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ORIGINAL ARTICLE

Correlation Among Pathology, Genotype, and Patient Outcomes in Glioblastoma

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Abstract

Glioblastomas are histologically and genetically heterogeneous. We have investigated to what extent histologic features reflect the genetic profile and whether they are predictive of clinical outcome. Key histologic characteristics, including major cell types (small cell, nonsmall cell), other components such as oligodendroglial components, gemistocytes, multinucleated giant cells, as well as necrosis and microvascular proliferation, of 420 cases of glioblastoma within a population-based study (1) were reassessed and correlated with patients' clinical outcome and key genetic alterations. *EGFR* amplification and $p16^{INK4a}$ homozygous deletion were significantly more frequent in small cell glioblastomas than in nonsmall cell glioblastomas (*EGFR*, 46% vs 26%, p = 0.0002; $p16^{INK4a}$ 39% vs 25%, p = 0.0167). Multivariate analyses with adjustment for age and gender showed that small cell glioblastomas had frequent *EGFR* amplification and $p16^{INK4a}$ deletion but infrequent *PTEN* mutations. An oligodendroglial component was detected in 20% of glioblastomas; these patients were significantly younger (54.4 ± 13.6 vs 59.2 ± 13.8 years; p = 0.0049) and survived longer (10.3 ± 8.3 vs 8.2 ± 8.4 months; p = 0.0647). However, multivariate analyses with adjustment for age and gender did not show the presence of an oligodendroglial component to be predictive of longer survival. After adjustment for age and gender, LOH 1p was associated with longer survival (hazard ratio, 0.7; 95% confidence interval [CI], 0.5–1.0), whereas LOH 10q was associated with shorter survival (hazard ratio, 1.4; 95% CI, 1.0–1.8) of patients with glioblastoma. Glioblastomas containing ≥5% multinucleated giant cells showed more frequent *TP53* mutation and infrequent *EGFR* amplification than those containing <5% multinucleated giant cells (*TP53*, 45% vs 24%, p = 0.0001; *EGFR*, 24% vs 42%, p = 0.0005). Vascular proliferation was observed in all glioblastomas, whereas large ischemic and/or pseudopalisading necrosis was observed in 366 of 420 (87%) cases. Glioblastomas with necrosis were associated with older age (59.2 ± 13.3 vs 51.6 ± 15.3 years; p = 0.0001) and shorter survival (7.9 ± 6.8 vs 12.9 ± 14.2 months; p = 0.0017). Multivariate analyses with adjustment for age and gender confirmed this observation (hazard ratio, 1.5; 95% CI, 1.1–2.0). Multivariate analysis with adjustment for age and gender showed that necrosis was significantly associated with wild-type *TP53* and absence of an oligodendroglial component. These results suggest that some histologic features in glioblastomas are associated with specific genetic alterations and with clinical outcome.

Key Words: *EGFR* amplification, Glioblastoma, LOH 1p, LOH 19q, Necrosis, Oligodendroglial component, $p16^{INK4a}$ deletion, Population-based study, *TP53* mutations.

INTRODUCTION

Glioblastoma is the most frequent and malignant intracranial brain tumor and is characterized by hypercellular anaplastic glioma cells with marked mitotic activity as well as the presence of necrosis and microvascular proliferation (2). Neoplastic cells frequently reveal pleomorphism showing different histologic features such as small homogeneous cells with scant cytoplasm, fibrillary-shaped cells, multinucleated giant cells, and cells with pleomorphic nuclei and cytoplasm (2).

Several studies have suggested that certain histologic features in glioblastomas are associated with patients' clinical outcome. The presence of necrosis has been considered a predictive factor for poor survival of patients with glioblastoma (3–5). The presence of oligodendroglial components has been reported to be predictive of longer survival of patients in some studies (6–8), although others did not show any difference (9).

There may also be an association between histologic features and specific genetic alterations. Burger et al (10) showed that small cell structure represents a histologic feature of most primary glioblastomas and is associated with *EGFR* amplification: 14 of 21 (67%) exclusively small cell neoplasms, 8 of 25 (32%) glioblastomas with both small cells and nonsmall cell areas, and 3 of 33 (9%) nonsmall cell glioblastomas had *EGFR* amplification. However, their study was based on a small number of cases and no validation with a large number of cases has been carried out.

In the present study, we reevaluated 420 cases of glioblastomas from a population-based study in the Canton of Zurich, Switzerland (1) for detailed histologic features of

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glioblastomas and correlated these with data on patients' outcome and key genetic alterations (*EGFR* amplification, *TP53* mutations, *PTEN* mutations, $p16^{INK4a}$ homozygous deletion, LOH 10q), which have been published previously (1, 11). We focused on major cell types (small cells or nonsmall cells) and other cell components, including oligodendroglial component, gemistocytes, multinucleated giant cells, as well as necrosis and vascular proliferation. Because LOH 1p and 19q are typical genetic alterations in oligodendrogliomas (12), and it was of interest to assess the correlation between oligodendroglial components in glioblastomas and such LOH, we also carried out analyses for LOH 1p and LOH 19q in glioblastomas in the present study.

