The cytokine transforming growth factor β (TGF-β) plays an important role in preventing tumor formation by blocking cell cycle progression. Accordingly, many cancers demonstrate mutations in TGF-β signaling components or enhanced expression of inhibitors of the TGF-β pathway such as Smad7. In this report we show that the oncoprotein HER2/Neu is able to collaborate with the ETS transcription factor ER81 to activate Smad7 transcription in breast, endometrial, and ovarian cancer cell lines. ER81 binds to two ETS sites within the Smad7 promoter, and mutation of one of these ETS sites greatly decreases Smad7 induction by HER2/Neu and ER81. Furthermore, we show that Smad7 activation involves the processing of signals from HER2/Neu to ER81 via the ERK mitogen-activated protein kinase pathway. Thus, we have uncovered a novel mechanism by which oncogenic HER2/Neu, in collaboration with ER81, can induce carcinogenesis through Smad7 up-regulation. Moreover, we show that TAK1, a TGF-β-activated protein kinase, stimulates ER81 via the p38 mitogen-activated protein kinase pathway and thereby induces the Smad7 promoter. This suggests that attenuation of TGF-β signaling by activating Smad7 transcription may proceed not only through TGF-β receptor-regulated Smad proteins but also through an independent pathway involving ER81 and TAK1.

The biological effects of the cytokine TGF-β are diverse, ranging from controlling cell growth and differentiation to immune modulation and apoptosis. One of the most widely studied roles of TGF-β is its function as a tumor suppressor, specifically its ability to block cell cycle progression through the G1/S transition. This is accomplished by facilitating p15INK4b and p21WAF1/CIP1 transcription, which in turn inhibits retinoblastoma protein phosphorylation (1, 2). TGF-β is also believed to down-regulate proto-oncogenes such as myc, a promoter of cell cycling (3–5). Thus, TGF-β growth surveillance is an important obstacle many cells must overcome to evolve into a malignancy and achieve unrestricted growth.

TGF-β signaling is propagated through the TGF-β receptors, a heterodimer consisting of transmembrane receptor serine/threonine kinases (6). When bound to ligand, the type II TGF-β receptor phosphorylates the type I receptor within the hetero-meric complex, which in turn phosphorylates receptor-regulated Smads (R-Smads), a family of transcription factors that are then free to associate with the common mediator Smad4 and translocate to the cell nucleus. There, R-Smad-Smad4 complexes can bind to specific DNA consensus sequences called Smad binding elements and thereby effect changes in gene transcription (7–12).

Many cancer cells display mutations in the TGF-β signaling pathway. Over 15% of colon carcinomas display mutations in the type II TGF-β receptor. The incidence of type II receptor mutations climbs to 90% if only those colon cancers with microsatellite instability are examined (13). Furthermore, 25% of ovarian cancers have a mutation in the type II receptor and 33% in the type I receptor (14), whereas mutations in type I TGF-β receptors are less frequent in cancers of the lung, breast, and colon (15, 16). Mutations have also been observed within the Smad family. About 50% of pancreatic and 15% of colorectal cancers have a mutation in Smad4 (6, 17), whereas Smad2 mutations are seen in less than 5% of lung and colon cancers (18).

Smad7 has been shown to inhibit TGF-β signaling (19–21), and, accordingly, Smad7 expression inhibits downstream effectors of TGF-β, such as p21WAF1/CIP1, in a dose-dependent manner (22). Whereas Smad7 possesses the domain responsible for binding to the type I TGF-β receptor, it lacks both the DNA binding domain and the serine motif present in R-Smads that becomes phosphorylated by the TGF-β receptor (23). In this way Smad7 acts as a competitive antagonist at the type I receptor, preventing R-Smad signaling. Smad7 has also been shown to play a key role in TGF-β receptor degradation by interacting with Smurf1 and Smurf2, two E3 ubiquitin ligases with whom Smad7 translocates from the nucleus to the type I receptor at the cell membrane upon TGF-β stimulation (24–26). Research into the mechanisms governing Smad7 transcription has revealed a negative feedback loop, because the Smad7 promoter harbors a Smad binding element that facilitates activation by R-Smad4 complexes (27, 28). Because Smad7 can inhibit the anti-proliferative TGF-β signaling, Smad7 overexpression may be an additional mechanism by which cells progress on their path to a transformed phenotype. Consistently, overexpression of Smad7 has been noted in pancreatic and colon cancers (29, 30).

