MiR-377 inhibits wear particle-induced osteolysis via targeting RANKL

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Introduction

Periprosthetic osteolysis caused by wear particles was still the main factor affecting the long-term efficacy of artificial joint replacement (Dyskova et al. , 2017)

There was increasing evidence that the activation of the monocyte-macrophage system induced by wear particles promoted the secretion of certain chemokines, thereby creating a favorable environment for osteoclast generation and original osteoclast activation, leading to osteolysis and subsequent aseptic loosening (Córdova et al. , 2015)

A previous study has reported that the miR-377 expressed in peripheral blood mononuclear cells (PBMCs) in patients with latent tuberculosis infection (LTBI) was upregulated by more than 3-fold when compared with that in healthy participants. (Meng et al. , 2014a)

This study was designed to explore the molecular functions and biological roles of miR-377 in PIO（particle-induced osteolysis）.

Methods

1.Isolation of macrophages from human peripheral blood and cell culture
2.BMMs isolation and in vitro osteoclastogenesis assay
3.Luciferase reporter assay
4.Cell transfection
5.Cell cytotoxicity
6.In vivo experiment
7.RNA extraction and qRT-PCR analysis
8.Protein extraction and western blot
9.ELISA measurement of TNF-α and IL-6
10.TRAP staining

Results

1 Decreased miR-377 expression was associated with PIO pathogenesis

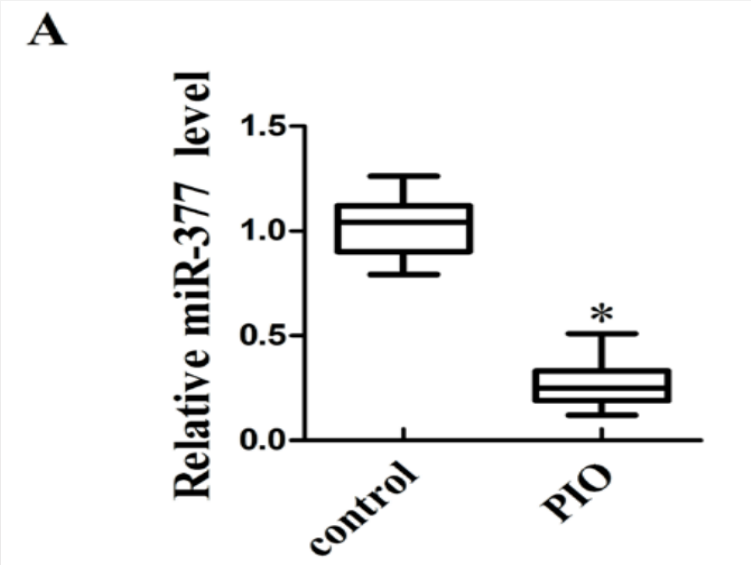
Parameter		n	miR-377 expression	P value
Gender	Male	9	0.54±0.077	0.898
	Female	21	0.55±0.089	
Age	< 60	5	0.59±0.082	0.320
	≥ 60	25	0.54±0.085	
Diagnosis	Rheumatoid arthritis	12	0.57±0.099	0.340
	Idiopathic osteoarthritis	10	0.53±0.081	
	Posttraumatic arthritis	8	0.53±0.066	0.310

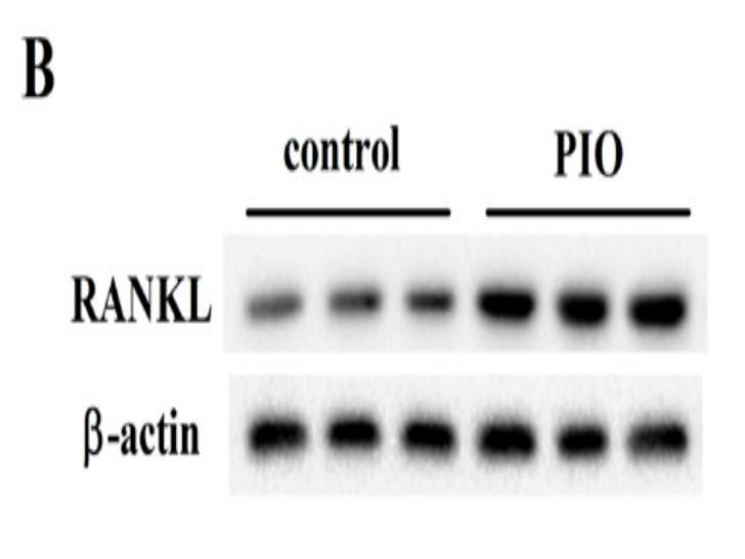
	<15±0.01	14	0.62±0.040	
No. of particles in joint prosthesis	≥15±0.01	16	0.48±0.042	0.000
	Metal stem	18	0.49±0.050	0.000
Prosthesis type	Cemented	12	0.63±0.043	0.640
Bone loss	>15%	3	0.52±0.094	
	≤15%	27	0.55±0.085	

