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Ferroptosis Mechanism in the "Secondary Injury" Phase of Osteoporotic Fractures: From Laboratory to Perioperative Intervention

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Abstract: Delayed healing of osteoporotic fractures is a common and challenging clinical problem, traditionally attributed to insufficient local blood supply. However, recent years have seen increasing attention on the critical role of ferroptosis during the "secondary injury" phase of osteoporotic fractures. Ferroptosis damages chondrocytes through iron overload and lipid peroxidation, having a significant impact on bone repair. This article explores the molecular mechanisms of ferroptosis, focusing on the role of osteoclasts in secreting free iron and the impact of changes in GPX4 and FSP1 expression on ferroptosis regulation, highlighting the significance of ferroptosis chondrocyte subpopulations in fracture healing. It also evaluates the application potential and existing controversies of perioperative intervention strategies such as iron chelators and vitamin K2, discussing the development trends of bone-targeted iron chelating nanoparticles and rapid detection technologies for ferroptosis evaluation. This review aims to provide new theoretical bases and intervention ideas for the treatment of clinical osteoporotic fractures, promoting solutions to delayed fracture healing.

Keywords: Ferroptosis; GPX4; FSP1; Iron chelator (DFO); Delayed healing of osteoporotic fractures; Perioperative intervention

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1. Introduction

Osteoporotic fractures are a common and serious health issue among the elderly, with increasing incidence and associated burden due to population aging. Osteoporosis significantly reduces bone strength, making older patients more likely to fracture, especially in the spine, hip, and long bones. These fractures not only have high disability rates but also severely impact patients' quality of life and survival rates. Current clinical data show that about 30% of patients with osteoporotic fractures experience delayed healing or nonunion after treatment, leading to limited

functional recovery and increased complications, putting a strain on both patients and the healthcare system [1]. Traditional research on fracture healing mechanisms has mainly focused on insufficient local blood supply, poor mechanical stability, and bone metabolism disorders, but these factors fail to comprehensively explain the complex issues encountered in the healing of osteoporotic fractures.

In recent years, studies have found that factors such as apoptosis, oxidative stress, and abnormal iron metabolism during the "secondary injury" phase of fracture healing significantly affect healing quality. In particular, ferroptosis, a novel form of iron-dependent programmed cell death, has gradually gained attention for its role in bone tissue. Ferroptosis triggers lipid peroxidation through iron overload, leading to cell membrane damage and cell death, thereby affecting bone cell function and the bone remodeling process. Basic and clinical studies have revealed that an iron metabolism imbalance is closely related to osteoporosis and delayed fracture healing, where iron overload not only inhibits osteoblast activity but also promotes osteoclast activation, leading to enhanced bone resorption and bone structure degradation [2,3]. Additionally, oxidative stress plays an important role during the "secondary injury" phase of osteoporotic fractures, with antioxidants such as tanshinone IIA showing potential in protecting bone marrow mesenchymal stem cells from oxidative damage and promoting bone repair [4].

In 2023, a study first confirmed the involvement of ferroptosis in the mechanism of delayed healing of osteoporotic fractures, marking a new phase in research in this field. The study indicated significant changes in the activity of ferroptosis-related signaling pathways, affecting the survival of bone cells and the bone healing microenvironment, suggesting that ferroptosis may become a new target for the treatment of osteoporotic fractures [3]. However, current clinical guidelines do not cover the mechanisms of ferroptosis and its perioperative intervention strategies, and clinical awareness and application of ferroptosis-related treatments are still in the early stages.

2. Mechanisms of ferroptosis in delayed healing of osteoporotic fractures

2.1. Osteoclast-mediated release of free iron and chondrocyte injury

The delayed healing process of osteoporotic fractures is closely related to abnormal osteoclast activity. In osteoporotic conditions, osteoclast activity is significantly enhanced, leading to excessive bone resorption and a large release of free iron. Free iron promotes local iron overload, inducing lipid peroxidation reactions within chondrocytes, becoming one of the key factors for the occurrence of ferroptosis. The iron-dependent lipid peroxidation reaction is the core mechanism of ferroptosis, primarily manifested by the accumulation of lipid peroxides, which subsequently leads to cell membrane damage, resulting in cell dysfunction or even death.

