Integrative analyses reveal RNA regulatory network in Ti particles induced inflammation

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Abstract

Introduction Wear particles induced inflammatory osteolysis is the most important initiating factors in the mechanism of aseptic loosening. However, the molecular network changes in this process remain largely elusive. Methods Here, we performed whole transcriptome analysis using Ti particles induced RAW264.7 cell model to identify specific genes and pathways. Results Sequencing results totally identified 159 mRNAs, 96 lncRNAs, 31 circRNAs, and 12 miRNAs were significantly differently expressed. Of these, we selected two of each RNA for qRT-PCR validation and the results were highly consistent with the RNA-seq data. GSEA analysis shows that upregulated gene sets were related to the three classical inflammation pathway, cytokine–cytokine receptor interaction, TNF, and NF-kappa B signaling pathway. The enriched genes included not only IL-1β and TNF-α, which were independently verified before sequencing, but also other inflammatory osteolysis-related genes such as Mmp9, Fas, and Ccl2. Co-differentially expressed RNAs were employed to construct the ceRNA co-regulatory network. Conclusion: The results revealed that 4 lncRNAs and 2 circRNAs formed a regulatory network to simultaneously regulate miR-3065-3p targeting Myo18a. The present study helps to comprehensively understand the molecular mechanisms and regulatory interaction networks during early inflammatory response.

Keywords

inflammatory, mRNA, lncRNA, circRNA, ti particles, myo18a

Introduction

Artificial joint replacement is the most effective treatment for advanced osteoarthritis, rheumatoid arthritis, and severe traumatic arthritis.¹ Surgical treatment can reconstruct joint function, restore joint range of motion, reduce pain, and improve patients’ quality of life. The most common and serious complication after artificial joint replacement is aseptic loosening. The physical and chemical reaction of the artificial prosthetic material produces a large number of wear particles. The activation, proliferation, and osteolytic changes of osteoclasts in the prosthesis boundary

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membrane induced by wear particles are the most important initiating factors of aseptic loosening.\textsuperscript{2,3} After the wear particles enter the joint cavity, they gather locally or are swallowed by mononuclear macrophages to secrete a large number of cytokines, including tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1\(\beta\) (IL-1\(\beta\)), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), prostaglandin E\(2\) (PGE\(2\)), and matrix metalloproteinasises (MMPs),\textsuperscript{4–7} eventually leading to aseptic loosening. Thus, the inflammatory response caused by wear particles is very important for this pathological process.

Besides mRNAs, which consist of 2% transcribed genome codes, other remaining part of the transcribed genome is known as noncoding RNAs (ncRNAs).\textsuperscript{8} Among the various types of ncRNAs, long ncRNA (lncRNA), circular RNA (circRNA), and microRNA (miRNA) have attracted increasing attention. These ncRNAs are upstream factors of mRNAs that directly or indirectly regulate the biological function of mRNAs and play important roles in disease development.\textsuperscript{9,10} lncRNA/circRNA may act as competing endogenous RNAs (ceRNAs) by competitively binding to miRNAs through their miRNA response elements (MREs), thus regulating the expression of miRNA targeting mRNAs.\textsuperscript{11} A growing body of evidence supports that ncRNA-mediated gene expression is critical in inflammatory osteolysis.\textsuperscript{12} Therefore, understanding the regulation of these ncRNAs and their coding RNAs may provide clinically valuable predictive tools for its effective treatments. Dou et al. attempted to describe the changes in the expression profiles of lncRNAs, mRNAs, circRNAs, and miRNAs during osteoclastogenesis.\textsuperscript{13} In this study, the transcriptional effects of Ti particles on the RAW264.7 cells were detected to study the mechanism of early inflammatory process caused by wear particles.

**Material and methods**

**Preparation of Ti particles**

Commercially pure Ti particles were purchased from Johnson Matthey Chemicals (Ward Hill, USA). The average diameter of the particles was 2.9 µm according to the manufacturer’s certificate of analysis. The particles were baked at 180°C for 6 h and then washed in 70% ethanol for 48 h to remove endotoxins. The endotoxin level of the particles was determined with a Limulus Amebocyte Lysate Assay (Walkersville, USA), and only endotoxin-free particles (<0.1 EU/mL) were used in this study.

