Correlation of chromosomal imbalances by comparative genomic hybridization and expression of EGFR, PTEN, p53, and MIB-1 in diffuse gliomas

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Abstract. The histological subclassification of gliomas is increasingly assisted by the underlying molecular genetics which has major importance in guiding clinical management of the disease. However, the assessment of several molecular events for improving clinical care remains a challenge. Herein, we report on comparative genomic hybridization (CGH) and immunohistochemical (IHC) assessment of EGFR, PTEN, p53, and MIB-1 expression in 13 oligodendrogliomas (10 WHO grade II, 3 WHO grade III), one oligoastrocytoma (WHO grade III) and 23 high-grade astrocytomas (3 WHO grade III, 20 glioblastoma multiforme). The most frequent imbalances in oligodendroglial tumors including the oligoastrocytic case were, in decreasing order of frequency, +7q, -1p, and -4q and in astrocytomas +7q, -10q, +7p, -9p, -10p, +20q, and +20p. Some individual imbalances were associated with increasing numbers of chromosomal changes, that were +7q in both oligodendrogliomas and astrocytomas, and -9p, -10q, +20p, and +20q in astrocytomas. The markers p53 and MIB-1 were significantly higher expressed in astrocytomas than in oligodendrogliomas and expression levels of p53 and EGFR were inversely associated within the astrocytic group. In addition, p53 overexpression correlated positively with +7q and negatively with -1p in the oligodendroglial group whereas EGFR overexpression correlated positively with -1p in the oligodendroglial and positively with +7p and -10p in the astrocytic group. Short overall survival was significantly associated with +7p and -10q in astrocytomas. Collectively, these results contribute to the increasing clinical relevance of assessing tumor biological markers in gliomas.

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Introduction

Diffuse gliomas are the most common primary central nervous tumors in adults and are histopathologically classified as oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. The WHO (World Health Organisation) grading system defines the spectrum from low to high grade gliomas comprising in the astrocytic lineage a wide range of genetically and clinically distinct tumors from grade I pilocytic astrocytomas, grade II diffuse astrocytomas, grade III anaplastic astrocytomas to grade IV glioblastoma multiforme (GBM) (1). The clinical management of gliomas remains a challenge, because of their infiltrative pattern of invasion, complete resection of all glial tumor components is almost impossible and adjuvant treatment still does not improve survival in most patients with the exception of pilocytic astrocytomas which can be successfully treated by complete tumor resection (2,3). Molecular genetic analysis has added useful information regarding the characteristic pattern of aberrations for the different histological subtypes. Oligodendrogliomas are commonly characterized by losses of 1p and 19q whereas astrocytomas have commonly gains of the entire or part of chromosome 7 and loss of 10. The importance of distinguishing oligodendrogliomas from astrocytomas depends on the predicted sensitivity to chemotherapy of oligodendrogliomas marked with 1p loss or combined 1p/19q losses (4). Other aberrations frequently occurring in both histological subtypes include gains at 8q, 12q and losses at 9p harbouring the known oncogenes *MYC*, *CDK4* and the tumor suppressor genes *CDKN2A* and *CDKN2B* (5). This summary report by Koschny and colleagues on >500 gliomas also demonstrated that the average number of copy alterations correlates with malignancy in oligodendrogliomas as well as in astrocytomas. Recent advances in molecular genetic approaches have led to the identification of cellular molecules and pathways severely affected in gliomas

