Insulin-like Growth Factor Binding Protein-related Protein 1 (IGFBP-rP1) Is a Potential Tumor Suppressor Protein for Prostate Cancer

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ABSTRACT

Insulin-like growth factor binding protein-related protein-1 (IGFBP-rP1) has been shown to have decreased expression in the progression from benign to malignant prostate epithelial cells (V. Hwa et al., J. Clin Endocrinol. Metab., 83: 4355–4362, 1998). The present study was undertaken to determine the effects of the re-expression of IGFBP-rP1 in a cell line from a model of human prostate cancer, M12, in which IGFBP-rP1 expression had been demonstrated to decrease from the parent epithelial cell, P69, to the malignant subline, M12. An IGFBP-rP1 cDNA encoding the protein was transfected into M12 cells in a plasmid that resulted in constitutive-expression of IGFBP-rP1. Clones of transfected M12 cells were selected for low (L) and high (H) levels of expression, and the plasmid vector alone was transfected into M12 as a control. After transfection, there was a marked alteration in the morphology of the M12 cells such that the H clones had an elongated appearance when compared with the M12 control cells. The M12 clones overexpressing IGFBP-rP1 had a dose-related increase in population doubling time, decreased colony formation in soft agar, an increased propensity to undergo apoptosis in response to 6-hydroxyurea, and decreased tumor formation in male athymic, nude mice. These data suggest that IGFBP-rP1 may have a suppressive effect on prostate cancer development.

INTRODUCTION

The IGF3 system has been shown to be an integral part of the development of prostate cancer in men (1–7). Recently, serum levels of IGF-I were suggested to be stronger predictors for the development of prostate cancer in men than were serum androgen levels (6). The IGF system in the prostate has classically consisted of IGF-II as the predominant ligand, the type 1 IGF receptor, the type 2 mannose 6 phosphate/IGF-II receptor, and five high affinity IGFBPs –2, –3, –4, –5, and –6 (1, 3–5, 8). Recently, a new group of IGFBP-related proteins has been described, including IGFBP-rP1, whose cDNA has been cloned by several investigators (8–14). The gene resides on human chromosome 4q12 and encodes a precursor protein of 282 amino acids (10, 11, 15). This protein is processed to a mature 27-kDa protein of 256 amino acids with one N-glycosylation site resulting in a mature protein of 32 kDa. IGFBP-rP1 has been found in a number of sources including human fibroblasts, leptomeningial and meningioma cells, muscle, osteosarcoma cells, human mammary tumor cell lines, and senescent mammary epithelial cells (11, 16–20). Originally termed mac25 in the meningioma and meningioma cell lines, the protein and cDNA were isolated from a number of different sources and, depending on function, IGFBP-rP1 has also been called tumor cell adhesion factor (TAF) or prostacyclin stimulating factor (PSF; Refs. 11, 16, 18–20). Subsequent sequence searches have revealed significant homology to the IGFBPs and follistatin protein families. When compared with the IGFBPs, specifically IGFBP-3, IGFBP-rP1 binds IGF-1 and IGF-II with 10-to-25-fold lower affinity (11). However, when compared with the NH2-terminal region of the IGFBPs, IGFBP-rP1 displays a 43% sequence homology in addition to containing 12 of the 12 conserved cysteines in this region (13).

Investigators are just beginning to elucidate the function of IGFBP-rP1. In fibroblasts, it increases the proliferative response to IGF and increases the production of prostacyclin (21, 22). In mammary glands, IGFBP-rP1 expression has been associated with differentiated cells (16). IGFBP-rP1 expression is increased in breast and prostate epithelial cell lines by TGF-b1 and retinoic acid (8, 16). Furthermore, in the breast and prostate epithelial cells, IGFBP-rP1 message and expression are decreased in malignant tissue and cell lines, which suggests that IGFBP-rP1 may have tumor suppressor activity (15, 16, 21, 23). This activity has been demonstrated functionally by the inhibition of clonal growth in p53-deficient osteosarcoma cell lines and through the induction of apoptosis when added to the media of cultured human breast carcinoma cells (20).

The purpose of this study was to determine the effect of IGFBP-rP1 on the growth of a tumorigenic human prostate epithelial cell line, M12.