A possible regulator of Smad7 transcription is AP-1 (31), a complex that consists of Fos and Jun proteins and appears to be involved in cell proliferation and apoptosis (32). Interestingly, TGF-β can transiently up-regulate mRNA levels of c-Jun (33). Moreover, different promoters of TGF-β-responsive genes contain AP-1 binding sites, and their mutation impairs the ability of TGF-β to activate these promoters (8, 11), which may be due
to the fact that AP-1 components interact with R-Smad and Smad4 proteins are the primary mediators of the TGF-β pathway. However, other signaling pathways may be involved in TGF-β responses. TAK1 is a member of the mitogen-activated protein kinase (MAPK) kinase kinase family whose protein kinase activity is stimulated in response to TGF-β and may therefore activate TGF-β-responsive genes independently of Smad proteins (36–38). Importantly, TAK1 stimulation leads to the down-regulation of cyclin D1 expression and inhibition of cell proliferation (39). Therefore, TAK1 may be responsible, in part, for the anti-proliferative effects of TGF-β.

Another important activator of MAPK pathways is HER-2 Neu, a transmembrane receptor kinase overexpressed in 20–30% of breast and ovarian cancers and 10–15% of endometrial cancers (40, 41). Clinically, HER2/Neu overexpression in tumors is associated with high grade, advanced stage, and poor prognosis. One downstream target of HER2/Neu is the ETS transcription factor ER81 (42, 43), which has a highly conserved ETS DNA binding domain (44). In particular, ER81 is overexpressed in breast cancer cells (45) and may collaborate with HER2/Neu in breast cancer formation.

Here, we show that HER2/Neu and ER81 synergistically activate the human Smad7 promoter. Thus, we have identified how the oncoprotein HER2/Neu can antagonize the action of the tumor suppressor TGF-β through Smad7 up-regulation. This may further our understanding of carcinogenesis and explain one way in which HER2/Neu and/or ER81 overexpression favors cancer formation. Moreover, ER81 and TAK1 are shown to be part of an alternative negative feedback loop of the TGF-β pathway involving Smad7 up-regulation.

EXPERIMENTAL PROCEDURES

Luciferase Promoter Constructs—A segment of the human Smad7 promoter (∼3100 to +1502) was cloned upstream of the luciferase gene within the pGL2-Basic plasmid (Promega). Truncations of this luciferase promoter construct were generated using restriction endonucleases. Within the pGL2-Basic plasmid (Promega). Truncations of this luciferase reporter construct, there was a 10-fold induction of promoter activity (see “Materials and Methods”). However, although similar to the very minimal promoter region responsible for HER2/Neu- and ER81-mediated activation, truncated promoter constructs were generated (Fig. 1A). Five progressive 5’ and 3’ truncations of the full promoter construct had little effect on the activation of the Smad7 promoter (compare “7b,” “7a,” “7h,” “7p” and “7s” to “7c,” Fig. 1B). However, although the Smad7 promoter region ∼180 to +336 displayed a similar behavior as the ∼3100 to +1502 region, further deletion of the 3’-end to +21 resulted in a dramatic loss in baseline and inducible promoter activity (see “7xs,” Fig. 1B); in fact, the very low activity of the 7xs construct was comparable to that seen with the empty pGL2-Basic vector. Thus, the Smad7 promoter region ∼180 to +336 appears to be responsible for the stimulation by HER2/Neu and ER81.

