Transforming Growth Factor-β Stimulates Parathyroid Hormone-related Protein and Osteolytic Metastases via Smad and Mitogen-activated Protein Kinase Signaling Pathways*

Sanna-Maria Kääkönen‡, Katri S. Selander‡, John M. Chirgwin§, Juan Juan Yin‡, Suzanne Burns‡, Wayne A. Rankin‡, Barry G. Grubbs‡, Mark Dallas‡, Yong Cui‡, and Theresa A. Guise‡‡

From the ‡Department of Molecular Medicine, University of Texas Health Science Center at San Antonio, Institute for Drug Development, Cancer Therapy and Research Center, and the §Veterans Administration Research Service, San Antonio, Texas 78245-3217

Transforming growth factor (TGF)-β promotes breast cancer metastasis to bone. To determine whether the osteolytic factor parathyroid hormone-related protein (PTHrP) is the primary mediator of the tumor response to TGF-β, mice were inoculated with MDA-MB-231 breast cancer cells expressing a constitutively active TGF-β type I receptor. Treatment of the mice with a PTHrP-neutralizing antibody greatly decreased osteolytic bone metastases. There were fewer osteoclasts and significantly decreased tumor area in the antibody-treated mice. TGF-β can signal through both Smad and mitogen-activated protein (MAP) kinase pathways. Stable transfection of wild-type Smad2, Smad3, or Smad4 increased TGF-β-stimulated PTHrP secretion, whereas dominant-negative Smad2, Smad3, or Smad4 only partially reduced TGF-β-stimulated PTHrP secretion. When the cells were treated with a variety of protein kinase inhibitors, only specific inhibitors of the p38 MAP kinase pathway significantly reduced both basal and TGF-β-stimulated PTHrP production. The combination of Smad dominant-negative blockade and p38 MAP kinase inhibition resulted in complete inhibition of TGF-β-stimulated PTHrP production. Furthermore, TGF-β treatment of MDA-MB-231 cells resulted in a rapid phosphorylation of p38 MAP kinase. Thus, the p38 MAP kinase pathway appears to be a major component of Smad-independent signaling by TGF-β and may provide a new molecular target for anti-osteolytic therapy.

Substantial data support major roles for bone-derived TGF-β¹ and tumor-derived parathyroid hormone-related protein (PTHrP) in the vicious cycle of local bone destruction that characterizes osteolytic metastases. Tumor-produced PTHrP stimulates osteoclastic bone resorption to result in bone destruction associated with breast cancer metastases (1, 2). Neutralizing antibodies to PTHrP not only decreased osteoclastic bone resorption but also inhibited the development of metastases to bone by the human breast cancer cell line, MDA-MB-231 (3). TGF-β, stored in bone matrix (4) and released locally in active form during osteoclastic resorption (5), stimulates PTHrP production by tumor cells (6–8). A dominant-negative TGF-β type II receptor (TβRIIΔcyt) stably expressed in the MDA-MB-231 breast cancer line rendered the cells unresponsive to TGF-β and inhibited TGF-β-induced PTHrP secretion and the development of bone metastases in a mouse model. This dominant-negative type II blockade was reversed by a constitutively active TGF-β type I receptor (TβRI(T204D)). Furthermore, transfection of the cDNA for PTHrP into the dominant-negative MDA-MB-231 line also increased PTHrP production and accelerated bone metastases (9). These published data establish that TGF-β in bone can promote osteolysis by increasing PTHrP secretion from breast cancer cells. They do not, however, exclude contributions from other TGF-β-responsive tumor factors. Here we demonstrate that PTHrP is the central mediator of TGF-β-induced osteolytic metastasis. We also show that TGF-β increases PTHrP secretion from MDA-MB-231 cells by signaling through both Smad and p38 MAP kinase pathways.

