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Antisense RNA to the type I insulin-like growth factor receptor suppresses tumor growth and prevents invasion by rat prostate cancer cells *in vivo*

(prostate adenocarcinoma III cells/gene therapy/tumorigenesis/plasminogen activators)

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ABSTRACT **Prostate carcinoma is the second leading cause of death from malignancy in men in the United States. Prostate cancer cells express type I insulin-like growth factor receptor (IGF-IR) and prostate cancer selectively metastazises to bone, which is an environment rich in insulin-like growth factors (IGFs), thereby supporting a paracrine action for cancer cell proliferation. We asked whether the IGF-IR is coupled to tumorigenicity and invasion of prostate cancer. When rat prostate adenocarcinoma cells (PA-III) were stably transfected with an antisense IGF-IR expression construct containing the $ZnSO_4$-inducible metallothionein-1 transcriptional promoter, the transfectants expressed high levels of IGF-IR antisense RNA after induction with $ZnSO_4$, which resulted in dramatically reduced levels of endogenous IGF-IR mRNA. A significant reduction in expression both of tissue-type plasminogen activator and of urokinase-type plasminogen activator occurred in PA-III cells accompanying inhibition of IGF-IR. Subcutaneous injection of either nontransfected PA-III or PA-III cells transfected with vector minus the IGF-IR insert into nude mice resulted in large tumors after 4 weeks. However, mice injected with IGF-IR antisense-transfected PA-III cells either developed tumors 90% smaller than controls or remained tumor-free after 60 days of observation. When control-transfected PA-III cells were inoculated over the abraded calvaria of nude mice, large tumors formed with invasion of tumor cells into the brain parenchyma. In contrast, IGF-IR antisense transfectants formed significantly smaller tumors with no infiltration into brain. These results indicate an important role for the IGF/IGF-IR pathway in metastasis and provide a basis for targeting IGF-IR as a potential treatment for prostate cancer.**

Prostate carcinoma is the most commonly diagnosed cancer in men and the second leading cause of death from malignancy in men in the United States (1). If diagnosed after the carcinoma metastazises, prostatic cancer is a fatal disease for which there is no cure (2). Prostate cancer preferentially metastazises to bone where insulin-like growth factors (IGFs) are two of the most abundant growth factors (3). Prostate cancer cells express type I insulin-like growth factor receptor (IGF-IR), which could faciliate the development of bone metastases because IGFs are required for cell growth.

The IGF-IR has been shown to play a central role in the mechanism of transformation (4). Human prostate cancer cells have been shown to express binding sites for insulin-like growth factor I (IGF-I) (5). Receptor studies have demonstrated the presence of specific binding sites for IGF-I on rat prostate adenocarcinoma cells (PA-III) (6). These cells were obtained from a spontaneously occurring tumor in an aged Lobund–Wistar (L–W) rat (7). The Pollard system of transplantable prostate tumors has been demonstrated to be an extremely useful model for the evaluation of antimetastatic and cytotoxic agents for the treatment of androgen-insensitive prostatic cancer (8, 9). Moreover, a recent study strongly supports the validity of the Pollard model of spontaneous prostate cancer in L–W rats (10). Therefore, we have applied our antisense strategy using an IGF-IR construct to study the effect of blocking the IGF-IR on tumorgenesis of transfected PA-III cells *in vivo*.

We have reported that treatment of rat glioblastoma (11) and mouse teratocarcinoma (12) with our antisense constructs to IGF-I resulted in complete regression of tumors in syngeneic animals. Furthermore, an identical antitumorigenic effect has been described for C6 glioblastoma cells that were treated with a similar antisense RNA strategy that targeted the IGF-IR (13). In this study, we demonstrate that the L–W rat prostate cancer cells, PA-III, express the IGF-IR; however, we could not detect expression of either IGF-I or IGF-II in these cells by Northern blot hybridization. We observed a decrease in urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) in tumor cells transfected with the IGF-IR construct. Plasminogen activators have been shown to play an important role in tumor growth and to contribute to the lysis of tumor-associated fibrin, which is a critical step in the generation of metastasis (14). More important, we have found that *in vivo* experiments with the PA-III cells transfected with IGF-IR antisense construct resulted in a delay of and significant inhibition of tumor growth in nude mice. These findings, taken together with our previous studies of antisense RNA to IGF-I, suggest a role for the IGF-IR in a metastatic androgen-independent rat prostate cancer and may provide the basis for the development of a therapeutic treatment for prostate cancer using antisense RNA to the IGF-IR.

