FGFR1 Mutations in Rosette-Forming Glioneuronal Tumors of the Fourth Ventricle

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Abstract
Rosette-forming glioneuronal tumors (RGNTs) are rare glioneuronal tumors of the fourth ventricle region that preferentially affect young adults. Despite their histologic similarity with pilocytic astrocytomas (PAs), RGNTs do not harbor KIAA1549-BRAF fusions or BRAF mutations, which represent the most common genetic alteration in PAs. Recently, mutations affecting the hotspot codons Asn546 and Lys656 of fibroblast growth factor receptor 1 (FGFR1) have been described in PAs. They are considered to be the most frequent mechanism of mitogen-activated protein kinase activation, alternative to KIAA1549-BRAF fusion and BRAF mutations. To uncover possible molecular similarities between RGNTs and PAs, we performed a mutational study of FGFR1 in 8 RGNTs. An FGFR1 N546K mutation and an FGFR1 K656E mutation were found in the tumors of 2 patients. Notably, the patient with an FGFR1 K656E mutated RGNT had undergone a resection of a diencephalic pilocytic astrocytoma with pilomyxoid features 5 years before the discovery of the fourth ventricle tumor; the mutational analysis uncovered the presence of the same FGFR1 K656E mutation in the diencephalic tumor. These results indicate that, in addition to histologic similarities, at least a subgroup of RGNTs may show close molecular relationships with PAs. Whether FGFR1 mutated RGNTs represent a specific subset of this rare tumor entity remains to be determined.

Key Words: Brain tumors, FGFR1, MAPK, Pilocytic astrocytoma, RGNT.

INTRODUCTION
Rosette-forming glioneuronal tumors (RGNTs) are rare glioneuronal tumors of the fourth ventricle region that preferentially affect young adults. They are characterized by 2 distinct histologic components: neurocytic cells arranged in rosettes and pilocytic astrocytoma (PA)-like features that include piloid cytology and, in some cases, Rosenthal fibers and eosinophilic granular bodies (1). The histogenesis of RGNTs is largely unknown (1, 2), and data on the molecular features of this rare tumor have been provided only in recent years.

Despite its histologic similarities with PAs, RGNTs do not harbor KIAA1549-BRAF or BRAF mutations, which represent the most common genetic alteration in PAs (3, 4). The hypothesis that RGNTs could represent a distinct molecular entity has also been supported by evidence of PIK3CA mutations (5, 6). Recently, fibroblast growth factor receptor 1 (FGFR1) mutations in hotspot codons Asn546 and Lys656 have been described in PAs and are considered to be the most frequent mechanism of mitogen-activated protein kinase (MAPK) pathway activation alternative to KIAA1549-BRAF fusion and BRAF mutations (7). To uncover possible molecular similarities between RGNTs and PAs, we performed a mutational analysis of FGFR1 in a series of 8 RGNTs.

MATERIALS AND METHODS
Tissue and Immunohistochemistry
Formalin-fixed and paraffin-embedded RGNT tissue specimens (n = 8) from 6 patients were included in the study. There were 3 male and 3 female patients; the age ranges was from 9 to 54 years (mean age, 26.2 years). The histologic material was retrieved from the archives at the Institute of Neuropathology, University of Bonn and the German Brain Tumor Reference Center (Bonn, Germany). The diagnosis of RGNT was confirmed by 2 neuropathologists (Torsten Pietsch and Marco Gessi). All cases were located in the fourth ventricle region. All cases lacked BRAF mutations or KIAA1549-BRAF fusions (3). Confirmatory immunohistochemical analyses were performed on a semiautomated immunohistochemical stainer (Tecan, Crailsheim, Germany) or a Ventana Benchmark XT automated immunostainer (Roche-Ventana, Darmstadt, Germany) with antibodies against microtubule-associated protein 2 (Sigma, St. Louis, MO), S-100 protein (Dako, Glostrup, Denmark), epithelial membrane antigen (EMA; Dako), glial fibrillary acidic protein (Dako), neurofilament protein (Dako), synaptophysin (Dako), and NeuN (Chemicon, Temecula, CA). Proliferation indices were evaluated with an antibody to the Ki67 antigen (MIB1, Dako). For immunohistochemistry with an antibody against phosphorylated-ERK protein (p-ERK), the slides, after microwave treatment (30 minutes in 0.01 mol/L sodium citrate, pH 6.0), were incubated in 3% H2O2 for 5 minutes to block endogenous peroxidase activity and washed with distilled water, followed by Tris-buffered saline–Tween 20. The slides were incubated in a serum-free blocking solution (CSA II kit; Dako) for 30 minutes at room temperature. After removing the blocking solution, the samples were incubated overnight at...
Figure 1. Histopathologic features and pyrosequencing analysis of an RGNT case harboring an FGFR1 N546K mutation. (A, B) The tumor showed classical histologic RGNT features with evidence of rosettes (A) that were synaptophysin positive (B). (C) Pyrosequencing analysis revealed an AAC->AAG substitution corresponding to an N546K mutation of the FGFR1 gene. (D) The relapsed tumor had a PIK3CA H1047R mutation (CAT->CGT; His->Arg) in addition to the FGFR1 N546K mutation.
FIGURE 2. Histopathologic features of the RGNT case harboring a K656E FGFR1 mutation. (A) The patient was operated on for a tumor in the diencephalon at the age of 7 years. (B, C) The histologic review of this tumor revealed a PA with pilomyxoid changes (B) but without RGNT-like areas or evidence of a neuronal component (C, synaptophysin immunostaining). (D-F) The tumor of the fourth ventricle (D) was diagnosed at the age of 12 years and showed the typical features of an RGNT (E) with evidence of synaptophysin-positive cores within the rosettes (F). (G, H) The diencephalic (G) and the fourth ventricle tumor (H) showed the same alteration in the pyrosequencing profile. Direct Sanger sequencing confirmed the presence of an AAG→GAG substitution corresponding to a K656E FGFR1 mutation.
4°C with a rabbit polyclonal p-ERK antibody (Cell Signaling, Frankfurt am Main, Germany) (dilution 1:200). After Tris-buffered saline–Tween 20 rinses, the bound antibody was detected using the CSA II biotin–free tyramide signal amplification system (Dako) and visualized with 3,3′diaminobenzidine. The slides were lightly counterstained with hematoxylin.