First, to determine whether PTHrP is the major mediator of TGF-β-induced osteolysis, mice were inoculated with an MDA-MB-231 clonal line overexpressing the constitutively active type I TGF-β receptor, TβRI(T204D), and treated with neutralizing PTHrP antibody or control IgG. The mice treated with PTHrP antibody had a significantly lower tumor burden than the control mice, suggesting that the major downstream effector of TGF-β in the development and progression of bone metastases was PTHrP.

TGF-β increases PTHrP expression by stabilizing mRNA as well as by transcriptional mechanisms (6–8, 10–12). The signaling pathways through which TGF-β increases PTHrP secretion in breast cancer cells have not been defined, although intracellular mediators known as Smads are indispensable for many of the responses to TGF-β (12–15). However, there is also accumulating evidence that TGF-β signals through other pathways.
ways (16–18). TGF-β activates MAP kinase pathway components Ras, ERK1/2, and JNKs (19–27). TGF-β-activated kinase 1 (TAK1) and its upstream activator TAK1-binding protein mediate some responses to TGF-β family members (16, 17). Activation by TGF-β of p38 MAP kinase, which is downstream of TAK1, has also been reported (28, 29). Recently, MAP kinase kinase-independent activation of p38α by a TAK1-binding protein-dependent mechanism was demonstrated (30).

To examine the signaling pathways by which the TGF-β increases the PTHrP production, wild-type and dominant-negative Smad2, Smad3, and Smad4 were stably overexpressed in MDA-MB-231 breast cancer cells, and the changes in PTHrP production were measured. The data supported both Smad-dependent and independent mechanism for the TGF-β stimulation of PTHrP production by breast cancer cells. Specific protein kinase inhibitors were used to determine the Smad-independent signaling of TGF-β to increase PTHrP production. This study indicated that the MAP kinase pathway, and specifically p38 MAP kinase, is a major component of this Smad-independent signaling by TGF-β and provides new molecular targets for anti-osteolytic therapy.

Plasmid Constructs, Transfections, and Production of Stable Cell Lines

The Smad2 wild-type (pcDNA3-FLAG-MADRB2) and dominant-negative (pcDNA3-FLAG-MADRB2/3S-A) cDNA expression plasmids were provided by Drs. Wranica and Attisano (University of Toronto, Toronto, Canada) (31); Smad4 wild-type (pcCMV5-DPC4-HA), Smad3 wild-type (pcCM5-FLAG-PDC4), Smad3 dominant-negative (pcCM5-FLAG-Smad33S-A), and Smad4 dominant-negative (pcCM5-FLAG-PDC4) plasmids were provided by Dr. Massague, (Memorial Sloan-Kettering Cancer Center, New York, NY) (32), and the dominant-negative cDNA constructs were modified at the intracytoplasmic tail by Dr. Kato, Tokyo, Japan (33). The Smad wild-type and dominant-negative cDNAs were recloned into the pcDNA3 expression vector (Invitrogen). The Smad plasmids and pcDNA3 (empty vector) were transfected into MDA-MB-231 cells using LipofectAMINE PLUSTM reagent (Invitrogen). Single colonies were isolated by limiting dilution in the presence of neomycin analogue G418 (Sigma). The clones were assessed by transient transfection with the 3TP-lux reporter construct, which contains three copies of a TGF-β-responsive plasminogen activator promoter linked to the lux reporter gene. The clones were selected and the cells were counted. The samples were stored at −80 °C until measured. MeSO4 (0.05% v/v) diluted in medium was used as a negative control.

The optimal inhibitor concentration and incubation time for MAP kinase inhibitors SB2020380, SB202190, and PD98059 were determined by time course and dose-response studies. Concentrations for p38 inhibitors SB203580 and SB202190 (1, 5, 10, 20, and 40 μM) and for MEK inhibitor PD98059 (2, 10, 20, and 40 μM) were evaluated. The concentrations of 10 and 20 μM were selected for p38 inhibitors and MEK inhibitor, respectively, based on the capacity to inhibit PTHrP production without affecting cell viability. Subsequently, incubation times of 6, 12, 24, 30, and 48 h after 3 h of preincubation were used to determine the optimal inhibitor incubation time without affecting cell viability. The results indicated that the 24-h incubation time was optimal for all of the MAP kinase inhibitors used.

