MET Signaling Regulates Glioblastoma Stem Cells

Kyeung Min Joo1,2, Juyoun Jin1,2, Eunhee Kim9, Kang Ho Kim1,2, Yonghyun Kim1, Bong Gu Kang1,2, Youn-Jung Kang1,2, Justin D. Lathia5, Kwang Ho Cheong9, Paul H. Song9, Hyunggee Kim1, Ho Jun Seol1,2, Doo-Sik Kong2, Jung-II Lee7, Jeremy N. Rich9, Jeongwu Lee8, and Do-Hyun Nam1,2

Abstract

Glioblastomas multiforme (GBM) contain highly tumorigenic, self-renewing populations of stem/initiating cells [glioblastoma stem cells (GSC)] that contribute to tumor propagation and treatment resistance. However, our knowledge of the specific signaling pathways that regulate GSCs is limited. MET is a cognate ligand for MET signaling, known to stimulate the survival, proliferation, and invasion of various cancers including GBM. Here, we identified a distinct fraction of cells expressing a high level of MET in human primary GBM specimens that were preferentially localized in perivascular regions of human GBM biopsy tissues and were found to be highly clonogenic, tumorigenic, and resistant to radiation. Inhibition of MET signaling in GSCs disrupted tumor growth and invasiveness both in vitro and in vivo, suggesting that MET activation is required for GSCs. Together, our findings indicate that MET activation in GBM is a functional requisite for the cancer stem cell phenotype and a promising therapeutic target. Cancer Res; 72(15): 3828–38. ©2012 AACR.

Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor with a median survival of 14.6 months despite maximal therapy (1). As included in classification criteria for GBMs, excessive and unstable blood vessel formation and tumor necrosis associated with hypoxia are key pathologic characteristics of GBMs (2). GBMs heavily infiltrate into the neighboring brain parenchyma and are almost uniformly resistant to standard therapeutic regimes such as irradiation and chemotherapy (3). These biologic characteristics of GBMs are major reasons of lethality and need to be targeted for therapy.

Cancer stem cell hypothesis posits that a subpopulation of cancer cells is highly enriched with tumorigenic potential (3–7). Compared with bulk tumor cells, glioblastoma stem cells (GSC) survive better against irradiation and chemotherapies, thereby contributing to therapeutic resistance and tumor recurrence (8, 9). In addition, GSCs frequently reside in perivascular and hypoxic regions, actively promoting angiogenesis and facilitating the survival in harsh environment (10–12). However, the underlying molecular pathways that govern these processes in GSCs are poorly understood.

On the basis of this background, we hypothesized that MET activation in GBM is a functional requisite for the cancer stem cell phenotype and a promising therapeutic target.
Materials and Methods

Isolation of primary GBM cells and establishment of short-term cultured GSCs

Following informed consent, glioblastoma specimens were obtained from patients undergoing surgery at the Samsung Medical Center in accordance with the Institutional Review Boards. Tumor specimens were classified as GBM based on World Health Organization criteria by examination of pathologists (2). Tumor specimens were enzymatically dissociated into single cells and cultured, following the procedures previously reported (26, 27).

Fluorescence-activated cell sorting analysis and sorting

Patient GBM-derived tumor cells were dissociated into single cells and labeled with the following antibodies: anti-MET (R&D Systems), anti-CD133–PE (Miltenyi Biotec), and anti-CD15–FITC (MMA clone, BD Biosciences; ref. 28). For MET staining and sorting, one million cells were labeled with 2 μg of antibodies at 4°C for 10 minutes. Antibodies against mouse immunoglobulin conjugated to PE or fluorescein isothiocyanate (FITC) were used as antibody isotype controls (BD Biosciences). The stained cells were analyzed on the FACS Aria (BD Biosciences). Cell-Quest Acquisition and Analysis software (BD Biosciences) was used to acquire and quantify the fluorescence signal distributions and intensities from individual cells.

Antibodies

The following antibodies were used as primary antibodies: MET (Zymed and Santa Cruz for detection of total MET protein; Cell Signaling for detection of phosphorylated MET proteins), Nestin, Sox2 (R&D Systems), GFAP (Dako), TuJ1 (Covance), O4 (Chemicon), α-tubulin and β-actin (Sigma), and AKT and Erk (Cell Signaling).

