

Expression Profile of Osteoclasts Following the Stimulation With Interleukin-23 in Mice

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ABSTRACT

Objectives: This study aims to analyze the expression profile of osteoclasts (OCs) following the stimulation with interleukin 23 (IL-23) in mice, which would imply the underlying effects of IL-23 on the function of OCs in inflammatory arthritis.**Materials and methods:** Mature OCs were induced from bone marrow mononuclear cells of 5 male mice (age 6 weeks; weighing 18-20 g) in the presence of macrophage-colony stimulating factor (50 ng/mL) and receptor activator of nuclear factor kappa B ligand (30 ng/mL) *in vitro*. The Agilent SurePrint G3 Mouse GE V2.0 Microarray was used to analyze the gene expression profile of OCs stimulated with IL-23 (30 ng/mL) or vehicle. The four major IL-23-modulated genes were validated by quantitative real-time polymerase chain reaction (qPCR) analysis.**Results:** The expression levels of 23 genes were up-regulated and 32 genes were down-regulated by IL-23 stimulation (fold change ≥ 1.5 and p value < 0.05). Among them, there were 37 genes with assigned gene symbols. Gene ontology analysis showed that the IL-23-regulated messenger ribonucleic acids (mRNAs) were related to positive regulation of leukocyte chemotaxis, chemokine-mediated signaling pathway and C-X-C chemokine receptors binding. The pathway analysis showed that the IL-23-regulated mRNAs were related to chemokine signaling pathway and cytokine-cytokine receptor interaction. The significant up-regulation of chemokine (C-X-C motif) ligand 1 and chemokine (C-X-C motif) ligand 2 induced by IL-23 was confirmed by qPCR. In addition, there were 18 long non-coding RNAs that were regulated by IL-23, while their function needs to be confirmed in the future.**Conclusion:** Expression levels of genes related to chemotaxis in OCs were up-regulated by IL-23 in mice, which imply that IL-23 may facilitate chemotaxis of OCs in inflammatory arthritis.**Keywords:** Chemokine (C-X-C motif) ligand 1, chemokine (C-X-C motif) ligand 2, interleukin-23, long non-coding ribonucleic acid, messenger ribonucleic acid, osteoclasts.

Interleukin (IL)-23 is a heterodimeric cytokine composed of a p19 subunit and an IL-12 p40 subunit, which is mainly produced by activated macrophages and dendritic cells.¹ Accompanying with IL-17, IL-23 displays pro-inflammatory properties²⁻⁴ and plays an important role in autoimmunity and chronic inflammation, which formed the IL-23/IL-17 axis.⁵ Previous studies showed that IL-23 was involved in the development of inflammatory arthritis such as rheumatoid arthritis (RA), psoriatic arthritis (PsA)

and ankylosing spondylitis (AS). The concentration of IL-23 is elevated in serum and synovial fluid of patients with RA, which was positively associated with the disease activity and severity.⁶⁻⁸ In addition, the variants of single nucleotide polymorphisms in the genes encoding IL-23 receptor and IL-23 were also associated with susceptibility to spondyloarthritis like PsA or AS.⁹⁻¹² Furthermore, IL-23 antagonist ustekinumab has excellent efficacy in PsA. It is well known that osteoclasts (OCs) are in charge of bone resorption while osteoblasts

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(OBs) are responsible for bone formation. The dynamic balance between OC and OB activities contributes to normal bone remodeling. Dysregulation of osteoclastogenesis that results in enhanced bone resorption is a characteristic feature of inflammatory arthritis. IL-23 may play an important role in osteoclastogenesis and maintenance of bone mass.¹³ Receptor activator of nuclear factor kappa B (RANK), which is expressed on OC precursors induces their differentiation into bone-resorbing mature OCs upon stimulation with RANKL and macrophage-colony stimulating factor (M-CSF).¹⁴⁻¹⁶ IL-23 has shown pro-osteoclastogenic capacity via both RANKL/RANK dependent and independent pathways, thereby aggravating joint damage and systemic bone loss.¹⁷⁻²⁰ In addition, IL-23 can induce OC differentiation in other ways. The work of Shin et al.²¹ has shown that IL-23 acts on human CD16⁺/myeloid DNAX activating protein of 12 kDa-associated lectin-1⁺/DNAX activating protein of 12 kDa⁺ OC precursor population to induce OC differentiation. Leukotriene B4 and its receptor BLT1 also have critical roles in IL-23 induced myelopoiesis and osteoclastogenesis.²² However, the underlying mechanism where IL-23 modulates the function of OCs in inflammatory arthritis is still unknown. Therefore, in this study, we aimed to analyze the expression profile of OCs following the stimulation with IL-23 in mice, which would imply the underlying effects of IL-23 on the function of OCs in inflammatory arthritis.

