Elevation of growth hormone secretagogue receptor type 1a mRNA expression in human growth hormone-secreting pituitary adenoma harboring G protein alpha subunit mutation

Tongjiang Xu, Fei Ye, Baofeng Wang, Chunlei Tian, Sheng Wang, Kai Shu, Dongsheng Guo, Ting Lei

Department of Neurosurgery, Tongji Hospital Tongji Medical College, Huazhong University of Science and Technology Wuhan, People's Republic of China

Correspondence to: Ting Lei, MD., PhD.
Department of Neurosurgery, Tongji Hospital Tongji Medical College,
Huazhong University of Science and Technology,
Jie Fang Avenue 1095, Wuhan 430030, People's Republic of China.
TEL: +86 027 83668989; FAX: +86 027 83662594; E-MAIL: tlei@tjh.tjmu.edu.cn

Submitted: 2009-11-11 Accepted: 2010-01-10 Published online: 2010-02-17

Key words: **Gsp mutation; GH-secreting pituitary adenomas; GHSR-1a mRNA; Ghrelin mRNA; Tumor size**

Neuroendocrinol Lett 2010; **31**(1): 147–154 **PMID:** 20150876 NEL310110A14

Abstract

OBJECTIVE: The purpose of this study was to investigate the relationship between the ghrelin or GHSR-1a mRNA levels and clinical characteristics and to confirm the effect of gsp mutations on ghrelin/GHSR-1a system in human GH-secreting pituitary adenomas.

METHODS: The expression levels of ghrelin, GHSR-1a mRNA were determined by SYBR green real-time fluorescent quantitative PCR. The gsp mutations in 43 cases of human GH-secreting pituitary adenomas were detected using PCR-DNA direct sequencing analysis. Clinical data were obtained from the medical records of 43 acromegalic patients who had GH and IGF-1 assays in the same laboratory.

RESULTS: The expression of GHSR-1a correlated positvely with tumor size (R=0.411, p=0.006) and invasiveness (p<0.05). In contrast, ghrelin mRNA levels correlated positively only with tumor size (R=0.331, p=0.030) but not with tumor invasiveness (p>0.05). The expression level of GHSR-1a mRNA was significantly higher in gsp positive adenomas than in negative adenomas (p<0.05). Whereas, there was no significant difference in the expression of ghrelin mRNA between gsp mutation-positive and -negative adenomas (p>0.05). Additionally there was a significant positve correlation between the ghrelin and GHSR-1a mRNA expression levels in gsp-positive (R=0.553, p=0.040) or -negative adenomas (R=0.489, p=0.007).

CONCLUSIONS: GHSR-1a correlated positively with tumor size and invasiveness, while ghrelin correlated positively only with tumor size. Gsp mutations may upregulate the expression of GHSR-1a mRNA and have no effect on ghrelin mRNA levels in human GH-secreting pituitary adenomas.

ORIGINAL ARTICLE

Abbreviations :

GH	- Growth hormone
GHSR-1a	- growth hormone secretagogue receptor type 1a
Gsα	- alpha-subunit of the stimulatory GTP-binding protein
IGF-1	- insulin-like growth fator-1
GHRHR	- growth hormone-releasing hormone receptor
sst1 and sst2	- somatostatin receptor subtype 1 and somatostatin receptor subtype 2
GHRH	- growth hormone releasing hormone
AC	- adenylate cyclase

INTRODUCTION

Human GH-secreting pituitary adenomas are benign neoplasms that have received a great deal of clinical attention due to adenoma location, size and/or inappropriate GH hypersecretion. Local compressive effects include visual disturbances, headache, cranial nerve disorders and/or pituitary failure (Morita *et al.* 2008).

Ghrelin is a 28-amino-acid, acylated peptide originately isolated from human and rat stomach as an endogenous ligand specific for the GHSR-1a (Kojima *et al.* 1999). Different levels of GHSR-1a mRNA and ghrelin mRNA are present in the normal pituitary and various types of pituitary adenomas, with the highest mean level of GHSR-1a mRNA in human GH-secreting adenomas (Kim *et al.* 2001). Ghrelin/GHSR-1a has a synergistical effect, along with GHRH, on GH secretion. In addition, recent studies have demonstrated that ghrelin/GHSR-1a exerts proliferative effect on pituitary somatotroph cell line (Nanzer *et al.* 2004). These evidences imply that locally produced ghrelin/GHSR-1a might play a critical role in the tumorigenesis and progression of GH-secreting pituitary adenomas through paracrine or autocrine modes of action. However, the relationships between the ghrelin or GHSR-1a mRNA levels and hormonal and tumor characteristics are still obscure.

Somatic mutations of Gsα protein (gsp) were discovered in GH-secreting pituitary adenomas and remain the only known major genetic alterations of sporadic human GH-secreting pituitary adenomas to date (Yasufuku-Takano *et al.* 2006; Sakai *et al.* 2008). The mutations are localized at amino acid residues 201 and 227 that are critical sites for the intrinsic guanosine triphosphatase activity of the protein and lead to constitutive activation of cAMP-PKA signaling pathway.

