J Neurooncol (2010) 96:321–329
DOI 10.1007/s11060-009-9967-4

LABORATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

Different molecular patterns in glioblastoma multiforme subtypes upon recurrence

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Received: 13 April 2009 / Accepted: 6 July 2009 / Published online: 31 July 2009

Abstract One of the hallmarks of glioblastoma is its inherent tendency to recur. At this point patients with relapsed GBM show a survival time of only few months. The molecular basis of the recurrence process in GBM is still poorly understood. The aim of the present study was to investigate the genetic profile of relapsed GBM compared to their respective primary tumors. We have included 20 paired GBMs. In all tumor samples, we have analyzed *p53* and *PTEN* status by sequencing analysis, *EGFR* amplification by semiquantitative PCR and a wide-genome fingerprinting was performed by microsatellite analysis. Among primary GBM, we observed twelve type 2 GBM, four type 1 GBM and four further GBM showing neither *p53* mutations nor *EGFR* amplification (non-type 1–non-type 2 GBM). Upon recurrence, we have detected two molecular patterns of tumor progression: GBM initially showing either type 1 or type 2 profiles conserved them at the time of relapse. In contrast, non-type 1–non-type 2 GBM acquired the typical pattern of type 2 GBM and harbor *EGFR* amplification without *p53* mutation. New *PTEN* mutations upon relapse were only detected in type 2 GBM. Additional LOH were more frequently identified in relapses of type 2 GBM than in those showing the type 1 signature. Taken together, our results strongly suggest that recurrences of GBM may display two distinct pattern of accumulation of molecular alterations depending on the profile of the original tumor.

Keywords Glioblastoma · Relapse · *p53* · *PTEN* · Microsatellite · Mutation

Abbreviations

GBM	Glioblastoma multiforme
TTR	Time to tumor relapse
MGMT	O^6-methyl-guanine-methyl-transferase
CFTR	Cystic fibrosis transmembrane regulator
KPS	Karnofsky performance score

Introduction

Glioblastoma multiforme is the most frequent primary brain tumor in adults. One of its characteristic features is the intrinsic tendency to recur despite aggressive therapy [1]. Upon recurrence, the median survival of patients is only few months [2] whereas the overall median survival time is 15 months. This has not significantly changed in the last 20 years despite of advances in surgery, radio- und chemotherapy [3, 4] A moderate improvement of the 2-year survival rate has been achieved after therapy with the alkylating drug temozolomide in GBM with hypermethylated *MGMT* compared to those with unmethylated gene (46% vs. 26%) [3, 5, 6].

GBM is one of the most intensively investigated human malignancy but the molecular mechanisms associated with recurrence are still poorly understood. Saxena et al. [7] reported a high rate of homozygous deletion of the genes $p16^{INK4}$ and $p15^{INK4a}$ in GBM relapses. In a further report,

Electronic supplementary material The online version of this article (doi:10.1007/s11060-009-9967-4) contains supplementary material, which is available to authorized users.

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this author investigated 10 GBM and their corresponding relapses and observed that recurrences displayed the pattern of genetic alterations frequently observed in the de novo pathway (GBM without a low grade precursor lesion) [8]. Hulsebos et al. [9] analyzed 12 match paired GBM and found in relapse cases new LOH at chromosome regions 1p36, 19q13, 10q23 and 1q25. In contrast to the former study, GBM relapses encompassing new *p53* mutation or *EGFR* amplification were not observed.

The identification of molecular features associated with recurrences of GBM is of major importance, since a better understanding of this process might provide clues for the development of efficient treatments. In the present analysis, we have examined the molecular signatures of forty tumors consisting in relapsed GBM and their corresponding primary neoplasms which had undergone surgery and radiochemotherapy. This included the status of *p53*, *PTEN* and *EGFR* as well as a wide genome LOH analysis with thirteen highly informative microsatellite markers at chromosome regions 17p13, 10q23, 1p35-36, 19q13, 13q14, 9p21.

