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Research paper

LncRNA PRNCR1 regulates CXCR4 expression to affect osteogenic differentiation and contribute to osteolysis after hip replacement

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ABSTRACT

Background: Osteogenic differentiation plays an essential role in the pathogenesis of osteolysis, which is a complication of hip orthroplasty. The roles of lncRNA PRNCR1 in osteogenic differentiation and osteolysis were dissected in this study.

Methods: The expression of PRNCR1, miR-211-5p and C-X-C chemokine receptor-4 (CXCR4) protein in wear particles and mesenchymal stem cells (MSCs) were determined by qRT-PCR and Western blot, separately. The osteogenic differentiation degree of MSCs was assessed by ALP activity detection and ARS staining. Binding and interaction between RNA and protein were determined with RIP and RNA pull-down assay, respectively. Interaction between miR-211-5p and CXCR4 was examined by Dual luciferase reporter assay.

Results: PRNCR1 and CXCR4 were up-regulated in wear particles around prosthesis and in MSCs treated with PMMA, while miR-211-5p was down-regulated. Repression of PRNCR1 weakened the inhibitory effect of wear particles on osteogenic differentiation. PRNCR1 positively regulated CXCR4 through inhibiting miR-211-5p. Wear particles increased CXCR4 level through miR-211-5p to inhibit osteogenic differentiation of MSCs. Wear particles down-regulated miR-211-5p level through PRNCR1 to influence osteogenic differentiation of MSCs.

Conclusion: LncRNA PRNCR1 up-regulates CXCR4 through targeting miR-211-5p, which affects osteogenic differentiation and thus contributing to osteolysis after hip replacement.

1. Introduction

Hip replacement is the reparation surgery for replacement of all or part hip joint with artificial hip joint (Learmonth et al., 2007), which has been used clinically for many years with better joint motion, early move around and decreased complications for staying long in bed (Shan et al., 2014). However, complications of hip replacement such as postoperative infection, dearticulation, deep venous thrombosis, osteolysis and prosthesis loosening are gradually increased, leading to prosthesis failure and bringing about additional suffering and burden for patients (Attinger and Siebenrock, 2014). Osteolysis is caused by wear particles produced by friction between components of prosthesis and bone interface, and it's the leading cause of prosthesis aseptic loosening (Howie et al., 2013). With increasing wear particles produced, the formation of osteoclast was activated, and over-weight osteoclasts caused much bone resorption, which eventually led to osteolysis (Sun et al., 2014; Gallo et al., 2014).

As a result of excess of bone resorption and deficiency of bone formation, osteolysis is closely connected with osteogenic differentiation, which refers to the whole process of bone formation from MSCs (Nombela-Arrieta et al., 2011). Osteolysis after hip replacement is regulated by many factors related to osteogenic differentiation. CXCR4 is the primary transmembrane receptor of CXCL12 (stromal cell-derived factor-1, SDF-1) (Kim et al., 2014), and the CXCL12/CXCR4 signal axis plays an important role in mediating bone morphogenetic protein 9-induced osteogenic differentiation of MSCs (Liu et al., 2013). A recent report revealed that metallic wear debris up-regulated CXCR4 expression in vitro and in vivo, in a dose-dependent manner, and the up-regulation was found in periprosthetic tissue from revision arthroplasty of failed metal-on-metal hip replacements, with radiographic evidence of osteolysis (Drynda et al., 2015). These studies demonstrated that CXCR4 was involved in osteolysis after hip replacement through mediating osteogenic differentiation of MSCs, which the specific regulatory mechanism remains unclear.

Previous study demonstrated that miR-211 acted as vital negative regulator of Runx2 to promote adipogenesis and suppress osteogenesis in bone mesenchymal stem cells (BMSCs) (Huang et al., 2010), and that miR-211 and autophagy-related gene 14 regulated osteoblast-like cell

Abbreviations: CXCR4, C-X-C chemokine receptor-4; MSCs, mesenchymal stem cells

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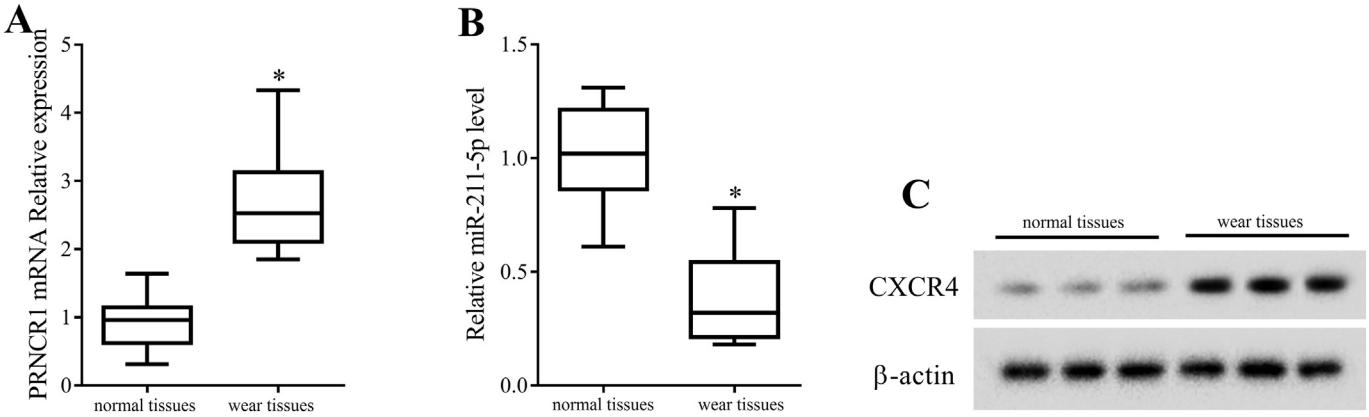

