The p38 MAPK Pathway Is Required for Cell Growth Inhibition of Human Breast Cancer Cells in Response to Activin*

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Activin, a member of the TGFβ family, inhibits cell growth in various target tissues. Activin interacts with a complex of two receptors that upon activation phosphorylate specific intracellular mediators, the Smad proteins. The activated Smads interact with diverse DNA binding proteins and co-activators of transcription in a cell-specific manner, thus leading to various activin biological effects. In this study, we investigated the role and mechanism of action of activin in the human breast cancer T47D cells. We found that activin treatment of T47D cells leads to a dramatic decrease in cell growth. Thus activin appears as a potent cell growth inhibitor of these breast cancer cells. We show that activin induces the Smad pathway in these cells but also activates the p38 mitogen-activated protein kinase pathway, further leading to phosphorylation of the transcription factor ATF2. Finally, specific inhibitors of the p38 kinase (SB202190, SB203580, and PD169316) but not an inactive analogue (SB202474) or the MEK-1 inhibitor PD98059 completely abolish the activin-mediated cell growth inhibition of T47D cells. Together, these results define a new role for activin in human breast cancer T47D cells and highlight a new pathway utilized by this growth factor in the mediation of its biological effects in cell growth arrest.

Activin, a member of the TGFβ family, regulates cell growth of various cell types. Activin interacts with a complex of two receptors (types I and II), both containing an extracellular domain, a single transmembrane region, and a large intracellular domain that contain a serine/threonine kinase domain. The type II receptor, which is constitutively phosphorylated (1), transphosphorylates the type I receptor (ALK4) upon ligand stimulation, on serine and threonine residues (2–4). The activated receptor complex then recruits the two receptor-regulated Smad2 and Smad3 (5–8). Following binding and phosphorylation by the activin type I receptor, Smad2 and Smad3 are released to the cytoplasm where they associate with the common-partner Smad4 before being translocated to the nucleus (8–11).

Both Smad3 and Smad4 but not Smad2 can directly bind DNA elements (Smad binding element) and activate the transcription of the target genes (12). However, the DNA binding affinity of the Smads is low (13), and they usually require the presence of other DNA binding proteins to efficiently interact with the promoters of their responsive target genes. As a result, the Smad binding elements are often found close to the DNA binding element of other transcription factors. Among those are the FAST family members, FAST1 (14) and FAST2 (15), TFE3 (16), Fox and Jun (17), Sp1 (18), CBP/p300 (19), Evi-1 (20), and ATF2 (21).

The Smad proteins are central elements in the activin receptor signaling pathway but are not the sole pathway activated by this receptor complex. Other members of the TGFβ superfamily have been shown to activate different signaling pathways, in addition to the Smads. TGFβ itself can activate a member of the MAPKKK family of kinases, TAK1 (TGF-activated kinase) (22). TAK1 then activates the stress-activated kinase p38 and the transcription factor ATF2, a member of the b-ZIP family of DNA binding proteins (21). In vitro studies also suggested that the transcription factor ATF2 could interact with the MH1 domains of two activin responsive Smads, Smad3 and Smad4 (21, 23). Both TGFβ and the Müllerian inhibiting substance (MIS) were also shown to mediate some of their biological effects through an NFκB-mediated pathway (24, 25). It is therefore conceivable that activin also utilizes other signaling pathways to transduce its signals.

Activin, its receptors, and the Smads are expressed in myoepithelial cells as well as in a certain number of human breast cancer cell lines (26, 27), suggesting a role for this growth factor and its downstream effectors, the Smads, in mammary cell growth and differentiation. Several reports have recently implicated TGFβ family members or their downstream signaling pathways in the regulation of breast cancer cell growth. Indeed, Smad4 can restore cell growth arrest in MDA-MB-468 cells, a breast cancer cell line in which the Smad4 gene is deleted (28). Genetic mutations or loss of expression of the activin and TGFβ receptors is also found in human breast cancers (29, 30). Finally, TGFβ and MIS mediates cell growth arrest in breast cancer by reducing NFκB DNA binding activity (24, 25), and activin itself can modulate cell growth of the breast cancer cells MCF7 (26).

