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IGF-1 signaling mediated cell-specific skeletal mechano-transduction

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Abstract

Mechanical loading preserves bone mass and stimulates bone formation, whereas skeletal unloading leads to bone loss. In addition to osteocytes, which are considered the primary sensor of mechanical load, osteoblasts and bone specific mesenchymal stem cells also are involved. The skeletal response to mechanical signals is a complex process regulated by multiple signaling pathways including that of insulin-like growth factor-1 (IGF-1). Conditional osteocyte deletion of IGF-1 ablates the osteogenic response to mechanical loading. Similarly osteocyte IGF-1 receptor (IGF-1R) expression is necessary for reloading-induced periosteal bone formation. Transgenic overexpression of IGF-1 in osteoblasts results in enhanced responsiveness to in vivo mechanical loading in mice, a response which is eliminated by osteoblastic conditional disruption of IGF-1 in vivo. Bone marrow derived stem cells (BMSC) from unloaded bone fail to respond to IGF-1 in vitro. IGF-1R is required for the transduction of a mechanical stimulus to downstream effectors, transduction which is lost when the IGF-1R is deleted. Although the molecular mechanisms are not yet fully elucidated, the IGF signaling pathway and its interactions with potentially interlinked signaling cascades involving integrins, the estrogen receptor, and wnt/β-catenin play an important role in regulating adaptive response of bone cells to mechanical stimuli. In this review, we discuss recent advances investigating how IGF-1 and other interlinked molecules and signaling pathways regulate skeletal mechano-transduction involving different bone cells, providing an overview of the IGF-1 signaling mediated cell-specific response to mechanical stimuli.

Keywords

IGF-1; IGF-1 receptor; osteocytes; osteoblasts; mesenchymal stem cells; mechanical stimuli; signaling

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Introduction

Bone is a dynamic tissue that is responsive to mechanical stimuli [1]. Physical activity promotes bone formation [2, 3], whereas skeletal unloading such as during spaceflight or sustained bed rest results in bone loss [4, 5]. Although the osteocyte is generally considered as the primary sensor of mechanical load [6], other cell populations including osteoblasts [7, 8] and mesenchymal stem cells [9, 10] are also involved. Mechanical loading can cause an immediate response in which osteoblasts are recruited from nondividing preosteoblasts and/or bone-lining cells and a delayed response involving proliferation and differentiation of preosteoblasts in the marrow stroma [11]. However, the underlying cellular and molecular mechanisms by which bone cells respond to the changes in mechanical loading remain to be fully determined. Multiple signaling pathways and genes have been shown to be involved in this process. That said, the insulin-like growth factor-1(IGF-1) signaling pathway is one of the key factors in the cellular response to mechanical stimuli in all the different cells involved.

IGF-1 and its receptor (IGF-1R) play a determinant role in the development of the embryonic skeleton and the acquisition of peak bone mass during postnatal growth in mammals [12]. The IGF system is comprised of type I and type II receptors, their ligands (IGF-1 and IGF-2), IGFBPs, and IGFBP proteases [13]. These proteins primarily mediate stimulation of somatic growth by promoting cell survival, proliferation, and differentiation. The growth-promoting effects of IGFs are primarily mediated via interactions with the type I IGF-1 receptor (IGF-1R). Global IGF-1R knockout embryos exhibit more severe skeletal defects than IGF-1 null animals and die shortly after death, suggesting that both IGF-1 and IGF-2 acting via their shared receptor are involved in regulating skeletal development in the embryonic stage.

Studies conducted in mechanically stimulated rat tibia in vivo, and in mechanically stimulated osteocytes in vitro, reveal increased expression of IGF-1 after a bout of increased loading [14, 15]. Conditional osteocyte knockout of IGF-1 also ablated the osteogenic response to mechanical loading, and this occurred despite normal IGF-1 expression in osteoblasts [16]. These data indicate the crucial role of IGF-1 signaling in the skeletal response to mechanical stimuli. In view of the other pathways that are stimulated by mechanical stimuli, it is likely that the skeletal response is a result of an integrated network of pathways that function to control bone mass and structure, rather than one pathway uniquely dedicated to this response to mechanical stimuli. In particular, although the insulin receptor stimulates many of the same pathways, little study has been made of the role of the insulin receptor in the skeletal response to mechanical load However, the focus of this review is on the role of IGF-1 mediated mechano-sensitive signaling events in the skeletal response to mechanical stimuli within the different subgroups of bone cells and on the crosstalk between IGF-1 and other signaling pathways mediating the skeletal response.

