

# Interleukin-32 Gamma as a New Face in Inflammatory Bone Diseases

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Interleukin-32 (IL-32), a recently identified pro-inflammatory cytokine, is involved in the pathogenesis and progression of infections, cancer, chronic inflammation, and autoimmune disease. IL-32  $\gamma$  is the most active isoform in cell death and cell activation among nine distinct isoforms of IL-32. IL-32  $\gamma$  potentiates both osteogenic and osteoclastogenic capacities, and is critical in the coupling of bone resorption and bone formation for maintenance of bone homeostasis. IL-32  $\gamma$  is strongly associated with inflammatory bone disorders such as rheumatoid arthritis, ankylosing spondylitis, and osteoporosis. In this review, we summarize current research on the role of IL-32  $\gamma$  in inflammatory bone disorders, highlighting this cytokine as a novel target for prognostic marker and control of these diseases. (*J Rheum Dis* 2017;24:14-20)

**Key Words.** IL-32  $\gamma$ , Osteogenesis, Rheumatoid arthritis, Ankylosing spondylitis, Osteoporosis

## INTRODUCTION

Bone is a target organ in diverse inflammatory diseases as inflammatory bone diseases can affect the pathologic bone changes by accelerating bone erosion in rheumatoid arthritis (RA) or abnormal bone formation in ankylosing spondylitis (AS). It has become clear that inflammatory mediators such as proinflammatory cytokines carry central roles in the pathogenesis of these diseases by altering bone metabolic status [1,2]. The defining features of a functional regulation by inflammatory cytokines in these diseases currently attract a great deal of interest to encourage their application for potential biomarker and therapeutic target. In this regard, a variety of inflammatory cytokines has been studied for their key roles in inflammatory bone diseases [1]. Recently, interleukin-32 (IL-32) has been suggested to be involved in the pathogenesis and progression of a number of inflammatory bone disorders [3-5].

IL-32, a recently identified proinflammatory cytokine

has been renamed from natural killer cells protein 4 (NK4) on the basis of its selective expression in IL-2 activated NK cells [6]. IL-32 is now defined as a multi-faceted cytokine involved in infections, cancer, chronic inflammation, and autoimmune disease [7-11]. Recent evidences have begun to shed light on the physiological role of IL-32  $\gamma$  in pathogenic changes of bone, thereby providing a means to grasp the underlying pathogenesis of human inflammatory bone diseases. The aim of this review is to emphasize the role of IL-32  $\gamma$  in bone-related diseases and to discuss the recent findings in the regulation of inflammatory bone disorders by IL-32  $\gamma$ .

## MAIN SUBJECTS

### General properties of IL-32

IL-32 is produced by immune and non-immune cells including NK cells, CD4<sup>+</sup> T cells, monocytes, epithelial cells, endothelial cells, and fibroblasts [12,13]. There are 9 different splice variants in humans: IL-32  $\alpha$ , IL-32  $\beta$ ,

**Received :** February 13, 2017, **Revised :** February 14, 2017, **Accepted :** February 14, 2017

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pISSN: 2093-940X, eISSN: 2233-4718

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IL-32  $\gamma$ , IL-32  $\delta$ , IL-32  $\epsilon$ , IL-32  $\xi$ , IL-32  $\eta$ , IL-32  $\theta$ , and IL-32  $\zeta$  [6], and different isoforms of IL-32 have diverse biological functions. IL-32 is now recognized as a proinflammatory cytokine produced in epithelial cells, NK cells, CD4<sup>+</sup> T cells, and synovial fibroblasts in response to various cytokines such as IL-1  $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ ), IL-6, IL-8, IL-12, and IL-18 [12-14]. Several microbial products such as lipopolysaccharide (LPS) and triacylated lipopeptide are also potent inducers of IL-32 [14,15]. Similarly, viral infection augments IL-32 production via toll-like receptor 3 (TLR3) [16].

