Bone morphogenetic protein 2 (BMP2), a member of the transforming growth factor-beta (TGF-β) superfamily, regulates a variety of cell fates and functions. At present, the molecular mechanism by which BMP2 induces apoptosis has not been fully elucidated. Here we propose a BMP2 signaling pathway that mediates apoptosis in mouse hybridoma NIH3T3 cells whose growth is interleukin-6 (IL-6)-dependent. BMP2 dose-dependently induces apoptosis in NIH3T3 cells even in the presence of IL-6. BMP2 has no inhibitory effect on the IL-6-induced tyrosine phosphorylation of STAT3, and the anti-caspase-3 gene expression which is known to be regulated by STAT3, suggesting that BMP2-induced apoptosis is not attributed to alteration of the IL-6-mediated caspase-3 pathway. We demonstrate that BMP2 induces activation of TGF-β-activated kinase (TAK1) and subsequent phosphorylation of p38 stress-activated protein kinase. In addition, forced expression of kinase-negative TAK1 in NIH3T3 cells blocks BMP2-induced apoptosis. These results indicate that BMP2-induced apoptosis is mediated through the TAK1-p38 pathway in NIH3T3 cells. We also show that NIH3T3-derived transfectants expressing Smad6 are resistant to the apoptotic signal of BMP2. Interestingly, this ectopic expression of Smad6 blocks BMP2-induced TAK1 activation and p38 phosphorylation. Moreover, Smad6 can directly bind to TAK1. These findings suggest that Smad6 is likely to function as a negative regulator of the TAK1 pathway in the BMP2 signaling, in addition to the previously reported Smad pathway.

BMP2-induced Apoptosis Is Mediated by Activation of the TAK1-p38 Kinase Pathway That Is Negatively Regulated by Smad6*

Received for publication, October 22, 1999, and in revised form, March 14, 2000
Published, JBC Papers in Press, March 23, 2000, DOI 10.1074/jbc.M90522199

Naoki Kimura‡‡, Ritsuko Matsuot, Hiroshi Shibuya*, Kinichi Takada*, and Tetsuya Taga‡‡

From the ‡Department of Molecular Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101-0062, Japan, the §Gene Search Program, Chugai Research Institute for Molecular Medicine, Inc., Nihari, Ibaraki 300-4101, Japan, and the ¶Division of Morphogenesis, Department of Developmental Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan

Bone morphogenetic protein 2 (BMP2), a member of the transforming growth factor-beta (TGF-β) superfamily, signals through the heterotetrameric complex of type I and II serine-threonine kinase receptors (1, 2). Downstream of the receptor complex, at least two distinct intracellular pathways have been suggested for mediating inductive signals from the cell membrane to the nucleus.

One pathway involves a family of transcription factors collectively known as Smads. Smad1, Smad5, or Smad8 are phosphorylated by activated type I BMP2/4 receptors and are then associated with a common signaling mediator, Smad4. The resultant heteromeric Smad complex is translocated into the nucleus where they activate transcription (3–5). Another pathway is mitogen-activated protein kinase (MAPK) cascade initiated by TAK1 (for TGF-β-activated kinase-1) (6). TAK1 was originally identified as a member of MAPK kinase kinase (MAPKKK) activated in response to TGF-β and BMP4 (6). More recently, it has been reported that TAK1 functions as a mediator of the MKK6-p38 pathway and the MKK–JNK pathway (7–9). However, a regulatory mechanism of the signal transduction pathway mediated by TAK1 remains to be clarified.

