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Cancer Genetics and Cytogenetics 180 (2008) 14–19

Cancer Genetics and Cytogenetics

TP53 codon 72 polymorphism in susceptibility, overall survival, and adjuvant therapy response of gliomas

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Received 4 June 2007; accepted 31 August 2007

Abstract *TP53* is a key tumor suppressor gene that encodes a transcriptional factor involved in several cellular mechanisms, including growth arrest, DNA repair, and induction of apoptosis. In addition to *TP53* gene mutations, a common polymorphism, Arg72Pro, has been involved in the carcinogenesis process. The Pro72 variant has been associated with a slower induction of apoptosis and may influence the risk of cancer development. The role of Arg72Pro polymorphism in glioma susceptibility is poorly characterized. With the objective of analyzing the role of the *TP53* Arg72Pro polymorphism in glioma risk, overall survival, and patient therapy response in a Portuguese population, we conducted a retrospective case–control study, including 171 patients with gliomas and 526 cancer-free individuals. The Arg72Pro genotype was assessed by the polymerase chain reaction–restriction fragment length polymorphism technique. No statistically significant differences were observed in the genotypic and allelic frequencies between glioma and control groups, and no statistically significant differences were observed with stratification of gliomas into distinct histological subtypes: astrocytic ($n = 115$), glioblastoma ($n = 75$), and oligodendroglial ($n = 54$) tumors. No significant association was observed between *TP53* Arg72Pro and patient overall survival, but Kaplan–Meier analysis of glioma patients harboring the Pro72 allele showed a significantly longer survival with adjuvant therapy. In this first assessment of the role of *TP53* Arg72Pro polymorphism in a large series of Portuguese glioma tumors, no association was observed with glioma susceptibility or overall survival, except for patients submitted to adjuvant therapy.

1. Introduction

Central nervous system tumors correspond to <2% of adult tumors, but they are the second most common among pediatric tumors [1,2]. These tumors are an important cause of morbidity and mortality, being the first leading cause of cancer-related death in childhood and the fourth among middle aged-men [1,2]. In Portugal, the incidence of central nervous system tumors is similar to that in other European countries [3].

Gliomas are the most common group of brain tumors; astrocytic tumors are the main histological type, followed by oligodendroglial and mixed oligoastrocytic tumors. Gliomas are classified into four grades of malignancy, according to the World Health Organization (WHO) classification [1]. Glioblastoma (WHO grade IV), the most malignant and frequent adult histological type, can be clinically and genetically subdivided into primary or de novo and secondary glioblastoma [1,4,5]. Primary glioblastomas correspond to ~95% of the cases, evolve rapidly without any evidence of a less malignant precursor lesion, and are characterized by *EGFR* amplification or overexpression [1,4,5]. Secondary glioblastomas develop more slowly, through progression of a less malignant lesion, and molecularly exhibit *TP53* mutations and *PDGFR* overexpression [1,4,5]. With

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0165-4608/08/$ – see front matter
doi:10.1016/j.cancergencyto.2007.08.019

the exception of pilocytic astrocytomas (WHO grade I), prognosis of glioma patients remains poor, especially in glioblastomas, with a mean survival time of ~1 year and an overall 5-year survival rate of <2% [1].

Little is known about the etiological factors of gliomas [6]. Several exogenous factors have been reported, such as the use of cell phones, exposure to high-tension wires, head trauma, and N-nitroso compounds, only ionizing radiation has been unequivocally implicated in glioma risk [6]. Genetically, only a small proportion of gliomas occur in a context of a familial cancer syndrome due to inherited high-penetrance mutations, such as Li–Fraumeni syndrome (*TP53* germline mutation), neurofibromatosis 1 syndrome (*NF1* germline mutation), neurofibromatosis 2 syndrome (*NF2* germline mutation), and Turcot syndrome (*MLH1* or *PMS2* and *APC* germline mutation) [1]. The great majority of gliomas arise sporadically, and current evidence suggests that low-penetrance genetic polymorphisms can play a role in susceptibility to sporadic tumors [7]. Polymorphisms of genes involved in DNA repair, cell cycle control, carcinogen metabolism, or immune response have been associated with higher susceptibility to glioma development, but without consistent findings [7].

