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Metallothionein-1 is associated with osteoarthritis disease activity and suppresses proinflammatory cytokines production in synovial cells

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ABSTRACT

Backgrounds: OA (Osteoarthritis) is a predominant degenerative disease, characterized by the synovial inflammation and cartilage destruction. The pathogenic mechanisms remain mostly unknown. There is an critical require for extra investigations to discover new therapeutic targets to prevent and treat OA disease, as there are currently no effective treatments except for the joint replacement.

Methods: The mRNA and protein levels of Metallothionein-1(MT-1) were quantified by qPCR and ELISA in peripheral blood mononuclear cells (PBMCs), serum and synovial cells (SCs) from erosive inflammatory OA (EIOA) and primary generalized OA (PGOA) patients. Age and sex matched healthy volunteers were recruited as healthy controls (HCs). The correlation between the MT-1 level and OA activity was assessed and the anti-inflammatory effects of MT-1 was determined *in vitro*.

Results: The mRNA and protein levels of MT-1 were significantly increased in the PBMCs and serum of EIOA patients compared with those of PGOA patients and HCs. Serum levels of MT-1 were positively correlated with VAS score, CRP, and ESR in OA patients. And the positive correlations were also identified between the MT-1 and IL-1β, TNF-α or IL-6 in synovial cells. Furthermore, the recombinant MT-1 protein could significantly inhibit the expression of IL-1β, TNF-α and IL-6 in PBMCs and SCs from EIOA patients *in vitro*.

Conclusion: The data had shown that the MT-1 was up-regulated in EIOA patients and positively correlated with the disease activity. The recombinant MT-1 could suppress the expression of pro-inflammatory cytokines in both PBMCs and synovial cells from EIOA patients. Therefore, the MT-1 might become a novel therapeutic target for OA treatment.

1. Introduction

OA (Osteoarthritis) is a predominant degenerative disease, characterized by the synovial inflammation and cartilage destruction [1–5]. The pathogenic mechanisms remain mostly unknown. There is an critical require for extra investigations to discover new therapeutic targets to prevent and treat OA disease, as there are currently no effective treatments except for the joint replacement.

OA could be divided into two different types, the primary generalized osteoarthritis (PGOA) and erosive inflammatory osteoarthritis (EIOA), based on the inflammation levels and clinical symptoms [6]. It has been demonstrated that the inflammatory cytokines, such as IL-6, IL-1β and TNF-α, play an important role in the pathogenesis of OA [7–9]. On the other hand, the anti-inflammatory cytokines, such as interleukin 10, have also been widely studies as their roles in suppressing the inflammation [10–13].

Metallothioneins (MTs) have been demonstrated as important regulator in the immune suppression process, including inhibiting the cytotoxic T cell function and inducing the regulatory T proliferation [14–17]. Although it has been demonstrated that the MT-1 is up-regulated in OA patients [18], the relationship between the MT-1 expression and OA remains unclear. In the current study, we investigated the expression levels of MT-1 between the PGOA and EIOA patients and the correlations between the MT-1 levels and clinical data. In addition, we also studied the suppressive function of MT-1 on the pro-inflammatory cytokines *in vitro*.

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Table 1
Demographic and clinical characteristics of OA and healthy controls.

Characteristics	OA patients (n = 360)	Healthy controls (n = 100)
Age (years)	63.65 ± 14.28	64.32 ± 14.37
Sex (male/female)	65/295	21/79
Disease duration (years)	4.62 ± 3.7	–
Anti-CenpB (%)	8.4	–
Anti-SSA60 (%)	7.1	–
ALB (g/L)	39.35 ± 5.21	–
ESR (mm/h)	29.75 ± 24.08	< 20
CRP (mg/L)	7.52 + 7.31	< 5
BUN (mmol/L)	5.62 ± 2.27	–
Cr (μmol/L)	94.85 ± 46.57	–
VAS	5.61 ± 2.19	–

2. Methods and materials

2.1. Patients recruitment

This study was approved by the Ethics Committee of the Third Hospital of Hebei Medical University, China. 360 OA patients with informed consent were recruited from the Third Hospital of Hebei Medical University. Patients were diagnosed as OA according to the classification criteria developed by the Osteoarthritis Criteria Subcommittee of the American Rheumatism Association [19]. The OA patients were further divided into two different types, the erosive inflammatory OA and primary generalized OA, according to the MRI, X-ray and clinical parameters [6]. Patients with infections, other rheumatic diseases, malignant tumors were excluded. Visual analogue scale (VAS) was used to assess the pain intensity [20]. 100 age and sex matched healthy volunteers were recruited as controls (HCs). Demographic and clinical information were listed in Table 1.

