REVIEW

Effects of myokines on bone

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The links between muscle and bone have been recently examined because of the increasing number of patients with osteoporosis and sarcopenia. Myokines are skeletal muscle-derived humoral cytokines and growth factors, which exert physiological and pathological functions in various distant organs, including the regulation of glucose, energy and bone metabolism. Myostatin is a crucial myokine, the expression of which is mainly limited to muscle tissues. The inhibition of myostatin signaling increases bone remodeling, bone mass and muscle mass, and it may provide a target for the treatment of both sarcopenia and osteoporosis. As myostatin is involved in osteoclast formation and bone destruction in rheumatoid arthritis, myostatin may be a target myokine for the treatment of accelerated bone resorption and joint destruction in rheumatoid arthritis. Numerous other myokines, including transforming growth factor-β, follistatin, insulin-like growth factor-I, fibroblast growth factor-2, osteoglycin, FAM5C, irisin, interleukin (IL)-6, leukemia inhibitory factor, IL-7, IL-15, monocyte chemoattractant protein-1, ciliary neurotrophic factor, osteonectin and matrix metalloproteinase 2, also affect bone cells in various manners. However, the effects of myokines on bone metabolism are largely unknown. Further research is expected to clarify the interaction between muscle and bone, which may lead to greater diagnosis and the development of the treatment for muscle and bone disorders, such as osteoporosis and sarcopenia.

Introduction

Muscles exert motor function by their contraction under neuronal regulation. Moreover, muscles regulate glucose metabolism through glucose uptake as a target of insulin. It is well-known that adipose tissues influence systemic metabolic control and bone metabolism through adipocytokines, such as leptin, adiponectin and tumor necrosis factor-α. Recently, accumulating evidence suggests that bone tissues affect glucose and energy metabolism as an endocrine organ. Muscles affect various anatomically distant organs, by exhibiting biological effects on target organs in an endocrine manner, by secreting myokines.

Many researchers have recently examined muscle/bone relationships in skeletal tissues, as muscle and bone are anatomically adjacent to each other. The influences of muscle tissues on bone cells may be equally as important as those of bone on muscle cells. In this review, I will summarize recent findings concerning the effects of myokines on bone.

Interaction between Muscle and Bone

Osteoporosis and sarcopenia are age-related systemic and progressive disorders, which impair the activities of daily living and quality of life in elderly people. Sarcopenia is characterized as systemic and progressive decreases in skeletal muscle mass, strength and function.[1] Numerous studies indicate that an increase in muscle mass is positively associated with an increase in bone mineral density (BMD), and a reduction in fracture risk.[2] Our previous study suggested that lean body mass (muscle mass), measured by dual-energy x-ray bone absorptiometry, is positively associated with BMD in the lumbar spine and femoral neck of postmenopausal women.[3] Moreover, several studies have suggested that muscle strength and function are correlated with BMD and fracture risk.[2]

Several factors simultaneously affect muscle and bone,[2,4] including genes, nutrition, mechanical stress, inflammation and endocrine factors, such as vitamin D, growth hormone and androgens. In the pathological state, muscle wasting and osteoporosis are observed in patients with glucocorticoid excess and diabetes. In addition, calcium ions are critical for both muscle contraction and bone formation. Clinical evidence suggests that there are interactions between muscle and bone, which may have physiological and pathological roles in humans.

Systemic humoral factors produced from muscle or bone tissues affect each other. Bone-derived factors such as sclerostin and osteocalcin affect muscle cells as humoral factors.[2,4] Weight-loss-induced increase of serum sclerostin

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levels is prevented by exercise in obese older adults, and changes in serum sclerostin levels were negatively correlated with muscle mass.[5] Moreover, trabecular bone volume and muscle mass were increased and decreased in sclerostin-deficient mice, respectively.[6] As canonical Wnt signaling regulates muscle regeneration and differentiation, sclerostin might affect skeletal muscles,[4] although the direct effects of sclerostin remained unclear. Concerning the effects of muscle on bone, muscle-derived local factors are considered to positively affect osteoblastic bone formation, as muscle supports fracture healing and osteoblastic differentiation.[2,4] Moreover, muscles influence bone through direct mechanical impact and an increase in vessel blood flow. In addition, numerous humoral factors, produced in muscle, affect distant bone tissues through the bloodstream.