The *TP53* tumor suppressor gene encodes a transcriptional factor, p53, which is involved in a diversity of cellular processes, including growth arrest, induction of apoptosis, DNA repair, and inhibition of angiogenesis [8]. The p53 protein is activated in response to various genotoxic and nongenotoxic stimulus, triggering the expression of several genes that affect cell cycle arrest (via the p21 pathway) and DNA repair (via the GADD45 pathway). If repair fails, apoptosis is triggered (via the bax pathway), hence, the denomination of p53 as the ''genome guardian'' [9]. Inactivation of *TP53* by somatic mutations is a key molecular event, detected in ~50% of all malignancies and in ~40% of gliomas [10].

Several studies have focused on *TP53* polymorphisms as predisposing factors for tumor development [11]. One such polymorphism is the *TP53* Arg72Pro, located in exon 4 at codon 72, involving a guanine to cytosine nucleotide exchange, which leads to nonconservative change of an arginine to proline. This single-nucleotide polymorphism is located in a proline-rich encoding region of *TP53* gene, which is important for growth suppression and apoptotic function of p53 protein, indicating that Arg72 and Pro72 variants have distinct biochemical and biological activities [11]. Arg72 is significantly more efficient than Pro72 in apoptosis induction, and Pro72 individuals have been shown to be at higher risk of develop cancer [12–18]. In some epithelial cancers, the Pro72 variant was associated with a worse survival than Arg72 [15,19]. On the other hand, Pro72 is shown to be a stronger inducer of transcription than Arg72 [12], and some studies suggest that Arg72 variant is more susceptible than Pro72 to degradation by human papillomavirus E6 protein [20].

Few reports have addressed the association of Arg72Pro polymorphism with susceptibility to glioma, and the results obtained are inconsistent [21–25]. Moreover, there are no reports concerning the association between this polymorphism and glioma patient outcome or adjuvant therapy response. Our objective was to determine whether *TP53* Arg72Pro polymorphism represents a predisposition factor for glioma risk and to identify the impact on patient survival and therapeutic response in a Portuguese population.

2. Materials and methods

2.1. Patient and control populations

In this retrospective analysis, tumor samples were obtained from patients treated at Hospital S. João, Porto, and at Hospital S. Marcos, Braga, Portugal, as previously described [26]. The study of Arg72Pro polymorphisms included 171 patients with sporadic gliomas of distinct histological subtypes (Table 1) and 526 cancer-free individuals. The cancer-free control group was randomly selected from blood donors at Hospital S. Marcos, Bragal, and from Clínica Laboratorial and Dr. Edgar Botelho Moniz, Santo Tirso. All patients and control subjects were from northwestern Portugal and of European-origin ethnic background. The mean age of the patient population was 49.5 years; the mean age of the control group was 38.1 years. The distribution by sex of the patients was matched between patients (52.6% male, 47.4% female) and the control group (50.2% male, 49.8% female).

The procedures followed in the present study were in accordance with the institutional ethical standards. All samples enrolled in the present study were deidentified and unlinked from their donors.

2.2. DNA preparation

Tumor DNA was obtained from paraffin-embedded sections, as previously described [26]. (For the great majority of glioma patients, no peripheral blood DNA was available.) Briefly, paraffin was removed by incubation in