DNA Extraction and Pyrosequencing

DNA from formalin-fixed, paraffin-embedded tumor tissue was extracted using the QIAamp DNA Mini Tissue Kit (Qiagen, Düsseldorf, Germany) according to the manufacturer’s instructions. FGFR1 hotspots (c.546 and c.656) were screened for the presence of mutations using a pyrosequencing assay. Briefly, 264-bp and 226-bp fragments of FGFR1 covering hotspot codons 546 and 656, respectively, were amplified using the following primers: FGFR1 (c.546) forward 5′- CGGACGACAGAAGACTT-3′, FGFR1 (c.546) reverse 5′-biotin-CCCAGATCCCGAGATAACACA-3′, FGFR1 (c.656) forward 5′-biotin-CTCGACGGGACATTCC-3′ and FGFR1 (c.656) reverse 5′-GGTGGCACTCCACTCACA-3′. For FGFR1 c.546 and c. 656 the pyrosequencing primers, Py-5 primes of the mutation. Polymerase chain reaction (PCR) products were treated with ExoSAP-IT (USB, Staufen, Germany) followed by direct sequencing using the Big Dye Prism DNA cycle sequencing kit (Applied Biosystems). The amplified products were purified for the presence of mutations using a pyrosequencing assay. The PCR products were purified using a PCR purification kit (Qiagen). Direct sequencing reactions were performed in duplicate (forward and reverse) as custom service by Eurofins MWG Operon (Ebersberg, Germany) using 30 ng of the PCR product.

RESULTS

The tumors were investigated by pyrosequencing for mutations of the 2 hotspots of the FGFR1 gene. The analysis revealed the presence of 2 different FGFR1 mutations in 2 tumors: an FGFR1 N546K mutation (AAC->AAG, Asn->Lys) was found in an RGNT affecting a 27-year-old woman (Fig. 1), and a K656E (AAG->GAG, Lys->Glu) was identified in a tumor arising in a 12-year-old boy (Fig. 2). The same mutations were also observed in the relapsing tumors of both cases. The other tumors were wild type at both codons 546 and 656 of FGFR1. Immunohistochemical analysis with an anti-p-ERK antibody revealed strong cytoplasmic and nuclear staining in mutated tumors, indicating MAPK pathway activation (not shown). Notably, the PIK3CA mutation analysis revealed the presence of a PIK3CA H1047R mutation (CAT->CGT, His->Arg) in the relapsed tumor of the FGFR1 N546K–mutated RGNT (Fig. 1D). The other RGNT cases included in this study were PIK3CA wild type. The results are summarized in the Table.

DISCUSSION

The histologic features, localization, and the indolent clinical behavior of RGNTs have raised the possibility that they do not represent a specific tumor entity but represent a variant of PA. This hypothesis has not so far received further support from the molecular data. The absence of KIAA1549-BRAF fusions and BRAF mutations in RGNTs has led us to consider RGNT as an unlikely member of the PA tumor group. The evidence of PIK3CA mutations, an event observed in diffuse gliomas, in 4 of 5 cases of RGNTs investigated (5, 6) further strengthened this interpretation. In this context, the evidence of FGFR1 mutations in our series seems unexpected but it may represent an important step in deciphering the biology of RGNTs and, more importantly, their possible relationship with PAs.

