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Stat1-Mediated Cytoplasmic Attenuation in Osteoimmunology

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Abstract Signal transducer and activator of transcription 1 (Stat1) is a critical mediator of gene transcription in type I interferon (IFN-α/β) signaling that is essential for host defense against viruses. In the skeletal system, type I IFNs (IFN-α/β) also play an important physiological role in the inhibition of receptor activator of NF-κB ligand (RANKL)-induced osteoclast differentiation and bone resorption: mice deficient in IFN signaling exhibit decreased bone mass accompanied by the activation of osteoclastogenesis. On the other hand, an unexpected increase in bone mass was observed in Stat1-deficient mice, indicating that Stat1 has a hitherto unknown function in the regulation of bone formation. Indeed, Stat1 was found to have a unique, non-canonical function as a cytoplasmic attenuator of Runx2, a key transcription factor for osteoblast differentiation. Thus, the loss of Stat1 results in excessive activation of Runx2 and osteoblast differentiation, thereby tipping the balance in favor of bone formation over bone resorption. This is an interesting example in which a latent transcription factor attenuates the activity of another transcription factor in the cytoplasm, and reveals a novel regulatory mechanism of bone remodeling by immunomodulatory molecules. Here, we summarize recent advances in the study of Stat1 and IFNs in the context of osteoimmunology, including latest reports that question whether the inhibitory function of Stat1 in chondrocytes is responsible for dwarfism in achondroplasia. J. Cell. Biochem. 94: 232–240, 2005.

Key words: Stat1; interferon; osteoblast; Runx2; transcription factor; osteoclast; RANKL

The immune and skeletal systems have various regulatory molecules in common including cytokines and transcription factors. Furthermore, immune cells are formed in the bone marrow, interacting with bone cells. Therefore, it is reasonable to consider that the physiology and pathology of one system may affect the other. It is worth noting that an abnormal activation of the immune system leads to bone destruction in diseases such as rheumatoid arthritis, and animal models deficient in immunomodulatory molecules often develop an unexpected skeletal phenotype. Thus, the crosstalk between immune and skeletal systems or the interdisciplinary field called "osteoimmunology" has attracted much attention in recent years [Arron and Choi, 2000; Baron, 2004].

The development and homeostasis of the vertebrate skeletal system depend on the balanced action of bone-forming osteoblasts and bone-resorbing osteoclasts [Karsenty and Wagner, 2002]. This balance must be tightly controlled by various regulatory systems including the endocrine, nervous, and immune systems.

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Tipping this balance in favor of osteoclasts leads to pathological bone resorption, as observed in various osteopenic diseases: autoimmune arthritis, periodontitis, postmenopausal osteoporosis, Paget's disease, and bone tumors. Therefore, the regulatory mechanisms of osteoclasts and osteoblasts are critical for understanding the health and disease of the skeletal system. Receptor activator of NF-κB ligand (RANKL) is a tumor necrosis factor family cytokine essential for the induction of osteoclastogenesis. RANKL was cloned as an activator of dendritic cells expressed by activated T-cells, and this molecule explicitly highlighted the close relationship between the immune and bone systems [Theill et al., 2002]. The intracellular signaling pathway activated by RANKL has been extensively studied. Briefly, the binding of RANKL to its receptor, RANK, results in the recruitment of TNF receptor-associated factor 6 (TRAF6), which activates the NF-κB and MAPK pathways, and also induces the expression of c-Fos. In cooperation with recently identified costimulatory signals for RANKL, the c-Fos and TRAF6 pathways induce and activate nuclear factor of activated T-cells c1 (NFATc1), the master transcription factor for osteoclastogenesis [Takayanagi et al., 2002a; Koga et al., 2004] (Fig. 1).

Both type I and II IFNs (IFN-α/β and IFN-γ, respectively) play crucial roles in the immune system in the context of host defense against

