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Biomarkers, Genomics, Proteomics, and Gene Regulation

Amplification of the *STOML3*, *FREM2*, and *LHFP* Genes Is Associated with Mesenchymal Differentiation in Gliosarcoma

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Gliosarcoma is a rare glioblastoma variant characterized by a biphasic tissue pattern with alternating areas that display either glial (glial fibrillary acidic protein–positive) or mesenchymal (reticulin-positive) differentiation. Previous analyses have shown identical genetic alterations in glial and mesenchymal tumor areas, suggesting that gliosarcomas are genetically monoclonal, and mesenchymal differentiation was considered to reflect the elevated genomic instability of glioblastomas. In the present study, we compared genome-wide chromosomal imbalances using array comparative genomic hybridization in glial and mesenchymal tumor areas of 13 gliosarcomas. The patterns of gain and loss were similar, except that the gain at 13q13.3-q14.1 ($\log_2$ ratio >3.0), containing the *STOML3*, *FREM2*, and *LHFP* genes, which was restricted to the mesenchymal tumor area of a gliosarcoma. Further analyses of 64 cases of gliosarcoma using quantitative PCR showed amplification of the *STOML3*, *FREM2*, and *LHFP* genes in 14 (22%), 10 (16%), and 7 (11%) mesenchymal tumor areas, respectively, but not in glial tumor areas. Results of IHC analysis confirmed that overexpression of STOML3 and FREM2 was more extensive in mesenchymal than in glial tumor areas. These results suggest that the mesenchymal components in a small fraction of gliosarcomas may be derived from glial cells with additional genetic alterations. ***(Am J Pathol 2012, 180: 1816–1823; DOI: 10.1016/j.ajpath.2012.01.027)***

Gliosarcoma is a rare variant of glioblastoma that constitutes approximately 2% of all glioblastomas.[1] Histologically, these tumors are characterized by a biphasic tissue pattern, with alternating areas displaying glial [glial fibrillary acidic protein (GFAP)–positive] and mesenchymal (reticulin-positive) differentiation.[1]

Despite the presence of these two distinct types of differentiation, previous genetic analyses have shown that glial and mesenchymal tumor areas are usually genetically identical in terms of *TP53* mutations, *PTEN* mutations, $p16^{INK4a}$ deletion, *CDK4* amplification, and *MDM2* amplification,[2,3] suggesting that gliosarcomas are genetically monoclonal. In two studies using conventional comparative genomic hybridization (CGH), although patterns of chromosomal imbalance were also largely similar at the genome-wide level in the glial and mesenchymal components, there were also gains and losses at several loci that were unique to either glial or mesenchymal tumor areas.[4,5] Therefore, it is unclear whether mesenchymal differentiation simply reflects the extensive genomic instability of glioblastomas, whether mesenchymal components are derived from glial cells with additional genetic alterations, or whether mesenchymal differentiation is caused by a mechanism similar to that involved in epithelial-mesenchymal transition (EMT) in epithelial neoplasms.[6,7]

In the present study, to explore the possibility that additional genetic alterations may lead to mesenchymal

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Figure 1. A: GFAP IHC is positive in glial tumor areas and negative in mesenchymal tumor areas. **B:** DNA was extracted from glial (Glial) and mesenchymal (Mes) tumor areas separately. The scraped borders were displayed with dotted lines (before) and solid lines (after DNA extraction).

differentiation in gliosarcoma, we assessed genome-wide chromosomal imbalances in glial and mesenchymal tumor areas from the same gliosarcomas using array CGH.

Materials and Methods

Tumor Samples

FFPE tissue samples from 64 cases of gliosarcomas and 10 primary (*de novo*) glioblastomas were obtained from the Department of Neuropathology, University Hospital Zurich, Zurich, Switzerland; the Institute of Neurology, University Hospital Frankfurt, Frankfurt, Germany; the Institute of Neuropathology, University Hospital Munster, Munster, Germany; the Institute of Neuroscience, Bordeaux, France; the Department of Neuropathology, University Hospital Rome, Rome, Italy; and the Department of Pathology, Gunma University, Gunma, Japan. The median ± SD age at histologic diagnosis of gliosarcomas was 59 ± 11 years (range, 32 to 82 years), and the sex ratio was 1.13 (male:female). Gliosarcomas from patients with recurrent disease were not included in this study.

