EUROPEAN JOURNAL OF CANCER 42 (2006) 888–894

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.ejconline.com

Growth hormone receptor overexpression predicts response of rectal cancers to pre-operative radiotherapy

Wu Xiaoyu, Wan Meifang, Li Gang, Xu Zhe, Chen Che, Liu Fukun, Li Jieshou*

Nanjing University School of Medicine, Department of General Surgery of Jinling Hospital, 305 Zhong-shan-dong Road, Nanjing 210002, JS, PR China

ARTICLE INFO

Article history:
Received 25 October 2005
Received in revised form 30 November 2005
Accepted 2 December 2005
Available online 3 March 2006

Keywords:
Growth hormone receptor
Rectal carcinoma
Pre-operative radiotherapy
Immunohistochemistry
Reverse transcription-polymerase chain reaction

ABSTRACT

In this study, we evaluated the possible role of Growth Hormone Receptor (GHR) expression pattern in determining rectal cancer radiosensitivity. We examined GHR expression in pre-treatment biopsy materials and post-operative specimens from 98 patients by immunohistochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR). GHR expression was evaluated for association with tumour radiosensitivity, which was defined according to Rectal Cancer Regression Grade (RCRG). IHC results demonstrated that GHR overexpression was significantly associated with a poor response to radiotherapy ($P < 0.001$, $r_s = 0.399$); RT-PCR detection of GHR expression on pre-radiation biopsy specimens also showed that GHR mRNA negative group had a higher radiation sensitivity ($P < 0.001$, $r_s = 0.398$). Compared with the pre-radiation biopsy specimens, the paired post-operative specimens showed a significantly up-regulated GHR expression in the reliquus cancer cells ($P < 0.001$). In conclusion, GHR expression levels may be an indicator for rectal cancer radiosensitivity before pre-operative irradiation. The administration of GHR antagonist may have the potential to increase rectal cancer radiosensitivity.

1. Introduction

Pre-operative radiotherapy is used in the treatment of advanced rectal carcinoma. It can downstage the tumour and increase the possibility of a sphincter sparing procedure.[1–3] Furthermore, pre-operative radiotherapy can reduce the rate of local recurrence and improve the chance of survival in patients with resectable rectal carcinoma.[4,5] However, not all rectal tumours respond well to radiation. It is documented that some tumours respond well to standard radiotherapy, while others remain non-responsive.[6,1] Some recent studies have shown that patients who respond to pre-operative radiotherapy with or without chemotherapy by demonstration of complete or near pathologic response, have lower rates of local recurrence. Their survival is also possibly improved when compared to non-responsive patients whose tumours are either partially or totally unresponsive.[7,8] Identification of predictive indicators of radiosensitivity is therefore of clinical significance in selecting patients best suitable for pre-operative radiotherapy and avoiding unnecessary pre-operative treatment.

Conditions with increased growth hormone (GH) levels, such as acromegaly, are associated with an increased risk of malignancy, especially in colorectal cancer.[9–11] This implies that GH/growth hormone receptor (GHR) may be involved in the pathogenesis of human colorectal cancer. Our prior study has provided evidence of GHR expression in human colorectal cancer, suggesting that GHR signalling may play a role in the development of human colorectal cancer.[12] Another study[13] demonstrated that GH could activate pathways involved in DNA repair processes by binding to GHR, which is thought to be one of the mechanisms for radioprotection.[14] Based on

* *Corresponding author:* Tel.: +86 25 80860034; fax: +86 25 84803956.
E-mail address: wuxiaoyu@medmail.com.cn (Z. Xu).
0959-8049/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.12.012

these preliminary data, we proposed that there could be a correlation between GH/GHR signalling and cellular response to radiation. In this study, we have attempted to clarify the possible role of the GHR expression in determining rectal cancer radiosensitivity before pre-operative irradiation.

2. Materials and methods

2.1. Materials

The paired pre-radiation biopsy specimens and post-operative specimens were obtained from 98 patients with rectal carcinoma by rectal enteroscopy, who underwent pre-operative radiation and surgical resection in our department. No distant metastasis of the lung was detected by plain chest X-ray or CT scan. Both the liver and the para-aortic lymph nodes were negative for metastasis on ultrasonography and CT scan. The total dose of radiation was 40 Gy (2 Gy × 20 times) in each case, and the radiotherapy span ranged from 4 to 5 weeks. Surgical intervention was performed 4–6 weeks after the radiotherapy. Informed consent was obtained from all patients in this study.

