

## Stat3 Signaling Cascade in the Differentiation, Growth and Functions of Bone Cells

Kylie A Corry<sup>1</sup>, Pei Zhang<sup>1</sup> and Jiliang Li<sup>1,2\*</sup><sup>1</sup>Department of Biology, Indiana University Purdue University Indianapolis, Indianapolis, USA<sup>2</sup>Center for Developmental and Regenerative Biology, Indiana University Purdue University Indianapolis, Indianapolis, USA\*Corresponding author: Jiliang Li, Department of Biology, Indiana University Purdue University Indianapolis 623 West Michigan Street, SL306 Indianapolis, IN 46202, USA, Tel: 317-278-1163; E-mail: [jilili@iupui.edu](mailto:jilili@iupui.edu)

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**Keywords:** Osteoporosis; Signal Transducer; Activator of Transcription 3; Osteoblast; Osteoclast; Reactive oxygen species Syndrome and osteoporosis that arise when this pathway becomes disrupted [5].

### Content

The human skeleton is a complex organ system comprised of a collection of many individual bones that are brought together by connective tissue to accomplish not only a biomechanical and supportive function, but also a homeostatic role. The skeleton plays a pivotal role in providing a metabolic supply for the body, supplying the body with calcium and phosphorous that is needed for a plethora of cellular processes [1]. To accomplish this dual role, bone cells have the capability to respond to both local cytokines and circulating hormones. Osteocytes, osteoclasts, and osteoblasts form the main cellular component of bone. Osteoclasts are bone resorbing cells while osteoblasts are bone forming cells. The adult skeleton goes through a continuous process of bone renewal called remodeling. Mediated by osteoclasts and osteoblasts, remodeling replaces old bone with new bone. Although it has long been known that these cells both arise from bone marrow progenitors, the extent to which the differentiation pathways interact and depend on one another has been studied only recently. It has been hypothesized that the local actions of cytokines play a crucial role in these intercellular signaling cascades [2]. In addition, exercise (mechanical loading on bone) has been shown to generate an anabolic effect via regulation of cytokine secretion and intracellular signaling pathways, such as Wnt signaling, in bone cells.

The balance between bone resorption and bone formation is a process that must be finely controlled. When this balance becomes askew, pathologies arise. Osteoporosis can be caused by various factors that result in a loss of this balance, with bone resorption reigning over bone formation. Osteoporosis is a system-wide skeletal disorder characterized by loss of bone mass and strength, ultimately leading to fragility fractures [3]. The underlying molecular pathways leading to osteoporosis are not well understood. It is important to recognize the heterogeneity of the numerous factors responsible for skeletal defects. Sex hormone deficiency, senescence, hyperthyroidism, corticosteroid excess, and certain autoimmune disorders all contribute to bone loss through distinct and independent mechanisms [4]. These pathological mechanisms ultimately converge at an imbalance in normal bone remodeling, resulting in the observed osteopenia and osteoporosis. Thus, in order to effectively select targets for therapeutic intervention of skeletal defects, it is fundamental to first elucidate the basic molecular interactions in bone formation and depletion. The following review will focus on how the signaling protein Stat3 and its signaling cascade mediate differentiation, growth, and maintenance of osteoprogenitor cells and their terminally differentiated lineage constituents. Also discussed are pathologies, specifically Hyper-IgE

Stat3 was first discovered in the 1990s to be part of the interferon signaling pathways as a member of the Stat family, a group of seven signal transducer and activator of transcription (Stat) proteins [6]. Despite the structural similarities amongst the Stat family, each participates in quite different cellular processes. All members of the Stat family have been identified in all bone tissues. Among the seven, Stat3, Stat5a and Stat5b have been shown to be directly involved in skeletal development. Interestingly, Stat1 and Stat3 have potential opposing effects on bone metabolism and healing. Our lab has worked on the role of Stat3 in bone cells for over five years. Stat3 is a 770-amino-acid-long protein encoded on chromosome 17. Stat3 is a highly conserved protein that was first cloned and purified from mouse livers. Stat3 has since been found to be critical during development in numerous mammals and invertebrates [6,7]. Although the significance of Stat3 signaling in the musculoskeletal system has not been well elucidated, emerging evidence suggests that Stat3 is involved in bone metabolism and bone response to mechanical loading.