It is difficult to explore the exact biological activities of each isoform because the surface receptor of IL-32 has yet to be identified; the IL-32 gene has not been identified in rodents either [10]. Thus, the physiological function of human IL-32  $\gamma$  had been explored using recombinant human IL-32  $\gamma$  (rhIL-32  $\gamma$ )-treated cells [12] and in murine models of various diseases by transgenically incorporating the human IL-32  $\gamma$  gene in mice (IL-32  $\gamma$  TG) [17,18]. In particular, IL-32  $\gamma$  is the representative human protein among the IL-32 isoforms, and has the highest biological activity for the stimulation of peripheral blood mononuclear cell or macrophage cytokine production [19,20]. Administration of rhIL-32  $\gamma$  leads to induction of TNF- $\alpha$ , IL-1  $\beta$ , IL-6, and IL-8 in THP-1 cells [21] as well as chemokine (C-X-C motif) ligand 1 (CXCL1) and chemokine (C-C motif) ligand 2 (CCL2) in macrophages [22]. Injection of rhIL-32  $\gamma$  into the knee joint of mice causes arthritis through elevated TNF- $\alpha$  expression [17]. Moreover, rhIL-32  $\gamma$  promotes apoptosis and IL-32  $\gamma$ -induced cytotoxicity is caspase-3 dependent [23,24]. IL-32  $\gamma$  is also involved in  $\alpha v \beta 3$  integrin-mediated angiogenesis, demonstrating the extracellular function of IL-32  $\gamma$ , although IL-32  $\gamma$  predominantly exerts intracellular functions as a

proinflammatory mediator [25].

### The role of IL-32 in pathogenicity

IL-32 induces various cytokines such as IL-1  $\beta$ , TNF- $\alpha$ , IL-6, and IL-8 [7,25] by activating p38 mitogen-activated protein kinases (p38MAPK) and NF- $\kappa$ B signaling pathways in macrophages and T cells [25]. It has been studied in various clinical fields ranging from infectious diseases, chronic inflammation, autoimmune diseases, and cancers [7-11] (Table 1).

#### 1) IL-32 in infectious diseases

IL-32 mainly acts on T cells and NK cells and exhibits antiviral properties by activating inducible nitric oxide synthase, IFN- $\gamma$ , and IL-6 [8,13]. IL-32 regulates inflammation and exerts antiviral properties in human cornea epithelial cells infected with Epstein-Barr virus (EBV), and also promotes macrophage survival directly from human immunodeficiency virus-1 (HIV-1). IL-32 also shows potent antiviral effects against vesicular stomatitis virus (VSV), influenza A virus (IAV), and herpes simplex virus 2 (HSV-2) [26-30]. Silencing of endogenous IL-32 leads to increase in HIV-1 production [27].

*Mycobacterium tuberculosis* and *Mycobacterium bovis* induce production of IL-32, which synergizes with toll-like receptor-2 and nucleotide-binding oligomerization domain-2 to produce cytokines and chemokines [31,32]. Transgenic (TG) mouse overexpressing human IL-32  $\gamma$  gene followed by infection with hypervirulent strain of *M. tuberculosis* exhibited the capability to promote host immunity against *M. tuberculosis*. The experimental evidence showing resistance of IL-32  $\gamma$  TG mice for LPS-mediated septic shock is through reduction of systemic cytokines release [33]. Taken together, IL-32 plays a beneficial host defense role against bacterial infection.

**Table 1.** Diseases with known interleukin (IL)-32 involvement in pathogenicity

Diseases	IL-32 expression	Study
Viral infection	Anti-viral responses against vesicular stomatitis virus (VSV), influenza A virus, herpes simplex virus 2 (HSV-2), Epstein-Barr virus (EBV), human immunodeficiency virus-1 (HIV-1)	[26-30]
Bacterial infection	Protection against <i>Mycobacterium tuberculosis</i> , <i>Mycobacterium avium</i> , <i>Mycobacterium leprae</i>	[31-33]
Cancer	Increased IL-32 level in gastric cancer, lung cancer, hepatocellular carcinoma, pancreatic cancer, clear cell renal cell carcinoma (CCRCC)	[34-38]
Chronic inflammation and autoimmune disease	Elevated IL-32 level in chronic obstructive pulmonary disease (COPD), chronic rhinosinusitis (CRS), inflammatory bowel disease	[9,13,39,40]
Inflammatory bone disease	Rheumatoid arthritis (RA), ankylosing spondylitis (AS), osteoporosis	[3-5]

## 2) IL-32 and tumor-related processes

IL-32 is considered a novel tumor marker as it plays a role in tumor-related processes [34,35]. IL-32 expression is highly elevated in human cancers including gastric cancer and lung cancer, in which it contributes to cancer progression by promoting growth, survival, invasiveness, and metastatic potentials of tumors [34,35]. IL-32 expression is also noted in hepatocellular carcinoma and pancreatic cancer [36,37]. Lee et al. [38] demonstrated the significance of IL-32 as a novel prognostic factor in patients with localized clear cell renal cell carcinoma (CCRCC). The patients with elevated IL-32 expression are more likely to have high recurrence rates and low survival rates compared to individuals with low IL-32 expression. Altogether, these evidence suggest that IL-32 is a crucial mediator in cancer.

