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BRIEF REPORT

Rare adult pilocytic astrocytoma of the septum pellucidum with novel RIN2::BRAF fusion

Xinglei Liu[1] · Xiaoxiao Dai[1] · Chungang Dai[1] · Qin Zhu[1] · Ailin Chen[1] · Yanming Chen[1] · Nan Chen[2,3] · Ping Chen[2,3] · Rong Rong[2,3] · Changjun Shi[2,3] · Sheng Xiao[4] · Jun Dong[1]

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Abstract
Pilocytic astrocytoma is mostly a pediatric tumor with the majority of patients under age 20. Although tumors can occur throughout neuraxis, most tumors are in the cerebellum and optic chiasm. Pilocytic astrocytoma in unusual locations is often associated with different genetic alterations than the classic *KIAA1549::BRAF* fusion. We report a rare adult pilocytic astrocytoma of the septum pellucidum that presented with progressive headache. A detailed genomic evaluation found a fusion between *BRAF* and a novel partner *RIN2*, a gene overexpressed in both low-grade glioma and glioblastoma. The *RIN2::BRAF* transcript encodes a chimeric protein containing a dimerization domain SH2 and an intact kinase domain, consistent with a prototypic oncogenic kinase rearrangement. In addition, we discuss the potential oncogenic mechanisms of *BRAF* signaling and its implication in targeted therapy with kinase inhibitors.

Keywords Adult pilocytic astrocytoma · Pellucid septum · *RIN2::BRAF* fusion

Background

Pilocytic astrocytoma is the most common glioma in children, with 75% of patients being 20 years old or younger [1]. These tumors can arise throughout the neuraxis; however, most of them are located at the cerebellum and optic chiasm. While pilocytic astrocytomas are WHO grade 1 benign tumors that are cured by complete surgical resection, approximately 20% of tumors are situated in pivotal or deep regions (e.g., brainstem) that may require adjuvant radiotherapy or chemotherapy. Adult pilocytic astrocytoma is rare, and its aggressive behaviors seem to increase with age [2].

The most common genetic alteration of pilocytic astrocytoma is an oncogenic fusion between the N terminus of KIAA1549 and the kinase domain of BRAF (KIAA1549::BRAF), resulting from a tandem duplication at chromosome 7q34. *KIAA1549*::*BRAF* is found in approximately 60% of pilocytic astrocytomas, often as a sole aberration. *BRAF* can also be fused with non-*KIAA1549* partner genes.

These non-*KIAA1549*::*BRAF* tumors often occur in unusual anatomical locations and may associate with malignancy tendency. We report here a rare adult pilocytic astrocytoma of the septum pellucidum with a *RIN2::BRAF* fusion. To our knowledge, this is the first case of *RIN2::BRAF* rearrangement in pilocytic astrocytoma.

Materials and methods

Targeted RNA next-generation sequencing (NGS)

Total RNA from fresh tumor tissue was extracted with TRIzol™ LS Reagent according to the manufacturer's instructions (Cat: 10,296,010, ThermoFisher, Invitrogen, USA). One hundred nanograms of total RNA was used for reverse transcription. End repairing and adaptor ligation were performed

Xinglei Liu, Xiaoxiao Dai, and Chungang Dai contributed equally to this work

✉ Jun Dong
dongjun@suda.edu.cn

[1] Department of Neurosurgery, The Second Affiliated Hospital of Soochow University, Suzhou 215004, China

[2] Suzhou Sano Precision Medicine Ltd, Suzhou, China

[3] Department of Biological Sciences, Xi An Jiaotong-Liverpool University, Suzhou, China

[4] Department of Pathology, Brigham and Women's Hospital, Boston, USA

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according to standard NGS protocols (Cat: E7771 and E6111, NEB, USA). PCR enrichment was performed using 390 gene-specific primers specific to a group of 63 genes commonly involved in solid tumors, and the enriched PCR products were sequenced in an Illumina NovaSeq 6000 platform (San Diego, USA). Sequencing results were analyzed with SeqNext software (JSI, Germany).