**Cell culture and treatments**

Murine macrophage RAW 264.7 cells (Cell Bank of Chinese Academy of Sciences, China) were cultured in DMEM (Hyclone, USA) containing 10% fetal bovine serum (Gibco, USA) and 1% antibiotics (100 U/mL penicillin-G and 100 pg/mL streptomycin) in a humidified atmosphere of 5% CO\(_2\) at 37°C. The cells were passaged at approximately 80% confluence by scraping and only early passages (p3–5) were used. The cells were plated onto different cell culture plates before stimulated with or without Ti particles (0.1 mg/mL). After 12 h, 24 h, and 36 h, the cells were harvested and stored at −80°C until subsequent study.

**RNA isolation and qualification**

Total RNA was extracted from specific cultured RAW 264.7 cells using TRIzol reagent (Invitrogen, USA) according to the manufacturer’s instructions. RNA concentration was measured in Qubit 2.0 Fluorometer (Life Technologies, USA), and RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, USA).

**RNA sequencing**

The chain-specific library was constructed by removing ribosomal RNA (rRNA).\textsuperscript{14} In brief, after removing the rRNA, the fragment was randomly interrupted into 250–300 bp. Through the fragment length selection, all the RNA, except rRNA and small RNA were obtained, including lncRNA, mRNA, circRNA, and so on. Then the quality of the library is evaluated by Agilent 2100. After passing the library test, PE150 (Pair end 150 bp) sequencing was carried out on Illumina HiSeq TM 2500 at Novogene. For small RNA sequencing, Multiplex Small RNA Library Prep Set for Illuma HiSeq TM 2500 at Novogene. Small RNA sequencing was performed using the R package (3.12.1). The p-values were adjusted using the Benjamini and Hochberg method. We used a standard q-value < 0.05 for DElncRNAs and DEmiRNAs, and p-value < 0.05 for DElncRNAs and DEcircRNAs.

**Differential expression analysis**

Differentially expressed mRNAs, lncRNAs, miRNAs, and circRNAs were performed using the R package (3.12.1). For GSEA for mRNAs was based on curated gene sets which summarized and represented specific well-defined pathway.
MSigdb was used for downloading reference gene sets. Gene sets with \( p \)-value < 0.01 and FDR \( q \)-value < 0.1 were considered as significant enrichments. KEGG pathway analysis was performed to identify the enriched pathways with KOBAS. The Benjamini and Hochberg method was used to correct the \( p \)-value, and \( q \)-value < 0.05 was considered as significant pathway.

**ceRNA co-expression network construction**

The co-expression network was constructed based on the correlation analysis between DEmRNAs and DEncRNAs. Cytoscape was used to draw the co-regulatory networks.

**Quantitative real time PCR (qRT-PCR)**

Total RNA was reverse transcribed to cDNA using the Prime Script RT Kit with gDNA Eraser (Takara, Japan). qRT-PCR was performed using the SYBR Premix Ex Taq kit (Takara, Japan) on a Light Cycler 480 System (Roche, SUI). The primer pairs used are presented in Table S1. \( \beta \)-Actin and U6 were used as housekeeping genes for normalization. The relative quantification values were calculated by the \( 2^{-\Delta\Delta CT} \) method.

**Statistical analysis**

Data were expressed as mean ± SEM of at least three independent experiments. Statistical analysis was performed using the two-tailed Students t-test. Values were considered significant at \( p < 0.05 \).

### Results

**Validation of Ti particles induced inflammatory response**

The RAW 264.7 cells were induced with Ti particles (0.1 mg/mL) for 12 h, 24 h, and 36 h, respectively. The expressions of \( IL-1\beta \) and \( TNF-\alpha \) were detected. \( IL-1\beta \) reached the highest level at 12 h after induction, which increased almost 30 folds and then decreased in varying degrees at 24 h and 36 h (Figure 1(a)). The expression of \( TNF-\alpha \) reached the highest level (about 3 folds) at 12 h and 24 h after induction, but the upregulation trend became very slight after 36 h (Figure 1(b)). These results indicated that the inflammatory response induced by Ti particles was more obvious in the early stage, so the time point chosen for whole transcriptome expression profiles was 12 h after the induction of Ti particles.

