

Expression of hepatocyte growth factor and its receptor c-Met in human pituitary adenomas

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Hepatocyte growth factor (HGF) and its receptor c-Met have been known as key determinants of growth and angiogenesis in some brain tumors like gliomas, meningiomas, and schwannomas. But little is known about their expression in pituitary adenomas. In this study, the expression of HGF and c-Met in pituitary adenomas of different histology types was investigated by immunohistochemistry, and correlative analysis of their expression with microvessel density (MVD), Ki-67 expression, and other clinicopathologic factors was made. The results showed that the expression of HGF and c-Met exists in 98% (64 of 65) and 92% (60 of 65) pituitary adenomas, respectively, and co-expression of them existed in 91% (59 of 65) adenomas. HGF had significant correlation with MVD (Spearman's correlation coefficient, $r = .31$, $P = .01$) and Ki-67 ($r = .32$, $P = .01$). c-Met had significant correlation with MVD ($r = .30$, $P = .02$) and Ki-67 ($r = .38$, $P = .00$). HGF and c-Met expression had no significant correlation with age or extrasellar extension. There were no significant differences in HGF and c-Met expression between pituitary adenomas of different histology types. The results indicate that HGF and c-Met are widely expressed in pituitary adenomas, and their expression correlates with MVD and Ki-67 expression.

Keywords: c-Met, hepatocyte growth factor, pituitary adenoma.

Hepatocyte growth factor (HGF) is a multifunctional tumor growth and angiogenic factor. The receptor tyrosine kinase c-Met is the only known receptor for HGF. HGF and c-Met are expressed in human brain tumors like gliomas, meningiomas, and

schwannomas, and their expression levels frequently correlate with poor prognosis.^{1–3} HGF:c-Met signaling pathway has been known to induce tumor cell proliferation, motility, invasion, and promote tumor angiogenesis.^{4–7} Approaches targeting HGF and c-Met have been proven to inhibit brain tumor growth and angiogenesis as well as tumor HGF and c-Met expression levels.⁸ So HGF and c-Met pathway targeted treatment may be a promising therapeutic strategy.

Though Kim et al.⁹ showed Met mRNA expression in functional and nonfunctional pituitary adenomas, little is known about protein expression of HGF and c-Met in pituitary adenomas. Herein, HGF and c-Met protein expression in pituitary adenomas of different histology types were investigated by immunohistochemistry, and correlative analysis of their expression with microvessel density (MVD), Ki-67 expression, and some other clinicopathologic factors was made.

Materials and Methods

About 10% of the neutral buffered formalin and paraffin embedded surgical specimens from 65 patients with pituitary adenomas who were admitted and operated on consecutively from October 2008 to May 2009 at Neurosurgery Department of Provincial Hospital Affiliated to Shandong University were included. Pituitary adenomas were classified according to hormone immunohistochemistry previously done by two pathologists at the pathology department of Provincial Hospital Affiliated to Shandong University. Patients' characteristics can be seen in Table 1.

The immunohistochemical study was performed using the strepto-avidin-biotin complex method. Primary antibodies used were as follows: Met (c-28) (rabbit polyclonal, sc-161, 1:50, Santa Cruz Biotechnology); HGF α (H-145) (rabbit polyclonal, sc-7949, 1:100, Santa Cruz Biotechnology); CD34 (BI-3c5) (rabbit polyclonal, sc-19621, 1:100, Santa

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Table 1. Expression of HGF, c-Met, Ki-67, MVD, and other clinicopathologic factors in pituitary adenomas of different histology types

Histology types of pituitary adenomas (number of adenomas)	Sex (male vs female)	Age (mean years)	Number of adenomas with extrasellar invasion	Ki-67 (\bar{x})	MVD (\bar{x})	HGF (-/+ / +/+ / +/+ / +/+ / +/+)	c-Met (-/+ / +/+ / +/+ / +/+)
Simple hormone immunopositive ²⁷	10/17	40	20	2.7	8.9	0/10/4/13	2/7/3/15
Plurihormone immunopositive ²⁸	6/22	41	21	3.9	9.4	1/3/3/21	2/2/4/20
Hormone immunonegative ¹⁰	5/5	53	8	3.7	10.4	0/1/1/8	1/1/1/7
Total (65)	21/44	43	49	3.3	9.3	1/14/8/42	5/10/8/42

Abbreviations: HGF, hepatocyte growth factor; MVD, microvessel density. Simple hormone immunopositive adenomas: pituitary adenomas with only 1 kind of hormone immunoreactivity including 10 adrenocorticotrophic hormone (ACTH) adenomas, 7 prolactin (PRL) adenomas, 5 growth hormone (GH) adenomas, 4 follicle stimulating hormone (FSH) adenomas, and 1 thyroid stimulating hormone (TSH) adenomas. Plurihormone immunopositive: pituitary adenomas with 2 or more kinds of hormone immunoreactivity including ACTH, PRL, GH, FSH, and TSH. Pituitary adenomas with extrasellar invasion: adenomas which extends beyond its capsule or involves neighboring structures like the dura, bone, blood vessels, and nerves seen from MRI. \bar{x} means the mean scores for Ki-67 and MVD in pituitary adenomas of different histology types. -/+ / +/+ / +/+ / +/+ refers to the number of adenomas with different immunoreactivity for HGF or c-Met: -, no immunopositive cells; +, <30% of tumor cells are immunopositive; ++, 30%–60% of tumor cells are immunopositive; +++, >60% of tumor cells are immunopositive.

