J Neurooncol
DOI 10.1007/s11060-010-0129-5

LABORATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

Prognostic significance of histological grading, p53 status, YKL-40 expression, and *IDH1* mutations in pediatric high-grade gliomas

Manila Antonelli · Francesca Romana Buttarelli · Antonietta Arcella · Sumihito Nobusawa · Vittoria Donofrio · Hiroko Oghaki · Felice Giangaspero

Received: 6 October 2009 / Accepted: 25 January 2010
© Springer Science+Business Media, LLC. 2010

Abstract The objective of this study was to evaluate, in a series of 43 pediatric high-grade gliomas (21 anaplastic astrocytoma WHO grade III and 22 glioblastoma WHO grade IV), the prognostic value of histological grading and expression of p53 and YKL-40. Moreover, mutational screening for *TP53* and *IDH1* was performed in 27 of 43 cases. The prognostic stratification for histological grading showed no difference in overall (OS) and progression-free survival (PFS) between glioblastomas and anaplastic astrocytomas. Overexpression of YKL40 was detected in 25 of 43 (58%) cases, but YKL-40 expression was not prognostic in terms of OS and PFS. p53 protein expression was observed in 13 of 43 (31%) cases but was not prognostic. *TP53* mutations were detected in five of 27 (18%) cases (four glioblastomas and one anaplastic astrocytoma). Patients with *TP53* mutation had a shorter median OS (9 months) and PFS (8 months) than those without mutations (OS, 17 months; PFS, 16 months), although this trend did not reach statistical significance ($p = 0.07$). *IDH1* mutations were not detected in any of the cases analyzed. Our results suggest that in pediatric high-grade gliomas: (i) histological grading does not have strong prognostic significance, (ii) YKL-40 overexpression is less frequent than adult high-grade gliomas and does not correlate with a more aggressive behavior, (iii) *TP53* mutations but not p53 expression may correlate with a more aggressive behavior, and (iv) *IDH1* mutations are absent. These observations support the concept that, despite identical histological features, the biology of high-grade gliomas in children differs from that in adults, and therefore different prognostic factors are needed.

Keywords Pediatric high-grade glioma · YKL-40 expression · *TP53* mutation · p53 expression · *IDH1* mutation

M. Antonelli · F. Giangaspero (✉)
Department of Experimental Medicine, Sapienza University, Rome, Italy
e-mail: felice.giangaspero@uniroma1.it

F. R. Buttarelli
Department of Neurological Sciences, Sapienza University, Rome, Italy

A. Arcella · F. Giangaspero
IRCCS Neuromed Pozzilli (Isernia), Pozzilli, Italy

S. Nobusawa · H. Oghaki
Section of Molecular Pathology, International Agency for Research on Cancer (IARC), Lyon, France

V. Donofrio
Department of Pathology Ospedale Pausilipon, AORN Santobono-Pausilipon, Naples, Italy

Introduction

Pediatric high-grade gliomas (pHGGs) are less frequent than their adult counterparts, but they account for 15% of all pediatric brain tumors, and show high mortality and morbidity [1]. They are anaplastic astrocytoma (WHO grade III) or glioblastoma (WHO grade IV), which are histologically indistinguishable from their counterparts in adults. The WHO grading system does not make any distinction between HGGs in children and in adults [2, 3]. In adults, there are two subtypes of glioblastomas, i.e. primary (de novo) glioblastomas and secondary glioblastomas which develop though progression from low-grade or anaplastic astrocytomas [4, 5]. Primary glioblastomas affect predominantly older patients (mean, 62 years) and frequently exhibit epidermal growth factor receptor

Published online: 21 February 2010

(*EGFR*) amplification, whereas secondary glioblastomas occur in relatively younger adults (mean 45 years) and are genetically characterized by frequent *TP53* mutations and *IDH1* mutations but infrequent *EGFR* amplification [4–6].

