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Up-regulated MiR-27-3p promotes the G1-S phase transition by targeting inhibitor of growth family member 5 in osteosarcoma

Pei Ye[a,1], Xueping Ke[b,1], Xuehui Zang[a], Hui Sun[a], Zhixing Dong[c], Jun Lin[a], Lihui Wang[a], Wenzhou Liu[a], Guiqiang Miao[a], Yongtao Tan[a], Weilai Tong[a], Haichang Xiao[a], Lihua Gao[a,*]

[a] Department of Orthopedics, Nanhai Affiliated Hospital of Southern Medical University, Foshan, Guangdong, 528000, China
[b] Department of Gastroenterology, the Liwan Hospital of the Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510175, China
[c] Department of Radiology, Nanhai Affiliated Hospital of Southern Medical University, Foshan, Guangdong, 528000, China

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ABSTRACT

Objective: MicroRNAs (miRNAs) play an essential role in regulating malignant progression of tumour cells by inhibiting translation or stability of messenger RNA. However, the expression pattern and regulatory mechanism of miR-27-3p in osteosarcoma remains unclear.

Methods: We examined the expression of miR-27-3p in 5 osteosarcoma cell lines compared with that in 2 normal osteocyte cell lines. Osteosarcoma cells U-2OS and MG-63 were transduced to up-regulate or down-regulate the expression of miR-27-3p. The 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide, or MTT, assay, colony formation assays, BrdUrd labelling, immunofluorescence, anchorage-independent growth ability assay and flow cytometry analysis were used to test the effect of miR-27-3p. Luciferase assays were added to verify the direct relationship between miR-27-3p and the predicted target gene inhibitor of growth family member 5 (ING5).

Results: The expression of miR-27-3p was significantly increased in examined osteosarcoma cell lines compared with that in normal osteocyte cell lines. Up-regulation of miR-27-3p significantly accelerated osteosarcoma cell growth via promoting G1-S transition. In addition, the opposite result was observed in miR-27-3p-down-regulated cells. Up-regulation of ING5 significantly attenuated the miR-27-3p-induced proliferation in osteosarcoma cells.

Conclusions: These data suggested that miR-27-3p could promote the G1-S phase transition that leads to proliferation by down-regulating the expression of ING5 in osteosarcoma.

1. Introduction

Osteosarcoma is one of the most common bone tumours in young patients [1]. Removal of the primary osteosarcoma and multidrug chemotherapy are still the basic treatment [2]. Although the advances in chemotherapy have substantially improved, the survival rate remains be in the plateau over the past two decades [3]. In term of side effects, most conventional chemotherapeutic drugs are life-threatening for managing osteosarcoma because of their renal toxicity and the possibility of haemorrhagic cystitis and hearing impairment. Therefore, it is urgent to find new therapies with safer and more sufficient anticancer properties. Biological targeted therapy is a rapidly evolving therapeutic modality [4].

Endogenous microRNAs (miRNAs), belonging to small noncoding RNA molecules, could bind to the partial sequence homology of the 3′-untranslated region of target messenger RNA (mRNA) and cause translation inhibition or mRNA degradation [5]. Studies show that miRNAs can form extensive regulatory networks with a complexity comparable to that of transcription factors [6]. Increasing evidence indicates that miRNAs are important regulators in the initiation and progression of cancers [7]. Some miRNAs act as oncogenes, such as miR-18b in ovarian cancer, miR-127 in gliomas cells, miR-23a in pancreatic cancer, and miR-93-3p in clear cell renal cell carcinoma [8–11]. In contrast, miR-15b in liver cancer cells, miR-33b in lung cancer cells, miR-204 in breast cancer, and miR-146a in colorectal cancer entirely act as antioncogenes [12–15].

Abbreviations: miRNAs, microRNAs; ING, inhibitor of growth; EMT, epithelial-mesenchymal transition; 3′ UTR, 3′-untranslated region; wt, wild-type; ORF, open reading frame; MTT, 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide

* Corresponding author at: Department of Orthopedics, Nanhai Affiliated Hospital of Southern Medical University, 40 Buddha Ping Road, Foshan, Guangdong, 528000, China.
E-mail address: guke360360@hotmail.com (L. Gao).

[1] These authors contributed equally to this work.

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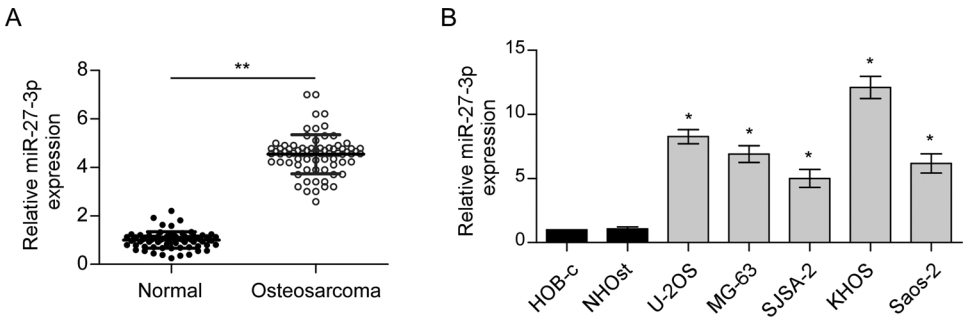

Fig. 1. MiR-27-3p is up-regulated in osteosarcoma. (A) Real-time PCR analysed the expression of miR-27-3p in the primary osteosarcoma tissues and in adjacent normal bone tissues. ** $P < 0.01$ versus Normal. (B) The expression of miR-27-3p was detected in human osteoblast cell line HOB-c, NHOst, and human osteosarcoma cell lines (U-2OS, MG-63, SJSA-2, KHOS and Saos). U6 small nuclear RNA was used as a normalised gene. Values are expressed as the means ± SD; n = 3. * $p < 0.05$ versus HOB-c.

