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ORIGINAL PAPER

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Genetically distinct and clinically relevant subtypes of glioblastoma defined by array-based comparative genomic hybridization (array-CGH)

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Abstract To optimize treatment strategies for patients with glioblastoma, a more precise understanding of the molecular basis of this disease clearly is necessary. Therefore, numerous studies have focused on the molecular biology of glioblastoma and its linkage to clinical behavior. Here we investigated 70 glioblastomas using the array-based comparative genomic hybridization (array-CGH) with GenoSensor Array 300 to identify recurrent DNA copy number imbalances associated with patient outcomes. Univariate log-rank analysis of array-CGH data revealed 46 copy number aberrations (CNAs) associated with outcome. Among them, 26 CNAs were associated with shortened survival whereas the remaining 20 CNAs correlated with good prognosis. A hierarchical cluster analysis disclosed two genetically distinct groups of glioblastomas (1 and 2; 56 and 14 tumors, respectively). Univariate log-rank test discerned significant difference in survival between both genetic subsets while the 5-year survival rate consisted of 0 for group 1 and 63% for group 2. Multivariate analysis revealed that unfavorable genetic signature is an independent prognostic factor increasing a risk of patient death (hazard ratio, 4.38; $P = 0.00001$). In conclusion, our current study suggests that glioblastomas can be subdivided into clinically relevant genetic subsets. Therefore, array-CGH screening of glioblastomas could provide clinically useful information and, perhaps, potentially improve the quality of treatment.

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Introduction

Glioblastoma remains a challenging and hopeless chapter in neurooncology [12, 15]. Median survival of patients with glioblastoma is approximately 12 months, and only 5% of patients live for >5 years [23, 24]. The prognostic value of various parameters has been examined in patients with glioblastomas, and the only consistent prognostic variables identified were patient age and Karnofsky performance score (KPS).

To optimize treatment strategies for patients with glioblastoma, a more precise understanding of the molecular basis of this disease clearly is necessary [18, 19]. Over the past several years, a variety of numerical chromosomal abnormalities, amplifications of potent oncogenes, and deletions/mutations of tumor suppressor genes (TSGs) have been implicated in the pathogenesis of these malignancies [6, 17–19, 22, 27, 35, 43]. Nevertheless, studies regarding the clinical significance of these genetic alterations for patients with glioblastoma have reported controversial results [2]. By novel matrix-based comparative genomic hybridization (CGH) technology (or array-CGH), the status of hundreds or even thousands of genomic targets can be studied simultaneously [38]. In the present study, we investigated 70 glioblastomas using the GenoSensor Array 300 to identify recurrent DNA copy number imbalances associated with patient survival. The cases included in the analysis had a wide range of survival times to facilitate identification of genetic alterations associated with clinical outcomes.

Materials and methods

Patient population and pathological analysis

In total, 70 adult patients who were treated in the Burdenko Neurosurgical Institute from 1 January 1995 to

1 January 2004 and who had newly diagnosed, supratentorial glioblastomas were included in this study. All diagnoses were confirmed by histological assessment of a tumor sample by two independent neuropathologists according to the current World Health Organization (WHO) histologic classification [19]. Approval to link laboratory data to clinical data was obtained by the Institutional Review Board.

The 70 patients included 37 men and 33 women, and patient age was from 19 to 73 (median 51.8 ± 12.3) years, with 34 patients (49%) younger than 50 years. All patients had undergone open surgery with either macroscopically total resection (38 patients) or subtotal tumor resection (32 patients) that was confirmed by either postoperative computed tomography (CT) (12 patients) or magnetic resonance images (MRI) (58 patients). Postoperative KPS was ≥70. All patients received postoperative radiotherapy with a total dose 56–62 cGy (mean 58 cGy) and chemotherapy with either nitrosourea compounds (14 patients) or temozolomide (56 patients). None of our patients had been treated in the settings of any prospective therapeutic trial.

The cases included in the analysis had a wide range of survival times, and five groups of patients were specially selected for these purposes: (a) 14 patients with a survival time of 3–6 months; (b) 14 patients with a survival time of 7–12 months; (c) 10 patients with a survival time of 13–18 months; (d) 16 patients with a survival time of 19–24 months (4 still alive); (e) 16 patients who lived at least 25 months (7 still alive). The 1-year survival rate for the entire cohort was 60%, the 3-year survival rate was 14%, and the 5-year survival rate was 9%.

Sample processing

Samples from each tumor were taken at the time of initial operation, immediately frozen in liquid nitrogen, and continuously stored at −80°C until used for microarray experiments. To confirm the presence of viable tumor (>90% of neoplastic cells), the cryosections of each handily dissected glioblastoma sample were stained with H&E and reviewed before DNA extraction. DNA was isolated according to a protocol that applies DNeasy tissue kit (Qiagen, Hilden, Germany). DNA concentration and quality were determined by absorption spectrophotometry, and the integrity and purity of DNA were analyzed on 1% agarose gel.