MATERIALS AND METHODS

Patients

We reexamined histologically 420 glioblastomas from a population-based study in the Canton of Zurich, Switzerland (1), corresponding to 75% of all glioblastomas that were histologically diagnosed between 1980 and 1994. Of these, 326 cases were diagnosed using biopsy and 94 cases were diagnosed at autopsy. The mean age of the patients was 58.2 ± 13.8 years and the male:female ratio was 243:177 (1.4:1). Tumors were considered primary (de novo) glioblastomas if a glioblastoma diagnosis was made at the first biopsy, without clinical or histologic evidence of a less malignant precursor lesion. The diagnosis of secondary glioblastoma was made only in cases with histopathologic evidence of preceding low-grade or anaplastic glioma (1, 11). Thirty-eight patients (28 primary and 10 secondary glioblastomas) received radiotherapy (2-Gy fractions and a total dose of 60 Gy) before glioblastoma diagnosis.

Reevaluation of Histologic Features of Glioblastomas

Major Cell Types

Glioblastomas were first subdivided into 3 groups based on their major cell types (i.e. small cell and nonsmall cell). Group 1 consisted of glioblastomas with almost exclusively highly cellular, monotonous small poorly differentiated cells with round to oval-shaped nuclei surrounded by scanty cytoplasm and high mitotic figures (Fig. 1A, B); group 2 consisted of glioblastomas in which the major cell type (>50%) is monotonous small cell, but other cell components such as pleomorphic neoplastic cells, which vary in size and shape of nuclei and cytoplasm (Fig. 1C), fibrillary cells, granular cells, cells resembling gemistocytes (Fig. 1D), multinucleated giant cells (Fig. 1E), and sarcomatous cells are also present in <50% of tumor areas; group 3 consisted of glioblastomas in which >50% of neoplastic cells are not small cells. Neoplastic cells usually consist of a mixture of pleomorphic neoplastic cells, fibrillary cells, granular cells, multinucleated giant cells, and gemistocytes.

Oligodendroglial Components

Oligodendroglial components were observed in both small and nonsmall cell glioblastomas and were composed of glioma cells that are characterized by "clear cytoplasm," a "perinuclear halo," and a "homogeneous round nucleus" resembling neoplastic cells in oligodendrogliomas (Fig. 1F, G), which may be seen in at least one focal area in glioblastomas. In tumors with an oligodendroglial component, typical glioblastoma features were observed in the other areas of tumors, eliminating the possibility of anaplastic oligodendrogliomas.

Microvascular Proliferation

When swollen endothelial proliferation was detected in the neoplasm on low-power view, this was considered positive for microvascular proliferation. All the glioblastomas in the present study showed microvascular proliferation. The degree of microvascular proliferation was classified into 2 categories: microvascular proliferation with glomeruloid structures and those without glomeruloid structures.

Necrosis

Large ischemic necrosis is defined as coagulation necrosis of glioma cells and necrotic debris (Fig. 1H). Pseudopalisading necrosis was surrounded by glioma cells in a pseudopalisading pattern (Fig. 1I). There was no sign of the typical histologic changes which may be associated with radiotherapy such as hyalinized vessels in any of the cases analyzed.

Quantitative Microsatellite Analysis for LOH 1p and LOH 19q

DNA was extracted from paraffin sections as previously described (10). Quantitative microsatellite analysis was performed using 2 microsatellite markers on each of chromosomes 1p and 19q. The following loci were analyzed: D1S214 and D1S2736 for chromosome 1p and D19S408 and D19S867 for chromosome 19q, as previously reported (13). These microsatellite markers were located within the areas of 1p36 and 19q13 that are commonly deleted in oligodendrogliomas (14) and glioblastomas (15, 16). Polymerase chain reaction (PCR) was performed in triplicate using an Mx3000p Real-time PCR system (STRATAGENE, La Jolla, CA) Genomic DNA (10–20 ng) was amplified in 6.25 μL with 2 × TaqMan Universal PCR Master Mix, 0.4 μmol/L of each primer, 60 nmol/L probe (21-bp oligomer complementary to the microsatellite CA repeat: 5,6-carboxyfluorescein [FAM]-TGT GTG TGT GTG TGT GTG TGT-3,6-carboxy-tetramethyl-rhodamine) in 12.5 μL per well in a 96-well plate, as previously reported (13). Primers and probes were purchased from Proligo Primers and Probes (Paris, France), and the TaqMan master mix was purchased from Applied Biosystems (Foster City, CA). Six reference primer sets for the reference pool were used in each plate in triplicate to normalize for differences in the amount of total input DNA. We calculated the PCR cycle number (Ct) value, δCt (Ct [microsatellite] – Ct [reference pool]), δδCt (δCt [tumor] – δCt [normal]) values and the relative copy number ($2^{-\delta\delta Ct}$) as previously reported (14). To calculate the average value of δCt (δCT [normal]), DNA was extracted from more than 10 samples of normal tissue in each reference marker. A tolerance interval (TI) with a confidence interval of 95% determined from the pooled standard deviation of normal DNA samples for each microsatellite marker was calculated as previously reported (14). According to this TI, LOH was defined as when copy numbers

