Molecular Genetic Analysis of Giant Cell Glioblastomas

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Glioblastomas (GBMs) are a heterogeneous group of tumors. Recently, distinct molecular genetic alterations have been linked to subgroups of patients with GBM. Giant cell (gc)GBMs are a rare variant of GBM characterized by a marked preponderance of multinucleated giant cells. Several reports have associated this entity with a more favorable prognosis than the majority of GBMs. To evaluate whether gcGBM may also represent a genetically defined subgroup of GBM, we analyzed a series of 19 gcGBMs for mutations in the TP53 gene for amplification of the EGFR and CDK4 genes and for homozygous deletions in the CDKN2A (p16/MTS1) gene. Seventeen of nineteen gcGBMs carried TP53 mutations whereas EGFR and CDK4 gene amplification was seen in only one tumor each and homozygous deletion of CDKN2A was not observed at all. The strikingly high incidence of TP53 mutations and the relative absence of other genetic alterations groups gcGBM together with a previously recognized molecular genetic variant of GBM (type 1 GBM). It is tempting to speculate that the better prognosis of gcGBM patients may result from the low incidence of EGFR amplification and CDKN2A deletion, changes known for their growth-promoting potential. (Am J Pathol 1997, 151:853–857)

Glioblastoma (GBM) is a clinically, histologically, and genetically heterogeneous entity. Clinically, GBMs have been subdivided into primary GBM (arising de novo) or secondary GBM (arising from malignant progression of lower-grade astrocytoma). Interestingly, secondary GBMs occur in younger patients and have a slightly better prognosis than primary GBMs.1 Histologically, GBM is heterogeneous, and a number of distinct variants have been defined, such as gliosarcoma or giant cell (gc)GBM.2 Increasing knowledge of the molecular genetic mechanisms underlying GBM formation has added another level for subdividing these tumors. Frequent molecular genetic alterations in GBM include mutations of the TP53 and RB genes, amplification of the EGFR, MDM2, and CDK4 genes, homozygous deletion of the CDKN2 (p16/MTS1) gene, and loss of heterozygosity (LOH) on chromosomes 10, 19q, and 22q.3–12 Some of these lesions occur in a nonrandom distribution, allowing molecular genetic differentiation of GBM variants.13 One subset is characterized by LOH 17p coinciding with TP53 mutations and absence of EGFR gene amplification; this variant has been termed type 1 GBM.14 Interestingly, those clinicopathological GBM subgroups with somewhat more favorable prognoses (young patients and patients with secondary GBM) fall within the genetic definition of type 1 GBM.15,16 On the other hand, GBMs with EGFR amplification and without LOH 17p have been termed type 2 GBM and usually occur in elder patients.14

One curious clinicopathological variant of GBM is giant cell glioblastoma gcGBM. The morphological hallmark of gcGBM is a prominent component of multinucleated giant tumor cells,2 hence the old term monstroszellular sarcoma for these tumors.17 In addition, gcGBMs often have well demarcated, surgically discernible borders and occur in a younger patient population. Several studies have shown that gcGBM is associated with a somewhat better prognosis than typical GBM.18,19 The associations of gcGBM with younger patients and with a more favorable prognosis suggest similarities with type 1 GBM. To evaluate whether gcGBM is genetically related to type 1 GBM, we examined a series of 19 gcGBMs for mutations in the TP53 gene, for amplification of the EGFR and CDK4 genes, and for homozygous deletions of the CDKN2A gene.

Materials and Methods

Tissue Samples

Tumor and blood samples were obtained from 19 patients with GBM treated at the University Hospitals of Bonn and Düsseldorf, Germany, and the University Hos...

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pital Zürich, Switzerland. All tumors were classified and graded according to the guidelines of the World Health Organization by two neuropathologists (A. von Deimling and G. Reifenberger). Genomic DNA from leukocytes and tumor tissues was extracted by standard methods.