Animals

The animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Female nude (BALB/c) mice 4 weeks of age were housed in laminar flow isolated hoods. Water supplemented with vitamin K and autoclaved mouse chow was provided ad libitum. Whole blood samples for ionized calcium concentration were obtained by retro-orbital puncture under metofane anesthesia, and the radiographs were taken under mouse anesthesia (10% ketamine and 20% xylazine in 0.9% NaCl). Tumor inoculation into the left cardiac ventricle was performed as described previously (3).

In Vivo Analytical Methods

3TP-lux in Response to TGF-β—Response to TGF-β was measured by transient transfection of the 3TP-lux reporter construct, which contains three copies of a TGF-β-responsive element from the plasminogen activator inhibitor-1 promoter linked to the firefly luciferase (3TP-lux), as well as PTHrP production in response to 5 ng/ml recombinant human TGF-β1 (R & D Systems Inc.) in serum-free Dulbecco’s modified Eagle’s medium after 24 h of incubation. More than 50 clones were characterized for each construct. The MDA-MB-231 clone overexpressing dominant-negative TGF-β type II receptor (TβRIIΔcyt) (35), which is truncated at the intracytoplasmic domain (34), was constructed as described previously (9). This clone, MDA-MB-231/TβRIIΔcyt, is unresponsive to TGF-β. Stable expression of a constitutively active TGF-β type I receptor (TβRI(T204D)) (35) into MDA-MB-231/TβRIIΔcyt reversed the dominant-negative TGF-β blockade. This stable clone, MDA-MB-231/TβRIIΔcyt + TβRI(T204D), demonstrated constitutive TGF-β activity, even in the absence of ligand (9).

Inhibitor Studies

The inhibitors were dissolved in MeSO4 (cell culture grade; Sigma) and used in the following concentrations: wortmannin (20 μM), herbimycin A (35 nM), KT5720 (100 nM), KT5823 (150 nM), SB203580 (10 μM), SB202190 (10 μM), and PD98059 (20 μM). All of the inhibitors except wortmannin (Sigma) were obtained from Calbiochem. The inhibitors diluted in normal growth medium were added to wells containing confluent MDA-MB-231 cells and incubated for 3 h. TGF-β (5 ng/ml) was added to serum-free medium containing the respective inhibitors and incubated for 24 h, after which the conditioned media were collected and the cells were counted. The samples were stored at −80 °C until measured. MeSO4 (0.05% v/v) diluted in medium was used as a negative control.

The mice were inoculated with a tumor cell suspension of clonal MDA-MB-231/TβRIIΔcyt + TβRI(T204D) cells into the left cardiac ventricle on day 0, after base-line radiographs, body weight, and blood Ca2+ were determined. The mice were treated with neutralizing PTHrP mAb directed against PTHrP1–34 (antibody-producing hybridoma, 3F5, obtained from Dr. T. J. Martin, St. Vincent’s Institute, Melbourne, Australia) or isotype matched control IgG (Sigma) (n = 8/group, 1 mg subcutaneously, at the time of tumor inoculation and 1 week later). Ca2+, weight, and radiographs were monitored weekly for 6 weeks, at which time the mice were sacrificed. All of the bones and soft tissues were fixed in formalin for histological analysis. The radiographs were analyzed as described earlier in this paper. Neutralizing PTHrP mAb was produced as ascitic fluid in BALB/c mice primed with Pristane (2,6,10,14-tetramethylpentadecane; Sigma) and purified by protein G-agarose chromatography (Amersham Biosciences).

