Fluorosis increases the risk of postmenopausal osteoporosis by stimulating interferon γ

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A B S T R A C T
Estrogen deficiency in postmenopausal women frequently activates osteoclasts (OC), accelerates bone resorption, and leads to osteoporosis (OP). Previous studies have demonstrated that interferon γ (IFNγ) could increase bone resorption and may be involved in postmenopausal OP. Fluorosis also increased the risk of fractures and dental fluorosis, and fluoride may enhance osteoclast formation and induce osteoclastic bone destruction in postmenopausal women, but the underlying mechanisms are as yet unknown. Here, we show that serum fluoride and IFNγ levels are negatively correlated with bone mineral density (BMD) in postmenopausal women residing in a fluorotic area. Estrogen suppresses IFNγ, which is elevated by fluoride, playing a pivotal role in triggering bone loss in estrogen-deficient conditions. In vitro, IFNγ is inhibited by estrogen treatment and increased by fluoride in Raw264.7 cell, an osteoclast progenitor cell line. In ovariectomized (Ovx) mice, estrogen loss and IFNγ promote OC activation and subsequent bone loss in vivo. However, IFNγ deficiency prevents bone loss in Ovx mice even in fluoride conditions. Interestingly, fluoride fails to increase IFNγ expression in estrogen receptor α (ERα)-deficient conditions, but not in ERβ-deficient conditions. These findings demonstrate that fluorosis increases the bone loss in postmenopausal OP through an IFNγ-dependent mechanism. IFNγ signaling activates OC and aggravates estrogen deficiency inducing OP. Thus, stimulation of IFNγ production is a pivotal “upstream” mechanism by which fluoride promotes bone loss. Suppression of IFNγ levels may constitute a therapeutic approach for preventing bone loss.

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1. Introduction

Osteoporosis (OP) is a common bone metabolic disease characterized by bone loss and microarchitectural disorder of bone, which increases the risk of fractures [1]. Estrogen plays an important role in bone metabolism, including the promotion of bone formation and the prevention of bone loss [2]. Circulating estrogen deficiency is a predominant cause of OP in postmenopausal (PM) women [3]. Bone mass can be regulated by estrogen. In one way, estrogen inhibits the activation of osteoclastic bone resorption [4], preventing OP, and, in the other way, estrogen promotes the activation of osteoclastic bone formation [5]. Although multiple factors contribute to the homeostasis between bone formation and resorption, the mechanisms underlying OP resulting from estrogen deficiency remain largely unknown. Interferon γ (IFNγ) [6], which stimulates osteoclast (OC) formation and bone resorption [7], is one of the factors that promotes OC formation and activation and leads to bone loss in PM women and ovariectomized (Ovx) rodents. However, the mechanism by which estrogen regulates the production of IFNγ remains undetermined.

Fluorine combines with other elements and molecules to form fluoride, leading to its wide distribution across the globe. The necessity of fluorine for human health is still an ongoing debate. In some studies, a small amount of fluoride is reported to protect teeth and strengthen bones [8]. In contrast, chronic fluoride intake increases the risk of fractures and dental fluorosis [9]. High doses of fluoride also lead to many adverse effects in children, including thyroid hypofunction, mental retardation, and even decreased birth rates [10–12]. The pathogenesis function of skeletal fluorosis is not fully understood. However, previous studies demonstrated the role of fluoride in bone metabolism. When rats were permanently stimulated with high doses of fluoride, the ratio of OPGL/OPG was significantly increased, which represented enhanced osteolysis and...
greater bone resorption activity [13,14]. Sreekishami et al. [15] found that F-derived reactive oxygen species (ROS) increased oxidative stress and contributed to bone resorption in postmenopausal women. However, it remains unknown whether cytokines, such as IFNγ, are involved in the influence of fluorine on OP in postmenopausal women. In this study, we investigated the effects of fluorine on ovx-induced bone loss. The data showed that fluorine increased the risk of postmenopausal OP by stimulating IFNγ. Both estrogen deficiency and fluorine failed to induce bone loss in mice with a specific blockade of IFNγ signaling. Furthermore, fluorine increased the expression of IFNγ and resulted in bone loss which was mediated by ERα, but not ERβ. These results suggest that suppression of IFNγ levels may constitute a new therapeutic approach for preventing bone loss.