Targeted DNA next-generation sequencing

Genomic DNA of fresh tumor specimen was isolated with QIAamp DNA Micro Kits (Cat: 56,304, Qiagen, Germany). Three hundred nanograms of DNA was fragmented with a Bioruptor Pico (Diagenode, Denville, NJ, USA) to 200–300 bp, multiplex library preparation was performed using the Rapid Plus DNA Lib Prep Kit for Illumina (RK20208, ABclonal) according to the manufacturer's specifications. The libraries were incubated with a pool of biotin-labeled bait oligos that targeted 638 genes commonly involved in tumors for 16 h. Targeted regions were pulled down with streptavidin beads, amplified by PCR, and sequenced as paired-end 150-bp reads on an Illumina NextSeq 6000 instrument. Reads were aligned to the reference genome (hg19) using BWA-MEM. Sequencing results for single-nucleotide variations (SNVs), insertion/deletion (Indels), copy number variations (CNVs), and structure variations (SVs) were analyzed with SeqNext software (JSI, Germany) and laboratory-developed pipelines (Sano Medical Laboratories, China).

Fluorescence in situ hybridization (FISH)

FISH was performed on 5-µm paraffin slides of tumor tissue with two colored split apart probes for *BRAF* (Betrue, China). The slides were deparaffinized in xylene, rehydrated, treated in 750 U/ml pepsin digest solution (Cat: P6887, Sigma-Aldrich, USA) for 10 min and incubated in 10% buffered formalin for 10 min. The slides and probes were separately denatured, and hybridization was performed at 37 °C overnight. Post-hybridization wash was done in 0.4 × SSC/0.3% NP-40 at 73 °C for 3 min and slides were counterstained with DAPI.

Reverse transcriptase PCR (RT-PCR) and Sanger sequencing

Total RNA was extracted with TRIzol™ LS Reagent according to the manufacturer's instructions (Cat: 10,296,010, ThermoFisher, Invitrogen, USA). cDNA was synthesized with random priming and SuperScript™ IV reverse transcriptase (Cat: 18,090,050, ThermoFisher, USA). PCR was performed with primers specific to *RIN2* and *BRAF* (*RIN2*-F: 5'-GCCCAGTGTGACATGCTTGA-3', *BRAF*-R: 5'-GACTTCCTTTCTCGCTGAGGT-3'). The PCR conditions were 95 °C 3 min for 1 cycle followed by 35 cycles of 95 °C 30 s, 58 °C 60 s, and 72 °C 60 s. One microliter of the first PCR product was re-amplified with nested primers (*RIN2*-F: 5'-AAGAAGAACAAGCAGCGCGA-3', *BRAF*-R: 5'-TGGTTGATCCTCCATCACCAC-3;). The PCR conditions were 95 °C 3 min for 1 cycle followed by 40 cycles of 95 °C 30 s, 58 °C 60 s, and 72 °C 60 s. The PCR product was analyzed by gel electrophoresis and directly Sanger sequenced.

Results

A 22-year-old male patient presented with a progressive headache. CT plain scan showed analogous oval iso-dense mass of 37 × 32 × 30 mm in the septum pellucidum of anterior horns of the lateral ventricles, leading to blockage of the foramen of Monro. On MRI, the mass was iso-signal in T1 sequences and hyperintense in T2-weighted images with diffuse enhancement (Fig. 1). Total tumor resection was performed via an endoscopic trans-right frontal horn approach, which had a clear boundary between the tumor and normal brain tissue. Tumor tissue sections showed a biphasic appearance of compact and loose patterns with Rosenthal fibers in compact areas. Eosinophilic granular bodies were frequently observed in both compact and loose areas. Nuclei were round to elongate with mild pleomorphism and occasional large cells with multiple nuclei were observed. Glomeruloid microvascular proliferation was observed. Mitotic activity and necrosis were not seen. Immunohistochemistry (IHC) showed strong positivity for GFAP, S100, and OLIG2, partial positive for p53 (~ 10–20%), and negative for CD34, NFP, and NeuN (Fig. 2). A diagnosis of pilocytic astrocytoma was made. Targeted RNA next-generation sequencing (NGS) on fresh tumor specimen was performed in an Illumina NovaSeq 6000 platform (San Diego, USA), which discovered a fusion transcript containing the 5'*RIN2* exon 10 and 3'*BRAF* exon 9 (Fig. 3A). Reverse transcriptase PCR (RT-PCR) was performed with primers specific to RIN2 and BRAF. A PCR product at the expected size was obtained (Fig. 3B) and directly sequenced, which confirmed the *RIN2::BRAF* rearrangement (Fig. 3C). Additional fluorescence in situ hybridization (FISH) with a *BRAF* probe showed signal split apart, consistent with a *BRAF* rearrangement (Fig. 3D). The reading frame of the *RIN2::BRAF* is intact, which encodes a chimeric protein containing Src homology 2 (SH2) domain, proline-rich domains (PRDs), RIN-homology (RH) domain, and vacuolar protein sorting-associated protein 9 (VPS9) domain from RIN2 and the kinase domain from BRAF (Fig. 3A). Based on the functional evaluation of other BRAF fusion proteins, RIN2::BRAF is likely constitutively activated by a ligand-independent dimerization. Wild-type RIN2 is a tetramer in the cytoplasm and SH2 is a known dimerization domain [3]. Therefore, the SH2 domain from RIN2::BRAF chimeric protein likely contributes to its dimerization.