Gliosarcomas were diagnosed according to the 2007 World Health Organization classification.[1] Histologically, tumors showed the typical biphasic pattern with alternating areas of glial and mesenchymal differentiation. The glial area was composed of anaplastic glial cells with GFAP expression. The mesenchymal component demonstrated bundles of spindle cells with malignant transformation and abundant connective tissue stained by reticulin, without GFAP expression.[1]

Survival data were also collected for 31 patients with gliosarcoma. Mean ± SD follow-up was 12.6 ± 8.5 months (range, 3.0 to 29.7 months), and 10 of 31 patients (32%) were still alive at the time this study was conducted.

DNA Extraction

We first selected 21 gliosarcomas in which mesenchymal and glial tumor areas were clearly recognized, and both areas were sufficiently large for manual microdissection to be practicable. DNA was separately extracted from these two tumor areas. To confirm that the areas had been correctly dissected, GFAP staining was performed on the same histologic sections from which tumor tissues were scraped for DNA extraction (Figure 1). Samples with a small amount of DNA (<1 μg) were amplified by whole genome amplification (WGA) using protocols we recently established.[8] Eight cases were excluded from the study owing to lack of sufficient DNA even after WGA, and, therefore, 13 samples were available for array CGH (5 with WGA and 8 without WGA).

DNA was extracted as described previously.[8] Briefly, tumor samples scraped from histologic slides were deparaffinized in xylene and rehydrated in ethanol. After overnight incubation in 1 mol/L sodium thiocyanate solution, samples were suspended in DNA extraction buffer composed of ATL buffer and proteinase K (DNeasy Mini kit; Qiagen, Valencia, CA) and were incubated for 60 hours. The samples were then incubated with RNase for 10 minutes, and ATL buffer was added. After incubation with a mixture of 450 μL of ATL buffer and 450 μL of 100% ethanol for 5 minutes, the samples were loaded onto DNeasy Mini spin columns (Qiagen). After washing with buffer AW1, purified genomic DNA was eluted with 21 μL of nuclease-free H_2O. DNA concentrations were determined by spectrophotometer (NanoDrop Technologies, Wilmington, DE). Absorption was measured at 230, 260, and 280 nm, and the DNA quality was evaluated by A_{260}/A_{230} and A_{260}/A_{280} ratios.

Whole Genome Amplification

WGA was performed using the REPLI-g FFPE kit (Qiagen) as the reference.[8] Briefly, purified genomic DNA in a total volume of 10 μL was heated to 95°C for 5 minutes for denaturation. After cooling the samples on ice for 5 minutes, 8 μL of FFPE buffer, 1 μL of ligation enzyme, and 1 μL of FFPE enzyme were added, and the samples were incubated at 24°C for 30 minutes, followed by heat inactivation at 95°C for 5 minutes. Thirty microliters of the reaction mix (29 μL of reaction buffer and 1 μL of Midi

Phi29 DNA polymerase) was added to the denatured DNA to a total volume of 50 μL. The mix was incubated at 30°C for 1 hour. After amplification, the Phi29 enzyme was inactivated by heating at 95°C for 10 minutes.

Array CGH

Samples after WGA were purified using the NucleoTraP CR kit (Macherey-Nagel, Düren, Germany) before DNA labeling, as described previously.[8,9] Genome-wide chromosomal imbalance was assessed using a CGH oligonucleotide microarray (105K; Agilent Technologies, Santa Clara, CA; 15.0 Kb average probe resolution) according to the manufacturer's instructions. Briefly, the sample (1 μg) and the sex-matched reference DNA were chemically labeled with ULS-Cy5 and ULS-Cy3, respectively, at 85°C for 30 minutes using an oligonucleotide array CGH labeling kit for FFPE samples (Agilent Technologies). The labeled samples were purified with the genomic DNA purification module (Agilent Technologies), combined, mixed with human Cot-1 DNA, denatured at 95°C using an oligonucleotide array CGH hybridization kit (Agilent Technologies), and applied to microarrays. After hybridization at 65°C for 40 hours, microarrays were washed in oligonucleotide array CGH wash buffer 1 at room temperature for 5 minutes and in wash buffer 2 at 37°C for 1 minute. After drying, the microarrays were scanned by a DNA microarray scanner (G2565BA; Agilent Technologies), and data ($\log_2$) were extracted from the raw microarray image files using Feature Extraction software version 9 (Agilent Technologies). Data were analyzed by DNA Analytics software version 3.5 (Agilent Technologies) using default filter settings. The aberration detection method 2 algorithm with fuzzy zero correction was used to define aberrant intervals.