DNA labeling and hybridization of the microarrays

Approximately 100 ng of DNA from glioblastoma samples (test DNA) and normal reference human DNA were labeled by random priming reaction with Cy3-dCTP and Cy5-dCTP (Perkin Elmer, Foster City, CA, USA), respectively, according to the protocols recommended by the manufacturer (Vysis, Inc., Abbott Laboratories S.A., Downers Grove, IL, USA). Briefly, DNA was denatured with ×2.5 random priming solution at 100°C for 10 min, mixed with the nucleotide GenoSensor mix, fluorescent nucleotides and Klenow fragment, and incubated at 37°C for 2 h. After DNAse treatment, unincorporated fluorescent nucleotides were removed by ethanol precipitation.

Aliquots of labeled DNA were mixed with the hybridization buffer, denatured at 80°C for 10 min, and incubated at 37°C for 1 h to allow blocking of repetitive sequences. The hybridization mixture was then introduced on genomic DNA microarray slides. We used the commercially available GenoSensor Array 300 (Vysis, Inc) contained 287 genomic targets which were cloned from P1 and BAC libraries. The GenoSensor Array 300 includes cancer amplicons/oncogenes, TSGs, loci of gains/deletions/duplications, telomeres, and markers added to reduce gaps (for detailed information see http://www.vysis.com). The microarray slides were incubated 66–72 h at 37°C. Posthybridization process included subsequent washing in 50% formamide/2× standard saline citrate solution (three times for 10 min each at 40°C) and 1× standard saline citrate solution (four times for 5 min room temperature). The microarray slides were counterstained with DAPI solution and covered with coverslip glasses.

Analysis of the Genosensor Array 300

Microarray slides were analyzed by using the GenoSensor Reader System (Vysis, Inc) according to the instructions recommended by the manufacturer. Fluorescent signals from tumor DNA (green) and reference DNA (red) were quantitatively detected and captured by the autoexposure system, and exposure times usually varied from 0.5 s to 2.0 s. Images were analyzed with the GenoSensor Reader software, which performed a segmentation and detection of the array targets. After background subtraction, the program calculated total intensity and intensity ratio of test and reference signals for each target (three replicate spots). Normalized ratio of each target indicated the degree of gain and loss copy number when compared with the sample's modal copy number.

We confirmed the quality of hybridization results by five control experiments using test and reference DNA from normal individuals. In all experiments, intensity ratios of hybridization signals were between 0.80 and 1.19. On the basis of the current and previous control experiments using the GenoSensor Array 300 [28, 34, 40], we determined loss, gain, and amplification of target DNA sequence copy numbers by a green/red ratio <0.8, >1.2, and >2.0, respectively.

Fluorescence in situ hybridization (FISH)

A two-color interphase fluorescence in situ hybridization (FISH) assay was performed on 5-μm-thick sections.

The following commercial fluorochrome-labeled probes were applied (all produced by Vysis, Inc): centromere (CEP)7/7p12(*EGFR*) dual color probe set, CEP9/9p21(*p16*) dual color probe set, CEP10/10q23(*PTEN*) dual color probe set, orange-labeled 20q13(*ZNF217*) locus probe, and green-labeled 22q11(*BCR*) locus probe. Pretreatment of slides, hybridization, posthybridization processing, and signal detection were performed as described previously [21].

Statistical analysis

For categorical data, the chi-squared test, Wilcoxon–Mann–Whitney test, and Fisher's exact test were used. Intraclass correlation analysis was used to assess the degree of associations between pairs of variables. Survival analysis was made using the Kaplan–Meier method. For pairwise comparisons of survival time distributions, the log-rank test was used. Multivariate analysis for survival was performed using the Cox proportional hazard models. Probability (P) values <0.05 were considered significant with a confidence interval (CI) at 95%. Unsupervised clustering using array-wise and uncentered hierarchical analysis with complete linkage was also performed. For this purpose, the mean ratio for each DNA target in each sample was converted to a score of 1(gain/amplification), 0 (balanced), or −1 (loss). Gain, loss, and balanced clones were represented in the final heat map as red, green, and black, respectively.

Table 1 Most frequently gained DNA clones (>30% of samples examined)

Clone name	Cytogenetic location	No. of tumors
TP73	1p36.33	23 (33%)
FGR (SRC2)	1p36.2-p36.1	34 (49%)
MSH2;KCNK12	2p22.3-p22.1	27 (39%)
D2S447	2q tel	23 (33%)
RASSF1	3p21.3	29 (41%)
C84C11/T3	5p tel	37 (53%)
CSF1R	5q33-q35	26 (37%)
NIB1408	5q tel	22 (31%)
G31341	7p22.3	52 (74%)
IL6	7p21	24 (34%)
EGFR	7p12.3-p12.1	51 (73%)
ELN	7q11.23	22 (31%)
RFC2;CYLN2	7q11.23	34 (49%)
ABCB1 (MDR1)	7q21.1	22 (31%)
CDK6	7q21-q22	22 (31%)
stSG48460	7q36.3	31 (44%)
7QTEL20	7q tel	22 (31%)
INS	11p15.5	22 (31%)
CDKN1C (p57)	11p15.5	34 (49%)
KAI1	11p11.2	38 (54%)
8M16/SP6	12p tel	24 (34%)
CCND2	12p13	22 (31%)
GLI	12q13.2-q13.3	26 (37%)
SAS; *CDK4*	12q13-q14	24 (34%)
AKT1	14q32.32	24 (34%)
FES	15q26.1	26 (37%)
PACE4C	15q26	28 (40%)
282M15/SP6	17p tel	26 (37%)
TOP2A	17q21-q22	22 (31%)
AFM217YD10	17q tel	29 (41%)
JAG1	20p12.1–p11.23	25 (36%)
20QTEL14	20q tel	22 (31%)
BCR	22q11.23	32 (46%)
PDGFB (SIS)	22q13.1	30 (43%)

