Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Overexpressing circular RNA hsa_circ_0002052 impairs osteosarcoma progression via inhibiting Wnt/β-catenin pathway by regulating miR-1205/APC2 axis

Zhen Wu [a], Wangping Shi [b], Chendi Jiang [c, *]

[a] The Orthopaedic Department of Tongde Hospital of Zhejiang Province, Hangzhou, 310012, PR China
[b] Department of Pharmacy, Zhejiang Medical & Health Group Hangzhou Hospital, Hangzhou, 310022, PR China
[c] Department of Orthopaedic Surgery, The Dingli Clinical Institute of Wenzhou Medical University, Wenzhou, 325000, PR China

ARTICLE INFO

Article history:
Received 11 May 2018
Accepted 28 May 2018
Available online xxx

Keywords:
hsa_circ_0002052
Osteosarcoma
Progression
miR-1205
APC2
Wnt/β-catenin

ABSTRACT

Circular RNAs (circRNAs) are a novel class of noncoding RNAs, whose importance in cancer has been gradually acknowledged. However, the functions of circRNAs in tumorigenesis have not been fully understood. In the present study, we identified a novel circRNA hsa_circ_0002052 significantly downregulated in osteosarcoma (OS) tissues and cell lines. Moreover, we found that hsa_circ_0002052 could act as a biomarker to indicate the prognosis of OS patients. Functionally, we showed that hsa_circ_0002052 overexpression significantly suppressed OS cell proliferation, migration and invasion while promoting apoptosis *in vitro*. Similarly, *in vivo* assay indicated that ectopic expression of hsa_circ_0002052 impaired OS cell growth. In terms of mechanism, we found that hsa_circ_0002052 inhibited miR-1205 while miR1205 targeted APC2, a negative regulator of Wnt/β-catenin signaling pathway. By releasing the inhibition of miR-1205 on APC2 expression, hsa_circ_0002052 suppressed the activation of Wnt/β-catenin signaling pathway, leading to attenuated OS progression. Taken together, our study for the first time revealed a suppressive circRNA hsa_circ_0002052 involved in OS progression. Our study suggested hsa_circ_0002052/miR-1205/APC2/Wnt/β-catenin axis might be a potential target for OS therapy.

1. Introduction

Osteosarcoma (OS) is the most frequent and aggressive bone cancer, with a high incidence among children and adolescents [1]. Although significant improvements achieved on surgical techniques and chemotherapeutic treatment recently, the outcomes of OS patients still remain unsatisfied [2]. The five-year survival rate of OS patients is only about 60% [3]. Especially, OS patients with advanced stage and metastasis show a lower survival rate of about 20% [3]. Thus, there is an urgent requirement to identify novel biomarkers for the diagnosis and prognosis of OS patients. Furthermore, to develop effective therapeutic strategies, it is critical to understand the molecular mechanism of OS progression.

Circular RNAs (circRNAs) are a class of conserved and stably closed RNAs derived from back-splicing of pre-mRNAs [4] and characterized with neither a 5′ cap nor a 3′ polyadenylated tail [5]. Previously, circRNAs as well as long noncoding RNAs (lncRNA) are termed as “junk molecule” [6,7]. However, more and more evidences show that circRNAs exert essential roles in multiple biological processes, such as tumorigenesis, by regulating survival, proliferation and metastasis [8]. Many circRNAs are reported to be aberrantly expressed in various tumor tissues [9]. For instance, Circular RNA circMAN2B2 facilitates lung cancer cell proliferation and invasion via miR-1275/FOXK1 axis [10]. Silencing circular RNA hsa_circ_0000977 suppresses pancreatic ductal adenocarcinoma progression by stimulating miR-874-3p and inhibiting PLK1 expression [11]. In OS, a few circRNAs are reported to promote tumor progression, such as circNASP [12], circ-NT5C2 [13], hsa_circ_0001564 [14], hsa_circ_0009910 [15] and UBAP2 [16]. However, whether circRNAs suppress OS progression remains unknown and the function of hsa_circ_0002052 requires to be investigated.

In our study, we identified a novel suppressive circRNA

* Corresponding author. Department of Orthopaedic Surgery, The Dingli Clinical Institute of Wenzhou Medical University, No. 32 Dajian Lane, Lucheng District, Wenzhou, 325000, Zhejiang Province, PR China.
E-mail address: 517679750@qq.com (C. Jiang).

https://doi.org/10.1016/j.bbrc.2018.05.184
0006-291X/

hsa_circ_0002052 in OS by bioinformatics analysis. We found that hsa_circ_0002052 expression was significantly downregulated in OS tissues and cell lines. Furthermore, hsa_circ_0002052 could serve as a prognostic biomarker for OS patients. Functionally, we showed that hsa_circ_0002052 overexpression inhibited OS cell proliferation, migration and invasion while promoting apoptosis. Mechanistically, we identified hsa_circ_0002052 as a miR-1205 sponge to facilitate APC2 expression and consequently inactivate Wnt/β-catenin signaling pathway, leading to attenuated OS progression. Taken together, our study revealed a new regulatory mechanism that hsa_circ_0002052/miR-1205/APC2/Wnt/β-catenin signaling pathway modulates OS progression.

