Overexpression of HGF/MET axis along with p53 inhibition induces de novo glioma formation in mice

Yuan Qin, Anna Musket, Jianqun Kou, Johanna Preiszner, Barbara R. Tschida, Anna Qin, Craig A. Land, Ben Staal, Liang Kang, Kirk Tanner, Yong Jiang, John B. Schweitzer, David A. Largaespada, and Qian Xie

Abstract

Background. aberrant MET receptor tyrosine kinase (RTK) activation leads to invasive tumor growth in different types of cancer. Overexpression of MET and its ligand hepatocyte growth factor (HGF) occurs more frequently in glioblastoma (GBM) than in low-grade gliomas. Although we have shown previously that HGF-autocrine activation predicts sensitivity to MET tyrosine kinase inhibitors (TKIs) in GBM, whether it initiates tumorigenesis remains elusive.

Methods. using a well-established Sleeping Beauty (SB) transposon strategy, we injected human HGF and MET cDNA together with a short hairpin siRNA against Trp53 (SB-hHgf.Met.ShP53) into the lateral ventricle of neonatal mice to induce spontaneous glioma initiation and characterized the tumors with H&E and immunohistochemistry analysis. Glioma sphere cells also were isolated for measuring the sensitivity to specific MET TKIs.

Results. Mixed injection of SB-hHgf.Met.ShP53 plasmids induced de novo glioma formation with invasive tumor growth accompanied by HGF and MET overexpression. While glioma stem cells (GSCs) are considered as the tumor-initiating cells in GBM, both SB-hHgf.Met.ShP53 tumor sections and glioma spheres harvested from these tumors expressed GSC markers nestin, GFAP, and Sox 2. Moreover, specific MET TKIs significantly inhibited tumor spheres’ proliferation and MET/MAPK/AKT signaling.

Conclusions. Overexpression of the HGF/MET axis along with p53 attenuation may transform neural stem cells into GSCs, resulting in GBM formation in mice. These tumors are primarily driven by the MET RTK pathway activation and are sensitive to MET TKIs. The SB-hHgf.Met.ShP53 spontaneous mouse glioma model provides a useful tool for studying GBM tumor biology and MET-targeting therapeutics.

Key Points

- We demonstrate that HGF/MET overexpression along with Trp53 inhibition induces spontaneous glioma in mice.
- We established an HGF/MET-driven glioma mouse model for studying GBM biology.
- We show that HGF/MET-driven GBMs are sensitive to MET inhibitors.
Importance of the Study
Elevated HGF/MET axis provokes RAS/RTK/PI3K pathway activation, the leading signaling alteration in primary GBM. MET overexpression is a marker of the “mesenchymal” subtype and is required for GSC maintenance and its invasive repopulation. While HGF or MET transgenic mice were shown to initiate liver or breast cancers, a glioma phenotype has not been reported, raising the question of whether the HGF/MET axis may drive glioma initiation. Here, we show that overexpression of HGF and MET, along with Trp53 inhibition, induces spontaneous glioma in mice. Moreover, the GSCs derived from these tumors are sensitive to MET and MEK inhibitors. These results not only provide the evidence that HGF/MET elevation may transform NSCs into GSCs, resulting in GBM initiation and progression, but also suggest that HGF-autocrine GBMs are primarily driven by the MET RTK pathway that may be inhibited by MET or MEK inhibitors.

Materials and Methods

Plasmid Vectors
Plasmids pT2/C-Luc/PGK-SB100 (the SB transposase expression plasmid coding luciferase as the reporter gene), pT/CAGGS-NRASV12 (a construct coding a mutant NRAS), and pT2/shP53/GFP4 (a short hairpin siRNA against Trp53 constructed with green fluorescent protein) were created as previously described. To construct human HGF and MET coding plasmids, the full-length human HGF or MET cDNAs were PCR amplified from PMOG or MG-pRS24 plasmids (provided by Dr. George Vande Woude, Van Andel Research Institute) with the primers engineered with SnaBI and XbaI compatible enzyme sites (FspI and AvrII). The hHGF and hMET cDNAs were...
further cloned into the pENTR1 Gateway vector using SnaBI and XbaI and then moved to the PT3.5-CAGG-DEST plasmid by an LR Clonase reaction (Invitrogen) as described previously. The SB constructs are summarized in Figure 1A. The primers are as follows: hMET forward—NNNTGCGAGCCACCATGAAG GCCCCCGC; hMET reverse—NNNCCTAGGCTATGATGTCTCCCAGAAGG; hHGF forward—NNNTGCGAGCCACCATGTGGGTGACCAAAC; hHGF reverse—NNNCCTAGGCTATGAC TGTGGTACC.