**Expression profiles of lncRNAs, mRNAs, circRNAs, and miRNAs**

Then we performed RNA sequencing to investigate the transcriptome-wide profiling, including mRNA, miRNA, lncRNA, and circRNA. We identified a total of 23,857 lncRNAs and 26,330 mRNAs in all samples. Compared to the control group, a total of 96 DElncRNAs, including 61 upregulated and 35 downregulated, were detected in the Ti group. A total of 159 DEmRNAs, including 99 upregulated and 60 downregulated, were identified between the two groups. Hierarchical clustering and Venn diagram of these DElncRNAs (Figure 2(a)) and DEmRNAs (Figure 2(b)) indicated an obvious discrimination.

![Figure 1](image-url). Validation of Ti particles induced inflammatory response. (a) \( IL-1\beta \) and (b) \( TNF-\alpha \) expressions were examined at 12 h, 24 h, and 36 h after Ti particle stimulation. Data are means ± SEM **\( p < 0.01 \), and ***\( p < 0.001 \). Ti, titanium; \( IL-1\beta \), interleukin-1\( \beta \); \( TNF-\alpha \), tumor necrosis factor-\( \alpha \).
functions of circRNAs have been reported as regulators of splicing and transcription, miRNA sponges, protein binding, and RNA transport. Among them, the function of miRNA sponges drew the most attention. We identified a total of 1360 mature circRNAs and 1029 mature miRNAs in all samples. In addition, 1041 circRNAs and 84 miRNAs were identified as novel circRNAs and miRNAs, respectively. Two groups showed a total of 31 DEcircRNAs (p-value < 0.05 and | log2 (foldchange)) > 1), including 15 upregulated and 16 downregulated, and 12 DEMiRNAs (p-value < 0.05), including 11 upregulated and 1 downregulated. Hierarchical clustering and Venn diagram of these DEcircRNAs (Figure 2(c)) and DEMiRNAs (Figure 2(d)) indicated an obvious discrimination. Blue and yellow color represented up and downregulated, respectively.

qRT-PCR validation of the selected differentially expressed profiles

To validate the differentially expressed profiles suggested by the sequencing results, qRT-PCR was performed to validate their expressions. Two dysregulated lncRNAs (Spaca6-210 and Btla-AS1), two dysregulated circRNAs (novel_circ_0001990 and novel_circ_0000814), two dysregulated miRNAs (mmu-miR-381-3p and mmu-miR-129-3p), and two dysregulated mRNAs (Mef2c and Ckb), with relatively high fold changes and low p-values, were selected for qRT-PCR validation. As shown in Fig. S1, the qRT-PCR results were consistent with the sequencing results, thus demonstrating high reliability of the gene profiles in our study.

Pathway-based GESA of mRNAs

As many genes had altered expression profiles after Ti particles induction, GSEA was performed to investigate the enriched transcription changes. The results showed 3 gene sets that were significantly downregulated and 17 gene sets that were significantly upregulated in the Ti group compared to the control group (FDR q-value < 0.1, Table 1). Two of the upregulated gene sets were related to TNF signaling (Figure 3(a)) and NF-kappa B signaling (Figure 3(b)). The altered genes included not only IL-1β and TNF-α, which we have verified before, but also other inflammatory-related genes such as Mmp9, Fas, and Ccl2.7,19,20

Pathway analysis of lncRNA, circRNA, and miRNA

KEGG pathway analysis was performed to identify the enriched pathways of ncRNAs. The top 20 enriched terms were shown in Fig. S2. According to the results, cytokine–cytokine receptor interaction pathway (mmu04060, p=0.002) and hematopoietic cell lineage pathway (mmu04640, p=0.014) were the most significant enriched pathways. In addition, other enrichment pathways that may be closely related to the inflammatory response included
osteoclast differentiation (mmu04380), rheumatoid arthritis (mmu05323), toll-like receptor signaling pathway (mmu04620), ECM-receptor interaction (mmu04512), TNF signaling pathway (mmu04668), and VEGF signaling pathway (mmu04370).

