Missense Mutation of the *MET* Gene Detected in Human Glioma

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Multiple mechanisms, such as gene mutations, amplifications, and rearrangements, as well as perturbed mitogen and receptor function, are likely to contribute to glioma formation. The *MET* (also known as *c-met*) proto-oncogene located at 7q31-34 has been shown to be amplified in human gliomas, and activating mutations within the tyrosine kinase domain of *MET* have been causally related to tumorigenesis in hereditary papillary renal cell carcinoma. To elucidate the role of *MET* gene in glioma formation, sporadic gliomas from 11 patients were examined for *MET* gene mutations and allelic duplications or deletions by polyermase chain reaction-single strand conformational polymorphism analysis and fluorescence *in situ* hybridization. Three of 11 sporadic gliomas showed a deletion of one copy of the *MET* gene, and a specific *MET* gene missense mutation in the remaining gene copy was detected in one of those tumors. The corresponding sequence in non-tumor DNA was normal in all cases. Three of 11 sporadic gliomas showed duplication of one copy of the *MET* gene, but none of them contained mutations. One tumor showed *MET* amplification without mutation. Three showed neither allelic change nor mutation. These data suggest that somatic *MET* gene mutation may play a role in the development of a subgroup of sporadic gliomas. However, *MET* mutations appear to be absent in the majority of sporadic gliomas.

Gliomas are the most frequent primary tumors of the central nervous system in humans (1). The most consistent chromosomal abnormalities in aggressive gliomas are gains of chromosome 7 or losses of chromosome 10, which are present in 80 to 90% of cases (2–4). As has been demonstrated in a number of other cancers, multiple oncogenes, such as *MET*, *SAS*, *N-MYC*, and *c-MYC*, have been implicated in glioma formation (5–8).

Increased MET-hepatocyte growth factor/scatter factor signaling, as well as *MET* amplifications, overexpression, or activation by point mutation, have been demonstrated in a number of tumors, both *in vivo* and *in vitro* (9–11). Di Renzo *et al.* (12) have shown elevated levels of *MET* mRNA in glioma tissues, and hepatocyte growth factor/scatter factor has been shown to stimulate growth, migration, and invasiveness of cultured glioma cell lines (8). *MET* gene amplification has been detected in some glioblastomas (GBMs) (13). Increased expression of *MET* has been shown to correlate with astrocytoma progression (14) and may be related to greater biologic aggressiveness found with GBMs (15).

Recently, mutations within the tyrosine kinase domain of *MET* have been implicated in tumorigenesis of both sporadic and hereditary papillary renal cell carcinoma (HPRC) (11, 16). Because a strong correlation between the enzymatic and biologic transforming activity of mutations in the tyrosine kinase domain of *MET* has been shown to relate tumorigenesis to the level of *MET* activation, we screened a variety of human gliomas for mutations in exons 15–19 of the *MET* gene by polyermase chain reaction (PCR)-based single strand conformational polymorphism (SSCP) analysis and

examined allelic deletion or duplication by fluorescence *in situ* hybridization (FISH) and PCR-based allelic quantitative analysis.

MATERIALS AND METHODS

Patients and Specimens

Surgical specimens were obtained from patients who underwent craniotomy and tumor resection in the Surgical Neurology Branch at the National Institutes of Health, as part of institutional review board-approved protocols, for which informed consent was obtained. At the time of tumor resection, specimens were frozen at $-70°$ C until later use.

Nine-micron thick sections were placed on glass slides and air dried. These slides were compared with sections that were stained with hematoxylin and eosin to guide the area of dissection. Histologic fields of interest also were compared with the original hematoxylin and eosin pathologic slides to ensure uniformity of tumor selection.

Specimens were suspended in 30 μL of DNA extraction solution, which contained 50 mM Tris-HCl, 1 mM ethylenediamine-tetraacetic acid, 1.0% Tween-20, and 0.1 mg/mL proteinase K (pH 8.0). This mixture was incubated overnight at 37° C; proteinase K was inactivated by heating at 95° C for 10 min (17).

