Journal of Pharmacological Sciences

Retraction

Retraction: Nuclear Receptors as Targets for Drug Development: Crosstalk Between Peroxisome Proliferator-Activated Receptor γ and Cytokines in Bone Marrow-Derived Mesenchymal Stem Cells

Ichiro Takada, Miyuki Suzawa, and Shigeaki Kato

***Journal of Pharmacological Sciences*, Vol. 97, pp. 184–189 (2005)**

The following review article by I. Takada, M. Suzawa, and S. Kato: Nuclear receptors as targets for drug development: crosstalk between peroxisome proliferator-activated receptor γ and cytokines in bone marrow-derived mesenchymal stem cells. J Pharmcol Sci 97, 184 – 189 (2005) has been retracted by agreement between the authors, the journal Editor in Chief, and The Japanese Pharmacological Society. The authors have requested the retraction based on their acknowledgement that their original article underlying this review is under doubt because of improper figure image arrangements.
The authors apologize to all concerned.

The Japanese Pharmacological Society, the publisher

Published online in J-STAGE
doi: 10.1254/jphs.097184RT

J Pharmacol Sci **97**, 184 – 189 (2005)

Journal of Pharmacological Sciences

Forum Minireview

Nuclear Receptors as Targets for Drug Development: Crosstalk Between Peroxisome Proliferator-Activated Receptor γ and Cytokines in Bone Marrow-Derived Mesenchymal Stem Cells

Ichiro Takada[1,*], Miyuki Suzawa[1], and Shigeaki Kato[1]

[1]*Institute of Molecular and Cellular Bioscience, University of Tokyo, Tokyo 113-0032, Japan and ERATO, Japan Science and Technology Agency, Japan*

Received October 25, 2004; Accepted December 20, 2004

Abstract. Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-dependent nuclear receptor and regulates adipogenesis and fat metabolism. PPARγ is activated by fatty acid derivatives and some synthetic compounds such as the thiazolidinediones. In addition, certain cytokines were known to affect the transactivation function of PPARγ. However, the molecular mechanism of the functional interaction between PPARγ and cytokine signaling remains unclear. We found that combined treatment of PPARγ and cytokines (IL-1 or TNF-α) inhibited adipogenesis and induced osteoblastgenesis in bone marrow-derived mesenchymal stem cells. Furthermore, we showed that the ligand dependent transactivation function of PPARγ was suppressed by IL-1 and TNF-α. This suppression was mediated through NF-κB activated by the TAK1/TAB1-NIK cascade, a downstream cascade triggered with IL-1 or TNF-α signaling. Thus, we have identified a molecular mechanism of functional cross-talk between PPARγ and cytokine signaling that may provide a theoretical basis for development of novel therapeutical strategies and design of novel compounds for treatment of obesity, diabetes, and some other chronic diseases.

Keywords: nuclear receptor, adipogenesis, cytokine, mesenchymal stem cell, transcription

Introduction

Peroxisome proliferator-activated receptor gamma (PPARγ) is a member of the nuclear receptor gene superfamily and represents an extremely important target for drug discovery. As it was the case for its heterodimeric partner (retinoid X receptor: RXR), PPARγ was originally discovered (1, 2) as an 'orphan' receptor (a protein belonging to the nuclear receptor superfamily, but without a known ligand). Subsequently, it has been found that the widely used anti-diabetic thiazolidinedione (TZD) drugs are ligands for PPARγ (3), and 15-deoxy-delta(12,14)-prostaglandin J2 was identified as a endogenous ligand for PPARγ (4). Practical significance of PPARγ ligands for treatment of diabetes have moved PPARγ studies to the forefront of both molecular and clinical therapeutics, and therefore, it become important to determine the roles of PPARγ in other major chronic human diseases such as atherogenesis and carcinogenesis (5).

Moreover, one of the PPARγ functions is to regulate adipocyte differentiation (6). Some adipocytes derived from the bone marrow stem cells have been shown to possess adipogenic, osteogenic, chondrogenic, myogenic, and neurogenic potential in vitro (7, 8). These cells differentiate into a particular cell type in response to multiple cell-membrane receptor-mediated signals and to lipid-soluble ligands of nuclear receptors (Fig. 1). However, effect of combination of these signaling pathways on bone marrow stem cell differentiation remains unclear. We have reported earlier that signaling pathways of many nuclear receptors cross-talk with those of cell membrane receptor-mediated signals (9, 10). So, this raises the possibility that the function of PPARγ in gene regulation may be modulated by cell-membrane receptor-mediated signaling.