MATERIALS AND METHODS

Materials. Tissue culture media [RPMI 1640, gentamicin, fungizone, geneticin (G418), and DNase] were obtained from Life Technologies (Grand Island, NY). Epidermal growth factor, dexamethasone, and the additive ITS were purchased from Sigma Chemical Co. (St. Louis, MO). IGFs-I and -II were gifts from Eli Lilly and Co. (Indianapolis, IN). FBS was obtained from Hyclone (Logan, UT). Tfx-50 reagent was obtained from Promega (Madison, WI). The BCA protein assay kit was from Pierce Biological (Rockford, IL.). The IGFBP-rP1 antibody used in this study was produced and characterized as described previously (11, 24). The IGFBP-rP1 cDNAs were obtained as previously described from a cDNA library made from the human breast carcinoma cell line, Hs578T (11). Two clones were obtained from this library, both of which contain an IGFBP-rP1 cDNA encoding for an IGFBP-rP1 protein that reacted with the IGFBP-rP1 antibody at the appropriate size of 32 kDa on a SDS-polyacrylamide gel. Horseradish peroxidase-linked donkey antirabbit IgG and ECL detection reagents were purchased from Amersham (Arlington Heights, IL). Each experiment was performed at least three separate times.

Cell Culture.

The derivation of the M12 cell line has been described previously by Bae et al. (25–27). Briefly, human prostate epithelial cells were immortalized with SV40-T antigen to produce the poorly tumorigenic P69SV40T (P69) cell line. P69 cells were injected s.c. into athymic nude mice, producing tumor nodules in 2 of 18 animals after 180 days (25). These nodules were reinplanted in athymic mice and after three passages resulted in the M12 cells, which demonstrated a short latency period of 7–10 days to tumor formation in 10 of 10 animals and were locally invasive and metastatic (25, 26). Cells were cultured in RPMI 1640 supplemented with 10 ng/ml epidermal growth factor, 0.02 mm dexamethasone, 5 μg/ml insulin, 5 μg/ml transferrin, 50 ng/ml selenium, fungizone, and geneticin at 37°C under 5% CO2. All of the cells used in these experiments were Mycoplasma free, as determined by the Mycoplasma PCR Primer Set (Stratagene, La Jolla, CA).
Fig. 1. A, Western immunoblot of media from high IGFBP-rP1-expressing clones (Ha, Hb, Hc), a low IGFBP-rP1 expressing clone (L), and M12-pcDNA control cells using an IGFBP-rP1 antibody demonstrating the 32-kDa IGFBP-rP1 protein. Cell number controlled for medium loading. B, Northern blot analysis of total cytoplasmic RNA from the same cell lines described in A, demonstrating the 1.1-kb IGFBP-rP1 transcript. An actin cDNA probe assessed loading of RNA.

Table 1 Doubling-time in hours of the M12-pcDNA control cells, low (L) and high (Ha) IGFBP-rP1-expressing clones

<table>
<thead>
<tr>
<th>Cell line</th>
<th>M12-pcDNA</th>
<th>L</th>
<th>Ha</th>
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<tr>
<td>Doubling time (h) ± SE</td>
<td>21 ± 4</td>
<td>27 ± 2.6</td>
<td>70 ± 4*</td>
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* P ≤ 0.001 versus M12-pcDNA cell.

Vector Preparation. The mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA) was used to prepare the pc-IGFBP-rP1 construct, which expressed the IGFBP-rP1 cDNA from the constitutive cytomegalovirus promoter. A 1.1-kb cDNA fragment containing the full-length coding sequence was ligated into the EcoRI and XhoI sites, oriented 5’ to 3’ of pcDNA3.1 using the Ready-To-Go T4 DNA Ligase kit (Pharmacia, Alameda, CA). Subcloning efficient DH5α Escherichia coli cells (Life Technologies) were transformed with the pcDNA3.1-IGFBP-rP1 ligation; ampicillin-resistant colonies were assayed by DNA mini-preparations and restriction digestion.

