FEBS Letters 372 (1995) 78–82 FEBS 16021

Concomitant expression of hepatocyte growth factor (HGF), HGF activator and c-met genes in human glioma cells in vitro

Takuzou Moriyama[a], Hiroaki Kataoka[a], Hirohito Tsubouchi[b], Masashi Koono[a,*]

[a]*Second Department of Pathology, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-16, Japan*
[b]*Second Department of Internal Medicine, Miyazaki Medical College, 5200 Kihara, Kiyotake, Miyazaki 889-16, Japan*

Received 6 June 1995; revised version received 25 July 1995

Abstract Three new cell lines of human glioblastoma have been established. These cells co-expressed hepatocyte growth factor (HGF) and its receptor, c-Met, genes in vitro. Reverse-transcriptase/polymerase-chain reaction study revealed that the cells also expressed gene for HGF activator, a recently cloned serine proteinase, suggesting that HGF might have a role in glioma cells in vitro as an autocrine factor. The activator mRNA was also detected in other well-established glioma cell lines, glioma tissues and normal brain. The concomitant expression of HGF, HGF activator and c-met was also detected in one glioblastoma case in vivo out of five tested.

Key words: Hepatocyte growth factor; Hepatocyte growth factor activator; c-Met; Glioma

1. Introduction

Cellular proliferation and migration participate in one of the important aspects of malignant phenotypes of the tumors, particularly of brain tumors which rarely metastasize but can kill the host by their proliferative and migratory activity in the brain that is located in a rigidly confined space, the skull. These processes are often mediated by polypeptide growth factors acting in either a paracrine or autocrine fashion. Hepatocyte growth factor (HGF) is a pleiotropic factor initially identified as a polypeptide growth factor for hepatocytes [1,2], and is indistinguishable from scatter factor (SF), a motility factor [3–6]. HGF functions as a mitogen for a variety of cell types, as a morphogen and as a motogen for some epithelial cells [6]. Although the roles of HGF in tumors have yet been clarified, the identification of its receptor as the c-met proto-oncogene product, which was originally described as an activated oncogene in a human osteosarcoma cell line [7], has accelerated efforts to determine the roles of HGF in tumors [6]. To generate the biological activity of HGF, the proteolytic conversion of the single-chain precursor form to the two-chain heterodimer active form is essential [8]. The conversion occurs after secretion and takes place in the extracellular environment [8,9]. This converting activity was first purified from fetal bovine serum [10] and designated as HGF activator, a novel serine proteinase. Recently, the HGF activator was also purified from human serum and its cDNA was cloned [11]. This HGF activator mRNA has been reported to be detected only in the liver with Northern blot analysis [11], but the expression of this gene in tumors has yet been examined. Urokinase-type plasminogen activator (u-PA) was also reported to be a candidate for the converting proteinase [12,13].

Gliomas are the most common brain tumors. Glioblastoma multiforme and anaplastic astrocytoma are high-grade gliomas of astrocytic origin and the prognosis for patients with glioblastoma is very poor. Recently a few reports described the expression of HGF and c-Met receptor in the normal brain tissue [14–16]. However, to date, little is known about the expression, functions and roles of these proteins in gliomas. We report here the expression not only of HGF and c-met, but also HGF activator mRNAs in cell lines and tissues of gliomas, particularly glioblastoma multiforme.