There is a growing body of evidence suggesting that gsp mutations modulate the signal transduction system in several ways in GH-secreting pituitary adenomas. In addition to constitutive activation of AC-cAMP-PKA signaling pathway, recent studies have shown that gsp mutations up-regulate GHRHR mRNA (Sakai *et al.* 2008) and induce higher expression of sst1 and sst2 mRNA levels (Kim *et al.* 2005) in GH-secreting adenoma cells. As a common genetic abnormality in GH-secreting adenomas, what a pathophysiological role does gsp mutations exert on the ghrelin/GHSR-1a system? Although, Kim *et al.* (2003) put forward that the expression of GHSR-1a mRNA was significantly lower in gsp mutation-positive than -negative adenomas, data is still scarce. Further studies are still essential to clarify this issue.

Therefore, in this study, we aim to examine the expression levels of GHSR-1a and ghrelin on a large scale in GH-secreting pituitary adenomas through the quantitative real-time PCR, and investigate the influence of aberrant signaling pathway induced by gsp mutations on ghrelin/GHSR-1a expression. Moreover, our interests also focus on the association between GHSR-1a or ghrelin expression levels and tumor clinical characteristics.

MATERIAL AND METHODS

Patients and adenoma samples

Written, informed consent was obtained from all 43 patients with acromegaly (26 women and 17 men; mean ± standard deviation of the age, 46.5±13.5 years; ranging 19–62 yrs) enrolled in this research. The serum samples were withdrawn before surgery and stored at −20 °C. Pituitary adenoma tissues obtained during transsphenoidal surgery were snap frozen in liquid nitrogen and then preserved at −80 °C until analysis. The diagnoses of all specimens in this study were confirmed immunohistochemically.

Extracting genomic DNA and PCR for detecting gsp mutations

Genomic DNA was extracted from adenoma tissues preserved at −80 °C using AxyPrep Multisource Genomic DNA Miniprep Kit (Cat. No. AP-MN-MS-GDNA-50, Axygen Biosciences, USA) according to the manufacturer's specifications. The target DNA was then amplified by PCR. The reaction contained 10×PCR buffer (Invitrogen, USA), 200 nM dNTP mix, 1.5 mM $MgCl_2$, 200 nM primers to human Gsα, 5′-CCACCAGAGGACTCTGAGCCCTCTT-3′(sense) and 5′-AGCGTGAGCAGCGACCCTGATCCCT-3′ (antisense), and Taq DNA polymerase (Invitrogen) in 50 ml total volume. Thermal cycling consisted of 5 minutes at 95 °C followed by 30 cycles of 60 seconds at 94 °C, 60 seconds at 60 °C, 90 seconds at 72 °C, and a final extension at 72 °C for 10 minutes.

Direct DNA sequencing for determining the presence of gsp mutations

The PCR product (425 bp) was purified by AxyPrep™ PCR Cleanup Kit (Cat. No. AP-PCR-50, Axygen Biosciences, USA) for sequencing. These samples were directly sequenced using ABI PRISM 3730 (Applied Biosystems, Foster City, CA). Preparation of primers and PCR conditions used to detect gsp mutation were the same as those described above. The PCR product was 425bp long using these primers.

Real-time Reverse Transcription PCR Analysis

Total RNA was isolated from the tissues preserved at –80 °C using Trizol (Invitrogen, USA) following the manufacture's protocols. Subsequently RNA was treated with deoxyribonuclease (Promega, USA) before the reverse transcription reaction to eliminate the contaminating genomic DNA.

The isolated RNA (2 μg) and oligo (dT) primers were utilized to synthesize single-stranded cDNA using a reverse transcription kit (TaKaRa, Japan) according to the manufacturer's instructions. The PCR was set up using Real-time PCR Master Mix (Cat. No. GMRS-001, GenePharma, Shanghai, China) along with 2 μl of cDNA and the gene-specific primers at a final concentration of 0.2 μM. Thermal cycling was carried out on MX-3000P Real-time PCR Instrument (Stratagen, USA) and SYBR green dye intensity was analyzed using mx3000p software. The human GHSR-1a and ghrelin primers utilized in these studies were described in Table 1. Thermal cycling conditions included pre-incubation at 50 °C for 2 minutes, 95 °C for 3 minutes followed by 40 PCR cycles at 95 °C for 30 seconds and 60 °C for 40 seconds. The expression of the eukaryotic 18S ribosomal RNA (18sRNA) gene was analyzed as endogenous control. A dissociation curve was run after each PCR to verify amplification specificity. Each reaction was performed in triplicate. To calculate the relative expression levels, we used the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen 2001).