Specifically, the excessive presence of free iron promotes the progression of lipid peroxidation reactions, leading to the downregulation of glutathione peroxidase 4 (GPX4) expression. GPX4, as an important intracellular antioxidant enzyme, can reduce lipid peroxides and protect the integrity of cell membranes; its reduced expression directly weakens the antioxidant capacity of chondrocytes, making them more susceptible to ferroptosis. The impaired function of chondrocytes hinders callus formation and bone repair, resulting in delayed fracture healing.

Multiple studies have confirmed the close relationship between iron overload and bone metabolism disorders. Iron generates a large amount of reactive oxygen species (ROS) through the Fenton reaction, exacerbating lipid peroxidation and inducing ferroptosis. Furthermore, iron overload may also exacerbate the development of osteoporosis by regulating the functional imbalance between osteoclasts and osteoblasts. The free iron released by osteoclasts not only directly affects chondrocytes but may also enhance inflammatory responses, indirectly

promoting bone resorption and bone loss, leading to a vicious cycle [5].

Therefore, osteoclast-mediated release of free iron induces chondrocyte ferroptosis by promoting lipid peroxidation reactions and downregulating GPX4 expression, which is one of the important mechanisms for delayed healing of osteoporotic fractures. This mechanism reveals the key role of ferroptosis in bone metabolism disorders, providing a theoretical basis and potential therapeutic targets for interventions targeting osteoclast activity and iron metabolism.

2.2. Changes in the expression and function of ferroptosis suppressor protein FSP1

Ferroptosis suppressor protein 1 (FSP1), as another important ferroptosis suppressor besides GPX4, has recently gained attention. A recent study reported that in an osteoporotic mouse model, FSP1 expression was significantly downregulated by about 40%. This finding suggests the important regulatory role of FSP1 in the processes of osteoporosis and fracture healing. FSP1 prevents ferroptosis by regulating the inhibition of lipid peroxidation reactions. The specific mechanism includes FSP1 acting as an NAD(P)H coenzyme-dependent reductase, promoting the reduction of coenzyme Q10 (CoQ10) to form reduced coenzyme Q10 (CoQ10H2), thereby capturing lipid peroxidation free radicals, inhibiting lipid peroxidation reactions, and maintaining cell membrane stability. The activity of FSP1 provides cells with an alternative defense pathway against ferroptosis beyond the GPX4 system, enhancing the cell's resistance to ferroptosis.

The downregulation of FSP1 expression leads to a weakened ability to inhibit lipid peroxidation, accelerating the occurrence of ferroptosis due to the accumulation of lipid peroxidation products. The significant reduction of FSP1 in osteoporotic mice further exacerbates the ferroptosis process in bone tissue cells, hindering bone repair and fracture healing. Additionally, the downregulation of FSP1 may lead to increased oxidative stress levels in the bone metabolic environment, promoting osteoclast activity and creating an unfavorable microenvironment for bone formation.

This mechanism not only deepens our understanding of the ferroptosis regulatory network but also offers new potential targets for treating delayed healing in osteoporotic fractures. Regulating FSP1 expression or inhibiting ferroptosis by activating the FSP1 pathway may become effective strategies to promote bone repair and improve osteoporosis [6].

2.3. Ferroptosis chondrocyte subpopulations and their impact on bone resorption

With the development of single-cell sequencing technology, significant breakthroughs have been made in the study of cellular heterogeneity during the delayed healing of osteoporotic fractures. Recent research has revealed a specific "ferroptosis chondrocyte subpopulation" that not only exhibits ferroptosis-related molecular characteristics but also secretes receptor activator of nuclear factor kappa-B ligand (RANKL), playing a key role in bone metabolism regulation.

