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Original contribution

Intramedullary gangliogliomas: histopathologic and molecular features of 25 cases☆

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Summary Gangliogliomas are uncommon glioneuronal tumors, which usually arise in the cerebral hemispheres and occasionally in the brain stem. Gangliogliomas occurring in the spinal cord are extremely rare. In this study, we analyzed the clinical, histopathologic, and molecular features of 25 spinal gangliogliomas. The cases included in our series affected mostly children and young adults (15 males and 10 females; mean age, 20 years; median age, 14 years; age range, 1-72 years) and were predominantly localized in the cervical and thoracic spine. From the clinical point of view (detailed follow-up available for 9 pediatric cases; mean follow-up: 2 years 10 months; range, 3 months to 5 years 10 months), most patients showed stable disease after subtotal resection. Radiotherapy was rarely used as adjuvant treatment. Histologically, gangliogliomas (WHO grade I) (21 cases) showed features largely similar to their supratentorial counterparts. Anaplastic gangliogliomas (World Health Organization grade III) (4 cases) showed features of anaplasia (including high cellularity and increased mitotic and proliferation activity). From a molecular point of view, only 2 tumors (2/19, 11%) harbored a *BRAF*^{V600E} mutation. In conclusion, although spinal gangliogliomas display histologic and clinical features similar to their supratentorial counterparts, they show a relatively low frequency of *BRAF*^{V600E} mutations, alteration otherwise common in hemispheric and brain stem gangliogliomas.

1. Introduction

Gangliogliomas (GGs) are uncommon neuroepithelial tumors, which are composed of glial cells as well as dysplastic neurons [1]. They account for 2% of the central nervous system (CNS) neoplasms [1]. Although they may occur throughout the CNS, they are typically hemispheric

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with a predilection for the temporal lobes, where they represent one of the most common epilepsy-associated tumors [1]. GGs are rarely diagnosed in the spinal cord [2-5], accounting for less than 10% of all intramedullary neoplasms in some series [6]. Although several cases have been described as case reports, no large cohort of spinal GG was analyzed in detail, focusing on their neuropathological and molecular features.

In this study, we investigated the clinical, histopathologic, and molecular features of 25 spinal GGs.

2. Materials and methods

2.1. Patients and tissues

Twenty-five formalin-fixed, paraffin-embedded tissue specimens of spinal GG were retrieved from the archive of the Institute of Neuropathology, University of Bonn medical Center, and the DGNN German Brain Tumor Reference Center, Bonn, Germany (between 2000 and 2015). Detailed follow-up (FU) information was available for 9 pediatric patients enrolled in the HIT-LGG 1996 protocol of the German Society of Pediatric Oncology and Hematology (GPOH).

2.2. Immunohistochemistry

Immunohistochemistry was performed on a semiautomated immunohistochemistry stainer (Tecan, Crailsheim, Germany) or a Ventana Benchmark XT Immunostainer (Roche Ventana, Darmstadt, Germany) with antibodies against glial fibrillary acidic protein (dilution 1:1000; Dako, Hamburg, Germany), microtubule-associated protein 2 (MAP2C) (1:20 000; Sigma, St Louis, MO), p53 protein (1:150; Dako), $BRAF^{V600E}$ protein (clone VE1, 1:200; Spring Bioscience, Pleasanton, CA), S-100 protein (1:2000; Dako), CD34 (1:1000; Dako), synaptophysin (1:200; Dako), NeuN (1:100; Dako), chromogranin A (1:1000; Dako), neurofilament (1:1000; Dako), H3.3K27M (1:500; Millipore, Temecula, CA) and trimethylated H3.3 (1:200; Cell Signaling, Danvers, MA), and Ki-67 (Mib-1, 1:500; Dako). The proliferation index was expressed as percentage of stained cells.

