Impact of *TGF-β1* -509C/T and 869T/C polymorphisms on glioma risk and patient prognosis

Vieira de Castro J [1,2], Gonçalves CS [1,2], Costa S [1,2], Linhares P [3], Vaz R [3], Nabiço R [4], Amorim J [4], Pereira M[1,2], Reis RM [1,2,5], Costa BM [1,2]

[1]Life and Health Sciences Research Institute, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal; [2]ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Campus de Gualtar, 4710-057, Braga, Portugal; [3]Department of Neurosurgery, Hospital São João, Alameda Professor Hernâni Monteiro, 4202-451, Porto, Portugal; [4]Department of Oncology, Hospital de Braga, Sete Fontes – São Victor, 4710-243, Braga, Portugal; [5]Molecular Oncology Research Center, Barretos Cancer Hospital, Rua Antenor Duarte Vilela, 1331 - Doutor Paulo Prata, Barretos, SP, 14780-000, Brazil

Running Title: *TGF-β1* -509C/T and 869T/C polymorphisms in glioma

Corresponding author: Bruno M Costa

E-mail address: bfmcosta@ecsaude.uminho.pt

Telephone: +351 253 604 872

Fax: +351 253 604 809

Abstract

Transforming growth factor beta (TGF-β) plays an important role in carcinogenesis. Two polymorphisms in the *TGF-β1* gene (-509C/T and 869T/C) were described to influence susceptibility to gastric and breast cancer. The 869T/C polymorphism was also associated with overall survival in breast cancer patients. In the present study we investigated the relevance of these *TGF-β1* polymorphisms in glioma risk and prognosis. A case-control study that included 114 glioma patients and 138 cancer-free controls was performed. Single nucleotide polymorphisms (SNPs) were evaluated by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). Univariate and multivariate logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence intervals (95% CI). The influence of *TGF-β1* -509C/T and 869T/C polymorphisms on glioma patient survival were evaluated by a Cox regression model adjusted for patients' age and sex, and represented in Kaplan-Meier curves. Our results demonstrated that *TGF-β1* gene polymorphisms -509C/T and 869T/C are not significantly associated with glioma risk. Survival analyses showed that the homozygous -509TT genotype associates with longer overall survival of glioblastoma (GBM) patients when compared with patients carrying CC+CT genotypes (OR, 2.41; 95% CI, 1.06-5.50; $p = 0.036$). In addition, the homozygous 869CC genotype is associated with increased overall survival of GBM patients when compared with 869TT+TC genotypes (OR, 2.62; 95% CI, 1.11-6.17; $p = 0.027$). In conclusion, this study suggests that *TGF-β1* -509C/T and 869T/C polymorphisms are not significantly associated with risk for developing gliomas, but may be relevant prognostic biomarkers in GBM patients.

Keywords: Glioma; Glioblastoma; Transforming growth factor beta 1; Single nucleotide polymorphisms; Risk; Prognosis

Introduction

During the last decades, the incidence and mortality of brain tumors have increased in most developed countries, mainly in the older age groups, with a slightly higher incidence in men than in women [1]. Gliomas, the most common primary tumors of the central nervous system (CNS), account for almost 80% of brain malignancies [2]. According to their histological characteristics, these tumors can be divided into 4 main subgroups: astrocytomas, oligodendrogliomas, oligoastrocytomas and ependymomas (the less common). Glioma tumors can also be divided into 4 grades of malignancy according to the World Health Organization (WHO) classification, being glioblastoma (GBM) the most common and biologically aggressive glioma type (grade 4) [3, 4]. Despite the advances in the field of neuro-oncology, the prognosis of glioma patients remains very poor [5], particularly for patients with GBM [6]. Few factors have been associated with increased glioma risk, including hereditary syndromes, such as Li-Fraumeni and Turcot syndromes, neurofibromatosis (type 1 and type 2) and tuberous sclerosis complex [7, 8], familial aggregation [7, 9], and exposure to high doses of ionizing radiation [7, 10, 11]. Some genome-wide association studies have showed that single nucleotide polymorphisms (SNPs) are associated with glioma susceptibility [12, 13]. However, other factors that may contribute to glioma susceptibility require additional investigation.

The transforming growth factor beta (TGF- β), a multifunctional cytokine, is involved in the regulation of several immunomodulatory processes that play a key role in numerous cellular processes, such as proliferation, differentiation, apoptosis, angiogenesis, tumor progression and extracellular matrix production [14]. TGF-β has three isoforms, TGF-β1, TGF-β2, and TGF-β3. These three isoforms bind and activate a membrane receptor serine/threonine complex (type I - TGFβRI and type II - TGFβRII). The intracellular signaling is initiated when TGFβRII phosphorylates TGFβRI, which in turn phosphorylates the transcription factors Smad2 or Smad3 that consequently bind Smad4. This complex is translocated from the cytoplasm to the nucleus, resulting in the transcriptional activation of TGF-β responsive genes that ultimately mediate the effects of TGF-β at the cellular level [15]. Deregulation of TGF-β signaling has been implicated in cancer, where TGF-β has been demonstrated to have a dual role. It may act as a strong inhibitor of proliferation of normal astrocytes and epithelial cells, being considered a tumor suppressor factor, but in some tumor types, including high-grade glioma, TGF-β acts as an oncogenic factor contributing to cell growth and invasion, and decreases host immune responses against tumor [16]. It was also demonstrated that TGF-β activity confers poor prognosis in glioma

