Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 8864–8868, July 1998
Medical Sciences

Growth hormone-releasing hormone antagonist MZ-5-156 inhibits growth of DU-145 human androgen-independent prostate carcinoma in nude mice and suppresses the levels and mRNA expression of insulin-like growth factor II in tumors

NAJIB LAMHARZI*†, ANDREW V. SCHALLY*†‡, MIKLÓS KOPPÁN*†§, AND KATE GROOT*

*Endocrine, Polypeptide, and Cancer Institute, Veterans Affairs Medical Center, and †Department of Medicine, Tulane University School of Medicine, New Orleans, LA 70146

Contributed by Andrew V. Schally, May 20, 1998

ABSTRACT **Insulin-like growth factors I and II (IGF-I and -II) are potent mitogens for various cancers, including carcinoma of the prostate. In several experimental cancers, treatment with antagonists of growth hormone-releasing hormone (GH-RH) produces a reduction in IGF-I and -II, concomitant to inhibition of tumor growth. To investigate the mechanisms involved, we treated male nude mice bearing xenografts of DU-145 human androgen-independent prostate cancer for 8 weeks with potent GH-RH antagonist MZ-5-156 at a dose of 20 μg/animal s.c. twice a day. Tumor growth, serum and tumor levels of IGF-I and -II, and the mRNA expression of IGF-I and -II in tumors were evaluated. After 8 weeks of therapy, final volume and weight of DU-145 tumors in mice treated with MZ-5-156 were significantly ($P < 0.01$) decreased compared with controls, and serum IGF-I showed a significant reduction. Treatment of nude mice bearing DU-145 xenografts with MZ-5-156 also significantly ($P < 0.01$) diminished by 77% the levels of IGF-II in tumor tissue compared with controls, but did not affect the concentration of IGF-I. Reverse transcription–PCR analyses revealed a high expression of IGF-II mRNA in DU-145 tumors. Treatment with GH-RH antagonist MZ-5-156 decreased the expression of IGF-II mRNA by 58% ($P < 0.01$) as compared with controls. Our work suggests that GH-RH antagonist MZ-5-156 may inhibit the growth of DU-145 human androgen-independent prostate cancers through a reduction in the production and mRNA expression of IGF-II by the tumor tissue. These findings extend our observations on the mechanism of action of GH-RH antagonists and may explain how GH-RH antagonists inhibit tumor growth.**

Carcinoma of the prostate is the most common malignant tumor in men (1). About 70% of human prostate cancers are testosterone-dependent (2). The present methods of treatment for advanced prostate cancer are palliative and based on androgen deprivation (2). However, after a period of remission, a relapse occurs because of acquisition of tumor growth autonomy. An incomplete understanding of prostate cancer pathophysiology has hampered the development of effective therapies for patients with hormone-refractory disease. Various growth factors may be responsible for the relapse. There is evidence that growth factors, such as epidermal growth factor (EGF), transforming growth factor α and β, and insulin-like growth factors I and II (IGF-I and -II), and some neurohormones such as luteinizing hormone-releasing hormone (LH-RH), somatostatin, and bombesin/gastrin-releasing peptide (GRP) can affect the function and growth of the prostate gland (3, 4).

IGF-I and IGF-II are produced by various tissues, are potent mitogens for several cell types, and regulate normal and malignant growth through endocrine, paracrine, or autocrine mechanisms (5–9). Most biological actions of IGFs are thought to be mediated through the IGF-I receptor (8, 10, 11), which has tyrosine kinase activity (12). The blockade of IGF-I receptor by a specific antibody inhibits autonomous cell growth in an IGF-I-producing small-cell lung carcinoma (SCLC) cell line (13). Similar results have been obtained in an IGF-II-producing neuroblastoma cell line (7). IGF-I and -II appear to be involved in the malignant transformation of cells and the progression of many tumors, including prostate cancer (14–18). Consequently, the blocking of the production of IGFs might lead to improvement in the treatment of advanced androgen-independent prostate cancer.

Recently, we developed various potent and specific antagonists of growth hormone-releasing hormone (GH-RH), such as [PhAc-Tyr^1, DArg^2, Phe(4-Cl)^6, Abu^{15}, Nle^{27}, Agm^{29}]hGH-RH(1–29) (MZ-5-156) (19, 20). By suppressing GH secretion, these compounds might decrease the synthesis of IGF-I in the liver or other tissues. GH-RH antagonists could also reduce the autocrine and paracrine production of IGF-I and/or IGF-II by various tumors (18, 21–24). In the present study, we investigated the effects of the GH-RH antagonist MZ-5-156 on tumor growth, IGF-I and IGF-II levels in serum and tumors, as well as the expression of IGF-I and IGF-II mRNA in the DU-145 human hormone-independent prostate cancer line xenografted into nude mice.

