Tazarotene-Induced Gene 1 (TIG1) Expression in Prostate Carcinomas and Its Relationship to Tumorigenicity

Chun Jing, Manal Abd El-Ghany, Carol Beesley, Christopher S. Foster, Philip S. Rudland, Paul Smith, Youqiang Ke

Background: Prostate cancer is the most common noncutaneous male cancer and one of the least understood malignant diseases. Identifying key genetic factors involved in the metastasis of prostate cancer cells is critical. In this study, we used selective subtractive differential gene display to identify a gene whose decreased expression may contribute to the growth and expansion of prostate cancer. Methods: We used 192 primer pair combinations and polymerase chain reaction technology to identify genes expressed in the benign prostate cell line PNT-2 but not in the malignant prostate cancer cell lines LNCaP, Du-145, PC-3, or PC-3M. The tazarotene-induced gene 1 (TIG1) was chosen for further study. TIG1 expression in normal tissues and cell lines was analyzed by northern blot and in normal and tumor prostate tissue sections by in situ hybridization. The in vitro invasiveness (migration through extracellular matrix) and in vivo tumorigenicity (growth in nude mice) were assessed for the highly malignant PC-3M cell line transfected with TIG1 or control cDNA. All statistical tests were two-sided. Results: TIG1 mRNA was expressed in a variety of normal tissues other than prostate tissue. TIG1 mRNA was detected in all 10 normal human prostate tissues and all 51 benign prostatic hyperplastic tissues analyzed but in only four of 51 malignant prostate tissues analyzed. Compared with vector-transfected cells, transfection of PC-3M cells with TIG1 decreased in vitro invasiveness from 14.7% to 3.7%, (mean difference = 11%; 95% confidence interval [CI] = 9.2% to 12.8%, P<.001), and decreased in vivo tumorigenicity from an average tumor weight of 1.31 g to 0.55 g, (mean difference = 0.76 g; 95% CI = 0.43 to 1.09 g, P<.001). Conclusion: TIG1 may be a tumor suppressor gene whose diminished expression is involved in the malignant progression of prostate cancer. [J Natl Cancer Inst 2002;94:482–90]
promote or suppress the tumorigenicity of the DNA recipient cells in vivo.

With the use of the subtractive selection strategy, our previous work demonstrated that the human cutaneous fatty acid-binding protein (C-FABP) gene is a metastasis-promoting gene (6) that induces metastasis by increasing the expression of the vascular endothelial growth factor gene (7). In this work, we used the subtractive selection strategy and identified another gene, which encodes the tazarotene-induced gene 1 (TIG1), a retinoic acid receptor-responsive gene that was originally isolated from the skin (8). We first compared the differential expression patterns of TIG1 between the benign and the malignant model cells and then verified the differences in a wider range of benign and malignant prostatic cells in culture. We have also examined its expression in a large number of benign and malignant prostatic tissue samples from humans. To analyze its possible role in suppressing the malignant phenotype, TIG1 was transfected into the highly malignant prostate cancer cell line PC-3M (9) to test whether the restoration of TIG1 expression can reduce the invasiveness and tumorigenicity of the transfectants.

**MATERIALS AND METHODS**

**Cell Lines**

The human cell lines used in this work, including the benign prostate cell line PNT-2 (10) and the malignant cell lines LNCaP (11), PC-3 (12), DU-145 (13), and PC-3M (9), have been well characterized and widely used. The benign and malignant natures of these cell lines were confirmed in our previous work (6). PC-3M-PSV was obtained by transfection of the PC-3M cells with the pSVneo plasmid DNA (Glaxo Wellcome, London, U.K.) alone by the calcium phosphate precipitation method (6). PC-3M-T1G1-P is a pool of transfected colonies generated by transfecting PC-3M cells with a TIG1-expressing plasmid pSV-TIG1. PC-3M-TIG1-1 is one of five single cloned cell lines derived from PC-3M-TIG1-P. Cells were passaged and grown in RPMI-1640 medium supplemented with 10% (vol/vol) fetal calf serum, penicillin (100 U/mL), streptomycin (100 mg/mL), and testosterone (5 ng/mL) at 37°C, as described previously (6).