and contributed thus to the understanding of the biological behaviour of gliomas (6). Epidermal growth factor receptor *(EGFR)* gene located on chromosome 7p11.2 is involved in controlling cell proliferation and is frequently overexpressed or mutated in astrocytomas leading to tumor progression, decreased apoptosis and tumor cell invasion. Gains or amplifications of chromosome 7 seem to be closely related with EGFR-immunoreactive tumor cells in gliomas (7). The phosphatase and tensine homologous gene *PTEN*, located on 10q23.3 acts as a phosphatidylinositol 3,4,5-triphosphate phosphatase and its tumor suppressor function is impaired in a variety of malignancies. *PTEN* is frequently affected by mutations in the coding region and by loss of heterozygosity (LOH) or structural rearrangements of chromosome 10 in gliomas (8). PTEN controls cell proliferation via the PI3 kinase/AKT pathway which becomes deregulated owing to PTEN inactivation (6). Immunohistochemical expression of PTEN has been shown to correlate with better prognosis (9). p53 acts under normal conditions as a tumor suppressor by its antimitotic quality. It is frequently mutated in gliomas which leads to abolished tumor suppressor function and to nuclear accumulation of the protein, which in turn is accessible to nuclear staining with p53 antibodies. The impact of p53 mutation on the prognosis of gliomas is subject of controversy (3) and it seems that p53 mutations are more related to secondary than to primary gliomas (10).

The aim of the present study was to evaluate genetic imbalances by CGH and immunohistochemistry in diffuse gliomas and comparing the results with clinicopathological features in order to contribute to an improved clinical management of gliomas.

Materials and methods

Patients and tumor specimens. Tumor tissue samples of 37 human glioma specimens were studied from patients who underwent neurosurgery at the Medical Faculty of Uludag University. The tumor specimens were collected between September 1997 and July 2003. Hematoxylin and eosin-stained sections from each specimen were assessed by neuropathologists (A.G., S.T.) according to the WHO classification system (1).

Comparative genomic hybridization. Genomic tumor DNA was isolated from snap-frozen tumor specimens in 26 cases and from dewaxed, formalin-fixed and paraffin-embedded specimens in 11 cases by proteinase K digestion and spin column purification (Qiagen, Hilden, Germany). CGH was performed as described previously (11). Briefly, labelling of tumor DNA with biotin-16-dUTP (Roche, Mannheim, Germany) and normal reference DNA with digoxigenin-11-dUTP (Roche) was performed by standard nick translation. The denatured DNA probes containing each 1.5 to 2 μg of tumor DNA and reference DNA and 80 μg of COT-1 DNA were hybridized for 3 days to normal metaphase spreads (Vysis, Downers Grove, IL). Subsequently, the slides were washed extensively, blocked with bovine serum albumin solution and incubated with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA) and rhodamine-conjugated antidigoxigenin (Roche). The slides were then washed again and finally mounted in an antifade medium (vectashield, Vector Laboratories) supplemented with actinomycin (12.5 μg/ml) and DAPI (4',6-diamidino-2-phenylindole) (1.25 μg/ml) for counterstain. Image acquisition was performed on a Zeiss Axioskop fluorescence microscope (Zeiss, Göttingen, Germany) equipped with three separate bandpass filters for DAPI, green, and red fluorescence spectrum and a high sensitive monochrome charge coupled device camera (Photometrics, Tucson, AZ). For each analysis, the averaged chromosome-specific green to red fluorescence ratios, from at least 10 well selected metaphases, were plotted using the Quips CGH software (distributed through Applied Imaging, Newcastle, UK). Relative copy number changes were interpreted as gains when the average green-to-red ratio exceeded 1.15 (1.5 for amplifications) and as losses when the