After initial activation of the receptors, BMP2 elicits multiple effects ranging from cell differentiation to regulation of early embryogenesis. Because there are tens of different cytokines present simultaneously in vivo, it is sometimes the case that two different signal pathways could cause the synergistic or antagonistic interplay in common target cells. We have recently reported that BMP2 and leukemia inhibitory factor (LIF) act in synergy on cultured fetal neural progenitor cells to induce differentiation into astrocytes, whereas BMP2 or LIF alone do not induce astrocyte development under the same culture conditions (10). LIF is a member of the interleukin-6 (IL-6) family of cytokines, which shares membrane glycoprotein gp130 as a common subunit in the receptor complex (11). Ligand binding to the receptor triggers the dimerization of gp130, activating gp130-associated cytoplasmic tyrosine kinase in the Janus kinase family and a downstream transcription factor, signal transducer and activator of transcription-3 (STAT3) (11). In the case of synergistic effect caused by BMP2 and LIF, respective downstream transcription factors Smad1 and STAT3 form a signal-dependent complex bridged by p300 in the nucleus, thereby exerting the synergy between BMP2 and LIF in astrocyte differentiation (10). Moreover, another group has demonstrated the inhibitory cross-talk between the Smad cascade and the signal from a receptor tyrosine kinase: activation of epidermal growth factor receptor leads to MAPK activation, which then phosphorylates Smad1 on serine residues, thereby inhibiting Smad1 translocation into the nucleus.
In the present study, we attempt to know the interaction of the signals mediated by IL-6 and those by BMP2 in mouse hybridoma MH60 cells whose growth is IL-6-dependent. We show here that BMP2 induces apoptosis in MH60 cells even in the presence of proliferative signals of IL-6. We show that phosphorylation of STAT3 and expression of its target gene bcl-2 are not affected by BMP2, suggesting that the cell death signaling pathway initiated by BMP2 is independent of the survival signaling by IL-6. The studies presented here further propose a mechanism of BMP2-induced apoptosis in MH60 cells, which appears to be mediated by the TAK1-p38 kinase pathway. In addition, we show that Smad6 unexpectedly blocks BMP2-induced apoptosis and suggest a mechanism by which Smad6 prevents cell death promoted by BMP2.

EXPERIMENTAL PROCEDURES

Plasmids—Wild type TAK1 cDNA was subcloned into pEF-BOSE-FLAG vector (13) to generate pEFBOSE-FLAG-TAK1. Mammalian expression vectors encoding HA-BMPR-IA, Myc-XIAP, and FLAG-TAB1 were described previously (14). HA-TAK1 (wild type) and HA-KNTAK1 (K581W) were also described previously (15). Myc-tagged and FLAG-tagged Smad6-expressing vectors were kind gifts of Drs. Miyazono and Imamura (16).

Cell Culture—Mouse Hybridoma MH60 cells were cultured in RPMI 1640 medium (Sigma) containing 10% fetal bovine serum, 2 ng/ml IL-6. COS7 cells were maintained in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% fetal bovine serum. To establish Smad6-overexpressing MH60 cell clones (MH60/Smad6), 20 μg of FLAG-tagged Smad6 expression vector or mock vector was cotransfected with 1 μg of pSV2-NEO using an electroporation method. Transfectants were selected by 500 μg/ml G418, and single cell clones were obtained by limited dilution. Expression of Smad6 was analyzed by Western blotting using anti-FLAG antibody (Sigma).

Reverse Transcriptase-Polymerase Chain Reaction—First strand cDNAs were synthesized from 1 μg of total RNA using superscript II (Life Technologies, Inc.). The respective first strand cDNAs were then used directly for amplification of bcl-2 and G3PDH genes by polymerase chain reaction (PCR). The PCR reaction was performed using cycling conditions appropriate for each gene.

DNA Fragmentation Assay—After the cultivation of cells in the presence of 50 ng/ml of BMP2 for 13 or 24 h, cells (5 × 10⁶) were lysed in a buffer containing 0.5% Triton X-100, 10 mM Tris, pH 7.4, and 10 mM EDTA. After treatment with RNase A and proteasine K, the size of DNA was analyzed by agarose gel electrophoresis.

Immunoblotting and Coimmunoprecipitation Analysis—To examine the STAT3 tyrosine phosphorylation, MH60 cells were starved for 1 h without IL-6 and then treated with IL-6 together with BMP2 or IL-6 or with BMP2 alone. After 10 min, the cells were solubilized with Nonidet P-40 lysis buffer (0.5% Nonidet P-40, 10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 5 mM EDTA, 2 mM NaF, 1 mM phenylmethylsulfonyl fluoride, and 5 μg/ml aprotinin). Lysates were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotted with anti-STAT3 antibody or anti-phospho-tyrosine antibody (4G10, Upstate). The proteins were detected by using an enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech).