Amplification of the STOML3*,* FREM2*, and* LHFP *Genes*

Amplification of the *STOML3*, *FREM2*, and *LHFP* genes was assessed by quantitative PCR using the *CF* sequence as a reference in pairs of glial and mesenchymal tumor areas from 64 gliosarcomas. Primer sequences were as follows: 5′-TCACCAGAGACTCCGTAACT-3′ (sense) and 5′-AGAAATGTTGCTTGATGGAC-3′ (antisense) for *STOML3* (PCR product, 109 bp), 5′-TCCAACCTCCTGGATTATAC-3′ (sense) and 5′-GACAAGCTGTACTGGTAAGGA-3′ (antisense) for *FREM2* (PCR product, 110 bp), and 5′-CCTGTGCATGATGAGAGTC-3′ (sense) and 5′-GTCACTATGGTGCAGATCCT-3′ (antisense) for *LHFP* (PCR product, 110 bp), and 5′-GGCACCATTAAAGAAAATATCATC TT-3′ (sense) and 5′-GTTGGCATGCTTTGATGACGCTTC-3′ (antisense) for the CF (PCR product, 79 bp). PCR reactions were performed in a total volume of 20 μL, with 10 μL of iQ SYBR green (Bio-Rad Laboratories, Hercules, CA), 6.4 μL of primer sets (1.25 μmol/L of each primer), and 20 ng of DNA, with cycling parameters as reported previously.[10] PCR was performed in triplicate on a 96-well optical plate using an iCycler iQ5 detection system (Bio-Rad Laboratories). The copy number calculation was performed using the comparative C_T method, as described previously.[10,11] To calculate the average δC_T [δC_T (normal)], DNA was isolated from 14 FFPE normal tissues. The gene copy numbers of the samples are calculated by the following formula: δC_T = [C_T (target) − C_T (reference)] and $\delta\delta C_T$ =

