Multicentric Glioma Develops via a Mutant IDH1-Independent Pathway: Immunohistochemical Study of Multicentric Glioma

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Key Words

Multicentric glioma · Mutant IDH1 · ATRX · 1p19q loss

Abstract

Multicentric gliomas are very rare. Due to differences in their tumor types they remain enigmatic. We focused on the pathogenesis of multicentric gliomas and compared their immunoprofile with that of solitary gliomas. This retrospective study included 6 males and 8 females with multicentric glioma (8 glioblastomas, 2 anaplastic astrocytomas, 4 diffuse astrocytomas). Their age ranged from 27 to 75 years and all were treated between 2004 and June 2015. The expression of mutant isocitrate dehydrogenase 1 (IDH1), α -thalassemia X-linked intellectual disability (ATRX), p53, phosphatase and tensin homolog (PTEN), and epidermal growth factor receptor (EGFR) was examined immunohistochemically; for 1p19q analysis we used fluorescence in situ hybridization (FISH). In all patients, immunohistochemical staining was negative for mutant IDH1 and cytoplasmic PTEN; only 1 patient (7.1%) manifested nuclear PTEN positivity. FISH for 1p19q codeletion was negative in all 9 examined samples; 5 of 14 specimens (35.7%) were p53-positive, 9 (64.3%) were EGFR-posi-

tive, and 4 (28.6%) were ATRX-negative. The MIB-1 labeling index was 0.9–15.6% for grades II and III, and ranged between 17.3 and 52.4% for glioblastoma. Our results suggest that the pathogenesis of multicentric gliomas is different from the mutant IDH1-R132H pathogenesis of lower-grade glioma and secondary glioblastomas. More studies are needed to confirm the molecular mechanisms underlying the pathogenesis of multicentric glioma. © 2016 S. Karger AG, Basel

Introduction

Multicentric glioma, defined as multiple, widely separated brain tumor masses in different lobes or different hemispheres, is not spread via pathways along commissural or cerebrospinal fluid channels or local metastases via satellite formation [1]. Among gliomas, multicentric glioma is very rare [1–5] and its incidence depends on the diagnostic criteria and techniques used [e.g. computed tomography, magnetic resonance imaging (MRI), autopsy].

Different from solitary gliomas, the pathogenesis of multicentric gliomas is not well understood [1, 2, 4–8]

because most of the earlier studies focused on imaging findings and clinical outcomes. Although no detailed molecular findings on multicentric glioma have been reported, new insights into the molecular background of brain tumors, including gliomas, have yielded a better understanding of glioma's biological nature [9, 10]. In glioblastoma patients, mutant isocitrate dehydrogenase 1 (IDH1) is the most powerful prognostic factor and is useful for the differentiation between primary and secondary glioblastomas [11–13]. Lower-grade gliomas are classified into 3 subgroups: types 1 and 2 are mutant IDH1- or mutant IDH2-positive, and type 3 is mutant IDH1- or mutant IDH2-negative [14, 15].

We profiled immunohistochemical staining of 14 multicentric gliomas and compared them with those of the better-understood solitary tumors. We report that the pathogenesis of multicentric gliomas is mutant IDH1-independent.

Patients and Methods

This retrospective study was approved by our institutional review board. To protect patient privacy, we removed all identifiers from our records upon completion of the analysis.

Our study subjects, 6 males and 8 females (age: 27–75 years), were treated in our institution between 2004 and July, 2015. The diagnostic criteria was based on Batzdorf and Malamud [1], and we excluded gliomatosis cerebri cases. When 1.5-tesla (until June 2006) or 3.0-tesla (after July 2006) MRI studies showed multiple brain tumors with no connecting signal alteration, the glioma type was confirmed histopathologically [16]. MRI included nonenhanced T1-weighted and T2-weighted fluid-attenuated inversion recovery (FLAIR), and transverse, sagittal, and coronal enhanced T1-weighted images acquired after the intravenous administration of a gadolinium-based contrast medium. The MR characteristics were defined as FLAIR-high (no gadolinium enhancement) and as focally, diffusely, and ring enhanced. In all but 2 patients the analysis was based on one lesional biopsy performed at the primary operation. In the other 2 patients it was based on the analysis of 2 different lesions, both of which were diagnosed as glioblastoma. All samples were stained immunohistochemically; 11 samples from 9 patients underwent fluorescence in situ hybridization (FISH) due to the limited availability of tissue. The tumor specimens were obtained by surgical biopsy in all cases and fixed in 10% formalin before being embedded in paraffin and stained with hematoxylin-eosin for standard histologic diagnosis.