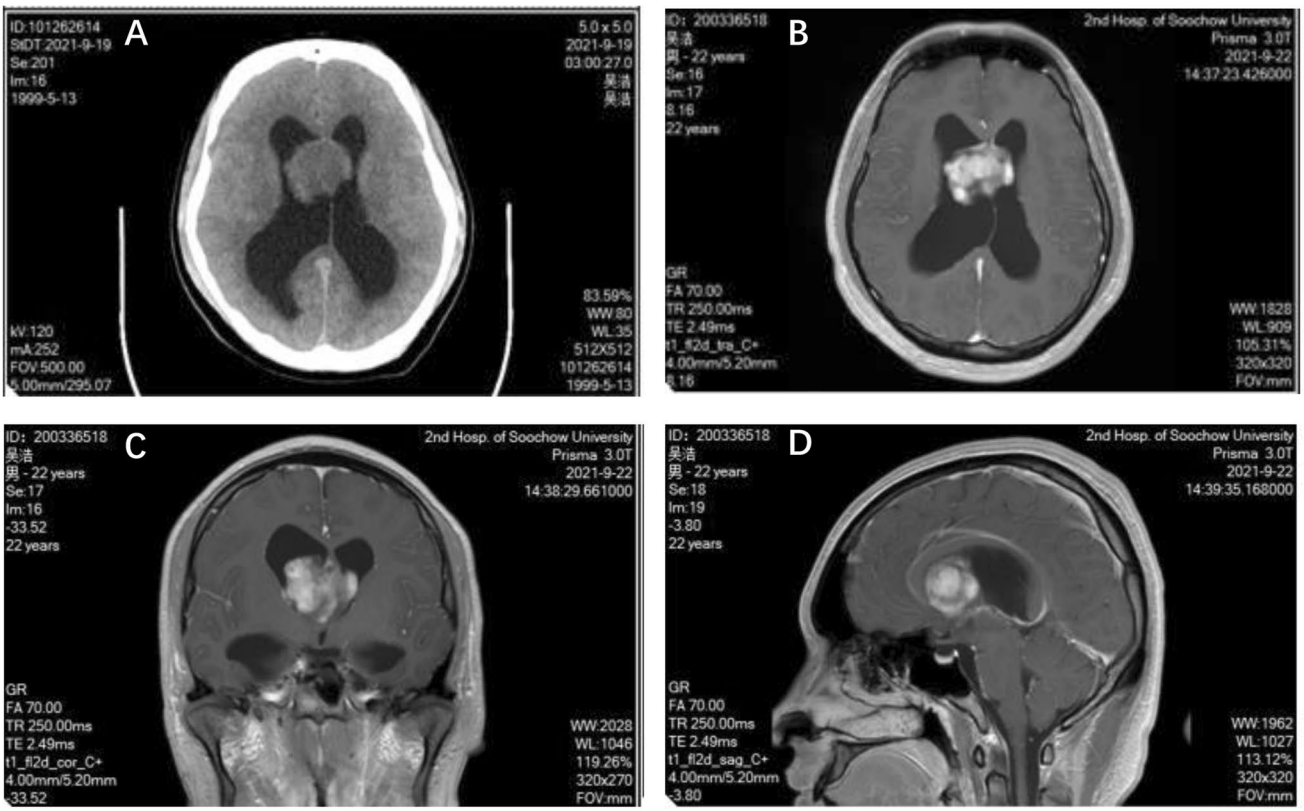

Fig. 1 Imaging studies of the pilocytic astrocytoma of the septum pellucidum. **A** CT plain scan showed a mass of 37 × 32 × 30 mm centered in the septum pellucidum with uneven density and dilated frontal horn. **B–D** MRI displayed a clumped T1W1 low, T2W2/FLAIR high, DWI slightly high, ADC iso-signal mass with clear boundary and significant enhancement