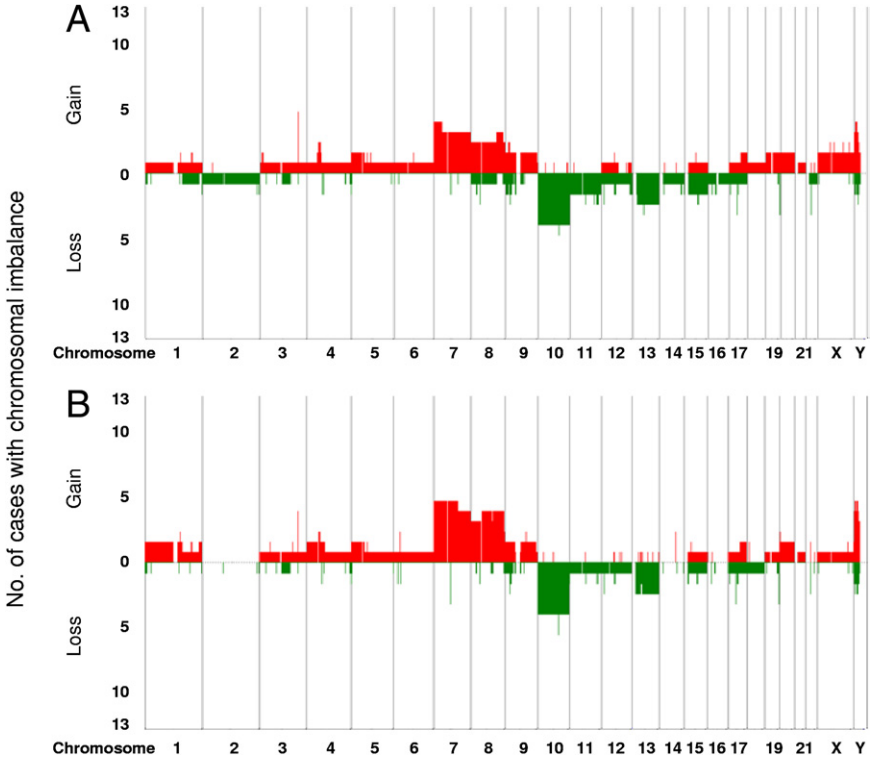

Figure 2. Genome-wide chromosomal imbalance ($\log_2$ ratio >1.0) in glial (**A**) and mesenchymal (**B**) tumor areas in gliosarcomas (n = 13). Note that the overall pattern of chromosomal imbalance is largely similar in glial and mesenchymal tumor areas.

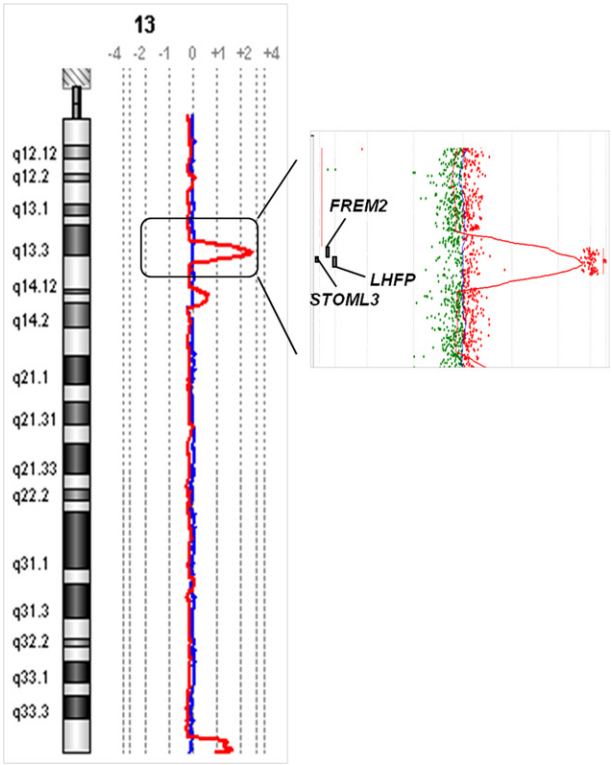

Figure 3. Array CGH showing gain at 13q13.3-q14.1 in a mesenchymal tumor area (red line) but not in a glial tumor area (blue line) of a gliosarcoma.

[δCt(tumor) – δC_T (normal)]. The relative gene copy numbers are calculated by the expression $2 \times 2^{-\delta\delta C_t}$. Using this method, a $\delta\delta C_t$ ratio, $2 \times 2^{-\delta\delta C_t}$ more than 2.88 was considered to indicate amplification.

IHC Analysis

Immunohistochemical (IHC) analysis was performed for 64 gliosarcomas and 10 primary (*de novo*) glioblastomas using the UltraVision Quanto detection system (Thermo Fisher Scientific Inc., Fremont, CA) according to the manufacturer's protocol. STOML3 antibody (1:250 dilution) and FREM2 antibody (1:100) were purchased from Atlas Antibodies (Stockholm, Sweden). Immunoreactivity was evaluated separately in mesenchymal and glial tumor areas of gliosarcomas and was scored as follows: – indicates <10%; +, 10% to 50%; ++, 51% to 90%; and +++, >90% of neoplastic cells with immunoreactivity. LHFP antibody for IHC on FFPE sections was not available.

Statistical Analyses

The Student's *t*-test was performed to analyze differences in mean ages between patients with tumors with or without genetic alterations. Fisher's exact test was used to assess group differences in the analysis of qualitative features in IHC analysis data. Pearson's correlation was used for analysis of the correlation between two variables. The Mann-Whitney nonparametric test was used for numerical variables. The Kaplan-Meier curve with the log-rank test was used for survival analysis. $P < 0.05$ was considered statistically significant.

