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Preclinical report

Inhibition of growth and metastases of MDA-MB-435 human estrogen-independent breast cancers by an antagonist of growth hormone-releasing hormone

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Antagonists of growth hormone-releasing hormone (GH-RH) inhibit the growth of various cancers by mechanism(s) that include the suppression of the insulin-like growth factors (IGF)-I and/or -II. In this study, nude mice bearing orthotopic implants of MDA-MB-435 human estrogen-independent breast carcinoma received 39 days of therapy with GH-RH antagonist JV-1-36 (20 μg/day). The treatment significantly inhibited tumor growth by 71.1% ($p < 0.01$) and nullified the metastatic potential of MDA-MB-435 cells. Four of eight control mice (50%) developed metastases in the lymph nodes and one (12.5%) in the lung, but none of the animals receiving JV-1-36 showed metastatic spread. GH-RH antagonist JV-1-36 inhibited the growth of MDA-MB-435 cells *in vitro*, while IGF-I stimulated it. However, mRNA for IGF-I or -II was not detected in MDA-MB-435 cells, indicating that the suppression of autocrine IGFs may not be involved in the antiproliferative mechanism. Using ligand competition assays with ^{125}I-labeled GH-RH antagonist JV-1-42, specific high-affinity binding sites for GH-RH were found on tumor membranes. Reverse transcription-polymerase chain reaction revealed the expression of mRNA for GH-RH receptor splice variant-1 in MDA-MB-435 tumors. Our results suggest that the antitumorigenic action of GH-RH antagonists on MDA-MB-435 breast cancer could be direct and mediated by tumoral GH-RH receptors. [© 2001 Lippincott Williams & Wilkins.]

Key words: Breast cancer therapy, growth hormone-releasing hormone antagonists, growth hormone-releasing hormone receptor, insulin-like growth factors.

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Introduction

Breast cancer is the second leading malignancy among women in the Western world with an annual mortality of about 45 000 in the US alone.[1] Recent progress in diagnosis and therapy increased the survival of women with estrogen-dependent breast cancer, but estrogen-independent tumors have a poor prognosis because the treatment options available are not completely satisfactory and new therapeutic modalities must be explored.[2–4]

Antagonists of growth hormone-releasing hormone (GH-RH) inhibit the growth of various cancers.[4,5] The inhibitory action of GH-RH antagonists appears to be mediated by several mechanisms.[5] The indirect effect of GH-RH antagonists is exerted by blocking the release of GH from the pituitary, which in turn leads to a suppression of the hepatic production of insulin-like growth factor (IGF)-I. This contributes to inhibition of those neoplasms that express IGF-I receptors and depend on IGF-I as a growth factor.[5] Recent findings also indicate that GH-RH receptors are present on many cancers and cancer cell lines including those of the breast, ovary, kidney, pancreas, prostate and lung.[6–9] Thus, the antitumor effect of GH-RH antagonists is also exerted through their action on tumoral GH-RH receptors, leading to a direct inhibition of malignant cell proliferation, by mechanisms dependent or independent of IGFs.[5–9] The direct effect of GH-RH antagonists on tumors *in vivo* is evident from the significant reduction in gene expression and concentrations of IGF-I and/or -II in osteosarcomas,[10] non-small cell lung carcinomas (non-SCLC),[11] glioblastomas,[12] and renal,[13] prostatic,[14] ovarian,[8] pancreatic[15] and colorectal[16] cancers xenografted into nude mice, after treatment with these analogs. Antagonists

of GH-RH also inhibit growth and suppress IGF-II production in various human cancer cell lines *in vitro*.[17] However, in the case of SCLC, the antiproliferative effect of GH-RH antagonists was not associated with an inhibition of the serum and tumor levels of IGFs, indicating that IGF-independent inhibitory mechanisms also exist.[18,19] Thus, in some cancers, GH-RH antagonists appear to block the effects of locally produced GH-RH[19–21] which may serve as an autocrine/paracrine growth factor for those tumors.[9,19]

MDA-MB-435 estrogen-independent breast cancer cell line exhibits a highly aggressive metastatic potential after implantation in an orthotopic site, the mammary fat pad (MFP), of nude mice.[22] In the present study we evaluated the effects of GH-RH antagonist JV-1-36[23] on the growth and the metastatic potential of MDA-MB-435 human estrogen-independent breast cancer cell line implanted orthotopically into female athymic nude mice. The contribution of direct antiproliferative mechanism mediated by tumoral GH-RH receptors, as well as the possible endocrine (indirect) and direct inhibitory pathways dependent on IGFs were also investigated.