Animal Models, Plasmid Injections, and Bioluminescence Imaging

FVB/N strain mice were purchased from the Charles River Corporation and breeding pairs were monitored each day at the Vivarium of the Van Andel Research Institute (VARI). Given previous observations that neonatal mice are tolerant to human neoantigen delivered within 24 h of birth and that glioma penetrance in mice is reduced in mice older than 3 days, only neonatal mice with age less than 2 days were used for the studies. Mixed plasmid injection is described in Supplementary Methods. Athymic/nude mice were used for testing glioma initiation and tumor growth inhibition using isolated glioma sphere cells, which is also described in Supplementary Methods. All studies involving animals were approved by the VARI and East Tennessee State University (ETSU) Institutional Animal Care and Use Committees.

Immunohistochemistry Staining of GBM Orthotopic Tumors

At the time of necropsy, mouse brains with tumors were dissected, fixed with 10% neutral buffered formalin...
(Sigma-Aldrich), and embedded into paraffin blocks for H&E and immunohistochemistry (IHC) staining using standard clinical techniques in the ETSU pathology laboratory (Supplementary Methods).

Cell Lines and Drugs
The SB-026 and SB-033 neurospheres were generated from mouse 26 and mouse 33, respectively, which received SB-hHgf.Met.ShP53 plasmid injections. To establish SB-026 and SB-033 spheres, tumor tissues were excised and isolated using accutase (Invitrogen) and grown in the DMEM/F12 serum-free medium supplemented with B27 (Invitrogen), EGF (20 ng/mL, R&D), bFGF (20 ng/mL, R&D), and 1% penicillin and streptomycin (Invitrogen). To eliminate the possible clonal selection effect by EGF or bFGF, both growth factors were withdrawn from the culture medium after sphere cells started to passage. M114 cells (NIH3T3 cells stably transfected with mouse Met and mouse Hgf)24 were grown in DMEM (Invitrogen) supplemented with 10% FBS. V-4084 and SGX523 are specific MET tyrosine kinase inhibitors (TKIs). V-4084 was kindly provided by Vertex Pharmaceuticals. SGX523, EGFR inhibitor erlotinib, and MEK inhibitor PD0325901 were purchased from Selleck. All compounds were dissolved in DMSO at 0.01 M and aliquots were stored at −80°C until use. To treat cells in vitro, stock solutions were serially diluted using culture medium at the indicated concentrations.

Immunofluorescent Staining
SB-026 and SB-033 cells were seeded at 5000 cells per well in chamber slides (Lab-Tek) for neurosphere formation. Antibodies and staining are described in Supplementary Methods.

RT-PCR and Western Blot Analysis
Total RNA extraction, PCR primers and analysis, Western blotting, and antibodies are all described in Supplementary Methods.

Cell Proliferation Assay
Cells were seeded into a 96-well plate at 5 × 10^3 cells per well and grown for 48 h at 37°C followed by treatment with V-4084 or SGX523 at the indicated concentrations. Triplicate wells were used for each concentration. After an additional 5 days, cell proliferation was determined using the CellTiter 96 aqueous (MTS) assay (Promega). After MTS reagent was added into each well and incubated for another 3 h at 37°C, the number of viable cells was measured by the absorbance (OD = 490) using a microplate reader (BioTek).