2.2. Cell isolation

Venous blood were collected in the morning. PBMCs were isolated by the Ficoll-Paque plus (TBD science, China) density gradient centrifugation within three hours. Synovial tissues and synovial fluid were collected from 12 osteoarthritis patients with erosive inflammation during surgeries. The synovial cells (SCs) were immediately isolated from the synovial tissues as described before [21].

2.3. Cell culture

Cells, including PBMCs and SCs, were cultured with RPMI 1640 (Gibco) medium plus 10% fetal calf serum (Gibco), 100 IU/ml penicillin, 100 μg/ml streptomycin. The cells (20×10^4 cells per well in p12 plate) were treated with or without human recombinant MT-1100 ng/ml (BioCrick) for 12 h, and stimulated with LPS (1 μg/ml) for 4 h.

2.4. RNA extraction and RT-PCR

Total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was prepared by using the iScript™ cDNA Synthesis kit (Bio-Rad, USA). RT-PCR amplification reaction was prepared with the SYBR Green PCR kit (Bio-rad, USA) and performed using the 7500 fast Real-Time PCR system (Applied Biosystems, USA). PCR products were verified by melting curve analysis. The primer sequences used for RT-PCR in this study are shown in Table S1. The relative expression of target genes MT-1, TNF-α, IL-1β and IL-6 was normalized to control housekeeping genes GAPDH and were reported using the $2^{-\Delta\Delta}$ct method.

2.5. ELISA

The MT-1 (Uscn Life Science, Wuhan) [22], TNF-α, IL-1β and IL-6 were quantified by ELISA kits (eBioscience) according to the manufacturer's instructions.

2.6. Statistical analysis

The results are presented as the mean ± SD. Spearman correlations test were used to assess the association between the MT-1 levels and OA clinical or the pro-inflammatory cytokines. Comparisons between groups were determined by using nonparametric Mann-Whitney U test. $P < 0.05$ was considered statistically significant Statistical analysis was performed with Graph Pad Prism 5.0 software.

3. Results

3.1. Up-regulated MT-1 in patients with EIOA but not PGOA

Totally 134 patients with EIOA (erosive inflammatory OA), 226 patients with PGOA (primary generalized OA), and 100 age and sex matched healthy volunteers (HCs) were recruited. The mRNA and protein levels of MT-1 were increased significantly in patients with EIOA when compared with HCs or PGOA (Fig. 1A, B). Furthermore, the serum levels of MT-1 were tightly associated with the OA clinical indexes of inflammation, including VAS, CRP (C-Reactive Protein) and ESR (Erythrocyte sedimentation rate) (Fig. 2A–C). Thus, the MT-1 may be involved in the pathogenesis of EIOA.