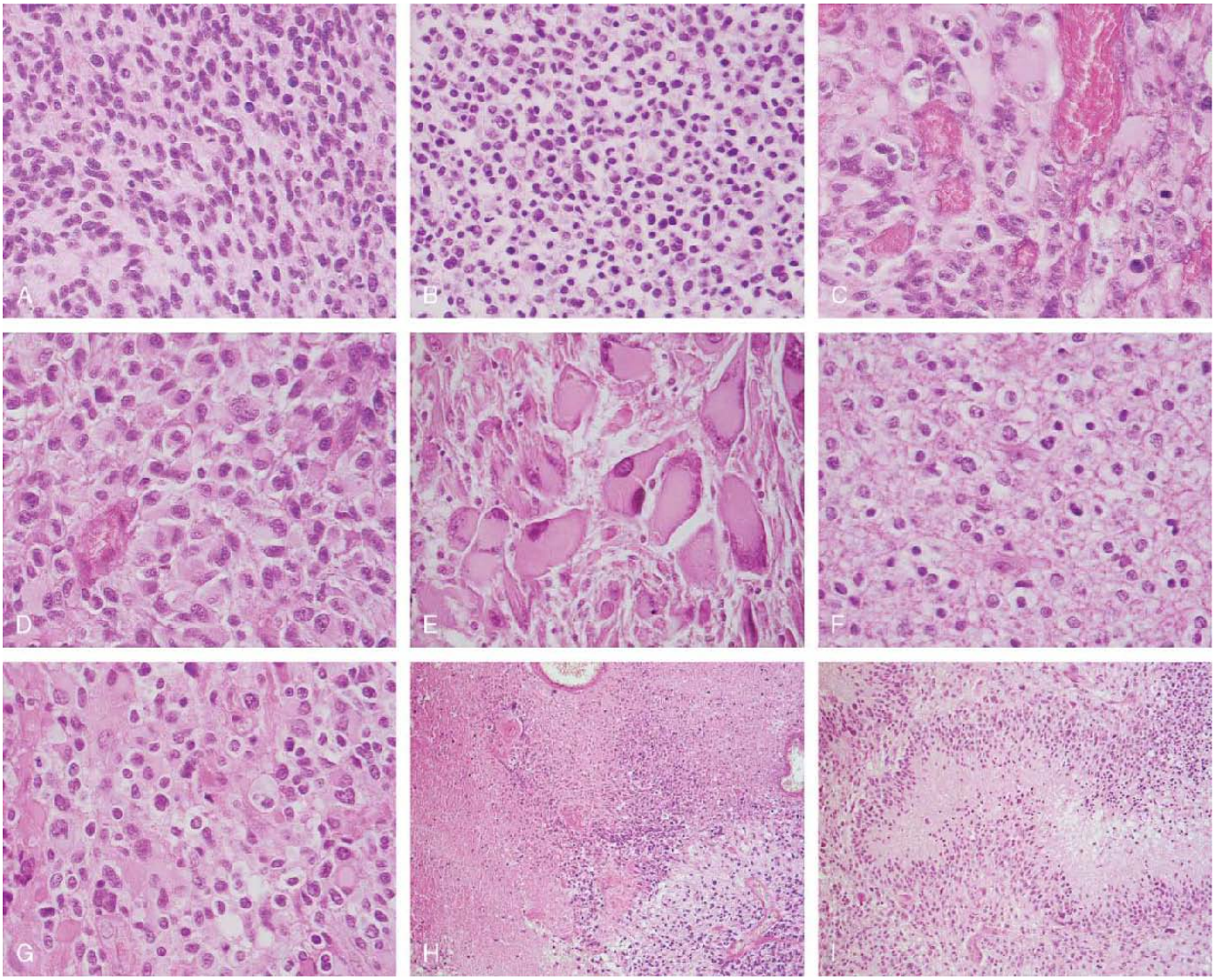

FIGURE 1. Histologic features of glioblastomas. Small homogeneous cells with high cellularity and high mitotic activities showing scant cytoplasm with round to oval nuclei **(A, B)**. Pleomorphic shaped cells with bizarre nuclei **(C)**. Cells resembling gemistocytes **(D)**. Multinucleated giant cells **(E)**. Oligodendroglioma-like cells characterized as homogeneous round nuclei with perinuclear halo and clear cytoplasm **(F, G)**. Large ischemic necrosis **(H)** and pseudopalisading necrosis **(I)**.

were less than 1.34 to 1.37. Losses on chromosomes 1p and 19q were determined as those occasions in which the samples were positive for both markers.

Statistical Analyses

Kaplan-Meier survival statistics were used to evaluate survival curves in each patient group. The log-rank (Mantel-Cox) test was used for comparing these survival curves.

Unpaired *t*-test was performed for comparison of patient's ages. The χ^2 test and Fisher exact test were performed to analyze relationships between histopathologic features and genetic alterations. Additionally, to analyze relationships between primary/secondary glioblastoma and histopathologic features, χ^2 test and Fisher exact test were used.

Multivariate Cox regression models were used to assess predictive factors of survival. Basic adjustment was made for age and gender, and additional adjustment included major histologic features and genetic alterations. Interactions of the predictor with time were added in the model when we found a violation of the proportionality assumption. Logistic regression models were used to analyze the associations between histologic features and genetic alterations.