2. Materials and methods

2.1. Cell culture

MGM-1 was derived from a primary tumor of left temporal lobe with histological diagnosis of glioblastoma multiforme, removed from a 64-year-old Japanese male in August 1992. The patient had not received other therapies before surgery. MGM-2 was derived from a primary tumor of left fronto-parietal lobe with histological diagnosis of glioblastoma multiforme, removed from a 37-year-old Japanese male in December 1993. The patient had not received other therapies before surgery. MGM-3 was derived from a recurrent tumor of right frontal lobe with histological diagnosis of glioblastoma multiforme, removed from a 73-year-old Japanese female in July 1994. Prior to the surgery, chemo- and radiation-therapy were done. For primary culture, fragments of the surgically resected tumors were rinsed repeatedly with phosphate-buffered saline, minced finely and digested in 0.2% collagenase (Wako Junyaku, Osaka, Japan), 1000 U/ml dispase (Sanko Junyaku, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DMEM) at 37°C for 2 h. Then the dispersed cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and antibiotics. The human glioma cell lines, U251 and KG-1-C, were obtained from RIKEN cell bank (Tsukuba, Japan) and cultured in the same medium. U251 was derived from a glioblastoma multiforme [17], and KG-1-C was from a mixed glioma and has features of oligodendroglioma [18]. A human pancreas adenocarcinoma cell line SUIT-2 [19] was kindly provided by Dr. T. Iwamura, First Department of Surgery, Miyazaki Medical College, Japan. A human rectal adenocarcinoma cell line, RCM-1, was established in our laboratory [20]. Conditioned media were harvested from the confluent cultures after 24 h cultivation.

2.2. RNA and DNA extraction

Total RNA was extracted from the tissue samples by Trizol (Gibco BRL, Gaithersburg, MD) followed by poly(A)$^+$ RNA selection by using oligo(dT)-Latex (Takara Shuzo, Shiga, Japan) according to the manufacturer's instructions. Poly(A)$^+$ RNA was extracted from the cultured cells by using FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). The chromosomal DNA was extracted by using DnaQuick DNA extraction kit (Dainippon Seiyaku, Osaka, Japan). Human whole brain poly(A)$^+$ RNA was purchased from Clontech (Palo Alto, CA).

2.3. Reverse-transcriptase/polymerase-chain reaction (RT-PCR)

For cDNA synthesis, 0.1 μg of poly(A)$^+$ RNA was reverse-transcribed with oligo(dT)$_{15}$ and SuperScript reverse transcriptase (Gibco BRL) according to the manufacturer's instruction. For PCR, the

*Corresponding author. Fax: (81) (985) 85-6003.

SSDI 0014-5793(95)00949-3

cDNA samples were brought to a final concentration of 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM dNTPs and 7 mM anti-Taq polymerase monoclonal antibody (Clontech). In addition, each sample contained 0.1 pmol of both reverse and forward primers and 2.5 U of Taq polymerase (Takara). PCR was carried out in a programmable heating block (Perkin Elmer Cetus, Norwalk, CT) using 5 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C (for HGF activator) or 58°C (for HGF and c-met) for 2 min and extension at 72°C for 2 min, followed by 30 cycles consisting of 94°C for 30 s, 55°C or 58°C for 1 min and 72°C for 2 min. Products were analyzed by 2.5% agarose gel electrophoresis. The products were also subcloned into TA cloning vector, pCR II (Invitrogen, San Diego, CA) and the nucleotide sequences were determined by the dideoxy chain-termination method.

2.4. Primers for PCR

The sequence of the forward primer for HGF activator, HGFA-s3 and HGFA-s4, are 5′-AGGACACAAGTGCCAGATTG-3′ and 5′-CGACGGACGTGACGCAGACC-3′, respectively, and correspond to bases 1593–1612 and 1409–1428, respectively, of the HGF activator sequence [11]. The sequences of reverse primers for HGF activator, HGFA-as2 and HGFA-as4, are 5′-TCGGACTTGCAGTCGAAGTA-3′ and 5′-GTTGATCCAGTCCACATAGT-3′, respectively, and are complementary to bases 1756–1775 and 1907–1926, respectively, of the HGF activator sequence [11]. The sequences of the forward primers for c-met, MET-s1 and MET-s2, are 5′-ATTCATCAGGCTGTGAA-GCG-3′ and 5′-ACAGTGGCATGTCAACATCGCT-3′, respectively, and are complementary to bases 1214–1233 and 2413–2434, respectively, of the c-met sequence [21]. The sequences of the reverse primers for c-met, MET-as1 and MET-as2, are 5′-CAGAGAGGCATTGACT-GCAG-3′ and 5′-GCTCGGTAGTCTACAGATTC-3′, respectively, and are complementary to bases 1575–1594 and 3049–3068 [21]. The sequences of the forward primers for HGF, HGF-s2 and HGF-s3, are 5′-GGGAAATGAGAAATGCAGCCAG-3′ and 5′-TCACGAGCAT-GACATGACTCC-3′, respectively, and are complementary to bases 1911–1932 and 1002–1022, respectively of the HGF sequence [22]. The sequences of the reverse primers for HGF, HGF-as2 and HGF-as3, are 5′-AGTTGTATTGGTGGGTGCTTC-3′ and 5′-AGCTTACTTGCA-TCTGGTTCC-3′, respectively, and are complementary to bases 2205–2225 and 1284–1304, respectively, of the HGF sequence [22].

