The Anti-proliferative Function of the TGF-β1 Signaling Pathway Involves the Repression of the Oncogenic TBX2 by Its Homologue TBX3*

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Background: The transcription factor TBX3 regulates the anti-proliferative function of the TGF-β1 pathway but its downstream target(s) responsible for this is unknown.

Results: TBX3 downstream of TGF-β1 signaling represses TBX2 to inhibit proliferation.

Conclusion: The down-regulation of TBX2 is required for the anti-proliferative function of the TGF-β1-TBX3 axis.

Significance: This study sheds light on the mechanisms involved in the anti-proliferative role of TGF-β1.

A growing body of work has shown that the highly homologous T-box transcription factors TBX2 and TBX3 play critical but distinct roles in embryonic development and cancer progression. For example, TBX2 and TBX3 are up-regulated in several cancers and recent evidence suggests that whereas TBX2 functions as a pro-proliferative factor, TBX3 inhibits cell proliferation but promotes cancer cell migration and invasion. While the molecular mechanisms regulating these functions of TBX2 and TBX3 are poorly understood we recently reported that the TGF-β1 signaling pathway up-regulates TBX3 expression to mediate, in part, its well described anti-proliferative and pro-migratory roles. The TBX3 targets responsible for these functions were however not identified. Here we reveal for the first time that the TGF-β1 signaling pathway represses TBX2 transcriptionally and we provide a detailed mechanism to show that this is mediated by TBX3. Furthermore, we implicate the down-regulation of TBX2 in the anti-proliferative function of the TGF-β1-TBX3 axis. These findings have important implications for our understanding of the regulation of TBX2 and TBX3 and shed light on the mechanisms involved in the anti-proliferative and pro-migratory roles of TGF-β1.

The transforming growth factor β1 (TGF-β1)2 pathway plays critical roles in a wide range of cellular processes including cell proliferation, apoptosis, differentiation, and the immune response. This multi-functional cytokine generally exerts its effects by binding and activating the TGFβ receptors I and II, which initiate a cascade of signaling events frequently involving Smad proteins and co-factors but which can also be Smad-independent (1). One of the well-known roles of TGF-β1 is its ability to inhibit the proliferation of epithelial cells but promote their migration (2). TGF-β1 has been shown to inhibit proliferation primarily by inducing a G1 cell cycle arrest through its ability to transcriptionally up-regulate expression of the cyclin-dependent kinase inhibitors p15Ink4b and p21Cip1 (3). Its pro-migratory effects, however, appear to be mainly due to its ability to regulate key players of epithelial-mesenchymal transition (EMT) (4). These anti-proliferative and pro-migratory roles of TGF-β1 are important in embryonic development, for example during branching morphogenesis of the mammary gland, as well as during oncogenesis (5, 6). We recently reported that TGF-β1 up-regulates expression of the T-box transcription factor, TBX3, through a mechanism involving the co-operation of Smad proteins and JunB. This was shown to be a critical downstream event for mediating TGF-β1-induced anti-proliferation and pro-migration of breast epithelial cells and keratinocytes but the TBX3 target genes responsible for this were not described (7).

