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ORIGINAL RESEARCH

Vertebral Osteoporosis in Systemic Lupus Erythematosus: A Possible Involvement of Inflammation-Related Osteoblast Ferroptosis

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Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by immune system dysregulation and the production of autoantibodies, leading to widespread inflammation and multi-organ damage. Despite clinical observations have shown that approximately 1.4–68.7% of SLE patients develop vertebral osteoporosis (OP), the underlying mechanisms remain poorly defined. This study utilized the MRL/*lpr* mouse model, which effectively replicates human SLE manifestations, to investigate the impact of SLE on vertebral bone homeostasis.

Methods: Female MRL/*lpr* mice were employed to investigate SLE-induced bone loss. The study comprehensively evaluated bone structural changes through micro-CT analysis, histological assessment, and bone metabolic markers. Specifically, we analyzed trabecular parameters (TV, BV, BV/TV, Tb.Th), inflammatory cytokine profiles (TNF-α, IL-6, IL-1β, IL-18), osteogenic markers (RUNX2, OSTERIX, ALP, OPG), osteoclastogenic indicators (TRAP, RANKL, CTSK), and ferroptosis-related proteins (FACL4, FTH1, GPX4).

Results: SLE progression in MRL/*lpr* mice led to significant vertebral bone loss and OP phenotype, evidenced by reduced bone volume fraction (BV/TV) and trabecular thickness (Tb.Th). The inflammatory microenvironment was characterized by elevated TNF-α and IL-6 levels, which disrupted bone homeostasis by suppressing RUNX2, OSTERIX, and OPG expression while enhancing RANKL signaling. Mechanistically, SLE induced ferroptosis through increased FACL4 and FTH1 expression coupled with decreased GPX4 levels, leading to impaired osteoblast function and enhanced osteoclast activity.

Conclusion: SLE-associated vertebral OP is mediated by inflammation-driven ferroptosis, disrupting the balance between bone formation and resorption, offering novel insights into potential therapeutic strategies for managing bone loss in SLE patients.

Keywords: systemic lupus erythematosus, vertebral osteoporosis, inflammation, ferroptosis, osteoblast dysfunction

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by immune system dysregulation and pathogenic autoantibody production, predominantly affecting women of childbearing age.[1,2] Beyond its well-documented manifestations of organ-specific inflammation and immune complex deposition, mounting evidence indicates that SLE patients face a significantly higher risk of skeletal complications, with cohort studies revealing alarming rates of bone mineral density reduction, with osteopenia occurring in 24–74% and osteoporosis (OP) in 1.4–68.7% of

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SLE patients.[3–5] Despite these compelling epidemiological associations, the molecular mechanisms underlying SLE-induced vertebral bone loss remain incompletely understood.

Vertebral bone homeostasis is maintained through a precisely orchestrated balance between bone formation and resorption, regulated by osteoblasts and osteoclasts respectively.[6] This delicate equilibrium is particularly susceptible to inflammatory disruption, with pro-inflammatory cytokines, particularly TNF-α and IL-6 emerging as critical mediators of pathological bone remodeling. These inflammatory factors exhibit dual pathogenic effects: suppressing osteoblast differentiation through downregulation of master transcription factors RUNX2 and OSTERIX, while simultaneously promoting osteoclastogenesis via RANKL-dependent pathways.[7,8] Our recent clinical observations and experimental studies with SLE model mice have demonstrated elevated inflammatory cytokine profiles across multiple tissues, suggesting a potential mechanistic link between SLE-associated inflammation and accelerated bone loss.[9,10]

Recent advances in cell death research have highlighted ferroptosis, an iron-dependent form of regulated cell death characterized by lipid peroxidation, as a significant contributor to various pathological conditions.[11] The involvement of ferroptosis in bone metabolism disorders has been increasingly recognized through the dysregulation of key molecular regulators, including FACL4,[12] FTH1,[13] and GPX4.[14] Ferroptosis appears to exert its detrimental effects on bone homeostasis through multiple mechanisms: compromising osteoblast viability and function while potentially enhancing osteoclast-mediated bone resorption.[15] This process is intimately connected to immune-mediated bone loss,[16] where inflammatory cytokines—particularly through the IL-6/JAK/STAT3 signaling axis—regulate hepcidin expression, highlighting the complex interplay between inflammation and systemic iron homeostasis.[17] Furthermore, inflammation-driven ferroptosis has been shown to impair bone formation by inhibiting both the osteogenic differentiation of bone marrow stromal cells (BMSCs) and the bone-forming capacity of mature osteoblasts.[18] However, the potential role of ferroptosis in mediating SLE-induced vertebral bone loss remains unexplored.

To address this critical knowledge gap, we utilized the MRL/*lpr* mouse model, which closely recapitulates human SLE manifestations, to investigate the mechanisms underlying SLE-induced vertebral bone loss. We hypothesized that SLE progression promotes vertebral bone loss through a cascade of events involving inflammation-driven ferroptosis, leading to impaired osteoblast function and enhanced osteoclast activity. Our findings provide new insights into the pathogenesis of SLE-associated bone loss and identify potential therapeutic targets. Understanding these mechanisms is crucial for developing more effective strategies to prevent and treat bone loss in SLE patients, ultimately improving their quality of life and long-term outcomes.

Materials and Methods

Animals and Experimental Design

Female MRL/*lpr* and their genetic background-matched MRL/*MpJ* controls (6 weeks of age) were obtained from the Center Animal House of Zhejiang Chinese Medical University. The MRL/*lpr* mice spontaneously develop lupus-like symptoms, while age-matched MRL/*MpJ* mice serve as controls.[19] All animals were maintained under specific pathogen-free (SPF) conditions at 23 ± 2°C with 40–60% relative humidity and a 12-hour light/dark cycle, with ad libitum access to standard laboratory chow and water. At 14 weeks of age, 6 mice were assigned to the experimental group (MRL/*lpr* group), while 6 age-matched female MRL/*MpJ* mice served as controls. At 14 weeks of age, all mice were euthanized with 0.3% sodium pentobarbital administered intraperitoneally, and the lumbar vertebrae were harvested for subsequent analyses. All animal procedures were conducted in accordance with institutional guidelines and approved by the Ethics Committee for the Use of Experimental Animals at Zhejiang Chinese Medical University (Approval No. IACUC-20211101-04).

