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Original Article

C-MET overexpression and amplification in gliomas

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Abstract: We investigated c-Met overexpression and *MET* gene amplification in gliomas to determine their incidence and prognostic significance. c-Met immunohistochemistry and *MET* gene fluorescence in situ hybridization were carried out on tissue microarrays from 250 patients with gliomas (137 grade IV GBMs and 113 grade II and III diffuse gliomas). Clinicopathological features of these cases were reviewed. c-Met overexpression and *MET* gene amplification were detected in 13.1% and 5.1% of the GBMs, respectively. All the *MET*-amplified cases showed c-Met overexpression, but *MET* amplification was not always concordant with c-Met overexpression. None of grade II and III gliomas demonstrated c-Met overexpression or *MET* gene amplification. Mean survival of the GBM patients with *MET* amplification was not significantly different from patients without *MET* amplification (P=0.155). However, GBM patients with c-Met overexpression survived longer than patients without c-Met overexpression (P=0.035). Although *MET* amplification was not related to poor GBM prognosis, it is partially associated with the aggressiveness of gliomas, as *MET* amplification was found only in grade IV, not in grade II and III gliomas. We suggest that MET inhibitor therapy may be beneficial in about 5% GBMs, which was the incidence of *MET* gene amplification found in the patients included in this study.

Keywords: Brain tumor, c-Met, glioblastoma, FISH, immunohistochemistry, target therapy

Introduction

Glioblastomas (GBMs) are the most common type of malignant primary brain tumor, comprising about 15% of all gliomas. Approximately 450 and 9000 new cases are diagnosed each year in Korea and the United States, respectively, according to the Brain Tumor Registry of Korea and Central Brain Tumor Registry of the United States (www.cbtrus.org). In South Korea, GBMs accounted for 5.9% of all primary CNS tumors and 33.5% of gliomas [1]. Despite the use of intensive treatment modalities GBM still has a poor prognosis. Receptor tyrosine kinase (RTK) c-Met (proto-oncogene proteins) overexpression and *MET* (hepatocyte growth factor receptor) gene amplification was found in GBMs as well as in other cancers, such as lung, pancreas, ovary, salivary gland, and breast cancers. c-Met overexpression and gene amplification promote malignancy and are associated with poor clinical outcome in gliomas [2, 3]. Aberrant *MET* activation can occur through various mechanisms, such as autocrine or paracrine stimulation, transcriptional regulation, ligand-independent or mutational activation [4, 5]. *MET* amplification can be a major driver after acquired resistance to epidermal growth factor receptor (EGFR) inhibitors, because of the cross-talk with other RTK family members [6]. Hepatocyte growth factor (HGF) may induce resistance to EGFR tyrosine kinase inhibitors in EGFR mutant lung cancer cells by Met/PI3K/Akt signaling [7]. However, one recent study reported a patient with *MET*-amplified GBM who showed clinical improvement when treated with the MET inhibitor crizotinib. Moreover, targeting the *MET* pathway potentiates the responsiveness of GBM to γ-radiation [8]. The purpose of our study is to investigate the positive rate, expression pattern, and significance of c-Met overexpression and *MET* gene amplification in gliomas.

Materials and methods

c-Met immunohistochemistry (IHC) and *MET* gene fluorescence in situ hybridization (FISH) were carried out on tissue microarrays made from formalin-fixed paraffin-embedded (FFPE)