Although we have shown that HER2/Neu and ER81 synergistically activate the Smad7 promoter, we wondered whether the small activation observed with HER2/Neu alone in RK13 cells could be accounted for by endogenous ER81 or related proteins. To this end we carried out transient transfections in the presence and absence of a dominant negative ER81 molecule consisting of the C-terminal fragment of ER81 (amino acids 333–477) that contains the ER81 DNA binding domain but is unable to activate transcription (46). The presence of this
Smad7 Activation by HER2/Neu, TAK1, and ER81

Fig. 1. ER81 and HER2/Neu synergistically activate the Smad7 promoter. A, scheme of Smad7 promoter truncations used. ETS core sites are highlighted by black boxes, and an arrow indicates the transcription start site. B, ER81 was co-transfected with HER2/Neu and the indicated Smad7 promoter reporter luciferase plasmids into RK13 cells. C, effect of dominant negative ER81-(333–477) on Smad7 promoter activation by HER2/Neu and ER81 in RK13 cells. D, activation of the Smad7 promoter construct was utilized and resultant luciferase activities are depicted. E, activation of the Smad7 promoter construct by HER2/Neu and ER81 in MCF7 cells. E, similar in HEC-1A cells.

dominant negative ER81 protein led to a dramatic reduction in the ability of HER2/Neu to activate the Smad7 promoter in the absence of ectopically expressed ER81 (Fig. 1C). Furthermore, the synergism in Smad7 stimulation observed upon co-expression of HER2/Neu and full-length ER81 was completely abrogated by ER81-(333–477). This implicates that transfected ER81 is necessary for efficient HER2/Neu-mediated activation of Smad7 reporter transcription.

We also tested whether HER2/Neu and ER81 synergized in the activation of the Smad7 promoter in various cell lines other than RK13. Indeed, under all conditions tested, HER2/Neu and ER81 together activated Smad7 transcription more efficiently than when overexpressed alone in the human breast cancer cell line MCF7 (Fig. 1D), the human endometrial cancer cell line HEC-1A (Fig. 1E), and in the human ovarian cancer cell line OVCAR3 (see Fig. 4A). Thus, HER2/Neu and ER81 are able to jointly stimulate the Smad7 promoter in a wide variety of cell lines.

-binding of ER81 to the Smad7 Promoter—Next we attempted to demonstrate binding of the ETS transcription factor ER81 to the Smad7 promoter. Within the Smad7 promoter region (−180 to +360) identified to confer inducibility by HER2/Neu and ER81, we noted only six ETS core sites of the sequence GGAA/T that are required for ETS proteins to bind to DNA (44). However, three of these are located in a very GC-rich region 100 bp upstream of the transcription start site and are therefore unlikely to be functional ER81 binding sites. Thus, we focused on the three other putative ETS core sites (see Fig. 1A), namely from −131 to −128 (ETS1 site), +50 to +53 (ETS2 site), and +55 to +58 (ETS3 site), none of which were part of a sequence perfectly matching the established ER81 consensus DNA binding site (52). Radioactively labeled double-stranded oligonucleotides corresponding to these three potential ETS sites within the Smad7 promoter were synthesized. Because of the proximity of ETS sites 2 and 3, only one common probe was used to investigate these two sites (ETS2/3) that are in opposite direction to each other (+50 GGAAGATCC +58, ETS core sites are underlined). In electrophoretic mobility shift assays, purified ER81 (249–477) protein, which contains the ETS DNA binding domain, failed to interact with the ETS1 site (Fig. 2A). However, ER81 readily associated with the ETS2/3 probe containing the closely juxtaposed ETS2 and ETS3 sites, and an anti-ER81 antibody elicited a respective supershift formation. Interestingly, much more DNA binding was observable in the presence of the anti-ER81 antibody that probably stabilizes DNA binding by relieving an intramolecular repression (42, 53). However, when the ETS2 site was mutated, ER81 binding was no longer observable (see ETSm2/m3 in Fig. 2A). Similarly, binding of ER81 was dramatically reduced upon mutation of the ETS3 site (see ETS2/m3), and mutation of both ETS sites (see ETSm2/m3) resulted in no binding of ER81. These results suggest that ER81 binds to both ETS sites 2 and 3, albeit with a higher affinity to the ETS2 site as residual supershifted complexes were observable with the ETS2/m3 but not ETS2/m3 oligonucleotide (Fig. 2A). Consistently, a 50-fold excess of cold ETSm2/3 oligonucleotide was inefficient to compete with 32P-labeled ETS2/3 oligonucleotide for ER81 binding, whereas cold ETS2/m3 oligonucleotide was nearly as efficient as cold ETS2/3 oligonucleotide in these competition experiments (Fig. 2B).