In the present study, we investigated the role and mechanism of action of activin in breast cancer cells. We show here for the first time that activin strongly inhibits cell growth of the
human breast cancer cell line T47D. In addition, our results indicate that activin induces the Smad pathway in these cells but also activates the p38 MAPK pathway. Activation of this pathway further leads to phosphorylation of the transcription factor ATF2. Furthermore, we show that specific inhibitors of the p38 MAPK pathway antagonize the activin-mediated cell growth arrest in T47D cells. Thus, this highlights for the first time the involvement of this p38 kinase pathway downstream of the activin receptor signal transduction pathways leading to cell growth arrest.

MATERIALS AND METHODS

Cell Culture and Proliferation Assay—T47D cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) in the presence of 10% fetal calf serum. For proliferation assay, cells were plated in triplicates in 96-well dishes, at 5000 cells/well in 2% FCS. Cells were stimulated or not with activin (0.5 nM) and grown over a 5-day period. Cell proliferation was assessed using direct cell counting, and the non-radioactive MTT cell proliferation assay for eukaryotic cells (Cell Titer 96, Promega G 4000). Absorbance was measured at 570 nm with a reference wavelength at 595 nm, using a Bio-tek Microplate reader.

Transfection and Reporter Assay—T47D cells (10⁶ cells) were transfected with a luciferase reporter (Bio-Rad Gene Pulser II) in 500 μl of phosphate-buffered saline (240 V and 975 microfarads) with 10 μg of each of the indicated cDNAs. Following transfection, cells were plated in 6-well dishes in DMEM (10% FCS) for 24-h recovery. The following day, cells were starved overnight in DMEM without serum and stimulated or not with activin (0.5 nM) for 16 h. Then, cells were washed once with phosphate-buffered saline and lysed in 250 μl of lysis buffer (1% Triton X-100, 15 mM MgSO₄, 4 mM EGTA, 1 mM dithiothreitol, 25 mM glycyl-glycine) on ice. The luciferase activity of each sample was measured using 45 μl of cell lysate (EG&G Berthold lumimeter) and normalized to the relative β-galactosidase activity.

RNA Protection Assay—RNase protection assay was performed using the hcc-2 template set and RiboQuant kit from PharMingen (San Diego, CA) according to the manufacturer’s instructions, with minor modifications. Radiolabeled antisense RNA probes were prepared by in vitro transcription of the hcc-2 templates with T7 RNA polymerase in vitro or not with activin (0.5 nM) and grown over a 5-day period. Modifications. Radiolabeled antisense RNA probes were prepared by

RESULTS

Activin Inhibits T47D Human Breast Cancer Cell Growth—Although activin and its receptors are expressed in a number of breast cancer cell lines, the role of activin in the regulation of breast cancer cell growth has not yet been fully investigated. To analyze the role of activin in regulating growth of human breast cancer cells, we utilized the human breast cancer cell line T47D, which endogenously expresses activin-responsive Smad2, Smad3, and Smad4 (31). Using a cell growth and viability assay (MTT assay), we show that activin treatment of T47D cells leads to a significant inhibition in their growth, apparent as early as day 2 and reaching 40% inhibition at day 3 (Fig. 1A). To verify that activin affects cell growth and not the metabolic rate of the cell, direct cell counting was also performed. As shown in Fig. 1B, activin stimulation of T47D cells for 3 days also results in clear cell growth inhibition. Therefore, activin appears as a potent cell growth inhibitor for T47D breast cancer cells.

Activin Modulates Cell Cycle Regulators in Breast Cancer Cells—TGFβ family members regulate cell growth through different mechanisms. They often mediate cell cycle arrest through up-regulation of the three cyclin-dependent kinase inhibitors p15INK4B, p21CIP1/WAF1, and p27 (32–34). Because activin exerts a strong effect on cell growth in T47D (Fig. 1), we analyzed its effects in modulating gene expression levels of different cyclin-dependent kinase inhibitors as well as of other cell cycle regulatory genes. For this, we examined the level of mRNA species of different cell cycle regulators, using a highly sensitive and specific ribonuclease protection assay. As shown in Fig. 2, T47D cells were stimulated for different periods of

15 min in the washing buffer, and immunoreactivity was normalized by chemiluminescence (Lumi-Light Plus Western blotting substrate, Roche Molecular Biochemicals) according to the manufacturer’s instructions and revealed using an Alpha Innotech Fluorochem imaging system (Packard Canberra).

FIG. 1. Activin induces cell growth arrest of the breast cancer cell line T47D. T47D cells were grown in 2% FCS DMEM over a 5-day period in the presence or absence of 0.5 nM activin. Cell proliferation was assessed by (A) MTT colorimetric assay in triplicates and (B) direct cell counting. Values are expressed in arbitrary units.

