Cancer Therapy: Preclinical

Transforming Growth Factor-β–Stimulated Clone-22 Is an Androgen-Regulated Gene That Enhances Apoptosis in Prostate Cancer following Insulin-Like Growth Factor-I Receptor Inhibition

Cynthia C.T. Sprenger,1,2 Kathleen Haugk,3 Shihua Sun,1 Ilsa Coleman,4 Peter S. Nelson,1,4 Robert L. Vessella,2,3 Dale L. Ludwig,5 Jennifer D. Wu,1 and Stephen R. Plymate1,3

Abstract

Purpose: Inhibition of insulin-like growth factor (IGF) signaling using the human IGF-I receptor monoclonal antibody A12 is most effective at inducing apoptosis in prostate cancer xenografts in the presence of androgen. We undertook this study to determine mechanisms for increased apoptosis by A12 in the presence of androgens.

Experimental Methods: The castrate-resistant human xenograft LuCaP 35 V was implanted into intact or castrate severe combined immunodeficient mice and treated with A12 weekly. After 6 weeks of tumor growth, animals were sacrificed and tumors were removed and analyzed for cell cycle distribution/apoptosis and cDNA arrays were done.

Results: In castrate mice, the tumors were delayed in G2 with no apoptosis; in contrast, tumors from intact mice underwent apoptosis with either G1 or G2 delay. Transforming growth factor-β–stimulated clone-22 (TSC-22) was significantly elevated in tumors from the intact mice compared with castrate mice, especially in those tumors with the highest levels of apoptosis. To further determine the function of TSC-22, we transfected various human prostate cancer cell lines with a plasmid expressing TSC-22. Cell lines overexpressing TSC-22 showed an increase in apoptosis and a delay in G1. When these cell lines were placed subcutaneously in athymic nude mice, a decreased number of animals formed tumors and the rate of tumor growth was decreased compared with control tumors.

Conclusions: These data indicate that IGF-I receptor inhibition in the presence of androgen has an enhanced effect on decreasing tumor growth, in part, through increased expression of the tumor suppressor gene TSC-22. (Clin Cancer Res 2009;15(24):7634–41)

We have shown that inhibition of insulin-like growth factor-I receptor (IGF-IR) with the fully human monoclonal antibody A12 resulted in a decreased rate of tumor growth for both androgen-dependent and androgen-independent human prostate cancer xenografts (1, 2). However, depending on whether the xenograft was castration-resistant, we saw either apoptosis for androgen-dependent xenografts grown in intact mice or a slowing of tumor growth due to G2 arrest without apoptosis for androgen-independent xenografts grown in castrate mice. Thus, inhibition of signaling through IGF-IR resulted in significant suppression of prostate tumor growth by both increased apoptosis and decreased proliferation (1). Furthermore, we showed that inhibition of IGF-IR signaling in androgen-dependent xenografts following castration significantly delayed progression to androgen-independent prostate cancer (3). A further suppression of androgen receptor transcriptional activity also occurred following A12 treatment (3). In this current study, we investigated whether the differences in apoptosis between androgen-dependent and androgen-independent human prostate cancer xenografts were due to the presence of androgen in the androgen-dependent animals or to a change in tumor phenotype.

We found an increase in transcript levels of several androgen-regulated genes when castrate-resistant LuCaP 35 V tumors were grown in intact mice. One androgen-regulated gene of particular interest was transforming growth factor-β (TGF-β)–stimulated clone-22 (TSC-22). TSC-22, which is regulated by androgens, TGF-β1, and PPAR-γ, has been associated with decreased tumor formation and thus is a putative tumor suppressor gene (4–10).
Translational Relevance

In this study, we show that androgen-regulated genes such as transforming growth factor-β–stimulated clone-22 (TSC-22) may be responsible for the induction of apoptosis following inhibition of the insulin-like growth factor-I receptor (IGF-IR) in androgen-dependent prostate cancer. The induction of TSC-22 by androgen in castrate-resistant prostate cancer xenografts that are placed in an intact host may reinitiate an apoptotic response to IGF-IR inhibition that is lost when the same xenografts are placed in castrate hosts. Clinically, both fully human monoclonal antibodies, such as A12, and small molecules in trial for prostate as well as other epithelial malignancies. The timing for the best effects of IGF-IR–targeted therapy is important to achieve optimal tumor regression. In this study, we have identified one factor, TSC-22, the expression of which in androgen-dependent disease could indicate a favorable response to early IGF-IR inhibition in prostate cancer.