Table 1. Clinicopathologic characteristics of glioblastoma patients

Characteristics	No.
Patients	137 (100%)
Sex	
Male	85 (62.0%)
Female	52 (38.0%)
Mean age (range)	47.9 (19-73) years
Mean overall survival	24.6 months
Surgical resection	
Gross total resection	69 (50.4%)
Partial resection	59 (43.1%)
Biopsy only	9 (6.5%)

glioma tissues obtained from 250 patients (137 GBMs and 113 grade II and III diffuse gliomas). We reviewed clinicopathological features of the 137 GBMs and compared these findings with other protein expression and molecular genetic factors to ascertain the significance of c-Met overexpression and *MET* gene amplification patterns. Clinical feature of the 137 GBM patients are summarized in **Table 1**. The mean age was 48 years (range: 19-73 years old) and the male to female ratio was 1.6:1. Patients underwent gross total resection (37.1% of patients), partial resection (53.6%), or biopsy only (9.3%). Grade II and III diffuse gliomas were composed of 13 diffuse astrocytomas, 36 anaplastic astrocytomas, 30 low-grade oligodendrogliomas, and 34 anaplastic oligodendrogliomas. This research was approved by the Institutional Review Board of Seoul National University Hospital (H-1010-059-434).

Immunohistochemistry (IHC)

Immunohistochemical staining was performed on FFPE tissues of 3 μm thickness. All IHC procedures were carried out using a Leica BOND-MAX (Leica, Heidelberg, Germany) according to routine laboratory procedures and the manufacturer's protocols. The primary antibodies, antigen retrieval methods, and dilution ratios used for IHC analysis are listed in **Table 2**. Positivity was measured by Aperio membrane algorithm after scanning with Aperio Scanscope, which appeared as positive %.

Fluorescence in situ hybridization (FISH)

Analysis of MET and EGFR gene status was carried out by FISH using Vysis probes: Tissue sections of 4 μm thickness were deparaffinized using xylene, incubated with 0.3% pepsin in 10 mM HCl at 37°C for 10 min, boiled with citrate buffer (pH 6.0) in a microwave, incubated in 1 M NaSCN for 35 min at 80°C, immersed in the pepsin solution, and fixed in 10% neutral-buffered formalin. Labeled locus-specific (LSI) EGFR/CEP7 dual-color probes (Abbott Molecular) and LSI *MET*/CEP9 dual color probes (Abbott Molecular) were used for recognizing the EGFR-gene and *MET* gene status according to the manufacturer's protocol. We applied the probe mixture to slides and incubated them in a humidified atmosphere with HYBrite™ (Abbott Molecular) at 73°C for 5 min for simultaneous denaturation of the probe and the target DNA. Next, we changed the temperature to 37°C for 19 hours to hybridize the probes and target DNAs. Slides were then soaked in 0.4× SSC buffer/0.3% NP-40 for 2 min at room temperature, followed by 2× SSC/0.1% NP-40 for 5 min at 73°C. The processing and analysis of the FISH studies were conducted as described previously [9, 10]. The signals on 100 non-overlapping intact nuclei were counted.

Statistical analysis

Kaplan-Meier analysis and other statistical analyses were carried out using SPSS version 21. Student t-test was used to calculate *P*-values.

Results

IHC and FISH results

Results of the IHC and FISH studies are shown in **Table 3** and **Figure 1**. c-Met overexpression was detected in 13.1% (18 of 137) of GBMs, but not in any of the 113 cases of grade II and III astrocytic and oligodendroglial tumors. All *MET*-amplified GBMs also had c-Met overexpression, but among the c-Met-overexpressing GBMs, only 38.9% (7/18) showed *MET* gene amplification. IHC showed that only one out of seven cases with *MET* gene amplification also had phosphatase and tensin homolog deleted on chromosome 10q (PTEN) (**Table 3**). c-Met overexpression or *MET* amplification was not associated with IDH mutation. Only 16.7% (3/18) of c-Met expressed GBM and 28.5% (2/7) of *MET* amplified GBM were positive for IDH1, respectively. Loss of PTEN expression was detected in 13.9% (20 of 137) of GBMs, but only four out of 18 c-MET expressed cases

Table 2. Primary antibodies used in this study

Name	Manufacturer	Antigen retrieval	Dilution
c-Met	Ventana (SP44)	RTU MILD/16'	Prediluted
PTEN	DAKO, Glostrup, Denmark	EDTA, microwave	1:100
IDH1 (H09)	Dianova, Hamburg, Germany	Microwave	1:50

(22.2%) and one out of seven cases (14.3%) with *MET* gene amplification had loss of PTEN expression.

