a pmirGLO Vector (Promega) to form the reporter vector XIST-wild-type (XIST-Wt). The