Materials and methods

Peptides and reagents

hGH-RH(1–29)NH_2 and GH-RH antagonist JV-1-36 ([PhAc-Tyr^1, D-Arg^2, Phe(4Cl)^6, Arg^9, Abu^{15}, Nle^{27}, D-Arg^{28}, Har^{29}]hGH-RH(1–29)NH_2) were synthesized by solid-phase methods as described.[23] For daily injections, peptides were dissolved in 0.1% dimethyl sulfoxide (DMSO) in sterile aqueous 10% propylene glycol (vehicle solution).

Animals

Female athymic (NCr*nu/nu*) nude mice, approximately 6 weeks old on arrival, were obtained from the National 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

MDA-MB-435 estrogen-independent breast carcinoma cell line was obtained from ATCC (Manassas, VA). MDA-MB-435 cells were cultured in minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, 0.25 μg/ml amphotericin B, 1 mM sodium pyruvate, 1:50 MEM vitamins, non-essential amino acids and 10% fetal bovine serum. The cells were grown at 37°C in a humidified 95% air/5% CO_2 atmosphere, passaged weekly and routinely monitored for mycoplasma contamination using a detection kit (Boehringer Mannheim, Mannheim, Germany). All culture media components were purchased from Gibco (Grand Island, NY).

In vivo studies

Orthotopic xenografts of MDA-MB-435 cells were initiated by injection of 2×10^6 cells into the right MFP of 16 female nude mice 8–10 weeks old as described previously[22] with a few modifications: mice were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL) and a 5-mm incision was made in the skin over the lateral thorax. The MFP was exposed and an inoculum of 2×10^6 cells resuspended in 30 μl saline was injected into the tissue through a 27-gauge needle. When tumors reached a volume of approximately 60–70 mm^3, nude mice were randomly divided into two experimental groups of eight animals each and received the following treatment as s.c. injections: group 1 (control), vehicle solution; group 2, GH-RH antagonist JV-1-36 at a dose of 20 μg/day per animal. The treatment was continued for 39 days. 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 (Metofane), sacrificed by decapitation and necropsy was performed for the detection of metastatic nodules. Body weights were recorded, and various organs were removed and weighed. Tumors were dissected, cleaned and weighed, and samples were stored at -70°C for molecular biology analysis.

Radioimmunoassays for GH, and IGF-I and -II

Serum GH was determined using materials provided by Dr AF Parlow (NIDDK's National Hormone Pituitary Program, Torrance, CA): mouse GH 10783B for standard curve and iodination, and anti-rat GH-RIA-5/AFP-411S antibody. The methods used for determination of IGF-I and -II levels in serum and breast tumor samples after acid–ethanol cryoprecipitation have been described in detail.[10,11,14] IGF-I was measured by using rat IGF-I standard (Diagnostic Systems Laboratories, Webster, TX) and goat anti-rat IGF-I antibody (Diagnostic Systems Laboratories) at the final