MATERIALS AND METHODS

Peptide and Reagents. GH-RH antagonist (MZ-5-156) PhAc-[D-Arg^2, Phe(p-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1–28)Agm [where PhAc is phenylacetyl, Phe(p-Cl) is parachlorophenylalanyl, Abu is α-aminoisobutyryl, Nle is norleucyl, and Agm is agmatine] was synthesized by solid-phase methods and purified in our laboratory (20). For daily injections, MZ-5-156 was dissolved in 0.1% dimethyl sulfoxide (DMSO) in sterile 10% propylene-glycol/saline solution (Sigma).

Animals. Male athymic (Ncr *nu/nu*) nude mice, approximately 6 weeks old on arrival, were obtained from the National

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GH-RH, growth hormone-releasing hormone; IGF, insulin-like growth factor; RT-PCR, reverse transcription–PCR; SCLC, small-cell lung carcinoma.

‡To whom reprint requests should be addressed at: Veterans Affairs Medical Center, 1601 Perdido Street, New Orleans, LA 70146.
§On leave from the Department of Human Anatomy, University Medical School of Pécs, Pécs, Hungary.

Cancer Institute (Bethesda, MD) and housed in laminar airflow cabinets under pathogen-free conditions with a 12-h light/12-h dark schedule and fed autoclaved standard chow and water ad libitum. Their care was in accord with institutional guidelines.

Cell Culture. The human androgen-independent prostatic carcinoma line DU-145 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). DU-145 cells were grown as a monolayer in RPMI 1640 medium (GIBCO) supplemented with 10% fetal bovine serum, antibiotics, and antimycotics at 37°C in a humidified 95% air/5% CO_2 atmosphere. Tumor cells growing exponentially were harvested by brief incubation with 0.25% trypsin-EDTA solution (GIBCO). Xenografts of DU-145 were initiated by s.c. injection of 1×10^7 cells into the left flanks of five male nude mice.

Experimental Protocol. DU-145 tumors resulting after 8 weeks of growth were aseptically dissected and mechanically minced; 3-mm^3 pieces of each tumor tissue were transplanted s.c. by trocar needle into male nude mice under methoxyflurane (Metofane, Pitman–Moore, Mundelein, IL) anesthesia. Six weeks after transplantation when tumors had grown to a volume between 40 and 50 mm^3, tumor-bearing mice were divided into two groups of eight animals each. The control group was injected only with 0.1% DMSO in 10% propyleneglycol/saline s.c. (vehicle solution) and the experimental group was treated with 20 μg of GH-RH antagonist MZ-5-156 s.c. twice a day. The treatment was continued for 8 weeks. The tumors were measured once a week with microcalipers, and the tumor volume was calculated as length $\times$ width $\times$ height $\times$ 0.5236 (25). At the end of the experiment, mice were anesthetized with methoxyflurane and sacrificed by decapitation, and trunk blood was collected. The serum was separated and analyzed by radioimmunoassay. Body weights were recorded and the tumors were carefully dissected, cleaned, and weighed, and samples of each tumor were taken for radioimmunoassay and molecular biology analyses.

Radioimmonoassay for IGF-I and IGF-II. To determine IGF-I and IGF-II levels in DU-145 prostate cancers, 100 mg of tumor tissue was homogenized in 1 ml of homogenization buffer (0.05 M Tris·HCl/0.005 M EDTA/0.005 M $MgCl_2$/30 μg/ml bacitracin, pH 7.6) and centrifuged at 2000 $\times$ *g* (20 min at 4°C). Protein determination in the supernatant was performed by using the Bio-Rad protein assay kit. All serum and reconstituted tumor samples were extracted by a modified acid–ethanol cryoprecipitation method reported previously (26, 27). This method eliminates most of the IGF-binding proteins that can interfere in the RIA. The extracted IGF-I in serum and tumor samples was measured by RIA using IGF-I (88-G4, Genentech) as a standard in the range of 2–1,000 pg/tube and also for iodination by the chloramine-T method. Antibody UB-2–495 (a gift from L. E. Underwood and J. Van Wyk, University of North Carolina, Chapel Hill) obtained from the National Institute of Diabetes and Digestive and Kidney Diseases was used at the final dilution of 1:14,000 in the RIA. IGF-II was measured by using human recombinant IGF-II (Bachem) in the range of 2–1,000 pg/tube. IGF-II was iodinated by lactoperoxidase method and purified by reverse-phase HPLC liquid chromatography using a Vydac C18 column. For the assay, Amano mAb generated against rat IGF-II (10 μg/ml) was used at a final dilution of 1:14,285 (Amano International Enzyme, Troy, VA). This antibody cross-reacts 100% with human and rat IGF-II and 10% with human IGF-I (28).

RNA Extraction. Total RNA was extracted from human DU-145 tumors by using RNAzolJ B (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. The RNA pellets were suspended in 50 μl of 10 mM Tris/1 mM EDTA buffer (pH 8.0) and quantified spectrophotometrically at 260 nm.

