

Growth Hormone Receptor Antagonist Administration Inhibits Growth of Human Colorectal Carcinoma in Nude Mice

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Abstract. Increasing evidence has accumulated in support of the hypothesis that growth hormone (GH) and insulin-like growth factors (IGFs) play a role in carcinogenesis. In order to test this hypothesis, female nude mice were xenografted with two different human colorectal cancer cell lines (COLO 205 and HT-29) and randomized to receive placebo or a GH receptor antagonist (GHRA) (B2036-PEG) every second day for 16 days. The tumour volume was measured in each animal throughout the study and by the end of the experiment the tumour weights were recorded. After 16 days of therapy in nude mice with the COLO 205 colorectal cancer, GHRA treatment caused a 39% reduction in tumour volume ($p < 0.02$) and a 44% reduction in tumour weight ($p < 0.01$). GHRA treatment equally reduced circulating IGF-I and IGF-BP-3 levels, while apoptosis was increased in the treatment group. Expression of IGF-I, IGF-II and the corresponding receptors in COLO 205 tumours was also decreased by the treatment. GHRA had no effect on the growth of the HT-29 colorectal cancer despite pronounced reduction in serum IGF-I. The present study thereby demonstrates a central role for the GH/IGF system in the pathogenesis of some colorectal cancers and suggests that specific GHR blockade may present a new concept in the treatment of colorectal cancer.

Colorectal cancer is the second most frequent cause of death from malignancies in the Western world (1), with a death rate second only to that of lung cancer (2-4). Many

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colorectal cancers are detected at a late stage when surgery cannot cure the disease and more than 40% of the patients with colorectal cancer develop metastases during the course of their illness (5). Despite advances in surgical treatment, chemotherapy and radiotherapy, these treatment modalities are not effective against disseminated colonic cancer (6, 7). The prognosis of disseminated colonic cancer is often poor and new treatment modalities are needed.

During the past few years, more findings have accumulated supporting a role for the growth hormone (GH)/insulin-like growth factor I (IGF-I)-axis in the growth of colorectal cancers (8-16).

Increased colon epithelial cell proliferation and a higher risk of developing colonic and other cancers were demonstrated in patients with acromegaly (12, 17-22), showing that elevated serum GH and IGF-I levels may be associated with a higher incidence of colon cancers and various other malignancies (10, 23, 24). GH can stimulate colonic epithelial cell growth either directly or indirectly by increasing IGF-I synthesis. The growth hormone receptor (GHR) is expressed in colonic epithelial cells (25) and GH has the ability to promote cell growth through induction of c-myc expression (26). IGF-I and IGF-II may also play a major role in the progression of colon tumours (27, 28). IGF-I stimulates cell proliferation (29) and IGF-I receptor expression has been demonstrated in normal colonic mucosal cells as well as in colonic carcinoma tissue (29, 30). Moreover, human colonic carcinoma cells are able to secrete IGF-I (31), which suggests additional stimulation of neoplastic cell proliferation by an autocrine mechanism.

IGF-II may have a regulatory role in the proliferation and differentiation of human colonic carcinoma cells (32). IGF-II mRNA was overexpressed in about one-third of colon cancers, while it was not detectable in normal colonic mucosa cells (33). Colon cancer cells also produce specific proteases that degrade IGF-binding proteins (IGFBP) secreted by

these cells, thus decreasing the protective effect of IGF-BPs against activation of the IGF-I receptor (IGF-IR) (33).

New therapeutic strategies for the treatment of colorectal cancers based on agonistic or antagonistic hormonal analogues have been explored, but GH/IGF-axis intervention in colon cancer is documented only in the case of somatostatin and its analogues (34-37).

Pegvisomant (B2036-PEG) is a newly developed GH receptor antagonist (GHRA) and, compared with the somatostatin analogues, it has a long duration of action and a high selective affinity, specifically binding to the GHR and further blocking the transmission of the intracellular signal system (38-40). B2036-PEG is a recombinant protein similar to natural human GH, but containing 9 amino acid substitutions. Pegvisomant has several covalently attached polyethylene glycol molecules that significantly prolong its half-life.