2.5. Enzyme immunoassay

Amount of HGF protein in the culture conditioned media were measured by the sandwich enzyme immunoassay [23] (EIA) (Otsuka Seiyaku, Tokyo, Japan). 50 μl of unconcentrated conditioned medium was used for each assay. FBS (10%) containing medium was used as a control. The assays were performed in duplicate and were presented as mean values. The amount of u-PA antigen in the conditioned media was also measured by the sandwich EIA kit (Biopool, Umeå, Sweden).

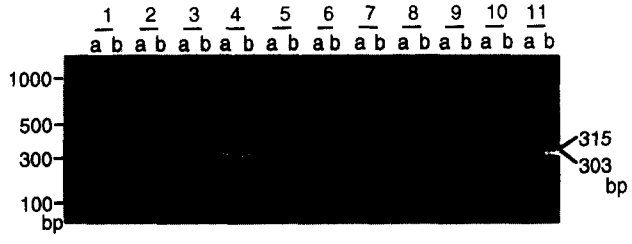

Fig. 1. RT-PCR for HGF mRNA. Poly(A)$^+$ RNA preparations derived from cultured cells (lanes 1–5) and tissues (lanes 6–11) were used for the reaction. Products (10 μl of each) were analyzed in 2.5% agarose gel electrophoresis. The 315 bp (a) and 303 bp (b) fragments were obtained with different combination of primers (HGF-s2–as2 and -s3–as3, respectively). Lane 1 = MGM-1; lane 2 = MGM-2; lane 3 = MGM-3; lane 4 = KG-1-C; lane 5 = U251; lane 6 = glioblastoma multiforme (GBM)-1; lane 7 = GBM-2; lane 8 = GBM-3; lane 9 = anaplastic astrocytoma (A.Astro.)-1; lane 10 = A.Astro.-2; lane 11 = normal brain (whole-brain).

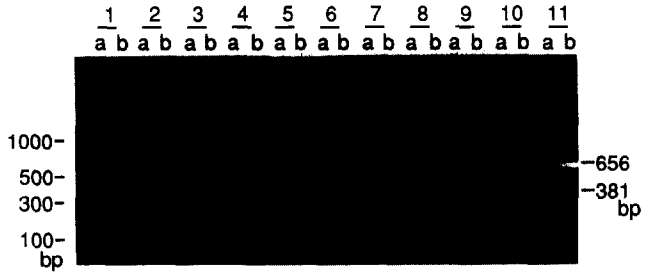

Fig. 2. RT-PCR for c-met mRNA. Poly(A)$^+$ RNAs derived from cultured cells (lanes 1–5) and tissues (lanes 6–11) were used. The 381 bp (a) and 656 bp (b) fragments were obtained with different combination of primers (MET-s1–as1 and -s2–as2, respectively). Lane 1 = MGM-1; lane 2 = MGM-2; lane 3 = MGM-3; lane 4 = KG-1-C; lane 5 = U251; lane 6 = GBM-1; lane 7 = GBM-2; lane 8 = GBM-3; lane 9 = A.Astro.-1; lane 10 = A.Astro.-2; lane 11 = normal brain.

3. Results

3.1. Secretion of HGF by glioma cells in vitro

Antigen levels of HGF in the conditioned media of four glioma cell lines were measured by EIA. As shown in Table 1, two cell lines (MGM-1 and KG-1-C) out of four produced detectable amounts of HGF into the conditioned media.