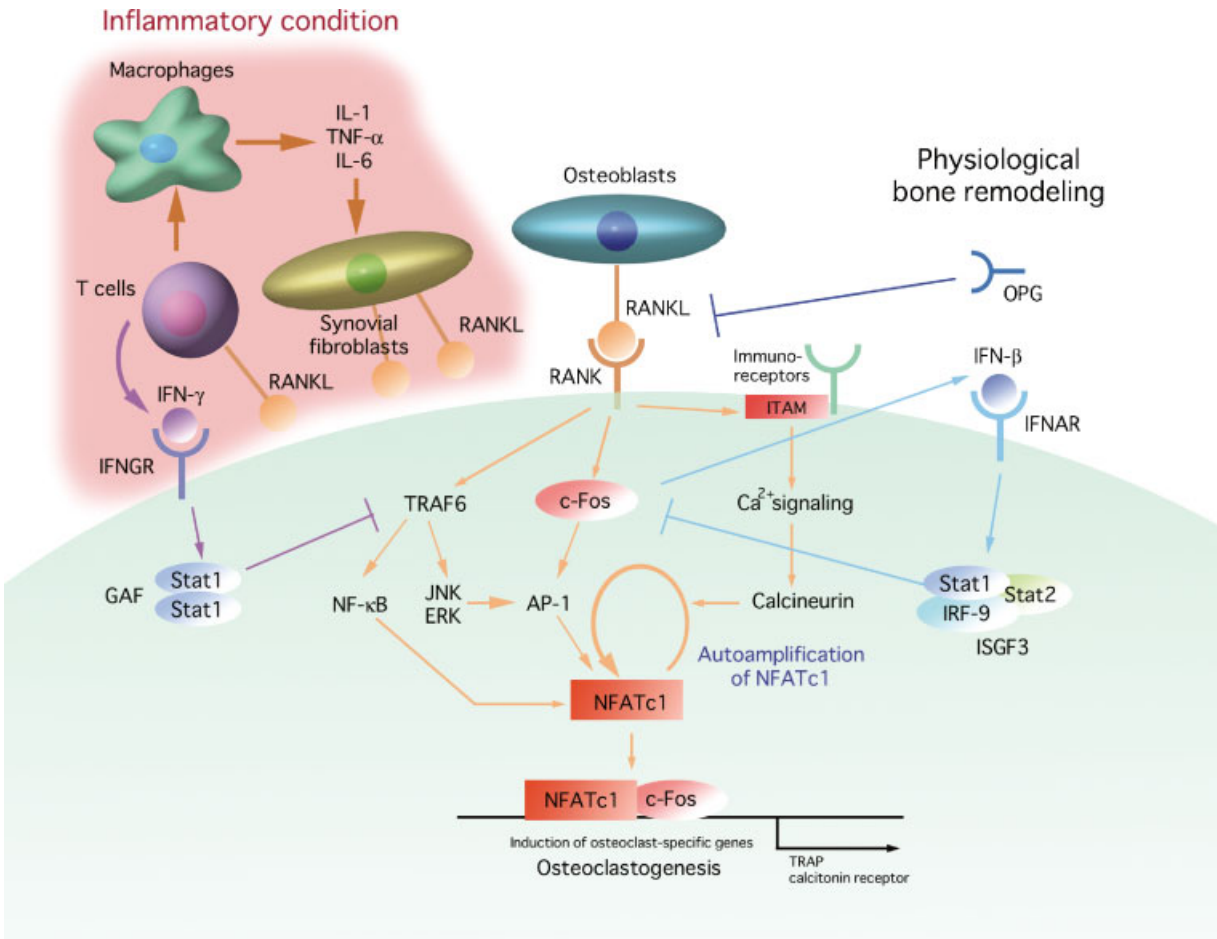

Fig. 1. Crosstalk between RANKL and IFN signaling in osteoclast differentiation. RANKL binds to its receptor, RANK, and activates essential signaling pathways for osteoclastogenesis including TRAF6, c-Fos, and calcium pathways. The transcription factor NFATc1 integrates these pathways, acting as a master switch for osteoclast differentiation. Recently, immunoglobulin-like receptors associated with ITAM-harboring adaptors have been identified as critical costimulatory molecules of RANKL. In the inflammatory condition, IFN-γ produced by activated T-cells inhibits RANKL signaling by the downregulation of TRAF6 expression. In addition to osteoprotegerin (OPG), IFN-β, which is induced by RANKL, is an important physiological regulator of RANKL signaling by inhibiting the expression of c-Fos. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pathogens [Taniguchi and Takaoka, 2002]. More recent works revealed that IFNs are also involved in the negative regulation of osteoclastogenesis by unique signaling cross-talks with RANKL [Takayanagi et al., 2002c] (Fig. 1). In the inflammatory condition, T-cells maintain bone homeostasis by counterbalancing the action of RANKL through the production of IFN-γ [Takayanagi et al., 2000b]. IFN-β also contributes to the regulation of bone remodeling as a RANKL-induced autoregulator of RANKL signaling [Takayanagi et al., 2002b]. Since both IFNs utilize Stat1 as an essential signaling transducer and transcription factor, it was naturally expected that Stat1 may be involved in the negative regulation of osteoclast differentiation. Another clue to the function of Stat1 in the skeletal system can be obtained from reports that Stat1 is involved in the negative regulation of chondrocyte proliferation downstream of fibroblast growth factor receptor 3 (FGFR3) [Su et al., 1997; Sahni et al., 1999]. Nonetheless, Stat1-deficient mice have an increased bone mass without any defects in enchondral bone formation, suggesting that Stat1 has yet an undetermined function in the skeletal system.