Immunohistochemical Staining

Immunohistochemical staining for all antibodies except p53 was performed in an automated immunostainer (BenchMark GX; Ventana, Tucson, Ariz., USA). Mutant IDH1, α -thalassemia X-linked intellectual disability (ATRX), and epidermal growth factor receptor (EGFR) were detected with the iView Detection Kit using cell conditioning (CC)-1. Mutant IDH1 and EGFR were incubated at 37°C for 1 h and 32 min, respectively, with neither amplification

nor blocking; ATRX was incubated at 37°C for 32 min with amplification and blocking. The primary antibodies were anti-human IDH1 R132H (1:100, Dianova, Hamburg, Germany), anti-ATRX (1:200; Sigma-Aldrich, St. Louis, Mo., USA), and anti-human wild-type EGFR (1:50; Dako, Glostrup, Denmark). The positive control for mutant IDH1 was oligodendroglioma (WHO grade II; IDH R132H-mutant, 1p/19q-codeleted), based on the 2016 WHO classification [17]. As for ATRX, the negative control was diffuse astrocytoma (WHO grade II; IDH R132H-mutant case) (online suppl. figure; see www.karger.com/doi/10.1159/000447951 for all online suppl. material).

Immunostaining for phosphatase and tensin homolog (PTEN) was done with the OptiView Detection Kit with CC-1 at 37°C incubation for 32 min, HQ universal linker, and HRP multimer for 8 min without amplification. Anti-human PTEN (1:100, Dako) was used as the primary antibody. The antibody for p53 was prepared using anti-human p53 protein (1:100, Dako) [18]. The antibody against Ki-67/MIB-1 was an anti-human Ki-67 antigen clone MIB-1 (1:25, Dako).

Immunohistochemical staining was evaluated according to established criteria. Positivity for mutant IDH1 was recorded when there was strong cytoplasmic staining [19], for p53 and ATRX when more than 10% of the tumor revealed nuclear staining [18, 20]. PTEN positivity was based on the cytoplasmic staining intensity and pattern [21], and EGFR positivity was based on the grade of membrane and/or cytoplasm staining [22]. The MIB-1 labeling index evaluation was based on nuclear staining of the tumor cell [23].

FISH

Sample slides (4 μ m in thickness) were deparaffinized and dehydrated with xylene and absolute alcohol ($\times 3$), respectively. At room temperature, they were pretreated with 0.2 N HCl for 20 min, washed twice with SSC buffer (Life Technologies, New York, N.Y., USA), and incubated at 80°C for 30 min in pretreatment solution (Abbott Molecular Inc., Abbot Park, Ill., USA). After washing with SSC they were incubated at 37°C for 5 min with pepsin (Dako). Hybridization was at 75°C for 5 min followed by 37°C for 16 h with Vysis 1p36/1q25 and 19q13/19p13 FISH probe kit (Abbott Laboratories, Ill., USA). After washing with 0.3% nonionic detergent (Nacalai Tesque Inc., Kyoto, Japan) at 37 and 65°C for 1 and 8 min, respectively, and the application of DAPI (Life Technologies), the slides were evaluated under an IX81 Olympus fluorescent microscope using Meta Imaging series software (Version 7.1).

The results were evaluated based on existing criteria [24].