To examine the p38 activation, MH60 cells were treated with BMP2 (20 ng/ml) for 40 min followed by starvation as described above. The cells were lysed with lysis buffer, and subjected to immunoblotting with p38 antibody (Santa Cruz Biotechnology) or phospho-specific p38 antibody (New England BioLabs), respectively.

For the coimmunoprecipitation assay, COS7 cells were transfected with expression constructs using a Trans-IT LT-1 polyamine transfection reagent (Qiagen) according to the manufacturer’s protocol. Cells were fixed with 3% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min followed by washing with PBS. Cells were permeabilized with 0.1% Triton X-100 for 5 min, washed again, and incubated in blocking solution (3% bovine serum albumin in PBS) at 4 °C for 2 h. Cells were incubated with monoclonal antibody against FLAG-epitope or polyclonal antibody against HA-epitope at 4 °C for 1 h in PBS containing 2% bovine serum albumin. Cells were washed three times with PBS and incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG (ImmunoResearch) or rhodamine-conjugated anti-rabbit IgG (Chemicon) for an additional 1 h. After three times washing, cells were counterstained with Hoechst 33258. Images were obtained using fluorescent microscopy (AX70, Olympus).

Protein Kinase Assay—Cells were lysed in extraction buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 12.5 mM β-glycerophosphate, 1.5 mM MgCl₂, 2 mM EDTA, 10 mM NaF, 2 mM dithiothreitol, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, and 20 μg/ml aprotinin) containing 0.5% Triton X-100. Endogenous TAK1 was precipitated using rabbit polyclonal antibody against TAK1-C-terminal peptide (8). Immunocomplexes were recovered with protein A-Sepharose and washed three times with washing buffer (20 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM MgCl₂) then twice with kinase buffer (20 mM Tris-HCl, pH 7.4, 10 mM MgCl₂). Immunoprecipitates were resuspended in kinase buffer containing 1 μg of the specific substrate his-MKK6. The kinase reaction was initiated by addition of 5 μCi of [γ-32P]ATP (NEN Life Science Products). After 2 min of incubation at 30 °C, reactions were terminated by adding SDS sample buffer followed by boiling for 5 min. Samples were separated by SDS-PAGE, dried, and visualized by autoradiography.

RESULTS

BMP2-induced Apoptosis in MH60 Cells—We have previously demonstrated that BMP2 and LIF exert a synergistic effect on the differentiation of neural progenitor cells (10). In an attempt to know whether a similar cooperative mechanism takes place in other cell systems, we tested the effect of BMP2 on the growth of IL-6-dependent mouse hybridoma MH60 cells. As shown in Fig. 1, BMP2 dose-dependently suppressed growth of MH60 cells in the presence of IL-6. To know the mechanism of this growth suppression induced by BMP2, we first examined whether BMP2 alters the cell cycle distribution and/or induces apoptosis on MH60 cells cultured with IL-6. BMP2 treatment for 24 h resulted in the cell cycle arrest in the G₁ phase (data not shown) and genomic DNA fragmentation (Fig. 2A). These observations indicate that treatment of BMP2 causes cell cycle arrest in the G₁ phase and apoptosis in MH60 cells.

Withdrawal of IL-6 for 24 h led to the apoptosis of MH60 cells (Fig. 2A) without apparently inducing cell cycle arrest in the G₁ phase (data not shown) but with decreased expression of bcl-2...
BMP2-induced Apoptosis Mediated by TAK1

FIG. 2. BMP2 induced apoptosis in MH60 hybridomas but did not interfere with the IL-6 signal transduction pathway. MH60 cells were incubated with IL-6 (2 ng/ml) or BMP2 (50 ng/ml) in a combination as indicated above the figure. A, DNA purified after 24-h culture was analyzed for fragmentation in an agarose gel. B, total RNA prepared after 15-h culture was used for reverse transcriptase-PCR analysis with primers specific for bcl-2 and G3PDH. C, lysates prepared from cells stimulated with cytokines for 15 min were subjected to antiphosphotyrosine or anti-STAT3 immunoblotting.

To our surprise, in the Smad6-overexpressing MH60 cells (MH60/Smad6), which are resistant to BMP2-induced apoptosis, activation of p38 and TAK1 did not occur after BMP2 stimulation (Fig. 4, A and B). These results indicate that Smad6 inhibits the activation of the TAK1-p38 pathway in BMP2 signaling.