dilution of 1:20 833. IGF-II was measured by using human recombinant IGF-II (Bachem, Torrance, CA) as a standard and Amano monoclonal antibody generated against rat IGF-II (10 μg/ml) at the 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.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from MDA-MB-435 tumors and cells by using RNAzolB (Tel-Test, Friendswood, TX) according to the manufacturer's instructions. Poly(A)$^+$ RNA was also purified from total RNA using oligo(dT)–cellulose (MicroPolyA-Pure mRNA isolation kit; Ambion, Austin, TX). Reverse transcription of mRNA into cDNA was performed as described previously.[8] The PCR amplification of the cDNAs for human glyceraldehyde-3-phosphate dehydrogenase (hGAPDH), GH-RH, IGF-I and -II, IGF receptors type I and II, and GH-RH receptors, was performed as follows: 1–5 μl of the cDNA was amplified in a 50-μl solution containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.7 mM $MgCl_2$ (for hGAPDH, IGF-I and -II, and IGF receptors I and II, and GH-RH) or 2 mM $MgCl_2$ (for GH-RH receptor), 200 μM of each dNTP, 2.5 U Taq DNA polymerase and 0.4 μM of each primer. The primers used for GH-RH receptors were 5′-CCT ACT GCC CTT AGG ATG CTG G-3′ (sense) and 5′-GCA GTA GAG GAT GGC AAC AAT G-3′ (antisense) for the first PCR, and 5′-GCA CCT TTG AAG CCA GAG AAG G-3′ (sense) and 5′-CAC GTG CCA GTG AAG AGC ACG G-3′ (antisense) for the second PCR.[6] The other primers used were described previously.[19] PCR consisted of 1 cycle at 95°C for 3 min, 58°C for 1 min and 72°C for 1 min, and subsequently 26 (hGAPDH), 30 (IGF receptors type I and II) or 35 (IGF-I and -II, and GH-RH) cycles of 95°C for 35 s, 58°C for 40 s and 72°C for 40 s, or 1 cycle at 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 90 s for GH-RH receptors, by using a Stratagene Robocycler 40 System. For the detection of GH-RH, IGF-I and -II, and GH-RH receptors, after the first round of PCR, 1 μl of PCR product (for GH-RH, and IGF-I and -II) or 5 μl of the diluted 1/10 GH-RH receptor primary PCR product, was subjected to a second round of PCR consisting of 28 cycles with the same profile as in the first round (for GH-RH, and IGF-I and -II), or 40 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 45 s (for GH-RH receptors). The number of cycles was determined in preliminary experiments to be within the exponential range of PCR amplification. Aliquots of each PCR product were electrophoresed on a 2% agarose gel (for hGAPDH, GH-RH, IGF-I and -II, and IGF receptors I and II) or 1.5% agarose gel (for GH-RH receptors), stained with ethidium bromide and visualized under UV light. For quantitation of PCR-amplified product a scanning densitometer (model GS-700; BioRad, Hercules, CA) coupled with the BioRad personal computer analysis software was used. All experiments were repeated at least twice and similar results were obtained. The relative mRNA levels of each gene were normalized versus the corresponding levels of hGAPDH.

GH-RH receptor binding studies

Receptors for GH-RH on MDA-MB-435 tumors from the control group were characterized by radioligand competition assay. Preparation of tumor membrane fractions and receptor binding studies of GH-RH were performed as reported in detail previously.[7]

Cell proliferation assay

MDA-MB-435 cells were seeded into 96-well plates at low density, corresponding to a confluence of 2–5%. After a recovery period of 24–48 h, the cell culture medium was removed and replaced with serum-reduced medium containing 5% FBS and the test compounds dissolved in 0.1% DMSO. Test compounds included IGF-I and -II (5, 15, 25 and 35 ng/ml) (Bachem, Torrance, CA), as well as hGH-RH(1–29)NH_2 and JV-1-36 (10^{-7}, 10^{-6} and 10^{-5} M), in octuplicate wells each. After another period of 95 h, when the cells reached a maximum confluence of 80–90%, the plates were fixed with glutaraldehyde and *in vitro* cell growth was estimated by the crystal violet method as described.[24] Results were calculated as %T/C, where T is the optical density of treated cultures and C is the optical density of control cultures. Each experiment was performed 3 times and similar results were obtained. The proliferation experiments were also repeated in serum-free medium, and cell growth was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide] assay as previously reported.[10,11] The serum-free medium was composed of Dulbecco's modified Eagle medium/F-12 Ham (1:1) with 1 mM sodium pyruvate, 1:50 MEM vitamins and 1:100 ITS+3 (insulin–transferrin–selenium with essential fatty acids; Sigma, St Louis, MO).