The evidence for the possible effect of the GH/IGF axis on the growth of human colon cancer cells prompted us to investigate B2036-PEG treatment *in vivo*. Two colon cancer cell lines, COLO 205 and HT-29, were selected in our study. HT-29 cells release carcino-embryonic antigen (CEA), while COLO 205 cells do not.

Materials and Methods

Animals. Female athymic nude BALB/c mice (BALB/cABom-Foxn1^{nu}, CA.Cg-Foxn1^{nu}) were obtained from M&B Taconic Ltd (Ry, Denmark) and maintained in the animal facility. The animals were approximately 6 weeks old with an initial body weight of 17-18 g when used for the experiments. The mice were housed in microisolators (filter top cages, Tecniplast, Buguggiata, Italy) under pathogen-free conditions with a 12-h light/12-h dark schedule and fed autoclaved standard chow (Altromin #1324, Lage, Germany) and water *ad libitum* throughout the experiment. All the animals were handled under sterile conditions and maintained in a separate facility. The Danish Experimental Animal Inspectorate approved the experimental protocol and all the animal experiments were performed according to the ethical standards required by the UKCCCR Guidelines (41).

Cell culture. The human colorectal adenocarcinoma cell lines COLO 205 (CCL-222) and HT-29 (HTB-38) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). COLO 205 cells were grown in RPMI 1640 media (Gibco™ Invitrogen A/S, Taastrup, Denmark) containing 1 mM sodium pyruvate and HT-29 cells were grown in McCoy's 5A media (Sigma M-6523, Vallensbaek, Denmark) containing 2 mM L-glutamine (Sigma G-3126) at 37°C in a humidified 5% CO₂ atmosphere. Both media contained 10% fetal bovine serum (Gibco™ Invitrogen A/S), 100 IU/ml penicillin and 100 g/l streptomycin. Tumour cells growing exponentially were harvested by brief incubation with 0.05% trypsin-EDTA (Gibco™ Invitrogen A/S) solution suspended in new medium for injection.

In vivo experimental protocol. Tumour xenografts were initiated by subcutaneous (s.c.) injection of 10⁷ cells into the left flank of nude mice. Three days later, the tumours had grown to a volume of

approximately 90-105 mm³ and 60-70 mm³ in COLO 205 and HT-29, respectively. The animals were divided into two groups with approximately equal average tumour volumes and randomly allocated to receive 0.5 ml saline or the GHRA (B2036-PEG) (Sensus, Austin, TX, USA) at a dose of 60 mg/kg subcutaneously every second day. Tumour volume, food consumption and body weight were measured twice a week. Blood glucose was determined once weekly until the termination of experiments. Animals with tumours that did not show exponential growth were excluded from the study.

On day 21 for the COLO 205 experiment and day 38 for the HT-29, mice were sacrificed after anesthesia with sodium barbital (50 mg/kg *i.p.*) and blood was drawn 3 min later from the retroorbital venous plexus for determination of serum IGF-I and IGF-BP-3. The serum was stored at -80°C until the measurements were performed. The animals were euthanised by cervical dislocation and tumour and liver were carefully removed and weighed. Pieces of liver and tumour were snap-frozen in liquid nitrogen; one-third for later determination of IGF-I, one-third for PCR analyses and the rest of the tumour sample was fixed in 4% buffered neutral formalin for histological examination.

Evaluation of tumour growth. The tumours were measured every 3-4 days with a digital caliper. Baseline tumour volumes were calculated using the following formula (42):

$$V = \pi/6 \times 0.67 \times [(d1 - 0.5) \times (d2 - 0.5)]^{3/2}$$

Where d1 was the longest diameter of the tumour and d2 was the perpendicular to d1. Tumour weight at the end of the study was also measured.