Statistical Analysis
Survival time was graphed using Prism 5 software (GraphPad Software) and analyzed by log-rank test (P < .05). The therapeutic efficacy of small molecule inhibitors was analyzed using the Student’s t-test (P < .05) as previously described.25

Results
Overexpression of HGF and MET Induces Spontaneous Glioma Growth in Mice
Using the SB transposon system, Wiesner et al.22 have previously shown that mixed injection of oncogene-coding plasmids into the lateral ventricle of neonatal mice induced spontaneous glioma formation. For this study, a modified SB vector (pT2/C-Luc/PKG-SB100) with enhanced transposase expression was used to test whether overexpression of HGF and MET induces glioma formation in mice. Given that p53 inhibition alone or in combination with EGFR/PI3K did not induce gliomas in mice,22 we used pT2/ShP53/GFP4, a plasmid with a small hairpin RNA against p53 that is linked to co-expression of green fluorescence protein (GFP), to enhance the oncogenic activity of HGF/MET axis (Figure 1A).5,8 This SB vector co-injected with an empty transposon and pT2/ShP53/GFP4 did not induce glioma in mice (Supplementary Table 1). To perform the injection, plasmids pT2/C-Luc/PKG-SB100, pT3.5/CAGGS-huHgf, pT3.5/CAGGS-huMet, and pT2/ShP53/GFP4 were mixed at 1:1:1:1 ratio (µg/µg) and intracerebrally injected into FVB/N neonatal mice (n = 14). All mice were subjected to BLI at 24 h post-injection as an indication for transient plasmid uptake and once every month thereafter for tumor initiation and growth (Figure 1B). After excluding 4 mice that died of post-surgical hydrocephalus or significant weight loss, 8 out of 10 remaining mice (80%) developed lethal glioma growth with median survival time at 130.5 days (SB-hHgf.Met.ShP53, Figure 1C). Given that RTK/RAS/PI3K signaling accounts for the top pathway alteration in GBM, and that MET activates RAS as part of its downstream signaling,8 we tested whether overexpression of NRAS along with HGF/MET axis may accelerate glioma tumor growth. With 2 independent injections, a total of 11 mice were analyzed, and all grew tumors with a median survival time that is significantly shorter than HGF/MET/ShP53 mice (Figure 1C, SB-hHgf.Met.NRas.ShP53 vs SB-hHgf.Met.ShP53, 88 vs 130.5 days, P = .0032).

SB-hHgf.Met.ShP53 Mice Grow Invasive Glioma Expressing GSC Markers
To characterize the SB-hHgf.Met.ShP53 tumors, we first sectioned the paraffin-embedded tumor blocks for histology analysis using H&E staining. We show tumor cells infiltrating into normal parenchyma without a clear border, demonstrating an invasive phenotype (Figure 2A–C). A high density of tumor cells accumulating around the blood vessels were also seen, showing a peri-vascular tumor growth (Figure 2B, squared and Figure 2C, arrowed). We also observed geographic necrosis in tumor sections (Figure 2D, arrowed) with nearby neoangiogenesis (Figure 2D, arrowed in red). Actual microvascular proliferation (endothelial), however, was not impressive.
While microvascular proliferation is commonly present in GBM, the cellular anaplasia, mitotic activity, and intrinsic tumor necrosis are sufficient for the diagnosis of GBM. Additional GBM hallmarks also illustrated include mitoses (Figure 2E) and tumor giant cells (Figure 2F).

We then performed IHC staining to analyze the HGF and MET expressions in these tumors. We show that SB-hHgf.Met.Shp53 tumors overexpress MET in tumor cells as compared with the surrounding normal brain cells, demonstrating an infiltrative border (Figure 3A). At the higher magnification, we show a high expression of MET localized at the tumor cell membrane, however, with overall expression varying from low to high within the tumors (Figure 3A), suggesting a heterogeneous nature of these tumors. HGF was also overexpressed in tumor cells relative to the normal tissues, showing a cytoplasmic location (Figure 3A).

Since MET is reported to play an essential role in maintaining GSC properties, we further stained SB-hHgf.Met.Shp53 tumors with glial fibrillary acid protein (GFAP), nestin, and Sox II, the neural stem cell (NSC) or progenitor cell markers indicating self-renewal and pluripotency (Figure 3B). We show that all 3 markers were expressed in the tumor sections. These results suggest that overexpression of HGF/MET may transform NSCs and progenitor cells into GSCs, which leads to GBM formation.