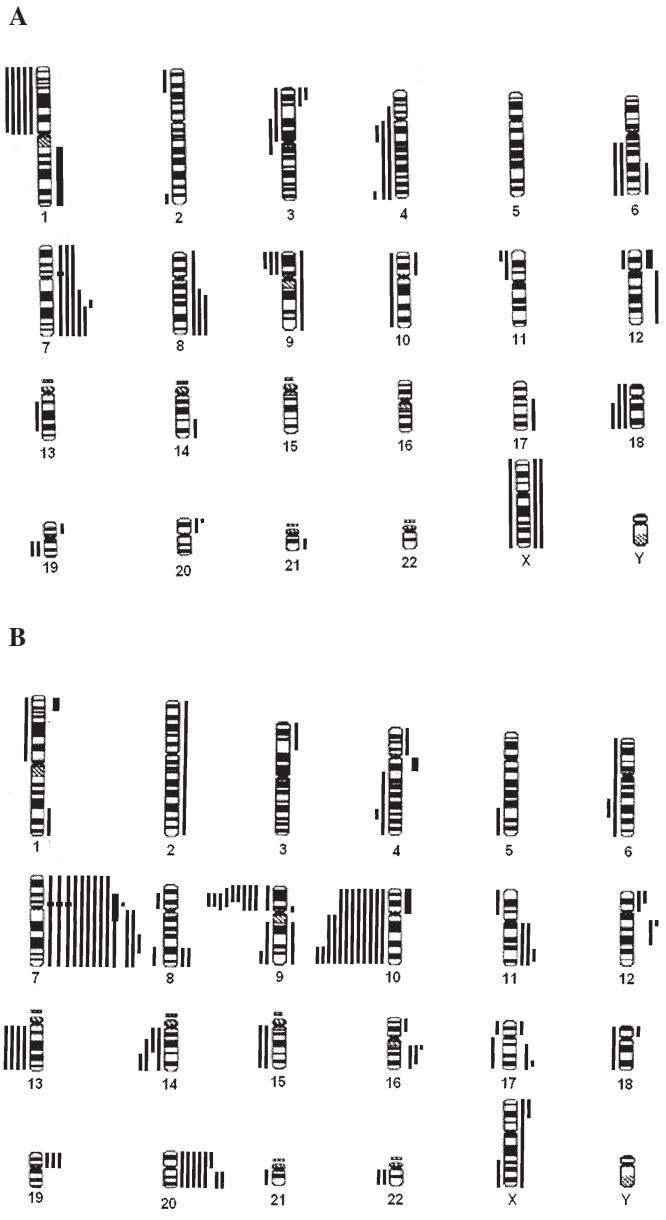

Figure 1. Overview of chromosomal imbalances in 14 oligodendroglial (A) and 23 high-grade astrocytic tumors (B). Bars on the left of each ideogram represent losses and on the right gains, corresponding to the chromosomal regions. Amplifications are in bold.

Table I. Clinicopathological characteristics and genomic imbalances of 14 oligodendroglial and 23 high-grade astrocytic cases.