Micro-CT Analysis

Vertebral bone microarchitecture was assessed using high-resolution micro-CT (Skyscan 1176, Bruker micro-CT N.V., Kontich, Belgium) with the following parameters: 50 kV voltage, 500 μA current, and 9 μm pixel resolution. Image reconstruction and analysis were performed using NRecon v1.6 and CTAn v1.15 software, respectively. Three-dimensional visualization was generated using CTVol v2.2 software. Quantitative assessment of bone morphologic

parameters was conducted on L3 vertebral bodies, including total volume (TV), bone volume (BV), bone volume fraction (BV/TV), and trabecular thickness (Tb.Th).

Histological Staining, Alkaline Phosphatase (ALP) Staining and Immunofluorescence (IF)

Lumbar vertebrae specimens were fixed in 4% buffered paraformaldehyde (72 hours), decalcified in 0.5 M EDTA (pH = 7.4, 3 weeks), and embedded in paraffin. Serial coronal sections (5 μm thickness) were prepared for histological analyses.

Histological analysis of the vertebral sections was evaluated using hematoxylin and eosin (H&E) staining. Osteoblast activity was assessed using a commercial ALP detection kit (CW0051S, CWBIO, Beijing, China) according to the manufacturer's instructions. ALP-positive areas within L3 vertebral bodies were analyzed and quantified using Image-Pro Plus software (version 6.0; Media Cybernetics Inc., Rockville, Maryland, USA) with triplicate measurements per sample.

For IF staining studies, the sections were incubated with the following primary antibodies at 4°C overnight: inflammatory markers: TNF-α (1:500, Ruiying Biological, Suzhou, China), IL-6 (1:500, Proteintech, Wuhan, China), IL-1β (1:500, Bioss, Woburn, MA, USA), IL-18 (1:500, Ruiying Biological); bone formation markers: RUNX2 (1:500, Abcam, Cambridge, UK), OSTERIX (1:500, Proteintech); bone resorption markers: RANKL (1:500, Ruiying Biological), CTSK (1:500, Bioss); ferroptosis markers: FACL4 (1:500, Bioss), FTH1 (1:500, Abcam), and GPX4 (1:500, Abcam). Sections were subsequently incubated with fluorescent-conjugated secondary antibodies (Sungene Biotech, Tianjin, China) and counterstained with DAPI. Images were captured using a fluorescence microscope (Carl Zeiss). Quantitative analysis was performed using Image-Pro Plus software.

TRAP Staining Assay

Osteoclast identification was performed using TRAP staining. Briefly, TRAP staining solution was freshly prepared by mixing 20 mL of buffer solution (anhydrous sodium acetate, acetic acid, sodium tartrate, and distilled water) with 1 mL AS-BI phosphate and ethylene glycol ether mixture. Sections were immersed in this primary solution at 37°C for 1 hour. A secondary staining solution was prepared by adding sodium nitrite dye (2 mL), p-rosaniline dye (2 mL), concentrated hydrochloric acid, and distilled water to 20 mL of preheated primary solution. Sections were transferred to the secondary solution and monitored until optimal wine-red coloration developed (approximately 5 minutes). Following triple distilled water rinses, sections were briefly counterstained with 0.02% fast green, dehydrated through graded ethanol series, cleared in xylene, and mounted with neutral balsam. TRAP-positive multinucleated cells (≥3 nuclei) were counted as osteoclasts. Quantitative analysis was performed on six randomly selected fields per section using Image-Pro Plus software.

Statistical Analysis

Data are presented as mean ± SEM. All analyses were conducted using GraphPad Prism software (San Diego, CA, United States). Statistical comparisons between MRL/*MpJ* and MRL/*lpr* groups were performed using unpaired two-tailed Student's *t*-test after confirming variance homogeneity (F-test). Statistical significance was set at $P < 0.05$.

Results

SLE Progression Contributes to Severe Vertebral Bone Loss and Osteoporotic Phenotype in MRL/Lpr Mice

To gain a comprehensive understanding of the intricate relationship between SLE progression and vertebral bone loss, we utilized the well-characterized MRL/*lpr* mouse model of SLE, with MRL/*MpJ* mice serving as controls. The SLE phenotype in MRL/*lpr* mice was confirmed through established biomarkers and histopathological criteria as previously described.[9] Initial histological examination of lumbar vertebrae using H&E staining revealed significant structural deterioration in the vertebral bodies of MRL/*lpr* mice, characterized by disrupted trabecular architecture and compromised bone integrity compared to MRL/*MpJ* controls (Figure 1A). To quantitatively assess these structural alterations, we performed high-resolution μCT analysis of the L3 vertebral