Characterization of GSC<sub>hgf/met</sub> Cells

With IHC staining, the SB-hHgf.Met.Shp53 tumors showed tumor-specific expression of nestin and Sox 2, but a heterogeneous expression of GFAP, a marker for astrocytes, which was more evident in some apparent reactive astrocytes than tumor cells (Figure 3B GFAP, arrowed). To confirm the GFAP expression in tumor cells, 2 neurosphere cell lines (SB-026 and SB-033) were generated from SB-hHgf.Met.ShP53 tumors and characterized for expression markers. Both SB-026 and SB-033 spheres expressed strong GFP (Figure 4A), indicating sufficient uptake of the ShP53/GFP plasmid. Since we did not generate wild-type p53 glioma spheres from this study, the levels of p53 suppression in SB-026 and SB-033 were tested by RT-PCR, showing a significant reduction as compared with M114 cells, the normal mouse fibroblast cells overexpressing mouse Hgf/Met (Figure 4B). Both Western blot (Figure 4C) and confocal analysis (Figure 4D) confirmed the expression of GFAP, nestin, and Sox 2 in SB026 and SB033 spheres, ruling
out reactive astrocytes as the only source of GFAP expression in SB-hHuHgf.Met.Shp53 tumors. Importantly, on intracranial injection, both SB-026 and SB-033 cells initiate glioma growth in nude mice. SB-026 sphere-derived intracranial tumors propagate GBM histological hallmarks including infiltrative border, perivascular tumor growth, and necrotic center. Mitoses and giant cells are also shown (Supplementary Figure 1). These results indicate that HGF-autocrine activation of MET RTK is the driving force that transforms NSCs into GSCs to initiate glioma growth in these mice.

SB-026 and SB-033 Are Sensitive to Specific MET TKIs

Using GBM and HCC models, we previously reported that tumor cells overexpressing both HGF and MET often propagate an HGF-autocrine activation of MET RTK, indicating sensitivity to MET TKIs. To test whether this applies to SB-026 and SB-033, we treated these cells with V-4084 and SGX523 for 5 days and monitored spheroid formation. We show that both V-4084 and SGX523 at 0.1–1 µM significantly reduced the size of SB-026 and SB-033 spheroids (Figure 5A). The 5-day survival rate of SB-026 and SB-033 after V-4084 and SGX523 treatment was quantified using MTS assay, with erlotinib (an EGFR inhibitor) and PD-325901 (a MEK inhibitor targeting MAPK downstream from MET) tested for comparison (Figure 5B and C). We show that V-4084 and SGX523 at 0.1 µM and higher concentration significantly inhibited SB-026 (V-4084 vs SGX523: 61.7% vs 51.9%, \( P < .001 \)) and SB-033 (V-4084 vs SGX523: 55.2% vs 55.1%, \( P < .001 \)) proliferation. In contrast, erlotinib (1 µM) only marginally inhibited SB-026 and SB-033 (15% and 11%, respectively, \( P < .05 \)), suggesting a nonspecific targeting. Notably, PD-0325901 inhibited both SB-026 and SB-033 at 0.01 µM (51.1% and 62.6%,
respectively, \( P < .001 \), showing a higher efficacy than MET TKIs. At the protein expression level, both SB-026 and SB-033 expressed HGF, MET, and p-MET, demonstrating an HGF-autocrine activation of MET. Both V-4084 (Figure 5D and E) and SGX523 (Figure 5F and G) treatment significantly inhibit MET/MAPK/AKT signaling regardless of additional HGF stimulation. We further tested whether V-4084 inhibited SB-033 tumor growth in vivo. While an initial single dose of V-4080 at 30 mg/kg for 8 days showed marginal inhibition, increasing dosing to 60 mg/kg for an additional 3 days significantly inhibited SB-033 tumor growth in mice (Supplementary Figure 2, 1595 vs 647 mm\(^3\), \( P < .05 \)). While the non-orthotopic model is limited by the lack of the appropriate microenvironment for tumor growth and therefore may not reproduce the therapeutic efficacy or pharmaceutical kinetics precisely, mechanistically, these results indicate that the HGF/MET axis is the driving force in SB-026 and SB-023 tumor growth and that targeting MET/MAPK signaling will be effective for treating HGF-autocrine GBMs.