Next we set out to demonstrate binding of full-length ER81 to the ETS2/3 probe using the lysates of transfected 293T cells. We conducted respective electrophoretic mobility shift assays in the presence of the anti-ER81 antibody that stabilizes DNA binding. However, even in the presence of the anti-ER81 antibody, we were not able to demonstrate binding of full-length ER81 isolated from cells transfected with ER81 alone (Fig. 2C). Rather, binding was only evident with lysates from 293T cells transfected with both ER81 and HER2/Neu, and this binding could be abrogated in competition experiments with cold ETS2/3 oligonucleotide, but not ETSm2/m3. Similarly, E74, a known binding site for ETS proteins, including ER81 (46), was able to efficiently compete for binding, whereas a mutated E74 site was not. In particular, note that one DNA-protein complex (marked “ETS?”) was also competed by unlabeled ETS2/3 and E74 oligonucleotides, suggesting that this complex may represent DNA binding of an endogenous ETS protein other than
ER81. Altogether, we conclude that ER81 can bind to the Smad7 promoter upon stimulation by HER2/Neu.

We wondered if ER81 binds to the juxtaposed ETS2 and ETS3 sites at the same time. To test this hypothesis we synthesized a double-stranded probe equal in length to the ETS2/3 probe, but replaced its two ETS sites with a single one, the E74 site (modE74 oligonucleotide). Because ER81 is known to associate with E74 as a monomer (46), we compared the migration of protein/DNA complexes seen with the modE74 and ETS2/3 probes (Fig. 2D). We found that the complex formed between ER81 and ETS2/3 migrated slower than the ER81/modE74 complex, indicative of binding of two ER81 molecules to one ETS2/3 oligonucleotide. Furthermore, because we did not detect a 1:1 complex of the ETS2/3 oligonucleotide with ER81, an exclusive simultaneous binding of ER81 to both the ETS2 and ETS3 sites of the Smad7 promoter appears to occur in vitro.

Next, we assessed the impact of the various ETS sites on the activity of the Smad7 promoter. Similar to the −180 to +336 promoter construct, a −128 to +336 construct was synergistically inducible by HER2/Neu and ER81 (see ΔETS1 in Fig. 3). Because the ETS1 site (−131 to −128) is absent in the −128 to +336 construct, we conclude that the ETS1 site is not required for Smad7 stimulation by ER81, consistent with the inability of ER81 to interact with the ETS1 site. Furthermore, mutation of the ETS2 site in the −180 to +336 promoter construct had no effect, whereas mutation of ETS site 3 severely compromised Smad7 promoter activity under all conditions tested (Fig. 3). Combined mutation of ETS sites 2 and 3 did not aggravate this phenotype much further. These results suggest that ETS site 3 is relevant for the activation of the Smad7 promoter, whereas ETS site 2 appears to be dispensable.