Materials and Methods

Xenografts and cell lines. The LuCaP 35 V androgen-independent human prostate xenograft, which only grows in vivo, was originally described by Corey et al. (11). The generation and characterization of the M12 cell line has been described previously (12–14). The PC-3 cell line was obtained from the American Type Culture Collection. Both cell lines were cultured in RPMI 1640 supplemented with 5% FCS, 10 ng/mL epithelial growth factor, 0.02 mmol/L dexamethasone, 5 μg/mL insulin, 5 μg/mL transferrin, 5 ng/mL selenium, fungizone, and gentamicin at 37°C with 5% CO2. The androgen receptor–positive LNCaP C4-2 subline was a gift from Dr. Robert Sikes (University of Delaware). These cells were grown in T-medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C with 5% CO2.

Cell transfection. A human full-length cDNA clone of TSC-22 (NM_006022) was obtained from Origene Technologies and subcloned into pcDNA 3.1 with a G418 resistance gene. M12, PC-3, and the LNCaP (NM_006022) was obtained from Origene Technologies and subcloned into pcDNA 3.1 with a G418 resistance gene. M12, PC-3, and the LNCaP

Animal studies. The growth of LuCaP 35 V in castrate mice and the responses to A12 treatment have been reported previously from our laboratory (1). The RNA used for microarray analysis in this study was collected immediately after the tumor was removed and was preserved in DEPC H2O at -80°C. For studies of LuCaP 35 V growth and response to A12 in noncastrate (intact) animals, tumor bits (20-30 mm3) of LuCaP 35 V were implanted subcutaneously into ten 6- to 8-week-old intact severe combined immunodeficient mice as described previously for the LuCaP 35 xenografts (1). When the implanted tumor reached a volume of 100 mm3, half the animals received A12 antibody intraperitoneally at a dose of 40 μg/kg body weight three times a week for up to 5 weeks and the other half received human IgG as a control. Animals were weighed twice a week. Blood samples were collected from orbital sinus weekly. Plasma was separated and prostate-specific antigen level was determined using the IMx Total PSA Assay (Abbott Laboratories). Tumors were measured twice weekly and tumor volume was estimated by the formula: volume = (l × w × h) / 2. After euthanization, tumors were collected, quartered, and treated as follows: (a) fixed in 10% neutral buffered formalin and embedded in paraffin, and sections (5 μm) were prepared for immunohistochemistry; (b) separated into single cells mechanically by mincing and filtering through 70 μm nylon sieves for flow cytometry; (c) minced and extracted for protein; (d) RNA extracted and cDNA prepared for microarrays as described previously (1, 15). For TSC-22 studies, groups of 10 male athymic nude mice were subcutaneously injected with 1 million cells each of either empty vector control cells (M12pc) or TSC-22–overexpressing cells (M12 TSC-22). Mice were monitored weekly for tumor formation; tumors were measured and tumor volume was estimated as described above. Animals were euthanized after 7 weeks and tumors were removed. All animal studies performed followed a University of Washington–approved Institutional Animal Care and Use Committee animal protocol.

cDNA microarray analyses. Custom PEDB cDNA microarrays were constructed as described previously using clones derived from the Prostate Expression Database (16), a sequence repository of human prostate expressed sequence tag data available to the public. The inserts of individual cDNA clones were amplified by PCR, purified, and spotted in duplicate on glass microscope slides (Gold Seal; Becton Dickinson) with a robotic spotting tool (GeneMachine OmniGrid 100). Labeling with Cy3 and Cy5 fluorescent dyes and hybridization to the microarray slides were essentially as described (17). Fluorescent array images were collected for both Cy3 and Cy5 using a GenePix 4000B fluorescent scanner (Molecular Devices). The image intensity data were gridded and extracted using GenePix PRO 4.1 software, and spots of poor quality determined by visual inspection were removed from further analysis. RNA from tumors from A12-treated castrate mice was pooled into two groups: (a) tumors that exhibited G2 arrest (8 tumors) and (b) tumors that exhibited no G2 arrest (10 tumors). These two pools were hybridized against a pool of 18 tumors from untreated castrate tumor controls. RNA from tumors from A12-treated intact mice was pooled into three groups: (a) G1 arrest with apoptosis (2 tumors), (b) G2 arrest with apoptosis (2 tumors), and (c) G1 arrest with no apoptosis (2 tumors). These three pools were hybridized against a pool of 4 untreated noncastrate tumor controls. RNA also was collected from laser-captured microdissections from 37 human prostate cancer tissue samples. Each sample yielded RNA from two areas: benign and cancer. Each of these experiments (LuCaP 35 V RNA and human prostate cancer RNA) was repeated with a switch in fluorescent labels to account for dye effects. Normalization of the Cy3 and Cy5 fluorescent signal on each array was done using GeneSpring 7.3 software (Agilent Technologies). A print-tip-specific Lowess curve was fit to the log-intensity versus log-ratio plot and 20.0% of the data was used to calculate the Lowess fit at each point. This curve was used to adjust the control value for each measurement. If the control channel