The Stat signaling cascade was originally identified as a receptor-activated pathway responsive primarily to interferon-gamma (IFN $\gamma$ ) and members of the interleukin-6 (IL-6) family. The intracellular domains of many cytokine receptors, mainly IL-6 receptors, are physically associated with tyrosine kinases of the Janus kinase (JAK) family. IL-6 belongs to the IL-6 family (or gp130 family), which is composed of IL-6, IL-11, oncostatin M, leukemia inhibitory factor, cardiotrophin-1, and novel neurotrophin-1/B-cell stimulatory factor-3 [8,9]. They are pleiotropic cytokines, sharing the glycoprotein chain gp130 as a common signal transducer [8,10,11]. When IL-6 and other cell signaling cytokines bind to their membrane-bound receptors, dimerization of the receptor occurs. The Janus kinase associated with these activated receptors becomes activated and phosphorylates its signature tyrosine residues. This phosphorylation creates docking sites, specifically composed of a Src homology domain, for subsequent recruitment of the Stat family of proteins. The recruited Stat protein is then tyrosine phosphorylated. The activated Stat protein then dimerizes, typically forming a homodimer. The dimerized Stat proteins can then translocate to the nucleus where they bind to enhancer elements or palindromic sequences in target genes. The end result of this signaling cascade is the induction of transcriptional activation [12-14]. This pathway can be down-regulated or inhibited through suppression of the cytokine signaling proteins that bind to the Jak-Stat membrane complex [14].

The cytokines of the IL-6 family, including IL-6, IL-11, and oncostatin M, which are expressed in osteoblasts and osteocytes, play a major role in regulating the differentiation of both osteoblasts and

osteoclasts through the activation of Stat3. IL-6 cytokines act via Stat activation and promote differentiation of committed osteoblasts [15]. IL-6 is produced by all cells of the osteoblast lineage in response to other signaling cytokines. In experimental studies using knock-in models, the up-regulation of IL-6 induces osteoclast formation and, as a result, increases osteoclastic bone resorption [16]. Increased osteoclastic bone resorption is often observed in diseases of bone metabolism, so it has been hypothesized that IL-6 could potentially be a target for treating such disorders [17].

Disruption of gp130 leads to defects in cartilage and bone development. Sims et al. [18] analyzed two lines of gp130 knock-in mice (gp130  $\Delta$ STAT/ $\Delta$ STAT and gp130 Y757F/Y757F). gp130 $\Delta$ STAT/ $\Delta$ STAT exhibited bone shortening as a result of reduced chondrocyte differentiation. In another study using gp130 $^{-/-}$  mice, osteoblasts were fewer in number in trabecular bone and exhibited widespread abnormalities in function [19]. Osteoblasts from these mice showed decreased mRNA and protein levels of alkaline phosphatase, both in vivo and in cultured osteoblasts. Since gp130  $\Delta$ STAT/ $\Delta$ STAT and gp130 $^{-/-}$  models are defective for all signals dependent on the cytoplasmic tail of gp130, including Stat3 activation, these results suggest that Stat3 is required for chondrocyte and osteoblast differentiation and function.

