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Original Article

Glioneuronal tumor with neuropil-like islands: a histological, immunohistochemical, and molecular study of three cases

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Abstract: In this study, we report three cases (male, aged 47, 47 and 53 years respectively) of glioneuronal tumors with neuropil-like islands (GTNI). Two of them had a cerebral tumor, and the other had a spinal tumor. For operations, all patients underwent macroscopically total resection. These three patients are in complete remission (at 33, 13 and 12.5 months, respectively) after surgery. Microscopically, the common feature was the presence of synaptophysin positive neuropil-like islands. No tumor had 1p deletion, 19q deletion and EGFR amplification. IDH1 gene mutation and TP53 gene mutation were both found in all cases. 1p/19q intact astrocytomas and oligoastrocytomas showed a high incidence of IDH1 mutation. Two of the presented patients (WHOII) who did not receive chemotherapy and radiotherapy after total resection have not showed any sign of recurrence of tumor, with a survival time of more than 33-months and 13-months. In conclusion, our results suggest that complete resection is a key prognostic factor in GTNI patients.

Keywords: Glioneuronal tumors with neuropil-like islands (GTNI), histological features, treatment, prognosis

Introduction

Glioneuronal tumors with neuropil-like islands (GTNI) are featured by "neuropil-like islands (NIs)" within dominating astroglial component [1]. Although rare, at present, at least 30 cases of GTNI occurring in the CNS have been reported. The pathophysiology and biological behavior of this tumor type remain not fully understood. Most cases in the literature have been located in the cerebrum [2-8]. Spinal cord localization rarely has been reported [9-11]. In this context, we describe the clinical, histological, immunohistochemical, genetic and radiological features of three GTNI cases (2 in the cerebrum and 1 in spinal cord).

Materials and methods

Initial biopsy specimens from three patients were reserved, then sliced and paraffin-embedded to be prepared for following study. The slices were stained with H&E and immunohistochemically with the following antibodies: glial fibrillary acid protein (GFAP, 1:300), synaptophysin (Syn, 1:2), anti-neuronal nuclei (NeuN, 1:100, Chemicon), Ki-67 (clone MIB-1, 1:50), epidermal growth factor receptor (EGFR, 1:100), S-100 (1:200), epithelial membrane antigen (EMA, 1:50), Olig-2 (1:300), anti-R132H-IDH1 antibody (1:200), TP53 (1:100) and anti-neuronal nuclei (NeuN, 1:100, Chemicon). All antibodies (except NeuN) were purchased from DAKO, Denmark. In addition, positive and negative controls were included and evaluated appropriately for each procedure.

Results

Case 1

This previously healthy, 47-year-old man was well until late 2012, when he had numbness and weakness in his right extremities for one