Clinical outcomes

It had been reported that the mean survival of GBM patients with *MET* amplification was not shorter than patients without *MET* amplification; however, in our cohort, the patients with c-MET positive GBM survived for longer (35.7 months versus 24.0 months for c-Met positive and c-MET negative GBMs, respectively, P=0.035, **Figure 2B**). Among *MET* gene-amplified GBMs, two were IDH1 positive, which were secondary GBMs, initially an anaplastic astrocytoma and a diffuse astrocytoma, respectively. At that time, neither c-Met overexpression nor *MET* gene amplification was detected (**Table 4**). Kaplan Meier survival analyses revealed that survival did not differ between patients with *MET* amplification and those without *MET* amplification (P=0.155, **Figure 2A**).

Discussion

GBM is the most common malignant glioma, comprising 15% of primary brain tumors. Outcomes for patients with GBM are dismal, due to its highly malignant nature and infiltrative growth. There is no suitable chemotherapy regimen, even though CCRT with temozolomide can extend the longevity of patients. Recently, personalized targeted therapies have been trialed in various tumors, such as non-small cell lung carcinoma, gastrointestinal stromal tumors, and melanoma. However, the genetic abnormalities found in gliomas, including GBMs, are remarkably different from extracranial malignant tumors. Therefore, more research is needed to identify suitable GBM tumor markers and therapeutic targets, such as the *MET* signaling pathway.

MET is a proto-oncogene that encodes a protein known as HGF receptor. c-Met is an integral plasma membrane RTK, which is normally expressed by epithelial cells [11]. Its ligand, HGF, is the only ligand to bind c-Met, and is usually secreted by mesenchymal cells. HGF binds to c-Met and activates the c-Met signaling pathway, resulted in fetal morphogenesis and increased cell motility, infiltrative growth, angiogenesis and resistance to apoptotic signals. Tumor cells can secrete HGF, and HGF has been shown to stimulate the motility and invasiveness of several types of cancer cells and to induce angiogenesis [12]. In tumors, HGF binding to c-Met triggers leads to tumor cell proliferation, invasion, survival, and angiogenesis by activating the RAS, PI3K, STAT, beta-catenin and NOTCH signal transduction pathways. Aberrant c-Met activation can occur through numerous mechanisms, such as autocrine or paracrine stimulation, or transcriptional activation [4]. The identification of tumor subgroups that are suitable targets for MET inhibitors is of great clinical importance, this question was addressed by Xie et al. [13]. They found that an HGF paracrine environment did not indicate sensitivity to MET inhibitors, although it may enhance GBM growth in vivo [4]. Among 18 cell lines investigated by in vivo and in vitro studies, only two cell lines (U87 and U87M2) expressed autocrine HGF at a significant level; however, c-Met expression was not affected by these autocrine HGF levels. HGF-autocrine GBMs bear an activated c-Met signaling pathway that may predict sensitivity to MET inhibitors [4]. The same study also suggests that serum HGF levels may serve as a biomarker for the presence of autocrine tumors and their responsiveness to MET therapeutics [4].

Somatic mutations/deletions of PTEN are commonly found in a wide variety of solid tumors, and are detected in 20-50% of GBMs [14, 15]. PTEN is known to co-regulate RTK-mediated gene expression in glioma models and influences their response to targeted kinase inhibitors [16]. Previous studies have suggested that the therapeutic efficacy of RTK inhibitors is predicted by PTEN activity, with PTEN loss rendering tumors [17, 18]. Therefore, here we investigated c-Met expression and *MET* gene amplification as well as PTEN loss in gliomas.