**Analysis of the EGFR and CDK4 Genes**

EGFR and CDK4 amplification was analyzed as previously described. In brief, parts of the EGFR or CDK4 gene were co-amplified with fragments of the interferon-γ (IFNG) or the adenine phosphoribosyltransferase (APRT) gene, respectively. One primer of each pair was labeled with 5-carboxy-fluorescein (Applied Biosystems, Foster City, CA) at the 5’ end. Differential polymerase chain reaction (PCR) was performed in a final volume of 10 μL containing 10 ng of DNA, 50 mmol/L KCl, 2.5 mmol/L MgCl₂, 10 mmol/L Tris/HCl, pH 8.3, 200 μmol/L of each dNTP, 0.1% gelatin, 20 pmol of each of the primers, and 0.25 U of Taq polymerase (Gibco BRL, Gaithersburg, MD). Initial denaturation at 95°C for 3 minutes was followed by 26 cycles on a thermocycler (Perkin Elmer 9600). Denaturation was facilitated at 95°C for 30 seconds, annealing at 59°C for 60 seconds, and extension at 72°C for 40 seconds. A final extension of 10 minutes at 72°C was used. Fluorescent PCR products were separated on 6% acrylamide gels and analyzed on a semiautomated DNA sequencer (Applied Biosystems model 373A). A quantitative analysis of the signal intensity was carried out with the Genescan program (Applied Biosystems Genescan version 1.2.1). EGFR/IFNG and CDK4/APRT ratios were determined in a series of lymphocyte DNAs. Ratios of more than twice the mean of EGFR/IFNG or CDK4/APRT plus 3 SD (in lymphocyte DNAs) were taken as evidence for gene amplification.

**Analysis of the CDKN2A Gene**

The PCR assay for the detection of CDKN2A deletions has been described previously. In brief, a 174-bp fragment of exon 1b in the CDKN2A gene was co-amplified with a 134-bp fragment of the APRT gene. One primer of each pair was labeled with 5-carboxy-fluorescein (Applied Biosystems) at the 5’ end. Differential PCR was performed in a final volume of 10 μL containing 10 ng of DNA, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 10 mmol/L Tris/HCl, pH 8.3, 200 μmol/L of each dNTP, 0.1% gelatin, 5 pmol of each primer, and 0.25 U of Taq polymerase (Gibco BRL). Initial denaturation at 94°C for 3 minutes was followed by 28 cycles on a thermocycler (Biometra, Goettingen, Germany). These included denaturation at 94°C for 40 seconds, annealing at 56°C for 55 seconds, and extension at 72°C for 55 seconds. A final extension step of 10 minutes at 72°C was used. Fluorescent PCR products were separated on a 6% polyacrylamide gel and analyzed on a semiautomated DNA sequencer (Applied Biosystems model 373A). A quantitative analysis of the signal intensity was carried out with the Genescan program (Applied Biosystems Genescan version 1.2.1).

**Analysis of the TP53 Gene**

Exons 5 to 8 of the TP53 gene were analyzed by single-stranded conformational polymorphism (SSCP) and direct sequencing. PCR products were electrophoretically separated on long acrylamide gels followed by silver staining as previously described. Variant SSCP bands were excised, re-amplified, and sequenced bidirectionally. Sequence analysis was carried out with a semiautomated sequencer (Applied Biosystems model 373A).

**Results**

Histopathological examination revealed malignant astrocytomas with multinucleated giant cells as well as undifferentiated cells. Necrosis was present in all cases. None of the tumors contained noteworthy areas of sarcomatous or xanthomatous morphology. Characteristic examples are shown in Figure 1. Ten of the nineteen patients were female, and nine were male. The average age of the patients was 46.2 years; excluding the two pediatric patients, the mean age was 50.5 years. None of the patients had a history of a preceding low-grade glioma.

Exons 5 to 8 of the TP53 gene were examined for mutations. Previous studies have shown that approximately 90% of TP53 mutations occur within this section of the gene. SSCP analysis and consecutive bidirectional sequence analysis revealed a total of 17 mutations among the 19 (90%) gcGBMs included in this study. Representative examples are shown in Figure 1. All mutations resulted in alterations on the protein level. We detected 10 transitions, 5 transversions, and 2 deletions of 3 bp each. Eight of these mutations have been reported in gliomas, three mutations occurred in codons previously described with other mutations, and six mutations were novel. Patients 7594 and 7596 carried the identical mutation verified by a second round of independent experiments with DNA from a previous unused stock. In 10 cases, constitutional DNA from peripheral blood leukocytes was available; none of these cases showed TP53 mutations in the germline. Nine of the constitutional DNA samples were informative for one or more polymorphic markers on chromosome 17p; five of nine cases, each of which had a TP53 mutation, exhibited loss of the wild-type allele. Mutations and resulting changes are summarized in Table 1.