## 3) Chronic inflammation and autoimmune disease

IL-32 also has diverse roles in mediating chronic inflammation and autoimmunity [10]. The expression level of IL-32 is elevated in RA patients and mice model with experimental inflammatory arthritis [5,11]. IL-32 is linked to disease severity in chronic obstructive pulmonary disease (COPD) [13]. It has been shown that IL-32 induces the production of proinflammatory cytokine, indicating the participation of IL-32 in the pathogenesis of chronic rhinosinusitis (CRS), the inflammatory disease of the nose and paranasal sinuses that affects approximately 15% of the total global population [9,39]. Elevated level of IL-32 was also observed in other autoimmune disease, including inflammatory bowel disease [40] and chronic obstructive pulmonary disease [13]. The expressions of IL-32 and TNF- $\alpha$  show high correlation in lung biopsies from patients with obstructive pulmonary disease [13].

## IL-32 $\gamma$ in bone resorption

The prominent role of IL-32 in inflammatory bone diseases as evidenced by in vitro and in vivo studies is as a potent osteoclastogenic factor. IL-32 can directly induce the differentiation of adherent peripheral blood monocytes into osteoclasts (OCs) even in the absence of soluble receptor activator of nuclear factor-kappa B ligand (RANKL); however, IL-32 alone is not sufficient for the formation of fully-active bone-resorbing OCs. The lack of bone resorption in IL-32-treated cells in the absence of RANKL may partly be due to the lack of F-actin ring formation and the accelerated release of IL-4 and IFN- $\gamma$ , which are

known inhibitors of OC maturation and activation by IL-32. Nevertheless, IL-32 can stimulate the release of proinflammatory mediators from several cell types such as epithelial cells, T cells, natural killer cell, and monocytes [41]. In this regard, there is one finding that highlights the significance of the interaction of IL-32 with IL-17 pathway in the synovium of patients with RA. IL-17 can stimulate IL-32 production in fibroblast-like synovial cells (FLSs) of RA patients and IL-32 in turn induces the production of IL-17 in CD4+ T cells. These reciprocal cellular responses can amplify the differentiation of OCs, independent of RANKL stimulation [42].

Accumulating evidence indicate that local elevation of IL-32  $\gamma$  in inflamed tissues of RA is associated with the pathogenesis of inflammatory bone diseases. Several groups have investigated the expression of IL-32  $\gamma$  in synovial tissue from RA patients [5,17,43]. IL-32  $\gamma$  is increased in RA synovium compared with osteoarthritis (OA) and healthy controls as evidenced by immunohistochemistry analysis. Elevated levels of IL-32  $\gamma$  in RA were also observed in synovial fluid, suggesting a positive association between synovial IL-32  $\gamma$  expression levels and disease severity [5].

The RA joints characterized by synovial inflammation and hyperplasia are the representative pathologic sites with progressive cartilage and bone destruction. In this inflamed synovium, activated macrophages are the predominant infiltrating cell type, and they promote inflammation through production of inflammatory cytokines such as TNF- $\alpha$  and IL-6. These conditions drive the differentiation of monocytic lineage cells to OCs and enhance bone resorbing activity and survival of OCs, contributing to bone destruction in nearby joints [1]. The increased content of IL-32  $\gamma$  in RA synovial tissues is tightly regulated by innate immunity-driven inflammatory mediators: TNF- $\alpha$ , IL-6, and chemokines. The fact that synovial staining of IL-32  $\gamma$  co-localizes with TNF- $\alpha$  staining in the inflamed site and that IL-32  $\gamma$  stimulates TNF- $\alpha$  production raises the possibility of the cooperative role of these two disparate cytokines in inflammatory response. The main cellular source of IL-32  $\gamma$  is discovered as FLSs. Functionally, it had been found that isolated RA-derived FLSs produce IL-32  $\gamma$  in response to TNF- $\alpha$ . In support of this, IL-32  $\gamma$  activity can amplify an inflammatory cascade in RA synovial tissue through autocrine loop [43]. This effect makes conditions more favorable for OC formation and attributes to the resorbing property of OC in RA joints.