Materials and methods

Patient population and tumor samples

All patients had undergone surgery with the goal of maximal possible tumor resection followed by fractionated radiotherapy (mean dose: 58 Gray) and chemotherapy. Forty paired tumors from twenty patients were available. Informed consent for samples and data analysis from each patient or the patient's caretaker was obtained.

Tumor samples were immediately frozen in liquid nitrogen after surgical resection and stored at −80°C. Tumor tissue was evaluated by experienced pathologists according to the 2000 WHO classification criteria. DNA from tumor specimens was isolated applying the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany).

LOH analysis

LOH of chromosome 10q was studied with markers covering reported deletions on 10q23-24: D10S215, D10S541 and the intragenic *PTEN* marker PTENCA. Analysis of LOH at 17p13 was performed with a *p53* intragenic localized marker. Allelic losses at 9p21 ($p16^{INK4a}$, $p14^{ARF}$ and *p15*) and 13q14 (*RB1*) were studied with primers D9S171, D9S1748, D9S1749, D13S153 and D13S267. Loss of heterozygosity at 1p35-p36 and 19q13 was assessed with the markers D1S468, D1S482, D19S112 and D19S412. Amplified PCR products were analyzed on a denaturing 6.5% Long Ranger polyacrylamide gel on an Automated Laser Fluorescence sequencing device and analyzed using the ALLELELINKS® 1.00 software (Amersham Pharmacia Biotech, Freiburg, Germany). Evaluation of LOH was performed as described [10].

Semiquantitative PCR-analysis

For studying amplification of *EGFR* a differential duplex-PCR with the *CFTR* gene marker was carried out, as described [11]. Briefly, we calculated the *EGFR/CFTR* ratios [x] from peripheral blood DNA of 20 healthy Caucasian adults. A value [$x + 3SD$] was considered as the cut-off level for the normal gene copy number. Ratios higher than [$x + 3SD$] were regarded as evidence of more than 2 copies of the *EGFR* gene.

Sequencing analysis of p53 and PTEN

Mutational analysis of *p53* and *PTEN* were performed in the DNA from both tumors and corresponding leukocytes. After amplifying all exons and intron-exon boundaries, we analyzed the PCR products on 1% agarose gel and excised and eluted them in sterile H_2O. Subsequently, they were subjected to cycle sequencing reactions using the Thermo Sequenase® Fluorescent Cycle Sequencing kit (Amersham Pharmacia Biotech). The cycle sequencing products were resolved using a denaturing 6.5% Long Ranger polyacrylamide gel on a sequencing device. *PTEN* primer sequences and PCR conditions were previously described [12]. *P53* primer sequences and PCR conditions are based on genome database entries (http://www.ncbi.nlm.nih.gov). Briefly, PCR reactions were carried out using a mixture containing 150 ng DNA, 10 mM Tris, 2 mM $MgCl_2$, HCl (pH 8.3), 0.2 mM dNTP, 10 mM concentrations of each primer and 0.5 unit of Taq DNA Polymerase. Temperature cycles and times for PCR reactions were: denaturation at 94°C for 30 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s. Each PCR reaction was preceded by a 3-min denaturation at 94°C, and the final cycle was followed by a 3-min extension at 72°C. The total number of cycles for PCR amplification was 25–30, depending on the sample DNA.

Statistical analysis

Student *t*-test, Chi-square test, Fisher's exact test and Mann–Whitney U-test, were performed to compare differences between groups depending on the analyzed variables. Spearman correlation test was used to evaluate the correlation between two parameters. Confidence interval (CI) was obtained through logistic regression. Survival studies were performed with the Kaplan Meier analysis and the log-rank test. A value of $P < 0.05$ was considered to be significant. Analyses were performed with the SPSS software (version 10, SPSS Inc., Chicago, IL, USA).