patients [17, 18]. Several studies have identified *TGF-β1* as a predictive cancer biomarker, particularly focusing on *TGF-β1* genetic polymorphisms [19-22]. In fact, it was demonstrated that polymorphisms in this gene contribute to breast and gastric cancers susceptibility [19, 23]. Additionally, studies demonstrated an association of *TGF-β1* 869T/C polymorphism with overall survival of breast cancer patients [24, 25]. The *TGF-β1* gene is located on chromosome 19q13 and two common polymorphisms of the *TGF-β1* gene have been extensively studied, the -509C/T (rs1800469) and the 869T/C (rs1800470, previously known as rs1982073; T29C and Leu10Pro) [22, 26, 27]. The -509C/T polymorphism is located in the promoter region of *TGF-β1* gene, which may potentially regulate *TGF-β1* transcription. The 869T/C polymorphism is located in exon 1 and could lead to a leucine-to-proline substitution at codon 10 [21, 26]. Some studies demonstrated that the -509T allele is associated with an increased transcriptional activity as compared to -509C allele [28], which leads to a higher serum concentration of TGF-β1 among TT homozygotes than in the CT heterozygotes [29]. Similarly, the 869C allele was associated with high serum concentrations of TGF-β1 [19, 30]. Moreover, some studies showed that -509C/T and 869T/C *TGF-β1* polymorphisms were able to affect TGF-β1 protein expression [31, 32]. Importantly, the circulating levels of this cytokine have been associated with cancer [33-35]. The relevance of *TGF-β1* polymorphisms has not been reported in gliomas. Thus, the aim of this case-control study was to investigate the relevance of *TGF-β1* -509C/T and 869T/C polymorphisms in glioma susceptibility and how specific polymorphic variants may influence the prognosis of patients.

Methods

Study population

In this case-control study, we enrolled 114 glioma patients from Portugal (Hospital of Braga, Braga, and Hospital São João, Porto) diagnosed between 2004 and 2013. The peripheral blood from these subjects was collected. Tumors were classified according to WHO [3], and clinico-pathological features are summarized in Table 1. The control group was randomly selected from blood donors at Hospital of Braga, and it included 138 cancer-free individuals. All subjects were of Caucasian ethnic background. The procedures followed in the present study were in accordance with institutional ethical standards.

Genotyping

Genomic DNA from glioma cases and controls was extracted from peripheral blood leukocytes by proteinase K/chloroform/isopropanol treatment [36]. The purified DNA was used to determine the genotypes of both polymorphisms, using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) methods. The PCR primers for -509C/T polymorphism were 5'-CCCGGCTCCATTTCCAGGTG-3' (forward) and 5'-GGTCACCAGAGAAAGAGGAC-3' (reverse), and for the 869T/C polymorphism were 5'-CCTCCCCACCACACCAG-3' (forward) and 5'-CCGCAGCTTGGACAGG-3' (reverse). The PCR was performed in a total volume of 25 µl containing 50 ng of DNA, 0.5 unit of KAPA Taq DNA polymerase (GRiSP), 1x KAPA Taq Buffer A containing MgCl2, 0.2 mM dNTP Mix, and 0.8 µM of each primer. For the -509C/T polymorphism, the DNA was initially denatured at 95°C for 7 min, followed by 11 cycles of 95°C for 30 s, 66-61°C for 30 s, and 72°C for 1 min, followed by 30 cycles of 95°C for 30 s, 61°C for 30 s, and 72°C for 1 min. The PCR was finished by a final extension cycle at 72°C for 8 min. Regarding 869T/C polymorphism, the PCR cycle conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 9 cycles of 95°C for 30 s, 68-64°C for 30 s, and 72°C for 30 s, followed by 30 cycles of 95°C for 30 s, 64°C for 30 s, and 72°C for 30 s. Finally, the PCR was completed by a final extension cycle at 72°C for 8 min. After confirmation of an amplified fragment of the expected size (808 bp for -509C/T and 235 bp for 869T/C) on 2% agarose gel, 8-12 µL of PCR products were digested overnight at 37 °C with the appropriate restriction enzymes. For the -509C/T polymorphism, 10 units of restriction enzyme Bsu36I (New England Biolabs) were used, and for the 869T/C 5 units of the restriction enzyme MspA1I (Fermentas) were applied. The DNA fragments were resolved on 2% agarose gel for -509C/T polymorphism and 4% agarose gel for 869T/C polymorphism and were detected by Greensafe Premium staining (Nzytech). For -509C/T polymorphism, the PCR product (808 bp) with C allele was digested into two fragments (617 and 191 bp), whereas the PCR product with T allele was not digested by Bsu36I. For 869T/C polymorphism, the PCR product (235 bp) with T allele was digested into four fragments (103, 67, 40, and 25 bp), and the PCR product with C allele was digested into five fragments (91, 67, 40, 25, and 12 bp).

Statistical analysis

Data analysis was performed using SPSS 22.0 software (SPSS, Inc.). Differences in allele and genotype frequencies were compared between glioma patients and cancer-free controls by the chi-square, and the frequency distribution of patients' age and sex were compared between glioma patients and cancer-free controls by the non-parametric Wilcoxon-Mann Whitney test. Additionally, the chi-square test was used to verify that the observed allele distribution, in the control group, was in Hardy-Weinberg equilibrium. Odds ratio (OR) and 95% confidence intervals (95% CI) were estimated by univariate and multivariate logistic regression analyses, adjusted for patients' age (as a continuous variable) and sex, to assess the risk for each glioma type conferred by a particular allele and genotype of each polymorphism. Patient survival curves were assessed by the Kaplan-Meier method for GBM. A Cox regression model adjusted for patients' age (as a continuous variable) and sex was applied to evaluate the effect of the *TGF-β1* genotypes on overall survival. Statistical significance was considered for p values < 0.05.