there were no observable differences of miR-377 mRNA expression in peripheral blood between different gender, age and diagnosis of patients undergoing arthroplasty (P>0.05)

there were remarkable differences in miR-377 expression among different numbers of particles in joint prosthesis (P=0.000), suggesting that miR-377 expression might be correlated to clinicopathologic features of PIO

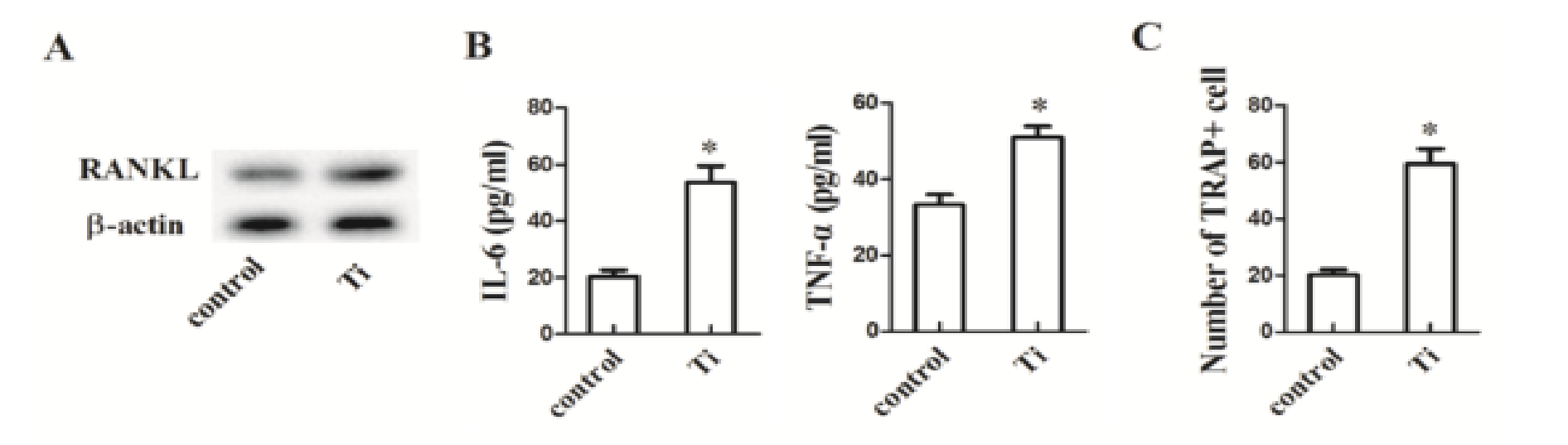
2 MiR-377 was downregulated while RANKL was upregulated in patients with PIO





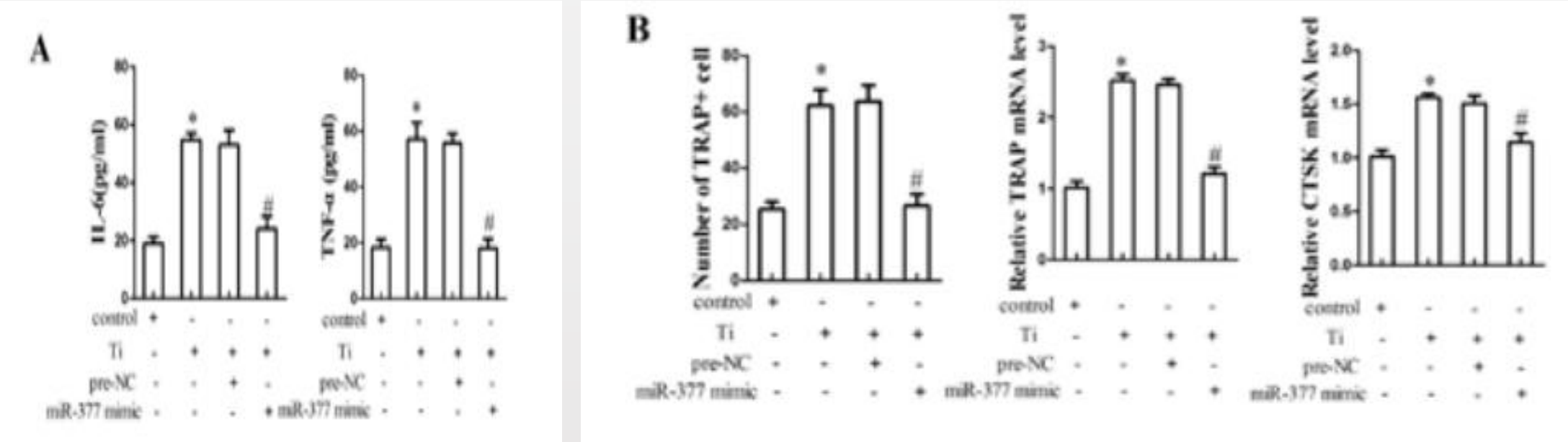
Effect of wear particles on miR-377 and RANKL expressions. The peripheral blood macrophages obtained from 15 patients undergoing hip revision after arthroplasty on account of prosthetic loosening and 15 healthy people

