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CLINICAL ARTICLE

Chordoid Glioma of the Third Ventricle: Immunohistochemical and Molecular Genetic Characterization of a Novel Tumor Entity

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Chordoid glioma of the third ventricle was recently reported as a novel tumor entity of the central nervous system with characteristic clinical and histopathological features (Brat *et al.*, *J Neuropathol Exp Neurol* 57: 283-290, 1998). Here, we report on a histopathological, immunohistochemical and molecular genetic analysis of five cases of this rare neoplasm. All tumors were immunohistochemically investigated for the expression of various differentiation antigens, the proliferation marker Ki-67, and a panel of selected proto-oncogene and tumor suppressor gene products. These studies revealed a strong expression of GFAP, vimentin, and CD34. In addition, most tumors contained small fractions of neoplastic cells immunoreactive for epithelial membrane antigen, S-100 protein, or cytokeratins. The percentage of Ki-67 positive cells was generally low (< 5%). All tumors showed immunoreactivity for the epidermal growth factor receptor and schwannomin/merlin. There was no nuclear accumulation of the p53, p21 (Waf-1) and Mdm2 proteins. To examine genomic alterations associated with the development of chordoid gliomas, we screened 4 tumors by comparative genomic hybridization (CGH) analysis. No chromosomal imbalances were detected. More focussed molecular genetic analyses revealed neither aberrations of the *TP53* and *CDKN2A* tumor suppressor genes nor amplification of the *EGFR*, *CDK4*, and *MDM2* proto-oncogenes. Our data strongly support the hypothesis that chordoid glioma of the third ventricle constitutes a novel tumor entity characterized by distinct morphological and immunohistochemical features, as well as a lack of chromosomal and genetic alterations commonly found in other types of gliomas or in meningiomas.

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Introduction

Brat *et al.* (4) recently reported on a series of eight patients with unique third ventricular tumors showing glial and chordoid features. The authors designated these tumors as chordoid gliomas and provided evidence that they represent a distinct clinicopathologic entity. Morphologically, chordoid gliomas are characterized by clusters and cords of epitheloid tumor cells with a prominent eosinophilic cytoplasm, relatively uniform nuclei and inconspicuous nucleoli. Similar to chordomas and chordoid meningiomas, the tumor cells are embedded in a mucinous matrix. Lympho-plasmacellular infiltrates are a regular feature. Mitotic activity is low and signs of anaplasia are absent (4). Immunohistochemical investigation showed strong immunoreactivity for glial fibrillary acidic protein (GFAP) and vimentin, while expression of epithelial membrane antigen (EMA), S-100 protein, and cytokeratins was either absent or weak (4). Ultrastructural studies revealed abundant intermediate filaments, focal projections resembling microvilli, scattered intermediate junctions and focal basal lamina formation, but neither well-

formed desmosomes and interdigitation of cell membranes nor cilia (4).

In the present study, we report on five patients with third ventricular chordoid gliomas. One of our patients was already included in the series of Brat *et al*. (4). The neuropathological characterization of a second case was published as an abstract (20). The remaining three patients were not reported on previously. We performed extensive immunohistochemical, molecular cytogenetic, and molecular genetic analyses on these tumors. Taken together, our results support the concept that chordoid glioma of the third ventricle represents a unique tumor entity among the neoplasms of the central nervous system.

Material and methods

Tumors. The five cases reported here were received for consultation at the Brain Tumor Reference Center of the German Society for Neuropathology and Neuroanatomy between 1992 and 1998. The tumors were from 2 male and 3 female patients, ranging in age from 32 - 65 years. From all tumors, formalin-fixed and paraffin-embedded surgical specimens were available. These were routinely processed and stained with hematoxylin and eosin (H&E), Gomori silver impregnation, and alcian blue / periodic acid Schiff (PAS).

Immunocytochemistry. All tumors were immunocytochemically evaluated on formalin-fixed paraffin sections for the expression of various differentiation antigens, including glial fibrillary acidic protein (GFAP), vimentin, protein S-100, epithelial membrane antigen (EMA), cytokeratins, desmin, neurofilaments, synaptophysin, and the glycoprotein CD34, as well as the proliferation-associated nuclear antigen Ki-67. In addition, expression of the tumor suppressor gene products p53, p21 (Waf1), and schwannomin/merlin, as well as the oncogene products Mdm2 and epidermal growth factor receptor (EGFR) was analyzed by immunohistochemistry. The indirect avidin-biotin peroxidase method was used for the detection of monoclonal mouse antibodies and the peroxidase-anti-peroxidase method for the detection of polyclonal rabbit antibodies as described (33). Antibody binding was visualized with 3'3-diaminobenzidine (Sigma, Deisenhofen, Germany). All sections were counterstained with hemalum.