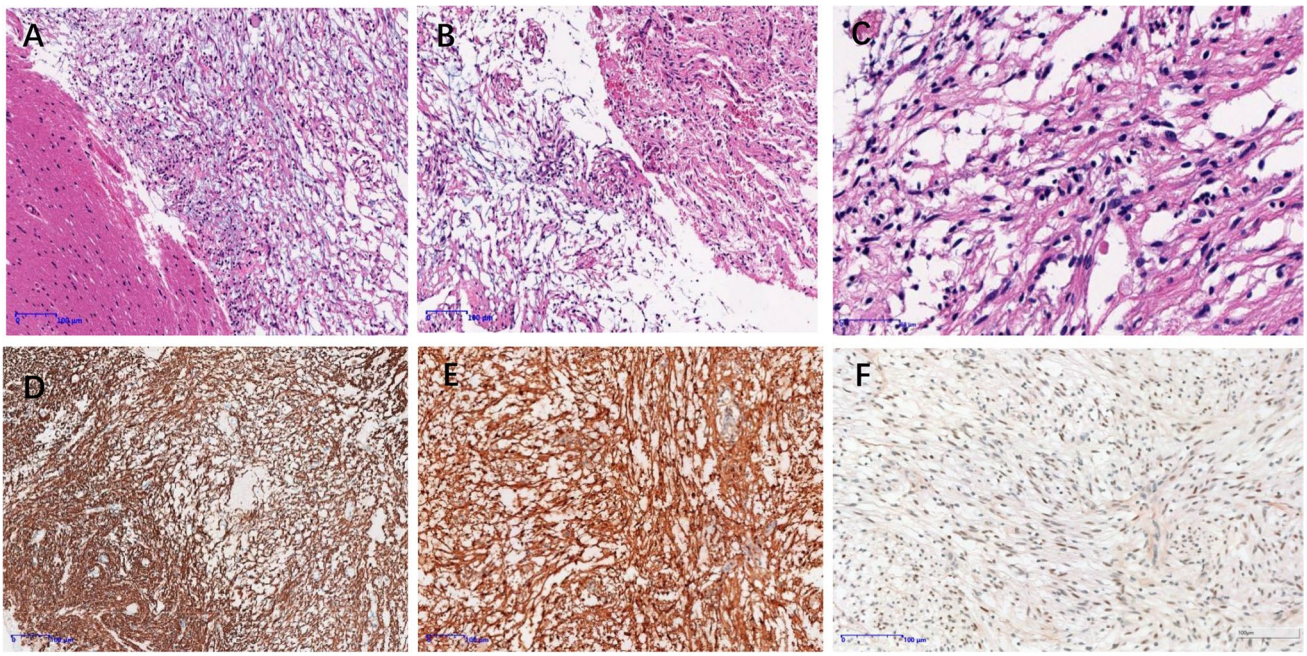

Fig. 2 H&E stain and IHC of the tumor tissue section. **A** Well-defined boundary between tumor tissue and normal brain tissue. **B** Biphasic structure of compact and loose patterns with Rosenthal fibers in compact areas. **C** Eosinophilic granular bodies were frequently observed (arrows). IHC showed positive staining for GFAP (**D**) and S-100 (**E**) and partial positive for p53 (10–20%; **F**)