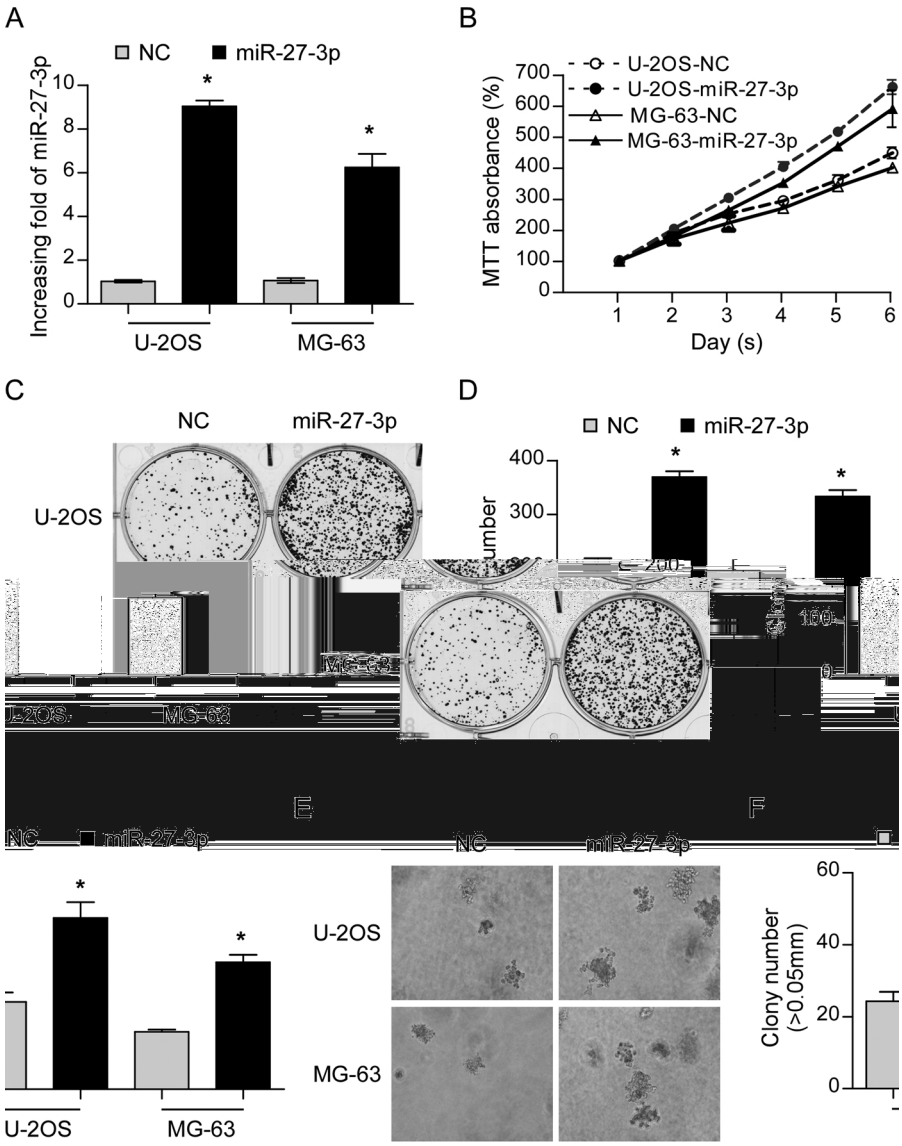

Fig. 2. Overexpression of miR-27-3p promoted the proliferation of osteosarcoma cells U-2OS and MG-63. (A) Increasing levels of miR-27-3p expression as assessed by real-time PCR. (B) MTT assays show that miR-27-3p-transfected cells grow faster than the vector-transfected cells. (C) Representative micrographs of crystal violet stained cell colonies. (D) Quantification of crystal violet stained cell colonies. (E) Representative micrographs of colonies in the anchorage-independent growth assay. Each bar represents the mean of three independent experiments. (F) Numbers of colonies in the anchorage-independent growth assay. Each bar represents the mean of three independent experiments. Values are expressed as the means ± SD; n = 3. * $p < 0.05$ versus NC, respetively.

miR-27a and miR-27b belong to the members of the miR-23-27-24 cluster and reportedly were highly expressed in endothelial cells [16]. Studies have identified important roles for miR-27 in various pathological changes, such as angiogenesis, mitochondrial network and brown adipogenesis [17–20]. MiR-27 was even identified to promote the differentiation of odontoblastic cells and accelerates mineralisation [21]. More recently, miR-27 displayed its critical role in the type of progression of cancers. Previous studies have demonstrated that miR-27 was overexpressed in invasive adenocarcinomas, and its expression increased linearly according to clinical stage [22]. Jiang J et al. found that miR-27 modified growth and invasion of non-small cell lung cancer cells partially by targeting Sp1 transcription factor [23]. Up-regulation of miR-27 increased the levels of the epithelial-mesenchymal transition-associated genes in gastric cancer [24]. Furthermore, miR-27 could tightly regulate the immune system and thus affected the malignant process of tumour. For example, miR-27 functions as a key regulator in Treg development, suggesting a proper regulation of miR-27 in regulatory cell-mediated immunological tolerance [25]. However, the specific functions and targets of miR-27 in osteosarcoma are largely unexplored. As the family members miR-27a and miR-27b differ in only