Myokines

Intracellular organelles, such as the endoplasmic reticulum, are richly expressed in the cytoplasm of muscle cells, and muscle tissues produce a large amount of proteins, which may have a physiological role. Myokines are skeletal muscle-derived cytokines and growth factors. Myokines have various physiological roles, including the regulation of glucose metabolism, vascularization and bone metabolism.[7] As the largest organ in the body, muscle is related to endocrine regulation of distant organs, such as bone, through the release of myokines into the bloodstream.

As shown in **Figure 1**, there are various kinds of muscle-derived humoral factors, including myostatin, transforming growth factor-β (TGF-β), bone morphogenetic proteins (BMPs), activin, follistatin, insulin-like growth factor (IGF)-I, fibroblast growth factor (FGF)-2, osteoglycin, FAM5C, irisin, interleukin (IL)-6, IL-7, IL-15, monocyte chemoattractant protein (MCP)-1, ciliary neurotrophic factor (CNTF), osteonectin and matrix metalloproteinase (MMP)-2. Here, I will describe the effects of these myokines on bone.

Myostatin

Myostatin, one of the TGF-β superfamily proteins, is a well-known myokine and potent suppressor of skeletal muscle mass and development, which is expressed mainly in skeletal muscle tissues. It binds to the activin receptor type IIB (ACVR2B), leading to the activation of activin-like kinase (ALK)4/5, and then phosphorylates Smad2 and Smad3, the TGF-β-specific Smads. Myostatin-deficient mice exhibit skeletal muscle hypertrophy,[8] and myostatin-overexpressing mice show a decrease in muscle mass.[9] There is also a negative correlation between exercise-induced muscle hypertrophy and myostatin expression.[10] These findings indicate that myostatin is a negative regulator of skeletal muscle mass. On the other hand, myostatin deficiency led to improved gains in bone strength with exercise in mice.[11] Moreover, immobilization, microgravity and inflammation enhance muscle and blood myostatin levels, which might be related to muscle wasting in these pathological states. With regard to the effects of myostatin on bone, osteogenic differentiation and *in vitro* calcification of bone marrow-derived mesenchymal stem cells are increased in myostatin-null mice,[12] suggesting that myostatin negatively affects osteoblast differentiation. In contrast, myostatin

enhances receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclast formation through Smad2-dependent regulation of the nuclear factor of activated T cells (NFATc1).[13] Indeed, myostatin enhanced RANKL-induced expression of osteoclast-related genes, including integrin $\alpha_V\beta_3$, DC-STAMP and calcitonin receptor in the same study. In addition, an *in vitro* study indicated that the paracrine and autocrine actions of myostatin are important for osteoclast differentiation. These findings suggest that myostatin exerts negative effects on bone mass through decreased bone formation and enhanced resorption.

Myostatin and its signaling might be a crucial target molecule for the treatment of both sarcopenia and osteoporosis. A clinical study found that myostatin gene polymorphism is associated with BMD,[14] and genome-wide association studies suggested that the myostatin gene is related to both osteoporosis and sarcopenia.[15] ACVR2B-Fc, a soluble myostatin decoy receptor, suppresses the binding of myostatin, some BMPs and activin to ACVR2B. Systemic administration of ACVR2B-Fc enhances hind limb skeletal muscle weight in osteogenesis imperfecta mice.[16] In addition, ACVR2B-Fc enhances muscle mass and bone formation indices in postmenopausal women.[17] These findings suggest that inhibiton of ACVR signaling might be effective for the treatment of both osteoporosis and sarcopenia. However, ACVR is a receptor for activin, which has numerous essential roles in various tissues. An inhibition of ACVR signaling might cause unexpected side effects in humans. Indeed, a recent study using ACVR2B-Fc in boys with muscular dystrophy was suspended because of the development of unexpected gum bleeds and nose bleeds.[18] On the other hand, Arounleut *et al.*[19] reported that myostatin propeptide-Fc, a myostatin inhibitor, did not affect BMD and bone strength in aged mice, although it increased muscle mass. These findings suggest that pharmacological inhibition using myostatin neutralization is not sufficient for the treatment of osteoporosis in mice.

The production of myostatin in tissues other than muscles has recently been reported. Myostatin is expressed during fracture repair and negatively regulates fracture callus size.[20] Dankbar *et al.*[13] revealed that myostatin is highly expressed in the synovial tissues of patients with rheumatoid arthritis (RA) and a mouse model of RA. Myostatin deficiency or inhibition with an antibody caused an improvement in arthritis, as well as increased grip strength and less bone destruction in a mouse model of RA.[13] These findings suggest that myostatin is involved in osteoclast formation and bone destruction in RA, and that myostatin may be a target myokine for the treatment of RA-associated accelerated bone resorption and joint destruction (**Figure 2**). Future studies are likely to further elucidate the therapeutic potential of myostatin inhibition.