Reverse Transcription–PCR (RT-PCR). One microgram of total RNA extracted from DU-145 prostate tumors was reverse-transcribed into single-strand cDNA by using Moloney murine leukemia reverse transcriptase according to the manufacturer's instructions (Perkin–Elmer). After an initial 2 min of denaturation step at 94°C, the RT reaction mixture was subjected to 27 cycles of PCR amplification by using specific primers for human IGF-I, IGF-II, and β-actin (Table 1) (29–31). These primers were synthesized by use of the standard model 394 DNA/RNA synthesizer (Applied Biosystems). The number of cycles was determined previously to be within the exponential range of PCR product amplification necessary for quantitative densitometry. Each cycle consisted of a 1-min denaturation step at 94°C, a 1-min annealing step at 54°C, and 2 min of elongation at 72°C. The last cycle was followed by 10 min of elongation at 72°C using a thermal cycler (Stratagene). Negative controls were run in parallel to control for cross-contamination of samples. A reaction was performed as described above but without the addition of reverse transcriptase as a test for the presence of contaminating genomic DNA in the RNA preparation from these tumors. Ten microliters of PCR-amplified product was resolved by electrophoresis on a 1.8% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

Southern Blot Analysis. After electrophoresis, the gel was treated with denaturation buffer, then by neutralization buffer, the PCR products were transferred to Hybond N^+ membrane by capillary transfer, and the cDNA was linked onto it by heating for 2 hr at 80°C. The prehybridization was realized at 37°C for 4 hr in buffer containing 50% formamide, 1 M NaCl, 1% SDS, and 100 μg/ml denatured and sonicated salmon DNA. The membrane then was hybridized in prehybridization buffer containing human ^{32}P-labeled IGF-II cDNA (1 kbp) or ^{32}P-labeled β-actin cDNA (1.1 kbp). Both cDNAs were purchased from the ATCC and were labeled by using a multiprime labeling system (Amersham). The blots were washed under stringent conditions, and the signals from samples were scanned and quantified by using an imaging densitometer (Model GS-700, Bio-Rad).

cDNA Sequencing. PCR product obtained after RT-PCR analysis using specific primers for IGF-II was sequenced by using modified protocols of ABI Big Dye Terminator chemistry (Genome Systems, St. Louis). The cDNA sample was analyzed by B. Blakey by using the ABI Prism 337 DNA sequencer at Genome Systems.

Statistical Analyses. All values are expressed as the mean $\pm$ SEM, and statistical analyses were performed by using Duncan's multiple range test.

Table 1. Oligonucleotide primers used in RT-PCR analysis for human IGF-I, IGF-II, and β-actin mRNA expression in DU-145 human androgen-independent prostate tumors

mRNA	Primers	Sequences (5′ . . . 3′)	Position	Target size	Ref.
IGF-I	Sense	ACATCTCCCATCTCTCTGGATTTCCTTTTGC	85–116	514 bp	29
	Antisense	CCCTCTACTTGCGTTCTTCAAATGTACTTCC	567–598		
IGF-II	Sense	AGTCGATGCTGGTGCTTCTCACCTTCTTGGC	270–300	538 bp	30
	Antisense	TGCGGCAGTTTTGCTCACTTCCGATTGCTGG	776–807		
β-actin	Sense	ATCTGGCACCACACCTTCTACAATGAGCTGCG	294–326	518 bp	31
	Antisense	GCAGCGGAACCGCTCACCGCCAATGGTGAT	782–812		

RESULTS

Effect of GH-RH Antagonist MZ-5-156 on Growth of DU-145 Tumors. After 6 weeks of treatment, the volume of DU-145 tumors in the group receiving MZ-5-156 was significantly ($P < 0.05$) decreased to 108.0 ± 29.0 mm^3 as compared with the control group (254.6 ± 71.5 mm^3) corresponding to a 57.4% reduction (Fig. 1). After 8 weeks, the final tumor volume was significantly ($P < 0.01$) diminished in animals treated with MZ-5-156 to 113 ± 47.9 mm^3 compared with controls, which measured 403 ± 45.0 mm^3, corresponding to a 71% decrease. At the end of the experiment, there also was a 66.8% ($P < 0.01$) reduction in tumor weight in the group injected with MZ-5-156 (112.5 ± 37.1 mg) as compared with the control group (338.0 ± 24.3 mg). There were no significant differences in body weights between the treated (32.7 ± 1.57 g) and untreated (32.8 ± 2.35 g) animals.

Effect of GH-RH Antagonist MZ-5-156 on IGF-I and IGF-II Levels in Serum and DU-145 Tumors at the End of the Treatment Period. The serum and tumor tissue levels of IGF-I and IGF-II in control animals and in nude mice treated with MZ-5-156 are shown in Table 2. In mice bearing DU-145 tumors, the serum level of IGF-I was significantly ($P < 0.05$) lowered by treatment with MZ-5-156 to 88.3 ± 6.7 ng/ml as compared with the control group (111.5 ± 6.5 ng/ml) whereas serum IGF-II levels were similar in both groups. The IGF-I concentration in DU-145 tumors was not affected significantly after therapy with MZ-5-156 (402 ± 77.4 pg/100 μg protein) as compared with the control group (331 ± 44.5 pg/100 μg protein). However, administration of MZ-5-156 significantly ($P < 0.05$) reduced the levels of IGF-II in DU-145 tumors to 304 ± 196 pg/100 μg protein at the end of the treatment period, as compared with the control group, which showed 1486 ± 252 pg/100 μg protein.