MATERIALS AND METHODS

This study was conducted at Shanghai Jiao Tong University Affiliated Sixth People's Hospital between January 2017 and January 2018. Bone marrow monocytes were obtained from 5 male C57BL/6 mice (age 6 weeks; weighing 18-20 g) and cultured in the presence of M-CSF (50 ng/mL) for four days, and then the cultures were further stimulated with soluble receptor activator of RANKL (30 ng/mL) in the presence of M-CSF (50 ng/mL) for six days. The cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP), using a commercial kit. All the multinucleated (more than or equal to three nuclei) TRAP positive cells were mature OCs. In the following experiments, the mature OCs were cultured in the presence of IL-23 (30 ng/mL) or

vehicle for four hours. The flow chart of the study was shown in Figure 1. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

The Agilent SurePrint G3 Mouse GE V2.0 Microarray (Agilent Technologies, Design ID: 074809, Agilent Technologies Inc., Santa Clara, CA, USA) was used to analyze the gene expression profiles of the six samples in this study (three samples from each group). Each glass slide was formatted with eight high-definition 60K array and screened for 56605 genes, most of which were messenger ribonucleic acids (mRNAs), and a few long non-coding RNAs (lncRNAs) as well. Total RNAs were quantified by the NanoDrop ND-2000 (Thermo Fisher Scientific, Wilmington, DE, USA)

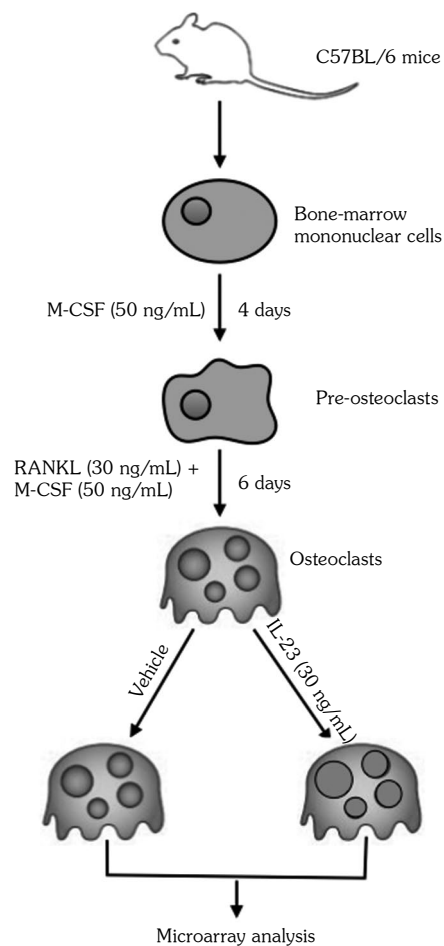


Figure 1. Flowchart of study.

M-CSF: Macrophage-colony stimulating factor; RANKL: Receptor activator of nuclear factor kappa B ligand; IL-23: Interleukin-23.

and the RNAs integrity was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA, USA). Based on the manufacturer's standard protocols, the sample labeling, microarray hybridization and washing were performed. In brief, total RNAs were transcribed to double stranded complementary deoxyribonucleic acids, then synthesized into cRNAs and labeled with cyanine 3 labeled analog of cytidine triphosphate (3-CTP). The labeled cRNAs were hybridized onto the microarray. After washing, the arrays were scanned by the Agilent Scanner G2505C (Agilent Technologies Inc., Santa Clara, CA, USA).

The volcano plot was applied to screen out the genes based on their expression levels between the two groups. After analyzing the differentially expressed genes between the two groups by t-test, the volcano plot was drawn according to statistical significance [$-\log_{10}(p \text{ value})$ as y-axis] and the magnitude of change [$\log_2(\text{fold change})$ as x-axis]. The hierarchical cluster analysis was performed to identify distinguishable mRNA expression profiles among all the samples. The differentially expressed mRNAs that have assigned gene symbol between the samples were shown as a heat map under hierarchical cluster analysis.

Gene ontology (GO) analysis was performed to explore the function of differentially expressed genes (<http://www.geneontology.org>). It contains three parts, which are biological process, cellular component and molecular function. The number of differential genes included in each GO category

was calculated, and the two-sided Fisher's exact test was performed to classify the GO category and the GO annotation list. The small p value indicated that the gene was enriched in this GO annotation. The cut-off of p value was set as 0.05 to select genes for subsequent studies. Kyoto Encyclopedia of Genes and Genomes was used to perform the pathway analysis. The enrichment was calculated in a similar manner as the GO analysis.

The expression levels of some differentially expressed mRNAs were verified with quantitative real-time polymerase chain reaction (qPCR). Total RNAs were extracted using TRIzol (Thermo Fisher Scientific Inc., Waltham, MA, USA) and then reversely transcribed. The expression level of mRNA was represented as fold change using $2^{-\Delta\Delta Ct}$ methods. The expression differences of mRNAs between two groups were analyzed by Student's t-test. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control to standardize mRNA expression level. The primer sequences used in this study were shown in Supplementary Table 1.

Statistical analysis

Feature Extraction software version 10.7.1.1 (Agilent Technologies Inc., Santa Clara, CA, USA) was used to analyze array images to get raw data. GeneSpring version 13.1 (Agilent Technologies Inc., Santa Clara, CA, USA) was employed to perform the basic analysis of the raw data. Firstly, the raw data were normalized with the quantile

