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Original Article

Local administration of IKK small molecule inhibitor may enhance fracture healing in osteoporosis patient

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Abstract: Osteoporosis is an inflammatory bone disease affecting millions of population worldwide, which often cause increased fracture risks and prolonged fracture healing. Growing evidence suggests that IKK-NF-κB signaling exert inhibitory influence on MSCs osteogenic differentiation and bone formation. Moreover, enhance the fracture healing process in osteoporosis patient. In the current work, IKK-NF- κB differentiated osteoblasts. Thus, manipulating local inflammatory IKK-NF-κB signaling was also found to suppress the anabolic effect of signaling in osteoporotic related fracture emerge as a promising therapy to we hypothesized to use locally delivered IKK small molecule inhibitor to augment the impaired fracture healing ability in osteoporosis patient via enhancing both MSCs osteogenic differentiation and osteoblast function.

Keywords: IKK inhibitor, osteoporosis, fracture healing, NF-κB signaling

Introduction

Osteoporosis is a sever bone disease characterized by low bone mass and microarchitectural deterioration of bone structure, resulting in bone fragility and an increase in susceptibility to fracture. In the United States alone, annually more than ten million people were affected by osteoporosis. Also, osteoporosis is an important cause of morbidity and mortality [1, 2]. Especially, osteoporosis related fracture remains a major public health concern throughout the world. Although the etiology of osteoporotic fractures is multifactorial, low bone mineral density (BMD) has been identified as one of the primary predictive risk factors [3-5]. Moreover, senior citizens have a 5- to 8-fold increased risk for all-cause mortality during the first 3 months after osteoporotic hip fracture [6]. Generally in osteoporotic pathology, the mineralization and the acid phosphate content of osteoporotic tissues is decreased [7, 8], which subsequently affects bone microarchitecture. Cross-linking of subchondral bone is decreased with a thinning of trabeculae from resorption, resulting in fewer and thinner connections. Reduction in the bone mass narrows the tolerable loading directions, which in turn may increase the fracture risk [8].

The transcription factor nuclear factor kappa B (NF-κB) is a major regulator of inflammation and host immune responses which can be activated by proinflammatory cytokines such as TNF and interleukin-17 (IL-17), LPS, and viral DNA in cases of inflammatory diseases and tissue injuries [9-14]. The IκB kinase (IKK) complex plays an essential role in NF-κB activation by phosphorylating and degrading IκBs [9-14]. Growing evidence suggests that proinflammatory cytokines exert inhibitory influence on MSCs osteogenic differentiation and bone formation [15-21]. Thus, to enhance fracture repair in inflammatory bone disease such as osteoporosis, it will likely be necessary to overcome inflammation- mediated inhibition of bone formation. Recently, Jia et al found that IKK-NF-κB signaling in differentiated osteoblasts has an anti-anabolic effect on bone formation. Furthermore, inhibition of IKK-NF-κB in differentiated osteoblasts significantly enhanced bone matrix formation and mineral density during postnatal bone growth [15, 17]. Thus, manipulating inflammatory IKK-NF-κB signaling holds great

promise to enhance the fracture healing process in osteoporosis patient.

Hypotheses

Local administration of IKK small molecule inhibitor in the fracture gap during open reduction surgery may enhance the post-operative fracture healing in osteoporosis patients by suppressing inflammation induced bone loss and promoting local bone formation. Both osteoblast function and MSCs osteogenic differentiation could be enhanced by locally inhibited IKK-NF-κB signaling which may augment the impaired fracture healing ability of osteoporosis patients.

Evaluation of our hypotheses

Fracture healing is a complex physiological process involves the coordinated participation of hematopoietic and immune cells within the bone marrow in conjunction with vascular and skeletal cell precursors, including mesenchymal stem cells (MSCs) recruited from the surrounding tissues and the circulation. Multiple factors regulate this molecular cascade by interacting with the osteoblast and chondroblast lineage through various processes namely migration, proliferation, chemotaxis, differentiation and extracellular protein synthesis. However, many scholars revealed that, under osteoporotic condition, fracture healing strength is impaired. Li and colleagues demonstrated that the phenotype of the cells associated with bone formation is altered in osteoporotic bone and reported abnormal calcified tissue within the fracture callus of osteoporotic fracture models [22]. By investigating the impact of aging and ovariectomy on the healing of femoral fractures in a osteoporotic rat model, Meyer et al reported that both aging and ovariectomy significantly compromise the process of fracture healing in female rats as judged by measurements of rigidity, breaking load and excessive mineral accumulation into the fracture callus [23]. Furthermore, Namkung et al showed that the bone loss in the early phase of fracture healing in rat osteoporotic model significantly reduced the fracture callus size, BMD, and mechanical strength, which is indicative of early failure of the repair process [24]. Accordingly, as aiming to evaluate the influence of osteoporosis in the middle and late periods of fracture healing in rat osteoporotic models, Wang et al found a lower callus bone mineral density and callus failure stress under osteoporotic condition. They observed that endochondral bone formation was delayed, while newly formed trabeculae were loosely and irregularly arranged, demonstrating an histomorphological impairment of fracture healing [25]. Similar findings were revealed by Qiao who reached the conclusion that fracture healing in the presence of osteoporosis results in compromised bone quality [26].

