Recurrent MET fusion genes represent a drug target in pediatric glioblastoma

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Pediatric glioblastoma is one of the most common and most deadly brain tumors in childhood. Using an integrative genetic analysis of 53 pediatric glioblastomas and five in vitro model systems, we identified previously unidentified gene fusions involving the MET oncogene in ~10% of cases. These MET fusions activated mitogen-activated protein kinase (MAPK) signaling and, in cooperation with lesions compromising cell cycle regulation, induced aggressive glial tumors in vivo. MET inhibitors suppressed MET tumor growth in xenograft models. Finally, we treated a pediatric patient bearing a MET-fusion-expressing glioblastoma with the targeted inhibitor crizotinib. This therapy led to substantial tumor shrinkage and associated relief of symptoms, but new treatment-resistant lesions appeared, indicating that combination therapies are likely necessary to achieve a durable clinical response.

Pediatric glioblastoma is a deadly childhood tumor that is characterized by a complex genomic landscape and profound heterogeneity1–3. Certain canonical pathways, however, such as the MAPK or phosphatidylinositol 3-kinase (PI3K) pathways, are frequently deregulated. The identification of recurrent mutations of histone H3-encoding genes (most commonly H3F3A) and chromatin modifiers such as ATRX also suggests that epigenetic deregulation has a prominent role in pediatric glioblastoma4,5. Standard treatment is based on nonselective radio- and chemotherapy, with marginal clinical benefit. No molecularly targeted therapy is currently used. Patient outcomes remain dismal, and new targets for individualized or molecularly stratified therapies are desperately needed.

We performed whole-genome sequencing of tumor and blood DNA from 53 samples (Supplementary Table 1), as well as from five pediatric glioblastoma cell lines covering the whole spectrum of recurrent H3.3 mutations, in the context of the International Cancer Genome Consortium (ICGC) PedBrain Tumor project (Fig. 1 and Supplementary Tables 2 and 3). Genome-wide DNA methylation analysis on our cohort revealed that, in addition to the previously described epigenetic subgroups6, five of the tumors molecularly resembled pleomorphic xanthoastrocytoma (PXA)7, a less aggressive brain tumor with heterogeneous appearance3 (Supplementary Fig. 1). The full tumor cohort is described in Supplementary Tables 1 and 2. Two of five PXA-like tumors carried lesions that are typical of PXAs (BRAF V600E and deletion of CDKN2A and CDKN2B)8,9. Moreover, one potential PXA (ICGC_GBM34) in an infant was found to harbor a gene fusion between Ets variant 6 (ETV6) and the neurotrophin receptor type 2 (NTRK2) gene. This fusion, and others involving NTRK-family genes, have been identified in 40% of infant glioblastomas10 and some lower-grade gliomas11,12. Analogous to the highly oncogenic FGFR1-TACC1 and FGFR3-TACC3 fusions in glioblastoma13, we found a previously unknown fusion of fibroblast growth factor receptor 2 (FGFR2) with CAP-GLY-domain-containing linker protein 2 (CLIP2) in a molecular PXA primary-relapse pair (ICGC_GBM19&50) (Supplementary Table 4). Three pediatric glioblastomas whose DNA methylation profiles clustered together with normal brain samples (suggesting high

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normal cell content) showed notably lower-than-expected mutant allele frequencies (Supplementary Fig. 2).

A full overview of genetic alterations is provided in Figure 1 and Supplementary Table 3. The most commonly altered pathway was cell cycle regulation, with mutations in TP53 or PPM1D, or homozygous deletion of CDKN2A and CDKN2B, being identified in 83% of all samples. We also detected numerous genetic lesions that are likely to result in aberrantly activated receptor tyrosine kinase (RTK)-PI3K-MAPK signaling, including activating mutations of RTKs (for example, EGFR) or downstream proteins (for example, NRAS, KRAS, BRAF and PIK3CA) and high-level gene amplifications of EGFR, PDGFR/KIT and MET (Fig. 1). ICGC_GBM17 harbored a previously unknown splice site mutation in intron 12 of NTRK3, likely resulting in an amino-terminally truncated protein that maintained the catalytically active NTRK3 kinase domain (Supplementary Fig. 3). Other common features included a high frequency of marked structural rearrangements (chromothripsis)\(^1\), hypermutated tumors (before any adjuvant therapy, and often associated with germline mismatch repair deficiency\(^2\)) and alterations in telomere maintenance. Analysis of five primary-recurrent tumor pairs revealed that known somatic driver events were typically shared between primary and recurrent lesions, with the exception of ICGC_GBM11 and ICGC_GBM71 (which were clinically reported as two anatomically distinct lesions; Supplementary Fig. 4). Notably, different BCOR gene mutations were detected in the primary tumor (ICGC_GBM4, BCOR F206fs) and both recurrent tumors (ICGC_GBM36, BCOR wild type and ICGC_GBM49, BCOR V1112fs) of one patient (Supplementary Fig. 4).