Essential Role of TAK1 Activation in the BMP2-induced Apoptosis in MH60 Cells—Catalytically inactive TAK1 (TAK1/KN) is known to inhibit the TAK1 signaling pathway (6). To determine the involvement of TAK1 in BMP2-induced apoptosis, we transiently transfected expression vectors encoding FLAG-tagged TAK1/KN (FLAG-TAK1/KN) or wild type control ((FLAG-TAK1(WT)) into MH60 cells. Expression of these proteins could be identified by immunostaining with anti-FLAG.
BMP2-induced Apoptosis Mediated by TAK1

**FIG. 5. Expression of the catalytically inactive TAK1 prevented BMP2-induced apoptosis.** MH60 cells were transfected with vectors encoding kinase-negative TAK1 (A and B) or FLAG-tagged wild type TAK1 (C and D). 24 h after transfection, cells were further cultured with BMP2 for 14 h. Cells expressing these proteins were detected by immunostaining with anti-FLAG antibody (A and C). Changes of the chromatin structure in the same transfected cells were monitored by Hoechst dye staining (B and D). Overexpression of the kinase-negative TAK1 prevented apoptosis induced by BMP2 (arrow in A and B), whereas normal MH60 cells (triangles), or cells overexpressing wild type TAK1 (arrow in C and D) show apoptotic morphology by treatment of BMP2.

Several recent studies have suggested that there is cross-talk between intracellular signaling pathways of different cytokines or growth factors. For example, epidermal growth factor and hepatocyte growth factor can antagonize the effects of BMP2 by inducing phosphorylation of Smad1 (12). On the other hand, LIF and BMP2 can elicit the synergistic interplay on differentiation of neural progenitor cells (10). In the later case, respective downstream transcription factors Smad1 and STAT3 form a complex bridged by p300 in the nucleus in a signal-dependent manner. In MH60 hybridoma cells, BMP2 opposes the proliferative effect of IL-6. However, our data suggest that BMP2 does not interfere with the IL-6 signal transduction pathway, partly because BMP2 does not alter phosphorylation of STAT3 and expression of bcl-2 gene induced by IL-6. Furthermore, we observed that BMP2 could induce apoptosis efficiently enough in MH60 cells regardless of the presence or absence of IL-6 (data not shown); therefore, BMP2 did not induce apoptosis in these cells more efficiently in the absence of IL-6. These results suggest that BMP2 promotes cell death by activating its own apoptotic pathway.

BMP2-induced apoptosis has been reported in vivo so far, for example, in rhombomeres 3 and 5 (24) and the interdigit field of the limb (25, 26) in developing chickens. In addition, another earlier study demonstrated that BMP2 was capable of inducing apoptosis in cultured mouse embryonic HS-72 cells whose growth is independent of IL-6 (27). Although the BMP2 signal transduction pathways and molecular nature of their components have been studied in detail, the signaling cascade by which BMP2 induces apoptosis both in vivo and in vitro are not fully understood.

BMP2 is a member of the TGF-β superfamily of cytokines whose signals have been suggested to be transduced from receptor serine/threonine kinases to the nucleus via at least two different signaling pathways involving transcription factors Smads and MAPKKK family kinase TAK1. TAK1 was originally identified as a TGF-β-responsive MAPKKK (6). No member of the TGF-β superfamily other than TGF-β and BMP4 have been reported so far to directly activate TAK1 (6). It was later shown that injection of Xenopus TAK1 (xTAK1) and its activator xTAB1 with bcl-2 in early embryos caused ventral-
BMP2-induced Apoptosis Mediated by TAK1

Fig. 7. Smad6 and TAK1 interacted in vivo. A, Myc-tagged Smad6 was expressed together with FLAG-tagged TAK1 in COS7 cells. Cell extracts were subjected to immunoprecipitation (IP) with anti-FLAG or anti-Myc antibody. Precipitates or lysates were separated by SDS-PAGE and analyzed by immunoblotting with anti-Myc antibody to detect Smad6 (upper panel) or anti-FLAG to detect TAK1 (lower panel). B, MH60/Smad6 transfectants and parental cells were lysed with lysis buffer and subjected to immunoprecipitation with anti-FLAG antibody to pull down Smad6. The precipitates were subjected to SDS-PAGE and subsequent immunoblotting with anti-TAK1 antibody to detect Smad6-TAK1 interaction.