Statistical analyses

Data are expressed as mean $\pm$ SE. Statistical analyses were performed using Duncan's new multiple range

test and Student's two-tailed *t*-test. All p values are based on two-sided hypothesis testing.[26].

Results

Effect of GH-RH antagonist JV-1-36 on the growth of MDA-MB-435 breast carcinoma

After 39 days of treatment, the mean tumor volume was significantly decreased in mice receiving JV-1-36 to 190.8 ± 56.5 mm^3, corresponding to a reduction of 71.1% ($p < 0.01$), as compared with the control group which measured 661.4 ± 104.7 mm^3 (Table 1 and Figure 1). The final tumor weight in the group treated with JV-1-36 was also significantly reduced by 48.5% ($p < 0.05$ versus controls) (Table 1). At the end of the experiment, no significant differences in body weights and in the weights of various organs such as lung, heart, liver and kidneys were observed between the groups, indicating that the treatment with JV-1-36 was not toxic to the tumor-bearing animals (data not shown).

Effect of GH-RH antagonist JV-1-36 on the metastatic incidence of MDA-MB-435 breast carcinoma

The metastatic incidence of orthotopically implanted MDA-MB-435 breast carcinoma was evaluated in mice receiving GH-RH antagonist JV-1-36. Four of eight control animals (50%) developed lymph node metastases, whereas no metastases were detected in any of the eight mice receiving JV-1-36 after 39 days of treatment. Distant hematogenous metastasis (in the lungs) was also detected in one of eight (12.5%) animals from the control group (Table 1).

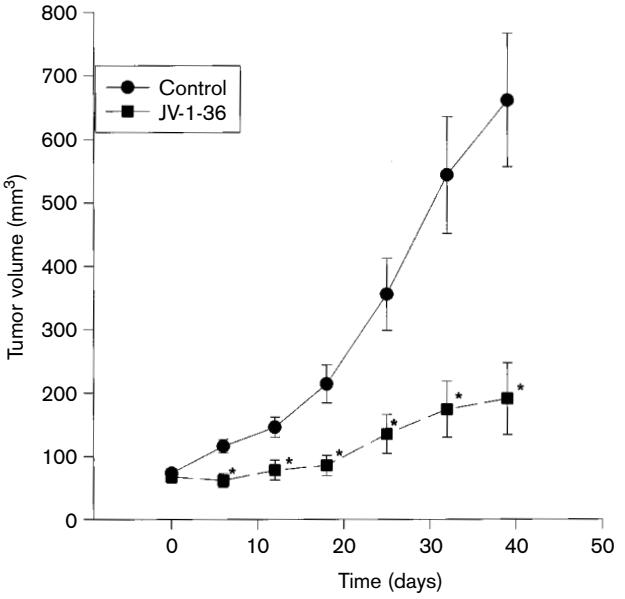

Figure 1. Tumor volumes in athymic nude mice bearing orthotopic xenografts of MDA-MB-435 breast cancer cell line during treatment with GH-RH antagonist JV-1-36 administered by daily s.c. injections at a dose of 20 μg/day per animal. Vertical bars represent SE. *$p < 0.01$ versus controls.

Serum levels of GH and IGF-I and -II, and tumor concentrations of IGF-I and -II

As shown in Table 2, the treatment with GH-RH antagonist JV-1-36 at the dose of 20 μg/day did not affect significantly the serum levels of GH, and IGF-I and -II, and the tumor concentrations of IGF-I and -II in nude mice bearing MDA-MB-435 tumors.