*Corresponding author. FAX: +81-3-5841-8477
E-mail: itakada@iam.u-tokyo.ac.jp

IL-1 and TNF-α suppress PPARγ-induced adipogenesis in the bone marrow

We focused on two cytokines, interleukin (IL)-1 and tumor necrosis factor (TNF)-α. In vivo, these cytokines are produced by osteoblastic cells and control osteoclastic bone resorption (11, 12). Both IL-1 and TNFα activate various intracellular signal transducers, including nuclear factor (NF)-κB. IL-1 and TNF-α are also known to suppress both in vivo and in vitro adipogenesis (13). Therefore, we examined whether these cytokines are able to inhibit transcriptional function of PPARγ and PPARγ-induced adipogenesis. Consistent with the results in earlier reports (13), we have found that adipogenesis in ST2 cells, a mesenchymal cell line derived from mouse bone marrow (14), can be induced by treatment with a synthetic PPARγ ligand, troglitazone (Tro). This adipogenesis was prevented by the presence of IL-1 and TNF-α in the media (Fig. 2A).

Consequently, ST2 cells treated with Tro and the cytokines differentiated into osteoblasts, indicating a switch of cytodifferentiation from adipogenesis into osteoblastgenesis (Fig. 2B). This finding of the cytokine inhibitory action on the adipogenesis in the ST2 cells suggested that the ligand-induced transactivation function of PPARγ might be suppressed by these cytokines.

The inhibitory actions of cytokines on the PPARγ function is mediated by the TAK1/TAB1-NIK-NFκB pathway

To investigate whether the transactivation function of PPARγ was attenuated by cytokines, we first examined whether cytokine treatment over a short period of time (12 h) caused a reduction in the transactivation function of PPARγ. The ligand-dependent transactivation function of PPARγ was markedly suppressed in ST2 cells by treatment with either IL-1 or TNF-α (Fig. 3A). As the cell membrane receptor for IL-1 and TNF-α trigger a number of known downstream signaling cascades (12), we examined the activity of several downstream signals by reporter expression assay. We found that TAK1 (transforming growth factor-beta-activated kinase 1) (15, 16); a MAP kinase kinase kinase (MAP3K); and TAB1 (TAK1-binding protein) (17), which is an activator of TAK1, suppressed the transcactivation function of PPARγ (Fig. 3A, lane 6). Consistent with these results, the use of a kinase-negative mutant of TAK1 (K63W) abrogated the suppressive effect of TAK1/TAB1, TNF-α, and IL-1 on PPARγ transactivation function (Fig. 3A, lanes 8 – 10).

Next we examined the activity of downstream signals of TAK1/TAB1 and found that NIK (NF-κB-inducing kinase) (18), activated via phosphorylation by TAK1/TAB1, suppressed the function of PPARγ (Fig. 3B, lane 9). A dominant negative form of NIK (629-947) abrogated the suppressive effects of TAK1/TAB1 induced by IL-1 or TNF-α treatment of ST2 cells (Fig. 3B, lane 10). Moreover, adipogenesis in the cell lines expressing the dominant-negative NIK mutant (629-947) remained Tro-sensitive, indicating that this NIK mutant failed to suppress the PPARγ transactivation.

We have also found that both IKK-α (I kappa B kinase-α) (19) and IKK-β (20), which are downstream of NIK and activate NF-κB, suppressed the ligand-dependent transactivation function of PPARγ (Fig. 3C, lanes 3 and 4). NF-κB (p65/p50) alone was also able to effectively suppress the ligand-dependent transactivation function of PPARγ in ST2 cells (Fig. 3C, lane 5). Using a co-immunoprecipitation assay with antibodies against PPARγ, p50, and p65, an association between the endogenous NF-κB complex and PPARγ was observed in ST2 cells, but only when the cells were treated with either TNF-α or IL-1 (Fig. 3D). By assessing the DNA binding activity of PPARγ/RXRα heterodimer by electrophoretic gel mobility shift assay (EMSA), we found that treatment with either cytokine and the overexpression of downstream factors and NF-κB caused significant reduction in the DNA binding activity of PPARγ/RXRα heterodimers to a consensus DNA response element (acyl-CoA-PPRE), suggesting direct association of PPARγ/RXRα with NF-κB in the nucleus. The direct association was further confirmed by interaction of in vitro translated proteins and glutathiore-*S*-transferase (GST)-fusion proteins. Futhermore, DNA binding of PPARγ/RXRα was inhibited by NF-κB or p65 alone (Fig. 3E), and the DNA binding C domain of PPARγ was mapped as a p65 interacting region, while the Rel-homology region (49-298) in p65 (21) appeared to directly interact with PPARγ.