Results

Recurrent genomic imbalances in glioblastoma detected by array-CGH

Each tumor examined displayed DNA copy number aberrations (CNAs), and mean number of CNAs per tumor was 63.2 ± 11.9 (range, 37–116). Mean number of copy number gains was 39.8 ± 8.3 (range, 14–69) and the frequent gains are outlined in Table 1. Sixty-seven tumors (96%) harbored at least one gained clone at chromosome 7 and complete gain of 7 was found in 17 cases. Mean number of copy number losses was 39.1 ± 6.7 (range, 22–67), and recurrent losses are outlined in Table 2. Sixty-five tumors (93%) showed at least one deleted region at chromosome 10, and complete loss of 10 was identified in 19 cases. There were no samples with completely balanced profiles for chromosomes 7 and 10 simultaneously. In addition, 41 tumors showed 81 high-level gains (Table 3) with mean number of amplifications per tumor of 1.87 ± 1.3 (range, 1–6).

We compared the results of array-CGH and FISH analyses in all samples examined (Fig. 1), and there was a high level of concordance between data from both techniques (Table 4). We found no significant differences in mean number of CNAs between patients who were younger or older than 50 years (63.4 ± 12.1 and 62.9 ± 11.8, respectively). Nevertheless, we found 40 CNAs (21 gains and 19 losses), which were different for patients younger and older than 50 years (Fisher's exact test, $P<0.05$; Table 5).

Genomic imbalances associated with glioblastoma clinical outcome

Unsupervised hierarchical cluster analysis including all 287 DNA clones revealed two major genetic groups of glioblastomas (data not shown). Tumors from group 1 ($n=25$) tended to reveal an entire gain of chromosome 7 and/or complete loss of chromosome 10 and also included the cases with loss of 22q and gains at 20q. In contrast, group 2 ($n=45$) was composed of tumors that frequently showed gains at 12q, 15q, 19, and 22q as well as losses at 11q and 17q. There were no differences in mean CNAs between these tumor subsets (Wilcoxon–Mann–Whitney test; $P=0.43$). Also, average age of patients and survival times did not differ by group (Wilcoxon–Mann–Whitney test; $P=0.57$).

Using univariate log-rank analysis of array-CGH data according to survival times, we identified 46 CNAs

Table 2 Most frequently lost DNA clones (>30% of samples examined)

Clone name	Cytogenetic location	No. of tumors
D1S500	1p31.1	26 (37%)
BIN1	2q14	25 (36%)
THRB	3p24.3	31 (44%)
ROBO1; D3S1274	3p12-p13	27 (39%)
APC	5q21-q22	29 (41%)
EGR1	5q31.1	24 (34%)
HTR1B	6q13	24 (34%)
D6S268	6q16.3-q21	31 (44%)
TIF1	7q32-q34	24 (34%)
LPL	8p22	23 (33%)
MTAP	9p21.3	24 (34%)
CDKN2A (p16); MTAP	9p21	36 (52%)
AFM137XA11	9p11.2	38 (54%)
TSC1	9q34	22 (31%)
SHGC-44253	10p tel	22 (31%)
D10S249; D10S533	10p15	22 (31%)
WI-2389; D10S1260	10p14-p13	27 (39%)
BMI1	10p13	37 (53%)
D10S167	10p11-q11	32 (46%)
EGR2	10q21.3	25 (36%)
DMBT1	10q25.3-q26.1	34 (49%)
FGFR2	10q26	28 (40%)
stSG27915	10q26.3	32 (46%)
RDX	11q22.3	44 (63%)
DRIM;ARL1	12q23	42 (60%)
RB1	13q14	30 (43%)
SNRPN	15q12	28 (40%)
FRA16D	16q23.2	22 (31%)
D17S125; D17S61	17p12-p11.2	23 (33%)
BRCA1	17q21	27 (39%)
NME1(NME23)	17q21.3	22 (31%)
RPS6KB1(STK14)	17q23	32 (46%)
StSG42796*(ZNF439)*	19p13.2	46 (65%)
ARHGAP8	22q13.3	22 (31%)

Table 3 Amplified DNA clones ($n=81$)