Co2+ Measurement—Ca2+ concentrations were measured in whole blood using a Calib Corning 634 ISE Ca2+/pH analyzer (Corning Medical and Scientific, Medfield, MA) as described previously (3).
Radiographs and Measurement for Osteolytic Lesion Area

The animals were x-rayed using a 43855A x-ray system (Faxitron, Buffalo Grove, IL) as described previously (3). All of the radiographs were evaluated without knowledge of the treatment groups. Quantitation of

![FIG.1](image1)

**A**, representative radiographs of hind limbs from mice 28 days after tumor inoculation. The arrows indicate osteolytic lesions.

**B**, osteolytic lesion number and area on radiographs of forelimbs and hind limbs from tumor-bearing mice. Tumor cells were inoculated on day 0. The arrows indicate the inoculation of neutralizing PTHrP mAb (black triangles) and control IgG (open triangles). The lesion number and area were statistically different from each other: \( p < 0.001 \) (***), \( p < 0.01 \) (**), and \( p < 0.05 \) (*) for lesion number and area, respectively. The values represent the means ± S.E. (n = 3/group).

**C**, bone histology from the midtibial metaphyses. Tumor (arrows) filled the marrow cavity and replaced normal cellular elements.

**D**, histomorphometric analysis of hind limbs from mice with osteolytic lesions. The data represent measurements from midsections of tibiae and femora of mice. Tumor area (mm²) from metastatic bone lesions is illustrated on the left, and osteoclast number/mm tumor adjacent to bone (tumor-bone interface) is illustrated on the right. These values were both significantly lower in PTHrP mAb-treated animals compared with control IgG-treated: \( p < 0.01 \) (**) and \( p < 0.001 \) (***). The values represent the means ± S.E.
lesion area was performed using image analysis software (Java, Jandel Video Analysis; Jandel Scientific, Corte Madera, CA). Bone Histology and Histomorphometry—Forelimb and hind limb bones were removed from mice at the time of sacrifice, fixed in 10% buffered formalin, decalcified in 14% EDTA, and embedded in paraffin. The sections were stained with hematoxylin, eosin, orange G, and phloxine. Total tumor area and osteoclast number/mm² of tumor/bone interface were measured in midsections of tibiae and femora without knowledge of experimental groups. Histomorphometric analysis was performed using OsteoMeasure System program (Osteometrics Inc., Atlanta, GA).

Statistical Methods

The results are expressed as the means ± S.E. The data were analyzed by analysis of variance followed by Tukey-Kramer post-test. p < 0.05 was considered statistically significant.

RESULTS

PTHrP Is the Central Effector of TGF-β in the Pathogenesis of Osteolytic Bone Metastases—Six weeks after tumor inoculation with the MDA-MB-231 stable clone expressing the constitutively active TGF-β type I receptor (MDA-MB-231/TβRⅡA(Mycyt + TβRI(T204D))), the number and the area of osteolytic lesions were significantly less in mice treated with PTHrP mAb compared with those treated with control IgG (Fig. 1, A and B). The tumor area was less, and osteoclasts were fewer in tumor-bearing mice treated with PTHrP mAb (Fig. 1, C and D). The body weights were greater in mice treated with PTHrP mAb compared with the control IgG (21.7 ± 0.7 g versus 17.7 ± 0.9 g; p = 0.009).

Overexpression of Wild-type and Dominant-negative Smads in MDA-MB-231 Cells—To test whether the major effect of TGF-β on PTHrP production is via the Smad proteins, we stably expressed wild-type and dominant-negative mutants of Smad2, Smad3, and Smad4 in MDA-MB-231. Wild-type Smad2, Smad3, and Smad4 clones, which had enhanced responses to TGF-β, and dominant-negative Smad clones, which had reduced response to TGF-β in the 3TP-lux reporter assay, were further characterized. Fig. 2 demonstrates that MDA-MB-231 stable clones that express dominant-negative Smad2, Smad3, or Smad4 did not respond to TGF-β in the 3TP-lux reporter assay.

