Transforming Growth Factor-β2 Is a Transcriptional Target for Akt/Protein Kinase B via Forkhead Transcription Factor*

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Tumors evade cell death by constitutively activating cell survival pathways and suppressing intrinsic death machinery. Activation of cell survival pathways leads to transcriptional repression of genes associated with cell death and activation of ones promoting anti-apoptosis. Akt/protein kinase B phosphorylates forkhead transcription factors and prevents their nuclear localization, leading to repression of genes involved in apoptosis, such as Fas ligand (FasL). Using bioinformatic approaches, we have identified three consensus sequences for forkhead transcription factor binding in transforming growth factor β2 (TGF-β2) promoter. TGF-β inhibits cell proliferation and induces apoptosis in many cell types, and acquisition of TGF-β resistance is linked to tumorigenesis. In this study, we show that activated Akt down-regulates TGF-β2 promoter, and sequences within the promoter that are related to consensus forkhead binding sites are necessary for repression. Forkhead factor FKHL1 binds in vitro to the three consensus sequences and can activate TGF-β2 promoter in normal and Akt-transformed cell lines. In human breast and pancreatic tumors, activated Akt expression correlated with down-regulation of TGF-β2 mRNA levels. A number of tumor cells expressing activated Akt were responsive to TGF-β addition, indicating the presence of intact TGF-β-signaling pathway. These results suggest that repression of TGF-β2 promoter activity in cells expressing activated Akt may play a role in promoting tumorigenesis and escape from the growth-inhibitory and/or apoptotic effects of TGF-β.

Akt/protein kinase B is a cellular homologue of the viral oncogene v-Akt of the transforming retrovirus Akt-8 (1). Akt is a serine/threonine kinase that plays a critical role in cell survival signaling, and its activation has been linked to tumorigenesis (for reviews, see Refs. 2 and 3). Up-regulation of Akt as well as its upstream regulator phosphatidylinositol 3-kinase has been found in many tumors, and the negative regulator of this pathway, PTE-N/MMAC, is a tumor suppressor (4). The mechanism by which Akt activity contributes to cellular transformation is generally thought to be by inhibiting apoptosis. Several substrates of Akt have been recently identified, and these include two components of the intrinsic cell death machinery, BAD and caspase-9, transcription factors of the forkhead family, and a kinase IKK-α, which regulates the NFκB transcription factor (for reviews, see Refs. 2 and 3). In the case of BAD and caspase-9, phosphorylation by Akt suppresses their proapoptotic functions, accounting at least in part for the potent survival functions of Akt. In addition, Akt phosphorylates proteins that control the cell cycle progression, such as glycogen synthase kinase 3 and p21WAF1 (5). It is clear that Akt is a focal point for deregulation in cancer and can impact on cell proliferation and survival of cancer cells through a growing list of key targets.

The best-characterized nuclear substrates of Akt are transcription factors of the Forkhead family, FKHR, FKHL1, and AFX (6). According to the new nomenclature these three proteins have been assigned to the FOXO subfamily of Forkhead transcription factors (7). Phosphorylation of forkhead transcription factor by Akt results in its retention in the cytoplasm and repression of its target genes (6, 8). Under conditions of growth factor deprivation, the phosphatidylinositol 3-kinase/Akt pathway is inactivated, and forkhead factor is unphosphorylated, leading to activation of genes associated with programmed cell death. Genes that might be targets for transcriptional regulation by the forkhead transcription factors have been identified in several systems. These include the insulin-like growth factor-binding protein 1 (IGFBP-1) (9), Fas ligand (FasL) (10), Bim (11), and p27 (12). Evidence that forkhead family members can regulate apoptosis stems from the observation that the non-phosphorylatable mutants of FKHR or FKHL1, which are potent transcriptional activators in the nucleus, trigger apoptosis in multiple cell types (8). In the case of FKHL1-induced apoptosis, a target gene suggested to be able to mediate this effect is the Fas ligand gene (FASL), but other genes involved in forkhead-induced apoptosis have yet to be identified. Hence, we set out to identify other candidate target genes that may be under the control of the Akt/forkhead pathway.