Effect of MZ-5-156 on IGF-II mRNA Expression in DU-145 Tumors. RT-PCR analysis was performed to detect the expression of IGF-I and IGF-II mRNA in DU-145 tumors. Using primers for human IGF-I, we could not find the expression of IGF-I mRNA in DU-145 tumors. In contrast, using specific primers for human IGF-II, we detected a high expression of IGF-II mRNA. The PCR product obtained after amplification was of the expected size (538 bp) (Fig. 2*a*). Amplification with specific primers for human β-actin (internal control) produced a single product of 518 bp (Fig. 2*b*). No PCR product was amplified from the negative control. Visual observations of the bands for IGF-II obtained after ethidium bromide staining revealed a low signal of the PCR products in the group treated with MZ-5-156 compared with the untreated group. Southern blot analysis confirmed the specificity of the PCR products for IGF-II (Fig. 3*a*) and β-actin (Fig. 3*b*). Additional confirmation that the PCR product corresponds to IGF-II was obtained by sequencing. Semiquantitative analysis of the developed bands for IGF-II was performed by densitometry. The signals obtained for IGF-II were standardized according to the signals obtained for β-actin. After treatment with GH-RH antagonist MZ-5-156, the mRNA level for IGF-II was decreased to 41.8 ± 7.1% ($P < 0.01$) as compared with controls accepted as 100 ± 6.1% (Fig. 4). This confirmed that the decrease in the level of IGF-II in DU-145 tumors observed after treatment with GH-RH antagonist MZ-5-156 is associated with the inhibition of mRNA for IGF-II.

Table 2. IGF-I and IGF-II levels in the serum and tumor tissue of nude mice bearing DU-145 human androgen-independent prostate xenografts at the end of the treatment period with GH-RH antagonist MZ-5-156

	Serum		Tumor	
Treatment group	IGF-I, ng/ml	IGF-II, ng/ml	IGF-I, pg/100 μg protein	IGF-II, pg/100 μg protein
Control	111.5 ± 6.5	35.1 ± 2.48	331 ± 44.57	1,486 ± 252
MZ-5-156	88.3 ± 6.7*	39.8 ± 4.95	402 ± 77.48	304 ± 196.2*

*$P < 0.05$ vs. control.

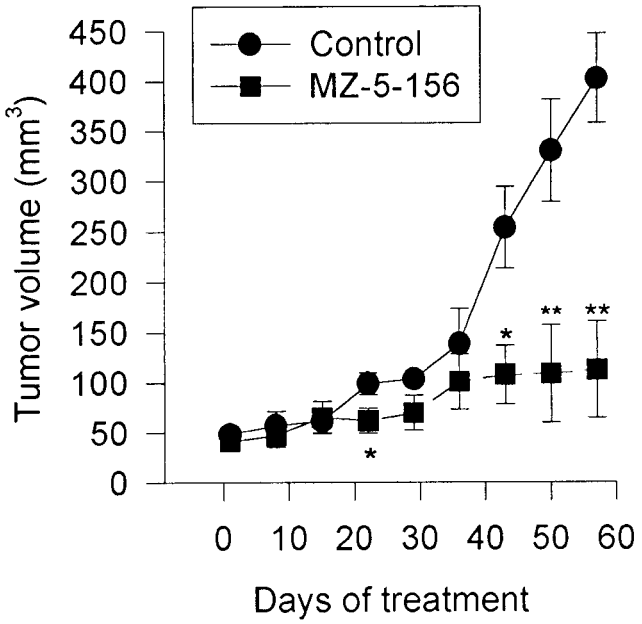

FIG. 1. Tumor volume in athymic mice bearing subcutaneously transplanted DU-145 human androgen-independent prostate cancers after treatment with the GH-RH antagonist MZ-5-156 administered s.c. at a dose of 20 μg per animal twice a day (b.i.d.). Treatment was started when tumors measured approximately 40–50 mm^3 and lasted for 8 weeks. Vertical lines indicate the SEM. *, $P < 0.05$; **, $P < 0.01$ versus control.