2.3. DNA extraction, *BRAF*^{V600E} mutation analysis, and *KIAA1549-BRAF* fusion status analysis

Hematoxylin and eosin–stained sections of each case were reviewed carefully before they were selected for DNA extraction. All samples selected contained at least 80% of viable tumor. DNA from 13 cases was extracted using the QIAamp DNA Mini Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer's instructions. We screened the hotspot codon 600 (exon 15) of the *BRAF* gene for mutations using a pyrosequencing assay as reported in detail elsewhere [7]. As a positive control for the screening, DNA from a *BRAF*^{V600E} mutated melanoma brain metastasis was used. The *KIAA1549-BRAF* fusion was studied using a multiplex ligation-dependent probe amplification (MLPA)–based approach [8]. Twelve cases with available DNA were investigated. The P370 MLPA kit (MRC Holland, Amsterdam, The Netherlands) was used. This kit contains multiple specific hybridization probes for both *KIAA1549* and *BRAF* genes, within and outside the parts of genes that undergo fusion. The formation of the fusion is associated to the generation of an extra copy that can be detected by a quantitative assessment of regions included and not included in the fusion. The MLPA analysis of these cases was performed along with 3 positive controls (pilocytic astrocytomas with known *BRAF* fusions) and 3 negative controls (normal cerebellar tissue), following the manufacturer's instructions. The results were analyzed using the Coffalyser.net software (MRC Holland).

3. Results

3.1. Clinical and neuroradiological features

The series included tumors from 25 patients (15 males and 10 females), comprising 7 adults and 18 children (mean age, 20.5 years; median age, 14 years, age range, 1-72 years). Two tumors were localized in the upper cervical spine extending into the medulla oblongata, 12 were cervical (6 of these affected also the thoracic spine), 5 were exclusively thoracic, and 4 were localized in the thoracolumbar segment or lumbar. Only 1 case was localized in the cauda equina. Detailed neuroradiology was available for 9 cases. Their magnetic resonance imaging scans showed mostly well-demarcated lesions, displaying some cystic features and presenting with variable, often strong contrast enhancement (Fig. 1). According to the clinical information available, 1 patient was affected by neurofibromatosis type 1 (NF-1), the others did not show clinical features of a tumor predisposition syndrome. For 9 pediatric patients, detailed FU data were available (mean FU, 2 years 10 months; FU range, 3 months to 5 years 10 months). Of the 9 patients, 7 did not show progression of the tumor residue, but 2 patients received radiotherapy after appearance of neurologic symptoms. One patient had a second surgical intervention a few months after the first resection due to clinical evidence of tumor progression without further relapse. One patient with GG of the cauda equina was previously diagnosed with a pleomorphic xanthoastrocytoma in the posterior fossa, for which he received radiotherapy and chemotherapy upon progression. The clinical data are briefly summarized in the Table.

3.2. Histopathologic features

The histologic specimens consisted of small biopsies (18 cases) or larger tumor resections (7 cases). All GGs (World

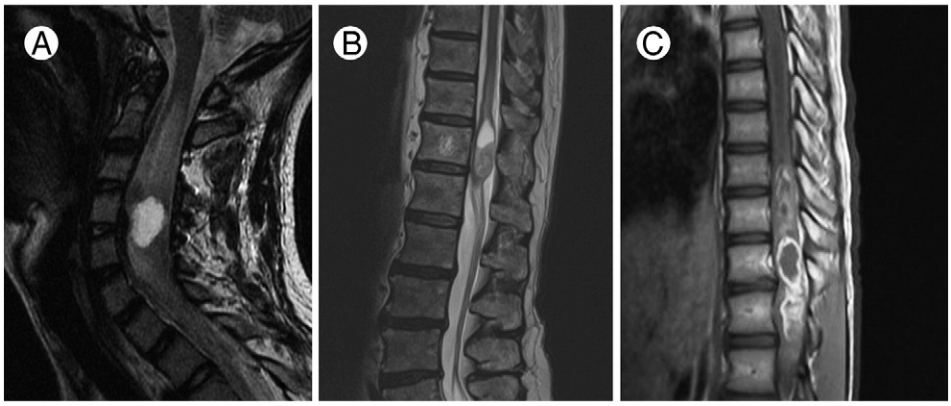

Fig. 1 Neuroradiological features of spinal gangliogliomas described in this series. A, A T1 hyperintense tumor at C4 level appeared well demarcated from surrounding CNS tissue (case 7). B, A contrast-enhancing tumor located at T12-L1 (case 11) shows partially cystic features. C, The magnetic resonance imaging scans display a partially cystic, partly solid, and contrast-enhancing intramedullary ganglioglioma at T12 level (case 6). The lesion is T1 hyperintense and contrast-enhancing with numerous cysts located centrally.