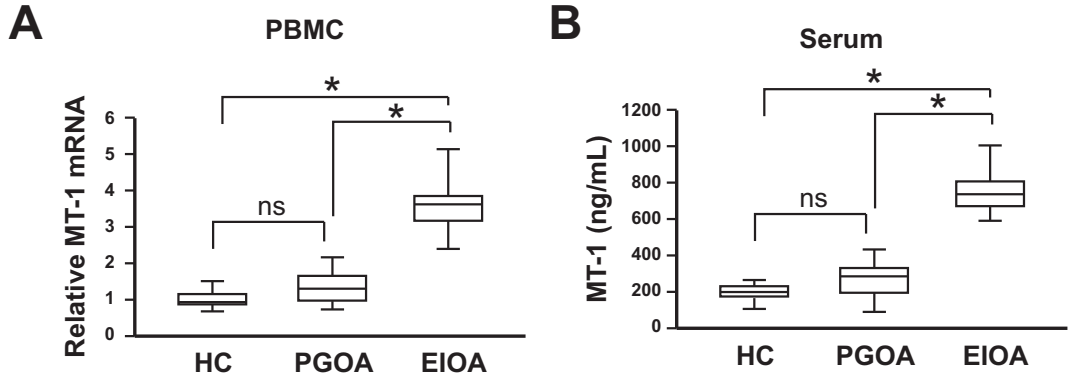

Fig. 1. Comparison of MT-1 mRNAs and protein levels between OA and HC. Expression of MT-1 mRNAs in PBMCs from PGOA (n = 226), EIOA (n = 134) and HCs (n = 100) was detected by RT-PCR (A). Serum MT-1 protein levels were determined by ELISA (B). * indicates $P < 0.05$. HC: healthy control; PGOA: primary generalized OA; EIOA: erosive inflammatory OA; OA: osteoarthritis.