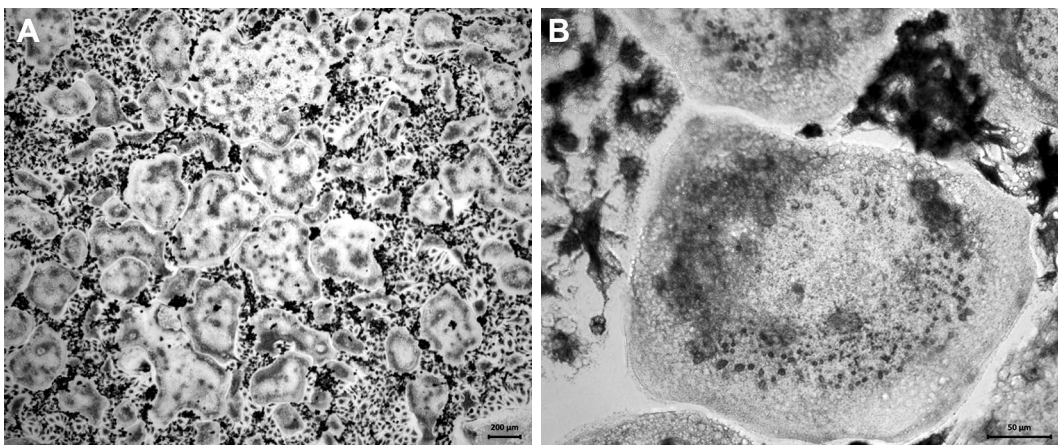


Figure 2. Osteoclast formation induced from mouse bone marrow monocytes. Tartrate-resistant acid phosphatase staining to identify mature osteoclasts. Scale bars, 200 μm in panel A and 50 μm in panel B.

algorithm. The probes with at least 100% of the values in anyone out of all conditions that had flags in “detected” were chosen for further data analysis. Differentially expressed genes were

then identified through fold change as well as p value calculated with t-test. The threshold set for up- and down-regulated genes was a fold change ≥ 1.5 and p value < 0.05 .

Table 1. Differentially expressed mRNAs with assigned gene symbols in osteoclasts following the stimulation with Interleukin-23

Gene symbol	Fold change	P value	Changes
Sf1	1.504	0.0067	DR
LOC105247398	1.504	0.0155	DR
Magel2	1.513	0.0065	DR
Kazn	1.524	0.0309	DR
Catsper4	1.525	0.0285	DR
Lrrc2	1.586	0.0081	DR
2310005E17Rik	1.603	0.0273	DR
Snhg7os	1.618	0.0497	DR
8030411F24Rik	1.644	0.0485	DR
Gm15401	1.666	0.0112	DR
Sbp	1.687	0.0172	DR
Vmn1r217	1.750	0.0050	DR
4930517J16Rik	1.772	0.0135	DR
Gm10866	1.773	0.0114	DR
Kcnj4	1.905	0.0105	DR
Gm28382	1.955	0.0493	DR
Gm12128	1.978	0.0426	DR
Yipf6	2.020	0.0312	DR
Plek2	2.058	0.0020	DR
Samd4	2.720	0.0169	DR
Arhgap28	1.509	0.0132	UR
Nfasc	1.516	0.0427	UR
Cxcl1	1.526	0.0121	UR
Shisa9	1.536	0.0013	UR
Trappc12	1.540	0.0317	UR
Fcrlb	1.541	0.0447	UR
Atat1	1.576	0.0253	UR
Cxcl2	1.599	0.0138	UR
Kifc1	1.617	0.0124	UR
Ndrp2	1.637	0.0388	UR
H1f0	1.650	0.0496	UR
2210018M11Rik	1.654	0.0141	UR
Spag1	1.663	0.0125	UR
Tcap	1.678	0.0288	UR
Dpf2	1.678	0.0093	UR
Arid4b	1.759	0.0127	UR
Olfir699	1.946	0.0334	UR

mRNAs: Messenger ribonucleic acids; DR: Down-regulated; UR: Up-regulated.

RESULTS

After the mouse bone marrow monocytes were cultured in the presence of M-CSF and RANKL, TRAP staining was used to identify the mature OCs. Mature OCs were successfully induced (Figure 2), and then stimulated with IL-23 or vehicle.

Based on the criteria of fold change ≥ 1.5 as well as p value < 0.05 , there were 55 mRNAs that were differentially expressed between the two groups. Among them, 23 genes were up-regulated and 32 genes were down-regulated, which were shown by volcano plot (Figure 3). Among these differentially expressed mRNAs, there were 37 mRNAs that had assigned gene symbols (Table 1). The relative expression levels of these 37 mRNAs were shown in a heat map with hierarchical clustering analysis (Figure 4).

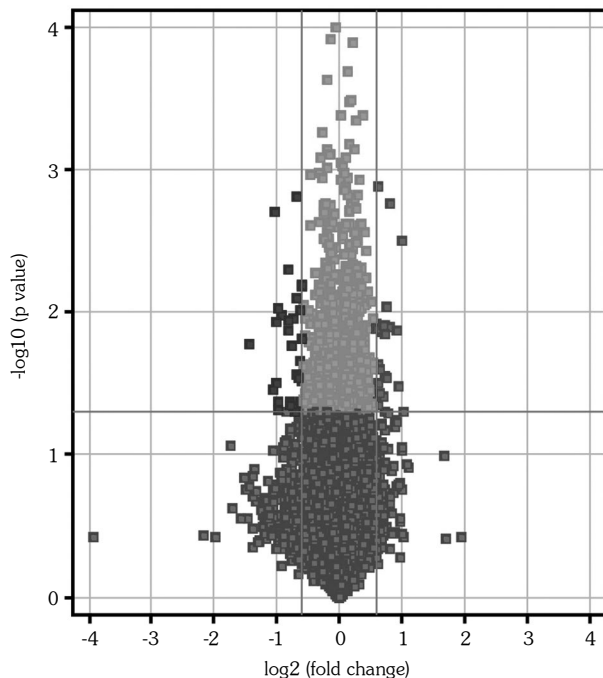


Figure 3. Volcano plot showing differentially expressed messenger ribonucleic acids. Vertical green lines correspond to a 1.5 fold up- and down-regulation while horizontal green line represents a p value of 0.05. The red dots and blue dots respectively represent the up-regulated and down-regulated messenger ribonucleic acids with more than 1.5 fold change and statistical significance. Statistical significance was defined as fold change ≥ 1.5 and p value < 0.05 between two groups.