Transfection. Cell lines overexpressing IGFBP-rP1 were produced by liposome-mediated transfection of the M12 cell line, using TfX-50 according to the manufacturer’s protocol and using 1.33 μg DNA per 5.5 × 10⁵ cells in a 60-mm tissue culture dish. Transfecting the M12 cells with pcDNA3.1 alone produced control cells. Both transfected and nontransfected cells were selected with G418 (800 mg/liter) for 5–7 days, until all of the nontransfected M12 cells died. Surviving transfected cells were maintained with G418 (200 μg/liter), and the formation of individual colonies was monitored. Visible colonies were then subcloned, using cloning rings, and each colony was transferred to a new well in a 12-well tissue culture plate. Cells were grown to confluence and split twice before the medium was collected (see “Western Immunoblots and Ligand Blots” section below) and total cytoplasmic RNA was isolated (see “mRNA Analysis” section below). Both the media and RNA were then analyzed for the degree of overexpression of IGFBP-rP1.

Western Immunoblots and Ligand Blots. Media from cells were collected and pelleted after 0, 4, and 8 h of treatment with 50 mM 6-hydroxyurea. Cells were then resuspended in SDS-PAGE loading buffer A [62.5 mM Tris (pH 6.8), 6 M urea, 10% glycerol, 2% SDS, 0.003% bromphenol blue, and 5% b-mercaptoethanol], sonicated for 20 s, and then heated at 65°C for 15 min. Approximately 50 μg of protein were run for each sample on a 7.5% SDS-polyacrylamide gel and then electroblotted to nitrocellulose. Western blots were incubated with the monoclonal antibody PARP (Oncogene, Cambridge, MA) at a 1:100 dilution in 5% nonfat dry milk and 0.3% Tween 20 in TBS overnight at 4°C. Bound antibody was detected using a horseradish peroxidase-linked sheep antimouse secondary antibody and the ECL detection system according to manufacturer’s protocol.

mRNA Analysis. Cells were grown to approximately 90% confluence, at which time total cytoplasmic RNA was isolated using an acid guanidinium thiocyanate/phenol/chloroform extraction method (30). Approximately 10 μg of each RNA preparation (quantitated by absorbance at 260 nm) was separated by electrophoresis on a 1% agarose gel and transferred to nitrocellulose (28). After concentration, proteins were redissolved in 500 μl of SDS sample buffer (0.5 M Tris (pH 6.8), 1% SDS, 10% glycerol, 0.003% bromphenol blue, and 8 M urea) by heating for 10 min at 100°C.

PARP Immunoblot. Both attached and floating cells were collected and pelleted after 0, 4, and 8 h of treatment with 50 mM 6-hydroxyurea. Cells were then resuspended in SDS-PAGE loading buffer A [62.5 mM Tris (pH 6.8), 6 M urea, 10% glycerol, 2% SDS, 0.003% bromphenol blue, and 5% b-mercaptoethanol], sonicated for 20 s, and then heated at 65°C for 15 min. Approximately 50 μg of protein were run for each sample on a 7.5% SDS-polyacrylamide gel and then electroblotted to nitrocellulose. Western blots were incubated with the monoclonal antibody PARP (Oncogene, Cambridge, MA) at a 1:100 dilution in 5% nonfat dry milk and 0.3% Tween 20 in TBS overnight at 4°C. Bound antibody was detected using a horseradish peroxidase-linked sheep antimouse secondary antibody and the ECL detection system according to manufacturer’s protocol.

Anchoragindependent Growth. For studies of anchorage-independent growth of M12-pcDNA and M12pc-IGFBP-rP1 lines, each well of a 12-well
plate was first layered with 0.6% agar/2X RPMI 1640. A top layer containing 5 × 10⁵ cells/well suspended in 2X RPMI supplemented with 5% FBS and 0.3% agar was then added. Plates were maintained at 37°C under 5% CO₂ for 14 days. Colonies greater than 50 μm in diameter were counted.

**DNA Fragmentation Assay.** Cells from culture dishes were collected and pelleted. Representative samples (2 × 10⁶ cells per sample) were suspended in a 1:1 loading buffer:RNase (10 ng/ml) and loaded into wells on a gel. The gel consisted of two parts with 1.5% agarose in Tris-borate-EDTA electrophoresis buffer (TBE) buffer below the comb and 1% agarose, 2% SDS, and 64 μg/ml proteinase K above the comb. Samples were then electrophoresed, and the DNA was visualized by staining with ethidium bromide.