3.2. Expression of HGF and c-met genes in gliomas

Expression of HGF gene was also confirmed by RT-PCR study. Four cell lines (MGM-1, -2, -3 and KG-1-C) out of five expressed the HGF mRNA. Both primer sets (HGF-s2–as2 and -s3–as3) yielded products of the expected sizes (Fig. 1.). The mRNA was detected even in MGM-3 cells in which HGF antigen was undetectable in the conditioned medium by EIA (Table 1). Next, we analyzed the expression of mRNA for c-Met protein, the receptor for HGF. All the cell lines expressed c-met gene (Fig. 2), and thus only U251 expressed c-met gene without co-expression of HGF gene. When analyzed in the surgically resected glioma tissues (three glioblastomas and two anaplastic astrocytomas), HGF mRNA was also detected with RT-PCR analysis, however, mRNA for c-met was detectable only in one case (GBM-3; Fig. 2, lane 8). On the other hand, in normal brain (whole-brain poly(A)$^+$ RNA), both mRNAs were clearly detected (Figs. 1 and 2).

3.3. Expression of HGF activator gene in gliomas

Finally, we examined the expression of HGF activator gene in the glioma cell lines and tissues. With RT-PCR study, all glioma samples, except for one anaplastic astrocytoma tissue, expressed the activator mRNA. Furthermore normal brain tissue (whole-brain) also expressed this gene. Both primer sets (HGFA-s3–as2 and -s3–as4) yielded products of the expected

Table 1
Secretion of HGF by cultured glioma cell lines

Cells	(ng/ml)	(ng/10^7 cells/24h)
MGM-1	0.56	2.77
MGM-3	<0.3	–
KG-1-C	1.81	140.5
U251	<0.3	–

Confluent cells were cultured in DMEM, 10% FBS, for 24 h and conditioned media were harvested and analyzed.

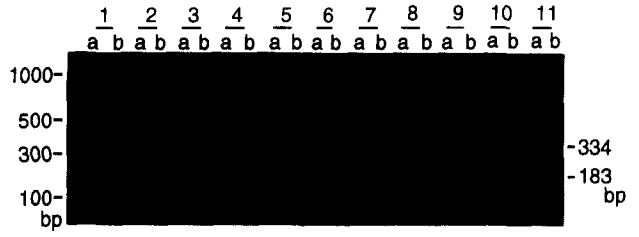

Fig. 3. RT-PCR for HGF activator mRNA. Poly(A)$^+$ RNAs derived from cultured cells (lanes 1–5) and tissues (lanes 6–11) were used. The 183 bp (a) and 334 bp (b) fragments were obtained with different combination of primers (HGFA-s3–as2 and -s3–as4, respectively). Lane 1 = MGM-1; lane 2 = MGM-2; lane 3 = MGM-3; lane 4 = KG-1-C; lane 5 = U251; lane 6 = GBM-1; lane 7 = GBM-2; lane 8 = GBM-3; lane 9 = A.Astro.-1; lane 10 = A.Astro.-2; lane 11 = normal brain.

sizes (183 bp and 334 bp, respectively) (Fig. 3, Table 2). Other primers sets, HGFA-s4–as2 and -s4–as4, were also examined in cases of KG-1-C and a glioblastoma tissue, and bands of expected sizes (367 bp and 518 bp, respectively) were also detected (Table 2). To confirm that the RT-PCR products are derived from mRNA, not from the contaminated genomic DNA, the genomic DNA preparation from MGM-1 and U251 cells were also amplified by using the same primer set (HGFA-s3–as2). This primer set generated ~ 280 bp product when the genomic DNA was amplified suggesting that between these primers there might exist ~ 100 bp intron sequence (Fig. 4A). Sunsequent sequencing of the ~ 280 bp product revealed that there is an additional sequence between the nucleotide no. 1636 and 1637 (numbers are according to Miyazawa et al. [11]) (Fig. 4B). This additional sequence had conserved sequences for introns, i.e. GT at the 5′-end and AG at the 3′-end (Fig. 4B). Furthermore, PCR of the genomic DNA preparation by using primers HGFA-s3 and -as4 generated products larger than 1000 bp, that could be re-amplified by an internal primer set such as HGFA-s3–as2 (Fig. 4A). Therefore it would be apparent that the 183 bp (HGFA-s3–as2) and 334 bp (HGFA-s3–as4) RT-PCR products were derived from mRNA for HGF activator, not from contaminated genomic DNA. In addition, both of the 183 and 334 bp products were sequenced and found