SSCP Analysis for MET Mutations

PCR-SSCP analysis was performed by the modification of the method of Orita *et al.* (18) with intronic sequences designed to amplify exons 15 to 19 from tumor and normal DNA. Each PCR sample contained 1.0 μL of template DNA, 10 pmol of each primer, 20 nmol each of dGTP, dATP, dTTP, dCTP, 15 mM $MgCl_2$, 0.1 unit of *Taq* DNA polymerase (Perkin-Elmer, Foster City, CA), 0.05 μL of [^{32}P] dCTP (6000 Ci/mmol), and 1 μL of 10× buffer in a total volume of 10 μL. PCR was performed for 35 cycles: denaturation at 94° C for 40 sec, annealing at 52 to 54° C for 40 sec, and extension at 72° C for 40 sec. The final extension was continued for 10 min. The labeled amplified DNA (2 μL) was mixed with an equal volume of formamide loading dye (95% formamide, 20 mM ethylenediamine-tetraacetic acid, 0.05% bromophenol blue, and 0.05% xylene cyanol). The samples were denatured for 5 min at 95° C and loaded onto a SSCP gel (MDE; FMC BioProducts, Rockland, ME) with 10% glycerol. Samples were electrophoresed at 8 W at room temperature overnight. After electrophoresis, the gels were transferred to 3-mm Whatman paper and dried, and autoradiography was performed with Kodak X-OMAT film (Eastman Kodak, Rochester, NY). After detection of a mutant allele, DNA was extracted

from the excised aberrant band of the SSCP gel by overnight incubation in 50 μL of distilled water at room temperature and subsequently reamplified by PCR. The PCR products were directly sequenced (Cycle Sequencing Kit, Perkin-Elmer), and normal and tumor DNA sequences were compared.

FISH

FISH was performed with touch preparations of frozen brain tumor specimens. The probe for chromosome 7 was cosmid c182b3, which contains a portion of the *MET* proto-oncogene (a gift of S. W. Scherer, Hospital for Sick Children, Toronto, Canada). A chromosome 7 specific α-satellite probe (Oncor, Gaithersburg, MD), biotin-labeled, was used as a control.

In situ hybridization and detection procedures were performed according to the technique described by Pack *et al.* (19). In brief, cosmid DNA was labeled by nick translation (Boehringer Mannheim, Indianapolis, IN) with digoxigenin-11-dUTP and ethanol precipitated in the presence of 50× herring sperm DNA (Boehringer Mannheim, Indianapolis, IN) and 50× Cot-1 human DNA (Boehringer Mannheim, Indianapolis, IN). The DNA pellet was re-suspended in Hybrisol solution [50% deionized formamide/10% dextran sulfate/2× standard saline citrate (SSC)] to a final concentration of 25 ng/mL. Slides were denatured in 70% formamide/2× SSC at 72° C for 2 min, dehydrated in a cold ($-20°$ C) ethanol series of 70, 80, 90, and 100% for 2 min each, then air-dried. Probes were denatured at 78° C for 10 min, then incubated for 30 min at 37° C for pre-annealing. A total of 250 μg of DNA probe was applied to the slide. Chromosome 7-specific, α-satellite repetitive DNA was denatured separately and mixed with the cosmid probe just before hybridization. Overnight hybridization was performed in a humidified chamber at 37° C.

Post-hybridization washes were at 45° C in 50% formamide/2× SSC (5 min × 3), 1× SSC (5 min × 2), and 0.1× SSC (5 min × 2). Detection was performed using avidin-FITC (Vector Laboratories, Burlingame, CA) and anti-digoxigenin rhodamine (Boehringer Mannheim, Indianapolis, IN) (40 min at 37° C) followed by washing in 4× SSC/0.1% Tween-20 at 45° C and counterstaining with DAPI-antifade (0.25 mg/mL).

Hybridization signals were scored using a Zeiss Axiophot epifluorescence microscope (Zeiss, Thornwood, NY), and two-color images were captured on a Photometrics charge-coupled device camera (Photometrics, Tucson, AZ) using IP Lab image software (Scanalitics Inc., Fairfax, VA). At least 100 interphases with strong hybridization signals were scored. Normal brain tissue control showed <6% of cells with one *MET* gene signal. Alterations of *MET* copy numbers were scored when present in at least 30% of cells.
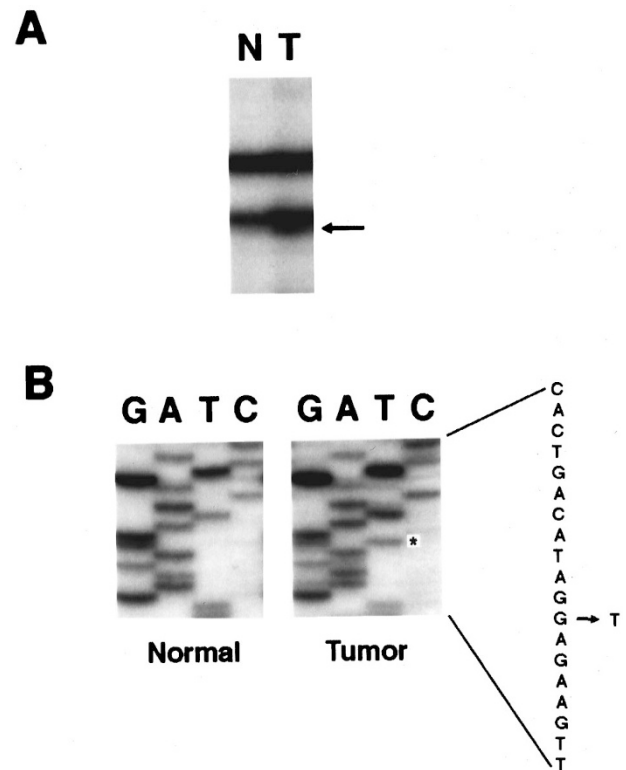
RESULTS