Health Organization [WHO] grade I) (21 cases) were characterized by low to moderate cellularity. The astrocytic tumor component showed variable cytology, mainly piloid or microcystic (Fig. 2A). In 2 cases (9%, 2/21), oligo-like features were present. We found no cases with an unequivocally biphasic architecture. The glial component was commonly glial fibrillary acidic protein and variably MAP2C positive. Eosinophilic granular bodies (EGBs) were found in 14 cases (66%, 14/21). In 7 cases (33%, 7/21), Rosenthal fibers were seen. In 3 cases (14%, 3/21), the tumor presented with abundant hyalinized vessels (Fig. 2B). Calcifications were seen in 3 GGs (14%, 3/21 cases). Lymphocytic inflammatory infiltrates were identified in 6 GGs (28%, 6/21). Nine cases (42%, 9/21) displayed an abundant neuronal component with dysmorphic ganglion cells (DGCs) (Fig. 2C). In the remaining GGs (WHO grade I), the DGCs were still easily identifiable also in the hematoxylin and eosin–stained slide. In only 1 case, this component was limited to a few cells. The morphology of dysplastic ganglion cells varied greatly. Most cells displayed irregular-shaped nuclei and variable, occasionally very large, plump cytoplasms (Fig. 2D) also with aggregated Nissl substance. In the large majority of GGs, single or small clusters of DGC appeared scattered within the tumor tissue. In 2 cases, large nodules of DGC were observed. Binucleated neurons were identified in 15 of 21 cases (Fig. 2D). In 1 case, DGC presented cytoplasmic vacuolizations. In only 1 case, the neuronal component showed discrete neurocytic cytology along with large dysplastic ganglion cells. DGC showed mainly perisomatic and/or cytoplasmic positivity for synaptophysin (Fig. 2E), although the perisomatic pattern appeared to be more common. Cytoplasmic expression of chromogranin A was seen in 17 cases (95%, 17/18) (Fig. 2G). In 15 GGs (71%, 15/21), MAP2C antibody stained significantly only the neuronal component (Fig. 2F). In a few cases (26%, 4/15), an abnormal cytoplasmic accumulation of phosphorylated neurofilaments was seen in DGC. Mitotic figures were very rare in GG, and the proliferation index varied between 1% and 4%. Four cases in our series were compatible with the diagnosis of anaplastic ganglioglioma (AGG) (WHO grade III). These tumors

Table Clinical features of spinal gangliogliomas included in this study

Case	Age (y)	Sex	Site	Diagnosis	*BRAF*^{V600E} status
1	17	M	C4-T1	AGG (WHO III)	NA [a]
2	9	M	MO-C4-6	GG (WHO I)	Mut
3	3	F	C3-5	GG (WHO I)	WT
4	13	M	MO-C2	GG (WHO I)	WT
5	4	M	T11-L1	GG (WHO I)	WT
6	14	F	T10-12	AGG (WHO III)	WT
7	13	F	C4	GG (WHO I)	WT
8	8	M	C7-T2	GG (WHO I)	Mut
9	49	M	T6-7	GG (WHO I)	NA
10	63	M	C3-C5	AGG (WHO III)	WT
11	68	F	T12-L1	GG (WHO I)	WT
12	6	M	L4-5	GG (WHO I)	NA
13	9	F	T10-L1	GG (WHO I)	WT [b]
14	6	F	C5-T4-5	GG (WHO I)	WT [b]
15	3	F	C6-7, T8-9	GG (WHO I)	WT
16	15	M	C5-T1	GG (WHO I)	WT
17	13	M	T10-L2	AGG (WHO III)	WT
18	31	M	T6-9	GG (WHO I)	WT
19	16	M	T3-7	GG (WHO I)	WT
20	17	F	C2-4	GG (WHO I)	WT
21	15	F	T5-6	GG (WHO I)	WT
22	36	F	C	GG (WHO I)	NA
23	20	M	C2-5	GG (WHO I)	NA
24	1	M	C1-T7	GG (WHO I)	WT
25	72	M	NA	GG (WHO I)	NA

Abbreviations: M, male; F, female; C, cervical; T, thoracic; L, lumbar; MO, medulla oblongata; AGG, anaplastic ganglioglioma; GG, ganglioglioma; WT, wild type; Mut, mutated; NA, not available.

[a] Patient affected by NF-1.

[b] Presence of extra copy of *KIAA1549* and *BRAF* genes suggestive of *KIAA1549-BRAF* fusion.