Results

The clinico-pathological features of the controls and cases are summarized in Table 1. For both *TGF-β1* -509C/T and 869T/C polymorphisms, 114 glioma patients and 138 cancer-free control individuals were analyzed. The statistical analysis of age distribution between control and glioma cases showed significant differences ($p \leq 0.001$). Regarding sex distribution, no significant differences were found between controls and cases ($p = 0.195$). The genotype and allele frequencies of the *TGF-β1* -509C/T and 869T/C polymorphisms in controls and glioma cases are shown in Table 2. The frequencies of the CC, CT, and TT genotypes of -509C/T were 39.1%, 44.9%, and 16.0% in cancer-free controls, and 36.8%, 47.4%, and 15.8% in glioma patients, respectively. Regarding the 869T/C polymorphism, the frequencies of the TT, TC, and CC genotypes were 34.8%, 46.4%, and 18.8% in controls, and 36.8%, 46.5%, and 16.7% in glioma cases, respectively. The distribution of -509C/T and 869T/C allele frequencies in the control group were in Hardy−Weinberg equilibrium ($p = 0.891$ and $p = 0.685$, respectively).

When assessing the allele frequencies of the *TGF-β1* -509C/T polymorphism by univariate analysis, we found that the C allele was not significantly associated with a higher risk for glioma (OR, 0.97, 95% CI

0.68-1.39; Table 2). Additionally, using TT genotype as reference, the OR analysis showed that the CC, CT and combined CC+CT genotypes were not significantly associated with increased risk for glioma (OR 0.95, 95% CI 0.45-1.98 for CC; OR 1.07, 95% CI 0.52-2.19 for CT; OR 1.01, 95% CI 0.51-2.00 for CC+CT; Table 2). Evaluating the *TGF-β1* 869T/C polymorphism by univariate analysis, the T allele was not significantly associated with a higher risk for glioma (OR 1.09, 95% CI 0.76-1.56, Table 2). Using CC genotype as reference, the OR analysis showed that the TT, TC and combined TT+TC genotypes were not significantly associated with increased risk for glioma (OR, 1.20, 95% CI 0.58-2.47 for TT; OR 1.13, 95% CI 0.57-2.27 for TC; OR 1.16, 95% CI 0.61-2.23 for TT+TC; Table 2). Taking into account that GBM were the most frequent subtype in our series (n = 85), we also compared the control group with GBM cases. Using similar analysis, a lack of association between both *TGF-β1* -509C/T and 869T/C allele or genotype variants and risk for developing GBM was observed (Table 2). Moreover, for both polymorphisms, a multivariate logistic regression model adjusted for sex and age as a continuous variable (Table 3) was applied. As expected, increased age was associated with increased risks for developing glioma and GBM. Similarly, female gender was associated with decreased risks (Table 3). Consistent with the results observed by the univariate analysis, no associations between each polymorphic variant and risk for developing gliomas or GBMs were found (Table 3).

We then evaluated whether these *TGF-β1* polymorphisms may have an impact in patients' survival. To do so, we focused exclusively in GBM patients with available survival data (n = 44), as glioma grade is a strong influencer of survival, precluding an analysis in the whole glioma dataset. Regarding -509C/T polymorphism, the Cox model showed that GBM patients carrying the TT genotype had significantly increased overall survival compared to those with the CC+CT genotypes (OR 2.41, 95% CI 1.06-5.50, Table 4; $p = 0.036$, Fig.1a). Moreover, patients with CT genotype alone presented a shorter overall survival when compared to those carrying TT genotype (OR 2.72, 95% CI 1.12-6.65, Table 4; $p = 0.028$, Fig. 1b). No significant differences in overall survival were found in GBM patients with *TGF-β1* -509CC versus TT genotypes (Table 4). Concerning the survival analysis for the *TGF-β1* 869T/C polymorphism, the Cox regression model demonstrated that TT+TC genotypes were significantly associated with shorter survival in GBM patients, as compared to the CC genotype (OR 2.62, 95% CI, 1.11-6.17, Table 4; $p = 0.027$, Fig.1c). These results were further supported when we compared patients with TC genotype with patients carrying the CC genotype (OR

2.71, 95% CI 1.12-6.54, Table 4; $p = 0.027$, Fig.1d). No significant differences in overall survival were found in GBM patients with *TGF-β1* 869CC versus TT genotypes (Table 4).

Discussion

Gliomagenesis is a complex and poorly understood process in which genetic and environmental factors play critical roles. Several studies have suggested that SNPs are the most common sources of human genetic variation, and they may contribute to individual's susceptibility to cancer, including glioma [37]. So far, SNPs of several genes have been studied and identified as putative biomarkers for glioma susceptibility. Some examples include genes encoding proteins involved in DNA repair pathways (*MGMT, PRKDC, ERCC1, XRCC1, APEX1, TP53, PARP1,* and *LIG1*) [38-41], cancer metabolism (*GST, CYP2D6, SOD2, SOD3, GPX1* and *NOS1*) [42, 43], growth pathways [44, 45], among others [46, 47]. Many association studies on the *TGF-β1* polymorphisms have been conducted in several types of cancer, including lung [27], prostate [20, 26], gastric [21], hepatocellular [22] and breast cancers [19, 24, 30, 48]. To the best of our knowledge, this is the first study to evaluate the *TGF-β1* -509C/T and 869T/C polymorphisms in glioma patients. This is particularly relevant as these two polymorphisms have been reported to affect TGF-β1 protein expression and influence the structure and function of TGF-β1 peptide which may contribute to cancer [31, 32].