DISCUSSION

Prostate cancer is the second-leading cause of cancer-related deaths among men in the United States (1). After an initial response to androgen-deprivation therapy, most patients with advanced prostate cancer relapse as the tumors acquire hor-

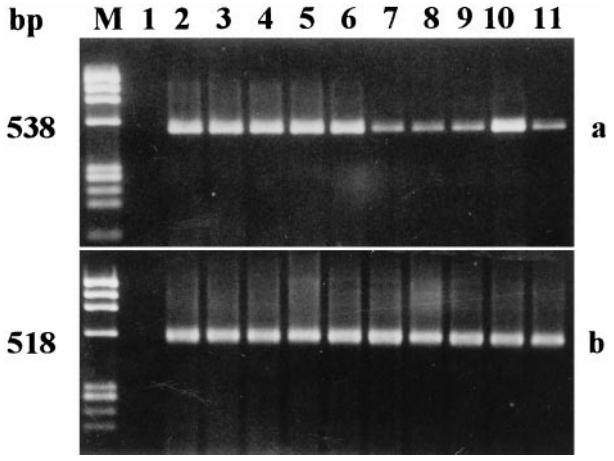

FIG. 2. RT-PCR analysis of human IGF-II (*a*) and β-actin (*b*) mRNA expression in DU-145 prostate tumors. PCR products were separated by 1.8% agarose gel electrophoresis and stained with ethidium bromide. The sizes of expected PCR products were 538 and 518 bp for hIGF-II and hβ-actin, respectively. Lanes: M (molecular weight marker), MX174 *Hae*III digest; 1, negative control; 2–6, samples from untreated animals; 7–11, tumor samples from animals treated with GH-RH antagonist MZ-5-156.

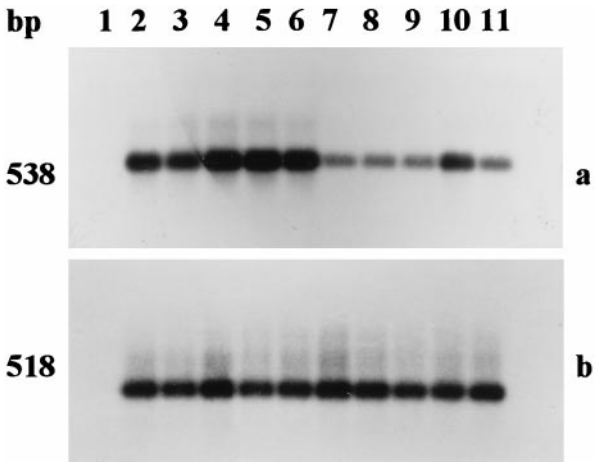

FIG. 3. Southern blot analysis of cDNA for human IGF-II (*a*) and β-actin (*b*) obtained after RT-PCR. The PCR products were separated by 1.8% agarose gel electrophoresis and transferred to Hybond N^+ membranes. Hybridizations were performed with cDNA probes specific for hIGF-II or hβ-actin. The blots were washed under stringent conditions and exposed to autoradiography. Numbering is the same as in Fig. 2.

mone independence and growth autonomy (2, 3, 32). The DU-145 cancer cell line, derived from a human adenocarcinoma metastatic to the brain, is androgen-independent (33). This cell line can be xenografted into nude mice and represents a suitable model for investigating the effects of various classes of antitumor peptides on androgen-independent prostate cancers *in vivo* and *in vitro* (21, 34).

The findings of this study with a recently synthesized GH-RH antagonist, MZ-5-156 (20), confirm and extend our previous reports, which showed that a less potent GH-RH antagonist, MZ-4–71 (19), inhibited the growth of androgen-independent PC-3 and DU-145 human prostate cancers xenografted into nude mice and Dunning R-3327-AT-1 prostate tumors in rats (21). We likewise have demonstrated that antagonist MZ-4–71 or MZ-5–156 could suppress *in vivo* proliferation in nude mice of various human cancer lines such as Caki-I renal adenocarcinoma (22), SK-ES-1 and MNNG/HOS osteosarcomas (23), and H-69 SCLCs and H-157 non-SCLCs (24).

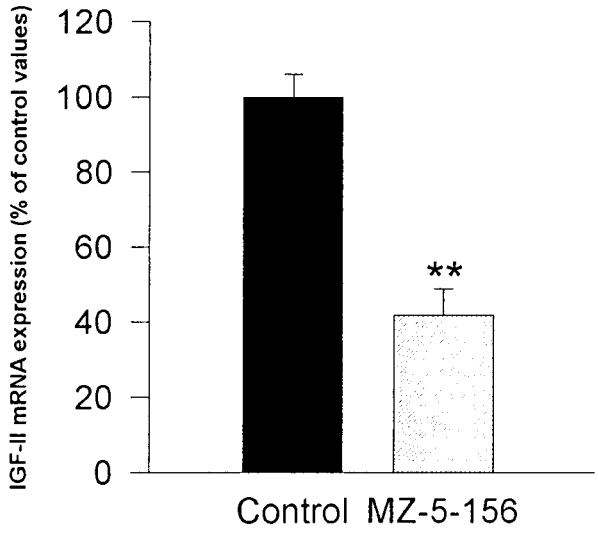

FIG. 4. Effect of GH-RH antagonist MZ-5-156 on the expression of mRNA for IGF-II in DU-145 human androgen-independent prostate cancers. After densitometry, the levels of mRNA for hIGF-II were standardized according to the levels of hβ-actin mRNA and are expressed as a percentage of control value. **, $P < 0.01$ versus control.