The T-box family of transcription factors are highly conserved in evolution and play critical roles in embryonic development. TBX3 is a member of the TBX2 subfamily and along with its closely related family member, TBX2, it plays an important role in the development of the limbs, heart, and mammary glands (8). TBX2 and TBX3 are overexpressed in a number of cancers, including breast, pancreatic, ovarian, liver, cervical, and melanoma (9–13). While there is evidence to suggest that they both repress the cell cycle regulators p19ARF and p21WAF1/CIP1/SDII, they have distinct functions in embryonic development and cancer (8–10, 14–19). For example, mouse studies examining the effects of loss of function mutations have shown that Tbx3 plays an important role during mammary placode induction while Tbx2 has no significant effects on this process (19–21). Furthermore, we and others have also shown that TBX2 and TBX3 play distinct roles in cancer progression, with TBX2 functioning as a powerful pro-proliferative factor and TBX3 playing a role in migration and invasion (22, 23).
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Together with our previously published data showing that TBX2 and TBX3 exhibit mutually exclusive expression in some melanoma cell lines (24), we therefore speculated that the anti-proliferative function of TGF-β1 may be due to its ability to down-regulate TBX2 expression. Indeed, here we show that in human breast epithelial and mouse melanoma cells, TGF-β1 negatively regulates TBX2 protein and mRNA levels at a transcriptional level and importantly, we demonstrate that this regulation is dependent on increased TBX3 levels. Using both in vitro and in vivo assays we reveal that TBX3 binds and regulates the TBX2 promoter in response to TGF-β1 through a T-element at −186 bp. Furthermore, we show that the down-regulation of TBX2 expression is responsible for the decrease in cell proliferation induced by TGF-β1 and that this is possibly mediated by increased p21 levels.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture Conditions—The normal human breast epithelial MCF-12A cells were maintained in complete medium consisting of DMEM/Ham’s F12 supplemented with 10% FBS, 100 units/ml penicillin, 0.1 μg/ml chlora toxin (Sigma), 0.5 μg/ml hydrocortisone (Calbiochem), 10 μg/ml insulin (Novorapid, Denmark), 20 ng/ml EGF (GIBCO), and 5% horse serum (Highveld Biological, Lyndhurst, UK). B16 mouse melanoma cells were maintained in RPMI 1640 (Highveld Biological, Lyndhurst, UK) supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. All cell lines were maintained in a 5% CO₂ humidified incubator at 37 °C.

For TGF-β1 treatment, we used 5 ng/ml rhTGF-β1 (R&D Systems), diluted in 4 mM HCl, 1 mg/ml BSA. For inhibition of de novo protein synthesis experiments, cells were pretreated with 30 μg/ml cycloheximide (Sigma-Aldrich) for 1 h and then treated with TGF-β1 for 8 h.

Plasmid Constructs—The human TBX2 promoter luciferase reporter constructs have been described previously (25). The pcDNA 3.1(+) containing the full-length human TBX2 cDNA was supplied by Prof. van Lohuizen (Antoni van Leeuwenhoek hospital, Amsterdam, The Netherlands). The TBX2 cDNA was then excised and the vector re-ligated to generate the pcDNA3.1 (+) empty vector, into which the human TBX3 cDNA was cloned using the Nhel and BamHI sites. The p21-pRe/CMV plasmid, which was prepared in the laboratory of Prof William Kaelin, was obtained from Addgene (Addgene plasmid 20814) (26). The TBX3 N-terminal construct was constructed by excising the C-terminal portion of the pCMV-TBX3 construct with BglIII and SpeI and re-ligated.

Western Blot Analysis—Cells were harvested and protein and mRNA prepared as described previously (27). Primary antibodies used were as follows: goat polyclonal anti-TBX2 antibody (sc-17880, Santa Cruz Biotechnology), rabbit polyclonal anti-TBX3 (42–4800, Zymed Laboratories Inc., Invitrogen), rabbit polyclonal anti-p21 (sc-397, Santa Cruz Biotechnology), and rabbit polyclonal anti-p38 (M0800, Sigma, Germany).

Quantitative Real-time PCR (qRT-PCR)—Cells were harvested, and mRNA prepared as described previously (28). Primers used to amplify the human TBX3 (QT00022484), TBX2 (QT00091266), and GUSB (QT00046046) were purchased from Qiagen.

Generation of Stable Cell Lines Expressing TBX2—To generate stably transfected cell lines, MCF-12A cells were transfected with either the empty expression vector pcDNA3.1 (+) or with this vector containing the full-length human TBX2 cDNA using X-tremeGENE HD DNA transfection reagent (Roche Diagnostics, Basel, Switzerland). Stable transfected cells were selected by 400 μg/ml G-418.

