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GASTRIC CANCER

Effects of recombinant human growth hormone on growth of human gastric carcinoma xenograft model in nude mice

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Abstract

AIM: To study effects of recombinant human growth hormone (rhGH) on growth of a human gastric carcinoma cell *in vivo*.

METHODS: Experimental mice were divided into control group, rhGH group, oxaliplatin (L-OHP) group and rhGH+L-OHP group. Cultured human gastric carcinoma cells BGC823 were inoculated into right axilla of nude mice and carcinoma xenograft model was established successfully. Inhibitory rate of xenograft tumor growth was estimated by measuring tumor volume; expression of proliferating cell nuclear antigen (PCNA), Bax and Bcl-2 proteins of xenograft tumor was detected using immunohistochemical S-P method.

RESULTS: Tumor growth inhibitory rate, the positive expression rate of PCNA, Bax and Bcl-2 were 49.3%, 58.2%, 65.2% and 59.2% in rhGH+L-OHP group respectively; 46.6%, 62.5%, 59.7% and 64.7% in L-OHP group; 5.0%, 82.7%, 23.2% and 82.2% in rhGH group and 0, 77.8%, 23.5% and 80.3% in control group. There was significant difference between rhGH+L-OHP group (or L-OHP group) and control group or rhGH group ($P < 0.05$), whereas there were no significant differences ($P > 0.05$) between L-OHP group and rhGH+L-OHP group and between rhGH group and control group.

CONCLUSION: rhGH does not accelerate the proliferation of human gastric cancer cell *in vivo*.

Key words: Human growth hormone; Stomach neoplasm; Nude mice

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INTRODUCTION

Recombinant human growth hormone (rhGH) can reverse many nutritional and metabolic abnormalities associated with severe catabolic states. In clinical studies rhGH has been shown to promote protein synthesis, improve nitrogen balance, accelerate wound healing[1-6], maintain host immune function and alleviate postoperation fatigue syndrome (POF)[7]. GH has become available for clinical use. It was reported that rhGH enhanced positive nitrogen balance in metabolic recuperation of postoperative patients who suffered from malignant tumor[8,9]. However, it is still controversial[10-12] whether rhGH should be applied in postoperative tumor patients since hGH promoted the proliferation of normal cells, as well as tumor cells. In the present study we investigated the effects of rhGH on human gastric cancer cell line BGC823 *in vivo* by inducing carcinoma xenograft model in nude mice, in an effort to clarify whether rhGH can be applied in postoperative patients with gastric cancer for metabolic intervention.

MATERIALS AND METHODS

Materials

B*ALB/C* nude mice were obtained from Shanghai Pharmaceutical Institute of Chinese Academy of Sciences. The mice were 6 wk old, with weights ranging between 12-22 g. Human gastric cancer cell line BGC823 was supplied by the Cell Bank of Shanghai Cell Biology Institute of Chinese Academy of Sciences. rhGH (Saizen) was supplied by Serono (Switzerland). Oxaliplatin (L-OHP) was purchased from Henrui Pharmaceutical Company, Jiangsu, China. Immuno-histochemical reagents, including proliferating cell nuclear antigen (PCNA), Bax and Bcl-2 were obtained from Maixin Reagents Company, Fujian, China.

Methods

At logarithmic growth phase, the cells of human gastric

Table 1 Volume and inhibitory rate of xenograft tumor growth (n = 6, mean ± SD)

Group	Volume (cm^3)	Inhibitory rate (%)
Control	0.406 ± 0.138	0
rhGH	0.383 ± 0.136	5
L-OHP	0.215 ± 0.087[a]	46.6
rhGH+L-OHP	0.208 ± 0.102[a]	49.3

[a]P < 0.05 *vs* control or rhGH.

cancer cell line BGC823 were digested by trypsin. Then the activity of the cells was examined (*Via* = 98.6%) and the cells were counted in hemocytometer using Trypan blue exclusion. The density of single cell suspension was adjusted to 1×10^{10}/L for further use.

Cell suspension (0.2 mL) was inoculated subcutaneously into right axilla of nude mice and all of carcinoma xenograft models were established within 3-7 d. Then the volume of tumor was measured. The experimental mice were randomly divided into 4 groups: control group, rhGH group, L-OHP group and rhGH+L-OHP group, 6 in each according to the drugs they received. From the 8th day of inoculation, the following drugs were administrated for 6 consecutive days: normal saline(NS) was subcutaneously injected in control group, 0.1 mL/d; rhGH group with rhGH, 2 IU/kg per day; L-OUP group with L-OHP by celiac injection, 1.3 mg/kg per day; and rhGH+L-OHP group with both rhGH and L-OHP of the same dosage as rhGH group and L-OHP group, respectively.