Measurement of serum IGF-I. Serum IGF-I was measured after extraction with acid-ethanol as previously described (43). The mixture was incubated for 2 h at room temperature, centrifuged and 25 ml of the supernatant was diluted 1:200 before analysis. Serum IGF-I levels were measured by RIA using a polyclonal rabbit antibody (Nichols Institute Diagnostics, San Capistrano, CA, USA) and recombinant human IGF-I as standard (Amersham Biosciences, Piscataway, NJ, USA). Mono-iodinated IGF-I (¹²⁵I-labelled [Tyr³¹]IGF-I) was obtained from Novo-Nordisk A/S (Bagsvaerd, Denmark). Intra- and intra-assay coefficients of variation were < 5% and < 10 %, respectively.

Measurement of tumour IGF-I and IGF-II. The IGF-I was extracted as previously described (44, 45). Briefly, 50 mg of the tissue was homogenized on ice in 1 M acetic acid (5 ml/g tissue) and further disrupted using another homogenizer. This extraction process was repeated twice for each sample. The supernatant was lyophilized and redissolved in 40 mM phosphate buffer, pH 8. For IGF-II extraction, 50 mg of tissue was homogenized on ice in 3.3 M formic acid-0.5% Tween 20 (5 ml/g tissue) and centrifuged at 40,000 g for 10 min at 4°C. (46). A 150-µl aliquot was heated to 90°C for 30 min. Reagent-grade acetone (350 µl) was added and the samples were vortexed and centrifuged at 3000 g. As with IGF-I, the supernatants were lyophilized and redissolved in 40 mM phosphate buffer, pH 8. The tissue extracts were stored at -20°C until IGF-I and IGF-II concentrations were measured in duplicate in diluted extracts by a non-competitive time-resolved immunofluorimetric assay for human IGF-I and IGF-II, as previously described (47). The cross-reactivity of IGF-I and IGF-II was less than 0.0002% and the detection limits were 0.0025 µg/L and 0.0010 µg/L for the IGF-I and IGF-II assays, respectively. Circulating mouse IGF-I and IGF-II were undetectable

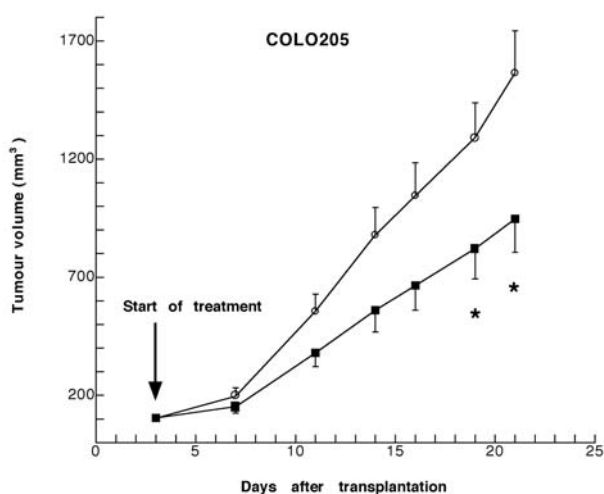


Figure 1. The effect of growth hormone receptor antagonist GHRA (B2036-PEG) on the growth of human colon adenocarcinoma tumour COLO 205 engrafted to nude mice. Mice were treated with B2036-PEG (■) or isotonic saline (○). Each curve represents the mean tumour volume per group. Vertical bars represent standard error, * $p < 0.05$ versus control.