**Systematic Differential Display (SDD)**

The procedures for SDD have been described previously (3). Briefly, in SDD the mRNA from different cellular sources is isolated and transcribed into double-stranded cDNA and cut by a four-base recognition enzyme (Mnl I). The 3' cDNA fragment from each cDNA is separated from other cDNA molecules and used as a template in the polymerase chain reaction (PCR). Before amplification, the 3' cDNA fragments are tagged with specifically designed short DNA adapters containing the primer sequences for PCR in such a way that the entire 3' cDNA fragments can be divided into 192 subsets and amplified by PCR using 192 different primer pairs in a systematic manner. The PCR products from each subset generated from benign and malignant cells can be separated in a denaturing polyacrylamide gel by electrophoresis. The differentially expressed mRNAs can be assessed by comparing the intensities of the bands of cDNA fragments on the denaturing gel by autoradiography.

In this article, the anchor primer used for first-strand cDNA transcription was 5'-CTGGCTGTG15'-3'. The nucleotide sequences of the 16 positive-strand primers at the nonbiotinylated end were 5'-ACAAGCCACCGGCGCATNN-3' (N = A, G, T, or C). Similarly, the 12 negative-strand primers at the biotinylated end were 5'-CTGGCTGTG12MN-3' (M = A, G, or C; N = A, G, T, or C). Thus, a total of 192 (12/16) primer combinations can be derived from these two primer groups and used for amplifying 192 possible cDNA subsets. With the use of the primers derived from these two primer groups, the entire cDNA population can be amplified and compared in a systematic manner. The 3' fragment of TIG1 cDNA described in this work was amplified by the specific primer pair 5'-ACAAGCCACCGGCGCATTTCTGGCTGTG12GN-3' for the positive strand at the 5' end and 5'-CTGGCTGTG12GC-3' for the negative strand at the poly(A) end.

The differentially expressed cDNA fragments detected by SDD were verified by a modified reverse-northern blot hybridization procedure (14), which can be used to validate a large number of fragments in a single round of hybridization.

**Northern Blot**

Total and poly(A) RNAs from cultured cells were prepared according to the manufacturer’s protocols with an RNaseasy kit and an Oligotex mRNA mini-kit (QIAGEN GmbH and QIAGEN, Hilden, Germany), respectively. Samples of total RNA (20 μg each) were subjected to electrophoresis under denaturing conditions with formaldehyde in 0.8% (wt/vol) agarose gels, transferred to nylon membranes (Hybond, Amersham, Little Chalfont, U.K.), and cross-linked by a brief exposure to a 302-nm UV light. The multiple-tissue northern blots with different tissue RNAs were purchased from Clontech Laboratories (Palo Alto, CA). The membranes were incubated in prehybridization buffer at 42°C for 4 hours and then hybridized with the radioactively labeled TIG1 cDNA probe for 16 hours at 42°C as described (15). Radioactivity bound to the washed membrane was detected by autoradiography against Kodak XAR films (Eastman Kodak, Rochester, NY) with intensifying screens. The RNA integrity was verified by the presence of an undegraded band following hybridization to a radioactively labeled β-actin probe.

**Determination of the Sequence of TIG1 cDNA**

The nucleotide sequences of the cDNA fragments of the candidate tumor-suppressing or promoting genes, including those of TIG1, were determined with an automated sequencer (377; Applied Biosystems, Foster City, CA). The 3' terminal fragment of the TIG1 cDNA was obtained from the SDD gel by a further round of amplification by PCR. Its 5' fragment was obtained by rapid amplification of cDNA ends (RACE) (16). To perform RACE, 1 μg of mRNA extracted from the benign human PNT-2 cells was transcribed into double-stranded cDNA with a cDNA synthesis kit (Boehringer-Mannheim Biochemica, Mannheim, Germany). A poly(A) tail was added to the 3' end of the reverse cDNA strand by terminal transferase (Life Technologies, Paisley, U.K.) in the presence of 1mM deoxyadenosine triphosphate (dATP), and the product was used as a template for RACE-PCR. The PCR was performed in a total volume of 20 μL, with 2 μL of template, 20 mM Tris–HCl (pH 7.6), 25 mM KCl, 0.05% Tween 20, 100 μg/mL bovine serum albumin (BSA), 1 mM MgCl2, 2.5 μM deoxynucleotide triphosphates (dNTPs), 200 μM of each primer, 0.2 U Taq DNA polymerase (PerkinElmer, Foster City, CA). The PCR consisted of 30 cycles at 94°C for 30 seconds, 56°C for 1 minute, and 72°C for 1 minute. The PCR products were subjected to 2% agarose gel electrophoresis, and the amplified bands were excised. The PCR products were purified with a Qiagen gel extraction kit and then transcribed into double-stranded cDNA with a cDNA synthesis kit (Boehringer-Mannheim Biochemica, Mannheim, Germany). The transcribed cDNA was used as a template in the polymerase chain reaction (PCR).