Clinical data

We collected preoperative data retrospectively for each patient. The size of pituitary adenomas was determined by measuring the maximum diameter with the preoperative magnetic resonance imaging. Adenomas were classified into four groups based on size by two experienced neurosurgeons specializing in pituitary adenomas surgery: micro (adenoma diameter less than 10 mm), intra (adenoma diameter 10 mm or greater with the adenoma mass confined to the sella turcica), supra (adenoma diameter less than 25 mm with the adenoma mass extending beyond the suprasellar area), and huge (adenoma diameter equal to or greater than 25 mm). The presence or absence of adenoma invasion of the cavernous sinus was determined though MRI. Additionally, the preoperative serum basal GH and IGF-1 concentrations were obtained in all patients.

Statistical Analysis

Quantitative data were expressed as mean ± standard deviation (SD). SPSS software for Windows (Version 15.0; SPSS, Inc., Chicago, IL) was used for statistical analysis. χ^2 test was performed to compare the sex ratio and adenoma invasiveness between gsp-negative and gsp-positive groups. An independent-sample student *t*-test (two-tailed) was performed to compare basal serum GH levels and IGF-I levels between the adenomas with or without gsp mutations. The Mann–Whitney test was used to compare the adenoma size between gsp-positive and gsp-negative groups. Correlations were analyzed by simple linear regression analysis. Differences were considered significant at $p<0.05$.

RESULTS

Expression of the GHSR-1a mRNA in human GH-secreting pituitary adenomas

The expression of the GHSR-1a mRNA was detected in all 43 adenoma tissues examined using the quantitative real-time PCR. There were no significant correlations between the GHSR-1a mRNA expression level and sex or age at the time of surgery of the patients. The expression level of GHSR-1a mRNA in GH-secreting adenoma was not correlated with plasma GH levels (R=0.206, p=0.185) or plasma IGF-1 levels (R=0.235, p=0.129).

However, the levels of GHSR-1a mRNA expression in GH-secreting adenoma correlated positively with the size of the adenomas (R=0.411, p=0.006) (Figure 1). Additionally, as is shown in Table 2, the expression level of GHSR-1a mRNA in invasive cases was significantly higher than that in non-invasive cases.

Tab. 1. Sets of primers designed to amplify human GHSR-1a and ghrelin.

Gene	Forward primer (5′-3′)	Reverse primer(5′-3′)
18sRNA	AGGGACAAGTGGCGTTCAGC	CGGACATCTAAGGGCATCAC
GHSR-1a	AATGCTGGCTGTAGTGGTGTTT	AGAAGAGGACAAAGGACACGAG
Ghrelin	CTGGAAGTCCGGTTCAACG	CCAGAGGATGTCCTGAAGAAAC

18sRNA, 18S-ribosomal RNA ; GHSR-1a, Growth hormone secretagogue receptor type 1a.

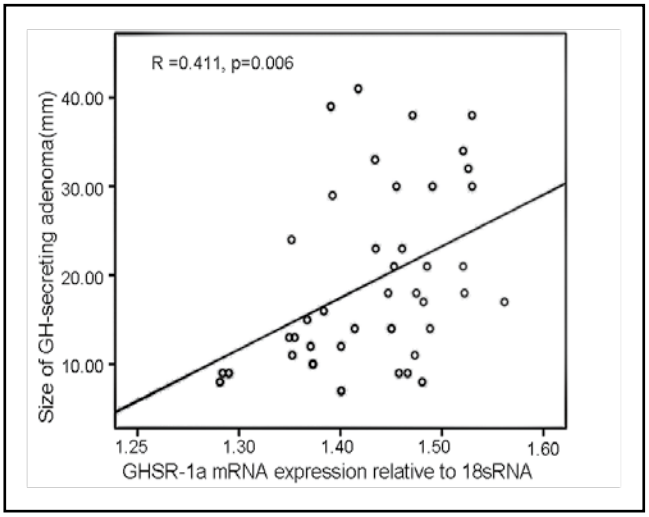


Fig. 1. Simple linear regression analysis reveals that the levels of GHSR-1a mRNA expression in human GH-secreting adenomas correlate positively with the adenoma size (R=0.411, p=0.006).

Tab. 2. The expression of GHSR-1a mRNA in GH-secreting pituitary adenomas (n=43, mean ± SD).

	n	GHSR-1a mean CT	18sRNA mean CT	ΔCT GHSR-1a-18sRNA	ΔΔCT ΔCT.Inv–ΔCT.Non-inv	$2^{-\Delta\Delta CT}$ GHSR-1a Inv /Non-inv
Inv	22	16.42	10.63	5.79	–1.38	2.60
Non-inv	21	17.91	10.74	7.17*	0.00	1.00

Inv, invasive cases; Non-inv, non-invasive cases; SD, standard deviation; GHSR-1a, Growth hormone secretagogue receptor type 1a; CT, the number of cycles required for the fluorescent signal to cross the threshold; ΔCT, the CT value of GHSR-1a subtract the CT value of 18sRNA; ΔΔCT, the ΔCT value of invasive group adenomas subtract the ΔCT value of non-invasive group adenomas; * $p<0.05$ compared with invasive cases.