http://www.peddb.org
was <10, then 10 was used instead. Data were filtered to remove values from poorly hybridized cDNAs with average foreground minus background intensity levels <300. Data from the two duplicate cDNAs spots on each PEDB chip were combined and the average ratios were used for comparative analyses. Ratios were filtered to include only clones whose expression was measurable in at least 75% of the samples. Differences in gene expression were determined using the SAM procedure (18). Gene expression differences with a false discovery rate of \( \leq 1\% \) were considered significant.

**Immunohistochemistry.** Tumor samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 \( \mu \)m onto slides. After deparaffinization and rehydration, antigens were retrieved with 0.01 mol/L citric acid (pH 6.0) at 95°C for 2 to 5 min. Slides were allowed to cool for 30 min followed by sequential rinsing with PBS. Endogenous peroxidase activity was quenched by an incubation with 0.3% H2O2 in methanol for 15 min. After blocking with 1.5% normal goat serum in PBS containing 0.05% Tween 20 for 1 h, slides were incubated with TSC-22 antibody (1 \( \mu \)g/mL) for 1 h followed by sequential incubation with biotinylated goat anti-mouse IgG for 30 min, peroxidase-labeled avidin for 30 min (Santa Cruz Biotechnology), and dianinobenzidine/hydrogen peroxide chromogen substrate (Vector Laboratories) for 5 to 10 min. Negative controls were done with TSC-22 antibody preabsorbed with TSC-22 protein (Abnova). All incubation steps were done at room temperature. Slides were counterstained with hematoxylin (Sigma) and mounted with Permount (Fisher Scientific).

**Western Blotting.** For cells in culture, cells were washed with PBS and lysed with cold lysis buffer [50 mmol/L HEPES (pH 7.5), 150 mmol/L NaCl, 1.5 mmol/L MgCl2, 1 mmol/L EGTA, and 10% Triton X-100] containing Phosphatase Inhibitor Cocktail II (Sigma) and protease inhibitors (Complete Mini Tablets; Roche). For assaying in vivo effects of A12, freshly prepared xenografts were minced and then washed with PBS and lysed as described for cell culture. Twenty-five micrograms of protein were resolved on 4% to 15% SDS-PAGE gels, transferred onto a nitrocellulose membranes, and probed with a 1:400 dilution of a rabbit polyclonal TSC-22 antibody (ProteinTech Group). The blot was washed and incubated with a horseradish peroxidase–conjugated secondary antibody (Pharmacia Biotech) for 1 h. Immunoreactive proteins were detected by enhanced chemiluminescence (Pharmacia Biotech). The membranes were stripped for 30 min in stripping buffer (Pierce) and reprobed with anti-\( \beta \)-actin antibody (Chemicon) as described above. Independent experiments validated that this stripping procedure did not lead to loss of signal.

**Apoptosis and cell cycle assay.** Apoptosis was measured by terminal deoxynucleotidyl transferase–mediated nick end labeling assay using the Apop-Direct kit (BD BioScience) as described previously (19). Briefly, differences in gene expression were determined using the SAM procedure (18).7 Gene expression differences with a false discovery rate of \( \leq 1\% \) were considered significant.

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Fig. 2. A, cDNA microarray expression values of androgen-regulated genes differentially expressed in apoptotic LuCaP 35 V tumors relative to nonapoptotic tumors from both intact and castrate animals. There were 28 genes that changed in the same direction in each pool that were significantly different from each nonapoptotic pool as well as had a false discovery rate of <1%. Identical gene listings (such as TSC-22) indicate that different areas of the sequence were spotted on the array. As expected, most androgen-regulated genes were increased in the intact versus castrate tumors; however, an additional set was increased in those tumors treated with A12 in which apoptosis occurred. *, P < 0.05, apoptosis versus no apoptosis.