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products were separated by electrophoresis through a 0.8% agarose gel and visualized after staining with ethidium bromide. The nucleotide sequence of the full-length TIG1 cDNA was obtained by assessing the sequence of the overlapping regions between the 3'-terminal fragment, which was recovered by PCR from the area containing the cDNA band in the SDD denaturing gel, and the 5' fragment, which was obtained by RACE.

Detection of TIG1 mRNA in Human Prostate Tissues by In Situ Hybridization

The TIG1 probe (the antisense strand) was transcribed with SP6 RNA polymerase using a PAGNB27 plasmid (a gift from Dr. R. Chanfraratna, Department of Biology and Chemistry, Allergan, Inc., Irvine, CA) constructed by inserting the full-length TIG1 cDNA into the pBluescript plasmid (Invitrogen, Groningen, The Netherlands) as a template, as described (17). The digoxigenin label was incorporated into the single-strand probe with a probe-labeling kit (Boehringer Mannheim Biochemica). In situ hybridization was performed on a nonradioactive in situ hybridization kit (Boehringer Mannheim Biochemica). After incubating the archival paraffin-embedded tissue sections with an alkaline phosphatase-conjugated antidigoxigenin antibody and the appropriate substrate, a blue/black nitrroblue tetrazolium precipitate was formed at sites of hybridization, which were then visible by light microscopy. All the sections used for the in situ hybridization were counterstained with methyl green to visualize the negatively stained cells. Five to 10 fields of approximately 100 cells each in two sequential sections from each tissue sample were examined. Samples with positive staining in less than 10% of the cells were classified as unstained (negative), those with positive staining in 10%–75% of the cells were classified as partially positive (part positive), and those with positive staining in more than 75% of the cells were classified as positive. These classifications were determined before the experiments began.

Sections from formaldehyde-fixed and paraffin-embedded prostate tissues were prepared according to standard routine procedures in our histologic pathology laboratory. Tissue sections were examined independently by two qualified pathologists and classified as normal, benign prostate hyperplasia (BPH), or carcinoma. Carcinomas were graded according to their combined procedures in our histologic pathology laboratory. Tissue sections were classified as positive. These classifications were determined before the experiments began.

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DNA Transfection and Assays for Invasion and Tumorigenicity

TIG1 cDNA was excised from the PAGNB27 construct by digestion with SalI and XhoI restriction enzymes. The cDNA was inserted into the pSVneo plasmid, which had been previously linearized by digestion with SalI and XhoI, to form a TIG1-expression construct, pSV-TIG1. Exponentially growing, highly malignant PC-3M cells were harvested, seeded at 0.5 to 0.75 x 10⁶ cells per 10 mL RPMI-1640 in 10-cm-diameter tissue culture dishes and transfected with 2 μg of pSV-TIG1 DNA or pSVneo vector DNA alone, using an Effectene Transfection Reagent kit (QIAGEN, Valencia, CA). After 18 hours, the resultant cells were passaged at a split ratio of 1:10 in selective medium consisting of RPMI-1640-supplemented fetal calf serum (10% vol/vol), penicillin (100 U/mL), streptomycin (100 mg/mL), and testosterone (5 ng/mL) containing 1 mg/mL geneticin G418, as described (19). Two weeks after the transfection, the cells that did not harbor the plasmid DNA had died, and those that had successfully taken up the pSVneo plasmid or pSV-TIG1 had formed colonies that ranged from 1 mm to 3 mm in diameter. The colonies generated by transfection with pSVneo DNA alone were pooled to form a mixture of control transfectants (PC-3M-PSV). Five colonies were isolated by cloning rings from those harboring pSV-TIG1 to form five independent transfectant cell lines (PC-3M-TIG1–1, PC-3M-TIG1–2, PC-3M-TIG1–3, PC-3M-TIG1–4, and PC-3M-TIG1–5). The remaining colonies harboring pSV-TIG1 DNA were pooled to form a transfectant pool (PC-3M-TIG1-P).