Cruz Biotechnology); Ki-67 (mouse monoclonal, M7240, 1:150, DAKO). Sections of gliomas were used as a positive control to prove the specificity of the antibodies. In negative control, primary antibodies were replaced by phosphate buffered solution.

Tumor-containing sections of 5 μ m thickness were baked at 60°C for 30 minutes, deparaffinized in xylene, and rehydrated in graded concentrations of ethanol. Heat-induced antigen retrieval (10 mM citrate buffer [pH 6.0] at 96°C for 15 minutes in a thermostat-controlled waterbath) was used. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide at 37°C for 15 minutes. Nonspecific binding of primary antibodies was blocked by normal serum from the same species as that of secondary antibodies at 37°C for 25 minutes. Immunostaining involved sequential applications of primary antibody at 4°C overnight, incubation at 37°C for 30 minutes, followed by biotinylated secondary antibodies (Zhongshan Goldenbridge Biotechnology) at 37°C for 30 minutes and strepto-avidin-biotin complex (Zhongshan Goldenbridge Biotechnology) at 37°C for 30 minutes. Diaminobenzidine was used as the enzyme substrate to observe the specific antibody localization, and hematoxylin (not in Ki-67 immunostaining) was used as a nuclear counterstain. In Ki-67 immunostaining, eosin was used as a cytoplasmic counterstain.

Sections were examined and scored for immunoreactivity for HGF, c-Met, MVD, and Ki-67 by an observer who was unaware of the histological diagnoses or clinical features. Scores for HGF and c-Met were recorded as follows: -, no immunopositive cells; +, <30% of tumor cells are immunopositive; ++, 30%–60% of tumor cells are immunopositive; +++, >60% of tumor cells are immunopositive. Scores for Ki-67 were recorded as the number of immunopositive cells per high-power microscope ($\times 400$). The number of immunopositive cells under 5 microscopes per slide with the highest cell counts was counted and the average was recorded. MVD was recorded as the

number of vessels or clusters of cells immunopositive for CD34 per high-power microscope ($\times 400$). Each immunostained cell or cell cluster that was clearly separated from adjacent microvessels was considered as a single countable microvessel. MVD under 5 microscopes per slide with the highest vascular counts were counted and the average was recorded.

Spearman rank correlation analysis (SPSS statistical software, version 11.5) was used in the analysis of correlation between HGF and c-Met expression, MVD, Ki-67 expression, age, sex, and extrasellar extension because the assumption for a parametric test (Kolmogorov–Smirnov test) was invalid except for age and MVD. The Kruskal–Wallis test was used to see whether HGF or c-Met was differentially expressed between adenomas of different histology types. Expression levels of HGF and c-Met in pituitary adenomas of different histology types are recorded in Table 1 and MVD, Ki-67, and patients' age are recorded as mean (\bar{x}) in Table 1. The results were reported as being statistically significant if *P* value was <.05.

Results

Results showed that HGF and c-Met expression existed in 98% (64 of 65) and 92% (60 of 65) of pituitary adenomas, respectively, and co-expression of HGF and c-Met existed in 91% (59 of 65) of pituitary adenomas. The number of adenomas with different immunoreactivity for HGF and c-Met can be seen in Table 1. There were no significant differences in c-Met or HGF expression between pituitary adenomas of different histology types. HGF and c-met expression in pituitary adenomas can be seen in Figs 1 and 2.

There was a significant correlation between HGF and c-Met expression ($r = .41, P = .00$). Both HGF and c-Met expression had significant correlation with MVD and Ki-67, but not with age or extrasellar invasion (for details, see Table 2). c-Met had a correlation with

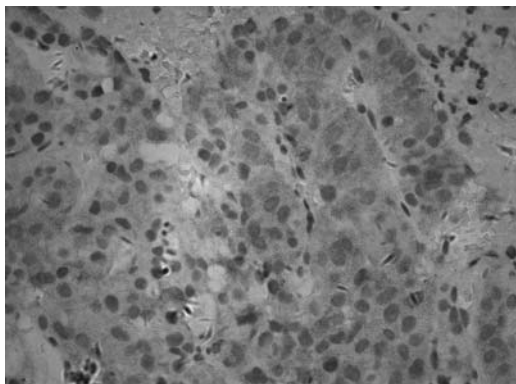


Fig. 1. HGF expression in follicle stimulating hormone adenoma ($\times 400$).