The following primary antibodies were used: rabbit polyclonal antibodies against GFAP and S-100 (both from Dako, Copenhagen, Denmark); mouse monoclonal antibodies against vimentin (V9, Roche Molecular Biochemicals, Mannheim, Germany), desmin (D33, Dako), pan-cytokeratins (Lu5, Roche), cytokeratin 5/6 (D5/16B4, Roche), cytokeratin 8 (C-51, Novocastra Laboratories Ltd, Newcastle upon Tyne, UK), cytokeratin 13 (K_S13.1, Progen, Heidelberg, Germany), cytokeratin 18 (K_S18.04, Progen), cytokeratin 19 (RCK108, Biogenex, San Ramon, CA), cytokeratin 20 (K_S20.8, Dako), synaptophysin (SY38, Roche), neurofilament 70 kd and 200 kd subunits (NF2F11, Biochrom, Berlin, Germany), EMA (E29, Dako), CD34 (QBEND10, Immunotech, Marseille, France), Ki-67 (MIB-1, Dianova, Hamburg, Germany), p53 (DO-7, Dako), p21 (EA10, Dianova), Mdm2 (IF2, Dianova), EGFR (E30, Merck, Darmstadt, Germany), and schwannomin/merlin (5990, kindly provided by Dr. S.-M. Pulst, Los Angeles, CA). All monoclonal antibodies were used at final concentrations of 1 - 2 μg/ml for an incubation period of 16 hours at room temperature. The polyclonal antibodies against GFAP and S-100 were diluted 1:2000. To enhance immunoreactivity for Ki-67, cytokeratin 5/6, cytokeratin 20, vimentin, p53, p21, and Mdm2, sections were pretreated by microwave heating in 10 mM citrate buffer pH 6.0 for 3 x 10 minutes. Pretreatment of the sections with pronase E (0.1% for 15 min at 37°C) was employed to enhance immunoreactivity for EGFR and cytokeratins 8, 13, and 18. Negative controls were performed by omission of the primary antibody and its substitution with either an irrelevant mouse monoclonal IgG antibody or an irrelevant rabbit polyclonal antibody.

DNA and RNA Extraction, and Single Strand Conformation Polymorphism (SSCP) / Heteroduplex Analysis. From four chordoid gliomas (T2-5), DNA was extracted from formalin-fixed and paraffin-embedded surgical tissue samples as reported previously (34). Histopathological evaluation of the tumor fragments taken for DNA extraction showed tumor cell contents of at least 80% in each case. The stereotactic biopsy specimens available from tumor T1 were too small to allow extraction of sufficient amounts of DNA. Exons 4 – 10 of the *TP53* gene and exons 1α, 2, and 3 of the *CDKN2A* gene were amplified by PCR using published oligonucleotide primers (34, 36). Two primer pairs were used to amplify overlapping fragments covering exon 1β of the *CDKN2A* gene. The respective primer sequences were: 5′-GTCCCAGTCTGCAGTTAAGG-3′ and 5′-TGAGCCGCGGGATGTGAA-3′ (amplifying a 192-bp fragment covering nucleotides 154-345 of the CDKN2A exon 1b sequence deposited in Genbank, accession no. L41934), as well as 5′-CCGCGAGTGAGGGTTTTCG-

3′ and 5′-CCTAGAATGGGCTAGAGACG-3′ (amplifying a 233-bp fragment covering nucleotides 306-538). SSCP/heteroduplex analysis of the respective PCR products was carried out as described (8, 34).

Differential PCR analyses. The gene dosages of *MDM2*, *CDK4*, *EGFR*, and *CDKN2A* were analysed by differential PCR using primer pairs described elsewhere (8, 34). Differential PCR reactions were performed for 30 cycles using primers for the individual target gene and a reference gene in the following combinations: *MDM2* and *GAPDH*, *CDK4* and *IFNG*, *EGFR* and *IFNG*, *CDKN2A* (exon 1α) and *9qSTS* (38), as well as *CDKN2A* (exon 1β) and *IFNG* (Figure 3). Positive controls for the differential PCR experiments included DNA from the glioblastoma cell line TP365MG (amplification of *MDM2* and *CDK4*), a glioblastoma with known *EGFR* amplification, and the glioblastoma cell line U118MG (homozygous *CDKN2A* deletion). Cell line U118MG was obtained from the American Type Culture Collection (ATCC). TP365MG was kindly provided by Prof. V. Peter Collins, Department of Histopathology, Addenbrooke's Hospital, Cambridge, UK. Peripheral blood leukocyte DNA was used as reference template with normal gene copy number.

The PCR products were separated on a 3% agarose gel and the ethidium bromide-stained bands were recorded using the Gel-Doc 1000 system (BioRad, Hercules, CA). Quantitative analysis of the signals obtained for the target and reference genes was performed with the Multi-Analyst software (version 1.0.2, BioRad). Only increases in the target/reference gene ratio of more than 3-times the ratio obtained for constitutional DNA were considered as evidence for gene amplification. *CDKN2A/9qSTS* ratios of less than 0.3 relative to constitutional DNA were considered as homozygous deletion.