Among our cases, *MET* amplification and c-Met overexpression was detected in 5.1% (7/137) and 13.1% (18 of 137) of GBMs, respectively,

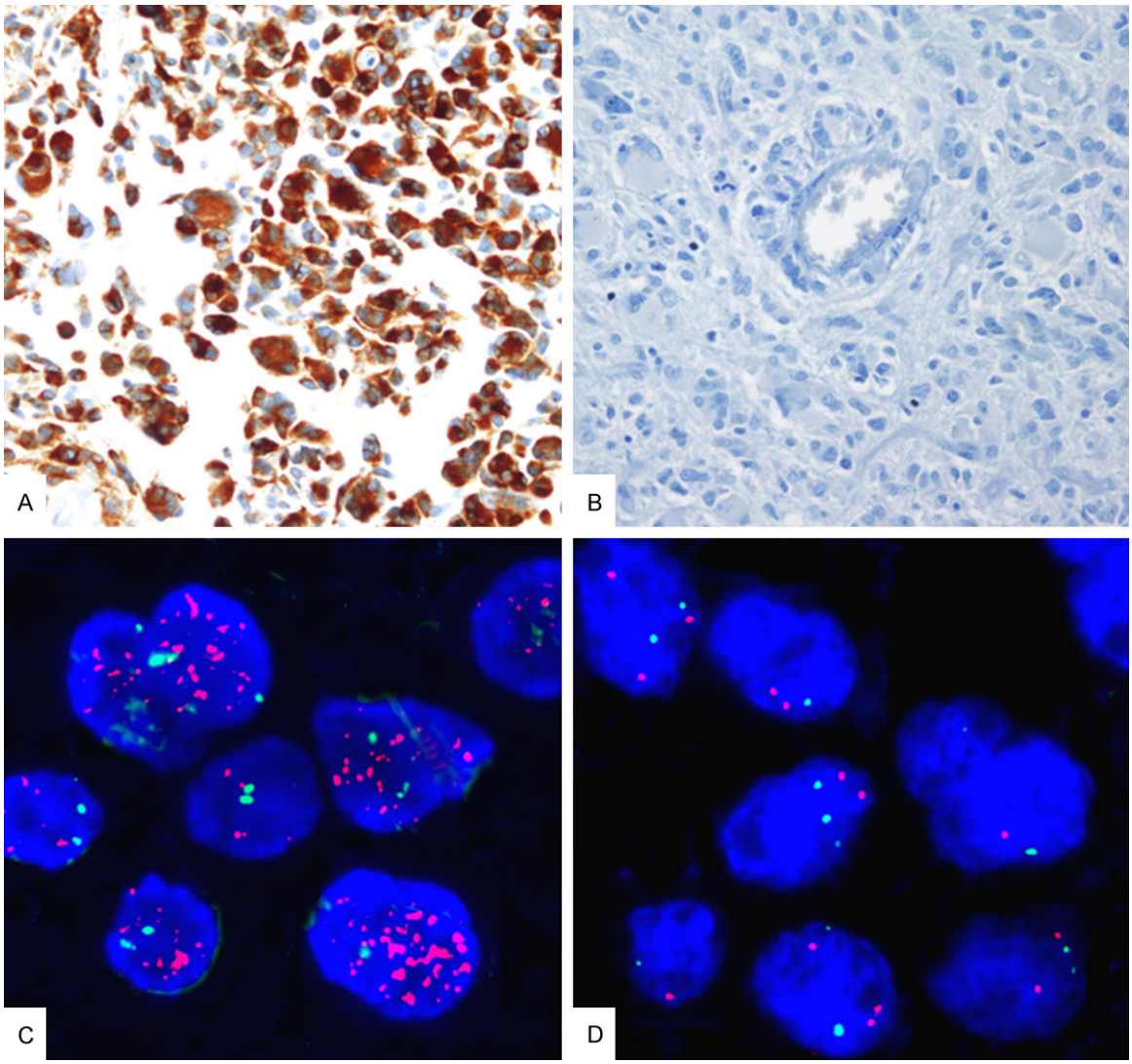

Figure 1. Representative images of glioblastomas with (A) diffuse c-Met overexpression, (B) lack of c-Met immunopositivity, (C) *MET* gene amplification, and (D) no *MET* gene amplification. (A, B: c-Met immunohistochemistry, ×400; C, D: Labeled locus-specific dual-color Vysis probe of *MET* gene (7q31), spectrum orange/CEP7 Spectrum green, ×1000).