Case[1]	Sex[2]/ Age	Histology[3]	Radio-/ Chemotherapy[4]	Sur- vival[5]	Follow-up[6] (months)	Immunohistochemistry[7] EGFR	PTEN	p53	MIB-1	CGH Gains[8]	Losses
G1	M/35	OD II		1	35	1	1	1	1	7, **7p11,** 20p	10
G2	M/3	OD II		0	55	1	1	1	1	no imbalances	
G3	M/48	OD II		0	50	1	1	1	1	no imbalances	
G4	M/41	OD II	RT	0	34	2	2	2	1	X, 7q31qter	6q14qter, 18q12qter
G5	M/39	OD II		0	56	1	1	1	1	no imbalances	
G6	F/48	OD II	CCNU	0	32	2	2	1	1		1p
G7	M/41	OD II	CCNU	0	38	3	1	1	2		1p, 9p13pter, 18, 19q
G8	F/29	OD II	RT	0	50	2	1	2	1	X, 3p24pter, 8q21qter, 20p13	2p21pter, 2q36qter, 6q14qter, 11p15, 13q13q31
G9	F/45	OD II	CCNU	0	37	1	1	1	1		1p13pter, 4p14qter
G10	M/43	OD II		1	43	2	3	1	1	19p	1p, 4q13q21, 18
G11 rec	F/57	OD III		1	126, 6	3	1	1	2	7, 17q, 21q	1p, 9p, 19q
G12	M/31	OD III	RT	0	45	1	1	2	2	**1q,** 7, 8q13qter, 9	
G13	M/32	OD III		0	48	1	1	2	1	3p22pter, 7q21qter, 10p, **12p**	3p14q13, 4q
G14	F/34	OA III	RT, Mp	1	38	1	3	3	2	6q22qter, 7q22, 8, 12q, 14q24qter	X, 3p, 4q34qter, 9p, 11p, 12p
G15	M/38	AA	RT, Mp+B	1	11	1	1	2	1	19p	
G16	F/63	AA	RT, Mp	0	14	2	3	3	2	3p21pter, 11q	11p12pter, 13q, 15q
G17	F/35	AA		0	61	1	1	2	2	no imbalances	
G18	F/57	GBM	RT, Mp	1	5	1	1	3	1	7, 20p	8q23qter, 10q, 14q24qter, 17q, 22q
G19	M/63	GBM	RT	0	28	1	1	1	1		6
G20	M/60	GBM		1	1	3	1	2	1	7, **7p11**	9p13p21, 10, 14q11q22
G21	M/56	GBM	RT	1	10	3	1	2	2	**1p36,** 7, 16q12	10, 14q, 22q
G22	M/65	GBM	RT, Mp	1	11	3	1	1	1	**7p14q11,** 7q21qter, 12q13q21, 20	8p11p21, 9p13p21, 10
G23	F/76	GBM		1	8	2	1	2	2	7, **7p11,** 20	9p11p21, 10, 13q
G24	F/51	GBM	RT	1	6	1	1	2	2	7, 12q13	10, 15q
G25	M/52	GBM	RT	1	20	1	1	3	1		17p
G26	M/43	GBM	RT, Mp	1	15	2	1	1	2	7, 19p, 20	6q21q23, 9, 10
G27	M/57	GBM	RT, Mp	1	15	3	3	1	1	no imbalances	
G28	F/31	GBM		1	1	1	1	2	2	16q	13q
G29	F/47	GBM	RT, Mp	1	13	1	3	2	2	no imbalances	
G30	F/26	GBM	RT	0	8	1	3	2	2	4p, 9p12qter, 11q, 12p12pter, 17p, 20q12	Xq23qter, 9p21pter, 10q24qter, 14q21qter
G31	F/70	GBM		1	4	3	3	1	2	2, 7q, 16p, 17q21qter, 20	9p, 9q33qter, 10q, 13q, 18
G32	M/60	GBM	RT, Mp+PCV	1	11	3	1	1	1	7p11	9p
G33	M/48	GBM	RT	1	8	2	2	2	2	7, 19p, 20	9p13p23, 10
G34	F/60	GBM	RT, PCV	1	8	1	1	2	2	X, 7	
G35	M/40	GBM	RT	1	45	1	2	2	2	7q, 8q23qter	1p, 4q13, 9p, 10q24qter
G36 rec	M/48	GBM	RT, Mp	0	72, 13	1	1	3	2	Xp21pter, 1q32qter, **4q12q13,** 7q22q32, 8q23qter, **10p,** 11q23q24, 12p, 16q12q23, 17q24, 18p, 20q, 21q	4q21qter, 5q31qter, 9p21pter
G37 rec	M/37	GBM	RT, Mp+TMZ	1	15	2	3	2	2	7, **7p11**	10

[1]rec, recurrence; [2]M, male; F, female; [3]OD, oligodendroglioma; OA, oligoastrocytoma; AA, anaplastic astrocytoma; GBM, glioblastoma multiforme; [4]RT, radiotherapy with 60 Gy total dose, except case G29 (30 Gy), CCNU, lomustine; Mp, muphoran, B, local bucladesine; PCV, procarbazine, lomustine, and vincristine; TMZ, temozolomide; [5]survival (0, alive; 1, died); [6]for G11 rec and G36 rec follow-up time of primary tumor is given firstly; G37 rec is recurrence of G1; [7]p53 and MIB-1 values for case G27 were derived from a recurrence; [8]amplifications are given by bold bars.

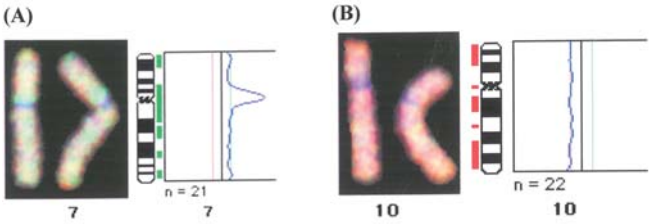

Figure 2. Example of chromosomal imbalances in GBM shown by a representative fluorescent karyogram and corresponding ideogram of case G22 for chromosome 7 (A) and 10 (B). This case is marked by gains/amplifications at 7p, 7q, 12q, and 20 as well as losses at 8p, 9p, and 10. The ideogram is shown along with its chromosome-specific green-to-red fluorescent ratio profiles and bars to the right represent gains/amplifications and bars to the left represent losses (n indicates number of analyzed chromosomes).

