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Interferon-β-induced miR-155 inhibits osteoclast differentiation by targeting SOCS1 and MITF

Jun Zhang [a,1], Hongying Zhao [b,1], Jinping Chen [a], Bing Xia [a], Yongming Jin [a], Wei Wei [a], Jianjian Shen [c], Yazeng Huang [a,*]

[a] Department of Orthopedics, Zhejiang Provincial People's Hospital, Hangzhou, PR China
[b] Department of Pharmacy, Zhejiang Provincial People's Hospital, Hangzhou, PR China
[c] School of Medicine, Zhejiang University of Traditional Chinese Medicine, Hangzhou, PR China

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ABSTRACT

IFN-β is induced via a c-fos dependent mechanism that is present downstream of the receptor activator of NF-κB ligand (RANKL)-RANK signal transduction cascade during osteoclast differentiation. Increased production of IFN-β in turn inhibits osteoclastogenesis. However, the mechanism by which IFN-β exerts its suppressive function remains unclear. In the present study, we found that miR-155, an IFN-β-induced miRNA, mediated the suppressive effect of IFN-β on osteoclast differentiation by targeting SOCS1 and MITF, two essential regulators of osteoclastogenesis. These findings have not only demonstrated that miR-155 inhibits osteoclast differentiation, but also provided a new therapeutic target for treatment of osteoclast-mediated diseases.

1. Introduction

Osteoclasts are multinucleated bone-absorbing cells derived from the monocyte-macrophage lineage under the presence of receptor activator of NF-κB ligand (RANKL) and M-CSF [1–4]. M-CSF promotes proliferation and survival of osteoclast progenitors. RANKL prompts the cells to differentiate along the osteoclast linage and acts as an activating and survival factor for mature osteoclasts [5–6]. RANKL exerts its effect on osteoclasts by binding to the receptor RNAK expressed on osteoclast precursors and mature osteoclasts. The interaction of RANKL and RANK results in recruitment of the adaptor molecules such as TNF receptor-associated factors and stimulates downstream signaling cascades, including three well-known MAPK pathways, PI3K, and NF-κB [7]. RANKL also leads to the induction of osteoclastogenic transcription factors, including c-Fos, Fra-1 and cytoplasmic, calcineurin-dependent NFAT1 (NFATc1) [8–11].

The maintenance of bone homeostasis is dependent on the balance between bone-forming osteoblasts and bone-resorbing osteoclasts [12–13]. Excessive bone resorption by osteoclasts is often associated with diseases accompanied by pathological bone loss, including osteoporosis and rheumatoid arthritis [14–17]. Thus, research into the mechanism of osteoclast differentiation is both biologically and clinically important.

IFN-β plays an inhibitory role during osteoclast differentiation. IFN-β is induced in response to osteoclast activation by RANK-RNAKL interaction [18]. Increased production of IFN-β is in turn expected to inhibit c-fos activity resulting in the inhibition of osteoclastogenesis. The effects of IFN-β are primarily mediated through Jak-Stat pathway, inhibitory effects related to osteoclastogenesis may be regulated through the interferon stimulated gene factor 3 (ISGF3) complex comprising of signal transducers and activators of transcription (STAT)1 and STAT2 and interferon regulatory factor 9 (IRF9) [18]. Protein kinase R (PKR) has been implicated to mediate the inhibition of c-fos protein by IFN-β [18–19]. CXCL11 may be another key intermediary in the inhibition of c-fos by IFN-β [20–21]. However, the other regulators mediated the suppressive effect of

Abbreviations: miRNA, microRNA; IFN-β, interferon-β; RANKL, receptor activator of NF-κB ligand; BMMs, bone marrow-derived macrophages; TRAP, tartrate-resistant acid phosphatase; SOCS1, suppressor of cytokine signaling1; MITF, microphthalmia-associated transcription factor; 3′UTRs, 3′untranslated regions

* Corresponding author. Address: Department of Orthopedics, Zhejiang Provincial People's Hospital, 158 Shangtang Road, Hangzhou 310014, PR China. Fax: +86 571 85893595.
E-mail address: yzhuang.zju@gmail.com (Y. Huang).

[1] These authors contribute equally to this work.

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IFN-β on osteoclast differentiation still need to be investigated.microRNAs (miRNAs) are small, noncoding RNAs that are involved in posttranscriptional repression by binding to minimal binding sites on target mRNAs [22]. Many miRNAs play roles in hematopoietic cells, including osteoclasts [23]. A specific miRNA, miR-155, is involved in the commitment of monocyte progenitors to macrophage differentiation and activation, by effectively interfering with the genetic network driving the alternative, osteoclast fate [24]. By targeting SOCS1, miR-155 regulates antiviral innate immunity and dendritic cell development, controls macrophage response to lipopolysaccharide, and acts as an oncomiRNA in breast cancer [25–28]. Whether miR-155 also controls osteoclast differentiation by targeting SOCS1 is unknown, although it is well-known that SOCS1 facilitates osteoclastogenesis by blocking the inhibitory effect of inflammatory cytokines and IFN-β on RANKL-mediated osteoclast differentiation signals [29–30]. In monocyte lineage, miR-155 was induced by IFN-β [28,31], playing an important role in monocyte proliferation, and in macrophage and dendritic cell differentiation [32]. However, the roles of miR-155 in the osteoclast trajectory, derived from the same precursor, were less known. Our data suggest that miR-155 is induced by IFN-β, and mediates the suppressive effect of IFN-β on osteoclast differentiation by targeting SOCS1 and microphthalmia-associated transcription factor (MITF), two positive regulators during osteoclastogenesis.