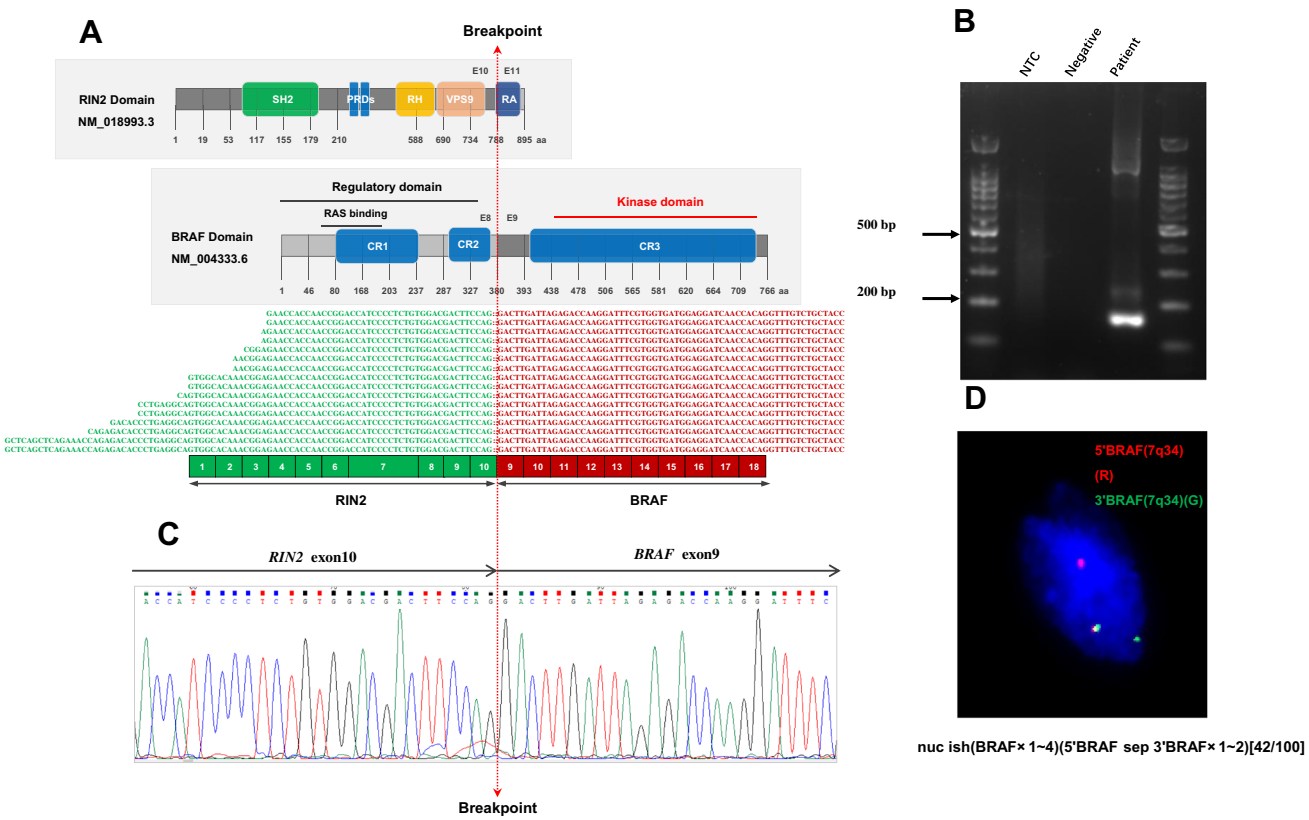

Fig. 3 Characterization of the *RIN2::BRAF* rearrangement. **A** RNA NGS showed an in-frame fusion between *RIN2* exon 10 and *BRAF* exon 9. The location of the breakpoints of corresponding proteins was marked. SH2, Src homology 2; PRDs, proline-rich domains; RH, RIN-homology; VPS9, vacuolar protein sorting-associated protein 9; CR1-3, conserved region 1–3. **B** RT-PCR amplified a fusion product of the expected size with primers specific for *RIN2* and *BRAF*. **C** Sanger sequencing of the PCR product confirmed the *RIN2::BRAF* fusion. **D** FISH with BRAF probe showed a signal separation between 5'BRAF (red) and 3'BRAF (green), consistent with BRAF rearrangement

Because p53 was positive by IHC in a subset of tumor cells, we performed a targeted DNA NGS, which showed a low-frequency TP53 mutation (5.5%). No other alterations, including chromosome copy number variations and loss of heterozygosity (LOH), were observed. A classic pilocytic astrocytoma carries a wild-type *TP53*. The clinical significance of the low-frequency *TP53* mutation in this patient is unclear. Due to his young age, *TP53* mutation from age-related clonal hematopoiesis is unlikely. The patient recovered completely after surgery, and follow-up showed no sign of recurrence 1 year post-operation.

Discussion

Aberrant BRAF activation is a dominant driver for several tumors including pilocytic astrocytoma, lung adenocarcinoma, malignant melanoma, thyroid cancer, colon cancer, and hairy cell leukemia [4–7]. BRAF activation leads to mitogen-activated protein kinase (MAPK) signaling, which plays major roles in cell proliferation, differentiation, and apoptosis [8]. Two common mechanisms for aberrant BRAF activation include *BRAF* V600E mutation and *BRAF* rearrangement. The *BRAF* V600E causes disruption of the auto-inhibitory interaction between the N-terminal conserved region 2 (CR2) and the kinase domain, leading to constitutive kinase activation [9]. BRAF rearrangements resulted in the formation of chimeric proteins containing N-terminal domains from the fusion partners and C-terminal tyrosine kinase domain from BRAF. The single-most common BRAF fusion partner is KIAA1549, which is seen in ~ 60% of pilocytic astrocytomas and can be seen in diffuse leptomeningeal glioneuronal tumor. Nine other fusion partners have been reported so far in pilocytic astrocytoma, with *RIN2* being the newly added member (Fig. 4A). RIN2 is a guanine nucleotide exchange factor (GEF) that activates Rab5 by promoting the exchange of free cytosolic