Results

Clinico-pathological characteristics of the patients

The male/female ratio was 1:1. The median age at diagnosis of primary tumor was 59.5 years (range: 27–69; SD: 12.25). The median time to tumor recurrence was 7.5 months (range: 3–24 months; SD: 5.1; 95%-CI: 5.3–7.4 months). With one only exception, all patients displayed at diagnosis a Karnofsky performance score (KPS) of >70 points. An association between age or KPS and TTR was not evidenced ($P = 0.35$, $\rho = 0.30$, Spearman correlation test). There were no significant associations between mutation of *p53* or *EGFR* amplification and either survival time ($P = 0.250$ and 0.127, respectively, log-rank test and Kaplan–Meier) or time to tumor relapse ($P = 0.210$ and 0.287, respectively, log-rank test and Kaplan–Meier).

Genetic analyses

In primary GBM we have observed *EGFR* amplification without mutations of *p53* in 12 cases, thus indicating a type II GBM profile. Four further cases showed *p53* mutations (Table 1, Fig. 1) in the absence of *EGFR* amplification, which was indicative of a type 1 GBM signature. In four

Table 1 Sequence variations of *p53* in type 1 glioblastomas

p53 sequence variations					
Case	Sequence variation	Base exchange	Aminoacid substitution	CpG site	Distribution
G3p	c.278C>T	CCT→TCT	Pro→Ser	No	Somatic mutations in 74 tumors Germline mut. in 2 Li-Fraumeni[a]
	c.266G>T	GGA→GTA	Gly→Val	No	Somatic mutations in 44 tumors No germline mutations[a]
G3r	c.278C>T	CCT→TCT	Pro→Ser	No	Somatic mutations in 74 tumors Germline mut. in 2 Li-Fraumeni[a]
	c.266G>T	GGA→GTA	Gly→Val	No	Somatic mutations in 44 tumors No germline mutations[a]
G7p	c.72C>G (Arg72Pro)	CCC→CGC	Arg→Pro	No	Validated polymorphism Higher risk lung cancer [34]
	IVS3+21_37dup (*P53*PIN3)				Validated polymorphism Higher risk of CRC [36]
	c.278C>T	CCT→TCT	Pro→Ser	No	Somatic mutations in 74 tumors Germline mut. in 2 Li-Fraumeni[a]
G7r	c.72C>G (Arg72Pro)	CCC→CGC	Arg→Pro	No	Validated polymorphism Higher risk lung cancer [34]
	IVS3+21_37dup (*P53*PIN3)				Validated polymorphism [a]Higher risk of CRC [36]
	c.278C>T	CCT→TCT	Pro→Ser	No	Somatic mutations in 74 tumors Germline mut. in 2 Li-Fraumeni[a]
G14p	c.14G>C	CTG→CTC	Leu→Leu	No	Not described mutation
	c.273G>A	CGT→CAT	Arg→His	Yes	Somatic mutations in 733 tumors Germline mut. in 16 Li-Fraumeni[a]
G14r	c.14G>C	CTG→CTC	Leu→Leu	No	Not described mutation
	c.273G>A	CGT→CAT	Arg→His	Yes	Somatic mutations in 733 tumors Germline mut. in 16 Li-Fraumeni[a]
G16p	c.273G>A	CGT→CAT	Arg→His	Yes	Somatic mutations in 733 tumors Germline mut. in 16 Li-Fraumeni[a]
	IVS2+38G>C				Validated polymorphism [41]
G16r	c.273G>A	CGT→CAT	Arg→His	Yes	Somatic mutations in 733 tumors Germline mut. in 16 Li-Fraumeni[a]
	IVS2+38G>C				Validated polymorphism [41]

[a] According to *p53* data bases entries, *mut* mutation, *CRC* colorectal cancer