2. Methods

2.1. Study population and design

Postmenopausal female volunteers (n = 35) with an average age of 60.5 years, with less than 10 years since menopause, were selected from the Xincheng district (F < 0.4 PPM), Hohhot, Inner Mongolia, China, as the normal group. Postmenopausal women (n = 35) with an average age of 58.3 years, with less than 10 years since menopause were selected as the study population from the endemic fluorotic village (F > 4 ppm), Wuliying village, Xincheng district, Hohhot, Inner Mongolia, China. All the procedures were approved by the Medical Ethics Review Group of Inner Mongolia Hospital.

Serum and urine F levels were measured with an F ion selective electrode orion-940 (Thermo, Waltham, MA, USA). Serum ALP, cathepsin K, and TRAP-5b activity were measured by the enzyme-linked immunosorbent assay (ELISA) method with reagent kits (Nanjing Jiancheng Biological Institute, Nanjing, China). Serum RANKL, CT, and OPG activity were measured by ELISA reagent kits (R&D systems, Shanghai, China). BMD of long femur was measured by dual-energy X-ray absorptiometry (DXA) fan-beam bone densitometer (EXA-3000, Osteosys, Seoul, Korea).

2.2. Animals and surgery

All experimental and surgical procedures were approved by the Animal Care Committee of Inner Mongolia Hospital and were conducted in accordance with the Chinese guidelines for the care and use of laboratory animals.

Balb/c athymic nude mice and wild type (WT) Balb/c mice were bred at the Center for Animal Experiments Laboratory of Inner Mongolia Hospital. C57BL/6 mice IFNγ−/− and WT mice were obtained from the Jackson Laboratory. Mice were either sham-operated or Ovx at the age of 8 weeks and euthanized at the age of 16 weeks [16,17]. Unoperated mice were also sacrificed at 16 weeks old to serve as controls. To investigate the role of fluoride on Ovx-induced bone loss, sham- and Ovx-operated mice were given deionized water with or without F ion (100 mg F ion/L from NaF) after surgery, which continued for 8 weeks.

2.3. Cell culture and treatment

Raw264.7 cells were cultured and treated by 10−6 M 17β-estradiol (E2) or 10−5 M NaF (Sigma-Aldrich Co., Louis, MO, USA) [18,19].

2.4. RNA extraction and QRT-PCR

Total RNA from BMMs and Raw264.7 cells were extracted according to the manufacturer’s instructions. 0.5 μg of total RNA was reverse-transcribed into cDNA using ReverTra Ace kits (Takara Biotechnology, Dalian, Liaoning, China). All real-time polymerase chain reaction (RT-PCR) reactions with SYBR® Green (TOYOBO, Osaka, Japan) were performed and analyzed with the ABI Prism 7500 instrument (Applied Biosystems, Foster City, CA). The following primers were used in this study: IFN-γ forward sequence (5′-3′): TGTTACTGCCAGGACCCATA, reverse sequence (5′-3′): CTTCCTGTAGGTCTTCCACA; β-actin forward sequence (5′-3′): TGGAATCCTGTGGCATCCATGAAAC, reverse sequence (5′-3′): TGGAATCCTGTGGCATCCATGAAAC, reverse sequence (5′-3′): CTTCCTGTAGGTCTTCCACA; β-actin forward sequence (5′-3′): TGGAATCCTGTGGCATCCATGAAAC, reverse sequence (5′-3′): TAAAACGCAGACTACGTAACAGTCCG.

2.5. Western blot detection

For the expression of IFN-γ detection, total protein from BMMs and Raw264.7 cells were extracted by radioimmunoprecipitation assay (RIPA) buffer. To testify the level of ERs protein, Raw264.7 were transfected using shRNA targeting ERα, ERβ, or control constructs (Santa Cruz Biotechnology, Santa Cruz, CA, USA) according to the manufacturer’s instructions. Anti-IFN-γ mAb (clone RMMG-1, 1:500) (Abcam, Cat NO. ab24979), anti-ER (clone AER314, 1:1000) (Cat NO. MAB447), and anti-ERβ (clone 9.88, 1:1000) (Cat NO. GR39) purchased from Millipore (Millipore, Billerica, MA, USA) were used as primary antibodies, respectively. Anti-β-actin (1:1000) purchased from Santa Cruz was used as an internal reference. Thirty micrograms of protein was used to perform western blot analysis.
2.6. CD4+ T cell preparation and adoptive transfer

As described [20], spleen cells were prepared from WT or IFN-γ−/− C57BL/6 mice. In order to detect cytokine production in CD4+ T cells, CD4+ T cells were isolated from spleens and sorted using a magnetic bead cell purification kit (Mouse CD4 T Lymphocyte Enrichment Set—DM, BD, Franklin Lakes, NJ, USA), and 2 × 10⁶ cells were injected intravenously into the lateral tail vein of Balb/c nude mice under sham−, Ovx− or Ovx + NaF-operation using established methods [21,22]. Four weeks after T cell transfer, serum CTx and BMD were detected [20].