Table 2
Summary of the RT-PCR experiments

	HGF -s2–as2/-s3–as3	c-met -s1–as1/-s2–as2	HGF activator -s3–as2/-s3–as4/-s4–as2
Cell lines (histology of the original tumor)			
MGM-1 (GBM)	+/+	+/+	+/+/n.t.
MGM-2 (GBM)	+/+	+/+	+/+/n.t.
MGM-3 (GBM)	+/+	+/+	+/+/n.t.
KG-1-C (mixed glioma)	+/+	+/+	+/+/+
U251 (GBM)	−/±	+/+	+/+/n.t.
SUIT-2 (Ad)	−/−	+/+	−/−
RCM-1 (Ad)	n.t.	n.t.	−/−
Tissues			
GBM-1	+/+	−/−	+/+/+
GBM-2	−/±	−/−	+/+/n.t.
GBM-3	+/+	+/+	+/+/n.t.
A.Astro.-1	+/+	−/−	+/+/n.t.
A.Astro.-2	−/±	−/±	±/−/n.t.
Normal brain (whole brain)	+/+	+/+	+/+/n.t.

GBM: Glioblastoma multiforme; A.Astro.: Anaplastic astrocytoma; Ad: Adenocarcinoma
+: single band of expected size; ±: only a faint band of expected size; −: no visible band of expected size; n.t.: not tested

to be completely identical to the sequence of HGF activator cDNA reported previously [11]. Two human adenocarcinoma cell lines, SUIT-2 and RCM-1, did not express detectable amounts of HGF activator mRNA.

The results of RT-PCR studies are summerized in Table 2. It should be emphasized that concomitant expression of HGF, its receptor c-Met, and HGF activator genes was found in four glioma cell lines out of five tested and in one glioma tissue out of five.

3.4. Secretion of u-PA by the cultured glioma cells

Secretion of u-PA protein, another candidate for the HGF activation [9,10], in the culture conditioned media of glioma cell lines was measured by EIA. All cell lines, except for MGM-2,