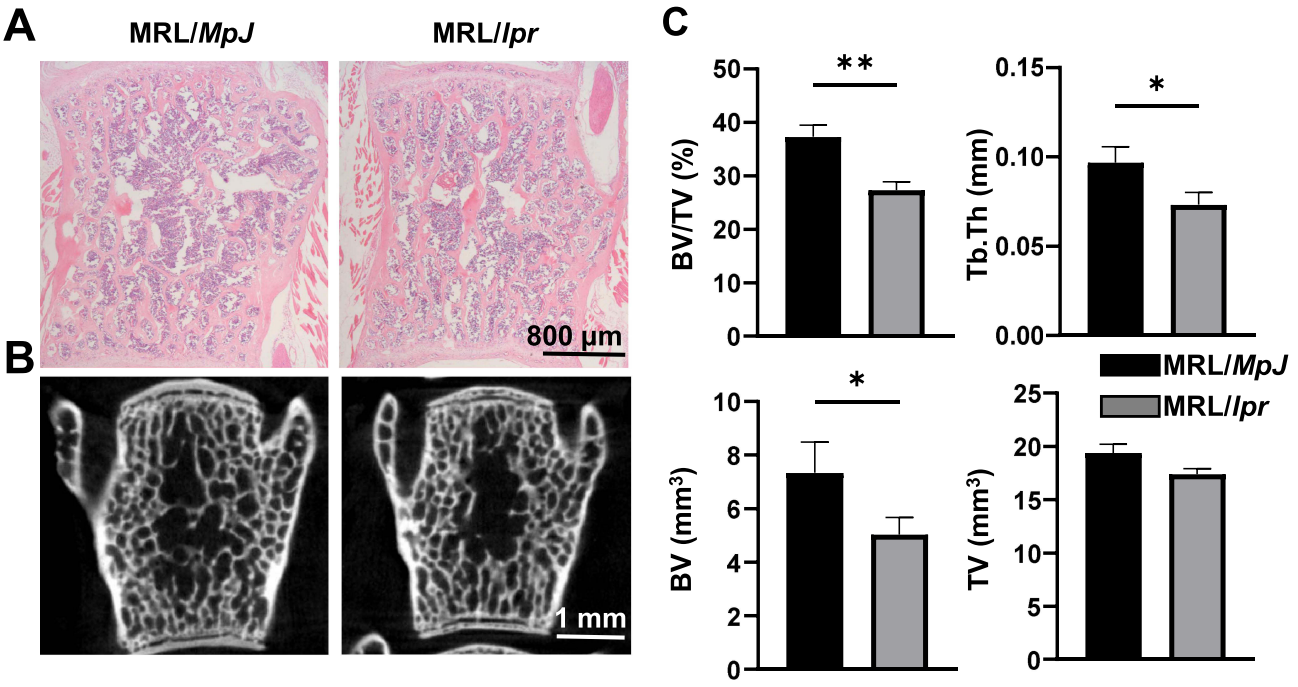

Figure 1 SLE promotes vertebral osteoporotic phenotype in MRL/*lpr* mice. (**A**) Representative H&E staining images of lumbar vertebrae showing altered trabecular bone architecture in MRL/*lpr* mice compared to MRL/*MpJ* controls. (**B** and **C**) High-resolution μCT analysis revealing compromised bone structural integrity in MRL/*lpr* mice, with representative 3D reconstructions of vertebral trabecular bone (**B**) and comprehensive quantification of key bone parameters (**C**) demonstrating significant reductions in BV/TV, Tb.Th, BV, and TV. Data represent mean ± SEM. *$p < 0.05$, **$p < 0.01$ (vs MRL/*MpJ* group), n = 6 per group.
Abbreviations: SLE, Systemic lupus erythematosus; H&E, Hematoxylin and eosin; μCT, Micro-computed tomography; TV, Trabecular volume; BV, Bone volume; BV/TV, Bone volume fraction; Tb.Th, Trabecular thickness; SEM, Standard error of mean.

bodies. Notably, MRL/*lpr* mice showed significant bone mass reduction (Figure 1B). Consistently, detailed bone morphological parameters demonstrated substantial decreases in both BV and BV/TV in MRL/*lpr* mice, while trabecular thickness (Tb.Th) was reduced by approximately 26% in MRL/*lpr* mice relative to controls, providing compelling evidence for SLE-induced bone loss (Figure 1B and C). In summary, SLE progression leads to significant bone loss and the development of an osteoporotic phenotype.

Enhanced Inflammatory Response in the Vertebral Body of MRL/Lpr Mice

Given the crucial role of inflammation in bone metabolism,[20] we next characterized the inflammatory microenvironment within vertebral tissues. IF analysis revealed a marked upregulation of pro-inflammatory cytokines in the vertebral body of MRL/*lpr* mice. Specifically, TNF-α showed a 2.1-fold increase, while IL-6 levels were dramatically elevated by 5.1-fold compared to controls. Although elevated trends were observed for IL-1β and IL-18, these changes did not reach statistical significance (Figure 2A–D). These findings indicate a robust inflammatory response in the vertebral micro-environment of SLE mice.

Impaired Osteogenic Differentiation in the Vertebral Body of MRL/Lpr Mice

Since disrupted osteogenesis is a major hallmark of vertebral bone loss and OP,[21] we comprehensively evaluated osteoblast function through multiple approaches. ALP staining results showed a significant reduction in ALP-positive cells within the vertebral bodies of MRL/*lpr* mice (Figure 3A). Supporting this observation, IF analysis demonstrated substantial decreases in key osteogenic markers: RUNX2 expression was reduced by 98%, OSTERIX by 51%, and OPG by 40% compared to MRL/*MpJ* mice (Figure 3B–D). These findings strongly suggest that SLE severely impairs osteoblastic differentiation and function, contributing to vertebral bone loss.