BMP2-induced apoptosis is most likely mediated by the TAK1-MAPK cascade (3–5). Smad6, one of the two known inhibitory Smad species (Smad6 and Smad7), blocks the Smad pathway by associating with type I receptors to inhibit Smad1 and Smad2 phosphorylation (16) or by sequestration of phosphorylated Smad1 from Smad4 (31). Interestingly, an overexpression of Smad6 blocks BMP2-induced apoptosis in M60 cells. In a similar approach, Ishiaki et al. (27) have previously shown that an overexpression of Smad6 suppressed BMP2-induced apoptosis in mouse B cell hybridoma HS-72 cells. They showed that Smad6 blocked BMP2-induced phosphorylation of Smad1/Smad5 in HS-72 cells, as has been demonstrated by other groups. However, because their studies did not explain how BMP2 could trigger apoptosis, it was not clear how Smad6 could prevent BMP2-induced apoptosis. Here we clearly show that BMP2-induced apoptosis is dependent on the TAK1-p38 cascade in MH60 cells and that Smad6 prevents the activation of TAK1-p38 cascade induced by BMP2. Therefore, Smad6 is likely to function as a negative regulator of the TAK1 pathway in the apoptotic signaling of BMP2, in addition to the previously known negative regulatory function in the Smad pathway.

It has been reported that Smad6 binds to the type I receptors for the TGF-β superfamily cytokines and inhibits the Smad signaling pathway (16). It is important to note that our results show that Smad6 physically interacts with TAK1. This was confirmed in a COS7 cell expression system and also in MH60/Smad6 transfectant clones, in the latter of which endogenous TAK1 was found to bind to Smad6. This suggests that Smad6 directly blocks the TAK1 activity by physical interaction. X-chromosome-linked inhibitor of apoptosis protein (XIAP) is a cytoplasmic molecule that is suggested to interact with BMP type I receptor (BMPR-I) and is involved in linking the BMP2-stimulated receptor to TAK1 (14). The possibility that association of Smad6 with BMPR-I releases XIAP from BMPR-I and thereby unlinks the receptor to TAK1 does not appear to be true, because association of BMPR-I with XIAP was not disrupted by binding of Smad6 to BMPR-I in COS7 cells (data not shown). Taken together with the previous finding that Smad6 is a negative regulator of the Smad signaling, our finding of the potential for Smad6 to also inhibit the TAK1-p38 pathway indicates that Smad6 serves as a general inhibitor of TGF-β superfamily cytokines.

Because the transcription of Smad6 mRNA is induced by TGF-β superfamily cytokines (32), Smad6 may completely block the signaling by the TGF-β superfamily in an autoregulatory negative-feedback manner. Recently, involvement of TAK1 in the signal transduction of cytokines outside the TGF-β superfamily has been suggested, for instance in the NF-κB-inducing kinase-IκB kinase cascade in the IL-1 signaling pathway (28) and in the NF-κB essential modulator like kinase MAPK-like cascade in the Wnt signaling pathway (33, 34). Therefore, our finding suggests that antagonistic cross-talk exists between BMP2 and other cytokines such as IL-1 and Wnt.

The molecular mechanism by which Smad6 inhibits TAK1 kinase activation remains to be elucidated. TAK1 is known to be activated by binding with TAB1, and it has been proposed that TAB1 binding to TAK1 induces an activating conformational change (15). We observed that Smad6 interacted with TAK1 but led to little disruption of the TAK1-TAB1 complex (not shown), implying that Smad6 may not compete with TAB1 for the binding to TAK1 but may block the catalytic site, or alternatively, interaction of Smad6 with the TAK1-TAB1 complex could cause the conformational change of TAK1 back to its inactive form again.