3 Ti particles stimulated pro-inflammatory cytokine secretion,upregulate d RANKL and promoted osteoclast activity in BMMs



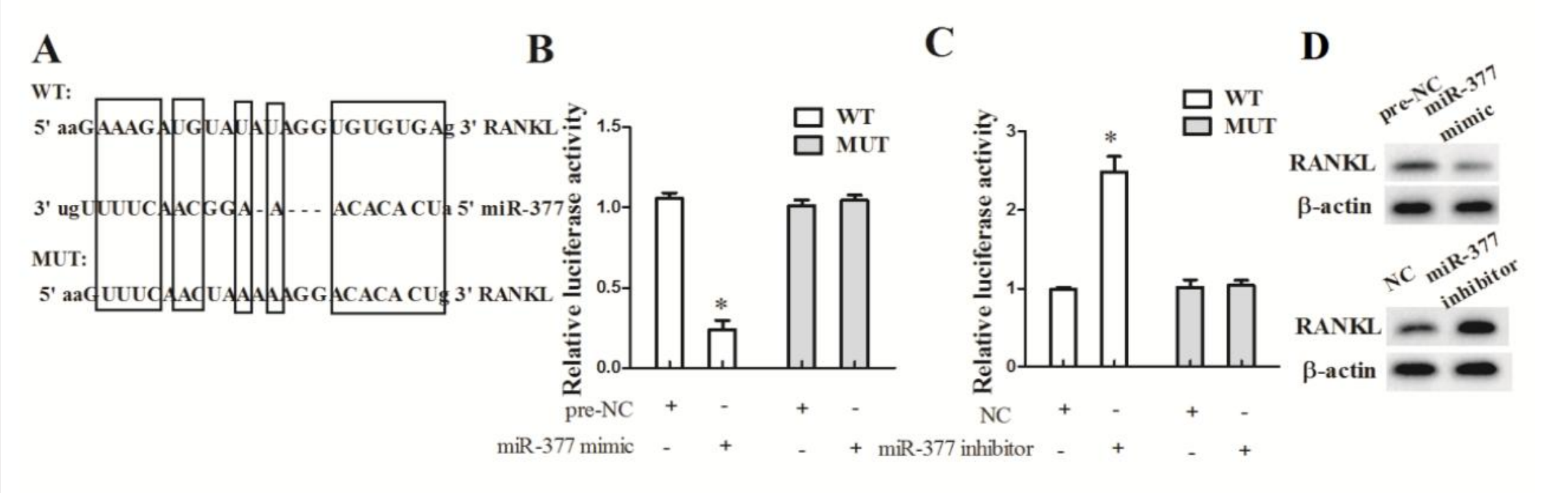
Effect of Ti particle treatment on RANKL expression, pro-inflammatory cytokine levels and osteoclast activity. The BMMs obtained from 15 healthy people were receiving the particle treatment