Results

Array CGH

The overall pattern of chromosomal imbalance in array CGH analysis was largely similar in glial and mesenchymal tumor areas (Figure 2). The imbalance commonly detected in >10-Mb chromosomal regions included gain at 7p22.3-q36.3 (8 of 13; 62%) and 20p13-q13.33 (3 of 13; 23%) and loss at 8q24.3 (3 of 13; 23%), 10p15.3-q26.3 (7 of 13; 54%), 11p15.5-q25 (4 of 13; 31%), and 13q11-q34 (6 of 13; 46%). Loss of several loci containing well-characterized tumor-associated genes was observed at 9p21.3-p21.2 (*CDKN2A*, *CDKN2B*) (3 of 13; 23%), 10p15.3-q26.3 (*PTEN*) (7 of 13; 54%), and 13q14.2 (*RB1*) (1 of 13; 8%).

We then focused on gain ($\log_2$ ratio >1.0) in mesenchymal but not in glial tumor areas. Direct comparison between glial and mesenchymal components of the same tumors showed the highest gain at 13q13.3-q14.1 ($\log_2$ ratio >3.0) containing the *STOML3*, *FREM2*, *COG6, C13orf23, LOC387921, LHFP*, and *UFM1* loci uniquely in the mesenchymal tumor area of a single gliosarcoma (Figure 3). Amplification of the *STOML3*, *FREM2*, and *LHFP* genes at 13q13.3 was confirmed by quantitative PCR in mesenchymal but not in glial tumor areas of this gliosarcoma.

Amplification of STOML3, FREM2, *and* LHFP *Is Characteristic of Mesenchymal Tumor Areas of Gliosarcomas*

We examined 64 gliosarcomas (57 tumors clearly containing both glial and mesenchymal tumor areas and 7 tumors largely presenting a mesenchymal phenotype) for

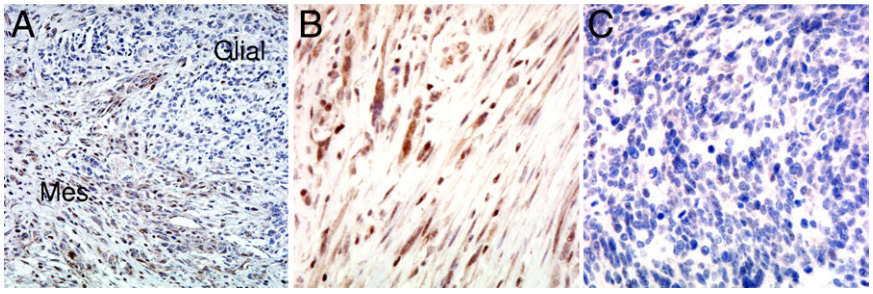

Figure 4. STOML3 IHC in gliosarcomas. **A:** Note that STOML3 immunoreactivity is positive in mesenchymal tumor areas (Mes) and negative in glial tumor areas (Glial). **B:** Higher magnification of a mesenchymal tumor area of gliosarcoma showing nuclear and cytoplasmic staining for STOML3. Original magnification, ×400. **C:** Higher magnification of a glial tumor area showing that STOML3 immunoreactivity is largely negative. Original magnification, ×400.

Table 1. Amplification and Overexpression of *STOML3, FREM2,* and *LHFP* in Glial and Mesenchymal Tumor Areas of Gliosarcomas

