The role of a single nucleotide polymorphism of MDM2 in glioblastoma multiforme

Laboratory investigation

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Object. Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, with a 5-year survival rate of < 5%. Aberrant function of TP53 is common in GBM. Although mutational inactivation of p53 is found in many cases, there remain tumors in which genetic alterations of p53 are absent. Negative regulators of the TP53 pathway such as MDM2, which directly inhibits TP53 expression and activity, may influence the pathogenesis of GBM. To understand its potential function in gliomagenesis, the authors analyzed a novel single nucleotide polymorphism (SNP) in the MDM2 promoter that enhances MDM2 expression.

Methods. The investigators isolated DNA from 98 patients with GBM and 102 healthy, cancer-free controls. A polymerase chain reaction analysis was performed to determine the MDM2 SNP309 genotype by using distinct primer pairs for the wild-type (T) and mutant (G) alleles.

Results. The frequency of the mutant MDM2 polymorphism was found to be higher (p = 0.0092) in patients with GBM (54.6%) compared with healthy controls (41.2%); the TT and GG genotypes were more common in healthy controls and patients with GBM (p = 0.0004 and p = 0.02, respectively). Although there was no association between the MDM2 SNP309 and overall survival, the GG genotype was associated with development of GBM at a younger age in patients with tumors harboring wild-type p53, which may mitigate the effect of the MDM2 SNP.

Conclusions. Although the MDM2 SNP309 does not portend decreased survival, the increased incidence of the mutant G allele in patients with GBM and its influence on age of onset suggest a potential role in the molecular pathogenesis of GBM, and may be a therapeutic target. (DOI: 10.3171/JNS/2008/109/11/0842)

Abbreviations used in this paper: ANOVA = analysis of variance; GBM = glioblastoma multiforme; PCR = polymerase chain reaction; SD = standard deviation; SNP = single nucleotide polymorphism.
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sificantly enhances MDM2 protein levels (Fig. 1A). The MDM2 SNP309 resulted in higher levels of MDM2 RNA and protein, and attenuation of the TP53 pathway, with acceleration of tumor formation in patients with Li-Fraumeni cancer syndrome, especially the formation of sarcomas. Recently, it was shown that in cells homozygous for the GG polymorphism, a complex forms between MDM2 and TP53 that is transcriptionally inactive, and that supports the oncogenic potential of the MDM2 SNP309. Based on the strong evidence in support of TP53 pathway dysfunction in the pathogenesis of GBM, and the significant role MDM2 plays in regulating TP53 function, we studied the incidence and clinical effect of MDM2 SNP309 in a cohort of patients with GBM.

Methods

Tissue and Clinical Information

This study consisted of 98 patients with GBM and 102 healthy, cancer-free controls whose data were obtained from the Brain Tumor and Neuro-Oncology Center's Institutional Review Board–approved tissue bank. This bank contains detailed clinical, pathological, molecular genetic, treatment, and outcome data on prospectively collected samples.

Blood was collected prospectively in patients with histologically confirmed GBM, without prior knowledge of the molecular pathological features of the tumor. The patient cohort was 57.1% male, and the age (mean ± SD) at diagnosis was 58.7 ± 13.9 years (range 23–88 years). Control patients (that is, those without cancer) were 70.1% male; their age (mean ± SD) was 39.7 ± 10.9 years. Patient information, including age at diagnosis, sex, and survival time, was obtained in a retrospective and blind review. Patients with GBM were treated uniformly with surgery (gross-total or near-total [≥ 95%] resections), chemotherapy (carmustine or temozolomide), and radiation therapy (59.4 Gy). This study was approved by the Cleveland Clinic's Institutional Review Board.

Isolation of DNA

The DNA in blood from healthy (cancer-free) con-
controls and patients with GBM was purified using the Qiamp DNA Blood Extraction Kit (Qiagen).