In this study, using bioinformatic approaches we have identified candidate target genes that contain forkhead binding sites in their promoter region. One candidate gene identified in this search is transforming growth factor β2 (TGF-β2). TGF-β is of particular interest because it is known to inhibit cell proliferation and induce apoptosis in many cell types (13). In mammals there are three isoforms of TGF-β, β1, β2, and β3, encoded by three different genes, all of which function through the same signaling pathways (14). TGF-β signaling is initiated by binding to two receptors, termed type I and type II, and mediated by a family of effector proteins known as Smads (15).

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§ The abbreviations used are: IGFBP-1, insulin-like growth factor-binding protein 1; TGF, transforming growth factor; TK, thymidine kinase; CAT, chloramphenicol acetyltransferase.
were used: anti-Akt, anti-phospho-Akt (Ser-473), anti-phospho-Akt CAT assays were performed as described previously (17).

enzymes and ligated into the Hin
er). The PCR product was digested with

Growth inhibition and/or apoptotic effects of TGF-

increases above a threshold level. The comparative CT method was used

derived as the cycle number at which the reporter fluorescence emission

which correlates inversely with the target mRNA levels, was meas-

(performer primer) and 5\'-CTCGCACTCAGATGCA-3\' (reverse primer)

expression vector encoding human FKHRL1 cDNA fused to the GST

A bacterial strain BL21 (DE3). The transfor-

determined by selection of binding sites from random sequence oligonucleotides (19). Recently, the binding sites for the fork-

transcription factors have also been experimentally de-

lected with 2'-3'IPAT and gel-purified. The binding reactions were

alyzed on a non-denaturing polyacrylamide gel as described previ-

17). For competition experiments, a 100-fold molar excess of

oligonucleotides corresponding to the forward binding sites from

TGF-β or IFGBP1 promoters or nonspecific oligonucleotides were used

in the reaction mixtures before the addition of the labeled probe.

RESULTS

Identification of Genes Containing Forkhead Factor Binding Sites in Their Promoters—We undertook a search for genes

containing forkhead factor binding sites in their promoter regions using computational methods (18). The forkhead factor

binding sequences from IFGBP1 and FasL promoters have been characterized (8, 9). The DNA binding specificities of four

of the FREAC proteins belonging to the forkhead family were determined by selection of binding sites from random sequence

oligonucleotides (19). Recently, the binding sites for the fork-

transcription factors have also been experimentally de-

(20, 21). We aligned the six known forkhead transcription factor binding sites and derived a consensus sequence for

for forkhead transcription factor binding (Fig. 1). The consensus sequence, WAARYAAAYW (W = A or T, R = A or G, Y = C or

was used to search the GenBank\textsuperscript{TM} data base. The informatics approach focused on genes with annotated gene struc-

predominantly in the 5'-flanking region. The GCG FIND-

PATTERNS program was used to match the consensus sequence of the forkhead factor binding site as described above.

We analyzed the human subset (93,412 sequences) of the primar-

ite division of GenBank\textsuperscript{TM} and identified 16,511 forkhead binding sites (or 6,406 sequences) that match the consensus sequence.

The GenBank\textsuperscript{TM} feature fields that define the tran-

script start site of a gene were used as coordinates to locate the matched sequences in the 5'-flanking region of a gene. The

Parsing of the GenBank\textsuperscript{TM} report was carried out using script

language PERL (22). This resulted in a forkhead target refer-

base that is composed of 437 genes with at least one forkhead binding site in the promoter region.\textsuperscript{2} A partial list of

genes that contain the forkhead factor binding sites is shown in

Table I. We selected TGF-β2 from this list to determine whether its transcription is under the control of Akt/protein

kinase B-signaling pathway. TGF-β is of particular interest since it is the prototype of a family of structurally related

\textsuperscript{2}A. A. Samatar, L. Wang, A. Mirza, S. Koseoglu, S. Liu, and C. C. Kumar, unpublished results.
cytokines capable of inducing diverse cellular responses, including apoptosis, differentiation, and cell cycle arrest.