Tumorsphere forming limiting dilution assay

Tumor cells were dissociated into single-cell suspensions, sorted for MET expression, and then plated into 96-well plates with various seeding densities (1–500 cells per well depending on the experiment, more than 30 wells for each condition).

Intracranial tumor cell injection into NOD/SCID Il2rg–/– mice

Unsorted GBM cells or the sorted MET\(^{\text{high}}\) and MET\(^{\text{low}}\) tumor cells were resuspended in 5 μL of HBSS and injected stereotactically into the striatum of adult nonobese diabetic/severe combined immunodeficiency mice lacking interleukin-2 gamma receptor (NOD/SCID Il2rg\(^{−/−}\)) mice by using a stereotactic device (Kopf instruments; coordinates: 2 mm anterior, 2 mm lateral, and 2.5 mm depth from the dura). Mice with neurologic signs were killed for the analysis of tumor histology and immunohistochemistry. All mouse experiments were carried out according to the guidelines of the Animal Use and Care Committees.

In vivo delivery of MET siRNA-polyelectrolyte–micelle complexes

For the conjugation of MET siRNA or nontargeting siRNA with polyethylene glycol 5000 (PEG5K), we followed the procedures as previously described (29). PEG-MET siRNA or PEG-control siRNA (0.5 mg/kg body weight) complexes were delivered through tail vein injections twice a week for 4 weeks.

Determination of intracranial tumor volumes

To estimate the size of intracranial tumor, we carried immunohistochemical analysis using an anti-human nuclei specific antibody (Chemicon) on the serial brain sections. Staining images were analyzed by ImageJ software (NIH), and the number of immunopositive cells and the total number of cells in a given field were calculated. The volume of the intracranial tumor was estimated by the largest width\(^2 \times \) length × 0.5.

Statistical analysis

All values are shown as mean ± SD. For group comparison, paired t test (2 group comparison) or ANOVA (more than 3 group comparison) were used. Kaplan–Meier survival analysis was done using Prism 4.0 software.

Results

MET-positive tumor cells are preferentially located in perivascular regions of human GBM specimens and coexpress GSC markers

We reasoned that components of a GSC regulator pathway would be preferentially expressed in perivascular regions and hypoxic edges, the proposed in vivo niches for GSCs (10). By immunohistochemical analysis using human GBM patient specimens (n = 93), we determined the expression patterns and intensities of various growth factor receptors, oncogenes, and stem cell–associated proteins.

High frequency of cells positive for MET (more than 10% of tumor cells) in GBM specimens was positively correlated with the shorter progression-free survival (PFS) and overall survival (OS) of the patients, suggesting a potential role of MET overexpression in malignancy (Fig. 1A and B). We also determined whether high expression of MET mRNA in GBM specimens portends poor patient survival by the analysis of The Cancer Genome Atlas (TCGA) database (Supplementary Fig. S1; ref. 19). Patients with high MET mRNA expression have shorter PFS and OS, however, the prognostic value was marginal although significant (Supplementary Fig. S1).

We found that MET protein expression displayed inter- and intratumoral heterogeneity. Interestingly, cells strongly stained for MET were predominantly located near blood vessels and hypoxic edges (Fig. 1C and D). To quantify the proximity between MET-positive cells and endothelial cells in primary GBM specimens, we carried out dual immunofluorescence for CD31 (endothelial marker) and MET using the frozen sections derived from 4 GBM patient specimens (Fig. 1E–G). MET-positive cells were located closely to the nearest blood vessels, compared with MET-negative cells (Fig. 1G). In addition, a subset of tumor cells express both GSC enrichment markers CD133/CD15 and MET (Supplementary Fig. S2; refs. 7, 28).
Figure 1. Expression of MET in primary human GBM specimens. A and B, Kaplan–Meier PFS (A) and OS (B) graphs of patients with high (red) and low expression of MET (blue). GBM sections from 93 patients were stained by using MET antibodies and categorized by the frequency of MET-stained cells (>10% of tumor cells as high expression group). P values were determined by log-rank test: \( P < 0.007 \) for A and \( P < 0.015 \) for B. C and D, representative microphotographs of immunohistochemical staining of MET protein in paraffin sections of GBM patient specimens. Immunopositive cells were visualized by brown DAB staining. Arrows indicate the stained cells. Ve indicates blood vessels, and Nec indicates the area of necrosis. Scale bar represents 20 \( \mu \text{m} \). E and F, representative microphotographs showing dual immunofluorescence analysis using MET antibody (green) and CD31 (red) in frozen sections of GBM patient specimens. 4’, 6-Diamidino-2-phenylindole (DAPI) was used to visualize nuclei. Scale bar represents 100 \( \mu \text{m} \). G, measurement of the average distance between MET-positive cells and blood vessels.