Table 1
Histological and clinical features of glioma patients

Tumor type	WHO grade	Cases, no.	Age, years, mean ± SD	Male/female, *n/n*
Astrocytic				
Pilocytic astrocytoma	I	6	24.8 ± 7.1	2/4
Diffuse astrocytoma	II	24	36.5 ± 13.2	9/15
Anaplastic astrocytoma	III	4	38.8 ± 12.9	4/0
Glioblastoma[a]	IV	75	57.2 ± 12.3	40/35
Gliosarcoma	IV	6	60.5 ± 10.3	3/3
Oligodendroglial				
Oligodendroglioma	II	22	38.1 ± 15.9	12/10
Anaplastic oligodendroglioma	III	32	53.3 ± 11.2	19/13
Oligoastrocytic				
Oligoastrocytoma	II	2	41.5 ± 19.1	1/1
TOTAL		171	49.5 ± 15.9	90/81

[a] All are primary de novo glioblastomas.

xylene, followed by ethanol washing, drying, and digestion in 100 μL of lysis buffer (500 mmol/L Tris-HCl, pH 8.5, and 1 mmol/L EDTA, pH 8.0, containing proteinase K to a final concentration of 0.5 mg/mL) at 55°C for 48 hours. After heat denaturation, DNA samples were stored at −20°C for subsequent molecular analysis. DNA of the control population was extracted from peripheral blood leukocytes according to the proteinase K–chloroform–isopropanol protocol [27].

2.3. TP53 Arg72Pro genotyping analysis

Analysis of *TP53* Arg72Pro polymorphism was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Briefly, amplification reaction was carried out in a total volume of 25 μL, consisting of 2 μL of DNA solution, 1 U *Taq* DNA polymerase (Bioron, Ludwigshafen, Germany), 1.5 mmol/L $MgCl_2$ (Bioron), 0.2 mmol/L of each dNTP (Fermentas, Burlington, ON, Canada), 0.1 μmol/L of both sense and antisense primers, and 1× enzyme buffer (Bioron). PCR amplification was performed in an Primus 96 Plus thermal cycler (MWG Biotech, High Point, NC), with an initial denaturation step at 94°C for 5 minutes, then amplified for 38 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 45 seconds, and final extension step at 72°C for 10 minutes. Primer sequences were: 5′-CTGCCCTGGTA GGTTTTCTG-3′ (reverse) and 5′-GAAGACCCAGGTCC AGATGA-3′ (forward), leading to a 152-bp PCR product.

The PCR product was then analyzed by RFLP. he DNA PCR products were digested overnight at 37°C with *Bsh*1236I (Fermentas), according to the manufacturer's instructions. The results of enzymatic digestion were visualized in a 3% agarose gel by electrophoresis. The results were the 152-bp fragment of uncut PCR product representing homozygotes proline, two fragments of 50 and 102 bp representing homozygotes arginine, and three fragments of 50, 102, and 152 bp representing heterozygotes for codon 72.

2.4. Statistical analysis

Analysis of data was performed using the SPSS version 14.0 computer software (SPSS, Chicago, IL). Chi-square analysis was used to compare categorical variables. A 5% level of significance was used in the analysis. The odds ratio (OR) and its 95% confidence interval (CI) were calculated to measure the association between *TP53* Arg72Pro polymorphism and glioma risk. Logistic regression analysis was used to calculate the adjusted OR and 95% CI for the influence of *TP53* genotypes in the risk of glioma, adjusted for age. Whenever appropriate, the observed number of each genotype was compared with that expected for a population in the Hardy–Weinberg equilibrium by using a goodness of fit χ^2 test.

Survival and adjuvant treatment (radiotherapy and/or chemotherapy) data was available for 117 of the 171 patients. The probabilities of survival were calculated, and the medians and life-tables were computed using the product–limit estimate of Kaplan–Meier. The curves were examined by the log-rank test, a statistical test for equality of survival distributions. Survival duration was defined as the time between diagnosis and death or the time to the most recent clinical evaluation of the patient.

3. Results

The frequencies of the Arg/Arg, Arg/Pro, and Pro/Pro genotypes in the control group were 56.7%, 37.5% and 5.9%, respectively, resulting in a Pro allele frequency of 0.25. The distribution of these genotype frequencies was in agreement with those calculated from the Hardy–Weinberg equilibrium model for controls ($P = 0.986$).