2. Materials and methods

2.1. Mice

C57BL/6 mice (6–8 weeks old) were purchased from SIPPR-BK Experimental Animal (Shanghai, China). *IFNb* and *IFNAR1* deficient mice were from Knockout Mouse Project (KOMP) Repository and were bred in specific pathogen-free conditions. All animal experiments were undertaken in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Zhejiang University (Hangzhou, China).

2.2. Reagents

Mouse RANKL and M-CSF were from PeproTech (Rocky Hill, NJ). Mouse recombinant IFN-β was from R&D (Minneapolis, MN). TLR3 ligand Poly I:C and anti-β-actin were from Sigma–Aldrich (St. Louis, MO). Anti-Socs1 and HRP-coupled secondary Abs were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Mitf was from LifeSpanBioSciences (Seattle, WA). The miR-155 mimic and its control mimic were from GenePharma (Shanghai, China). The transfection reagents Jet-Endo™ and INTERFERin™ were from Polyplus Transfection (Illkirch, FRANCE). siRNAs targeting Socs1 and Mitf were from Dharmacon (ThermoFisher Scientific, Lafayette, CO). VSV virus (Hazelhurst strain) from was from American Type Culture Collection (ATCC) (Manassas, VA).

2.3. Cell culture and transfection

HEK293T cells and Murine macrophage cell line RAW264.7 were obtained from the ATCC (Manassas, VA) and maintained at 37 °C (5% CO_2, 95% air) in culture medium consisting of DMEM supplemented with 10% FBS. INTERFERin™ was used to transfect siRNAs into RAW 264.7 cells according to the manufacturer's instructions.

2.4. Generation of BMMs and osteoclastogenesis

Femurs and tibiae were separated from C57BL/6 mice. After soaking the bones in 70% ethanol for 2 min and rising in PBS, both end were cut, and the bone marrow was flushed out with PBS using a 25-gauge syringe. Erythrocytes were lysed using M-lysis buffer (R&D Systems, Minneapolis, MN). To prepare osteoclast precursors, bone marrow cells were cultured in α-MEM supplemented with 10% FBS overnight. The next day, non-adherent cells were harvested and cultured with α-MEM supplemented with 10% culture supernatant from CMG14–12 cells as the source of M-CSF. After 3 days of culturing, floating cells were removed, and attached cells were used as osteoclast precursor cells (BMMs). For in vitro osteoclast differentiation, BMMs were cultured in 24-well plates (5×10^4 cells/well) in α-MEM in the presence of M-CSF and RANKL for 4 days and stained for tartrate-resistant acid phosphatase (TRAP) activity using a leukocyte acid phosphatase kit (Sigma–Aldrich, St.Louis, MO). In osteoclastogenesis condition, the concentration of M-CSF and RANKL is 50 ng/ml and 100 ng/ml, respectively.

2.5. RNA quantification

Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Real-time quantitative RT-PCR analysis was performed using the LightCycler (Roche) and SYBR RT-PCR kits (Takara). The relative expression level of miRNAs was normalized to the internal control U6 by using $2^{-\Delta\Delta Ct}$ cycle threshold method [33]. The relative expression level of Socs1 and Mitf was normalized to the level of β-actin expression in each sample.

2.6. Plasmid construction

The Socs1 3′UTR and Mitf 3′UTR luciferase reporter constructs were made by amplifying the mouse Socs1 3′UTR and Mitf 3′UTR sequence by PCR and cloning into the *Mlu*I and *Spe* I sites of

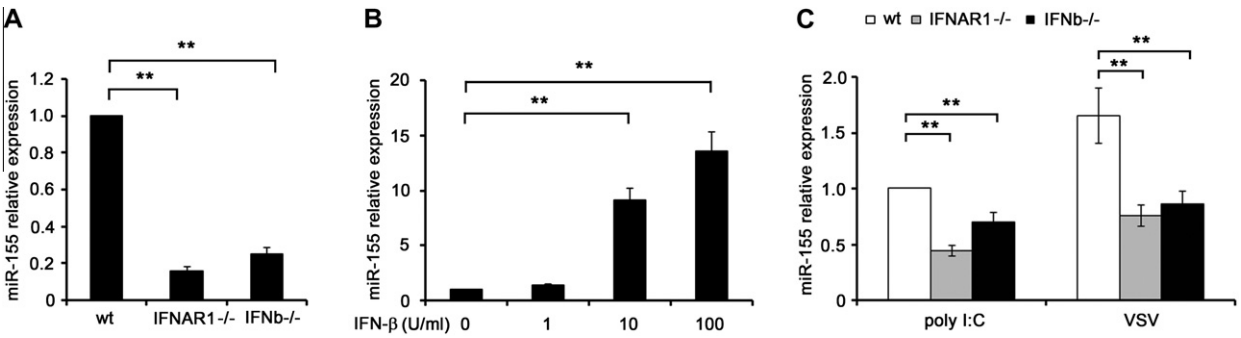

Fig. 1. IFN-β induced miR-155 in bone marrow-derived macrophages (BMMs). (A) BMMs from wild type (wt) mice, IFNAR1 deficient (IFNAR1−/−) mice or IFNb deficient (IFNb−/−) mice were cultured in osteoclastogenesis condition for 4 days, qPCR assays were performed to detect the miR-155 expression.(B) Wild type BMMs were treated with indicated doses of IFN-β in osteoclastogenesis condition for 12 h. qPCR assays were performed to detect the miR-155 expression. (C) BMMs from wt, IFNAR1−/− or IFNb−/− mice was stimulated with 10 μg/ml Poly I:C or infected with VSV (MOI = 0.1) in osteoclastogenesis condition for 24 h, qPCR assays were performed to detect the miR-155 expression. Data are mean ± S.E.M of three independent experiments. $^{**}P < 0.01$.