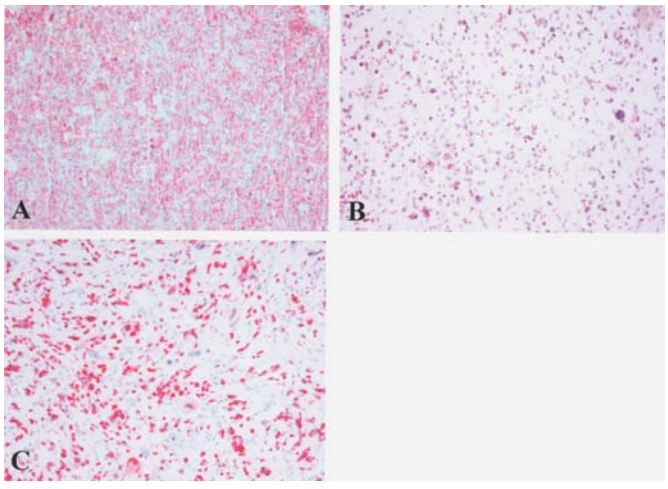

Figure 3. EGFR overexpression in case G21 (score 3) (A), PTEN overexpression in case G16 (score 3) (B), and p53 overexpression in case G18 (score 3) (C). Magnification, x200.

corresponding ratio was <0.85. Exceptionally, in cases with only trends not reaching the aforementioned thresholds, deviations from normal were classified as gains or losses when the 95% confidence interval varied beyond the ratio of 1.0. Chromosomal regions 13p, 14p, 15p, 21p, 22p, Y, telomeres and constitutive heterochromatic regions at 1q, 9q, and 16q known to produce false results by CGH were excluded from all analyses (11).

Immunohistochemistry. IHC evaluations were performed for p53 (mouse monoclonal, clone DO-7, dilution 1:50, microwave pre-treatment; Dako, Hamburg, Germany), PTEN (mouse monoclonal, clone 28H6, dilution 1:50, microwave pre-treatment; NeoMarkers, Fremont, CA, USA), EGFR (mouse monoclonal, clone E30, dilution 1:1500, protease pre-treatment; Dako), and the Ki-67 antigen (Clone MIB-1, dilution 1:50, microwave pre-treatment; Dako) using the DAK ChemMate™ detection kit (Dako) for visualization. Levels of p53, PTEN and EGFR staining were expressed as the product of labelling index (LI) and percentage of positive-stained tumor cells from 10 high power random fields. The labelling index was scored into categories, 1 (<10%, no or minimal reactivity corresponding with normal brain tissue

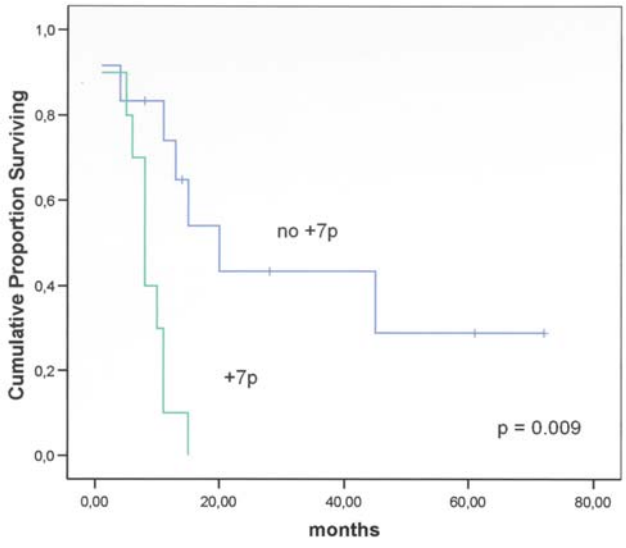

Figure 4. Kaplan-Meier plot of survival rates revealing worse prognosis of astrocytic cases with +7p compared with cases showing no +7p. Case G37 rec, the recurrence of G1, was excluded from this analysis.

reactivity), 2 (10 = LI < 50%), or 3 (LI = 50%). For p53 and PTEN only nuclear staining and for EGFR only membranous staining was considered. The MIB-1 score was derived from the percentage of positive-stained tumor cells in 10 high power fields and categorized into 1 (=5%) or 2 (>5%).