Detailed analyses of osteoblasts derived from Stat1-deficient mice demonstrate that Stat1 functions as a cytoplasmic attenuator of Runx2, which is an essential transcription factor for osteoblastic bone formation [Ducy et al., 2000]. This novel function of Stat1 does not require tyrosine 701 that is phosphorylated when Stat1 becomes a transcriptional activator [Levy and Darnell, 2002]. Here we will focus on the IFN-independent function of Stat1 in Runx2-mediated osteoblast differentiation in the context of a new mode of transcriptional attenuation.

RANKL AND IFN-γ IN ARTHRITIC BONE DESTRUCTION

The molecular mechanism by which abnormal immune responses destroy bone in autoimmune arthritis has long been a mystery. Although there was circumstantial evidence that osteoclasts generated from synoviocytes play a critical role [Takayanagi et al., 1997], it was not until RANKL was cloned and found to be overexpressed in arthritic joints that researchers widely accepted that osteoclasts play a critical role in arthritic bone destruction [Takayanagi et al., 2000a]. Finally, recent studies have provided genetic evidence that RANKL or osteoclasts are indispensable for inflammatory bone destruction [Pettit et al., 2001; Redlich et al., 2002], although it remains unclear whether synovial mesenchymal cells or T-cells are the major RANKL-expressing cells in arthritis.

We hypothesized that T-cells have a negative regulatory mechanism to counterbalance the action of RANKL, since abnormal bone resorption is not observed during normal T-cell responses despite the expression of RANKL. Using mice lacking a receptor component for IFN-γ, we found that IFN-γ produced by T-cells strongly suppresses osteoclastogenesis by interfering with the RANKL signaling pathway [Takayanagi et al., 2000b]. Thus, activated T-cells not only positively regulate, but also negatively affect osteoclastogenesis. The inhibitory effect of IFN-γ on osteoclastogenesis was not observed in *Stat1*$^{-/-}$ cells, showing that Stat1 is essential for this effect. Furthermore, the IFN-γ inhibition of osteoclastogenesis is rescued by overexpressing the RANK adaptor protein TRAF6 in precursor cells, indicating that TRAF6 is the target critical for the IFN-γ action.

The mechanism of arthritic bone destruction initiated by T-cells is summarized in Figure 2. It is worth noting that RANKL is abundantly expressed on synovial fibroblasts stimulated by inflammatory cytokines such as IL-1 or TNF-α in addition to RANKL on T-cells. Interestingly, despite a significant T-cell infiltration in arthritic joints, IFN-γ expression in these T-cells is suppressed. Therefore, the paucity of IFN-γ and the enhanced expression of RANKL may underlie the activation of osteoclastogenesis in arthritis.

NEGATIVE FEEDBACK REGULATION OF RANKL SIGNALING BY IFN-β

During the course of a genomewide screening of target genes induced by RANKL, we detected multiple IFN-α/β-inducible genes in osteoclast precursor cells. This led us to investigate the bone phenotype of mice deficient in an IFN-α/β receptor component, IFNAR1 [Takayanagi et al., 2002b]. We found that these mice spontaneously develop marked osteopenia (low bone mass) accompanied by enhanced osteoclastogenesis. We found that RANKL induces the IFN-β gene in

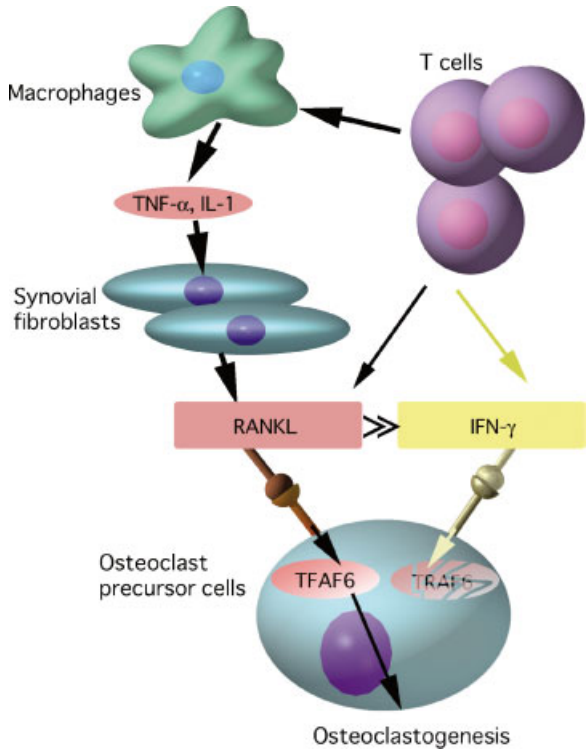

Fig. 2. Mechanism of arthritic bone destruction. Activated T-cells stimulate macrophages to secrete proinflammatory cytokines such as TNF-α and IL-1, which strongly induce RANKL on synovial fibroblasts. In addition, T-cells themselves express RANKL, although this contribution remains unclear. In contrast, T-cells produce a very low level of IFN-γ, a potent RANKL inhibitor produced by T-cells. This imbalance may be responsible for the aberrant activation of osteoclast formation in arthritis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