RANKL is the main regulatory factor for osteoclast formation and activity, and its excessive expression promotes osteoclast activation and bone resorption. The RANKL secreted by the ferroptosis chondrocyte subpopulation enhances osteoclast activity, forming a positive feedback loop: increased osteoclast activity releases more free iron, promoting more chondrocytes to undergo ferroptosis, thereby producing more RANKL, exacerbating bone resorption and bone loss.

This finding provides a new perspective on cellular heterogeneity in delayed healing of osteoporotic fractures and reveals the complex interplay between ferroptosis and bone metabolism. Ferroptosis not only affects the

survival of bone cells as a mode of cell death but also regulates bone metabolic signals through specific cell subpopulations, influencing the balance between bone resorption and formation. This mechanism suggests that interventions targeting the ferroptosis chondrocyte subpopulation and its RANKL secretion may break the positive feedback loop of bone resorption and promote fracture healing. Future research could focus on the molecular characteristics and regulatory mechanisms of this subpopulation to develop more precise therapeutic strategies to improve the clinical prognosis of osteoporotic fractures [7].

3. Intervention strategies for ferroptosis mechanisms in the perioperative period

3.1. Local application and effects of the iron chelator DFO

Deferoxamine (DFO), a classic iron chelator, is widely used to treat iron overload-related diseases because of its strong ability to bind iron ions. In recent years, the potential application of DFO in the repair of osteoporotic fractures has attracted attention. A study published in 2023 in *Bioactive Materials* reported that continuous application of local sustained-release DFO gel at the fracture site for 7 days significantly increased callus density by 32%, effectively promoting the bone healing process. This local application of DFO effectively chelates excess free iron, reducing iron-catalyzed lipid peroxidation reactions, inhibiting ferroptosis in chondrocytes, thereby improving the microenvironment for bone repair and promoting the regeneration and repair of bone tissue.

Mechanistically, DFO inhibits ferroptosis by lowering the intracellular excess iron ion content, reducing the generation of iron-dependent free radicals, and blocking cell membrane damage caused by lipid peroxidation. Additionally, DFO may also activate the cell's antioxidant defense system, further alleviating oxidative stress damage and protecting bone cell function. The advantage of local application is that it avoids the risk of systemic iron deficiency, reducing interference with systemic iron metabolism, making it suitable for precise interventions at the fracture site during the perioperative period, maximizing the bone repair-promoting effect of DFO.

Moreover, drug delivery technologies for DFO are continuously innovating, such as using chitosan nanoparticles as carriers for sustained release, improving local drug concentration and duration of action, further enhancing the therapeutic effect and safety of DFO. These advancements provide new strategies and directions for intervening in the ferroptosis mechanism in osteoporotic fractures during the perioperative period, making DFO a key candidate for targeted treatment [8,9].

3.2. Protective role of vitamin K2 in activating the NRF2/GPX4 axis

Vitamin K2, a fat-soluble vitamin, has been shown to play a crucial role in bone metabolism and antioxidant activity. A study published in 2024 in *Bone Research* revealed that vitamin K2 can promote the expression of GPX4 by activating the nuclear factor erythroid 2-related factor 2 (NRF2) signaling pathway, significantly enhancing the antioxidant capacity of cells, thereby inhibiting the occurrence of ferroptosis and helping protect against delayed healing in osteoporotic fractures.

The specific mechanism is that NRF2, as an important transcription factor within cells, plays a central role in regulating antioxidant responses. Vitamin K2 can promote the nuclear translocation of NRF2, activating the expression of its downstream target gene *GPX4*. GPX4 is a key antioxidant enzyme in the ferroptosis process, capable of catalyzing the reduction of lipid peroxides, preventing lipid peroxidation chain reactions, and protecting cell membranes from damage. Through the activation of the NRF2/GPX4 axis by vitamin K2, intracellular oxidative stress levels are reduced, ferroptosis is effectively inhibited, and bone cell function is maintained,

promoting the bone healing process.

