Case Report
Gene alteration of rosette-forming glioneuronal tumor in a suprasellar lesion

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Abstract: Background: Rosette-forming glioneuronal tumor (RGNT) in suprasellar lesions is exceedingly rare, and its clinicopathological features are distinctive. Case report: A 55-year-old woman was admitted with headache and visual field disturbance. Magnetic resonance imaging (MRI) revealed a mass lesion in a suprasellar lesion accompanied by obstructive hydrocephalus. Following surgery, pathological examinations demonstrated a rosette-forming glioneuronal tumor. The patient has been free from recurrence for 27 months after surgery without adjuvant therapy. Pathological findings: The specimen exhibited nuclear and cytoplasmic pleomorphism. The nuclei varied in size, shape, and coarseness. Variability was also observed in the eosinophilic granular bodies, Rosenthal fibers and spindle-shaped tumor cells. GFAP, S-100 and vimentin were found to be immunohistochemically positive. Genetic alterations: We detected IDH1 R132H mutation without IDH2 mutation in this case. We failed to find alterations of the MAPK pathway including BRAF, FGFR1 and KRAS alterations. Discussion: The histologic features and clinical history of RGNT resemble those of pilocytic astrocytoma (PA). However, the genetic alterations of the present RGNT were quite different from those of PA.

Keywords: Rosette-forming glioneuronal tumor, suprasellar lesion, MAPK pathway, BRAF, FGFR1

Introduction

Rosette-forming glioneuronal tumor (RGNT) of the fourth ventricle was designated as a new tumor entity in the 2007 issue of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System. This glioneuronal tumor was found to be rare, with a slowly growing neoplasm, and corresponded to WHO grade I. RGNT arose in the midline, occupying fourth ventricular lesions, with possible extension into the surrounding structures including the cerebellar vermis and cerebellopontine angle [1].

Histologically, these tumors exhibited two major components. The first consisted of uniform neurocytes involved in the formation of neurocytic rosettes as well as perivascular pseudorosettes. The second component consisted of spindle to stellate astrocytes with elongate to oval nuclei and moderately dense chromatin in a dense background of fibrillary processes. Occasional Rosenthal fibers and eosinophilic granular bodies were identified [1, 2]. These pathological findings of the glial components resembled those of pilocytic astrocytoma (PA).

Alterations of the mitogen-activated protein kinase (MAPK) pathway have been found to be frequent in PA. The major gene alterations observed in the MAPK pathway were gene fusions between KIAA1549 and BRAF and point mutation involving the oncogene BRAF (B-Raf Proto-Oncogene) [3-6]. The frequencies of KIAA1549-BRAF fusion gene and BRAF mutation, which showed V600E, were 51-75% [7-10] and 7-9% [9, 11-13]. Such gene alterations in diffuse astrocytomas were detected at rates of 17.6% (3/17) and 6% (1/17) [9]. Further, these gene alterations were good clinical factor in pediatric lower grade gliomas, including PA [6]. Recent studies have described upstream alterations of the MAPK pathway, Fibroblast...
growth factor receptor 1 (FGFR1) and Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) mutation in PA [14, 15].

In contrast, there were no genetic alterations of BRAF, including KIAA1549-BRAF fusion gene and BRAF mutation, with only a small number of RGNT studies [16-18]. Recently, Gessi M. et al. reported the detection of FGFR1 mutations in 2 cases out of 6 RGNTs [19].

We present here an unusual case of RGNT arising from a suprasellar lesion with 3rd ventricle obstruction, resulting in hydrocephalus. This is the first report to evaluate the major gene alteration of the MAPK pathway, including KIAA1549-BRAF fusion gene, BRAF, FGFR1 and KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog) mutation, and major gene alteration of lower grade glioma including IDH1 (isocitrate dehydrogenase 1), IDH2 (isocitrate dehydrogenase 2) mutation and ATRX (alpha thalassemia/mental retardation syndrome X-linked) expression status in the same patient.

**Case report**

A 55-year-old woman had been in excellent health until she presented at another clinic with progressive headache followed by visual field disturbance. A computed tomography (CT) scan revealed an intracranial mass lesion, and she was then admitted to our hospital after day 22 from the appearance of symptoms.