PC-3M-PSV, PC-3M-TIG1–P, and PC-3M-TIG1–1 cells were assessed for their ability to invade an extracellular matrix using an in vitro invasion assay (20) with a modification (21). To test their ability to form tumors in vivo, we suspended PC-3M-PSV and PC-3M-TIG1–1 cells in phosphate-buffered saline (PBS) mixed with equal volumes of Matrigel (Sigma, Dorset, U.K.) and then injected (2 x 10⁶ cells in 0.25 mL of the mixture) subcutaneously into the left shoulder region of 8- to 10-week-old, male BALB/c (nu/nu) nude mice. All mice were killed after 32 days when at least one tumor that developed in a mouse reached 10% of its body weight (a limit set by the U.K. Home Office). At this time, all the resultant tumors were removed and weighed. The tumor tissues were then subjected to RNA extraction (22) for the detection of TIG1 mRNA. Animal experiments were conducted under U.K. Coordinating Committee on Cancer Research guidelines with Home Office Project License PPL 40–1515 to Philip S. Rudland.

Statistical Methods

In vitro invasiveness was measured as the percentage of cells invading the extracellular membrane matrix taken from eight separate measurements. The differences in the sizes of tumors developed from the control (PC-3M-PSV) and TIG1 transfectant cells (PC-3M-TIG1–1) in the nude mice were measured by comparing their mean weights. Data are reported as mean ± 95% CI, generated according to the Excel 97/98 Book system (Microsoft, Redmond, WA), and statistical significance in the differences in invasiveness or tumor weight were determined by the Student’s t test. The differences in TIG1 mRNA expression among normal, BPH, and malignant prostate tissues were examined by Fisher’s exact test, using the Statistical Package for the Social Sciences, Version 10.0 (SPSS, Chicago, IL). P values of less than .05 were considered to be statistically significant. All statistical tests were two-sided.

RESULTS

Expression of TIG1 in Benign and Malignant Prostate Epithelial Cells

When SDD was used to assess genes that are differentially expressed between the benign prostate cell line PNT-2 and the malignant prostate cell line LNCaP, 73 candidate cDNA fragments were identified. The 50th differential cDNA fragment (F50) was detected readily in the benign PNT-2 cells but not in the malignant LNCaP cells (Fig. 1, A, arrow). When F50 cDNA was recovered from the denaturing gel, amplified by a further
round of PCR with the same primer pair, and subjected to nucleotide sequence analysis, F50 contained a cDNA fragment of 155 base pairs in length (excluding the primer sequences at both ends).

To confirm that F50 was differentially expressed between benign and malignant prostate cancer cells, we used F50 as a probe in northern blot analysis. F50 detected a 17.5-kb mRNA transcript in the benign PNT-2 cells but not in any of the four malignant cell lines examined, including the weakly malignant cell line LNCaP and the highly malignant cell lines Du-145, PC-3, and PC-3M (Fig. 1, B; left panel).

On the basis of the sequence data of the F50 poly(A) terminal cDNA fragment, a PCR primer for amplifying the reverse strand of the cDNA was designed: 5'-CAGTCTAAAGGAGAC-CACTTGTATTG-3'. This primer and a poly(T)30 primer were used to amplify the 5' terminal fragment of the F50 cDNA by RACE (16). The nucleotide sequence of the PCR product was analyzed and compared with that of the fragment recovered from the SDD gel to identify any overlapping regions. By making this comparison, we identified the full-length cDNA represented by F50, which was found to be 862 bp in length. A search of GenBank and European Molecular Biology Laboratory (EMBL) databases revealed that the nucleotide sequence of this cDNA was identical to that of the cDNA for TIG1 (8), also known as retinoic acid receptor responsive 1 gene (RARRES1) (23).