Table 3. c-Met overexpression (measured by IHC) and *MET* gene amplification (measured by FISH) in a cohort of glioblastoma patients

c-Met IHC	*MET* FISH		Total	PTEN IHC		Total
	Amplification	No amplification		Loss	No loss	
Positive	7 (5.1%)	11 (8.0%)	18 (13.1%)	1 (0.7%)	7 (5.1%)	8 (5.8%)
Negative	0	119 (86.9%)	119 (86.9%)	19 (13.9%)	110 (80.3%)	129 (94.2%)
Total	7 (5.1%)	130 (94.9%)	137 (100%)	20 (14.6%)	117 (85.4%)	137 (100%)

IHC: immunohistochemistry, FISH: fluorescence in situ hybridization.

but in none of 113 grade II and III gliomas. c-Met overexpression and *MET* gene amplification were not perfectly concordant. Only 38.9% (7/18) of c-Met immunopositive cases showed *MET* gene amplification. The incidence of c-Met overexpression in our study is far lower than reported by Kong et al., who reported c-Met overexpression rate in 29.0% of GBMs [3]. Pierscianek et al. studied *MET* copy number gain using quantitative polymerase chain reac-

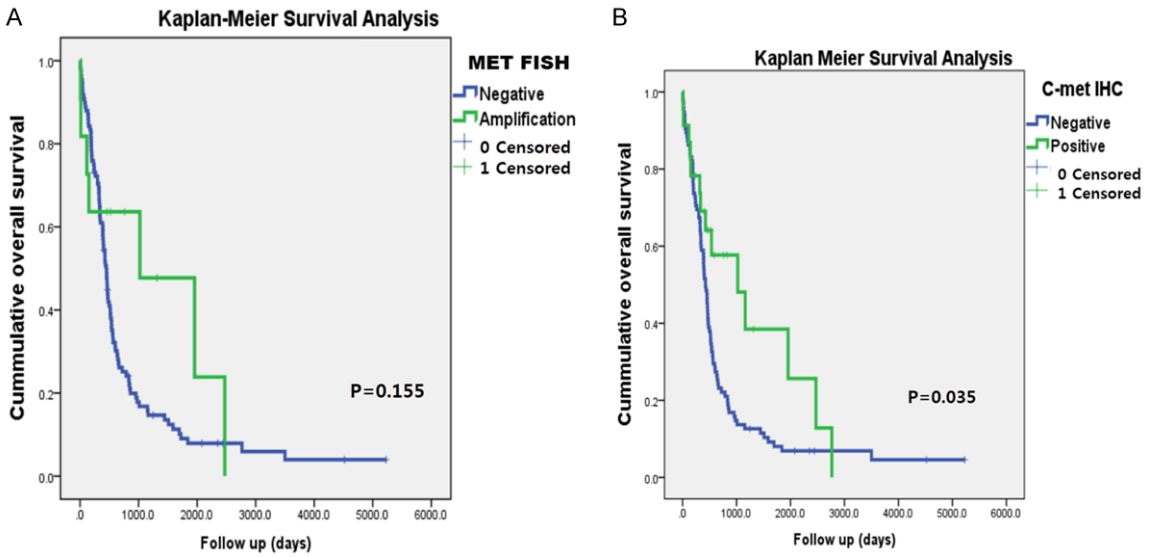

Figure 2. (A) Kaplan-Meier survival analysis revealed that patient survival was not related with *MET* gene amplification, but that (B) patients with c-Met overexpression had better survival than those without c-Met overexpression.