Most pediatric glioblastomas seem to occur de novo [3]. However, in contrast with adult primary glioblastomas, they exhibit frequent *TP53* mutations and infrequent *EGFR* amplification [7, 8]. Cytogenetics studies [9] and more recent gene-expression profiling [10–12] have also demonstrated that adult and pediatric high-grade gliomas have distinct molecular profiles, suggesting intrinsic biological differences between the two groups.

Numerous biomolecular markers have been explored to stratify adult patients with malignant gliomas in prognostic subgroups [13]. Among these YKL-40 protein overexpression is a negative prognostic marker probably related to increased resistance of tumor cells to radiation [14]. However, YKL-40 expression has not been investigated, so far, in pHGG. Alteration of the p53 pathway, in terms both of *TP53* mutations and overexpression, which is an important characteristic in the malignant progression, has not been found helpful in prognostic stratification of adult malignant astrocytoma [13, 15, 16]. In contrast in pHGGs in which this type of tumor progression is not present, TP53 mutations and p53 overexpression seem to be related to an adverse prognosis [7]. Recent studies have indicate that IDH1 mutations are a strong predictor of more favorable prognosis and a highly selective marker of secondary glioblastomas [6, 17]. However few studies have so far specifically investigated IDH1 mutations in a large series of pHGG to verify its prognostic value [18]. The objective of this study was to evaluate, in a series of 43 pHGG (21 anaplastic astrocytoma WHO grade III and 22 glioblastoma WHO grade IV), the prognostic value of histological grading and expression of p53 and YKL-40. Moreover mutational screening for TP53 and IDH1 was performed in 27 of 43 cases to evaluate the effect, if any, of such mutations in the prognosis of pHGG.

Materials and methods

Patients and tissue samples

We studied 43 patients with malignant diffuse gliomas (anaplastic astrocytoma WHO grade III and glioblastoma WHO grade IV). Anaplastic ependymoma or malignant mixed glio-neuronal tumors were not included in the study. The mean age was 9.4 years (range, 1 month to 17 years); eight patients were infants (0–3 years). The cases were re-reviewed by two neuropathologists (M.A. and F.G.) and classified according to the WHO classification as follows: 21 anaplastic astrocytomas WHO grade III and 22 glioblastomas WHO grade IV. The female:male ratio was 1.2:1. The median age of children with grade IV glioma was similar to that with grade III (10 years vs. 9 years).

Immunohistochemistry

Monoclonal antibodies for p53 (DO-7; Dako, Carpinteria, California, USA; dilution 1:300), and polyclonal anti-YKL-40 antibody (Quidel Corporation, San Diego, CA;, USA; dilution 1:100) were used. Sections were deparaffinized and rehydrated through a graded series of xylene–ethanol and incubated for 15 min in 3% hydrogen peroxide to inhibit endogenous peroxidases. Antigen retrieval was performed by boiling the slides for 15 min in citrate buffer. Tissue sections were incubated with the primary antibody. The sections were then incubated with biotinylated secondary antibody for 20 min at room temperature. This was followed by incubation for 20 min with avidin–biotin peroxidase complex (Dako). Visualization was performed using 3,3-diaminobenzidine. Tissue sections were counterstained with hematoxylin, dehydrated, and mounted. As a negative control the primary antibodies were omitted.

Staining for YKL-40 was scored using a three-tiered system: 2+, strongly positive staining of most tumor cells in at least one medium-power microscopic field (×100); 1+, weak/patchy staining in tumor cell; 0, no staining [14]. Immunoreactivity for p53 was scored only in cells with dense nuclear staining according to Pollack et al. [7]. Tumors were categorized as expressing little or no p53 (a grade of 0 or 1), similar to normal brain tissue, or as overexpressing p53, with staining observed in a sizable subgroup of cells (25–50%; grade 2), most cells (50–75%; grade 3), or nearly all cells (>75%; grade 4) in the high-power field in areas with maximum staining.