Using both univariate and multivariate statistical analyses, our results showed that none of the *TGF-β1* -509C/T and 869T/C polymorphisms are significantly associated with glioma susceptibility. These data fit well with previous studies in other tumor types in which 869T/C was not associated with breast cancer risk [48], and -509C/T polymorphism was not associated with an increased risk of colorectal cancer [49]. While in our dataset we included solely patients of Caucasian background, future studies should evaluate how these *TGF-β1* polymorphisms may have relevance in other ethnic backgrounds, as previously suggested for many other polymorphisms [50-53].

It has been described that TGF-β1 contributes to cell growth, angiogenesis, and invasion, is highly active and confers poor prognosis in high-grade glioma patients [16-18]. Therefore, it is conceivable that patients carrying the T allele of the -509C/T polymorphism and patients with the C allele of the 869T/C polymorphism may have reduced cancer survival, since both these alleles are associated with an elevated *TGF-*

β1 levels. Contrarily, in our study, GBM patients carrying TT genotype of the -509C/T polymorphism and patients with CC genotype of 869T/C polymorphism presented longer overall survival. This is in agreement with a previous work where it has been shown that breast cancer patients carrying the CC genotype of the 869T/C polymorphism presented a longer overall survival [24]. Therefore, the TT genotype of the -509C/T polymorphism and the CC genotype of the 869T/C polymorphism have the potential to be used as predictive marker of better survival in patients with GBM. Additionally, taking into account that -509T allele has been suspected to increase the transcription of *TGF-β1*, patients that present this variant may be more suited for an anti-TGF-β1 monoclonal antibody therapy (Metelimumab) [54]. It remains, however, to be seen if -509C/T and 869T/C polymorphisms are in a linkage disequilibrium and if it is functionally relevant. For instance, it has been shown that these two *TGF-β1* polymorphisms (-509C/T and 869T/C) are in strong linkage disequilibrium in breast cancer patients, although it remains to be determined which of the two polymorphisms is functionally significant and affect survival [25].

In conclusion, this study shows that *TGF-β1* -509C/T and 869T/C polymorphisms do not confer susceptibility to develop glioma but may have an impact in the survival of GBM patients. Specifically, the *TGF-β1* -509TT and 869CC genotypes can be used as predictive markers of improved survival. In the future, additional studies with larger datasets will be needed to extend and validate these novel findings.

Acknowledgments

This work was supported by Fundação para a Ciência e Tecnologia, Portugal (PTDC/SAU-GMG/113795/2009 and SFRH/BPD/33612/2009 to B.M.C.; SFRH/BD/88121/2012 to J.V.C.; SFRH/BD/92786/2013 to C.S.G.; PTDC/SAU-ONC/115513/2009 to R.R.).

Conflict of Interest

The authors declare that they have no conflict of interest.

Table 1 Clinico-pathological features of gliomas and controls

Groups (WHO grade)	No. Cases	Age, y (mean ± SD)	Male/Female Ratio
Controls	138	40.9 ± 12.1	1.2
Gliomas (2-4)	114	58.3 ± 12.9	1.7
Astrocytomas (2-4)	97	58.9 ± 12.7	2.2
Astrocytomas (2-3)	8	52.3 ± 13.3	1
Diffuse astrocytoma (2)	5	54.8 ± 13.8	0.67
Anaplastic astrocytoma (3)	2	55.0 ± 9.9	1
Gliosarcomas (4)	4	61.3 ± 9.1	All males
Glioblastoma (4)	85	59.5 ± 12.7	2.1
Oligodendrogliomas (2-3)	16	53.1 ± 12.3	0.45
Oligodendroglioma (2)	4	46.3 ± 9.7	All females
Anaplastic Oligodendroglioma (3)	10	54.7 ± 12.9	0.67

Table 2 Univariate analysis of the association between -509C/T and 869T/C polymorphisms and risk for each glioma group

Polymorphism	Control	Glioma (WHO grades 2-4)	[a]OR (95% CI)	Glioblastoma (WHO grade 4)	[a]OR (95% CI)
***TGF-β1* -509C/T**					
Genotypes					
TT	22	18	-	16	-
CC	54	42	0.95 (0.45-1.98)	28	0.71 (0.32-1.57)
CT	62	54	1.07 (0.52-2.19)	41	0.91 (0.43-1.94)
CC+CT	116	96	1.01 (0.51-2.00)	69	0.82 (0.40-1.66)
Alleles					
T	0.384	0.395	-	0.429	-
C	0.616	0.605	0.97 (0.68-1.39)	0.571	0.85 (0.57-1.25)
***TGF-β1* 869T/C**					
Genotypes					
CC	26	19	-	17	-
TT	48	42	1.20 (0.58-2.47)	30	0.96 (0.45-2.05)
TC	64	53	1.13 (0.57-2.27)	38	0.91 (0.44-1.89)
TT+TC	112	95	1.16 (0.61-2.23)	68	0.93 (0.47-1.84)
Alleles					
C	0.420	0.399	-	0.424	-
T	0.580	0.601	1.09 (0.76-1.56)	0.576	1.10 (0.75-1.64)

[a]Odds ratio (OR) with 95% confidence intervals (CI).

