Biochemical and Biophysical Research Communications xxx (2017) 1–11

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p

Sheng Zhou [a], Ling Yu [a], Min Xiong [b, *], Guo Dai [a, **]

[a] Department of Orthopedics, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, PR China
[b] Department of Orthopedics, Affiliated Dongfeng General Hospital, Hubei University of Medicine, Shiyan 442008, Hubei, PR China

ARTICLE INFO

Article history:
Received 20 November 2017
Accepted 8 December 2017
Available online xxx

Keywords:
LncRNA SNHG12
miR-195-5p
Notch2
Osteosarcoma

ABSTRACT

Osteosarcoma is the most common primary malignant bone tumor and has a high fatality rate in children and adolescents. Recently, an increasing amount of evidence has demonstrated that lncRNAs have crucial roles in regulating biological characteristics in malignant tumors. Therefore, this research was carried out to uncover the biological function and the potential molecular mechanism of SNHG12 in osteosarcoma. In this study, we found that SNHG12 was significantly upregulated in both osteosarcoma tissues and cell lines and osteosarcoma patients with high levels of SNHG12 tended to have a poor prognosis. We evaluated the biological function of SNHG12 in 143B and U2OS cells and show that the downregulation of SNHG12 suppressed cell proliferation by blocking cell cycle progression at the G0/G1 phase and weakened cell invasion and migration abilities. Dual-luciferase reporter and RIP assays were conducted to confirm that SNHG12 functioned as a ceRNA, modulating the expression of Notch2 by sponging miR-195-5p in osteosarcoma. We further demonstrate that Notch2 played a crucial role in activating the Notch signaling pathway. In conclusion, SNHG12 might serve as a valuable biomarker and prognosis factor in osteosarcoma patients. The SNHG12/miR-195-5p/Notch2-Notch signaling pathway axis might become a novel therapeutic for osteosarcoma.

1. Introduction

Osteosarcoma is the most common primary malignant bone tumor and has a high fatality rate in children and adolescents [1]. Although the incidence of osteosarcoma is relatively low (about 6% of all childhood cancers) compared with other malignant tumors, the five-year survival rate is significantly poor [2]. During the first half of the 20th century, the five-year survival rate for osteosarcoma patients was less than 20% due to the highly invasive nature of this disease and the rapid lung metastasis that occurs during the first stage of its progression [3]. Despite the recent advances in multimodal therapeutics of osteosarcoma, the outcomes are still unsatisfactory [4]. Therefore, it is urgent for us to explore new therapeutic targets and novel approaches to osteosarcoma.

Long non-coding RNAs (lncRNAs) are longer than 200 nucleotides and highly conserved across mammalian species [5,6]. Recently, numerous studies have uncovered that lncRNAs are related to multiple cellular biological processes, such as cell cycle, cell invasion, and cell migration, and especially to cancer development and progression [7–9]. Accumulating evidence also indicates that the ectopic expression of lncRNAs correlates with tumorigenesis and metastasis in a variety of tumors, including osteosarcoma [10,11]. For example, Liu et al. [12] not only showed that the lncRNA HOXA11-AS was significantly upregulated in gastric cancer but also demonstrated that HOXA11-AS promoted gastric cancer progression and metastasis by partially regulating β-catenin and KLF2. Zhang et al. [13] found high levels of lncRNA FOXC2-AS1 and its antisense transcript FOXC2 in osteosarcoma and further proved that FOXC2-AS1 promoted doxorubicin resistance by increasing FOXC2 in osteosarcoma. Therefore, lncRNAs may serve as novel and effective therapeutic targets for cancer.

LncRNAs have been shown to regulate gene expression through complicated manners, including gene imprinting, dosage compensation and transcriptional or post-transcriptional processing [14]. In addition to acting directly on the target gene, competing endogenous RNAs (ceRNAs), a novel regulatory mechanism, have been identified in numerous studies and are characterized as

* Corresponding author.
** Corresponding author.
E-mail addresses: zhou415098@sina.com (M. Xiong), daiguo720@sina.com (G. Dai).

https://doi.org/10.1016/j.bbrc.2017.12.047
0006-291X/

lncRNAs that acts as molecular sponges for microRNAs (miRNAs) to regulate the target genes of miRNAs [15,16]. Lu et al. [17] reported that the downregulation of lncRNA00673 suppressed the expression of ZEB1 by sponging miR-150-5p, thus inhibiting the progression of lung cancer. Similarly, lncRNA CRNDE was proven to function as a ceRNA to regulate the level of E2F3 via the sponging of miR-145 in gastric cancer [18].

Recently, small nucleolar RNA host gene 12 (SNHG12) was reported by numerous studies to be upregulated in various types of cancers, including osteosarcoma [19–21]. Furthermore, Lan et al. [22] demonstrated that SNHG12 functioned as a ceRNA for miR-199a/b-5p to promote tumorigenesis and metastasis in hepatocellular carcinoma. Whether SNHG12 also functions as a ceRNA to regulate the tumorigenesis and metastasis of osteosarcoma is still unknown. Therefore, it is urgent to investigate this hypothesis.

2. Materials and methods

2.1. Tissue sample collection

We collected 25 samples of osteosarcoma and matched adjacent non-tumor tissues from Renmin Hospital of Wuhan University and 6 samples were collected from Dongfeng General Hospital between June 2009 and June 2012. All tissues were obtained and then stored at −80 °C in liquid nitrogen immediately until use. The detailed parameters of all the patients are shown in Table 1. The patients that participated in our research did not undergo any chemotherapy, radiotherapy or immunotherapy before surgery. Patients were followed up every 3–5 months until 5 years post-surgery.