Glycoprotein 130 (gp130) may regulate bone resorption through Stat3. In order to show that Stat3 is a downstream effector of gp130, O'Brien et al. [2] conditionally knocked out Stat3 in an osteoblastic cell line. Expression of this dominant-negative Stat3 protein completely eliminated osteoclast formation, specifically the formation of osteoclasts in relation to IL-6 and its receptor [2]. Therefore, Stat3 is required for many of the cellular processes and responses to gp130 activation. Furthermore, the same cytokines that activate the gp130-mediated signaling cascade also stimulate the up-regulation of RANK ligand (RANKL) production by osteoblasts. Osteoclast precursors express a surface-bound receptor, RANK, for this ligand. Osteoclastogenesis requires this interaction between bone forming and bone resorbing cells. This means that enhancing the Stat3 signaling pathway directly affects osteoblasts while indirectly enhancing the formation of osteoclasts [20,21].

In order to elucidate Stat3's role in bone metabolism in vivo, studies have been carried out in conditional knockout models using the Cre-lox Pre combination system. Universal Stat3 deficiency is lethal at embryonic day seven, a loss that cannot be compensated for by other Stat proteins. Itoh et al. [22] bred a strain of mice that had a point mutation in the tyrosine at position 759 (gp130F759/F759). This specific tyrosine residue provides the binding site for cytokines that act as suppressors of the Jak-Stat3 pathway. When this domain is floxed and conditionally knocked down, this pathway runs unopposed [21]. The gp130F759/F759 mice showed increased number and thickness of trabeculae and also increased total bone volume. Since the suppressor of the gp130-mediated Stat3 signaling cascade was knocked out, the Jak-Stat3 pathway was enhanced. Conversely, Itoh et al. [22] conditionally knocked down the Stat3 protein in osteoblasts using a type I collagen promoter. The control and experimental groups underwent bone histomorphometry of the vertebrae and tibiae, bone mineral density scans, and osteoblast isolation. These mice failed to show reduced bone size, suggesting that the knockout of Stat3 does not noticeably affect chondrocyte signaling. Quantitative analysis of various bone parameters revealed that these mice had reduced total bone volume and trabecular thickness as well as increased trabecular separation. The specific targeting of Stat3 in vivo using the type I

collagen promoter did not seem to affect osteoclast number or the amount of eroded surface per bone surface, suggesting that osteoclast function was normal. This study clearly demonstrated, however, that Stat3 signaling plays a crucial role in bone formation in vivo, specifically in osteoblasts [22].

Besides its action on gene transcription through binding specific gene-promoter sequences in the nucleus, Stat3 may also regulate bone homeostasis and mechanically induced bone formation via its involvement in regulation of cell respiration in mitochondria. It is known that an increase in reactive oxygen species (ROS) causes the bone loss observed in old age; the same effect may contribute to osteoporosis. Stat3 is present in the mitochondria of cells, where it plays a role in ensuring efficient functioning of the electron transport chain [23]. The electron transport chain acts to generate energy through the process of oxidative phosphorylation. When this is disrupted, ROS are formed. This raises interesting questions on what effect the inappropriate function of the electron transport chain would have when Stat3 is knocked down. To test this, Zhou et al. [24] generated bone-selective Stat3 knockout mice, in which Stat3 is specifically inactive in osteoblasts and osteocytes, and performed in vivo mechanical loading on their right ulnas. These bone cell-specific Stat3-deficient mice had significantly reduced body stature and weight, with femur lengths showing the most marked disparities. Osteoblasts from the calvaria were cultured and assayed to evaluate the different levels of ROS compared to those of wild-type mice. The ROS levels were shown to be significantly higher in the Stat3knockout mice compared to those of the control mice [24]. The high levels of ROS appear to negatively affect in vivo bone formation in response to mechanical stress, as evidenced by the reduction of load-induced bone formation in these conditional Stat3KO mice in comparison with the controls. Loss-of-function of Stat3 in osteoblasts and osteocytes decreases bone mineral density and strength, as well as reduces the osteogenic response following mechanical loading.