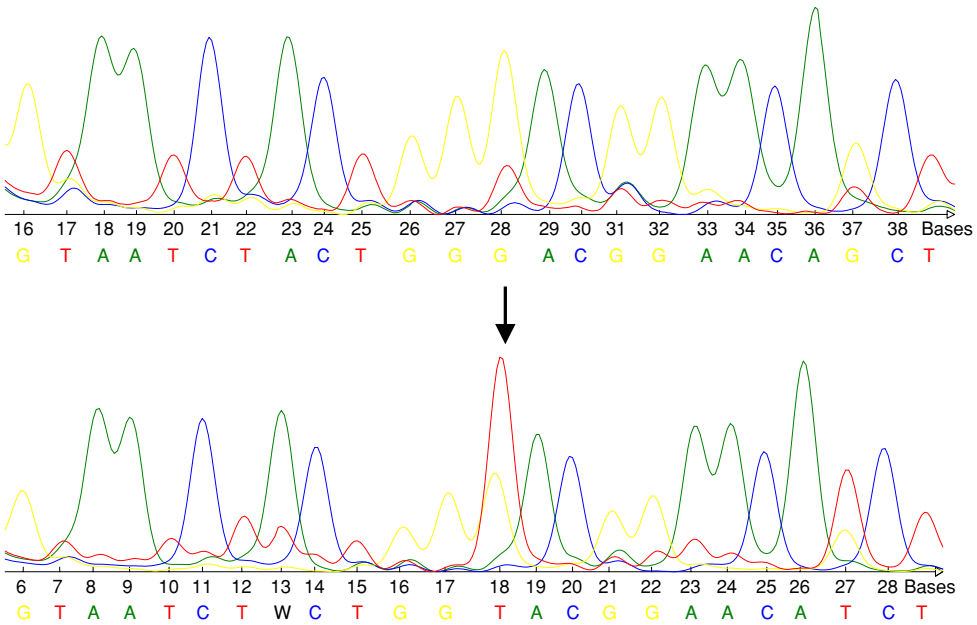

Fig. 1 *Upper row*: wild type sequence of *p53*. *Lower row*: base exchange mutation (GGA→GTA) of *p53* (exon 8, codon 266), predicting the aminoacid substitution Gly→Val. The arrow indicates the base exchange

GBM neither *p53* mutations nor *EGFR* amplification were identified (non-type 1–non-type 2 GBM).

At the time of recurrence, every type 1 GBM and type 2 GBM conserved their genetic pattern concerning *p53* mutational status and *EGFR* amplification. On the other hand, relapses of non-type 1–non-type 2 GBM showed in all cases a new *EGFR* amplification, thus acquiring the signature of type 2 GBM. Additional *PTEN* mutations in relapse cases were observed only among type 2 GBM (Table 2). Overall, additional LOH were observed more frequently in relapses of type 2 GBM (83.3%), than in those of type 1 (50%) or non-type 1–non-type 2 tumors (25%). Further results are shown in Table 3.

Among *p53* mutations there were one C:G to A:T transition mutation at CpG site (codon 273), one C>T transition at non-CpG site (codon 278), two transversion mutations at non-CpG sites (G>C at codon 14 and G>T at codon 266, Fig. 1) as well as one validated polymorphism in exon 4 (G>C at codon 72). Moreover, we have observed two validated intronic polymorphisms in intron 3 (IVS3+21_37dup) and intron 2 (IVS2+38G>C). One of the mutations (c.14G>C) has not been previously described in *p53* data bases (http://www.p53.iarc.fr, http://www.p53.free.fr). A detailed characterization of *p53* sequence variations is shown in Table 1.

Concerning *PTEN* mutations, we have identified four transition mutations in exons 2, 5, 6 and 7, in all cases predicting aminoacid substitution. Furthermore, there was an acceptor site splice mutation at the conserved junction nucleotides (IVS8-2A>G). Finally, we have observed two transversion mutations in exon 5 (c.105G>T) and exon 4 (c.75C>A, Fig. 2) as well as four intronic polymorphisms (Table 2). The transition in exon 6 (c.177A>G), the splicing mutation and two of the polymorphisms have not been previously reported (Table 2).