RNA sequencing revealed fusion transcripts that were the results of structural rearrangements in most samples (27 of 42, 64%; Supplementary Table 4). These often involved known cancer-associated genes, such as FGFR2, NTRK2 and PIK3R2. The most frequently affected gene was MET, which encodes an oncogenic tyrosine kinase. We detected two previously unknown fusions of MET that retained the carboxy-terminal kinase domain. In ICGC_GBM1, the MET kinase domain was fused to TRK-fused gene (TFG), which was previously described to form chimeric proteins with other RTKs, such as NTRK1 in papillary thyroid carcinoma or ALK in anaplastic large-cell lymphoma\(^3,4\). The pediatric glioblastoma cell line SJ-G2 (ICGC_GBM41) harbored a CLIP2-MET fusion (Fig. 2a). For the first time in the pediatric setting, to the best of our knowledge, we also identified two primary pediatric glioblastomas with a PTPRZ1-MET fusion\(^5\). In this variant, expression of full-length MET was
driven from the highly active PTPRZ1 promoter, leading to MET overexpression (Supplementary Fig. 5a,b). In two additional tumors without RNA-seq data, copy number analysis suggested the presence of a PTPRZ1-MET fusion, which we subsequently confirmed by PCR (ICGC_GBM43 and ICGC_GBM71). Dual-color fluorescence in situ hybridization detected the PTPRZ1-MET fusion in tumor sections of ICGC_GBM11, ICGC_GBM15 and ICGC_GBM71 (Supplementary Fig. 5c). ICGC_GBM43 (H3.3 G34R) was the only histone H3.3-mutant tumor harboring a MET fusion and amplification. Notably, all pediatric glioblastomas bearing a MET fusion were found to have impaired cell cycle regulation as a result of TP53 mutation or homozygous deletion of the CDKN2A and CDKN2B locus (Fig. 1). DNA methylation and gene expression did not show MET-fusion-bearing pediatric glioblastomas as clustering separately. None of the tumors expressed the short variant of MET lacking exons 7 and 8, which was recently described in 6% of high-grade gliomas.

Overexpression of hemagglutinin (HA)-tagged TFG-MET or PTPRZ1-MET in HEK293T cells resulted in the phosphorylation of tyrosines Y1234 and Y1235 in the activation loop of the kinase domain (Fig. 2b). Moreover, this overexpression induced strong activation of MAPK signaling, as indicated by substantially elevated pERK levels (Fig. 2b), and resulted in cell rounding and detachment (Supplementary Fig. 6a). Notably, the amino-terminally truncated fusions showed much higher downstream activity than the full-length, PTPRZ1-driven variant (Fig. 2b).

To further characterize induced transcriptional changes in a model that better mimics the presumed origins of pediatric glioblastoma, we generated expression profiles of TFG-MET-overexpressing normal human astrocytes. Transduced cells displayed a phenotypic change that was indicative of transformation (Supplementary Fig. 6a,b), as well as altered expression of multiple factors involved in MAPK signaling (for example, MAP2K3, MAP2K6 and DUSP14) and downstream transcription factors (for example, FOS and JUN) (Supplementary Fig. 6c and Supplementary Table 5).