Brain tissue containing normal and neoplastic cells was obtained at the time of surgery, snap-frozen, and frozen sections were cut. Selective tissue microdissection was used to obtain discrete populations of normal and neoplastic cells, which were analyzed for mutations of the *MET* proto-oncogene. There were seven patients with GBM multiforme, three with low-grade oligodendrogliomas, two with anaplastic astrocytomas, and one with anaplastic oligodendroglioma (Table 1).

We analyzed exons 15, 16, 17, 18, and 19, the most commonly affected regions of the *MET* gene, for mutations via SSCP and sequencing. One of 11 cases (Case 3) exhibited an aberrant band in SSCP (Fig. 1A). On sequencing the aberrant band (exon 17), a GGA to GTA mutation, resulting in glycine to valine substitution in codon 1137 was confirmed (Fig. 1B). The mutation was not detected in normal DNA from the same patient (Fig. 1B).

We also analyzed all the tumors for the changes in the number of *MET* allelic copies in gliomas by FISH using cosmid c182b3 containing *MET* gene. Three of 11 sporadic gliomas showed a deletion of one copy of the *MET* gene (Cases 3, 5, and 10), and a specific *MET* gene mutation in the remaining gene copy was detected in one of those tumors (Case 3). Three of 11 sporadic gliomas showed a duplication of one copy of the *MET* gene but none of them showed mutation (Cases 6, 7, and 8). One glioma (Case 11) showed *MET* amplification exhibiting 20 to 100 copies of *MET* signal in each affected cell. Three of 11 did not show either allelic change or mutation (Cases 1, 2, and 4). One of 11 (Case 9) was not analyzed because of the poor condition of the cell morphology in the touch preparation (Table 1).

DISCUSSION

Multiple mechanisms, such as gene mutations, amplifications, and rearrangements, as well as perturbed mitogen and receptor function, are likely to contribute to development of gliomas. Mutations in *p53* (20), *Rb* (21), *p16* (22), *MMAC1* (23) and *DCC* (24) genes have been correlated with the initiation and progression of human gliomas. Amplification and/or activation of oncogenes, such as the genes for the epidermal growth factor receptor (25), transforming growth factor-α (26), *MET* (8), N-*myc* (5), *c-myc* (6), and *gli* (27) are thought to contribute to glioma formation by elevated expression of proteins that participate in mitogenic signalling pathways.


FIGURE 1. Detection of somatic mutation for *MET* gene in a sporadic glioma of the brain. **A**, the SSCP gel shows an aberrant band (**arrow**) at the lower allele in the tumor (T) compared with the normal (N). **B**, the sequencing gel shows missense mutation GGA → GTA (G1137V) (**asterisk**) in a sporadic glioma.

TABLE 1. *MET* **Gene Mutation and Allelic Changes in 11 Gliomas**

Case	Sex/Age	Diagnosis	Mutation	Allelic Change in Gliomas by FISH
1	F/21	LG oligo	—	—
2	F/50	LG oligo	—	—
3	M/42	LG oligo/Anaplastic oligo	G1137V (exon 17)	Deletion
4	M/27	Anaplastic astro	—	—
5	F/24	Anaplastic astro/GBM	—	Deletion
6	M/57	GBM	—	Trisomy
7	M/51	GBM	—	Trisomy
8	F/54	GBM	—	Trisomy
9	M/67	GBM	—	NA
10	M/44	GBM	—	Deletion
11	F/54	GBM	—	Amplification[a]