Statistical analysis. Individual imbalances were defined as gain, including amplification, or loss involving a chromosome arm. The Fisher exact test for contingency tables, Wilcoxon sum test and Kruskal-Wallis test were used to evaluate significant differences in frequencies of most common chromosomal imbalances and clinicopathological factors including IHC data. The association of the most common individual imbalances and clinicopathological factors with overall survival rates was tested with the log-rank test for censored data and visualized using the Kaplan-Meier method. The p-values for accepting significance (p=0.05) were not adjusted for multiple comparisons. Statistical analysis was performed using the SPSS program (SPSS, Chicago, IL, USA).

Results

Clinicopathological and follow-up data. Thirty-seven glial cases were incorporated in this study comprising 13 oligodendrogliomas (10 WHO grade II; 3 WHO grade III), one oligoastrocytoma (WHO grade III) and 23 high-grade astrocytomas (3 anaplastic astrocytomas, WHO grade III; 20 GBM, WHO grade IV). In the oligodendroglial group including the oligoastrocytic case, nine patients were male and 5 female (mean age, 37.6 years, ±12.6) and in the astrocytic group 13 patients were male and 10 female (mean age, 51.4 years, ±12.9). Thirty-four were primary and 3 were recurrent tumors, altogether obtained from 36 patients. Seven of 11 oligodendroglial and 18 of 23 astrocytic cases received either radio- and/or chemotherapy which in all instances was applied postoperatively. Mean follow-up time was 49.1 months (range, 32-126 months, ±18.3) in the oligodendroglial and

Table II. Correlation of chromosomal imbalances and clinicopathological features.

Variables	Group[a]	p-value	Test[b]
Overall imbalances vs. individual imbalances			
Overall changes vs. +7q	OD	0.026	WT
Overall changes vs. +7q	AC	0.001	WT
Overall changes vs. -9p	AC	0.001	WT
Overall changes vs. -10q	AC	0.001	WT
Overall changes vs. +20p	AC	0.007	WT
Overall changes vs. +20q	AC	<0.001	WT
Individual imbalances vs. individual imbalances			
+7p vs. +7q	AC	0.003	FET
+7p vs. -10p	AC	<0.001	FET
+7p vs. -10q	AC	0.012	FET
+7q vs. -10p	AC	0.006	FET
+7q vs. -10q	AC	0.001	FET
+7q vs. +20p	AC	0.019	FET
-9p vs. -10q	AC	0.036	FET
-9p vs. +20q	AC	<0.001	FET
-10p vs. -10q	AC	0.001	FET
-10q vs. +20p	AC	0.014	FET
+20p vs. +20q	AC	0.003	FET
Individual imbalances vs. clinicopathological factors			
-1p vs. +EGFR	OD	0.025	WT
-1p vs. -p53	OD	0.049	WT
+7q vs. +p53	OD	0.039	WT
+7p vs. +EGFR	AC	0.046	WT
-10p vs. +EGFR	AC	0.024	WT
+7p vs. short-term survival	AC	0.009	LR
-10q vs. short-term survival	AC	0.039	LR
Clinicopathological factors vs. clinicopathological factors			
+MIB1	AC	0.045	FET
+p53	AC	0.031	WT
+p53 vs. -EGFR	AC	0.046	KW

[a]OD, oligodendrogliomas; AC, astrocytomas. [b]FET, Fisher exact test; WT, Wilcoxon test; LR, log-rang test; KW, Kruskal-Wallis test.

17.0 month (range, 1-72 months, ±23.5) in the astrocytic group. Seven of 11 patients in the oligodendroglial and 5 of 23 patients in the astrocytic group were alive at the end of follow-up period.