FIG. 2. T47D cells were stimulated for different periods of
time with activin (0.5 nM). Total RNA from unstimulated or stimulated cells were extracted and hybridized with multiple antisense probes for human cell cycle regulators (p15, p16, p18, p19, p21, p27, as well as for p53, p57, p107 p130, the retinoblastoma protein (Rb), and the two housekeeping genes L32 and glyceraldehyde-3-phosphate dehydrogenase). As shown in Fig. 2, a modest but reproducible ligand-dependent increase in the mRNA level of p21\textsuperscript{CIP1/WAF1} was observed. This is consistent with a microarray analysis of T47D cells treated for 8 h with activin, which shows a 1.7-fold increase in p21\textsuperscript{CIP1/WAF1} mRNA level.\textsuperscript{2} mRNA levels for p15\textsuperscript{INK4B} were also consistently increased upon activin treatment, although at a lower level than p21\textsuperscript{CIP1/WAF1}. This experiment was repeated three times and showed consistent results. None of the other cell cycle regulators (p130, Rb, p107, p53, p57, p27, p19, p18, or p16) or housekeeping genes (L32 or glyceraldehyde-3-phosphate dehydrogenase) mRNA levels showed any significant or reproducible difference in response to activin (Fig. 2). Our attempts to detect p15\textsuperscript{INK4B} and p21\textsuperscript{CIP1/WAF1} protein levels in these cells were unsuccessful, probably due to the low level of expression of these two proteins. This suggests that at least part of the activin effect on cell growth arrest in T47D cells is mediated through up-regulation of p15\textsuperscript{INK4B} and p21\textsuperscript{CIP1/WAF1}.

**Activin Induces Smad2 Phosphorylation in T47D Cells**—To further examine the role of the Smad pathway in T47D cells, we first examined the activation state of Smad2, following activin stimulation. Cells were starved for an overnight period and treated with 0.5 nM activin for a different period of time, as indicated in Fig. 3. Total cell lysates were separated by SDS-polyacrylamide gel electrophoresis, and resolved proteins were transferred to a nitrocellulose membrane for Western blotting analysis. The membrane was probed with a specific antibody to phospho-Smad2 that recognizes the two phosphorylated serine residues in the C-terminal end of the MH2 domain of Smad2 (SSXS). As shown in Fig. 3, activin treatment of T47D cells leads to a clear phosphorylation of Smad2, as early as 15 min following ligand stimulation of the cells. The membrane was stripped and reprobed with a polyclonal antibody that recognizes both Smad2 and Smad3 (Fig. 3, lower panel) and subsequently with a monoclonal antibody to Smad4 (Fig. 3, lower panel) and shows equal levels of all Smads in all samples. These data indicate that the Smad pathway is functional in T47D cells and is activated in response to activin stimulation.

**Activin Induces 3TPLux and ARE-Lux Promoters in T47D Cells**—To further examine the activation of the activin receptor/Smad pathway in T47D cells, we analyzed the ability of activin to induce two activin receptor/Smad-responsive promoter constructs (3TPLux and ARE-Lux). T47D cells were transiently co-transfected as shown under “Materials and Methods” with the promoter construct 3TPLux or ARE-Lux and an expression vector encoding the co-activator Fast1. As shown in Fig. 4A, activin treatment of T47D cells led to a 2.6- and 2.7-fold induction of 3TPLux and ARE-Lux, respectively. Furthermore, T47D cells were also co-transfected with 3TPLux or ARE-Lux/Fast1 and an expression vector encoding a constitutively active form of the activin type I receptor (ALK4 TAD). This point mutation replaces threonine 206 by an aspartic acid and renders the receptor constitutively active even in the absence of ligand or type II receptor (4). As shown in Fig. 4B, ALK4 TAD mimics activin effects on the activation of the two promoter constructs, leading to a 3.2- and 2.7-fold induction of 3TPLux and ARE-Lux/Fast1, respectively. Finally, to confirm the involvement of the Smad pathway, a dominant negative form of Smad3, which lacks the MH2 domain (Smad3\textsuperscript{ΔC}), was transfected in T47D cells together with 3TPLux. Deletion of the C-terminal domain of Smad3 results in the loss of homo- and heterodimerization with the wild type Smad4 as well as in its ability to induce a reporter construct (35). Cells were stimulated or not with activin and, as seen in Fig. 4C, overexpression of Smad3\textsuperscript{ΔC} completely blocks activin-induced 3TPLux activity. Together, these results confirm that the activin receptor/

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\textsuperscript{2} J.-J. Lebrun, unpublished data.
was normalized to the relative constructs were stimulated with activin 16 h. The luciferase activity responsive promoter constructs 3TP-Lux or ARE-Lux/Fast1 reporter T47D cells.