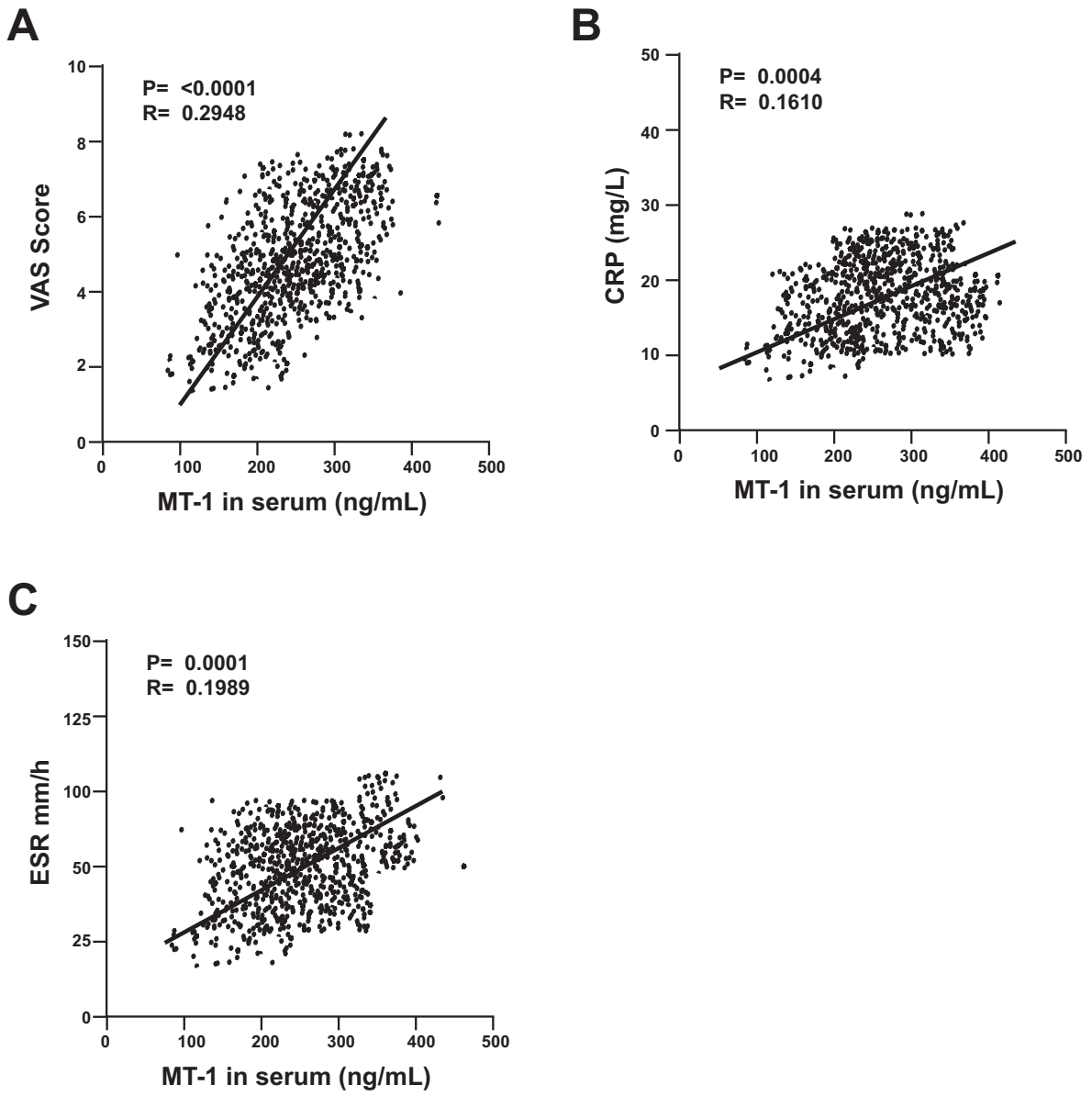

Fig. 2. Correlations of serum MT-1 levels with OA clinical values. Serum MT-1 levels in OA were positively correlated with VAS (A), CRP (B) and ESR (C). VAS: visual analogue scale; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

3.2. MT-1 inhibits the pro-inflammatory cytokines in EIOA patients

It has been demonstrated that the pro-inflammatory cytokines play important roles during the OA development [9]. Therefore, their mRNA and protein levels were assessed in PBMCs treated with or without human recombinant MT-1. When compared with HCs, the mRNA and protein levels of IL-1β, TNF-α, and IL-6 were significantly increased in EIOA patients (Fig. 3A–F), but not in the PGOA patients. Furthermore, the recombinant MT-1 protein could significantly suppress the mRNA and protein levels of these pro-inflammatory cytokines (Fig. 3A–F).

3.3. MT-1 positively correlated with the pro-inflammatory cytokines produced by synovial cells from EIOA patients

To further determine whether the MT-1 levels were related to the inflammation of EIOA, the synovial fluid and synovial cells were isolated from the EIOA patients. The data showed that the mRNA and protein levels of MT-1 were up-regulated in the synovial cells when compared with the peripheral venous blood from the same patients with EIOA (Fig. 4A, B). Furthermore, a positive correlation was discovered between the MT-1 and TNF-α, IL-1β and IL-6 produced by synovial cells (Fig. 5A–C).

MT-1 suppresses the expression of inflammatory cytokines in synovial cells from EIOA patients.

To investigate whether the recombinant MT-1 protein suppresses the expression of the inflammatory cytokines in synovial cells, the synovial cells were we isolated and cultured from the EIOA patients. Data showed that the mRNA and protein levels of TNF-α, IL-1β and IL-6 were significantly reduced by MT-1 in synovial cells (Fig. 6A–F). Thus, the MT-1 had the anti-inflammatory effects in OA inflammation.

4. Discussion

Although it has been demonstrated that the MT-1 was up-regulated in the OA patients [18], the function of MT-1 and its correlation with clinical characteristics remains mostly unclear. Here, we studied the expression pattern of MT-1 in EIOA and PGOA patients, and demonstrated the immune suppression function of MT-1 in osteoarthritis.