Immunohistochemistry

The mice were killed on the 7 d of drug administration and inhibitory rate of xenograft tumor growth was estimated by measuring tumor volume and expressions of PCNA, Bax and Bcl-2 proteins were detected by using immunohistochemical technique. Definition of positive PCNA cells was that cytoblast was nigger-brown stained and that for positive Bax and Bcl-2 cells was brown yellow stained in cytoplasm and cell membrane.

Statistical analysis

Data were expressed as mean ± SD and analyzed by variance analysis with SPSS11.0. Statistical significance was considered at $\alpha = 0.05$.

RESULTS

Inhibitory rate of xenograft tumor growth

Length(a) and breadth(b) of xenograft tumor were measured and the volume was counted using the formula: $V = ab^2/2$, as Oscieka described[13]. Xenograft tumor grew more slowly and tumor growth inhibitory rate was larger in rhGH+ L-OHP group and L-OHP group compared with control group and rhGH group ($P < 0.05$), whereas there was no significant difference between control group and rhGH group or between L-OHP and rhGH+L-OHP groups (Table 1). Tumor growth inhibitory rate (%) = [(volume in control group-volume in experiment group)/ volume in control group] × 100%

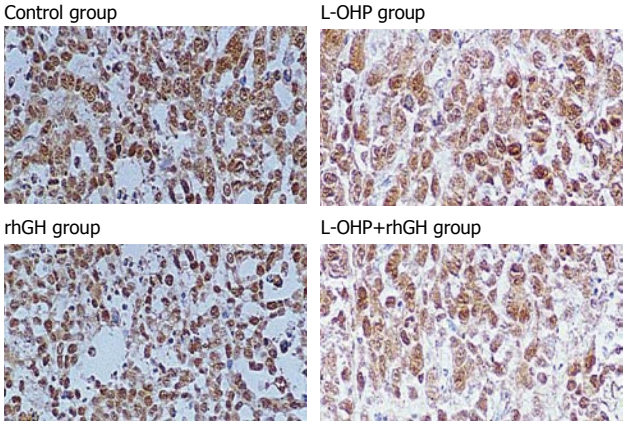

Figure 1 Positive expression of PCNA of human gastric cancer cell (SABC × 100).

Detection of PCNA, bax and Bcl-2 proteins using immunohistochemical technique

Positive expression of PCNA of human gastric cancer: There were more cells whose cytoblasts were nigger-brown stained in control group and rhGH group compared with L-OHP group and rhGH+L-OHP group.

Positive expression of Bcl-2 of human gastric cancer: There were more cells whose cytoplasm and cell membrane were brown yellow stained in control group and rhGH group compared with L-OHP group and rhGH+L-OHP group. In contrast, positive expression of Bax was seen more in L-OHP group and rhGH+L-OHP group (Figures 1, 2, and 3).

PCNA and Bcl-2 expressions were obviously decreased, while Bax distinctly increased in rhGH+L-OHP group and L-OHP group compared with control group and rhGH group ($P < 0.05$), but there was no significant difference between control group and rhGH group or between L-OHP and rhGH+L-OHP group (Table 2).

DISCUSSION

Nude mouse was a mutant mouse found and cultured in 1966. Because it has no thymus and completely lacks lymphocyte function and has no repellent reaction to heterogeneous transplant, it is especially adapted to animal heterogeneous transplant and human tumor heterogeneous transplant. In addition, karyotype, histological form and tumor biological characters of transplant tumor is the same as original tumor. Therefore, tumor model of nude mice is an ideal model for studying human malignant tumor. In our study, tumor xenograft model was successfully established through inoculation of human gastric carcinoma cells BGC823 into right axilla of nude mice. All tumor xenograft grew obviously.

In recent years, many studies reported that rhGH did not promote tumor growth[14-17]. Researchers from home and abroad found that rhGH accelerated improvement of patients by tentatively applying rhGH to patients with gastrointestinal malignant tumor after operation for metabolic intervention. rhGH did not promote tumor

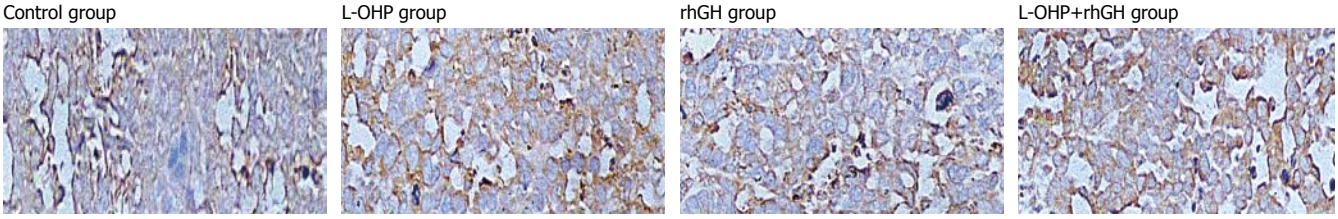

Figure 2 Positive expression of Bax of human gastric cancer cell (SABC × 200).