2.2. Cell culture

The human osteosarcoma cell lines (143B, U2OS, MG63 and HOS) and normal human osteoblast cell line (hFOB) were purchased from the Cell Bank of Type Culture Collection (Shanghai, China). All cell lines were cultured in DMEM (HyClone, Logan, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, NY, USA), 100 U/ml penicillin and 100 μg/ml streptomycin in an atmosphere of 5% CO2 at 37 °C.

Table 1
Correlation between SNHG12 levels and clinical features in osteosarcoma patients.

Variable	Total No. (n = 31)	Relative SNHG12 expression		P value
		Low (n = 15)	High (n = 16)	
Gender				
Male	17	8	9	0.870
Female	14	7	7	
Age (years)				
< 20	22	11	11	0.779
≥ 20	9	4	5	
Histologic subtype				
Osteoblastic	16	7	9	0.298
Chondroblastic	10	4	6	
Fibroblastic	5	4	1	
Anatomical site				
Femure	13	7	6	0.926
Tibia	11	5	6	
Humerus	4	2	2	
Others	3	1	2	
Tumor grade				
Low	12	9	3	0.018[a]
High	19	6	13	
Enneking stage				
I	8	6	2	0.038[a]
II	15	8	7	
III	8	1	7	
Tumor size (cm)				
< 8	17	12	5	0.006[b]
≥ 8	14	3	11	
Metastasis				
No	10	8	2	0.015[a]
Yes	21	7	14	

[a] P<0.05.
[b] P < 0.01.

2.3. Plasmid construction and cell transfection

Small interfering RNA (siRNA) targeting SNHG12 or Notch2 was obtained from GenePharma (Shanghai, Chian). miR-195-5p mimic, miR-195-5p inhibitors and pCDNA3.1-Notch2 were purchased from GeneCreate (Wuhan, China). We transfected si-SNHG12 or si-Notch2 into 143B and U2OS cells by using GenMute (SignaGen Laboratories, Rockville, USA). Lipofectamine 2000 (Invitrogen, Carlsbad, USA) was used to transfect miR-195-5p mimic, miR-195-5p inhibitors and pCDNA3.1-Notch2 into 143B and U2OS cells. All above procedures followed the manufacturer's instruction. The sequences of si-SNHG12 were as follows: sense 5′-GCAGUGUGCUACUGAACUUTT-3′, antisense 5′-AAGUUCAGUAGCACACUGCTT-3’; The siRNA sequences were for Notch2: sense 5′-CACCUGAGUUGGAUGAUAUTT-3′, antisense 5′-AUAUCAUCCAACUCAGGUGTT-3’; miRNA-195-5p inhibitors sequence: 5′-GCCAAUAUUUCUGUGCUGCUA-3’.

2.4. RNA extraction and qRT-PCR assays

Total RNA was extracted from tissues and cell lines using TRIzol reagent (Invitrogen, Carlsbad, USA). Reverse transcription reactions were conducted via Takara RNA PCR Kit (Takara, Kyoto, Japan). Quantitative real-time polymerase chain reactions (qRT-PCR) were performed using a SYBR Green detection system (Takara, Kyoto, Japan). All above operations followed the manufacturer's instructions. The PCR results were analyzed to determine the Ct values of amplified products. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. U6 was used as the internal control. The PCR primer sequences used in this study are listed as follows: SNHG12, forward 5′-TCTGGTGATCGAGGACTTCC-3′ and reverse 5′-ACCTCCTCAGTATCACACACT-3′; miR-195-5p, forward 5′-GAATTCGCCTCAAGAGAACAAAGTGGAG-3′ and reverse 5′-AGATCTCCCATGGGGGCTCAGCCCCT-3′; Notch2, forward 5′-GGGACCCTGTCATACCCTCT-3′ and reverse 5′-GAGCCATGCTTACGCTTTCG-3′; U6, forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′; and GAPDH, forward 5′-TGTTCGTCATGGGTGTGAAC-3′ and reverse 5′-ATGGCATGGACTGTGGTCAT-3′.

2.5. Western blot analysis

Total proteins was extracted from transfected cells by using Radio Immunoprecipitation Assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Then, all proteins were separated by a 10% sodium dodecyl sulfate polyacrylamide (SDS/PAGE) gel and transferred onto a polyvinylidene fluoride membrane (PVDF, Millipore, Billerica, USA). The PVDF membrane was blocked in 5% non-fat dried milk for 2 h at room temperature and then incubated with primary antibody at 4 °C overnight. After being washed with TBST buffer five times, the PVDF membrane was incubated with horseradish peroxidase-conjugated secondary antibody (Proteintech Group, Inc., Chicago, USA) for 1 h at room temperature. Then, the PVDF membrane was washed three times with TBST buffer again. Finally, the proteins were detected by using an enhanced chemiluminescent kit (ECL kit, Santa Cruz Biotechnology, Santa Cruz, USA). The detailed information of primary antibodies is listed as follows: anti-CDK4, anti-CDK6, anti-CCND1, anti-Notch2, anti-

Please cite this article in press as: S. Zhou, et al., LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.12.047