Gene ontology and pathway analyses was performed to explore the potential function of the 37 differentially expressed mRNAs. The most significantly enriched GO annotations in the classification of biological process, cellular component and molecular function among the differentially expressed mRNAs were respectively shown in Figure 5a-c. Accordingly, the differentially expressed mRNAs were related to cell chemotaxis, chemokine-mediated signaling pathway, cell junction and C-X-C chemokine receptors (CXCR). The pathway analysis showed that the IL-23-regulated mRNAs were related to tumor necrosis factor (TNF) signaling pathway, chemokine signaling pathway, cytokine-cytokine receptor interaction and nuclear factor (NF)-kappa B signaling pathway (Figure 6). The network of the differentially enriched genes was shown in Figure 7.

Considering the observed fold changes, the calculated p values, and the gene function, we selected the following mRNAs to validate the array results: N-myc downstream-regulated gene 2 (*Ndr2*), sterile alpha motif domain containing protein 4 (*Samd4*), chemokine

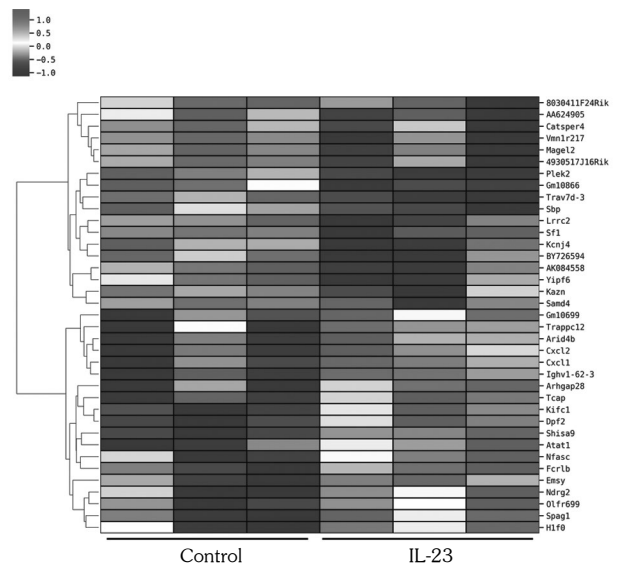


Figure 4. Heat map under hierarchical cluster analysis of differentially expressed messenger ribonucleic acids. Hierarchical cluster showed that differentially expressed genes ultimately clustered into two major branches, including up-regulated genes, which were shown in red, and down-regulated genes, which were shown in blue. Color depth represents relative gene expression levels. IL-23: Interleukin-23.