The mechanism of osteoporosis is still unclear. Yet many evidences pointed to the differentiation of Mesenchymal stem cells (MSCs) and the subsequent balance of osteogenic and adipogenic lineages. In osteoporosis scenario, bone formation decreases possibly because osteoblasts decrease with age [27]. Osteoblasts originate from MSCs residing in bone marrow together with hematopoietic stem cells [28, 29]. These two stem cell types cooperate through direct cell-to-cell interactions and release of cytokines and growth factors [30, 31]. Since osteoblast numbers might relate to progenitor numbers, D'ippolito and colleagues hypothesized that the number of MSCs (with osteogenic potential) residing in the bone marrow of high turnover rate could be associated with age-related osteoporosis [32]. They concluded that the bone-marrow micro-environment alters with age, resulting in cell-to-cell and cell-to-matrix interactions that may be unfavorable for MSCs proliferation, however, in turn favors MSCs differentiation toward adipogenic lineage. Moreover, an inverse relationship between marrow adipocytes and osteoblasts has been noticed with aging [28, 33]. Supportively, Ye et al confirmed this osteogenic and adipogenic inverse relationship by depletion of histone demethylases KDM4B and KDM6B [34]. In accordance, in osteoporotic patients, decreased trabecular bone volume is usually accompanied by increased bone marrow adipose tissue [16]. Early histomorphometric observations suggested that adipose replacement of the marrow functional cell population stands for one cause of the change in bone cell dynamics which contributing to osteoporosis [35]. These findings suggest that the tendency to the adipocyte differentiation pathway occurs at the expense of osteoblast numbers and osteogenic function [16], which may contribute to the decrease in bone volume, and hence

mechanical strength and may also negatively affect bone formation during fracture healing [36]. Also, Bergman et al demonstrated that defects in the number and proliferative potential of MSCs may lead age-related defects in osteoblast number and function [37]. Similar finding has been reported from clinical settings, Rodriguez's team demonstrated that MSCs derived osteoporotic postmenopausal women exhibit differential mitogenic response to IGF-1 with diminished ability to differentiate into the osteogenic lineage, suggest that osteoporotic MSCs have a compromised ability to produce mature bone forming cells [38]. Thus, both clinical and in vitro observations document an inverse relationship between adipogenic and osteogenic lineages. As a result, searching of effective therapy to strengthen the fracture healing of osteoporotic patient is of practical urge.

In the recent decades, chronic inflammation has been found to be associated with osteoporosis and aging-related bone loss [20, 39, 40]. In general, NF-κB signaling is activated during inflammatory processes [11]. Growing evidence suggests that NF-κB plays an indispensable role in aging-related disorders, including aging-related bone loss and osteoporosis [17, 41-43]. Of note, in 2009, Jia et al demonstrated the inhibition of endogenous IKK/NF-κB signaling in differentiated osteoblasts significantly strengthens trabecular bone mass and bone mineral density in young mice [15]. Since gene knockout of major IKK/NF-κB components results in embryonic lethality, they make use of the osteoblast-specific bone gamma carboxyglutamate protein 2 (Bglap2) promoter to drive the dominant negative mutant of IKK-γ in mature osteoblasts in mice in an attempt to address whether NF-κB regulates mature osteoblast function without affecting osteoblast differentiation. Of interest, their datum showed that the inhibition of IKK/NF-κB in differentiated osteoblasts maintains bone formation and prevents osteoporotic bone loss induced by ovariectomy (OVX) in adult mice model. Furthermore, Krum's team reported that the inhibition of IKKβ can suppress inflammatory bone loss by inhibiting osteoclast formation in arthritis animal model [17]. Taken together, these findings strongly indicate IKK/NF-κB signaling plays not only a negative role of in the regulation of mature osteoblast function, but also a positive role in osteoclast regulation both of which renders great promise to local delivered IKK small molecule inhibitor to augment the bone formation and limit the local bone resorption in osteoporotic fracture healing. On the other hand, IKK/NF-κB signaling was also reported to inhibit in vitro osteogenic differentiation of MSCs. Jia et al demonstrated that proinflammatory cytokines TNF and IL-17 stimulated IKK-NF-κB signaling while substantially impaired in vitro osteogenic differentiation of MSCs [44]. Moreover, they reported the presence of IKK small molecule inhibitor, IKKVI, largely enhanced osteogenic differentiation of MSCs. This finding shows the IKK-NF-κB signaling also downregulate the osteogenic differentiation of MSCs suggesting that local administration of IKK small molecule may also aid the osteoporotic fracture healing by promoting more robust local MSCs osteogenic differentiation which may substantially elevate the local osteoblast number in the fracture healing wound with augmented bone formation ability.

Conclusion

In summary, the above mentioned research findings show that IKK-NF-κB signaling plays an important role in regulating osteoclast and osteoblast function and MSCs differentiation. IKK-NF-κB signaling suppress the mature osteoblast function while enhancing osteoclast bone resorption function. Also, IKK-NF-κB signaling inhibit the osteogenic differentiation of MSCs which may even weaken the bone formation power in osteoporosis patient. Accordingly, the local administration of IKK small molecule inhibitor in the fracture gap during open reduction surgery in osteoporotic fractures may overcome all these negative facts and hold great promise to provide strengthened post-operative fracture healing ability especially in osteoporosis fracture cases.

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Disclosure of conflict of interest

None.

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