TP53 and *IDH1* mutations

DNA was extracted from paraffin-embedded sections as previously described [4]. PCR-SSCP analysis was carried out to prescreen for mutations in exon 4 of the *IDH1* gene [15], and in exons 5–8 of *TP53* as previously described [4]. Briefly, PCR was performed in a total volume of 10 µl, consisting of 1 µl DNA solution (approximately 100 ng/µl), 0.5 U platinum Taq DNA polymerase (Invitrogen, Cergy Pontoise, France), 0.1 mCi [α-^{33}P]dCTP (ICN Biomedicals, Costa Mesa, CA, USA; specific activity, 3000 Ci/mmol), 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.25 mM of each primer, 10 mM Tris–HCl, pH 8.3, and 50 mM KCl in a thermal cycler (Biometra, Archamps, France) with an initial denaturing step at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 56–64°C for 30 s, extension at 72°C for 40 s, and a final extension at 72°C for 10 min. PCR products

(10 µl) were mixed with 20 µl loading buffer (0.02 M NaOH, 95% formamide, 20 mM EDTA, 0.05% xylene cyanol, and bromophenol blue), denatured at 95°C for 10 min and quenched on ice, and then 5.5 µl of this mixture was electrophoresed on a 12.5% polyacrylamide non-denaturing gel containing 10% glycerol at 45 W for 2.5 h with cooling by a fan. Gels were dried at 80°C and autoradiographed for 24–36 h. Samples with variant bands in SSCP analyses were further analyzed by direct sequencing on an ABI 3100 Prism DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the Big Dye terminator cycle sequencing kit (ABI Prism, Applied Biosystems).

Statistical analysis

The primary clinical endpoints were overall survival time (OS) and progression-free survival (PFS). Overall survival was determined from the date of diagnosis to the date of death or last follow-up. Progression-free survival was determined from the date of diagnosis to the date of relapse. Patients alive at last follow-up or without documented time to progression at last follow-up were considered censored. OS and PFS curves were estimated with use of the two-sided log-rank test.

Results

Patient characteristics and histological grading

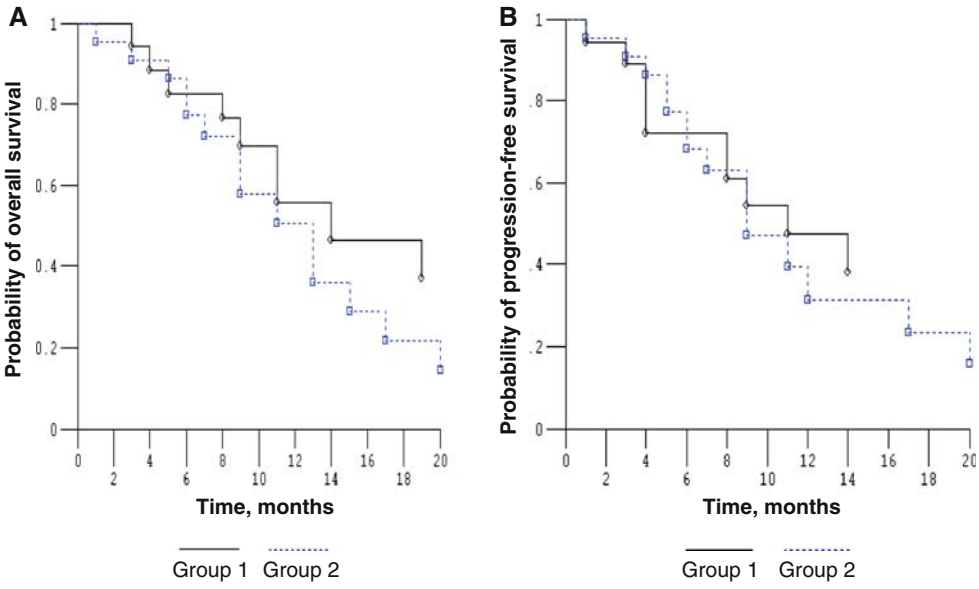
The median overall survival of patients with pHGGs was 13 months (range 0.1–89), and the median PFS was 11 months (range, 0.1–62 months). Glioblastomas showed a shorter median survival (13 months: range 0.2–60 vs. 14 months: range, 3–89 months) and progression-free survival (9 months: range, 0.7–60 vs. 14 months: range, 0.1–62 months) than anaplastic astrocytomas, but the difference was not statistically significant ($P = 0.9$) (Fig. 1).