TFG-MET-overexpressing cells were subsequently treated with the MET inhibitors foretinib, SGX523 or crizotinib, which abrogated MET-fusion-induced MAPK activation (Fig. 2b,c). In CLIP2-MET-expressing SJ-G2 cells, foretinib reduced cell viability in a concentration-dependent manner, resulting in a half-maximum inhibition concentration (IC50) of 0.8 μM. In three pediatric glioblastoma cell lines without MET fusion, the IC50 was substantially higher (2–13.5 μM; Supplementary Fig. 6d). Although the methylated MGMT promoter may suggest sensitivity to alkylating therapy, temozolomide (TM) or carmustine (BCNU)-driven variant (ICGC_GBM15 and ICGC_GBM71). Dual-color fluorescence in situ hybridization detected the PTPRZ1-MET fusion in tumor sections of ICGC_GBM11, ICGC_GBM15 and ICGC_GBM71 (Supplementary Fig. 5c). ICGC_GBM43 (H3.3 G34R) was the only histone H3.3-mutant tumor harboring a MET fusion and amplification.

Figure 2 Oncogenic MET fusions. (a) Schematics of wild-type MET and fusion proteins identified in pediatric glioblastomas. The MET polypeptide precursor is composed of the extracellular domain (ED), the transmembrane domain (TM), the juxtamembrane domain (JM), the kinase domain (KD) and the carboxy-terminal domain (CT). Tyrosine phosphorylation (pY) and Erk (pErk) activation (for example, MAP2K3, MAP2K6 and DUSP14) and downstream transcription factors (for example, FOS and JUN) (Supplementary Fig. 6c and Supplementary Table 5).

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phosphorylated MET and Erk (Fig. 3c). A contribution of off-target viral mutagenesis to tumorigenesis in this system can be excluded on the basis of previously published data21 and our own unpublished observations (J. Gronych).

As a result of the rapid growth of RCAS-driven tumors and the lack of reliable techniques for oral drug delivery in infant mice (younger than postnatal day 10), we used luciferase-labeled RCAS-TFG-MET-driven
mouse tumor cells that were allografted into the striatum of 6–8-week-old immunocompromised mice to assess pharmacological MET inhibition in vivo. We split the mice into two groups with similar distributions of luminescence intensity 1 week after transplantation and subsequently subjected them to a 60 mg per kg foretinib versus vehicle 1 d on/1 d off treatment regimen. Foretinib treatment significantly...
decelerated MET-fusion-driven tumor growth (P < 0.0001; Supplementary Fig. 6g), leading to prolonged overall survival (Fig. 3d).

To confirm our results, we established a xenograft model using luciferase-labeled SJ-G2 cells that endogenously harbor the CLIP2-MET fusion, which we transplanted intracranially into C.B-17 SCID immunocompromised mice. Tumor growth was monitored once a week by intravitral bioluminescence imaging (Fig. 3e). Although all of the vehicle-treated animals showed a clear increase in luminescence signal, the signal in foretinib-treated animals stagnated or even decreased under treatment. All foretinib-treated animals were still alive at the end of the 2-week treatment, whereas six out of seven of the untreated animals had died (median survival: vehicle, 17 d; foretinib, 26 d; P = 0.0001; Fig. 3f). Western blot analysis showed reduced ERK phosphorylation in tumor tissue of the foretinib-treated animals, indicating that target inhibition was successful and downstream signaling was reduced (Fig. 3g).

Finally, we were able to translate these findings into clinical application in the pilot phase of the INFORM personalized oncology program22 (German Clinical Trials Register ID: DRKS00007623). Whole-exome, low-coverage whole-genome (for copy number annotation) and RNA sequencing were performed on a recurrent lesion from an 8-year-old male patient treated 3 years previously for a group 3 medulloblastoma (INF_51_XT1). This revealed the presence of a PTPRZ1-MET fusion in the recurrent tumor, and subsequent molecular and histological review indicated that this lesion was in fact a cerebellar glioblastoma (that is, most likely a radiation-induced secondary glioblastoma) (Fig. 4a). On this basis, the patient received treatment with crizotinib (a Food and Drug Administration–approved kinase inhibitor with activity against MET), which has been shown to induce tumor regression of a MET-amplified glioblastoma23. Magnetic resonance imaging (MRI) evaluation 2 months after treatment initiation revealed a partial response of the primary lesion with concomitant relief of symptoms. However, several new treatment-resistant lesions were also observed. Rapid progression of those lesions ultimately resulted in the death of the patient (Fig. 4b). No autopsy material was available for studying the resistance mechanism.

