Hiroshima J. Med. Sci.
Vol. 61, No. 1, 1~6, March, 2012
HIJM 61–1

Growth Hormone Receptor Expression in Brain Tumors

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ABSTRACT

Growth hormone (GH) is essential for quality of life in both children and adults, but it is also believed to enhance the growth of various neoplasms. However, the role of GH in the brain, particularly in brain tumors, has yet to be established. To clarify these problems from the perspective of receptor expression, we examined GH receptor (GHR) expression in brain tumors using immunohistochemistry and the correlation between GHR expression and clinical features. Surgical specimens obtained from patients with brain tumors (106 pituitary adenomas, 12 craniopharyngiomas, 13 germ cell tumors, 6 medulloblastomas, and 12 malignant gliomas) were examined immunohistochemically for GHR expression. The GHR positive rate was lower in malignant tumors than in benign tumors (59% in pituitary adenomas, 73% in craniopharyngiomas, 23% in germ cell tumors, and 0% in medulloblastomas and gliomas). GHR staining in pituitary adenomas was weaker than that in normal pituitary gland. Among the GH-producing pituitary adenomas, there was no difference in size between GHR-positive and -negative tumors. However, among the non-GH-producing adenomas, GHR-positive tumors were significantly smaller. Thus, immunohistochemical GHR expression may have, at least in part, a negative impact on tumor growth potential in brain tumors.

Key words: *Growth hormone, Growth hormone receptor, Brain tumor, Pituitary adenoma*

Growth hormone (GH) is regarded as an essential factor for quality of life and for correct body composition[13)]. However, patients with brain tumors, such as pituitary adenomas, craniopharyngiomas, germ cell tumors, medulloblastomas or astrocytic tumors, require surgery and/or subsequent radiation therapy, which results in deficiencies in pituitary hormones such as GH. GH deficiency can result in growth impairment in children, and increases the risk of cardiac disease, obesity and dyslipidemia in adults[2)]. Therefore, GH replacement therapy is a widely accepted therapeutic measure for GH deficiency in both children and adults[19)]. In contrast, hyper-secretion of GH also induces several diseases, including hypertension, diabetes mellitus and cancer[4,12)].

The action of GH is mediated via GH receptor (GHR), which is widely expressed in GH target cells. GHR is a single transmembrane domain protein that belongs to the family of cytokine receptors. When GH binds to the GHR, this induces a homodimerization of the receptor, subsequently activating Janus kinase (JAK) 2 and other downstream molecules, including members of the signal transducers and activators of transcription (STAT) protein family, resulting in activation of cell growth. Expression of GHR is widely distributed throughout the body[17)] and the degree of GHR expression is thought to be related to tumor aggressiveness in some extracranial tumors, as many previous studies have examined the expression of GHR and its contribution to tumorigenesis in various extracranial cancers, such as colon[22,23)], liver[9)] and breast cancer[10)]. Thus, GHR expression is believed to be a trigger for carcinogenesis in some extracranial tumors.

GH is secreted from the pituitary gland, and this secretion is regulated by the hypothalamus and pituitary gland. Thus, GHR expression is observed in the hypothalamus and pituitary cells. In addition, GHR is ubiquitously expressed in the

brain, and GH is thought to act via GHR binding in neural growth and development, neurotransmission, behavior and psychology. The brain is therefore thought to be a target site for GH action[11]. Similarly in some extracranial tumors, GHR expression in the central nervous system is supposed to have a positive effect on tumor aggressiveness, particularly under hyper-GH secretory conditions. Several previous reports have examined GHR expression in intracranial tumors (mainly pituitary tumors)[5,6,15,16,21]. Lincoln et al reported that immunohistochemical GHR expression is observed in the cytoplasm and nucleus in some gliomas and pituitary adenomas[16]. Uchino et al reported that some recurrent brain tumors (craniopharyngioma, glioma, germ cell tumors) after GH replacement therapy exhibited immunohistochemical GHR positivity[21]. However, these reports did not examine the effects of GHR on clinical features, such as tumor growth potential and hormonal secretion.