Table 3 Multivariate logistic regression analysis of the association between -509C/T and 869T/C polymorphisms and risk for each glioma group

Polymorphism	Control	Glioma (WHO grade 2-4)	[a]OR (95% CI)	Glioblastoma (WHO grade 4)	[a]OR (95% CI)
***TGF-β1* -509C/T**					
Genotypes					
TT	22	17	-	15	-
CC	54	40	1.14 (0.45-2.98)	27	0.76 (0.27-2.13)
CT	62	52	1.11 (0.45-2.75)	39	0.82 (0.30-2.21)
CC+CT	116	92	1.13 (0.48-2.63)	66	0.79 (0.31-2.01)
Alleles					
T	0.384	0.394	-	0.426	-
C	0.616	0.606	1.08 (0.68-1.70)	0.574	0.89 (0.53-1.48)
Age			**1.12 (1.09-1.15)**		**1.14 (1.10-1.18**)
Sex					
Male	76	69	-	58	-
Female	62	40	**0.40 (0.20-0.77)**	27	**0.24 (0.11-0.52)**
***TGF-β1* 869T/C**					
Genotypes					
CC	26	17	-	15	-
TT	48	40	1.36 (0.55-3.34)	29	0.89 (0.33-2.41)
TC	64	52	1.12 (0.47-2.66)	37	0.80 (0.31-2.07)
TT+TC	112	92	1.22 (0.55-2.73)	66	0.84 (0.35-2.02)
Alleles					
C	0.420	0.394	-	0.414	-
T	0.580	0.606	1.19 (0.75-1.87)	0.586	1.09 (0.64-1.84)
Age			**1.12 (1.09-1.15)**		**1.14 (1.10-1.18)**
Sex					
Male	76	69	-	58	-
Female	62	40	**0.39 (0.20-0.76)**	27	**0.24 (0.11-0.52)**

[a]Odds ratio (OR) with 95% confidence intervals (CI), adjusted for age (as a continuous variable) and sex. Bold-faced values indicate significant differences at 5% level.

Table 4 Multivariate COX regression analysis of the association between -509C/T and 869T/C polymorphisms and survival in grade 4 gliomas

Polymorphism	n	[a]OR (95% CI)
***TGF-β1* -509C/T**		
Genotypes		
TT	12	-
CC	16	2.09 (0.83-5.31)
CT	16	**2.72 (1.12-6.65)**
CC+CT	32	**2.41 (1.06-5.50)**
Age		1.02 (0.99-1.05)
Sex		
Male	31	-
Female	13	1.13 (0.52-2.45)
***TGF-β1* 869T/C**		
Genotypes		
CC	12	-
TT	14	2.43 (0.88-6.70)
TC	18	**2.71 (1.12-6.54)**
TT+TC	32	**2.62 (1.11-6.17)**
Age		1.01 (0.99-1.04)
Sex		
Male	31	-
Female	13	1.28 (0.57-2.89)

[a]Odds ratio (OR) with 95% confidence intervals (CI), adjusted for age (as a continuous variable) and sex. Bold-faced values indicate significant differences at 5% level.

Figure Legend

Fig. 1 Effect of *TGF-β1* -509C/T and 869T/C polymorphisms in the survival of glioblastoma patients

Kaplan-Meier overall survival curves for *TGF-β1* -509C/T **(a** and **b)** and 869T/C **(c** and **d)** polymorphisms. In the -509C/T polymorphism, Cox regression analysis showed that the group of glioblastoma patients harboring CC+CT genotypes (**a**, $p = 0.036$) or patients with CT genotype (**b**, $p = 0.028$) had statistically significant shorter overall survivals when compared to patients with TT genotype. Regarding the 869T/C polymorphism, the group of glioblastoma patients with TT+TC genotypes (**c**, $p = 0.027$) or patients with TC genotype (**d**, $p = 0.027$) had significantly shorter overall survivals than patients with CC genotype. Tick marks indicate censored data

References

1 Boyle P, Levin B: World cancer report 2008. IARC Press, International Agency for Research on Cancer, 2008.

2 Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M: Epidemiology and molecular pathology of glioma. Nature clinical practice Neurology 2006;2:494-503; quiz 491 p following 516.

3 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P: The 2007 who classification of tumours of the central nervous system. Acta neuropathologica 2007;114:97-109.

4 Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA, Jr., Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW: An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-1812.

5 Butowski NA, Sneed PK, Chang SM: Diagnosis and treatment of recurrent high-grade astrocytoma. J Clin Oncol 2006;24:1273-1280.

6 Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-996.

7 Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, Kruchko C, McCarthy BJ, Rajaraman P, Schwartzbaum JA, Sadetzki S, Schlehofer B, Tihan T, Wiemels JL, Wrensch M, Buffler PA: Brain tumor epidemiology: Consensus from the brain tumor epidemiology consortium. Cancer 2008;113:1953-1968.

8 Reuss D, von Deimling A: Hereditary tumor syndromes and gliomas. Recent Results Cancer Res 2009;171:83-102.

9 Wrensch M, Lee M, Miike R, Newman B, Barger G, Davis R, Wiencke J, Neuhaus J: Familial and personal medical history of cancer and nervous system conditions among adults with glioma and controls. Am J Epidemiol 1997;145:581-593.

10 Hocking B: Occupational exposure to ionizing and non-ionizing radiation and risk of glioma. Occup Med (Lond) 2008;58:148-149; author reply 149.

11 Ron E, Modan B, Boice JD, Jr., Alfandary E, Stovall M, Chetrit A, Katz L: Tumors of the brain and nervous system after radiotherapy in childhood. N Engl J Med 1988;319:1033-1039.