PPARγ coactivator is involved in PPARγ-NF-κB complex

We then studied the molecular mechanism of the NF-κB recruitment to PPARγ in the bone marrow cell nuclei with a number of potential coactivators and corepressors for PPARγ. No direct association of NF-κB with either p160 family proteins (SRC-1/TIF2/AIB1) (22), DRIP205/TRAP220 (23), or NCoR/SMRT (24, 25), was detected. However, we found that PGC-2 (PPAR gamma coactivator-2), a PPARγ AF-1-specific co-activator (26), was associated with endogenous NF-κB activated by IL-1 and TNF-α. This cytokine-induced interaction was detected when the complex was

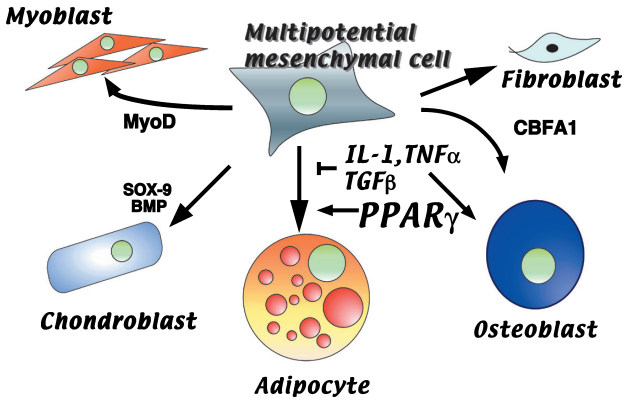

Fig. 1. Diagramatic depiction of bone marrow-derived mesenchymal stem cell differentiation paradigm and related signals. MyoD, myoblast determination; CBFA1, core binding factor alpha 1; SOX-9, SRY box-9; BMP, bone morphogenic protein; TGFβ, transforming growth factor-β.

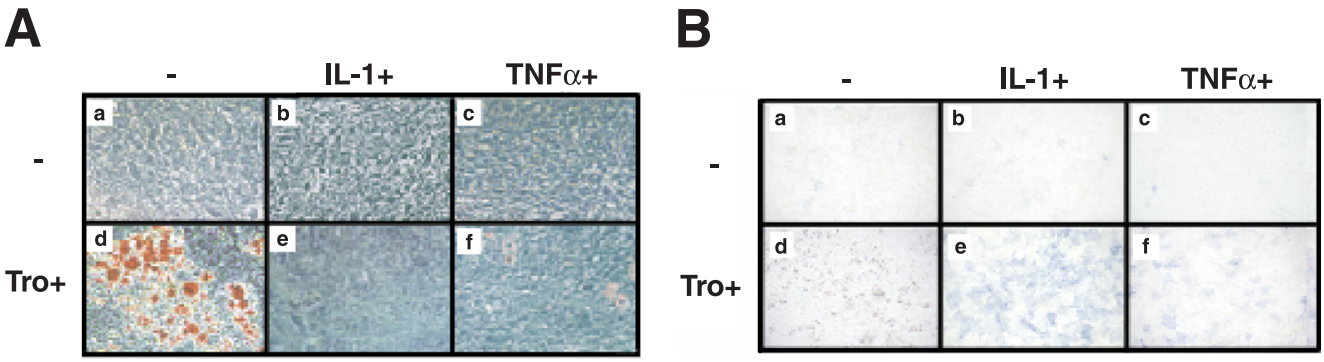

Fig. 2. Inhibitory effects of IL-1 and TNF-α on the troglitazone-induced adipogenesis. A: Weak treatment (for 1 week) using the synthetic PPARγ ligand troglitazone (Tro) adipogenesis of ST2 cells (d, e, f). The presence of either IL-1 or TNF-α prevented ligand-induced adipogenesis (b, c, e, f). Differentiated adipocytes were detected by accumulated lipid stained red by Oil-Red O staining. B: Combined treatment of cytokines and troglitazone induces osteoblastgenesis in ST2 cells. Cells were treated with cytokines and troglitazone as in panel A, and differentiated osteoblasts were stained by alkaline phosphatase.