We therefore immunohistochemically examined GHR expression in several types of intracranial tumor. We also analyzed the correlation between GHR expression and biological/clinical parameters of the tumors, with a particular focus on pituitary adenomas. Since detailed clinical data, such as tumor size and endocrinological function were available from patients with pituitary adenomas, we were able to evaluate the effect of GHR expression on tumor growth or on hormonal secretion. As pituitary adenomas are histologically and clinically benign, we also examined GHR expression in malignant tumors (medulloblastomas and malignant gliomas) in order to evaluate the correlation between GHR expression and tumor aggressiveness.

MATERIALS AND METHODS

This study included surgically obtained tissue from patients with brain tumors: pituitary adenomas (n=106) (30 clinically non-functioning adenomas, 41 GH-producing adenomas, 23 prolactin (PRL)-producing adenomas, and 12 adrenocorticotropic hormone (ACTH)-producing adenomas), craniopharyngiomas (n=12), germ cell tumors (n=13), medulloblastomas (n=6) and malignant gliomas (n=12) (6 each of WHO grade 3 and grade 4). Surgeries were performed at Hiroshima University Hospital between 2000 and 2006. Magnetic resonance imaging (MRI) and endocrinological and histopathological data were further obtained from patients with pituitary adenoma for statistical analysis. The study protocol was approved by our institutional review board, and written consent from patients was waived. To protect patient privacy, we removed all identifiers from our records upon completion of our analyses.

All tumor specimens were fixed in 10% formalin before paraffin processing and were stained with hematoxylin and eosin to confirm the histological diagnosis. The primary antibody used to analyze GHR was a mouse monoclonal antibody (MAB 263, ab11380; Abcam Inc., Cambridge, MA), which was used at a 1:100 dilution. Specimens (4 μm) were mounted on gelatin-coated slides and were deparaffinized using a 15-min xylene treatment. To block endogenous peroxidase, slides were immersed for 30 min in 3% hydrogen peroxidase in methanol. Each specimen was rinsed 3 times for a total of 15 min in phosphate-buffered saline (pH 7.5) with gentle stirring. Specimens were then incubated overnight with primary antibody at 4°C. Negative controls were prepared by omitting the primary monoclonal antibody. Normal pituitary gland (from a cadaver) was used as a positive control. In cases of pituitary adenoma, all tumor specimens were stained with anterior pituitary hormone (GH, PRL, thyroid-stimulating hormone, ACTH, luteinizing hormone and follicle-stimulating hormone) antibodies for immunohistochemical diagnosis.

Tumors were divided into two groups based on degree of GHR staining; negative (little or no staining) and positive (strong staining as compared to negative controls) (Fig. 1). GHR staining was evaluated and categorized by three medical doctors (K.S., S.H. and Y.K.) who were blinded to clinical and pathologic data. Tumor classifications were also assigned by all the three doctors. For pituitary adenomas, the correlation between GHR expression and tumor characteristics was then examined by comparing the GHR-negative and -positive groups with respect to the following clinical factors: age; gender; hormone secretion; hormone value; and tumor size.

In all patients with parasellar tumors (pituitary adenomas, craniopharyngiomas, and neurohypophyseal germ cell tumors), anterior pituitary functions were evaluated using provocation tests before surgery, as described previously[20]. GH secretion was evaluated using induced hypoglycemia (by intravenous injection of 0.1 U/kg body weight insulin), or arginine administration test (by intravenous drip of 30 g L-arginine) for patients whose medical conditions contraindicated hypoglycemia (age ≥ 65 years, hypopituitarism, diabetes mellitus or coronary disease).

In order to estimate the tumor growth of patients with pituitary adenomas, we evaluated pre-operative tumor size based on the maximum diameter on axial, coronal and sagittal MRI. Generally, tumor growth potential was examined by MIB-1 index, but the MIB-1 indices for most pituitary adenomas were < 1%; thus, the MIB-1 index was insufficient for statistical analysis.