12 Shete S, Hosking FJ, Robertson LB, Dobbins SE, Sanson M, Malmer B, Simon M, Marie Y, Boisselier B, Delattre JY, Hoang-Xuan K, El Hallani S, Idbaih A, Zelenika D, Andersson U, Henriksson R, Bergenheim AT, Feychting M, Lonn S, Ahlbom A, Schramm J, Linnebank M, Hemminki K, Kumar R, Hepworth SJ, Price A, Armstrong G, Liu Y, Gu X, Yu R, Lau C, Schoemaker M, Muir K, Swerdlow A, Lathrop M, Bondy M, Houlston RS: Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 2009;41:899-904.

13 Wrensch M, Jenkins RB, Chang JS, Yeh RF, Xiao Y, Decker PA, Ballman KV, Berger M, Buckner JC, Chang S, Giannini C, Halder C, Kollmeyer TM, Kosel ML, LaChance DH, McCoy L, O'Neill BP, Patoka J, Pico AR, Prados M, Quesenberry C, Rice T, Rynearson AL, Smirnov I, Tihan T, Wiemels J, Yang P, Wiencke JK: Variants in the cdkn2b and rtel1 regions are associated with high-grade glioma susceptibility. Nat Genet 2009;41:905-908.

14 Massague J: Tgfbeta in cancer. Cell 2008;134:215-230.

15 Derynck R, Zhang YE: Smad-dependent and smad-independent pathways in tgf-beta family signalling. Nature 2003;425:577-584.

16 Kaminska B, Kocyk M, Kijewska M: Tgf beta signaling and its role in glioma pathogenesis. Adv Exp Med Biol 2013;986:171-187.
17 Rich JN: The role of transforming growth factor-beta in primary brain tumors. Front Biosci 2003;8:e245-260.
18 Penuelas S, Anido J, Prieto-Sanchez RM, Folch G, Barba I, Cuartas I, Garcia-Dorado D, Poca MA, Sahuquillo J, Baselga J, Seoane J: Tgf-beta increases glioma-initiating cell self-renewal through the induction of lif in human glioblastoma. Cancer Cell 2009;15:315-327.
19 Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC: A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res 2003;63:2610-2615.
20 Cai Q, Tang Y, Zhang M, Shang Z, Li G, Tian J, Jiang N, Quan C, Niu Y: Tgfbeta1 leu10pro polymorphism contributes to the development of prostate cancer: Evidence from a meta-analysis. Tumour Biol 2014;35:667-673.
21 Chang WW, Zhang L, Su H, Yao YS: An updated meta-analysis of transforming growth factor-beta1 gene: Three polymorphisms with gastric cancer. Tumour Biol 2014;35:2837-2844.
22 Migita K, Miyazoe S, Maeda Y, Daikoku M, Abiru S, Ueki T, Yano K, Nagaoka S, Matsumoto T, Nakao K, Hamasaki K, Yatsuhashi H, Ishibashi H, Eguchi K: Cytokine gene polymorphisms in japanese patients with hepatitis b virus infection--association between tgf-beta1 polymorphisms and hepatocellular carcinoma. J Hepatol 2005;42:505-510.
23 Li K, Xia F, Zhang K, Mo A, Liu L: Association of a tgf-b1-509c/t polymorphism with gastric cancer risk: A meta-analysis. Ann Hum Genet 2013;77:1-8.
24 Gonzalez-Zuloeta Ladd AM, Arias-Vasquez A, Siemes C, Coebergh JW, Hofman A, Witteman J, Uitterlinden A, Stricker BH, van Duijn CM: Transforming-growth factor beta1 leu10pro polymorphism and breast cancer morbidity. Eur J Cancer 2007;43:371-374.
25 Shu XO, Gao YT, Cai Q, Pierce L, Cai H, Ruan ZX, Yang G, Jin F, Zheng W: Genetic polymorphisms in the tgf-beta 1 gene and breast cancer survival: A report from the shanghai breast cancer study. Cancer Res 2004;64:836-839.
26 Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J: A gain of function tgfb1 polymorphism may be associated with late stage prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:759-764.
27 Kang HG, Chae MH, Park JM, Kim EJ, Park JH, Kam S, Cha SI, Kim CH, Park RW, Park SH, Kim YL, Kim IS, Jung TH, Park JY: Polymorphisms in tgf-beta1 gene and the risk of lung cancer. Lung Cancer 2006;52:1-7.
28 Luedecking EK, DeKosky ST, Mehdi H, Ganguli M, Kamboh MI: Analysis of genetic polymorphisms in the transforming growth factor-beta1 gene and the risk of alzheimer's disease. Human genetics 2000;106:565-569.
29 Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD: Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93-97.
30 Kaklamani VG, Baddi L, Liu J, Rosman D, Phukan S, Bradley C, Hegarty C, McDaniel B, Rademaker A, Oddoux C, Ostrer H, Michel LS, Huang H, Chen Y, Ahsan H, Offit K, Pasche B: Combined genetic assessment of transforming growth factor-beta signaling pathway variants may predict breast cancer risk. Cancer Res 2005;65:3454-3461.
31 Watanabe Y, Kinoshita A, Yamada T, Ohta T, Kishino T, Matsumoto N, Ishikawa M, Niikawa N, Yoshiura K: A catalog of 106 single-nucleotide polymorphisms (snps) and 11 other types of variations in genes for transforming growth factor-beta1 (tgf-beta1) and its signaling pathway. J Hum Genet 2002;47:478-483.