YKL40 and p53 immunohistochemistry

YKL40 protein expression was detected in 25/43 (58%) cases (Fig. 2). A slight predominance of positive cases was observed in glioblastomas compared with anaplastic astrocytomas (63% vs. 52%) (Table 1). In addition to tumor cells, YKL-40 immunoreactivity was also detected in blood vessels, in extracellular matrix, in neurons, and in neutrophilis. For the outcome analysis, tumors were classified according to the presence (1+, 2+) or absence (0) of YKL40 expression. Statistical analysis comparing the two groups with different YKL-40 expression did not show prognostic stratification in terms of OS and PFS ($P = 0.9$). p53 expression (p53 immunoreactivity on >25% of cells) was observed in 13/43 of cases (31%) (Fig. 3; Table 2). Statistical analysis comparing the two groups did not show differences in OS and PFS ($P = 0.8$).

TP53 and *IDH1* mutations

Of 27 tumors in which the quality of the DNA was sufficient for genetic analyses, five (18%) had *TP53* mutations in exon 6 and exon 8. Four were glioblastomas and one anaplastic astrocytoma. The age of patients with *TP53* mutations ranged from 4 to 17 years. Patients with *TP53*



Group 1 : Anaplastic astrocytoma (gradeIII)
Group 2 : Glioblastoma (gradeIV)

Fig. 1 Estimates of overall survival (**a**) and progression-free survival (**b**) for grade III gliomas and grade IV gliomas does not show a significant statistical difference in terms of survival between the two groups

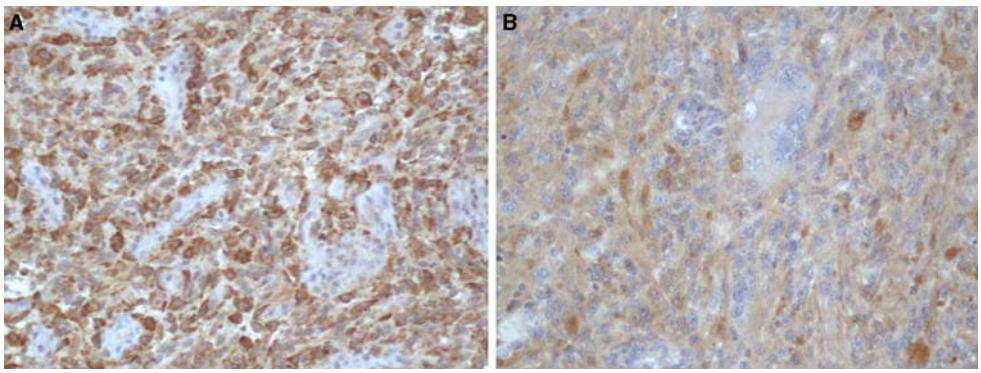


Fig. 2 Expression of YKL40 in neoplastic cells and matrix. **a** strongly positive staining of most tumor cells (score 2) **b** example of weak/patchy staining in tumor cells (score 1)

Table 1 YKL40 protein expression in pediatric high-grade gliomas

	YKL 40-negative (Score 0)	YKL 40-positive (Score 1+/2+)	Total
Anaplastic astrocytoma	10	11	21
Glioblastoma	8	14	22
Total	18	25	43

mutations had a median OS of 9 months and PFS of 8 months. Among patients without mutations, OS and PFS were longer (17 and 16 months respectively), but this trend did not reach statistical significance ($P = 0.07$; Fig. 4). No *IDH1* mutation was detected in any of the 27 cases analyzed.