A panel

an equal amount of the p38 kinase in all lanes (Fig. 5 and reprobed with an antibody directed against p38 and shows decreases to return to basal level. The membrane was stripped and reprobed with an antibody directed against the phosphorylated form of the p38 kinase (pp38) (upper panel). The membrane was stripped and reprobed with an antibody directed against the phosphorylated ATF2 (pATF2) (upper panel). The membrane was stripped and reprobed with an anti-ATF2 (ATF2) antibody (lower panel).

B panel

We then analyzed the activin effects on the phosphorylation of the transcription factor ATF2, a downstream target of the p38 kinase. Total cell lysates were analyzed by immunoblot using specific antibodies directed against the phosphorylated form of the p38 kinase (p38) (upper panel). The membrane was stripped and reprobed with an anti-p38 (p38) antibody (lower panel). B, similarly, total cell lysates were analyzed by immunoblot using a specific antibody directed against the phosphorylated ATF2 (pATF2) (upper panel). The membrane was stripped and reprobed with an anti-ATF2 (ATF2) antibody (lower panel).

C panel

As shown in Fig. 5B (upper panel), there is a time-dependent phosphorylation of the transcription factor ATF2 following activin treatment of the cells. The phosphorylation of ATF2 correlates with the activation of the p38 MAPK and shows a maximum phosphorylation at 40 min. The membrane was stripped and reprobed with an anti-ATF2 antibody and shows an equal amount of the transcription factor in all lanes (Fig. 5B, lower panel). Together, these data demonstrate that the p38 MAPK/ATF2 pathway is activated in T47D cells in response to activin.

The p38 Kinase Inhibitor PD169316 Blocks Activin-induced p38 and ATF2 Phosphorylation—To further confirm the involvement of the p38 MAPK pathway downstream of the activin receptor, T47D cells were treated with a specific p38 kinase inhibitor (PD169316) or Me2SO as a control. Cells were then stimulated or not with activin for 30 min, and total cell lysates were analyzed by Western blotting using different antibodies directed against phosphop38 or p38, phospho-ATF2 or ATF2. As shown in Fig. 6A (upper panel), in the presence of Me2SO, activin induces phosphorylation of the kinase p38, confirming the previously seen results (Fig. 5). However, in the presence of the specific p38 kinase inhibitor PD169316, this activin effect on p38 phosphorylation is abolished (Fig. 6A, upper panel). The membrane was stripped and reprobed with an antibody directed against p38 and shows an equal amount of proteins in all lanes (Fig. 6A, lower panel). Similarly, as shown in Fig. 6B, upper panel, activin induces phosphorylation of the transcription factor ATF2 in the presence of Me2SO, but this activin-induced effect is abolished in the presence of the p38 kinase inhibitor.

Smad pathway is functional in breast cancer cells.

Activin Activates the p38 Kinase Pathway in T47D Cells—Recently, the p38 mitogen-activated protein kinase (MAPK) pathway was shown to regulate gene expression in response to TGFβ (23). To assess the role of this pathway in activin-mediated cell growth inhibition of breast cancer cells, T47D cells were starved overnight and stimulated with 0.5 nM activin. As shown in Fig. 6A (upper panel), there is a time-dependent phosphorylation of the transcription factor ATF2 following activin treatment of the cells. The phosphorylation of ATF2 correlates with the activation of the p38 MAPK and shows a maximum phosphorylation at 40 min. The membrane was stripped and reprobed with an anti-ATF2 antibody and shows an equal amount of the transcription factor in all lanes (Fig. 5B, lower panel). Together, these data demonstrate that the p38 MAPK/ATF2 pathway is activated in T47D cells in response to activin.

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kinase inhibitor PD169316. An equal amount of protein in all lanes was ensured by stripping and reprobing of the membrane with an anti-ATF2 antibody (Fig. 6, lower panel). The membrane was stripped and reprobed with an anti-p38 (p38) antibody (lower panel).