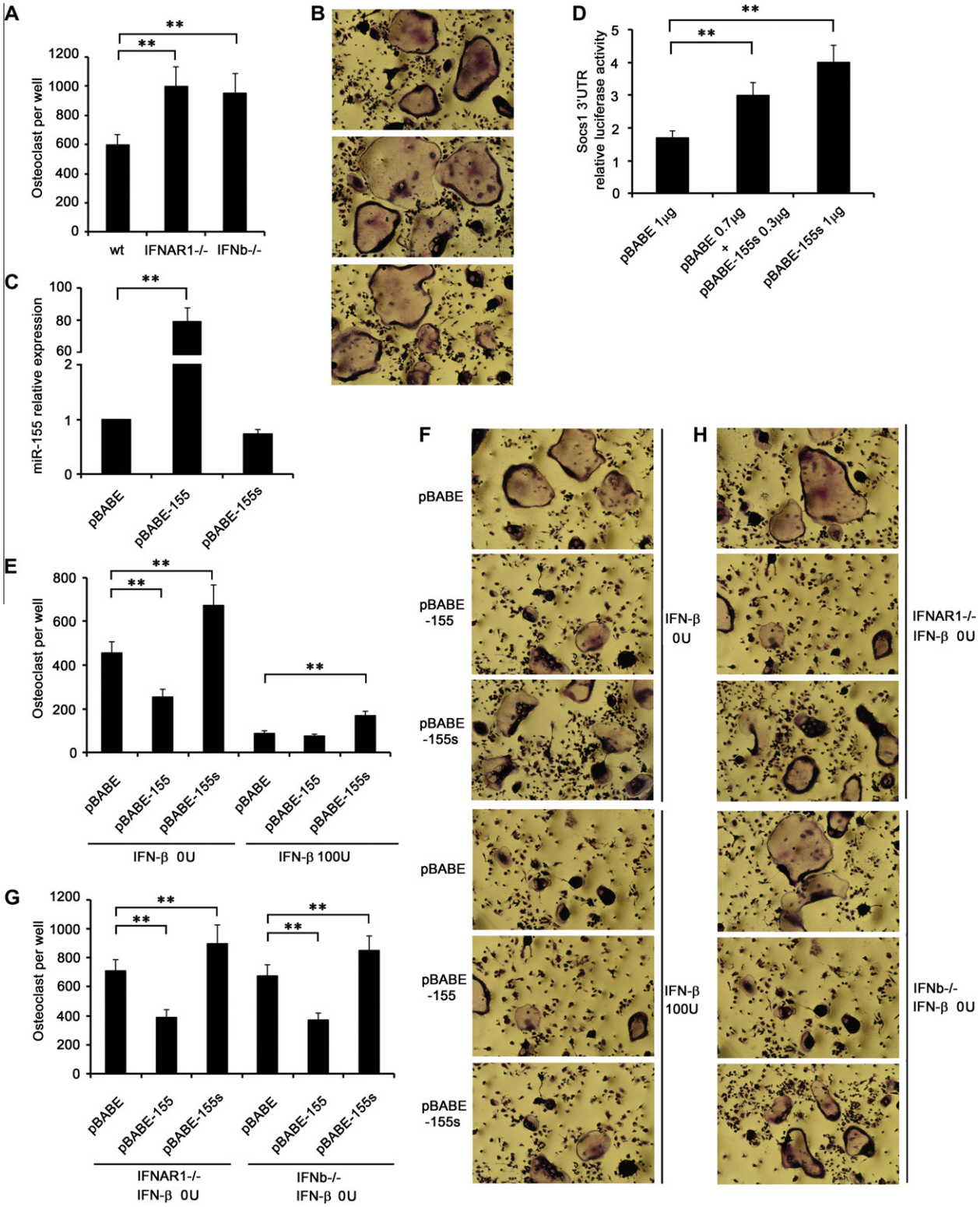

Fig. 2. miR-155 mediated the suppressive effect of IFN-β on osteoclast differentiation. (A–B) BMMs from wt, IFNAR1−/− or IFNb−/− mice were cultured in osteoclastogenesis condition for 4 days. TRAP-positive multinucleated cells were counted as osteoclasts. (C) BMMs were incubated for 8 h with the pBABE, pBABE-155 or pBABE-155s viral supernatants in the presence of M-CSF (50 ng/ml) and polybrene (6 μg/ml). After the viral supernatant was removed, BMMs were further cultured in osteoclastogenesis condition for 24 h. qPCR assays were performed to detect the miR-155 expression. (D) HEK293T cells were cotransfected with pTK-*Renilla* luciferase plasmids, Socs1 3′UTR reporter construct and indicated retroviral construct, 24 h later, the *firefly* luciferase activity was measured and normalized to *Renilla* luciferase activity. (E–H) wild type, IFNAR1−/− or IFNb−/− BMMs were incubated for with the pBABE, pBABE-155 or pBABE-155s viral supernatants as described in (C). After the viral supernatant was removed, BMMs were further cultured in osteoclastogenesis condition with or without IFN-β for 4 days. TRAP-positive multinucleated cells were counted as osteoclasts. Data of (A), (C), (E) and (G) are mean ± S.E.M of three independent experiments. Data of C is mean ± SD ($n = 6$) of one representative experiment. Similar results were observed in three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