B, results of microarray data for TSC-22 including LuCaP 35 V xenografts grown in intact mice. TSC-22 is increased in the xenografts grown in intact mice when compared with castrate mice and TSC-22 expression is increased even further when A12 is added (P < 0.05).

C, immunohistochemistry for TSC-22 protein in representative LuCaP 35 V xenografts. 1, negative control with TSC-22 antibody preabsorbed with TSC-22 protein; 2, untreated intact mouse; 3, A12-treated castrate mouse; 4, A12-treated intact mouse. Note the increase in staining for TSC-22 in tumors from intact mice (2 and 4) compared with castrate mice (3; magnification, ×40). A representative higher-magnification image (×200) is shown for the tumors from intact mice showing positive cytoplasmic staining for TSC-22 in apoptotic host tumors, which have weak staining.

D, Western blot of levels of TSC-22 in apoptotic LuCaP 35 V tumors versus nonapoptotic LuCaP 35 V tumors from intact mice. β-Actin was used as a loading control.
$1 \times 10^6$ cells from the single-cell suspension were fixed with 10% neutral buffered formalin followed by 70% ethanol alcohol at $-20^\circ$C for 30 min. After several washes, cells were permeabilized with 0.1% Triton X-100 and incubated with FITC-conjugated dUTP and terminal deoxynucleotidyl transferase enzyme at 37°C for 1 h followed by an incubation with a propidium iodide/RNase buffer ($100 \mu g/mL$ propidium iodide and $50 \mu g/mL$ RNase) at room temperature for 60 min. Cell cycle was determined by staining separate cell aliquots with propidium iodide as described previously (1). Samples were analyzed by flow cytometry using a BD FACScan (BD Bioscience). Data were analyzed with CellQuest software. Apoptosis was also measured via Western blots using antibodies against the intact and cleaved forms of poly(ADP-ribose) polymerase and caspase-3 and -7 (Cell Signaling Technology). The presence of cleavage products with a concomitant decrease in levels of intact protein for any of these three proteins indicates apoptosis has occurred.

**Cell proliferation assay.** Cells were seeded in a 96-well plate at 2,500 per well in RPMI T&S for 24 h before addition of 1 or 5 ng/mL TGF-β, 10⁻⁸ DHT, 20 ng/mL IGF-I, 40 μg/mL A12, DHT + IGF, DHT + A12, IGF + A12, or DHT + IGF + A12. When A12 was used, it was added 1 h before addition of other factors. Proliferation was quantified after 72 h by a colorimetric MTS tetrazolium (MTT) assay using the Cell Titer 96 AQ assay kit (Promega) according to the manufacturer's protocol. Eight replicates were done in each experiment and each experiment was done three times.

**Real-time PCR.** M12, PC3, and LNCaP C4-2 cells were treated with IGF-I (1, 10, and 50 ng/mL), DHT, and A12 (40 μg/mL) for 3 h. Then, RNA was collected and real-time PCR was done on an Applied Biosystems 7900HT Fast Real-time PR System using ABI SYBR Green 2× MasterMix (ABI). Primers used were IGF-IR forward (5'-GAAGTGGAACCCTC-3'), TSC-22 forward (5'-GAAATGTTGTCCACAAGAGTGTC-3'), and TSC-22 reverse (5'-TGCTGAGGAGACATTCGGCTG-3').

**Results**

**Growth and prostate-specific antigen levels of LuCaP 35 V tumors in castrate and noncastrate mice.** A12 treatment significantly reduced tumor volume and prostate-specific antigen levels of LuCaP 35 V tumors grown in noncastrate animals.
Further, LuCaP 35 V tumor volumes were significantly lower in the A12-treated intact mice than in the A12-treated castrate mice ($P < 0.05$; Fig. 1A). This decrease in tumor volume was accompanied by a significant decrease in serum prostate-specific antigen in the treated intact mice compared with the treated castrate mice ($P < 0.05$; Fig. 1B).