TGF-β-induced PTHrP Production Is Only Partially Smad-dependent—Stable MDA-MB-231 clones that express wild-type or dominant-negative Smad forms were characterized for PTHrP production in response to TGF-β using a two-site PTHrP immunoradiometric assay (Fig. 3). Compared with parental cells and empty vector controls, the MDA-MB-231/TβRⅡA(Mycyt cell line was unresponsive to TGF-β, as reported previously (9). In contrast, overexpression of the dominant-negative Smads (Smad2(3S-A), Smad3(3S-A), Smad3(D407E), or Smad4(1–514)) failed to completely suppress TGF-β-induced PTHrP secretion by MDA-MB-231 cells. On the other hand, overexpression of wild-type Smad2, Smad3, and Smad4 enhanced PTHrP production in response to TGF-β. These data suggest that TGF-β signaling to induce PTHrP production is mediated only partially through the Smad pathway.

MAP Kinase Pathway Is a Major Component of the Smad-independent Signaling by TGF-β—The data suggested that both Smad-dependent and -independent pathways mediated the effect of TGF-β to stimulate PTHrP production by MDA-MB-231 breast cancer cells. We investigated the Smad-independent pathways by treating parental MDA-MB-231 with specific signaling pathway inhibitors. Protein kinase A (KT5720), protein kinase G (KT5823), tyrosine kinase (herbimycin A), and phosphatidylinositol 3-kinase (wortmannin) inhibitors did not affect basal or TGF-β-induced PTHrP secretion (Fig. 4). However, the p38 MAP kinase inhibitors, SB203580 and SB202190, and the MEK1/2 inhibitor, PD98059, significantly reduced both basal and TGF-β-stimulated PTHrP production dose-dependently (Fig. 4). Therefore, these inhibitors were tested in the context of the Smad dominant-negative blockade. In empty vector MDA-MB-231 clones, TGF-β-stimulated PTHrP was effectively decreased by both p38 inhibitors; SB202190 was more potent (Fig. 5A). The reduction of TGF-β-stimulated PTHrP production by the MEK inhibitor, PD98059, was likely due to decreased basal PTHrP production because the ratio of TGF-β-stimulated PTHrP to untreated was similar to the control. The cells expressing the dominant-negative TGF-β type II receptor (MDA-MB-231/TβRⅡA(Mycyt)) did not respond to TGF-β and secreted a low amount of PTHrP that was not affected by either p38 or MEK1/2 inhibitors (Fig. 5B). p38 inhibition by SB202190 significantly reduced basal and TGF-β-induced PTHrP production by an MDA-MB-231 clone that expressed the constitutively active TGF-β type I receptor (TβRⅡA(Mycyt + TβRI(T204D))), whereas MEK inhibition by PD98059 reduced the basal PTHrP synthesis (Fig. 5B, inset).

The effects of MAP kinase inhibition on PTHrP production by Smad dominant-negative clones are shown in Fig. 5 (C–F). In the presence of Smad2 dominant-negative blockade, p38 inhibition decreased the basal and TGF-β-induced PTHrP production. MEK inhibition reduced basal PTHrP production, but the ratio of TGF-β-stimulated PTHrP to control was similar to no inhibition (Fig. 5C). The combination of p38 inhibition and Smad2 dominant-negative blockade totally inhibited the TGF-β induction of PTHrP, comparable with that observed in the dominant-negative type II TGF-β receptor. Similar results were obtained with the Smad3 and Smad4 dominant-negative clones (Fig. 5, D–F). However, the clone that expressed the dominant-negative Smad3(D407E) displayed less responsiveness to TGF-β in the presence of p38 inhibition than the Smad3(3S-A) clone (Fig. 5E).