The contributions of myostatin's direct action and its muscle mass-mediated secondary action on bone cannot be clearly discriminated at present, and both actions might be involved in its effects on bone.

TGF-β Superfamily Molecules

TGF-β superfamily molecules other than myostatin are produced from muscle tissues. BMPs and TGF-β are important regulators of bone and cartilage formation.[21] These factors, released from muscle tissues, have physiological roles as
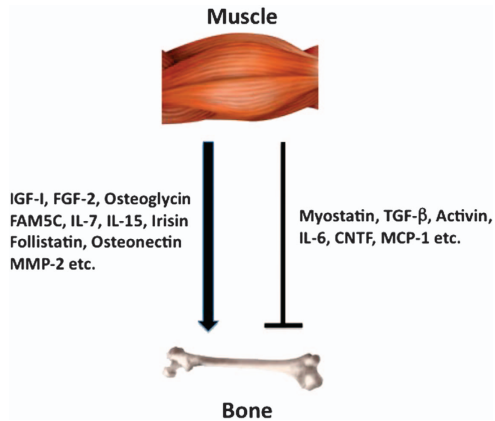
Figure 1 Various myokines affect bone. Numerous muscle-derived factors (myokines) positively or negatively affect bone metabolism, which may have physiological and pathological roles in the interactions between muscle and bone.
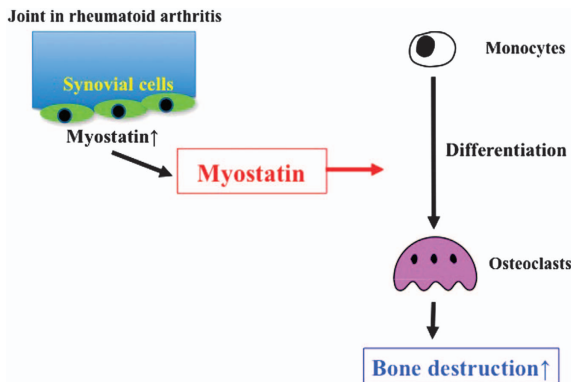

Figure 2 Role of myostatin in joint and bone destruction of rheumatoid arthritis. Exposure of synovial fibroblasts to inflammatory cytokines causes the upregulation of myostatin in patients with RA. Myostatin enhances RANKL-induced osteoclast differentiation in autocrine and paracrine manners. Myostatin stimulates osteoclast formation through increased Smad2-dependent nuclear translocation of NFATc1 and subsequent upregulation of osteoclast differentiation genes.[13]

Figure 3 Role of irisin in the linkage of muscle to bone. Irisin is a myokine produced from skeletal muscle after exercise. It induces a browning response in white adipose tissues and protects from insulin resistance. In bone, irisin increases osteoblast differentiation through canonical Wnt-β-catenin, p38 MAPK and ERK pathways. On the other hand, irisin suppresses osteoclast differentiation through the suppression of RANKL/NFATc1 pathways.

, paracrine and endocrine factors. BMP-2 and BMP-4, especially, are crucial for the maintenance of osteogenesis. Loss of Smad1/5/8, BMP-specific Smads, induces severe chondrodysplasia and decreased bone formation. As BMPs are mainly produced from bone tissues, the significance of BMPs produced from muscle remains unknown.

Activin, a ligand for ACVR2B, which is a receptor of myostatin, is expressed in most tissues including muscle and bone. Activin has been shown to enhance osteoclast formation *in vitro*.[22] Recent findings suggest that activin negatively affects BMP-induced osteoblastic differentiation, although an enhancement of osteoblast differentiation by activin has previously been reported.[23] The impact of local activin may be more important than that of activin as a humoral factor in both muscle and bone.