Tab. 3. The expression of ghrelin mRNA in GH-secreting pituitary adenomas (n=43, mean ± SD).

	n	Ghrelin mean CT	18sRNA mean CT	ΔCT Ghrelin-18sRNA	ΔΔCT ΔCT.Inv–ΔCT.Non-inv	$2^{-\Delta\Delta CT}$ Ghrelin Inv /Non-inv
Inv	22	25.16	10.63	14.53	–0.71	1.64
Non-inv	21	25.98	10.74	15.24*	0.00	1.00

Inv, invasive cases; Non-inv, non-invasive cases; SD, standard deviation; CT, the number of cycles required for the fluorescent signal to cross the threshold; ΔCT, the CT value of Ghrelin subtract the CT value of 18sRNA; ΔΔCT, the ΔCT value of invasive group adenomas subtract that of non-invasive group adenomas; *, p>0.05 compared with invasive cases.

Expression of the ghrelin mRNA in human GH-secreting pituitary adenomas

A similar procedure was performed for assessment of the expression level of ghrelin mRNA. Real-time PCR revealed that ghrelin mRNA was present in all 43 adenoma tissues. Age and sex did not correlate significantly with the expression of ghrelin mRNA in the GH-secreting adenomas. The level of ghrelin mRNA expression was not correlated with plasma GH levels (R=0.079, p=0.613) or plasma IGF-1 levels (R=–0.247, p=0.110).

As expected, there was a significant positive correlation between ghrelin mRNA and the size of the GH-secreting adenomas (R=0.331, p=0.030) (Figure 2), but there was no significant difference in the expression of Ghrelin mRNA between the invasive and non-invasive cases (p>0.05) (Table 3).

Detection of gsp mutations in the adenoma genomic DNA

We initially investigated the expression of gsp mutations in 43 human GH-secreting adenomas. The mutations were detected in 14 out of the 43 adenomas in the present research. Of these 14 adenomas, nine were a CGT-to-TGT mutation at codon 201 (Arg201Cys), which replaced Arg to Cys; two were a CGT-to-CAT mutation at codon 201 (Arg201His); three were a CAG-to-CTG mutation at codon 227 (Gln227Leu) (Figure 3). The prevalence of gsp mutations in this study was 32.6%.

Next, we analyzed the clinical characteristics between the gsp-positive and gsp-negative adenomas. It was found there was no significant difference in age, sex, basal serum GH and IGF-I levels between the patients with and without the gsp mutations (Table 4), and also there was no significant difference in adenoma size between the two groups (p= 0.987) (Figure 4). Subsequently, we examined whether the gsp mutations were correlated with the tumor invasiveness, and no statistical difference was identified between gsp-positive and -negative adenomas (p=0.332) (Figure 5).

Elevation of the GHSR-1a mRNA expression in adenomas harboring gsp mutations, but not the ghrelin mRNA

The influence of gsp oncogene on the expression level of GHSR-1a mRNA and Ghrelin mRNA was investigated. The ΔCT values of the GHSR-1a and 18sRNA in 43 human GH-secreting pituitary adenomas were shown in Table 5, which indicated that the GHSR-1a mRNA level was approximately 2.03 fold higher in gsp positive group than in gsp-negative group (p<0.05).

However, there was no significant difference in the ghrelin mRNA expression between the adenomas with or without gsp mutations. The ΔCT values of the ghrelin and 18sRNA in two groups were presented in Table 6, which demonstrated that the ghrelin mRNA expression was approximately 1.40 fold higher in the gsp positive group than in gsp-negative group (p>0.05).

Comparison of the expression levels of ghrelin mRNA and GHSR-1a mRNA

Next, the relationship between the mRNA expression level of ghrelin and GHSR-1a was evaluated. Simple linear regression analysis revealed that there was a significant direct correlation between the levels of ghrelin mRNA and GHSR-1a mRNA expression in gsp-negative adenomas (R=0.489, p=0.007) (Figure 6A). Similarly, direct correlation was also found in gsp positive adenomas (R=0.553, p=0.040) (Figure 6B).

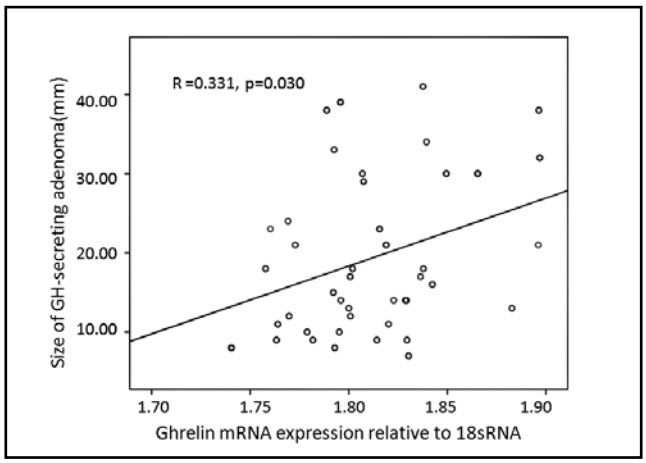


Fig. 2. Simple linear regression analysis reveals that the levels of ghrelin mRNA expression in GH-secreting adenomas correlate positively with the adenoma size (R=0.331, *p*=0.030).