The data suggested that TGF-β signals via p38 MAP kinase to induce PTHrP production in MDA-MB-231 cells. We confirmed the involvement of p38 MAP kinase in TGF-β-signaling in parental MDA-MB-231 cells using Western blotting and antibodies specific for nonphosphorylated and phosphorylated p38 MAP kinase. The relative phosphorylation of p38 MAP kinase was increased in MDA-MB-231 cells treated with 5 ng/ml of TGF-β compared with control. The TGF-β-induced phosphorylation of p38 MAP kinase occurred as early as 10 min post-treatment and peaked at 1 h with a 5.5-fold increase (Fig. 6).

DISCUSSION

The role of TGF-β in malignancy is multifunctional (38, 39). TGF-β inhibits growth of epithelial and some tumor cells. Certain carcinomas have inactivated or deleted TGF-β receptors or Smad proteins (40). In contrast, TGF-β can also promote invasiveness and metastases (41–44). Our previous studies support an additional role for TGF-β in malignancy to promote breast cancer osteolysis by stimulating tumor production PTHrP (9). Expression of a dominant-negative type II TGF-β receptor in MDA-MB-231 cells blocked TGF-β-stimulated PTHrP in vitro and the development and progression of bone metastases in vivo. This was reversed by overexpression of either a constitutively active type I TGF-β receptor or PTHrP. These experiments suggested that the effects of TGF-β to promote breast cancer metastases to bone were mediated by tumor production of PTHrP, but they did not exclude other effects of TGF-β. The experiments presented here provide evidence that the central effector of TGF-β on the development and progression of bone metastases is PTHrP. Mice inoculated with an MDA-MB-231 clonal line that expressed the constitutively active type I TGF-β receptor, TβRⅡA(T204D), had extensive osteolysis that was prevented by treatment with a neutralizing monoclonal antibody directed against PTHrP(1–34).
How does TGF-β mediate its effects on tumor cells in the bone microenvironment to promote osteolysis? TGF-β released from bone matrix as a consequence of osteoclastic bone resorption (5) binds to the type II TGF-β serine-threonine kinase receptor on the surface of tumor cells. This complex recruits and phosphorylates the type I receptor. The heterotetrameric TGF-β receptor complex in turn phosphorylates the receptor-regulated Smad2 and Smad3, which then form hetero-oligomers with Smad4 that translocate into the nucleus, where they assemble with transcription complexes and regulate the expression of target genes.

Overexpression of receptor-regulated Smad2 and Smad3 and the co-Smad 4 increased TGF-β responsiveness in various cell lines (33, 45–49). Our results are consistent with these findings in that the overexpression of wild-type Smads increased the TGF-β-stimulated PTHrP production in MDA-MB-231 cells. Lindeman et al. (12) have reported that TGF-β activates PTHrP expression in the same breast cancer cell line by up-regulating transcription from PTHrP P3 promoter through Smad3/ETs1 synergism. In our study dominant-negative Smads only partially suppressed TGF-β-induced PTHrP production. There are several possible explanations for this. First,
TGF-β induces tumor PTHrP via Smad and MAP kinase pathways. The effects of TGF-β on PTHrP production in MDA-MB-231 breast cancer cells have been extensively studied, and the use of dominant-negative Smad and MAP kinase inhibitors has revealed complex signaling pathways. In this study, we investigated the role of Smad and MAP kinase pathways in TGF-β-induced PTHrP production.

The Smad mutant constructs may not have functioned as complete dominant-negatives. This possibility is unlikely because the efficacy of the Smad dominant-negative constructs has been widely demonstrated in transient transfection assays (31–33). Our results show that the stable Smad dominant-negative clones were not responsive to TGF-β in the 3TP-lux reporter assay. Thus, the dominant-negative constructs expressed in these cell lines are sufficient completely to block TGF-β responsiveness through the Smad pathway. Second, expression of the dominant-negative Smad could have induced compensatory expression of endogenous Smad proteins. This explanation might account for the situation with Smad2 and Smad3 dominant-negatives, but because the Smad4 is the common Smad that is crucial for nuclear localization, an up-regulation of Smad2 or Smad3 in the Smad4 dominant-negative cell line would not alter signal transduction. In addition, overexpression of Smad3(D407E) hinders the phosphorylation of Smad2 by TGF-β type I receptor (33).