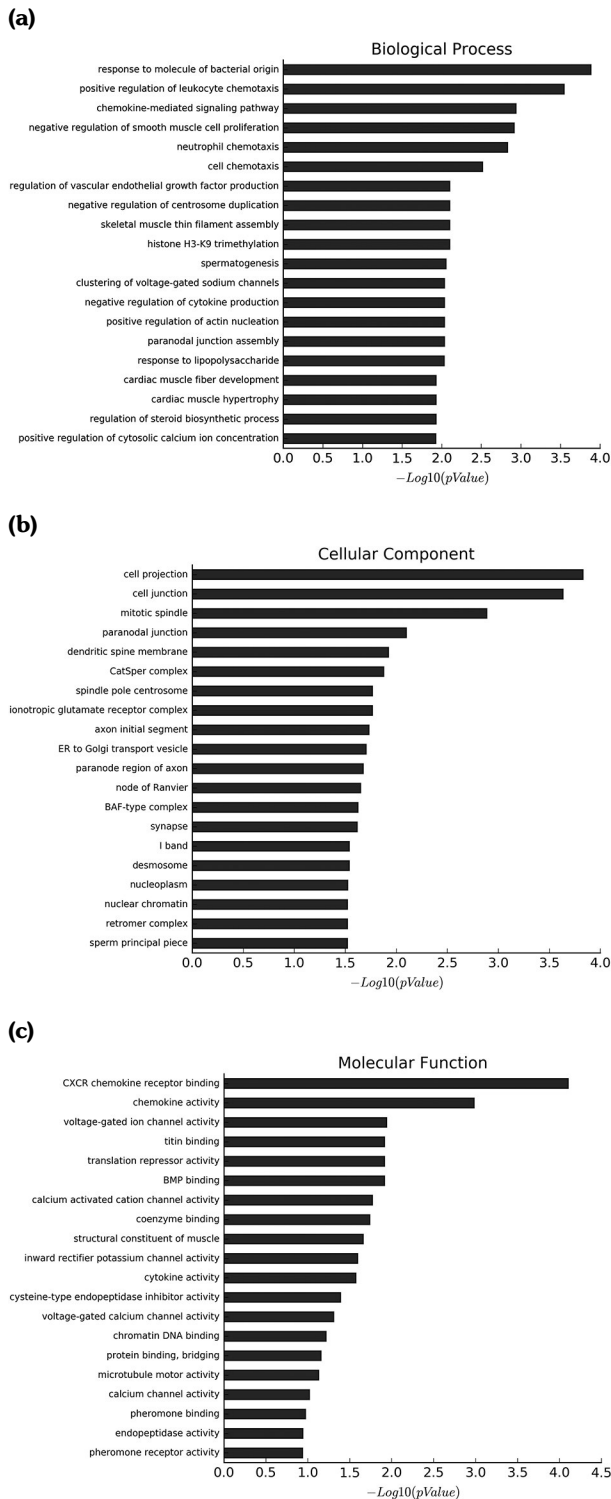


Figure 5. Most significantly enriched gene ontology annotations among differentially expressed messenger ribonucleic acids. **(a)** Top-ten significant changes in gene ontology biological process classification. **(b)** Top-ten significant changes in gene ontology cellular component classification. **(c)** Top-ten significant changes in gene ontology molecular function classification.

(C-X-C motif) ligand 1 (Cxcl1) and chemokine (C-X-C motif) ligand 2 (Cxcl2). The expression trends of all these genes were consistent with microarray data. However, the differences of *Ndrg2* and *Samd4* did not reach statistical significance ($p > 0.05$). The expression levels of *Cxcl1* and *Cxcl2* were significantly up-regulated under the stimulation of IL-23 (Figure 8).

There were 18 differentially expressed lncRNAs between the two groups, which consisted of four up-regulated lncRNAs and 14 down-regulated lncRNAs according to the criteria of fold change

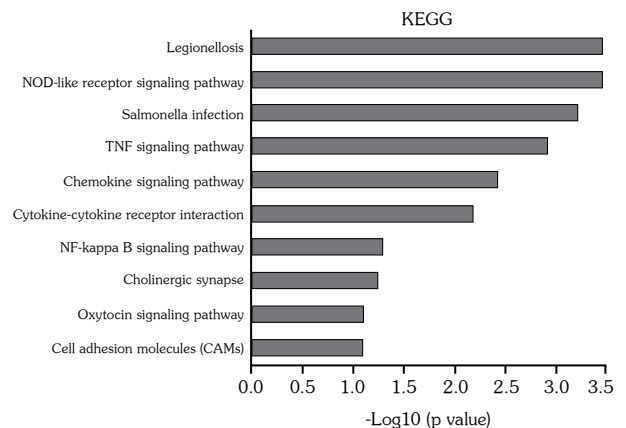


Figure 6. Top-ten significantly enriched pathways among differentially expressed messenger ribonucleic acids. KEGG: Kyoto Encyclopedia of Genes and Genomes; NOD: Nucleotide-binding oligomerization domain; TNF: Tumor necrosis factor; NF: Nuclear factor.

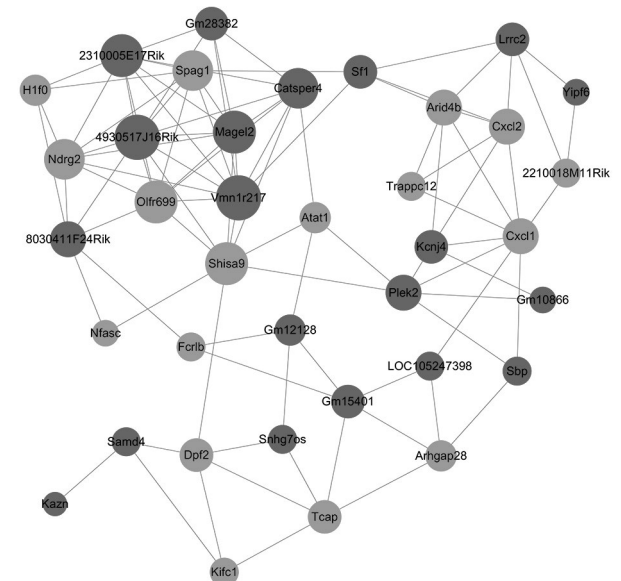


Figure 7. Network of differentially enriched genes.