One of the most well studied pathologies involving Stat3 is Hyper-IgE Syndrome, also called Job's Syndrome. The main etiology of Hyper-IgE Syndrome is a deficiency in the expression of the Stat3 protein. This syndrome was first characterized by Davis in 1966 and was initially defined as eosinophilia, eczema, and recurrent skin and pulmonary infections [25]. Hyper-IgE Syndrome is a rare disease, affecting an estimated one in 100,000 people. It shows an equal distribution between male and female patients and does not seem to be localized to one geographic region. In 2007, genetic studies of patients with Hyper-IgE Syndrome identified a dominant-negative Stat3 mutation, revealing the genetic basis of this pathology. This syndrome is characterized by elevated levels of immunoglobulin E (IgE) and has immunological and infectious manifestations [26]. These patients also show low levels of IL-6-dependent phosphorylation and subsequently low nuclear translocation of the Stat3 protein. With correct diagnosis and appropriate care, these patients tend to improve with time. Preventative treatment for the coinciding infections includes antibiotics and prophylaxis for bacterial infections [26]. As expected with a deficiency in Stat3, specific bone abnormalities in patients with Hyper-IgE syndrome are also observed, including prominent foreheads, broad nasal bridges, and abnormally larger spacing between the eyes. These patients often suffer from frequent fractures, cranio synostosis, and osteoporosis. Clinical studies indicate that Stat3mutation leads to an increase in osteoclast number and bone resorption, suggesting that Stat3 plays a role in osteoclast recruitment and activity. In addition, patients with Hyper-IgE syndrome manifest recurrent pathological fractures in the long bones and ribs, features

not commonly seen in postmenopausal females, who typically experience osteoporotic fractures at femoral heads and vertebral bodies. These findings indicate that the Stat3 mutation has a detrimental effect, independent of bone mineral content, on bone quality, which is likely related to mineralization. Overall, these clinical findings suggest that Stat3 mutations cause increased bone resorption and abnormal bone formation.

Currently, a prominent topic in bone biology research relates to how we can potentially replace bone that has been lost due to inflammatory and degenerative diseases like rheumatoid arthritis and osteoporosis. As discussed above, cell-signaling cytokines are known to manage the balance between bone resorption and bone formation by regulating the differentiation of osteoblasts and osteoclasts, the bone forming and bone resorbing cells. By locally inducing differentiation of osteoblasts, researchers hope to develop a tool to increase bone formation systemically and during fracture repair. Based on past research and current observations, Stat3 seems to be a good candidate for this.

The future research direction includes identification of downstream genes directly affected by Stat3 in bone cells. The IL-6 family of cytokines, activators of the Jak-Stat pathway, stimulates this ligand secretion and thus osteoclastogenesis [27]. Less understood is the direct role of Stat3 in osteoclastogenesis and osteoclast activity. This can be achieved by using a conditional knockout mouse model in which Stat3 inactivation occurs specifically in osteoclasts. Our ongoing studies suggest that Stat3 may have stage-dependent effects in the processes of osteoclastogenesis. Stat3 deficiency in both osteoblasts and osteocytes decreases bone response to loading. It is unclear if suppression of load-induced bone formation is primarily caused by Stat3 deficiency in osteoblasts or osteocytes, as osteocytes have been considered to be the cells that sense the mechanical forces in bone tissues. Future studies using osteocyte-specific Stat3KO mice would help to differentiate the role of Stat3 in osteoblasts and osteocytes.