	STOML3		*FREM2*		*LHFP*
Case no.	Amplification (Glial/Mes)	IHC (Glial/Mes)	Amplification (Glial/Mes)	IHC (Glial/Mes)	Amplification (Glial/Mes)
1	−/−	−/−	−/−	−/−	−/−
2	−/−	−/−	−/−	−/−	−/−
3	−/amp	−/++	−/amp	−/−	−/amp
4	−/amp	−/−	−/amp	−/−	−/−
5	−/−	−/−	−/−	−/+	−/−
6	−/−	−/−	−/−	−/−	−/−
7	−/−	−/−	−/−	+/−	−/−
8	−/−	−/−	−/−	−/+	−/−
9	−/−	−/−	−/−	−/−	−/−
10	−/amp	+/++	−/−	−/+	−/−
11	−/−	+++/+	−/−	−/−	−/−
12	−/amp	−/+++	−/amp	−/+++	−/−
13	−/amp	++/+++	−/−	−/+++	−/−
14	−/−	−/−	−/−	−/−	−/−
15	−/−	−/+++	−/−	−/+	−/−
16	−/−	+/+	−/−	−/−	−/−
17	−/−	+/+++	−/amp	++/++	−/amp
18	−/−	−/−	−/−	+/−	−/−
19	−/−	−/−	−/−	++/−	−/−
20	−/−	−/+++	−/amp	+/++	−/−
21	−/−	+/+	−/−	+/++	−/−
22	ND/−	ND/+	ND/−	ND/−	ND
23	−/−	−/−	−/−	−/−	−/−
24	−/−	++/++	−/−	−/−	−/−
25	−/−	−/−	−/−	−/++	−/−
26	−/−	−/+++	−/amp	+/++	−/amp
27	−/−	−/−	−/−	−/+	−/−
28	−/−	−/−	−/−	−/−	−/−
29	−/−	−/−	−/−	−/−	−/−
30	−/−	−/+	−/−	−/−	−/−
31	−/−	−/−	−/−	−/−	−/−
32	−/−	−/+	−/−	−/−	−/−
33	−/−	−/+++	−/−	+/+++	−/−
34	−/amp	++/++	−/amp	−/+	−/−
35	−/amp	−/++	−/−	−/++	−/−
36	−/−	+/+	−/−	+/+	−/−
37	−/−	+/++	−/−	+/−	−/−
38	−/−	−/−	−/−	−/−	−/−
39	−/−	+/++	−/−	−/+	−/−
40	ND/amp	ND/++	ND/−	ND/++	ND
41	ND/−	ND/+	ND/−	ND/−	ND
42	−/−	+/+++	−/−	−/−	−/−
43	−/−	+/−	−/−	+/+	−/−
44	−/−	−/−	−/−	−/++	−/−
45	−/−	−/−	−/−	−/−	−/−
46	−/−	−/−	−/−	−/−	−/−
47	ND/amp	ND/++	ND/−	ND/+++	ND/amp
48	−/−	−/−	−/−	−/−	−/amp
49	−/amp	+/++	−/−	−/+++	−/−
50	−/−	−/−	−/−	−/+	−/−
51	−/−	++/++	−/−	−/−	−/−
52	ND/−	ND/+	ND/−	ND/−	ND
53	−/−	−/−	−/−	−/−	−/−
54	−/−	−/+++	−/−	−/+	−/−
55	−/amp	−/+++	−/−	+/++	−/−
56	−/amp	+/++	−/−	+/++	−/−
57	−/−	−/−	−/−	+/+	−/−
58	−/−	−/−	−/−	−/−	−/−
59	ND/−	ND/−	ND/−	ND/−	ND
60	−/−	−/++	−/−	−/+	−/−
61	−/−	−/−	−/amp	−/−	−/−
62	−/amp	++/+++	−/amp	+/+++	−/amp
63	−/−	−/−	−/−	+/+	−/−
64	ND/amp	ND/++	ND/amp	ND/−	ND/amp

The fraction of STOML3− or FREM2−positive cells in IHC was evaluated as follows: − indicates less than 10%; +, 10% to 50%; ++, 51% to 90%; and +++, greater than 90% of positive cells.

amp, amplification; Glial, glial tumor area; Mes, mesenchymal tumor area; ND, not determined.