osteoclast precursor cells and that IFN-β inhibits their differentiation by interfering with the RANKL-induced expression of c-Fos, an essential transcription factor for osteoclastogenesis. Interestingly, unlike the induction by virus, IFN-β gene induction by RANKL is not dependent on interferon regulatory factor (IRF)-3/IRF-7, but on c-Fos. Thus, a unique autoregulatory mechanism operates, wherein RANKL-induced c-Fos induces its own inhibitor. The inhibitory effect of IFN-β on osteoclastogenesis was not observed in *Stat1*$^{-/-}$ or *IRF-9*$^{-/-}$ cells, indicating that the interferon-stimulated gene factor 3 (ISGF3) complex containing Stat1 and IRF-9 is essential for this effect (see below).

NOVEL ROLE OF Stat1 IN BONE REMODELING

Despite the distinct mechanisms of regulating osteoclast differentiation by IFN-β and IFN-γ, Stat1 is critically involved in both mechanisms to suppress RANKL signaling (see Fig. 1). These mechanisms are consistent with the canonical activation pathway of Stat1 dependent on its phosphorylation: Stat1 resides in the cytoplasm in the latent form, and the binding of IFNs to their receptors results in the activation of Jak family kinases, which phosphorylate tyrosine 701 of Stat1. Phosphorylated Stat1 leads to the activation of transcription factors, which include ISGF3 (a heterotrimeric complex consisting of Stat1, Stat2, and IRF-9) and IFN-γ-activated factor (GAF; a Stat1 homodimer) [Stark et al., 1998; Taniguchi et al., 2001] (Fig. 3). It was also reported that unphosphorylated Stat1 is involved in the transcriptional control [Chatterjee-Kishore et al., 2000], but there has been no evidence that Stat1 has a physiological function in the cytoplasm.

To investigate the physiological function of Stat1 in the skeletal system, we examined the bone phenotype of *Stat1*$^{-/-}$ mice [Kim et al., 2003]. Consistent with the observation that Stat1 is required for the IFN-β-mediated inhibition of osteoclastogenesis, we found an increased osteoclast number and enhanced osteoclastic bone resorption in *Stat1*$^{-/-}$ mice. However, the *Stat1*$^{-/-}$ mice had an increased bone mass, unlike the *IFNAR1*$^{-/-}$ mice that exhibited osteopenia. This unexpected bone phenotype of the *Stat1*$^{-/-}$ mice prompted us to examine the status of bone-forming osteoblasts in these mice. Bone morphometric analysis revealed a notable increase in bone formation rate and other osteoblast parameters, such as osteoid surface/thickness and osteoblast surface, suggesting that excessive osteoblast differentiation is responsible for the increased bone mass. In vitro osteoblast differentiation was significantly enhanced in the absence of Stat1. These results indicate that Stat1 interferes with osteoblast differentiation and bone formation. In view of the well-known fact that Stat1 is activated in response to IFNs and other cytokines, such as leukemia inhibitory factor (LIF), interleukin (IL)-6, and oncostatin M, we examined whether these cytokines are involved in the above described osteoblast phenotype, but no such evidence was obtained. Although it remains unclarified how FGF regulates osteoblast differentiation, it is interesting that the expression of FGFR3 is downregulated and the FGF-18-stimulated proliferation is accelerated in *Stat1*$^{-/-}$ osteoblasts [Xiao et al., 2004].