**Tumor Formation in Vivo.** Groups of 4–10 nude, athymic male mice were injected s.c. with 10⁶ cells. A mouse was injected with either a control (M12-pcDNA) clone, one of three high-IGFBP-rP1-expressing clones (Ha, Hb, Hc), or a low expressing clone (L). The clones were selected from G418-resistant colonies. The low expressing clone was chosen as a comparison because it expressed very low levels of IGFBP-rP1 compared with the control cells as well as the high expressing clones (Fig. 1). Mice were then monitored biweekly for tumor formation and weight gain/loss for a duration of 8 weeks. If tumors were present, tumor volume was calculated using the formula: \( V = \frac{1}{2} \times l \times w^2 \) (where \( l = \text{length} \) and \( w = \text{width of tumor} \)). Statistical analyses using Kruskal-Wallis (for comparing the rate of tumor formation) and the Mann-Whitney U test (for comparing tumor volumes) were then performed. After 8 weeks, the mice were sacrificed, and their tumors were removed. These tumors were then digested with 0.1% collagenase (Type I) and 50 μg/ml DNase (Worthington Biochemical Corp., Freehold, NJ; Ref. 27). Dispersed cells were plated in ITS medium/5% FBS at 37°C for 24 h to allow attachment. After 24 h, cultures were switched to serum-free medium (see "Materials and Methods").

**RESULTS**

**Transfection of the M12 Cell Line with the IGFBP-rP1 cDNA.** After transfection, several clones were picked for low and high levels of expression of IGFBP-rP1. In addition, a clone transfected with the empty pcDNA 3.1 vector (M12pcDNA) was used as the control. The expression of IGFBP-rP1 protein and mRNA for the control and for clones selected for low (L) and high (Ha, Hb, Hc) expression of IGFBP-rP1 is shown in Fig. 1.

**Effect of IGFBP-rP1 Expression on the Growth Characteristics of M12 Cells.** Table 1 demonstrates the growth characteristics of each clone. As the level of expression of IGFBP-rP1 increased, there was a progressive increase in the doubling time of the cell line when grown in the absence of serum and growth factors. Although the parent M12 cells grow well in serum-free defined medium (27), the clones expressing the highest levels of IGFBP-rP1 (as represented by the Ha clone) developed a requirement for serum for early-phase growth when sparsely plated.

**Effect of IGFBP-rP1 Expression on Morphology.** After transfection, the M12, the controls, and the low expressing clones had the characteristic cuboidal morphology associated with epithelial cells; those clones overexpressing IGFBP-rP1, however, were elongated with an increased cytoplasmic/nuclear ratio (Fig. 2). This morphological change was associated with the level of overexpression of IGFBP-rP1. No clones containing the control pcDNA vector in M12 cells, in which we have previously expressed other binding proteins, have assumed this elongated appearance (29). In addition, the high expressing clones, even after becoming confluent, retain an elongated morphology. When this appearance has been described in the LNCaP human prostate cancer cell line or in breast cancer cell lines, it has been associated with increased apoptotic potential (16, 31).

**Effect of IGFBP-rP1 Expression on Apoptosis of M12 Cells.** When exposed to the apoptosis-inducing agent 6-hydroxyurea (50 mM; 6-h treatment), the cells overexpressing IGFBP-rP1 readily un-
deteriorate apoptosis compared with the control cells as determined by DNA fragmentation in agarose gels in which an equal number of cells (2 × 10^6) was loaded per sample (Fig. 3). In the high expressing clones, those cells undergoing apoptosis appeared in the cells collected from the plate and not in the media. Furthermore, when the various clones were treated for 16 h with 6-hydroxyurea, DNA fragmentation appeared in only the media of control cells but in both the media and plated cells for clones overexpressing IGFBP-rP1 (data not shown).

Apoptosis was confirmed as the mechanism for DNA fragmentation in cells overexpressing IGFBP-rP1 by demonstration of PARP cleavage (Fig. 4). In the high expressing clones, PARP cleavage, indicated by the presence of both an intact 115-kDa band and the cleaved 85-kDa band, was evident even without 6-hydroxyurea treatment. In the control cells, evidence of PARP cleavage did not appear until 8 h after 6-hydroxyurea treatment; the intensity of the 85-kDa band, however, was markedly lower than the 8-h band for the high expressing clones. Furthermore, the low expressing clone (L) did not exhibit any PARP cleavage (Fig. 4) even after 24 h of 6-hydroxyurea treatment (data not shown).