Generation of MCF-12A Cell Line Overexpressing TBX3—The MCF-12A cells were transiently transduced using adenovirus with human TBX3 cDNA cloned into the vector component of an Adeno-X Tet-off expression system 1 (Clontech). The virus was purified using the Adeno-X Maxi purification kit (Clontech). All steps were performed according to the manufacturer’s instructions.

Transfection and Luciferase Assays—Transient transfections were performed using X-tremeGENE HD DNA transfection reagent (Roche Diagnostics) according to the manufacturer’s instructions. MCF-12A cells were plated at 1 × 10⁵ cells per well of a 12-well plate, 24 h before transfection. Cells were co-transfected with 400 ng of a TBX2-luciferase reporter plasmid plus 100 ng of the TBX3 expression plasmids or corresponding amounts of an empty-vector plasmid. Luciferase activities were measured using the Luminoskan Ascent luminometer (Thermo Labsystems, Franklin, MA). All transfections were performed in duplicate and at least three independent experiments were done to confirm reproducibility. Firefly luciferase values were expressed relative to empty-vector control.

Site-directed Mutagenesis—Site-directed mutagenesis was performed on the −218/+33 bp TBX2 promoter-luciferase reporter or pCMV-TBX3 expression construct as a template using PFU polymerase reagents (Promega). Mutations were introduced as follows (only sense-strand presented and mutations indicated with lowercase letters): T-element (-186): 5’-GCTGAGGCTTCCaAaaCTTCTCCAGGCC-3’; TBX3 DNA binding domain mutant (R133G): 5’-CAttACCAaGT-CGGGAgGGaGGAaTGTCCTCCATTAAAG-3’. The integrity of each mutant construct was verified using agarose gel electrophoresis and sequencing.

siRNA Sequences and Transfection—Transient knockdown of TBX2 and TBX3 was achieved by siTBX2#1 (S102656563), siTBX2#2 (S103238802), siTBX3#1 (S100083503), and siTBX3#2 (S103100426) purchased from Qiagen. Knockdown of p21 was achieved using ON-TARGETplus SMARTpool Human CDKN1A siRNA (sip21#1) (Thermo Scientific) and sip21#2 (S100299810) from Qiagen. The cells were transfected with siRNAs to TBX2, TBX3, p21 or a control (non-silencing) siRNA (1027310; Qiagen) using HiPerFect (Qiagen) according to the manufacturer’s instructions.

Chromatin Immunoprecipitation Assays (ChIP)—ChIP assays were carried out as previously described (10). Briefly, MCF-12A cells treated with TGF-β1 for 24 h were fixed in 1% formaldehyde and the chromatin extracted, sonicated, and immunoprecipitated using antibodies against TBX3 (sc-17871) or IgG (negative control, Santa Cruz Biotechnology). DNA precipitated was analyzed by qRT-PCR using human TBX2-specific primer pairs or a nonspecific promoter region (GUSB, Qiagen). Cross- ing values (Ct) of TBX3 precipitated DNA were normalized against the Ct values of IgG. Fold enrichment was determined...
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results suggest that TGF-β1 represses basal and TGF-β1 mRNA and protein levels, suggesting that TBX3 represses basal and TGF-β1 regulated TBX2 levels.