Comparative Genomic Hybridization (CGH) analysis. CGH analysis was carried out according to the protocol described in detail elsewhere (21). Image acquisition and processing was performed with the Cytovision System version 3.1 (Applied Imaging Inc., Sunderland, Tyne and Wear, UK). Mean ratio profiles were determined from the analysis of 12 metaphase spreads. The threshold values used to distinguish between the balanced and imbalanced state of the average ratio profiles were 0.75 (lower threshold) and 1.25 (upper threshold) in accordance with previously reported CGH analysis protocols (13). CGH experiments were carried out along with control experiments in which differently labeled normal DNAs were hybridized to normal metaphase chromosomes. The control experiments showed no diagnostic alterations.

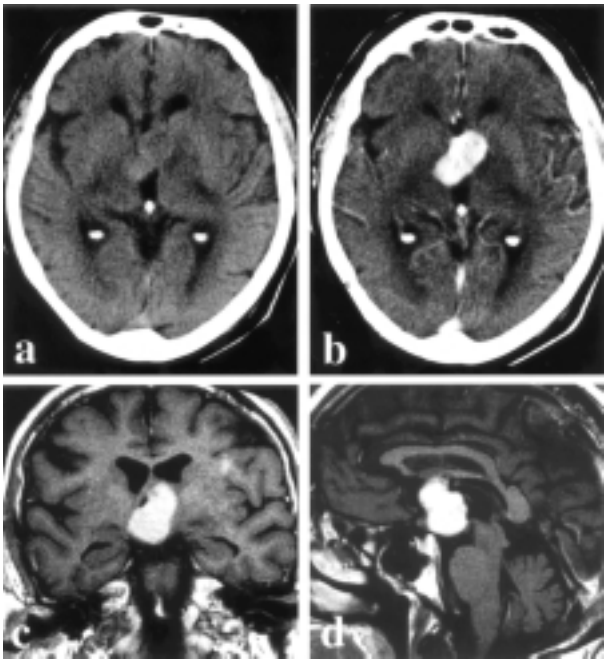

Figure 1. Typical neuroradiological appearence of a chordoid glioma (tumor T4). **a-b**) CT scans before (a) and after (b) contrast enhancement show a well demarcated, homogeneously enhancing mass lesion in the third ventricle. **c-d**) Frontal (c) and parasagittal (d) magnetic resonance images after administration of gadolineum-DTPA. Note a solid, well demarcated midline tumor with homogeneous contrast enhancement.

Case Reports

Case 1. A 56-year-old woman presented with a 6-months history of headaches and fatigue. She also noted occasional episodes of flimmer scotoma. Neurological examination was normal. However, an MRI-scan showed a suprasellar, well demarcated, space occupying lesion (volume: 23 x 19 x 20 mm), which was located above the optic chiasm and extended into the third ventricle. The tumor demonstrated strong contrast enhancement after gadolineum-DTPA administration. The lesion was stereotactically biopsied and originally diagnosed as chordoid meningioma with aberrant expression of GFAP. The patient then underwent a partial resection of the tumor through a transventricular approach, followed by gamma knife radiosurgery. In addition, an atrio-ventricular shunt was implanted because of a hydrocephalus of the right ventricle. In the postoperative phase, the patient developed an organic psychosyn-