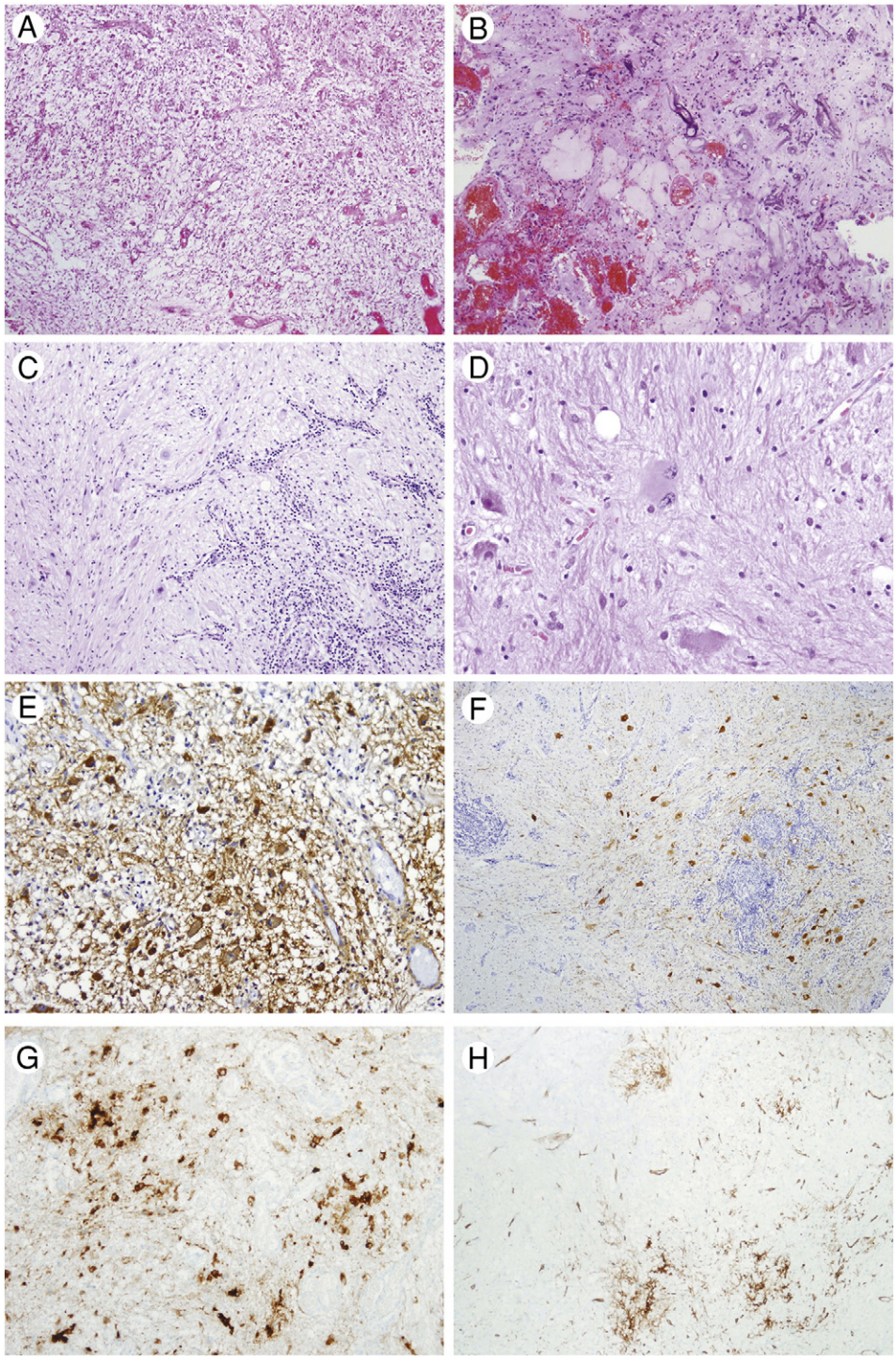

Fig. 2 Histopathologic and immunohistochemical features of spinal gangliogliomas included in this study. A, The tumors display mostly a glial "touch" with a predominant astrocytic component often with piloid cytology (case 6). B, Unusual angiomatous features and calcifications can be identified in a minority of cases (case 9). C, Occasionally, the tumor may show dense inflammatory infiltrates (case 22). C, Dysplastic ganglion cells (DGCs) can be easily observed in all cases, scattered within the tumor tissue (case 22) or arranged in small clusters. D, The DGC appears bizarre, irregularly shaped, occasionally binucleated (case 15), rarely vacuolated. The DGCs express synaptophysin (E), MAP2C (F) (case 22), and chromogranin (G) (case 5). A cytoplasmic or perisomatic staining pattern can be seen. H, In approximately 60% of cases, a variable number of CD34-positive "satellite cells" can be detected (case 4).