Clone name	Cytogenetic location	Number
D1S214	1p36.31	1
D1S418	1p13.1	1
WI-5663; WI-13414	1q21	1
LAMC2	1q25-q31	1
PTGS2(COX2)	1q31.1	2
AKT3	1q44	2
ITGA4	2q31-q32	1
MLH1	3p21.3-p23	2
TP63	3q27-q29	2
PDGFRA	4q11-q13	2
D6S268	6q16.3-q21	2
MYB	6q22-q23	1
G31341	7p22.3	1
EGFR	7p12.3-p12.1	24
ABCB1(MDR1)	7q21.1	1
CDK6	7q21-q22	3
SERPINE1	7q21.3-q22	1
MET	7q31	2
MYC	8q24.12-q24.13	1
MLL	11q23	1
8M16/SP6	12p tel	1
CDK2;ERBB3	12q13	2
GLI	12q13.2-q13.3	4
SAS;CDK4	12q13-q14	7
MDM2	12q14.3-q15	1
D13S327	13q34	2
IGH(D14S308)	14q tel	2
IGF1R	15q25-q26	1
ERBB2 (HER-2)	17q11.2-q12	1
CTDP1	18q23	2
129F16/SP6	19p tel	2
20PTEL18	20p tel	1
CSE1L(CAS)	20q13	1
ZNF217(ZABC1)	20q13.2	1
TNFRSF6B(DCR3)	20q13	1

significantly associated with outcome (Table 6). Among them, 26 CNAs were associated with shortened survival (unfavorable CNAs) whereas the remaining 20 imbalances correlated with good prognosis (favorable CNAs). *EGFR* amplification was also analyzed but showed no association with outcome.

A hierarchical cluster analysis was performed repeatedly using only 46 prognostically significant CNAs. The final dendrogram shows two distinct groups of tumors (Fig. 2). All samples having no unfavorable CNAs were discerned in group 2 ($n=14$) whereas all tumors from group 1 ($n=56$) revealed these imbalances. All tumors from group 2 showed at least one unfavorable CNA in contrast to 11 glioblastomas from group 1. No differences in histopathological features could be discerned between both genetic subsets. Also, the mean number of CNAs did not differ between the clusters (Wilcoxon–Mann–Whitney test; $P=0.38$), but the patients from group 1 tended toward higher mean age (54.3 vs 44.7 years; Wilcoxon–Mann–Whitney test; $P=0.06$). There was a difference in median survival between both subsets (13.1 months for group 1 compared with 34.5 months for group 2, log-rank test, $P=0.0001$), and the survivors were found predominantly within group 2 (9 out of 11). Univariate log-rank test revealed significant difference in OS between both genetic subsets ($P<0.00001$) while the 5-year survival rate consisted of 0 for group 1 and 63% for group 2 (Fig. 3). There were no differences in survival time between the minor clusters within group 1.

Additionally, we analyzed the prognostic value of various clinical parameters. Because patient age is a continuous variable, the cutoff age that subdivided patients into distinct survival groups was calculated as described previously [20]. Consequently, the cutoff age of 50 years was the strongest predictor of outcome (5-year survival rate, 16% for younger patients compared with 2% for older patients; log-rank test; $P=0.00001$). We were unable to draw any significant association between overall survival and other clinical variables, including tumor location (frontal vs nonfrontal), volume of resection (total vs subtotal), and regimens of chemotherapy.

To consider whether two outlined genetic subsets of glioblastomas are independent prognostic indicators when clinical factors (age, gender, tumor location, and extent of resection) are known, we performed a multivariate regression analysis. Consequently, it was found that age >50 years (hazard ratio, 4.47; $P=0.00001$) and unfavorable genetic signature (hazard ratio, 4.38;