The EGFR/INFG ratio for control DNAs was 1.15 ± 0.29. Eighteen gcGBMs presented with ratios compatible with normal gene dosage. The EGFR/INFG ratio for patient 7578 was 8.0, arguing for an amplification of the EGFR gene. The CDK4/APRT ratio for control DNAs was 1.1 ± 0.18. Eighteen gcGBMs presented with ratios compatible with normal gene dosage. The CDK4/APRT ratio for patient 7902 was 9.4, supporting amplification of the CDK4 gene. The CDKN2A/APRT ratio for control DNAs was 1.05 ± 0.17. Eighteen gcGBMs showed CDKN2A/APRT ratios within the limits of normal gene dosage. Patient 2268 exhibited a CDKN2A/APRT ratio of 0.6, indicating the loss of one CDKN2A allele.
Discussion

The search for clinical and histopathological criteria of prognostic relevance in GBM patients has led to the identification of several parameters. For instance, young age has been established as one of the most important independent favorable prognostic factors. Secondary GBM, i.e., disease evolving from lower-grade tumors, may also be associated with longer survival. Histopathological findings indicating a somewhat better prognosis include the presence of abundant multinucleated giant cells, indeed, most proliferation studies of GBMs have shown that large, multinucleated cells are in general not proliferating. Another favorable prognostic factor may be intratumor lymphocytic infiltration, but this observation has been questioned by other investigators. Patients with gcGBM often combine three of these clinicopathologically favorable prognostic factors: multinucleated cells, younger age, and frequent lymphocytic tumor infiltration. Such observations raise the possibility that gcGBM has specific underlying molecular genetic changes.

Our data show that gcGBM is almost always molecular genetic type 1 GBM, with frequent TP53 mutations but rare EGFR amplification and CDKN2A deletion. In this series, 17/19 (90%) tumors exhibited TP53 mutations compared with an incidence of 30% reported in unselected GBM. With 9/17 TP53 alterations being novel mutations, the mutational spectrum differs from a previous report compiling these lesions in brain tumors. The high incidence of TP53 mutations in gcGBM may also provide an explanation for the abundance of giant multinucleated cells. Due to the function of p53 protein in centrosome duplication and in p53-dependent growth arrest, lack of p53 protein may result in genomic instability with pathological mitotic spindles and multiple and bizarre nuclei. For instance, giant polyploid cells develop in the testes of p53-deficient mice, perhaps secondary to a lack of p53-mediated surveillance of recombination intermediates in spermatocytes. In gliomas, aneuploidy is associated with TP53 mutations. Furthermore, recent studies have shown that etynitrosoureainduced brain tumors in p53-deficient mice are almost all...

Figure 1. Histopathology and molecular data of three patients with gcGBM. All patients exhibit the typical morphological aspect of gcGBM (upper panels). H&E. magnification, x200. The corresponding mutations in the TP53 gene are shown in the lower panels. Sense strands are shown for patients 2268 and 5396, and antisense strand is shown for patient 7578.
GBMs with bizarre tumor cells, reminiscent of human gcGBM; this contrasts strikingly with ethylnitrosourea induction of brain tumors in normal mice, which results in a wide variety of tumors, many characterized by small cells. In light of these observations, it is tempting to speculate that the prominent giant cells in gcGBM may be directly related to inactivation of p53.

Only 1 of 19 tumors exhibited EGFR amplification, far less than the incidence of 30 to 40% reported in unselected GBMs. Likewise, only 1 of 19 tumors showed amplification of the CDK4 gene, somewhat less than the 11 to 15% of GBMs noted to have CDK4 amplification in unselected GBMs. Furthermore, homozygous deletion of the CDKN2A gene was not detected in any gcGBM, whereas this alteration is found in 30 to 60% of unselected GBMs. As EGFR amplification and CDKN2A deletions are cardinal features of type 2 GBM, our data demonstrate that gcGBMs are distinct from type 2 GBM.

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M, male; F, female; n, no TP53 alteration detected in exons 5 to 8; xxx, three deleted nucleotides.

*Patient with hemizygous deletion of CDKN2A.
†Patient with EGFR amplification.
‡Patient with CDK4 amplification.

Table 1. Molecular Genetic Findings in 19 Patients with Giant Cell Glioblastoma

Acknowledgment

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References