**Discussion**

Aberrant HGF/MET signaling activation is known to promote cancer initiation and malignant progression.\(^ {28,29} \) Genetically engineered mouse models have been widely used to test the tumor initiation role of HGF/MET in different cancer types. Previously, overexpression of HGF alone resulted in significant liver enlargement and triggers hepatocellular adenomas and/or carcinomas in mice.\(^ {30,31} \) Knocking in mutant *Met*
in mice induced diverse mammary carcinomas mimicking human basal breast cancer. Overexpression of Met alone or in combination with beta-catenin is sufficient for developing hepatocellular carcinoma. However, none of these mouse models showed brain tumor phenotypes. Although HGF/MET expression in human primary brain tumors is found to increase with the grade of malignancy and is a prognostic marker of short survival time in GBM, whether the HGF/MET axis directly triggers brain tumor formation has not been tested. In this study, we show that overexpression of HGF/MET, along with p53 suppression, induced de novo glioma formation in mice. These tumors infiltrate the brain and the...
perivascular spaces, are mitotically active, and show intrinsic, geographic necrosis and neoangiogenesis, pathological hallmarks that recapitulate human GBM histology. These results suggest that expression of HGF or MET is not sufficient for initiating brain tumors, while a co-expression of both ligand and receptor especially preconditioned with loss of function of tumor-suppressor genes is likely required. Although the SB-based gene transfer has the potential to cause insertional mutagenesis, the tumors developed in this model did not likely result from randomized mutations for 2 reasons: (1) the negative control plasmids did not induce any glioma in mice (Supplementary Table 1) and (2) the SB033 tumors are sensitive to V-4084, a MET-specific inhibitor, demonstrating a level of MET dependency (Supplementary Figure 1).

During normal CNS development, NSCs are primarily located in the hippocampus and subventricular zone. These are undifferentiated precursor cells defined by their capacity for self-renewal and multipotency, which may further amplify into multi-lineage progenitor cells and differentiate into neurons, astrocytes, and oligodendrocytes to populate the nervous system. However, GSCs within a brain tumor may arise from normal NSCs or neural progenitor cells that harbor genetic alterations such as mutations and oncogene overexpression.1,36,37 Previous studies have shown that loss of Pten in combination with p53 converted neural and glioma stem cells into glial tumors.38,39 While shown that loss of Pten in combination with p53 converted tumors for sensitivity to anti-HGF or anti-MET neutralizing binding to HGF, our results only demonstrate a MET de-binding in the MET RTK domain rather than blocking MET TKIs. These results suggest that HGF-autocrine activation may transform NSCs into GSCs, and ultimately, initiate glioma growth and progression in these mice (Figure 6). MET activation is essential in maintaining GSC’s stem-like phenotype.14,15 The fact that MET TKIs V-4084 and SGX523 and MEK inhibitor PD-0325901 significantly inhibited SB-026 and SB-033 proliferation in vitro and tumor growth in vivo support the application of targeting MET/MAPK signaling pathway for treating gliomas with HGF/MET overexpression. Because MET-targeted inhibitors are all designed to block the ATP binding in the MET RTK domain rather than blocking MET binding to HGF, our results only demonstrate a MET dependency in this model. However, whether the tumors are driven by an HGF-autocrine activation remains undetermined. Therefore, it would be necessary to further test the tumors for sensitivity to anti-HGF or anti-MET neutralizing antibodies to further distinguish the HGF dependency.29