MATERIALS AND METHODS

Cell Culture. The rat prostate adenocarcinoma cell line PA-III, a generous gift of Morris Pollard (University of Notre Dame, South Bend, IN), was maintained in routine tissue culture in Dulbecco's modified Eagle medium (GIBCO/BRL) supplemented with 10% fetal bovine serum (HyClone). Culture medium for transfected PA-III cells was supplemented with hygromycin B at 500 μg/ml (Calbiochem) to maintain selection pressure. Twenty-four hours before injection into 6-week-old male athymic nude mice (6×10^5 cells or 1.2×10^6 cells), PA-III cells were washed and maintained in serum-free medium plus 50 μM $ZnSO_4$. The human breast cancer cell line T47D was purchased from American Type Culture Collection

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Abbreviations: IGF-IR, type I insulin-like growth factor receptor; uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; PA-III, rat prostate adenocarcinoma cells; CMV, cytomegalovirus.

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and cultured in Richter's Medium (Biofluids, Rockville, MD) that was supplemented with 10% fetal bovine serum (HyClone).

Reverse Transcription–PCR and cDNA Clones. The cDNA probe for human type I IGF receptor was amplified from total RNA obtained from T47D cells using the reverse transcription–PCR method (15). The PCR reactions were performed with the following IGF-IR specific primers: IGFIR 1, 5′-AGAATGAAGTCTGGCTCCGG-3′ (nt 42–63) and IGFIR 2, 5′-GCAGCACTCATTGTTCTCGGTGC-3′ (nt 716–738), according to the cDNA sequence of human IGF-IR (16). The PCR product generated for human IGF-IR was subcloned into the *Hin*cII site of the vector pGEM3zf$^+$ (Promega). The entire cDNA sequence of both strands was determined by using the Sequenase system (United States Biochemical). The clone pIGF-I harboring a human hepatic cDNA for IGF-I and the placental cDNA probe for IGF-II were kindly provided by Martin Jansen (State University of Utrecht, The Netherlands) (17, 18). The cDNA probe pTAM2.5-a for mouse tPA was purchased from American Type Culture Collection (19). The cDNA for human uPA was kindly provided by A. Shevelev (Case Western Reserve University) (20). The cDNA fragment for chicken β-actin was obtained from Richard Hanson (Case Western Reserve University).

RNA Isolation and Hybridization. Total RNA was isolated from cells with Trizol reagent (GIBCO/BRL) according to the manufacturer's instructions. For the selection of poly(A)$^+$ RNA, the Messagemaker reagent assembly (GIBCO/BRL) was used. Total RNA and poly(A)$^+$ RNA were separated on a denaturing agarose gel and transferred to a Hybond-N nylon membrane (Amersham). The cDNA probes were labeled with [^{32}P]dCTP (NEN) using the random hexanucleotide primer method (21) and hybridized to Northern blots in 5× standard saline citrate (SSC), 5× Denhardt's solution, 10% (wt/vol) dextran sulfate, 0.1% (wt/vol) SDS, and 100 μg/ml denatured salmon sperm DNA at 65°C for 18 h. The filters were washed at room temperature for 15 min in 2× SSC followed by 5 to 15 min in 0.5× SSC and 0.5% (wt/vol) SDS at 65°C. Filters were exposed to x-ray film for 6 to 24 h. X-ray films were analyzed with the laser densitometer SciScan 5000 (United States Biochemical) and normalized relative to β-actin mRNA.