Activated Akt Expression Leads to Transcriptional Repression of TGF-β2—Our analysis showed that TGF-β2 promoter contains three consensus forkhead binding sites at −212, −480, and −579 positions from the transcription start site (Table I) (23). The core forkhead consensus binding site is not found in other isoforms of TGF-β such as TGF-β1 and -β3. The three putative forkhead binding sites in TGF-β2 promoter matched perfectly with the consensus sequence (Fig. 2a). The sequences also matched the experimentally derived consensus site for FOXO sub-family (21). To determine whether TGF-β2 promoter activity is regulated by activated Akt in mammalian cells, a plasmid containing TGF-β2 promoter linked to CAT reporter gene was transiently transfected into NIH 3T3 cells, and its response to activated Akt expression was measured. Two forms of Akt expression vectors were tested. One encodes myristoylated Akt (pEE-Myr-Akt), in which a membrane-targeting myristoylation sequence and an EE epitope tag were fused to Akt-coding sequences at the N terminus. The other encodes a mutant form (pHA-Akt-AA, with a hemagglutinin epitope tag at the N terminus) in which the two phosphorylation sites (Thr-308 to and Ser-473) have been mutated to generate an inactive version of the protein (25). Coexpression of constitutively active Myr-Akt in NIH 3T3 cells leads to strong repression of TGF-β2 promoter, whereas the kinase-dead mutant of Akt had very little effect (Fig. 2b). Western blot analysis of total cell lysates using epitope-specific antibodies indicates (Fig. 2d) that the two Akt proteins are expressed at equal levels in this transient transfection experiment. Next, we transfected TGF-β2-CAT plasmid into parental Rat-1 and myristoylated Akt-transformed Rat-1 (Myr-Akt-Rat-1) cells. As a control, plasmid pLW2 containing herpes immediate early promoter linked to the CAT reporter gene was transfected into these cells. Fig. 2c shows that TGF-β2-CAT reporter gene expression was selectively repressed in Myr-Akt-Rat-1 cells compared with parental Rat-1 cells. In contrast, transfection of a deletion mutant of TGF-β2 (−28, TGF-β2-CAT) lacking all three forkhead binding sites into Rat-1 and Myr-Akt-transformed Rat-1 cells showed basal CAT activity in both cell lines. Roughly a 3.5-fold higher amount of deletion mutant was transfected in this experiment. This result shows that deletion of region containing forkhead binding sites in TGF-β2 promoter leads to loss of Akt responsiveness. Together these results show that TGF-β2 promoter is repressed in cells expressing activated AKT, and the region containing the three forkhead binding sites is required for its repression.

Consensus Forkhead Factor Binding Sites Mediate Repression of TGF-β2 Promoter by Activated Akt—To determine whether the three forkhead binding sites in TGF-β2 promoter can confer Akt responsiveness to heterologous promoters, we isolated the region containing the three forkhead binding sites (−579 to −212) in TGF-β2 promoter by PCR methods. This region, which does not contain the binding sites for other known transcription factors, was subcloned into plasmid pBlCAT2 containing minimal TK promoter linked to CAT reporter gene (26). This plasmid, designated 3XFH-TK-CAT, was transiently transfected into NIH 3T3 cells along with myr-Akt and Akt kinase dead mutant expression vectors. Co-expression of myr-Akt, but not the kinase dead mutant of Akt, resulted in strong repression of the 3XFH-TK-CAT expression (Fig. 3a). Similarly, CAT gene expression by this plasmid was also strongly repressed in myr-Akt-Rat-1 cells compared with parental Rat-1 cells (Fig. 3b).

Forkhead Factor Binds to the Three Consensus Sequences in TGF-β2 Promoter in Vitro and Activates in Vivo—to analyze the ability of forkhead transcription factor to bind in vitro to the putative forkhead binding sites in TGF-β2 promoter, we synthesized oligonucleotides corresponding to the three forkhead factor binding sites in TGF-β2 promoter. Electrophoretic mobility shift assays were performed using GST-tagged FKHL1 protein purified from an E. coli expression system. The results indicate specific binding of forkhead protein to the three forkhead consensus binding sites which can be competed out by unlabeled oligonucleotides and also by oligonucleotides corresponding to forkhead binding site in IGFBP-1 promoter.
Nonspecific oligonucleotides did not compete for the mobility shift in these experiments, suggesting that forkhead factor binds specifically to these sites. Similar results were seen with all three forkhead binding sites, although the forkhead binding site 3 bound less protein compared with sequences corresponding to binding sites 1 and 2 (Fig. 4).