While we show in this study that overexpression of HGF/MET induces gliomagenesis, it is not clear whether HGF or MET alone may be sufficient. Specifically, MET amplification occurs in approximately 4% of GBM patients.8 Although early studies have not reported that HGF or MET transgenic mouse models develop glioma as a phenotype,30,31,34 it is possible that the dose of HGF or MET transgenes in previous models was not high enough to initiate tumors in the brain. Since the SB transposon system allows for the quantification of the concentration of plasmid DNA for injection, it is worthwhile to apply injections to deliver a higher dose of human HGF or MET, either alone or in combination with deletions of glioma suppressor genes, such as TP53, PTEN, or NF1.5 Such studies may elucidate how ligand-independent MET activation may regulate GBM initiation and progression (Figure 6). With the SB transposon system as a tool, another important application is to study how HGF-autocrine activation may crosstalk with other tumor-driver genes or suppressor genes in determining the therapeutic response to anticancer treatments. We have shown that introducing NRAS into the HGF/MET/ ShP53 axis may accelerate glioma growth and significantly shorten the survival time of the animals (Figure 1C). Thus, future studies may test how other GBM core pathway components, such as p53 and RB signaling pathways, may regulate HGF/MET axis in gliomagenesis and progression.8 Recently, CRISPER/cas9 has become a powerful technology for gene editing. Combining the use of CRISPER/cas9 and SB approaches has been shown to be an effective approach in screening for tumor-driver genes and therapeutic targets for functional validation.40 Future studies may use CRISPER/cas9 to improve this SB-hHgf.Met.ShP53 mouse model in multi-target gene delivery as a better system for studying brain tumor biology and therapeutic strategies.

In conclusion, using a well-established SB transposon strategy, we demonstrate that mixed injection of plasmid DNAs coding human HGF and MET, along with microRNA-mediated siRNA against p53, results in de novo glioma formation in mice. These tumors show invasive phenotypes that resemble human GBM pathology. Moreover, the neurospheres isolated from SB-hHgf.Met.Shp53 tumors expressed human HGF and MET along with NSC markers including nestin, GFAP, and Sox 2 and are sensitive to specific MET TKIs. These results suggest that HGF-autocrine activation may transform NSCs into GSCs that ultimately lead to GBM initiation. Successful targeted therapy relies on clinically relevant preclinical models. MET inhibitors are being evaluated in clinical trials against multiple cancer types including brain tumors.28,41 Although MET-targeted therapy in GBM patients has not been successful, a recent clinical trial has shown that a combination of MET and VEGFR inhibitors (onartuzumab plus bevacicuzumab vs placebo plus bevaciuzumab) significantly improved both progression-free survival and overall survival in the mesenchymal subtype of recurrent GBM patients with high HGF expression.42 This result supports our previous findings that HGF is a predictive marker for MET-targeted therapy and that developing biomarker analysis for patient stratification is necessary in future clinical trials targeting MET.20,21 While our previous studies have used immune-compromised mice bearing human GBM cell lines or PDXs, the SB-hHgf.Met.ShP53 mouse glioma model provides an immune-competent microenvironment for studying GBM tumor biology and MET-targeting therapeutics and will be of value for studying cancer immunotherapy against malignant glioma.
**Supplementary Data**

Supplementary data are available at *Neuro-Oncology Advances* online.

**Keywords**

genetically engineered mouse model | glioblastoma | glioma initiating cells | HGF/MET signaling | targeted therapy

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**Conflict of interest statement.** D.A.L. is the co-founder and co-owner of several biotechnology companies including NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immusoft, Inc.), B-MoGen Biotechnologies, Inc. (recently acquired by the Bio-Techne corporation), and Luminary Therapeutics, Inc. He consults for Genentech, Inc., which is funding some of his research. D.A.L. holds equity in and serves as the Chief Scientific Officer of Surrogen, a subsidiary of Recombinetics, a genome-editing company. The business of all these companies is unrelated to the contents of this manuscript. All other authors declare no conflict of interest.

**Figure 6.** Proposed mechanism of MET RTK alteration induced gliomagenesis and therapeutics. During normal neurogenesis, neural stem cells are primarily located in the hippocampus and subventricular zone where they proliferate into multi-lineage progenitor cells and differentiate into mature neural cells. When preconditioned with loss of function of tumor-suppressor genes, such as TP53 mutation, MET oncogenic activation such as HGF-autocrine activation may transform NSCs or progenitor cells into GSCs, which ultimately lead to glioma initiation. While these tumors are primarily driven by MET RTK signaling and are sensitive to MET-targeting therapy, the extensive clinical literature indicating the accumulation of many other molecular abnormalities in clinically evident infiltrating gliomas (especially glioblastoma) likely requires consideration of combining MET-targeting therapy with other targeted therapies to overcome resistance and recurrence.
Authorship Statement

Y.Q., A.M., J.K., B.T., A.Q., C.L., B.S., L.K., and Q.X. performed the majority of the experiments and analysis. B.T. constructed the Sleeping Beauty plasmids. J.P. performed pathological staining and J.B.S. reviewed slides. K.T. provided material. D.A.L. and Q.X. performed study design and data interpretation. Q.X. wrote the paper with feedback from Y.Q., K.T., J.B.S., and D.A.L. All authors read and approved the manuscript.