**Effect of IGFBP-rP1 Expression on Anchorage-independent Growth of M12 Cells.** After 14 days of growth in soft agar, the number of colonies formed and the colony size were analyzed. There were significantly (P < 0.0001) fewer colonies formed by the clones overexpressing IGFBP-rP1 compared with the control clones and the low expressing clones (Fig. 5). There was no significant difference between the number of colonies formed by the low expressing clones versus the control cells. In addition, not only were there fewer colonies formed by the high expressing clones, the colonies were significantly (P < 0.0001) smaller then those colonies formed by the low expressing clones and the control cells (Fig. 6). Again, there was no significant difference in colony size between the low expressing clones and the control cells. The decrease in colony growth in soft agar was consistent with the increase in expression of IGFBP-rP1.

**Tumor Formation in Vivo.** Mice were monitored biweekly for tumor formation over an 8-week period. Tumor formation, expressed as the percentage of mice developing tumors over an 8-week period, was then determined (Table 2). By the 8th and final week of the experiment, 17 (100%) of 17 mice that received injections of the low expressing clone and 14 (78%) of 18 mice given injections of control clones had tumors, but only 6 (22%) of 27 mice that were given injections of high clones had tumors. When the number of mice forming tumors in each group was compared, there was a significant difference (df = 2; P < 0.001) between the number of mice which formed tumors versus those that did not among the various clones [controls, L, H (a, b, and c)]. In addition, over the duration of the experiment, there was a significant difference (df = 6; P < 0.001) in the rate of tumor formation between the controls, L, and Ha clones (Table 2).

When tumor volume was compared at the 8th and final week of the experiment, there was a significant difference (P < 0.0001) in volume among the various clones (control, L, and Ha; Fig. 7). Specifically, those tumors formed by the mice injected with high expressing clones

<table>
<thead>
<tr>
<th>Table 2 Rate of tumor formation after s.c. injection of M12pcDNA, low (L), and high (Ha, Hb, Hc) clones in male athymic, nude mice</th>
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<tr>
<td>2 weeks n (%)</td>
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<tr>
<td>M12pcDNA</td>
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<tr>
<td>L</td>
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<td>Hb</td>
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<td>Hc</td>
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*The rate of formation was significantly different (df = 6; *, P < 0.001) over the duration of the 8-week experiment.
were significantly smaller than those formed by the control cells ($P < 0.01$) as well as those formed by the low expressing clones ($P < 0.001$). In addition, when comparing tumors formed by control cells versus low expressing clones, control cell tumors were significantly smaller than tumors formed by low expressing clones ($P < 0.001$).

After 8 weeks, tumors were removed, and the tumor-derived cells were grown in tissue culture. Media and RNA were collected from these tumor-derived cells to determine whether IGFBP-rP1 levels after tumor formation compared with the preinjection levels. Both mRNA and protein levels of IGFBP-rP1, after tumor formation, were similar to those of preinjection, with the tumors formed by the mice that had received injections of high expressing clones retaining a high level of IGFBP-rP1 expression compared with the controls and low clones (Fig. 8).

Those mice that failed to develop tumors during the 8-week experiment were then observed over the next 5 months. During that time period, the remaining 4 mice that had been given injections of M12pc control cells all developed tumors and were sacrificed, whereas the 21 mice who had received overexpressing clones (Ha, Hb, and Hc) remained tumor-free.

**DISCUSSION**

The IGF system has been demonstrated to play an integral role in both the function of the normal prostate and the development and progression of prostate cancer (1–5). Classically, the influence of IGFBPs on the prostate has been either to enhance or inhibit the activity of IGF ligands. Recently, IGFBP-independent activities of some of the binding proteins have been described, especially for IGFBP-3 and proteolytic fragments of IGFBP-3 (1, 32–36). These data suggest that peptide sequences in the IGFBP molecule can have direct effects on cells at either the cell membrane or the nuclear level. We have demonstrated previously that IGFBP-rP1 expression is increased by the two proapoptotic agents, TGF-$\beta$ and retinoic acid (8). IGFBP-rP1 expression in vivo was decreased in malignant prostate epithelium, and its expression was decreased in the progression to malignancy in the P69SV40-T human prostate cancer cell model (8). These data suggest the possibility that IGFBP-rP1 may have tumor suppression activities in prostate epithelium. The results from the present set of studies are consistent with this hypothesis. We have shown that overexpression of IGFBP-rP1 in the metastatic human prostate epithelial cell line, M12, results in an increased doubling time for the cells, an alteration in cell morphology that is consistent with increased sensitivity to the induction of apoptosis, an actual increase in apoptosis in response to 6-hydroxyurea, and decreased colony formation in soft agar. Furthermore, when M12 cells overexpressing IGFBP-rP1 were injected into male athymic, nude mice, the rate of tumor formation decreased in addition to the tumor volume decreasing in those mice that did form tumors. These data are consistent with previous data from our lab and others (8, 23) demonstrating a loss of IGFBP-rP1 expression in the progression from a benign to a malignant state in both prostate and breast cancer.