RESULTS

Major Cell Types

Of 403 glioblastomas that could be assessed for major cell types, 83 (21%) showed exclusively monotonous small cells in most of the tumor area (group 1, see "Materials and Methods"). One hundred seven cases (27%) showed predominant small cells (>50%), but contained also other cell types (group 2). In the remaining 213 cases, the major cell types were not small cells, but neoplastic cells, usually consisting of a mixture of pleomorphic neoplastic cells,

TABLE 1. Correlation Between Histologic Features and Age and Survival of Patients With Patients With Glioblastoma

Histologic Features	Number of Cases	(percent of all cases)	Mean Age (years)	Survival (months)
Major cell type				
Small cells	190	(47%)	58.2 ± 14.2	8.5 ± 7.4
Nonsmall cells	213	(53%)	58.3 ± 13.7	8.6 ± 9.2
Oligodendroglial component				
Present	80	(20%)	**54.4 ± 13.6***	**10.3 ± 8.3†**
Absent	323	(80%)	**59.2 ± 13.8**	**8.2 ± 8.4**
Multinucleated giant cells				
≥5%	167	(41%)	57.7 ± 15.4	8.0 ± 8.7
<5%	236	(59%)	58.7 ± 12.8	9.0 ± 8.1
Gemistocytes				
≥5%	204	(51%)	58.9 ± 12.6	8.8 ± 8.4
<5%	199	(49%)	57.6 ± 15.1	8.4 ± 8.3
Necrosis				
Present	366	(87%)	**59.2 ± 13.3‡**	**7.9 ± 6.8§**
Absent	54	(13%)	**51.6 ± 15.3**	**12.9 ± 14.2**
Vascular proliferation				
With glomeruloid	304	(72%)	57.5 ± 13.7	8.8 ± 7.6
Without glomeruloid	116	(28%)	60.0 ± 13.7	7.8 ± 9.9

Bold letters indicate significant difference.
*, p = 0.0049.
†, p = 0.0647.
‡, p = 0.0001.
§, p = 0.0017.

fibrillary cells, granular cells, multinucleated giant cells, and gemistocytes (group 3). The mean age, survival of patients, and data on genetic alterations were similar between groups 1 and 2. Groups 1 and 2 were considered as small cell glioblastomas and group 3 as nonsmall cell glioblastomas in subsequent analyses (Table 1). Multivariate analyses with adjustment for age and gender showed that small cells as the major cell type in glioblastomas do not affect patients' survival (Table 2).

Oligodendroglial Component

Oligodendroglial components were detected at least focally in 80 of 403 (20%) of the glioblastomas analyzed. Patients with glioblastomas containing an oligodendroglial component were significantly younger (mean, 54.4 years) than those without (59.2 years; p = 0.0049; Table 1). The presence of oligodendroglial components in glioblastomas was significantly associated with longer survival of patients (mean survival 10.3 vs 8.2 months; Table 1, Fig. 2). For glioblastomas having an oligodendroglial component, the median survival of patients was 8.1 months, and for those without an oligodendroglial component, median survival was 6.0 months. However, after adjustment for age and gender, multivariate analyses showed no predictive value of an oligodendroglial component in glioblastomas (Table 2).

Multinucleated Giant Cells

Patients with glioblastomas containing ≥5% or <5% multinucleated giant cells showed similar age and survival (Table 1). Multivariate analyses after adjustment for age and gender showed that patients with glioblastomas containing ≥5% multinucleated giant cells had poorer survival, but this tendency was no longer seen after adjustment for age, gender, and genetic alterations (Table 2).

Fifteen of 403 (3.7%) glioblastomas showed a predominance of bizarre multinucleated giant cells in more than 20% of all neoplastic cells and were diagnosed as giant cell glioblastomas. All of these were clinically classified as primary glioblastomas. Patients with giant cell glioblastomas had a mean age of 43.6 ± 18.8 years, significantly younger than those with other glioblastomas (58.8 ± 13.3 years; p < 0.0001). Patients with giant cell glioblastomas had longer survival (12.4 ± 16.2 months) than those with other glioblastomas (8.4 ± 7.9 months), but the difference was not significant (p = 0.1053).

Gemistocytes

Glioblastomas with gemistocytes in ≥5% of all neoplastic cells and those with ≥20% gemistocytes were not significantly different from those with gemistocytes in <5% of all neoplastic cells with respect to patients' age, survival, or genetic alterations (Table 1).

Microvascular Proliferation

All glioblastomas showed microvascular proliferation. Microvascular proliferation with glomeruloid structures was detected in 304 cases (72%). The mean age and mean survival of patients with glioblastoma with or without glomeruloid structure were similar (Table 1).

TABLE 2. Multivariate Analysis for the Effect of Various Histologic Features and Genetic Alterations on Death Rates of Patients With Glioblastoma

Characteristic	Number of Cases	Hazard Ratio (95% CI) Model 1*	Hazard Ratio (95% CI) Model 2†
Necrosis	366 (87%)	**1.5 (1.1–2.0)**	**1.8 (1.0–3.2)**
Oligodendroglial components (yes)	80 (20%)	0.8 (0.7–1.1)	
Major cell types (small cells)	190 (47%)	0.9 (0.7–1.1)	
Multinucleated giant cells (≥5%)	167 (41%)	**1.2 (1.0–1.5)**	1.0 (0.7–1.4)
TP53 mutations	113 (32%)	0.8 (0.7–1.1)	
PTEN mutations	73 (25%)	0.9 (0.7–1.2)	
$p16^{INK4a}$ deletion	95 (31%)	0.9 (0.7–1.2)	
EGFR amplification	121 (35%)	1.1 (0.9–1.4)	
LOH 10q	130 (63%)	**1.4 (1.0–1.8)**	**1.3 (1.0–1.8)**
LOH 1p	37 (16%)	**0.7 (0.5–1.0)**	**0.6 (0.4–0.9)**
LOH 19q	16 (7%)	0.7 (0.4–1.2)	

Bold letters indicate statistical significance.
*, Adjusted for age and gender.
†, Adjusted for age, gender, and all other significant factors in model 1.
CI, confidence interval.