2.2. Immunohistochemical staining of GHR

Tissue specimens were fomalin-fixed, paraffin-embedded and consecutively sliced into 4 μm-thick sections for immunohistochemistry. The sections were immunohistochemically stained with labelled streptavidin–biotin peroxidase method (LSAB2 Kit; Dako Japan Inc., Kyoto, Japan) with the following primary antibody: mouse monoclonal antibody GHR (Novocastra Laboratories Ltd., United Kingdom, dilution 1:80). The slides were immersed in 0.3% hydrogen peroxide/methanol for 10 min to deplete endogenous peroxidase. Then, nonspecific binding sites were blocked with 0.3% normal goat serum for 10 min, to which the primary antibody was applied, and the sections were incubated at 4 °C overnight. After being washed with phosphate-buffered saline (PBS) (0.01 mol/L pH 7.4), biotinylated goat anti-mouse IgG was applied onto the tissue sections and incubated at room temperature for 10 min. After being washed with PBS, a streptavidin peroxicase reagent was applied and incubated at room temperature for 10 min. Finally, the reaction product was visualized using developing colour by incubating the slides in a solution of 0.3% hydrogen peroxide and AEC chromogen. The sections were counterstained slightly with hematoxylin. Negative controls included parallel sections treated without the primary antibody, in addition to negating an adjacent section of the same block in which the primary antibody was replaced by PBS. Normal liver tissue treated under the same condition was used as a positive control of GHR expression.

To quantify the GHR expression in the various samples examined, a semi-quantitative scoring system was used. According to the staining intensity of tumour cells, the immunoreactions were graded as negative (0), faint yellow staining (1), brown staining (2), or dark brown staining (3), and as 0% (0), 0–10% (1), 10–50% (2) and 50–100% (3) according to the percentage of the positive cells. By multiplying the two scores, the IHC expression score of each case was obtained: 0 was determinated as −; 1,2,3 as +; 4,6 as ++; 9 as +++. All cases were evaluated by two pathologists who were blind to this study. At least five high-power fields were observed for each section, with the total number of cells exceeding 1000.

2.3. Assessment of tumour radiosensitivity

Tumour response to radiotherapy was evaluated by assessing the shrinkage and fibrosis of the resected tumour samples and their hematoxylin and eosin (HE) stained slides. According to RCRG from Wheeler and colleagues,[15] tumour radiosensitivity included three grades: RCRG 1: Sterilization or only microscopic foci of adenocarcinoma remaining, with marked fibrosis; 2: Marked fibrosis but presentation of macroscopic disease; and 3: Little or no fibrosis with abundant macroscopic disease. The RCRG was assessed by two pathologists who were completely blind to this study.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Freshly frozen rectal cancer specimens (10 mg each) were minced and total RNA was extracted with SV total RNA Isolation Kit (Promega Company, Madison, USA), and processed according to the manufacturer's instructions. For the tumour samples, necrotic and ulcerative portions were removed and the presence of at least 80% of tumour cells was verified histologically. RNA was quantified by absorbance at 260 nm. The quality of RNA was tested on agarose gel by analyzing the integrity of the UV visualized, ethidium bromide stained 28S and 18S rRNAs.

RT-PCR System Kit was from Promega Company (Madison, USA). The key points of the RT-PCR method are summarized as follows: 3 μg of each sample was used for RT-PCR to generate GHR and Glyceraldehyde-3-phosphate dehydrogenase (GADPH) PCR products of 247 and 452 bp, respectively. Semi-quantitative PCR was performed for 28 cycles for both GHR and GAPDH. Optimal amplification cycles were determined based on the linear relationship between the amount of PCR product detected and the number of amplification cycles. We used the following primer pairs: for GHR sense 5′-GAA TGG AAA GAA TGC CCT GA-3′ and antisense 5′-GTG GTG CTT CCC ATC TCA CT-3′; for GAPDH sense 5′-ACC ACA GTC CAT GCC ATC AC-3 and antisense 5′-TCC ACC ACC CTG TTG CTG TA-3′. The reactions were performed in 50 μl under the following conditions: initial denaturation at 95 °C for 2 min followed by 28 cycles of 95 °C for 50 s, 58 °C for 50 s, 72 °C for 40 s, and an additional cycle with extension at 72 °C for 7 min. The final PCR products were analyzed by electrophoresis, stained with ethidium bromide and photographed. The digital images were analyzed using Scion Image Software. The relative levels of GHR mRNA were normalized to GAPDH transcripts from the same reaction. Each sample was repeated for three times, and the average was calculated.