Therefore, F50 is a section of TIG1. Searching the National Center for Biotechnology Information’s (NCBI’s) Online Mendelian Inheritance in Man (OMIM) database revealed that TIG1 is located on the short arm of chromosome 3, somewhere between 3p-13 and 3p-12.

The TIG1 cDNA contained an open reading frame coding for a putative protein of 228 amino acids (Fig. 1, C).
Carcinomas

Tissues

486 ARTICLES Journal of the National Cancer Institute, Vol. 94, No. 7, April 3, 2002

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uronic acid binding motif: CSAVFK (amino acids 125–133; the bold letters represent three key amino acids that bind to the hyaluronic acids).

Expression of TIG1 in a Variety of Different
Normal Tissues

When mRNAs extracted from a variety of normal tissues were hybridized with the TIG1 cDNA probe by northern blotting, TIG1 mRNA was shown to be expressed in 14 of the 16 tissues examined (Fig. 1, D). From the intensities of images of the TIG1 mRNA bands on the northern blot, relatively high levels of TIG1 mRNA were expressed in prostate, heart, lung, liver, colon, and small intestine compared with levels expressed in skeletal muscle, kidney, spleen, thymus, placenta, pancreas, testis, and uterus. The expression of TIG1 mRNA in brain and blood leukocytes could not be detected by northern blot analysis.

Expression of TIG1 in Normal, Benign, and Malignant
Prostate Tissues

We next assessed expression of TIG1 mRNA in normal, benign, and malignant prostate tissues by in situ hybridization. When TIG1 cDNA was used as a probe to hybridize to the tissue mRNAs in a large number of clinical prostate tissues, the TIG1 mRNA was expressed in normal prostate and BPH tissues. By contrast, TIG1 mRNA was expressed in only a very few carcinoma tissues with combined Gleason scores of 2–3 (Table 1) and not expressed in the carcinoma tissues with Gleason scores of 4–10. Among the normal tissues (obtained from subjects without previous prostatic disease) examined, all 10 (100%) were positive for TIG1 mRNA. Among the 51 BPH tissues, 12 (23.5%) were positive for TIG1 mRNA and 39 (76.5%) were partially positive. By contrast, among the 51 malignant tissues, 47 (92.2%) were negative for TIG1 mRNA by in situ hybridization, and only four (7.8%) were partially positive. When the samples were classified according to their combined Gleason scores, all four partially positive malignant tissues were found among the 15 carcinoma tissues with Gleason scores of 2–3. All of the remaining 36 carcinoma tissues with Gleason scores of 4–10 were negative for TIG1 mRNA by in situ hybridization. Compared with mRNA expression in BPH tissues, TIG1 mRNA expression in carcinoma tissues was statistically significantly reduced (Fisher’s exact test, P < .001).

The TIG1 mRNA signal was consistently detected in normal prostate epithelial cells but not in the basal cells (Fig. 2, A). Similar patterns were observed in the positive BPH tissues (23.5%). For the partially positive BPH samples (76.5%), the TIG1 mRNA signal was predominately detected in a heterogeneous staining pattern, with stronger signals observed in normal epithelium and less intense signals observed in the benign hyperplastic cells (Fig. 2, B). In some samples, although no TIG1 mRNA signal was detected in the malignant carcinomas, a signal was detected in the adjacent nonmalignant BPH cells within the same tissue section (Fig. 2, C). A representative negatively stained section from a poorly differentiated, highly malignant carcinoma is shown in Fig. 2, D.

Table 1. Expression of TIG1 mRNA in human prostate tissues*

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Total No.</th>
<th>Negative †</th>
<th>Partially Positive</th>
<th>Positive</th>
</tr>
</thead>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Normal prostate</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BPH</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>Carcinomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With Gleason score 2–3</td>
<td>15</td>
<td>11</td>
<td>73.3</td>
<td>4</td>
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<tr>
<td>Total</td>
<td>51</td>
<td>47</td>
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*TIG1 = tazarotene-induced gene 1; mRNA = messenger RNA; BPH = benign prostate hyperplasia.