References

1. Xie Q, Mittal S, Berens ME. Targeting adaptive glioblastoma: an overview of proliferation and invasion. *Neuro Oncol*. 2014;16(12):1575–1584.
2. Johnson DR, Omuro AMP, Ravelo A, et al. Overall survival in patients with glioblastoma before and after bevacizumab approval. *Curr Med Res Opin*. 2018;34(5):813–820.
3. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2008–2012. *Neuro Oncol*. 2015;17(Suppl 4):iv1–iv62.
4. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*. 2006;9(3):157–173.
5. Verhaak RG, Hoadley KA, Purdom E, et al.; Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17(1):98–110.
6. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chromoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006;5:67.
7. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760.
8. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2006;444(7120):756–760.
9. HGF/MET overexpression induces GBM in mice. *Neuro-Oncology Advances*. Singapore: Springer Nature; 2018:129–154.
10. Rath P, Lal B, Ajala O, et al. In vivo c-met pathway inhibition depletes human glioma xenografts of tumor-propagating stem-like cells. *Transl Oncol*. 2013;6(2):104–111.
11. Boccaccio C, Comoglio PM. The MET oncoprotein in glioblastoma stem cells: implications as a diagnostic marker and a therapeutic target. *Cancer Res*. 2013;73(11):3193–3199.
12. Prados MD, Byron SA, Tran NL, et al. Toward precision medicine in glioblastoma: the promise and the challenges. *Neuro Oncol*. 2015;17(8):1051–1063.
13. Wiesner S, Decker S, Larson J, et al. De novo induction of genetically engineered brain tumors in mice using plasmid DNA. *Cancer Res*. 2009;69(2):431–439.
14. Xie Q, Bradley R, Kang L, et al. Hepatocyte growth factor (HGF) autocrine activation predicts sensitivity to MET inhibition in glioblastoma. *Proc Natl Acad Sci U S A*. 2012;109(2):570–575.
15. Johnson J, Ascieto ML, Mittal S, et al. Genomic profiling of a hepatocyte growth-factor-dependent signature for MET-targeted therapy in glioblastoma. *J Transl Med*. 2015;13:306.
16. Rath P, Lal B, Ajala O, et al. In vivo c-met pathway inhibition depletes human glioma xenografts of tumor-propagating stem-like cells. *Transl Oncol*. 2013;6(2):104–111.
17. Boccaccio C, Comoglio PM. The MET oncoprotein in glioblastoma stem cells: implications as a diagnostic marker and a therapeutic target. *Cancer Res*. 2013;73(11):3193–3199.
18. Prados MD, Byron SA, Tran NL, et al. Toward precision medicine in glioblastoma: the promise and the challenges. *Neuro Oncol*. 2015;17(8):1051–1063.
19. Wiesner S, Decker S, Larson J, et al. De novo induction of genetically engineered brain tumors in mice using plasmid DNA. *Cancer Res*. 2009;69(2):431–439.
20. Xie Q, Bradley R, Kang L, et al. Hepatocyte growth factor (HGF) autocrine activation predicts sensitivity to MET inhibition in glioblastoma. *Proc Natl Acad Sci U S A*. 2012;109(2):570–575.
21. Johnson J, Ascieto ML, Mittal S, et al. Genomic profiling of a hepatocyte growth-factor-dependent signature for MET-targeted therapy in glioblastoma. *J Transl Med*. 2015;13:306.
22. Wiesner SM, Decker SA, Larson JD, et al. De novo induction of genetically engineered brain tumors in mice using plasmid DNA. *Cancer Res*. 2009;69(2):431–439.
23. Moriarity BS, Rahrmann EP, Beckmann DA, et al. Simple and efficient methods for enrichment and isolation of endonuclease modified cells. *PLoS One*. 2014;9(5):e96114.