The results of her physical examination were essentially normal apart from bitemporal hemianopsia based on the Goldmann visual field test.

A CT scan demonstrated a mass lesion in a suprasellar lesion with 3rd ventricle obstruction, resulting in hydrocephalus.

Magnetic resonance imaging (MRI) was performed, and the T1-weighted image revealed a 3 × 3 × 4 cm low intensity lesion in a suprasellar lesion and 3rd ventricle wall, with homogeneous contrast enhancement (Figure 1).

Her serum levels of human chorionic gonadotropin, α-fetoprotein, carcinoembryonic antigen, and placental alkaline phosphatase were negative, and she exhibited normal pituitary hormone levels.

On day 25 after symptom appearance, the suprasellar lesion tumor was removed, employing the interhemispheric trans lamina terminalis approach.

Macroscopically, the tumor was reddish, soft, and vascular-rich. After the surgery, no other neurological deficit was evident, and the patient did not require care in her daily life. Pathological examinations demonstrated the tumor to be a rosette-forming glioneuronal tumor.

Adjuvant therapy including chemotherapy and radiotherapy was not administered. There has been no sign of recurrence for 27 months after the operation.
Rosette-forming glioneuronal tumor

Pathological findings

The specimen displayed nuclear and cytoplasmic pleomorphism. Morphologically, the nuclei varied in size, shape, and coarseness or dispersion of chromatin, but no mitosis, necrosis or endothelial proliferation was observed. The tumor exhibited two components. The first consisted of neurocytes that had perivascular pseudorosettes. The other contained glial elements including eosinophilic granular bodies, Rosenthal fibers and spindle-shaped tumor cells (Figure 2). A lobulated pattern with intersecting connective tissue, as found in the normal pineal gland structure, was observed in the same section. Glial fibrillary acid protein (GFAP) and S-100 protein were immunohistochemically positive. Vimentin was also positive. Synaptophysin was immunohistochemically positive in the focal area. The MIB-1 labeling index (LI) was 2.9%. Epithelial membrane antigen (EMA) was immunohistochemically negative. There was no expression of the macrophage marker, CD34. Immunoreactivity of ATRX was positive.

Genetic alterations

Genomic DNA of this case was isolated from formalin-fixed paraffin-embedded tumor tissues, as described previously [20].

Screening for IDH1 and IDH2 mutations was carried out by Sanger sequencing as described previously [21-23].

Screening for BRAF FGFR1 and KRAS mutations were also carried out by Sanger sequencing. FGFR1 mutation revealed two hot spots
Figure 4. Sanger sequencing for IDH2 R132G, BRAF V600E, FGFR1 N546K, FGFR1 K656E, KRAS R73M, KRAS E63K, KRAS Q22K and KRAS L19F. All were wild-type.

(c1638C>A, pN546K and c1966A>G, pK656E). KRAS mutation had 4 hot spots (c57G>C, pL19F and c64C>A, pQ22K at exon 2; and c187G>A, pE63K and c218G>T, pR73M at exon 3). The primer sequences were 5'-AGC CTC AAT TCT TAC CAT CCA-3' (forward) and 5'-GAA GAC CTC ACA GTA AAA ATA GGT G-3' (reverse) for BRAF (PCR product, 120 bp); 5'-AGA GAG GCC TTG GGA CTG AT-3' (forward) and 5'-GAT GAA GAT GAT CGG GAA GC-3' (reverse) for FGFR1 pN546K (PCR product, 140 bp); 5'-CTC AGA TGA AAC CAC CAG CA-3' (forward) and 5'-CCT GGT GAC AGA GGA CAA TG-3' (reverse) for FGFR1 pK656E (PCR product, 135 bp); 5'-TGT GGT AGT TGG AGC TGG TG-3' (forward) and 5'-GTT CCT GCA GTA ATA TGC -3' (reverse) for KRAS exon 2 (PCR product, 134 bp); and 5'-TGT GTT TCT CCC TTC TCA GGA -3' (forward) and 5'-AAA GAA AGC CCT CCC CAG T -3' (reverse) for KRAS exon 3 (PCR product, 146 bp). The PCR was carried out using a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA) in a total volume of 10 μl, consisting of PCR buffer (20 mM Tris-HCl, pH 8.0; 40 mM NaCl; 2 mM sodium phosphate; 0.1 mM EDTA; 1 mM DTT; stabilizers; 50% glycerol), 3 mM MgCl₂, dNTPs (250 μM each), sense and antisense primers (0.3 μM each for BRAF, FGFR1 pN546K and FGFR1 pK656E), 0.5 units of iPlatinum® Taq DNA polymerase (Invitrogen) and DNA (approx. 40 ng). Initial denaturing at 95°C for 5 min was followed by 40 cycles of denaturing at 95°C for 45 sec, annealing at 58°C for 45 sec, and extension at 72°C for 45 sec. A final extension step at 72°C for 5 min was added. Sequencing reactions were performed on an ABI 3100 PRISM DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a Big Dye Terminator cycle sequencing kit (ABI PRISM, Applied Biosystems), as previously reported [23]. The quality of all PCR products had been checked by electrophoresis with 2% agarose gel before undertaking the sequencing.