TBX3 Binds to the TBX2 Promoter at ~186 bp in Response to TGF-β1 Treatment—We next wanted to confirm that TBX3 can directly repress TBX2 in vivo and to this end we performed a chromatin immunoprecipitation (ChIP) assay. Cross-linked chromatin was prepared from TGF-β1-treated and control MCF-12A cells and DNA immunoprecipitated with an antibody to TBX3 was subjected to qRT-PCR with primers spanning the ~186 bp putative T-element in the TBX2 promoter. The results obtained show that compared with the untreated control, the binding of TBX3 to the TBX2 promoter was enhanced by 8.5-fold in the presence of TGF-β1 (Fig. 4A). To determine whether TBX3 binds specifically to the ~186 bp T-element in the TBX2 promoter, we performed a DNA affinity immunoblot (DAI) using nuclear extract isolated from TGF-β1-treated 186 bp in Response to TGF-β1 treatment in both cell lines from 8 h in MCF-12A cells and 4 h in B16 cells (Fig. 1, A and B). This robust repression was also observed in WI-38 normal human fibroblasts (data not shown). Quantitative real-time PCR (qRT-PCR) experiments performed on cells treated as above demonstrate a corresponding decrease in TBX2 mRNA levels that precedes the decrease in protein levels (Fig. 1, C and D). To investigate if this regulation is transcriptional, we tested whether the ~1604 bp TBX2 promoter is responsive to TGF-β1 treatment using a luciferase assay and the results showed a time-dependent decrease in reporter activity in response to TGF-β1 (Fig. 2A). To distinguish the need for de novo protein synthesis, cells were pre-treated with cycloheximide (CHX), a protein synthesis inhibitor, which abolished the repression of TBX2 mRNA and protein levels by TGF-β1 (Fig. 2, B and C). Together these results suggest that TGF-β1-mediated repression of TBX2 is transcriptional and that it requires the production of nascent protein.

The Down-regulation of TBX2 by TGF-β1 Is Mediated by TBX3 in Breast Epithelial Cells—To narrow down the region of the TGF-β1 is the ChIP of interest, and ΔCt2 is the IgG.

DNA Affinity Immunoblot Assay—Biotinylated DNA oligos were: WT: 5’-GCTGAGGCTTCCGACACTCCAG-3’; MT: 5’-GCTGAGGCTTCCaAaCTTCCGAG-3’; Consensus: 5’-CTTAGGAAATTCACCTAGG-TGTTAATTCCCT-3’. For each DNA-binding reaction, nuclear extract was incubated with biotinylated DNA probe in binding buffer (20 mM Tris-HCl pH 7.6, 50 mM NaCl, 1 mM MgCl₂, 0.2 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, and 10 ng/µl poly(dl-dC)). The beads were washed with binding buffer and boiled in protein loading buffer (125 mM Tris-HCl, pH 6.5, 0.4% SDS, 10% β-mercaptoethanol, and 20% glycerol). Proteins bound to the biotinylated probes were analyzed by SDS-PAGE, followed by immunoblotting using goat polyclonal antibodies to TBX3 (sc-17871) antibody (Santa Cruz Biotechnology).

Growth Curves—Short-term growth of cells was compared over a 3-day period, as described previously (29). 0.5 × 10⁴ cells were seeded in triplicate in 24-well plates on day 0 and treated with vehicle or 5 ng/ml TGF-β1 for 2–3 days.

Statistical Analysis—Statistical analysis was performed by using the 2-sample t test (Excel).

RESULTS

TGF-β1 Transcriptionally Represses TBX2 Expression in MCF-12A Breast Epithelial and B16 Melanoma Cells—TBX2 and TBX3 are highly homologous T-box factors with distinct oncogenic roles. We recently reported that TBX3 is transcriptionally activated by the TGF-β1 signaling pathway (7), and we therefore examined whether the expression of TBX2 is also responsive to this pathway. To this end we exposed MCF-12A breast epithelial and B16 mouse melanoma cells to TGF-β1 or vehicle over a time course spanning 1–36 h. Western blot analyses show that TBX2 levels decrease substantially in response to TGF-β1 treatment in both cell lines from 8 h in MCF-12A cells and 4 h in B16 cells (Fig. 1, A and B). This robust repression was also observed in WI-38 normal human fibroblasts (data not shown). Quantitative real-time PCR (qRT-PCR) experiments performed on cells treated as above demonstrate a corresponding decrease in TBX2 mRNA levels that precedes the decrease in protein levels (Fig. 1, C and D). To investigate if this regulation is transcriptional, we tested whether the ~1604 bp TBX2 promoter is responsive to TGF-β1 treatment using a luciferase assay and the results showed a time-dependent decrease in reporter activity in response to TGF-β1 (Fig. 2A). To distinguish the need for de novo protein synthesis, cells were pre-treated with cycloheximide (CHX), a protein synthesis inhibitor, which abolished the repression of TBX2 mRNA and protein levels by TGF-β1 (Fig. 2, B and C). Together these results suggest that TGF-β1-mediated repression of TBX2 is transcriptional and that it requires the production of nascent protein.