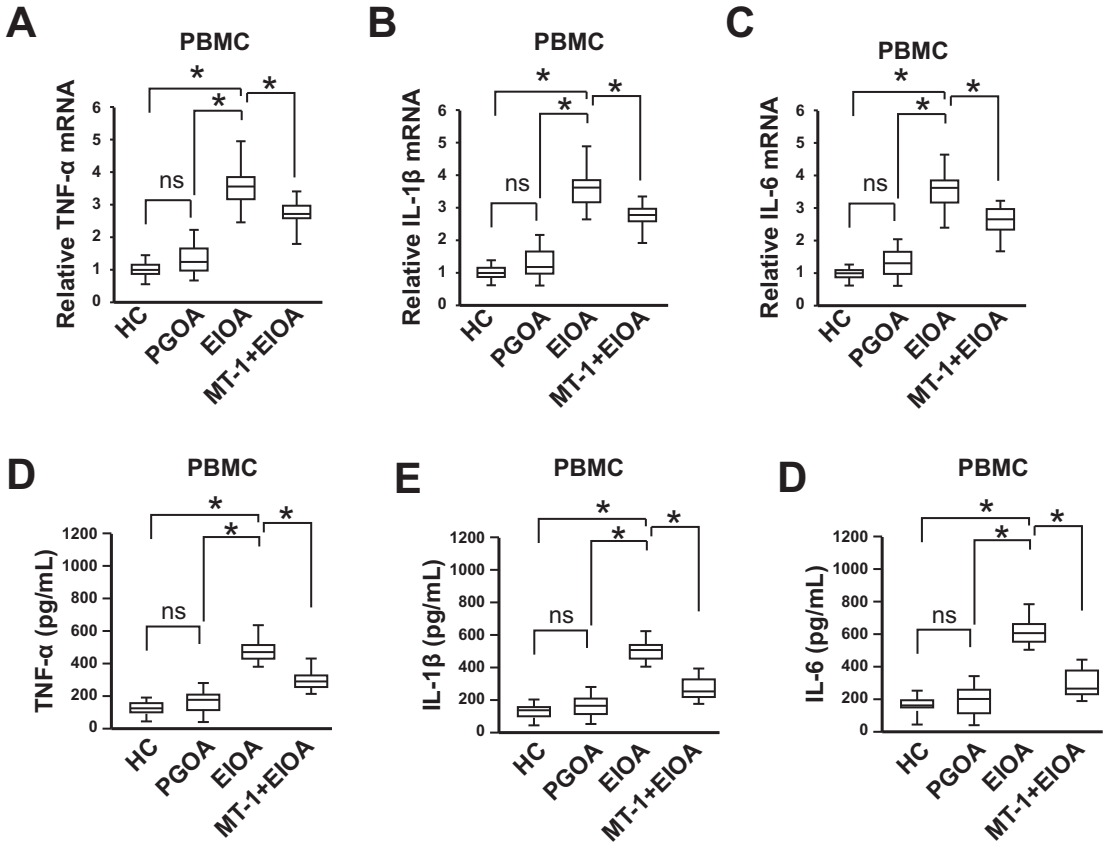

Fig. 3. MT-1 inhibits the expression of pro-inflammatory cytokines in PBMCs from EIOA patients. The mRNAs expression of TNF-α (A), IL-1β(B) and IL-6 (C) in PBMCs from EIOA patients (n = 30) and HCs (n = 30) were analyzed by RT-PCR, the supernatants produced by PBMCs were collected for measuring TNF-α(D), IL-1β (E) and IL-6 (F) protein levels by ELISA. HC: healthy control; PGOA: primary generalized OA; EIOA: erosive inflammatory OA; OA: osteoarthritis.

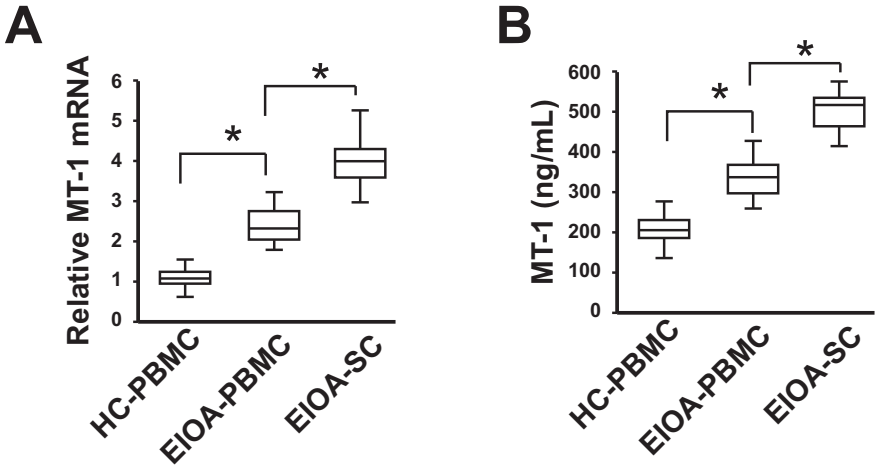

Fig. 4. Comparison of MT-1 mRNAs and protein levels between PBMCs and Synovial cells (SCs) from EIOA patients. PBMCs and SCs were collected from EIOA patients (n = 12). the relative mRNA (A) and protein levels (B) were detected by RT-PCR and ELISA, respectively. HC: healthy control; EIOA: erosive inflammatory OA; OA: osteoarthritis; PBMC: peripheral blood mononuclear cell; SC: synovial cell.