**Genotyping Protocol**

The **MDM2** SNP309 genotype was amplified by PCR, in which 2 distinct primer pairs were used for each of the alleles. The primer pair used to amplify the 121-bp wild-type (T) allele was as follows: forward −GGATTTCCGAGGCTCTC and reverse −TCCGGACCTCCCGGCAGA. The pair used to amplify the 168-bp mutant allele (G) was as follows: forward −GGATTTCGGACGTGGCT and reverse −ATCCGGACCTCCCGGCAG (Fig. 1B). The PCR assays were performed using 100 ng of genomic DNA with FailSafe PCR System – Premix H (Epicentre Biotechnologies). The samples were subjected to 35 cycles of PCR (initial denaturation 94°C for 2 minutes, then 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds). The PCR products were resolved on a 2% agarose gel (Shelton Scientific), and then they were stained with ethidium bromide. The PCR assays were performed in duplicate, with 100% fidelity of PCR results between the first and second runs for all cases and controls.

**Mutational Analysis of p53**

Immunopositivity of TP53 was used as a marker of the presence of p53 mutations, as described by several other groups.18,21 Less than 5% staining represented the presence of wild-type p53 alleles; > 5% staining represented the presence of p53 mutation(s).

**Statistical Analysis**

Student t-tests, chi-square, ANOVA, and Kaplan–Meier analyses were performed using GraphPad Prism, version 4.03; Kaplan–Meier was used to estimate overall survival, and survival between groups was compared using the log-rank test. Baseline characteristics were compared between groups by using the Student t-test.

**Results**

The results of **MDM2** SNP309 genotyping in healthy controls and patients with GBM are summarized in Table 1. The difference between the 3 genotypes (TT vs TG vs GG) was significant when comparing patients with GBM to healthy controls (p = 0.0003, chi-square test). The TT genotype was more common in healthy controls (p = 0.0004; chi-square test) and the GG genotype was more common in patients with GBM. As shown in Table 2, the frequency of the G allele was also higher in patients with GBM than in healthy controls (p = 0.0092, chi-square test). Kaplan–Meier analysis showed no significant difference in overall survival among TT, TG, and GG genotypes in patients with GBM (mean survival 15.2 months for TT, 12.9 months for TG, and 19.8 months for GG; p = 0.3, log-rank test [Fig. 2]). The mean age at GBM diagnosis was similar in patients with the TT, TG, and GG genotypes (p = 0.9, ANOVA).

Next, patients were analyzed based on p53 mutational status. All patients with the GG genotype (6 evaluable cases) possessed wild-type TP53 expression levels. Among the patients with the TT genotype for whom we have information (44 individuals), we found no significant association between age at diagnosis and either p53 status (p = 0.09, Student t-test) or survival, as shown in Fig. 3 (p = 0.3, log-rank test).

Analysis of the patients without p53 mutations (27 evaluable cases) showed no difference in age at diagnosis for patients with wild-type p53, comparing all 3 genotypes (p = 0.1, ANOVA). However, patients with the GG genotype were diagnosed at a younger age than those with TT or TG (Fig. 4A). Patients with a GG genotype were significantly younger at the time of GBM diagnosis (mean age

### TABLE 1

**Genotypic frequency of MDM2 SNP309 in 102 healthy controls and 98 patients with GBM**

<table>
<thead>
<tr>
<th>Genotype Distribution</th>
<th>Patients w/ GBM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>TT</td>
<td>23 (22.6)</td>
</tr>
<tr>
<td>TG</td>
<td>74 (72.5)</td>
</tr>
<tr>
<td>GG</td>
<td>5 (4.9)</td>
</tr>
<tr>
<td>total patients</td>
<td>102</td>
</tr>
</tbody>
</table>

* Values are expressed as the mean ± SD.
† There was a significant difference in the distribution of genotypes between normal controls and patients with GBM: the TT genotype was more common in normal controls, and the GG genotype was more common in patients with GBM, according to the two-tailed chi-square test.