Discussion

In adult glioblastomas, YKL-40 protein expression, which is observed in 77% of cases, is significantly associated with increased resistance to radiotherapy and reduced overall survival [14]. The biological function of YKL-40 in cancer and the mechanism by which it reflects cancer aggressiveness are poorly understood. YKL-40 has been shown to initiate the phosphoinositide 3-kinase (PI-3 K)/Akt pathway and the Ras signaling pathway including the mitogen-activated protein kinase (MAPK) pathway, which are important pathways associated with mitogenesis and cell survival [19, 20]. In addition, YKL-40 induces stimulation of proliferation of fibroblasts [19], and modulation of collagen formation [21] facilitating tumor invasion and metastasis. Such mesenchymal functions found support in microarrays analysis in high-grade gliomas [22]. In this study, adult HGG associated with poor prognosis had an upregulated YKL40 gene together with others genes activated in mesenchymal tissues. In this study, we found YKL-40 expression in 58% of pHGG with a slightly higher percentage in glioblastomas (63%) than in anaplastic astrocytomas (52%), However, YKL40 expression was not prognostic for OS and PFS. Such lack of correlation may be because of the intrinsic molecular differences between adult and pediatric malignant gliomas [23].

It has been reported that p53 protein expression is more frequent in pediatric malignant gliomas than in adult cases [24]. One study reported p53 expression in 75% of pHGGs, whereas the *TP53* mutation was observed in 38% of cases [25]. Pollack et al. found *TP53* mutations in 33%, and p53 expression in 36% of pHGGs, and expression of p53 was associated with shorter overall survival of patients [7]. Suri et al. [26] showed p53 protein expression in 53% of pediatric glioblastomas. In this study, we found p53 protein expression in 31% of cases, and *TP53* mutations in 18% of cases. Although the data did not reach statistical significance, there was a trend toward an association between *TP53* mutation and shorter overall survival.

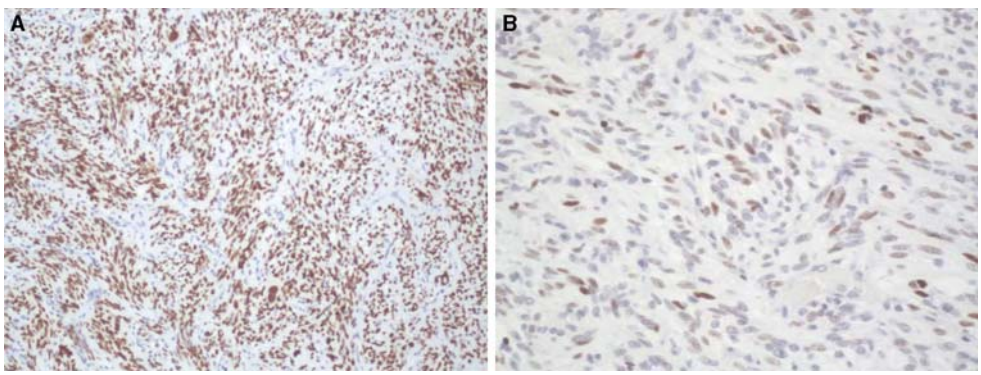


Fig. 3 Expression of p53 protein in pediatric high-grade gliomas in more than 75% of cells (**a**) and in less than 25% (**b**)

Table 2 p53 protein expression in pediatric high-grade gliomas

	p53 expression normal (Score 0, +1)		p53 overexpression (Score 2+, 3+, 4+)			Total
	0	1+	2+	3+	4+	
Anaplastic astrocytoma	13	4	0	4	0	21
Glioblastoma	13	0	0	5	4	22
Total	26	4	0	9	4	43