Inflammation plays an important role in OA symptoms, such as joint pain, swelling and stiffness [8,9,23]. Among those pro-inflammation factors, the IL-1β, TNF-α and IL-6 could up-regulated each other to boost the inflammatory response in OA [7,9,24,25]. Therefore their mRNA and protein levels of were determined in OA patients and the data showed that they were significantly up-regulated in EIOA patients than in PGOA patients and HCs. And their up-regulation was tightly associated with the OA development which is in accordance with previous investigations [9].

To further study the association between the MT-1 and EIOA disease, the synovial fluid and synovial cells were isolated from EIOA patients. The MT-1 was significant up-regulated in synovium when compared to the blood from the same EIOA patient. In addition, the MT-1 was positively correlated with the levels of TNF-α, IL-1β and IL-6 in synovial cells from EIOA patients. These data indicated that the MT-1 might be tightly associated with the levels of pro-inflammatory cytokines in EIOA. Furthermore, the MT-1 could significantly suppress the inflammatory response in synovial cells, indicating the anti-inflammatory effect of MT-1.

Metallothioneins (MTs) are a group of small, intracellular cysteine-rich, metal-binding proteins and there are totally 4 isoforms (MT-1, MT-2, MT-3 and MT-4) have been discovered in human [26–28]. Although

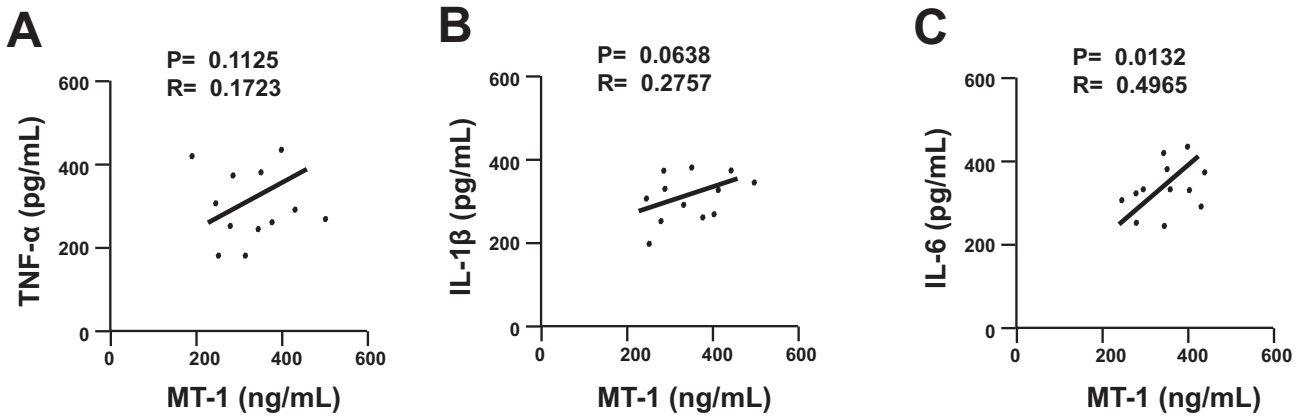

Fig. 5. Correlation between MT-1 and pro-inflammatory cytokines in synovial cells from EIOA patients. Synovial MT-1 levels were positively correlated with TNF-α (A), IL-1β (B), and IL-6 (C) produced by SCs from EIOA patients (n = 12).

most studies include both MT-1 and MT-2 together as they both are expressed in most type of cells and may have function redundancy [14–16], the most studied type of MTs is MT-1, especially in the immune regulation function [17,18,22]. However, it has been demonstrated latterly, that the MT-2 might play a more important role in autoimmune disease development [18,29]. Thus more studies might focus on the function of MT-2 and also the differences between the MT-1 and MT-2 in the future.