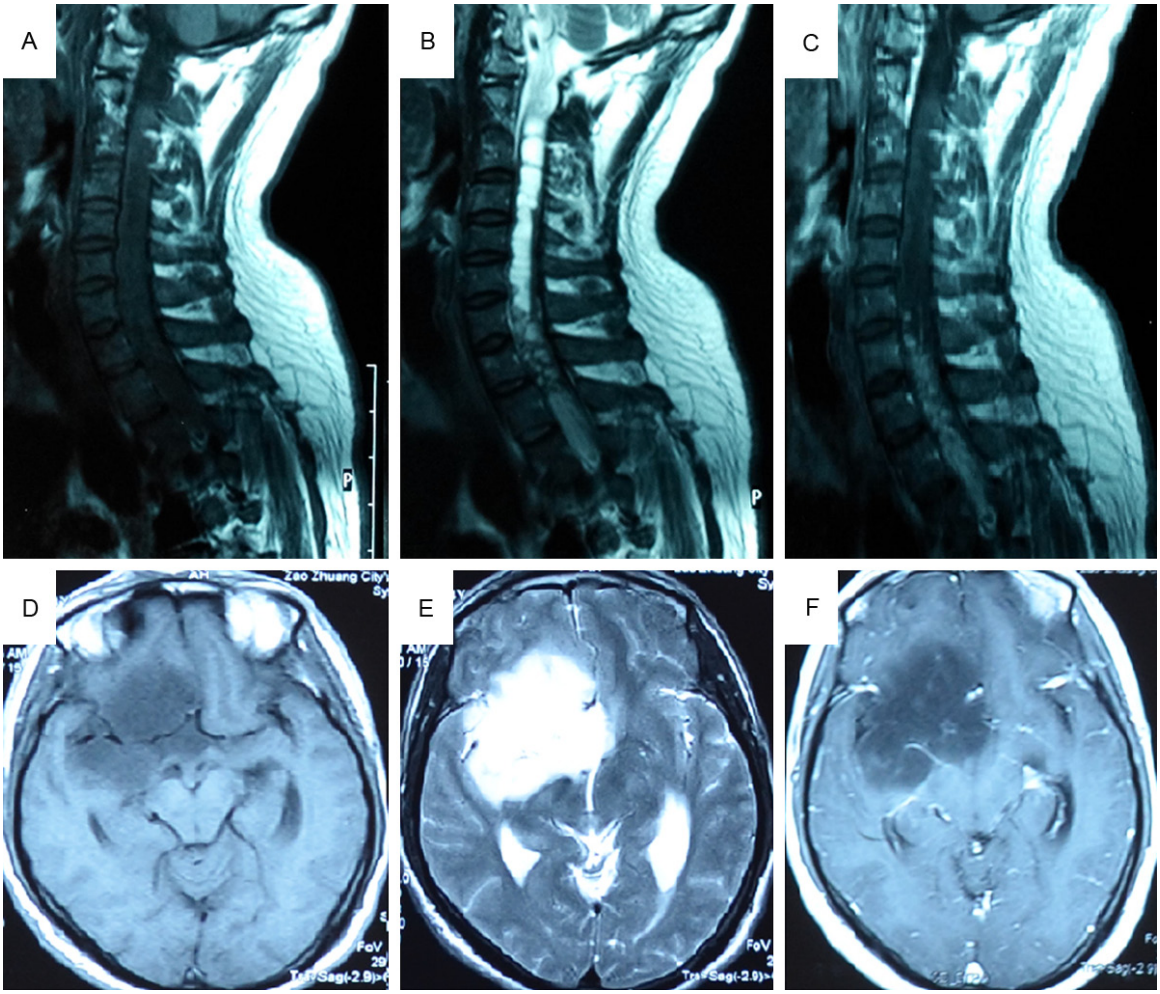

Figure 1. Neuroradiologic characteristics. Shown in panels (A-C) are the MRI features of case 2 as seen at presentation in T1-weighted, T2-weighted and post-gadolinium T1-weighted studies, respectively. These demonstrate a solid lesion that was low intensity on a T1-weighted image (A) and high intensity on a T2-weighted image (B) between T1 and T6, and an enhanced lesion with gadolinium between T1 and T6 level (C). Shown in panels (D-F) are the MRI features of case 3 as seen at presentation in axial T1-weighted, T2-weighted and post-gadolinium T1-weighted studies, respectively. These demonstrate a solid lesion that was hypointense on T1-weighted MRI (D), hyperintense on T2-weighted MRI in right frontotemporal, insular lobe and basal ganglia region (E), and no gadolinium enhancing was found (F).

month. For the ensuing 2 weeks the patient was troubled with periodic seizure. His medical history and neurological examination was unremarkable. Magnetic resonance images (MRI) demonstrated a solid, T1-hypointense and T2-hyperintense, non-enhanced mass that involved the left fronto-parietal lobe with ventricular compression and midline shift. On November 21th, 2012, the patient underwent left frontal craniotomy, which revealed a gray-white lesion in the left frontal lobe cortex. The major part of the lesion was located in the left frontal lobe, with expansion to the precentral gyrus. The tumor was macroscopically totally removed.