Analysis of mRNA expression for GH-RH, IGF-I and -II, and IGF-I and -II receptors in MDA-MB-435 tumors

The expression of mRNAs for GH-RH, IGF-I and -II, and IGF-I and -II receptors in MDA-MB-435 cells cultured *in vitro* or tumors grown in nude mice was evaluated by RT-PCR. As shown in Figure 3(a), mRNA for IGF-I and -II receptors was found in MDA-MB-435 cells and xenografts. However, no mRNA for GH-RH, and IGF-I and -II (Figure 3b) was detected. The levels of mRNA for IGF-I and -II receptors were also analyzed in MDA-MB-435 tumors of nude mice receiving GH-RH antagonist JV-1-36, but no differences were found as compared to untreated controls (data not shown).

GH-RH receptor analysis

Using complete displacement analyses with ^{125}I-labeled GH-RH antagonist JV-1-42 as radioligand, our ligand competition assays revealed the presence of high-affinity (K_d=0.42 ± 0.12 nM) binding sites for GH-RH with a maximal binding capacity of 123.6 ± 14.9 fmol/mg protein in membrane fraction of MDA-MB-435 tumors from the control group.

The expression of mRNA for GH-RH receptors was evaluated by RT-PCR. Using gene-specific primers for amplifying cDNAs for splice variants of GH-RH receptors,[6] we detected a PCR product of 720 bp in MDA-MB-435 tumors (Figure 4). This PCR product corresponded to splice variant 1 (SV_1) of GH-RH receptors.[6] The major part of the nucleotide sequence (nucleotides 77–1383) of SV_1 has more than 99% identity with the corresponding sequence of pituitary GH-RH receptor cDNA derived from exon 4–13. However, the first 334 nucleotides of SV_1 are completely different from those in pituitary.[6]

Table 1. Effect of treatment with GH-RH antagonist JV-1-36 on the growth and metastatic potential of MDA-MB-435 human estrogen-independent breast cancer cell line orthotopically implanted in female athymic nude mice

Treatment group	Initial tumor volume $(mm)^3$	Final tumor volume $(mm)^3$	Tumor weight (g)	Tumor burden (mg/g body weight)	Incidence of lymph node metastases	Incidence of distant metastases
Control	73.6±7.5	661.4±104.7	0.70±0.07	24.7±2.6	4/8	1 (lung)/8
JV-1-36	67.7±10.0	190.8±56.5[a]	0.36±0.01[b]	12.2±3.2[b]	0/8	0/8

Values are mean±SEM.
[a] $p<0.01$ versus controls.
[b] $p<0.05$ versus controls.

Table 2. Serum levels of GH, IGF-I and -II, and tumor concentrations of IGF-I and -II in nude mice bearing MDA-MB-435 human breast tumors and treated with GH-RH antagonist JV-1-36 (20 μg/day)

Treatment group	Concentration in serum (ng/ml)			Concentration in tumors (pg/100 μg protein)	
	GH	IGF-I	IGF-II	IGF-I	IGF-II
Control	3.42±0.18	210±23	14.6±0.70	168±8.0	45.0±3.9
JV-1-36	3.85±0.48	222±8.1	17.0±0.70	155±19	34.8±7.0

Effect of GH-RH antagonist JV-1-36, GH-RH, and IGF-I and -II on the proliferation of MDA-MB-435 cells *in vitro*

MDA-MB-435 cells cultured *in vitro* were also exposed to various concentrations of GH-RH antagonist JV-1-36, hGH-RH(1–29)NH_2, IGF-I and II, and their proliferation was monitored by using the crystal violet method. As shown in Figure 2(a), IGF-I at 25 ng/ml significantly stimulated the growth of MDA-MB-435 cells by about 17% ($p<0.05$ versus control). In contrast, GH-RH antagonist JV-1-36 at 10^{-5} M inhibited the proliferation of MDA-MB-435 cells by about 25% ($p<0.05$ versus control). The inhibition of proliferation was already apparent at 10^{-7} M JV-1-36 (Figure 2b). hGH-RH(1–29)NH_2 and IGF-II added to the culture medium at 10^{-7} to 10^{-5} M and at 5–35 ng/ml, respectively, had no significant effect on the proliferation of MDA-MB-435 cells (data not shown). These experiments were also repeated in serum-free medium and cell proliferation was followed by the MTT assay. Under these conditions, similar results were obtained as for the serum-reduced medium (data not shown).