pMIR-REPORT construct (Ambion). The mutant Socs1 and Mitf 3′UTR luciferase reporter constructs were generated by converting miR-155 binding site "AGCATTAA" into "ACCTTAAT".

2.7. Statistical analysis

Statistical significance was determined by Student's *t* test, with *P* values of ≦0.05 considered to be statistically significant.

3. Results

3.1. IFN-β induced miR-155 during osteoclastogenesis

IFN-β negatively regulates the osteoclast differentiation, which is an important regulatory mechanism for bone homeostasis [18]. miR-155 suppresses the RANKL-induced osteoclast differentiation by repressing the expression of MITF, a transcription factor essential for osteoclast differentiation [24]. It also has been reported that miR-155 is induced in primary murine macrophages after exposure to the cytokine IFN-β [31]. After culturing the bone marrow-derived macrophages (BMMs) in the presence of M-CSF and RANKL for 4 days, the BMMs were differentiated into tartrate-resistant acid phosphatase (TRAP) positive osteoclasts. The wild type osteoclasts expressed much more miR-155 than the *IFNAR1*−/− or *IFNb*−/− osteoclasts (Fig. 1A). To determine if IFN-β could induce miR-155 during osteoclast differentiation, we added increasing dose of IFN-β in osteoclastogenesis conditions. Increasing miR-155 induction could be observed after treating with increasing doses of IFN-β (Fig. 1B). Given that TLR3-triggered and VSV-infected macrophages produce large amount of IFN-β [34–35]. We stimulated the BMMs with poly I:C or infected with VSV during osteoclast differentiation. The *IFNAR1*−/− and *IFNb*−/− BMMs expressed less miR-155 than wild type BMMs, no matter stimulated with poly I:C or infected with VSV (Fig. 1C). Taken together, induction of miR-155 was IFN-β dependent during osteoclast differentiation.

3.2. miR-155 suppressed osteoclast differentiation

IFN-β inhibits osteoclast differentiation and induction of miR-155 was IFN-β dependent. Therefore, we hypothesized that miR-155 mediated the suppressive effect of IFN-β on osteoclastogenesis. Consistent with the reported data [18], we found that *IFNAR1*−/− and *IFNb*−/− BMMs could differentiate into more osteoclasts than the wild type BMMs (Fig. 2A and B). In order to overexpress miR-155 in BMMs, we constructed pBABE-155 by inserting part of the primary mir-155 into pBABE vector, a