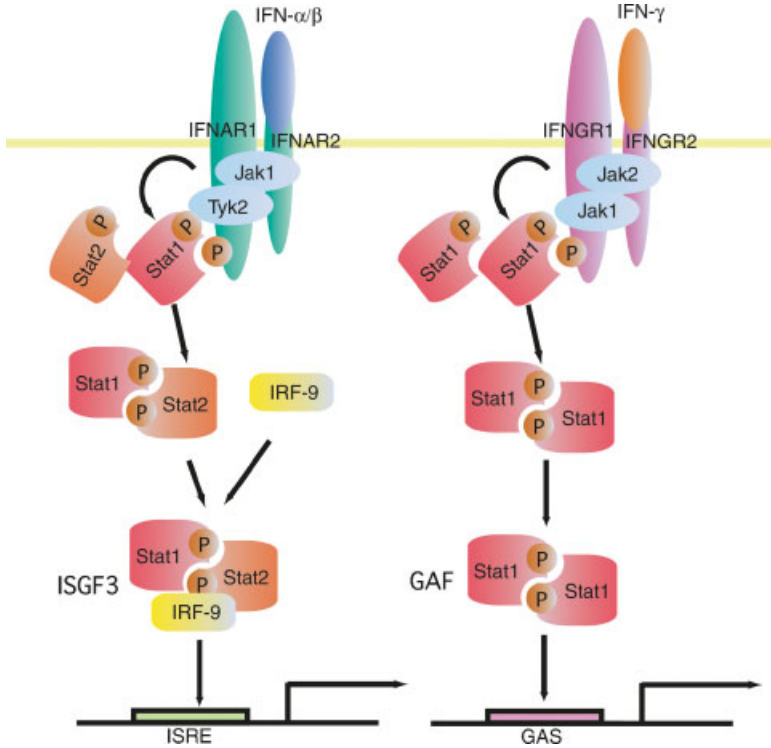

Fig. 3. Canonical mechanism of Stat1 activation by IFNs. The binding of IFN-α/β to its receptor complex induces the activation of Jak family tyrosine kinases, Jak1 and Tyk2, resulting in the phosphorylation of Stat1 and Stat2. This leads to the formation of a heterotrimeric complex, ISGF3, consisting of Stat1, Stat2, and IRF-9 and GAF (Stat1 homodimer). IFN-γ activates Jak1 and Jak2, which phosphorylates Stat1 and mainly induces GAF. The typical pathways are depicted in the schematic. ISGF3 and GAF bind to the interferon-stimulated response element (ISRE) and IFN-γ activation site (GAS) on the promoter of IFN-inducible genes, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CYTOPLASMIC ATTENUATION OF Runx2 BY Stat1

To gain insights into the mechanism by which Stat1 interferes with the osteoblast differentiation program, we examined the role of Stat1 in signaling events induced by bone morphogenetic protein (BMP)-2, a potent osteogenic cytokine. Briefly, BMP-2 binds to its receptor, which induces the phosphorylation of Smad family proteins such as Smad1, Smad5, and Smad8, resulting in the formation of a trimetric complex with Smad4 [Massague, 2000]. The Smad complex translocates to the nucleus and cooperates with another class of transcription factor, Runx2, to activate the transcription of osteoblast-specific genes [Ito and Miyazono, 2003]. The expression of Stat1 resulted in a strong inhibition of the Runx2-dependent activation of the *osteocalcin* promoter, without affecting the Smad1-dependent promoter activation, suggesting that Stat1 selectively interferes with the transcriptional activity of Runx2. Indeed, an electrophoretic mobility shift assay (EMSA) using the Runx2-binding probe revealed that the DNA-binding activity of Runx2 is upregulated in $Stat1^{-/-}$ osteoblasts [Kim et al., 2003]. Although a study using Runx2 transgenic mice suggested that the abnormal expression of Runx2 at the late stage of osteoblast differentiation may affect bone formation [Liu et al., 2001], $Stat1^{-/-}$ mice provide an interesting model in which the temporal Runx2 expression is normal but its activity is enhanced.

Is the inhibitory effect of Stat1 on Runx2 activity dependent on its activation by phosphorylation? In the context of the signaling of IFNs and other cytokines, Stat1 is activated by phosphorylation at tyrosine 701. Interestingly, the phosphorylation of tyrosine 701 of Stat1 was barely detectable in osteoblasts irrespective of BMP-2 stimulation. In addition, the inhibitory effect on Runx2 was exerted by a mutant form of Stat1 in which tyrosine 701 is converted to phenylalanine, showing that the inhibition of Runx2 activity by Stat1 is independent of phosphorylation.