Next we wanted to see if forkhead transcription factor could activate TGF-β2 promoter in vivo. Previous studies show that forkhead, when not phosphorylated by Akt, is an activator of transcription, and phosphorylation by Akt leads to its retention in cytoplasm, resulting in repression of forkhead target genes (8). A mutant of FKHRL1 in which all three putative Akt phosphorylation sites have been converted to alanines localizes to the nucleus and induces transcription of genes even in the presence of activated Akt. To determine whether forkhead transcription factor expression can regulate TGF-β2 promoter activity in vivo, we cotransfected TGF-β2-CAT plasmid along with expression plasmids encoding wild type or mutant FKHRL1 into Myr-Akt-transformed Rat-1 cells, ZR-75, and LNCaP tumor cells. ZR-75 is a human breast carcinoma cell line, whereas LNCaP cell line is derived from prostate carcinoma. Both these cells are null for PTEN/MMAC gene, indicating that Akt activation in these cells is primarily due to inactivation of PTEN/MMAC tumor suppressor gene product (27). TGF-β2-CAT activity is repressed in Myr-Akt-Rat-1, ZR-75, and LNCaP cells (Fig. 5a). Cotransfection of wild type or mu-
tant FKHRL1 expression plasmids stimulated TGF-β2-CAT activity in all three cell types (Fig. 5a). The mutant FKHRL1 protein was slightly more active than the wild type protein in stimulating TGF-β2-CAT activity in these cell lines (Fig. 5a).

Next, we cotransfected plasmid p3XFHTK-CAT into ZR-75 and LNCaP cells along with FKHRL1 expression plasmids. Wild type and mutant FKHRL1 protein stimulated CAT activity in both cell types (Fig. 5b). These results show that the TGF-β2 promoter is repressed in tumor cells in which Akt is activated primarily due to inactivation of PTEN/MMAC tumor suppressor gene product, and forkhead factor can activate the promoter activity in vivo.

To further confirm that activated Akt expression mediates the repression of TGF-β2-CAT activity in tumor cells, we treated LNCaP cells with phosphatidylinositol 3-kinase inhibitor LY294002 for 24 h. As shown in Fig. 5c, TGF-β2-CAT and 3XFHTKCAT activities were significantly activated after treatment with LY294002, indicating that inhibition of Akt activation by treatment with a phosphatidylinositol 3-kinase inhibitor leads to activation of TGF-β2 promoter activity. Taken together, these results demonstrate that TGF-β2 is a transcriptional target for Akt signaling via forkhead transcription factor.

**FIG. 3.** Forkhead binding sites in TGF-β2 promoter can confer Akt responsiveness to heterologous promoters. The region containing the three forkhead binding sites (−579 to −212) in TGF-β2 promoter was PCR-amplified and subcloned into the HindIII-BamHI sites of the plasmid pBLCAT2 containing minimal TK promoter linked to CAT reporter gene. A, the resulting plasmid p8XHFTK-CAT was transfected into NIH 3T3 cells along with Myr-Akt and Akt K6 expression plasmids, and CAT assays were performed on cell lysates as described in the legend to Fig. 1. A, plasmid p3XFHTK-CAT was transfected into NIH 3T3 cells along with Myr-Akt and Akt K6 expression plasmids, and CAT activity in these cells was determined 48 h later. Plasmid pLW2 was transfected as a positive control in these cells to show equal expression of this promotor in both cells.

**FIG. 4.** Forkhead transcription factor (FKHRL1) binds to the three consensus sequences from TGF-β2 promoter in vitro. Oligonucleotides corresponding to the three consensus Forkhead binding sites (FBS) sites from the TGF-β2 promoter were end-labeled with [γ-32P]ATP and subjected to electrophoretic mobility assay using 100 ng of GST-FKHRL1 fusion protein purified from an E. coli expression system. For competition experiments, a 100-fold molar excess of unlabeled FBS oligonucleotides or the consensus site from IGFBP1 promoter or nonspecific oligonucleotides were added to the reaction mixes 10 min before adding the labeled oligos. Purified GST protein was also included as a control. The DNA-protein complexes were resolved on a 4% non-denaturing polyacrylamide gel in 0.5× Tris-buffered EDTA running buffer at 4 °C. The gels were dried and exposed to x-ray film overnight.