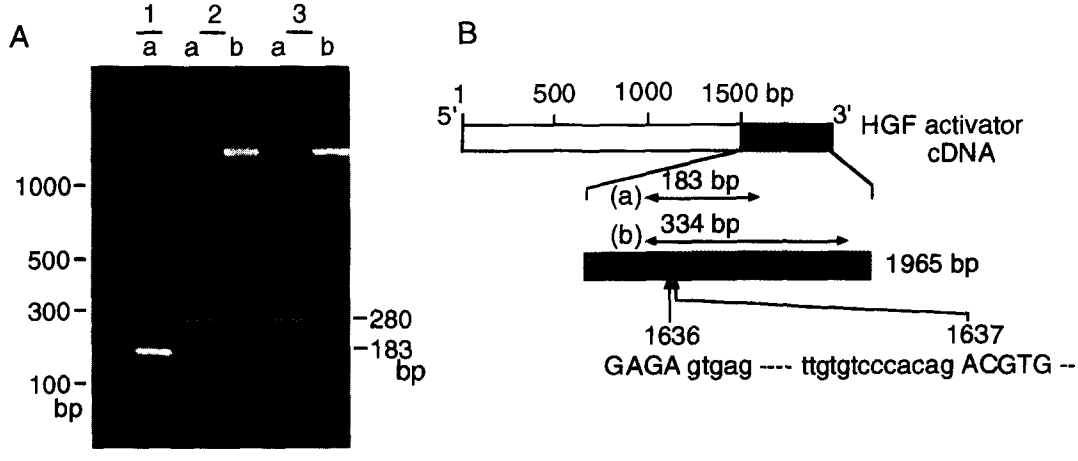

Fig. 4. PCR of the genomic DNA preparation with the primer sets for HGF acitvator (A). Lane 1 shows control RT-PCR product (183 bp) by using liver total RNA. Lane 2 and 3 show PCR products by using the genomic DNA derived from MGM-1 and U251, respectively. One μg of genomic DNA was used as template. The primer sets used are HGFA-s3–as2 (a) and HGFA-s3–as4 (b). Subsequent sequencing of the ~280 bp products (lanes 2a and 3 a) revealed the presence of a presumed intron sequence between nucleotide no. 1636 and 1637 of HGF activator cDNA sequence [11] (B).

Table 3
Secretion of u-PA by cultured glioma cell lines

Cells	(ng/ml)	(ng/10^7 cells/24h)
MGM-1	91.0	450.1
MGM-2	n.d.	n.d.
MGM-3	18.3	225.0
KG-1-C	0.08	6.2
U251	7.7	30.3

Confluent cells were cultured in DMEM, 10% FBS, for 24 h and conditioned media were harvested and analyzed. n.d. = not detectable.

secreted detectable amount of u-PA (Table 3). Particularly, MGM-1 and -3 cells secrete a large amount.

4. Discussion

This study describes the RT-PCR analysis of gene expression for HGF activator, HGF and c-met in human gliomas and normal brain. Of these, to the best of our knowledge, expression of HGF activator has not been examined in the tumor cells and the brain tissue so far. In the present communication we report for the first time that glioma cells express HGF activator gene, and that the normal brain tissue also express this gene.

HGF is a multifunctional, heparin-binding glycoprotein initially identified as a potent mitogen for hepatocytes [1,2]. The c-met proto-oncogene product, a membrane-bound tyrosine kinase, was demonstrated to represent the high affinity HGF receptor suggesting the roles of HGF in tumor progression [6]. HGF is thought to be mainly a paracrine factor produced by mesenchymal cells [6], however, an autocrine activity was reported in osteosarcoma cells very recently [24]. Furthermore, creation of HGF autocrine loop in carcinoma cells induced invasiveness and increased tumorigenicity [25]. Human HGF is synthesized as a 728-amino acid precursor followed by removal of the N-terminal 31 amino acid residues as a signal peptide. HGF is then secreted as a single-chain inactive form [6]. It has been shown that proteolytic conversion of the single-chain form to the two-chain heterodimer is essentially required for biologic activity [8]. The conversion occurs in the extracellular environment after secretion [8,9]. Therefore the converting proteinase must be a key player in the regulation of HGF activity particularly in cellular microenvironment. HGF activator is a recently reported serine proteinase which shows homology to blood coagulation factor XII, and is thought to be synthesized by the liver [11]. It cleaves the bond between Arg^{494} and Val^{495} in the single-chain HGF to generate the active two-chain form [8,11]. Another candidate for the HGF-activation is u-PA [12,13].

In the present communication, we described that cultured glioma cells express both HGF and its receptor c-Met protein genes. The co-expression of these genes in the glioma cells would lead to a functional interaction of the products in vitro. Furthermore, the cells also expressed HGF activator gene and secreted u-PA protein. The surgically resected glioma tissues also expressed HGF and HGF activator genes. In addition, elevated level of u-PA in glioblastomas has also been reported recently [24]. The production of HGF activator and u-PA, in addition to HGF and c-Met, by glioma cells themselves might have a great advantage of creating an autocrine loop of HGF in the glioma cell microenvironment. Interestingly, although all glioma cell lines expressed c-met in vitro, only one out of five glioma tissues examined in this study expressed notable amount of c-met mRNA in vivo (Table 2). Two possibilities can be considered to explain this finding. First, the expression of c-met is induced in vitro, and thus is an artifact of the tissue culture environment. Second, the tissue culture environment selects for a minor population of cells that express c-met in vivo. In either case, the concomitant expression of c-met, HGF and its activators may somehow provide an advantage of adapting to the in vitro condition by creating the autocrine loop and thus the frequent expression of c-met in vitro may be a feature of the tissue culture environment. However, we have found at least one case of glioblastoma that co-express c-met, HGF and HGF activator in vivo suggesting that the creation of the autocrine loop can also occur even in vivo in limited cases of glioma.