†Sections (4 μm) of human prostate tissue samples were prepared from archival paraffin blocks and processed for nonradioactive in situ hybridization with an antisense TIG1 digoxigenin-labeled single-stranded riboprobe. After incubation of the sections with an alkaline phosphatase-conjugated antidigoxigenin antibody, the sites of hybridization were identified by the formation of a nitroblue tetrazolium blue/black precipitate. All sections were counterstained with methyl green. Five to 10 fields of approximately 100 cells in each of the two sections from each tissue sample were examined. Samples with less than 10% of the cells containing hybridization signals were classified as negative, those with 10–75% of the cells containing hybridization signals were classified as partially positive, and those with more than 75% of the cells containing hybridization signals were classified as positive. These classifications were predetermined before the experiments.
The Effect of TIG1 on In Vitro Invasiveness of Prostate Cancer Cells

To test the possible inhibitory effect of TIG1 on the malignant property of prostate cancer cells, a TIG1 expression vector (pSV-TIG1) was transfected into the highly malignant PC-3M cells to yield a transfectant pool (PC-3M-TIG1-P) and five individual clonal cell lines (PC-3M-TIG1–1, PC-3M-TIG1–2, PC-3M-TIG1–3, PC-3M-TIG1–4, and PC-3M-TIG1–5). A control vector (pSVneo DNA) was transfected into PC-3M cells to generate a pool of control transfectants (PC-3M-PSV). Northern blot analysis showed that PC-3M-PSV control transfectants did not express TIG1 mRNA, although both PC-3M-TIG1-P and PC-3M-TIG1–1 expressed a level of TIG1 mRNA similar to that detected in the benign PNT-2 prostate cancer cell line (Fig. 1, B, middle and left panels). In the hope of finding the cell line with the highest level of TIG1 expression, slot blot analysis was conducted to measure the TIG1 expression in the five individual clonal cell lines. However, the result showed that there were no differences in or among the TIG1 mRNA levels expressed among the five separate clones (data not shown); thus, a representative clone (PC-3M-TIG1–1) was chosen at random for the invasion analysis.

We next measured the in vitro growth rates of the parental PC-3M cells and the PC-3M-PSV, PC-3M-TIG1–P, and PC-3M-TIG1–1 cells by directly counting cell numbers daily for 16 days. No apparent difference in growth rates between the control and transfectant cells was observed (data not shown).

We next compared the ability of the parental malignant PC-3M cells, the control transfectants, and the TIG1 transfectants to invade an extracellular matrix in vitro in 48 hours. A similar fraction of both the parental malignant PC-3M cells (13.5%; 95% CI = 11.9% to 15.1%) and control PC-3M-PSV cells (14.7%; 95% CI = 12.93% to 16.48%) invaded the extracellular matrix. However, when compared with control transfectant cells, only 3.7% (95% CI = 3.19% to 4.21%) of PC-3M-TIG1-P and 4.2% (95% CI = 3.59% to 4.81%) of PC-3M-TIG1–1 cells invaded the extracellular matrix—a statistically significantly reduction (Student’s t test, P < .001, n = 8) (Fig. 3, A).

The Effect of TIG1 on Tumorigenicity of Prostate Cancer Cells

To test the possible effect of TIG1 expression on tumor formation, we injected the control PC-3M-PSV cells or transfectant PC-3M-TIG1–1 cells subcutaneously into the left shoulder regions of BALB/c (nu/nu) nude mice (10 per group). Tumors developed by day 6 in all 20 mice. However, at autopsy 32 days after the injection, the average weight of the 10 tumors from the control group was 1.31 g (95% CI = 0.99 to 1.64 g) and the average weight of those from the experiment group was 0.55 g (95% CI = 0.49 to 0.61 g)—a statistically significant difference (Student’s t test, P < .001, n = 10) (Fig. 3, B).