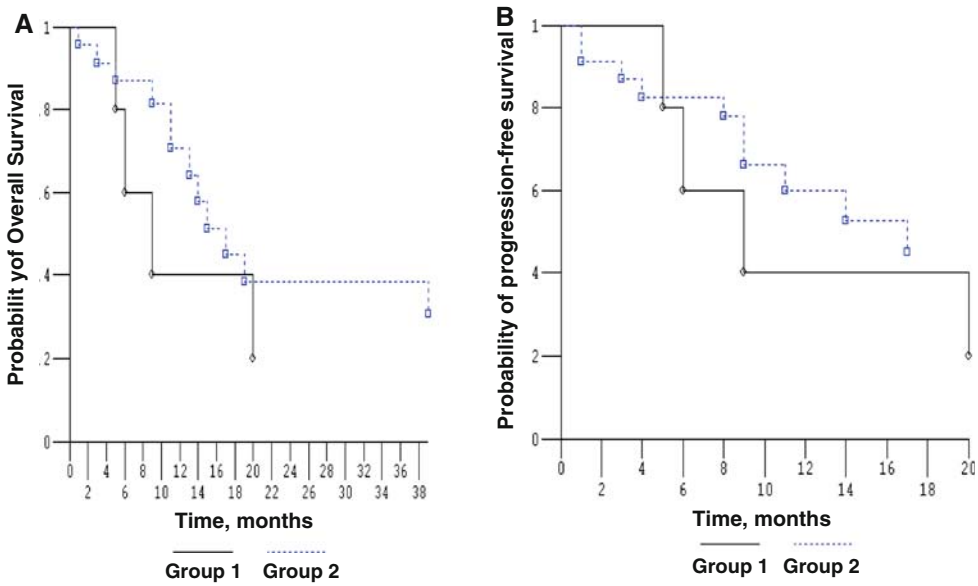


Fig. 4 Kaplan-Meier and log-rank tests show trends toward longer survival (**a**) and progression-free survival (**b**) in patients with wild-type *TP53*

Frequency of TP53 mutations in high-grade gliomas in children in our study was lower than in previous studies [7, 25, 26]. Such lower frequency is unlikely to be because of the methods we used to detect mutations (SSCP followed by DNA sequencing). We used the same methods in previous studies in which a high frequency of TP53 mutations was detected in low-grade diffuse astrocytomas and secondary glioblastoma [4, 27]. However, comparing our study with that of Pollack et al., which is numerically more relevant, the difference is not statistically significant ($p = 0.08$). Moreover, such discrepancies may be because of relatively small sample size and different age groups of patients between these two studies. As Pollack et al. [7] reported, frequency of TP53 mutations may be age-dependent. They showed that frequency of TP53 mutations were much lower (2/17; 12%) in younger children (<3 years) than in older children (24/60; 40%). We also carried out statistical analysis of patients in different age groups and again there were no significant differences between our study and the study by Pollack et al. ($p = 0.1329$).

In a recent genome-wide analysis, somatic mutations at codon 132 of the isocitrate dehydrogenase 1 (*IDH1*) gene were detected in 12% of adult glioblastomas [28]. Subsequent studies have shown that *IDH1* mutations are a highly selective and very frequent (>80%) molecular marker of secondary glioblastomas and their precursor lesions [6, 17, 29]. In adult glioblastomas, *IDH1* mutations have been associated with *TP53* mutations, young age, and longer survival [6, 17, 30, 31].

Few studies which included small numbers of pHGG did not find any IDH1 mutations in this age group [17, 29] De Carli et al. [18] reported that four of 73 (5%) diffuse gliomas in children carried an *IDH1* mutation, and that children with tumors carrying *IDH1* mutations were older than those with mutation-negative tumors (median age, 16 years vs. 7 years). Our study also included older children (six cases were >16 years old), but *IDH1* mutations were not detected in any of these cases. Taken together, these observations suggest that *IDH1* mutations are very rare, if not absent, in pediatric high-grade gliomas.

In this study we observed the lack of prognostic significance in terms of OS and PFS between anaplastic astrocytomas and glioblastomas. This lack of correlation has been reported in previous studies [32]. However others

have confirmed the prognostic significance of grading also in pHGG as in adult patients [33]. Further studies in large series are needed to confirm or not the prognostic value of the grading in pHGGs.