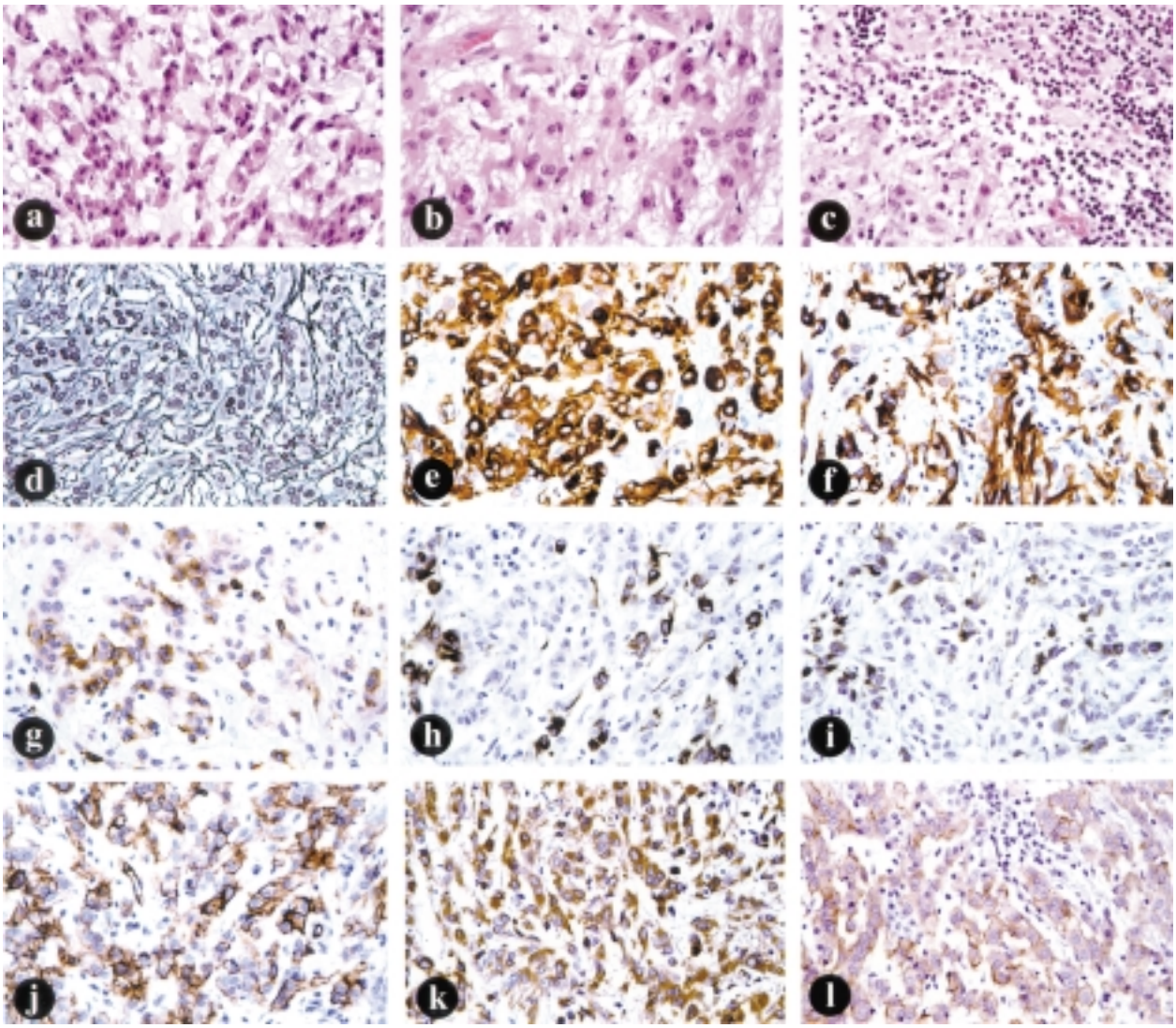

Figure 2. Histopathological and immunohistochemical features of chordoid gliomas (a: T1; b-c, e-g: T4; d, h-l: T2). **a-b**) Epitheloid tumor cells with abundant eosinophilic cytoplasm, scant processes, and inconspicuous nuclei are forming chordoma-like cords and clusters in a myxoid matrix. H&E. **c**) Reactive infiltrates consisting of small lymphocytes and plasma cells are frequent within the tumor and along its borders. H&E. **d**) Gomori silver impregnation shows a fine network of reticulin fibers surrounding clusters of cohesive tumor cells. **e-g**) Chordoid gliomas strongly express vimentin (e) and GFAP (f), whereas EMA immunoreactivity is restricted to a small fraction of tumor cells (g). **h-i**) Occasional tumor cells may be positive for cytokeratin polypeptides, such as cytokeratin 18 (h) and cytokeratin 8 (i). **j**) All chordoid gliomas from our series showed strong staining for CD34. **k-l**) Immunoreactivity for schwannomin/merlin (k) and epidermal growth factor receptor (l) in a chordoid glioma. All immunohistochemical sections (e-l) are counterstained with hemalum.

drome with impaired short term memory and somnolent episodes. However, these psycho-organic symptoms gradually disappeared over the following months. The last available neuroradiological follow-up at 3.5 years after diagnosis revealed a stable residual lesion without further growth.

Case 2. The clinical and neuroradiological data from this 31-year-old female patient were reported on in detail elsewhere (case 5 in the series of Brat *et al.*, ref. 4).

Case 3. A 53-year-old woman presented with a 20-year history of visual disturbances. She had gained nearly 50 kg of body weight over the past 10 years. Neurological examination revealed bitemporal hemianopia. Otherwise, no neurological abnormalities were found. An MRI scan showed a 2.5 x 2.5 x 2 cm large midline

tumor in the suprasellar region. The tumor was microsurgically resected via a bifrontal osteoplastic craniotomy. The postoperative course was uneventful. Follow-up data are not available.

Case 4. A 65-year-old man was admitted to his local hospital with a 1-day history of speech problems and facial weakness on the right side. His medical history was uneventful except for deep venous thrombosis in his left thigh several years ago. Clinical examination revealed arterial hypertension and a slight right sided peripheral facial nerve palsy, which spontaneously resolved over the following weeks. There were no further neurological abnormalities. All laboratory findings were normal. However, a cranial computerized tomography (CCT) scan was performed and surprisingly showed a well demarcated, contrast enhancing midline tumor in the suprasellar / third ventricular region (Figure 1a-b). In line with the CCT findings, an MRI scan revealed a homogeneously contrast enhancing, well circumscribed, space occupying midline lesion in the suprasellar region, which partially obstructed the third ventricle (Figure 1c-d). On sagittal sections, the tumor had no direct contact to the dorsum sellae or the clivus (Figure 1d). In order to obtain a definite diagnosis, a stereotactic biopsy was performed and the tumor was histopathologically classified as chordoid glioma. The patient underwent craniotomy and the tumor was totally resected. The early post-operative course was uneventful. However, the patient died only a few days after the operation due to fulminant pulmonary thromboembolism. An autopsy was not performed.