Table 4. Clinical outcome of seven glioblastomas patients with *MET* amplification

Sex/ Age	Operation	Initial Dx	Final Dx	Recur	PFS (months)	OS (months)	Outcome
F/40	GTR	GBM, conventional	GBM-IDHw	No	5.1	5.1	DOD
M/65	Biopsy	GBM, conventional	GBM-IDHw	No	3.9	3.9	DOD
M/49	GTR	GBM, conventional	GBM-IDHw	Yes	81.2	82.4	DOD
M/77	Biopsy	GBM, conventional	GBM-IDHw	No	15.4	15.4	DOD
F/21	PR	GBM, epithelioid	GBM-IDHw	Yes	7.7	43.8	Alive
F/41	PR	LO	AA→GBM-IDHm	Yes	89.6 (from oligo to GBM)	124 (from LO to DOD)	DOD
						29 (from GBM to DOD)	DOD
F/47	PR	DA	DA→GBM-IDHm	Yes	19.8 (from DA to AA)	65.2 (from DA to DOD)	DOD
					40 (from AA to GBM)	7 (from GBM to DOD)	

GBM: glioblastoma, IDHw: isocitrate dehydrogenase wild type, IDHm: IDH mutant, GTR: gross total removal, PR: partial removal, PFS: progression free survival, OS: overall survival, DOD: dead of disease, LO: low grade (grade II) oligodendroglioma, DA: diffuse astrocytoma, AA: anaplastic astrocytoma.

tion and found that *MET* gain was present in 47% of primary GBMs, 44% of secondary GBMs, 38% of diffuse astrocytomas, and 16% of oligodendrogliomas; however, a prognostic association was found only in diffuse astrocytomas, in which *MET* gain was associated with shorter survival [2]. However, *MET* gain was not associated with survival in GBM patients [2]. Therefore, the prognostic value of *MET* gain or amplification remains controversial.

In our cohort, c-Met overexpression or *MET* amplification was not associated with IDH1 mutation. Loss of PTEN expression was detected in 13.9% (20 of 137) of GBMs, but only four out of 18 c-MET expressed cases and one out of seven cases with *MET* gene amplification had loss of PTEN expression; such PTEN lost cases may have increased susceptibility to c-Met inhibitors. Paradoxically, GBM patients with c-Met overexpression had better survival than those without c-Met expression (P=0.035). However *MET* gene amplification was not associated with patient survival (P=0.155) in our patients. Therefore, early recognition of GBMs with c-Met overexpression and *MET* amplification and a trial of treatment with a MET inhibitor may be necessary next steps for research and provision of personalized targeted therapy. However, *MET* amplification was not detected in our series of grade II and III gliomas, suggesting that MET inhibition may not be a viable therapeutic option in these cases.

Conclusion

This study clearly showed that *MET* amplification is not associated with poor prognosis in GBMs, but is partially associated with the aggressiveness of gliomas, as *MET* amplification was found only in grade IV GBMs, not in grade II or III gliomas. We predict that *MET* might play a key role in tumorigenesis and cancer progression in about 5% of GBMs. Accordingly, our study implies that the administration of a MET inhibitor may be a promising targeted therapy for GBMs with *MET* amplification. Further studies are required to verify the exact incidence of *MET* gene amplification and to determine if a MET inhibitor is a precision medicine for gliomas.

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Disclosure of conflict of interest

None.

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References

[1] Lee CH, Jung KW, Yoo H, Park S and Lee SH. Epidemiology of primary brain and central nervous system tumors in Korea. J Korean Neurosurg Soc 2010; 48: 145-152.

[2] Pierscianek D, Kim YH, Motomura K, Mittelbronn M, Paulus W, Brokinkel B, Keyvani K, Wrede K, Nakazato Y, Tanaka Y, Mariani L, Vital A, Sure U and Ohgaki H. MET gain in diffuse astrocytomas is associated with poorer outcome. Brain Pathol 2013; 23: 13-18.

[3] Kong DS, Song SY, Kim DH, Joo KM, Yoo JS, Koh JS, Dong SM, Suh YL, Lee JI, Park K, Kim JH and Nam DH. Prognostic significance of c-Met expression in glioblastomas. Cancer 2009; 115: 140-148.