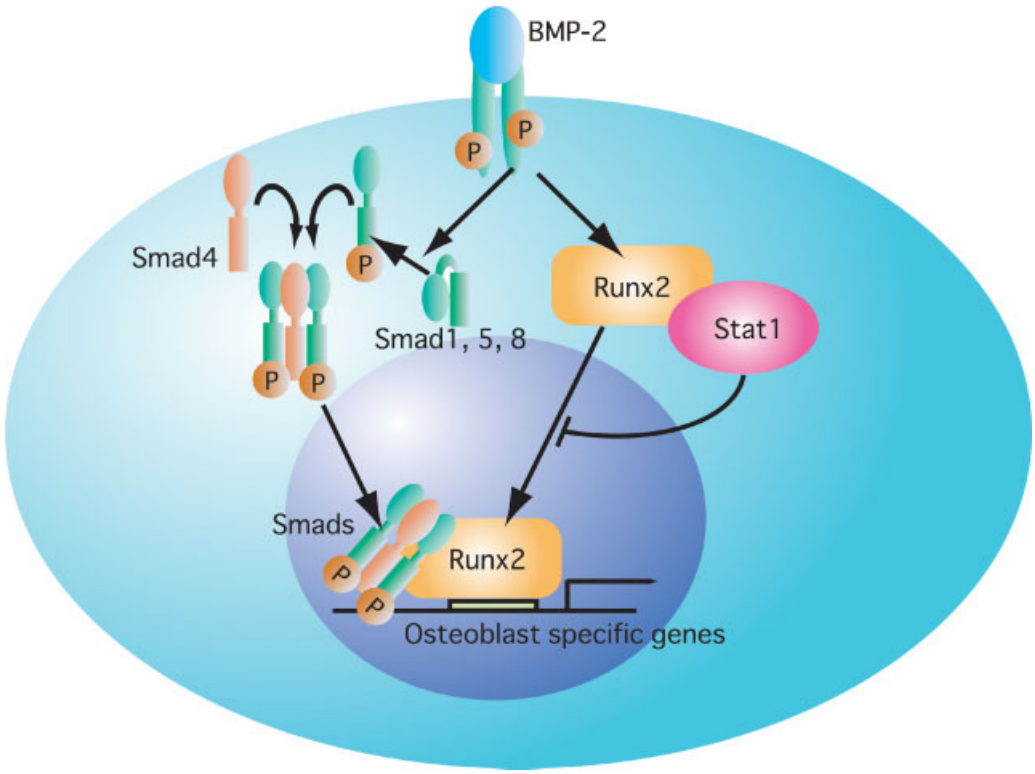

Fig. 4. A schematic model of Stat1-mediated cytoplasmic attenuation. Smads and Runx2 are among the transcription factors activated downstream of BMP-2. Stat1 has no effects on Smad-dependent transcriptional activity, but strongly inhibits Runx2-dependent activity. Stat1 inhibited the nuclear localization of Runx2 by their physical association in the cytoplasm. This effect is independent of Stat1 phosphorylation or IFN signaling. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Finally, we investigated the association between Stat1 and Runx2, and found that the inhibitory effect of Stat1 on Runx2 is dependent on the physical interaction between the two molecules. The overexpression of Runx2 resulted in the nuclear localization of the protein, but the coexpression of Stat1 with Runx2 maintained Runx2 in the cytoplasm, inhibiting the nuclear localization and transcriptional activity of Runx2. Thus, unphosphorylated Stat1 residing in the cytoplasm associates with Runx2 and attenuates the activity of Runx2 (Fig. 4). The mode of action of Stat1 is unique, in that it interferes with another transcription factor, Runx2, in the cytoplasm in its transcriptionally latent form.

FUNCTION OF Stat1 IN CHONDROCYTES

An activating mutation in FGFR3 results in human achondroplasia, one of the commonest congenital diseases characterized by dwarfism [Rousseau et al., 1994]. Several studies proposed that the inhibitory effect of FGF-activated Stat1 on chondrocyte proliferation causes impaired endochondral ossification and skeletal malformation in achondroplasia [Su et al., 1997; Sahni et al., 1999, 2001]. Notwithstanding, there was no severe abnormality in the gross development of skeletons, longitudinal bone length, or growth plate anatomy in *Stat1*$^{-/-}$ mice, suggesting that the physiological contribution of Stat1 to endochondral bone formation is minimal.

Although Stat1 is involved in the inhibitory effect of FGF on chondrocyte proliferation, recent studies suggest that dwarfism in achondroplasia is not associated with Stat1, but with the activation of the MAPK pathway. Notably, the constitutive activation of the MAPK pathway results in an achondroplasia-like phenotype in mice, and transgenic mice carrying an activating mutation (G374R) in FGFR3 develop an achondroplasia-like phenotype even in an Stat1-deficient background [Murakami et al., 2004]. Furthermore, the overexpression of the C-type natriuretic peptide (CNP) in chondrocytes rescues achondroplasia by suppressing

the activation of the MAPK pathway without affecting the Stat1 pathway [Yasoda et al., 2004]. These results show that the MAPK pathway is more important in terms of the regulation of the hypertrophic differentiation of chondrocytes, and the Stat1-mediated inhibition of cell proliferation may not be responsible for the abnormal enchondral ossification in achondroplasia, although further studies are necessary to clarify the molecular basis of this discrepancy.