Although the expression of TIG1 mRNA did not affect the tumor incidence, it was associated with an approximately 2.4-fold reduction in the average size of tumors developed from the highly malignant PC-3M cells. When RNA extracted from the tumors tissues was subjected to northern blotting, TIG1 mRNA was detected in the tumors produced by PC-3M-TIG1–1 cells but not in those produced by the control PC-3M-PSV cells (Fig. 1, B, right panel), further confirming that the reduction of tumor sizes was associated with the expression of TIG1 mRNA.

**DISCUSSION**

In this work, we have identified TIG1 as a potential tumor suppressor gene for human prostate cancer. We also demonstrated that the decreased expression of TIG1 was associated with an increase in the malignant characteristics of both prostate cell lines and tissues. Moreover, we have found that the expression of TIG1 was absent in all malignant prostate cell lines examined, in all carcinoma tissues with Gleason scores of 4–10,
and in the majority (73%) of the prostate tissues with Gleason scores of 2–3 (Table 1). Restoration of TIG1 expression by transfection of an expression vector in the highly malignant PC-3M cells, which do not express TIG1 mRNA, greatly reduced their invasiveness in vitro and their tumorigenicity in nude mice. Indeed, when compared to tumors produced by the control transfectants, the average tumor size of TIG1-expressing PC-3M cells at autopsy was reduced by 2.4-fold (Fig. 3, B). Thus, TIG1 may be one of those genes whose diminished expression contributes to the malignant progression of prostate cancer. Because TIG1 is also expressed in many other normal tissues (Fig. 1, D), it would be interesting to examine TIG1 expression in cancers of these other tissues.

Prostate cancer is a heterogeneous and multifocal disease with clinical and morphologic complexities. An important aspect regarding prostate cancer carcinogenesis is its malignant progression at an early stage. Only some prostate cancer precursors, such as high-grade intraepithelial neoplasia, can be identified by morphologic criteria. Furthermore, prostate cancer may also arise from premalignant lesions that cannot be recognized by morphologic means, such as those thought to be associated with BPH (24–27). Thus, the identification of new molecular markers to detect putative premalignant lesions would be valuable for the early diagnosis of prostate cancer. In this work, by in situ hybridization we detected TIG1 mRNA expression in all normal and BPH prostate tissues (Table 1). We also found that only 23.5% of the BPH samples had high TIG1 mRNA expression, whereas the remaining 76.5% BPH samples had moderate expression. It is not clear whether the 76.5% of samples with reduced levels of TIG1 mRNA expression have a higher potential to undergo malignant progression. Further investigations are needed to decide whether the reduced TIG1 expression is associated with putative premalignant lesions and how useful TIG1 expression would be as a possible molecular marker to identify such lesions.

Loss of function of tumor suppressor genes is an important mechanism for the malignant progression of cancer cells. However, the mechanisms involved in loss-of-function genetic events are complicated. In diploid human cells, mRNA expression levels reflect the combined transcription of both copies (alleles) of a particular gene. Inheritance of one mutant copy of a tumor suppressor gene may predispose an individual to cancer, especially if the remaining wild-type allele is lost in somatic cells, resulting in cells completely devoid of the tumor suppressor gene product. Thus, somatic allele mutations have been found in a number of tumor suppressor genes (28,29). However, a more recent investigation (30) suggests that, for some tumor suppressor genes, the presence of predisposed somatic mutations in one or both alleles may not be necessary because expression at insufficient levels may inhibit the tumor suppressive function. This dosage insufficiency for tumor suppression may be caused by a variety of alterations, including haploinsufficiency (31) caused by the deletion of a single allele, by the complete loss of both copies of that region of the chromosome by DNA deletion (32), by epigenetic silencing through methylation (33), or by some other mechanism (30).