Construction of co-expression networks

To speculate on the functions of lncRNAs acting as miRNA targets, a network among DElncRNAs, DEmiRNAs, and DEmRNAs was reconstructed. As shown in Figure 4, the network was composed of 26 DElncRNAs, 6 DEmiRNAs, 16 DEmRNAs, and 60 edges. It was reported that miR-3065 was related to odontoblastic differentiation and postmenopausal osteoporosis.21,22 Moreover, we found that BC049715-OT4, Lmf2-206, Snx24-OT3, and 2010111I01Rik-AS1 may function as ceRNAs to suppress the inhibitory effects of mus-miR-3065-3p on Myo18a, which is closely involved in suppressing inflammatory responsiveness of macrophages via a mechanism that modulates CD14 trafficking.23 Accumulating studies have shown that circRNAs play roles as a miRNA sponge by competitively binding MRE.11,18 The circRNA–miRNA–mRNA network also predicted that two novel circRNAs (novel_circ_00019943 and novel_circ_0000280) may exist as ceRNAs to suppress mus-miR-3065-3p targeting Myo18a. Together, these results demonstrate that certain lncRNAs/circRNAs-miR3065-Myo18a network might have a regulatory role in early inflammatory response. These RNA interactions provided a novel perspective on the mechanisms of this process.

Discussion

The application of artificial joint replacement reconstructed the function of diseased joints and significantly improved the life quality of patients.1 With the extension of the service life of artificial joints, the problem of aseptic loosening has gradually emerged and become the main reason affecting the long-term effect of artificial joints. The inflammatory response caused by wear particles is the initiating factor for aseptic loosening. Particles produced by prosthesis could induce inflammation and release a variety of inflammatory factors (TNF-α, IL-1β, VEGF, etc.). Previous studies have found that Ti particle-treated RAW 264.7 cells can upregulate TNF-α, IL-1β, and IL-6.24–26

| KEGG pathway                              | MSigDB    | SIZE | ES       | NES | NOM p-val | FDR q-val |
|-------------------------------------------|-----------|------|----------|-----|-----------|-----------|
| Retinol metabolism                        | MMU00830 | 38   | 0.700248 | 2.216328 | 0     | 0         |
| Cytokine–cytokine receptor interaction    | MMU04060 | 166  | 0.504727 | 2.047488 | 0     | 0.000558  |
| TNF signaling pathway                     | MMU04668 | 105  | 0.542818 | 2.04425 | 0     | 0.000372  |
| Complement and coagulation cascades       | MMU04610 | 56   | 0.584845 | 2.024369 | 0     | 0.000279  |
| NF-kappa B signaling pathway              | MMU04064 | 82   | 0.513728 | 1.878065 | 0     | 0.010338  |
| Malaria                                   | MMU05144 | 33   | 0.585125 | 1.806957 | 0.001838 | 0.020366  |
| Rheumatoid arthritis                      | MMU05323 | 66   | 0.504589 | 1.797109 | 0.003617 | 0.021111  |
| NOD-like receptor signaling pathway       | MMU04621 | 54   | 0.526303 | 1.783983 | 0     | 0.026631  |
| Mineral absorption                        | MMU04978 | 37   | 0.553304 | 1.75795  | 0.005587 | 0.030092  |
| Chemokine signaling pathway               | MMU04062 | 146  | 0.438796 | 1.737788 | 0     | 0.034121  |
| Amoebias                                  | MMU05146 | 81   | 0.473714 | 1.721326 | 0     | 0.041433  |
| Hematopoietic cell lineage                | MMU04640 | 65   | 0.479388 | 1.69708  | 0     | 0.0554    |
| African trypanosomiasis                   | MMU05143 | 23   | 0.587539 | 1.661698 | 0.009124 | 0.051585  |
| Collecting duct acid secretion            | MMU04966 | 24   | 0.561601 | 1.655969 | 0.005747 | 0.0543    |
| Bladder cancer                            | MMU05219 | 34   | 0.535621 | 1.654824 | 0.005792 | 0.055185  |
| Tuberculosis                              | MMU05152 | 143  | 0.411361 | 1.631357 | 0.001712 | 0.06221   |
| Proteoglycans in cancer                   | MMU05205 | 168  | 0.390754 | 1.593805 | 0     | 0.083408  |
| Propanoate metabolism                     | MMU00640 | 28   | −0.66919 | −2.03628 | 0     | 0.009966  |
| Glycosaminoglycan degradation             | MMU00531 | 16   | −0.67544 | −1.80072 | 0.002114 | 0.05153   |
| ABC transporters                          | MMU02010 | 34   | −0.50164 | −1.63032 | 0.017058 | 0.097416  |
| Huntington’s disease                      | MMU05016 | 172  | −0.38595 | −1.63347 | 0     | 0.104271  |
| Citrate cycle (TCA cycle)                 | MMU00020 | 29   | −0.54723 | −1.68262 | 0.010846 | 0.115241  |
| Parkinson’s disease                       | MMU05012 | 114  | −0.41163 | −1.63824 | 0     | 0.119339  |
| Other glycan degradation                  | MMU00511 | 16   | −0.64754 | −1.70237 | 0.006749 | 0.125352  |
| Valine, leucine, and isoleucine degradation| MMU00280 | 48   | −0.48642 | −1.63934 | 0.012739 | 0.146676  |
| Fatty acid metabolism                     | MMU01212 | 49   | −0.44714 | −1.51906 | 0.015184 | 0.224382  |
| Longevity regulating pathway—multiple species | MMU04213 | 55   | −0.41883 | −1.49257 | 0.028037 | 0.248363  |
These cytokines can activate osteoclasts and then trigger osteolysis around the prosthesis. Aseptic inflammation, as the upstream reaction of osteolysis, plays a key role in the whole pathological process, but the specific molecular mechanism is still unclear. We detected IL-1β and TNF-α expression levels at different time point after Ti particles induction. The results indicated a fast-inflammatory response induced by Ti particles.