HER2/Neu Activates the Smad7 Promoter via the ERK

MAPK Pathway—Because the ovarian cancer cell line OVCAR3 endogenously overexpresses HER2/Neu (54), this represented a model by which to characterize the mechanisms
Fig. 4. The Ras-Raf—ERK MAPK pathway is crucial for HER2/Neu-mediated activation of the Smad7 promoter. A. OVCAR3 cells were co-transfected with ER81, HER2/Neu, and the Smad7 luciferase reporter plasmid in the presence of vehicle (MeSO/DMSO) or the HER2/Neu inhibitor AG825. B. Similar, the effect of the phosphatidylinositol 3-kinase inhibitor LY294002 (LY) or the ERK MAPK pathway inhibitor U0126 on Smad7 promoter activity, assessed with antibodies against Smad7 and Smad5, or the constitutively active Ras molecule (Ras-G12V) and the Smad7 reporter plasmid. C. Analogous with a constitutively active Raf molecule, BXB. D. RK13 cells were co-transfected with the Smad7 reporter plasmid and the 6xA mutant of ER81 in which all MAPK-dependent phosphorylation sites have been mutated to alanine residues.

To further demonstrate this point, we assessed whether HER2/Neu could be replaced by components of the HER2/Neu—Ras—Raf—ERK MAPK pathway. To this end, we utilized a constitutively active Ras molecule, Ras-G12V (49), or a constitutively active Raf molecule, BXB (48). Indeed, both of these oncogenic signaling molecules were capable of synergizing with ER81 to stimulate the Smad7 promoter (Fig. 4, C and D). Moreover, the 6xA mutant of ER81, in which all six ERK MAPK-dependent phosphorylation sites in ER81 have been mutated to alanine residues (42, 59), was unable to stimulate the Smad7 promoter, nor did it support HER2/Neu to do so (Fig. 4E). Collectively, our results suggest that phosphorylation of ER81 upon activation of the Ras→Raf→ERK MAPK signaling pathway by oncogenic HER2/Neu plays a pivotal role in the activation of the Smad7 promoter.

Activation of Smad7 Transcription by ETS Proteins—More than 20 transcription factors belonging to the ETS family have been described (44), raising the possibility that other ETS proteins aside from ER81 might stimulate the Smad7 promoter. Thus, we analyzed the ability of various members of the ETS protein family to activate the Smad7 promoter in conjunction with HER2/Neu. First, we focused on PEA3 and ERM, two proteins that are highly related to ER81 and form a subfamily of ETS proteins (45). Indeed, ERM was even more potent than ER81 in activating the Smad7 promoter upon HER2/Neu co-expression, whereas PEA3 was not as efficient (Fig. 5). We then assessed Elk1, an ETS protein that is inducible by ERK MAPK phosphorylation (44, 60). However, HER2/Neu was even slightly less able to activate the Smad7 promoter in the presence of Elk1 than in its absence. Similarly, ER71 (52, 61) and Elf1, the latter one not being activated by ERK MAPKs (51), were unable to synergize with HER2/Neu. Thus, induction of Smad7 promoter activity by HER2/Neu can occur via the related ER81, ERM, and PEA3 ETS proteins but not other ETS proteins tested.

Role of AP-1 in Smad7 Stimulation by HER2/Neu—Previously, it has been reported that AP-1 might be necessary for TGF-β-mediated activation of the rat Smad7 promoter (31). Also, the AP-1 component c-Jun was shown to bind to the mouse Smad7 promoter at a half-site (TGAC) of the consensus sequence for AP-1 binding (12). A homologous AP-1 half-site exists in the human Smad7 promoter at −166 to −163. Sequence analysis of the human Smad7 promoter from −180 to +336 revealed two other potential AP-1 sites of the sequence TGAG at +180 to +183 and +188 to +191 (see Fig. 6). Furthermore, AP-1 transcription factors might be regulated by HER2/Neu through MAPKs (32, 56). As such, we investigated whether AP-1 plays a role in Smad7 promoter regulation by HER2/Neu.

To this end, we deleted the upstream AP-1 site (ΔAP1), mutated both downstream AP-1 sites (mut-AP1), or did both (Δmut-AP1). None of these promoter constructs behaved significantly different from the wild-type (−180 to +336) Smad7 promoter.
construct upon stimulation with HER2/Neu in the presence or absence of ER81 (Fig. 6). Thus, AP-1 appears not to be directly involved in HER2/Neu-dependent Smad7 transcription.