Results

MRI and histological findings are summarized in table 1. Of the 14 patients; 8 were diagnosed as glioblastoma (grade IV), 2 as anaplastic astrocytoma (grade III), and 4 as diffuse astrocytoma (grade II). Two patients presented with 4 lesions and the others with 2–3 lesions. Half of the patients showed different imaging characteristics among the lesions. In one patient with 4 lesions, one was ring enhanced and the others were diffusely enhanced. In other

Table 1. Patient data

Case	Age, years	Sex	Location	MRI characteristics	Pathologic diagnosis
1	75	M	R. temporal L. parietal	Ring enhanced FLAIR-high	Glioblastoma
2	68	F	R. temporal L. parietal R. parietal L. temporal	Diffuse enhanced Diffuse enhanced Diffuse enhanced Diffuse enhanced	Glioblastoma
3	73	M	R. temporal R. frontal R. parietal Corpus callosum	Ring enhanced Diffuse enhanced Diffuse enhanced Diffuse enhanced	Glioblastoma
4	61	F	R. parietal L. parietal L. temporal	Ring enhanced Ring enhanced FLAIR-high	Glioblastoma
5a 5b	66	F	L. parietal L. frontal	Ring enhanced Ring enhanced	Glioblastoma Glioblastoma
6	54	F	L. temporal R. frontal	Ring enhanced FLAIR-high	Glioblastoma
7	65	M	R. frontal R. parietal	Ring enhanced Ring enhanced	Glioblastoma
8a 8b	44	F	R. frontal L. frontal	Ring enhanced Ring enhanced	Glioblastoma Glioblastoma
9	71	M	Corpus callosum R. parietal R. temporal	Ring enhanced Ring enhanced Ring enhanced	Anaplastic astrocytoma
10	70	F	R. frontal R. temporal R. parietal	Focal enhanced FLAIR-high FLAIR-high	Anaplastic astrocytoma
11	56	M	L. frontal L. parietal L. occipital	FLAIR-high FLAIR-high FLAIR-high	Diffuse astrocytoma
12	27	F	L. thalamus L. frontal	FLAIR-high FLAIR-high	Diffuse astrocytoma
13	31	M	L. frontal R. temporal	Focal enhanced FLAIR-high	Diffuse astrocytoma
14	58	F	L. temporal L. thalamus	FLAIR-high FLAIR-high	Diffuse astrocytoma

patients, 2–3 lesions manifested either as both ring enhanced and FLAIR-high ($n = 3$) or focally enhanced and FLAIR-high ($n = 1$).

The immunohistochemical staining results are summarized in table 2. All patients were negative for mutant IDH1 (16 tumor specimens). One patient (7.1%) mani-

fested nuclear positivity for PTEN; it was negative in the cytoplasm of all 16 tumor samples. FISH results for 1p19q codeletion were negative in all 9 studied cases (11 tumor specimens); p53 was positive in 5 of 14 patients (35.7%; 6 of 16 samples) and ATRX was negative in 4 of 14 patients (28.6%, 6 of 16 specimens). EGFR was overexpressed in 9

Table 2. Results of immunohistochemical staining and FISH analysis

Case	Immunohistochemical staining							FISH 1p19q code- letion
	MIB-1 LI, %	mutant IDH1	ATRX	p53	EGFR	PTEN (cytoplasmic)	PTEN (nuclear)	
1	17.3	–	+	+	–	–	–	–
2	19.4	–	+	–	–	–	–	ND
3	40.2	–	–	–	+	–	–	ND
4	45.9	–	+	–	–	–	–	–
5a	12.1	–	+	–	+	–	–	–
5b	17.8	–	–	–	+	–	–	–
6	16.5	–	+	+	+	–	–	–
7	26.9	–	–	+	–	–	–	–
8a	18.4	–	+	+	+	–	–	–
8b	52.4	–	+	+	+	–	–	–
9	15.3	–	+	–	+	–	–	ND
10	15.6	–	+	–	+	–	–	–
11	1.4	–	–	–	+	–	–	–
12	6.2	–	+	–	–	–	+	ND
13	0.9	–	+	–	–	–	–	ND
14	1.6	–	+	+	+	–	–	–

ND = Not done.

of 14 patients (64.3%, 6 of 16 samples). The MIB-1 labeling index ranged between 0.9 and 15.6% for grade II and III tumors, and in the range of 17.3–52.4% for glioblastoma.