In our present study, we totally identified 96 DElncRNAs, 159 DEmRNAs, 31 DEcircRNAs, and 12 DEmiRNAs. qRT-PCR validation was consistent with the sequencing results, indicating high reliability of the profiles. Furthermore, GSEA was performed to investigate the enriched transcription changes. TNF signaling and NF-kappa B signaling were two of the top upregulated gene sets. Schwarz et al. also identified the effects of TNF-α/NF-κB signaling in the proinflammatory response to titanium particles in vitro and in vivo.27,28 This not only validate the key roles of TNF signaling and NF-kappa B signaling but also reflect that our model is feasible.

Myo18a, a divergent member of the myosin family, appears to regulate cell migration, cellular response to interferon-gamma, and macrophage activation.29,30 The Myo18a gene encodes two splice isoforms in macrophages, namely, SP-R210L and SP-R210S,31 indicating that it is subject to cell type–dependent alternative splicing. Various studies have reported that Myo18a involved in unconventional myosin processes including lung surfactant function, Golgi function via an interaction with GOLPH3, and cancers.33 Interestingly, Myo18a was also a susceptibility gene of Alzheimer’s disease based on a genome-wide association study.34 It remains uncertain whether fascinating Myo18a has potential roles in aseptic inflammation. Increasing evidence indicates that lncRNAs and circRNAs are extensively targeted by miRNAs and that they function as ceRNAs.35,36 For instance, LncRNA KCNQ1OT1 could ameliorate particle-induced osteolysis through inducing macrophage polarization by inhibiting miR-21a-5p.37 Chen et al. found that circRNA_28313/miR-195a/CSF1 axis could modulate osteoclast differentiation to affect OVX-induced bone absorption.38 So, we further constructed lncRNA/circRNA–miRNA–mRNA co-expression networks in this study. The networks indicate that four DElncRNAs (BC049715-OT4, Lmf2-206, Snx24-OT3, and 2010111I01Rik-AS1) and two DEcircRNAs (novel_circ_00019943 and novel_circ_0000280) may function as ceRNAs to suppress the inhibitory effects of mus-miR-3065-3p on Myo18a. Taken together, we suspected that certain lncRNAs/circRNAs-Mir3065-my018a networks may participate in inflammatory response. Undoubtedly, there are still some limitations that need to be specified. First, our CeRNA analysis identified regulatory networks, results that require further experimental validation. Furthermore, the inflammatory response

![Figure 3. Pathway-based gene set enrichment analysis (GESA) of mRNAs. (a) TNF signaling pathway and target genes. NES = 2.044, p-val < 0.001, FDR q-val < 0.001. (b) NF-kappa B signaling pathway and target genes. NES = 1.878, p-val < 0.001, FDR q-val = 0.010. ctrl, control; Ti, titanium.](image-url)
of unstimulated RAW 264.7 cells was weak compared to primary and human cells. These findings still require further validation in primary cells and clinical samples in the future. Although these findings still needed further functional experiments’ validation, it did give us some novel insight on the mechanism of this process.