Role of IGF-1 in the mesenchymal stem cell response to mechanical stimuli

Mesenchymal stem cells (MSCs) represent a class of cells from bone marrow and periosteum that can be isolated and expanded in culture while maintaining their in vitro

capacity to form bone, cartilage, fat, and other tissues. Mechanical unloading leads to bone loss in part due to impaired osteoblastogenesis of bone marrow derived MSCs (BMSCs) [17]. Cells isolated from the marrow compartment of the femoral head of bone that had undergone significant bone resorption in microgravity showed substantial down-regulation of gene expression markers for early mesenchymal differentiation but not for stem cell markers, supporting the idea that mechanical unloading of mammalian tissues in microgravity is a strong inhibitor of tissue growth and regeneration mechanisms, acting at the level of early mesenchymal stem cell differentiation [18].

Accumulating evidence indicates that the cell fate of MSCs is influenced and regulated by mechanical signals, thereby biasing differentiation of MSCs toward bone [9, 19, 20]. Small-magnitude stress significantly upregulated Runx2 and Collagen I expression and downregulated PPARγ and C/EBPα expression in BMSCs cultured in adipogenic medium [19]. Six weeks of low magnitude mechanical signals increased the overall marrow-based stem cell population by 37% and the number of MSCs by 46%. Concomitant with the increase in stem cell number, the differentiation potential of MSCs in the bone marrow was biased toward osteoblastic and against adipogenic differentiation [9]. The osteogenic potential of BMSCs is retained by low-magnitude vibrations during disuse, an attribute that may have contributed to an enhanced recovery of bone morphology during reambulation [20].

In vitro studies showed that cyclic mechanical stretch (CMS) orchestrates the expression of genes involved in osteogenic differentiation and promotes osteoblastogenesis of BMSCs through regulating HDAC1 and the Sonic Hedgehog signaling pathway [17, 21]. Even BMSCs from ovariectomized rats could be induced toward osteogenic differentiation by intermittent mechanical loading [22]. Flow shear stress (FSS) can also induce a variety of responses such as recruitment, proliferation and osteogenic differentiation of BMSCs, thus promoting bone formation [23]. Low intensity vibrations signal enhanced BMSC proliferation, osteogenic differentiation, and upregulation of genes associated with cytoskeletal structure [24]. These responses to mechanical stimuli are reduced with aging, and the age-related loss of osteogenic potential in BMSCs can be attributed in part to the impairment in IGF-1 signaling in BMSCs. On the other hand IGF-1 overexpression in aging BMSCs facilitates the formation of cell clusters in scaffolds, increases cell survival inside the cell clusters, induces the expression of osteoblast markers, and enhances the biomineralization of cell clusters [25].

Though no in vivo study directly investigated whether the IGF-1 signaling pathway plays a role in the cellular response of BMSCs to mechanical stimuli, some in vitro studies demonstrated the role IGF-1 signaling played in this process. BMSCs from unloaded rats exhibit resistance to the anabolic effects of IGF-1 by failure of IGF-1 to stimulate the activation of its receptor, and subsequently that of Ras, ERK1/2 (p44/42 MAPK), and Akt despite unchanged IGF-1R levels and normal binding of IGF-1 to its receptor [26]. Concomitant with the resistance to IGF-1, BMSCs from unloaded bone exhibit a decrease in the expression of β1 and β3 integrin subunits [27], which are two subunits of the cell surface receptor integrin family, and have been shown to be involved in the mechano-transduction in bone cells [28–30]. Pretreatment of BMSCs with an integrin inhibitor, echistatin, blocked

IGF-1 signaling. Recovery of IGF-1 signaling in BMSCs from unloaded rats followed the recovery of integrin expression induced by skeletal unloading. Selective targeting of integrin subunits with siRNA oligonucleotides revealed that integrin β3 and possibly β1 function as regulators of IGF-1 receptor function in rat BMSCs, maintaining responsive to mechanical stimulation [31].

