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Clinical Study

Role of the *GNAS1* T393C polymorphism in patients with glioblastoma multiforme

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ABSTRACT

The T393C polymorphism of the *GNAS1* locus, which encodes the Gαs protein, has recently been found to be associated with patient outcome in various malignancies. We investigated the association between *GNAS1* genotype and survival among patients suffering from glioblastoma multiforme (GBM). One hundred and sixty-two patients with GBM were retrospectively investigated. Inclusion criteria were availability of DNA and, for surviving patients, a follow-up of at least 24 months. The results were analysed based on clinical data, type of surgical intervention, adjuvant therapy, and 2-year survival. At the 2-year follow up, 79.6% of patients had died. Two-year survival rates were as follows: CC-homozygous patients, 15.8%; CT-heterozygous patients, 23.1%; and TT-homozygous patients, 18.2% ($p = 0.461$). Subgroup analysis revealed different 2-year survival rates in the group that underwent stereotactic biopsy, with 0% for CC-homozygous, 2.8% for CT-heterozygous, and 15.4% survival for TT-homozygous patients, but the differences were not statistically significant ($p = 0.229$). Our results indicate that there is no association between the *GNAS1* T393C polymorphism and 2-year survival among patients with GBM.

1. Introduction

Glioblastoma multiforme (GBM) is the most common and most malignant primary brain tumour, with incidences of 7.3 per 100 000 people per year in the USA[1,2] and 5–6 per 100 000 people per year in Europe.[3] GBM can be differentiated into primary and secondary GBM. The primary type is more common and arises de novo. Older patients are more prone to developing primary GBM, and the malignancy presents at diagnosis as a full-blown tumour, without clinical, radiological, or histopathological evidence of a precursor lesion.[4,5] Secondary GBM is found in younger patients and slowly progresses from a low-grade diffuse tumour (World Health Organization grade II) or anaplastic astrocytoma (World Health Organization grade III).[4–6]

Despite modern techniques, such as intraoperative tumour visualisation, complete resection of GBM remains impossible due to its infiltrating nature.[7,8] Therefore, the prognosis for patients remains poor and relapse is inevitable. The treatment of choice is surgical reduction, followed by radiotherapy with concomitant and adjuvant temozolomide chemotherapy. Median survival is 14.6 months.[9]

GBM originates sporadically, and only a few genetic factors are known to play a role.[10] The most important prognostic genetic marker is *MGMT* (*O*-6-methylguanine-DNA methyltransferase)-promoter methylation status. Patients with methylation of the *MGMT*-promoter have a better outcome after temozolomide chemotherapy, with a median survival of 21.7 months.[11,12] Clinical parameters that impact on prognosis in patients with GBM include young age, good Karnofsky performance status, and radical microsurgical resection.[13] Nevertheless, due to diffuse invasion, microvascular proliferation, necrosis and intense anti-apoptosis, GBM seems to be resistant to all cancer therapies and remains incurable.[14–18]

Common proteins associated with apoptosis and survival in GBM patients are G-proteins.[10,19,20] Heterotrimeric G-proteins communicate signals from a large family of receptors to several distinct intracellular signalling pathways, thereby controlling a broad range of biological processes including cell growth, transcription and motility.[21,22] The gene *GNAS1* encodes the ubiquitously expressed Gαs subunit of heterotrimeric G-proteins. In vitro experiments suggest that increased expression of Gαs is associated with enhanced apoptosis and that the second messenger cyclic AMP, which functions downstream of the G-proteins, plays a major role in pro-apoptotic processes.[23–27] We have recently shown that the common T393C single nucleotide polymorphism (SNP) of the *GNAS1* gene is significantly associated with clinical course among patients with various malignancies. Patients carrying the T-allele have prolonged survival compared with patients harbouring the C-allele. This association is possibly mediated by

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an increase in the apoptotic rate in patients harbouring the T-allele.[28–35] To date, the impact of the *GNAS1* T393C polymorphism on survival in GBM has not been investigated. Thus, the aim of the present study was to determine the influence of this polymorphism on prognosis in GBM.

2. Patients and methods

The study was conducted according to the Declaration of Helsinki and approved by the local ethics committee of the University Hospital of Essen. Informed consent was obtained from all patients.

2.1. Study population

The study population comprised 162 Caucasian patients who underwent surgery for primary GBM at the Department of Neurosurgery, University Hospital Essen, Germany, between 2002 and 2005. Inclusion criteria for this study were a histopathological diagnosis of GBM, the availability of tumour material, DNA extraction, and the potential for tracking follow up for at least 24 months. Tumour material was obtained by means of neurosurgical procedures such as gross total resection (GTR) or stereotactic biopsy (SB). The decision between GTR and SB was made according to clinical and neuroradiological parameters such as tumour localisation and extension, patient age, and Karnofsky performance status (KPS). GTR was defined as at least a 95% resection of the contrast-enhanced tumour manifestation, as confirmed by postoperative MRI within 72 hours following surgery.

2.2. DNA preparation

Several 10–20-μm tumour sections (overall weight not exceeding 25 mg) from routinely processed paraffin blocks were placed in a 1.5-mL microfuge tube. The samples were dewaxed in 1 mL xylene on a shaker incubator at 45 °C for 5 minutes. After centrifugation at full speed for 5 minutes at room temperature, the supernatant was removed. Pellets were washed in 1 mL ethanol and again centrifuged at full speed for 5 minutes. The supernatant was removed, and the open microfuge tubes were incubated at 45 °C for 2–5 minutes until the ethanol had evaporated. DNA was purified using the commercially available QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The tissue pellet was resuspended in 180 μL Buffer ATL/20 μL proteinase K and incubated overnight on a shaker incubator at 56 °C. Further processing of the samples was conducted in accordance with the recommendations of the supplier.