Discussion

Novel treatment modalities for patients with estrogen-independent breast cancer must be explored.[2–4] The present study shows that GH-RH antagonist JV-1-36 inhibits significantly the growth of orthotopic xenografts of MDA-MB-435 human estrogen-independent breast cancer cell line in nude mice and suppresses the metastases.

The precise molecular mechanism by which GH-RH antagonists inhibit tumor growth remains to be elucidated. The antitumor action of GH-RH antagonist is due, in part, to the inhibition of the release of growth hormone from the pituitary and the resulting suppression of the hepatic production of IGF-I. Several IGF-dependent cancers are inhibited by this indirect action of GH-RH antagonists.[4,5] Thus, the possibility exists that the antitumor action of JV-1-36 on MDA-MB-435 breast tumors *in vivo* might have been partially due to this endocrine effect because MDA-MB-435 cells express receptors for IGFs and their proliferation is stimulated *in vitro* by IGF-I. However, the suppression of the GH–IGF-I axis probably made only a minor contribution to the inhibition of tumor growth observed, because in the present study the GH-RH antagonist JV-1-36 was used at a low dose that did not significantly affect the levels of GH and IGF-I in the serum and tumors. In a like manner, GH-RH antagonists have been previously shown to inhibit the growth of SCLC,[18,19] pancreatic,[15] colorectal[16] and ovarian cancers[8] in nude mice, without significantly decreasing the concentration of serum IGF-I.

Much recent evidence shows that besides the inhibition of the GH-RH–GH–IGF-I axis, antagonistic analogs of GH-RH inhibit tumor growth by direct action upon the cells.[9] GH-RH receptors were detected on MDA-MB-435 tumors and cells by