4 MiR-377 overexpression decreased PIO-induced inflammation and inhibited osteoclast activity



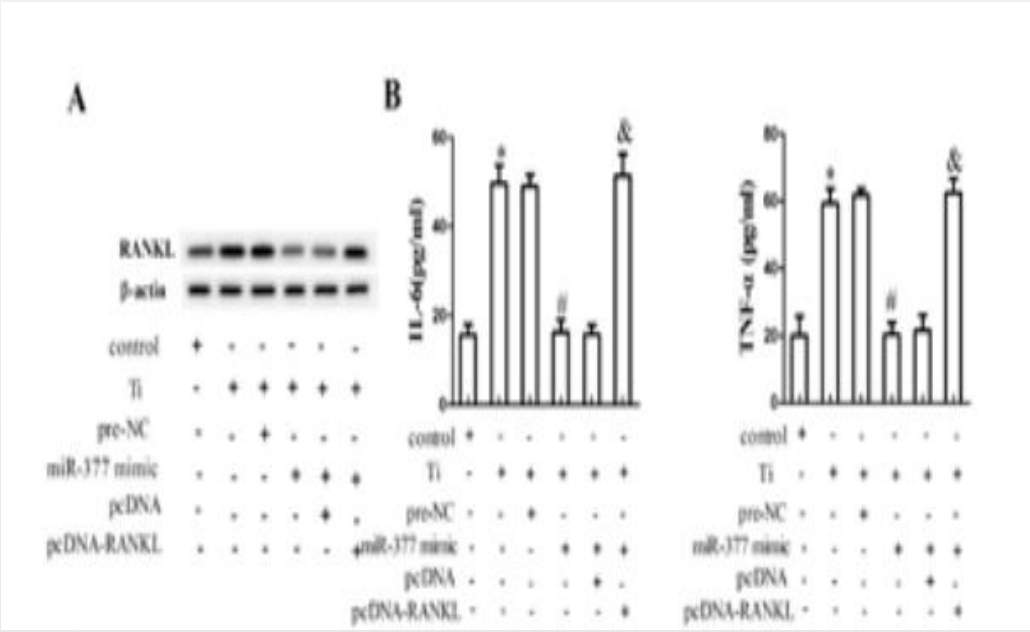
Effect of miR-377 on particles-induced inflammation and osteoclast activity. BMMs were divided into control, Ti, Ti+pre-NC and Ti+miR-377 mimic

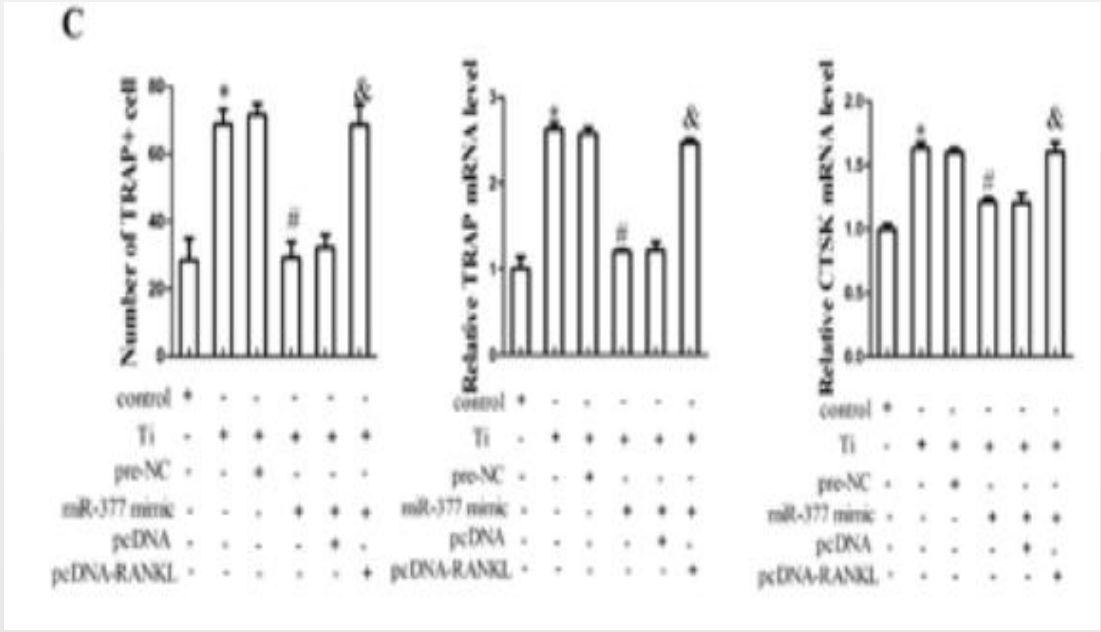
5 RANKL was a downstream target of miR-377



The interaction between miR-377 and RANKL.
(A) Potential binding sites of miR-377 and RANKL was predicted by bioinformatical analysis. HEK-293 cells co-transfected with miR-377 mimic/pre-NC and WT/Mut vector of RANKL: (B and C) The relative luciferase activity was determined using the luciferase reporter assay;
(D) The protein expression of RANKL.