Transfection. The nontransfected PA-III cells are referred to as the parental cell line. Transfection of PA-III rat prostate cancer cells was accomplished using Lipofectin (GIBCO/BRL) according to the supplier's instructions with either the control (no insert) or the vectors that contained the IGF-IR cDNA insert in the antisense direction. After transfection, the cells were cultured in nonselective OPTI-MEM I reduced serum medium (GIBCO/BRL) for 24 h. Hygromycin was then added at the optimal concentration (predetermined to be 500 μg/ml for PA-III cells). For the isolation of single cell transfectants, clonal rings (Nalge) were used, and at least five single cell clones each of PA-III cells, containing either the control vectors or the antisense vectors, were further expanded under continued hygromycin selection.

Construction of the Episome-Based Plasmids pIGF-IRAS. For IGF-I receptor inhibition in PA-III cells, we assembled an episome-based vector pMT/EP with the $ZnSO_4$-inducible mouse metallothionein-1 (MT-1) promoter we described previously (22). The second episome-based vector pCEP4 (Invitrogen) used for the transfection experiments contains the cytomegalovirus (CMV) promoter that drives the expression of the insert in a constitutive manner. The human IGF-IR cDNA fragment containing 696 bp (from nucleotide position 42 in exon 1 to nucleotide position 738 in exon 3) was cloned into the *Hin*dIII/*Bam*HI sites of the pMT/EP and pCEP4 vectors, respectively, in the antisense orientation (see Fig. 1). The directional cloning of the IGF-IR cDNA insert was confirmed by restriction mapping.

Tumor Growth in Nude Mice. Untransfected and transfected PA-III cells were tested for tumorigenesis *in vivo* by s.c. injection of 6×10^5 cells or 1.2×10^6 cells through a 22-gauge needle over the right shoulder of 6-week-old athymic nude mice. In a second experiment, 1.2×10^6 cells were inoculated over the abraded calvaria of nude mice through a 27-gauge needle (23). Tumor diameters were measured at regular intervals with calipers and tumor volume in cm^3 were calculated by the formula: volume $\doteq$ (width)^2 × length/2. Data are presented as mean ± SE.

RESULTS

To determine whether PA-III cells express IGF-I or IGF-II, poly(A)$^+$ RNA obtained from the parental cells was analyzed by Northern blot hybridization. Strong hybridization signals were seen with the placental control RNA, whereas IGF-I and IGF-II were not detectable in RNA from the PA-III cells (data not shown).

As described, the eukaryotic expression vectors pMT/EP with a $ZnSO_4$-inducible metallothionein promoter and pCEP4 with a constitutive cytomegalovirus (CMV) promoter were used for expression of the antisense form of human IGF-IR in PA-III cells. The 0.7-kb cDNA fragment for IGF-IR was generated by reverse transcription-PCR on total RNA prepared from the human breast cancer cell line T47D and subcloned into the episomal vectors in the antisense orientation (Fig. 1). The presence of the IGF-IR cDNA insert in the antisense direction was confirmed by restriction mapping of the vector DNA and by restriction analysis on DNA isolated from transfected PA-III cells (data not shown).

After transfection of PA-III cells with vectors containing the IGF-IR cDNA in the antisense direction or control vectors without an insert, several clones were selected and maintained in culture for further growth. To induce IGF-IR antisense RNA expression, the derived PA-III cell transfectants harboring the vectors with the inducible metallothionein promoter were cultured for 24 hr in serum-free medium plus 50 μM $ZnSO_4$, whereas the PA-III cells transfected with the constitutive CMV promoter were treated with serum-free medium only. Total RNA (20 μg) from transfected PA-III cell clones was analyzed by Northern blot hybridization with a radioactive labeled IGF-IR cDNA probe. Fig. 2 demonstrates a strong expression of the 1.1-kb IGF-IR antisense transcript in five different selected PA-III clones. The PA-III cells transfected with the inducible metallothionein-1-promoter maintained their high expression of IGF-IR antisense RNA for up to 60 days in culture under selection pressure with hygromycin, whereas PA-III cells transfected with the constitutive CMV-promoter lost their ability to produce antisense RNA to IGF-IR after 10 days in selective medium (data not shown).