To further assess the contribution of IGF-1R in the response of BMSCs to mechanical stimuli, Tahimic et al. performed an in vitro study using BMSC cultures from IGF-1R$^{flox/flox}$ animals that allows for cre-mediated deletion of this gene. They found that BMSCs with intact IGF-1R displayed enhanced activation of this receptor and increased ERK phosphorylation in response to shear stress, whereas in BMSCs in which IGF-1R was deleted by adenovirus-Cre recombinase activity exhibited a significant reduction in shear stress-induced ERK phosphorylation. This established that IGF-1R was required for the transduction of a mechanical stimulus to downstream effectors such as ERK [32]. Though measurements of IGF-1 concentrations in culture medium before and after shear stress stimulation in normal or IGF1-R KO cells were not made, these results indicated that BMSC could probably secrete IGF-1 itself and then bind to IGF-1R and activate the downstream pathways. However, these in vitro studies do not totally tell the story in vivo, whereby the IGF-1 could be produced by osteocytes, osteoblasts or BMSC. More research will be needed to clarify the mechanism of IGF-1/IGF-1R mediated mechanical response in BMSC.

These data indicate that the modulation of IGF-1 signaling by the integrin pathway is a potential mechanism by which biomechanical forces are translated into signals that regulate osteogenic differentiation, proliferation and survival of BMSCs. However, at this point no studies have been reported that deletion of IGF-1R from MSCs in vivo alters the response of the skeleton to mechanical stimulation. Those studies are in progress in the authors' laboratory.

Role of IGF-1 in the osteoblast response to mechanical stimuli

Decreased osteoblast function has been thought to play a pivotal role in the process of unloading-induced bone loss. Both in vivo and in vitro studies have provided evidence that osteoblasts subjected to spaceflight were delayed in their differentiation with down-regulation of type I collagen and osteocalcin gene expression, thereby inhibiting matrix formation and maturation [33–35]. On the other hand, it has been shown that appropriate mechanical stimuli promote osteoblast differentiation and osteogenic function. Applying orbital shear stress with suitable magnitude and duration generated an anabolic response as osteoblast metabolism and functional markers such as alkaline phosphatase activity and osteocalcin protein production were enhanced [36]. Similarly, fluid shear stress or cyclic compression stimulate osteoblast differentiation [7, 37–39]. Low fluid shear stress plays an important role in the activation of osteoblasts with enhanced cell proliferation, increased calcium deposition, and increased expression of osteoblastic markers [37]. Similar findings were observed with cyclic compressive load [39].

IGF-1 is a well-studied anabolic regulator of osteoblast proliferation and differentiation during the process of osteogenic differentiation in vitro and fracture healing or postnatal

bone development in vivo [40–43]. While osteoporotic osteoblasts showed increased tyrosine phosphorylation of the IGF-1 receptor in the basal state and blunted stimulation of receptor phosphorylation by IGF-1 [44], persistence of TGF-β1 inhibited osteoblast differentiation via suppression of IGF-1 expression and subsequent down-regulation of the PI3K/Akt pathway [14]. Targeted overexpression of IGF-1 in osteoblasts with an osteocalcin [46] or collagen 1 [47] promoter increases bone formation, with more profound changes in cancellous bone than cortical bone. Mice in which the IGF-1 receptor is specifically deleted in mature osteoblasts have a mineralization defect in vivo, and lead to decreased bone mass and deterioration of microstructure of trabecular bone but not cortical bone in femur when 6 weeks old [48]. Although the number of colony forming units in BMSC cultures from such mice is normal, the subsequent mineralization of the colonies was markedly decreased. Moreover, loss of IGF-IR in mature osteoblasts blocked the stimulatory effects of PTH given in vivo on the gene expression of early (RUNX2, ALP) and late (osteocalcin) osteoblast differentiation markers in bone, and osteoblast differentiation and mineral matrix production in vitro [49]. Considering that osteoblasts may have a significant effect on promoting BMSC proliferation and osteogenic differentiation [50], it is reasonable to propose that in vivo knockout of IGF-1R in osteoblast may impact the osteogenic ability and responsiveness of BMSC to PTH stimulation in vivo and finally exert decreased osteoblast differentiation and mineral matrix production in vitro. However, the precise role of the IGF-IR in the mature osteoblast in mediating these actions of on BMSC remains for future study.

An important role of IGF-1 signaling in the local effect of skeletal unloading on metaphyseal bone formation is indicated in a skeletal unloading rat model, in which mRNA levels for IGF-1 and IGF-1R in the metaphyseal bone decreased at 4–7 days, then rose above normal at 14 days of suspension. These biphasic changes in IGF-1 mRNA levels were strongly correlated with collagen 1 and osteocalcin mRNA levels [51]. Furthermore, our previous study showed that skeletal unloading induces resistance to IGF-1 administration in vivo with respect to bone formation [26]. On the other hand, transgenic mice overexpressing IGF-1 in osteoblasts exhibit a fivefold increase in periosteal bone formation in response to low magnitude loading. In contrast, there was no significant increase in periosteal bone formation in similarly treated wild-type animals, suggesting that IGF-1 and mechanical load act synergistically to stimulate periosteal bone formation [52].