showed a higher cellularity in comparison with grade I GG (Fig. 3A and B). They also displayed increased cellular pleomorphism and presence of mitoses (Fig. 3B, D). EGBs were found in 3 cases. Necrosis could be identified in 1 case. In all AGGs, the DGCs were clearly visible with neuronal cells mostly intermingled with the glial component (Fig. 3C,

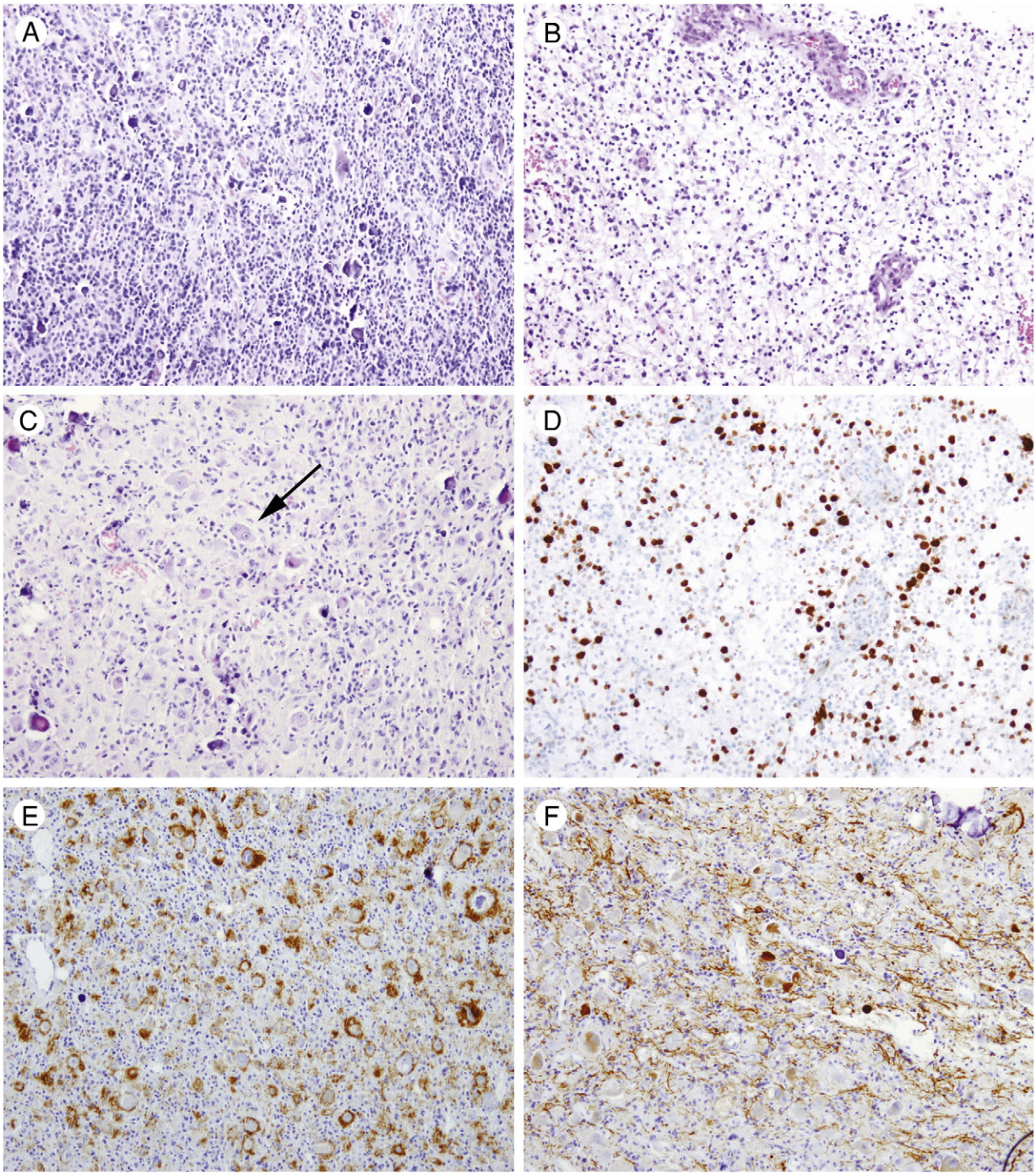

Fig. 3 Histopathologic and immunohistochemical features of spinal anaplastic gangliogliomas included in this study. Anaplastic gangliogliomas show increased cellularity (A and B), also reminiscent of high-grade glioma (B), note the vascular proliferations (case 1). C, Besides an anaplastic glial component, DGCs are seen (arrow, binucleated DGC) (case 17). D, The mitotic count and proliferation activity in anaplastic ganglioglioma appear clearly increased. DGCs express synaptophysin (E) and, occasionally, neurofilaments (F) (case 17).

E-F). Binucleated DGCs were identified in 3 of 4 cases and expressed chromogranin A as well as synaptophysin (Fig. 3E). Notably, a nuclear positivity for mutant H3.3K27M protein and negativity for trimethylated H3.3 was detected in 2 AGGs (2/3 cases investigated, cases 10 and 17).

3.3. *BRAF*^{V600E} and *KIAA1549-BRAF* fusion status analysis

BRAF^{V600E} status was investigated in 19 cases (19/25, 76%) using immunohistochemistry (6 cases) or using a

pyrosequencing-based assay (13 cases). Notably, we identified only 2 tumors (2/19; 11%) in our series harboring a *BRAF*^{V600E}. These tumors affected a 9-year-old patient with a lesion extending from the medulla oblongata to the C6 level and an 8-year-old boy with a tumor from C7 to T2, respectively.

In 2 cases (13 and 14), extra copies of *KIAA1549* containing exons 11 and 4 as well as exons 17 and 14 of *BRAF* were observed and were highly indicative for the presence of a *KIAA1549-BRAF* fusion. Three cases did not show any alteration. That suggests the absence of fusions. Four displayed inconclusive results. One case revealed gain of the whole chromosome 7; in another case, an isolated gain of the whole *BRAF* gene was detected.

4. Discussion

GGs occurring in the spinal cord are extremely rare [6]. In accordance with the available data from the literature, our results confirm that spinal GGs may affect a broad spectrum of age but seem to have a slight predilection for children, adolescents, or young adults [2,4]. Infants or small children appear to be rarely affected. With regard to localization, spinal GGs arise preferentially in the cervical and thoracic spinal cord, whereas they seem to be rather uncommon in the lumbar segment as well as in the cauda equina [2-4]. Frequently, these tumors involve multiple spinal segments including the craniospinal junction and the medulla