The post-operative course was uneventful. Oxcarbazepine was used in combination with other medications to control seizures. No recurrence of the tumor has been observed on radiologic images at 33 months' follow-up.

Case 2

This 47-year-old man presented with a history of discomfort in chest and back for 7 years, a history of chronic pain for 1 year in the lumbar and back region and continuous intermittent numbness of the right leg for 6 months. More seriously, he can barely walk without help. A

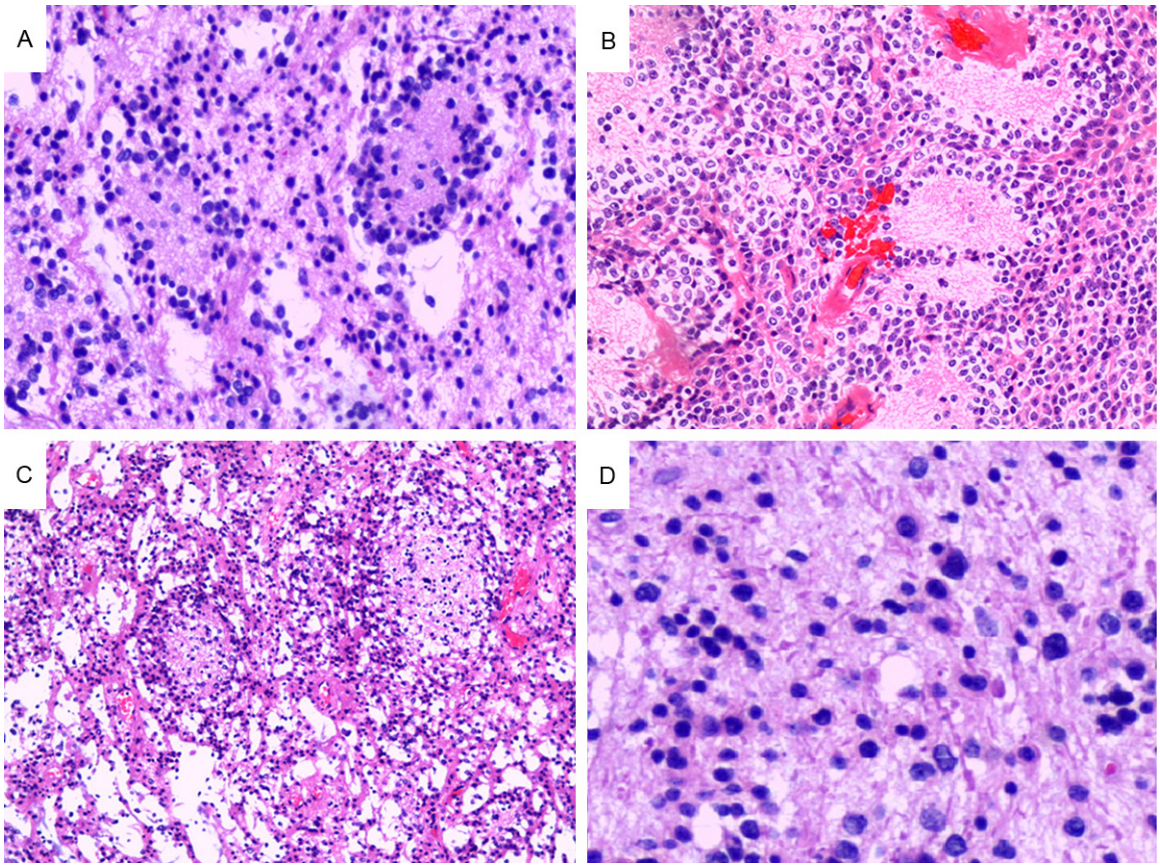

Figure 2. Histologic features. A-C. Demonstrate lower and higher magnification of the glioneuronal tumor with "rosetted" neuropil islands, as seen in case 1 (Hematoxylin and eosin (H&E) ×100), case 2 (H&E, ×200) and case 3 (H&E, ×100). D. Taken from case 1, demonstrates the glial element consisted of diffuse astrocytoma. H&E, ×400.