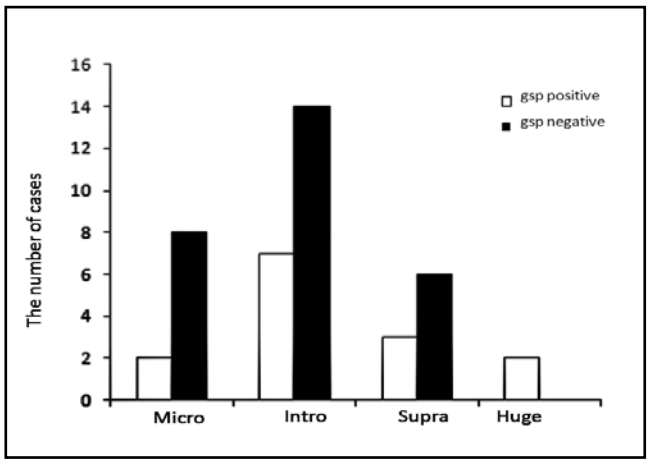


Fig. 4. Gsp-positive and gsp-negative adenomas classified by adenoma size.

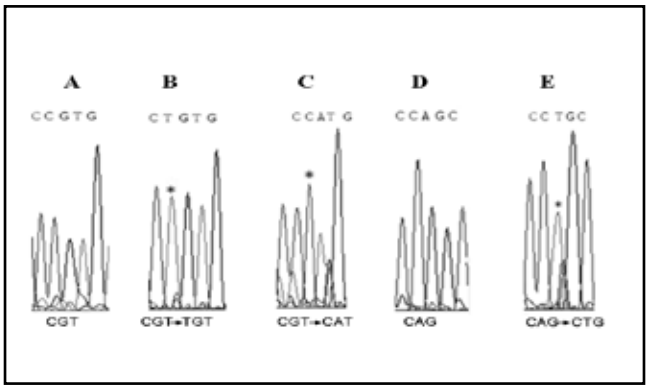


Fig. 3. Direct sequencing of gsp mutations detected by PCR. The information strands are shown. *denotes the mutation. A: Wild-type codon 201 (CGT). B: CGT→TGT mutation. C: CGT→CAT mutation. D: Wild-type codon 227 (CAG). E: CAG→CTG mutation.

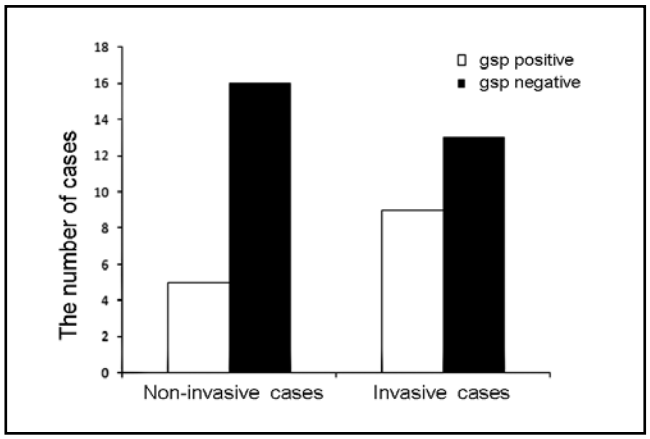


Fig. 5. Number of cases with and without invasion of the cavernous sinus for gsp-positive and gsp-negative adenomas.

Tab 4. The analysis of the clinical characteristics between the gsp-positive and gsp-negative human GH-secreting pituitary adenomas (n=43).

	Sex (M/F)	Age (year)	Basal GH μg/l	Basal IGF-1 (μg/l)
gsp (+) (n=14)	5/9	43.2 ± 11.8	95.3 ± 78.7	636.9 ± 273.8
gsp (–) (n=29)	13/16	44.7 ± 13.9	85.0 ± 118.8	707.2 ± 262.4
p-value	0.573*	0.657†	0.771†	0.421†

(+) present; (–) absent. * by Fisher's exact test. † by Student's t-test.

DISCUSSION

The human GHSR-1a, a 7 transmembrane domain G protein–coupled receptor, was identified and cloned by Howard *et al.* (1996). With the further advance of research, GHSR-1a was found to be widely distributed in various types of pituitary adenomas and other neoplasms. Skinner *et al.* (1998) have demonstrated that GHSR-1a mRNA expression was 200-fold higher in somatotroph tumors than in normal pituitary tissue, suggesting that this receptor may be a promoting factor in tumourigenesis. However, the association between GHSR-1a or ghrelin expression and clinical characteristics is still not well known. In the present study, we found that GHSR-1a mRNA was correlated positively with the tumor size and invasiveness, while the ghrelin mRNA correlated positively only with tumor size in Chinese GH-secreting pituitary adenomas patients. These observations were not consistent with the reports by Kim *et al.* (2001). They displayed that the ghrelin mRNA expression was correlated negatively with the tumor size, whereas there was no significant correlation between GHSR-1a mRNA levels and tumor size in GH-secreting adenomas (n=13). In our opinion, the discrepancy might be due to their small sample size.