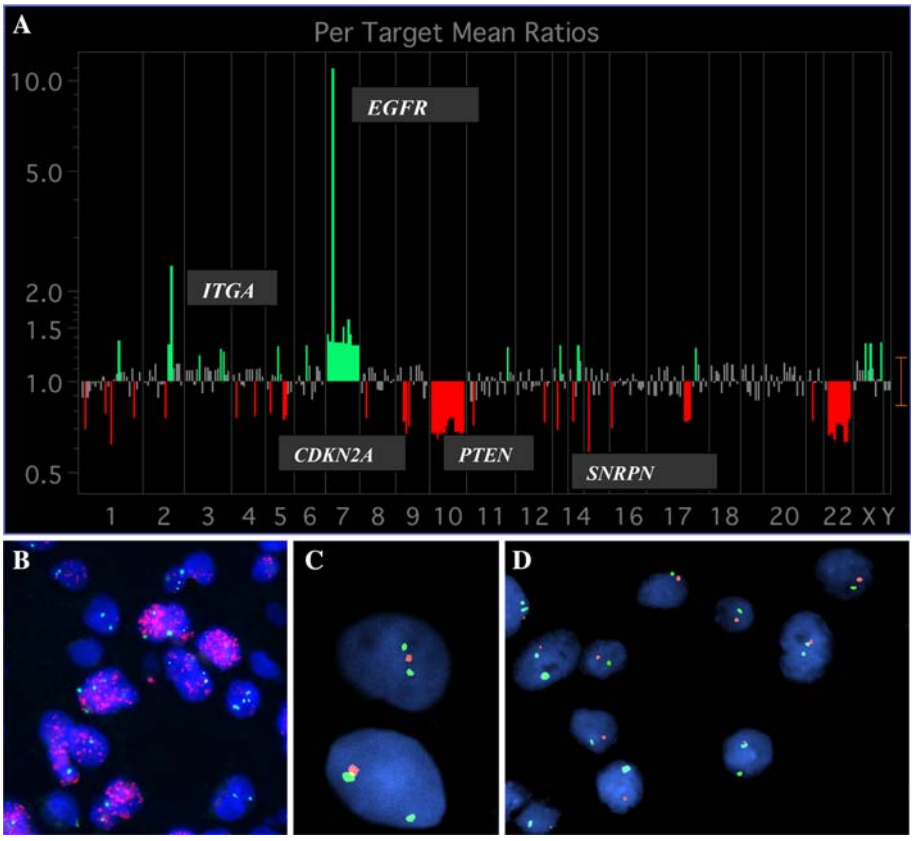

Fig. 1 Array-based comparative genomic hybridization (array-CGH) (**a**) validation by fluorescence in situ hybridization (FISH) (**b**–**d**). Array-based cytogenetic profile of glioblastoma patient (woman; 68 years; survival time, 22 months) displays 73 copy number aberrations (gains in *green* and losses in *red*), including *EGFR* amplification (**b**; *red signals and clouds*), loss of *CDKN2A* (**c**; *single red signals*), and loss of *PTEN* (**d**; *single red signals*). In addition, amplification of *ITGA4* locus at 2q31-q32 and deletion of *SNRPN* gene at 15q12 could be seen

$P = 0.00001$) are independent prognostic variables increasing a risk of patient death.

Discussion

Recurrent genomic imbalances in glioblastomas

During the last several years, glioblastomas have been analyzed by using array-CGH that allowed identifying of novel aberrant loci and candidate genes involved in their tumorigenesis [5, 10, 25, 29, 32]. Our data identified numerous gains/amplifications comprising predominantly the genes involved in signal transduction pathways, cell-cycle activation, and mitosis, or code for viral oncogenes, growth factors, and receptors. Some of these genes (*PDGFRA, EGFR, CDK6, MET, CDK2, SAS/CDK4, MDM2, GLI, CCND2, TOP2A, AKT1, IGFR1, PDGFB*) have been previously found to be gained in glioblastomas [18, 19]. Additionally, we found frequent gains linked to oncogenes in various tumors but not previously associated with glioblastomas. The role of *TP73* in cancer is complex because two isoforms originate from its different promoters, one of which is a potent oncogene [26]. *FGR* is a viral oncogene homolog involved in Akt signaling pathway that is frequently affected in glioblastomas [16]. *MSH2* is a mismatch repair gene, but its amplification has been identified in cervical carcinoma [14]. *JAG1* encodes for ligand of Notch-1 and was found to be overexpressed in human gliomas and cell lines [31]. *CSF1R* and *FES* are homologs of feline sarcoma viral oncogenes involved in the biology of various human tumors [33]. *PACE4C* encodes for proprotein convertase enzyme (furin) that has been associated with aggressive behavior in lung and breast cancers [4].

Table 4 Correlations between the data obtained by array-based comparative genomic hybridization (array-CGH) and fluorescence in situ hybridization (FISH) techniques

Array-CGH clone	FISH probe	ICC (*r*)	*P* value
EGFR	7p12/*EGFR*	1.00	< 0.00001
CDKN2A/MTAP	9p21/*p16*	0.93	0.00004
ZNF217(ZABC)	20q13/*ZNF217*	0.87	0.00006
BCR	22q11/*BCR*	0.81	0.00008
PTEN	10q23/*PTEN*	0.73	0.0001

ICC intraclass correlation coefficient

Our results identified frequent losses on chromosomes 9, 10, and 13, with deletions of *CDKN2A, DMBT1*, and *RB1* described previously [18, 19]. The frequency of *PTEN* deletion (28%) corroborated with studies that used the GenoSensor Array 300 [28, 34, 40] but differed from data obtained by high-resolution microarrays [25]. This discrepancy may be explained by differences in the design of microarrays, number of tumors examined, and patient selection. Frequent deletions of other TSGs, such as *BIN1, ROBO1, APC, TSC1, FRA16D, BRCA1*, were also found in the current