2.5. Statistical analysis

The Kendall's tau-b and the Spearman test were used to examine the correlation between various clinical or pathologic parameters and tumour GHR expression. The correlation

was evaluated using Spearman test. P values less than 0.05 were considered statistically significant. All calculations were performed by SPSS 10.0 for windows.

3. Results

General information of the cancer patients are summarized in Table 1. There were 52 men and 46 women, ranging in age from 25 to 77 years (median, 56 years). According to the endoscope inspection, the tumour area ranged from 1 to 49 cm^2 (median, 12 cm^2). Twenty-nine tumours were well differentiated, 45 tumours moderately differentiated, and 24 tumours poorly differentiated. Pre-treatment tumour stage was determined by a CT scan of chest, abdomen, and pelvis in all patients, with a following pelvic MRI scanning or transrectal ultrasonography (TRUS) or both. According to pTMN classification, 25 patients had Stage I disease, 37 patients Stage II and 36 patients Stage III.

3.1. *Immunohistochemistry*

The results of GHR immunostaining were summarized in Table 1. The results of the two pathologists were almost the same with only one exception. The consistency was 99%. The score from one pathologist was 1 and that from the other was 2. They met together for a discussion and finally agreed on 1.

A variable degree of cytoplasmic staining of tumour cells was observed in 81 of all 98 pre-radiation biopsy specimens analyzed (Fig. 1b). Seventeen of all 98 tumours were negative (Fig. 1a). A significant correlation was found between GHR expression on pre-radiation biopsy specimens and pre-treatment tumour stage ($P = 0.036$, $r_s = 0.212$). There was no significant correlation between GHR expression and differentiation ($P = 0.075$), age ($P = 0.693$), gender ($P = 0.843$) and tumour size ($P = 0.290$).

Compared with the pre-radiation biopsy specimens, the paired 93 (except five post-radiation sterilized cases) post-operative specimens showed a significantly increased GHR protein expression in the reliquus cancer cells ($P < 0.001$, Fig. 2a and b). Most of them score 3+ (56/98, 57.1%) or 2+ (24/98, 24.5%). Even for the 17 negative cases (pre-radiation biopsy specimens), the post-radiation remained cancer also showed a GHR protein expression in nine cases.

Table 1 – Correlation between immunohistochemistry GHR expression on pre-radiation biopsy specimens and clinico-pathological parameters

Clinico-pathological parameters	Cases	GHR				P
		0	1	2	3	
Age						
<56	48	9	11	14	14	
≧56	50	8	10	19	13	0.693
Gender						
Male	52	10	9	20	13	
Female	46	7	12	13	14	0.843
Stage						
I	25	8	5	7	5	
II	37	6	10	10	11	
III	36	3	6	16	11	0.036*
Differentiation						
Well	29	6	9	9	5	
Moderately	45	9	8	13	15	
Poorly	24	2	4	11	7	0.075
Size (cm^2)						
≦12	55	11	11	18	15	
>12	43	6	10	15	12	0.290

Well, well differentiation; moderate, moderate differentiation; poor, poor differentiation; *difference, $P < 0.05$.

3.2. *Reverse transcription-polymerase chain reaction (RT-PCR)*

Amplified fragments of the expected sizes (247 bp) were detected in 85 of all 98 pre-radiation biopsy specimens. The RT-PCR result showed consistency compared with the IHC detection of GHR expression ($P < 0.001$), all 81 tumour tissues with GHR protein expression in IHC were positive in RT-PCR; only two tumours graded as "0" in immunohistochemical analyses expressed weak positive GHR mRNA; and 15 cases were negative in both RT-PCR and IHC.

In some post-operative samples, especially some RCRG 1 cases, the tumour tissue that remained after irradiation was too scarce to be detected by RT-PCR, therefore only 60 samples were examined. Compared with the paired pre-radiation specimens, the post-operative specimens showed significantly increased GHR mRNA expression (Fig. 3, detailed data not shown), which is consistent with the IHC result.

3.3. *Tumour radiosensitivity*

According to the RCRG from Wheel and colleagues,[15] variable responses to radiotherapy were seen in 98 post-radiation rectal cancer specimens (Fig. 1c–f). The results of the two pathologists were exactly the same. Table 1 shows RCRG 1 in 31 cases, RCRG 2 in 33 cases and RCRG 3 in 34 cases. In five RCRG 1 cases, the tumours were sterilized (complete regression) after pre-operation radiotherapy. In HE-stained slides, cancer cells were found to be completely replaced by fibrosis, necrosis, or calcified tissue.