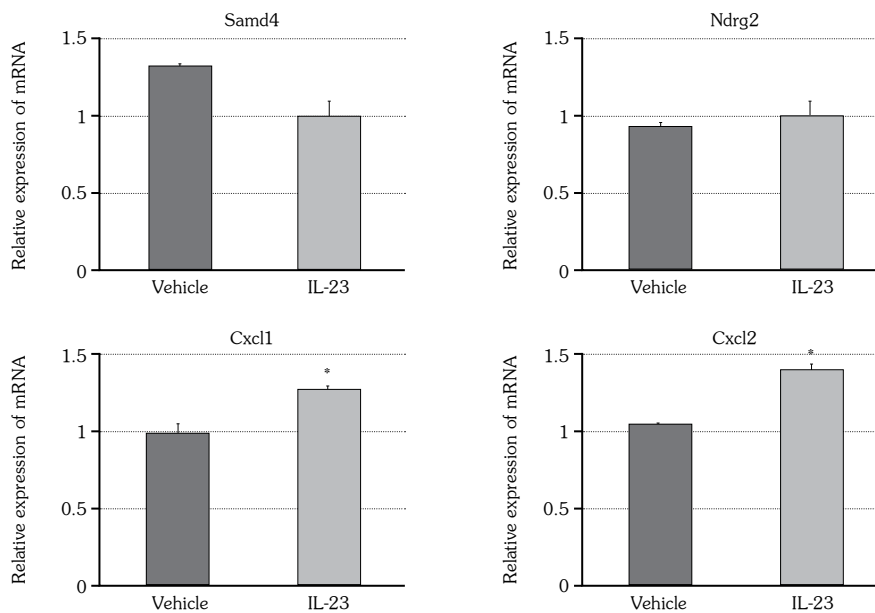


Figure 8. Validation of differentially expressed messenger ribonucleic acids using real-time polymerase chain reaction. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control to standardize messenger ribonucleic acid expression level. Relative expression levels of sterile alpha motif domain containing protein 4, N-myc downstream-regulated gene 2, chemokine (C-X-C motif) ligand 1 and chemokine (C-X-C motif) ligand 2 were evaluated in osteoclasts following stimulation with interleukin 23 (n=3) or vehicle (n=3). Values are mean±standard error of mean. * p value<0.05; mRNA: Messenger ribonucleic acid; Samd4: Sterile alpha motif domain containing protein 4; IL-23: Interleukin-23; Ndr2: N-myc downstream-regulated gene 2; Cxcl1: Chemokine (C-X-C motif) ligand 1; Cxcl2: Chemokine (C-X-C motif) ligand 2.

≥ 1.5 as well as p value <0.05 (Table 2). The hierarchical clustering analysis was also applied, as shown in the Figure 9. The co-expression networks were constructed with all of the differentially expressed lncRNAs and mRNAs in this study. The lncRNAs and mRNAs that had Pearson correlation coefficients ≥ 0.7 and p value <0.05 were selected, and subsequently a network was constructed (Figure 10).

DISCUSSION

Although the role of IL-23 in OC formation has been investigated in some researches, the potential effects of IL-23 on the function of OCs and the mechanism of it involved in the pathogenesis of inflammatory arthritis are still unknown. In an attempt to elucidate these issues, we performed a preliminary study to analyze the impact of IL-23 on the gene expression profile in

OCs. As a result, we found that expression levels of more than 37 genes in OCs were modulated by IL-23, implying that IL-23 may also modulate the function of mature OCs.

Functional group analyses were performed to reveal the biological function of the genes regulated by IL-23. According to GO analysis, the IL-23 regulated genes are mainly involved in chemokine-mediated signaling pathway, cell chemotaxis, CXCR chemokine receptor binding and chemokine activity. Consistent with this result, the pathway analysis identified that the IL-23 regulated genes were mainly associated with the inflammatory signaling pathways like TNF, chemokine, NF-kappa B and cytokine receptor interaction. In all these above biological functions and signaling pathways, the most enriched genes were Cxcl1 and Cxcl2. Their expression levels were also confirmed by the following qPCR validation.

Table 2. Differentially expressed lncRNAs in osteoclasts following the stimulation with Interleukin-23

Gene symbol	Fold change	P value	Changes
Gm15401	1.666	0.0112	DR
LOC105247398	1.504	0.0155	DR
lincRNA:chr8:85952719-85958369	1.543	0.0427	DR
lincRNA:chr16:30200995-30205965	1.547	0.0219	DR
2310005E17Rik	1.603	0.0273	DR
Snhg7os	1.618	0.0497	DR
lincRNA:chr11:22500336-22520995	1.654	0.0464	DR
lincRNA:chr5:113594167-113645342	1.712	0.0451	DR
lincRNA:chr13:98513868-98517366	1.713	0.0431	DR
Gm28382	1.955	0.0493	DR
lincRNA:chr7:108253956-108254385	1.977	0.0095	DR
Gm12128	1.978	0.0426	DR
lincRNA:chr6:90436386-90469399	2.018	0.0119	DR
lincRNA:chr6:91928958-91936887	2.099	0.0351	DR
lincRNA:chr17:26911032-26912366	1.544	0.0232	UR
lincRNA:chr13:99870660-99877535	1.582	0.0367	UR
lincRNA:chr10:117021051-117038683	1.891	0.0134	UR
lincRNA:chr17:34018371-34019394	1.995	0.0032	UR

lncRNAs: Long non-coding ribonucleic acids; DR: Down-regulated; UR: Up-regulated.

Chemokine (C-X-C motif) ligand 1 and Cxcl2 belong to CXC chemokine family with 78% homology of their sequence.²³ They were first

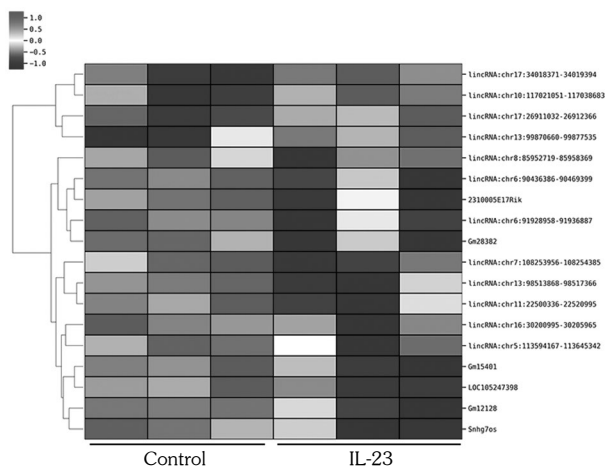


Figure 9. Heat map under hierarchical cluster analysis of differentially expressed lncRNAs. Hierarchical cluster showed that differentially expressed genes ultimately clustered into two major branches, including up-regulated genes, which were shown in red, and down-regulated genes, which were shown in blue. Color depth represents relative gene expression levels.