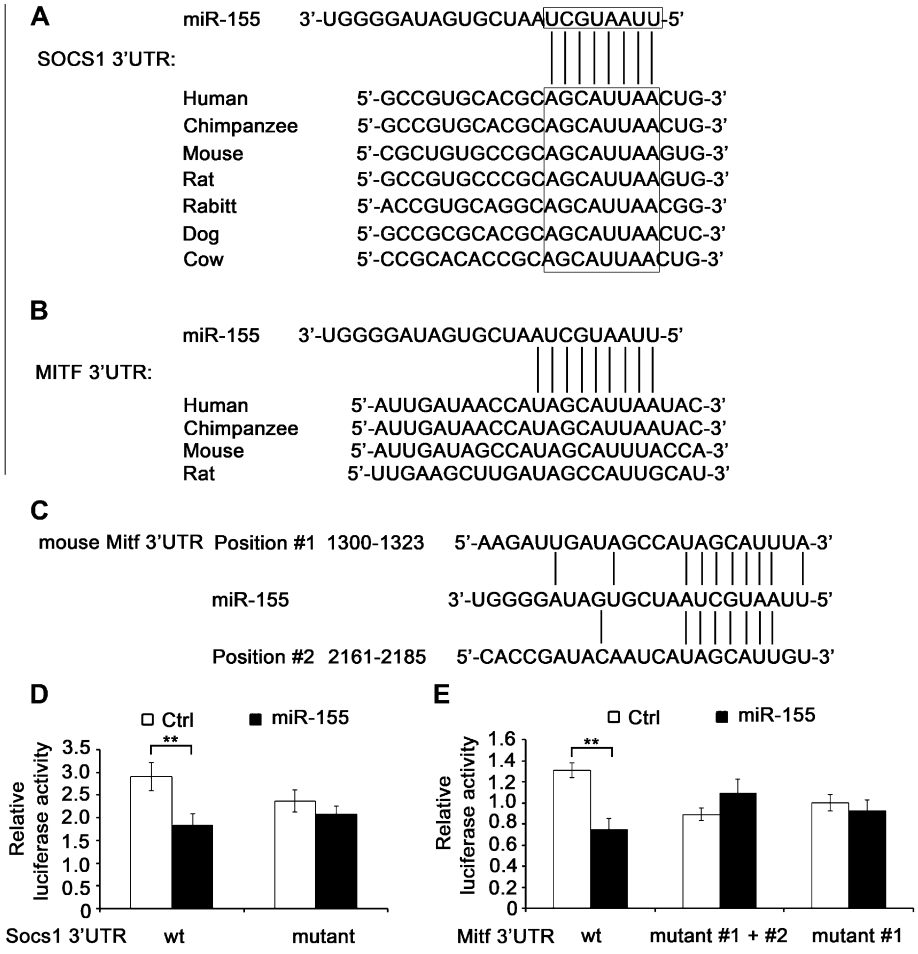

Fig. 3. Socs1 and Mitf were potential targets of miR-155. (A) The seed region of miR-155 was well complementary to SOCS1 3′UTR. The binding sites in SOCS1 3′UTR were very conserved among mammals. (B) The seed region of miR-155 was complementary to MITF 3′UTR. The binding sites in MITF 3′UTR were conserved among mammals. (C) Two potential sites in mouse Mitf 3′UTR could be targeted by miR-155. (D) HEK293T cells were cotransfected with 80 ng wild type (wt) or mutant Socs1 3′UTR *firefly* luciferase reporter plasmids, 10 ng pTK-*Renilla* luciferase plasmids, together with control (Ctrl) mimics or miR-155 mimics, (final concentration: 20nM) as indicated. After 24 h, *firefly* luciferase activity was measured and normalized to *Renilla* luciferase activity. (E) Wild type (wt) or the double mutant (mutant #1 + #2) or the position #1 mutant (mutant #1) Mitf 3′UTR was cotransfected with pTK-*Renilla* and miRNA mimics, and then the relative luciferase activity was measured as described in (D). Data are mean ± SD ($n = 6$) of one representative experiment. Similar results were observed in three independent experiments. $^{**}P < 0.01$.

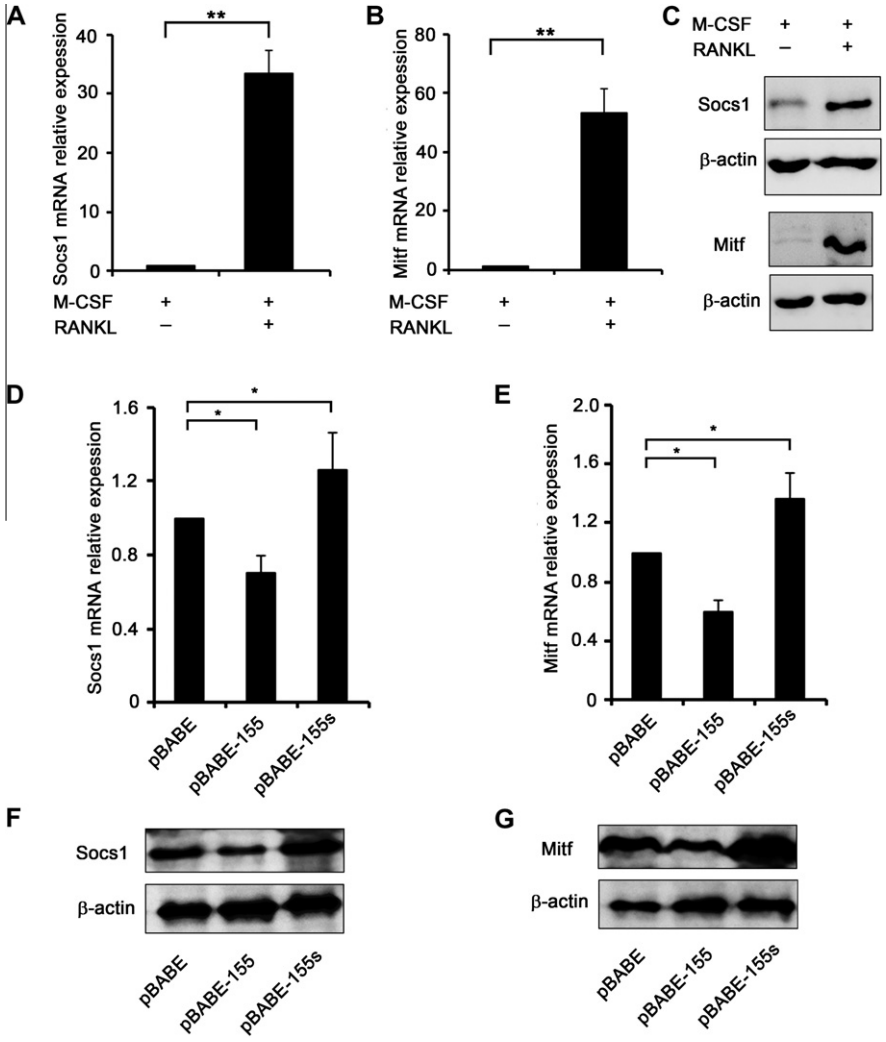

Fig. 4. miR-155 inhibited Socs1 and Mitf expression during osteoclast differentiation. (A–C) wild type BMMs were incubated in the presence of M-CSF (50 ng/ml) with or without RANKL (100 ng/ml) for 24 h, Socs1 and Mitf expression were detected by qPCR (A–B) and Western blot (C). (D–G) wild type BMMs were incubated for 8 h with the pBABE, pBABE-155 or pBABE-155s viral supernatants in the presence of M-CSF (50 ng/ml) and polybrene (6 μg/ml). After the viral supernatant was removed, BMMs were further cultured in osteoclastogenesis condition for 4 days. The Socs1 and Mitf mRNA expression were detected by qPCR (D–E) and protein expression were detected by Western blot (F–G). Data of (A), (B) and (D) are mean ± S.E.M of three independent experiments, $^{**}P < 0.01$. Data of (C), (F) and (G) are shown as one representative experiment. Similar results were observed in three independent experiments.