Oncogenic activation of MET signaling is found in numerous human malignancies, including cancer of the hematopoietic system, carcinomas, sarcomas and glioblastomas24,25. In pediatric glioblastomas, MET gene amplification has been described in about 3–7% of tumors10,26, whereas we found that MET fusions were present in up to 10% of cases. Notably, the structural alterations in ICGC_GBM11 and ICGC_GBM71, representing two lesions from one patient, suggest that distinct PTPRZ1-MET fusions arose separately in each tumor. High clonality of the PTPRZ1-MET fusion, as indicated by our fluorescence in situ hybridization data, further underlines the likely tumor-initiating character of this fusion, which seems to rely on extremely high expression of full-length MET driven by the PTPRZ1 promoter. The amino-terminally truncated versions, however, represent cytosolic, constitutively active forms of MET, which escape normal downregulation27. The latter are analogous to the oncogenic TPR-MET fusion that was originally identified in mutagen-treated osteosarcoma cells28. A recent screen of data of the Cancer Genome Atlas interrogating multiple tumor types (http://54.84.12.177/PanCanFusV2/)29 revealed a small number of MET fusions, including one TFG-MET fusion, in breast, lung and thyroid cancer, suggesting that MET rearrangements may have a broader role across tumor entities.

Our data suggest that MET-fusion-induced tumorigenesis is dependent on additional genetic lesions affecting cell cycle regulation. All seven pediatric glioblastomas bearing a MET fusion harbored mutations of TP53 or deletions of CDKN2A and CDKN2B. Accordingly, overexpression of TFG-MET in neural progenitor cells induced aggressive glial brain tumors in Cdkn2a- or Trp53-deficient mice, but not in wild-type animals.

**Figure 4** Translation of MET inhibitor treatment into a clinical setting. (a) Immunohistochemical staining for hematoxylin and eosin, GFAP and pMET (Y1234/Y1235) of the primary medulloblastoma (top) and the PTPRZ1-MET-expressing pediatric glioblastoma (bottom) in patient INF_51_XT1. Scale bars represent 100 µm. (b) All images represent axial T1-weighted MRI scans with contrast enhancement of patient INF_51_XT1 at baseline and at indicated time points after initiation of treatment. Crizotinib was administered orally at 2 × 250 mg/d (equivalent to 2 × 280 mg/m², published recommended phase II dose33) for 11 weeks, with a pause from days 13 to 16. Marked tumor shrinkage corresponding to a partial response at the site of the main lesion was observed (arrows). New lesions also developed during the course of treatment (arrowheads). Scale bars represent 5 cm.
On the basis of our preclinical allograft and xenograft data, pharmacological MET inhibition was immediately translated into clinical application by treating a child with a PTPRZ1-MET fusion-driven pediatric glioblastoma with a MET inhibitor, leading to a relief of symptoms over a period of 2 months and substantial volume reduction of the primary tumor. Unfortunately, new lesions that developed rapidly under crizotinib monotherapy ultimately led to a fatal outcome. Given that acquired resistance to MET inhibition is a well-known challenge in the treatment of numerous cancers, combinatorial inhibition of multiple RTKs, as recently described in diffuse intrinsic pontine gliomas, might represent a promising therapeutic option.

In conclusion, our results highlight a new recurrent mechanism of tumorigenesis in pediatric glioblastoma and underline the importance of individualized molecular diagnosis for cancer patients as a basis for optimal personalized therapy. Our data also provide strong rationale for the systematic analysis of MET inhibitors in future pediatric glioblastoma clinical trials.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Short-read sequencing and methylation data are available at the European Genome-phenome Archive (http://www.ebi.ac.uk/ega/), hosted by the European Bioinformatics Institute under the accession number EGASA0001001139.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS


COMPETING FINANCIAL INTERESTS

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Dynamic reprogramming of signaling upon met inhibition

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ONLINE METHODS

Patient and tumor samples. Informed consent from all patients as well as an ethical vote (Ethics Committee of the Medical Faculty of Heidelberg) was obtained according to the ICGC guidelines. Tumor tissues were subjected to neuropathological review to confirm histology and tumor cell content.