**TAK1 and ER81 Synergistically Activate the Smad7 Promoter**—Because TGF-β has been shown to activate Smad7 transcription (19, 20), we asked the question whether the TGF-β-stimulated MAPK kinase kinase, TAK1 (37), also stimulates the Smad7 promoter via the MAPK target, ER81. Indeed, TAK1 was able to stimulate the Smad7 (−180 to +336) promoter construct and synergized with ER81 to do so (Fig. 7A). In contrast, a kinase-defective mutant, TAK1-K63W (37), was unable to activate the Smad7 promoter. Moreover, TAK1 relies on endogenous ETS proteins to stimulate Smad7 transcription as the dominant negative ER81-(333–477) protein suppressed TAK1-dependent Smad7 promoter stimulation (Fig. 7B). Furthermore, TAK1 was no longer able to stimulate the Smad7 promoter when both of the ER81 binding sites (ETS2 and ETS3) were mutated (Fig. 7C). Thus, transfected ER81 and TAK1 are capable of synergistically stimulating the Smad7 promoter.

To elucidate which MAPK pathway is involved in TAK1- and ER81-dependent Smad7 up-regulation, we employed specific inhibitors of distinct MAPK pathways. Neither U0126, an inhibitor of the ERK MAPK pathway (58), nor SP600125, an inhibitor of Jnk MAPKs (62, 63), reduced TAK1- and ER81-dependent Smad7 promoter activity (Fig. 7D), whereas SB202190, an inhibitor of p38 MAPKs (64), did so. Thus, TAK1 appears to selectively employ the p38 MAPK pathway. Consistently, overexpression of MEK6-DD, a constitutively active stimulator of p38 MAPKs (50), synergized with ER81 to stimulate the Smad7 promoter (Fig. 7E), and joint expression of a p38 MAPK and MEK6-DD was even more effective.

**DISCUSSION**

In this investigation we have shown that the oncprotein HER2/Neu can activate the human Smad7 promoter in a variety of different cell lines. This activation can be mediated by the binding of the ETS transcription factor ER81 to the Smad7 promoter and involves the activation of ER81 via the Ras→Raf→ERK MAPK pathway (Fig. 8). Moreover, we have shown that TAK1 and ER81 synergize in Smad7 promoter activation involving the p38 MAPK pathway. As such, ER81 may be a crucial mediator of Smad7 regulation, which is important both in tumor formation as well as in the attenuation of TGF-β signaling.

Electrophoretic mobility shift assays demonstrated that the ER81 DNA binding domain readily interacts with a region of exon 1 of the Smad7 gene encompassing two closely separated ETS sites, ETS2 and ETS3. Mutation of either ETS2 or ETS3 severely compromised binding of ER81 to the Smad7 promoter, suggesting that ER81 can bind as a dimer. Consistently, the electrophoretic mobility of ER81 bound to the ETS2/3 oligonucleotide is much slower than the one of ER81 bound to a modified E7 oligonucleotide of equal length (see Fig. 2D) with which ER81 interacts as a monomer (46). Also, the ability of the anti-ER81 antibody to strongly enhance DNA binding may partially be explained by the fact that it promotes, due to its bivalent nature, the dimerization of ER81. One reason why ER81 cannot efficiently bind to the ETS2 or ETS3 site alone may be the fact that nucleotides flanking their ETS core sequences (see sequences under “Experimental Procedures”) do not exactly match the established consensus binding site for ER81, (G/A)(G/C)(C/A/GGA(A/T)(G/A)(T/C) (52).
Interestingly, electrophoretic mobility shift assays performed with cell lysates derived from transfected cells showed binding of full-length ER81 to the ETS2/3 oligonucleotides only when the cells were co-transfected with HER2/Neu and ER81. This suggests that DNA binding of ER81 is intramolecularly inhibited by N-terminal amino acids that contain its phosphorylation sites, similar to what has been observed for the related ETS protein PEA3 (65, 66). HER2/Neu induces the phosphorylation of ER81 within its N terminus (42) and may thereby alleviate this intramolecular repression.