retroviral vector. To achieve inhibiting the function of endogenous miR-155, we constructed pBABE-155s by inserting 7 repeats of miR-155 sponge sequence into pBABE vector. A robust upregulation of miR-155 in pBABE-155-tranduced BMMs, while no significant downregulation of miR-155 in pBABE-155s-transduced BMMs (Fig. 2C). It was reported that miRNA sponge absorbed endogenous miRNA rather than degrading them [36], and Socs1 is one of the miR-155 targets [25–28]. We found that increasing the luciferase activity of Socs1 3′UTR reporter could be found after transfecting with increasing dose of pBABE-155s vectors into HEK293T cells (Fig. 2D). Therefore, pBABE-155s could inhibit the function of endogenous miR-155. We transduced BMMs with pBABE-155 or pBABE-155s retroviruses, and then cultured those BMMs in osteoclastogenesis condition. pBABE-155 suppressed osteoclasts differentiation while pBABE-155s facilitated it. After adding IFN-β into the osteoclastogenesis condition, the osteoclast differentiation was suppressed significantly, the suppression of pBABE-155 was abolished. However, pBABE-155s could slightly reverse the suppressive effect of IFN-β on osteoclast differentiation (Fig. 2E and F). We found similar results in IFNAR1−/− and IFNb−/− BMMs as wild type BMMs (Fig. 2G and H). That was to say, pBABE-155 could suppress osteoclasts differentiation while pBABE-155s facilitate it without the activation of IFNAR-Jak-Stat signaling. In conclusion, miR-155 could suppress osteoclast differentiation and mediate the suppressive effect of IFN-β on osteoclastogenesis.

3.3. Socs1 and Mitf were potential targets of miR-155

By using bioinformatic tools for miRNA target prediction (Targetscan [http://www.targetscan.org] and miRanada [http://www.microrna.org]), we found that there were miR-155 binding sites in the 3′UTRs of SOCS1 and MITF. The miR-155 binding site in SOCS1 3′UTR was highly conserved in mammals (Fig. 3A). Although the miR-155 binding site in MITF 3′UTR was not so highly conserved in mammals as SOCS1 3′UTR (Fig. 3B), MITF was reported as a target of miR-155 [24]. There were two potential miR-155 binding sites in mouse Mitf 3′UTR and the position #1 was more complementary to the seed region of miR-155 (Fig. 3C). miR-155 inhibited the luciferase activity of wild type Socs1 3′UTR reporter while has no effect on mutant Socs1 3′UTR reporter in which the miR-155 binding site was mutated (Fig. 3D). Similarly, miR-155 inhibited the luciferase activity of wild type