SUMMARY AND FUTURE DIRECTIONS

In this review, we summarized our current understanding of IFN signaling and its downstream transcription factors in the regulation of bone remodeling. The series of studies places both IFN-α/β and -γ systems in the context of osteoimmunology, indicating that these cytokines are critical not only for immune responses but also for bone homeostasis in both physiological and pathological conditions.

In particular, the new role of Stat1 in the inhibition of Runx2 is interesting in that this and other transcription factor members crosstalk in the cytoplasm. Runx2 has been identified as the central transcription factor for bone formation, but how its function is regulated has been largely unknown. There was no acceleration of ossification in newborn *Stat1*$^{-/-}$ mice, suggesting that the physiological regulatory function of Stat1 is not important for the skeletal development but critical for bone remodeling at the postnatal stage. Recently, Bialek et al. [2004]. have identified the Twist transcription factor as a negative regulator of Runx2 during the development of the skeletal system. Taken together, we conclude that the Runx2 function is regulated by distinct mechanisms between embryonic development and remodeling of the skeletal system. These findings suggest the importance of the temporal and spatial regulation of Runx2 for the normal development and homeostatic regulation of the skeletal system; Twist regulates Runx2 in the nucleus during embryogenesis and Stat1 regulates Runx2 in the cytoplasm in the adult bone.

Runx family transcription factors play an important role in various biological systems such as hematopoiesis, bone metabolism, and oncogenesis [Li et al., 2002; Ichikawa et al., 2004]. In addition, accumulating evidence suggests that the Runx family regulates the differentiation of immune cells [Taniuchi et al., 2002]. Interestingly, single nucleotide polymorphism (SNP) in Runx binding sites is associated with the onset of several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, and psoriasis [Prokunina et al., 2002; Helms et al., 2003; Tokuhiro et al., 2003]. The unexpected bone phenotype revealed an intriguing crosstalk between Stat1 and Runx2, indicating that the regulation of bone metabolism by immunomodulatory molecules extends beyond the regulation of osteoclastic bone resorption. The regulation of Runx by Stat family members may shed light on new aspects of an intricate network linking the immune and skeletal systems and provide a novel therapeutic strategy for pathological conditions affecting both systems.

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REFERENCES

Arron JR, Choi Y. 2000. Bone versus immune system. Nature 408:535–536.

Baron R. 2004. Arming the osteoclast. Nat Med 10:458–460.

Bialek P, Kern B, Yang X, Schrock M, Sosic D, Hong N, Wu H, Yu K, Ornitz DM, Olson EN, Justice MJ, Karsenty G. 2004. A twist code determines the onset of osteoblast differentiation. Dev Cell 6:423–435.

Chatterjee-Kishore M, Wright KL, Ting JP, Stark GR. 2000. How Stat1 mediates constitutive gene expression: A complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene. EMBO J 19: 4111–4122.

Ducy P, Schinke T, Karsenty G. 2000. The osteoblast: A sophisticated fibroblast under central surveillance. Science 289:1501–1504.

Helms C, Cao L, Krueger JG, Wijsman EM, Chamian F, Gordon D, Heffernan M, Daw JA, Robarge J, Ott J, Kwok PY, Menter A, Bowcock AM. 2003. A putative RUNX1 binding site variant between SLC9A3R1 and NAT9 is associated with susceptibility to psoriasis. Nat Genet 35:349–356.

Ichikawa M, Asai T, Saito T, Yamamoto G, Seo S, Yamazaki I, Yamagata T, Mitani K, Chiba S, Hirai H, Ogawa S, Kurokawa M. 2004. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. Nat Med 10:299–304.

Ito Y, Miyazono K. 2003. RUNX transcription factors as key targets of TGF-β superfamily signaling. Curr Opin Genet Dev 13:43–47.

Karsenty G, Wagner EF. 2002. Reaching a genetic and molecular understanding of skeletal development. Dev Cell 2:389–406.

Kim S, Koga T, Isobe M, Kern BE, Yokochi T, Chin YE, Karsenty G, Taniguchi T, Takayanagi H. 2003. Stat1 functions as a cytoplasmic attenuation of Runx2 in the transcriptional program of osteoblast differentiation. Genes Dev 17:1979–1991.

Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. 2004. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. Nature 428:758–763.

Levy DE, Darnell JE, Jr. 2002. Stats: Transcriptional control and biological impact. Nat Rev Mol Cell Biol 3: 651–662.

Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. 2002. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109:113–124.

Liu W, Toyosawa S, Furuichi T, Kanatani N, Yoshida C, Liu Y, Himeno M, Narai S, Yamaguchi A, Komori T. 2001. Overexpression of Cbfa1 in osteoblasts inhibits osteoblast maturation and causes osteopenia with multiple fractures. J Cell Biol 155:157–166.