### TABLE 2

**Allelic frequency of MDM2 SNP309 in 204 healthy controls and 196 patients with GBM**

<table>
<thead>
<tr>
<th>Allelic Frequency</th>
<th>Normal Controls (%)</th>
<th>Patients w/ GBM (%)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>120 (58.8)</td>
<td>89 (45.4)</td>
<td>—</td>
</tr>
<tr>
<td>G</td>
<td>84 (41.2)</td>
<td>107 (54.6)</td>
<td>0.0092</td>
</tr>
<tr>
<td>total patients</td>
<td>204</td>
<td>196</td>
<td></td>
</tr>
</tbody>
</table>

* The mutant G allele was also found more frequently in patients with GBM than in normal controls.
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56.3 years; p = 0.02, Student t-test) compared with patients with homozygous wild-type (TT genotype) GBMs (mean age 73.3 years). Among patients with wild-type p53 there was no significant difference in survival associated with any MDM2 SNP309 genotype, as seen in Fig. 4B and C (p = 0.2, log-rank test), although there was a trend toward improved survival among patients with the GG genotype. The mean survival times were 13.3, 11.1, and 21 months among patients with the TT, TG, and GG genotypes, respectively.

Discussion

Although specific molecular abnormalities, such as epidermal growth factor receptor amplification, p53 mutations, chromosome 1p and 19q deletions, or alterations in p16 or PTEN are often considered in the assessment of GBM, a direct relationship between any one of these factors and the prognosis has yet to be determined unequivocally in a large, unbiased series of patients. At present, the most useful prognostic factors in GBM (age, Karnofsky performance score, and extent of tumor necrosis) are only surrogate biological markers. The incidence of SNPs, which tremendously increase the genetic diversity between individuals, adds another layer of complexity to the risk and course of GBM. The clinical implications of these polymorphisms in the pathogenesis of GBM have yet to be determined.

The functional consequence of the MDM2 SNP309 and its clinical significance was first described in 2004 in patients with Li-Fraumeni cancer syndrome. In these patients, who already carry a greatly increased risk of tumor formation due to germline mutations in p53, the MDM2 SNP309 GG genotype was associated with a younger age at tumor onset (principally for sarcomas) and an increased frequency of primary tumors over a lifetime. Amplification of MDM2 has previously been reported in almost 10–15% of primary GBMs. This molecular alteration has only been described in wild-type p53 tumors, suggesting that MDM2 overexpression is an alternative mechanism
of TP53 inactivation. However, when compared with patients with p53 mutated tumors, overexpression of MDM2 in GBM was found to be a negative prognostic marker, associated with a shorter survival time. The presence of MDM2 SNP309 and MDM2 amplification both increase MDM2 protein levels. This suggests that the MDM2 SNP309 may have clinical implications in the pathogenesis of GBM by a mechanism comparable to that of gene amplification.

Based on these findings, we investigated the incidence and effect of MDM2 SNP309 in patients with GBM. Our results show a significantly increased frequency of the MDM2 SNP309 G allele in patients with GBM when compared with healthy (cancer-free) controls. This suggests that MDM2 SNP309 may be a risk factor for GBM. Similar findings have been reported in other forms of cancer, including sporadic endometrial carcinoma, gastric carcinoma, and hepatocellular carcinoma associated with chronic hepatitis C. For example, in a study of 758 patients with esophageal squamous cell carcinoma, the GG genotype was associated with an increased risk of developing poorly differentiated or advanced tumors when compared with TT or TG genotypes, with a concomitant deleterious effect on survival. A meta-analysis of 14,770 cancer patients with a variety of solid tumors (none with gliomas) confirmed an increased risk of cancer, compared with controls, in patients with SNP309 G/G (odds ratio 1.17, confidence interval 1.04–1.33). In nearly every study reviewed (19 of 25 studies), p53 tumors were not seen or were uncommon. In our study, however, the presence of the G allele did not portend a worse prognosis based on overall survival, as has been reported for these other tumor types.

In the 27 patients with wild-type p53 tumors, however, we did find an association between MDM2 SNP309 and age at diagnosis of GBM. Those patients with the GG genotype were diagnosed at a significantly younger age than those with the TT genotype. These findings coincide with those presented by Bond et al. in their original description of MDM2 SNP309, and the reports of MDM2 SNP309 in other tumor types. In 2006, Menin et al. showed that, in patients with wild-type p53 tumors, even a single G allele was associated with a younger age at diagnosis of colorectal cancer. In our study, we did not identify a significant difference in age at diagnosis between patients with TT and TG genotypes, which suggests that extremely high levels of MDM2 are required (which would more likely be the consequence of two G alleles) for the abnormal MDM2 SNP309 allele to have a clinical effect to offset the relative advantage of a younger age at onset of GBM.