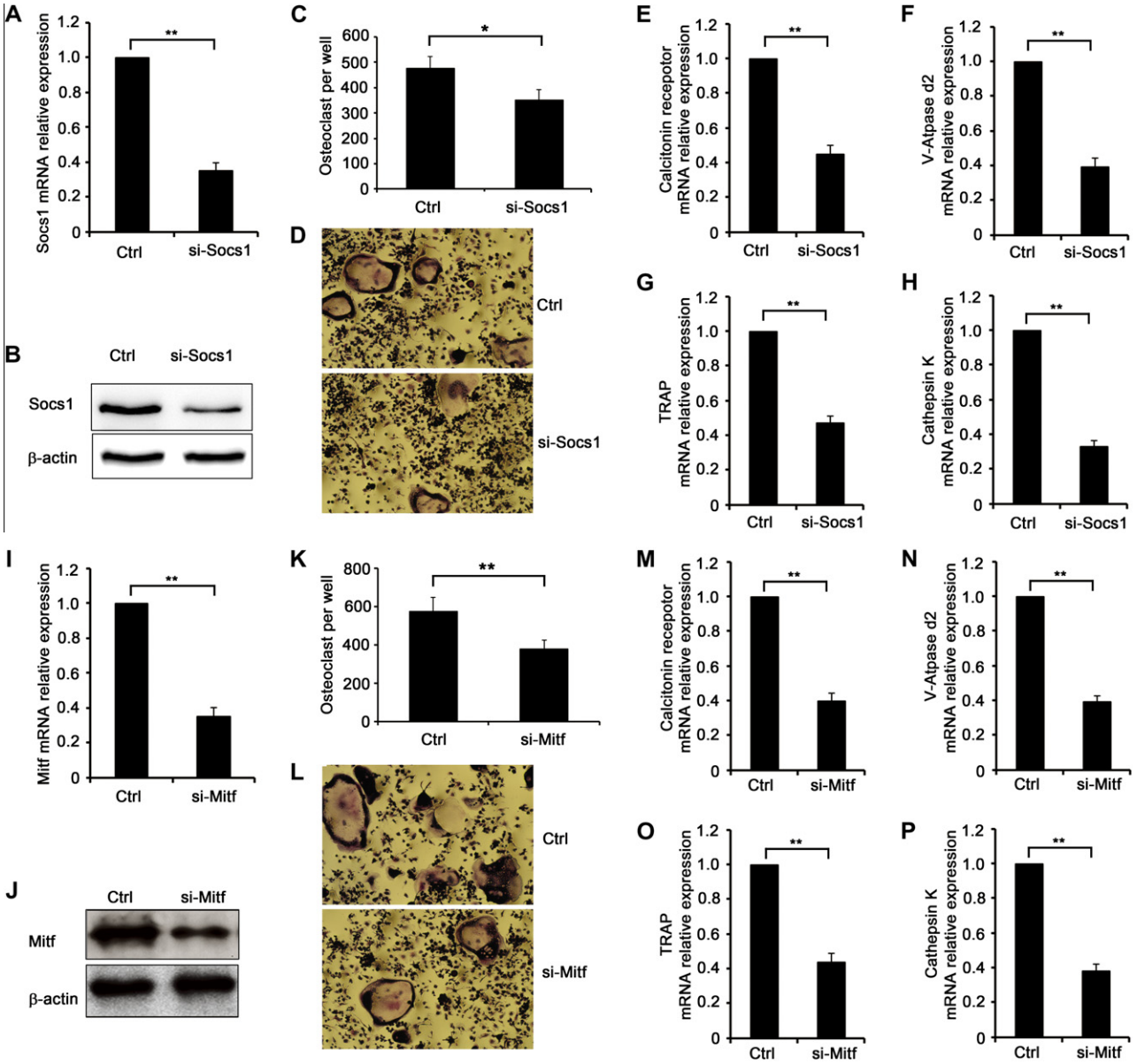

Fig. 5. Socs1 and Mitf facilitated osteoclast differentiation. (A–D) wild type BMMs were transfected with Socs1 siRNA (si-Socs1) or their control siRNA (Ctrl) for 8 h, then the cells were incubated in osteoclastogenesis condition for 4 days, the Socs1 expression were detected by qPCR (A) and Western blot (B). TRAP-positive multinucleated cells were counted as osteoclasts (C–D). (E–H) Calcitonin receptor (E), V-Atpase d2 (F), TRAP (G) and Cathepsin K (H) mRNA from cells described in (A–D) were detected by qPCR. (I–L) wild type BMMs were transfected with Mitf siRNA (si-Socs1) or their control siRNA (Ctrl) for 8 h, then the cells were incubated in osteoclastogenesis condition for 4 days, the Mitf expression were detected by qPCR (I) and Western blot (J). TRAP-positive multinucleated cells were counted as osteoclasts (K–L). (M–P) Calcitonin receptor (M), V-Atpase d2 (N), TRAP (O) and Cathepsin K (P) mRNA from cells described in I–L were detected by qPCR. The western blot data are shown as one representative experiment. The qPCR and TRAP-positive osteoclast data are mean ± S.E.M of three independent experiments, $^{**}P < 0.01$.

Mitf 3′UTR reporter while has no effect on mutant Mitf 3′UTR reporters in which both miR-155 binding sites or only position #1 binding site was mutated (Fig. 3E). According to the results of Mitf 3′UTR reporter, it seemed that position #1 binding site could be targeted by miR-155, although it was not perfectly complementary to the seed region of miR-155.

3.4. miR-155 inhibited Socs1 and Mitf expression during osteoclast differentiation

To address whether miR-155 regulates Socs1 and Mitf expression during osteoclast differentiation. First, we detected the Socs1 and Mitf expression from BMMs after one day treated with or without RANKL. Significant induction of both Socs1 and Mitf was found in RANKL-treated groups (Fig. 4A–C). Next, we infected BMMs with pBABE-155 or pBABE-155s retrovirus. After one day culturing in osteoclastogenesis condition, pBABE-155 suppressed Socs1 and Mitf expression while pBABE-155s facilitated their expression (Fig. 4D-G). Our data indicated that miR-155 could target Socs1 and Mitf during osteoclastogenesis, therefore, miR-155 might inhibit osteoclast differentiation by targeting Socs1 and Mitf, thus at least partially mediate the suppressive effect of IFN-β on osteoclastogenesis.

3.5. Socs1 and Mitf facilitated osteoclast differentiation

To further determine the roles of miR-155 in the regulation of osteoclast differentiation. We used siRNAs to interfere with the

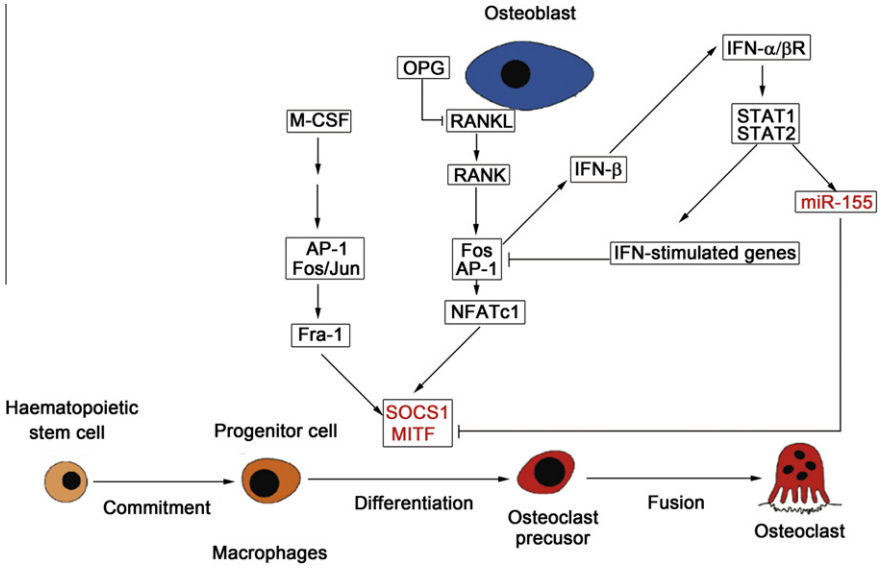

Fig. 6. miR-155 was involved in the signaling networks of osteoclast differentiation.