The present work also shows that the treatment with GH-RH antagonist MZ-5-156 decreases serum IGF-I and IGF-II levels in DU-145 tumors. This is in agreement with our previous observations that GH-RH antagonist MZ-4–71 reduced serum GH and IGF-I levels and decreased the concentration of IGF-I and IGF-II in PC-3 prostate tumors (21). However, no significant effect was observed on the level of IGF-I in DU-145 prostate tumors after treatment with MZ-5-156.

Using molecular biology analyses, we showed that IGF-I mRNA is not expressed in DU-145 tumors, whereas a high level of IGF-II mRNA is detected in these tumors. Figueroa, *et al.* (35) demonstrated that DU-145 and PC-3 prostate cancer cell lines express IGF-II mRNA but not IGF-I mRNA. Angelloz-Nicoud and Binoux (36) determined that PC-3 cells secreted IGF-II but not IGF-I. Similarly, Iwamura *et al.* (37) could not detect the secretion of IGF-I in DU-145 and PC-3 cell lines. In contrast, Pietrzkowski *et al.* (38) reported that DU-145 and PC-3 cells maintain an autocrine production of IGF-I.

Elevated levels of IGF-II and, less commonly, IGF-I have been observed in different tumors (9, 10). A high level of IGF-II mRNA has been reported in Wilms' tumor, rhabdomyosarcoma, hepatoblastoma, liposarcoma, colon carcinoma, pheochromocytoma, some neuroblastomas, leiomyomas, and leiomyosarcomas (10). It has been shown that prostatic stromal cells from patients with benign prostatic hyperplasia express higher levels of mRNA for IGF-II than normal prostate cells (39). Our work shows that treatment with GH-RH antagonist MZ-5-156 leads to a significant decrease in IGF-II and its mRNA in DU-145 prostatic tumors. The correlation between the decrease in the level of IGF-II and its mRNA after treatment with MZ-5-156 suggests that the reduction in IGF-II is the result of inhibition of gene transcription or a change in the stability of the mRNA for IGF-II. In addition, the tumor growth inhibition after treatment with GH-RH antagonists is likely to be the result of the inhibition of the autocrine or paracrine production of IGF-II in DU-145 prostate cancers.

The mechanisms of action by which GH-RH antagonists inhibit tumor growth and suppress the level of IGF-II and its mRNA in tumors are still unknown. GH-RH antagonists could affect the growth of prostate cancers through indirect or direct pathways (18). The indirect mechanism would operate through a suppression of the GH release from the pituitary and the resulting inhibition of the hepatic production of IGF-I (18). We have shown that GH-RH antagonists decrease the level of IGF-I and GH in serum of nude mice bearing prostatic and renal cancers, osteosarcomas, and SCLC and non-SCLC xenografts (21–24). These results suggest that the inhibitory effect of GH-RH antagonist on tumor growth *in vivo* could be a result, in part, of suppression of GH and IGF-I secretion. However, the results of this investigation and previous studies (18, 21, 22, 24) demonstrate that a major decrease in tumor IGF-II levels also is found in Caki-I renal carcinomas, PC-3, and DU-145 prostate cancers, and H-157 non-SCLC xenografted into nude mice after therapy with GH-RH antagonists. In addition, a significant reduction in concentration of IGF-I was produced in osteosarcomas (23), renal cancers, and PC-3 prostate tumors as well as in non-SCLCs (21, 22, 24) after treatment of nude mice with MZ-4–71 or MZ-5-156. A strong suppression of IGF-II mRNA expression in DU-145 tumors after treatment with MZ-5-156 points to a likely direct effect of GH-RH antagonist on tumors. Ongoing *in vitro* studies on the effect of GH-RH antagonists on DU-145 and other tumors should help clarify the mechanism of action. The results will be reported elsewhere (V. Csernus and A.V.S., unpublished data). Investigations are in progress to identify the peptide receptors in tumors that respond to GH-RH antagonists and that could be different from the pituitary GH-RH receptors (H. Kiaris, G. Halmos, and A.V.S., unpublished data). GH-RH is a member of the family of peptides that includes glucagon, secretin, vasoactive intestinal peptide (VIP), gastric inhibitory

peptide, and pituitary adenylate cyclase-activating peptide (PACAP) (40, 41). These peptides demonstrate considerable amino acid sequence homology (40, 41). The human and rat GH-RH receptors are homologous to secretin and VIP receptor proteins (42). VIP_1 and PACAP receptors have been identified in certain tumors (43). GH-RH can compete for binding to common receptor proteins, such as the VIP receptor, and produce similar biologic responses in many target organs (40, 41, 44, 45). GH-RH antagonists might also bind with reduced affinity to some of these receptors in tumors. Further studies are required to determine the receptors involved in the action of GH-RH antagonists on tumors.