Mutations of gsp were discovered in human GH-secreting pituitary adenomas and remained the only known major genetic alterations of sporadic GH-secreting pituitary adenomas to date (Sakai *et al.* 2008; Grossman 2009). In this research, gsp mutations were

Tab. 5. The expression of GHSR-1a mRNA in 43 GH-secreting pituitary adenomas (n=43, mean ± SD).

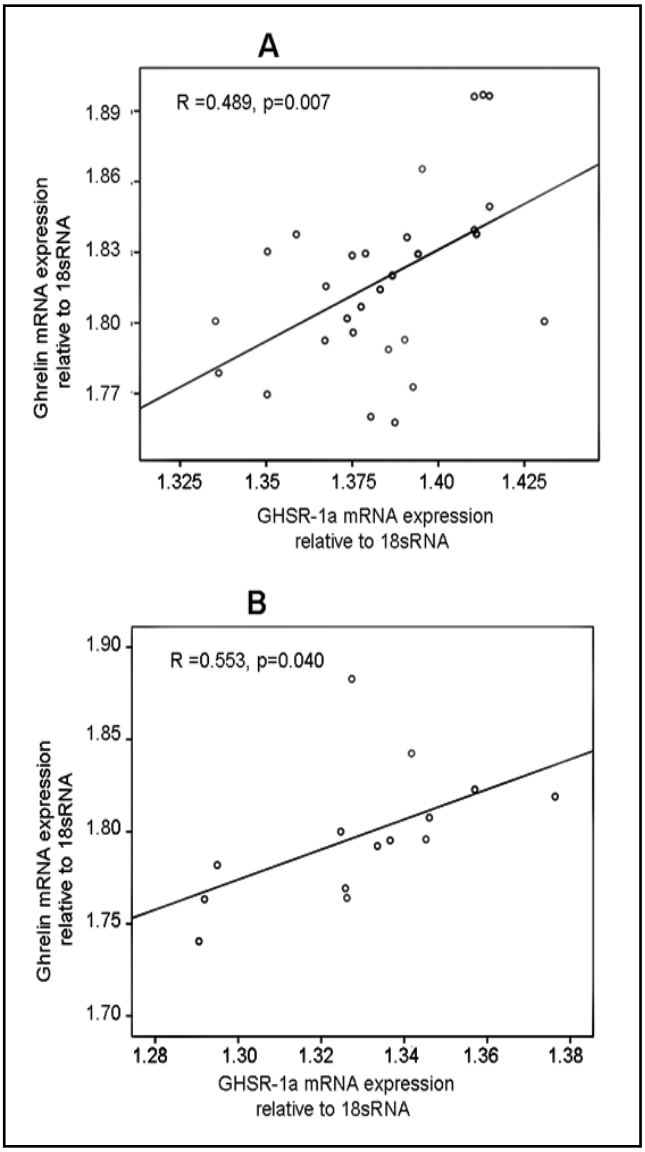
	n	GHSR-1a mean CT	18sRNA mean CT	ΔCT GHSR-1a-18sRNA	ΔΔCT ΔCT gsp(+)-ΔCT gsp(–)	$2^{-\Delta\Delta CT}$ GHSR-1a gsp(+)/gsp(–)
gsp (+)	14	17.07	11.24	5.83	-1.02	2.03
gsp (–)	29	17.86	11.01	6.85*	0.00	1.00

(+), present; (–), absent; SD, standard deviation; GHSR-1a, Growth hormone secretagogue receptor type 1a; CT, the number of cycles required for the fluorescent signal to cross the threshold; DCT, the CT value of GHSR-1a subtract the CT value of 18sRNA; DDCT, the DCT value of gsp (+) group adenomas subtract that of gsp (-) group adenomas; *p<0.05 compared with gsp (+) group adenomas.

Tab. 6. The expression of ghrelin mRNA in 43 GH-secreting pituitary adenomas (n=43, mean ± SD).

	n	Ghrelin mean CT	18sRNA mean CT	ΔCT Ghrelin-18sRNA	ΔΔCT ΔCT gsp(+)-ΔCT gsp(–)	$2^{-\Delta\Delta CT}$ Ghrelin gsp(+)/gsp(–)
gsp (+)	14	25.34	11.24	14.10	-0.49	1.40
gsp (–)	29	25.60	11.01	14.59*	0.00	1.00

(+), pesent; (-), absent; SD, standard deviation; CT, the number of cycles required for the fluorescent signal to cross the threshold; DCT, the CT value of Ghrelin subtract the CT value of 18sRNA; DDCT, the DCT value of gsp (+) group adenomas subtract that of gsp (-) group adenomas; *p>0.05 compared with gsp(+) group adenomas.



detected in 14 out of 43 GH-secreting adenomas, with the prevalence of 32.6%, which was in consistent with previous studies (Lei *et al.* 1996; Buchfelder *et al.* 1999; Kan *et al.* 2003); whereas it was reported much lower among the Japanese patients (4.4% (Hosoi *et al.* 1993) and 9.3% (Yoshimoto *et al.* 1993)). There was no significant difference with respect to adenoma size, invasiveness, or serum levels of GH and IGF-I in patients with or without gsp mutations, confirming the reports of others (Lei *et al.*; Buchfelder *et al.* 1999; Bai *et al.* 2000).