Case 5. This 35-year-old man became clinically apparent with an organic psychosyndrome. Neuroradiological examination showed a well demarcated, contrast enhancing tumor in the anterior part of the third ventricle. The tumor was completely resected. Unfortunately, the patient died 2 weeks after the operation due to pulmonary thromboembolism. No autopsy was performed. Histopathological and immunohistochemical findings of this case were reported at the 43rd Annual Meeting of the German Society for Neuropathology and Neuroanatomy, Homburg/Saar, 1998 (20).

Results

Histopathological findings. The five tumors of our series showed a remarkably uniform histopathological appearence. All tumors were moderately cellular and consisted of oval to polygonal, sometimes elongated, epitheloid tumor cells with abundant eosinophilic cytoplasm and scant processes (Figure 2a-b). Tumor cell nuclei were mostly round and isomorphous. Nucleoli were inconspicuous. Mitotic activity was generally low. Microvascular proliferations and necroses were absent. Reminiscent of the findings in chordomas and chordoid meningiomas, the tumor cells formed cohesive cords and clusters in a coarsely fibrillar, in larger areas strongly mucinous and partially vacuolated tumor matrix (Figure 2a-b). However, the tumor matrix was not as strongly mucinous and vacuolated as in chordomas, and physaliphorous cells were generally absent. Furthermore, none of the tumors showed characteristic features of meningiomas, such as whorl formations, psammoma bodies or nuclear pseudoinclusions. All tumors con-

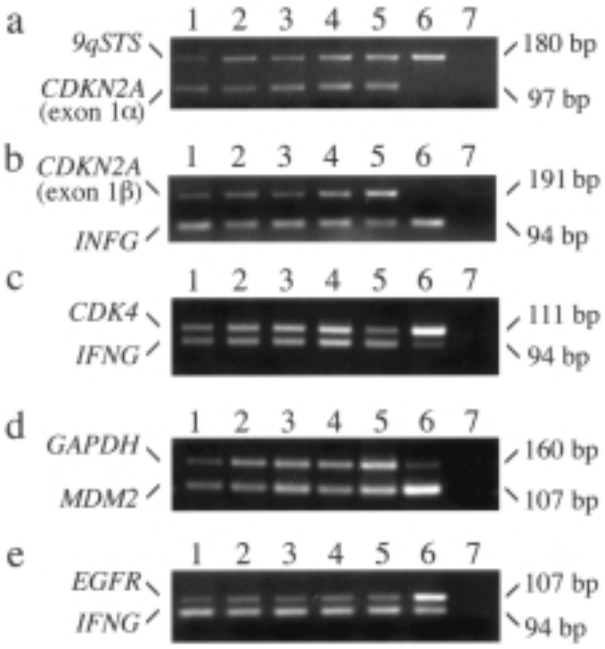

Figure 3. Molecular genetic findings in four chordoid gliomas. Shown are differential PCR analyses for deletions of *CDKN2A* exon 1α (**a**) and *CDKN2A* exon 1β (**b**), as well as for amplifications of *CDK4* (**c**), *MDM2* (**d**), and *EGFR* (**e**). The individual lane numbers correspond to: lanes 1 - 4, chordoid gliomas T2 - T5; lane 5, constitutional control (leukocyte DNA); lane 6, positive control (U118MG in a-b, TP365MG in c-d, and a glioblastoma with known *EGFR* amplification in e); lane 7, negative (water) control. **a-b**) None of the chordoid gliomas showed evidence for homozygous deletion of *CDKN2A* exon 1α or exon 1β, i.e. the densitometrically determined target gene to reference gene ratios normalized to constitutional DNA were > 0.3 for all tumors. c-e) None of the chordoid gliomas showed evidence for gene amplification of *CDK4*, *MDM2*, and *EGFR*, i.e. the normalized target gene to reference gene ratios were < 3 in all instances. The fragment sizes of the individual PCR products are indicated on the right.

Case	Age	Sex	Ki-67	GFAP	VIM	CD34	EMA	CK	S-100	DES	NF	SP
T1	56	f	<1%	+++	++++	+++	+	-	+++	-	-	-
T2	31	f	<3%	++++	++++	+++	+	+ (8, 18, 19)	+	-	-	-
T3	53	f	<1%	+++	++++	++++	+	+ (8, 13, 18, 19)	-	-	-	-
T4	65	m	<1%	++++	++++	++++	++	+ (5/6, 19)	-	-	-	-
T5	35	m	<5%	++++	++++	++++	++	+ (8, 18, 19)	++	-	-	-

Abbreviations used: f, female; m, male; GFAP, glial fibrillary acidic protein; VIM, vimentin; EMA, epithelial membrane antigen; CK, cytokeratins (the individual cytokeratin subtypes expressed are indicated in brackets); DES, desmin; NF, neurofilaments; SP, synaptophysin. Immunoreactivity was semiquantitatively scored as follows: -, negative; +, individual positive tumor cells (< 10%); ++, moderate fraction of positive tumor cells (< 50%); +++, high fraction of positive tumor cells (> 50%); ++++, very high fraction of positive tumor cells (> 90%).