To test whether transfected PA-III cells expressing high levels of IGF-IR antisense RNA could affect the expression of endogenous IGF-IR mRNA, we analyzed poly(A)$^+$ RNA from the cell clones and compared it with poly(A)$^+$ RNA from

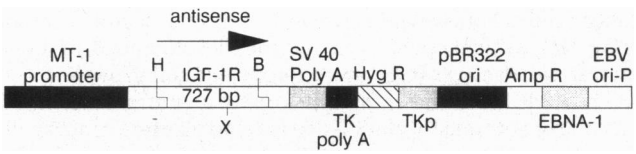

FIG. 1. Schematic representation of the episomal vector construct pIGF-IRAS. The 727-bp-long human IGF-IR fragment was cloned into an episomal vector in the antisense orientation containing either a $ZnSO_4$-inducible metallothionein promoter (MT-1) or a constitutive CMV promoter (not shown). SV 40, simian virus 40; Amp R, ampicillin resistance gene; Hyg R, hygromycin resistance gene; pBR 322 ori, pBR 322 origin of replication; EBNA-1, EBV-encoded nuclear antigen-1 (EBV, Epstein–Barr virus); EBV oriP, EBV origin of replication; TKp, thymidine kinase promoter; TK poly(A), thymidine kinase poly(A); H, *Hin*dIII; B, *Bam*HI; X, *Xho*I.

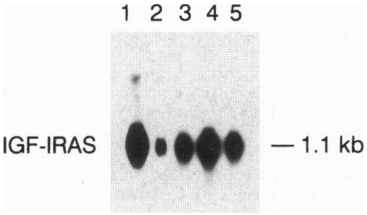

FIG. 2. Expression of antisense transcripts for IGF-IR (IGF-IRAS) by $ZnSO_4$ (lanes 1 and 3–5) or by a constitutive CMV promoter (lane 2) in cultured PA-III rat prostate cancer cells. Total RNA (20 μg) was used per lane, hybridized with the ^{32}P-labeled IGF-IR cDNA fragment and the membrane was exposed to an x-ray film for 24 hr at −80°C. The molecular size of IGF-IRAS is shown on the right [727 bases IGF-I receptor sequence plus ≈450 bases simian virus 40 poly(A) sequence].

PA-III cells transfected with the vector alone by Northern blot hybridization. We found that the expression of IGF-IR mRNA in the antisense-transfected clones was reduced by 65% to 86% relative to the control-transfected clone (Fig. 3 *A* and *E*). Rehybridization of the same membrane with radioactive-labeled cDNA probes for human uPA and mouse tPA revealed an inhibition of 65%-95% for uPA mRNA (Fig. 3 *C* and *F*) and 60%-85% for tPA mRNA (Fig. 3 *B* and *F*) in the IGF-IR antisense-transfected clones. The filter was rehybridized with a cDNA probe for chicken β-actin to verify the integrity and amount of poly(A)$^+$ RNA in the samples (Fig. 3 *D–F*).