Follistatin increases muscle mass and muscle regeneration after injury presumably by antagonizing activin and/or myostatin-induced phosphorylation of Smad2/3 and subsequent ACVR signaling.[24] In addition, follistatin might modulate bone metabolism by affecting activin and myostatin signaling in bone cells. Low-magnitude mechanical stress positively affects both muscle and bone through a periosteum
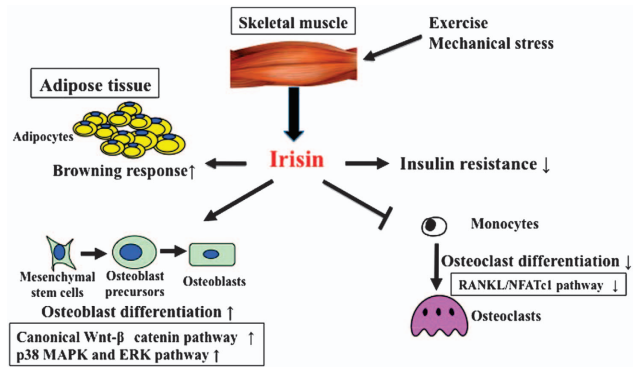
direction-dependent permeability-related mechanism.[25] Muscle wasting and osteoporosis are serious problems in immobilization. These findings suggest that mechanical stress influences both muscle and bone.[2,4] Muscle wasting and osteopenia is observed after long durations of space flight in astronauts.[26,27] Our preliminary study suggested that follistatin may be a myokine produced from muscle in response to gravity change, and further study is currently in progress in our laboratory (Manuscript in preparation).

Irisin

Irisin is a novel myokine produced from skeletal muscle following exercise, and it is characterized by the induction of a browning response in white adipose tissues. Exercise enhances the expression of irisin in muscle tissues through a PGC-1α-dependent mechanism.[28] Irisin is considered to be protective against insulin resistance, metabolic syndrome and cardiovascular disease in humans.[29] These findings suggest that irisin may be a myokine, which is important for metabolic regulation (**Figure 3**).

In bone, irisin increases osteoblast differentiation through the Wnt-β-catenin pathway in MC3T3-E1 cells.[30] Moreover, Qiao *et al.*[31] revealed that irisin enhances proliferation, differentiation, alkaline phosphatase activity and mineralization in cultured osteoblasts through the activation of p38 mitogen-activated protein kinase and extracellular signal-regulated kinase. On the other hand, irisin suppresses osteoclast formation through the inhibition of RANKL/NFATc1 in RAW264.7 cells. In an *in vivo* study, recombinant irisin administration increased cortical, but not trabecular, bone mass and strength primarily through the stimulation of bone formation, but with a parallel reduction in osteoclast number in male mice.[32] In the same study, irisin treatment upregulated the levels of osteogenic genes, including ATF4, Runx2, Osterix, low-density lipoprotein-related protein 5, β-catenin, alkaline phosphatase and type I collagen in bone marrow stromal cells (**Figure 3**). In humans, a previous study showed that serum irisin levels are correlated with the prevalence of osteoporotic fractures in postmenopausal women with osteopenia.[33] Loffler *et al.*[34] reported that acute strenuous exercise but not long-term elevations in physical activity increases serum irisin levels in children and adults.

These findings suggest that irisin might be a useful marker for the assessment of muscle/bone disorders and metabolic disease. However, the importance of serum irisin in humans has recently been questioned. Serum irisin levels were measured by enzyme-linked immunoassays using antibodies with poor specificity. Moreover, the start codon of the human irisin-coding gene, membrane protein fibronetin type III domain containing protein 5 (FNDC5), is mutated, resulting in translation with very low efficiency to the protein.[35] On the other hand, a study by Jedrychowski et al. used a quantitative mass spectrometry and unbiased assay for the detection of human irisin in plasma to show that human irisin is regularly translated from its non-canonical start codon.[36] This study claimed the importance of human irisin, and found that its synthesis was increased by physical activity. Further studies are necessary to determine whether irisin would be a useful diagnostic marker and therapeutic target for clinical use.[37]