2.7. Analysis of skeletal morphology

Sham or Ovx mice subjected to drinking deionized water with or without F ions were necropsied. Then, hind limbs were removed and fixed with 70% ethanol. BMD and bone histomorphometric analysis were measured by dual-energy X-ray absorptiometry as described [17,18].

2.8. Statistical analysis

Data are expressed as the means ± SD. Statistical analyses were performed using Student's t-test or one-way analysis of variance (ANOVA).

### Table 1

Biochemical markers of postmenopausal women residing in a nonfluorotic and a fluorotic area (Values are mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonfluorotic (n = 35)</th>
<th>Fluorotic (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum fluoride (ppm)</td>
<td>0.05 ± 0.03</td>
<td>0.16 ± 0.11*</td>
</tr>
<tr>
<td>Urinary fluoride (ppm)</td>
<td>0.27 ± 0.28</td>
<td>0.51 ± 0.42*</td>
</tr>
<tr>
<td>Serum ALP (U/L)</td>
<td>8.46 ± 1.26</td>
<td>6.84 ± 1.18*</td>
</tr>
<tr>
<td>Serum TRAP-5b (U/L)</td>
<td>3.13 ± 0.12</td>
<td>4.33 ± 0.21*</td>
</tr>
<tr>
<td>Serum RANKL (ng/L)</td>
<td>413.25 ± 33.16</td>
<td>514.25 ± 115.26*</td>
</tr>
<tr>
<td>Serum OPG (pmol/L)</td>
<td>7.12 ± 1.08</td>
<td>4.35 ± 1.15*</td>
</tr>
<tr>
<td>Serum CT (ng/L)</td>
<td>183.21 ± 42.22</td>
<td>125.52 ± 36.83*</td>
</tr>
<tr>
<td>Serum cathepsin K (ng/L)</td>
<td>15.64 ± 2.29</td>
<td>20.02 ± 2.46*</td>
</tr>
<tr>
<td>Bone density (g/cm²)</td>
<td>0.35 ± 0.06</td>
<td>0.30 ± 0.07*</td>
</tr>
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</table>

*Compared with the Nonfluorotic, p < 0.05.

3. Results

3.1. Biochemical markers of postmenopausal women residing in nonfluorotic and fluorotic areas

The biochemical parameters in the two groups of postmenopausal women were shown in Table 1. In the fluorotic group, the fluoride levels in serum and urine were simultaneously increased (0.16 ± 0.11 and 0.51 ± 0.42 ppm, respectively), and were significantly higher than those in the nonfluorotic group (0.05 ± 0.03 and 0.27 ± 0.28 ppm, respectively). However, the level of BMD in the fluorotic group was lower than that of the nonfluorotic group (0.30 ± 0.07 vs. 0.35 ± 0.06 g/cm², P < 0.05). Serum levels of CT and ALP, the markers of bone formation, were both decreased in the fluorotic group compared with the nonfluorotic group (P < 0.5). In contrast, the markers of bone resorption, serum TRAP-5b and cathepsin K, were both increased in the fluorotic group compared with the nonfluorotic group (P < 0.5). RANKL is a powerful bone resorption inducer through interaction with RANK, and OPG is a strong inhibitor of osteoclastic differentiation. In our study, serum RANKL levels in the fluorotic group were increased (514.25 ± 115.26 ng/L), but OPG was decreased (4.35 ± 1.15 pg/ml) compared with the nonfluorotic group (413.25 ± 33.16 ng/L and 7.12 ± 1.08 pg/ml, respectively). All these results suggested that fluorotic conditions promoted bone resorption and increased the risk of osteoporosis in postmenopausal women.

3.2. IFNγ mediates fluoride and/or estrogen-dependent bone loss

To investigate whether IFNγ is involved in the fluoride-inducing OP, we analyzed serum fluoride, IFNγ, and BMD levels. The serum fluoride and IFNγ levels were both increased in the postmenopausal women residing in fluorotic areas, compared with those residing in nonfluorotic areas (Fig. 1AB; P < 0.05). However, the BMD was decreased in the fluorotic group (Fig. 1C; P < 0.05). Furthermore, we calculated the relationship among serum fluoride, IFNγ, and BMD in these two groups. The results demonstrated that the serum fluoride level was negatively correlated with BMD in the fluorotic group (Fig. 1D; P < 0.05). Additionally, serum IFNγ was
positively correlated with serum fluoride but negatively correlated with BMD (Fig. 1E,F; P < 0.05).