Table 1. Summary of patient data and immunohistochemical findings.

tained reactive infiltrates consisting of small lymphocytes and mature plasma cells (Figure 2c). Formation of lymph follicles with germinal centers was not seen. In cases where surrounding brain tissue fragments were present, these appeared to be well demarcated from the tumor and demonstrated marked reactive astrogliosis with formation of Rosenthal fibers. On special stains, all tumors contained reticulin and collagen fibers embracing small groups and clusters of tumor cells (Figure 2d). There were focal areas of reactive fibrosis. The extracellular matrix was alcianophilic and partially PAS positive.

Immunohistochemical results. The results of our immunohistochemical studies for the expression of various differentiation antigens are summarized in Table 1. All tumors showed strong expression of vimentin and GFAP in the majority of neoplastic cells (Figure 2e-f). In addition, we found widespread immunoreactivity for the hematopoietic stem cell and vascular endothelium associated glycoprotein CD34 (Figure 2j). Expression of EMA was generally restricted to minor subpopulations of tumor cells (Figure 2g). A small percentage of pan-cytokeratin (Lu-5) immunopositive tumor cells was detected in 4 of 5 tumors. Cytokeratin subtyping revealed that one tumor (T4) contained individual tumor cells positive for cytokeratins 5/6 and 19, while three tumors (T2, T3, and T5) demonstrated minor fractions of tumor cells positive for cytokeratins 8, 18 and 19 (Figure 2h-i). Three tumors (T1, T2, and T5) contained some S-100 positive tumor cells. All chordoid gliomas were negative for synaptophysin, neurofilaments, and desmin. The focal synaptophysin positivity originally reported for tumor T5 (20) may be related to a different immunohistochemical protocol used. The Ki-67 (MIB-1) proliferation fraction was < 1% in T1, T3, and T4, while T2 contained approximately 2 - 3% Ki-67 positive tumor cells. Tumor T5 showed focal clusters with increased Ki-67 labelling rate, however, the total percentage of Ki-67 positive glioma cells was still less than 5%. Immunohistochemical analyses of selected oncogene products and tumor suppressor proteins revealed strong expression of schwannomin/merlin and weak to moderate expression of EGFR in the majority of neoplastic cells in all five tumors (Figure 2k-l). There was no pathological accumulation of p53, p21, and Mdm2 proteins (Table 2).

CGH and molecular genetic results. CGH analysis of 4 chordoid gliomas from our series (T2-5) revealed no diagnostic alterations, i.e. a balanced state of CGH profiles was found in all instances. Tumors T2-5 were additionally subjected to molecular genetic analyses for aberrations of the *TP53*, *CDKN2A*, *EGFR*, *CDK4*, and *MDM2* genes (Table 2). SSCP/heteroduplex analysis of exons 4 - 10 of the *TP53* gene and exons 1α, 1β, 2, and 3 of the *CDKN2A* gene revealed no aberrantly migrating bands in any of the tumors. Differential PCR analysis of *CDKN2A* gene copy number (exon 1α and exon 1β) yielded no evidence for homozygous gene deletion (Figure 3a-b). None of the tumors showed amplification of the proto-oncogenes *EGFR*, *MDM2*, or *CDK4* by differential PCR analysis (Figure 3c-e).

Discussion

The characteristic clinicopathologic features of chordoid gliomas of the third ventricle have been described in detail by Brat *et al.* (4), who reported on a series of eight patients with this rare type of central nervous system tumor. Vajtai *et al.* (39) recently published the biopsy and postmortem findings in a 60-year-old woman with a third ventricular chordoid glioma. Another patient with a suprasellar tumor showing very similar histopathological and immunohistochemical features to

Case	CGH	*TP53* Mut	*CDKN2A* Del/Mut	*EGFR* Amp	*CDK4* Amp	*MDM2* Amp	p53 (%)	p21 (%)	EGFR	NF2
T1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	<1	+++	++++
T2	-	-	-	-	-	-	0	<1	++++	++++
T3	-	-	-	-	-	-	<1	<1	++++	++++
T4	-	-	-	-	-	-	0	<1	++++	++++
T5	-	-	-	-	-	-	0	<1	+++	++++

Abbreviations used: CGH, comparative genomic hybridization; Mut, mutation; Del, homozygous deletion; Amp, amplification; n.d., not done. Results of immunostainings for p53 and p21 are given as percentage of positive tumor cells. Immunoreactivity for EGFR and merlin/schwannomin (NF2) was semiquantitatively scored as follows: -, negative; +, individual positive tumor cells (< 10%); ++, moderate fraction of positive tumor cells (< 50%); +++, high fraction of positive tumor cells (> 50%); ++++, very high fraction of positive tumor cells (> 90%).

