Bone 66 (2014) 267–276

Contents lists available at ScienceDirect

Bone

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

Original Full Length Article

MiR-126-5p regulates osteolysis formation and stromal cell proliferation in giant cell tumor through inhibition of PTHrP

Wang Zhou [a,1], Huabin Yin [a,1], Ting Wang [a,1], Tielong Liu [a], Zhenxi Li [a], Wangjun Yan [a], Dianwen Song [a], Haiyan Chen [b], Jia Chen [a], Wei Xu [a], Xinghai Yang [a,*], Zhipeng Wu [a,*], Jianru Xiao [a,*]

[a] Department of Bone Tumor Surgery, Changzheng Hospital, Second Military Medical University, Shanghai, China
[b] Division of Rheumatology, Zhongda Hospital, Dongnan University, Nanjing, China

ARTICLE INFO

Article history:
Received 18 March 2014
Revised 3 June 2014
Accepted 17 June 2014
Available online 25 June 2014

Keywords:
PTHrP
miR-126-5p
Giant cell tumor
Osteolysis
Osteoclast differentiation

ABSTRACT

Parathyroid hormone-related protein (PTHrP) has been identified to play a crucial role in osteolysis formation and stromal cell (GCTSC) proliferation in giant cell tumor (GCT). MiR-126-5p is an intronic miRNA identified as tumor suppressor in many tumors, but its role in GCT is poorly understood. We found that miR-126-5p was decreased in GCT and could directly regulate PTHrP expression. Furthermore, miR-126-5p could control osteoclast (OC) differentiation, GCTSC proliferation and osteolysis formation in GCT through negative regulation of PTHrP. Thus, these results suggest that miR-126-5p could directly target PTHrP and have a tumor suppressor function in GCT.

Introduction

Giant cell tumor of bone (GCT) is an aggressive skeletal tumor which is composed of three major cell types: osteoclast-like multinucleated giant cells, spindle-like stromal cells, and monocytic round cells [1–4]. Spindle-like stromal cells of GCT (GCTSC) which originate from mesenchymal stem cells in the bone marrow are neoplastic component of GCT [5]. In our previous study we found that complete resection and inhibition of bone resorption could significantly reduce GCT recurrence [6]; however, recurrence rates of 18% to 60% remain high and malignant transformation as well as lung metastasis occasionally happens [7–9]. Osteolytic lesion formation in GCT is a complex process involving many factors but is still not fully understood. Thus further understanding the biology of this tumor is critically important.

PTHrP shares the same N-terminal end as parathyroid hormone (PTH) and plays its roles through binding to and activating PTH/PTHrP type 1 receptor (PTH1R) [10–12]. PTHrP is considered to be highly expressed and play a vital role in GCT [13–15]. It can increase receptor activator of NF-κB ligand (RANKL), MMP-13 and IL-8 expression to stimulate osteoclastic bone resorption in GCT [15,16]. Furthermore, PTHrP can induce GCTSC proliferation by protecting them from apoptosis [13].

MiRNAs are small, evolutionarily conserved non-coding RNA molecules that act as post-translational regulators of gene expression [17,18]. MiRNAs are confirmed to play an essential role in a great many tumors through regulation of their target genes [19,20]. MiR-126-5p is an intronic miRNA located in the epidermal growth factor-like domain 7 (EGFL7) gene and has been identified as a tumor suppressor in many tumors such as prostate cancer, melanoma, breast cancer and non-small cell lung cancer [21–25]. However, the specific role of miR-126-5p in GCT remains unknown.

In this study, we demonstrated that miR-126-5p could directly regulate PTHrP expression and further control the biological behavior of GCT. An inverse correlation was found for miR-126-5p and PTHrP in GCTSCs; furthermore, miR-126-5p overexpression significantly decreased GCTSC proliferation and osteolytic lesion in GCT. Our study shows a novel mechanism for PTHrP regulation, and that miR-126-5p is a tumor suppressor for GCT.

Materials and methods

Cell lines and reagents

For primary cell culture, bone marrow-derived monocyte (BMM) cells isolated from C57/BL6 mice and GCTSCs isolated from GCT samples were cultured as described previously [26]. Human Embryonic Kidney 293 cells (HEK293) and human osteosarcoma cell line MG-63 were

* Corresponding authors.
E-mail addresses: cnspineyang@163.com (X. Yang), eaglewzp@sina.com (Z. Wu), jianruxiao83@163.com (J. Xiao).
[1] Wang Zhou, Huabin Yin and Ting Wang contributed equally to this work, and all should be considered first author.

http://dx.doi.org/10.1016/j.bone.2014.06.021
8756-3282/

bought from ATCC (Virginia, USA). HEK293 and MG63 cells were maintained in DMEM (GIBCO) supplemented with 10% fetal bovine serum (HyClone) in the cell incubator (37 °C, 5% CO_2). BMM and GCTS cells were maintained in α-MEM (GIBCO) supplemented with 10% fetal bovine serum.

The antibodies of PTHrP (sc-9680), PTH1R (sc-12722), IL-8 (sc-376750), RANKL (sc-7628), MMP-13 (sc-30073), GAPDH (sc-32233) were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Transfections were performed using FuGENE HD (Promega, USA) according to the manufacturer's instructions. Macrophage colony-stimulating factor (M-CSF) was from R&D Systems (Minneapolis, USA).