endogenous Socs1 and Mitf to mimic the function of miR-155. si-Socs1 could downregulate Socs1 expression during osteoclast differentiation, both in mRNA level and protein level (Fig. 5A and B). The osteoclast differentiation was also inhibited after silencing the endogenous Socs1 (Fig. 5C and D). Meanwhile, we found that several osteoclast marker genes such as Calcitonin receptor, V-Atpase d2, TRAP and Cathepsin K were downregulated after knocking down Socs1 (Fig. 5E-H). Similarly, we found that osteoclast differentiation was suppressed and osteoclast marker genes were downregulated after knocking down Mitf (Fig. 5I–P). The above data not only proved Socs1 and Mitf were positive regulators of osteoclast differentiation, but also implied miR-155 could regulate osteoclast differentiation by targeting Socs1 and Mitf. M-CSF and osteoblast provide signals that lead to expression of several essential transcription factors in osteoclastogenesis, such AP-1, Fos and NFATc1. These transcription factors drive the expression of their downstream genes and activate the differentiation of progenitor cells into osteoclast precursor. Meanwhile, Fos-dependent IFN-β negatively regulates the osteoclastogenesis via the IFN-stimulated genes such as PKR [18–19]. IFN-β also induced miR-155, which inhibited osteoclast differentiation by targeting SOCS1 and MITF, two positive regulators induced by RNAKL and M-CSF and play important roles in osteoclastogenesis (Fig. 6).

4. Discussion

IFNs exert their antiviral, antitumor or immune-regulatory functions by inducing downstream interferon stimulated genes via JAK-STAT pathways. However, the expression levels of some nod-coding RNAs such as miRNAs are also changed after IFNs treatment, and then those miRNAs may convey the effects of IFNs. For instances, IFN-β-treated hepatocytes rapidly modulate the expression of numerous cellular miRNAs which have sequence-predicted targets within the hepatitis C virus genomic RNA. The introduction of synthetic miRNA-mimics corresponding to those IFN-β-induced miRNAs reproduces the antiviral effects of IFN-β on HCV replication and infection [37]. Previous studies have shown that miR-155 was induced by IFN-β and IFN-β suppressed osteoclast differentiation [18,24], our present results have indicated that miR-155 mediated the suppressive effect of IFN-β on osteoclast differentiation. However, lots of other miRNAs are also responsive to IFN-β stimulation according to our unpublished microarray data, whether other IFN-β-responsive miRNAs involve in osteoclastogenesis still need to be investigated.

SOCS1 and MITF promote osteoclast differentiation by inhibiting IFN-β downstream signaling [29–30] and cooperating with NFATc1 [38–39], respectively. However, in order to maintain proper osteoclast numbers and avoid osteoclast-mediated diseases, negative regulation of osteoclast differentiation is also necessary. In the present study, we have demonstrated that miR-155, an IFN-β inducible miRNA, could target SOCS1 and MITF to convey the suppressive effect of IFN-β on osteoclastogenesis. Considering that SOCS1 and MITF are also responsive to IFN-β, therefore, IFN-β-inducible miRNAs modulate the expression of IFN-β-responsive genes, which indicates that miRNAs act as fine modulators to maintain bone homeostasis. Several interferon stimulated genes have been reported to control the osteoclast differentiation [19–21]. Our study has added miR-155, which is induced by IFN-β, as a new member involved in osteoclastogenesis.

An endotoxin-induced model of inflammatory bone destruction was applied to assess the in vivo efficiency of IFN-β for the suppression of osteoclast-mediated pathological conditions. It was indicated that daily administration of IFN-β into the inflamed site had markedly inhibited osteoclast formation and bone resorption, which suggested that IFN-β indeed had a beneficial effect against bone destruction, most likely by downregulating osteoclastogenesis [40]. However, the prospect of using IFN-β for treatment of bone disorders is still in its nascent stage of development. Given that the important roles of miR-155 in inflammatory response regulation, Ryan M. O'Connel and his colleagues are developing miR-155-based drugs to treat autoimmune inflammation [32]. Considering the suppressive effect of miR-155 on osteoclastogenesis and the advantages of miRNA-based drugs, using miR-155-based drugs is an alternative way for treatment of the osteoclast-mediated diseases.

Although both our results and the previous report have indicated miR-155 inhibits osteoclast differentiation in vitro [24], the systemic roles of miR-155 in bone remodeling are unknown. Given that miR-155 plays important roles in immune systems and the miR-155 knockout mice are available [41], the roles of miR-155 in osteoclastogeneis in vivo should be determined in further studies.

5. Disclosures

The authors have no financial conflicts of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.06.047.

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