To date, little is known about the biological roles of HGF in gliomas [6]. Expression of HGF gene has been reported in the normal brain tissue, particularly in neurons [16]. Immunohistochemically, HGF and its receptor c-Met protein were reported to be positive in astrocytes and microglia, respectively [14,15]. Amplification of c-met gene has recently been described in glioblastoma cell line and tissues [27–29], however, expression of HGF and c-met mRNAs in glioma has yet been clarified so far. DiRenzo et al. [30] described that one out of four glioma tissues examined expressed c-met mRNA, and this positive case was malignant glioma infiltrating the brain. In fact, we have found a chemotactic activity of HGF for U251 cells which expressed c-met in vitro (unpublished observation). Therefore, it is of interest to know whether the expression of c-met in vivo is a prognostic parameter in glioma.

In conclusion, although the biological functions of HGF were yet uncertain in glioma cells, the observations described here provide evidence for the hypothesis that HGF is acting as an autocrine factor in glioma cells in vitro and in limited cases of primary glioma in vivo. Further efforts would be necessary to explore the potential relevance of HGF, HGF activator and c-met in gliomas, particularly glioblastoma multiforme. Furthermore, the finding that HGF activator is also expressed in the normal brain would accelerate the efforts to determine the roles of HGF in the brain.

Acknowledgements: We thank Drs. K. Nabeshima and H. Itoh for valuable discussion, and Dr. Y. Yamakawa for providing surgical specimens. This work was supported by a grant from the Special Coordination Funds of the Science and Technology agency, Japan.

References

[1] Gohda, E., Tsubouchi, H., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Miyagaki, H., Hashimoto, S. and Daikuhara, Y. (1988) J. Clin. Invest. 81, 414–419.
[2] Nakamura, T., Nawa, K., Ichihara, A., Kaise, N. and Nishino, T. (1987) FEBS Lett. 224, 311–316.
[3] Stoker, M., Gherardi, E., Perryman, M. and Gray, J. (1987) Nature 327, 239–242.
[4] Gherardi, E., Gray, J., Stoker, M., Perryman, M. and Furlong, R. (1989) Proc. Natl. Acad. Sci. USA 86, 5844–5848.
[5] Weidner, K.M., Arakaki, N., Hartmann, G., Vandekerckhove, J., Weingart, S., Rieder, H., Fonatsch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y. and Birchmeier, W. (1991) Proc. Natl. Acad. Sci. USA 88, 7001–7005.
[6] Rubin, J.S., Bottaro, D.P. and Aaronson, S.A. (1993) Biochim. Biophys. Acta 1155, 357–371.