This mechanism provides a new therapeutic approach for non-iron chelator drug interventions in ferroptosis, especially suitable for the later stages of perioperative management and long-term management of osteoporosis patients. Compared to iron chelators, the application of vitamin K2 is safer, with fewer side effects, suitable for chronic use, and its multiple regulatory functions on bone metabolism and antioxidant activity further enhance its clinical value. Future development of vitamin K2 and its analogs is expected to become an emerging drug for intervening in the ferroptosis mechanism in osteoporotic fractures [10].

3.3. Controversies and challenges in perioperative iron metabolism regulation

Despite the important potential of iron metabolism regulation in intervening in the ferroptosis mechanism during the perioperative period, its clinical application still faces many controversies and challenges. First, systemic iron chelators like DFO can effectively reduce body iron load but may affect normal blood cell production, leading to adverse reactions such as anemia. Therefore, the risks and benefits must be weighed when applying them perioperatively to avoid excessive iron deficiency, causing other complications. Second, there are also differences in the choice of iron supplementation methods during the perioperative period. Intravenous iron can quickly correct iron deficiency but carries risks of allergic reactions and iron overload; oral iron supplements are convenient but have unstable absorption and more gastrointestinal side effects. Currently, there is a lack of unified clinical guidelines to clarify the best strategies for regulating iron metabolism during the perioperative period, resulting in considerable variations in practice. Additionally, perioperative patients often have complex conditions and variable iron metabolism states, making it urgent to accurately assess iron load and ferroptosis status and develop individualized intervention strategies. Future large-scale, multicenter clinical trials are needed to systematically evaluate the safety and efficacy of different iron metabolism regulation plans, clarify the optimal time window, dosage, and administration methods for ferroptosis intervention during the perioperative period, and integrate iron metabolism regulation into the comprehensive management system of the perioperative period to improve patient prognosis quality [11].

4. Future prospects and technological innovations in ferroptosis mechanism research

4.1. Development of bone-targeted iron-chelating nanoparticles

Using nanocarriers can effectively improve the stability and bioavailability of iron chelators. For example, functionalized polydopamine nanoparticles have good iron chelation ability and biocompatibility, enabling targeted delivery to bone tissue through surface modification. Their pH-dependent iron ion release characteristics are suitable for the acidic environment of fracture sites, promoting local inhibition of ferroptosis and improving the fracture healing process [12]. Additionally, nanoparticles mediating iron ion chelation can also synergistically scavenge reactive oxygen species, reducing oxidative stress and further protecting bone cells from ferroptosis damage [13]. Current research is also exploring iron-chelating nanosystems using biodegradable materials like chitosan, which not only have good drug-carrying capacity but can also achieve long-lasting treatment by regulating drug release rates [9,14]. In the future, developing multifunctional nanocarriers that simultaneously carry factors promoting bone formation and iron chelators is expected to become an innovative treatment method for the delayed healing of osteoporotic fractures. In summary, the development of bone-targeted iron-chelating nanoparticles represents a cutting-edge direction for ferroptosis intervention strategies. Achieving precise targeted

delivery and controlled release to bone tissue through nanotechnology not only enhances the therapeutic effects of iron chelators but also significantly reduces systemic side effects, holding great clinical translational potential and providing new ideas and methods for the treatment of osteoporotic fractures [15].