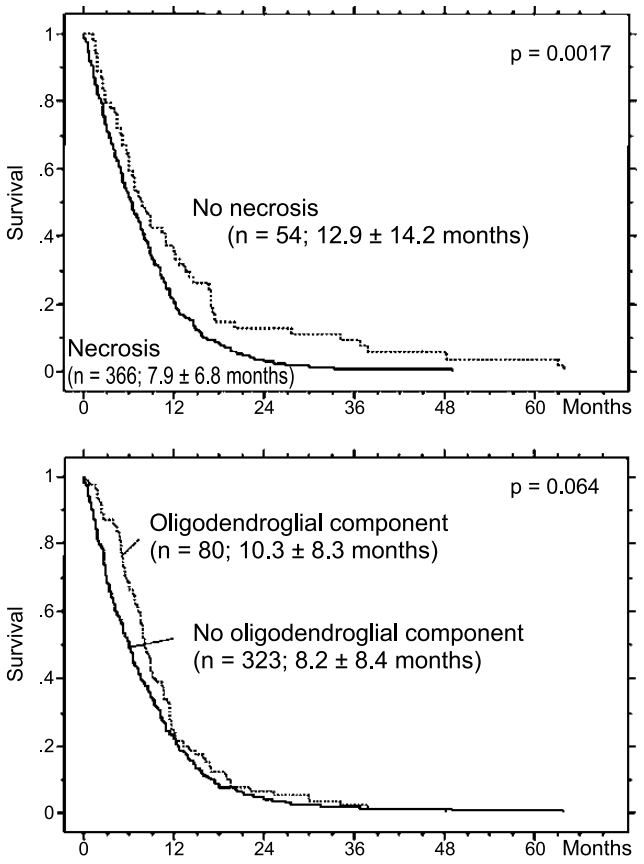

FIGURE 2. Survival of patients with glioblastoma. Kaplan-Meier curves show that the presence of necrosis is associated with shorter survival **(A)**, whereas the presence of an oligodendroglial component is associated with longer survival **(B)**.

Necrosis

Large ischemic necrosis and/or pseudopalisading necrosis was detected in 366 of the 420 glioblastomas analyzed (87%). The mean age of patients with glioblastoma with necrosis was 59.2 years, which was significantly older than those without necrosis (51.6 years; p = 0.0001; Table 1). Patients with glioblastoma showing necrosis had mean survival of 7.9 months, significantly shorter than those without necrosis (12.9 months; p = 0.0017; Table 1, Fig. 2). Multivariate analyses with adjustment for age and gender showed that necrosis was a significant predictive factor for poor survival of glioblastoma patients (hazard ratio, 1.5; 95% confidence interval [CI], 1.1–2.0) (Table 2).

Association Between Histologic Features in Glioblastomas

Multivariate analyses with adjustment for age and gender showed a significant positive association between glioblastomas with an oligodendroglial component and small cell glioblastomas (odds ratio [OR], 1.7; 95% CI, 1.0–2.8) and an inverse association between an oligodendroglial component and necrosis (OR, 0.4; 95% CI, 0.2–0.8). Multivariate analyses with adjustment for age and gender showed a significant positive association between glioblastomas with multinucleated giant cells and necrosis (OR, 1.9; 95% CI, 1.0–3.8) and an inverse association between multinucleated giant cells and small cell glioblastomas (OR, 0.2; 95% CI, 0.1–0.2).

LOH 1p and LOH 19q in Glioblastomas

LOH 1p was assessed in 231 glioblastomas. Of these, 37 (16%) showed LOH at both D1S214 and D1S2376. LOH 19q was assessed in 218 glioblastomas. Of these, 16 cases (7.3%) showed LOH at both D19S408 and D19S867. For 209 cases, data on both LOH 1p and LOH 19q were available. Of these, 43 (21%) showed LOH 1p and/or 19q (i.e. LOH 1p, LOH 19q, or both LOH 1p and LOH 19q). The mean ages of patients with glioblastomas showing LOH 1p and/or 19q and those without LOH 1p and/or 19q were similar (56.5 ± 12.2 vs 54.5 ± 13.4 years).

Survival of patients who had glioblastomas with LOH 1p was longer than that of patients without LOH 1p (13.2 ± 10.8 months vs 9.6 ± 7.4 months; p = 0.0536). Multivariate analyses after adjustment for age and gender showed that LOH 1p was associated with longer survival of patients (hazard ratio, 0.7; 95% CI, 0.5–1.0) (Table 2). Univariate

TABLE 3. Frequencies of Genetic Alterations in Glioblastomas With Different Histologic Features (univariate analysis)

	Histologic Features							
	Necrosis		Oligodendroglial Component		Major Cell Type		Multinucleated Giant Cells	
	Present	Absent	Present	Absent	Small cells	Nonsmall Cells	≥5%	<5%
TP53 mutations	29%	**51%***	38%	31%	29%	35%	**45%§**	24%
PTEN mutations	25%	24%	22%	26%	19%	29%	28%	23%
$p16^{INK4a}$ deletion	32%	24%	35%	30%	**39%†**	25%	26%	35%
EGFR amplification	36%	24%	41%	33%	**46%‡**	26%	24%	**42%‖**
LOH 10q	63%	60%	61%	65%	67%	61%	60%	66%
LOH 1p/19q	20%	15%	21%	19%	24%	15%	18%	20%

Bold letters indicate significant difference.
*, p = 0.0056.
†, p = 0.0167.
‡, p = 0.0002.
§, p = 0.0001.
‖, p = 0.0005.

and multivariate analyses showed that there were no significant differences in survival of patients who had glioblastomas with and without LOH 19q.