To assess the effect of IGF-IR suppression on tumorgenicity of PA-III cells, male nude mice were inoculated s.c. with 1.2×10^6 of viable parental, control-transfected cells (vector without insert, pMT/EP) or IGF-IR antisense-RNA expressing cells (pIGF-IRAS) (Table 1). Animals in all groups developed local tumors; however, in animals injected with IGF-IR antisense-transfected PA-III cells, tumor appearance was dramatically delayed and tumor weight in these animals was 85% less than in the control groups (Fig. 4 *A Left* and *B Left*). In a second *in vivo* experiment, 6×10^5 PA-III cells treated as above were injected s.c. into nude mice. All animals in the control groups developed rapidly growing tumors, whereas 9 out of 12 nude mice injected with IGF-IR antisense-transfected PA-III cells had significantly smaller tumors and 3 mice remained tumor-free (Table 1). As depicted in Fig. 4 *A Left* and *B Left*, the remaining 9 out of 12 animals injected with IGF-IR antisense-transfected cells formed tumors that showed an inhibition of ≈95% in tumor growth and weight. In contrast, in animals injected with control cells (parental and pMT/EP, respectively) massive, solid tumors could be observed (Fig. 5 *A* and *B*, arrows), whereas IGF-IR antisense-expressing cells showed barely visible tumors (Fig. 5*C*, arrow) or no tumor formation (Fig. 5*D*).

To further investigate the metastatic behavior of PA-III cells, we inoculated 1.2×10^6 PA-III cells over the abraded calvaria of nude mice. A similar tumor growth curve to that achieved with subcutaneous injection of the PA-III cells was apparent. The control cells (vector without insert) formed rapidly growing tumors (Fig. 6*A*), whereas the IGF-IR antisense-transfected cancer cells showed a remarkable attenuation of tumor growth (Fig. 6*B*). Moreover, invasion of PA-III control cells into the brain of nude mice was observed (Fig. 6*C*, arrow), whereas the brains of animals injected with IGF-IR antisense RNA expressing PA-III cells remained tumor-free (Fig. 6*D*).

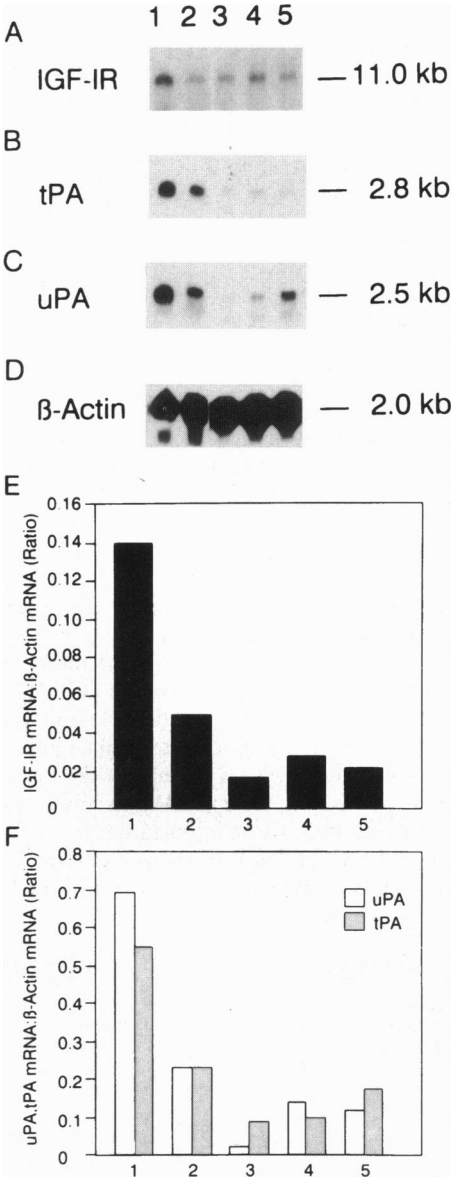

FIG. 3. Northern blot analysis of mRNA from transfected PA-III rat prostate cancer cells. (*A*) Poly(A)$^+$ RNA (12 μg per lane) derived from control-transfected (lane 1) and several IGF-IR antisense-transfected PA-III cell clones (lanes 2–5) was analyzed using an IGF-IR cDNA as a hybridization probe. (*B–D*) Rehybridization of the same filter was performed with cDNA probes for tPA, uPA, and β-actin, respectively. The mRNA signals were scanned using the laser densitometer SciScan 5000, and the difference in the expression of mRNA transcripts was calculated relative to the β-actin standards. Results of the analysis for IGF-IR (*E*) and for tPA and uPA (*F*) are shown in the bar graphs. The numbers under the bar graphs correspond to the numbers used in *A–D*.