Our present study and others (27) show BMP2-induced apo-
optosis in the mouse hybridomas that were derived from plasma
cytomas, the tumors of terminally differentiated B lineage cells. We are in the process of testing the effect of BMP2 on human-equivalent tumors, i.e. multiple myeloma. It is of much interest to note that (i) growth of myelomas are often IL-6-dependent; (ii) myeloma cells produce IL-6, IL-6 receptor (IL-6R), and soluble IL-6R (35); and (iii) patients with multiple myeloma often exhibit osteoporosis, presumably because of promotion of osteoclast de-
velopment by IL-6 and soluble IL-6R produced by myeloma cells. BMP2 is known to play an important role in inducing the bone formation. In the bone marrow, BMP2 produced by osteoblasts is suggested to be stored in a latent form bound to bone matrix as in the case of TGF-β (36, 37). When bone resorption is initiated by osteoblasts, BMP2 is released from the deposit in the calcified bone matrix, and thereafter it stimulates the differentiation of osteoblasts. In addition, osteoclasts activated by IL-6 and soluble IL-6R, which are produced by myelomas, may be growth-inhibited by the released BMP2. Because the bone marrow provides an important hematopoietic microenvironment, bone formation and hematopoiesis are closely related there. For instance, Kajkenova et al. (38) has previously shown that myeloid progenitors and IL-6 production, M. Hagiwara for recombinant MKK6 protein, K. Yasukawa for Ninomiya-Tsuji for the anti-TAK1 antibody and for encouraging discus-
sion, M. Hagiwara for recombinant MKK6 protein, K. Yasukawa for Ninomiya-Tsuji for the anti-TAK1 antibody and for encouraging discus-

Acknowledgments—We thank Yamanouchi Pharmaceutical Co., Ltd. for providing recombinant human BMP2, K. Matsumoto and J. Ninomiya-Tsuji for the anti-TAK1 antibody and for encouraging discus-