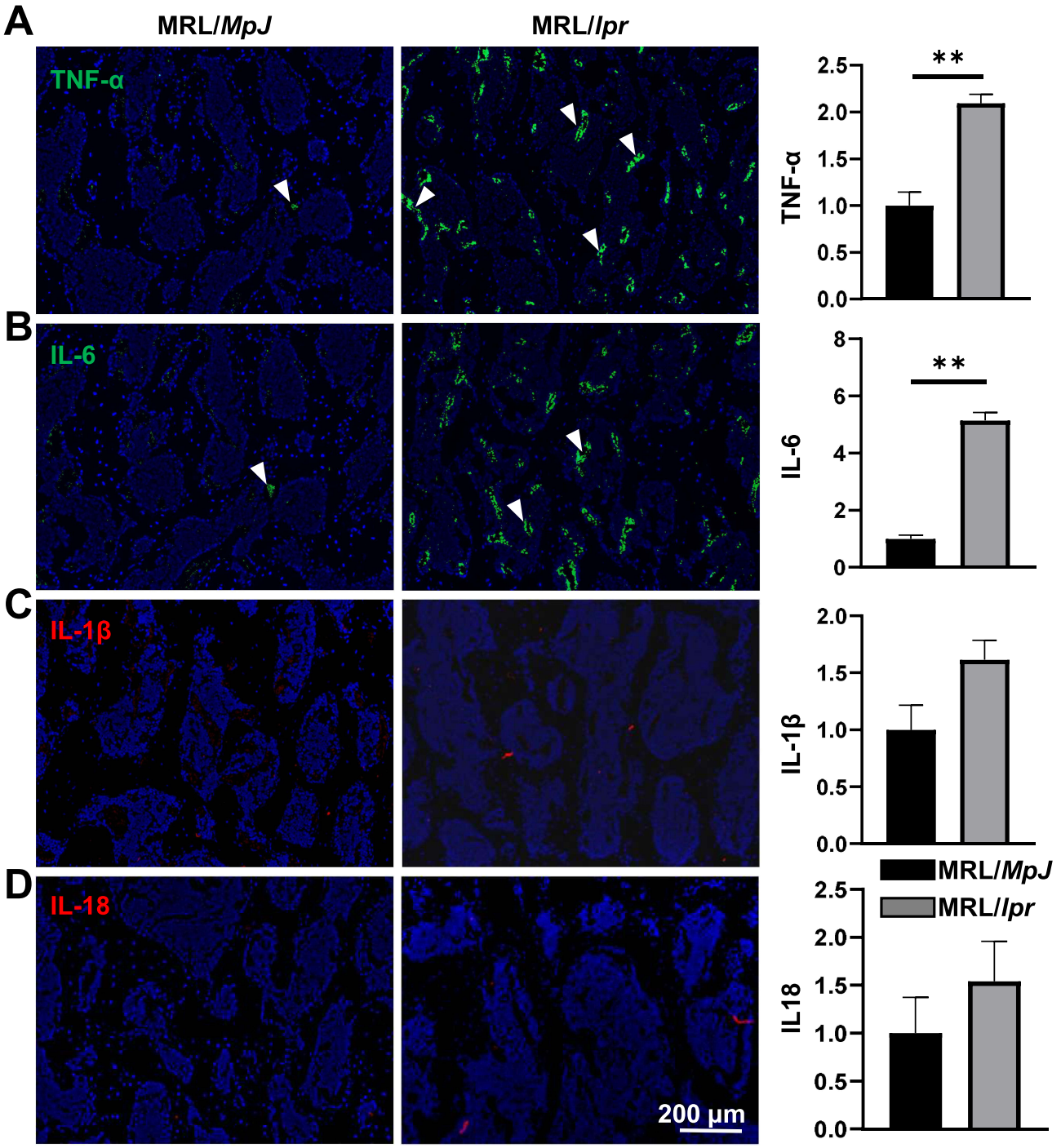

Figure 2 SLE triggers a pro-inflammatory microenvironment in vertebral bone tissue of MRL/*lpr* mice. (**A**–**D**) IF analysis of inflammatory cytokine expression in vertebral bodies: TNF-α (**A**), IL-6 (**B**), IL-1β (**C**), and IL-18 (**D**), revealing significantly elevated levels in MRL/*lpr* mice compared to MRL/*MpJ* controls. White arrowheads indicate representative areas of high cytokine expression. Nuclear counterstaining was performed with DAPI (blue). Data represent mean ± SEM. $^{**}p < 0.01$ (vs MRL/*MpJ* group), n = 6 per group.

Abbreviations: SLE, Systemic lupus erythematosus; TNF-α, Tumor necrosis factor alpha; IL-6, Interleukin 6; IL-1β, Interleukin 1 beta; IL-18, Interleukin 18; DAPI, 4′,6-diamidino-2-phenylindole; SEM, Standard error of mean.

Increased Osteoclast Activity in the Vertebral Body of MRL/Lpr Mice

Considering that bone homeostasis through the balance between bone formation and resorption, we next assessed osteoclast activity. TRAP staining demonstrated significantly increased osteoclast number in the vertebral bodies of MRL/*lpr* mice (Figure 4A). Consistent with enhanced osteoclastogenesis, IF analysis showed elevated expression of both

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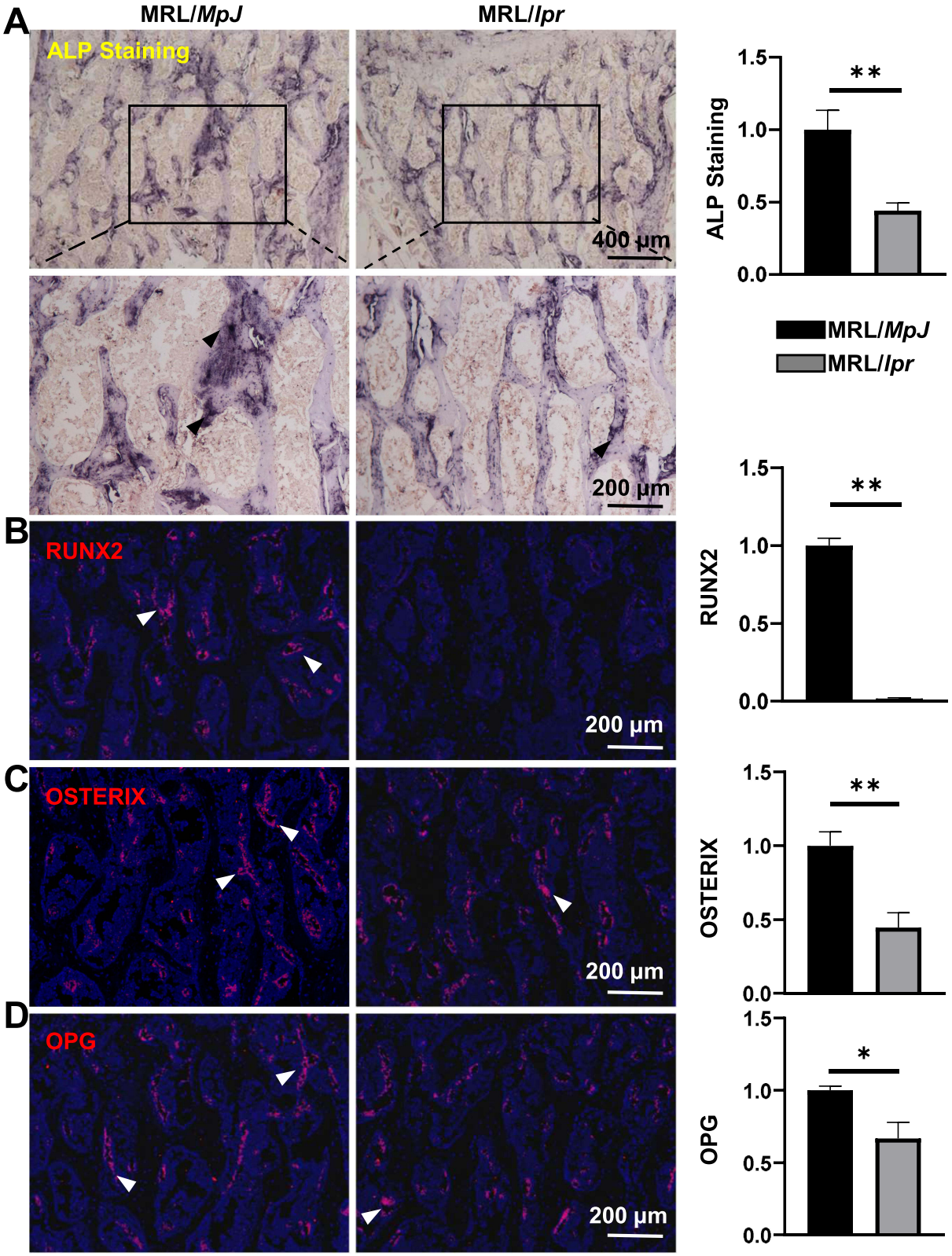