Representative cases of diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma are shown in figures 1–3, respectively.

Discussion

We focused on molecules that play an important role in the pathogenesis of glioma and included mutant IDH1, ATRX, EGFR, p53, and the 1p19q codeletion status [10, 11, 20, 25–27]. IDH1 mutations are thought to occur at the early stage of most lower-grade gliomas and secondary glioblastomas [11, 12, 27–29], contrary to primary glioblastomas where negativity for mutant IDH1 expression is primarily observed [10, 30]. None of our patients with grade IV or lower-grade multicentric gliomas expressed mutant IDH1. Furthermore, we found no ATRX immunonegativity, although the majority of secondary glioblastomas and lower-grade astrocytomas exhibit ATRX mutation [11, 31, 32].

Our findings suggest that the pathogenesis of multicentric lower-grade gliomas and multicentric glioblastomas is mutant IDH1 (R132H)-independent as opposed to

the common solitary low-grade glioma, and that they most likely exhibit characteristics of primary glioblastoma. A study of 258 glioblastomas by Liu et al. [33] reported that multicentric glioblastoma was dominated by the mesenchymal subtype, without mutation in IDH1 and ATRX, contrary to the common proneural or G-CIMP subtype. They also have poorer survival than the solitary ones. Although different methods were applied, our IDH1 and ATRX profiles in glioblastoma were comparatively consistent with the above study.

Considering the characteristic impact of 1p19q codeletion in oligodendroglioma [11, 17, 25], we performed FISH analysis of viable cases. None of the analyzed cases showed 1p19q codeletion, indicating that multicentric gliomas are not of oligodendroglial origin [11, 34, 35].

We also found that most of our multicentric gliomas showed no or low p53 staining. This result was in contrast to other diffuse astrocytomas and secondary glioblastomas with mutant IDH1 [11, 36–38].

Studies have classified lower-grade gliomas (grades II and III) into 3 subgroups: type 1 = mutant IDH1- or mutant IDH2-positive with 1p19q codeletion (oligodendroglial tumor group), type 2 = mutant IDH1- or mutant IDH2-positive with ATRX and/or p53 mutation (astrocytic tumor group), and type 3 = mutant IDH1- or mutant IDH2-negative, i.e. wild-type IDH1/IDH2 with EGFR activation or PTEN inactivation [14, 15]. All of our

Fig. 1. Diffuse astrocytoma in a 27-year-old female. **a, b** Note two solid masses in the left thalamus and the left frontal lobe on FLAIR images. **c, d** Neither mass is enhanced on the T1-weighted gadolinium-enhanced scans. **e** Tissue biopsy of the mass in the left thalamus revealed a low-cellularity astrocytic tumor (HE). **f** Mutant

IDH1-R132H immunohistochemical staining was negative. ATRX staining was positive (**g**) and p53 was negative (**h**). Nuclear PTEN staining was positive (**i**), and EGFR appeared negative (**j**). All magnifications $\times 400$.

Fig. 2. Anaplastic astrocytoma in a 70-year-old female. There are 3 high-intensity masses in the right frontal-, parietal-, and temporal lobe on FLAIR images. **a, b** The right frontal mass shows focal enhancement on the T1-weighted gadolinium-enhanced scan. **c** Tissue biopsy of the frontal mass revealed predominantly anaplastic and hyperchromatic nuclei with atypical mitoses (HE).

d Mutant IDH1-R132H staining was negative. Immunohistochemical staining for ATRX was positive (**e**); p53 and PTEN were stain-negative (**f, g**). There was diffuse EGFR overexpression (**h**). FISH showed no codeletion of chromosome 1p and 19q (**i, j**). All magnifications $\times 400$.