REFERENCES
1. Massague, J., and Weis-Garcia, F. (1996) Cancer Surv. 27, 41–64
2. ten Dijke, P., Miyazono, K., and Heldin, C. H. (1996) Curr. Opin. Cell Biol. 8, 139–145
3. Heldin, C. H., Miyazono, K., and ten Dijke, P. (1997) Nature 390, 465–471
4. Derynck, R., Zhang, Y., and Peng, X. H. (1998) Cell 95, 757–770
5. Massague, J. (1998) Annu. Rev. Biochem. 67, 753–791
6. Yamaguchi, K., Shirakabe, K., Shibuya, H., Irie, K., Oishi, I., Ueno, N., Taniguchi, T., Nishida, E., and Matsumoto, K. (1995) Science 270, 2088–2091
7. Meriguchi, T., Kuroyanagi, N., Yamaguchi, K., Gotou, Y., Irie, K., Kano, T., Shirakabe, K., Muro, Y., Shibuya, H., Matsumoto, K., Nishida, E., and Hagiwara, M. (1996) J. Biol. Chem. 271, 13675–13679
8. Shirakabe, K., Yamaguchi, K., Shibuya, H., Irie, K., Matsuda, S., Moriguchi, T., Gotou, Y., Matsumoto, K., and Nishida, E. (1997) J. Biol. Chem. 272, 8141–8144
9. Yao, Z., Zhou, G., Wang, X. S., Brown, A., Diener, K., Gan, H., and Tan, T. H. (1999) J. Biol. Chem. 274, 2118–2125
10. Nakashima, K., Yanagisawa, M., Arakawa, H., Kimura, N., Hisaume, T., Kawabata, M., Miyazono, K., and Taga, T. (1999) Science 284, 479–482
11. Taga, T., and Kishimoto, T. (1997) Annu. Rev. Immunol. 15, 797–819
12. Kretzschmar, M., Doody, J., and Massague, J. (1997) Nature 389, 618–622
13. Ninomiya-Tsuji, J., Narazaki, M., and Taga, T. (1997) FEBS Lett. 403, 79–82
14. Yamaguchi, K., Nagai, S., Ninomiya-Tsuji, J., Nishita, M., Tamai, K., Irie, K., Ueno, N., Nishida, E., Shibuya, H., and Matsumoto, K. (1999) EMBO J. 18, 179–187
15. Shibuya, H., Yamaguchi, K., Shirakabe, K., Tonegawa, A., Gotou, Y., Ueno, N., Irie, K., Nishida, E., and Matsumoto, K. (1996) Science 272, 1179–1182
16. Isamu, T., Takei, T., Nishihara, A., Oeda, E., Hanai, J., Kawabata, M., and Miyazono, K. (1997) Nature 389, 622–626
17. Fukuda, T., Hibi, M., Yamanaka, Y., Takahashi-Tezuka, M., Fujitani, Y., Yamaguchi, T., Nakajima, K., and Hiran, T. (1996) Immunity 5, 449–460
18. Xia, Z., Dickens, M., Rangead, J., Davis, R. J., and Greenberg, M. E. (1995) Science 270, 1326–1331
19. Butterfield, L., Storey, B., Maas, L., and Heasley, L. E. (1997) J. Biol. Chem. 272, 10110–10116
20. Verheij, M., Rose, R., Lin, Y. H., Yao, B., Jarvis, W. D., Grant, S., Bigger, M. J., Szabo, E., Zou, L. I., Kyriakis, J. M., Haimovitz-Friedman, A., Fuku, Z., and Kolesnick, R. N. (1996) Nature 380, 75–79
21. Zanke, B. W., Boudreau, K., Rubie, E., Winnett, E., Tibbles, L. A., Zou, L., Kyriakis, J., Liu, F. F., and Woodgett, J. R. (1996) Curr. Biol. 6, 606–613
22. Raingeaud, J., Gupta, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) J. Biol. Chem. 270, 7420–7426
23. Shibuya, H., Iwata, H., Masuyama, N., Gotoh, Y., Yamaguchi, K., Irie, K., Matsumoto, K., Nishida, E., and Ueno, N. (1998) EMBO J. 17, 1019–1028
24. Graham, A., Francis-West, P., Brockell, P., and Lumsden, A. (1994) Nature 372, 684–686
25. Yokouchi, Y., Sakiyama, J., Kameda, T., Iba, H., Suzuki, A., Ueno, N., and Kurisuwa, A. (1996) Development 122, 3725–3734
26. Zou, H., and Niewander, L. D. (1996) Science 273, 738–741
27. Ishiaki, A., Yamato, K., Hashimoto, S., Naka, A., Tanaki, K., Nonaka, K., ten Dijke, P., Sugino, H., and Nishihara, T. (1999) J. Biol. Chem. 274, 13637–13642
28. Ninomiya-Tsuji, J., Kishimoto, K., Miyayma, A., Inoue, J., Cao, Z., and Matsumoto, K. (1999) Nature 398, 252–256
29. Zhou, G., Lee, S. C., Yao, Z., and Tan, T. H. (1999) J. Biol. Chem. 274, 20333–20338
30. Le-Niculescu, H., Bonfoco, E., Kasuya, Y., Claret, F. X., Green, D. R., and Kari, M. (1999) Mol. Cell. Biol. 19, 751–763
31. Hata, A., Lagna, G., Massague, J., and Hemmati-Brivanlou, A. (1998) Genes Dev. 12, 186–197
32. Takek, M., Imamura, T., Sampath, T. K., Takeda, K., Ichijo, H., Miyazono, K., and Kawabata, M. (1998) Biochem. Biophys. Res. Commun. 244, 26–29
33. Meneghini, M. D., Ishitani, T., Carter, J. C., Hisamoto, N., Ninomiya-Tsuji, J., Lup, C. J., Hamill, D. R., and Bowerman, K. (1999) Nature 399, 793–797
34. Ishitani, T., Ninomiya-Tsuji, J., Nagai, S., Nishita, M., Meneghini, M., Barker, N., Waterman, M., Bowerman, B., Clevers, H., Shibuya, H., and Matsumoto, K. (1999) Nature 399, 788–802
35. Treson, S. P., and Anderson, K. C. (1998) Curr. Opin. Hematol. 5, 42–48
36. Mostov, K. and Werb, Z. (1997) Science 276, 219–220
37. Suzawa, M., Takeuchi, Y., Fukumoto, S., Kato, S., Ueno, N., Miyazono, K., Matsumoto, T., and Fujita, T. (1999) Endocrinology 140, 2125–2133
38. Kajkenova, O., Lecka-Czerniak, B., Gubri, I., Hauser, S. P., Takahashi, K., Parfitt, A. M., Jitka, R. L., Mangelas, S. C., and Lipschitz, D. A. (1997) J. Bone. Miner. Res. 12, 1772–1779