DNA sequencing. Paired-end library preparation was conducted using Illumina v2 protocols. Genomic DNA (~1 µg) was fragmented to an insert size of ~300 bp with a Covaris device, and size selection was performed using agarose gel excision. Deep sequencing was carried out with Illumina HiSeq 2000 instruments.

RNA sequencing. RNA integrity was evaluated by using a Bioanalyzer 2100 instrument (Agilent). Stranded paired-end libraries were prepared from 1 µg RNA using the Ribo-Zero Gold Kit (Epipcent). One library per lane was sequenced on a HiSeq 2000 instrument with 2 × 51 bp reads. Gene fusion events were detected by RNA-seq reads mapping to the human NCBI37/hg19 reference assembly using SOAPFuse43 and TopHat2-Fusion35. High-confidence events were retained after filtering of common artifacts and visual inspection of RNA-seq coverages on fused exons. Fusion transcripts were annotated on the basis of the Ensembl gene annotation (v70). For fusion transcript validation, 50 ng of total RNAs were reverse transcribed, and fused transcripts were amplified using the dART 1-Step RT-PCR Kit (EURx® #E0803-02) using primers located upstream and downstream of the transcript breakpoints. RT-PCR products were separated and visualized on 2.5% TBE-agarose gel, excised and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research). Capillary Sanger sequencing of 30 ng RT–PCR product was performed with 15 pmol primer (Eurofins MWG Operon).

Mapping and analysis. According to the ICGC The Cancer Genome Atlas Pan Cancer Whole Genome workflow, reads were mapped to the 1000 genomes phase 2 assembly of the human reference genome GRCh37 (NCBI build 37.1, downloaded from ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/refer-ence/phase2-reference_assembly_sequence) using BWA36 version 0.7.8 mem with option -T 0. The biobambam package37 was used to sort the output and to mark PCR duplicates during merging of the per-lane BAM files.

High-level copy number gains were identified by read depth plots and custom Perl scripts.

For detection of single nucleotide variants (SNVs), we used our in-house SNV detection pipeline based on SAMtools mpileup and bcftools36 version 0.1.19 with parameter adjustments to allow calling of somatic variants with low allele frequency as described previously39 and heuristic filtering, also as described previously11.

Short insertions and deletions (indels) were identified with Platypus version 0.7.4 (ref. 39) by providing the tumor and control BAM files. To be of high confidence, somatic calls (control genotype 0/0) are required to have the Platypus filter flag PASS or pass custom filters allowing for low variant frequency using a similar scoring scheme as for SNVs. In detail, we discard candidates with the badReads flag, with allelleBias or strandBias if the variant allele frequency is less than 10%, and indels that have more than two of the remaining flags. In addition, combinations of Platypus non-PASS filter flags, bad quality values, low genotype quality, very low variant counts in the tumor and presence of variant reads in the control were not tolerated.

All mutations are annotated with ANNOVAR40 version November 2014, with the Gencode version 19 gene model. Additional information is included by overlapping the genomic positions with dbSNP version 141, 1,000 genomes phase 1 integrated calls 20101123 and COSMIC version 66. Only somatic, high confidence SNVs and indels were considered for further analysis. We then extracted nonisolated coding SNVs, SNVs at splice sites and indels that fall into a coding gene or splice site. For defining potential somatic SNVs in the cell lines, we discard SNVs and indels known from 1,000 genomes or with the dbSNP 141 COMMON = 1 flag.

RNA-seq reads were mapped with STAR version 2.3.0e41 using an index of the 1,000 genomes reference sequence with Gencode version 17 transcript annotations. The output was converted to sorted BAM with SAMtools and duplicates were marked with Picard version tools (http://broadinstitute.github.io/picard/, version 1.90).