### Table: Distance from the nearest vessel (\( \mu \text{m} \pm \text{S.D} \))

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Distance from the nearest vessel (( \mu \text{m} \pm \text{S.D} ))</th>
<th>( T ) test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{MET}_{\text{high}} )</td>
<td>( \text{MET}_{\text{low}} )</td>
</tr>
<tr>
<td>541</td>
<td>18.2 (± 13.7)</td>
<td>67.3 (± 28.8)</td>
</tr>
<tr>
<td>609</td>
<td>28.5 (± 21.3)</td>
<td>77.0 (± 37.4)</td>
</tr>
<tr>
<td>586</td>
<td>18.2 (± 12.7)</td>
<td>35.9 (± 16.5)</td>
</tr>
<tr>
<td>381</td>
<td>19.2 (± 8.1)</td>
<td>27.1 (± 14.8)</td>
</tr>
</tbody>
</table>
To obtain more quantitative results, we determined the expression level of MET in the freshly dissociated cells obtained from 23 different GBM patients by fluorescence-activated cell sorting (FACS) analysis (Fig. 2A). The percentages of MET-positive cells vary between the samples (3.7%–76.4% of bulk cells; SD 16.1%). In 15 of 23 samples, the percentages of MET-positive cells are between 10% to 30% of the bulk cells. The cells dissociated from nontumor epileptic brain specimens did not have any distinct MET-positive population (data not shown). To determine whether there are distinct subpopulations expressing both MET and CD133 and/or CD15, we carried out dual FACS staining on the freshly isolated GBM cells derived from 14 different patients (Fig. 2A and Supplementary Table S1). CD133- and/or CD15-positive cells were enriched (2- to 7-fold) in MET-positive subpopulation compared with the bulk tumor cells. Conversely, MET-positive cells were enriched in CD133+ or CD15+ populations (Supplementary Table S1).

To corroborate these findings, we extended our analysis to a previous characterized GSCs derived from human GBM specimens (26, 28). GSCs and their derivatives used in this study are described in supplementary information (Supplementary Table S2). We fractionated these short-term cultured GSCs by FACS and carried out immunoblot analysis (Fig. 2B). MET protein was highly expressed in CD133+ (or in CD15+) subpopulations compared with the corresponding negative populations in all 3 GSCs (Fig. 2B). Total MET and activated MET (Y1234) were preferentially expressed in GSC-enriched population compared with GSC-depleted cells (Fig. 2C). Collectively, these data indicated that MET-positive GBM cells have activated MET signaling and that they significantly overlap with CD133+ and/or CD15+ GBM populations.

**MET**<sup>high</sup> cells sustain *in vitro* growth and are highly clonogenic compared with MET<sup>low/−</sup> cells

To determine whether MET<sup>high</sup> GBM cells have cellular characteristics associated with GSC phenotypes, we used FACS using an antibody recognizing the extracellular domain of MET protein and prospectively isolated MET<sup>high</sup> and MET<sup>low/−</sup> subpopulations from the patient GBM specimen 448. We operationally defined MET<sup>high</sup> cells as the cells with top 10% to 20% highest intensities out of total MET-positive cells. After confirming the purity and viability of these sorted populations by FACS reexamination, we first determined the growth kinetics of each subpopulation by culturing these cells in the standard stem cell culture condition. MET<sup>high</sup> cells readily formed neurosphere-like aggregates and continued to proliferate, whereas MET<sup>low/−</sup> cells failed to sustain active cell growth *in vitro* (Fig. 3A–C).