3.4. *Relation between GHR expression and tumour radiosensitivity*

The correlation between GHR expression in pre-radiation biopsy specimens and tumour radiosensitivity is summarized in Table 2. There was a significant correlation between GHR expression (IHC detection) and tumour radiosensitivity ($P < 0.001$, $r_s = 0.399$). The sensitivity and specificity for radiosensitive (RCRG 1–2) in the GHR low expression (IHC score 0–1) group were 48.3% and 81.6%, respectively. RT-PCR detection of GHR expression on pre-radiation biopsy specimens also showed that the GHR mRNA negative group had a higher radiation sensitivity ($P < 0.001$, $r_s = 0.398$), and all the negative 15 cases showed radiosensitive (RCRG 1, 11; RCRG 2, 4). The sensitivity and specificity for radiosensitive (RCRG 1–2) in

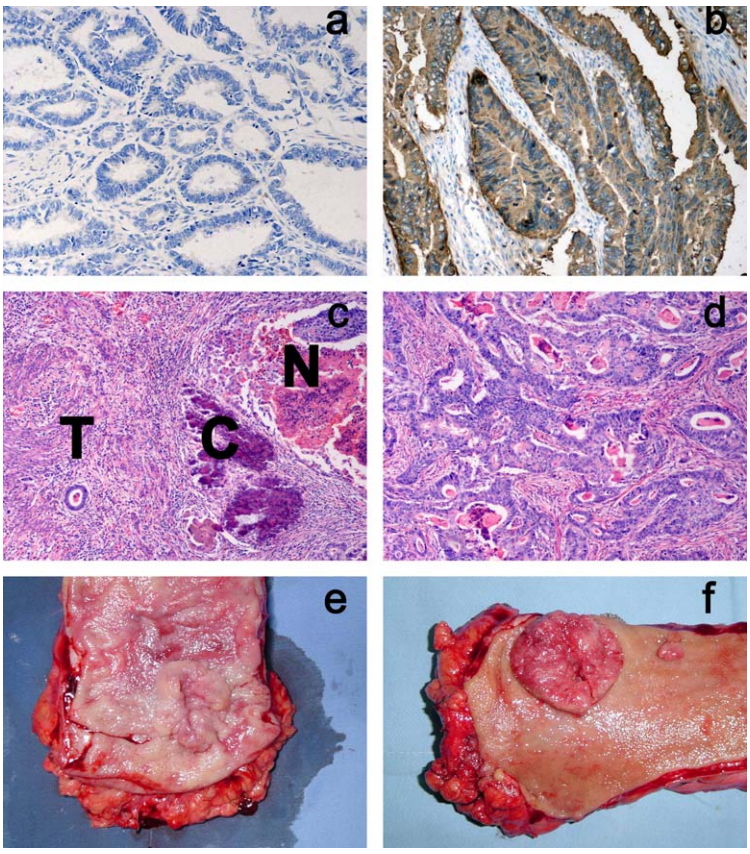

Fig. 1 – GHR immunostaining of rectal cancer (200×) and histopathological response to radiation (100×). (a), (c) and (e) are from a radiosensitive sample: (a) negative cytoplasmic immunostainings of rectal cancer cells was seen on the pre-radiation biopsy specimen; (c) correspondingly, good histopathological response to radiation therapy was seen on the post-operative sample. Most of the entire lesion was replaced by fibrosis and necrosis, T: relic tumour glandular tube; C: calcification; N: necrosis. (e) The post-operative macroscopic sample showed that the original tumour ulcer shrank and the raised periphery of the ulcer became flat and soft, the hyperemia by inflammation was seen in the adjacent normal mucous. (b), (d) and (f) are from the radioresistant sample: (b) strong cytoplasmic immunostaining of rectal cancer cells was seen on the pre-radiation biopsy specimen; (d) correspondingly, poor histopathological response to radiation therapy was seen on the post-operative sample. Most of the tumour remained, and little fibrosis was seen among the tumour cells. (f) Marked tumour remained in the post-operative macroscopic sample.