4.2. Application prospects of rapid test strips for ferroptosis evaluation in the perioperative period

Core biomarkers of ferroptosis include iron ion levels, lipid peroxidation products (such as malondialdehyde, 4-HNE), and the expression status of key regulatory proteins (such as GPX4, SLC7A11). By combining nanomaterials and biosensing technology, highly sensitive biosensors can be designed for rapid detection of these biomarkers. Existing studies have shown that magnetic and optical signal carriers like nano-ferrite particles have excellent performance in biological imaging and sensing fields [16], and this technology can be referenced for the development of rapid detection platforms. Combining artificial intelligence and big data analysis, a perioperative ferroptosis scoring system can integrate patient clinical data, biochemical indicators, and imaging information to achieve individualized risk assessment and treatment decision support [17]. Utilizing machine learning models for pattern recognition and prognosis prediction of ferroptosis-related indicators is expected to improve diagnostic accuracy and treatment safety. Furthermore, the portability and ease of use of rapid test strips make them suitable for operating rooms and intensive care environments, allowing real-time monitoring of patients' ferroptosis status and timely adjustment of treatment plans to reduce postoperative complication rates. In the future, with the continuous discovery of biological markers and advancements in nanosensing technology, the application prospects of rapid test strips for ferroptosis evaluation in the perioperative period are broad, becoming an important tool for promoting precise treatment of osteoporotic fractures during the perioperative period [17,18].

4.3. Multidisciplinary integrated research promoting in-depth analysis of ferroptosis mechanisms

Ferroptosis, as a novel regulatory mode of cell death, involves iron metabolism, lipid metabolism, oxidative stress, and various signaling pathways. Researching its complex biological characteristics and clinical relevance requires deep integration of multiple disciplines. The future development trend of ferroptosis mechanism research is to combine molecular biology, cell biology, bone metabolism, and clinical medicine to construct a systematic panorama of ferroptosis.

Cutting-edge technologies such as single-cell sequencing provide powerful means to reveal the heterogeneity of ferroptosis cells, allowing for the analysis of specific gene expression and regulatory networks in different cell subpopulations during ferroptosis [19]. Gene editing technologies (such as CRISPR/Cas9) can precisely intervene in key regulatory factors to verify their functions in the ferroptosis process, advancing mechanism research towards in-depth exploration of causal relationships [20].

At the same time, research on the association between ferroptosis and osteoporosis, fracture healing, and perioperative complications requires close integration of clinical data and basic research. For example, analyzing molecular biomarkers from clinical samples, combined with imaging and functional assessments, can reveal the specific mechanisms of ferroptosis in skeletal pathology, providing scientific evidence for clinical diagnosis and treatment [21].

Multidisciplinary integration can also promote the development of new therapeutic strategies, such as drug screening based on ferroptosis mechanisms, design of nanoparticle drug delivery systems, and formulation of

precise intervention plans during the perioperative period. Cross-disciplinary collaboration will drive the clinical translation of ferroptosis research results, accelerate innovations in the treatment of osteoporotic fractures, and improve patient prognosis and quality of life [22]. In summary, through multidisciplinary integration and advanced technology applications, the in-depth analysis of ferroptosis mechanisms will continue to advance, potentially providing a new theoretical basis and treatment strategies for the prevention and treatment of osteoporotic fractures, helping translate basic research into clinical use.

5. Conclusion

An important role of ferroptosis mechanisms in the delayed healing of osteoporotic fractures marks a significant shift in fracture repair research. Traditionally, delayed fracture healing has been attributed mainly to factors such as insufficient blood supply, while discovering ferroptosis adds a new perspective to this complex process, showing how abnormal iron metabolism and ferroptosis signaling pathways critically affect bone tissue repair. This enriches the theoretical framework of bone metabolism regulation. It also offers new targets and strategies for clinical diagnosis and treatment. Looking ahead, advancing the clinical application of ferroptosis mechanisms needs teamwork across multiple disciplines. First, accelerating the development of bone-targeted drugs using nanotechnology and biomaterials for precise drug delivery and controlled release; second, developing detection technologies for ferroptosis that are rapid and sensitive, combining molecular markers with imaging techniques for real-time monitoring and assessment of the fracture healing process. Furthermore, closely integrating basic research with clinical trials will help reconcile different viewpoints in various studies and find the most valuable intervention strategies for clinical use. Multi-center, large-sample clinical studies will provide strong evidence for the safety and efficacy of ferroptosis-related treatments, helping to include them in standard treatment for osteoporotic fractures.

Disclosure statement

The authors declare no conflict of interest.

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