Mutation of ETS site 2 in the Smad7 promoter had no effect on its stimulation by HER2/Neu and ER81, whereas promoter stimulation was severely reduced upon mutation of ETS site 3. This does not conform to our in vitro DNA binding assays in which mutation of either ETS site compromised binding of ER81 to the Smad7 promoter. However, even a weak interaction of ER81 with the Smad7 promoter in vivo may suffice to activate transcription, yet will not be observable in in vitro DNA binding assays that rely on a relatively stable binding of protein to DNA. As such, ETS site 3 may still recruit ER81 in vivo even when ETS site 2 is mutated, and we can also not exclude that protein-protein interactions of ER81 with other Smad7 promoter bound proteins help in this in vivo recruitment of ER81 to the Smad7 promoter.

We have shown previously that ERK MAPKs activate ER81 through direct phosphorylation as well as phosphorylation through protein kinases such as RSK and MSK that are themselves activated by ERK MAPKs (42, 59, 67). Mutation of all these phosphorylation sites abolished the ability of ER81 to synergize with HER2/Neu in Smad7 promoter stimulation, indicating that the ERK MAPK pathway is involved in HER2/Neu mediated Smad7 up-regulation. Indeed, inhibition of the ERK MAPK pathway with U0126 suppressed Smad7 promoter stimulation, and oncogenic Ras or Raf, known activators of the ERK MAPK pathway, were also able to synergize with ER81 in Smad7 regulation. Thus, HER2/Neu-dependent activation of the Smad7 promoter proceeds via the Ras→Raf→ERK MAPK pathway and ER81. However, ER81 may not be the sole effector of HER2/Neu, especially considering that the related ETS protein ERM also synergized with HER2/Neu in the activation of Smad7 promoter activity.

ER81 is not only activated by ERK MAPK but also by p38 MAPK (42, 68). TAK1 is an MAPK kinase kinase that is capable of activating the p38 MAPK pathway (39, 69–73), and consistently TAK1 appears to activate ER81 and thereby the Smad7 promoter selectively via the p38 MAPK pathway. Smad7 is known to participate in a negative feedback loop in which TGF-β activation results in R-Smad translocation to the nucleus where Smad7 transcription is induced by R-Smad-Smad4 complexes and the produced Smad7 protein subsequently inhibits TGF-β signaling (19, 20). Our data showing Smad7 activation by TAK1 and ER81 suggests an additional negative feedback loop, as TGF-β is known to induce TAK1 activity (37). Thus, activation of ER81 by p38 MAPK can contribute to increased Smad7 transcription upon TGF-β stimulation and represents an additional means by which the strength of the many cellular responses to TGF-β is modulated.

HER2/Neu overexpression has been demonstrated in many cancers and is linked to advanced stage and poor prognosis (40, 41). Furthermore, ER81, or the related ETS proteins ERK and PEA3, appear to be overexpressed in HER2/Neu-overexpressing human breast cancers, and transgenic mice overexpressing HER2/Neu in mammary tissue accordingly display a coordinate up-regulation of ER81, ERM, and PEA3 (42, 45, 74). Also, both ER81 and PEA3 have been shown to activate HER2/Neu transcription, thereby establishing a positive feedback loop (53, 75).