The Down-regulation of TBX2 by TGF-β1 Is Mediated by TBX3 in Breast Epithelial Cells—To narrow down the region of the TGF-β1 signaling pathway (7), and we therefore examined whether the expression of TBX2 is also responsive to this pathway. To this end we exposed MCF-12A breast epithelial and B16 mouse melanoma cells to TGF-β1 or vehicle over a time course spanning 1–36 h. Western blot analyses show that TBX2 levels decrease substantially in response to TGF-β1 treatment in both cell lines from 8 h in MCF-12A cells and 4 h in B16 cells (Fig. 1, A and B). This robust repression was also observed in WI-38 normal human fibroblasts (data not shown). Quantitative real-time PCR (qRT-PCR) experiments performed on cells treated as above demonstrate a corresponding decrease in TBX2 mRNA levels that precedes the decrease in protein levels (Fig. 1, C and D). To investigate if this regulation is transcriptional, we tested whether the ~1604 bp TBX2 promoter is responsive to TGF-β1 treatment using a luciferase assay and the results showed a time-dependent decrease in reporter activity in response to TGF-β1 (Fig. 2A). To distinguish the need for de novo protein synthesis, cells were pre-treated with cycloheximide (CHX), a protein synthesis inhibitor, which abolished the repression of TBX2 mRNA and protein levels by TGF-β1 (Fig. 2, B and C). Together these results suggest that TGF-β1-mediated repression of TBX2 is transcriptional and that it requires the production of nascent protein.
The 186 bp T-element was mutated (Fig. 4C). To identify the functional domains of the TBX3 protein that are responsible for this regulation, the wild type 218 bp TBX2 promoter luciferase construct was co-transfected with vectors expressing either a WT TBX3 protein or a TBX3 protein in which the DNA binding site was mutated or only a N-terminal TBX3 protein.
lacking the dominant repression domain. The data obtained indicate that the TBX3 DNA binding domain and C terminus containing a repression domain are important for its ability to repress the TBX2 promoter (Fig. 4D). All TBX3 constructs were expressed at similar levels (Fig. 4, C and D, right).