Survival of patients who had glioblastomas with both LOH 1p and 19q (6 cases) was 20.5 ± 21.5 months, which was longer than that for patients without either LOH 1p or 19q (9.5 ± 6.6 months; p = 0.0506).

Association Between Histologic Features and Genetic Alterations

Major Cell Types

Small cell glioblastomas showed a significantly higher frequency of *EGFR* amplification (46%) and $p16^{INK4a}$ homozygous deletion (39%) than nonsmall cell glioblastomas (Table 3). After adjustment for age and gender, multivariate analyses revealed that small cell glioblastomas were characterized by frequent *EGFR* amplification, frequent $p16^{INK4a}$ deletion, and infrequent *PTEN* mutations (Table 4).

Oligodendroglial Component

There was no difference in the frequency of genetic alterations between glioblastomas with or without an oligodendroglial component (Table 3). Only one of the 6 glioblastomas with both LOH 1p and LOH 19q contained an oligodendroglial component. Multivariate analyses with adjustment for age and gender also showed no significant association with any of the genetic alterations (Table 4).

Multinucleated Giant Cells

Glioblastomas containing ≥5% multinucleated giant cells were associated with frequent *TP53* mutations (45%) and infrequent *EGFR* amplification (24%; Table 3). These findings were confirmed by multivariate analyses adjusted for age and gender (Table 4).

Microvascular Proliferation

There was no significant difference in frequency of any of the genetic alterations analyzed between glioblastomas with or without glomeruloid structures (Table 3).

Necrosis

TP53 mutations were more frequently detected in glioblastomas without necrosis than in glioblastomas with necrosis (51% vs 29%; p = 0.0056) (Table 3). Multivariate analyses with adjustment for age and gender also showed the association of glioblastomas with necrosis with infrequent *TP53* mutations (OR, 0.4; 95% CI, 0.2–0.8) (Table 4).

Histologic Features of Primary and Secondary Glioblastomas

Secondary glioblastomas, which developed through progression from low-grade or anaplastic gliomas, more frequently contained oligodendroglial components (42%) than primary (de novo) glioblastomas (18%, p = 0.0138; Table 5). Large ischemic necrosis was significantly more frequent in primary (311 of 396 [79%]) than in secondary

TABLE 4. Association Between Histologic Features and Genetic Alterations in Glioblastomas (multivariate logistic regression analyses)

		Dependent Variable			
		Necrosis (Y)	Oligodendroglial Components (Y)	Major Cell Type: Small Cells (Y)	Multinucleated Giant Cells(≥5%)
Covariates	**Number of Cases**	**OR (95% CI)†**	**OR (95% CI)†**	**OR (95% CI)†**	**OR (95% CI)†**
TP53 mutations					
No*	241	1	1	1	1
Yes	113	**0.4 (0.2–0.8)**	1.3 (0.7–2.2)	0.7 (0.5–1.1)	**2.5 (1.6–4.1)**
PTEN mutations					
No*	223	1	1	1	1
Yes	73	0.9 (0.4–1.9)	0.8 (0.4–1.5)	**0.6 (0.3–1.0)**	1.3 (0.7–2.2)
$p16^{INK4a}$ deletion					
No*	208	1	1	1	1
Yes	95	1.2 (0.6–2.5)	1.3 (0.7–2.3)	**1.8 (1.1–3.0)**	0.6 (0.4–1.1)
EGFR amplification					
No*	228	1	1	1	1
Yes	121	1.7 (0.8–3.7)	1.4 (0.9–2.5)	**2.4 (1.5–3.8)**	**0.4 (0.3–0.7)**
LOH 10q					
No*	77	1	1	1	1
Yes	130	1.0 (0.4–2.7)	0.9 (0.5–1.8)	1.3 (0.7–2.3)	0.8 (0.4–1.5)
LOH 1p/19q					
No*	166	1	1	1	1
Yes	43	1.4 (0.4–4.3)	1.1 (0.6–2.3)	1.8 (0.9–3.6)	0.9 (0.5–1.8)

Bold letters indicate statistical significance.
*, Reference category.
†, Adjusted for age and gender.
CI, confidence interval.

TABLE 5. Histologic Features of Primary and Secondary Glioblastomas

Histologic Features	Primary Glioblastoma	Secondary Glioblastoma	p Value
Small cells (≥50%)	178/379 (47%)	12/24 (50%)	0.8347
Oligodendroglial component (yes)	70/379 (18%)	**10/24 (42%)**	0.0138
Gemistocytes (≥5%)	187/379 (49%)	12/24 (50%)	>0.9999
Multinucleated giant cells (≥5%)	223/379 (59%)	13/24 (54%)	0.6738
Necrosis (yes)	**351/396 (89%)**	15/24 (63%)	0.0014
Glomeruloid vascular proliferation (yes)	288/396 (73%)	16/24 (67%)	0.4901

Bold letters indicate significant difference.

glioblastomas (13 of 24 [54%], p = 0.0105). Pseudopalisading necrosis was significantly more frequent in primary (177 of 396 [45%]) than in secondary glioblastomas (5 of 24 [21%], p = 0.0319). Large ischemic necrosis and/or pseudopalisading necrosis was also significantly more frequent in primary (89%) than secondary glioblastomas (63%, p = 0.0014; Table 5).