Figure 3 SLE suppresses osteoblast differentiation and function in the vertebral bone of MRL/*lpr* mice. (**A**) ALP enzymatic staining showed markedly reduced osteoblastic activity in MRL/*lpr* vertebrae, with high-magnification insets highlighting the differences in ALP-positive areas. Black triangles indicate the high expression. (**B**–**D**) IF analysis of key osteogenic transcription factors and markers: RUNX2 (**B**), OSTERIX (**C**), and OPG (**D**), demonstrating significant downregulation in MRL/*lpr* mice. White arrowheads indicate positive expression signals. Nuclear counterstaining with DAPI (blue). Data represent mean ± SEM. *$p < 0.05$, **$p < 0.01$ (vs MRL/*MpJ* group), n = 6 per group.
Abbreviations: SLE, Systemic lupus erythematosus; ALP, Alkaline phosphatase; RUNX2, Runt-related transcription factor 2; OSTERIX, Sp7 transcription factor; OPG, Osteoprotegerin; DAPI, 4′,6-diamidino-2-phenylindole; SEM, Standard error of mean.

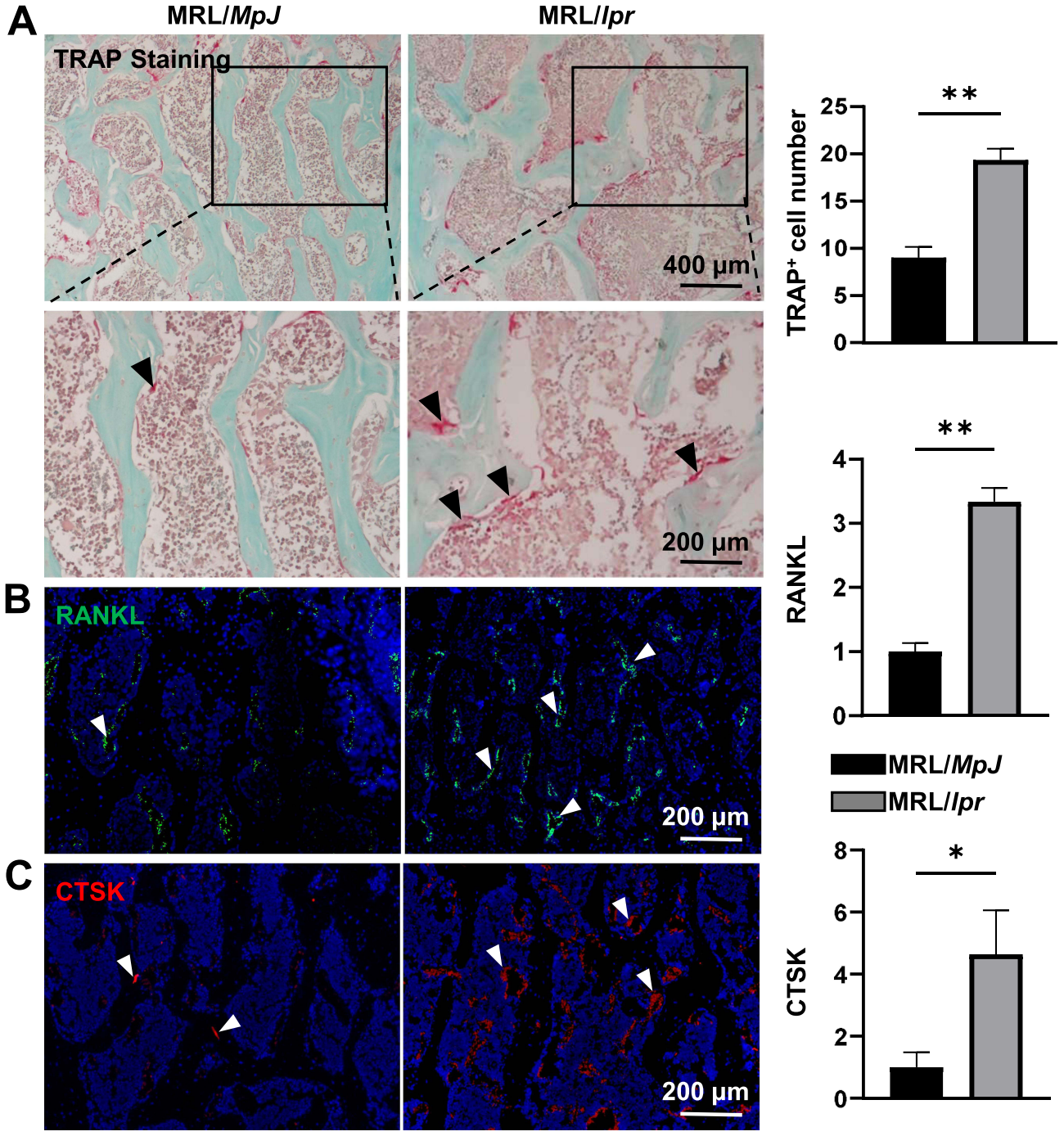

Figure 4 SLE enhances osteoclastogenesis in vertebral bodies of MRL/*lpr* mice. (**A**) TRAP histochemical staining revealed increased osteoclast formation in MRL/*lpr* vertebrae, with high-magnification insets showing TRAP-positive multinucleated osteoclasts (black arrowheads). (**B** and **C**) IF analysis of osteoclast-regulatory factors: RANKL (**B**) and CTSK (**C**), showing significant upregulation in MRL/*lpr* mice. White arrowheads indicate positive signals. Nuclear counterstaining with DAPI (blue). Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01 (vs MRL/*MpJ* group), n = 6 per group.

RANKL (3.3-fold increase), a key osteoclast differentiation factor, and CTSK (4.6-fold increase), a terminal differentiation marker of osteoclasts (Figure 4B and C). These results indicate that SLE promotes osteoclast differentiation and activity in vertebral bone tissue.

