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LAB. INVESTIGATION-HUMAN/ANIMAL TISSUE

No association of *MDM2* SNP309 with risk of glioblastoma and prognosis

Soufiane El Hallani · Yannick Marie · Ahmed Idbaih · Mathieu Rodero · Blandine Boisselier · Florence Laigle-Donadey · François Ducray · Jean-Yves Delattre · Marc Sanson

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Abstract The *MDM2* SNP309 variant has been shown to increase MDM2 expression and to be associated with tumor formation. In glioblastomas, the P53/MDM2 pathway is of crucial importance and *MDM2* amplification is related to poor prognosis. However, we show here that *MDM2* SNP309 is not associated with glioblastoma risk, and is not a prognostic factor.

Keywords Glioblastoma · MDM2 · Polymorphism · Prognosis

Introduction

With a median survival of 12–15 months, glioblastoma multiforme (GBM) is the most frequent and the most malignant subtype of glioma [1]. The majority of glioblastomas are sporadic, and little is known about the environmental and genetic risk factors in this context. One of the critical molecular steps involved in gliomagenesis involves the p53 pathway which plays a central rule in oncogenesis [2]. Out of the most frequent molecular alterations, the amplification of *MDM2* was shown to be the only one related to prognosis in glioblastoma [3]. *MDM2* encodes an ubiquitin protein ligase responsible for p53 degradation, and acts therefore as an oncogene. Recently, a frequent single nucleotide polymorphism (SNP 309 T/G) of the *MDM2* promoter has been shown to increase MDM2 RNA and protein, by increasing the affinity with the Sp1 factor, and therefore downregulating p53 pathway [4].

Based on a large case-control analysis of GBM patients and controls, we investigated here the role of *MDM2* SNP309 as potential risk factor and/or prognostic marker of GBM, and the potential correlation with p53 expression and *MDM2* amplification in the tumor.

Patients and methods

Patients were selected according to following criteria: histologic diagnostic of glioblastoma without previous history of glial tumor, age ≥18 years, clinical data and follow-up available on the neuro-oncology database, available blood DNA and written informed consent obtained. Controls DNA were obtained from unrelated cancer-free and healthy Caucasian volunteers (laboratory staff and healthy visitors to the same hospital).

Blood DNA was extracted according to standard procedures. The primers and probes, designed according to the Custom Taqman SNP genotyping Assay protocol (Applied Biosystems), were as follows: forward primer 5′-CGGGAG

S. El Hallani · Y. Marie · A. Idbaih · B. Boisselier · F. Laigle-Donadey · F. Ducray · J.-Y. Delattre · M. Sanson
INSERM, U711, Biologie des Interactions Neurones & Glie, Paris 75013, France

S. El Hallani · Y. Marie · A. Idbaih · B. Boisselier · F. Laigle-Donadey · F. Ducray · J.-Y. Delattre · M. Sanson
Faculté de Médecine, Université Pierre et Marie Curie, Paris 75013, France

A. Idbaih · F. Ducray · J.-Y. Delattre · M. Sanson (✉)
AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Neurologie Mazarin, 47 boulevard de l'Hôpital, Paris 75013, France
e-mail: marc.sanson@psl.ap-hop-paris.fr

M. Rodero
Laboratoire d'Immunologie Cellulaire, INSERM U543, Paris 75013, France

TTCAGGGTAAAGGT-3′, reverse primer 5′-ACAGGCA CCTGCGATCATC-3′, VIC-labelled probe 5′-CTCCCGC GCCGAAG-3′, and FAM-labelled probe 5′-TCCCGCGC CGCAG-3′. The PCR reaction, performed in 15 µl with 20 ng genomic DNA, 1× Taqman universal PCR master mix, forward and reverse primers (450 nmol/l each), 200 nmol/l VIC-labelled probe, 200 nmol/l FAM-labelled probe, was as follow: 95°C for 15 min, 50 cycles of 92°C for 30 s, 60°C for 1 min. Completed PCR plates were read on an Mx3000P sequence detector and analyzed using the Mx Pro Q-PCR Software (Stratagene).