**FIG. 5.** Forkhead binding sites in TGF-β2 promoter can confer Akt responsiveness to heterologous promoters. The region containing the three forkhead binding sites (−579 to −212) in TGF-β2 promoter was PCR-amplified and subcloned into the HindIII-BamHI sites of the plasmid pBLCAT2 containing minimal TK promoter linked to CAT reporter gene. A, the resulting plasmid p8XHFTK-CAT was transfected into NIH 3T3 cells along with Myr-Akt and Akt K6 expression plasmids, and CAT assays were performed on cell lysates as described in the legend to Fig. 1. A, plasmid p3XFHTK-CAT was transfected into NIH 3T3 cells along with Myr-Akt and Akt K6 expression plasmids, and CAT activity in these cells was determined 48 h later. Plasmid pLW2 was transfected as a positive control in these cells to show equal expression of this promotor in both cells.

**FIG. 6.** Forkhead transcription factor (FKHRL1) binds to the three consensus sequences from TGF-β2 promoter in vitro. Oligonucleotides corresponding to the three consensus Forkhead binding sites (FBS) sites from the TGF-β2 promoter were end-labeled with [γ-32P]ATP and subjected to electrophoretic mobility assay using 100 ng of GST-FKHRL1 fusion protein purified from an E. coli expression system. For competition experiments, a 100-fold molar excess of unlabeled FBS oligonucleotides or the consensus site from IGFBP1 promoter or nonspecific oligonucleotides were added to the reaction mixes 10 min before adding the labeled oligos. Purified GST protein was also included as a control. The DNA-protein complexes were resolved on a 4% non-denaturing polyacrylamide gel in 0.5× Tris-buffered EDTA running buffer at 4 °C. The gels were dried and exposed to x-ray film overnight.
and MDAMB-231 cells (Fig. 6a). The fold repression observed in these cells ranged from 100- to 150-fold. There was no change in TGF-β1 mRNA levels in these cell lines (Fig. 6a). We also determined the relative levels of TGF-β1 and -β2 mRNAs in these experiments and observed that in Cfpac-II and MDAMB-231 cells TGF-β2 mRNA is expressed 5-fold higher than TGF-β1 (Fig. 6a). Recently we carried out a genome-wide gene expression analysis of breast tumor cell lines using microarrays that contain 60,000 cDNAs including TGF-β1 and TGF-β2 (18). Fig. 5b shows the results obtained using microarray experiments, indicating that TGF-β2 mRNA levels are down-regulated in T47D, MCF-7, ZR-75–1, BT-474, and BT-20 cells but not in MDAMB-231 and HMEC21 (normal mammary epithelial) cells. TGF-β1 mRNA levels did not change appreciably in different tumor cell lines (results not shown). These results were also confirmed using Taqman analysis (Fig. 6b).

Analysis of total cell extracts by Western blot using phospho-(Ser-473)Akt-specific antibodies showed that Miapaca-2, Aspc-1, Panc-1, T47D, BT-20, ZR-75–1, BT-474, and MCF-7 cell extracts showed significant levels of phosphorylated Akt protein but not Capan-1, Cfpac-II, and MDAMB-231 cells (Fig. 6c). These results demonstrate that expression of activated Akt in tumor cells is accompanied by selective down-regulation of TGF-β2 mRNA level.

**TGF-β Sensitivity in Tumor Cells Expressing Active Akt—** What is the biological significance of repression of TGF-β2 promoter in tumor cells expressing Akt? Loss of TGF-β sensitivity is frequently observed in human tumors derived from
cells that are normally sensitive, and the extent of TGF-β resistance often correlates with malignancy (14, 28). Our results suggest that repression of TGF-β at the transcriptional level by forkhead transcription factor may be an additional mechanism to escape from the antiproliferative and/or apoptotic effects of TGF-β. This would predict that tumor cells expressing activated Akt may have a normal TGF-β-signaling pathway and be responsive to exogenous addition of TGF-β.

To investigate this, we tested several pancreatic and breast tumor cells for their sensitivity to TGF-β. For this purpose, a cell viability assay was used to see if the growth of the tumor cells was inhibited by exogenous addition of TGF-β. The results shown in Fig. 7, a and b, indicate that Panc-1, MCF-7, BT-474, and MDA MB-435 S cells, which express activated Akt, retain a normal TGF-β-signaling pathway and are sensitive to TGF-β. In contrast, Miapaca-2, AspC-1, Capan-1, and MDA MB-231 were resistant to TGF-β, since these cells contain defective TGF-β-signaling components (29–32). Miapaca-2 cells are characterized by a lack of expression of TGF-β-type RII receptor and are reported to be resistant to the growth inhibitory effects of TGF-β. Similarly, Capan-1 cells have been shown to express a truncated form of Smad4 mRNA (33). Panc-1 cells express normal levels of Smad4 mRNA and are responsive to TGF-β (29). These results indicate that some of the tumor cell lines expressing activated Akt retain an intact TGF-β signaling, and the escape from the effects of TGF-β may be primarily due to repression at the transcriptional level.
DISCUSSION