Based on previous reports that implicate TP53 pathway dysfunction as well as MDM2 overexpression as common molecular aberrations in GBM, it is biologically plausible that MDM2 SNP309 may play a role in tumor progression. At the molecular level it has been shown that in MDM2 SNP309 cells, MDM2 and TP53 form a transcriptionally inactive complex that associates with chromatin, thereby inhibiting the downstream effects of TP53 activation. This may be a potential mechanism of deregulation of the TP53 tumor-suppressor function in gliogenesis, because astrocytes may become susceptible to the development of an oncogenic phenotype in GBM; it may also help explain why TP53 is dysfunctional in the absence of sequence-verified mutations in some primary GBMs.

It is also important to note the numerous p53-independent interactions of MDM2. For example, it has been suggested in several studies that MDM2 interacts with p21, a universal inhibitor of cyclin kinases, which are necessary for cell cycle progression. Levels of p21 are thought to be directly enhanced by TP53; in this way, p21 serves as a downstream effector in the p53 tumor suppressor pathway and ultimately promotes cell cycle arrest. It has also been shown that in cells overexpressing MDM2, p21 levels are significantly decreased. Direct physical interaction between MDM2 and p21 promotes TP53-independent pro-oncogenic degradation of p21. Degradation of p21 resulting from increased MDM2 levels associated with MDM2 SNP309 may lead to uninhibited progression through the cell cycle and may promote tumorigenesis in GBM. The coexistence of the GG genotype with only wild-type p53 could indicate that normal TP53 function is important for TP53 regulators to have an observable effect on the integrity of the p53 pathway.

Although limited by the small number of some subpopulations within the larger group of 98 patients with GBM, all of whom were treated uniformly (with aggressive surgery, chemotherapy, and radiation), our results provide preliminary evidence that MDM2 SNP309 may be a risk factor in GBM. Although it does not appear that MDM2 SNP309 is a strong predictor of overall survival on its own, nevertheless, because of its relatively high incidence in the normal population, further studies in a larger series of patients are needed to determine more accurately its prognostic and therapeutic implications in patients with GBM. We did not observe a significant effect on overall survival associated with the presence of a G allele. However, the younger age at diagnosis seen in patients with the GG genotype and wild-type TP53 tumors (where younger age is a favorable factor in terms of response to therapy and overall survival in patients with GBM) may have masked the direct effect of MDM2 SNP309 on overall survival. Similarly, the influence of p53 mutations on prognosis in GBM may be positive or negative, depending on the age of the patient and many other molecular features of the tumor. Levine and colleagues (see Atwal et al.) have suggested that the evolutionarily recent development of MDM2 SNPs (including SNP309) represents a form of “selective pressure” on the wild-type TP53 pathway. Although we did not sequence p53, TP53 immunopositivity as a marker of p53 mutation is a clinically accepted model of assessing a patient’s p53 mutational (and, more importantly, its functional) status. Finally, the ability of MDM2 to promote TP53 degradation cannot be overlooked, although this was not possible to assess in the current study. Arva et al. recently demonstrated that although MDM2 and TP53 form a complex in cells that are homozygous for MDM2 SNP309, MDM2 does not result in the degradation of TP53. Thus, the relationship between TP53 immunopositivity and mutational status may be unaffected by MDM2 SNP309. This issue deserves further exploration.
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Conclusions

We have identified a potential role for the MDM2 SNP309 in the pathogenesis of GBM as a modifier of age at diagnosis in patients with wild-type p53 GBMs. Although larger studies are needed to confirm these results, similar findings have been reported in other tumor types, including soft-tissue sarcomas and colorectal carcinoma. Although considerable advances have been made in delineating the molecular mechanisms underlying gliomagenesis, improvements in patient outcomes are still possible. Thus, MDM2 SNP309 appears to be a low-penetration susceptibility marker for GBM in some patients. Understanding the influence of SNP variations in GBM has been relatively unexplored and may open doors to novel therapeutic interventions that more effectively alter the aggressive course of GBM, particularly for patients with decreased TP53 due to heightened MDM2.

Disclaimer

The authors do not report any conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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References


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