24. Shinomiya N, Gao CF, Xie Q, et al. RNA interference reveals that ligand-independent met activity is required for tumor cell signaling and survival. *Cancer Res*. 2004;64(21):7962–7970.
25. Kou J, Musich PR, Staal B, et al. Differential responses of MET activations to MET kinase inhibitor and neutralizing antibody. *J Transl Med*. 2018;16(1):253.
26. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131(6):803–820.
27. Lee J, Kottiariova S, Kottiaryov Y, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell*. 2006;9(5):391–403.
28. Theuwke D, Kou J, Fulmer M, Xie Q. The HGF/MET signaling and therapeutics in cancer. In: Shinomiya N, Kataoka H, Xie Q, eds. *Regulation of Signal Transduction in Human Cell Research*. Singapore: Springer Nature; 2018:155–182.
29. Shinomiya N, Xie Q, Vande Woude G. Met activation and carcinogenesis. In: Shinomiya N, Kataoka H, Xie Q, eds. *Regulation of Signal Transduction in Human Cell Research*. Singapore: Springer Nature; 2018:129–154.
30. Sakata H, Takayama H, Sharp R, Rubin JS, Merlino G, LaRochelle WJ. Hepatocyte growth factor/scatter factor overexpression induces growth, abnormal development, and tumor formation in transgenic mouse livers. *Cell Growth Differ*. 1996;7(11):1513–1523.
31. Xie Q, Su Y, Dykema K, et al. Overexpression of HGF promotes HBV-induced hepatocellular carcinoma progression and is an effective indicator for met-targeting therapy. *Genes Cancer*. 2013;4(7–8):247–260.
32. Gravel CR, DeGroot JD, Su Y, et al. Met induces diverse mammary carcinomas in mice and is associated with human basal breast cancer. *Proc Natl Acad Sci U S A*. 2009;106(31):12909–12914.
33. Tward AD, Jones KD, Yant S, et al. Distinct pathways of genomic progression to benign and malignant tumors of the liver. *Proc Natl Acad Sci U S A*. 2007;104(37):14771–14776.
34. Wang R, Ferrell LD, Fazouri S, Maher JJ, Bishop JM. Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice. *J Cell Biol*. 2001;153(5):1023–1034.
35. Koochekpour S, Jeffers M, Rulong S, et al. Met and hepatocyte growth factor/scatter factor expression in human gliomas. Cancer Res. 1997;57(23):5391–5398.
36. Tang Y, Yu P, Cheng L. Current progress in the derivation and therapeutic application of neural stem cells. Cell Death Dis. 2017;8(10):e3108.
37. Zhang P, Lathia JD, Flavahan WA, Rich JN, Mattson MP. Squelching glioblastoma stem cells by targeting REST for proteasomal degradation. Trends Neurosci. 2009;32(11):559–565.
38. Zheng H, Ying H, Yan H, et al. Pten and p53 converge on c-Myc to control differentiation, self-renewal, and transformation of normal and neoplastic stem cells in glioblastoma. Cold Spring Harb Symp Quant Biol. 2008;73:427–437.
39. Zheng H, Ying H, Yan H, et al. p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. Nature. 2008;455(7216):1129–1133.
40. Gao M, Liu D. CRISPR/Cas9-based Pten knock-out and Sleeping Beauty transposon-mediated Nras knock-in induces hepatocellular carcinoma and hepatic lipid accumulation in mice. Cancer Biol Ther. 2017;18(7):505–512.
41. Wen PY, Schiff D, Cloughesy TF, et al. A phase II study evaluating the efficacy and safety of AMG 102 (riptumumab) in patients with recurrent glioblastoma. Neuro Oncol. 2011;13(4):437–446.
42. Cloughesy T, Finocchiaro G, Belda-Iniesta C, et al. Randomized, double-blind, placebo-controlled, multicenter phase II study of onartuzumab plus bevacizumab versus placebo plus bevacizumab in patients with recurrent glioblastoma: efficacy, safety, and hepatocyte growth factor and O6-methylguanine-DNA methyltransferase biomarker analyses. J Clin Oncol. 2017;35(3):343–351.