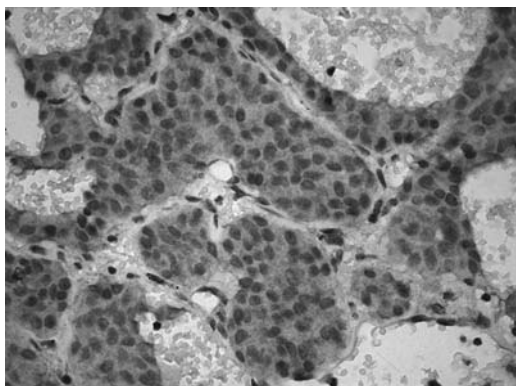


Fig. 2. c-Met expression in adrenocorticotrophic hormone adenoma ($\times 400$).

Table 2. Correlational analysis of HGF and c-Met expression with MVD, Ki-67, age, sex, and extrasellar invasion

Clinicopathological factors	HGF		c-Met	
	r^*	p^\dagger	r	p
MVD	.31	0.01	.30	0.02
Ki-67	.32	0.01	.38	0.00
Age	.05	0.70	-.05	0.68
Sex	.13	0.32	.26	0.04
Extrasellar invasion	-.03	0.79	-.20	0.10

Abbreviations: HGF, hepatocyte growth factor; MVD, microvessel density.

*Spearman rank correlation coefficient (r) is considered significant when $P < .05$.

†Permutation test (p) is used to test the significance of Spearman correlation coefficient.

sex ($r = .26$, $P = .04$). c-Met expressions in male and female patients were 81% (19 of 21) and 98% (43 of 44), respectively.

Discussion

To our knowledge, this is the first study that investigated HGF and c-Met expression in pituitary adenomas. The

study shows that HGF and c-Met are widely expressed in pituitary adenomas, and their expression significantly correlate with tumor angiogenic and proliferative factors. This implies that HGF and c-Met may have a role in the angiogenesis and tumorigenesis of pituitary adenomas.

Tumor angiogenesis, the formation of new blood vessels from pre-existing vessels, is essential for tumor growth. Angiogenesis, evaluated as tumor MVD, has significant association with metastasis, poor prognosis, and recurrence in breast, brain, bladder, prostate, and gastric cancers.¹⁰⁻¹⁵ HGF and c-Met are involved in various processes of brain tumor angiogenesis, including inducing proliferation and migration of tumor endothelial cells, enhancing vascular endothelial growth factor expression, and inducing endothelial tubule formation and angiogenesis.⁸ HGF and c-Met expression correlate with angiogenesis in breast, bladder, gastric, and soft tissue tumors.¹⁶⁻¹⁹ Inhibitors targeting HGF or c-Met have been shown to inhibit tumor angiogenesis.^{20,21} The present study shows that HGF and c-Met expression in pituitary tumors significantly correlate with MVD ($P < .05$). This implies that HGF and c-Met may have a role in pituitary angiogenesis.

HGF and c-Met are involved in the processes of tumorigenesis, including promoting tumor cell proliferation,^{22,23} invasion,²⁴⁻²⁶ and metastasis.²⁷ HGF and c-Met expression correlate with tumor growth, invasion, metastasis, and poor prognosis in bladder,²⁸ breast,²⁹ liver,³⁰ and lung³¹ cancers, and gliomas.^{32,33} Therapeutic agents targeting HGF and c-Met have been shown to inhibit tumor growth and improve survival.³⁴⁻³⁶ Ki-67 is a proliferative marker, and its expression correlates with poor prognosis in brain tumors like gliomas, ependymomas, and pituitary adenomas.³⁷⁻⁴⁴ The study shows that HGF and c-Met expression in pituitary tumors significantly correlate with Ki-67 expression ($P < .05$). This implies that HGF and c-Met may have a role in pituitary tumorigenesis.

Most pituitary adenomas are benign, but many of them have aggressive growth and invade important neighboring structures like blood vessels, nerves, dura, and bone, which makes complete surgical resection impossible and tumor recurrence is often observed after surgery. HGF and c-Met expression have no significant differences between invasive and noninvasive adenomas. The result implicates that HGF and c-Met expression in pituitary adenomas may not be related to invasive behaviors.

In the present study, age had no significant correlation with HGF or c-Met, which is in accord with previous results.⁴⁵⁻⁴⁷ c-Met expression had a correlation with sex ($r = .26$, $P = .04$), but this is different from the previous results⁴⁵⁻⁴⁷ and the underlying mechanisms are unknown. No significant differences exist in HGF and c-met expression between pituitary adenomas of different histology types. So HGF and c-met expression in pituitary adenomas may not be related to hormone immunoreactivity.

In conclusion, HGF and c-Met are widely expressed in human pituitary adenomas, and their expression

levels correlate with angiogenic and proliferative markers, but not with age, extrasellar invasion, or histology types. However, more investigations should be done about their roles in human pituitary adenomas.

Conflict of interest statement. None declared.

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