5. Conclusion

The MT-1 plays an important role in protecting EIOA from excessive inflammatory response. Thus, MT-1 might present a novel treatment target for OA inflammation.

Data accessibility statement

All data have been presented in the figures. And other related information are available under request to the corresponding author.

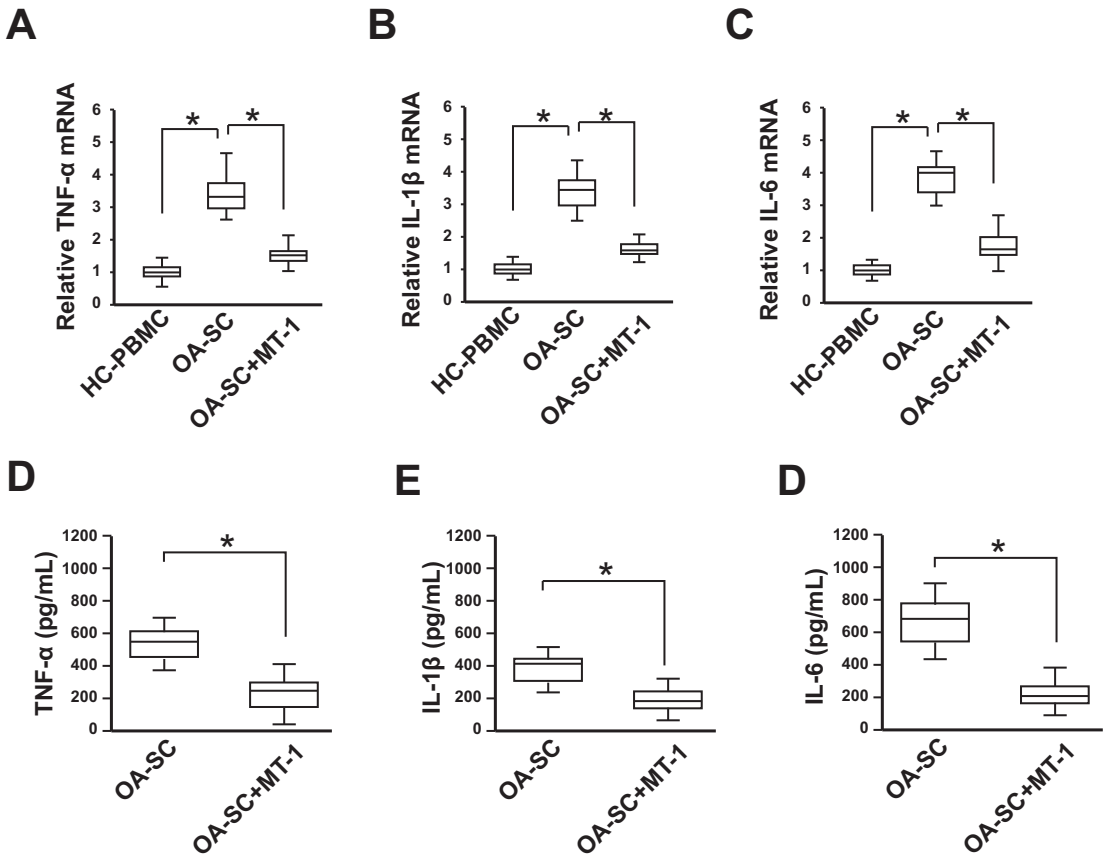

Fig. 6. MT-1 suppresses the expression of inflammatory cytokines in synovial cells from EIOA patients. Synovial cells (n = 12) were treated with or without recombinant MT-1. The relative mRNA expression of TNF-α (A), IL-1β (B) and IL-6 (C) were analyzed by RT-PCR, the protein levels of these cytokines produced by SCs were analyzed by ELISA (D–F). HC: healthy control; OA: osteoarthritis; PBMC: peripheral blood mononuclear cell; SC: synovial cell.

Authors' contributions

C.W. collected the samples, supervised the project and wrote the manuscript; Z.G. conducted the qPCR; S·H conducted the ELISA; S.H. conducted the cell culture and treatment; G.Z analyzed the data.

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Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105815.

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