The BRAF fusion reverse transcriptase polymerase chain reaction (RT-PCR) result was confirmed by fluorescence in situ hybridization (FISH) analysis in this case.

We detected IDH1 R132H mutation in the present RGNT case (Figure 3). IDH2, FGFR1 and
KRAS were wild-type in this case (Figure 4). We were unable to detect KIAA1549-BRAF fusion using FISH analysis (Figure 5).

Discussion

As described above, rosette-forming glioneuronal tumor (RGNT) of the fourth ventricle was designated as a new tumor entity in the 2007 issue of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System. The median age at diagnosis was 27 years (range, 6-79 years) [24]. The histological features of RGNT of the fourth ventricle were described by Komori, who noted that the tumors exhibited two major components [2]. In our case, the histological findings also demonstrated two components. One was a glial component, including Rosenthal fibers and eosinophilic granular bodies, and typically exhibited features of PA. The other component consisted of neurocytes, including perivascular pseudorosettes. On histopathological examination, the present case displayed typical features of RGNT.

RGNT lesions were originally considered to be exclusive to the fourth ventricle. Schlamann A. et al. summarized 85 cases of RGNT, of which 80% (68 cases) typically arose from posterior fossa lesions [24]. There were some unusual cases that originated from outside the typical location, such as in the pineal region, optic chiasm, spinal cord and septum pellucidum [25-29]. Two cases were reported with intraventricular dissemination via cerebrospinal fluid pathways [30, 31]. The present report describes the first case arising from a suprasellar lesion with 3rd ventricle obstruction, so that physical examination revealed bitemporal hemianopsia.

On typical neuroradiological feature of RGNT appearance on MRI has been described as iso-low intensity on T1-weighted images and high intensity on T2-weighted images, usually enhancement being observed [24, 32]. Thus, RGNTs have displayed various degrees of contrast enhancement, including patchy enhancement [33], solid enhancement [28-30, 34], and ring enhancement [27]. The tumor was found to be a solid (37%) or mixed solid/cystic lesion (41%) [24]. In addition, calcification was noted in 21.2% of RGNT [35]. In the present case, MRI showed low intensity on T1 weighted images with solid gadolinium enhancement and high intensity on T2 weighted images. The neuroradiological findings also demonstrated typical features of RGNT.

RGNT is considered to have a favorable outcome (WHO grade I). The progression free survival (PFS) rate at 2 years after diagnosis was 100% [24]. On the other hand, some cases were reported to display disease progression including local recurrence and dissemination [30, 36]. According to Schlamann A. et al., clinical data revealed 4 cases (7.7%) out of 52 with disease progression, and 3 cases (5.8%) who died [24]. The standard treatment for RGNT is surgical resection without adjuvant therapy. Zhang J. et al. reported that 3 cases (7.3%) underwent radiotherapy and no chemotherapy was received [37]. Although most published cases show stable outcomes without gross total resection, we must perform careful follow-up and consider radiotherapy when the tumor exhibits progression.