In the present research, it was found that GHSR-1a mRNA was significantly higher in gsp-positive adenomas than in gsp-negative adenomas. It has been reported that an intravenous GHRH infusion rapidly increased pituitary GHS-R mRNA levels in rat and treatment with GHRH at any dose tested did not affect GHS-R mRNA levels in primary pituitary cells (Kineman *et al.* 1999). The authors interpreted this phenomenon that additional factors, either central or systemic, are required for the stimulatory actions of GHRH on GHS-R synthesis. Then they confirmed that the acute stimulatory effect of GHRH on GHS-R synthesis is independent of the central and peripheral actions of GH. As mentioned above, gsp oncogene inhibits the activity of GTPase and keeps the AC-cAMP-PKA signaling pathways in a continuous turned-on state, mimicking the biological effects of GHRH on the cell membrane signal transduction system. Therefore we speculate that the

Fig. 6. Simple linear regression analysis of the correlation in the expression of GHSR-1a mRNA and ghrelin mRNA in GH-secreting pituitary adenomas with or without gsp-mutations. **A**: There is a significant positive correlation in gsp-negative adenomas (R =0.489, p=0.007). **B**: There is also a significant positive correlation in gsp-positive group (R =0.553, p=0.040).

elevated expression of GHSR-1a mRNA in gsp-positive adenomas is associated with the same factors, either central or systemic, which were induced by the abnormal activation of AC-cAMP-PKA signaling pathways. Additionally, Naoyuki Sakai *et al.* (2008) demonstrated that gsp mutations up-regulate GHRHR mRNA in GH-secreting pituitary adenoma cells, which may also play a key role in the stimulatory effect of gsp oncogene on GHSR-1a mRNA expression.

In contrast, we found that the ghrelin mRNA expression did not differ between gsp-positive and -negative adenomas, which was in accordance with the report by Kim et al that ghrelin mRNA expression showed no significant difference between gsp mutation-positive and -negative GH-producing adenomas, although the level of ghrelin mRNA in gsp mutation-positive adenomas tended to lower than that in gsp mutation-negative adenomas (Kim *et al.* 2003). This suggests that gsp mutations, which led to the aberrant activation of adenylyl cyclase, do not affect the ghrelin on the mRNA levels in GH-secreting adenoma cells.

The co-expression of ghrelin and the GHS-R-1a mRNA was confirmed in a lot of normal and adenomas tissues; however in current reports the relationship between them were contrary among some researches. In our study, we quantified the expression level of ghrelin and GHS-R-1a mRNA in GH-secreting pituitary adenomas, and found that there was a positive correlation between them, either in gsp-positive or gsp-negative adenomas. In 2006, Dixit *et al.* (2006) demonstrated that the expression of ghrelin and GHS-R-1a mRNA in several human astrocytoma cell lines. Ghrelin may exert autocrine/paracrine effects in promoting cell motility and invasion by up-regulating the GHSR-1a expression. In addition, Machado *et al.* (2007) reported that GHS (GHRP-6) stimulated the GHSR-1a mRNA overexpression in a patient with ectopic ACTH production by a lung carcinoid tumor. Therefore we deduce that ghrelin may enhance the expression of GHSR-1a mRNA in the adenomas or (and) hypothalamus of the GH-secreting pituitary adenomas, whereby to promote GH hypersecretion, cell proliferation and to accelerate the tumorigenicity and progression of these adenomas.

CONCLUSION

In summary, our work demonstrate that GHSR-1a correlated positively with tumor size and invasiveness, while ghrelin correlated positively only with tumor size in human GH-secreting pituitary adenomas. The most important finding of this research is that gsp mutations have a positive effect on GHSR-1a mRNA expression and ghrelin may promotes the GHSR-1a mRNA synthesis in human GH-secreting pituitary adenomas. Further investigation of the underlying molecular mechanism for these phenomena is warranted.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Natural Science foundation of China (No. 30672161). We are grateful to Xie RF for her excellent technical assistance in this research.