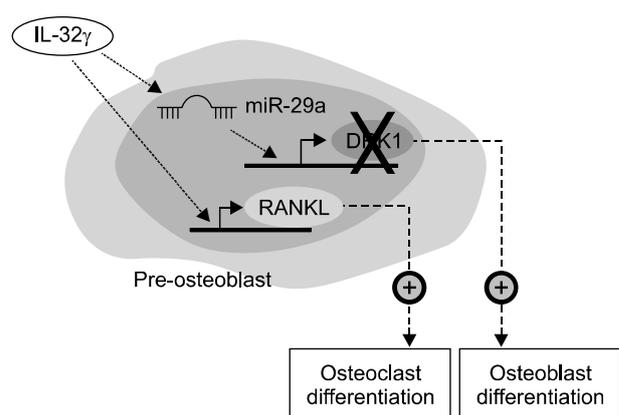
IL-32  $\gamma$  is recognized to be a strong stimulator of OC differentiation and activation via nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) activity and has synergistic effects with RANKL on osteoclastogenesis. Kim et al. [5] reported that IL-32  $\gamma$  increases the differentiation of CD14+ monocytes to OCs as well as the activity of the OCs in the presence of soluble RANKL stimulation. On the other hand, IL-32  $\gamma$  suppresses the transcription of osteoprotegerin (OPG). Thus, IL-32  $\gamma$  provides osteoclastogenetic environment in RA patients by promoting OC differentiation and its activity with RANKL stimulation and OPG reduction [5]. Therefore, inhibition of IL-32 may delay or inhibit the inflammation or tissue destruction.

### New aspects of IL-32 $\gamma$ on osteogenesis

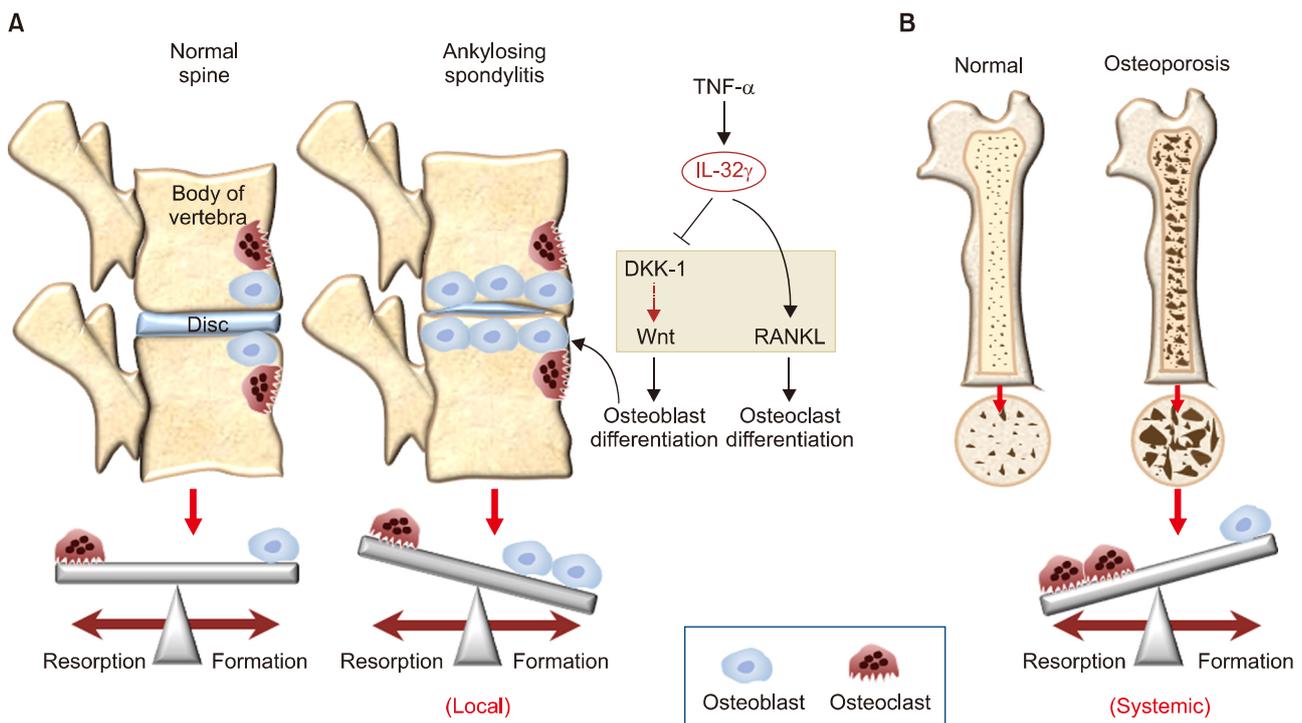
As discussed above, the investigation of IL-32  $\gamma$  functions has been focused on OC biology, or more specifically, bone resorption. Functionally, it has been found that TNF- $\alpha$  stimulates IL-32  $\gamma$  production from RA-FLSs and osteoblasts (OBs) and in turn, IL-32  $\gamma$  promotes OC formation by increasing RANKL production from these cells, consequently contributing to inflammatory bone loss [5,17]. These contradictory reports suggest that IL-32  $\gamma$  may have a dual function, depending on disease circumstances. The higher levels of IL-32  $\gamma$  in joints of AS relative to those of other inflammatory bone diseases are associated with enhanced OB differentiation and abnormal bone formation [4]. Recent findings show that IL-32  $\gamma$  potentiates both osteogenic and osteoclastogenic pathways by promoting OB differentiation directly and by upregulating RANKL production, respectively, supporting the idea that IL-32  $\gamma$  plays a role in the coupling of OC-mediated bone resorption to OB-mediated bone formation for maintenance of bone homeostasis [3].