32 Li X, Yue ZC, Zhang YY, Bai J, Meng XN, Geng JS, Fu SB: Elevated serum level and gene polymorphisms of tgf-beta1 in gastric cancer. Journal of clinical laboratory analysis 2008;22:164-171.
33 Blobe GC, Schiemann WP, Lodish HF: Role of transforming growth factor beta in human disease. N Engl J Med 2000;342:1350-1358.
34 Elliott RL, Blobe GC: Role of transforming growth factor beta in human cancer. J Clin Oncol 2005;23:2078-2093.
35 Derynck R, Akhurst RJ, Balmain A: Tgf-beta signaling in tumor suppression and cancer progression. Nat Genet 2001;29:117-129.
36 Mullenbach R, Lagoda PJ, Welter C: An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet 1989;5:391.
37 Wu GY, Hasenberg T, Magdeburg R, Bonninghoff R, Sturm JW, Keese M: Association between egf, tgf-beta1, vegf gene polymorphism and colorectal cancer. World J Surg 2009;33:124-129.
38 Lima-Ramos V, Pacheco-Figueiredo L, Costa S, Pardal F, Silva A, Amorim J, Lopes JM, Reis RM: Tp53 codon 72 polymorphism in susceptibility, overall survival, and adjuvant therapy response of gliomas. Cancer Genet Cytogenet 2008;180:14-19.
39 Liu Y, Scheurer ME, El-Zein R, Cao Y, Do KA, Gilbert M, Aldape KD, Wei Q, Etzel C, Bondy ML: Association and interactions between DNA repair gene polymorphisms and adult glioma. Cancer Epidemiol Biomarkers Prev 2009;18:204-214.
40 Wang LE, Bondy ML, Shen H, El-Zein R, Aldape K, Cao Y, Pudavalli V, Levin VA, Yung WK, Wei Q: Polymorphisms of DNA repair genes and risk of glioma. Cancer research 2004;64:5560-5563.
41 Wiencke JK, Aldape K, McMillan A, Wiemels J, Moghadassi M, Miike R, Kelsey KT, Patoka J, Long J, Wrensch M: Molecular features of adult glioma associated with patient race/ethnicity, age, and a polymorphism in o6-methylguanine-DNA-methyltransferase. Cancer epidemiology, biomarkers & prevention 2005;14:1774-1783.
42 Elexpuru-Camiruaga J, Buxton N, Kandula V, Dias PS, Campbell D, McIntosh J, Broome J, Jones P, Inskip A, Alldersea J, et al.: Susceptibility to astrocytoma and meningioma: Influence of allelism at glutathione s-transferase (gstt1 and gstm1) and cytochrome p-450 (cyp2d6) loci. Cancer research 1995;55:4237-4239.
43 Zhao P, Zhao L, Zou P, Lu A, Liu N, Yan W, Kang C, Fu Z, You Y, Jiang T: Genetic oxidative stress variants and glioma risk in a chinese population: A hospital-based case-control study. BMC Cancer 2012;12:617.
44 Costa BM, Ferreira P, Costa S, Canedo P, Oliveira P, Silva A, Pardal F, Suriano G, Machado JC, Lopes JM, Reis RM: Association between functional egf+61 polymorphism and glioma risk. Clin Cancer Res 2007;13:2621-2626.
45 Costa BM, Viana-Pereira M, Fernandes R, Costa S, Linhares P, Vaz R, Pinheiro C, Lima J, Soares P, Silva A, Pardal F, Amorim J, Nabico R, Almeida R, Alegria C, Pires MM, Carvalho E, Oliveira P, Lopes JM, Reis RM: Impact of egfr genetic variants on glioma risk and patient outcome. Cancer Epidemiol Biomarkers Prev 2011;20:2610-2617.
46 Jiang H, Lian M, Xie J, Li J, Wang M: Three single nucleotide polymorphisms of the vascular endothelial growth factor (vegf) gene and glioma risk in a chinese population. J Int Med Res 2013;41:1484-1494.
47 Pandey JP, Kaur N, Costa S, Amorim J, Nabico R, Linhares P, Vaz R, Viana-Pereira M, Reis RM: Immunoglobulin genes implicated in glioma risk. Oncoimmunology 2014;3:e28609.

48 Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, Paulweber B, Bahadori B, Samonigg H: The l10p polymorphism of the transforming growth factor-beta 1 gene is not associated with breast cancer risk. Cancer Lett 2003;201:181-184.

49 Qi P, Ruan CP, Wang H, Zhou FG, Zhao YP, Gu X, Gao CF: -509c>t polymorphism in the tgf-beta1 gene promoter is not associated with susceptibility to and progression of colorectal cancer in chinese. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland 2010;12:1153-1158.