Antagonists of GH-RH are needed clinically for tumor therapy because somatostatin analogues do not adequately suppress GH and IGF-I levels (2, 17, 18, 46, 47). The combination of GH-RH antagonists and somatostatin analogues could achieve a stronger tumor growth inhibition (18, 46, 47). In addition, some tumors do not express somatostatin receptors or the preferred subtype of somatostatin receptors to allow a response to somatostatin analogs (2). The suppression of production of IGF-I in the liver and tumors and/or autocrine/paracrine secretion of IGF-II by the tumors might inhibit cancer proliferation. These considerations suggest the merit of further development of antitumor agents based on GH-RH antagonists.

In conclusion, our findings indicate that the inhibition of growth of DU-145 human androgen-independent prostate cancers in nude mice by the GH-RH antagonists may be mediated through a reduction in the levels and mRNA expression of IGF-II in tumors. These results reinforce the view that GH-RH antagonists could be used for the treatment of prostate cancers and other cancers that are influenced by IGFs.

We thank Patricia Armatis for her technical assistance. The gifts of materials used in the RIA from the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases are appreciated. The sequencing of the IGF-II cDNA was performed by Brandon Blakey (Genome Systems). The work described in this paper was supported by the Medical Research Service of the Veterans Affairs Department, CaP Cure Foundation Research Award, and a grant from ASTA Medica (Frankfurt on Main, Germany) (all to A.V.S.).