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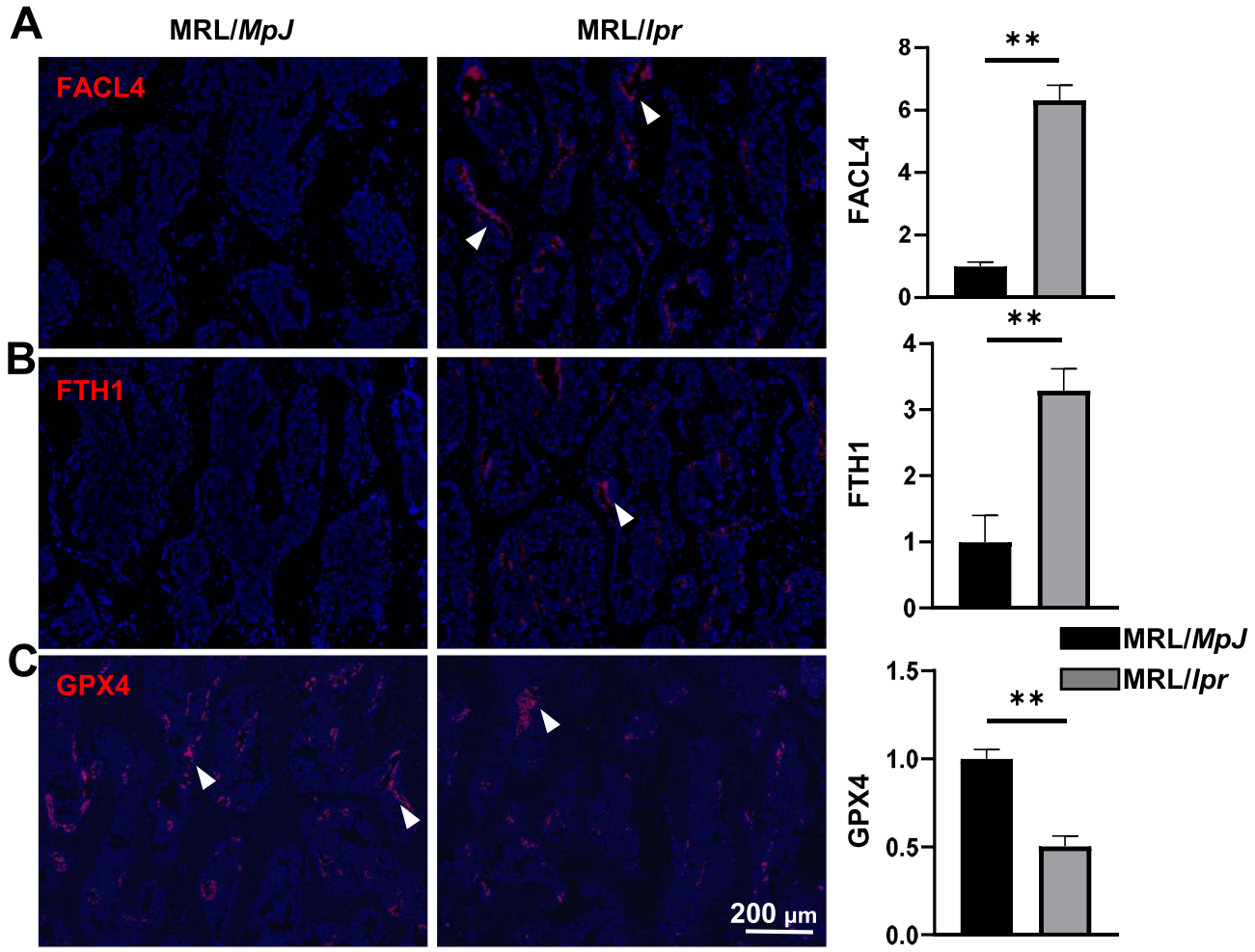

Figure 5 SLE induces osteoblast ferroptosis within vertebral bodies of MRL/*MpJ* mice. (**A–C**) IF analysis of ferroptosis-associated markers: increased FACL4 (**A**) and FTH1 (**B**) expression, coupled with decreased GPX4 (**C**) levels in MRL/*lpr* vertebrae, indicating enhanced ferroptotic cell death. White arrowheads indicate representative expression signals. Nuclear counterstaining with DAPI (blue). Data represent mean ± SEM. **$p < 0.01$ (vs MRL/*MpJ* group), n = 6 per group.
Abbreviations: SLE, Systemic lupus erythematosus; TRAP, Tartrate-resistant acid phosphatase; RANKL, Receptor activator of nuclear factor kappa-B ligand; CTSK, Cathepsin K; DAPI, 4′,6-diamidino-2-phenylindole; SEM, Standard error of mean.

Activation of Ferroptosis Signaling in the Vertebral Body of MRL/Lpr Mice

To further delineate the molecular mechanisms underlying SLE-induced bone loss and OP progression, and considering the recently established roles of ferroptosis in both SLE pathogenesis and bone metabolism disorders,[22,23] we investigated whether ferroptosis contributes to vertebral bone loss in our SLE model. IF analysis revealed significant alterations in key ferroptosis-related proteins within vertebral body tissues. Specifically, the expression of iron overload-related proteins FTH1 and FACL4 were markedly increased in osteoblasts of MRL/*lpr* mice, showing 6.3-fold and 3.3-fold elevations compared to MRL/*MpJ* controls, respectively (Figure 5A and B), whereas the expression of GPX4, a critical regulator protecting against lipid peroxidation was suppressed by approximately 50% in MRL/*lpr* mice (Figure 5C). These coordinated changes in ferroptosis markers suggest that ferroptosis activation may serve as a crucial mechanism mediating SLE-induced vertebral bone loss and OP progression.

Discussion

SLE represents a complex autoimmune disorder with diverse manifestations beyond its classical presentations of lupus nephritis,[24] arthritis,[25] and cardiovascular complications.[26] Among these, musculoskeletal disorders have emerged as significant concerns,[3,27] with clinical evidence demonstrating a markedly increased risk of bone loss and OP in SLE patients, particularly affecting vertebral sites.[28–31] Recent cohort studies report alarming rates of bone density reduction,

with osteopenia occurring in 24–74% and OP in 1.4–68.7% of SLE patients.[3–5] The predilection for vertebral involvement, characterized by pronounced bone loss in the lumbar spine followed by the femoral neck,[32] aligns with our experimental findings. In this study, using the MRL/*lpr* mouse model, we demonstrate that SLE progression induces significant structural deterioration in vertebral bone, characterized by reduced bone volume fraction and compromised trabecular architecture. Moreover, we identified a specific inflammatory signature in vertebral tissue, marked by selective elevation of TNF-α and IL-6. Importantly, we uncovered a previously unrecognized role of ferroptosis in SLE-induced bone loss, evidenced by the coordinated increase in ferroptosis markers FACL4 and FTH1, and GPX4. These findings provide new insights into the pathogenesis of SLE-associated bone loss and suggest potential therapeutic opportunities through targeting both inflammation and ferroptosis pathways (Figure 6).