The examination of the role of local IGF-1 in mediating the response to mechanical load was undertaken in an osteoblast-specific IGF-1 conditional knockout model using type I collagen Cre recombinase. Four-point bending resulted in a significant increase in periosteal bone formation in wild-type mice but not in the IGF-1 conditional knockouts, indicating that mechanical loading-induced periosteal bone expansion is dependent on local IGF-1 production in bone. Moreover, axial compression resulted in significant gains in trabecular bone volume, thickness, and density of wild-type mice but not in the conditional knockouts [53]. We further assessed the skeletal response of osteoblast-specific IGF-1R deficient mice to unloading and reloading, and found that the recovery of periosteal bone formation with reloading was completely inhibited in the conditional IGF-1R knockout mice [54]. Another study indicated that in osteoblasts conditional disruption of miR17-92 cluster, which is activated by IGF-1 stimulation, results in reduced periosteal bone formation and bone anabolic response to exercise [55].

In vitro data support these vivo observations. Inhibition of IGF-1 with the antagonist IGFBP-4 blunted fluid-flow stress-induced proliferation in the osteoblastic cell line MC3T3-E1 [22]. Furthermore, IGF-1 and pulsatile fluid flow activate mTOR, thereby stimulating the rate of mRNA translation in osteoblasts. The known anabolic effect of mechanical loading and IGF-1 on bone may thus be partly explained by mTOR-mediated enhanced protein synthesis in osteoblasts [56]. An additional synergistic interaction between shear stress and IGF-1 in the stimulation of osteoblastic proliferation involved an integrin-dependent up-regulation of IGF-1R phosphorylation through an inhibition of the recruitment of SHP-1 and/or SHP-2 to IGF-1R as well as an inhibition of the SHP-1 and/or SHP-2-mediated IGF-1R dephosphorylation. These findings not only confirm that the integrin activation is essential for the IGF-1 mitogenic pathway, but also provide mechanistic insights into the cross-talk between the integrin and IGF-1 signaling pathways in the underlying molecular mechanisms of enhanced bone formation in response to mechanical loading [57].

Estrogen receptor alpha (ERα) has been implicated in the response of bone to mechanical loading. Osteoblast conditional knockout of ERα in female mice results in decreased cortical and cancellous bone mass, but the response to mechanical loading was increased. Male mice with similar deletion of ERα actually showed increased bone mass but comparable response to mechanical loading as littermate controls [58]. Partial loss of both estrogen and IGF-1 significantly reduced cortical but not the trabecular bone response to mechanical loading, providing in vivo evidence of the above crosstalk in mediating the bone response to loading. [59]. When osteoblast-like cells, either primary cultures of normal mouse long bone-derived osteoblasts or those of the UMR-106 cell line, experience even a single short period of dynamic strain capable of stimulating proliferation in vitro and new bone formation in vivo, they show a rapid PI3K-mediated activation of Akt, Akt mediated inhibition of GSK-3, and increased levels of active nuclear β-catenin. IGF-1R-mediated activation of Akt by both strain and IGF-1 involves ER. Inhibition of ER by the selective ER modulator ICI 182,780 increases the concentration of des-(1–3) IGF-1 necessary to stimulate IGF-1R-mediated activation of Akt. Interestingly, in this context estradiol inhibits ER-related activation of IGF-1R because it reduces basal and des-(1–3) IGF-1-dependent phosphorylation of Akt. [60]

Role of IGF-1 in the osteocytic response to mechanical stimuli

Osteocytes are embedded in the mineralized matrix and are known to play a particularly important role in coordinating local bone remodeling [61, 62]. Their location, deep within the mineralized matrix, and their structural organization of a cellular network, make them ideal to sense mechanical stimuli and to transfer that signal to the surrounding cells. As such they are considered to be the primary mechanosensors, which locally coordinate adaptive (re)-modelling responses [6, 63, 64]. Tail suspension induced an increase in the prevalence of apoptotic osteocytes and RANKL-positive osteocytes in cancellous bone of the lumbar vertebrae [65]. Osteocyte apoptosis plays a central and controlling role in triggering osteocyte RANKL production and the activation of new resorption leading to bone loss in disuse [66].