Clonogenic growth as neurospheres is an *in vitro* indicator of self-renewal in normal neural stem/progenitor cells (NSC) and GSCs. To determine the clonogenic potentials of MET<sup>high</sup> and MET<sup>low/−</sup> populations, we carried out limiting dilution assays (LDA) using both 448 freshly isolated GBM cells and 206 short-term cultured GSCs (Fig. 3D). FACS-sorted MET<sup>high</sup> and MET<sup>low/−</sup> cells were plated into 96-well plates with various seeding densities and allowed to grow. Neurospheres were readily formed by MET<sup>high</sup> cells but not by MET<sup>low/−</sup> cells (Fig. 3D). In addition, we carried out a competitive growth assays in which MET<sup>high</sup> and MET<sup>low/−</sup> cells were differentially labeled with GFP and red fluorescence protein (RFP), respectively, mixed at various ratios, and then cultured for additional 2 weeks. Even when the initial fraction of MET<sup>high</sup> GFP cells was 10%, more than 85% of the resultant cells after the culture were GFP positive (Fig. 3E). Taken together, these data indicated that MET<sup>high</sup> GBM cells are highly clonogenic and sustain *in vitro* growth of total population.

To determine whether the HGF/MET signaling pathway is functional in GSCs, we examined the activation status of MET downstream effectors (Supplementary Fig. S3A). GSCs displayed basal activation of MET, suggesting the presence of an autocrine loop with further response to the addition of exogenous HGF. Treatment with exogenous HGF further increased activated MET (Y1234 and Y1349), AKT (S473), and ERK (16). Consistent with this, we detected high levels of the secreted HGF protein in GSC-conditioned media (7 of 11 GSCs) by ELISA assays (Supplementary Fig. S3B). Finally, we determined whether HGF could induce the proliferation of GBM cells by

---

**Figure 2.** Expression of MET in primary human GBM specimens and GBM-derived GSCs. A, flow cytometry data of MET-, CD133-, and CD15-positive cells in freshly isolated patient GBM cells. B and C, immunoblot analysis using CD133 and/or CD15 cell lysates from short-term cultured GSCs (B) and GSC-derived xenograft tumors (C). The numbers represent the designated names for each GSC. Actin was used as a loading control.
Cancer Research
Cancer Res; 72(15) August 1, 2012

The brains of highly immunodeficient NOD/SCID Il2rg<sup>−/−</sup> mice and monitored tumor formation (30). MET<sup>high</sup> cells from 4 GBMs (448 and 464, freshly isolated patient GBM cells; 822 and 206, short-term cultured GSCs) generated tumors more efficiently than their corresponding MET<sup>low</sup> cells (Fig. 4A and B). Of note, all of the above GBM cells express HGF (data not shown). To estimate the relative enrichment of tumorigenicity in MET<sup>high</sup> cells compared with MET<sup>low</sup> cells, we carried out in vivo tumorigenicity titration assays (Fig. 4C). Varying numbers of MET<sup>high</sup> and MET<sup>low</sup> cells isolated from primary GBM specimen (559 and 905) were injected into brains of NOD/SCID Il2rg<sup>−/−</sup> mice. Two of 5 mice injected with 100 MET<sup>high</sup> cells developed tumors and so did all the mice (κ = 5) that had 1,000 MET<sup>high</sup> cells (median survival of 88 days). In contrast, all the mice that received 100 MET<sup>low</sup> cells and 4 of 5 mice injected with 1,000 MET<sup>low</sup> cells showed no sign of tumor development 6 months after the injection (Fig. 4C). Kaplan–Meier survival analysis further showed significant survival differences between animals receiving these subpopulations (Fig. 4D and E).

Similar to the parental GBM tumor, 448 MET<sup>high</sup> cell-derived xenograft tumors harbored a mixture of MET<sup>high</sup> cells and MET<sup>low</sup> cells, indicating the reconstitution of tumor heterogeneity (ref. 31; Supplementary Fig. S4). In addition, immunohistochemical analysis on the xenograft tumors revealed that MET<sup>high</sup> cells were preferentially located near the blood vessels and infiltrating edges of tumors (Fig. 4F–H). Robust expression of tumor-derived HGF was also detected in xenograft sections by immunohistochemical analysis, suggesting an active HGF/MET signaling in these tumors (Supplementary Fig. S5).