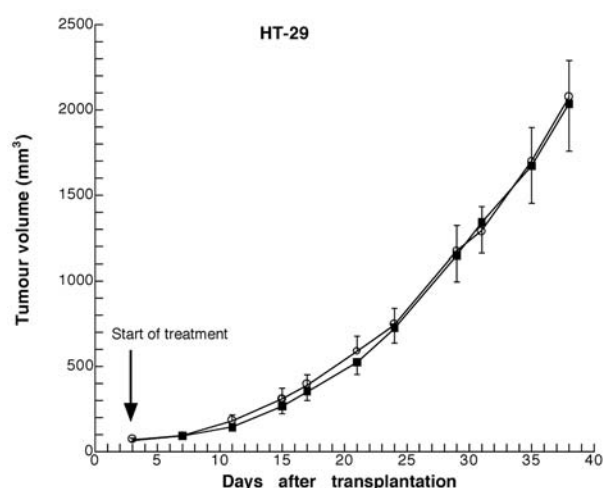


Figure 2. The effect of GHRA (B2036-PEG) on the growth of human colon adenocarcinoma tumour HT-29 engrafted to nude mice. Mice were treated with B2036-PEG (■) or isotonic saline (○). Each curve represents the mean tumour volume per group. Vertical bars represent standard error.

in these assays; accordingly, tumor IGF levels were not influenced by entrapped serum. Intraassay and interassay coefficients of variation were less than 5% and less than 10%, respectively.

Measurement of serum IGFBP-3. SDS-PAGE and Western ligand blotting (WLB) were performed according to the method of Hossenlopp (48), as previously described (49). Serum (2 μ l) was subjected to SDS-PAGE (10% polyacrylamide) under non-reducing conditions. The electrophoresed proteins were transferred by electroelution onto nitrocellulose paper (Schleicher & Schuell, Munich, Germany), and the membranes were incubated overnight at 4°C with 500,000 cpm 125 I-labelled IGF-I (specific activity 2,000 Ci/mmol) in 10 ml 10 mmol/l Tris-HCl buffer containing 1% bovine serum albumin and 0.1% Tween (pH 7.4). The membranes were washed with Tris-buffered saline, dried overnight and the nitrocellulose sheets were subsequently autoradiographed with Kodak X-AR film and exposed to Du Pont-NEN (Boston, MA, USA) enhancing screens at -80°C for 3-7 days. The specificity of the IGFBP bands was ensured by competitive co-incubation with unlabelled IGF-I purchased from Bachem (Bubendorf, Switzerland). On WLB (with 125 I-IGF-I as ligand), IGFBP-3 appears as a 38-42 kDa doublet band corresponding to the intact acid-stable IGF-binding subunit of IGFBP-3. WLBs were quantified by densitometry using a Shimadzu CS-9001 PC dual-wavelength flying spot scanner.

RNA isolation and reverse transcriptase-PCR analysis. Total RNA was extracted from samples of tumour tissue, using 500 μ l TRIZOL reagent (GIBCO BRL, Invitrogen A/S, 2630 Taastrup, Denmark) according to the manufacturer. The amount and the quality of isolated RNA was assessed by measurement of the optical density at 260 and 280 nm. From each sample, 1 μ g of RNA was used for

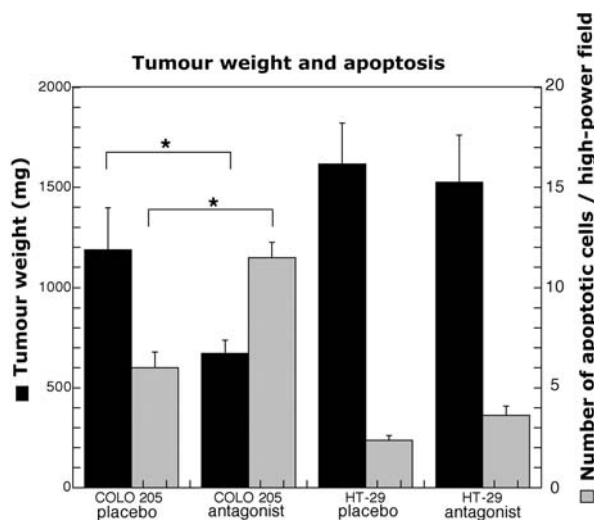


Figure 3. The effect of GHRA (B2036-PEG) on the tumour weight (■) and number of apoptotic cells in the tumour (■) in nude mice engrafted with COLO 205 or HT-29 colon adenocarcinoma cells. Bars represent mean tumour weight and mean number of apoptotic cells in tumour tissue. Vertical bars represent standard error, * $p < 0.05$ versus control, ** $p < 0.01$ versus control.