**Cell cycle and apoptosis in xenografts.** In contrast to the studies in castrate animals in which LuCaP 35 V animals treated with A12 had smaller tumors due to an arrest in G2, 60% of the LuCaP 35 V tumors treated with A12 in intact animals underwent both apoptosis and cell cycle arrest, either G1 or G2 (Fig. 1C-F).

**Transcriptional program of LuCaP 35 V tumors treated with A12.** To identify factors that may be responsible for apoptosis, we examined those androgen-regulated genes that were differentially regulated in tumors undergoing apoptosis versus non-apoptotic tumors from either intact or castrate mice and their regulation in each set of animals by A12 (Fig. 2A). In the intact group that underwent apoptosis, A12 elicited a significant increase in several androgen-regulated genes, including TSC-22, which is of particular interest because it has been associated with the induction of apoptosis and it is a potential tumor suppressor gene for salivary, glial, and prostate cancers (4–7, 9, 20, 21). As would be expected for an androgen-induced gene, TSC-22 mRNA levels were significantly higher in xenografts grown in intact mice (both treated and untreated) compared with xenografts grown in treated castrate mice ($P < 0.01$; Fig. 2B). Immunohistochemistry on tumors showed a decrease in TSC-22 levels in the castrate hosts compared with intact hosts (Fig. 2C). Using Western blot, we then showed an increase in TSC-22 protein levels for tumors that underwent apoptosis following A12 treatment (Fig. 2D).

**TSC-22 expression in benign and malignant human prostate glands.** To determine the clinical relevance of TSC-22 in prostate cancer, we examined laser capture–dissected prostate RNA from 37 patients with prostate cancer (Gleason 6-10; ref. 22). Benign and malignant glands were dissected from each individual, so each gland served as its own control. Amplified cDNA made from the RNA was then examined by cDNA microarray analysis.
as described in Materials and Methods. Analysis of TSC-22 showed a significant decrease in mRNA expression compared with normal tissue in 30 of 37 glands (P < 0.0001; Fig. 3). This finding is consistent with a recent report using immunohistochemistry showing that TSC-22 protein was also significantly decreased in malignant versus benign prostate epithelium (10).

In vitro effects of TSC-22 in the M12, LNCaP C4-2, and PC-3 human prostate cancer cell lines. Because TSC-22 is an androgen-regulated and potentially an IGF-IR negatively regulated gene, we treated the androgen receptor–positive LNCaP C4-2 cell line with both DHT and IGF-I and then exposed the cells to either A12 or IgG. DHT exposure significantly increases levels of IGF-IR in androgen receptor–positive prostate cancer cells, including the LNCaP cell lines, but has no effect in androgen receptor–negative lines such as PC3 (23, 24). In LNCaP C4-2 cells grown in the absence of androgen, TSC-22 is detectable, but levels are markedly lower than in cells grown in the presence of androgen. Real-time PCR showed a significant decrease in the mRNA levels of TSC-22 following IGF-I treatment and this decrease was blocked by the addition of A12 (Fig. 4A). Proliferation assays on these cells following treatment with DHT and A12 showed that A12 blocked the DHT and DHT + IGF-I induced increase in proliferation (Fig. 4B). Finally, when we used small hairpin RNAs to knockdown TSC-22 levels in the LNCaP C4-2 cells, we saw decreased apoptosis following DHT + A12 treatment (Fig. 4C). Thus, the in vitro data with the androgen receptor–positive LNCaP C4-2 line reflected what we observed with the LuCap 35 V xenografts in vivo. For the two lines that do not express an androgen receptor, M12 and PC3, no TSC-22 to very little was detected with PCR or Western blot and levels were not altered by exposure to DHT, IGF-I, or A12 (data not shown); because both of these lines are androgen receptor–negative and are poorly responsive to exogenous IGF-I, these results were expected.

Because TSC-22 expression has been associated with increased apoptosis in other cancers, we overexpressed TSC-22 in the M12, LNCaP C4-2, and PC-3 cell lines (Fig. 5A) and then evaluated the effect of increased expression of TSC-22 on response to A12 and TGF-β treatment. Elevated expression of TSC-22 increased apoptosis in response to A12 and TGF-β treatment as shown by the significantly increased amount of cleaved caspase-3 and -7 and poly(ADP-ribose) polymerase and decreased levels of intact protein (P ≤ 0.01; Fig. 5B) and by flow cytometry (Fig. 5C). Further, all of the cell lines overexpressing TSC-22 had significantly decreased cell proliferation (P < 0.05; Fig. 5D).