**Ectopic Expression of TBX2 Is Able to Rescue TGF-β1-inhibited Cell Proliferation**—We next investigated whether overexpression of TBX2 in MCF-12A cells could rescue the inhibition of cell proliferation by TGF-β1. Briefly, a pcDNA-TBX2 expression vector or the empty control vector was transfected into MCF-12A cells and G418-resistant clones were pooled for subsequent analysis. Overexpression of TBX2 was confirmed by Western blotting (Fig. 5A), and growth curves show that while cell proliferation was significantly reduced by TGF-β1 in the control cells, overexpressing TBX2 was sufficient to rescue this phenotype (Fig. 5B). This was confirmed by flow cytometry, which showed that TGF-β1 treatment induced a significant G1 cell cycle arrest in the control cells but had no effect on the cell cycle profile of the TBX2-overexpressing cells (Fig. 5C). These results correlated with changes in the protein and mRNA levels of the cyclin-dependent kinase inhibitor p21 (Fig. 5A and E). Importantly, the increase in p21 mRNA and protein levels in TGF-β1-treated cells are abrogated when TBX2 is overexpressed suggesting that TBX2 may be the upstream regulator of p21 in the TGF-β1 pathway. Indeed, when TBX2-overexpressing cells were transfected with a p21 expression vector they had a decreased proliferative ability, which could not be further reduced by TGF-β1 treatment (Fig. 5D). Interestingly, TBX2 overexpression failed to have any significant effect on the TGF-β1-induced activation of p15 mRNA levels (Fig. 5F), another cell cycle inhibitor which has been shown to mediate a G1 arrest in response to TGF-β1 (30). Together, these data imply that the anti-proliferative effect of TGF-β1 on MCF-12A cells occurs primarily through up-regulation of p21, since the continued repression of p21 in TBX2-overexpressing cells allowed the cells to proliferate even in the presence of TGF-β1 and despite the up-regulation of p15. To confirm this, p21 expression was knocked down using a siRNA approach and growth curve analyses performed in the presence and absence of TGF-β1. As expected, cells in which p21 were effectively knocked down proliferated faster than the control cells in the absence of TGF-β1 (Fig. 6A). Importantly, the p21-depleted cells also failed to show a significant decrease in cell proliferation in response to TGF-β1. In addition, when the MCF-12A cells were depleted of TBX2 the untreated cells had a significant decrease in cell proliferation which could not be significantly further decreased in response to TGF-β1 treatment depleted TBX2 in (Fig. 6B). These data together with Western blot results from TBX3 overexpressing cells (Fig. 6C) suggest that the down-regulation of TBX2 by TBX3 mediates the anti-proliferative effect of TGF-β1 through allowing up-regulation of p21.
**DISCUSSION**

The TGF-β1 signaling pathway controls a wide spectrum of cellular functions and deregulated TGF-β signaling has been linked to severe human diseases, including cardiovascular disease, skeletal, and muscular disorders and cancer (31). TGF-β1 is a potent inhibitor of cell proliferation, which is thought to

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**FIGURE 3.** The down-regulation of TBX2 by TGF-β1 is mediated by TBX3. A, luciferase assay of MCF-12A cells transfected with 400 ng of human TBX2 5′-deletion promoter constructs and treated with 5 ng/ml TGF-β1 or vehicle. B, luciferase assay of MCF-12A cells co-transfected with increasing amounts of Smad3/4 expression vectors and 400 ng of −218 bp TBX2 promoter reporter construct. C, alignment of the T-element on −186 bp of TBX2 promoter of human, chimpanzee, mouse and zebrafish. D and E, upper panels: siTBX3#1 lower panels: siTBX3#2. Serum-starved MCF-12A cells were transfected with either 50 nm siControl or siTBX3 for 48 h, followed by 2 days of TGF-β1 treatment and subjected to Western blotting (D) or qRT-PCR analysis (E). Bars, S.D. *, p < 0.05; **, p < 0.001.
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FIGURE 4. TGF-β1 repression of the TBX2 promoter is mediated by a T-element at −186 bp. A, MCF-12A cells were treated with 5 ng/ml TGF-β1 for 24 h and chromatin immunoprecipitation assays performed with antibodies against TBX3 or IgG (negative control). Immunoprecipitated DNA was assayed by qRT-PCR with primers against the TBX2 proximal promoter. B, biotinylated DNA probes of the TBX2 promoter containing the consensus T-element or homologous WT or MT T-element were immobilized on streptavidin beads, and incubated with nuclear extracts from MCF-12A cells treated with 5 ng/ml TGF-β1 for 24 h. The DNA-bound protein complexes were isolated and analyzed by Western blotting using antibodies to TBX3. C, luciferase assay of MCF-12A cells co-transfected with 100 ng of TBX3 expression vector or empty control vector and 400 ng of either TBX2 WT or T-element mutated (MT) promoter luciferase reporter. D, luciferase assay of MCF-12A cells co-transfected with 400 ng of TBX2 promoter luciferase reporter and 100 ng of an empty control vector or WT TBX3 or DNA binding domain mutant (DBM) or TBX3 N-terminal truncated expression vector. Western blots (C, D, right) show equal expression of TBX3 protein. Bars, S.D. * p < 0.05; ** p < 0.001.
result from its ability to induce a G1 cell cycle arrest through the up-regulation of the cyclin-dependent kinase inhibitors (cdkis), p15, and p21 (30). However, it can also stimulate invasiveness by promoting an epithelial to mesenchyme transition. Understanding how TGF-β1 coordinates its effects on the cell cycle and invasion in different cell types is a key issue, both for development and disease.