The multivariate logistic regression adjusted for age and gender confirmed the difference in frequency of oligodendroglial components (p = 0.0338) and large ischemic necrosis and/or pseudopalisading necrosis (p = 0.0167) between primary and secondary glioblastomas.

Histologic and Genetic Features in Glioblastomas From Long-Term Survivors

Thirty-seven patients with glioblastoma survived more than 18 months (long-term survivors), whereas 195 patients died within 6 months after glioblastoma diagnosis (short-term survivors). The mean age of long-term survivors was significantly less than that of short-term survivors (50 years vs 62 years; p < 0.0001). An oligodendroglial component was significantly more frequent in glioblastomas from long-term survivors than in those from short-term survivors (29% vs 14%; p = 0.0437). There was no significant difference in frequencies of genetic alterations between long-term and short-term survivors, except for LOH 10q, which was less frequently observed in long-term survivors (50%) than in short-term survivors (73%; p = 0.0605).

DISCUSSION

In the present study, we reevaluated the detailed histologic features of 420 glioblastomas diagnosed in the Canton of Zurich, Switzerland, between 1980 and 1994 in a population-based study (10). This is, therefore, not only the largest histologic evaluation of glioblastomas, but also the first population-based analysis of correlation between histologic features, clinical outcome, and genetic alterations in glioblastomas.

It is known that one of the major histologic features of glioblastomas is the presence of homogeneous small neoplastic cells. On the basis of 71 cases of glioblastomas, Burger et al (17) showed that patients with glioblastomas composed of homogeneous small neoplastic cells had shorter survival. In contrast, in a study of 97 glioblastomas, Matthias et al (18) did not find different survival of patients with glioblastoma with or without areas of better differentiation. In the present study, univariate and multivariate analyses both showed that small cells as the major cell type in glioblastomas are not predictive of survival.

Burger et al (9) reported that *EGFR* amplification was associated with a small cell phenotype in glioblastomas. They found that 67% of exclusively small cell neoplasms, 32% of glioblastomas with both small and nonsmall cell areas, and 9% of nonsmall cell glioblastomas had *EGFR* amplification (9). Perry et al (19) also reported that small cell glioblastomas showed frequent *EGFR* amplification (72%) and LOH 10q (100%), whereas none of these showed loss of 1p/19q. In the present study, with a large number of cases, small cell glioblastomas had significantly more frequent *EGFR* amplification and $p16^{INK4a}$ homozygous deletion, but infrequent *PTEN* mutations, suggesting that their genetic bases may differ from that of other glioblastomas.

We also found that 20% of glioblastomas contained, at least focally, an oligodendroglial component. This result is similar to the finding by He et al (8) of 25 glioblastomas with an oligodendroglial component among 142 malignant gliomas (17%). Several studies have shown longer survival of patients with glioblastomas containing an oligodendroglial component (5, 6). Hilton et al (6), in a study with 107 patients, showed that glioblastomas with an oligodendroglial component were associated with longer survival (median survival, 70 weeks) than those without (27 weeks). Similarly, Kraus et al (7) reported that the median survival of 12 patients with glioblastoma with an oligodendroglial component was 26 months, suggesting that these tumors are associated with better prognosis. In contrast, He et al (8) reported that the age (median, 54 years) and survival (median, 11.5 months) of 25 patients with glioblastoma containing an oligodendroglial component did not differ from those of patients with ordinary glioblastoma. Some studies reported that patients with glioblastoma having oligodendroglial features were younger than those with ordinary glioblastoma (5), but other studies failed to confirm this difference (6, 8). In the present study, we found that glioblastomas containing an oligodendroglial component developed in significantly younger patients. Univariate analysis revealed a tendency to longer survival of patients with glioblastoma containing an oligodendroglial component. We also showed that oligodendroglial components were significantly more frequent in glioblastomas that developed in long-term survivors (>18 months) than in short-term survivors (<6 months). However, multivariate analyses with adjustment for age and gender did not show the presence of an oligodendroglial component to be predictive of longer survival.

In a study with 97 glioblastomas, Schmidt et al (18) reported that neither LOH 1p nor LOH 19q was associated with better survival, but a combination of LOH 1p/19q, was associated with significantly better survival. In the present study, we showed that the LOH 1p/19q combination in

glioblastomas was associated with longer survival (20.5 ± 21.5 months) and that the presence of LOH 1p was predictive of longer survival of glioblastoma patients in both univariate and multivariate analyses.