The MRL/*lpr* mouse model, characterized by Fas (CD95) mutations that accelerate autoimmune responses, effectively recapitulates key pathological features of human SLE, including autoantibody production, systemic inflammation, and notably, musculoskeletal manifestations such as arthritis and joint inflammation,[9,33] making it an ideal model for investigating SLE-associated bone disorders. Our micro-CT analysis revealed significant structural deterioration in the vertebral bone of MRL/*lpr* mice, characterized by reduced BV/TV ratio and decreased trabecular thickness. These findings closely mirror the patterns of vertebral bone loss in SLE patients.[34] The compromised bone microarchitecture observed in our study suggests that SLE-induced bone loss may preferentially affect metabolically active trabecular bone

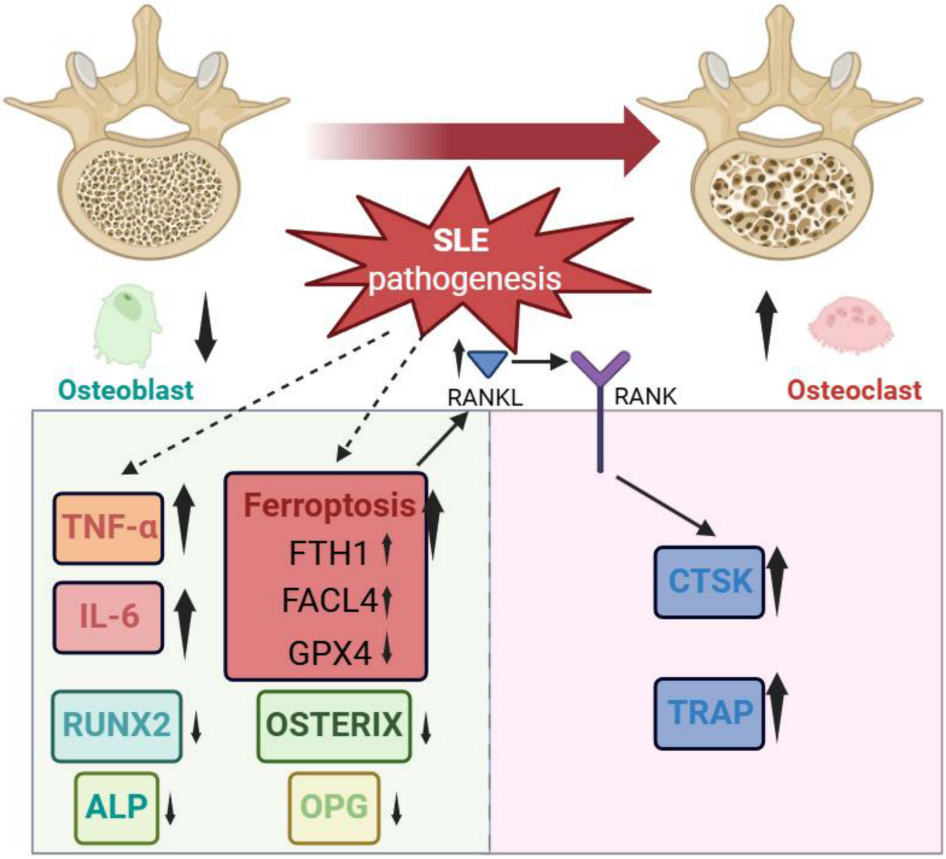

Figure 6 Proposed mechanism underlying SLE-induced vertebral bone loss. Schematic illustration depicting how SLE pathogenesis promotes vertebral OP phenotype through dual mechanisms: (1) Enhanced inflammation and ferroptosis leading to impaired osteoblast differentiation and function (marked by decreased RUNX2, OSTERIX, ALP, and OPG), and (2) Increased RANKL production stimulating osteoclast differentiation and activation (evidenced by elevated TRAP and CTSK). These molecular alterations collectively result in compromised bone mass and microarchitecture in the vertebral bodies of SLE model mice.

Abbreviations: SLE, Systemic lupus erythematosus; RANKL, Receptor activator of nuclear factor kappa-B ligand; RANK, Receptor activator of nuclear factor kappa-B; TNF-α, Tumor necrosis factor alpha; IL-6, Interleukin 6; FTH1, Ferritin heavy chain 1; FACL4, Fatty acid-CoA ligase 4; GPX4, Glutathione peroxidase 4; RUNX2, Runt-related transcription factor 2; OSTERIX, Sp7 transcription factor; ALP, Alkaline phosphatase; OPG, Osteoprotegerin; CTSK, Cathepsin K; TRAP, Tartrate-resistant acid phosphatase.

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sites. This site-specific vulnerability could explain the high prevalence of vertebral fractures reported in SLE patients, even in those with relatively short disease duration.[35]

The inflammatory microenvironment emerges as a critical regulator of bone homeostasis,[6] with TNF-α and IL-6 serving as critical mediators.[36,37] Recent studies have demonstrated that TNF-α directly suppresses osteoblast differentiation by inhibiting RUNX2 and OSTERIX expression through NF-κB-dependent mechanisms while simultaneously enhancing RANKL production.[38] Similarly, IL-6 disrupts the OPG/RANKL axis by promoting RANKL expression while suppressing OPG production, thereby creating a microenvironment favoring osteoclastogenesis.[39] Consistent with these studies, our findings demonstrated markedly elevated levels of TNF-α (2.1-fold) and IL-6 (5.1-fold) in vertebral tissue of MRL/*lpr* mice, accompanied by significant downregulation of RUNX2 (98% reduction) and OSTERIX (51% reduction), alongside enhanced RANKL signaling (3.3-fold increase).