We also measured the levels of IFNγ mRNA and protein in BMMs harvested from sham-operated and Ovx WT mice with or without F ion-containing water for 8 weeks after surgery (Fig. 2A,B). Compared with sham-operated mice, IFNγ mRNA and protein in Ovx BMMs were both increased (1.93-fold and 1.98-fold, respectively). Ovx + F significantly enhanced the mRNA and protein levels of IFNγ compared with Ovx (1.15-fold and 1.28-fold, respectively). After Ovx, the BMD, as measured by dual-energy X-ray absorptiometry, was decreased compared with sham mice (Fig. 2C,D), and Ovx + F exhibited more powerful inhibition effects on BMD than Ovx (Fig. 2E, F).

In addition, we tested the differences of bone morphometric parameters such as BV/TV, Ob.S/BS, Oc.S/BS, and Tb.N, Tb.Sp, Oc.N/B.Pm, Oc.S/BS, and ES/BS among different groups (Table 2). Compared with sham-operated mice, in Ovx and sham + F mice groups, it was demonstrated that BV/TV, Tb.Th, and Tb.N were reduced, and Tb.Sp was also reduced. Osteoclastogenesis indicators, such as ES/BS, Oc.N/B.Pm, and Oc.S/BS, were increased. Ob.S/BS, the indicator of osteoblastic activity, was decreased. Furthermore, the Ovx + F group exhibited a more powerful effect on these parameters than that in Ovx mice. The results confirmed that fluoride mediated bone loss, and IFNγ may be involved in fluoride-induced OP in PM women.

### 3.3. Silencing of IFNγ signaling prevents estrogen- and/or fluoride-dependent bone loss

To investigate the role of IFNγ in estrogen- and/or fluoride-dependent bone loss, we used IFNγ-knock-out (KO) mice with either sham- or Ovx-operation, and made them drink deionized water with or without F ions after surgery to analyze BMD, serum CT, and bone morphometric parameters. The results demonstrated that BMD were not influenced in either sham-IFNγ+/− or Ovx-IFNγ+/− operated mice (Fig. 3A, B). That drank deionized water with or without F ion after Ovx could not influence the BMD level (Fig. 3C–E).

In addition, serum C-terminal telopeptide (CTX), a marker of bone resorption was elevated in WT but not in IFNγ-KO mice following Ovx (Fig. 3F). Bone morphometric parameters such as BV/TV, Ob.S/BS, Oc.S/BS, Tb.N, Tb.Sp, Oc.N/B.Pm, Oc.S/BS, and ES/BS were not different in Ovx-IFNγ+/− or Ovx-IFNγ+/− + F operated mice compared with sham-IFNγ+/− operated mice (data not shown).

To eliminate the interference of autoimmunity of C57BL/6 mice and investigate the contribution of IFNγ signaling in T cells to the bone loss induced by E deficiency and/or fluoride, Balb/c nude mice...
Table 2
μ-CT analysis of femur morphometric indices (mean ± SD) after sham operation or ovx.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + F</th>
<th>Ovx</th>
<th>Ovx + F</th>
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<tbody>
<tr>
<td>BV/TV (%)</td>
<td>15.2 ± 1.4</td>
<td>12.4 ± 0.9*</td>
<td>10.1 ± 1.3**</td>
<td>7.5 ± 0.8***#</td>
</tr>
<tr>
<td>ES/BS (%)</td>
<td>5.7 ± 0.7</td>
<td>8.3 ± 1.0*</td>
<td>10.2 ± 1.3**</td>
<td>13.8 ± 1.8**#</td>
</tr>
<tr>
<td>Tb. Th (μm)</td>
<td>49.1 ± 3.5</td>
<td>38.7 ± 4.2*</td>
<td>34.5 ± 2.1**</td>
<td>29.7 ± 2.0**#</td>
</tr>
<tr>
<td>Tb. Sp (μm)</td>
<td>528.5 ± 23.5</td>
<td>609.4 ± 35.1*</td>
<td>655.1 ± 40.5**</td>
<td>744.9 ± 38.2**#</td>
</tr>
<tr>
<td>Tb. N (/mm)</td>
<td>59 ± 6</td>
<td>45 ± 4*</td>
<td>38 ± 5**</td>
<td>27 ± 4**#</td>
</tr>
<tr>
<td>Ob.s/BS (%)</td>
<td>28.4 ± 3.1</td>
<td>38.9 ± 2.9*</td>
<td>43.5 ± 3.7**</td>
<td>52.6 ± 4.2**#</td>
</tr>
<tr>
<td>Oc.s/BS (%)</td>
<td>40.2 ± 2.2</td>
<td>49.1 ± 3.8*</td>
<td>58.5 ± 4.4**</td>
<td>69.3 ± 3.7**#</td>
</tr>
<tr>
<td>Oc. N/B. Pm (/100 mm)</td>
<td>322.4 ± 32.5</td>
<td>415.2 ± 34.7*</td>
<td>474.4 ± 40.5**</td>
<td>573.7 ± 39.6**#</td>
</tr>
</tbody>
</table>