Here we have dissected the interrelated roles of the TBX2 and TBX3 transcription factors, which are widely implicated in cancer progression, in the response to TGF-β1. Previously we demonstrated that the TGF-β1-mediated growth arrest observed in melanoma could be bypassed by ectopic TBX2 expression (36), and that TBX3, which can promote invasiveness by repressing E-cadherin expression (24), is a downstream
target of the TGF-β1 signaling pathway and plays an important role in exerting its anti-proliferative and pro-invasive effects (7). However, how TBX3 might exert its anti-proliferative function, and what suppresses TBX2 expression was not known. In this study we show that when the TGF-β1 pathway is stimulated in breast epithelial and melanoma cells, TBX2 expression is repressed through the direct binding of TBX3 to a half T-box element in the TBX2 promoter. Our results are also consistent with TBX2 being a powerful pro-proliferative factor (22, 23), and indicate that the down-regulation of TBX2 blocks proliferation through the de-repression of the TBX2 target gene, p21, a cdki that initiates the TGF-β1-induced G1 arrest (32). Together these data provide an explanation for how the TGF-β1 pathway exerts its anti-proliferative effect and reveal an upstream mechanism involved in regulating TBX2 and TBX3 in contexts where they are differentially expressed. Moreover, since overexpression of TBX2 can override the anti-proliferative effects of TGF-β1 without affecting p15 levels, up-regulation of p15 is unlikely to be required for the effect of TGF-β1 on the proliferative ability of these cells, though it may be involved in other TGF-β1-mediated functions.

TBX2 and TBX3 are highly homologous T-box transcription factors which have distinct roles during embryonic development and in breast cancer and melanoma cell lines where they are both overexpressed (7, 19–22). Importantly, whereas TBX2 is essential for promoting cell proliferation, TBX3 inhibits cell proliferation but is required for cell migration. These results together with data from the current study suggest an interesting
The interplay between TBX2, TBX3, and TGF-β1 signaling in normal epithelial cells (Fig. 7). Briefly, in this context TGF-β1 signaling activates TBX3 expression in a Smad/JunB dependent manner and TBX3 then represses TBX2 transcription, which allows for the de-repression of p21 and a G1 cell cycle arrest. Interestingly, malignant cells acquire immortalizing mutations that enable them to evade the anti-proliferative effects of TGF-β1 signaling. For example, one of the mechanisms by which TGF-β1 exerts its anti-proliferative effects is through inhibiting expression of MYC that leads to the de-repression of p21. Many malignant cells however have mutations that result in the constitutive expression of MYC and hence these cells are resistant to the anti-proliferative effects of TGF-β1. It is therefore possible that cancers in which TBX2 is overexpressed will similarly disregard the TGF-β1 anti-proliferative signals. Intriguingly, the TBX3 promoter has a full consensus MYC binding site, raising the possibility that MYC will impact the TBX3-TBX2 axis identified here. The expression of MYC is usually down-regulated by TGF-β1 treatment, and ectopic expression of MYC in mouse keratinocytes desensitized the cells to TGF-β1 mediated growth inhibition (33–35). Furthermore, sustained MYC expression in ovarian cancers coincides with resistance to the anti-proliferative effects of TGF-β1 (36). Although, the precise relationship between MYC and TBX2 and TBX3 expression remains to be determined, there is no doubt that TBX2 and TBX3 are likely to represent key effectors of TGF-β1 signaling in multiple cell types.

Acknowledgments—The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

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