Table 5 DNA copy number aberrations associated with patient age

Name of clone	Cytogeneic location	< 50 years ($n = 34$) (%)	> 50 years ($n = 36$) (%)	P value[a]
Gains				
LAMC2	1q25-q31	6	28	0.02
AKT3	1q44	6	28	0.02
U32389	2p tel	32	14	0.04
5QTEL70	5q tel	29	8	0.02
ABCB1(MDR1)	7q21.1	18	44	0.02
CDK6	7q21-q22	21	42	0.03
TIF1	7q32-q34	6	38	0.006
StSG48460	7q36.3	34	58	0.02
D9S913	9p tel	3	17	0.03
ATM	11q22.3	3	17	0.03
ERBB2(HER-2)	17q11-q12	34	6	0.02
RPS6KB1(STK14)	17q23	3	19	0.03
YES1	18p11.3-p11.2	9	25	0.03
DCC	18q21.3	32	9	0.02
INSR	19p13.2	3	22	0.02
CCNE1	19q12	6	31	0.01
CSE1L(CAS)	20q13	6	25	0.02
ZNF217(ZABC1)	20q13.2	0	17	0.01
TNFRSF6B(DCR3)	20q13	0	17	0.01
PCNT2(KEN)	21q22-qter	9	25	0.03
GSCL	22q11.21	21	0	0.002
Losses				
FGR(SRC)	1p36.2-p36.1	3	22	0.02
BIN1	2q14	50	22	0.01
3PTEL25	3p tel	26	8	0.03
THRB	3p24.3	62	28	0.01
PIK3CA	3q26.3	44	11	0.008
D4S114	4p16.3	26	8	0.03
EGR1	5q31.1	53	17	0.01
D6S414	6p12.1-p21.1	3	19	0.03
MYB	6q22-q23	24	6	0.02
TIF1	7q32-q34	59	11	0.003
GATA3	10p15	12	39	0.005
D10S167	10p11-q11	29	61	0.02
PTEN	10q23.3	15	39	0.02
10QTEL24	10q tel	9	25	0.03
BRCA2	13q12-q13	3	19	0.03
D13S25	13q14.3	3	19	0.03
D15S11	15q11-q13	18	3	0.03
SNRPN	15q12	12	67	0.002
FRA16D	16q23.2	44	19	0.02

[a]Fisher's exact test

series (for review, see [9]). Additionally, frequently deleted clones *EGR1* and *EGR2* both encode for transcription factors that were defined as regulators of multiple tumor suppressors [3, 42]. Frequent loss of *LPL* was found in prostate carcinoma [8] whereas inactivation of *SNRPN* and *HTR1B* has been described in germ-cell and renal-cell tumors, respectively [7, 39]. Downregulated in metastases (*DRIM*) is a gene the decreased expression of which correlated with metastatic capability of cancers [44]. In addition, a few STS clones at 9p11.2, 17p12-p11.2, and 19p13.2 were found to be frequently deleted, thus suggesting the presence of TSGs in these regions.

In summary, the cytogenetic signature of glioblastomas displays recurrent DNA copy number imbalances with frequent gains of the clones harboring potent oncogenes and deletions of TSGs loci. These findings give evidence for a pronounced genomic instability in glioblastomas that compiles the molecular background of their biological aggressiveness.

Age-related genomic imbalances in glioblastomas

Patient age is an important prognostic factor for glioblastomas [23, 24], and age-related genetic alterations in these tumors have been established [18, 19, 43]. Consequently, we found a set of CNAs, the frequency of which differed for patients younger and older than 50 years. Gains at 7q, 19p, 19q, and 20q as well as losses at 10p and 10q tended to distribute among older patients, which is in keeping with cytogenetic studies published previously [22, 27, 43]. Loss of *SNRPN* was found almost exclusively in samples from older patients. This gene encodes for small ribonuclear protein, and deletion of *SNRPN* is critical for development of Prader-Willi syndrome. Tumors from younger patients frequently harbored deletions of TSGs (*BIN1, EGR1*, and *FRA16D*) and losses of potent oncogenes (*THRB, TIF1, MYB, PIK3CA*). *TIF1* encodes for ligand-activated transcriptional factor. Glioblastomas from older patients usually showed gain of this locus, along with other clones on 7q, whereas

Table 6 DNA copy number imbalances associated either with shortened survival [unfavorable copy number aberrations (CNAs)] or with prolonged survival (favorable CNAs)

Unfavorable CNAs				Favorable CNAs			
Clone name	Location	No of tumors	*P* value[a]	Clone name	Location	No of tumors	*P* value[a]
Gains							
*FAF1;*D1S427	1p32.3	6	0.03	*MYCL1*	1p34.3	14	0.03
LAMC2	1q25-q31	12	0.004	SGC34236	2q13	7	0.002
AKT3	1q44	12	0.004	6QTEL54	6q tel	14	0.004
2PTEL27	2p tel	9	0.008	*EXT1*	8q24.11	17	0.02
p44S10	3p14.1	7	0.04	*INS*	11p15.5	22	0.03
TP63	3q27-q29	13	0.01	*HRAS*	11p15.5	8	0.003
CTSB	8p22	9	0.02	*GARP*	11q13.5	12	0.03
D9S913	9p tel	7	0.02	*UBE3A;*D15S10	15q11	20	0.01
H18962	9q tel	15	0.04	stSG30213	16q tel	5	0.008
FGF4;FGF3	11q13	10	0.01	*DCC*	18q21.3	14	0.0007
MLL	11q23	14	0.02				
TCL1A	14q32.1	16	0.02				
CDH1	16q22.1	5	0.04				
THRA	17q11.2	14	0.02				
YES1	18p11.3	12	0.02				
JUNB	19p13.2	12	0.02				
Losses							
CASP8	2q33-q34	11	0.005	*CHL1;*3PTEL01	3p26.1	8	0.03
D5S23	5p15.2	17	0.03	*RBP1;RBP2*	3q21-q22	8	0.002
D6S434	6q16.3	10	0.02	*TERC*	3q26	14	0.001
E2F5	8p22-q21	16	0.03	*DDX15*	4p15.3	7	0.001
HRAS	11p15.5	19	0.001	*MYB*	6q22-q23	10	0.003
U11838	12q tel	11	0.04	AF170276	9p tel	8	0.006
WI-5214	15q tel	18	0.006	AFM137XA11	9p11.2	38	0.01
HIC1	17p13.3	14	0.001	*ATM*	11q22.3	11	0.03
D17S125	17p12-p11.2	23	0.008	D19S238E	19q13.4	13	0.003
LLGL1	17p12-p11.2	9	0.01	*PCNT2(KEN)*	21q22-qter	6	0.03