Table 2. Summary of results obtained by CGH analysis, molecular genetic studies and immunohistochemistry for the expression of tumor suppressor gene products and oncoproteins.

the chordoid gliomas described by Brat *et al.* (4) has been reported by Wanschitz *et al.* (40). However, in contrast to the ultrastructural findings in 4 chordoid gliomas from the series of Brat *et al.* (4), this particular tumor showed very rare, small but well developed desmosomes on electron microscopy (40). Therefore, the authors classified this neoplasm as a peculiar variant of meningioma with expression of GFAP, rather than as a glioma. Cenacchi *et al.* (6) recently reported on three patients with tumors of the third ventricle showing a chordoid morphology, diffuse immunoreactivity for GFAP and vimentin, as well as focal expression of EMA and cytokeratins. Ultrastructural examination revealed hemidesmosomes and desmosome-like junctions. The authors therefore proposed the term "chordoid neoplasms of the third ventricle" for these tumors (6). In our experience, however, the glial morphological and immunohistochemical characteristics of these tumors are much more prominent than the limited meningothelial features detectable at the ultrastructural level in some cases. In line with Brat *et al.* (4) and Vajtai *et al.* (39), we therefore refer to these tumors as "chordoid gliomas", i.e. regard them as a distinct type of glial neoplasm rather than as a rare variant of meningioma. However, the histogenesis of chordoid gliomas and the reason for their preferential manifestation in the third ventricular region remain to be elucidated.

All third ventricular chordoid gliomas reported in our series and the series of Brat *et al.* (4) occured in adult patients (mean age at operation: 48 years, range 31 - 70 years). Similarly, the patients reported by Vajtai *et al.* (39), Wanschitz *et al.* (40), and Cenacchi *et al.* (6) were all adults between 24 and 60 years of age at the time of operation. Neuroradiologically, chordoid gliomas generally present as well circumscribed, contrast enhancing lesions of the suprasellar/third ventricular region. A cystic component may be seen in a minority (4). Although the neuroradiological features of third ventricular chordoid gliomas appear to be quite uniform, neuroimaging on its own does not allow a definite diagnosis of these neoplasms. This can only be achieved by histopathological and immunohistochemical evaluation of tumor tissue obtained by either stereotactic biopsy or open resection. With respect to grading and prognosis, the available data suggest that chordoid gliomas are biologically benign, non-infiltrating tumors of low growth potential. However, their location in the third ventricle with attachment to hypothalamic and suprasellar structures may preclude complete resection, and this may result in recurrent tumor growth and less favorable prognosis. Furthermore, 5 of 16 reported patients (4, 6, 39, this study) died in the early postoperative phase due to medical complications such as pulmonary thromboembolism or bronchopneumonia. The reason for this surprisingly high postoperative mortality rate is unclear.

On H&E stained sections, the gross histopathological appearence of chordoid gliomas closely resembles that of chordoma or chordoid meningioma. However, there are some notable features that allow morphological differentiation of these distinct entities. For example, chordoid gliomas generally lack physaliphorous cells and show no tendency for bone infiltration, both of which are typical features of chordomas. In line with Brat *et al.* (4), our tumors demonstrated no morphological hallmarks of meningiomas, such as whorl formations, psammoma bodies, or nuclear pseudoinclusions. The lymphoplasmacellular infiltrates seen in chordoid gliomas are much less prominent than those observed in chordoid meningiomas associated with Castleman syndrome (19). Formation of lymphoid follicles with ger-

	GFAP	VIM	CD34	EMA	S-100	CK
Chordoid glioma	+	+	+	+/-	+/-	+/-
Chordoid meningioma	-	+	-	+	-	-
Chordoma	-	+	-	+	+	+
Pilocytic astrocytoma	+	+	-	-	+	-
Ependymoma	+	+	-	+/-	+	-

Abbreviations used: +, positive; -, negative; +/-, positive in some cases, but immunoreactivity usually restricted to a low fraction of tumor cells; GFAP, glial fibrillary acidic protein; VIM, vimentin; EMA, epithelial membrane antigen; CK, cytokeratins.

Table 3. Immunohistochemical differential diagnosis of chordoid glioma, chordoid meningioma, chordoma, pilocytic astrocytoma, and ependymoma.

minal centers, which may be seen in chordoid meningiomas (19), is not a feature of chordoid gliomas. With respect to electron microscopic findings, Brat *et al.* (4) detected no ultrastructural features characteristic of either chordoma or meningioma in four chordoid gliomas (4). Other authors, however, reported on the presence of desmosomes and/or desmosome-like junctions in some tumors (6, 40).