The Mann-Whitney U test for continuous

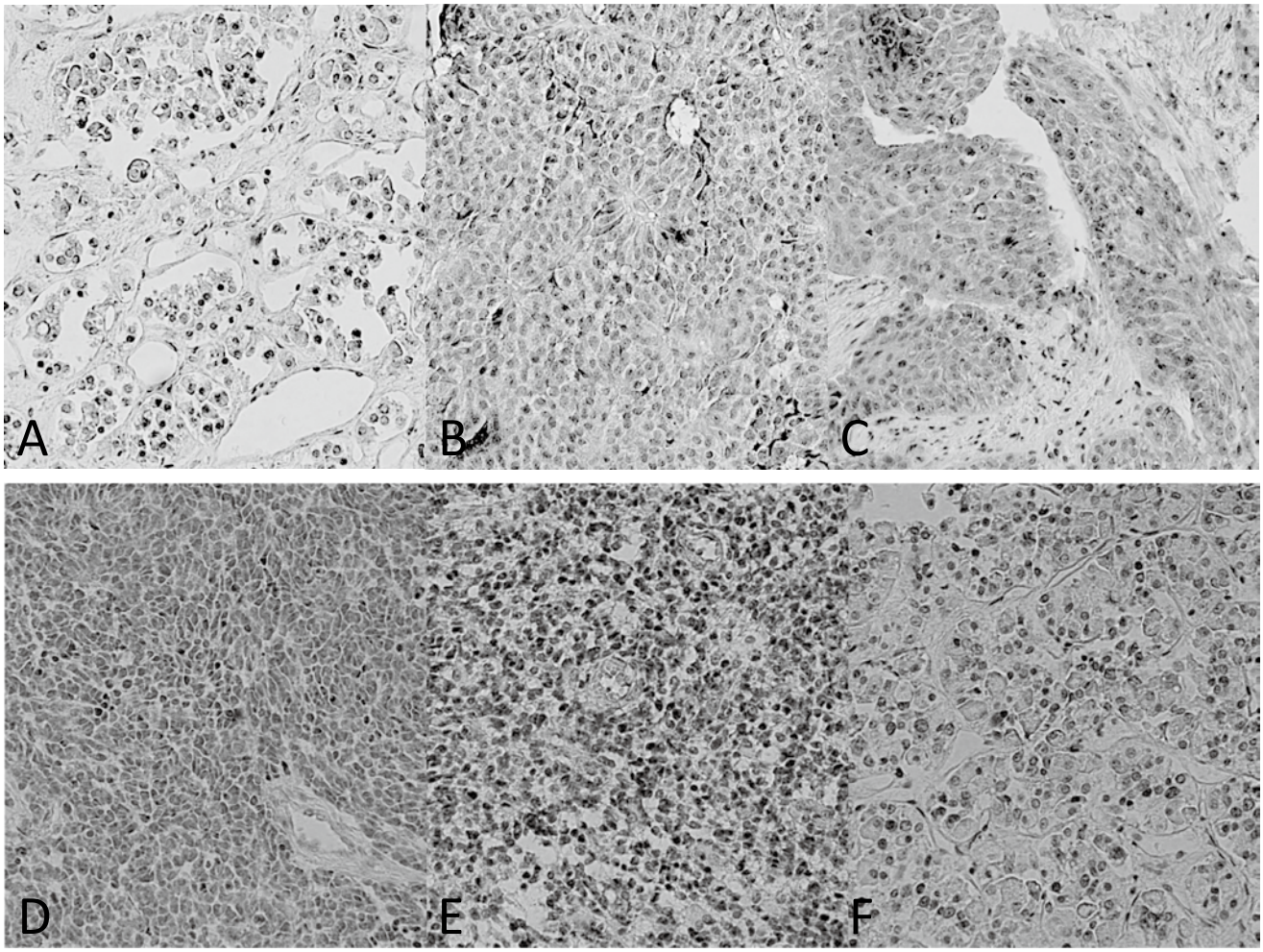

Fig. 1. Representative brain tumors, analyzed by immunohistochemistry for GHR expression using a GHR-specific antibody (B-E), are shown (× 100).
Normal pituitary tissues stained with (A) or without (F) this antibody were used as positive and negative controls, respectively. Normal pituitary tissues showed strong, cell-specific, immunopositivity (A). Pituitary adenomas showed mainly cytoplasmic staining for anti-GHR antibody, while the staining intensity of pituitary adenomas was weaker than that of normal pituitary cells (B). Some craniopharyngiomas showed strong cytoplasmic staining in squamous tumor cells (C). Medulloblastomas (D) and malignant gliomas (E) showed no apparent GHR staining.

variables and Fisher's exact test for categorical variables were used to examine the correlation between various clinicopathological parameters and GHR expression in pituitary adenomas, and p values of < 0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS version 16.0 for Windows (SPSS, Chicago, IL).