reverse transcription in 30 μ l of a reaction mixture containing 50 mM Tris, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.5 mM dTTP, 30 pmol random hexamers and 200 U MMLV-reverse transcriptase (GIBCO BRL). The samples were incubated for 75 min at 37°C followed by incubation for 15 min

Table I. Serum IGF-I, serum IGFBP-3 and serum CEA levels in nude mice bearing COLO 205 or HT29 tumours. Controls are nude mice without tumours. The values are given as a mean for each group of animals treated either with GHRA (B2036-PEG) or saline.

Group	Control	COLO 205 placebo	COLO 205 GHRA	HT-29 placebo	HT-29 GHRA
IGF-I	286 ± 9	205 ± 17	74 ± 7 *	326 ± 13	143 ± 19 *
IGFBP-3	520 ± 1.37	460 ± 0.52	230 ± 1.7**	690 ± 1.6	590 ± 2.0 **
s-CEA	n.d.	n.d.	n.d.	638 ± 102.8	532.3 ± 100.1

Data are expressed as mean ± SEM in µg/l for IGF-I and in pixel intensity for IGFBP-3; * $p < 0.05$ versus control, ** $p < 0.01$ versus placebo, n.d. not detected.

Table II. mRNA expression of mRNA of IGF-I, IGF-I, IGF-IR, IGF-IIR and GAPDH (control) in tumour tissue of mice treated with GHRA or saline.

Group	COLO 205 placebo	COLO 205 GHRA	HT-29 placebo	HT-29 GHRA
mRNA				
GAPDH	116 ± 2.4	114 ± 3.8	42.2 ± 2.6	39.9 ± 2.7
IGF-I	69.9 ± 5.0	41.6 ± 3.7**	36.0 ± 3.2	44.3 ± 1.5 *
IGF-II	74.6 ± 4.6	36.1 ± 4.7 **	28.8 ± 5.2	45.1 ± 2.6 **
IGF-IR	12.2 ± 0.8	8.6 ± 0.6 **	6.9 ± 0.7	9.4 ± 1.1
IGF-IIR	33.6 ± 1.6	29.2 ± 1.5	27.5 ± 2.4	35.0 ± 1.2**

Data are expressed as optical density mean ± SEM; * $p < 0.05$ versus placebo, ** $p < 0.01$ versus placebo.

Table III. Tumour IGF-I and IGF-II levels in nude mice bearing COLO 205 or HT29 tumours. The values are given as a mean for each group of animals treated either with GHRA (B2036-PEG) or saline.

Group	COLO 205 placebo ng/g	COLO 205 GHRA ng/g	HT-29 placebo ng/g	HT-29 GHRA ng/g
IGF-I	n.d.	n.d.	n.d.	n.d.
IGF-II	n.d.	n.d.	5.43 ± 0.77	6.63 ± 0.75

Data are expressed as ng/g tumour tissue mean ± SEM; n.d. not detectable.