1. Parker, S. L., Tong, T., Bolden, S. & Wingo, P. A. (1996) *CA Cancer J. Clin.* **46,** 5–27.
2. Schally, A. V. & Comaru-Schally, A. M. (1997) in *Cancer Medicine*, eds. Holland, J. F., Frei, E., III, Bast, D. R., Jr., Kufe, D. E., Morton, D. L. & Weichselbaum, R. R. (Williams & Wilkins, Baltimore), pp. 1067–1085.
3. Schally, A. V., Comaru-Schally, A. M. & Gonzales-Barcena, D. (1990) *Biomed. Pharmacother.* **46,** 465–471.
4. Reubi, J. C., Waser, B., Schaer, J. C. & Markwalder, R. (1995) *J. Clin. Endocrinol. Metab.* **80,** 2806–2814.
5. Sekyi-Otu, A., Bell, R. S., Ohashi, C., Pollak, M. & Andrulis, I. L. (1995) *Cancer Res.* **55,** 129–134.
6. Bergmann, U., Funatomi, H., Yukoyama, M., Beger, H. G. & Korc, M. (1995) *Cancer Res.* **55,** 2007–2011.
7. El-Badry, O. M., Minniti, C., Kohn, E. C., Houghton, P. J., Daughaday, W. H. & Helman, L. J. (1990) *Cell Growth Differ.* **1,** 325–331.
8. Daughaday, W. H. & Rotwein, P. (1989) *Endocrine Rev.* **10,** 68–91.
9. Daughaday, W. H. (1990) *Endocrinology* **127,** 1–4.
10. Rechler, M. M. & Nissley, S. P. (1985) *Annu. Rev. Physiol.* **47,** 425–442.
11. Rosenfeld, R. G. (1987) in *Acromegaly*, eds. Robbins, R. J. & Melmed, S. (Plenum, New York), pp. 45–53.
12. Fournier, B., Ferralli, J. M., Price, P. A. & Schlaeppi, J. M. (1993) *J. Endocrinol.* **136,** 173–180.
13. Nakanishi, Y., Mulshine, J. L., Kasprzyk, P. G., Natale R. B., Maneckjee, R., Avis, I., Treston, A. M., Gazdar, A. F., Minna, J. D. & Cuttitta, F. (1988) *J. Clin. Invest.* **82,** 829–839.
14. Toretsky, J. A. & Helman, L. J. (1996) *J. Endocrinol.* **149,** 367–372.
15. Westley, B. R. & May, F. E. B. (1995) *Br. J. Cancer* **72,** 1065–1066.
16. Tennant, M. K., Thrasher J. B., Twomey, P. A., Drivdahl, R. H., Birnbaum, R. S. & Plymate, S. R. (1996) *J. Clin. Endocrinol. Metab.* **81,** 3783–3792.
17. Chan, J. M., Stampfer, M. J., Giovannuci, E., Gann, P. H., Ma, J., Wilkinson, P., Hennekens, C. H. & Pollak M. (1998) *Science* **279,** 563–566.
18. Schally, A. V., Kovacs, M., Toth, K. & Comaru-Schally, A. M. (1998) in *Growth Hormone Secretagogues in Clinical Practice*, eds. Bercu, B. B. & Walker, R. F. (Dekker, New York), pp. 145–162.
19. Zarandi, M., Horvath, J. E., Halmos, G., Pinski, J., Nagy, A., Groot, K. & Schally, A. V. (1994) *Proc. Natl. Acad. Sci.* **91,** 12298–12302.
20. Zarandi, M., Kovacs, M., Horvath, J. E., Toth, K., Halmos, G., Groot, K., Nagy, A., Kele, Z. & Schally, A. V. (1997) *Peptides* **18,** 423–430.
21. Jungwirth, A., Schally, A. V., Pinski, J., Halmos, G., Groot, K., Armatis, P. & Vadillo-Buenfil, M. (1997) *Brit. J. Cancer* **74,** 1585–1592.
22. Jungwirth, A., Schally, A. V., Pinski, J., Groot, K., Armatis, P. & Halmos, G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 5810–5813.
23. Pinski, J., Schally, A. V., Halmos, G., Szepeshazi, K., Zarandi, M. & Armatis, P. (1995) *J. Natl. Cancer Inst.* **87,** 1787–1794.
24. Pinski, J., Schally, A. V., Jungwirth, A., Groot, K., Halmos, G., Armatis, P., Zarandi, M. & Vadillo-Buenfil, M. (1996) *Int. J. Oncol.* **9,** 1099–1105.
25. Janik, P., Brian, P. & Hartmann, N. R. (1975) *Cancer Res.* **35,** 3698–3700.
26. Daughaday, W. H., Mariz, I. K. & Blethen, S. L. (1980) *J. Clin. Endocrinol. Metab.* **51,** 781–788.
27. Breier, B. H., Gallaher, B. W. & Gluckman, P. D. (1991) *J. Endocrinol.* **128,** 347–357.
28. Tanaka, H., Asami, O., Hayano, T., Sasaki, I., Yoshitake, Y. & Nishikawa, K. (1989) *Endocrinology* **124,** 870–877.
29. Steenbergh, P. H., Koonen-Reemst, A. M., Cleutjen, C. B. & Sussenbach, J. S. (1991) *Biochem. Biophys. Res. Commun.* **175,** 507–514.
30. Rall, L. B., Scott, J. & Bell, G. I. (1987) *Methods Enzymol.* **146,** 239–248.
31. Ponte, P., Ng, S. Y., Engel, J., Gunning, P. & Kedes, L. (1984) *Nucleic Acids Res.* **12,** 1687–1696.
32. Crawford, D. E. (1990) *Oncology* **66,** 1035–1038.
33. Stone, K. R., Mickey, D. D., Wunderli, H., Mickey, G. H. & Paulson, D. F. (1978) *Int. J. Cancer* **21,** 274–281.
34. Jungwirth, A., Pinski, J., Galvan, G., Halmos, G., Szepeshazi, K., Cai, R.-Z., Groot, K., Vadillo-Buenfil, M. & Schally, A. V. (1997) *Eur. J. Cancer* **33,** 1141–1148.
35. Figueroa, J. A., Lee, A. V., Jackson, J. G. & Yee, D. (1995) *J. Clin. Endocrinol. Metab.* **80,** 3476–3482.
36. Angelloz-Nicoud, P. & Binoux, M. (1995) *Endocrinology* **136,** 5485–5492.
37. Iwamura, M., Sluss, P. M., Casamento, J. B. & Cockett, A. T. K. (1993) *Prostate* **2,** 243–252.
38. Pietrzkowski, Z., Mulholland, G., Gomella, L., Jameson, B. A., Wernicke, D. & Baserga, R. (1993) *Cancer Res.* **53,** 1102–1106.
39. Cohen, P., Peehl, D. M., Baker, B., Liu, F., Hintz, R. L. & Rosenfeld, R. G. (1994) *J. Clin. Endocrinol. Metab.* **79,** 1410–1415.
40. Ciampani, T., Fabbri, A., Isidori, A. & Dufau, M. L. (1992) *Endocrinology* **131,** 2785–2792.
41. Christophe, J., Svoboda, M., Dehaye, J. P., Winand, J., Vandermeers-Pire, M. C., Vandermeers, A., Cauvin, A., Gourlet, P. & Robberecht, P. (1989) in *Peptide Hormones as Prohormones: Processing, Biological Activity, Pharmacology*, ed. Martinez, J. (Halsted, New York), pp. 211–242.
42. Mayo, K. (1992) *Mol. Endocrinol.* **6,** 1734–1744.
43. Gourlet, P., Vandermeers, A., Vertongen, P., Rathe, J., De Neef, P., Cnudde, J., Waelbroeck, M. & Robberecht, P. (1997) *Peptides* **18,** 1539–1545.
44. Robberecht, P., Waelbroeck, M., Coy, D., De Neef, P., Camus, J. C. & Christophe, J. (1986) *Peptides* **7,** Suppl 1, 53–59.
45. Laburthe, M. & Couvineau, A. (1988) *Ann. N. Y. Acad. Sci.* **527,** 296–313.
46. Pollak, M. N., Polychronakos, C. & Guyda, H. (1989) *Anticancer Res.* **9,** 889–891.
47. Pollak, M., Sem, A. W., Richard, M., Tetenes, E. & Bell, R. (1992) *J. Natl. Cancer. Inst.* **84,** 966–971.