amplification of the *STOML3*, *FREM2*, and *LHFP* genes. *STOML3* amplification was restricted to mesenchymal tumor areas (14 of 64 mesenchymal tumor areas; 22%), and no glial tumor areas showed *STOML3* amplification (0 of 57, 0%; $P < 0.0001$) (Figure 4A). *FREM2* amplification was observed in mesenchymal tumor areas only in 10 of 64 cases (16%) but in none of the 57 glial tumor areas analyzed ($P = 0.002$). Similarly, *LHFP* amplification was seen in mesenchymal tumor areas only in 7 of 64 cases (11%), whereas no glial tumor areas revealed *LHFP* amplification (0 of 57, 0%; $P = 0.014$). Furthermore, direct comparison of $2^{-\delta\delta C_t}$ (mesenchymal area)/$2^{-\delta\delta C_t}$ (glial area) values for cases showing amplification or nonamplification in mesenchymal areas showed significant differences for *STOML3* (mean, 3.58/1.02 for amplification/nonamplification; $P < 0.0001$), *FREM2* (mean, 1.86/1.01 for amplification/nonamplification; $P < 0.0001$), and *LHFP* (mean, 1.47/0.97 for amplification/nonamplification; $P = 0.007$). There was no significant difference in age at diagnosis in patients with gliosarcoma with or without amplification of these genes. Amplification of the *STOML3*, *FREM2*, or *LHFP* genes was not detected in any of 10 ordinary glioblastomas (not gliosarcoma) analyzed.

STOML3 *and* FREM2 *IHC*

Cytoplasmic and nuclear STOML3 expression was detected by IHC in >50% of neoplastic cells in 25 of 64 gliosarcomas (39%) analyzed. STOML3 expression was more extensive in mesenchymal than in glial tumor areas except for 2 cases (Figure 4 and Table 1). STOML3 expression was significantly correlated with *STOML3* gene amplification in gliosarcomas (0.34; 95% CI, 0.11 to 0.55; $P = 0.006$; Figure 5).

Cytoplasmic FREM2 expression was detected by IHC analysis in more than 50% of neoplastic cells in 16 of 64 gliosarcomas (25%) analyzed. FREM2 expression was more extensive in mesenchymal than in glial tumor areas except in 4 cases (Table 1). There was a non–statistically significant correlation between *FREM2* amplification and expression.

We also performed STOML3 and FREM2 IHC in 10 ordinary glioblastomas. Only one glioblastoma showed immunoreactivity to *STOML3* (70% of neoplastic cells) and one to *FREM2* (20% of neoplastic cells).

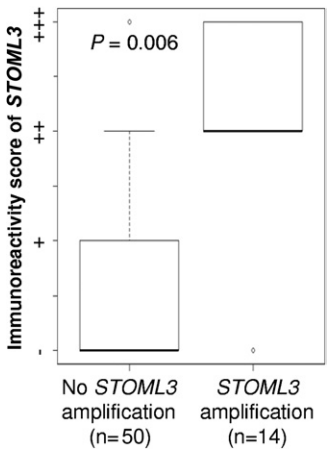

Figure 5. Correlation between amplification and overexpression of the *STOML3* gene in mesenchymal tumor areas of gliosarcomas. The **boxes** represent interquartile range, and **whiskers** represent maximal points that are not outliers, which are shown as the **white circle**. The **bold line** represents the median. Overexpression was assessed by IHC and was scored as follows: – indicates <10%; +, 10% to 50%; ++, 51% to 90%; and +++, >90% of neoplastic cells with immunoreactivity.

STOML3, FREM2, *or* LHFP *Overexpression/ Amplification and Patient Survival*

Overexpression of the *STOML3*, *FREM2,* or *LHFP* genes was not prognostic of survival in patients with gliosarcoma (data not shown). We compared survival of 138 patients with glioblastoma with and without *STOML3* expression in The Cancer Genome Atlas database (*http://www.cbioportal.org/public-portal*, last accessed August 9, 2011).[12] Mean survival in patients with glioblastoma with STOML3 overexpression was 5.9 months (95% CI, 3.35 to NA months), which was significantly shorter than that in patients without STOML3 overexpression (14.3 months; 95% CI, 12.6 to 18.0 months; $P = 0.015$).

Discussion

EMT is a critical step during embryogenesis and is also considered to play a significant role in cancer invasion and metastasis.[13,14] The migratory and invasive phenotype and stem cell characteristics of cancer cells may result from EMT.[15] Several signaling pathways and molecules seem to be involved in EMT, including transforming growth factor β and RTK/Ras signaling, autocrine factors and Wnt, Notch, Hedgehog, and nuclear factor κB–dependent pathways.[16,17] Loss of E-cadherin is a critical step for EMT, leading to the breakdown of cell-cell adhesion and acquisition of invasive growth properties in cancer cells.[18] Down-regulation of E-cadherin may be caused by several transcription factors, such as Twist, dEF1/Zfh1 family (Zeb1, SIP1), and Snail family (SNAI1/Snail, SNAI2/Slug),[19–22] or gene mutations.[18] Little is known as to whether similar mechanisms operate in glioma. It has been shown that Twist1 promotes invasion through mesenchymal changes in glioblastoma,[23] and SNAI2/Slug promotes growth and invasion in glioma,[24] suggesting that glial to mesenchymal transitions may play a role in invasion in gliomas.