Osteoglycin and FAM5C

Fibrodysplasia ossificance progressiva (FOP) is a genetic disease, which is characterized by progressive heterotopic ossification in skeletal muscle and caused by a mutation that results in constitutive activation of BMP-type I receptors (ALK2).[38] We speculated that the gene change suppressed by the conversion of muscle into bone might give us a clue to find muscle-derived humoral bone anabolic factors. We therefore extracted osteoglycin and FAM5C based on comprehensive microarray analysis between FOP-causing mutation-transfected mouse myoblastic C2C12 cells and controls. Osteoglycin is the seventh member of the small leucin-rich proteoglycan family. It belongs to class III of the small leucin-rich proteoglycans. Osteoglycin is included in the mechanosensitive genes that mediate the bone anabolic response to mechanical loading in mice.[39] In addition, osteoglycin is one of the secretome components that is differentially upregulated during skeletal myogenesis in mouse myoblastic C2C12 cells.[40] We reported that osteoglycin decreased the expression of early-stage osteoblast differentiation genes, such as Runx2 and Osterix in mouse osteoblasts, but enhanced mineralization and the expression of late-stage osteoblast phenotype-related genes, such as alkaline phosphatase, type I collagen and osteocalcin.[41] A reduction in endogenous osteoglycin using siRNA exerted the opposite effects to those of osteoglycin overexpression and recombinant osteoglycin addition in osteoblasts. Moreover, osteoglycin suppressed osteoblastic differentiation induced by BMP-2 in mouse mesenchymal C2C12 cells. These findings suggest that osteoglycin is a factor that inhibits osteoblast differentiation of premature osteoblasts and enhances osteoblast phenotype in well-differentiated osteoblasts. The conditioned medium from osteoglycin-overexpressed myoblasts enhanced late osteoblast differentiation, and osteoglycin was detected in cultured supernatant from myoblasts and human serum.[41] We further revealed that osteoglycin enhances TGF-β-specific Smad3/4-responsive transcriptional activity, as well as the expression of type I collagen independently of TGF-β as one of the mechanism of osteoglycin effects in osteoblasts.[41] These findings suggest that osteoglycin is one humoral bone anabolic factor produced from muscle. Our recent study revealed that active vitamin D and its derivative eldecarcitriol increase the production of osteoglycin in myoblasts, and then osteoglycin increased osteoblast differentiation as a muscle-derived humoral factor in vitro.[42]

FAM5C is associated with cell proliferation, migration and atherosclerosis. In our study, FAM5C overexpression, as well as the conditioned medium from FAM5C-overexpressed myoblasts, enhanced the osteoblast phenotype of differentiated osteoblasts, but suppressed osteoblastic differentiation induced by BMP-2 in mouse myoblastic cells.[43] FAM5C is also detected in human serum. These findings suggested that FAM5C might be a muscle-derived humoral factor.

Interleukins

Numerous interleukins and chemokines are produced from muscles following exercise. IL-6 is abundantly expressed in muscle, and muscle cells produce and release IL-6 in response to exercise and muscle contraction.[44] It is well-known that IL-6 affects bone metabolism, as well as glucose and energy metabolism. IL-6 stimulates bone resorption in concurrence with the soluble IL-6 receptor, and it might be related to the pathogenesis of postmenopausal osteoporosis.[45] Mechanically loaded myotubes enhance osteoclast formation via IL-6 release in vitro.[46] On the other hand, IL-6 enhances osteoblast differentiation at the early stage and IL-6-deficient mice exhibit low bone mass,[44] suggesting that IL-6 may partly be a positive regulator of osteoblastic bone formation. However, controversy exists about the effects of IL-6 on osteoblast differentiation. Although IL-6 is expressed in various cells, including hematopoietic cells, stromal cells and osteoblasts, it may be related to bone metabolism by an enhanced release from muscle tissues in response to exercise and inflammation pathologically.

LIF is released from muscle tissue and regulates bone formation, although the controversy exists about the effects of LIF on osteoblast differentiation.[47] Local effects may be greater on bone cells, compared with its effects as a myokine.

IL-7 influences both osteoblasts and osteoclasts.[48] Although muscle-derived IL-7 might modulate bone metabolism, Aguila et al. reported that osteoblast-specific overexpression of IL-7 rescues osteopenia in IL-7-deficient female mice.[48] These findings suggest that osteoblast-derived IL-7 is important for the regulation of bone mass.

IL-15 overexpression in muscle tissue, as well as systemic elevation of IL-15 levels, leads to an increase in bone mass, although it decreases visceral and body fat masses,[49] suggesting that IL-15 may be a myokine, which affects both bone and fat metabolism.

CNTF is part of the IL-6 family of cytokines. CNTF-deficient female mice exhibit larger bone mass and activated osteoblast activity.[50] Johnson et al.[51] reported that CNTF, abundantly expressed in mouse muscle tissues, suppresses osteoblast differentiation in vitro. Therefore, CNTF may be a negative regulator of bone formation as a myokine. The existence of its soluble receptor, sCNTFR, is necessary for the full suppressive action of CNTF in osteoblast differentiation, similar to the action of IL-6, and the level of CNTFR is elevated as the differentiation of osteoblast progresses.[50]

A cytokine array of mouse myotube (C2C12)-conditioned medium identified MCP-1 (CCL-2) as one of the most abundant myokines. MCP-1 is a chemokine and a chemoattractant for

osteoclast precursors and monocytes/macrophage. A recent study indicated that MCP-1 is involved in bone anabolic action by parathyroid hormone in mice.[52] Although the significance of MCP-1 in its physiological state in bone is unknown, MCP-1 may have significant roles in altering bones, in the pathological and pharmacological states.