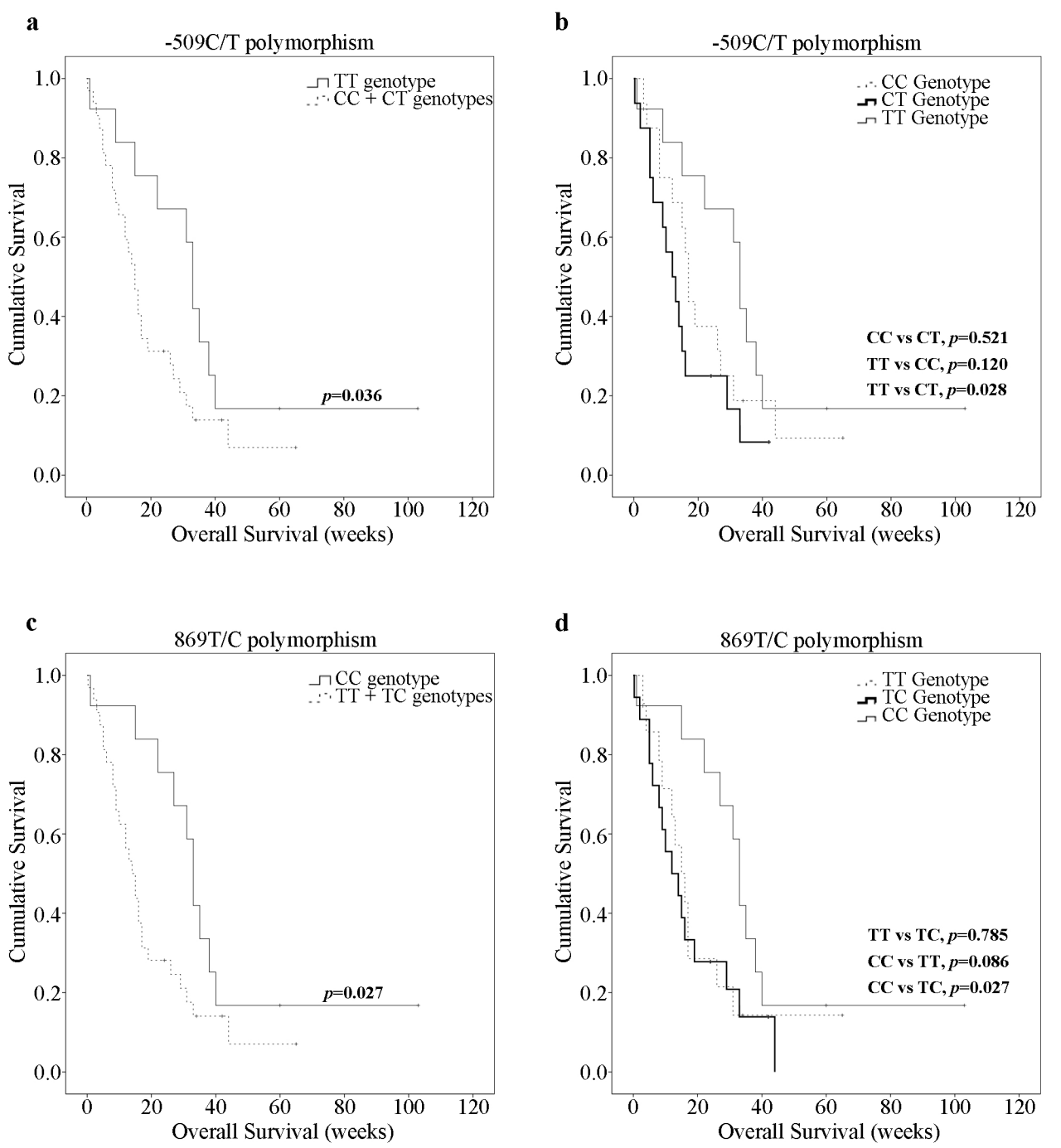
50 Crivello A, Giacalone A, Vaglica M, Scola L, Forte GI, Macaluso MC, Raimondi C, Di Noto L, Bongiovanni A, Accardo A, Candore G, Palmeri L, Verna R, Caruso C, Lio D, Palmeri S: Regulatory cytokine gene polymorphisms and risk of colorectal carcinoma. Ann N Y Acad Sci 2006;1089:98-103.

51 Macarthur M, Sharp L, Hold GL, Little J, El-Omar EM: The role of cytokine gene polymorphisms in colorectal cancer and their interaction with aspirin use in the northeast of scotland. Cancer Epidemiol Biomarkers Prev 2005;14:1613-1618.

52 Saltzman BS, Yamamoto JF, Decker R, Yokochi L, Theriault AG, Vogt TM, Le Marchand L: Association of genetic variation in the transforming growth factor beta-1 gene with serum levels and risk of colorectal neoplasia. Cancer Res 2008;68:1236-1244.

53 Zhang Y, Liu B, Jin M, Ni Q, Liang X, Ma X, Yao K, Li Q, Chen K: Genetic polymorphisms of transforming growth factor-beta1 and its receptors and colorectal cancer susceptibility: A population-based case-control study in china. Cancer Lett 2009;275:102-108.

54 Benigni A, Zoja C, Corna D, Zatelli C, Conti S, Campana M, Gagliardini E, Rottoli D, Zanchi C, Abbate M, Ledbetter S, Remuzzi G: Add-on anti-tgf-beta antibody to ace inhibitor arrests progressive diabetic nephropathy in the rat. Journal of the American Society of Nephrology : JASN 2003;14:1816-1824.


a
-509C/T polymorphism
TT genotype
CC + CT genotypes
p=0.036
Cumulative Survival
Overall Survival (weeks)
b
-509C/T polymorphism
CC Genotype
CT Genotype
TT Genotype
CC vs CT, p=0.521
TT vs CC, p=0.120
TT vs CT, p=0.028
Cumulative Survival
Overall Survival (weeks)
c
869T/C polymorphism
CC genotype
TT + TC genotypes
p=0.027
Cumulative Survival
Overall Survival (weeks)
d
869T/C polymorphism
TT Genotype
TC Genotype
CC Genotype
TT vs TC, p=0.785
CC vs TT, p=0.086
CC vs TC, p=0.027
Cumulative Survival
Overall Survival (weeks)