In this study we have shown that TGF-β2 promoter contains three consensus binding sites for forkhead transcription factor and is down-regulated by activated Akt expression. We have shown that repression is mediated by the forkhead binding sites and that forkhead transcription factor FKHRL1 can bind to these sites in vitro and regulate TGF-β2 promoter in vivo. TGF-β2 promoter is repressed in tumors expressing activated Akt, and inhibition of Akt activation leads to its derepression. TGF-β2 mRNA levels were found to be down-regulated in several human breast and pancreatic tumor cell lines expressing activated Akt. In addition, we have shown that human tumor lines expressing activated Akt retain a normal TGF-β-signaling pathway and remain sensitive to exogenous addition of TGF-β.

TGF-β is a potent inducer of growth inhibition in several cell types, and TGF-β-signaling pathway has been implicated in tumor suppression (16, 34). TGF-β has also been recently reported to play a role in apoptosis in a number of cell types including hepatocytes and several hepatoma cells (35–37). Increased levels of Smad3 or Smad4 can induce apoptosis, and dominant negative interference with Smad3 function protects against apoptosis (38). The loss of responsiveness to the antiproliferative effects of TGF-β results in unrestrained cell growth and correlates with the malignant progression of several tumors including breast carcinomas, hepatomas, gastric...
colonic carcinomas, and skin tumors as well as B and T lymphomas (16, 34). The role of TGF-β signaling as a tumor suppressor pathway is best illustrated by the presence of inactivating mutations in genes encoding components of the TGF-β-signaling pathway (16, 34). A substantial portion of colorectal and pancreatic cancers harbor inactivating mutations in the genes encoding the TGF-β type II receptor or its mediators, Smad2 and Smad4 (39). However, such alterations cannot account for the majority of cases in which TGF-β resistance is lost. Therefore, TGF-β transcriptional repression must also be achieved by other mechanisms. In particular, direct regulation of TGF-β at the transcriptional level has not been documented in human tumors. Our studies show that TGF-β2 is a transcriptional target for activated Akt via forkhead transcription factor.

Previous studies show that TGF-β is a tumor suppressor that shows true haploid insufficiency in its ability to protect against tumorigenesis, and mice with one inactivated allele of the gene encoding TGF-β show increased propensity for developing carcinoma (40). Thus, repression of TGF-β synthesis at the transcriptional level may indeed favor malignant transformation.

A large body of evidence has accumulated to suggest that pathways leading to transcriptional repression of genes associated with apoptosis. A major role of TGF-β in tumors is to induce apoptosis. A large body of evidence has accumulated to suggest that pathways leading to transcriptional repression of genes associated with apoptosis. A major role of TGF-β in tumors is to induce apoptosis.

Thus, tumors have developed mechanisms to evade cell death by constitutively activating cell survival pathways mediated by phosphatidylinositol 3-kinase/Akt. Activation of cell survival pathways leads to transcriptional repression of genes associated with cell death and activation of ones promoting anti-apoptosis. A large body of evidence has accumulated to suggest that TGF-β plays two distinct roles in promoting tumorigenesis (16). First, it is necessary for cells to lose responsiveness to TGF-β and thereby circumvent the antiproliferative effects of TGF-β. These resistant lines often then activate TGF-β expression, which is thought to act on neighboring cells to stimulate angiogenesis, inhibit immune responses, and thereby promote tumorigenic processes. Thus, transcriptional regulation of different TGF-β isoforms at different stages of tumor development may provide a mechanism for differential regulation of TGF-β expression. In this study we have shown that TGF-β2 is transcriptionally repressed in tumor cells expressing activated Akt. Human tumor cells expressing activated Akt were found to be sensitive to TGF-β addition, indicating the presence of normal TGF-β-signaling pathway. Repression of TGF-β2 promoter in tumors expressing activated Akt may contribute toward tumorigenesis at earlier stages of tumor development and escape from growth inhibitory and/or apoptotic effects of TGF-β.

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