Lee et al. [4] first reported the pathogenic role of IL-32  $\gamma$  in AS, and more specifically, OB differentiation using IL-32  $\gamma$  TG mice. Pro-inflammatory cytokine IL-32  $\gamma$  is accumulated in inflamed joints in patients with AS higher than in those with RA or OA. Lee et al. [3] has recently suggested the functional mechanism of osteogenesis mediated by systemic IL-32  $\gamma$  and the relationship between IL-32  $\gamma$  and Dickkopf-1 (DKK-1) in bone metabolism. Systemic overexpression of human IL-32  $\gamma$  TG mice resulted in the promotion of bone formation and the prevention of trabecular bone loss caused by aging and estrogen-deficiency. Systemic IL-32  $\gamma$  promotes the expressions of OB-specific genes and RANKL production in

vitro. Lee et al. [3] investigated differential regulation of microRNAs by IL-32  $\gamma$  to elucidate the molecular mechanism of IL-32  $\gamma$ -mediated downregulation of DKK-1. Interestingly, the expression level of miR-29a in primary OBs from IL-32  $\gamma$  TG mice was significantly higher than that in wild-type (WT) group (Figure 1). AS patients have typical characteristics which exhibit the formation of bony spurs and ankylosis (Figure 2). It is likely that elevated level of IL-32  $\gamma$  in peripheral and axial tissues from AS patients might lead to abnormal excessive bone formation. IL-32  $\gamma$  TG mice have higher potency of osteogenic differentiation compared to WT mice, and the administration of recombinant IL-32  $\gamma$  proteins also enhanced OB differentiation in vitro. Investigations on the molecular mechanism revealed that IL-32  $\gamma$  reduced the DKK-1 gene expression in OBs and enhanced the osteogenic capacity (Figure 2). Indeed, DKK-1 produced by OBs, a potent Wnt pathway inhibitor, suppresses OB differentiation. DKK-1-overexpressing mice display osteopenia phenotype due to reduced OB abundance and decreased bone formation [44]. These studies provide evidence that the enhanced OB differentiation from IL-32  $\gamma$  TG mice is closely related to the suppression of DKK-1 gene expression (Figure 2). Hence, IL-32  $\gamma$  may act as a key regulator that controls DKK-1 expression, leading to modulation of the Wnt/ $\beta$ -catenin signaling during the pathogenesis of AS, which could act as a promising novel molecular target with potential to prevent atypical new bone



**Figure 1.** Molecular mechanisms of interleukin (IL)-32  $\gamma$ -mediated bone metabolism. IL-32  $\gamma$  increases the level of Dickkopf-1 (DKK-1)-targeting miR-29a, leading to osteoblast differentiation and subsequent increase in bone formation. Simultaneously, IL-32  $\gamma$  enhances receptor activator of nuclear factor-kappa B ligand (RANKL) production to activate osteoclast differentiation.