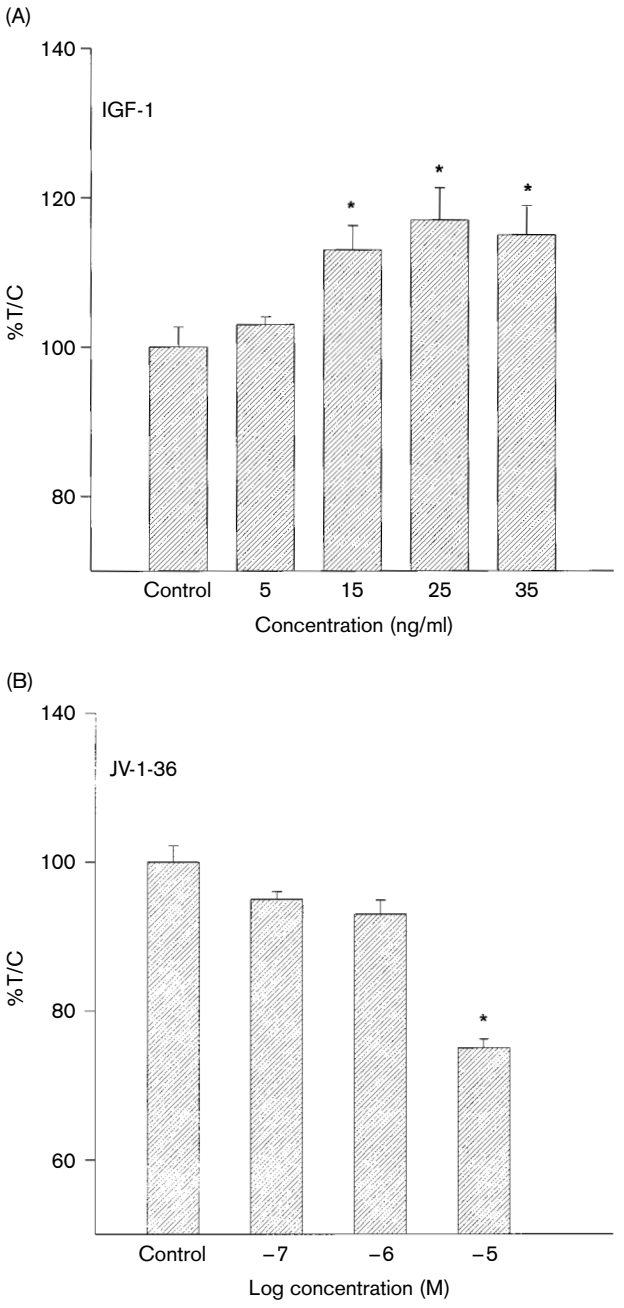

Figure 2. Effect of IGF-I (a) and JV-1-36 (b) on the proliferation of MDA-MB-435 human estrogen-independent breast cancer cell line cultured *in vitro*. The proliferation was evaluated by the crystal violet assay. Vertical bars represent SE. *$p < 0.05$ versus controls.

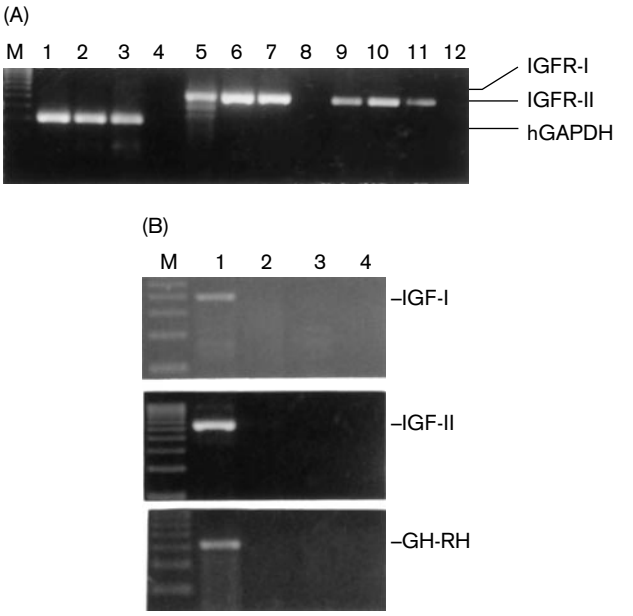

Figure 3. (A) RT-PCR analysis for hGAPDH (lanes 1–4), IGFR-I (lanes 5–8) and IGFR-II (lanes 9–12) mRNA expression in MDA-MB-435 human estrogen-independent breast cancer cells cultured *in vitro* or tumors grown in nude mice. mRNA template was isolated from H-69 SCLC (lanes 1, 5 and 9; positive control), MDA-MB-435 xenografts (lanes 2, 6 and 10) and MDA-MB-435 cells cultured *in vitro* (lanes 3, 7 and 11). Lanes 4, 8 and 12, negative controls; lane M, DNA molecular weight marker. (B) RT-PCR analysis of GH-RH, and IGF-I and -II mRNA expression in MDA-MB-435 human estrogen-independent breast cancer cells cultured *in vitro* or tumors grown in nude mice. Lane M, DNA molecular weight marker; lane 1, positive control (adult liver for IGF-I, and H69 SCLC for IGF-II and GH-RH); lane 2, MDA-MB-435 xenografts; lane 3, MDA-MB-435 cells cultured *in vitro*; lane 4, negative control. All PCRs yielded products of the expected size of 207 bp for hGAPDH, 395 bp for IGF-I, 538 bp for IGF-II, 322 bp for GH-RH, 447 bp for IGFR-I and 428 bp for IGFR-II.

radioligand binding and RT-PCR methods. ^{125}I-labeled GH-RH antagonist JV-1-42[7] was bound to a high-affinity, low-capacity binding site for GH-RH in the membrane fraction of MDA-MB-435 tumors. By using primers specific for the recently described tumoral GH-RH receptor splice variants (SV),[6] the expression of mRNA for the SV_1 was found in MDA-MB-435 tumors. In contrast, other primers that recognize the pituitary GH-RH receptors did not yield any visible band by RT-PCR, indicating that the pituitary form of GH-RH receptors is not expressed by the tumors (data not shown). Our recent studies demonstrate that human extrapituitary tissues and several cancers and cancer cell lines express the mRNA for multiple SV forms, i.e. SV_1, SV_2, SV_3 and SV_4, of the GH-RH receptor gene.[6–8] All SV have a retained intronic sequence at their 5′ end and lack the first three exons, as compared to the pituitary GH-RH receptor.[6,7] The deduced protein sequence of SV_1, the only SV expressed by MDA-MB-435 tumors, differs from that of the pituitary GH-RH receptor in the N-terminal extracellular domain.[6] Since all the transmembrane