Because LOH 1p and LOH 19q are genetic hallmarks of oligodendrogliomas (12, 13), it was of interest to examine whether the presence of an oligodendroglial component is associated with frequent LOH 1p/19q in glioblastomas. Schmidt et al (18) reported that patients with glioblastomas showing LOH 1p/19q did not exhibit morphologic features reminiscent of oligodendroglioma. Similarly, Kraus et al (7) carried out genetic analyses of 13 glioblastomas with an oligodendroglial component and detected LOH 1p in only 3 tumors (23%), one of which additionally showed LOH 19q (8%). In contrast, He et al (8) reported higher rates of LOH 1p (40%) and LOH 19q (60%) among 25 glioblastomas with an oligodendroglial component. In the present study, using markers for loci located within the common deletion (1p36 and 19q13) in which LOH has been observed frequently in oligodendrogliomas (13, 14) and also is commonly deleted in glioblastomas (15, 16), we found no correlation between oligodendroglial components and the presence of LOH 1p/19q in glioblastomas in both univariate and multivariate analyses.

Multinucleated giant cells are a cell type frequently encountered in glioblastomas. Glioblastomas characterized by predominance of large, bizarre, multinucleated giant cells are termed giant cell glioblastomas (20). This rare variant is genetically characterized by frequent *TP53* mutations (78%) and infrequent *EGFR* amplification (6%) (20). The present study demonstrated that the presence of ≥5% multinucleated giant cells is associated with frequent *TP53* mutations and infrequent *EGFR* amplification in multivariate analyses.

Several studies have shown that the presence of necrosis is predictive of poor prognosis of patients with glioblastoma. Pierallini et al (2) found that patients with necrosis (>35% of tumor) had a significantly shorter survival time. Hammoud et al (3) revealed by multivariate analysis that the strongest prognostic variable was the amount of tumor necrosis on a preoperative scan ($p < 0.001$) with median survival of 42, 24, 15, and 12 months for tumor necrosis grades of 0 (7 patients), I (11 patients), II (9 patients), and III (21 patients), respectively. Burger et al (17) reported that patients with glioblastoma with necrosis showed significantly higher risk of death than those without necrosis (66.5 vs 214 per 1,000 patient-months; $p = 0.028$). Barker et al (4) reported that of 275 patients with supratentorial glioblastoma containing endothelial proliferation, 88% had tumor necrosis. The presence of necrosis was correlated with older age of patients and was associated with significantly shorter survival (4). In the present study, the mean age of patients with glioblastoma with necrosis was 59.2 years, significantly older than those without necrosis (51.6 years; $p = 0.0001$), and survival of patients with glioblastoma with necrosis was 7.9 months, significantly shorter than those without necrosis (12.9 months; $p = 0.0017$). Multivariate analyses with adjustment for age and gender also indicated that the presence of necrosis is a significant predictive factor for poor survival of patients with glioblastoma.

Glioblastomas may develop de novo (primary glioblastoma) or through progression from low-grade or anaplastic astrocytoma (secondary glioblastoma) (1, 10). These glioblastoma subtypes constitute distinct disease entities that affect patients at different age and evolve through different genetic pathways. Primary glioblastomas develop in older patients and typically show LOH on the entire chromosome 10, *EGFR* amplification/overexpression, and *PTEN* mutations (10, 11). Secondary glioblastomas develop in younger patients and typically contain *TP53* mutations as an early alteration and LOH 10q as a late event (10, 11). Histologic features may also differ between primary and secondary glioblastomas. We have previously reported that large ischemic necrosis was significantly more frequent in primary glioblastomas than secondary glioblastomas (21). The present study confirmed these results on necrosis at the population level and further showed a tendency for a more frequent oligodendroglial component in secondary than in primary glioblastomas, whereas small cell phenotypes were observed at similar frequency in both glioblastoma subtypes. In addition, we observed a significant association between the presence of necrosis and absence of *TP53* mutations in glioblastomas. This can probably be explained at least in part by the infrequency of *TP53* mutations in primary glioblastomas that more frequently show necrosis.

Survival of patients with glioblastoma is still extremely poor, despite advances in surgical and clinical neurooncology. In a meta-analysis of 12 randomized clinical trials, the overall survival rate of patients with high-grade glioma was 40% at 1 year (22). At the population level, survival of patients with glioblastoma was even worse; observed survival rates were 42.6% at 6 months and 17.7% at 1 year (10). However, some patients survive longer, and several studies have focused on identification of histologic and genetic features of glioblastomas from long-term glioblastoma survivors. Scott et al (23) reported that 5 glioblastomas in patients who survived for >3 years after diagnosis did not show differences with respect to the presence of necrosis, vascular proliferation, lymphocytic infiltration, nuclear pleomorphism, and nuclear size compared with 286 cases of all glioblastomas. Burton et al (24) reported that the presence of necrosis or microvascular proliferation was not different between long-term (>3 years) and short-term glioblastoma survivors (<1.5 years) (24). McLendon et al (25) reported that intermediate fibrillary components were more frequent and small cell components less frequent in 17 cases of glioblastoma from patients who survived for more than 5 years. Burton et al (24) compared genetic alterations in glioblastomas from long-term survivors (>3 years; 41 patients) and those from short-term survivors (<1.5 years; 48 patients). Nuclear p53 expression was significantly more frequent in the long-term survivor group (85% vs 56%). Kraus et al (26) showed no differences in *TP53* mutation, *PTEN* mutation, *CDKN2A* deletion, *EGFR* overexpression among 21 long-term (>24 months) and 21 short-term (<6 months) survivors of glioblastoma. In the present study, patients with glioblastoma who survived more than 18 months were younger patients (mean, 50 years) and their tumors contained oligodendroglial components

more frequently (27% vs 14%; p = 0.0437). LOH 10q was the only genetic alteration that tended to be less frequent in glioblastomas from long-term survivors.

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