RESULTS

The positive staining rates for GHR in pituitary adenomas and craniopharyngiomas were 59% and 73%, respectively, but the positive rates were 23% in germ cell tumors and 0% in medulloblastomas and gliomas (Fig. 1, Table 1). The degree of GHR staining in pituitary adenoma cells was similar to or weaker than that in normal pituitary gland (positive control) (Fig. 1).

A summary of GHR expression and the clinical features of patients with pituitary adenomas (Table 2) confirmed no significant differences in gender, tumor type (hormone production) or hormone value in hormone-producing adenomas between GHR-positive and -negative tumors. However, the patients in the GHR-positive group were significantly younger, and tumor size was smaller, as compared to the GHR-negative group.

In order to determine whether circulating GH influences GHR expression and enhances tumor growth, we examined the correlation between tumor size and GHR expression by comparison with the type of secreted hormone (none, GH, PRL, and ACTH). No correlations were seen between GHR expression and tumor size in GH-producing adenomas. However, among non-GH-

Table 1. The ratio of GHR expression in brain tumors

Type of tumor		GHR positivity	
Pituitary adenoma	(n=106)	59%	(n=63)
Craniopharyngioma	(n=11)	73%	(n=8)
Germ cell tumors	(n=13)	23%	(n=3)
Medulloblastoma	(n=6)	0%	(n=0)
Malignant glioma	(n=12)	0%	(n=0)

GHR: Growth hormone receptor

Table 2. Summary of GHR expression and clinical features in pituitary adenomas

		Total	GHR (+)	GHR (−)	p value
No. of Cases		106	64	42	
Mean age (y.o.)		47.8 ± 16.5	44.9 ± 16.6	52.1 ± 15.6	0.026*
Gender	Male	41	21	20	0.12
	Female	65	43	22	
Type of tumor	NF	30	14	16	0.23
	GHoma	41	24	17	
	PRLoma	23	16	7	
	ACTHoma	12	9	3	
Mean hormone value in hormone producing adenomas					
GHoma	GH (ng/ml)	33.3 ± 41.6	35.5 ± 42.7	31.8 ± 41.5	0.78
	IGF-1 (ng/ml)	1018 ± 345.9	1008.3 ± 353.3	1033.5 ± 344.8	0.82
PRLoma	PRL (ng/ml)	971.8 ± 2394	233.9 ± 152.2	2658.6 ± 4022.7	0.16
ACTHoma	ACTH (pg/ml)	157.1 ± 102.9	176.7 ± 111.4	98.1 ± 39.7	0.1
	cortisol (mg/dl)	27.8 ± 9.8	27.3 ± 8.5	29.3 ± 15.4	0.85
Mean size (mm)		20.2 ± 11.2	18.1 ± 10.0	23.7 ± 12.4	0.018*

NF: clinically non-functioning pituitary adenoma, GHoma: GH-producing pituitary adenoma, PRLoma: PRL-producing pituitary adenoma, ACTHoma: ACTH-producing pituitary adenoma, IGF-1: insulin-like growth factor type-1. *Significantly different between the GHR (+) and (−) groups ($p<0.05$)

Table 3. GHR expression and tumor size (mm) in pituitary adenomas

		No. Cases	Overall	GHR (+)	GHR (−)	p value
GH		41	19.9 ± 10.0	19.8 ± 10.6	20.1 ± 9.9	0.91
Non-GH		65	20.4 ± 11.9	16.5 ± 9.6	26.3 ± 12.9	0.00072*
	NF	30	25.0 ± 7.3	23.8 ± 6.6	26.1 ± 8.8	0.42
	PRL	23	14.4 ± 8.7	10.9 ± 3.1	22.3 ± 12.1	0.049*
	ACTH	12	20.3 ± 19.6	14.9 ± 13.7	36.3 ± 29.1	0.1
Total		106	20.2 ± 11.2	18.1 ± 10.0	23.7 ± 12.4	0.018*

GH: GH-producing adenomas, NF: clinically non-functioning adenomas, PRL: PRL-producing adenomas, ACTH: ACTH-producing adenomas

*Significantly different between the GHR (+) and (−) groups ($p<0.05$)

secreting pituitary adenomas, particularly among PRL-secreting adenomas, GHR-positive tumors were significantly smaller in size than GHR-negative tumors (Table 3).