The p38 Kinase Inhibitors Antagonize Activin-induced Cell Growth Arrest in Breast Cancer Cells—To evaluate the contribution of the p38 kinase pathway in activin-mediated cell growth inhibition in T47D cells, we used different p38 kinase-specific inhibitors (SB202190, SB203580, PD169316) or an inactive analogue (SB202474) and an MEK1/ERK1/2 inhibitor (PD98059) as controls, in both MTT (Fig. 7A) and direct cell counting assays (Fig. 7B). T47D cells were cultured in DMEM, 2% serum for 3 days and stimulated or not with 0.5 nM activin in the presence or absence of 10 μM of the different p38 kinase-specific inhibitors, SB202190, SB203580, and PD169316, or the inactive analogue SB202474 and the MEK1/ERK1/2 inhibitor PD98059 as controls. Cell growth was assessed by (A) MTT colorimetric assay in triplicates and (B) direct cell counting. Values represent means and standard deviations of five separate experiments and are expressed as percentage of inhibition compared with the control. C, T47D cells were pretreated with Me2SO or with the p38 inhibitors (SB202190, PD169316, or SB203580) at 10 μM for 45 min before being stimulated with activin for 15 min. Total cell lysates were analyzed by immunoblot using specific antibodies directed against the phosphorylated form of Smad2 (pSmad2, upper panel), the phosphorylated form of p38 kinase (pp38, middle panel), or p38 kinase (p38, lower panel).

p38 kinase inhibitors, we observed no significant inhibitory effect on Smad2 phosphorylation under the same conditions. Thus it is likely that the antagonistic effect exerted by the p38 inhibitors on activin-induced cell growth arrest is mediated through inhibition of the p38 kinase pathway downstream of the activin receptor.

As activin is potent cell growth inhibitor in many different cell lines, the effect of the p38 kinase inhibitors were also analyzed in several activin-responsive cell lines such as K562, Chinese hamster ovary, and MCF7. Interestingly, the activin
inhibitors could reverse the activin effects on cell growth arrest in all cell lines tested (data not shown). This indicates that the contribution of the p38 MAPK pathway to activin-mediated cell growth arrest is critical.

Activin Effect on Cell Growth Arrest Is Not Mediated through the MEK1/ERK1/2 MAPK Pathway—The absence of effect of the MEK1/ERK1/2 inhibitor PD98059 on activin-mediated cell growth arrest (Fig. 7) suggests that activin does not modulate the MAPK MEK1/ERK1/2 pathway to arrest cell growth. The MEK1/ERK1/2 pathway is known to be involved in cell proliferation in response to various growth factors. To confirm that activin does not modulate or inhibit activation of this pathway in response to growth factors, T47D cells were starved overnight and stimulated with EGF (20 ng/ml) for different periods of time in the absence or presence of 1 nM activin (Fig. 8). Total cell lysates were then analyzed by Western blotting using an antibody directed against phospho-ERK1/2 (a-ERK). As shown in Fig. 8 (upper panel), EGF very rapidly and transiently induces the phosphorylation of ERK1/2 (p42/p44). However, activin co-stimulation of the cells does not affect EGF-induced ERK1/2 phosphorylation. The membrane was stripped and reprobed with an anti-ERK antibody and shows an equal amount of MAPK in all samples. Together this indicates that the activin effect on cell growth arrest in T47D cells is not mediated through the MEK1/ERK1/2 MAPK pathway.

DISCUSSION

Members of the TGFβ family of growth factors are important factors in regulating cell growth inhibition; hence, it is critical to characterize their intracellular signaling mechanisms. Although it is known that activin signals through activation of Smad proteins, the activation of other intracellular signaling pathways and their contribution to activin-mediated cell growth inhibition remain to be characterized. In this paper we have examined the role of activin in mediating cell growth inhibition of breast cancer cells. Our results indicate that activin induces the Smad pathway in T47D cells and emphasize the involvement of the p38 MAPK pathway in activin-induced cell growth inhibition of these breast cancer cells.