Expression levels were determined per gene and sample as reads per kilobase of exon model per million reads (RPKM). As the gene model, RefSeq was used. For each gene, overlapping annotated exons from all transcript variants were merged into nonredundant exon units with a custom Perl script. Nonduplicate reads with mapping quality above zero were counted for all exon units with coverageBed from the BEDTools package32. The read counts were summarized per gene, and then divided by the combined length of its exon units (in kilobases) and the total number of reads (in millions) counted in total by coverageBed.

SNVs and indels were annotated with RNA information by generating a pileup of the DNA variant position in the RNA BAM file with SAMTools. The respective genes, as well as genes in high-level copy number gains, were assigned their expression values.

Chromothripsis was scored in accordance with the criteria outlined previously43.

DNA methylation profiling. For genome-wide assessment of DNA methylation, ICGC_GBM samples (n = 51) were analyzed using the Illumina HumanMethylation450 BeadChip according to the manufacturer’s instructions at the DKFZ Genomics and Proteomics Core Facility. In addition, 450k DNA methylation data of 64 reference pediatric glioblastomas described previously46, 16 normal brain samples as well as seven PAXas was included. DNA methylation probes were filtered as previously described47. For unsupervised hierarchical clustering, we selected the 2,052 most variably methylated probes across the data set (s.d. > 0.3). Samples were clustered using 1-Pearson correlation coefficient as the distance measure and average linkage (x-axis). Methylation probes were reordered by hierarchical clustering using Euclidean distance and average linkage (y axis).

Gene expression profiling and classification. Differentially expressed genes (t test: P < 0.01) were identified by comparing TFG-MET-overexpressing and empty vector transduced normal human astrocytes (NHAs). Classification of tumor samples studied on the Affymetrix U133 Plus2.0 expression array was performed as described previously48 using the 840 gene Cancer Genome Atlas signature44.

In vitro studies of MET-fusion-expressing HEK293T and SJ-G2 cells. HEK293T and human SJ-G2 glioblastoma cells (authors’ long-term stocks) were cultured in high glucose Dulbecco’s Modified Eagle’s Medium (DMEM) (Life Technologies) supplemented with 10% FCS (GIBCO) and penicillin/streptomycin (GIBCO) at 37 °C and 5% CO2. Cells were trypsinized upon reaching a confluency of 80%. Foretinib (Selleck Chemicals) and temozolomide (University Hospital Pharmacy Heidelberg) was dissolved in DMSO and stored at −20 °C. 3 h before cell harvest, cell culture medium, including inhibitors, was replaced. Cell lines were checked for genotype and for mycoplasma contamination.

Coding sequences of wild-type MET, PTPRZ1-MET or TFG-MET were cloned from tumor cDNA into the pcDNA3.1 vector (Life Technologies) introducing a HA tag. HEK293T cells were transfected using TransTet-LTI transfection reagent (Mirus).

To determine the IC50 of foretinib and temozolomide, pediatric glioblastoma cell lines were seeded in a 96-well format (opaque-walled) and incubated for 24 h in DMEM supplemented with 10% FCS (GIBCO) and penicillin/streptomycin (GIBCO) at 37 °C and 5% CO2. Subsequently, cells were treated with the indicated concentration of foretinib and temozolomide for an additional 24 h before replacing the drug-containing medium and incubated for another 24 h at 37 °C and 5% CO2. Cell viability was determined in triplicates by using the CellTiter-Glo luminescence cell-viability assay (Promega) according to the manufacturer’s instructions using a Mithras LB 940 Microplate Reader. Quantification of anchorage-independent growth of SG-G2 cells was determined by using the CytoSelect 96-well cell transformation assay (Cell Bios) according to the manufacturer’s instructions after the indicated time period. Foretinib- or DMSO-containing DMEM medium was replaced every 24 h.