DISCUSSION

Our previous work demonstrated that transfection of rat C6 glioblastoma cells with an episome-based vector expressing antisense IGF-I RNA abolished the tumorigenicity of these cells *in vivo* (24). We report here the application of a similar approach for a highly metastatic rat prostate cancer; however, we have targeted the IGF-IR, because neither IGF-I nor IGF-II were expressed by the rat PA-III cells. Recent studies have suggested the possibility that, if the IGF-IR was obligatory for transformation, a decrease in the number of IGF-IR could bring about reversal of an already transformed phenotype (25). We obtained high expression of IGF-IR antisense RNA in transfected rat PA-III prostate cancer cells when using a construct with the $ZnSO_4$-inducible mouse metallothionein-1 promoter, whereas the expression of IGF-IR antisense RNA was significantly lower in cells transfected with the construct containing the constitutive CMV-promoter,

Table 1. Tumor take rate of parental and transfected PA-III cells in nude mice

	Cells ($\times 10^6$)	Tumor take
s.c. injection*		
PA-III parental	1.2	11/11
PA-III pMT/EP	1.2	5/5
PA-III pIGFIRAS/MT3A	1.2	5/5
s.c. injection†		
PA-III parental	0.6	6/6
PA-III pMT/EP	0.6	12/12
PA-III pIGFIRAS/MT3A	0.6	9/12
Calvaria injection‡		
PA-III pMT/EP	1.2	2/2
PA-III pIGFIRAS/MT3A	1.2	3/3

*Animals were killed 28 days postinjection.
†Animals were killed 29 days postinjection. Three out of 12 nude mice injected with IGF-IR antisense-transfected cells (pIGF-IRAS/MT3A) remained tumor-free for 60 days of observation.
‡Mice were anesthesized by an i.p. injection of sodium pentobarbital at 30 mg/kg. Tumor cells were then inoculated over the abraded calvaria. Animals were killed 33 days after injection.

which could be due to a disadvantage for growth or to apoptotic cell death *in vitro* (26).

Antibody-blocking experiments with the monoclonal antibody (alpha-IR-3) that is specific for the IGF-IR have suggested that the IGF-IR plays an essential role for tumor growth *in vivo*. Previous studies demonstrated that alpha-IR-3 suppressed tumor formation *in vivo* in human rhabdomyosarcoma (27), human breast cancer (28), and Wilms' tumor (29). These animal experiments indicate an important role for IGF-IR and provide a basis for targeting IGF-IR as a potential treatment for certain cancers. A recent study using an IGF-IR mutant