Abnormalities in the signaling pathways of activin/TGFβ have been clearly linked to various cancers, including breast cancer (37). We analyzed activin effects on the regulation of cell growth of human breast cancer cells. Using the human breast cancer cell line T47D, we found that activin has a profound and significant effect on the growth of these cells. We further investigated how activin triggers its effects in this cell line. Activin treatment of T47D cells leads to rapid phosphorylation of the receptor-regulated Smad2. Furthermore, both activin or the constitutively active form of the activin type I receptor (ALK4TΔD) induce the two promoter constructs 3TPLux and ARE-Lux, and this effect is completely abolished in the presence of an overexpressed dominant negative form of Smad3 (Smad3ΔC). All together, these results suggest that the activin receptor/Smad pathway is activated and can regulate the activin response in breast cancer cells, confirming the central role played by the Smad proteins in the mediation of the activin response.

The p38 MAPK is involved in regulating cellular responses to stress and cytokines (38–41). p38 kinase is activated and phosphorylated at the Threonine180-Tyrine182 site by the two closely related dual specificity protein kinases MKK3 and MKK6 (42, 43). The activated p38 kinase has been shown to phosphorylate several transcription factors such as ATF2 (44), Max (45), and Elk-1 (46) and indirectly cAMP-response element-binding protein via activation of Nrf2 (47), STAT1 (48), and MEF-2 (49). The p38 pathway is activated in response to TGFβ in C2C12, Mv1LU, and 293 cells (21, 23). TGFβ can induce phosphorylation of both p38 and the transcription factor ATF2 in these cell lines. In addition, p38 and ATF2 can contribute to the activation of the synthetic reporter construct 3TPLux in these cells, but the physiological significance of this pathway in the mediation of the TGFβ effects remains unclear. We show here that activin induces the p38 kinase pathway in T47D cells leading to phosphorylation of both the p38 kinase and the transcription factor ATF2. Furthermore, we show that the p38/ATF2 pathway is required to transduce the activin effects on cell growth inhibition. Indeed, different specific p38 kinase inhibitors, but not their inactive analogue or the MEK inhibitor can totally reverse the activin effect on cell growth inhibition. This highlights a new role for the p38 kinase pathway in the control of cell growth and proliferation downstream of the activin/TGFβ superfamily of growth factors. TGFβ family members often require the presence of parallel or synergistic pathways to the Smads to carry out their full biological effects and diversity of the Smad-interacting partners may contribute to signal specificity (50). In future studies, it will be interesting to examine the level of interaction between the Smad and the p38 kinase pathways in response to activin in T47D cells, because in vitro studies have suggested that the Smads could physically interact with the transcription factor ATF2 (21, 23).

Signaling by the MAPK family is organized hierarchically in three different steps. MAPK, such as p38, are phosphorylated by MAPK kinases (MAPKKS), such as MKK3 and MKK6, in the case of p38. The MAPKKS are themselves activated and phosphorylated by the MAPKK kinases (MAPKKS), such as MLK, TAK, and ASK1 kinases, which act as MAPKKS. Finally, the MAPKKS are regulated by cell surface receptors or other external stimuli (51, 52). It will be interesting to identify the upstream kinases and other partner proteins involved in the activin-mediated p38 activation that are acting between the activin receptor complex and the p38 kinase in the signaling cascade. Recent reports indicated that TAK1, a member of the MAPKKK family, is activated by several cytokines, including TGFβ (22) and bone morphogenetic protein (53). TAK1 is a potent activator of p38 kinase (54). It will be interesting, therefore, to determine whether or not TAK1 also lies downstream of the activin receptor complex signaling cascade.

It was also recently shown that the Müllerian Inhibitory substance (MIS) represses the growth of breast cancer cells by regulating the NFκB pathway (25). The TGFβ effect on cell growth inhibition of breast cancer has also been shown to be associated with a reduced NFκB activity (24). This suggests that different members of the TGFβ superfamily may regulate cell growth by utilizing different signaling pathways in the same target tissues. Interestingly, TAK1 was also shown to lead to NFκB activation (55), suggesting a potential role for this...
factor downstream of the activin receptor.

Our results indicate that activin strongly represses the cell growth of T47D breast cancer cells. Further characterization of the downstream target genes that are modulated in T47D cells in response to activin will greatly enhance our understanding of its mechanism of action on cell growth regulation. Our data suggest that at least some of these targets could be the cyclin-dependent kinase inhibitors. However, it will remain to be determined whether other cell cycle regulators as well as apoptosis regulators are also regulated by activin in these cells. Indeed, identification of the target genes, involved in the regulation of cell cycle and/or apoptosis, will be important in shedding light on the activin receptor mechanism of action in breast cancer cells.

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