**MET<sup>high</sup> cells are efficient in tumor formation regardless of CD133 expression**

Our data showed that some of MET<sup>high</sup> cells coexpress CD133 (Fig. 2 and Supplementary Table S1). To determine whether MET expression alone can enrich GSCs, we fractionated GBM cells into 4 subpopulations (CD133<sup>+</sup>/MET<sup>high</sup>, CD133<sup>+</sup>/MET<sup>low</sup>, CD133<sup>−</sup>/MET<sup>high</sup>, and CD133<sup>−</sup>/MET<sup>low</sup>) and determined their tumor formation efficiencies. Both CD133<sup>+</sup>/MET<sup>high</sup> and CD133<sup>+</sup>/MET<sup>low</sup> cells, derived from the freshly isolated GBM cells (060) and GBM xenografts (559 and 464), were highly efficient in tumor formation, whereas CD133<sup>−</sup>/MET<sup>low</sup> and CD133<sup>−</sup>/MET<sup>low</sup> cells were not (Fig. 5A). In vitro clonogenic potential of each subpopulation correlated with in vivo tumorigenicity of the corresponding cells (Fig. 5B). These data suggested that MET, but not CD133, is a major determinant for GSC enrichment at least in these GBMs.

**MET inhibition decreases the survival, migration, and clonogenicity of GSCs**

To interrogate the role of MET in GSC biology, we inhibited MET by short hairpin RNA (shRNA)–mediated knockdown and evaluated its effects on GSCs. To ensure the specific knockdown of MET, we used 2 independent sequences of shRNA directed against MET mRNA. Both MET shRNA constructs led to a significant reduction in MET protein compared with the
nontargeting shRNA control (Supplementary Fig. S6). First, we determined the cell growth/survival of MET knockdown GBM cells. Compared with the control, MET knockdown cells showed a significant decrease in the proliferation index (Supplementary Fig. S6B and C). MET knockdown cells were largely in G0/G1 phase without notable increase in sub-G1 populations, suggesting that cell-cycle arrest is a main contributor of the decreased cell proliferation. MET knockdown significantly decreased the clonogenicity of various GSCs, determined by LDA (Supplementary Fig. S6D and E). We also tested whether pharmacologic inhibition of MET signaling can decrease GSC clonogenicity. GSCs treated with SU11274, a widely used MET kinase inhibitor (25, 32), were less efficient in tumor-sphere formation compared with the control (Fig. 6A).
HGF is an effective chemokine for glioma cell migration (13, 33). To test whether MET signaling mediates invasive growth of GSCs, we carried out transwell-based in vitro migration assays (Supplementary Fig. S7A and B). We plated control shRNA- or MET shRNA–transduced GSCs in serum-free media and added serum (or HGF)-containing media on the other side of the transwell membranes to stimulate migration. MET knockdown decreased the number of migrating/invading cells up to 70% compared with the control (Supplementary Fig. S7A and B). In addition, we carried out ex vivo brain slice assays that simulate in vivo migration behavior much better than transwell assays (Supplementary Fig. S7C). RFP-expressing GSCs were treated with a chemical MET inhibitor PHA665752 for one day, mixed with GFP-expressing GSCs treated with the vehicle control, and then implanted into the cortex region of 300-μm thick brain slices. Three days after, more than 90% of the migrated cells were GFP positive, suggesting that MET inhibition decreases glioma cell invasion (Supplementary Fig. S7C and D). Together, these data showed that MET signaling is required for the self-renewal, proliferation, and migration of GSCs.

**In vivo MET targeting via liposome-conjugated siRNA increases the survival of tumor bearing mice**

By using GSC-derived tumors as a clinically relevant model system, we wanted to test whether MET targeting in vivo could provide therapeutic benefit. Polyelectrolyte complex micelles are stable in vivo and efficient for intracranial delivery of siRNA because of the highly lipophilic nature of brain tissues (29). We conjugated MET siRNA or nontargeting siRNA with polyethylene glycol 5000 (PEG5K) and mixed with small liposomes (29). Intracranial tumors were generated from 3 freshly isolated GBM cells (559 and 578, 5 for each group; 464, 4 for each group). These cells express both MET and HGF at high levels, determined by Western blot analysis and HGF ELISA (data not shown). Once tumors were established in the mouse brains, we initiated the administration of either PEG–MET siRNA or PEG–control siRNA (0.5 mg/kg body weight) intravenously twice a week for 4 weeks. Compared with the tumors from the control siRNA-treated mice, tumor cells in MET siRNA-treated mice revealed a significant decrease in MET staining positivity (Fig. 6B). After 4 weeks of treatment, we monitored the size of tumors by MRI (Fig. 6C) and brain histology section (Fig. 6D and Supplementary Fig. S8). All of MET siRNA–treated groups showed a significant decrease in tumor volumes, indicating the efficacy of in vivo MET targeting in intracranial GSC-derived tumors (Fig. 6E).