The results of *EGFR* amplification analysis and wide-genome LOH are shown in Table 3 and Supplementary Table 1 (*EGFR/CFTR* ratios). Interestingly, GBM cases harboring mutations of *p53* always displayed LOH at 17p13 as well. Similarly, tumors featuring *PTEN* mutations showed LOH at 10q23, at least in one of the microsatellites used to analyze this region.

Discussion

GBM mostly exceed in its occurrence and mortality beyond any other primary brain tumor in adults [13]. On molecular level, two subsets of GBM have been recognized on the basis of genetic make-up and clinical features [14, 15]. Type 2 (de novo) GBM occurs commonly in elderly patients and exhibit overexpression and/or amplification of *EGFR*. Type 1 (secondary) GBM shows typically histological progression from a previous low grade astrocytoma and affects young patients. Paradigmatically, mutations of *p53* will be observed (for review see [15]). Most of GBM cases belong to the type 2 group and genetic events are mutually exclusive for *EGFR* and *p53*. Moreover, there are GBM without either *EGFR* amplification or *p53* mutations (non-type 1–non-type 2 GBM) [16, 17]. During the past decade, accumulated evidence pointed to that type 1 and type 2 GBM constitute distinct disease entities developing through different genetic pathways [13, 14], showing different RNA and protein patterns [18, 19] and also probably differing in response to treatment [15].

Table 2 Sequence variations of *PTEN* in first and relapse glioblastomas

PTEN sequence variations				
Case	Sequence variation	Mutation effect	Aminoacid substitution	Distribution
G1p	c.44C>T	GGC→GGT	Gly→Gly	Bladder cancer [39]
	c.105G>T	TGT→TTT	Cys→Phe	Gliomas [38]
G1r	c.44C>T	GGC→GGT	Gly→Gly	Bladder cancer [39]
	c.105G>T	TGT→TTT	Cys→Phe	Gliomas [38]
G2p	c.177A>G	TAT→TGT	Tyr→Cys	Not described mutation
	IVS8+32G>T			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
G2r	c.177A>G	TAT→TGT	Tyr→Cys	Not described mutation
	IVS8+32G>T			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
G4	c.105G>T	TGT→TTT	Cys→Phe	Gliomas [38]
	IVS8+32G>T			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
G4r	c.105G>T	TGT→TTT	Cys→Phe	Gliomas [38]
	IVS8+32G>T			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
G5p	IVS5-28_29ins C			Not described polymorphism
	IVS2-96A>G			Not described polymorphism
G5r	IVS5-28_29ins C			Not described polymorphism
	c.88A>G	TAT→TGT	Tyr→Cys	Head neck squamous cell cancer [42]
G11p	IVS5-28_29ins C			Not described polymorphism
G11r	IVS5-28_29ins C			Not described polymorphism
	c.88A>G	TAT→TGT	Tyr→Cys	Head & neck squamous cell cancer [42]
	IVS8-2A>G	Splice mutation		Not described mutation
G13p	IVS5-28_29ins C			Not described polymorphism
G13r	IVS5-28_29ins C			Not described polymorphism
	IVS8-2A>G	Splice mutation		Not described mutation
	c.75C>A	CAT→AAT	His→Asn	Not described mutation
G17p	IVS4 109_110 ins TCTTA			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
	c.177A>G	TAT→TGT	Tyr→Cys	Not described mutation
G17r	IVS4 109_110 ins TCTTA			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
	c.177A>G	TAT→TGT	Tyr→Cys	Not described mutation
	IVS5-28_29ins C			Not described polymorphism
G19p	IVS8+32G>T			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
G19r	IVS8+32G>T			Validated polymorphism
				Cowden/Bannayan-Riley syndromes [40]
	c.252A>G	GAT→GGT	Asp→Gly	Glioma [43]

After multimodal therapy, GBM almost always recur. Although this is a capital feature of GBM, only few investigations with series of match paired patients are available from the literature and available data is partially contradictory [7–9, 20, 21]. We aimed to investigate whether molecular subtypes of GBM also show different profiles at the time of relapse.