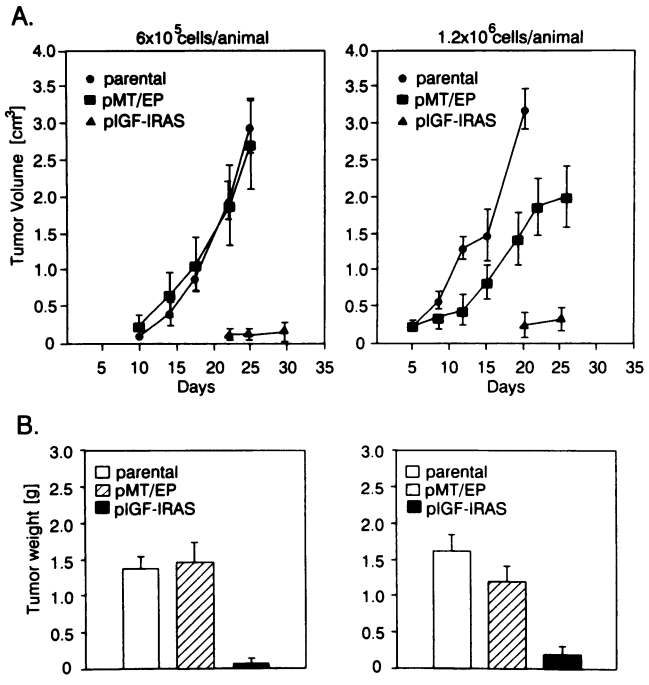

FIG. 4. Suppression of tumorigenesis by IGF-IR antisense-transfected PA-III cells *in vivo*. Either 6×10^5 (*A Left* and *B Left*) or 1.2×10^6 (*A Right* and *B Right*) parental, control-transfected (pMT/EP without an insert) and IGF-IR antisense-transfected PA-III cells were injected s.c. into male nude mice (see Table 1 for number of animals per group). (*A*) Tumor diameters were measured at regular intervals for up to 29 days with calipers and tumor volume was calculated. Each data point represents the mean ± SE of each group of mice. (*B*) The mice were killed 29 days postinjection, the tumors were excised, and the tumor weights were determined.

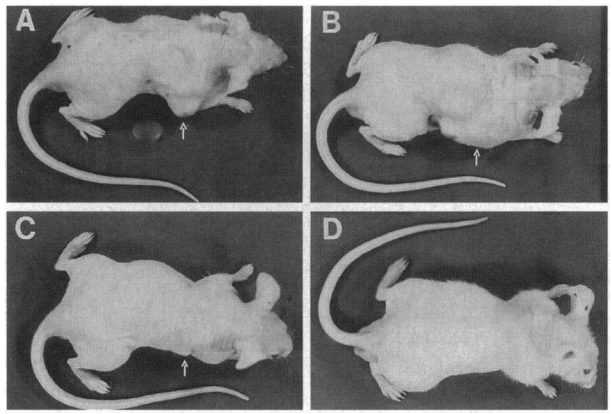

FIG. 5. Tumor growth in nude mice. Tumors derived from parental PA-III cells (*A*), control-transfected (pMT/EP) cells (*B*), and PA-III cells transfected with the construct expressing IGF-IR antisense RNA (*C*). Arrows indicate the positions of the tumors. (*D*) This mouse was injected with IGF-IR antisense-transfected PA-III cells and did not develop a tumor.

showed that a truncated form of IGF-IR could abrogate ligand-dependent cellular transformation and tumorigenesis mediated by wild-type IGF-IR (30).

We found that there was a significant reduction in the expression of endogenous IGF-IR mRNA in antisense-transfected PA-III cells *in vitro* that correlated to the delay and inhibition of tumorigenicity *in vivo*. Furthermore, three of the animals remained tumor-free until killed at 60 days postinjection. This could be due to a higher degree of apoptosis or to an immune response attributed to natural killer cells or B cells present in nude mice (31). Studies performed using antisense oligonucleotides with rat C6 glioblastoma cells support our findings (32). These investigators demonstrated a quantitative relationship between the level of IGF-IR and tumorigenesis in nude mice or syngeneic rats that is correlated to the extent of *in vivo* apoptosis and not to the extent of *in vitro* inhibition of growth. The same mechanism may be responsible for the dramatic delay and inhibition that we observed with the IGF-IR antisense treatment of rat PA-III prostate cancer cells.