physical examination demonstrated impaired sensation in the lower extremity. Examinations showed a spinous tenderness and percussion pain, which was located in the thoracic vertebra between T1 and T6. The muscle strength was scored grade I for the right ankle flexion, extension and right digitorum flexion, extension; grade III for right hip flexion, extension and right knee flexion, extension and grade IV for the left hip flexion, extension; left knee flexion, extension; left ankle flexion, extension and left digitorum flexion, extension.

MRI demonstrated a solid lesion that was low intensity on a T1-weighted image and high intensity on a T2-weighted image between T1 and T6 (**Figure 1A**, **1B**) and an enhanced lesion with gadolinium between T1 and T6 level (**Figure 1C**). There was no calcification on computed tomography. There were no abnormal findings in other regions. The lesion was almost total resected using a midline approach, with T1 to T6 laminectomy. The macroscopic appearance of the spinal lesion was soft and gelatinous, with a pale gray color and well-demarcated boundaries. Most of the lesion was intramedullary, soft and fresh with a gray-brown color. The patient's neurologic symptoms were improved slightly after the operation. No evidence of recurrence of tumor has been observed in radiologic images during a 13-month follow-up.

Case 3

This 53-year-old man with no significant past medical history presented with a 2-month history of progressively worsening headache. His neurological examination was unremarkable. MRI revealed an extension of the lesion in the right fronto-temporal, insular and basal ganglia region. The lesion was hypointense on T1-weighted, hyperintense on T2-weighted and no positivity on gadolinium enhanced MRI was