In summary, our results suggest that in pediatric high-grade gliomas:

1 histological grading does not have as strong prognostic significance as in adults;
2 YKL-40 overexpression is less frequent compared with adult high-grade gliomas and does not correlate with a more aggressive behavior;
3 *TP53* mutations but not p53 expression may correlate with a more aggressive behavior; and
4 *IDH1* mutations are absent.

These observations support the concept that, despite identical histological features, the biology of high-grade gliomas in children differs from that in adults, and therefore different prognostic factors are needed.

Acknowledgments This study was in part supported by "Il Fondo di Giò Onlus". Manila Antonelli is supported by a grant from Associazione Italiana per la Lotta al Neuroblastoma. We thank Maura Massimino and Maria Luisa Garrè for their assistance with the clinical information, and Alessandra Angiolosanto and Anne-Marie Camus for technical assistance.

References

1. Kaatsch P, Rickert CH, Kühl J, Schüz J, Michaelis J (2001) Population-based epidemiologic data on brain tumors in German children. Cancer. Dec 15:3155–3164
2. Kim TS, Halliday AL, Hedley-Whyte ET, Convery K (1991) Correlates of survival and the Daumas–Duport grading system for astrocytomas. J Neurosurg 74:27–37
3. Louis DN, Ohgaki H, Wiestler OD, Cavanee WK (eds) (2007) WHO classification of tumours of the central nervous system. IARC, Lyon
4. Ohgaki H, Dessen P, Jourde B, Horstmann S, Nishikawa T, Di Patre PL, Burkhard C, Schüler D, Probst-Hensch NM, Maiorka PC, Baeza N, Pisani P, Yonekawa Y, Yasargil MG, Lütolf UM, Kleihues P (2004) Genetic pathways to glioblastoma: a population-based study. Cancer Res 1:6892–6899
5. Ohgaki H, Kleihues P (2007) Genetic pathways to primary and secondary glioblastoma. Am J Pathol 170:1445–1453
6. Nobusawa S, Watanabe T, Kleihues P, Ohgaki H (2009) IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res 15:6002–6007
7. Pollack IF, Finkelstein SD, Woods J, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, Boyett JM, Finlay JL, Sposto R (2002) Expression of p53 and prognosis in children with malignant gliomas. N Engl J Med 346:420–427
8. Rood BR, Macdonald TJ (2005) Pediatric high grade glioma: molecular genetics clues for innovative therapeutic approaches. J Neurooncol 75:267–272
9. Rickert CH, Sträter R, Kaatsch P, Wassmann H, Jürgens H, Dockhorn-Dworniczak B, Paulus W (2001) Pediatric high-grade astrocytomas show chromosomal imbalances distinct from adult cases. Am J Pathol 158:1525–1532
10. Faury D, Nantel A, Dunn SE, Guiot MC, Haque T, Hauser P, Garami M, Bognár L, Hanzély Z, Liberski PP, Lopez-Aguilar E, Valera ET, Tone LG, Carret AS, Del Maestro RF, Gleave M, Montes JL, Pietsch T, Albrecht S, Jabado N (2007) Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. J Clin Oncol 25:1196–1207
11. Haque T, Faury D, Albrecht S, Lopez-Aguilar E, Hauser P, Garami M, Hanzély Z, Bognár L, Del Maestro RF, Atkinson J, Nantel A, Jabado N (2007) Gene expression profiling from formalin-fixed paraffin-embedded tumors of pediatric glioblastoma. Clin Cancer Res 13:6284–6292
12. Johnson R, Wright KD, Gilbertson RJ (2009) Molecular profiling of pediatric brain tumors: insight into biology and treatment. Curr Oncol Rep 11:68–72
13. Rivera AL, Pelloski CE, Sulman E, Aldape K (2008) Prognostic and predictive markers in glioma and other neuroepithelial tumors. Curr Probl Cancer 32:97–123
14. Pelloski CE, Mahajan A, Maor M, Chang EL, Woo S, Gilbert M, Colman H, Yang H, Ledoux A, Blair H, Passe S, Jenkins RB, Aldape KD (2005) YKL-40 expression is associated with poorer response to radiation and shorter overall survival in glioblastoma. Clin Cancer Res 1:3326–3334
15. Cunningham JM, Kimmel DW, Scheithauer BW, O'Fallon JR, Novotny PJ, Jenkins RB (1997) Analysis of proliferation markers and p53 expression in gliomas of associated origin: relationship and prognostic value. J Neurosurg 86:121–130
16. Danks RA, Chopra G, Gonzales MF, Orian JM, Kaye AH (1995) Aberrant p53 expression does not correlate with the prognosis in anaplastic astrocytoma. Neurosurgery 37:246–254
17. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360:765–773
18. De Carli E, Wang X, Puget (2009) IDH1 and IDH2 mutations in glioma. N Engl J Med 360:2248–2249
19. Recklies AD, White C, Ling H (2002) The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. Biochem J 1:119–126
20. Pelloski CE, Lin E, Zhang L, Yung WK, Colman H, Liu JL, Woo SY, Heimberger AB, Suki D, Prados M, Chang S, Barker FG 3rd, Fuller GN, Aldape KD (2006) Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma. Clin Cancer Res 1:3935–3941
21. Malinda KM, Ponce L, Kleinman HK, Shackelton LM, Millis AJ (1999) Gp38 k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. Exp Cell Res 250:168–173
22. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9:157–173
23. Pfister S, Witt O (2009) Pediatric gliomas. Recent Results Cancer Res 171:67–81
24. Pollack IF, Finkelstein SD, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, Finlay JL, Sposto R (2001) Age and TP53 mutation