Comparative genomic hybridization. Eleven of 14 oligodendrogliomas and 20 of 23 astrocytomas revealed chromosomal aberrations (Fig. 1A and B; Table I). The average number of chromosomal aberrations was 4.8 (range, 0-13; SD±3.8) in oligodendrogliomas and 5.7 (range, 0-16; SD±4.4) in astrocytomas. In both, oligodendrogliomas and astrocytomas, the mean number of gains (2.3 and 3.1, respectively) was similar to the mean number of losses (2.5 and 2.6). However, the pattern of genomic imbalances differed between the groups. Whereas +7q (43%), -1p (36%), and -4q (29%) were the most frequent imbalances in oligodendrogliomas, +7q (57%), -10q (52%), +7p (48%), -9p (43%), -10p (35%), +20q (30%), and +20p (26%), were the most common imbalances in astrocytomas. Recurrent high-level amplifications encompassing the 7p11 region were observed in a tumor evolving from astrocytoma II to GBM (cases G1 and G37 R) and in 3 other GBM cases. Fig. 2 depicts a CGH karyogram and ideogram of case G22 including a proximal 7p amplification.

Immunohistochemistry. Nuclear p53 overexpression was detected in 5 (4 score 2, 1 score 3) oligodendrogliomas and 17 (13 score 2, 4 score 3) astrocytomas. Pronounced nuclear expression of PTEN was revealed in 4 (2 score 2, 2 score 3) oligodendrogliomas and 8 (2 score 2, 6 score 3) astrocytomas. Elevated membranous EGFR expression was noted for 6 (4 score 2, 2 score 3) oligodendrogliomas and 11 (5 score 2, 6 score 3) astrocytomas and MIB-1 positivity in at least 5% of the cells was recorded for 4 oligodendrogliomas and 15 astrocytomas. Fig. 3 depicts representative microscopic images of EGFR, PTEN, and p53 overexpression in astrocytic cases.

Correlation of CGH and clinicopathological data. Some of the most common individual imbalances correlated significantly with the increasing number of overall imbalances, that were +7q in the oligodendroglial (p=0.026) as well as in the astrocytic group (p=0.001) and furthermore, -9p (p=0.001), -10q (p=0.001), +20p (p=0.007), and +20q (p<0.001) in the astrocytic group (Table II). Several individual imbalances in the astrocytic group showed a combined appearance, that were +7p, +7q, -10p, and -10q in any dual combination (p<0.05) and furthermore, +7q/+20p (p=0.019), -9p/-10q (p=0.036), -9p/+20q (p<0.001), -10q/+20p (p=0.014), and +20p/+20q (p=0.003). Among IHC markers, p53 overexpression correlated positively with +7q (p=0.039) and negatively with -1p (p=0.049) in the oligodendroglial group. Moreover, increased EGFR expression correlated positively with -1p (p=0.025) in the oligodendroglial and with +7p (p=0.046) and -10p (p=0.024) in the astrocytic group. In general, MIB-1 (p=0.045) and p53 (p=0.031) were more highly expressed in astrocytomas than in oligodendrogliomas, and p53 and EGFR expression was inversely correlated (p=0.046) within the astrocytic group. The log-rank test disclosed comparably shorter overall survival rates for astrocytic cases implemented with +7p (p=0.009) (Fig. 4) or with -10q (p=0.039).

Discussion

Several molecular genetic studies have established a pattern of chromosomal aberrations in gliomas which is increasingly assisting a genetically based classification scheme adjunct to the established histopathological criteria. In this respect, the present study could complement some notable results to the tumor biology of diffuse gliomas.