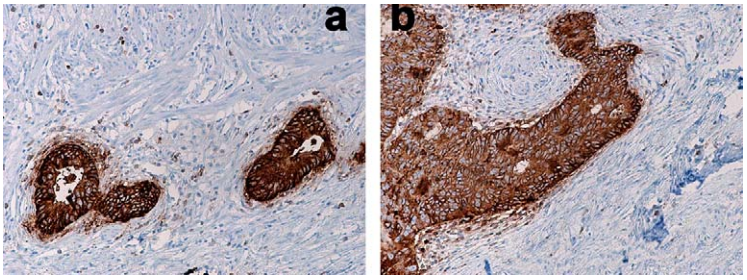

Fig. 2 – GHR immunostaining in the post-radiation rectal specimens. Regardless of radiosensitivity, strong cytoplasmic immunostaining was often seen in the post-radiation remained cancer cells (a) from the radiosensitivitive sample: few tumour glandular tubes remained; (b) from the radioresistant sample: most of the tumour remained; Fibrosis was seen around the remained tumour glandular tubes.

the RT-PCR negative group were 23.4% and 100%, respectively. The semi-quantitative RT-PCR results showed a stronger correlation between GHR mRNA expression and tumour radiosensitivity ($P < 0.001$, $r_s = 0.561$, data not shown).

4. Discussion

Mitotic or clonogenic cell death is considered to be the major mechanism by which most solid tumours respond to clinical

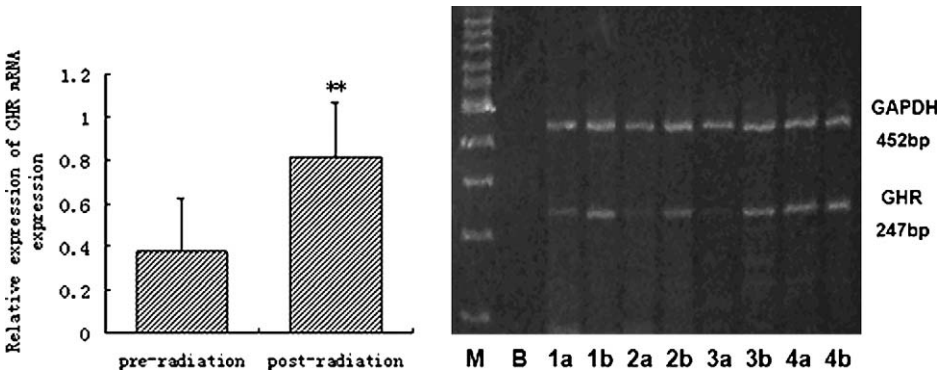

Fig. 3 – GHR mRNA expression by RT-PCR in the paired pre-radiation and post-radiation samples. Lanes M: Marker; B: blank control; 1–4a: pre-radiation samples; 1–4b: post-radiation samples; and **: significantly difference. $P < 0.01$ compared with the paired pre-radiation specimens, the post-operative specimens showed a significantly increased GHR mRNA expression ($P < 0.001$).

radiotherapy.[16] With increased knowledge about cell cycle regulation, apoptosis and DNA repair, several attempts have been made to identify molecular markers capable of predicting radiation sensitivity of tumours. It has been shown that the presence of a mutated *p53*, which is believed to be involved in the regulation of cell cycle and apoptosis, usually predicts tumour resistance to radiation.[17–22] Recent studies have also shown that there is a relation between immunohistochemical expression of Ku70 and tumour radiosensitivity in rectal cancer. Ku70 is a DNA protein kinase, which plays a specific role in the repair pathway for DNA damage.[23–25] However, whether or not these constituents may be used to predict radiotherapy response is still undetermined. Our study is one of those attempts to find out a new specific molecular marker for radiotherapy sensitivity.

This was the first report examining the relation between GHR expression and tumour radiosensitivity in rectal cancer. Our results demonstrated that GHR overexpression was significantly associated with a poor response to radiotherapy. The exact mechanism about this relation remains unknown, as there is a lack of relative studies on the correlation between GH/GHR signalling and radiotherapy. Using Chinese hamster ovary cells (CHO-4) which express GHR stably, Olga M and colleagues[13] demonstrated that the protective action of GH on radiation-induced injury was mediated by GHR, and the increased survival in response to radiation induced by GH correlated with an enhanced ability of the cells to repair damaged DNA. This result was also consistent with another study[26] in rat liver showing that GH induced the expression of several genes implicated in the control of DNA damage and in the response of cells to stress: namely, *GADD45*, which inhibits mitotic growth;[27] and *APEN*, which is both a DNA repair enzyme[28] and an activator of several transcription factors.[29] Interaction of GH with its receptor induces the activation of signalling cascades including the *JAK2* tyrosine kinase,[30] *MAPK*,[31,32] insulin receptor substrate (IRS)-1 and IRS-2,[33,34] Phosphatidylinositol 3-kinase,[35] Src homologous and collagen-like protein, *Grb2*,[36] Protein kinase C and phospholipase A2[37] and so on. Some of these pathways, particularly those driven by *JAK2* and *MAPK*, can activate transcription mediated by *STAT3* and *STAT5* as well as *SRF* and *c-fos*[38–40] and probably activate expression of genes like *GADD45* and *APEN*, which are involved in radiation-induced DNA repair. Since one of the mechanisms for radioprotection involves induction of biochemical and enzymatic pathways that prevent radiation-induced damage and/or promote DNA repair[14], GH/GHR signal may have a role in defending against cell stress and DNA damage by radiation. Therefore, the GHR overexpression in rectal cancer pre-radiation specimens may imply the stronger ability of tumour cells to repair radiation-induced DNA damage, which leads to increased tumour cell survival and the poor response to radiotherapy.