In our genetic analysis, we have observed two patterns of tumor recurrence (Fig. 3): GBM type 1 and type 2 retained upon recurrence their genetic alterations affecting

Table 3 Review of genetic changes of primary and relapsed glioblastomas

Case	Sex	Age*	Treatment	TTR	Mut. *p53*	Ampl. *EGFR*	Mut. *PTEN*	Loss of heterozygosity (LOH)					
								1p35	9p21	13q14	19q13	10q23	17p13
G1p	m	60	no ther	3	n	y	y						
G1r	m	60	no ther		n	y	y						
G2p	m	60	RT	6	n	n	y						
G2r	m	60	A,V		n	y	y						
G3p	f	62	RT	7	y	n	n						
G3r	f	62	A,V		y	n	n						
G4p	m	64	RT	3	n	y	y						
G4r	m	64	no ther		n	y	y						
G5p	m	49	RT	9	n	y	n						
G5r	m	49	A,V		n	y	y						
G6p	f	57	RT/ A,V	10	n	n	n						
G6r	f	58	A,V		n	y	n						
G7p	f	31	no ther	9	y	n	n						
G7r	f	32	RT/A,V		y	n	n						
G8p	m	39	RT	12	n	n	n						
G8r	m	40	no ther		n	y	n						
G9p	m	69	RT/A,V	16	n	n	n						
G9r	m	70	A,V		n	y	n						
G10p	m	46	RT	17	n	y	n						
G10r	m	47	A,V		n	y	n						
G11p	m	37	RT	5	n	y	n						
G11r	m	38	no ther		n	y	y						
G12p	f	65	RT	6	n	y	n						
G12r	f	65	A,V		n	y	n						
G13p	f	55	RT	8	n	y	n						
G13r	f	56	no ther		n	y	y						
G14p	f	68	RT	9	y	n	n						
G14r	f	69	A,V		y	n	n						
G15p	f	64	RT	8	n	y	n						
G15r	f	65	no ther		n	y	n						
G16p	f	27	RT	24	y	n	n						
G16r	f	29	T		y	n	n						
G17p	m	61	RT	8	n	y	y						
G17r	m	62	A,V		n	y	y						
G18p	f	59	RT/T	12	n	y	n						
G18r	f	60	T		n	y	n						
G19p	m	67	RT/T	9	n	y	n						
G19r	m	67	T		n	y	y						
G20p	f	58	RT/T	14	n	y	n						
G20r	f	59	T		n	y	n						

TTR time to tumor relapse (months), *Mut* mutation, *Ampl* amplification, *p* primary (first tumor), *r* relapse (first relapse), *m* male, *f* female, *no ther* no adjuvant therapy performed, *RT* radiotherapy, *A* ACNU (nimustine), *V* VM26 (teniposide), *T* temozolomide. *Black boxes* loss of heterozygosity, *grey boxes* retention of heterozygosity, *white boxes* not informative marker

* Age (years) at first operation

p53 and *EGFR*. In contrast, all relapses of non-type 1–non-type 2 GBM showed a new *EGFR* amplification, thus they acquired a type 2 GBM profile. Accumulation of additional genetic alterations upon relapse included *PTEN* mutations in four type 2 GBM (but not in the other subtypes). Additional LOH were more frequently observed in relapses of type 2 GBM (in 10/12 cases) compared to relapses of type 1 GBM and non-type 1–non-type 2 GBM (Table 3).