In line with previous studies (4, 6, 39, 40), we found an invariably strong expression of GFAP and vimentin in our series of chordoid gliomas. Immunoreactivity for EMA, protein S-100, and cytokeratins was additionally detected in the majority of tumors, however, was usually restricted to a minority of tumor cells. Cytokeratin subtyping revealed that three tumors contained individual tumor cells immunoreactive for cytokeratins 8, 18, and 19, which are the subtypes expressed in simple ductal and glandular epithelia (27). Only one tumor of our series contained a small number of tumor cells positive for cytokeratins 5/6, i.e. the cytokeratin polypeptides typically expressed in squamous epithelia (27). This particular tumor also harboured individual cells positive for cytokeratin 19. In contrast to chordoid gliomas, chordomas are strongly and uniformly positive for cytokeratins (1, 9-10, 23, 26). Vimentin, EMA and protein S-100 are also strongly expressed in virtually all chordomas (9-10, 23). Although some authors reported on GFAP immunoreactivity in chordomas (18, 42), we and others found these tumors consistently GFAP negative (9, 24, 26, 33). These differences are likely explained by the use of different anti-GFAP antibodies, which may or may not cross-react with epitopes on other intermediate filament proteins. With respect to the cytokeratin polypeptides expressed in chordomas, an invariably strong coexpression of cytokeratins 8 and 19 has been reported in both chordomas and fetal notochord (15, 29-30). The majority of chordomas also show immunoreactivity for cytokeratin 18 (15, 29), while a minor fraction demonstrated focal immunoreactivity for cytokeratin 5 (29). Thus, chordoid glioma and chordoma express similar cytokeratin subtypes but differ markedly in the expression level and the percentage of positive tumor cells.

An intriguing finding is the strong expression of CD34 in all five chordoid gliomas of our series. CD34 is a sialylated transmembrane glycoprotein expressed on hematopoietic stem cells and progenitor cells, vascular endothelia, and some mesenchymal cell types (37). CD34 has also been reported to be transiently expressed on murine neural cells during early neurulation (22). Previous studies on human central nervous system tumors have revealed immunoreactivity for CD34 in neurofibromas, meningeal hemangiopericytomas, a small fraction of meningiomas, solitary fibrous tumors of the meninges, as well as in benign glioneuronal tumors and hamartomatous lesions associated with temporal lobe epilepsy (3, 5, 7). Our study adds chordoid gliomas to the list of CD34 positive central nervous system neoplasms. We also examined 7 chordomas and 2 meningiomas with chordoid features for CD34 expression but found these tumors consistently negative (data not shown). Similarly, the common forms of glioma not associated with chronic temporal lobe epilepsy, including pilocytic and diffuse astrocytomas, oligodendrogliomas, mixed gliomas, and ependymomas, are consistently CD34 negative (3, 7). Thus, the strong expression of CD34 together with GFAP and vimentin in chordoid gliomas represents an unique immunohistochemical pattern that facilitates the differential diagnosis of these neoplasms (Table 3). The functional role of CD34 in chordoid gliomas and other types of neural tumors remains to be elucidated.

To investigate genomic alterations in chordoid gliomas, we examined 4 tumors from our series by CGH analysis. CGH is a powerful and comprehensive molecular cytogenetic technique that allows the detection of gains and losses of genetic material across the entire genome at a resolution of 10-20 Mbp for low copy number imbalances (2, 12, 17). High copy number changes can be detected if the product of amplicon size and copy number amounts to at least 2 Mbp (16, 32). None of the tumors of our series showed any detectable chromosomal losses, gains or high copy number amplifications. In line with the CGH results, differential PCR analysis revealed no evidence for amplification of the proto-oncogenes *EGFR* (7p21), *CDK4* (12q13-q14), and *MDM2* (12q15). In addition, we found neither mutations of the *TP53* gene nor p53 protein accumulation in our

tumor series. None of the tumors showed mutations or homozygous deletion of the *CDKN2A* gene. These five genes were investigated because they are frequently aberrant in diffuse astrocytomas of adulthood (for review see refs. 11, 28). Thus, the lack of detectable alterations of these genes in chordoid gliomas indicates that the molecular pathogenesis of chordoid gliomas differs from that of the common diffuse astrocytomas. Furthermore, the absence of deletions on chromosome arm 22q by CGH analysis and the strong expression of schwannomin/merlin suggest that inactivation of the *NF2* gene at 22q12, an important alteration in the majority of meningiomas (35, 41), is not usually involved in chordoid gliomas. In contrast to astrocytomas and meningiomas, genetic alterations in chordomas have been poorly investigated so far. However, the available cytogenetic data indicate that chordomas frequently carry complex structural and numerical chromosome aberrations (14, 25, 31), which thus represents yet another feature distinguishing chordomas from chordoid gliomas.

In summary, we report on clinical, neuroradiological, neuropathological, and molecular genetic findings in a series of 5 chordoid gliomas of the third ventricular region. Our immunohistochemical and molecular genetic results strongly support the proposal of Brat *et al.* (4) that third ventricular chordoid glioma represents a novel tumor entity. These neoplasms are characterized by distinctive clinicopathologic and immunohistochemical features, as well as a lack of chromosomal and genetic alterations commonly found in other types of gliomas and meningiomas.

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