MDM2 amplification was detected on tumor DNA as previously described [3].

p53 expression was detected on 5 µm section formalin-fixed and paraffin embedded tissues as previously reported [3]. Overexpression of p53 was defined as a moderate to strong staining of more than 10% of nuclei.

The independence of alleles (Hardy-Weinberg equilibrium) was ensured using the χ^2 test at one degree of freedom for each polymorphism. χ^2 test (or Fisher's exact test when one subgroup was <5) was used to compare the genotype distribution between the two groups (GBM and controls). Overall survival was defined as the time between the diagnosis and death or last follow-up. The survival curves were obtained by Kaplan-Meier methods and compared by Log rank test. *t*-Test was used to compare the mean age of onset.

Results

A total of 254 selected patients (168M/86F = 1.95; median age = 56.5 [19.2–83.6]) and 238 healthy controls (156M/82F = 1.9) were analyzed. Treatment consisted in surgery (72 biopsies, 64 partial removals and 112 complete surgeries), followed by radiotherapy (43), radio + chemotherapy (149), chemotherapy alone (32), or supportive care (30). Median survival was 13.5 months (range: 0.13–191.8). The frequencies of the *MDM2* SNP309 variants for both the control and GBM population are reported in Table 1. Both populations met the Hardy-Weinberg equilibrium ($P = 0.55$ and $P = 0.82$ respectively). There was no difference between the two populations neither for the genotype frequencies, nor for allele frequencies. The same result was obtained when considering separately the female and male populations.

We investigated then a possible correlation between *MDM2* variants and the detection of p53 protein or *MDM2* amplification in tumor samples: *MDM2* genotype frequencies did not differ according to p53 immunohistochemistry ($P = 0.79$) or *MDM2* amplification ($P = 0.23$). However the T allele showed a borderline association with *MDM2* amplification (Table 1). Median survival for the whole population of GBM was 13.5 months. Overall survival did not differ between the *MDM2* SNP309 variants (Fig. 1a). Since *MDM2* SNP309 has been suspected to affect tumorigenesis in a gender dependent manner, we analyzed separately male and female population for survival: again there was no difference of survival when considering separately males and females for each polymorphism (Fig. 1b and c). Lastly, there was no correlation between age of onset and genotype: T//T (53.7 years +/– 11.6), G//T (57.5 years +/– 11.7) and G//G (55.3 years +/– 11.8).

Discussion

The *MDM2* (SNP309 T → G) variant results in the up-regulation of MDM2 synthesis, accelerated tumor

Table 1 Comparison of genotype and allele frequencies in patients and controls, and correlation with p53 expression and *MDM2* amplification

Population	Number of individuals	Number per genotype (%)			Pearson χ^2 *P* value	Number per allele (%)		Pearson χ^2 *P* value
MDM2 SNP 309		T//T	T//G	G//G		T	G	
Healthy controls	238	109 (45.8)	96 (40.2)	33 (13.8)		314 (65.9)	162 (34.1)	
Male	156	73 (46.8)	63 (40.4)	20 (12.8)		209 (66.9)	103 (33.1)	
Female	82	36 (43.9)	33 (40.3)	13 (15.8)		105 (64.0)	59 (36.0)	
GBM	254	98 (38.6)	114 (44.9)	42 (16.5)	0.26	310 (61.0)	198 (39.0)	0.11
Male	168	64 (38.1)	74 (44.1)	30 (17.8)	0.22	202 (60.1)	134 (39.9)	0.07
Female	86	34 (39.6)	40 (46.5)	12 (13.9)	0.71	108 (62.8)	64 (37.2)	0.81
GBM P53 IHC-analyse	155							
Normal expression	61	22 (36.1)	26 (42.6)	13 (21.3)		70	52	
Overexpression	94	37 (39.5)	41 (43.4)	16 (17.1)	0.79	115	73	0.51
GBM *MDM2* amplification	174							
No amplification	166	62 (37.3)	69 (41.6)	35 (21.1)		193 (58.1)	139 (41.9)	
Amplification	8	5 (62.5)	3 (37.5)	0	0.23	13 (81.25)	3 (18.75)	0.07