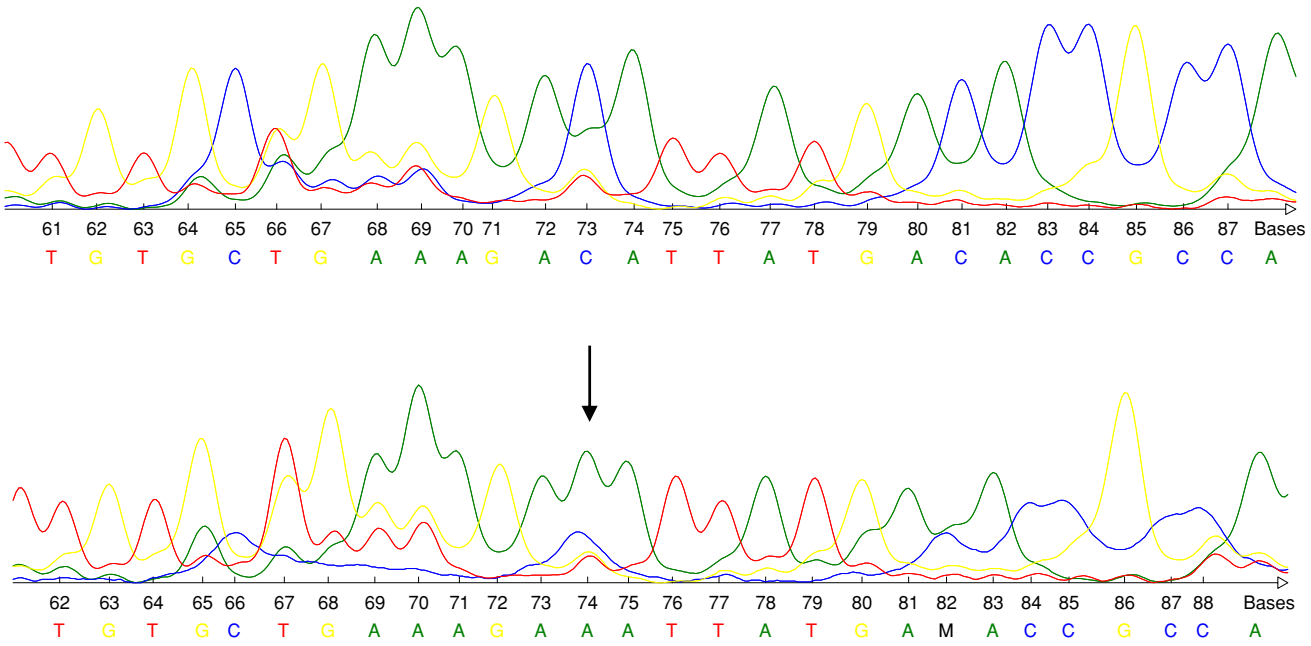

Fig. 2 *Upper row*: wild type sequence of *PTEN*. *Lower row*: base exchange mutation (CAT→AAT) of *PTEN* (exon 4, codon 75) predicting the aminoacid substitution His→Asn. The arrow indicates the base exchange

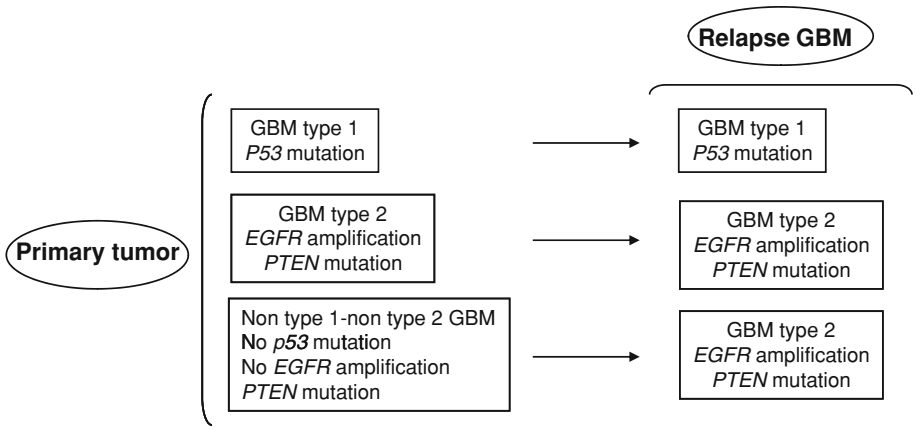

Fig. 3 Graphic showing two molecular patterns of GBM recurrence

The accumulation of alterations during the clonal expansion of transformed glial cells is well known in GBM, confer tumor cells a growth advantage and underlies the genetic heterogeneity of GBM [22, 23]. This phenomenon contributes to explain the molecular differences between primary tumors and relapses [9, 24, 25].