A third explanation for the incomplete blockade by dominant-negative Smads of TGF-β stimulation of PTHrP production is that TGF-β signaling in breast cancer cells utilizes both Smad and Smad-independent signaling pathways. TGF-β can activate components of MAP kinase pathways, mainly JNK, ERK, and p38 pathways (50–52), and recent studies suggest that some effects of TGF-β on cancer cells are Smad-independent (53). To test this third possibility, we screened a variety of protein kinase inhibitors for their effects on basal and TGF-β-stimulated PTHrP production in MDA-MB-231 parental cell line. Under the conditions tested, none of the inhibitors showed cellular toxicity or reduced cell numbers (data not shown). We found that only p38 MAP kinase inhibitors substantially reduced TGF-β-stimulated PTHrP production, whereas MEK1/2 inhibition reduced basal PTHrP production in MDA-MB-231 cells. Other protein kinase inhibitors had no effect on PTHrP production.

Thus, the third explanation is the most likely. TGF-β stimulates PTHrP production through both Smad-dependent and MAP kinase signaling pathways. This explanation was also supported by using cell lines stably expressing dominant-negative Smads in combination with MAP kinase inhibitors (Fig. 5). The combined inhibition of Smad and p38 MAP kinase signaling eliminated TGF-β stimulation of PTHrP production as effectively as the overexpression of dominant-negative TGF-β type II receptor. The MEK inhibitor reduced the basal PTHrP production in Smad dominant-negative clones to nearly undetectable concentrations. In addition, TGF-β increased the phosphorylation of p38 MAP kinase in a time-dependent fashion consistent with relevant MAP kinase activation (Fig. 6), further supporting the role of p38 MAP kinase in TGF-β-induced PTHrP production. The MAP kinase inhibitors had no additional effect on PTHrP production in the MDA-MB-231 clone overexpressing TGF-β dominant-negative type II receptor. These data indicate that the effects of TGF-β on MAP kinase pathways are mediated through the TGF-β receptor.

The results suggest that MDA-MB-231 breast cancer cells use both Smad and MAP kinase pathways to regulate PTHrP production. There is also evidence for MAP kinase involvement in other indicators of the metastatic phenotype in MDA-MB-231 cells. For example, activated p38 MAP kinase is essential for matrix invasion by breast cancer cells such as MDA-MB-231 (54). Similarly, both Smad and p38 MAP kinase pathways are activated by activin, a member of TGF-β gene family in the human breast cancer T47D cell line (55).

Although the inhibitor SB203580 was originally reported to be highly specific for p38 (56), the serine kinase activities of the TGF-β type I and II receptors can be inhibited weakly by the same compound (57). Because 400–800-fold higher concentrations of SB203580 are needed to inhibit TGF-β receptor activity compared with p38 activity (58), it is highly unlikely that the concentrations of SB203580 used in this study would be suffi-
cient to directly inhibit the TGF-β receptor kinase. The other p38 inhibitor, SB202190, has been reported to induce apoptosis in Jurkat cells through inhibition of p38β MAP kinase (59). However, no reductions of cell number in the SB202190-treated cells or in any other inhibitor-treated cells were observed after the pretreatment and 24 h of incubation when compared with the controls. Both compounds inhibit the p38α and β isoforms but not the γ and δ isoforms (60, 61). Whether TGF-β promotes PTHRp production via p38α or β forms or both in MDA-MB-231 cells remains to be studied.

The interactions between MAP kinase and Smad signaling pathways downstream of the TGF-β receptor may be complex. Smad2 and Smad3 are direct substrates for phosphorylation by active type I receptor. Because none of three dominant-negative Smads 2 and 3 are direct substrates for phosphorylation by aggregan gene. Future studies are needed to clarify the up-regulation of RANK ligand (65). TGF-β mediates breast cancer osteolysis. Metastatic breast cancer cells in the controls. Both compounds inhibit the p38MAP kinase (59).

is crucial in designing effective therapy for osteolytic metastases. Identification of the molecular mechanisms responsible for osteolytic metastases is crucial in designing effective therapy for osteolytic metastases of cancer.