Transduction of NHAs. The bicistronic retroviral vector pMIBerry has been described previously49. To generate a pMIBerry vector encoding C-terminally HA-tagged TFG-MET, this cDNA was amplified from pLVX/TFG-MET-puro.
Western blotting. Protein extract of cell pellets were generated by using RIPA buffer (Sigma-Aldrich) including the Halt Phosphatase Inhibitor cocktail (Thermo Scientific). Electrophoretic separation of protein samples was performed using 4–12% gradient NuPAGE Bis-Tris Precast Gels (Life Technologies) followed by protein transfer to a polyvinylidene fluoride membrane using Phusion polymerase (Finnzymes) and the oligonucleotides XhoITFG–METtwd 5′- AATTCTCGAGATGACCGACGCTTGGACTAATGACGCTAGG AGCTAATCT-3′ and BamHITFG–METrev 5′- TTAAGAGATCTCTAGACT AGGCGTACGAGCACGCTATAAGGATAGT-3′ using the standard protocol supplied by the manufacturer. PCR amplicons were gel purified, digested with XhoI and BamHI and subcloned into XhoI/BamHI linearized pMIBerry. Sanger sequencing confirmed the TFG–MET cDNA sequence.

Fluorescence in situ hybridization (FISH). Dual-color interphase fluorescence in situ hybridization was performed on FFPE-embedded tissue sections using a PTPTRZ1 (RP11-207K20; green) and MET (RP11-95120; red) specific probe. For each tumor, 200 interphase nuclei were analyzed microscopically.

Animal studies. All animal experiments were conducted in accordance with legal regulations and approved by the regional council (Regierungspräsidium Tübingen; G-4/11, G-238/12, G-163/14). Mice were housed in IVC caging in the Center for Preclinical Research of the DKFZ and monitored daily for the presence of tumor-related symptoms. Sample sizes were chosen to minimize the number of animals required to get significant results.

Xenograft and preclinical studies. Cells derived from Ntv-a; Cdkn2afl/fl–; Ptenfl/fl animals injected with RCAS–TFG-MET virus or SJ-G2 tumor cells were labeled with luciferase using pGF lentivirus and subsequently GFP-positive cells were FACs sorted. 500,000 SJ-G2 cells or 100,000 TFG-MET-RCAS cells were transplanted into the striatum of 6–8-week-old female C.B-17 SCID (coordinates 2.5 mm lat., 1 mm caud., 3 mm ventr. relative to bregma; animals obtained from Charles River or Janvier Labs, respectively). Animals received pre-emptive Carprofen analgesia and were anesthetized with Isoflurane. Post-surgically, analgesia was continued with Carprofen. For luciferase imaging, animals were injected with 100 μl Luciferin solution (15 mg/ml, Promega) and imaged using an IVIS100 or IVIS Lumina luminescence imager with an exposure time of 5 min. For the treatment studies, Foretinib was dissolved in DMSO and then diluted in 5 mg/ml hydroxypropyl methylcellulose/0.05% SDS. Animals were randomized to treatment or control strata according to their luminescence signals. 60 mg/kg Foretinib or vehicle was administered nonblinded by oral gavage every other day starting at day 7 after surgery. Kaplan–Meier analysis was done using GraphPad Prism, and statistical significance was calculated using a log-rank test.

MRI. MRI was undertaken on a 9.4T horizontal bore NMR scanner (BioSpec 94/20 USR, Bruker BioSpin GmbH, Ettlingen, Germany) with the CryoProbe head coil. A 15 slice T1-weighted RARE (rapid acquisition with relaxation enhancement) sequence, after an intraperitoneal injection of 100 μl of a 1:10 dilution of Omniscan (0.5 mmol/ml, GE Healthcare Buchler GmbH), was acquired. The MRI parameters were as follows: TR/TE = 1,000/6 ms, matrix = 200 × 150, resolution = 0.1 × 0.1 mm, slice thickness/gap = 0.3/0.3 mm, NA = 2; RARE factor = 1; total acquisition time = 5 min.

RCAS-based tumor model. For tumor induction using RCAS-based somatic gene transfer, the HA-tagged TFG-MET fusion was PCR amplified and cloned into the RCASBP(A) backbone using Clal and NotI restriction sites. Virus production was done in DF-1 chicken fibroblasts by transfection using FuGene HD (Promega) according to the manufacturer’s protocol. Ntv-a; Ntv-a; Cdkn2afl/fl–; Ptenfl/fl, Ntv-a; Trp53fl/fl or or Ntv-a; Trp53fl/fl pups were injected at postnatal day 0 with 100,000 virus-producing cells into the left cerebral hemisphere using a Hamilton syringe.