As a part of one previous investigation we had characterized the promoter methylation status of the DNA repair gene *MGMT* in all type 1 GBM (Table 3, cases G3, G7, G14 and G16), non-type 1–non-type 2 GBM (Table 3, cases G2, G6, G8 and G9) as well as eight type 2 GBM here described [26]. This analysis together with the present results of *p53* and *EGFR* have evidenced similar rates of *MGMT* methylation for the three GBM subtypes, and they are in accordance with the overall methylation rates reported in glioblastoma [5, 27]. Typically, *MGMT* was methylated in the first GBM and remained always methylated upon relapse. Thus, our results also confirmed the observation that, in the pathogenesis and progression of GBM, *MGMT* becomes hypermethylated at an early stage [28, 29] and remains methylated through progression, a situation which was has been described in small adenomas and carcinomas of colorectal origin as well [30]. The *p53* mutational analysis in those type 1 GBM with methylated *MGMT* showed G:C to A:T transition within CpG site (c.273 CGT→CAT) in two cases. Further mutations of *p53* in tumors with methylated *MGMT* (1 case) occurred in non-CpG sites. From *p53* data bases entries, approximately 52% of mutational events are transition mutations and, of this subset, about 72% are G:C to A:T transitions (http://www.p53.iarc.fr, http://www.p53.free.fr) [31], although the signature of the mutational spectrum varies according to

tumor type. It was previously suggested a link [29] between *MGMT* methylation and the presence of G:C to A:T transition mutations in *p53* in gliomas. In the present study we could confirm the former in two patients (cases G14p, G14r, G16p and G16r, Table 1); in these cases, G:C to A:T transitions could be attributable to a inactivation in *MGMT* that allows O^6-methylguanine to persist and be read as an adenine. Our observations contrast with a recent investigation about GBM reporting only 4% of mutations of *p53* in tumors with methylated *MGMT* within CpG sites [32] although we should take in mind the size of our type 1 GBM collective, clearly too short to state a definite tendency.

Strikingly, we have identified in cases G7p and G7r the exonic *p53* polymorphism c.72C>G (Arg72Pro) together with the intronic polymorphism IVS3+21_37dup (*P53*PIN3). Several investigations have pointed the association between *p53* polymorphisms and an increased risk for different cancers: for instance, in the case of the exonic c.72 variant such association was suggested since the p53 protein with Arg72 is more efficient in inducing apoptosis than the one with the Pro72 variant [33]. In clinical studies, the variants c.72 Pro/Pro and c.72 Arg/Pro have been associated with an elevated risk of lung cancer [34, 35]. Recently, intron 3 duplication (IVS3+21_37dup) has been found to be associated with increased risk of colorectal cancer [36]. However, the authors could not determine whether this intron 3 duplication alone influences mRNA stability or if this effect requires the codon Pro72 variant. To our knowledge, the occurrence of both polymorphisms in GBM patients has not been reported before and pointed to a possible role in brain cancer as well.

With respect to *PTEN*, we have identified mutations only in type 2 GBM. In four cases, *PTEN* mutations occurred in both the first GBM and the relapse. In four further cases, new mutations were observed in the recurrences but not in the first tumor. The rate and pattern of mutations are in accordance with previous reported data [37]. All tumors with *PTEN* mutations also harbored LOH at the chromosome region 10q23, which pointed to the inactivation of this tumor suppressor gene and make patent its contribution to the tumorigenesis and progression in GBM.

Taken together, our results strongly suggest that GBM relapses, compared to the corresponding first tumor, accumulate additional molecular alterations and may develop along two distinct pathways, which contribute to delineate the different profiles of type 1, type 2 and non-type 1–non-type 2 GBM.

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