## References

1. Favus M (2006) Primer on the metabolic bone diseases and disorders of mineral metabolism. Washington, DC: The American Society for Bone and Mineral Research.
2. O'Brien CA, Gubrij I, Lin SC, Saylor RL, Manolagas SC (1999) STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-kappaB ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. *J Biol Chem* 274: 19301-19308.
3. Raisz LG (2005) Pathogenesis of osteoporosis: concepts, conflicts, and prospects. *J Clin Invest* 115: 3318-3325.
4. Avioli LV (1991) Heterogeneity of osteoporotic syndromes and the response to calcitonin therapy. *Calcif Tissue Int* 49 Suppl 2: S16-19.
5. Deng H.-w. and Y.-z. Liu (2005) Current Topics in Bone Biology Singapore: World Scientific Publishing Company.
6. Takeda K, Akira S (2000) STAT family of transcription factors in cytokine-mediated biological responses. *Cytokine Growth Factor Rev* 11: 199-207.
7. Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, et al. (1994) Molecular cloning of APRE, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell* 77: 63-71.
8. Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L (1998) Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 334: 297-314.
9. Senaldi G, Varnum BC, Sarmiento U, Starnes C, Lile J, et al. (1999) Novel neurotrophin-1/B cell-stimulating factor-3: a cytokine of the IL-6 family. *Proc Natl Acad Sci U S A* 96: 11458-11463.
10. Heinrich PC, Behrmann I, Haan S, Hermans HM, Müller-Newen G, et al. (2003) Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 374: 1-20.
11. Kishimoto T, Akira S, Narazaki M, Taga T (1995) Interleukin-6 family of cytokines and gp130. *Blood* 86: 1243-1254.
12. Schindler C, Darnell JE Jr (1995) Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem* 64: 621-651.
13. Darnell JE Jr, Kerr IM, Stark GR (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264: 1415-1421.
14. Nishimura R, Moriyama K, Yasukawa K, Mundy GR, Yoneda T (1998) Combination of interleukin-6 and soluble interleukin-6 receptors induces differentiation and activation of JAK-STAT and MAP kinase pathways in MG-63 human osteoblastic cells. *J Bone Miner Res* 13: 777-785.
15. Bellido T, Borba VZ, Roberson P, Manolagas SC (1997) Activation of the Janus kinase/STAT (signal transducer and activator of transcription) signal transduction pathway by interleukin-6-type cytokines promotes osteoblast differentiation. *Endocrinology* 138: 3666-3676.
16. Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, et al. (1993) Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. *Proc Natl Acad Sci U S A* 90: 11924-11928.
17. Hirano T, Ishihara K, Hibi M (2000) Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene* 19: 2548-2556.
18. Sims NA, Jenkins BJ, Quinn JM, Nakamura A, Glatt M, et al. (2004) Glycoprotein 130 regulates bone turnover and bone size by distinct downstream signaling pathways. *J Clin Invest* 113: 379-389.
19. Shin HI, Divieti P, Sims NA, Kobayashi T, Miao D, et al. (2004) Gp130-mediated signaling is necessary for normal osteoblastic function in vivo and in vitro. *Endocrinology* 145: 1376-1385.
20. Sims NA (2009) gp130 signaling in bone cell biology: multiple roles revealed by analysis of genetically altered mice. *Mol Cell Endocrinol* 310: 30-39.
21. Atsumi T, Ishihara K, Kamimura D, Ikushima H, Ohtani T, et al. (2002) A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. *J Exp Med* 196: 979-990.
22. Itoh S, Udagawa N, Takahashi N, Yoshitake F, Narita H, et al. (2006) A critical role for interleukin-6 family-mediated Stat3 activation in osteoblast differentiation and bone formation. *Bone* 39: 505-512.
23. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, et al. (2009) Function of mitochondrial Stat3 in cellular respiration. *Science* 323: 793-797.
24. Zhou H, Newnum AB, Martin JR, Li P, Nelson MT, et al. (2011) Osteoblast/osteocyte-specific inactivation of Stat3 decreases load-driven bone formation and accumulates reactive oxygen species. *Bone* 49: 404-411.
25. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, et al. (2007) Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 448: 1058-1062.
26. Chandresris MO, Melki I, Natividad A, Puel A, Fieschi C, et al. (2012) Autosomal dominant STAT3 deficiency and hyper-IgE syndrome: molecular, cellular, and clinical features from a French national survey. *Medicine (Baltimore)* 91: e1-19.
27. Kwan Tat, Padrines M, Théoleyre S, Heymann D, Fortun Y (2004) IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. *Cytokine Growth Factor Rev* 15: 49-60.

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