Ovx vs. sham, or Ovx + F vs. Sham + F, *P < 0.05, **P < 0.01.
Ovx + F vs. Ovx, #P < 0.05.

Fig. 3. Silencing of IFNγ signaling prevents estrogen- and/or fluoride-dependent bone loss. A, B. BMD of femurs divided equally longitudinally from sham-IFNγ−/− or Ovx or Ovx-IFNγ−/− mice. C, D. BMD of femurs divided equally longitudinally from Ovx-IFNγ−/− or Ovx-IFNγ−/− + F mice. E, F. Average BMD (E) and serum CTx (F) from WT or IFNγ−/− mice suffered from sham-, Ovx- or Ovx + F-operated. G, H. Transfer of T cells insensitive to IFNγ into nude mice leads to bone loss (G) and increases bone resorption (H). All data are means ± SD (n = 6). *P < 0.05; NS, not significant.
were reconstituted using purified WT and IFNγ−/− spleen T cells. There were no differences in BMD and serum CTx of sham-, Ovx-, and Ovx + F-operated nude mice. However, BMD was significantly decreased in Ovx nude mice compared with sham nude mice following adoptively transferred T cells from WT mice. When T cells from WT mice were transferred into Ovx nude mice that drank water containing F ion, bone loss was more serious. Subsequently, when IFNγ−/− T cells were transferred into sham-, Ovx- or Ovx + F-operated nude mice, BMD and serum CTx were not influenced in these three groups. Together, these results implied that T cells rendered insensitive to IFNγ are also insensitive to the bone loss effect of estrogen and/or fluoride. Thus, IFNγ signaling in T cells plays a pivotal role in the mechanism of estrogen- and/or fluoride-dependent bone loss.

3.4. Fluoride-dependent osteoprotection requires ERα in vitro

To confirm whether fluoride-enhanced bone loss was mediated by ERs in vitro, Raw264.7 cells were treated with estrogen or NaF. We found that estrogen could inhibit IFNγ in both mRNA and protein levels (Fig. 4A, B); however, IFNγ expression was increased by NaF significantly. Subsequently, we obtained two independent shRNAs that effectively targeted ERα (Fig. 4C), and two shRNAs targeted ERβ (Fig. 4D). ERα knockdown in osteoclasts prevented E2-dependent IFNγ suppression, but not ERβ, as detected by immunoblotting of extracts from ERα knockdown cell lines (Fig. 4E, F). In addition, we used NaF-treated Raw264.7 cells and tested IFNγ protein levels. Interestingly, we found that NaF-inducing IFNγ was mediated by ERα, but not ERβ (Fig. 4G, H). These results suggested that estrogen-inhibited and fluoride-induced IFNγ expression were both mediated by ERα, but not ERβ.

4. Discussion

Until now, there is controversy about whether fluorine is essential for human health. Small amounts of fluoride were considered as protective of teeth and bone strength. By contrast, long-term or excessive fluoride intake was reported to be harmful