Intriguingly, while TNF-α and IL-6 showed pronounced elevation, IL-1β and IL-18 showed no significant changes in the vertebral tissue of MRL/*lpr* mice. This unexpected finding might be explained by the temporal dynamics of inflammatory responses in SLE. Recent studies have suggested that different inflammatory mediators exhibit distinct temporal patterns during disease progression, with some cytokines showing early, transient peaks followed by normalization.[40,41] Additionally, emerging evidence indicates that pyroptosis, a process that releases IL-1β and IL-18, occurs rapidly and may have already peaked before our analysis time point. This hypothesis is supported by recent findings showing that pyroptosis-related cytokine release often precedes the sustained elevation of TNF-α and IL-6 in various inflammatory conditions.[42,43] Furthermore, the complex regulatory networks controlling cytokine production in SLE might involve feedback mechanisms that selectively modulate different inflammatory mediators, potentially explaining the differential expression patterns observed in our study.

Previous studies have shown that SLE leads to the aggregation of lupus autoantigens in apoptotic cells and excessive production of inflammatory cytokines in various tissues, significantly influencing the development of SLE-related manifestations such as renal damage and cardiovascular complications.[44–46] Elevated serum IL-6 and TNF-α levels have been associated with the severity of lupus nephritis.[47] Our recent work with MRL/*lpr* mice confirmed that excessive inflammation in cardiac and renal tissues promotes metabolism dysfunction, accelerating lupus nephritis and cardiovascular disease progression.[10,48] In the context of bone metabolism, our current findings demonstrate that SLE creates a distinct inflammatory microenvironment in the vertebral tissue characterized by increased IL-6 and TNF-α levels. These cytokines disrupt bone homeostasis through multiple mechanisms: TNF-α induces osteoblast apoptosis,[36] while IL-6 inhibition enhances osteogenic gene expression, such as OCN.[37] Moreover, both cytokines promote osteoclastogenesis and bone resorption,[49] creating a pathological state that favors bone loss. This dual action - suppressing bone formation while enhancing resorption - identifies these inflammatory mediators as key drivers of secondary secondary OP in SLE. Therefore, our findings thus establish the central role of the SLE-induced inflammatory microenvironment in disrupting vertebral bone homeostasis, potentially offering new therapeutic targets for preventing bone loss in SLE patients.

Recent studies have highlighted ferroptosis as a critical regulator of inflammatory responses in various pathological conditions.[50] This iron-dependent cell death pathway initiates a self-amplifying inflammatory circuit through multiple mechanisms: ferroptosis triggers the release of pro-inflammatory mediators (IL-1β, IL-6, and TNF-α), while TNF-α activates NF-κB signaling, leading to enhanced inflammation and oxidative stress through leukocyte recruitment and ROS generation.[51] The pathological significance of this process is evidenced in rheumatoid arthritis, where TNF-α shows a strong correlation with ferroptosis,[52] and by the identification of IL-6 and TNF-α, as key regulators of ferritin synthesis.[17] Emerging evidence has implicated ferroptosis and its key markers (FACL4, FTH1, and GPX4) in various SLE-related complications, particularly lupus nephritis and cardiovascular manifestations.[53,54] In parallel, ferroptosis has been increasingly recognized as a vital mechanism in bone metabolism disorders, where osteoblast ferroptosis significantly contributes to bone loss and OP development.[55] Specifically, studies have shown that increased FACL4 and FTH1 expression, coupled with GPX4 suppression, leads to impaired osteoblast viability and function, ultimately resulting in reduced bone formation. In line with these observations, our investigation revealed significant alterations in ferroptosis markers within vertebral tissue of MRL/*lpr* mice, characterized by markedly increased expression of FACL4 (3.3-fold) and FTH1 (6.3-fold), alongside decreased levels of the protective protein GPX4 (50% reduction). These coordinated

changes in ferroptosis-related proteins, occurring in the context of elevated inflammatory cytokines, suggest a novel pathogenic mechanism whereby SLE-induced inflammation triggers osteoblast ferroptosis, thereby accelerating bone loss.

This newly identified mechanism provides potential therapeutic opportunities for preventing SLE-associated bone loss through targeted inhibition of ferroptosis pathways, either alone or in combination with anti-inflammatory strategies.

Several limitations of our study warrant consideration. First, although the MRL/*lpr* mouse model effectively mirrors human SLE manifestations, validation in other lupus-prone models, such as NZB/W F1 mice,[56] would help establish the generalizability of our findings. Second, our observations at 14 weeks provide a snapshot of established disease, but the temporal relationship between inflammation and ferroptosis activation remains unclear. Given the chronic nature of both SLE and OP, longitudinal studies across different disease stages would better elucidate how inflammatory responses trigger ferroptotic cell death and subsequent bone loss. Additionally, while we demonstrated associations between inflammatory markers and ferroptosis-related proteins, intervention studies using specific ferroptosis inhibitors would help establish direct causality. Finally, although animal models are invaluable for mechanistic studies, validation using human samples is essential for determining whether similar molecular pathways operate in SLE patients' bone tissue. Future studies addressing these limitations will provide a more comprehensive understanding of SLE-related bone loss and help develop more effective therapeutic strategies. We will next implement therapeutic intervention studies using specific ferroptosis inhibitors (eg, ferrostatin-1, liproxstatin-1) to establish direct causality between inflammatory activation and osteoblast ferroptosis. Additionally, we plan to validate our findings in human samples by analyzing bone biopsies and serum markers from SLE patients with varying degrees of bone mineral density to determine the clinical relevance of these pathways.

Conclusions

In conclusion, our findings reveal a novel mechanistic link between SLE progression and vertebral OP, demonstrating that SLE-induced inflammation promotes osteoblast ferroptosis while enhancing osteoclast activity, ultimately leading to compromised bone mass. This previously unrecognized pathogenic mechanism not only advances our understanding of SLE-associated skeletal complications but also identifies ferroptosis as a potential therapeutic target. Further validation of these findings using additional lupus-prone models and clinical samples will strengthen their translational relevance and support the development of more effective therapeutic strategies for SLE-associated bone loss.

Ethics Approval

This research adhered to the principles outlined in the Declaration of Helsinki, and all experimental protocols were reviewed and approved by the Ethics Committee for Animal Research at Zhejiang Chinese Medical University (Approval No. IACUC-20211101-04).

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Disclosure

The authors report no conflicts of interest in this work.

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