[a]Log-rank test

tumors from their younger counterparts usually disclosed *TIF1* as a minimally deleted region at 7q32–q34. Thus, our findings allow one to suggest that glioblastomas from various age groups represent molecularly distinct diseases that can develop along substantially different genetic pathways.

Prognostically relevant genetic subsets of glioblastomas

A few array-CGH glioblastoma studies included survival analysis, but the results obtained are controversial [25, 28, 29]. Our current study suggests that glioblastomas can be subdivided into clinically relevant genetic subsets. The 5-year survival in our patient cohort was greater than that reported for the entire population of glioblastomas [23, 24]. The reasons for such difference are likely related to selection bias because the cases included in this study showed a wide range of survival times. Given the strong relationship between older age and survival in glioblastomas, our cohort of patients was younger than the median age of patients with these tumors.

Current study disclosed a set of CNAs predicting for patient outcome. Most prognostically significant CNAs are not associated with well-defined chromosomal imbalances in glioblastomas. Some of these CNAs were also found to be correlated with patient age, thus explaining partly the age-related differences in outcomes of these tumors.

Unfavorable copy number gains comprised potent oncogenes (*AKT3, p44S10, FGF4, FGF3, MLL, TCL1A, THRA, YES1, JUNB)* that were reported to be overrepresented in solid tumors and hematological malignancies (for review, see [9]). Additionally, *TP63* was found frequently amplified in squamous cell carcinomas [13]. *LAMC2* and *CTSB* encode for laminin5 gamma2 chain and cathepsin B, respectively; both have been established as promoters of tumor invasiveness [1, 36]. Unfavorable losses targeted genes associated with cell death and suppression of tumor growth. *CASP8* encodes for a key enzyme of the top of the apoptotic cascade [41]. *HIC1* is a putative TSG encoding a transcription factor that has been found inactivated in various tumors [30]. *LLGL1* is a human homolog of Drosophila suppressor gene, and its experimental inactivation leads to malignant transformation of primordial cells in the brain [11]. *HRAS* allelic deletion was found to be a common abnormality in various tumors, and these findings suggest that a putative TSG closely linked to *HRAS* on 11p15.5 is involved in carcinogenesis. Yokota et al. [45] found that allelic deletions of *HRAS* were correlated with progression and metastases of carcinomas and sarcomas.