**Irradiation induces MET upregulation and MET targeting decreases the clonogenicity of surviving GBM cells**

Irradiation treatment decreases tumor burden and prolong the survival of GBM patients. However, patients eventually succumb because of tumor recurrence. Given the observations that GSCs preferentially survive radiation and that MET targeting disrupted GSC self-renewal and clonogenicity, we hypothesized that combination of radiation and MET inhibition in GSCs might be an effective therapeutic approach (9, 34, 35). Cells of a short-term cultured GSC (822) were irradiated in vitro with doses of 5 or 10 Gy and harvested 2 days later. We found about a 3-fold increase in the number of MET-positive cells and upregulation of MET protein in irradiated cells compared with nonirradiated control (Fig. 7A and B). To determine the clonogenic potentials of METhigh and METlow/− GBM cells after radiation treatment, we sorted METhigh and METlow/− cells from the freshly isolated 448 GBM cells, exposed them to radiation, and immediately seeded at the clonal density for LDA assays.

<table>
<thead>
<tr>
<th>GBM</th>
<th>Cell No.</th>
<th>Subpopulations (No. of mice with tumor/fatal mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD133+/METhigh</td>
</tr>
<tr>
<td>060</td>
<td>10,000</td>
<td>3/3</td>
</tr>
<tr>
<td>559</td>
<td>1,000</td>
<td>5/5</td>
</tr>
<tr>
<td>464</td>
<td>10,000</td>
<td>5/5</td>
</tr>
<tr>
<td>464</td>
<td>1,000</td>
<td>4/4</td>
</tr>
</tbody>
</table>
MET<sup>high</sup> cells remained highly clonogenic after radiation treatment, whereas irradiated MET<sup>low</sup>/C0 cells were significantly impaired in the ability to generate subsequent colonies (Fig. 7C). To determine whether MET is directly involved in the clonal regrowth after radiation, we carried out the similar clonogenic assays by MET knockdown or treatment with SU11274. Cells with MET inhibition were hardly able to generate spheres after radiation treatment, suggesting that MET inhibition significantly sensitizes GBM cells to radiation (Fig. 7D and Supplementary Fig. S9).

The above data raise the possibility that MET upregulation after radiation can be a prognostic biomarker for the GBM patients who have received radiation therapy. We acquired 14 matched sets of pre- and postirradiated (recurrent) tumor specimens from the same GBM patients (Supplementary Table S3). All the patients were diagnosed as de novo primary GBMs and received radiation as a front-line therapy after initial surgical resection. We determined the expression level of MET protein by immunohistochemical analysis on the sections from pre- and postirradiated GBM specimens. In 4 sets, MET expression in postirradiated GBM specimens was significantly increased (>2-fold increase in the number of MET-positive cells) compared with the untreated specimens. The remaining 10 sets showed either unchanged or modest changes in MET expression. Notably, the patients showing the significant MET induction had worse prognosis (median survival of 55 days after recurrence) than the other group (median survival of 189 days; P < 0.01; Fig. 7E), suggesting that MET upregulation after radiation is associated with aggressive growth of recurrent tumors.

Discussion

GSCs remains controversial because of unresolved questions with regard to the frequency of these cells, the surface
markers by which they can be identified, and the nature of the cell(s) of origin (31, 36). Previous reports have identified a series of GSC enrichment markers, including CD133, CD15, CD44, and A2B5 (7, 28, 37, 38). Although useful for the prospective isolation of putative GSCs, it is unclear whether these markers have distinct functions pertinent to GSC phenotypes (39, 40). Therefore, our screening approach in search for a GSC regulator was focused on the protein expression pattern in patient-derived GBM specimens, based on the hypothesis that GSCs are highly enriched in perivascular regions of human GBMs in situ (41).