Fig. 1. Altered expression of lncRNA PRNCR1, miR-211-5p and CXCR4 in wear particles around prosthesis. (A) The expression of PRNCR1 in wear particles around prosthesis ($n = 12$) and normal tissues around prosthesis ($n = 12$) was quantified by qRT-PCR. *$P < 0.05$ vs. normal. (B) The expression of miR-211-5p in wear particles around prosthesis ($n = 12$) and normal tissues around prosthesis ($n = 12$) was quantified by qRT-PCR. *$P < 0.05$ vs. normal. (C) The expression of CXCR4 protein in wear particles around prosthesis ($n = 12$) and normal tissues around prosthesis ($n = 12$) was analyzed by Western blot, with β-actin served as control.

differentiation of human induced pluripotent stem cells (Ozeki et al., 2017). The miR-21 expression also has been reported to be significantly up-regulated in the particle-induced osteolysis animal model (Zhou et al., 2012). MiR-211-5p is one of the members of miR-211 family that related to cell proliferation, apoptosis and drug sensitivity in hepatocellular carcinoma (Jiang et al., 2017), and we predicted that it may also play a role in osteogenic differentiation. Furthermore, bioinformatics analysis showed the complementary base pairs between miR-211-5p and CXCR4, indicating the potential binding site and interplay between them. In addition, up-regulation of lncRNA PRNCR1 has been noted in colorectal cancer, which promoted cell cycle and cell proliferation (Yang et al., 2016), while its role in bone metabolism has not been noted. But it's a breakthrough that the complementary base pairs was discovered between PRNCR1 and 3′UTR of miR-211-5p by the bioinformatics analysis, which strongly hinting the close interaction between them.

We initiated this study to explore expression levels of lncRNA PRNCR1, miR-211-5p and CXCR4 in osteolysis after hip replacement, and to evaluate whether they interact with each other and exert influence on osteogenic differentiation in periprosthetic osteolysis, aiming to offer a theoretical reference for prevention and treatment of osteolysis after hip replacement.

2. Materials and methods

2.1. Patient samples

From January 2016 to June 2016, 20 patients who received a revision surgery because of aseptic loosening of prosthesis after hip replacement were enrolled in this study. All the patients agreed and signed the documented informed consent for tissue donation for study before samples collection. Wear particles and normal tissues around the prosthesis of these patients were taken and stored at −80 °C before analysis. This research was approved by the ethics committee of the Changzhou first people's hospital, the Third Affiliated Hospital of Soochow University and performed in accordance with the Helsinki Declaration.

2.2. Reverse transcription and quantitative real-time PCR (qRT-PCR)

Total RNA from wear particles and normal tissues around prosthesis or MSCs was isolated by using Trizol reagent (Invitrogen) according to its standard instruction. Total RNA was reverse-transcribed with the M-MLV Reverse Transcriptase Kit (Promega). The obtained cDNA was mixed with SYBR Select Master Mix (Thermo Fisher) and amplified by using an ABI 7900-fast thermocycler (Applied Biosystems). Primers were designed and synthesized by Sangon Biotech (Shanghai, China). The relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

2.3. Protein extraction and Western blotting

The tissues or cells were treated with RIPA lysis buffer (Beyotime Biotechnology) for 30 min on ice, and the lysates were centrifuged at 12,000*g* for 10 min at 4 °C. The supernatants were collected and concentration of proteins was measured with a BCA Protein Assay Kit (Thermo Fisher). Expression of proteins was analyzed by Western blot. Proteins were separated by SDS-PAGE with electrophoresis system and transferred into the polyvinylidene difluoride (PVDF) membrane (Bio-Rad). The membrane was then blocked with 5% skimmed milk for 1 h at RT, and then incubated with primary antibodies including anti-CXCR4 antibody (Abcam, 1:500) and anti-β-actin antibody (Abcam, 1:1000) at 4 °C for overnight. The membrane was incubated with HRP-bounded antibodies for 1 h and the target proteins were visualized by ECL Plus Western Blotting Substrate (Thermo Fisher). The β-actin protein was used as control to quantify protein level.

2.4. Particle preparation

Polymethylmethacrylate (PMMA) with a mean diameter of 0.33 μm (0.33 ± 0.019 μm) was severed as wear particles in this study, which was purchased from a commercial source (Polysciences). The particles were washed in 70% ethanol solution and heat sterilized. For decontamination from endotoxins, a limulus assay (Endosafe) was performed on PMMA particles. PMMA particles were then suspended in sterile phosphate-buffered saline (PBS) solutions and stored at 4 °C.

2.5. Cell culture

The MSCs and HEK-293 cells were purchased from American Type Culture Collection (ATCC). The MSCs were divided into two groups: control, and 0.3% v/v PMMA, and they were maintained in the complete Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen), 200 ng/mL BMP2, and cultured at 37 °C in a humidified atmosphere with 5% CO_2, for 14 days. The 200 ng/mL BMP2 was used for osteogenic induction. The medium was refreshed thrice a week.

The HEK-293 cells were widely used in the luciferase reporter assay for its high-density transfection (Backliwal et al., 2008), also in research concerning osteogenic differentiation (Gu et al., 2017). The HEK-293