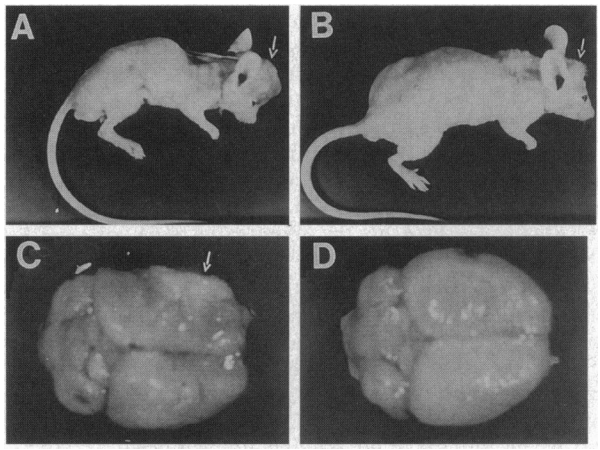

FIG. 6. Tumor formation in nude mice resulting from PA-III rat prostate cancer cells inoculated over the abraded calvaria of nude mice. Arrows indicate the positions of the tumors. (*A*) A mouse inoculated with control-transfected PA-III cells with pMT/EP construct lacking the IGF-IR insert. (*B*) An animal inoculated with PA-III cells transfected with the construct expressing IGF-IR antisense RNA. (*C*) The brain from a mouse that received an injection of control-transfected cells over the abraded calvaria. A large macroscopic tumor mass (arrow) invaded into the brain parenchyma, whereas (*D*) the brain from an animal injected with IGF-IR antisense-transfected cells appeared normal.

In the present study, we observed a significant reduction in the expression of the plasminogen activators, tPA and uPA, in antisense-transfected IGF-IR PA-III cells. These serine proteases have been shown to be involved in tumor invasion *in vivo* (33). Indeed, urokinase overproduction results in increased skeletal metastasis by human prostate cancer cells (34). It has been reported that uPA expressed by PA-III prostate cancer cells is implicated in the osteoblastic process through its protease action by hydrolyzing IGF binding proteins, which results in increased bioavailibility of IGFs (35). Our data indicate that a decrease in IGF-IR gene expression can down-regulate plasminogen activator expression in rat PA-III cells. The reduction in the expression of these proteases that occurs following transfection of the PA-III cells with the IGF-IR antisense RNA suggests that the invasive and metastatic processes of the cancer cells can be altered by a decrease in the level of plasminogen activators that may be mediated through the IGF-IR. A recent study has reported that IGF-I and epidermal growth factor can up-regulate plasminogen activator expression in rat astrocytes (36). While it is clear that growth factors can alter plasminogen activator levels, the correlation between the IGF/IGF-IR pathway and plasminogen activator expression remains to be elucidated.

Prostate cancer selectively spreads to the cancellous bones of the axial skeleton, where it is the only malignancy to consistently produce osteoblastic lesions (37). Bone has been shown to be a major source of IGFs with humans having the highest concentration of total somatomedins (38). Studies have shown that human prostate and ovarian cancer cell lines grow by an autocrine loop in which IGF-I activates its receptor and interference with activation of the receptor leads to cessation of growth *in vitro* (39, 40). However, it has been postulated that a paracrine regulatory loop exists between PA-III cells and osteoblasts, which provide a rich source of IGFs supporting the growth of the cancer cells (41). In our experiment in which we placed control-transfected PA-III cells over the abraded calvaria of nude mice, we observed an osteoblastic response with invasion of tumor cells into the adjacent brain tissue. In contrast, the IGF-IR antisense-transfected PA-III cells formed significantly smaller tumors that did not penetrate into the brain. These results further demonstrate the importance of the IGF-IR in tumor invasion because the transfected cells were confined to the bone. A recent study that demonstrated the loss of the metastatic phenotype in murine carcinoma cells expressing an antisense RNA to the IGF-IR supports our findings (42).

The results of this study could provide a basis for designing new strategies for treatment of prostate cancer that would involve targeting the IGF-IR at the cellular level by preventing expression of IGF-IR in prostate cancer cells. Such an approach would target tumorigenesis arising by a paracrine feedback from the neighboring microenvironment. Interfering with the IGF-IR on prostate cancer cells could block the access of IGFs from the microenvironment to the tumorigenic cells either in the prostate itself or at metastatic sites, thereby eliminating the mitogenic/transforming influence of IGFs from these sources. Furthermore, the reduction in the expression of plasminogen activators following IGF-IR transfection could alter the metastatic potential of the cancer cells.

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