Our results also showed a significantly increased GHR expression in the post-radiation rectal cancer samples when compared with the paired pre-radiation specimens. One way to explain the result might be that tumour cell selection in an often non-homogeneous tumour, with a higher fraction of the sensitive cells being sterilised making the resistant cell in relative numbers more present than before, which shows a higher GHR expression. Another possibility may be due to tumour response to radiation. Via feedback regulation of GHR expression, the tumour cells protect against radiation injury by increasing the ability of DNA repair. Thus, those tumour

Table 2 – Relationship between GHR expression on pre-radiation biopsy specimens and tumour radiosensitivity in rectal carcinoma

GHR expression	Cases	RCRG			P	r_s
		1	2	3		
IHC						
0	17	11	4	2		
1	21	9	7	5		
2	33	7	14	12		
3	27	4	8	15	<0.001**	0.399
RT-PCR						
+	83	20	29	34		
–	15	11	4	0	<0.001**	0.398

RCPG, Rectal Cancer Regression Grade; IHC, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction; **significant difference, $P < 0.01$.

cells with impaired feedback loop may be eliminated during the radiotherapy procedure. Possibly this could explain why almost all the remaining tumour cells after irradiation showed strong GHR expression (Fig. 2). These observations strongly suggest that blocking GH/GHR signalling could probably increase radiosensitivity, since the feedback up-regulation of GHR expression does occur during the radiotherapy procedure. Pegvisomant, a GHR antagonist, has been used clinically, especially in acromegaly patients and recently, Pegvisomant was also investigated as a possible antineoplastic agent.[41] Preliminary studies have suggested a marked inhibition of metastasis of colonic tumours in animals when Pegvisomant was combined with more standard cytotoxic drugs.[42] Other animal studies in which human colon cancer models were used, also showed that Pegvisomant can powerfully inhibit tumour growth.[43] Thus, our results propose the prospect of allied clinical use of GHR antagonist administration and radiotherapy in patients with rectal carcinoma, which could increase overall response rates.

In our study, a correlation was observed between GHR expression on pre-radiation biopsy specimens and pre-treatment tumour stage, which indicates a role for GHR signalling in human rectal cancer. However, the exact mechanisms for this correlation need to be further studied.

In conclusion, our study indicates that there is a significant correlation between GHR expression and tumour radiosensitivity by immunohistochemistry and RT-PCR. Examination of GHR expression on pre-radiation biopsy specimens may predict the radiosensitivity of rectal cancer before pre-operative irradiation. Further studies are needed to further understand the mechanisms involved, including how GH/GHR signalling cascades respond to radiotherapy in normal or tumour tissues in vitro and in vivo. The administration of GHR antagonist could probably increase the radiosensitivity of rectal cancer. As GHR antagonists are commercially available, the combined clinical use of GHR antagonist with radiotherapy in rectal cancer patients is suggested in clinical trials in an attempt to increase overall response rates.

Conflict of Interest statement

None declared.

Acknowledgement

We thank professor Zhou Xiaojun and Shi Qunli for their professional pathological technology support.