frequency in childhood malignant gliomas: results in a multi-institutional cohort. Cancer Res 15:7404–7407
25. Cheng Y, Ng HK, Zhang SF, Ding M, Pang JC, Zheng J, Poon WS (1999) Genetic alterations in pediatric high-grade astrocytomas. Hum Pathol 30:1284–1290
26. Suri V, Das P, Jain A, Sharma MC, Borkar SA, Suri A, Gupta D, Sarkar C (2009) Pediatric glioblastomas: a histopathological and molecular genetic study. Neuro Oncol 11:274–280
27. Okamoto Y, Di Patre PL, Burkhard C, Horstmann S, Jourde B, Fahey M, Schüler D, Probst-Hensch NM, Yasargil MG, Yonekawa Y, Lütolf UM, Kleihues P, Ohgaki H (2004) Population-based study on incidence, survival rates, and genetic alterations of low-grade diffuse astrocytomas and oligodendrogliomas. Acta Neuropathol 108:49–56
28. 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 (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 26(321):1807–1812
29. Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C, von Deimling A (2008) Analysis of the IDH1 codon 132 mutation in brain tumors. Acta Neuropathol 116:597–602
30. Ichimura K, Pearson DM, Kocialkowski S, Bäcklund LM, Chan R, Jones DT, Collins VP (2009) IDH1 mutations are present in the majority of common adult gliomas but are rare in primary glioblastomas. Neuro Oncol (Epub ahead of print)
31. Sanson M, Marie Y, Paris S, Idbaih A, Laffaire J, Ducray F, Hallani SE, Boisselier B, Mokhtari K, Hoang-Xuan K, Delattre JY (2009) Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J Clin Oncol (Epub ahead of print)
32. Sanders RP, Kocak M, Burger PC, Merchant TE, Gajjar A, Broniscer A (2007) High-grade astrocytoma in very young children. Pediatr Blood Cancer 49:888–893
33. Wolff JE, Gnekow AK, Kortmann RD, Pietsch T, Urban C, Graf N, Kühl J (2002) Preradiation chemotherapy for pediatric patients with high-grade glioma. Cancer 94:264–271 2002