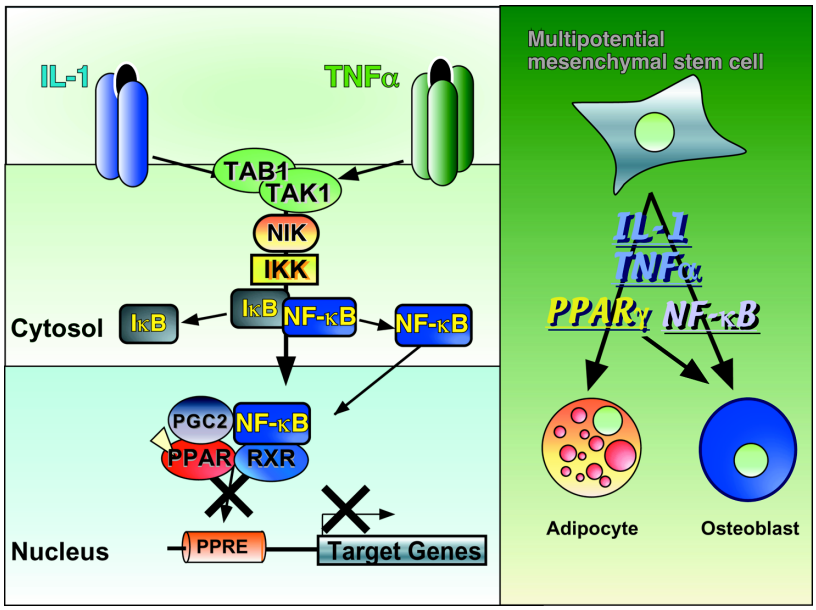

Fig. 4. Schema of the proposed molecular mechanism of adipogenesis inhibition by TNF-α and IL-1 through suppression of PPARγ function by NF-κB activated via the NIK-TAK1/TAB1-mediated cascade.

sequentially immunoprecipitated with PGC-2, then with PPARγ, suggesting that NF-κB associates with PGC-2 bound PPARγ. Direct interaction of PGC-2 with either NF-κB or PPARγ was further demonstrated in a GST-pull down assay (Fig. 3F). These findings suggest that in the nucleus, NF-κB is recruited to PPARγ through direct association with PGC-2, forming a tripartite molecular complex, resulting in the inhibition of PPARγ DNA binding.