REFERENCES

1. Sause WT, Pajak TF, Noyes RD, Dobelbower R, Fischbach J, Doggett S, et al. Evaluation of pre-operative radiation therapy in operable colourectal cancer. *Ann Surg* 1994;**220**:668–75.
2. Holm T, Cedermark B, Rutqvist LE. Local recurrence of rectal adenocarcinoma after "curative" surgery with and without pre-operative radiotherapy. *Br J Surg* 1994;**81**:452–5.
3. Marsh PJ, James RD, Schofield PF. Adjuvant pre-operative radiotherapy for locally advanced rectal carcinoma. Results of a prospective, randomized trial. *Dis Colon Rectum* 1994;**37**:1205–14.
4. Martijn H, Voogd AC, van de Poll-Franse LV, Repelaer van Driel HJ, Rutten HJ, Coebergh JW. Improved survival of patients with rectal cancer since 1980: a population-based study. *Eur J Cancer* 2003;**39**:2073–9.
5. Folkesson J, Birgisson H, Pahlman L, Cedermark B, Glimelius U, Gunnarsson U. Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. *J Clin Oncol* 2005;**23**:5644–50.
6. Rodel C, Sauer R. Neoadjuvant radiotherapy and radiochemotherapy for rectal cancer. *Recent Results Cancer Res* 2005;**165**:221–30.
7. Bouzourene H, Bosman FT, Seelentag W, Matter M, Coucke P. Importance of tumour regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with pre-operative radiotherapy. *Cancer* 2002;**94**:1121–30.
8. Wheeler JM, Dodds E, Warren BF, et al. Pre-operative chemoradiotherapy and total mesorectal excision surgery for locally advanced rectal cancer: correlation with rectal cancer regression grade. *Dis Colon Rectum* 2004;**47**(12):2025–31.
9. Melmed S, Ho K, Klibanski A, Reichlin S, Thorner M. Clinical review 75: recent advances in pathogenesis, diagnosis, and management of acromegaly. *J Clin Endocrinol Metab* 1995;**80**:3395–402.
10. Renehan AG, O'Connell J, O'Halloran D, Shanahan F, Potten ST, O'Dwyer ST, Shalet SM. Acromegaly and colourectal cancer: a comprehensive review of epidemiology, biological mechanisms, and clinical implications. *Horm Metab Res* 2003;**35**(11-12):712–25.
11. Jenkins PJ, Besser M. Acromegaly and cancer: a problem. *J Clin Endocrinol Metab* 2001;**86**:2935–41.
12. Yang X, Liu F, Xu Z, Li J. Growth hormone receptor expression in human colourectal cancer. *Zhong hua wai ke xue za zhi* 2005;**43**(5):321–2.
13. Madrid O, Varea S, Sanchez-Perez I, Gomez-Garcia L, De Miguel E, Gomez De Segura IA, et al. Growth hormone protects against radiotherapy-induced cell death. *Eur J Endocrinol* 2002;**147**(4):535–41.
14. Dittmann KH, Gueven N, Mayer C, Rodemann HP. The radioprotective effect of BBI is associated with the activation of DNA repair-relevant genes. *Int J Radiat Biol* 1998;**74**:225–30.
15. Wheeler JM, Warren BF, Mortensen NJ, et al. Quantification of histologic regression of rectal cancer after irradiation. *Dis Colon Rectum* 2002;**45**:1051–6.
16. Ross GM. Induction of cell death by radiotherapy. *Endocr Relat Cancer* 1999;**6**:41–4.
17. McIlwrath AJ, Vasey PA, Ross GM, Brown R. Cell cycle arrests and radiosensitivity of human tumour cell lines: dependence on wild-type p53 for radiosensitivity. *Cancer Res* 1994;**54**:3718–22.
18. Hamada M, Fujiwara T, Hizuta A, Gochi A, Naomoto Y, Takakura N, et al. The p53 gene is a potent determinant of chemosensitivity and radiosensitivity in gastric and colourectal cancers. *J Cancer Res Clin Oncol* 1996;**122**:360–5.
19. Bergqvist M, Brattstrom D, Gullbo J, Hesselius P, Brodin O, Wagenius G. p53 status and its in vitro relationship to radiosensitivity and chemosensitivity in lung cancer. *Anticancer Res* 2003;**23**:1207–12.
20. Desai GR, Myerson RJ, Higashikubo R, Birnbaum E, Fleshman J, Fry R, et al. Carcinoma of the rectum. Possible cellular predictors of metastatic potential and response to radiation therapy. *Dis Colon Rectum* 1996;**39**:1090–6.
21. Fu CG, Tominaga O, Nagawa H, Nita ME, Masaki T, Ishimaru G, et al. Role of p53 and p21/WAF1 detection in patient selection