In this study, we found that the expression of TIG1 was completely abrogated in 92.2% of the malignant prostate carcinomas tested. The loss of mRNA could be accounted for by deletion of the TIG1 gene at the genomic DNA level or by complete transcriptional suppression at the mRNA level. Genomic DNA deletion (including single allele deletion) (31,32) and mRNA transcriptional suppression, such as epigenetic silencing through methylation (33), are common mechanisms through which the majority of tumor suppressor genes with predisposed mutations lose their control on tumorigenicity. For a tumor suppressor gene with a single allele mutation, a second mutation in the other allele is the major mechanism through which the gene loses its function in tumor suppression. These mutations may not necessarily affect or reduce the expression of the gene at either the mRNA or protein level. Although mRNA for a tumor suppressor gene without a mutation is frequently absent or expressed at greatly reduced levels in cancer cells (31–33), mRNA for a tumor suppressor gene with a predisposed mutation is frequently overexpressed or expressed in an altered way (34–36). The pattern of TIG1 mRNA expression in prostate carcinoma tissues exhibits a similarity to that of the nonmutational tumor suppressor genes; it is therefore more likely that TIG1 is a tumor suppressor gene without associated predisposed mutations than a tumor suppressor gene with a mutated allele predisposition.

When analyzed by the NCBI OMIM program to assess chromosomal location, TIG1 (also known as RARRES1) was found to be located in the short arm of chromosome 3, between 3p13 and 3p12. Interestingly, several previous studies on the molecular mechanisms of malignant diseases have suggested imbalances of the chromosomal region that contains the TIG1 gene or of the regions overlapping the TIG1 gene. Nonrandom deletions in the short arm of chromosome 3 have been described in primary lung cancer (37). Chromosome 3 loci deletions were found in 60% of non-small-cell lung cancers (38). Mapping studies (39) of the chromosome 3 deletion show three discrete 3p deleted regions (3p14, 3p21, and 3p24–25) in lung and head and neck cancers. Thus, it has been suggested that the short arm of chromosome 3 harbors several tumor suppressor genes, which may be of diagnostic and therapeutic importance (39,40). Extensive loss of heterozygosity (LOH) studies in carcinomas of the lung, breast, cervix, kidney, and head and neck suggest that either a single tumor suppressor gene or a group of different ones reside in 3p loci and contribute to the pathophysiology of the respective cancers (41,42). More recently, 89% of microdissected prostate tissues had LOH in the 3p12–22 and 3p24–26 regions of the short arm of chromosome 3 (43). Because the TIG1 gene is located between 3p12 and 3p13, the 3p12–22 deleted region contains the area harboring the TIG1 gene. Combining the results from these previous investigations (41–43) and the results described in our present study, we suggest that TIG1 may be one of the possible tumor suppressor genes that is frequently deleted in malignant prostate carcinoma cells.

TIG1 or RARRES1 is a retinoic acid receptor-responsive gene that was originally isolated from the skin and whose expression is increased by the synthetic retinoid tazarotene, which is effective in the treatment of hyperproliferative dermatologic diseases, such as psoriasis (8). The tumor-suppressing activity of TIG1 may be related to its ability to react to retinoid stimulation. Retinoids can exert potent growth inhibitory and cell differentiation activities by binding to specific nuclear receptors. These receptors are members of the steroid thyroid hormone receptor superfamily of transcriptional regulators that can regulate vital cellular processes, such as growth, differentiation, and malignant progression (44). Tazarotene is a synthetic retinoid, and another tazarotene-induced gene (TIG3) is reported to be a class II tumor
suppressor through interaction with retinoids (45). Like TIG3, the expression of TIG1 is also inducible by tazarotene. Thus, it may also play a tumor suppressor role by acting as a retinoid mediator of the cell. Computer analysis showed that the putative TIG1 protein, including that expressed in prostate tissue, is a transmembrane molecule and its sequence contains a hyaluronic acid binding motif. Thus, it is possible that TIG1 functions as a cell adhesion molecule whose expression on the cell surface may lead to better cell-to-cell contact, and this may partially contribute to the decreased invasiveness. More studies are needed to mechanistically determine how TIG1 suppresses tumorigenicity of prostate and possibly other cancer cells.

REFERENCES


NOTES

Chun Jing and Manal Abd El-Ghany contributed equally to this work.

Supported by two research project grants awarded to Y. Ke by North West Cancer Research Fund (U.K.).

Manuscript received August 31, 2001; revised January 31, 2002; accepted February 14, 2002.