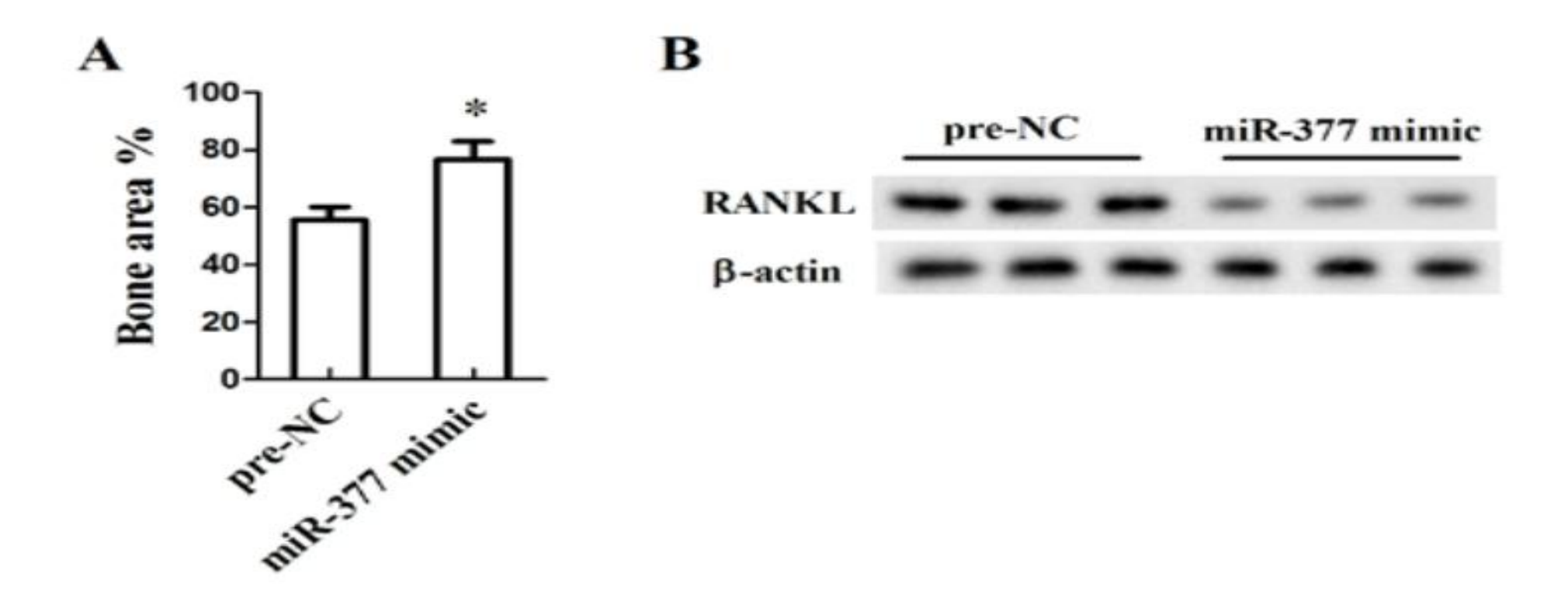
6 MiR-377 overexpression led to a decrease in RANKL expression, pro-inflammatory cytokines and osteoclast activity





The functional role and molecular mechanism of miR-377 in PIO. BMMs were divided into control, Ti, Ti+pre-NC, Ti+miR-377 mimic, Ti+miR-377 mimic+pcDNA and Ti+miR-377 mimic+pcDNA-RANKL

7 MiR-377 ameliorated PIO by targeting RANKL in vivo



Effect of miR-377 on PIO in vivo. Mouse model of calvarial osteolysis injected of Ti particles-treated BMMs following transfection of pre-NC or miR-377 mimic

Discussion

In this study, we investigated the potential role of miR-377 in aseptic loosening induced by wear metal particles. We found a marked decrease in the expression of miR-377 in peripheral blood samples from patients with >15 particles in joint prosthesis as compared with that of patients with <15 particles.We also showed that there was a significant decrease in miR-377 expression and an increased RANKL expression in macrophages derived from patients undergoing hip revision due to prosthetic loosening.

The protein expression of RANKL, IL-6 and TNF-α levels and the number of TRAP+ cells in BMMs were upregulated with M-CSF/RANKL/Ti particles treatment, while miR-377 overexpression led to an opposite effect.RANKL might be a downstream target of miR-377.

MiR-377 downregulated target gene RANKL, resulting in PIO inhibition. MiR-377 relieved PIO by negatively regulating RANKL.

In summary, miR-377/RANKL axis played a key role in osteoclast differentiation,and provided a potential therapeutic application in patients with PIO.

Thanks for your attention