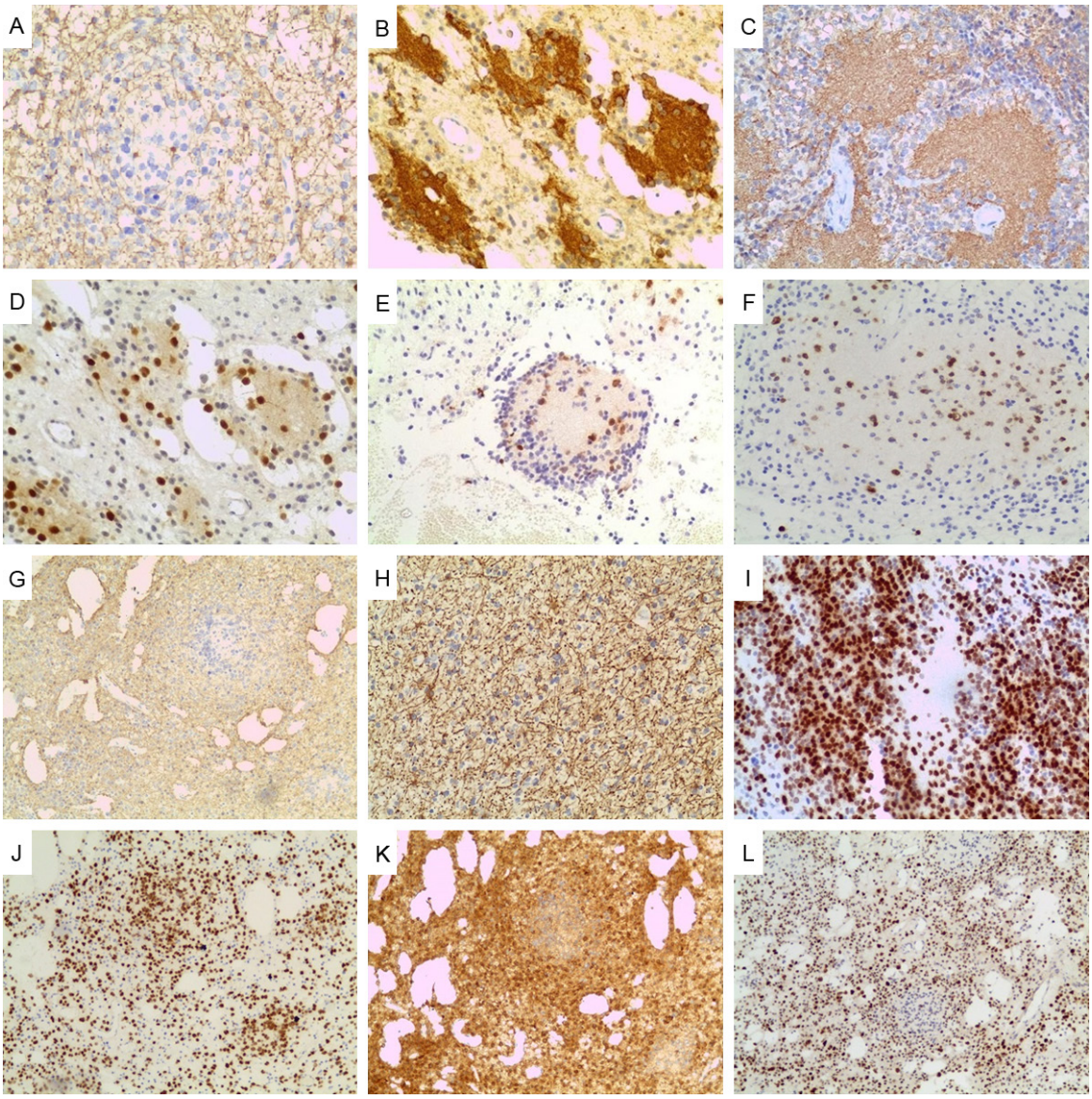

Figure 3. Immunohistochemical features. In panels (A) (case 3), the neuropil-like island was negative for GFAP; And exhibited uniformly strong matrix labeling for synaptophysin in case 1 (B) and case 2 (C) respectively. It was demonstrated oligodendrocyte-like cells, arranged around a neuropil-like island, immunolabeling were positive immunostaining with the neuronal markers NeuN in case 1 (D), case 2 (E) and case 3 (F) respectively. The gliomatous were strong positive immunostaining with GFAP in case 1 (G), case 2 (H). The gliomatous were strong positive immunostaining with Olig-2 in case 2 (I) and case 3 (J). And IDH1 genes mutation analyse was found in case 3 (K). TP53 genes mutation analysis was found in case 3 (L).

found (**Figure 1D-F**). The lesion was macroscopically totally removed using a right frontotemporal craniotomy. The macroscopic appearance of the lesion was soft and gelatinous, with a pale gray color and blurry boundaries. The patient subsequently received involved field radiotherapy with a total dose of 6000 cGy, followed by six cycles of TMZ. No sign of recurrence of tumor has been observed in MRI at 12.5-months' follow-up.

Histological and immunohistochemical findings

All tumors were diffusely infiltrating with surrounding tissue and, as further described below, exhibited both a neurocytic and a gliomatous component. In the neurocytic component, neurocytes formed neurocytic rosettes (**Figure 2A-C**). The nuclei of the tumor cells were small and round, and no atypia or mitosis

was detected. The prominent feature of GTNI is the presence of "rosetted" neuropil islands surrounded by neurocytic cells. The neurocytic cells sometimes linked with neighboring rosettes together (**Figure 2A**). The counterpart of the neurocytic component was the gliomatous component (**Figure 2D**). The cells had much more pleomorphism. No calcifications, necrosis, lymphoid infiltration, endothelial vascular proliferation or rosenthal fibers were observed in the three samples. The proportion of gliomatous component was higher in 3 than in patients 1 and 2. The presence of cytologically atypical and mitotically areas are both found in patient 3.