Patient sample collection

The diagnosis of GCT of bone was established by biopsy prior to surgical excision. All patients with GCT received extended curettage, with no adjuvant therapy. Specimens were obtained at the time of surgery and a pathologist verified the diagnosis of GCT post-operatively. Tissue samples from ten cases of GCT of bone were used in this study and all experiments were performed in triplicate or as otherwise stated for all ten patient specimens. This research was approved by the Research Ethics Board of the Changzheng Hospital, Second Military Medical University (Shanghai, China).

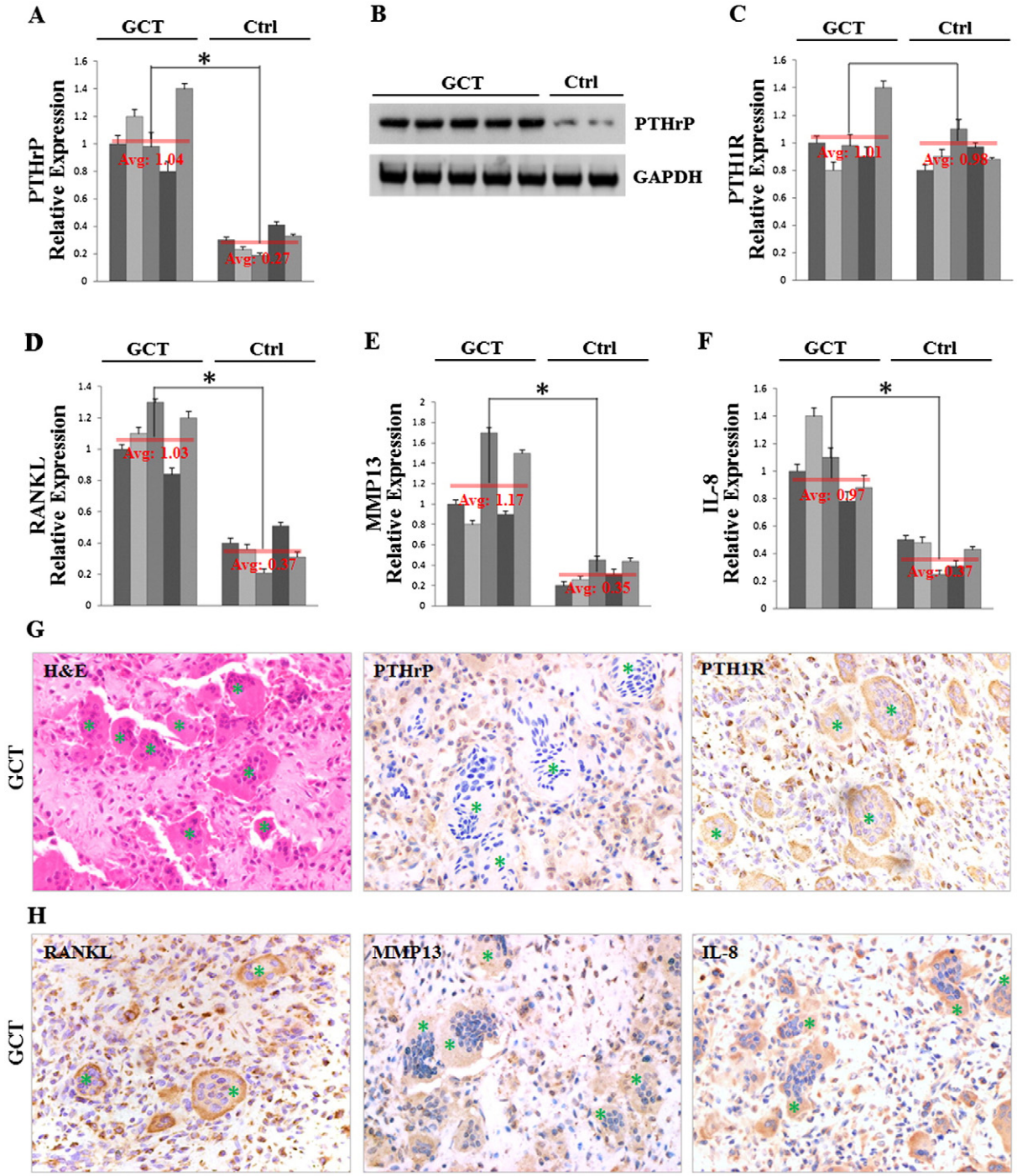

Fig. 1. Expression of PTHrP in GCT. A, C–F. qRT-PCR analysis of PTHrP, PTH1R, RANKL, MMP-13, and IL-8 expression in GCT samples and para-tumor normal bone tissues. B. Western blot analysis of PTHrP expression in GCT samples and para-tumor normal bone tissues. G. H&E staining and immunolocalization of PTHrP and PTH1R in human specimens of GCT formalin-fixed-paraffin-embedded tissue. Giant cells are marked with asterisks. H. Immunolocalization of RANKL, MMP-13 and IL-8 in human GCT specimens.