at 95°C. Polymerase chain reaction was subsequently performed, using 2 µl reverse transcriptase solution in a reaction mixture of 20 µl, containing 20 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.5 mM dTTP, 2.5 U DyNAzyme II DNA polymerase (Finnzymes, Espoo, Finland) and 500 pmol of each of the specific primers for human IGF-I, IGF-II, IGFIR1, IGFIR2 and GAPDH (50). The sequence for the primers were: Human-IGF- I: IGF-I-85: 5'-ACATCTCCCATCTCTCTGGATTT CCTTTTGC-3' and IGF-I-598: 5'-CCCTCTACTT GCGTTCTTCAAATGTACTTCC-3'. Human-IGF-II: IGF-II-270: 5'-AGTCGATGCTGGTGCTTCTCACCTTCTTGGC-3' and IGF-II-807~5'-TGCGGCAGTTTTGCTCACTTCCGATTGCTGG-3'. Human GAPDH: GAPDH-U: 5'-TAAAGGCCAAAAGG GTCATCATCTC-3' and GAPDH-D: 5'-ACTATAGGTAGAG GCAGGGATGATGTT-3'. Human-IGFR1: IGFIR1: 5'-GTGTACGTTCTGATGATGTTGGAG-3' and 5'-GCCCCGT GTCATCAGTTCCATGAT-3'. Human-IGFR2: 5'- GAGTGGC TGATGGAAGAGAT-3' and 5'-GGAGAATGTGTAGACTG AGG-3'.

Amplification of cDNA was performed in a Hybaid Thermocycler with two initial cycles at 95°C for 60 sec, 54°C for 60 sec and 73°C for 60 sec. These cycles were followed by a number of rounds at 94°C for 45 sec, 54°C for 45 sec and 45 sec at 73°C. In the final cycles, the samples were incubated for 2 min at 73°C. The PCR products were separated by gel electrophoresis in a 2% agarose gel, containing ethidium bromide. All PCR reactions gave rise to clean products of the expected size. The intensity of the visualized bands was measured in arbitrary absorbance units by

a BIO-RAD UV-gel camera and used as an estimate of mRNA amount. To ensure a dose-dependent PCR for the quantitative PCR protocol, pilot experiments were performed to determine the optimal number of cycles for each primer set: pools of cDNA from both GHRA- and placebo-treated COLO 205 and HT-29 tissue were subjected to IGF-I, IGF-II and GAPDH-PCR with different cycle numbers. This optimizing step was determined for samples from both cell types separately. The number of cycles which gave rise to a weak band was chosen for evaluation of the samples in the final setup. Optimal cycle numbers for IGF-I-PCR were 33 for COLO 205 tissue samples and 28 for HT-29 tissue. For IGF-II, it was 39 for COLO 205 and 35 for HT-29 samples. Finally, the cycle numbers for GAPDH were 27 in both COLO 205 and HT-29 tissue. Negative control reactions in the PCR analysis included RNA samples treated without MMLV-RT and samples without RNA, which did not give rise to DNA amplification.

Apoptosis assay. The Boehringer Mannheim In Situ Cell Death Detection, POD kit (Mannheim, Germany) was used for the TUNEL assay. For quantitative analysis, 3 random fields were counted at 200x magnification. The number of apoptotic cells per field was counted. TUNEL-positive-stained cells were considered to be apoptotic cells. Two independent observers counted the apoptotic cells in each field. The percentage of immunoreactive cells was determined by dividing the number of apoptotic cells by the total number of cells in each field. Statistical analysis was conducted using a Mann-Whitney test.

Determination of Carcino-Embryonic Antigen (CEA) in serum. The amount of CEA in serum from mice inoculated with COLO 205 and HT-29 was determined by a clinical assay kit (Wallac, Turku, Finland).

Statistical methods. All data are expressed as the mean \pm SEM and statistical analyses of the tumour data were performed using *t*-test. All *p* values are based on two-sided hypothesis testing.