IGF-I and FGF-2

IGF-I, which is highly expressed in muscle, is a crucial growth factor for muscle growth, as well as bone development and the preservation of bone mass. The application of mechanical loading stimulates the expression of IGF-I in skeletal muscle. IGF-I enhances bone remodeling through the acceleration of osteoblastic bone formation and osteoclastic bone resorption.[2,53] Several studies indicated that serum IGF-1 levels are positively associated with BMD and a predictive factor for osteoporotic fractures. Thus, the IGF-I pathway is crucial for the maintenance of bone metabolism. Although both circulating and bone-derived IGF-I proteins are physiologically necessary for bone metabolism, muscle-derived IGF-I may also have a role in the regulation of bone remodeling for a large volume of muscle in the body. IGF-binding proteins (IGFBPs) are related to the action of IGF-I. IGFBPs, such as IGFBP-2 and IGFBP-5, are produced in muscle tissues. There are conflicting reports about the effects of IGFBP-2 and IGFBP-5 on bone metabolism. Although IGFBP-2 exerts negative effects on IGF-I-mediated bone anabolic action dependently and independently of IGF-I, a human cohort study revealed that serum IGFBP-2 levels were the most robust negative predictors of muscle mass, when muscle mass was positively related to trabecular BMD.[54] IGFBP-5 also supports bone anabolic action dependently and independently of IGF-I, although the roles of muscle-derived IGFBP-5 in bone remain unknown.

FGFs exert various cellular functions such as cell proliferation. FGF-2, an effective growth factor to enhance bone formation, fracture repair, bone/cartilage regeneration and chondrogenesis, is released from muscle tissues after injury and strong exercise.[55] FGF-2 is abundantly produced in various tissues, including muscle and bone. Although muscle-derived FGF-2 might have some role in bone metabolism physiologically, bone-derived FGF-2 is more important as a paracrine factor.

Other Myokines

Osteonectin, a 43-KDa extracellular noncollagenous bone matrix glycoprotein, is involved in cell differentiation, vessel formation and growth factor binding. A previous study revealed that osteonectin levels were increased in plasma and muscle tissue of human and mice after exercise.[56] Osteonectin is abundantly expressed in bone, and osteonectin-deficient mice exhibit severe osteopenia with decreased trabecular connectivity and bone mineral content, suggesting that osteonectin is a positive regulator of bone formation.[57]

MMP-2 is abundant in both muscle and bone. Its secretion from muscle cells is elevated with exercise and mechanical load,[55] and MMP-2-deficient mice exhibit osteopenia and delayed fracture healing.[58] However, the role of muscle-derived MMP-2 in bone metabolism remains unknown.

Mechanically loaded myotubes secrete soluble factors, which affect osteoclast formation.[59] Although osteocytes have a central role during mechanical stress in bone during the linkage of bone to muscle, muscle produces unknown factors that preserve osteocyte viability in response to glucocorticoid treatment.[60]

Prospects

Muscle secretes various myokines, which regulate bone metabolism. The search for physiological and pathological roles of muscle-derived humoral factors is an interesting research topic. However, as numerous factors affect both muscle and bone in the same manner, and muscle mass change greatly affects bone metabolism, it will be difficult to distinguish myokine-specific effects from local paracrine action and other organ-derived effects scientifically. Identification of muscle-specific myokines may provide efficacious targets for diagnostic biomarkers and the development of therapeutic drug. Indeed, myostatin has been noted as a target for the treatment of muscle and bone disorders, especially sarcopenia and osteoporosis. Moreover, anti-myostatin signaling may provide a strategy for the treatment of inflammatory disease. In the near future, progress in myokine research, and the discovery of novel muscle-specific myokines, may increase the efficacy of treatments for skeletal disorders. To my knowledge, there is no evidence that demonstrates the direct contribution of myokines, discussed in this review, in bone discriminating muscle mass-mediated effects on bone, as those myokines affect both muscle and bone cells.

Conflict of Interest

The author declares no conflict of interest.

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