2. Materials and methods

2.1. Patient samples

Human primary breast cancer and their matched normal adjacent tissues were obtained from 54 cases of patients who received radical surgery at The Dingli Clinical Institute of Wenzhou Medical University. The diagnosis was based on pathological confirmation. None of the patients had received preoperative radiotherapy, chemotherapy or any other medical intervention. The tissues specimens were immediately snap-frozen and stored at −80 °C for further analysis. Written informed consent was collected from all patients and the study was approved by the ethics boards of The Dingli Clinical Institute of Wenzhou Medical University.

2.2. Cell culture and transfection

Human OS cell lines (HOS, U2OS, MG63 and 143B) and human osteoblast cell line hFOB1.19 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells cultured using Dulbecco's modified Eagle's medium (DMEM, Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen, MD, USA) and 100 U/ml penicillin and 100 U/ml streptomycin (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cell lines were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C.

For construction of stably hsa_circ_0002052 overexpressing cell lines, human hsa_circ_0002052 was synthesized and cloned into pcD-ciR vector (Geenseed Biotech, Guangzhou, China). Then, OS cells were transfected with this plasmid, followed by selected with G418.

2.3. Cell proliferation assay

The Cell Counting Kit-8 (CCK-8) method was utilized to detect cell proliferation. Transfected cells were seeded into 96-well plates at a density of 2000 cells per well in triplicate. Cell viability was measured by the CCK-8 system (Dojindo, Japan) at 24, 48, 72 and 96 h after seeding in accordance with the manufacturer's instructions.

2.4. Transwell assay

Cell migration and invasion were evaluated by using transwell cell culture chambers of 8 μm pore size according to the manufacturer's instruction. For migration assays, 1×10^5 cells were seeded in the top chamber without Matrigel (For invasion assay, the top chamber coated with Matrigel). The number of migrating or invasion cells was calculated under the microscope in ten random fields and shown as the average per field.

2.5. RNA isolation and qRT-PCR

Total RNA was isolated using the Trizol reagent (Invitrogen), followed by removal of DNA with the TurboDNase Kit (Ambion). Quantification of extracted RNA was performed using NanoDrop. cDNA synthesis was performed using PrimeScriptRT reagent KIT (Takara) using 1000 ng of total RNA. QRT-PCR was performed using the SYBR Select Master Mix (Applied Biosystems) on an ABI 7900 system (Applied Biosystem). The level of GAPDH was used as a control. The Ct value was calculated based on the ΔΔCt-method. Fold change of gene expression was expressed as $2^{-\Delta\Delta Ct}$ method.

2.6. Target prediction

Circular RNA Interactome (https://circinteractome.nia.nih.gov) was used to predict the potential miRNAs targeting hsa_circ_0002052. The potential targets of miR-1205 were predicted using the TargetScan (http://www.targetscan.org).

2.7. Apoptosis analysis

The apoptosis ratio was analyzed using the Annexin V-FITC Apoptosis Detection Kit (Beyotime, China). Cells were harvested and resuspended in binding buffer containing Annexin V-FITC and PI according to the manufacturer's instructions. The samples were analyzed by flow cytometry (BD Biosciences, USA). Experiments were performed three times.

2.8. Xenograft nude mouse model

To examine the effect of hsa_circ_0002052 expression in tumor formation, male BALB/c nude mice (4–6 weeks old) were used for all *in vivo* xenograft studies. All animal experiments were approved by the Animal Care and Use Committee of The Dingli Clinical Institute of Wenzhou Medical University. Exponentially growing U2OS cells were injected subcutaneously into the flanks of nude mice (2×10^6 cells per animal). Tumors size was measured every 1 week by caliper to determine tumor volume. All mice were killed 4 weeks after seeding of tumor cells, and the tumor weights measured.

2.9. Luciferase reporter assay

OS cells were seeded into a 24-well plate. Cells were co-transfected with wild-type or mutated hsa_circ_0002052 or APC2 3′-UTR reporter plasmids, and with miR-1205 mimics or negative controls. Luciferase assays were conducted 24 h after transfection using the Dual Luciferase Reporter Assay System (Promega, WI, USA).

2.10. Statistical analysis

All experiments were conducted at least three times. Data were displayed as mean ± SD. Analyses were performed with SPSS 20.0 software (SPSS, Chicago, IL, USA). Student's t-test was used for differences between two groups and one-way ANOVA for multiple comparisons. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Hsa_circ_0002052 was downregulated in OS tissues

In order to explore the function of circRNAs in OS progression, we first analyzed a microarray dataset (GSE96964). As shown in

Please cite this article in press as: Z. Wu, et al., Overexpressing circular RNA hsa_circ_0002052 impairs osteosarcoma progression via inhibiting Wnt/β-catenin pathway by regulating miR-1205/APC2 axis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.184