In vivo, osteocytes increase their transcriptional and metabolic activities in response to short loading periods [67, 68]. Loading increases their dentin matrix protein 1 (DMP1) and matrix extracellular phosphoglycoprotein (MEPE) expression, controlling bone matrix mineral quality [69, 70] and increasing the levels of related proteins involved in mechanically induced bone formation [14, 71]. Osteocytes express substantial amounts of IGF-1 [72]. IGF-1 is a critical mediator of PTH/PTHrP receptor signaling in the osteocyte regulation of periosteal bone formation and intracortical remodeling [73, 74]. Conditional disruption of the IGF-1 gene in osteocytes impairs developmental bone growth that is associated with suppression of bone turnover [75]. Mechanical loading leads to a twofold increase in IGF-1 mRNA expression in osteocytes [14].

Osteocytes are descendants of mature osteoblasts. Therefore the previously described studies using osteoblast specific IGF-1 knockout animals also indicated, to some extent, the role of deleting IGF-1 in osteocytes. It is reasonable to assume that deletion of the IGF-1 gene in mature osteoblasts would also delete the IGF-1 gene in osteocytes. Thus, osteoblast specific knockout of IGF-1 could not definitely determine whether the changes in skeletal response were due to IGF-1 deficient osteoblasts or IGF-1 deficient osteocytes, or both.

To evaluate whether osteocyte-derived IGF-1 functions as a key determining factor of the osteogenic response to loading, Lau et al [16] generated osteocyte specific IGF-1 conditional knockout mice and corresponding wild-type (WT) littermates by crossbreeding IGF-1 floxed mice with Dmp1-Cre transgenic mice. When an equivalent loading strain in the form of a two-week four-point bending exercise was applied to the tibia, the loading induced a robust periosteal bone formation response in WT littermates as expected, but this loading regimen failed to elicit an osteogenic response in osteocyte IGF-1 conditional KO mice. Furthermore, conditional disruption of the IGF-1 gene in osteocytes blocked the loading-induced expression of early mechanoresponsive genes such as cyclooxygenase-2 (Cox2) and c-Fos indicating that osteocyte-derived IGF-1 is a very early upstream regulator presumably of MSCs in the context of mechanotransduction [16].

Another regulator involved in the IGF-1 mediated osteocyte response to mechanical stimuli is sclerostin produced from its gene SOST. Sclerostin is secreted by osteocytes, and its expression is modulated in response to mechanical stimuli [76]. This osteocyte-specific protein inhibits bone formation, both in vitro and in vivo, by directly reducing proliferation and differentiation of osteoblasts via inhibition of the canonical Wnt signaling pathway. Sclerostin acts by binding the low-density lipoprotein receptor 5 and 6 and inhibiting Wnt-β-catenin signaling. Sclerostin levels are elevated during reduced loading as achieved by bed rest, paralysis or hind-limb unloading and reduced during increased loading [77–78].

However, loading could only reduce SOST expression in wild type mice but not in the osteocyte IGF-1 conditional KO mutants. Moreover, IGF-1 deficient osteocytes had reduced loading-induced upregulation of Wnt10b [16]. This suggests that osteocyte-derived IGF-1 is a mediator of the loading-induced activation of the canonical Wnt signaling mechanism, considered a critical factor in the bone response to mechanical stimuli [80–82].

Taken together, it has been proposed that mechanical stimuli upregulate IGF-1 expression in osteocytes, then the osteocyte-derived IGF-1 acts as an autocrine effector to rapidly upregulate Wnt10b expression and suppress SOST expression. The secreted osteocyte-derived IGF-1 additionally acts on bone surface osteoblasts and likely the MSC in the periosteum to activate their IGF-1 signaling pathway [16]. The elevated Wnt10b production and the reduced release of sclerostin then activate the canonical Wnt signaling in osteoblasts. The collective consequence of these actions leads to a synergistic increase in bone formation. Thus, osteocyte-derived IGF-1 appears to be an integral component of the circuitry for the normal activation of osteoblasts and osteoprogenitors in response to mechanical loading.