In vivo tumorigenicity of the M12 TSC-22 cells. To assess the effect of TSC-22 expression on tumorigenicity of prostate cancer cells, we injected male athymic nude mice subcutaneously with either 1 × 10⁶ empty vector control M12 cells (M12pc) or the stably transfected M12 TSC-22 cells. Seven weeks post-injection, 4 of 10 animals (40%) injected with M12 TSC-22 cells had developed tumors compared with 8 of 10 animals (80%) injected with M12pc cells. Further, the tumors in the TSC-22–injected animals were significantly smaller than those in the animals injected with the control cells (P < 0.001; Fig. 6).

Discussion

Therapy directed toward inhibition of IGF-IR is currently in clinical trials for several malignancies. Preclinical data suggest that it may be a successful adjunctive therapy for solid tumors, including prostate cancer (1–3). In preclinical prostate xenograft studies, we have shown that when the fully human monoclonal IGF-IR antibody A12 is used as a single-agent therapy, the response to IGF-IR inhibition differs depending on whether the tumor is androgen-dependent (grown in an intact host) or androgen-independent (grown in a castrate host; refs. 1, 3). In this study, to determine if it was the innate change in the tumor as it proceeded from androgen-dependent to androgen-independent or the decrease in androgens resulting from castration that accounted for the difference in response to A12, we grew the castrate-resistant LuCap 35 V xenograft in an intact host and showed that the xenograft underwent apoptosis when exposed to A12. These data suggest that genes regulated by the androgen receptor–driven transcription program, in some fashion, sensitize the cells to undergo apoptosis after inhibition of the IGF-IR.

The first of these potential genes is the IGF-IR itself. Others and we have shown that the IGF-IR is increased in prostate cancer cells by activation of the androgen receptor (1, 23, 24). However, there is no evidence that the level of androgen receptor in and of itself determines whether a cell will undergo apoptosis versus cell cycle arrest. Rather, these data suggest that the presence of higher levels of androgen in the intact mice makes the cells more sensitive to IGF-IR inhibition and thus to apoptosis. Based on the results of the microarray data, several candidate genes that are known to be associated with tumor suppression, including TSC-22, were upregulated in the tumors undergoing apoptosis (8). Thus, TSC-22 is likely part of a group of factors responsible for the effects described in this article.

TSC-22 has been associated with cellular differentiation, shown to be associated with apoptosis in head and neck epithelial cancers, and to act as a transcription repressor in hepatocellular and lymphoid tumors (5, 20, 25–27). TSC-22 was first identified in prostate cancer as a gene that was upregulated in response to thiazolidinediones in primary prostate cancer cultures (9). Rentsch et al. then showed a decrease in TSC-22 protein expression in human prostate cancer (10). In this study, we have shown a decrease in TSC-22 transcripts in laser capture–microdissected prostate cancer compared with normal epithelium from the same gland.
Nelson et al. first showed that TSC-22 was a gene that was significantly increased by androgens in the prostate epithelium (8). In this study, we show that TSC-22 is increased in the LNCaP 35 V xenografts by androgen and further increased by the addition of the human monoclonal IGF-IR antibody A12. Further, we present in vitro data showing that TSC-22 is negatively regulated by IGF-1 at the mRNA level and that addition of A12 blocks this decrease. In vivo, the increase in TSC-22 was greatest in those xenografts where apoptosis was clearly evident, suggesting that TSC-22 can induce an apoptotic response in prostate cancer similar to that seen in other tumors. We confirmed this finding in vitro by showing a decrease in apoptosis in LNCaP cells expressing a TSC-22 small hairpin RNA and an increase in apoptosis following A12 treatment in three human prostate cancer cell lines transfected with TSC-22 expression plasmids. TSC-22 expression also decreased in vitro proliferation of these prostate cancer cell lines and decreased in vivo tumorigenicity of the M12 TSC-22 cells compared with the M12pc control cells. These results support the role of TSC-22 as an androgen and potentially IGF-IR–regulated tumor suppressor in prostate.

This study shows that an increase in TSC-22 expression is part of the mechanism by which IGF-IR inhibition in prostate cancer xenografts induces apoptosis. It further indicates that inhibition of IGF-IR as a therapeutic strategy may be more effective in the presence of androgen or in androgen-dependent disease rather than in the castrate patient with androgen-independent disease.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

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