The distribution of the three different *TP53* codon 72 genotypes (Arg/Arg, Arg/Pro, and Pro/Pro) in both groups (cases and controls) is presented in Table 2. First, we compared the distribution of the *TP53* Arg72Pro genotypes in glioma with the control group; no statistical difference was found ($P > 0.05$). Likewise, the distribution of the Pro allele variant between case and control groups was not statistically different ($P > 0.05$) (Table 2).

We further stratified glioma cases by the histological subtypes astrocytic (grade I, II, III, and IV), glioblastoma (grade IV), and oligodendroglial (grade II and III) tumors and determined their genotype distribution. The genotype frequencies of the three histological groups were not statistically different from that of the control group ($P > 0.05$) (Table 2), and no statistical difference was found in the allelic distribution ($P > 0.05$) of these groups. Assessment of other stratification groups—diffusely infiltrating astrocytic tumors (grade II, III, IV), low-grade astrocytic tumors (grade I and II), and high-grade astrocytic tumors (grade III and IV)—revealed no significant differences (data not shown).

Kaplan–Meier curves were performed, to evaluate the possible association between the Arg72Pro polymorphism and overall patient survival. No statistical difference was observed ($P > 0.05$) for the gliomas ($n = 117$) for either 5-year or 10-year analysis (Fig. 1). Likewise, no significant associations were observed for the glioblastoma and oligodendroglioma subtypes (data not shown).

We also assessed the role of the Arg72Pro polymorphism with patient response to adjuvant treatment (radiotherapy or chemotherapy or both; $n = 42$). In the 5-year analysis, the median survival time in months of gliomas in patients with adjuvant therapy was 13 ± 20.4 SD for the Arg/Arg genotype, 24 ± 33.7 SD for the Arg/Pro genotype, and 57 ± 30.2 SD for the Pro/Pro genotype. Kaplan–Meier curves showed a tendency of longer survival associated with patients exhibiting Pro/Pro genotype ($P = 0.076$) (data not shown) and a significantly longer survival in patients harboring the Pro allele (Arg/Pro + Pro/Pro, $P = 0.027$) (Fig. 2). Upon histological stratification, only the oligodendroglioma

Table 2
Frequency of *TP53* Arg72Pro genotypes in glioma patients and healthy individuals

	Group 1, controls		Group 2, patients			
Genotype	No.	%	No.	%	*P* value	OR (95% CI)
Gliomas						
Arg/Arg	298	56.7	101	59.0	Ref.	
Arg/Pro	197	37.5	56	32.7	0.355	0.84 (0.58–1.22)
Pro/Pro	31	5.9	14	8.2	0.400	1.33 (0.68–2.60)
					0.580[a]	0.91 (0.64–1.29)[a]
					0.289[b]	1.42 (0.74–2.74)[b]
Arg allele	793	75.4	258	75.0	Ref.	
Pro allele	259	24.6	86	25.0	0.887	1.02 (0.76–1.37)
Astrocytomas[c]						
Arg/Arg	298	56.7	70	60.9	Ref.	
Arg/Pro	197	37.5	37	32.2	0.315	0.80 (0.52–1.24)
Pro/Pro	31	5.9	8	6.9	0.822	1.10 (0.48–2.49)
					0.408[a]	0.84 (0.56–1.27)[a]
					0.666[b]	1.19 (0.53–2.67)[b]
Arg allele	793	75.4	177	77.0	Ref.	
Pro allele	259	24.6	53	23.0	0.614	0.92 (0.64–1.30)
Glioblastomas						
Arg/Arg	298	56.7	47	62.7	Ref.	
Arg/Pro	197	37.5	24	32.0	0.333	0.77 (0.46–1.30)
Pro/Pro	31	5.9	4	5.3	0.717	0.82 (0.28–2.42)
					0.325[a]	0.78 (0.47–1.28)[a]
					0.846[b]	0.90 (0.31–2.62)[b]
Arg allele	793	75.4	118	78.7	Ref.	
Pro allele	259	24.6	32	21.3	0.379	0.83 (0.54–1.28)
Oligodendrogliomas						
Arg/Arg	298	56.7	31	57.4	Ref.	
Arg/Pro	197	37.5	18	33.3	0.676	0.88 (0.48–1.61)
Pro/Pro	31	5.9	5	9.3	0.393	1.55 (0.56–4.28)
					0.915[a]	0.97 (0.55–1.70)[a]
					0.329[b]	1.63 (0.61–4.38)[b]
Arg allele	793	75.4	80	74.1	Ref.	
Pro allele	259	24.6	28	25.9	0.765	1.07 (0.66–1.72)