In this study, we identified a distinct fraction of cells expressing high level of MET in various human primary GBMs and showed that these subpopulations have key characteristics of GSCs. Through extensive in vivo limiting dilution tumor formation assays, we have shown that METhigh GBM cells are...
highly enriched with tumor stem/propagating populations, supporting that MET is a key regulator of GSCs. We showed the importance of MET in GSC biology by showing that MET inhibition disrupts the clonogenicity, radioresistance, and tumorigenicity of GSCs. Co-option with angiogenesis and tumor cell survival in hypoxia are critical for tumor growth, and MET signaling is known to play important roles on these processes. Therefore, high MET expression in GSCs can be viewed as an adaptive response of cancer cells to their micro-environment (42, 43).

Numerous small molecule inhibitors and antagonistic antibodies targeting the HGF/MET pathway are at advanced stages of clinical development (44–48). Understanding of the molecular determinant(s) of response and resistance to HGF/MET targeting therapeutic agents is one of the most critical unmet needs in clinical research. A few of biomarkers to predict therapeutic response have been proposed, including genomic amplification of MET, HGF levels, or MET expression (total and phosphorylated MET), and PTEN status (33, 49, 50). Robustness of these biomarkers in GBM needs further validation. For example, genomic MET amplification seems to predict sensitivity to MET inhibition in gastric cancers but less likely in GBMs (33). A recent paper reported that HGF autocrine status may correlate with MET activity in GBM and predict sensitivity to MET inhibitors (33). We have determined the correlation between tumor-derived HGF and sensitivity to MET inhibition. Our ongoing studies so far indicated that high HGF-producing GBM cells seem to be more sensitive to MET inhibition compared with the little or no HGF-expressing GBM cells, consistent with the previous report (data not shown; ref. 33). Further studies are warranted to determine the role of HGF in GSC biology, the effects of HGF targeting on GSC self-renewal and tumorigenicity, and the correlation between HGF and GSC sensitivity to MET inhibition in vivo.

In conclusion, we show that MET is an enrichment marker for GSCs and a functional requisite for cancer stem phenotype. Not only will these data provide a clue for a more thorough understanding of cancer stem cell biology but also they further implicate MET as a promising therapeutic target.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: K.M. Joo, J. Lee, D.-H. Nam
Development of methodology: K.M. Joo, J. Jin, B.G. Kang, Y.-J. Kang, H. Kim, J. Lee, D.-H. Nam
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.M. Joo, J. Jin, Y. Kim, J.J. Lathia, H.J. Seol, J. Lee, D.-H. Nam
Writing, review, and/or revision of the manuscript: K.M. Joo, Y. Kim, P.H. Song, J.N. Rich, J. Lee
Administrative, technical, or material support (i.e., reporting and organizing data, constructing databases): K. M. Joo, J. Jin, K.H. Kim, H. Kim
Study supervision: K.H. Cheng, D.-H. Nam

Grant Support
This work was supported by the National Research Foundation of KOREA (NRF) grant funded by the Korea government (MEST, No.20090093731; D.-H. Nam), the grant for the Future-based Technology Development Program (2010-002032) funded by NRF of the MEST, Republic of Korea (D.-H. Nam), Cleveland Clinic Foundation grant (J. Lee), and Case Western Reserve University/Cleveland Clinic CTCSC Grant UL1 RR024989 and RE350-5510 (J. Lee).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 14, 2011; revised April 18, 2012; accepted May 3, 2012; published OnlineFirst May 22, 2012.

References

www.aacrjournals.org Cancer Res; 72(15) August 1, 2012 3837


MET Signaling Regulates Glioblastoma Stem Cells

Kyeung Min Joo, Juyoun Jin, Eunhee Kim, et al.


Updated version Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-11-3760
Supplementary Material Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2012/05/22/0008-5472.CAN-11-3760.DC1

Cited articles This article cites 50 articles, 12 of which you can access for free at: http://cancerres.aacrjournals.org/content/72/15/3828.full.html#ref-list-1
Citing articles This article has been cited by 4 HighWire-hosted articles. Access the articles at: /content/72/15/3828.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.