oblongata. From a clinical point of view, spinal GGs are characterized by an indolent biologic behavior. According to our limited data, patients with spinal GG seem to have stable disease after surgical intervention or are stabilized by further intervention or radiotherapy upon progression. However, given the small number of patients, we cannot definitively assess the effectiveness of these strategies. The use of chemotherapy has been mainly used for AGGs, which usually show more aggressive clinical behavior [2,4,9]. Unfortunately, we had no FU data regarding the patients with AGG included in this study.

Histologically, spinal GGs are largely similar to their hemispheric counterparts. Our analysis identified, however, some differences, such as the absence of significant lymphocytic infiltrates and the variable presence of CD34-positive "satellite" cells, frequently encountered in epilepsy-associated GG. Although the glial cell component could be easily detected, the correct identification of the DGC component of a spinal GG could be challenging. In fact, preexisting normal neurons in spinal cord nuclei could be easily misinterpreted as DGC. In our experience, the presence of bizarre cytology and binucleation, together with an inhomogeneous chromogranin and synaptophysin positivity with a perisomatic or cytoplasmic staining pattern, strongly supports the diagnosis of GG, although this staining pattern has been also described in normal neurons in the spinal cord [10,11]. Their limited number may also cause diagnostic difficulties. In fact, the frequency of these cells may vary within the tumor tissue ranging from many (as observed in most cases of our series) to very few. If the DGCs are rare, the differential diagnosis with a pilocytic astrocytoma, which to some extent may overrun normal tissue and entrap normal neurons [1], can be difficult or impossible. Notably, in a recent study [12], posterior fossa and spinal GG (7 and 4 cases of the 27 cases reported, respectively) with limited number of DGC have been defined as "pilocytic astrocytoma with gangliocytic component," due to their great similarities with pilocytic astrocytomas. This group of tumors presented a biphasic pattern of growth and frequent Rosenthal fibers besides a focal intratumoral ganglion cell component. From a genetic point of view, all these cases harbored, like pilocytic astrocytomas, *KIAA1549-BRAF* fusions. Although the confusion between pilocytic astrocytoma and GG (both are grade I tumors according to the 2007 WHO classification) [1] may have no significant implications in the management of these patients, the distinction between GG and diffuse astrocytoma (WHO grade II) infiltrating the surrounding nerve tissue may be noteworthy. The neuroimaging could be helpful in this distinction.