Abbreviations: CI, confidence interval; OR, odds ratio; Ref., reference value.
[a] Pro/Pro + Arg/Pro genotype frequencies vs. Arg/Arg genotype.
[b] Pro/Pro genotype frequency vs. Arg/Arg + Arg/Pro genotypes.
[c] Types I, II, III, and IV in the WHO classification [1].

subgroup showed a similar longer survival associated with harboring the Pro allele ($P = 0.044$). No statistical difference was observed in the 10-year analysis (data not shown).

4. Discussion

Genetic polymorphisms are known to play a role on cancer susceptibility, and their role on glioma risk is starting to be evaluated [7]. In particular, polymorphisms involved in DNA repair enzymes (e.g., *PRKDC*, alias *XRCC7*; *ERCC1*, *ERCC2*), growth factors (e.g., *EGF*), immune response (*IL4* and *IL13*), and metabolism (e.g., *GSTT1*) have been associated with glioma susceptibility [7,26,28–31].

In the present study, we evaluated the role of Arg72Pro polymorphism of the *TP53* gene in susceptibility to developing glioma in a Portuguese population. The results showed no significant difference in the genotypic and allelic frequencies between gliomas and controls, and no significant association was detected between the genotype and allele distributions and tumor histological type. These findings are in agreement with previous reports in gliomas from distinct geographic regions [21,22,24,32]. At variance with these results, Parhar et al. [23], reported a significant association between the Arg/Pro genotype and an increased risk for high-grade astrocytic tumors. These authors also found a significant difference in Arg/Pro genotype distribution between high-grade astrocytic tumors and non-astrocytic tumors, including oligodendroglial and oligoastrocytic tumors [23]. In the present study, we found no differences between these type of tumors. Notably, Malmer et al. [25] recently found that the Arg72Pro polymorphism alone had no impact on glioma risk, but in combination with two other single-nucleotide polymorphisms of *TP53* gene (one at the promoter and the other in intron 6) there was an association of risk haplotypes and protective haplotypes in the *TP53* gene for glioblastomas [25].

Several explanations can be offered for the discrepancies observed. (1) The populations analyzed were different. Beckman et al. [33] were the first to note a significant

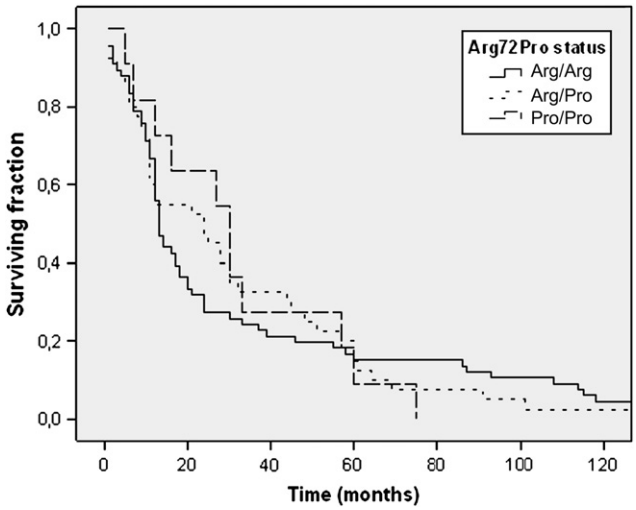

Fig. 1. Kaplan–Meier survival curves of glioma tumor patients ($n = 117$) as a function of *TP53* codon 72 genotypes ($P = 0.968$).