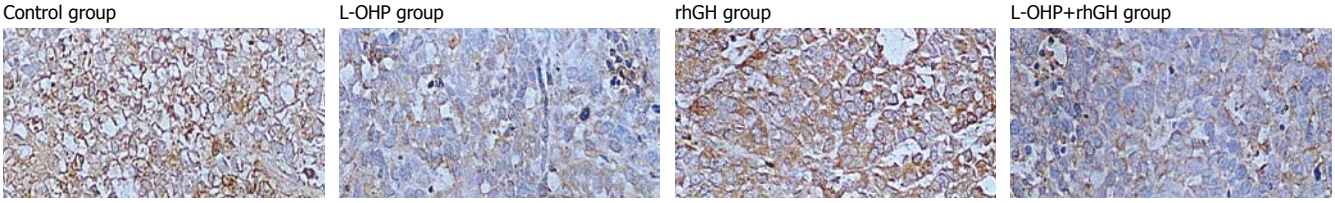

Figure 3 Positive expression of Bcl-2 of human gastric cancer cell (SABC × 100).

Table 2 Percentages of positive cells of PCNA, Bax and Bcl-2 expression

Group	PCNA	Bax	Bcl-2
Control	77.8 ± 6.3	23.5 ± 4.3	80.3 ± 8.9
rhGH	82.7 ± 9.4	23.2 ± 5.7	82.2 ± 7.4
L-OHP	62.5 ± 7.2[a]	59.7 ± 12.8[a]	64.7 ± 6.2[a]
rhGH+L-OHP	58.2 ± 5.2[a]	65.2 ± 11.9[a]	59.2 ± 9.2[a]

[a]$P < 0.05$ *vs* control or rhGH.

growth and metastasis in all their reported cases. Juergen *et al*[18] showed that postoperative treatment with rhGH in short term did not promote recurrence of tumor in 75 patients with gastrointestinal malignant tumor. Others carried out short-term nutritional treatment with rhGH in patients with tumor completely excised or other anticancer therapy[19].

In the present study, in comparison of rhGH+L-OHP and L-OHP groups with control and rhGH groups, PCNA and Bcl-2 apparently decreased and Bax obviously increased ($P<0.05$), while there was no significant difference between control group and rhGH group or between L-OHP and rhGH+L-OHP groups. It suggests that rhGH did not increase gastric cancer cell growth. In addition, tumor grew more slowly and tumor inhibitory rate was higher in L-OHP and rhGH+L-OHP groups, compared with control group and rhGH group, whereas there were no distinct differences between control group and rhGH group or between L-OHP and rhGH+L-OHP groups. These results also indicate that rhGH did not accelerate gastric cancer growth.

This study showed rhGH did not accelerate growth of a human gastric carcinoma xenograft model in nude mice. The results coincide with Fiebig's study that rhGH did not promote tumor (renal cancer and lung cancer) growth in nude mice[20] and also our former experiment *in vitro*[21]. In addition, our former study results showed tumor inhibitory rate did not change correspondingly with increase of the dose of rhGH. On the other hand, Blanck *et al*[22] proved rhGH did not promote liver cancer and renal cancer growth in rat tumor model. The mechanism that rhGH does not promote tumor growth is unknown. The possible mechanisms are (1) rhGH enhanced immunity of body[23]. Some researchers found GH and insulin-like growth factor-1 (IGF-1) increased the activity of natural killer cell (NK)[24,25]; (2) hGH directly or indirectly stimulate cell toxic effect[23]; (3) GH receptor expression reduced in tumor cell[22,26]; (4) IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3) were increased because of therapy of GH and IGFBP counteracted proliferation effect of IGF-I. Furthermore, IGFBP accelerated apoptosis independently of IGF-1[27]. Moreover, IGFBP-3 has been reported to inhibit the development of colonic tumors in experimental models and may hold promise as an adjuvant therapy for patients with neoplasm[28].

To sum up, our study demonstrates that postoperative treatment with rhGH in short term does not promote gastric cancer growth in patients with gastric cancer and it is safe using rhGH with chemotherapeutics.

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