[4] Xie Q, Bradley R, Kang L, Koeman J, Ascierto ML, Worschech A, De Giorgi V, Wang E, Kefene L, Su Y, Essenburg C, Kaufman DW, DeKoning T, Enter MA, O'Rourke TJ, Marincola FM and Vande Woude GF. Hepatocyte growth factor (HGF) autocrine activation predicts sensitivity to MET inhibition in glioblastoma. Proc Natl Acad Sci U S A 2012; 109: 570-575.

[5] Uchinokura S, Miyata S, Fukushima T, Itoh H, Nakano S, Wakisaka S and Kataoka H. Role of hepatocyte growth factor activator (HGF activator) in invasive growth of human glioblastoma cells in vivo. Int J Cancer 2006; 118: 583-592.

[6] Xu L, Kikuchi E, Xu C, Ebi H, Ercan D, Cheng KA, Padera R, Engelman JA, Janne PA, Shapiro GI, Shimamura T and Wong KK. Combined EGFR/MET or EGFR/HSP90 inhibition is effective in the treatment of lung cancers codriven by mutant EGFR containing T790M and MET. Cancer Res 2012; 72: 3302-3311.

[7] Kang XH, Xu ZY, Gong YB, Wang LF, Wang ZQ, Xu L, Cao F and Liao MJ. Bufalin Reverses HGF-Induced Resistance to EGFR-TKIs in EGFR Mutant Lung Cancer Cells via Blockage of Met/PI3k/Akt Pathway and Induction of Apoptosis. Evid Based Complement Alternat Med 2013; 2013: 243859.

[8] Lal B, Xia S, Abounader R and Laterra J. Targeting the c-Met pathway potentiates glioblastoma responses to gamma-radiation. Clin Cancer Res 2005; 11: 4479-4486.

[9] Jeon YK, Park K, Park CK, Paek SH, Jung HW and Park SH. Chromosome 1p and 19q status and p53 and p16 expression patterns as prognostic indicators of oligodendroglial tumors: a clinicopathological study using fluorescence in situ hybridization. Neuropathology 2007; 27: 10-20.

[10] Kim MA, Lee HS, Lee HE, Jeon YK, Yang HK and Kim WH. EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. Histopathology 2008; 52: 738-746.

[11] Mughal A, Aslam HM, Sheikh A, Khan AM and Saleem S. c-Met inhibitors. Infect Agent Cancer 2013; 8: 13.

[12] Lamszus K, Laterra J, Westphal M and Rosen EM. Scatter factor/hepatocyte growth factor (SF/HGF) content and function in human gliomas. Int J Dev Neurosci 1999; 17: 517-530.

[13] Xie Q, Gao CF, Shinomiya N, Sausville E, Hay R, Gustafson M, Shen Y, Wenkert D and Vande Woude GF. Geldanamycins exquisitely inhibit HGF/SF-mediated tumor cell invasion. Oncogene 2005; 24: 3697-3707.

[14] Ohgaki H and Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 2005; 64: 479-489.

[15] Kim B, Myung JK, Seo JH, Park CK, Paek SH, Kim DG, Jung HW and Park SH. The clinicopathologic values of the molecules associated

with the main pathogenesis of the glioblastoma. J Neurol Sci 2010; 294: 112-118.

[16] Abounader R, Reznik T, Colantuoni C, Martinez-Murillo F, Rosen EM and Laterra J. Regulation of c-Met-dependent gene expression by PTEN. Oncogene 2004; 23: 9173-9182.

[17] Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, Riggs BL, Horvath S, Liau LM, Cavenee WK, Rao PN, Beroukhim R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL and Mischel PS. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005; 353: 2012-2024.

[18] Bianco R, Shin I, Ritter CA, Yakes FM, Basso A, Rosen N, Tsurutani J, Dennis PA, Mills GB and Arteaga CL. Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. Oncogene 2003; 22: 2812-2822.