Massague J. 2000. How cells read TGF-β signals. Nat Rev Mol Cell Biol 1:169–178.

Murakami S, Balmes G, McKinney S, Zhang Z, Givol D, de Crombrugghe B. 2004. Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. Genes Dev 18:290–305.

Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravallese EM. 2001. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 159: 1689–1699.

Prokunina L, Castillejo-Lopez C, Oberg F, Gunnarsson I, Berg L, Magnusson V, Brookes AJ, Tentler D, Kristjansdottir H, Grondal G, Bolstad AI, Svenungsson E, Lundberg I, Sturfelt G, Jonssen A, Truedsson L, Lima G, Alcocer-Varela J, Jonsson R, Gyllensten UB, Harley JB, Alarcon-Segovia D, Steinsson K, Alarcon-Riquelme ME. 2002. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet 32:666–669.

Redlich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, Steiner G, Smolen JS, Wagner EF, Schett G. 2002. Osteoclasts are essential for TNF-α-mediated joint destruction. J Clin Invest 110:1419–1427.

Rousseau F, Bonaventure J, Legeai-Mallet L, Pelet A, Rozet JM, Maroteaux P, Le Merrer M, Munnich A. 1994. Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. Nature 371:252–254.

Sahni M, Ambrosetti DC, Mansukhani A, Gertner R, Levy D, Basilico C. 1999. FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. Genes Dev 13: 1361–1366.

Sahni M, Raz R, Coffin JD, Levy D, Basilico C. 2001. STAT1 mediates the increased apoptosis and reduced chondrocyte proliferation in mice overexpressing FGF2. Development 128:2119–2129.

Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. 1998. How cells respond to interferons. Annu Rev Biochem 67:227–264.

Su WC, Kitagawa M, Xue N, Xie B, Garofalo S, Cho J, Deng C, Horton WA, Fu XY. 1997. Activation of Stat1 by mutant fibroblast growth-factor receptor in thanatophoric dysplasia type II dwarfism. Nature 386:288–292.

Takayanagi H, Oda H, Yamamoto S, Kawaguchi H, Tanaka S, Nishikawa T, Koshihara Y. 1997. A new mechanism of bone destruction in rheumatoid arthritis: Synovial fibroblasts induce osteoclastogenesis. Biochem Biophys Res Commun 240:279–286.

Takayanagi H, Iizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, Koshihara Y, Oda H, Nakamura K, Tanaka S. 2000a. Involvement of receptor activator of nuclear factor κB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 43:259–269.

Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, Takaoka A, Yokochi T, Oda H, Tanaka K, Nakamura K, Taniguchi T. 2000b. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature 408:600–605.

Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. 2002a. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell 3:889–901.

Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, Yokochi T, Oda H, Nakamura K, Ida N, Wagner EF, Taniguchi T. 2002b. RANKL maintains bone homeostasis through c-Fos-dependent induction of *interferon-β*. Nature 416:744–749.

Takayanagi H, Kim S, Taniguchi T. 2002c. Signaling crosstalk between RANKL and interferons in osteoclast differentiation. Arthritis Res 4 Suppl 3:S227–S232.

Taniguchi T, Takaoka A. 2002. The interferon-α/β system in antiviral responses: A multimodal machinery of gene regulation by the IRF family of transcription factors. Curr Opin Immunol 14:111–116.

Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. 2001. IRF family of transcription factors as regulators of host defense. Annu Rev Immunol 19:623–655.

Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae SC, Komori T, Ito Y, Littman DR. 2002. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. Cell 111:621–633.

Theill LE, Boyle WJ, Penninger JM. 2002. RANK-L and RANK: T-cells, bone loss, and mammalian evolution. Annu Rev Immunol 20:795–823.

Tokuhiro S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, Suzuki M, Nagasaki M, Ohtsuki M, Ono M, Furukawa H, Nagashima M, Yoshino S, Mabuchi A, Sekine A, Saito S, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K. 2003. An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. Nat Genet 35:341–348.

Xiao L, Naganawa T, Obugunde E, Gronowicz G, Ornitz DM, Coffin JD, Hurley MM. 2004. Stat1 controls postnatal bone formation by regulating fibroblast growth factor signaling in osteoblasts. J Biol Chem 279:27743–27752.

Yasoda A, Komatsu Y, Chusho H, Miyazawa T, Ozasa A, Miura M, Kurihara T, Rogi T, Tanaka S, Suda M, Tamura N, Ogawa Y, Nakao K. 2004. Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. Nat Med 10: 80–86.