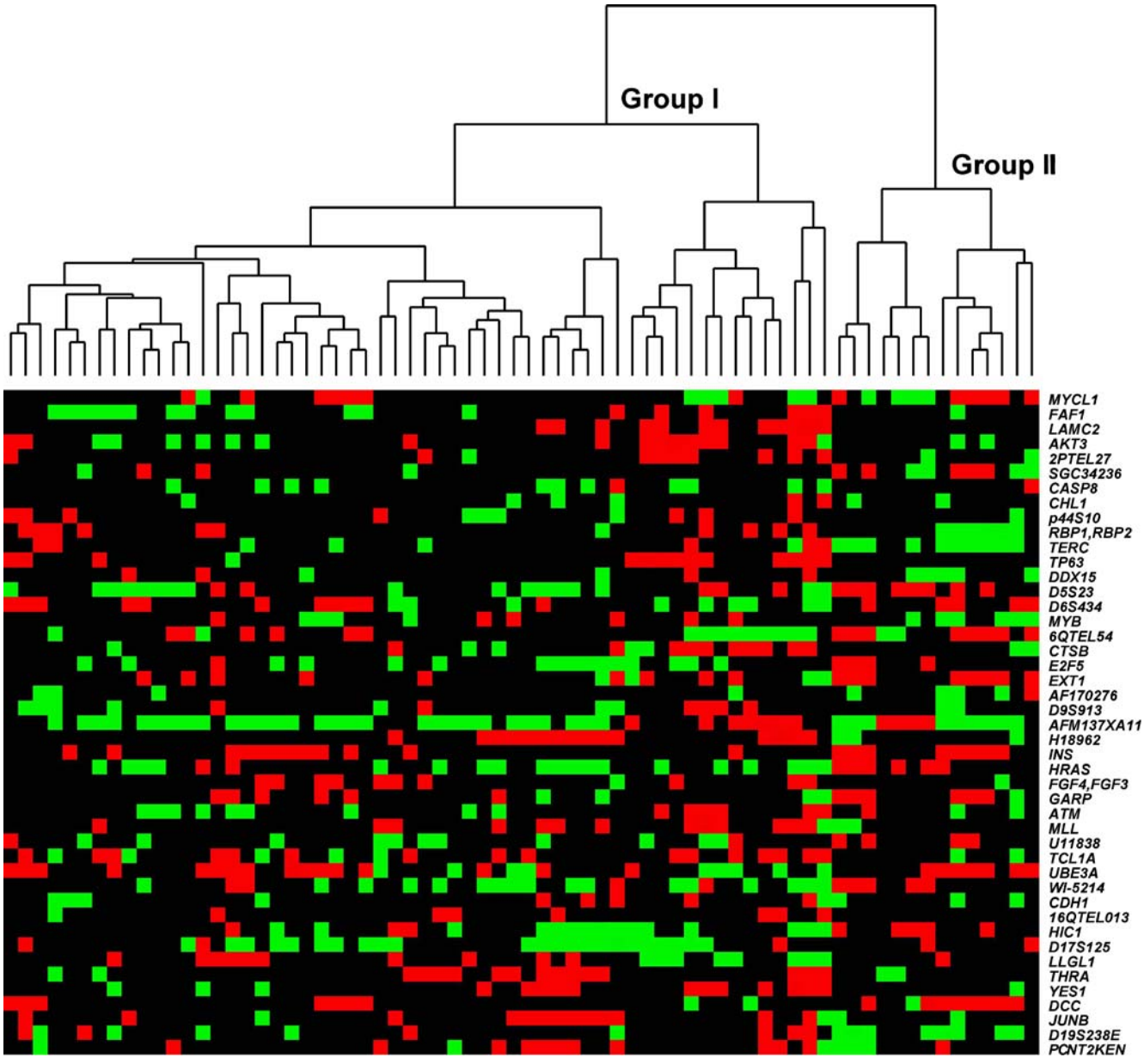

Fig. 2 Unsupervised cluster analysis of array-based comparative genomic hybridization (array-CGH) data from 70 glioblastomas (*each column* represents one case). We calculated cutoff assigned values of 1, 0, and −1 for gain, balanced, and loss, respectively, and clustered the samples including only 46 prognostically relevant copy number aberrations. *Each row* represents one clone, and clone identifiers are showed on the *right*. The latter includes 45 clones because *HRAS* locus is presented twice (as negative indicator being deleted and as positive one being gained). Losses are displayed in *green*, and gains are in *red*. The classification tree on top displays two major genetic subtypes for glioblastomas (groups 1 and 2, respectively)

The biologic significance of favorable CNAs remains to be defined. These imbalances comprised gains of putative TSGs (*EXT1, UBE3A*, *DCC*) and losses of potent oncogenes (*TERC*, *MYB*, *PCNT2*). Association of *INS* with glioblastoma survival has been identified [28]. Previous deletion mapping studies gradually narrowed down the 19q region involved in gliomas, and the frequent region of deletion was reported to be located at 19q13.3 [37]. However, we found here that glioblastoma prognosis was associated with loss of distal region at 19q13.4. Association of loss of the 9p21/*CDKN2A* locus with glioblastoma adverse outcomes has been discussed widely [6, 17–19, 21, 22, 27, 35, 43]. Surprisingly, we found that losses of subtelomere and pericentromeric regions at 9p were associated with favorable survival. From the current awareness of glioblastoma molecular biology, it is difficult to explain such associations; therefore, these data need to be elaborated further.

Combined cluster analysis allowed one to subdivide an entire tumor cohort on two genetically different subtypes with striking differences in outcomes, thus supporting the notion that glioblastomas might com-

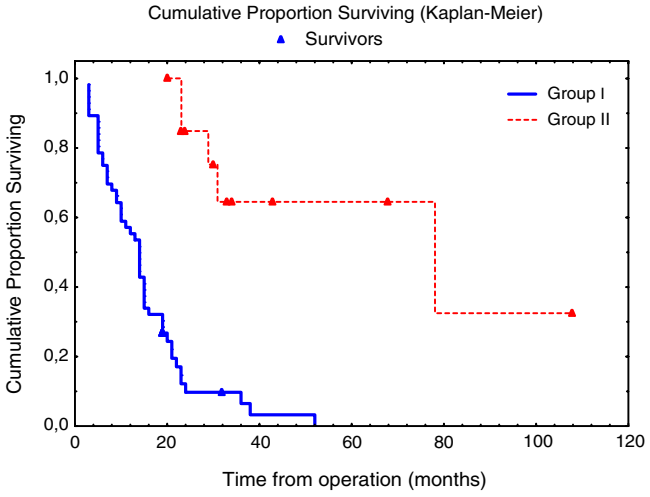

Fig. 3 Graph displaying Kaplan–Meier survival curves for glioblastoma patients in two genetic groups (1 and 2) defined by array-based comparative genomic hybridization (array-CGH) (log-rank test; $P < 0.0001$)

prise molecularly distinct tumor entities despite their histological similarity.

Conclusions

Genomic profiling of glioblastomas using the GenoSensor Array 300 identified several genetic alterations that might be associated with biologic behavior of these malignancies. Detailed mutational analyses and functional studies will be required to elucidate the role that each of these alterations might play in glioblastoma pathogenesis and clinical course.

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