lncRNA: Long non-coding ribonucleic acid; IL-23: Interleukin-23.

identified as major chemokines produced by endotoxin-treated macrophages and they function as inducers of inflammation.²⁴ Both act through binding to the receptor CXCR2, a G protein-coupled receptor,²⁵ to induce cellular adherence or cell-cell fusion.²⁶ In addition to functioning as chemoattractants during inflammation and potentially as inducers of pre-OC fusion, they were also identified as necessary for OC formation. According to the study of Hardaway et al.,²⁷ Cxcl1 and Cxcl2 chemokines stimulate OCs differentiation *in vitro*. Treatment with either recombinant Cxcl1 or Cxcl2 enhanced OC maturation compared to control conditions. Similar to the present study, Ha et al.²⁸ also found that the expression of Cxcl2 in OC precursors could be enhanced by RANKL, and it has critical roles in osteoclastogenesis *in vitro* and in bone erosion *in vivo*. In addition, Cxcl1 and Cxcl2 also participate in lipopolysaccharide (LPS)-induced OC formation. It was found that they were significantly up-regulated in response to LPS and blockade of Cxcl1 and Cxcl2 nearly ablated OC formation induced by LPS stimulation.²⁹ All these data suggest that Cxcl1 and Cxcl2 not only function as chemoattractants

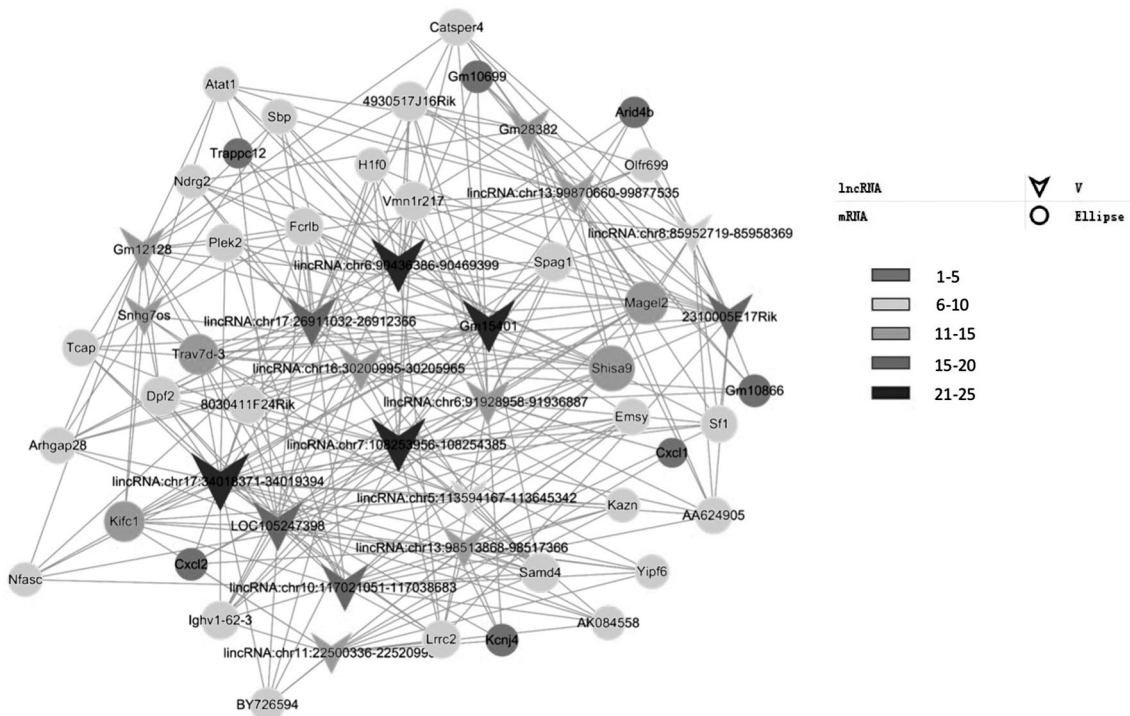


Figure 10. Co-expression networks of all differentially expressed long non-coding ribonucleic acids and messenger ribonucleic acids. A circle represents a messenger ribonucleic acid and an inverted triangle represents a long non-coding ribonucleic acid. Line between them represents significant co-expression of them. K-core was used to show number of co-expression genes associated with this gene. Different colors represent different ranges of k-core. Size of circle or triangle also represents number of co-expression genes associated with this gene.

lncRNA: Long non-coding ribonucleic acid; mRNA: Messenger ribonucleic acid.

for leukocytes during inflammation but also play important roles in OC formation. In our study, the expression of Cxcl1 and Cxcl2 in OCs were significantly up-regulated by IL-23. This result suggests that through modulating the expression of Cxcl1 and Cxcl2 in OCs, IL-23 might enhance the leukocyte chemotaxis as well as OC formation, thus promote the inflammatory and bone resorption in the pathogenesis of inflammatory arthritis.