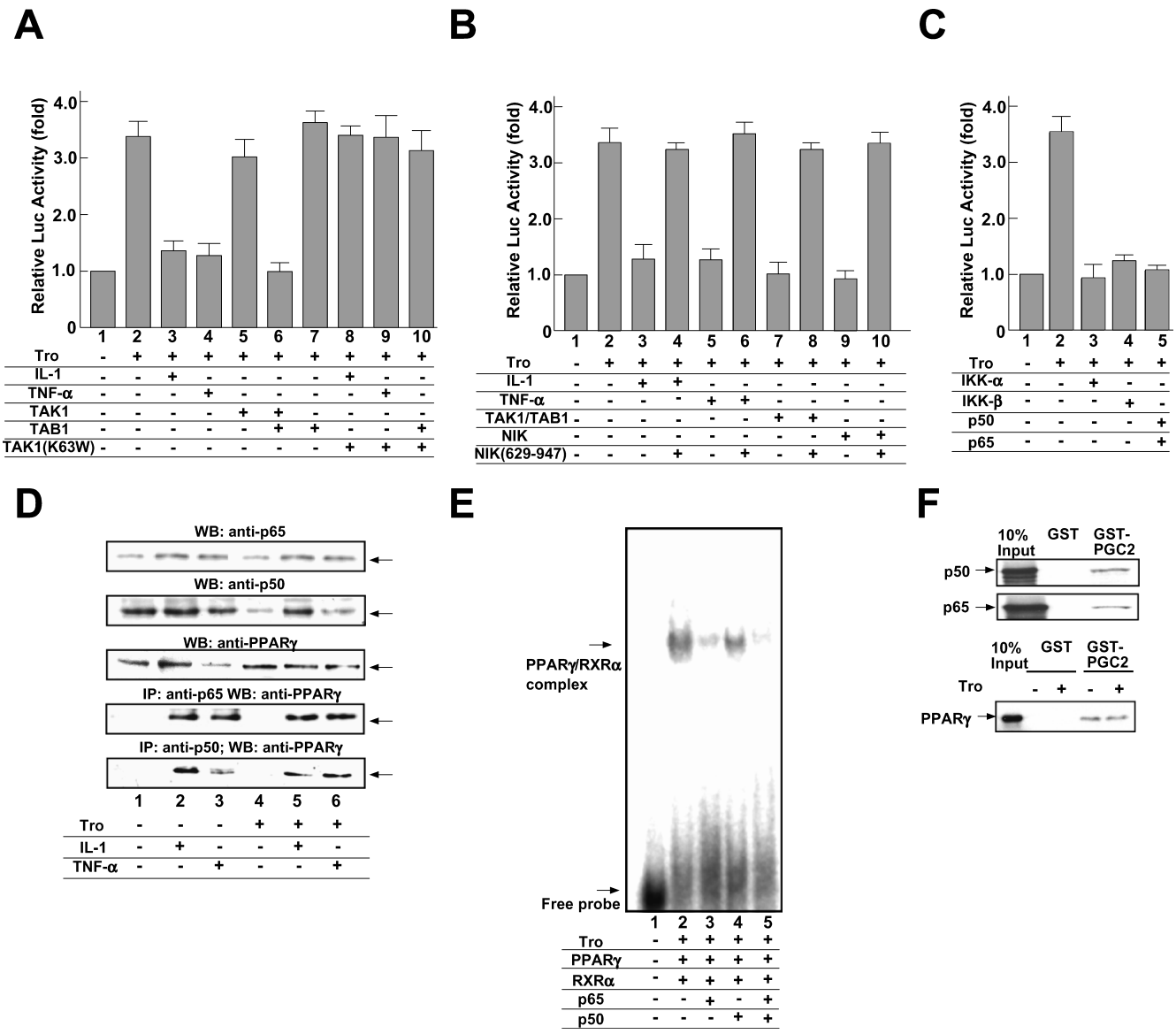

Fig. 3. TAK1/TAB1/NIK mediates suppression of PPARγ function, and NF-κB prevents DNA binding by the PPARγ/RXRα heterodimer. A: Troglitazone (Tro)-induced transactivation function of PPARγ was suppressed by IL-1 or TNF-α and TAK1/TAB1, a downstream signal inducer of IL-1 and TNF-α signaling. ST2 cells were transfected with the indicated expression vectors along with the mouse acyl-CoA oxidase-PPRE-tk–luciferase reporter plasmid, and further incubated for 16 h with the indicated cytokines in the absence or presence of cognent ligand. Similar results were obtained using 293T cell lines (data not shown). B: Suppression of PPARγ function by NF-κB-inducing kinase (NIK) that acts downstream of TAK1/TAB1. NIK (629-947) is a dominant-negative form of NIK. C: PPARγ function was suppressed by NIK-activated IKK-α, IKK-β, and NF-κB. The indicated expression vectors of signal inducers downstream of NIK were transfected to ST2 cell lines. D: NF-κB associates with PPARγ in the nucleus. Endogenous liganded PPARγ and endogenous NF-κB complex were coimmuno-precipitated with the indicated p65 or p50 antibodies following Western blotting. Cytokine treatment induced association of PPARγ with NF-κB complex in the nucleus. E: Suppression of PPARγ DNA binding by NF-κB in vitro. In vitro translated p50, p65, PPARγ, and RXRα were used for EMSA (Electro Mobility Shift Assay). F: Interaction of PGC-2 with either NF-κB (p50, p65) or PPARγ was examined by a GST pull-down assay.

Conclusion

Our results indicate that suppression of PPARγ function by IL-1 or TNF-α leads to increased sensitivity of mesenchymal stem cells to other cytodifferentiation factors and to development of cell types other than adipocytes, such as osteoblasts and osteoclasts (27). Previous reports showed that the activated PPARγ signaling in the progenitor cells of monocyte/macrophage lineage inhibits osteoclastogenesis through the suppression of NF-κB function (28, 29). In the nucleus, NF-κB appears to directly interact with the DNA binding domain of PPARγ, as well as with PGC-2 bound AF-1 A/B domain, resulting in the blocking of DNA binding of PPARγ/RXR heterodimers to target gene promoters. Moreover, as the functional interaction between PPARγ AF-1 domain and PGC-2 has been shown to play a significant role in fat cell differentiation (26), the interaction with NF-κB may interfere with their adipogenic function. It also appears that complexes formed by ligand-bound PPARγ, NF-κB, PGC-2, and possibly some other unknown factors lead to a mutual inactivation of both transcriptional factors, probably through suppression of their function (Fig. 4). Identification of associated proteins would lead to better understanding of coupled transcriptional regulation by distinct classes of transcriptional factors in the same complex and may provide insight for development of novel strategies for therapeutical modulation of their activity.