**Figure 2.** The effects of interleukin (IL-32)  $\gamma$  on bone remodeling in ankylosing spondylitis and osteoporosis. (A) Normal spine remodeling is balanced by the interplay between bone-forming osteoblasts and bone-resorbing osteoclasts. In the spinal joints of patients with ankylosing spondylitis, locally elevated IL-32  $\gamma$  suppresses Dkkoprf-1 (DKK-1), a Wnt inhibitor in the synovium, which enables differentiation of osteoblast and subsequent new abnormal bone formation; this effect overshadows the effect of IL-32  $\gamma$  on receptor activator of nuclear factor-kappa B ligand (RANKL)-mediated osteoclast differentiation. (B) Diminished level of systemic IL-32  $\gamma$  in osteoporosis patients results in elevated expression of DKK-1, which leads to low bone mass and high fracture risk; however, there are no significant differences in bone marrow IL-32  $\gamma$  level between osteoporotic hip fracture patients and no-fracture patients. TNF- $\alpha$  : tumor necrosis factor- $\alpha$  .

formation in AS patients [4].

Furthermore, osteoporotic fracture patients exhibited lower levels of IL-32  $\gamma$  in blood than did patients with no fractures, a phenomenon that was accompanied with higher DKK-1 level [3]. There was no significant alteration in the bone marrow level of IL-32  $\gamma$  between osteoporotic hip fracture patients and no-fracture patients; this indicates that reduction in systemic IL-32  $\gamma$ —rather than locally expressed IL-32  $\gamma$ —may be responsible for the onset of osteoporosis. Abnormal local accumulation of IL-32  $\gamma$  in inflamed tissues in RA and AS patients leads to bone-related pathogenic deterioration, even though the serum level of IL-32  $\gamma$  in these patients does not significantly differ from that of healthy population. Nevertheless, reduction in serum IL-32  $\gamma$  level may be related to diminished bone regeneration in patients with osteoporosis, which highlights the distinction in the physiological significance of circulating and localized IL-32  $\gamma$  . These studies suggest that systemic IL-32  $\gamma$  may exhibit

rather potent anabolic effects than catabolic effects on bone metabolism due to its ability to inhibit DKK-1 gene expression in OBs. Systemic IL-32  $\gamma$  may play a protective role for bone loss, providing clinical evidence of a negative correlation between IL-32  $\gamma$  and DKK-1 [3]. Alterations in IL-32  $\gamma$  level may represent a novel and promising biomarker and a target for potential therapeutic applications in inflammatory bone diseases for clinical outcomes in inflammatory bone pathologic condition.

## CONCLUSION

IL-32 has diverse roles in mediating the pathogenesis of infections, cancer, chronic inflammation, and autoimmune disease. Recent studies suggest that IL-32  $\gamma$  plays a critical role in the coupling of bone resorption to bone formation for maintenance of bone homeostasis. IL-32  $\gamma$  is associated with the pathogenesis of inflammatory bone

disorders such as RA, AS, and osteoporosis. IL-32  $\gamma$  promotes OC formation by increasing RANKL production from RA-FLSs, consequently contributing to inflammatory bone loss in RA. Locally elevated IL-32  $\gamma$  is also associated with abnormal excessive bone formation in AS and enhances osteogenic effects. Alterations in systemic IL-32  $\gamma$  and DKK-1 can be used as a prognostic marker in inflammatory bone diseases such as osteoporosis. An understanding of the role of IL-32  $\gamma$  in inflammatory bone diseases will aid in developing novel prognostic/therapeutic agents.

## ACKNOWLEDGMENTS

This work was supported by grants from the Basic Science Research Program funded by the Ministry of Education, Science, and Technology (2016R1DA1B039-33855 and NRF-2014R1A1A2056099).

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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