The mechanism(s) by which IGFBP-rP1 may have its effect on tumorigenesis has not been elucidated, but there are several potential routes by which this activity could be mediated.

The first of these could be by binding IGF ligands in a classical IGFBP activity. We and others have demonstrated that the findings in this study can be reproduced in this same cell line by the overexpression of IGFBP-4, an inhibitory IGFBP (29). However, it would be somewhat unusual for IGFBP-rP1 to exert its tumor suppression effects as an inhibitory binding protein because: (a) it has a much lower affinity for the IGF ligands than the other five IGFBPs produced by the prostate, plus all of the high affinity IGFBPs (except for IGFBP-3) are increased in malignant compared with benign prostate epithelium; (b) IGFBP-rP1 enhances the activity of IGF on proliferation in several cell types, including prostate; and (c) a Western ligand blot, with $^{125}$I-labeled IGF-II as the ligand, demonstrated an increase in IGFBP-2 in the tumorigenic lines and a loss of IGFBP-2 expression in the IGFBP-rP1 overexpressing line [Ha clone (data not shown)]. This result would be consistent with what has been reported by our lab and others in prostate as well as in glioblastoma and other epithelial cancers. Also, there is less IGF binding in the poorly tumorigenic line than in the more aggressive cell lines, which further supports the notion that IGFBP-rP1 is acting in an IGF-independent fashion. Therefore, it seems unlikely that the effects on tumor suppression are due to the inhibition of IGF activity by the sequestration of the ligand from its receptor (11, 37).

A second possible mode of activity for IGFBP-rP1 that would be associated with tumor suppression was suggested because of its sequence homology with follistatin (11, 37). In the benign prostate, activin—a member of the TGF-$\beta$ family—is produced by stromal and epithelial cells and is proapoptotic for the epithelium, like other members of the TGF-$\beta$ family (38, 39). Follistatin, an inhibitory binding protein for activin, is normally produced only by the stromal cells; however, in the malignant prostate, follistatin is produced in the epithelium and binds activin, thereby inhibiting the proapoptotic effects of activin (38, 39). Through its homology to follistatin, it is possible that IGFBP-rP1 could interfere with the follistatin-activin complex and allow activin to become active. This would be an interesting, but complex, mechanism of action. It is of interest, however, that activin causes a morphological change in malignant human prostate epithelial cells that is similar to that shown here with overexpression of IGFBP-rP1 (40).

A third mechanism of IGFBP-rP1 tumor suppression may be a direct effect on nuclear cycling or apoptotic functions. Oh et al. (41) have shown that there is a nuclear localization sequence in the IGFBP-rP1 structure, and IGFBP-rP1 localizes to the nucleus in human breast cancer cell lines. Whether it subsequently binds to macromolecules and functions as a transcription factor has not been determined.

Finally, the mechanism of loss of IGFBP-rP1 expression in prostate cancer has not been elucidated. Although IGFBP-rP1 is located on human chromosome 4q12, deletions of chromosome 4 in prostate
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cancer specimens have only been identified on chromosomal arm 4p (26, 27).

In summary, we have described the loss of expression of a potential tumor suppressor gene in prostate cancer. This study demonstrated that re-expression of this gene in a human prostate cancer cell line results in an increase in cell doubling time, a decrease in colony formation in soft agar, a marked change in epithelial morphology along with an increased sensitivity to apoptosis, and finally decreased tumor formation and size in vivo. Furthermore, because it is unlikely that IGFBP-rP1 exerts its effects through binding IGF ligands but rather through a specific sequence that is IGFBP-like, a new group of tumor suppressor genes could potentially be identified once the specific sequences responsible for IGFBP-rP1 action are identified.

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