References

1 Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature. 1990;347:645–650.

2 Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell. 1992;68:879–887.

3 Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem. 1995;270: 12953–12956.

4 Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-Deoxy-delta(12,14)-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell. 1995; 83:803–812.

5 Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circ Res. 2004;94:1168–1178.

6 Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. 1994;8:1224–1234.

7 Nuttall ME, Gimble JM. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Pharmacol. 2004;4:290–294.

8 Ballas CB, Zielske SP, Gerson SL. Adult bone marrow stem cells for cell and gene therapies: implications for greater use. J Cell Biochem Suppl. 2002;38:20–28.

9 Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science. 1995;270: 1491–1494.

10 Yanagisawa J, Yanagi Y, Masuhiro Y, Suzawa M, Watanabe M, Kashiwagi K, et al. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. Science. 1999;283:1317–1321.

11 Konig A, Muhlbauer RC, Fleisch H. Tumor necrosis factor alpha and interleukin-1 stimulate bone resorption in vivo as measured by urinary [3H]tetracycline excretion from prelabeled mice. J Bone Miner Res. 1988;3:621–627.

12 Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004;15: 49–60.

13 Ron D, Brasier AR, McGehee RE Jr, Habener JF. Tumor necrosis factor-induced reversal of adipocytic phenotype of 3T3-L1 cells is preceded by a loss of nuclear CCAAT/enhancer binding protein (C/EBP). J Clin Invest. 1992;89:223–233.

14 Ogawa M, Nishikawa S, Ikuta K, Yamamura F, Naito M, Takahashi K, et al. B cell ontogeny in murine embryo studied by a culture system with the monolayer of a stromal cell clone, ST2: B cell progenitor develops first in the embryonal body rather than in the yolk sac. EMBO J. 1988;7:1337–1343.

15 Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao Z, Matsumoto K. The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. Nature. 1999;398:252–256.

16 Takaesu G, Kishida S, Hiyama A, Yamaguchi K, Shibuya H, Irie K, et al. TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. Mol Cell. 2000;5:649–658.

17 Shibuya H, Yamaguchi K, Shirakabe K, Tonegawa A, Gotoh Y, Ueno N, et al. TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. Science. 1996;272:1179–1182.

18 Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF-kappaB induction by TNF, CD95 and IL-1. Nature. 1997;385:540–544.

19 Regnier CH, Song HY, Gao X, Goeddel DV, Cao Z, Rothe M. Identification and characterization of an IkappaB kinase. Cell. 1997;90:373–383.

20 Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IkappaB kinase-beta: NF-kappaB activation and complex formation with IkappaB kinase-alpha and NIK. Science. 1997;278:866–869.

21 Chen FE, Huang DB, Chen YQ, Ghosh G. Crystal structure of p50/p65 heterodimer of transcription factor NF-kappaB bound to DNA. Nature. 1998;391:410–413.

22 Onate SA, Tsai SY, Tsai MJ, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science. 1995;270:1354–1357.

23 Rachez C, Suldan Z, Ward J, Chang CP, Burakov D, Erdjument-Bromage H, et al. A novel protein complex that interacts with the vitamin D3 receptor in a liganddependent manner and enhances VDR transactivation in a cellfree system. Genes Dev. 1998;12:1787–1800.

24 Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature. 1995;377:397–404.
25 Chen JD, Evans RM. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature. 1995;377:454–457.
26 Castillo G, Brun RP, Rosenfield JK, Hauser S, Park CW, Troy AE, et al. An adipogenic cofactor bound by the differentiation domain of PPARgamma. EMBO J. 1999;18:3676–3687.
27 Suzawa M, Takada I, Yanagisawa J, Ohtake F, Ogawa S, Yamauchi T, et al. Cytokines suppress adipogenesis and PPAR-gamma function through the TAK1/TAB1/NIK cascade. Nat Cell Biol. 2003;5:224–230.
28 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature. 1998;391:79–82.
29 Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature. 1998; 391:82–86.