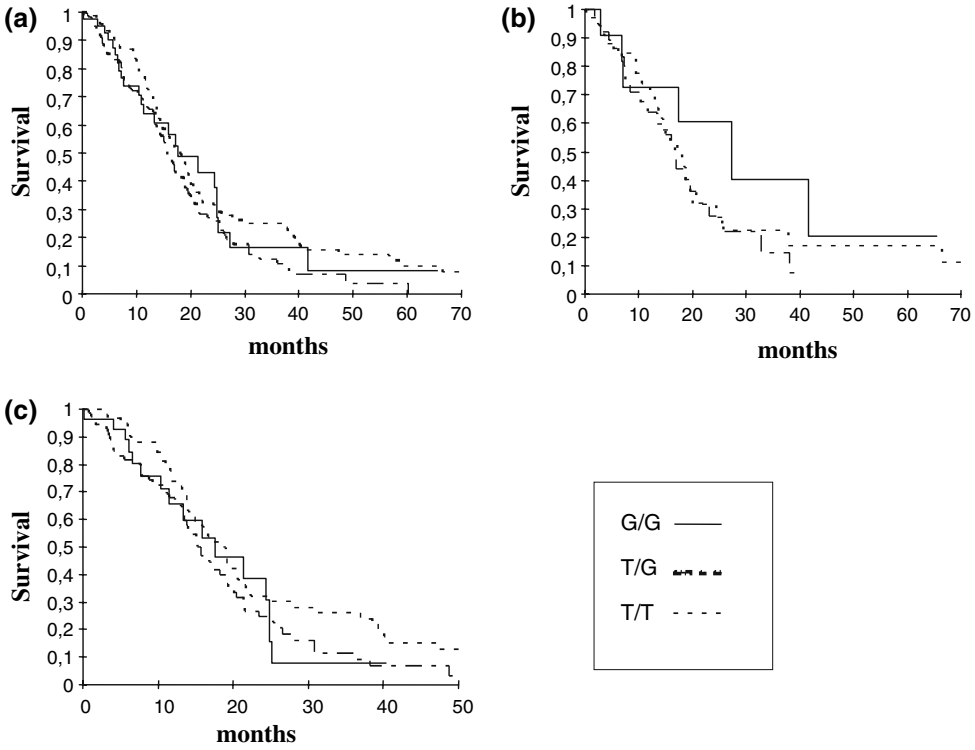

Fig. 1 Survival curves show no correlation of survival with the genotype (**a**) in the whole population ($P = 0.15$), (**b**) in the female population ($P = 0.42$) and (**c**) in the male population ($P = 0.17$)

formation in Li-Fraumeni syndrome and increased risk of sporadic sarcoma [4], endometrial cancer [5], gastric carcinoma [6], hepatocellular carcinoma [7], oesophageal squamous cell carcinoma [8] and bladder cancer [9]. In contrast, no association has been found here with glioblastoma, like in ovarian [10] and breast cancer [10–14], head and neck carcinoma, colorectal cancer [15], and lung cancer [16, 17] (in this case however positive but contradictory results were obtained by two independent studies [18, 19]). Since the *MDM2* G allele increases the affinity with the Sp1 factor which is positively influenced by the oestrogen signalling, its impact may be restricted to the female population, as recently suggested [18, 20]. However our study does not suggest that the *MDM2* SNP309 is associated with an increased risk of glioblastoma, neither in male nor in female population.

A few studies investigated the prognostic value of *MDM2* 309G variant, showing an association with poor survival in gastric carcinoma [6], and no correlation in ovarian cancer [21]. The fact that *MDM2* amplification in glioblastoma is associated with poor survival [3] prompted us to evaluate the prognostic impact of *MDM2* polymorphism on survival and to correlate it with *MDM2* amplification. Our results suggest that *MDM2* SNP309 polymorphism is not a prognostic factor in GBM, but they show a borderline correlation between *MDM2* amplification and SNP309 T alleles that needs further confirmation.

MDM2 309G variant has been correlated in breast and colon cancer with age of disease [22–24], particularly in women [20] suggesting that in could accelerate tumor formation, possibly in a gender specific manner. However, our results do not support this hypothesis in GBM.

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