Fig. 3. Glioblastoma in a 44-year-old female. Two masses in the left and right frontal lobe were high-intense on FLAIR images (**a**) with ring enhancement on T1-weighted gadolinium-enhanced scans (**b**). Tissue biopsy of the mass in the left (**c**) and right frontal lobe (**d**) (HE) showed pleomorphic and anaplastic tumor nuclei with frequent mitoses. Immunohistochemical staining results were the same for both lesions; mutant IDH1-R132H was negative in the

left (**e**) and right (**f**) frontal tumor. ATRX was positive in the left (**g**) and right mass (**h**) as was p53 (left, **i**; right, **j**). PTEN staining was negative (left, **k**; right, **l**). EGFR was overexpressed (left, **m**; right, **n**). FISH analysis of chromosome 1p (left, **o**; right, **p**) and 19q (left, **q**; right **r**) showed no codeletion in both lesions. All magnifications $\times 400$.

(For figure see next page.)

cases were negative for mutant IDH1 staining. Most of them showed neither ATRX immunonegativity nor 1p19q codeletion, and were negative for p53 staining. Based on the above typing of lower-grade gliomas, our current findings categorize multicentric gliomas as type 3 and suggest the involvement of a pathway different from that of mutant IDH-positive lower-grade gliomas and secondary glioblastomas. This type was also categorized by Gorovets et al. [39] as a preglioblastoma subclass, featuring wild-type IDH1 with molecular and clinical characteristics similar to primary glioblastoma [14, 39]. Despite their similar characteristics, the relationship between lower-grade glioma type III and primary glioblastoma has not been conclusively determined. There is evidence that different mechanisms are involved in wild-type IDH1 lower-grade gliomas and tumors manifesting mutant IDH1 or IDH2 [14, 15, 39]. This suggests that multicentric gliomas exhibit characteristics, due to their mutant IDH1 negativity, that are different from those of mutant IDH1 gliomas.

In primary glioblastoma, EGFR is strongly expressed due to its growth-factor-related activation of PI3K by which phosphatidylinositol (3,4,5)-triphosphate (PIP3) is formed from phosphatidylinositol (3,4)-bisphosphate (PIP2) phosphorylation and is activated downstream of Akt. Therefore, it is involved in cellular functions such as cell cycle progression and proliferation [40–43]. Cytoplasmic PTEN dephosphorylates PIP3 into PIP2 [44], resulting in the disruption of functions elicited by Akt regulation [40, 41, 45, 46]. Nuclear PTEN regulates entry into the cell cycle from G1 and mediates cell-cycle arrest and apoptosis [47, 48]. In most of our cases, EGFR was overexpressed and PTEN was negative, indicating high EGFR activity and destabilization of the modulation functions of PTEN. EGFR activation and PTEN deletion are changes in the RTK/PI3K signaling that are seen in

primary glioblastomas [49]. Regardless of tumor grading, such alterations are related to type III criteria [14, 15, 39].

Glioblastoma has different characteristics and a different pathogenesis from the solitary ones [33]. In lower-grade multicentric gliomas, the predominant characteristics and pathogenesis are those of primary glioblastoma. This phenomenon differentiates multicentric glioma from the majority of mutant IDH1-immunopositive solitary lower-grade gliomas and secondary glioblastomas. Our findings suggest that the nature of multicentric gliomas is different from that of solitary gliomas and might require different management and therapeutic approaches.

Our study has some limitations. Our sample size was small and in all but 2 patients only one lesion was sampled. We did not perform DNA sequencing for mutant IDH1 analysis and cannot rule out the possibility of other minor IDH mutations: mutant IDH2 and rare-type mutant IDH1, e.g. R132C, R132L, R132S, R132G, including wild-type IDH1. We also did not perform sequence analysis of ATRX and TP53. Further studies including DNA sequencing are necessary to draw more reliable conclusions considering complex mechanisms involved in the gene status and protein expression of gliomagenesis.

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Disclosure Statement

The authors do not report any conflicts of interest.

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