Acknowledgments—We thank Rami Käkönen for technical assistance; Drs. Massagué, Wranza, Attisano, and Kato for plasmid constructs or both in MDA-MB-231 cells remains to be studied.

The interactions between MAP kinase and Smad signaling pathways downstream of the TGF-β receptor may be complex. Smad2 and Smad3 are direct substrates for phosphorylation by active type I receptor. Because none of three dominant-negative Smads 2 and 3 are direct substrates for phosphorylation by aggregan gene. Future studies are needed to clarify the up-regulation of RANK ligand (65). TGF-β mediates breast cancer osteolysis. Metastatic breast cancer cells in the controls. Both compounds inhibit the p38MAP kinase (59).

is crucial in designing effective therapy for osteolytic metastases. Identification of the molecular mechanisms responsible for osteolytic metastases is crucial in designing effective therapy for osteolytic metastases of cancer.

Acknowledgments—We thank Rami Käkönen for technical assistance; Drs. Massagué, Wranza, Attisano, and Kato for plasmid constructs or both in MDA-MB-231 cells remains to be studied.

The interactions between MAP kinase and Smad signaling pathways downstream of the TGF-β receptor may be complex. Smad2 and Smad3 are direct substrates for phosphorylation by active type I receptor. Because none of three dominant-negative Smads 2 and 3 are direct substrates for phosphorylation by aggregan gene. Future studies are needed to clarify the up-regulation of RANK ligand (65). TGF-β mediates breast cancer osteolysis. Metastatic breast cancer cells in the controls. Both compounds inhibit the p38MAP kinase (59).

is crucial in designing effective therapy for osteolytic metastases. Identification of the molecular mechanisms responsible for osteolytic metastases is crucial in designing effective therapy for osteolytic metastases of cancer.

Acknowledgments—We thank Rami Käkönen for technical assistance; Drs. Massagué, Wranza, Attisano, and Kato for plasmid constructs or both in MDA-MB-231 cells remains to be studied.

The interactions between MAP kinase and Smad signaling pathways downstream of the TGF-β receptor may be complex. Smad2 and Smad3 are direct substrates for phosphorylation by active type I receptor. Because none of three dominant-negative Smads 2 and 3 are direct substrates for phosphorylation by aggregan gene. Future studies are needed to clarify the up-regulation of RANK ligand (65). TGF-β mediates breast cancer osteolysis. Metastatic breast cancer cells in the controls. Both compounds inhibit the p38MAP kinase (59).

is crucial in designing effective therapy for osteolytic metastases. Identification of the molecular mechanisms responsible for osteolytic metastases is crucial in designing effective therapy for osteolytic metastases of cancer.

Acknowledgments—We thank Rami Käkönen for technical assistance; Drs. Massagué, Wranza, Attisano, and Kato for plasmid constructs or both in MDA-MB-231 cells remains to be studied.
TGF-β Induces Tumor PTHrP via Smad and MAP Kinase Pathways

Transforming Growth Factor-β Stimulates Parathyroid Hormone-related Protein and Osteolytic Metastases via Smad and Mitogen-activated Protein Kinase Signaling Pathways

Sanna-Maria Käkönen, Katri S. Selander, John M. Chirgwin, Juan Juan Yin, Suzanne Burns, Wayne A. Rankin, Barry G. Grubbs, Mark Dallas, Yong Cui and Theresa A. Guise

doi: 10.1074/jbc.M202561200 originally published online April 18, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M202561200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 65 references, 36 of which can be accessed free at [http://www.jbc.org/content/277/27/24571.full.html#ref-list-1](http://www.jbc.org/content/277/27/24571.full.html#ref-list-1)