The present study is the first array CGH analysis to separately assess genome-wide chromosomal imbalance in glial and mesenchymal tumor areas in the same gliosarcomas. In line with the results of previous conventional CGH and genetic analyses in several selected genes, we showed that genome-wide chromosomal imbalance was similar between glial and mesenchymal tumor areas of gliosarcomas.[2–5] In the present study, we focused on genes at 13q13.3-q14.1, where significant gain was restricted to mesenchymal tumor areas only (not glial tumor areas) in 1 of 13 gliosarcomas analyzed. Several known genes are situated at this locus, including *STOML3*, *FREM2*, *COG6*, *C13orf23*, *LOC387921*, *LHFP*, and *UFM1*. We selected *STOML3*, *FREM2*, and *LHFP*

genes for further analyses based on the level of gain and their known functions.

We found amplification of the *STOML3*, *FREM2*, and *LHFP* genes in 22%, 16%, and 11%, respectively, of 64 gliosarcomas analyzed. Amplification of these genes was restricted to mesenchymal tumor areas only and was absent in glial tumor areas in any of the gliosarcomas analyzed. Mesenchymal tumor area–specific amplification of the *STOML3* and *FREM2* genes was associated with overexpression as confirmed by IHC analysis. These results suggest that mesenchymal differentiation in a small fraction of gliosarcomas may be due to additional genetic alterations.

STOML3 encodes a protein related to stomatin that regulates the activity of ion channels.[25] It is essential for touch sensation of mouse skin.[26,27] The STOML3 protein is expressed in receptor neurons of the olfactory epithelium, suggesting that it has some function in olfactory neurons.[28] LHFP is a frequent translocation partner with HMGIC (at 12q15) in lipomas.[29,30] The *Fras1/Frem* gene family, including the *FREM2* gene, encodes extracellular matrix proteins that are localized in epithelial basement membranes in eyelids, limbs, kidneys, lungs, gastrointestinal tract, and the central nervous system.[31,32] The *Fras1/Frem* gene family plays critical roles in epithelial-mesenchymal interaction during embryonic development.[33] Timmer et al[34] showed that loss of *FREM2* function results in defects in developmental events associated with morphogenetic rearrangements of the vasculature and of tissues arising from all germ layers in mice. *FREM2* mutations are known to cause epidermal adhesion defects[33] and are associated with Fraser syndrome, which is not linked to Fras1.[33,35]

In The Cancer Genome Atlas data (*http://tcga-portal.nci.nih.gov/tcga-portal/AnomalySearch.jsp*, last accessed August 9, 2011),[12] *FREM2* gain was found in 1 of 372 (0.3%) glioblastomas; *LHFP* gain and overexpression were found in 1 of 372 (0.3%) and 109 of 424 (26%) glioblastomas, respectively; and *STOML3* gain and overexpression were found in 1 of 372 (0.3%) and 4 of 138 (3%) glioblastomas, respectively, and *STOML3* overexpression was associated with poor prognosis in patients with glioblastoma. Thus, overexpression and amplification of the *STOML3*, *FREM2*, and *LHFP* genes seem to be rare in ordinary glioblastomas.

In summary, amplifications of the *STOML3*, *FREM2*, and *LHFP* genes are additional genetic alterations associated with the mesenchymal phenotype in a small fraction of gliosarcomas. It remains to be shown whether these genes or other genes co-amplified at 13q13.3-q14.1 are directly associated with glial-mesenchymal transition in glioblastomas. The relatively low frequency of chromosomal copy number abnormalities specific to mesenchymal tumor areas also suggests that other mechanisms, such as epigenetic regulation, may be operative in mesenchymal differentiation in gliosarcomas.

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