difference in the allelic distribution of Arg72Pro variants among Nigerian and Swedish populations, and subsequent studies showed that latitude could explain different allelic population frequencies [34]. Siddique et al. [35] found a preferential expression of either Arg72 or Pro72 allele variants, depending on the ethnicity of the population. (2) The DNA source (peripheral blood lymphocyte or tumor tissue) used in the studies was different. If tumor tissue is used, as in our study, allele retention due to mutation or loss of heterozygosity cannot be excluded. Nonetheless, the present findings are not necessarily influenced significantly by the possible allele retention that may have occurred in few of our cases. (3) Tumor histological stratification among studies was not uniform. (4) The contribution of environmental factors, which independently or together with gene interaction can modulate tumor risk, is usually disregarded [36].

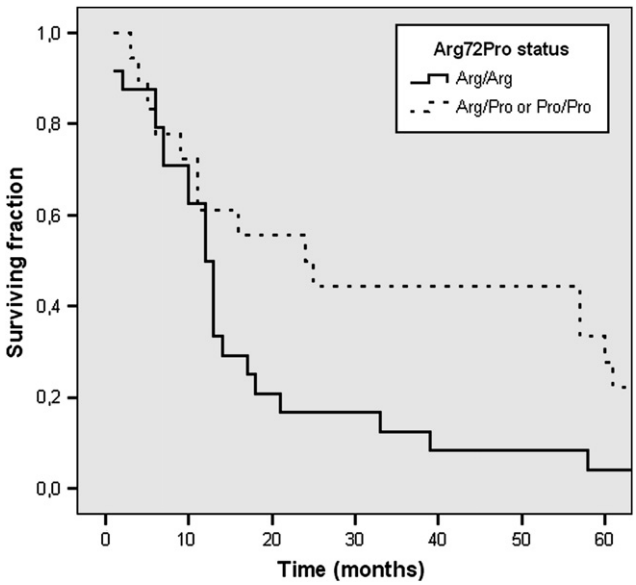

Fig. 2. Kaplan–Meier survival curves of glioma tumor patients treated with adjuvant therapy ($n = 42$) as a function of *TP53* codon 72 genotypes ($P = 0.027$).

In the present study, we also evaluated the role of the *TP53* Arg72Pro polymorphism in the overall survival of glioma patients. The evaluation did not show any statistically significant difference in overall survival among the individuals with different genotypes. When we analyzed the survival time of patients submitted to adjuvant therapy, however, we found that the patients harboring the Pro allele (Arg/Pro+Pro/Pro) had a significantly better response. This is at variance with results in other malignancies, such as head and neck, lung, breast, and ovarian cancer, for which a better outcome was seen in patients harboring the Arg allele who received adjuvant therapy [19,37–39]. A recent study showed that, under hypoxic conditions, cancer cells harboring the Pro allele had a survival disadvantage compared with cells harboring the Arg allele [40]. Notably, *TP53* Arg72Pro polymorphisms might play a role in the microenvironment severe hypoxia that characterizes gliomas [41].

In conclusion, this is the first analysis of *TP53* Arg72Pro polymorphism in Portuguese gliomas. We did not observe an association of *TP53* Arg72Pro polymorphism with glioma risk and overall patient survival. A significant association was, however, observed in glioma patient response to adjuvant therapy. Further studies are warranted to elucidate the role of this polymorphism in combination with other genetic variants of *TP53* gene and additional cancer-related genes, and to clarify the association of *TP53* Arg72Pro with response of glioma patients to adjuvant therapy.

Acknowledgments

We would like to thank the Immunochemotherapy Department of S. Marcos Hospital at Braga, Portugal, and Clínica Laboratorial and Dr. Edgar Botelho Moniz at Santo Tirso, Portugal, for their helpful assistance in the management of controls.

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