Results

Tumour xenografts were established in all the injected mice in this experiment, however, 20-30 % of the tumours were excluded after one week because of lack of exponential growth after initial establishment. GHRA treatment resulted in significant reductions in the tumour volume in COLO 205 tumours, with this effect becoming evident by day 14 of therapy ($p < 0.05$) and remaining significant at the termination of the experiment on day 21 (Figure 1). At the end of the COLO 205 experiment, the mean tumour volume of placebo was $1565.23 \pm 218.37 \text{ mm}^3$ and the GHA group $947.07 \pm 100.80 \text{ mm}^3$, with 39% reduction in the final tumour volume ($p = 0.01$), as well as 44% reduction in the final tumour weight ($1187.75 \pm 211.91 \text{ mg}$ vs $663.42 \pm 65.40 \text{ mg}$) ($p < 0.01$) (Figure 3). In the HT-29 experiment, no significant inhibition was observed either in the tumour volume ($p = 0.46$) (Figure 2) or in the tumour weight ($p = 0.39$) (Figure 3).

Serum IGF-I was significantly reduced in the animals with both COLO 205 and HT-29 tumours, indicating that the GHRA could reduce the secretion of IGF-I to the serum (Table I). When looking at the expression of IGF-I in the tumours, COLO 205 tumours had a significantly lower expression of IGF-I mRNA and IGF-II mRNA. The mRNA expression of both IGF-I and IGF-II, on the contrary, were significantly increased in the HT-29 tumours when treated with GHRA (Table II). mRNA expression of the receptors for both IGF-I and IGF-II were significantly reduced in animals with COLO 205 tumours treated with GHRA, while in the HT-29 tumours in GHRA-treated animals the expression of IGFR1 was increased and IGFR2 expression unchanged (Table II). At the protein level only IGF-II could be detected in HT-29 tumours, as the level of IGF-II in COLO205 and IGF-I in both tumour types was below the detection limit (Table III). In COLO 205 tumours the number of cells undergoing apoptosis was increased significantly in the GHRA-treated group, compared to the HT-29 tumours where there was no difference in the amount of apoptosis in the tumours from treated and untreated animals (Figure 3).

CEA in serum from mice bearing HT-29 tumours were not affected by the GHRA treatment (Table I). No significant differences in body weight, food consumption or blood glucose were observed between the GHRA-treated and placebo groups (data not shown).

Discussion

GH has a broad spectrum of biological actions. It exerts effects directly and indirectly through local production of IGF-I on a large variety of cells including neoplastic cells (51). The biological actions of GH are mediated by binding to a specific high affinity cell surface GHR and further activating tyrosine kinase, protein kinase C, and mitogen-activated protein or extracellular signal-regulated kinase, which are involved in the intracellular signalling mechanism of GH. By recognizing the potential role that GH may play in various pathophysiological conditions, a series of highly specific GHRA have been developed for potential therapeutic use by substitution of several amino acids in the third α -helix of GH, which is a critical region responsible for its growth promoting activity (52, 53). B2036-PEG was newly designed and synthesized with enhanced selective affinity and prolonged half-life in circulation (54, 55). This substance has been found effective in the treatment of acromegaly by directly inhibiting the effect of overproduction of GH (56, 57). The drug has also been explored as an exogenous administration to STZ-induced diabetic mice and here there was a significant inhibitory effect on diabetic renal / glomerular hypertrophy and urinary albumin excretion (UAE) mediated by a decreased renal IGF-I through GHR blockade (39, 58, 59). Recently, B2036-PEG has been evaluated for an anticancer effect in a model of human meningiomas transplanted to nude mice. The slowly growing meningiomas showed significant growth delay when treated with B2036-PEG compared to the untreated controls (60, 61). Here the antitumour effect of B2036-PEG was explained by a decrease of IGF-I in the circulation and/or surrounding tissue, since the meningioma specimens were found to have no autocrine IGF-I production. In the present study with colon cancer cell lines in nude mice, a significant decrease of IGF-I and IGF-BP-3 in the circulation was observed corresponding to the decrease observed in other studies with nude mice (16, 61). In this study, treatment with GHRA was able to decrease the IGF-I serum level and growth inhibition was observed in the COLO 205 colon cancer cell line, but not in the HT-29 cancer. This might be explained by the fact that the expression of IGF-I and IGF-II in the COLO 205 tumours was significantly decreased in the treatment group compared to the controls, indicating that the GHRA could suppress the expression of IGF-I and IGF-II as well as their receptors in the tumours. The treatment also decreased IGF-I receptor expression, which could explain the increase in apoptosis as previously described (62). In contrast, the HT-29 colon cancer cell line demonstrated an increased expression of IGF-I and IGF-II; particularly IGF-II and its receptor were up-regulated. The amount of IGF-II was found to be the same in the HT-29 tumours from the GHRA-treated animals as in the untreated controls.