IDH mutations are frequent and very early genetic alterations in the pathogenesis of lower grade gliomas, including diffuse astrocytic and oligodendroglial tumors [22, 38]. The frequencies of IDH1 mutation in WHO grade II diffuse astrocytomas vary among different studies (47-90%) [22, 38, 39]. On the other hand, Yan H. et al. found that the frequency of IDH1 mutations in PA is 0% (0/21) [22]. IDH1 mutation is a significant prognostic marker of favorable outcome in patients with glioblastomas and anaplastic astrocytomas [23, 40], but the prognostic value of IDH1 mutation in lower grade gliomas appears to be less clear. We demonstrate
here from an RGNT case carrying IDH mutations that we can detect IDH1 mutation (R132H) without IDH2 mutations in RGNT. IDH mutation status is evidently different between RGNT and PA.

Loss of ATRX expression is frequent in IDH mutant astrocytoma and mutually exclusive with 1p/19q co-deletion glioma (i.e., molecular evidence of oligodendroglioma). Loss of ATRX expression has been reported in one third of pediatric glioblastomas and 7% of adult glioblastomas [41, 42], associated with a better clinical outcome in a retrospective cohort of grade II, III and IV gliomas [43]. Yamada et al. found that pleomorphic xanthoastrocytoma (PXA) corresponding to WHO grade II, also showed loss of ATRX expression [44]. In contrast, ATRX expression is retained in all PA cases [45]. This result is consistent with the IDH mutation status in PA. In our RGNT case also, ATRX expression is retained with IDH1 mutation.

The important alterations of the MAPK pathway include KIAA1549-BRAF fusion and BRAF mutation. The frequency of KIAA1549-BRAF fusion is 66-75% in PAs [10, 46], but nil in high grade gliomas [10]. Such fusion does not appear to be specific to WHO grade I astrocytomas, whereas there are reports in pilomyxoid and diffuse astrocytoma cases [5]. Recent studies have identified BRAF V600E mutation in lower grade gliomas [13, 44, 47]. Schindler et al. indicated that the highest frequencies of this mutation were in PXA cases (66%) and PXA with anaplasia cases (65%). The frequency of BRAF V600E mutation was reported to be 18% in gangliogliomas, and 9% in PAs [13]. In our case, BRAF is wild-type, and both KIAA1549-BRAF fusion and BRAF mutation are absent.

The fibroblast growth factor receptor (FGFR) tyrosine kinase family consists of four members (FGFR1-4). FGFR signalling has evolved to become a highly complex growth factor signalling pathway, reflecting the multitude of physiological functions that are controlled by FGFR signalling [48]. Dysfunctions of this pathway including gene amplification and missense mutation have been described in several cancers [14, 15, 49-52]. FGFR1 mutations at 2 hotspots (c1638C>A, pN546K and c1966A>G, pK656E) have been reported in PAs [14, 15]. FGFR1 (located 8p11.23-11.22) represents an important gene mediated activation of the RAS, MAPK1/ERK2, MAPK3/ERK1 and MAPK pathway. Such missense mutations can lead to function and regulation that are considered to be damaged using a predictive deleterious score in SIFT or PolyPhen 2 tools (http://browser.1000genomes.org/index.html). A recent study has indicated that FGFR1 mutations were detected in 2 cases out of 6 RGNTs, of which one case had pN546K and the other had pK656E [19]. Jones et al. have reported the MAPK pathway alterations in PAs: BRAF alterations (gene fusion and missense mutation) were noted in 82/96, FGFR1 mutation in 4/96, and KRAS mutation in 2/96 cases. They found that the MAPK pathway alterations affected 100% of PAs [14]. The histological features and clinical history of RGNT resemble those of PA.

However, in our case, BRAF fusion and mutation and FGFR1 and KRAS mutations were absent in the RGNT. These findings imply that genetic alterations of the MAPK pathway may be quite different between PA and glioneuronal tumors including RGNT. IDH1 status also differs between PA and RGNT. Thus, although PA and RGNT are similar in their clinical features, their genetic features are different, suggesting that the modes of tumorigenesis may differ between PA and RGNT.

Disclosure of conflict of interest
None.

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