Considering the statistical significance as well as the gene function, other two genes including NdrG2 and Samd4 were also selected to validate the array results. Consequently, the expression level trends of these genes were consistent with microarray data, whereas the differences did not reach statistical significance. NdrG2 belongs to the NDRG family, which is a family of differentiation-related genes including the proteins NdrG1-4. Highly expressed in the tissue of brain, salivary glands, and skeletal muscle, NdrG2 has

been identified as a regulator of dendritic cell differentiation from monocytes.³⁰ Besides the role of regulation on cell growth, apoptosis, and neurodegeneration,³¹⁻³³ it also plays an important role in modulating the OC differentiation.³⁴ Kang et al.³⁴ showed that NDRG2 overexpression inhibited the differentiation of U937 cells into OCs in response to stimulation of M-CSF and RANKL. In the present study, the expression of NdrG2 in OCs was up-regulated by the stimulation of IL-23. According to the role of NdrG2 on OC differentiation previously reported, we speculate that increased expression of NdrG2 is a kind of negative feedback to the increase of OC differentiation under the stimulation of IL-23.

Sterile alpha motif domain containing protein 4 is a translational repressor which works by directly binding to RNA. It has been identified as a regulator for skeletogenesis by down-regulation of OB and chondrocyte differentiation.³⁵ To our knowledge, the effects of Samd4 on the OC

differentiation have not been revealed in previous studies. Here, we identified that the expression of *Samd4* was decreased in OCs stimulated with IL-23. This result suggests that *Samd4* might play a role in OC differentiation and participate in the development of disease.

The lncRNAs expression profile of OCs following the stimulation with IL-23 was analyzed at the same time. Recently, the biological function of non-coding RNAs has attracted more attention. With the development of sequencing technology and microarray, a large number of non-coding RNAs including lncRNAs have been identified. Increasing evidence revealed that lncRNAs might have interaction with mRNA or microRNA, thus playing important roles in regulation of genes expression. Several lncRNAs have been identified to contribute to inflammatory arthritis such as RA.³⁶ In this study, we identified that lncRNAs of OCs were regulated following the stimulation with IL-23, and some of them had co-expression with differentially expressed mRNAs. These results revealed that lncRNAs of OCs were also regulated by IL-23, suggesting that they may play a role in the pathogenesis of inflammatory arthritis. However, due to the limited number of lncRNAs that can be screened by the microarray used in this study, the functions of these differentially expressed lncRNAs have not been studied further. The role of lncRNAs in inflammatory arthritis and underlying mechanism needs to be confirmed by further studies.

The current study revealed that IL-23 regulated the gene expression profile including mRNAs and lncRNAs in OCs, thus IL-23 might modulate the function of OCs in inflammatory arthritis. Deciphering the precise molecular mechanisms of these coding and non-coding RNAs function in inflammatory arthritis would be vital to provide insight into the pathogenesis of disease and to explore new potential targets for therapy.

There are several limitations of this study that should be noted. The main limitation was that the functional experiments were not performed to validate the results. Functional experiments will be carried out in the future to elucidate the potential mechanism. In addition to this is the relatively small sample size. Thus these data may be confirmed in larger-scale studies. Lastly, the expression levels of differentially expressed

lncRNAs were not verified. Due to the limited lncRNA data contained in the current microarray, the differentially expressed lncRNAs were not further analyzed. The lncRNAs expression profile of OCs following the stimulation with IL-23 and their interaction with mRNAs can be explored in future studies.

In conclusion, we revealed the expression profile of OCs including mRNAs and lncRNAs following the stimulation of IL-23 in mice. The expression of *Cxcl1* and *Cxcl2* in OCs were significantly regulated by IL-23, suggesting that IL-23 might promote the inflammatory and bone resorption in the pathogenesis of inflammatory arthritis through enhancing the leukocyte chemotaxis as well as OC formation. Several lncRNAs of OCs were also regulated by IL-23, which needs to be confirmed by further studies. Although these results are preliminary findings at this stage, they might be valuable in providing targets for further research on the disease pathogenesis.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Supplementary Table 1. Primer sequence used in verification of differentially expressed mRNAs

Gene	Forward	Reverse
Ndr2	5'-ACACCTTATGGCTCGGTCAC-3'	5'-TCTCTTGCATATCCCCGAAC-3'
Samd4	5'-GAACCCACGACAGTACCAGA-3'	5'-TTTGTGGGGAAGTATGCACG-3'
Cxcl1	5'-CTGGGATTACCTCAAGAATC-3'	5'-CAGGGTCAAGGCAAGCCTC-3'
Cxcl2	5'-CCAACCACCAGGCTACAGG-3'	5'-GCGTCACACTCAAGCTCTG-3'
GAPDH	5'-GCACAGTCAAGGCCGAGAAT-3'	5'-GCCTTCTCCATGGTGGTGAA-3'

Ndr2: N-Myc downstream regulated gene 2; Samd4: Sterile alpha motif domain containing protein 4; Cxcl1: Chemokine (C-X-C motif) ligand 1; Cxcl2: Chemokine (C-X-C motif) ligand 2; GAPDH: Glyceraldehyde phosphate dehydrogenase.