## REFERENCES

1. Datto, M. B., Li, Y., Panus, J. F., Howe, D. J., Xiong, Y., and Wang, X. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5545–5549
2. Hannon, G. J., and Beach, D. (1994) Nature 371, 257–261
3. Pietinen, J. A., Haltt, J. T., Stein, R. W., and Moses, H. L. (1999) Proc. Natl. Acad. Sci. U. S. A. 97, 3758–3762
4. Pietinen, J. A., Stein, R. W., Moran, R., Yaciuk, P., Schlegel, R., Lyons, R. M., Pittelkow, M. R., Munger, K., Howley, P. M., and Moses, H. L. (1999) Cell 61, 775–778
5. Liu, X., Sun, Y., Weinberg, R. A., and Lodish, H. F. (2001) Cytokine Growth Factor Rev. 12, 1–8
6. Massague, J. (1998) Annu. Rev. Biochem. 67, 753–791
7. Denujro, S., Irob, S., Vivien, D., ten Dijke, P., Huet, S., and Gauthier, J. M. (1998) EMBO J. 17, 3091–3100
8. Yingling, J. M., Datto, M. B., Wong, C., Frederick, J. P., Liberati, N. T., and Wang, X. F. (1997) Mol. Cell. Biol. 17, 7019–7028
9. Jonk, L. J., Irob, S., Heldin, C. H., and ten Dijke, P., and Kruijer, W. (1998) J. Biol. Chem. 273, 21145–21152
10. Zawel, L., Dui, J. L., Buckhaults, P. R., Zhou, S., Kinzler, K. W., Vogelstein, B., and Kern, S. E. (1998) Mol. Cell 1, 611–617
11. Wong, C., Dui, J. L., Buckhaults, P. R., Zhou, S., Kinzler, K. W., Vogelstein, B., and Kern, S. E. (1998) Mol. Cell. Biol. 19, 1821–1830
12. Brosdin, G., Ahgren, A., ten Dijke, P., Heldin, C. H., and Heuchel, R. (2000) J. Biol. Chem. 275, 29023–29030
13. Parsons, R., Myers, L. L., Liu, B., Wilson, J. S., Markowitz, S. D., Kinzler, K. W., and Vogelstein, B. (1999) Cancer Res. 59, 5548–5550
14. Lynch, M. A., Nakashima, R., Song, H., DeGraff, W. L., Wang, D., Enomoto, T., and Weghorst, O. M. (1998) Cancer Res. 58, 4227–4232
15. Kim, S. J., Im, Y. H., Markowitz, S. D., and Yang, J. Y. (2000) Cytokine Growth Factor Rev. 11, 159–169
16. Chao, T., Triplet, J., Dehner, B., Hustin, B., Colligan, B., Pemberton, J., Graff, J. R., and Carter, J. H. (2001) Cancer Res. 61, 4679–4682
17. Massague, J., Blain, S. W., and Lo, R. S. (2000) Cell 103, 295–309
18. Eiglmayer, G. J., Kinzler, K. W., Vogelstein, B., and Thiagalingam, S. (1997) Cancer Res. 57, 2578–2580
19. Afrakhte, M., Maren, A., Jossan, S., Itoh, S., Sampath, K., Westerman, B., Heldin, C. H., Heldin, N. E., and ten Dijke, P. (1996) Biochem. Biophys. Res. Commun. 240, 505–511
20. Nakao, A., Afrakhte, M., Maren, A., Nakayama, T., Christian, J. L., Heuchel, R., Itoh, S., Kawabata, M., Heldin, N. E., Heldin, C. H., and ten Dijke, P. (1997) Nature 390, 631–635
21. Hayashi, H., Abdullah, S., Qu, Y., Cai, J., Xu, Y. Y., Grinnell, B. W., Richardson, M. A., Topper, J. N., Gimbrone, M. A., Jr., Wrana, J. L., and Falb, D. (1997) Cell 80, 1165–1172
22. Pardali, K., Kurisaki, A., Maren, A., ten Dijke, P., Kardassis, D. S., and Moustakas, A. (2000) J. Biol. Chem. 275, 29244–29256
23. Zimmerman, C. M., and Paspgett, R. W. (2000) Gene (Amst.) 249, 17–30
24. Kavvak, P., Rasmussen, R. K., Causing, C. G., Bonni, S., Zhu, H., Thomsen, G. H., and Wrana, J. L. (2000) Mol. Cell 6, 1365–1375
25. Ehisa, T., Fukuchi, M., Murakami, G., Chiba, T., Tanaka, K., Imamura, T.,...