Immunohistochemical study showed that the neuropil-like islands were generally negative for GFAP (**Figure 3A**) and exhibited uniformly strong matrix labeling for Syn (**Figure 3B**, **3C**, case 1 and 2) in cases. All three cases demonstrated oligodendrocyte-like cells, which were arrayed around a neuropil-like island, with the neuronal markers NeuN positive immunostaining (**Figure 3D-F**). Meanwhile, the gliomatous component was positively immunostained with GFAP (**Figure 3G**, **3H**, case 1 and 3), Olig-2 (**Figure 3I**, **3J**, case 2 and case 3), EGFR and S-100 protein. And IDH1 gene mutations were found in case 1, 2 and 3 (**Figure 3K**, case 3) and TP53 gene mutations were found in case 1, 2 and 3 (**Figure 3I**, case 3). Demonstrable proliferative activity was virtually restricted to the glial component of each tumor. The glial components in case 3 exhibited very high mitotic activities and the corresponding MIB-1 labeling index was 15-20%. Mitotic activities were not seen in the sample from patients 1 and 2.

According to MIB-1 index and the morphological changes, the final diagnosis of case 1 and 2 was glioneuronal tumor with rosetted neuropil-like islands-WHO Grade II. And final diagnosis of case 3 was glioneuronal tumor with rosetted neuropil-like islands-WHO Grade III.

Genetics findings (molecular findings)

MGMT promoter status was assessed by methylation-sensitive high resolution melting (MS-HRM). Genomic DNA was extracted from formalin-fixed and paraffin-embedded tissues using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. PCR amplification and HRM were performed on LC480 (Roche Applied Science) as adapted from the published protocol by Wojdacz and Dobrovic [12].

Dual-color fluorescence in situ hybridization (FISH) was performed using LSI 1p36/LSI 1q25 and LSI 19q13/19p13 dual-color probe (Vysis/Abbott Molecular) for losses of 1p36/19q13. The EGFR gene copy number was determined by FISH using the LSI EGFR/CEP 7 (Vysis/AbbottMolecular). Fluorescent signals were visualized and quantitated under fluorescence microscope. A minimum of 100 non-overlapping intact nuclei were assessed by hybridization. At least 30% or more increase in nuclei number is necessary for a signal to be scored as a deletion. Amplification of EGFR was asserted when ratio of EGFR signal to CEP7 signal was greater than or equal to two. Percentage of the cells showing deletion and amplification was estimated separately and independently for two component parts of this tumor.

The methylation level of the MGMT promoter was 8%-20% in case 3. No 1p/19q loss of heterozygosity (LOH) or EGFR gene amplification was found in all cases.

The methylation level of the MGMT promoter was 8%-20% in case 3. And the methylation level of the MGMT promoter in case 1 and case 2 were 0-2% and 2-5%, respectively. No 1p/19q loss of heterozygosity (LOH) or EGFR gene amplification was found in all three cases. The IDH1 gene mutations and TP53 gene mutations were all found in case 1, 2 and 3.

Discussion

GTNI is a rare neoplasm harboring circumscribed loci of neuronal differentiation and diffusely infiltrating astroglial and oligodendrocyte-like components. It was added, as a novel lesion, to the World Health Organization classification of tumors of the central nervous system in 2007 [1]. The neuronal component demonstrates immunoreactivity with neurocytic markers, such as Syn and NeuN, whereas the glial component displays strong immunoreactivity for GFAP, Olig-2 and S-100 protein. The prominent feature of GTNI is the presence of "rosetted" neuropil islands surrounded by neurocytic cells [13]. Thus, the diagnostic grade of

the GTNI is mainly determined by the glial compartment. For such a tumor, the mitotic activity and MIB-1 labeling were always important. Necrosis, lymphoid infiltration or endothelial vascular proliferation was not observed in any of the tumor in patients 1, 2 and 3. Mitotic activities were not seen in the sample from patients 1 and 2. The glial components in case 3 exhibited high mitotic activities and the corresponding MIB-1 labeling index was 15%-20%.