Anaplastic GGs are rare in the spinal cord, and only a few cases have been previously described [9]. The anaplastic features were detectable exclusively in the glial component and included presence of mitoses, increased proliferative activity, necrosis, and/or vascular proliferations. In these cases, the differential diagnosis with high-grade astrocytic tumors may be very difficult. The clinical history of the patient (ie, information about previously resected spinal low-grade GG) and some histologic features, such as presence of EGBs and binucleated synaptophysin-positive ganglion cells within a high-grade tumor, may point toward the presence of AGG. In our series, besides one patient with clinical history of previously resected spinal GG (case 1), the other lesion showed presence of EGBs and presence of binucleated synaptophysin-positive ganglion cells. In particular, binucleation could be very helpful to identify anaplastic GGs being, in our opinion, a very unusual feature to be found in normal neurons entrapped within the tumor.

Notably, 2 AGGs showed positivity for antibody against mutated H3.3K27M protein, indicating the presence of an underlying mutation, *H3F3A*^{K27M}, which is typically found in midline high-grade gliomas affecting children and adults [13]. As already indicated by the evidence of *TP53* mutations, *CDKN2A* deletions in AGG [1], it cannot be excluded that such cases may share to some extent other molecular features with high-grade gliomas, especially in midline lesions. Glioneuronal tumors with malignant features harboring both *H3F3A*^{K27M} and *BRAF*^{V600E} mutation have been recently described [14]. Whether patients with spinal AGG have different clinical outcome in comparison with high-grade gliomas has to be determined.

In case of a spinal tumor with poorly differentiated or clearly malignant neuronal component, the presence of a neuroblastoma or ganglioneuroblastoma must be considered in the differential diagnosis.

Although spinal GGs share several histologic features with their supratentorial counterparts, they apparently display different molecular features. According to our data, *BRAF* mutations seem to be rare in spinal GG. Notably, we found only 2 $BRAF^{V600E}$ mutated cases. In the study of Gupta et al [12] all 4 spinal GGs, classified as "pilocytic astrocytoma with gangliocytic component," showed a *KIAA-BRAF* fusion, but no $BRAF^{V600E}$ mutations. Although the analysis was limited to 12 cases of our series, we also identified 2 tumors harboring an extra copy of *KIAA1549* and *BRAF*, highly indicative for the presence of a *KIAA1549-BRAF* fusion.

BRAF, a member of the serine/threonine kinase protein family, is as an immediate downstream effector of RAS in the RAS/RAF/MEK/ERK signaling cascade, a signal transduction pathway that modulates various intracellular processes, including cell proliferation and survival [15]. The most frequent mutation is the $BRAF^{V600E}$ which leads to a significant increase of BRAF kinase activity. $BRAF^{V600E}$ mutations have been found in pilocytic astrocytomas (15%-20% of cases), WHO grade II to IV diffuse astrocytomas (5% of cases), and pleomorphic xanthoastrocytomas (in 60%-70% of cases) as well as in GGs [16-18]. The incidence of *BRAF* mutations in supratentorial GGs varies from study to study [16,17] ranging from 15% to 60%. In brainstem GG, the incidence of $BRAF^{V600E}$ ranges from 40% to 50% of cases [12,19]. The presence of $BRAF^{V600E}$ mutation seems to be associated with shorter progression-free survival in pediatric patients [19,20] with GG.

Spinal GGs seem to be rare in patients affected by NF-1, and only a few cases have been reported [21,22]. In our series, we reported 1 additional case of an AGG arising in the cervical-thoracic region of a young NF-1 patient. Spinal GGs are also exceedingly rare in patients with neurofibromatosis type 2, and only 1 neurofibromatosis type 2–associated GG has been described to date [23].

In conclusion, although spinal GGs display histologic and clinical features similar to their supratentorial counterparts, they show a relatively low frequency of $BRAF^{V600E}$ mutations, an alteration otherwise common in hemispheric and brain stem GGs.

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