LG Oligo, low-grade oligodendroglioma; astro, astrocytoma; GBM, glioblastoma; NA, not analyzed.
[a] Amplification: 20 to 100 copies of *c-met* signal.
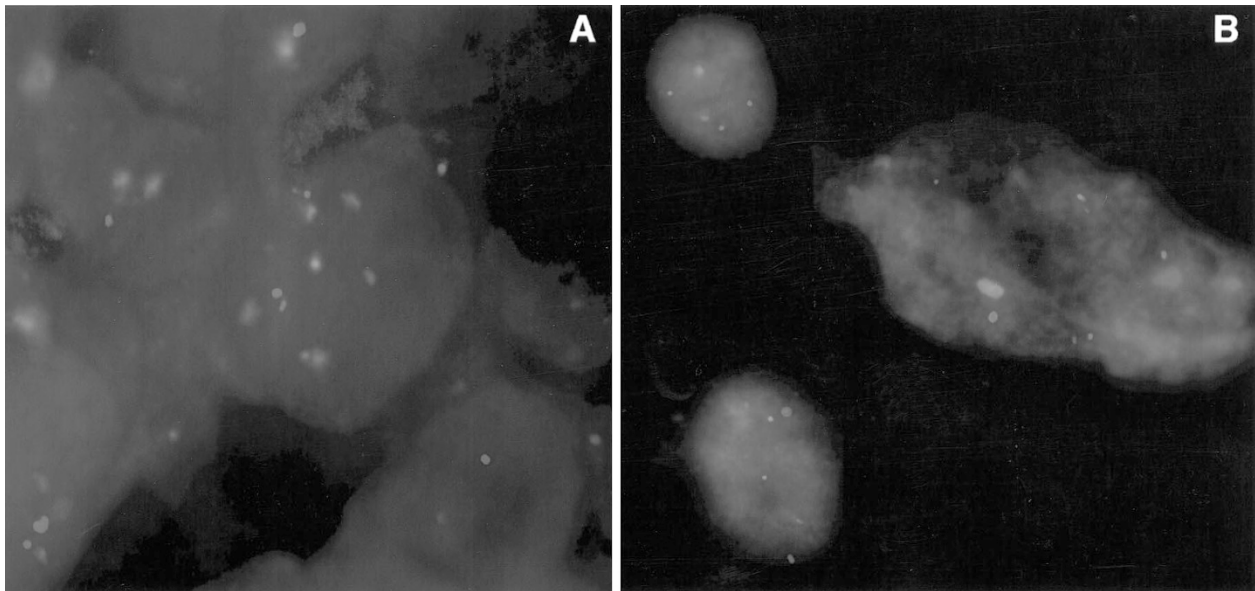
FIGURE 2. Representative results of FISH in sporadic gliomas. **Green signal**, α-satellite centrometric marker; **red signal**, chromosome 7-specific cosmid probe containing *MET* gene (cosmid c182b3). **A** and **B**, interphase touch preparations of sporadic gliomas. **A**, allelic deletion of one copy of the *MET* gene detected in a sporadic glioma. **B**, sporadic glioma showing three copies of chromosome 7.

MET tyrosine kinase receptor, the product of the *c-met* proto-oncogene, has been implicated in a variety of human cancers. Chromosome 7 abnormalities in HPRC has shown that mutations of the *MET* gene (particularly in exons 17, 18, and 19) and nonrandom duplication of the mutant *MET* allele in the trisomy 7 found in HPRC appears to be a mechanism in human tumorigenesis related to *MET* (11, 16, 28).

Our data showed a missense mutation in 1 of 11 cases. The missense mutation in exon 17 (Case 3) is one of the most commonly mutated regions of the tyrosine kinase domain of *MET*. Mutation studies on known mutations identified in papillary renal carcinoma exhibited increased levels of tyrosine phosphorylation and enhanced kinase activity in response to exogenous ligands when compared with wild-type *MET* (29). Moreover, NIH 3T3 cells expressing mutant *MET* molecules formed foci *in vitro* and were tumorigenic in nude mice.

The tumors showing duplication involving the *MET* locus were high-grade tumors (*i.e.*, GBMs) in our series. Although three cases with duplication of chromosome 7 did not reveal a mutant allele, *MET* amplification may be still associated with glioma formation, as shown in previous studies (8, 10).

The deletion of the *MET* locus on chromosome 7, as demonstrated by FISH data in the patient with a missense mutation (Case 3), may suggest the exclusive expression of the mutant allele by overrepresentation of the mutant allele (30) or loss of suppression effect of the wild-type allele, as shown in defective serine/threonine kinase gene (31).

Frequent allelic loss on chromosome 7q31 has been described for multiple cancers, including breast (32), gastric (33), and prostate (32) cancers. Likewise, allelic deletion without mutation of *MET* in our series may suggest the presence of a novel putative tumor suppressor gene at chromosome 7 near the *MET* locus, which may be involved in glioma formation.

This study represents the first investigation of the role of *MET* mutations and deletions in glioma tumorigenesis. Our results demonstrate that, although activating mutations are not common (1 of 11 tumors in this series), deletion, aneuploidy, and amplification of the *MET* gene are common (7 of 11 tumors) in tumor cells and may be a contributing factor in glioma formation, progression, or both. Furthermore, PCR-based allelic quantification, combined with FISH analysis, permits more sensitive analysis of the manner in which heterogenous alterations of the *MET* proto-oncogene may influence tumorigenesis.

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