REFERENCES

1 Bai X, Lei T, Liu K. (2000). Clinical and biochemical characteristics of growth hormone-secreting pituitary tumors. J Tongji Med Univ. **20**: 212–213.
2 Buchfelder M, Fahlbusch R, Merz T, Symowski H, Adams EF. (1999). Clinical correlates in acromegalic patients with pituitary tumors expressing GSP oncogenes. Pituitary. **1**: 181–185.
3 Dixit VD, Weeraratna AT, Yang H, Bertak D, Cooper-Jenkins A, Riggins GJ, et al. (2006). Ghrelin and the growth hormone secretagogue receptor constitute a novel autocrine pathway in astrocytoma motility. J Biol Chem. **281**: 16681–16690.
4 Grossman AB. (2009). The molecular biology of pituitary tumors: a personal perspective. Pituitary. **12**: 265–270.
5 Hosoi E, Yokogoshi Y, Hosoi E, Horie H, Sano T, Yamada S, et al. (1993). Analysis of the Gs alpha gene in growth hormone-secreting pituitary adenomas by the polymerase chain reaction-direct sequencing method using paraffin-embedded tissues. Acta Endocrinol (Copenh). **129**: 301–306.
6 Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, et al. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. Science. **273**: 974–947.
7 Kan B, Esapa C, Sipahi T, Nacar C, Ozer F, Sayhan NB, et al. (2003). G protein mutations in pituitary tumors: a study on Turkish patients. Pituitary. **6**: 75–80.
8 Kim E, Sohn S, Lee M, Park C, Jung J, Park S. (2005). Effect of gsp oncogene on somatostatin receptor subtype 1 and 2 mRNA levels in GHRH-responsive GH3 cells. Pituitary. **8**: 155–162.
9 Kim K, Arai K, Sanno N, Osamura RY, Teramoto A, Shibasaki T. (2001). Ghrelin and growth hormone (GH) secretagogue receptor (GHSR) mRNA expression in human pituitary adenomas. Clin Endocrinol (Oxf). **54**: 759–768.
10 Kim K, Sanno N, Arai K, Takano K, Yasufuku-Takano J, Teramoto A, et al. (2003). Ghrelin mRNA and GH secretagogue receptor mRNA in human GH-producing pituitary adenomas is affected by mutations in the alpha subunit of G protein. Clin Endocrinol (Oxf). **59**: 630–636.
11 Kineman RD, Kamegai J, Frohman LA. (1999). Growth hormone (GH)-releasing hormone (GHRH) and the GH secretagogue (GHS), L692,585, differentially modulate rat pituitary GHS receptor and GHRH receptor messenger ribonucleic acid levels. Endocrinology. **140**: 3581–3586.
12 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. **402**: 656–660.
13 Lei T, Adams EF, Buchfelder M, Fahlbusch R. (1996). Relationship between protein kinase C and adenylyl cyclase activity in the regulation of growth hormone secretion by human pituitary somatotrophinomas. Neurosurgery. **39**: 569–75; discussion 575–576.
14 Lei T, Yang B, Shu K, Dong F, Xue D, LI Ling, Jiang X. (2002). Analysis of the clinical biochemical characteristics of the growth hormone-secreting pituitary adenoma. Chin J Neurosurg Dis Res. **1**: 164–166.
15 Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. **25**: 402–408.

16 Machado MC, Sa SV, Goldbaum TS, Catania M, Campos VC, Correa-Giannella ML, et al. (2007). In vivo response to growth hormone-releasing peptide-6 in adrenocorticotropin-dependent Cushing's syndrome by lung carcinoid tumor is associated with growth hormone secretagogue receptor type 1a mRNA expression. J Endocrinol Invest. **30**: 334–340.
17 Morita K, Takano K, Yasufuku-Takano J, Yamada S, Teramoto A, Takei M, et al. (2008). Expression of pituitary tumour-derived, N-terminally truncated isoform of fibroblast growth factor receptor 4 (ptd-FGFR4) correlates with tumour invasiveness but not with G-protein alpha subunit (gsp) mutation in human GH-secreting pituitary adenomas. Clin Endocrinol (Oxf). **68**: 435–441.
18 Nanzer AM, Khalaf S, Mozid AM, Fowkes RC, Patel MV, Burrin JM, et al. (2004). Ghrelin exerts a proliferative effect on a rat pituitary somatotroph cell line via the mitogen-activated protein kinase pathway. Eur J Endocrinol. **151**: 233–240.
19 Sakai N, Kim K, Sanno N, Yoshida D, Teramoto A, Shibasaki T. (2008). Elevation of growth hormone-releasing hormone receptor messenger ribonucleic acid expression in growth hormone-secreting pituitary adenoma with Gsalpha protein mutation. Neurol Med Chir (Tokyo). **48**: 481–7; discussion 487–488.
20 Skinner MM, Nass R, Lopes B, Laws ER, Thorner MO. (1998). Growth hormone secretagogue receptor expression in human pituitary tumors. J Clin Endocrinol Metab. **83**: 4314–4320.
21 Yasufuku-Takano J, Takano K, Morita K, Takakura K, Teramoto A, Fujita T. (2006). Does the prevalence of gsp mutations in GH-secreting pituitary adenomas differ geographically or racially? Prevalence of gsp mutations in Japanese patients revisited. Clin Endocrinol (Oxf). **64**: 91–96.
22 Yoshimoto K, Iwahana H, Fukuda A, Sano T, Itakura M. (1993). Rare mutations of the Gs alpha subunit gene in human endocrine tumors. Mutation detection by polymerase chain reaction-primer-introduced restriction analysis. Cancer. **72**: 1386–1393.