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Fig. 4. Fluoride-dependent osteoprotection requires ERα in vitro. A, B. Raw264.7 cells treated with E2 or NaF, IFNγ protein levels determined by immunoblot. C, D. Raw264.7 cells transfected with shRNA targeting ERα (shERα) or ERβ (shERβ) or nontarget control shRNA (shControl) and IFNγ protein levels determined by immunoblot. E, F. Raw264.7 cells transfected with shRNA targeting ERα (shERα) or ERβ (shERβ) treated with (+) or without (−) E2. IFNγ expression determined by immunoblot. G, H. Raw264.7 cells transfected with shRNA targeting ERα (shERα) or ERβ (shERβ) treated with (+) or without (−)NaF. IFNγ expression determined by immunoblot. All data are means ± SD (n = 6). *P < 0.05; **P < 0.01.
for human health, leading to such issues as dental fluorosis, imbalance of bone metabolism (bone formation/bone resorption), and finally bone damage [23–27]. Previous studies have shown that the bone metabolism characteristics of rats with chronic fluoride intake were bone resorption, abnormal expression of OPG mRNA and OPG-L mRNA [28], and high F levels inducing osteoclastic bone activation in rats [13]. Goudou et al. [29] demonstrated that F-derived ROS contributed to bone resorption in postmenopausal women. However, the likely mechanisms of fluoride on bone metabolism are still not known.

In this study, we demonstrated that fluoride upregulated the production of IFNγ, which played a pivotal role in the mechanism of oxv-inducing bone loss. Fluoride increased bone resorption and decreased bone mass via IFNγ in osteoclasts. IFNγ could reportedly induce TNF-producing T cells [30,31] following Ovx via a complex mechanism [6]. Here, we found significantly increased serum IFNγ in the postmenopausal women residing in fluorotic areas compared with nonfluorotic areas (Fig. 1). Additionally, serum CT and ALP, the markers of bone formation, were both decreased in the fluorotic group compared with the nonfluorotic group. In contrast, the markers of bone resorption, serum RANKL, TRAP-5b and cathepsin K, were increased in the fluorotic group compared with the nonfluorotic group (Table 1). All these results suggested that fluorotic conditions promoted bone resorption, inhibited bone formation, and increased the risk of OP in postmenopausal women.

To further investigate whether IFNγ was involved in fluoride-mediated bone loss, we measured the levels of IFNγ mRNA and protein in BMMs harvested from sham-operated and Oxv WT mice with or without F ion-containing water for 8 weeks after surgery. We found that in the group of Oxv-operated mice drinking F ion-containing water, IFNγ mRNA and protein in BMMs harvested were both increased compared with the Ovx-operated mice that did not drink F ion-containing water. Also, Oxv + F exhibited more powerful inhibition effects on BMD than Oxv (Fig. 2). Meanwhile, bone morphometric parameters such as BV/TV, Ob.S/BS, Oc.S/BS, and Tb.N, Tb.Sp, Oc.N/B.Pm, Oc.S/BS, and ES/BS between different groups (Table 2) were detected. Results confirmed that fluoride-mediated bone loss and IFNγ may be involved in fluoride-induced OP in PM women. These results were supported by previous studies [7,16].

In the last few decades, estrogen has been considered to play an important role in homeostasis bone formation and resorption. In postmenopausal women, estrogen deficiency is the main cause of OP, which is characterized as one of the predominant trends of bone resorption. Estrogen regulated IFNγ expression and bone mass via ERα, but not ERβ [18]. To confirm whether fluoride-enhanced bone loss was mediated by ERα in vitro, ERα or ERβ was knocked down by specific siRNA in Raw264.7 cells, respectively, and cells were treated with NaF. Interestingly, we found that NaF-induced IFNγ was mediated by ERα, but not ERβ (Fig. 4). These results suggested that estrogen-inhibited and fluoride-induced IFNγ expression were both mediated by ERα, but not ERβ.

Many cytokines are involved in OP, including TGF-β and TNF-α. According to Nanes and Pacifi’s reports, TNF-α could upregulate osteoclast formation but TGF-β could inhibit OP [20]. IFN-γ reported in this article is only a factor in OP. In the immune system, T cells can secrete a large number of cytokines. IFN-γ, −g, −−T cells still produce other cytokines, except IFN-γ, which are likely participating in OP pathological processes. Thus, when IFN-γ, −g−−T cells were transferred into the sham nude mice, the histogram showed that the BMD in this group was slightly lower than that in the sham nude and sham nude + WT T cells groups.

To our knowledge, this research is the first to report on fluoride increasing the risk of postmenopausal OP by stimulating IFNγ. When mice showed specific blockades of IFNγ signaling, both estrogen deficiency (oxv-operation) and fluoride failed to induce bone loss. Furthermore, this study found that fluoride increased the expression of IFNγ and resulted in bone loss, which was mediated by ERα, but not ERβ. All these results suggest that suppression of IFNγ levels may constitute a new therapeutic approach for preventing bone loss.

Conflict of interest

No conflict of interest has been declared by the authors.

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