for pre-operative radiotherapy in rectal cancer patients. *Dis Colon Rectum* 1998;**41**:68–74.
22. Bohnke A, Westphal F, Schmidt A, El-Awady RA, Dahm-Daphi J. Role of p53 mutations, protein function and DNA damage for the radiosensitivity of human tumour cells. *Int J Radiat Biol* 2004;**80**:53–63.
23. Wilson CR, Davidson SE, Margison GP, Jackson SP, Hendry JH, West CM. Expression of Ku70 correlates with survival in carcinoma of the cervix. *Br J Cancer* 2000;**83**:1702–6.
24. Komuro Y, Watanabe T, Hosoi Y, Matsumoto Y, Nakagawa K, Saito S, et al. Prediction of Tumour Radiosensitivity in Rectal Carcinoma Based on p53 and Ku70 Expression. *J Exp Clin Cancer Res* 2003;**22**:223–8.
25. Komuro Y, Watanabe T, Hosoi Y, Matsumoto Y, Nakagawa K, Tsuno N, et al. The expression pattern of Ku correlates with tumour radiosensitivity and disease free survival in patients with rectal carcinoma. *Cancer* 2002;**95**:1199–205.
26. Thompson BJ, Shang CA, Waters MJ. Identification of genes induced by growth hormone in rat liver using cDNA arrays. *Endocrinology* 2000;**141**:4321–4.
27. Zhan Q, Lord KA, Alamo I, Hollander MC, Carrier F, Ron D, et al. The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. *Mol Cell Biol* 1994;**14**:2361–71.
28. Rothwell DG, Barzilay G, Gorman M, Morera S, Freemont P, Hickson ID. The structure and functions of the HAP1/Ref-1 protein. *Oncol Res* 1997;**9**:275–80.
29. Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S, Prives C. Identification of redox/repair protein Ref-1 as a potent activator of p53. *Gene Dev* 1997;**11**:558–70.
30. Argetsinger LS, Carter Su C. Growth hormone signalling mechanisms: involvement of the tyrosine kinase JAK2. *Horm Res* 1996;**45**(Suppl. 1):22–4.
31. Love DW, Whatmore AJ, Clayton PE, Silva CM. Growth hormone stimulation of the mitogen-activated protein kinase pathway is cell type specific. *Endocrinology* 1998;**139**: 1965–1971.
32. Moller C, Hansson A, Enberg B, Lobie PE, Norstedt G. Growth hormone (GH) induction of tyrosine phosphorylation and activation of mitogen-activated protein kinases in cells transfected with rat GH receptor cDNA. *J Biol Chem* 1992;**267**:23403–8.
33. Argetsinger LS, Hsu GW, Myers MG, Billestrup N, White MF, Carter Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. *J Biol Chem* 1995;**270**: 14685–92.
34. Argetsinger LS, Norstedt G, Billestrup N, White MF, Carter Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor utilize insulin receptor substrate-2 in intracellular signalling. *J Biol Chem* 1996;**271**:29415–21.
35. Jeay S, Sonenshein GE, Kelly PA, Postel Vinay MC, Baixeras E. Growth hormone exerts antiapoptotic and proliferative effects through two different pathways involving nuclear factor-kappaB and phosphatidylinositol 3-kinase. *Endocrinology* 2001;**142**:147–56.
36. VanderKuur J, Allevato G, Billestrup N, Norstedt G, Carter Su C. Growth hormone-promoted tyrosyl phosphorylation of SHC proteins and SHC association with Grb2. *J Biol Chem* 1995;**270**:7587–93.
37. Argetsinger LS, Carter Su C. Mechanism of signalling by growth hormone receptor. *Physiol Rev* 1996;**76**:1089–107.
38. Wang YD, Wood WI. Amino acids of the human growth hormone receptor that are required for proliferation and Jak-STAT signalling. *Mol Endocrinol* 1995;**9**:303–11.
39. Pircher TJ, Flores Morales A, Mui AL, Saltiel AR, Norstedt G, et al. Mitogen-activated protein kinase inhibition decreases growth hormone stimulated transcription mediated by STAT5. *Mol Cell Endocrinol* 1997;**133**:169–76.
40. Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Silvennoinen O. Signalling through the hematopoietic cytokine receptors. *Ann Rev Immunol* 1995;**13**:369–98.
41. Friend KE. Cancer and the potential place for growth hormone receptor antagonist therapy. *Growth Horm IGF Res* 2001;**11**(Suppl. A):S121–3.
42. Dagnaes-Hansen F, Duan H, Rasmussen LM, Friend KE, Flyvbjerg A. GH receptor antagonist treatment inhibits growth of human colourectal carcinoma, COLO205 in nude mice. *Growth Horm IGF Res* 1999;**9**(Suppl.):340.
43. Dagnaes-Hansen F, Duan H, Rasmussen LM, Friend KE, Flyvbjerg A. Growth hormone receptor antagonist administration inhibits growth of human colourectal carcinoma in nude mice. *Anticancer Res* 2004;**24**:3735–42.