Conclusion

In conclusion, our present study presented profiles of dysregulated lncRNAs, mRNAs, circRNAs, and miRNAs in Ti particles induced inflammatory response. The results revealed that 4 lncRNAs and 2 circRNAs formed a
regulatory network to simultaneously regulate miR-3065-3p targeting Myo18a. These findings may establish a foundation for future functional research on early aseptic inflammation.

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**Declaration of conflicts of Interest**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Supplemental material**

Supplemental material for this article is available online.

**References**

1. Hiligsmann M, Cooper C, Arden N, et al. Health economics in the field of osteoarthritis: an expert’s consensus paper from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Seminars in Arthritis and Rheumatism* 2013; 43(3): 303–313.

2. Abu-Amer Y, Darwech I and Clohisy JC. Aseptic loosening of total joint replacements: mechanisms underlying osteolysis and potential therapies. *Arthritis Research & Therapy* 2007; 9(Suppl 1): S6.

3. Drees P, Eckardt A, Gay RE, et al. Mechanisms of disease: molecular insights into aseptic loosening of orthopedic implants. *Nature Clinical Practice Rheumatology* 2007; 3(3): 165–171.

4. Redlich K, Hayer S, Ricci R, et al. Osteoclasts are essential for TNF-α-mediated joint destruction. *Journal of Clinical Investigation* 2002; 110(10): 1419–1427.

5. Chang K, Hong-Shong Chang W, Yu Y-H, et al. Pulsed electromagnetic field stimulation of bone marrow cells derived from ovariectomized rats affects osteoclast formation and local factor production. *Bioelectromagnetics* 2004; 25(2): 134–141.

6. Nakagawa M, Kaneda T, Arakawa T, et al. Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. *FEBS Letters* 2000; 473(2): 161–164.

7. Delaissé J-M, Andersen TL, Engsig M-T, et al. Matrix metalloproteinases (MMP) and cathepsin K contribute differently to osteoclastic activities. *Microscopy Research and Technique* 2003; 61(6): 504–513.

8. Li W, Notani D and Rosenfeld MG. Enhancers as non-coding RNA transcription units: recent insights and future perspectives. *Nature Reviews Genetics* 2016; 17(4): 207–222.

9. Eidem TM, Kugel JF and Goodrich JA. Noncoding RNAs: regulators of the mammalian transcription machinery. *Journal of Molecular Biology* 2016; 428(12): 2652–2659.

10. Morris KV and Mattick JS. The rise of regulatory RNA. *Nature Reviews Genetics* 2014; 15(6): 423–437.

11. Kartha RV and Subramanian S. Competing endogenous RNAs (ceRNAs): new entrants to the intricacies of gene regulation. *Frontiers in Genetics* 2014; 5: 8.

12. Ghayor C and Weber FE. Epigenetic regulation of bone remodeling and its impacts in osteoporosis. *International Journal of Molecular Sciences* 2016; 17(9).

13. Dou C, Cao Z, Yang B, et al. Changing expression profiles of lncRNAs, miRNAs, circRNAs and miRNAs during osteoclastogenesis. *Scientific Reports* 2016; 6: 21499.

14. Parkhomchuk D, Borodina T, Amstislavskiy V, et al. Transcriptome analysis by strand-specific sequencing of complementary DNA. *Nucleic Acids Research* 2009; 37(18): e123.

15. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences* 2005; 102(43): 15545–15550.

16. Mao X, Cai T, Olyarchuk JG, et al. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. *Bioinformatics* 2005; 21(19): 3787–3793.

17. Kohl M, Wiese S and Warscheid B. Cytoscape: software for visualization and analysis of biological networks. *Methods in Molecular Biology* 2011; 696: 291–303.

18. Lasda E and Parker R. Circular RNAs: diversity of form and function. *RNA* 2014; 20(12): 1829–1842.

19. Gruber R. Osteoimmunology: Inflammatory osteolysis and regeneration of the alveolar bone. *Journal of Clinical Periodontology* 2019; 46(Suppl 21): 52–69.

20. Craig MJ and Loberg RD. CCL2 (Monocyte Chemoattractant Protein-1) in cancer bone metastases. *Cancer and Metastasis Reviews* 2006; 25(4): 611–619.