Oligodendrogliomas are characterized by -1p or combined -1p/-19q and these findings were a hallmark in defining a genetic basis for histological subclassfication of gliomas

(12). Further studies could support the significance of these non-random aberrations (13,14) and demonstrate a close relationship of -1p/-19q with chemotherapy sensitivity (4). These correlations led to a genetic routine diagnosis for -1p/-19q allelic losses that predict a favourable clinical course for patients. In accordance with these findings we found -1p as one of the most significant findings in oligodendrogliomas. Furthermore, we found a relationship between -1p and low expression of p53 which resembles findings of inverse correlation of p53 mutations and loss of heterozygosity of 1p (15,16). It has been assumed that classic oligodendrogliomas are correlated with -1p/-19q whereas oligodendrogliomas with astrocytic features are more related with p53 mutations (17). In contrast to other studies, -19q was only detected in two of our oligodendroglial cases which might be attributed to sample bias or limitation of the CGH method which often is hampered to establish a suitable profile for the critical chromosome 19 (18). FISH or LOH studies would be the methods of choice to unravel minute deletions on 19q (19). Tumor heterogeneity might also account for the failure to detect more frequently -19q as for instance, only the recurrent case of G10 (not presented here) displayed -19q. It should be noted that 6 of our oligodendroglial and astrocytic cases revealed no imbalances despite repeated CGH analysis. This holds with other CGH studies on gliomas which likewise reported a respective number of non-informative cases even with microdissected tumor samples (20).

We encountered most frequently +7q in oligodendrogliomas; however, this imbalance correlated neither with -10 nor with +7p11, that specifically targets the *EGFR* gene locus. Gains of 7 or 7q were recurrently observed in CGH studies of oligodendrogliomas (14,21,22) and seemingly represent an alternative pathway in oligodendroglial pathogenesis. All 11 informative oligodendrogliomas in our study were either affected by -1p or +7q, or -1p/+7q. A promising target gene on 7q might be the *CDK6*, which seems to be frequently overexpressed in medulloblastomas (23) whereas for -1p/-19q no classical tumor suppressor or a chemotherapy-sensitive target gene has been identified (17,24).

The most characteristic imbalances in our astrocytic group were +7 and -10 contributing to the established pattern of genomic aberration in the astrocytic lineage (25). Gain of 7p are commonly associated with amplification of the *EGFR* gene locus and related with EGFR immunopositivity. The significance of this relationship was also demonstrated in our astrocytic series and furthermore, +7p revealed to be the most negative prognostic marker in this group, which might be of clinical relevance in view of the emerging therapeutic options targeting EGFR and EGFR signalling pathways (3). EGFR and p53 overexpression disclosed a negative association in the astrocytic group which is consistent with some studies also noting an inverse correlation of these markers, yet their assumed mutual exclusivity remains to be further clarified (3).

Amplification of 7p11 was detected only in one oligodendroglioma (case G1) which disclosed no positive EGFR immunostaining. However, the tumor recurred 20 months later as a secondary GBM (case G37 rec) which although showing essentially the same imbalances revealed increased immunopositivity for EGFR, PTEN, MIB-1, and p53. Low-grade oligodendrogliomas developing rapidly to GBM are rare (26). LOH of chromosome 10 is the most common aberration in high-grade gliomas and identified as a negative prognostic factor (27). Besides *PTEN*, other candidate genes affected by -10 aberrations include *LGI1* (leucine-rich gene-glioma inactivated, 1) of which the expression in malignant gliomas compared to normal brain tissue is severely reduced (28), and *DMBT1* (deleted in malignant brain tumour-1), which seems to be affected in a minor set of gliomas (29). Gains of 20p and/or 20q were only mentioned marginally for their possible involvement in glial pathogenesis (30), though the putative serine/threonine kinase gene *STK15* located at 20q13 has been recognized as a possible candidate gene involved in glial tumorigenesis (31,32). Other common regions of deletions in astrocytomas include -9p. The CDKN2 locus located at 9p21 encodes for the cell cycle control genes *CDKN2A* and *CDKN2B* which are frequently targeted in various tumors. Recently, it has been reported that patients with high-grade gliomas characterized by -9p and -10q benefit from Temozolomide, an alkylating chemotherapeutic agent (33). Considering this, it would be a diagnostic challenge to identify those gliomas with therapeutical susceptibility depending on the otherwise negative prognostic markers -9p/ -10q.

In summary, the present study identified significant interrelationships of CGH results and clinicopathological features in diffuse gliomas which in light of the emerging molecular biological influence on the clinical management of gliomas anticipate further recognition.

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