Upon review of MRI published in previous reports, we found that the cerebral examples of GTNI were absent or weakly present of contrast enhancement [14]; whereas, all spinal examples had enhanced avidly [9-11]. The tumor of patient 2, which was located in spine (T1-T6), was found to have very interesting neuroimaging characteristics. On T1 weighted post-gadolinium sequences, a diffuse enhancement was noted in the spinal cord. While the tumors of patient 1 and 3, located in cerebrum, were found with no gadolinium enhancement. All the MRI features were consistent with those in previous reports [9, 10, 11, 14, 15].

Perry and co-workers previously reported four cases of oligo-dendrogliomas with neurocytic differentiation, arguing for a possible correlation between such kind of tumor and glio-neuronal tumors with neuropil-like islands [16]. Although it was reported that GTNI shared some morphologic features (1p/19q loss) with recently reported cases of oligo-dendroglioma with neurocytic differentiations, these two kinds of tumors appeared differently at the molecular genetic level [2]. It was reported that 1p/19q LOH was found in 1 out of 8 GTNI tumors [2]. The positivity rate of 1p/19q LOH was not high at all. In our cases, no evidence of 1p/19q LOH and EGFR gene amplification was found in all cases. IDH1 gene mutations and TP53 gene mutations have been found in some GTNI cases [8, 17]. It was thought that combined with the frequent p53 immunoreactivity and general absence of 1p/19q chromosomal co-deletions previously documented, 1p/19q intact astrocytomas and oligoastrocytomas always showed a very high incidence of IDH1 mutations [18]. Twelve cases of GTNI were assembled and IDH mutation were found in all cases (100% incidence) [18]. In our 3 cases, strong nuclear immunolabeling of p53 and IDH1 gene mutations were found and absence of 1p/19q deletion by FISH assay were also confirmed, which is consistent with those in previous reports.

Although morphological, immunehistochemical, and molecular features have been intensively investigated in recent years, clinical features, current treatment approaches, and prognoses of GTNI are still elusive. Most of the patients reported in the literature have been treated by partial resection of the tumor due to its diffusely permeating growth pattern. Therefore, they are likely to recur locally and exhibit an unfavorable clinical prognosis, after few years of the diagnosis [5, 8]. Radiotherapy and chemotherapy as adjuvant treatments after resection are important for these patients with incomplete resection. Complete surgical resection followed by adjuvant (chemo- and radiation) therapy is a choice for treating GTNI [4, 19]. Fraum TJ and co-workers advocate the use of a safe adjuvant chemotherapeutic regimen with TMZ, used concomitantly and subsequently to radiotherapy, especially for GTNI cases [20]. We advocated all patients adopting chemotherapy with TMZ and radiation therapy after the surgery, in particular for case 3 with high-grade histological feature (WHOШ). However, neither radiation therapy nor chemo-therapy was performed for case 1 and 2, because of financial reasons for further therapy. Only case 3 received chemotherapy and radiotherapy (minimal dose for 4 weeks), and hasn't showed any symptom of recurrence 12.5-months later. Strikingly, case 1 and 2 without any further therapy, haven't showed any sign of recurrence of tumor either, with a survival time of more than 33-month and 13-month. This observation indicating that even though adjuvant (chemo- and radiation) therapy was a recommended method of choice for treating GTNI, the complete surgical resection was a more important key factor.

In conclusion, the combination of chemotherapy (with TMZ) and radiotherapy (with proven efficacy) against high-grade GTNI (case 3) should be considered as an adjuvant treatment. As one could see from our cases of patient 1 and 2, gross total resection and even without adjuvant chemo-/radio-therapy has resulted in a 33-months' and 13-months' relapse-free survival. So, our findings further

confirm that complete resection is a key prognostic factor in GTNI patients. To better understand and further investigate the real biology of this tumor in three cases, a longer follow-up would be needed.

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Disclosure of conflict of interest

None.

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