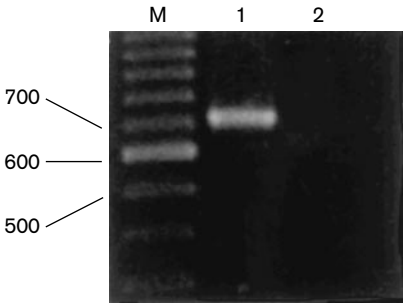

Figure 4. RT-PCR analyses of GH-RH receptor mRNA expression in MDA-MB-435 breast cancers grown in nude mice. Lane M, molecular weight markers. Lane 1, MDA-MB-435 breast tumor. Lane 2, negative control. PCR products were separated by electrophoresis in 1.5% agarose gel and stained with ethidium bromide. The PCR product was of the expected size of 720 bp (SV_1).

domains, extracellular and intracellular loops, and C-terminal intracellular domain characteristic of pituitary GH-RH receptors are also present in SV_1, this might produce a retained functionality, but different ligand binding affinity of the tumoral SV_1 receptor.

GH-RH antagonist JV-1-36 inhibits the proliferation of MDA-MB-435 cells *in vitro*, suggesting that the major mechanism responsible for the inhibition of tumor growth is the direct effect of this peptide on the cancer cells. Although the effect of JV-1-36 *in vitro* was significant only at 10^{-5} M concentration, the inhibition of proliferation was already apparent at 10^{-7} M. A non-specific toxic action of JV-1-36 at the higher concentrations is not likely, since the chemically related peptide hGH-RH(1–29)NH_2 and other GH-RH analogs with smaller antagonistic potency did not affect proliferation at equimolar doses (data not shown). In addition, no evidence of toxicity was found in any of the cancer models tested using JV-1-36 and other GH-RH antagonists with similar structures.

In many cancers, the antitumor effect of GH-RH antagonists is accompanied by the reduction of IGF-I and/or -II concentrations and mRNA levels in tumors and cell lines.[5,8–17] However, our present study indicates that in the MDA-MB-435 breast cancer model, the antiproliferative action of GH-RH antagonist JV-1-36 is exerted by a mechanism that is IGF independent. MDA-MB-435 cells do not produce mRNA for IGF-I and -II at levels detectable by the RT-PCR methods, and the treatment with JV-1-36 did not decrease the already low IGF-I or -II content of the tumors, which probably originated from the blood circulation. Consequently, the present observations reinforce the conclusion of previous studies with the H-69 SCLC model, which showed that an interference with the IGF system is not obligatory for the antiproliferative action of GH-RH antagonists.[18,19]

Elimination of the lymphatic and hematogenous spread of MDA-MB-435 cells after treatment with GH-RH antagonist JV-1-36 was also observed in this study. The most likely explanation for this observation is that the treatment with GH-RH antagonists prevents the dissemination of the cancers by arresting the growth of primary tumors.

Conclusion

In conclusion, our findings suggest that GH-RH antagonists such as JV-1-36 inhibit the growth of MDA-MB-435 estrogen-independent breast cancer and suppress the metastases, mainly by a direct antiproliferative action on the malignant cells that does not involve an interference with tumoral autocrine/paracrine IGFs. This direct effect appears to be mediated by tumoral GH-RH receptors that bind GH-RH antagonists specifically and with a high affinity. Antagonistic analogs of GH-RH could be considered for the development of new methods for treatment of advanced estrogen independent breast cancers.

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