2.3. Genotype determination

Genotypes of the T393C polymorphism were determined using polymerase chain reaction (PCR) using the following primers: forward 5′-CTCCTAACTGACATGGTGCAA-3′ and reverse 5′-TAAGGCCACACAAGTCGGGGT-3′ (Eurofins MWG Operon, Ebersberg, Germany). After denaturation at 94 °C, 35 cycles of DNA amplification were performed using *Taq* PCR Mastermix (Eppendorf, Hamburg, Germany) at 94 °C for 45 seconds, 58 °C for 40 seconds, and 72 °C for 45 seconds. The 345-bp PCR products were digested using the restriction enzyme *Fok*I and analysed on a 2% agarose gel. O'GeneRuler was used as a size marker (MBI, Burlington, ON, Canada). The unrestricted products (345 bp) represented the TT genotype; the completely restricted products (259 and 86 bp) represented the CC genotype (Fig. 1). Re-genotyping 50 randomly selected samples to control for genotype failures revealed 100% concordance with the previously obtained results.

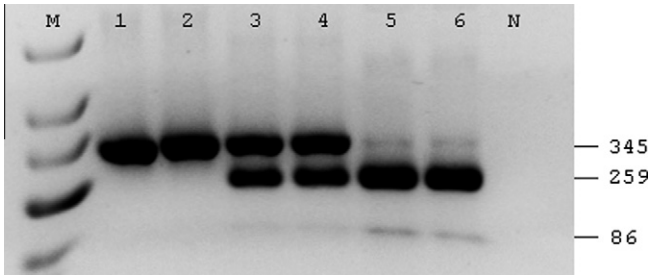

Fig. 1. Restriction analysis of *GNAS1* T393C polymerase chain reaction products. Equal amounts of digested PCR products (10 μL) were loaded on a 2% agarose gel. Lanes 1–6 show DNA from six individuals with glioblastoma multiforme. Lanes 1 and 2, TT genotype (345 bp); lanes 3 and 4, TC genotype (345 + 259 bp); lanes 5 and 6, CC genotype (259 + 86 bp); M, size marker; N, negative control.

2.4. MGMT-promoter methylation analysis

MGMT-promoter methylation was analysed using methylation-specific PCR after bisulfite modification, as previously reported.[36] In brief, 1 μg DNA was denatured using NaOH and modified with sodium bisulfite. DNA samples were then purified using the Wizard DNA Clean-Up System (Promega, Madison, WI, USA), treated with NaOH, precipitated with ethanol, and resuspended in water. Next, the DNA was subjected to PCR using the following reaction conditions: DNA was denatured at 95 °C, followed by 35 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 30 seconds, using primers specific for unmethylated and methylated DNA (Eurogentec, Liège, Belgium). The primer sequences used to detect unmethylated *MGMT*-promoter sequences were 5′-TGTGTTTTAGAATGTTTTGTGTTTTGAT-3′ and 5′-CTACCACCATCC CAAAAAAAAACTCCA-3′. The primer sequences used to detect methylated *MGMT*-promoter sequences were 5′-GTTTTTAGAAC GTTTTGCGTTTCGAC-3′ and 5′-CACCGTCCCGAAAAAAAACTCCG-3′.[37] CpGenome Universal Methylated DNA (Millipore, Bedford, MA, USA) was used as a positive control and DNA from normal lymphocytes was used as a negative control for methylated alleles of *MGMT*. Controls without DNA were used for each PCR set as negative control. PCR products were separated on 2% agarose gels and ethidium bromide-stained bands were recorded and evaluated (Fig. 2).

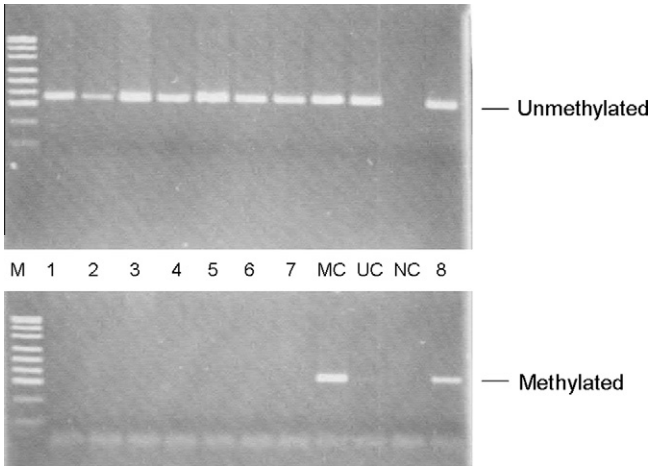

Fig. 2. *MGMT*-promoter methylation analysis. Equal amounts of polymerase chain reaction products (10 μL) were loaded on a 2% agarose gel. Lanes 1–8 show DNA from eight individuals with glioblastoma multiforme. Lanes 1–7, *MGMT*-promoter methylation negative; 8, *MGMT*-promoter methylation positive; M, size marker; MC, positive methylated control; UC, positive unmethylated control; NC, negative control.