[7] Cooper, C.S., Park, M., Blair, D.G., Taisky, M.A., Huebner, K., Croce, C.M. and Vande Wonde, G.F. (1984) Nature 311, 29–33.
[8] Naka, D., Ishii, T., Yoshiyama, Y., Miyazawa, K., Hara, H., Hishida, T. and Kitamura, N. (1992) J. Biol. Chem. 267, 20114–20119.
[9] Mizuno, K., Takehara, T. and Nakamura, T. (1992) Biochem. Biophys. Res. Commun. 189, 1631–1638.
[10] Shimomura, T., Ochiai, M., Kondo, J. and Morimoto, Y. (1992) Cytotechnology 8, 219–229.
[11] Miyazawa, K., Shimomura, T., Kitamura, A., Kondo, J., Morimoto, Y. and Kitamura, N. (1993) J. Biol. Chem. 268, 10024–10028.
[12] Naldini, L., Tamagnone, L., Vigna, E., Sachs, M., Hartmann, G., Birchmeier, W., Daikuhara, Y., Tsubouchi, H., Blasi, F. and Comoglio, P.M. (1992) EMBO J. 11, 4825–4833.
[13] Naldini, L., Vigna, E., Bardelli, A., Follenzi, A., Galimi, F. and Comoglio, P.M. (1995) J. Biol. Chem. 270, 603–611.
[13] Miyazawa, K., Shimomura, T., Kitamura, A., Kondo, J., Morimoto, Y. and Kitamura, N. (1993) J. Biol. Chem. 268, 10024–10028.
[14] DiRenzo, M.F., Bertolotto, M., Olivero, M., Putzolu, P., Crepaldi, T., Schiffer, D., Pagni, C.A. and Comoglio, P.M. (1993) Oncogene 8, 219–222.
[15] Yamada, T., Tsubouchi, H. Daikuhara, Y., Prat, M., Comoglio P.M., McGeer, P.L. and McGeer E.G. (1994) Brain Res. 637, 308–312.
[16] Jung, W., Castren, E., Odenthal, M., Vande Woude, G.F., Ishii, T., Dienes, H.-P., Lindholm, D. and Schirmacher, P. (1994) J. Cell Biol. 126, 485–494.
[17] Vaheri, A., Ruoslahti, E., Westermark, B. and Ponten, J. (1976) J. Exp. Med. 143, 64–72.
[18] Miyake, E. (1979) Acta Neuropathol. 46, 51–55.
[19] Iwamura, T., Katsuki, T. and Ide, K. (1987) Jpn. J. Cancer Res. 78, 54–62.
[20] Kataoka, H., Nabeshima, K., Komada, N. and Koono, M. (1989) Virchows Arch. B 57, 157–165.
[21] Park, M., Dean, M., Kaul, K., Braun, M.J., Gonda, M.A. and Vande Woude, G. (1987) Proc. Natl. Acad. Sci. USA 84, 6379–6383.
[22] Miyazawa, K., Tsubouchi, H., Naka, D., Takahashi, K., Okigaki, M., Arakaki, N., Nakayama, H., Hirono, S., Sakiyama, O., Takahashi, K., Gohda, E., Daikuhara, Y. and Kitamura, N. (1989) Biochem. Biophys. Res. Commun. 163, 967–973.
[23] Tsubouchi, H., Niitani, Y., Hirono, S., Nakayama, H., Gohda, E., Arakaki, N., Sakiyama, O., Takahashi, K., Kimoto, M., Kawakami, S., Setoguchi, M., Tachikawa, T., Shin, S., Arima, T. and Daikuhara, Y. (1991) Hepatology 13, 1–5.
[24] Ferracini, R., DiRenzo, M.F., Scotlandi, K., Baldini, N., Olivero, M., Lollini, P., Cremona, O., Campanacci, M. and Comoglio, P.M. (1995) Oncogene 10, 739–749.
[25] Bellusci, S., Moens, G., Gaudino, G., Comoglio, P., Nakamura, T., Thiery, J.-P. and Jauanneau, J. (1994) Oncogene 9, 1091–1099.
[26] Landau, B.J., Kwaan, H.C., Verrusio, E.N. and Brem, S.S. (1994) Cancer Res. 54, 1105–1108.
[27] Wullich, B., Müller, H.W., Fischer, U., Zang, K.D. and Meese, E. (1993) Eur. J. Cancer 29A, 1991–1995.
[28] Muleris, M., Almeida, A., Dutrillaux, A.M., Pruchon, E., Vega, F., Delattre, J.Y., Poisson, M., Malfoy, B. and Dutrillaux, B. (1994) Oncogene 9, 2717–2722.
[29] Wullich, B., Sattler, H.-P., Fischer, U. and Meese, E. (1994) Anticancer Res. 14, 577–580.
[30] DiRenzo, M.F., Narsimhan, R.P., Olivero, M., Bretti, S., Giordano, S., Medico, E., Gaglia, P., Zara, P. and Comoglio, P.M. (1991) Oncogene 6, 1997–2003.