DISCUSSION

The aim of this study was to clarify the clinical significance of GHR expression in brain tumors. The results suggest that GHR expression in

histologically malignant tumors (gliomas, medulloblastomas) is much weaker than that in benign tumors (pituitary adenomas, craniopharyngiomas). Moreover, our study of pituitary adenomas indicates that GHR-positive tumors, particularly among non-GH-producing adenomas, are significantly smaller in size than GHR-negative tumors.

Generally, neoplastic cells exhibit stronger immunohistochemical staining for GHR (in terms either of the proportion of positive cells or staining intensity) than normal tissue cells[16]. However, pituitary adenoma cells show weaker GHR staining than normal pituitary cells[15], which exhibit strong cell specific immunopositivity[18]. Beuschlein et al and Kola et al have reported that GHR mRNA expression in pituitary adenomas is lower than in normal pituitary tissue[5,15], particularly in non-functioning and GH-producing adenomas[5]. The immunohistochemical data of the present study is consistent with these previously reported observations. Thus, in brain tumors, as typified by pituitary adenomas, GHR expression is thought to be weaker than that in normal tissue cells.

Interaction between GH and GHR is thought to influence cell growth[14], and GHR-positive pituitary adenoma cells have high potential for tumor growth under high circulating concentrations of GH. Thus, we examined the relationship between GHR expression and tumor size after grouping based on hormone production. Contrary to our expectations, a correlation between GHR expression and tumor size was not observed in patients with GH-producing adenomas. Instead, non-GH-producing tumors showed a negative correlation between GHR expression and tumor size. Previous reports have implicated GH in the tumorigenicity of malignant tumors in other organs[9,10,14,22,23]. However, based on *in vitro* studies, Clausen et al. reported that GH does not consistently influence cell proliferation or hormone secretion in pituitary adenomas[6]. Thus, neither normal levels of GH secreted from normal pituitary cells nor excess levels of GH produced by tumor cells enhance tumor growth.

There are two isoforms of GHR messenger RNA: full-length GHR (GHRfl), which is responsible for the growth-promoting actions of GH through the JAK2/STAT5 signaling pathway, and truncated GHR (GHRt), which is a short membrane-anchored form of GHR that lacks 97.5% of the functional intracellular domain and binds GH without subsequent intracellular activation[8]. In addition, like many cytokine receptors, GHR exists in a soluble GH-binding protein (GHBP) form that corresponds to the extracellular domain of GHR, and GHBP is a negative regulator of GH in plasma[1,7]. GHRt is known to have a greater potential for generating soluble GHBP than GHRfl. Thus, GHRt is supposed to inhibit GH action mediated by GHRfl in a dominant-negative manner[3]. Clausen et al demonstrated that GHRt expression was present in 9 of 12 culture cells derived from pituitary adenomas[6]. Because the anti-GHR antibody used in the present study was able to recognize only the extracellular domain (not the intracellular domain) of GHR[1,3], it is possible that the antibody detected both isoforms of GHRfl and GHRt. This may explain why GHR expression did not influence tumor growth (tumor size) despite GH hyper-secretion.

In the present study, GHR staining was primarily seen in the cytoplasm, although GHR is thought to act as a transmembrane receptor. Numerous previous reports have also confirmed the localization of GHR staining in the cytoplasm. After GH binds to GHR, a dimer is formed, leading to signal transduction[1]. GHR is then internalized, followed by degradation or recycling/exocytosis[1]. Meanwhile, surface GHR is rapidly turned over; thus, the main localization of GHR staining appears to be in the cytoplasm.

We examined the correlation between GHR expression of, and clinical findings for, brain tumors. However, due to the small number of sampled tumors other than pituitary adenomas, the results could not be analyzed statistically. Further, GHR expression was the only factor examined immunohistochemically in this study. Thus, future studies are necessary in order to clarify the interaction between GHR and tumor growth in brain tumors.

In conclusion, although the GH/GHR pathway is believed to accelerate the tumorigenesis of extracranial tumors, the levels of immunohistochemical GHR expression in brain tumors and pituitary adenomas may have, at least in part, a negative impact on the biological aggressiveness of these tumors.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid for Research on Hypothalamo-Pituitary Dysfunction from the Japanese Ministry of Health Labor and Welfare (to K.A.), and by Kiban Research Grant B (to K.A. and K.K.) and Kiban Research Grant C (to S.H.) from the Japanese Ministry of Education, Culture, Science and Technology.

(Received October 25, 2011)
(Accepted January 5, 2012)

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