The FGFR family members, including FGFR1 to FGFR4, are tyrosine kinases that share a common structure consisting of extracellular immunoglobulin-like domains (9). In cancers, for example, breast and lung carcinoma, FGFR1 (8p21) is more

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<th>Case</th>
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Note: F, female; M, male; PA, pilocytic astrocytoma with pilomyxoid features; RGNT, rosette-forming glioneuronal tumor; RR, recurrent tumor; wt, wild type.
commonly activated through amplification (9). Although occasional FGFR1 mutations have been observed in adult glioblastoma (10), FGFR1 mutations in 2 hotspots (codons for Asn546 and Lys656) have recently been described in PA and considered a mechanism of MAPK activation alternate to KIAA1549-BRAF fusion and BRAF mutations (7). Both K656E (AAG->GAG, Lys->Glu) and N546K mutation (AAC->AAG, Asn->Lys) reported here have been described previously (7). The Asn546Lys mutation seems to alter FGFR1 autophosphorylation, resulting in increased kinase activity and transforming potential, whereas the p.Lys656Glu variant is also transforming in vitro (7). FGFR1 was found to be highly expressed in the ventricular zone of the developing human brain, enabling proliferation of multipotent stem cells (11). FGFR1 mutations have been mostly found in PAs located in the midline (thalamus and brainstem), which presumably originate from the periventricular brain tissue (7). Conversely, FGFR1 mutations appear to be very rare in PAs of the fourth ventricle region (7). Rosette-forming glioneuronal tumors are considered to be derived from precursor cells of the subependymal plate or the cerebellar parenchyma with the capacity for both neuronal and glial differentiation (12, 13).

Although evidence of FGFR1 mutations in RGNTs may suggest close molecular similarities with PAs, the possibility that the FGFR1–mutated RGNTs represent a defined subgroup of tumors remains to be determined. Furthermore, in view of the small number of cases investigated here, we cannot draw any conclusions about the possible clinical significance of the presence of FGFR1 mutation in RGNTs. Although RGNTs are classified as grade I by the World Health Organization and have an excellent prognosis on surgical resection, local recurrences have been reported in some patients. Both FGFR1–mutated tumors in our series relapsed 1 and 4 years after the first surgery, respectively. Interestingly, one of the relapsing tumors concomitantly had a PIK3CA H1047R mutation. This mutation, along with the PIK3CA E542K, has been previously described in RGNTs (5, 6), as well as in glioblastoma, anaplastic oligodendroglioma, and medulloblastoma (5). Unlike high-grade gliomas, it is unclear as to whether PIK3CA mutations could be clinically prognostic in such tumors (5). In comparison with the other RGNTs harboring PIK3CA mutations (5), we found such a mutation exclusively in a tumor relapse. Given the coexistence of FGFR1 and PIK3CA mutations in this RGNT, we hypothesize that concomitant activation of the MAPK and PIK3/Akt/mTOR pathways may have resulted in a biologic advantage for tumor cells, thereby enabling tumor relapse.

The analysis of histopathologic features and clinical data of mutated cases revealed, however, some further intriguing data about the possible relationship between PAs and RGNTs. Although the FGFR1 N546K–mutated case showed typical RGNT features in the primary and recurrent lesion samples, the FGFR1 K656E–mutated tumor showed classical RGNT architecture in the primary lesion and the presence of a largely predominant PA-like histology in the recurrent tumor sample. Moreover, review of the patient’s clinical history revealed that this patient had undergone resection of a midline diencephalic tumor (Fig. 2A) 5 years before the diagnosis of the fourth ventricle RGNT, which was followed by fractional radiotherapy (55 Gy). The histologic review of this tumor revealed a PA with pilomyxoid changes (Figs. 2B, C) without evidence of a neuronal component or RGNT-like features that were detected in the fourth ventricle lesion (Figs. 2D–F). Notably, the FGFR1 mutation analysis of the diencephalic tumor uncovered the same K656E mutation (Fig. 2G, H). Unfortunately, because a peripheral blood sample from this patient was unavailable, a mutational analysis to investigate the FGFR1 germline status of the patient was not possible. The fourth ventricle lesion could be explained as a late intraventricular dissemination of the diencephalic tumor, according to the clinical follow-up data. On the other hand, the RGNT-like features of the fourth ventricle tumor also could be interpreted as a “phenotypic variation” of a FGFR1–mutated relapsing PA.

In conclusion, we describe for the first time the presence of FGFR1 mutations in 2 RGNT cases. The possible presence of FGFR1 mutations in other glioneuronal tumors has to be further investigated in larger series, including cases of gangliogliomas and dysembryoplastic neuroepithelial tumor, in particular, in those with evidence of MAPK pathway activation.

REFERENCES