Proposed model of IGF-1 mediated network for the cellular response to mechanical stimuli

Based on the data presented in this review, we propose that bone mesenchymal stem cells, osteoblasts, and osteocytes all respond to mechanical stimuli, in both an independent and interacting manner. Osteoclasts likewise participate in the skeletal response to mechanical load [83], and IGF1 is important for their differentiation [84], but the role of IGF-1 signaling pathways in osteoclasts has not been evaluated with response to mechanical stimuli. Thus they have not been considered further in this review. The degree to which these responses share similarities in signaling or differ is not yet fully resolved, nor are the interactions between the different cell populations fully determined. We propose that IGF-1 signaling plays a key role in these responses in all cell types involved as well as in their interactions. We present a model (Figure) of an IGF-1 mediated network for the cellular response to mechanical stimuli, which incorporates a cell-specific response and a cascade of responses initiated by osteocytes that stimulates the response in osteoblast and mesenchymal stem cells. IGF-1 and its receptor, interacting with integrin β3, activates downstream effectors including Ras, ERK1/2 (p44/42 MAPK), and Akt in these cells in response to mechanical stimuli. ER and mTOR crosstalk with the IGF-1 signaling pathway to further promote and/or regulate this response. Interaction between IGF-1 and the Wnt signaling pathway likewise plays a critical role in the response to mechanical stimuli. The degree to which these responses in osteoblasts and mesenchymal stem cells require IGF-1 or other factors from the osteocyte remains uncertain.

Summary and Conclusion

Numerous studies have demonstrated that the IGF-1 signaling pathway plays a key role in the cellular response of mesenchymal stem cells, osteoblasts and osteocytes to mechanical stimuli. IGF-1R is required for the transduction of a mechanical stimulus to downstream effectors. However, unloaded bone marrow osteoprogenitors treated with IGF-1 fail to respond with respect to IGF-1R activation and its downstream signaling in part due to decreased integrin expression. Transgenic overexpression of IGF-1 in osteoblasts results in enhanced responsiveness to mechanical loading in mice, which is eliminated by osteoblastic conditional disruption of IGF-1 in vivo. Conditional osteocyte knockout of IGF-1 ablates the osteogenic response to mechanical loading, and osteocyte IGF-1R expression is necessary

for reloading-induced periosteal bone formation in mice. The IGF signaling pathway interacts with a number of other effector molecules and signaling cascades such as integrins, the estrogen receptor, and wnt/β-catenin and their downstream effectors all of which play an important role in regulating the response of bone cells to mechanical stimuli. The IGF-1 signaling pathway has emerged as central to the skeletal response to mechanical stimuli. However, the details of the bone cell specific molecular events, especially the cascades of this network of IGF-1 signaling interacting with the other signaling pathways we mentioned above, and the importance and details of the interactions between different bone cells including osteoclasts in the skeletal response to mechanical loading remain to be elucidated. Further studies using various knockdown strategies to test the functional role of these components are necessary to better understand the nature of these events.

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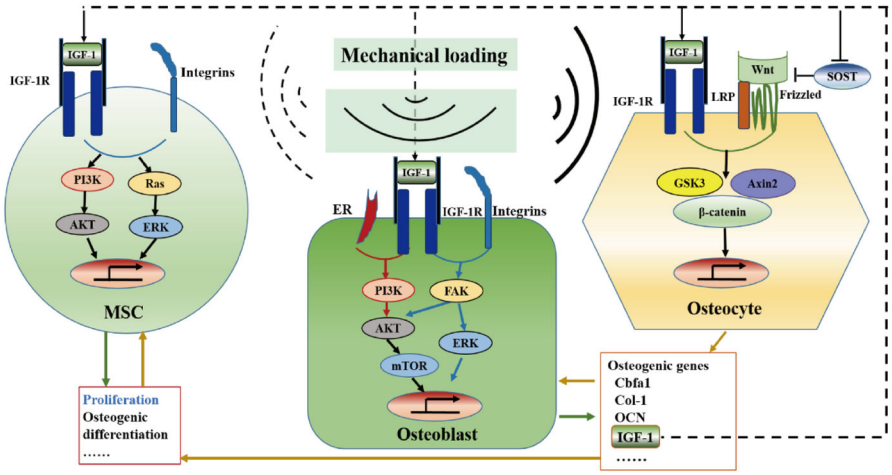

Figure. Model of the role of IGF-1 signaling in the response of bone cells to mechanical loading

The IGF-1R is activated by its ligand IGF-1 and by mechanical loading. Its activation is further regulated by a number of factors including the estrogen receptor-alpha (ER) and certain integrins, as well as its interaction with the Wnt signaling pathway. Downstream effectors of the IGF-1R include the P13K/Akt/mTOR pathway, the Ras/Raf/MAPK pathway, and in association with Wnt the β-catenin pathway. In addition to stimulating the expression of a number of proliferation and differentiation genes involved with osteogenesis, expression of IGF-1 is also increased in osteocytes, and this serves to enhance the stimulation by mechanical loading as well as to activate cells such as osteoblasts and mesenchymal stem cells that may not be directly activated by mechanical loading.