21. Lin C, Zhang Q, Yu S, et al. miR-3065-5p regulates mouse odontoblastic differentiation partially through bone morphogenetic protein receptor type II. *Biochemical and Biophysical Research Communications* 2018; 495(1): 493–498.

22. Lin C, Yu S, Jin R, et al. Circulating miR-338 cluster activities on osteoblast differentiation: potential diagnostic and therapeutic targets for postmenopausal osteoporosis. *Theranostics* 2019; 9(13): 3780–3797.

23. Yang L, Carrillo M, Wu YM, et al. SP-R210 (Myo18A) isoforms as intrinsic modulators of macrophage priming and activation. *PloS One* 2015; 10(5): e0126576.
24. Zhang Y, Yu S, Xiao J, et al. Wear particles promote endotoxin tolerance in macrophages by inducing interleukin-1 receptor-associated kinase-M expression. *Journal of Biomedical Materials Research Part A* 2013; 101A(3): 733–739.

25. Liu Z, Li Y, Guo F, et al. Tetrandrine inhibits titanium particle-induced inflammatory osteolysis through the nuclear factor-κB pathway. *Mediators of Inflammation* 2020; 2020: 1926947.

26. Zhao Y-p, Wei J-l, Tian Q-y, et al. Progranulin suppresses titanium particle induced inflammatory osteolysis by targeting TNFα signaling. *Scientific Reports* 2016; 6: 20909.

27. Schwarz EM, Lu AP, Goater JJ, et al. Tumor necrosis factor-α/nuclear transcription factor-κB signaling in periprosthetic osteolysis. *Journal of Orthopaedic Research* 2000; 18(3): 472–480.

28. Roebuck KA, Vermes C, Carpenter LR, et al. Down-regulation of procollagen α1(I) messenger RNA by titanium particles correlates with nuclear factor κB (NF-κB) activation and increased Rel A and NF-κB1 binding to the collagen promoter. *Journal of Bone and Mineral Research* 2001; 16(3): 501–510.

29. Hsu R-M, Hsieh Y-J, Yang T-H, et al. Binding of the extreme carboxyl-terminus of PAK-interacting exchange factor β (βPIX) to myosin 18A (MYO18A) is required for epithelial cell migration. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 2014; 1843(11): 2513–2527.

30. Matsui K, Parameswaran N, Bagheri N, et al. Proteomics analysis of the Ezrin interactome in B cells reveals a novel Association with Myo18at. *Journal of Proteome Research* 2011; 10(9): 3983–3992.

31. Dippold HC, Ng MM, Farber-Katz SE, et al. GOLPH3 bridges phosphatidylinositol-4- phosphate and actomyosin to stretch and shape the Golgi to promote budding. *Cell* 2009; 139(2): 337–351.

32. Walz C, Haferlach C, Hänel A, et al. Identification of aMYO18A-PDGFRB fusion gene in an eosinophilia-associated atypical myeloproliferative neoplasm with a t(5;17)(q33-34;q11.2). *Genes, Chromosomes and Cancer* 2009; 48(2): 179–183.

33. Farber-Katz SE, Dippold HC, Buschman MD, et al. DNA damage triggers golgi dispersal via DNA-PK and GOLPH3. *Cell* 2014; 156(3): 413–427.

34. Lee YH and Song GG. Genome-wide pathway analysis of a genome-wide association study on Alzheimer’s disease. *Neurological Sciences* 2015; 36(1): 53–59.

35. Xiong Y, Zhang J and Song C. CircRNA ZNF609 functions as a competitive endogenous RNA to regulate FOXP4 expression by sponging miR-138-5p in renal carcinoma. *Journal of Cellular Physiology* 2019; 234(7): 10646–10654.

36. Zhao Y, Wang H, Wu C, et al. Construction and investigation of LncRNA-associated ceRNA regulatory network in papillary thyroid cancer. *Oncology Reports* 2018; 39(3): 1197–1206.

37. Gao X, Ge J, Li W, et al. LncRNA KCNQ1OT1 ameliorates particle-induced osteolysis through inducing macrophage polarization by inhibiting miR-21a-5p. *Biological Chemistry* 2018; 399(4): 375–386.

38. Chen X, Ouyang Z, Shen Y, et al. CircRNA_28313/miR-195a/CSF1 axis modulates osteoclast differentiation to affect OVX-induced bone absorption in mice. *RNA Biology* 2019; 16(9): 1249–1262.