Overexpression of IGF-II has been clearly demonstrated to correlate with an aggressive phenotype in colon cancer (27, 63). Kawamoto *et al.* (64) found a positive correlation between IGF-II expression and size in invasive colon cancer, indicating that IGF-II may play a significant role in tumorigenesis, as well as in progression and the metastatic behaviour. The lack of response in the HT-29 tumours could be related to a major autocrine production of IGF-II, making the tumour inaccessible to the treatment. The absence of an effect of the GHRA on the growth of HT-29 was shown by the unchanged number of apoptotic cells and by the production of CEA, which has been correlated with the tumour burden (65). The significantly increased expression of IGF-I, IGF-II and their receptors in the HT29 tumours in mice treated with GHRA compared to the control group is, however, difficult to explain. If the GHRA had an inhibitory effect on the expression of IGF-I in local murine stromal cells, the increased expression of IGF-I, IGF-II and their receptors in the tumour cells could be a compensatory mechanism. In other words, more IGF-I and, especially, IGF-II had to be produced by the human tumour cells in order to promote their own growth and the growth of the stromal connective supportive tissue. Although the expression of IGF-II was up-regulated in the treated HT-29 tumours, the total amount of IGF-II in the tumours was similar in the control and treatment groups. To test this hypothesis of compensatory expression of IGF, measurements of the stromal (murine) expression of IGF-I and IGF-II had to be done. However, as hepatic production of IGF-I was suppressed, it is likely that the local production by the murine stromal tissue was also suppressed. IGF-II synthesis is, however, not regulated by GH (66). It is well established that tumour growth and invasion are profoundly influenced by the microenvironment and *vice versa*, and it must be realized that a treatment regime may have impact on both stromal and cancer tissue.

In the area of cancer research, somatostatin analogues have been subjected to some experimental studies. Clinical trials have shown antitumour activity not only mediated by specific somatostatin receptors, but also by inhibiting the release and action of GH as well as some gastrointestinal hormones like gastrin (67). Somatostatin analogues also influence the paracrine- and autocrine-mediated effects of growth factors including IGF-I and epidermal growth factor (EGF). Due to the multiple actions of somatostatin, side-effects in the form of disturbance of metabolism will be difficult to avoid (34-37). Theoretically, a GHRA is suggested to be more selective and, therefore, with fewer metabolic perturbations, may contribute to a target therapy to the tumour in which GHR is expressed at a higher level. GHRH-antagonist (GHRHA) has also been used for the treatment of colon cancer including HT-29 (68). The growth of HT-29 xenografted to nude mice and the autocrine

synthesis of IGF-II were effectively inhibited by low doses of GHRH antagonists that did not significantly suppress the serum levels of IGF-I (68), indicating the important role of IGF-II in the growth and the progression of HT-29.

In the present study, an inhibitory effect of a GHRA on tumour growth was observed in nude mice inoculated with COLO 205, but not with mice inoculated with another colorectal cell line, HT-29. This indicates that some colorectal cancers are sensitive to treatment with GHRA, while others are not. The inhibitory effect of the GHRA was relative, since the tumour growth was not totally blocked, but effects on all the examined parameters were observed.

In conclusion, the present study indicates that the growth of some colonic cancer can be modified through the manipulation of the GH-axis, and that tumours not having their own production of IGF-I and IGF-II are most likely to respond to the treatment. The treatment may, as such, be considered as an adjuvant to other treatments in colon cancer.

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