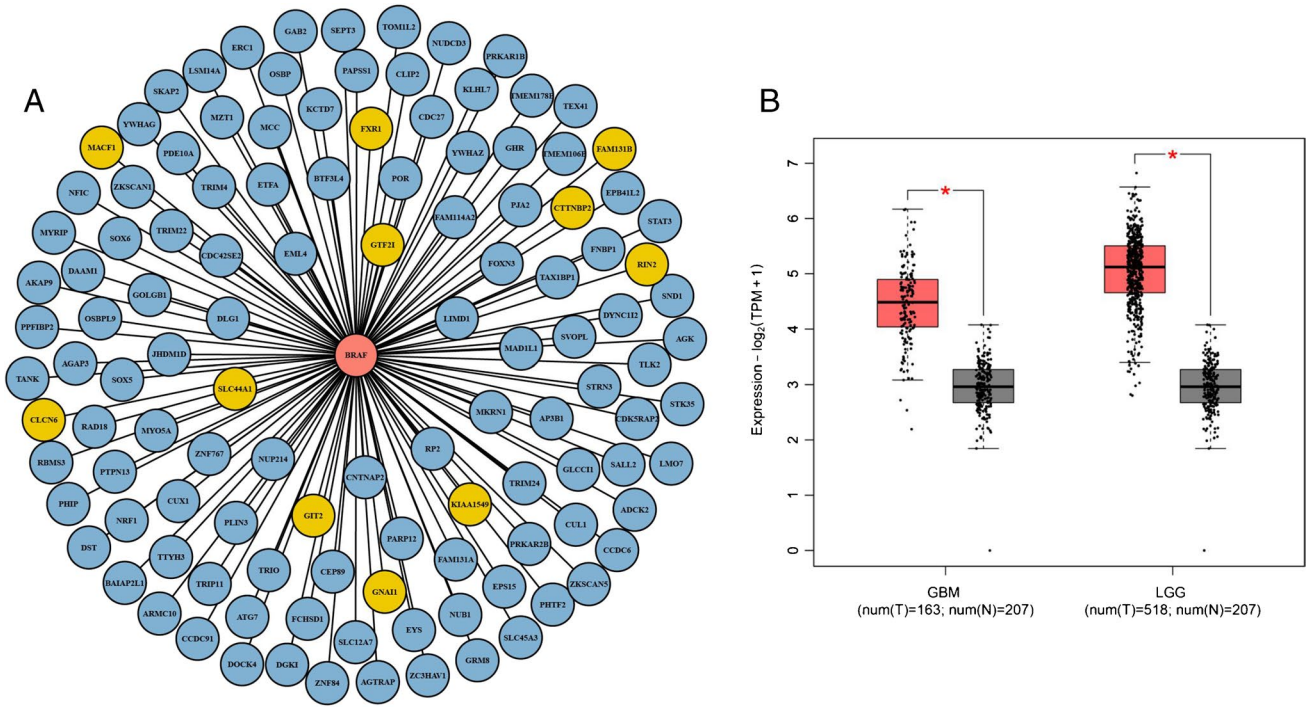

Fig. 4 **A** 119 fusion partners of *BRAF* are documented in tumors, with 11 being observed in pilocytic astrocytoma (marked yellow). **B** *RIN2* overexpression in low-grade glioma and glioblastoma from TCGA database

GTP for bound GDP (from inactive Rab5-GDP to active Rab5-GTP). Rab5 is a small GTPase important for endocytosis. Loss of RIN2 causes a genetic disorder, RIN2 syndrome, or MACS syndrome (for macrocephaly, alopecia, Cutis laxa, and scoliosis). The pathogenesis of the disease involves insufficient Rab5 signaling and defective trafficking of elastin and collagens from the endoplasmic reticulum to Golgi to the cell membrane. As a member of the Ras family, Rab5 also plays roles in tumors, particularly in tumor cell migration and invasion [10, 11]. Not much is known about the role of RIN2 in cancer; however, evaluation of the TCGA database showed consistent *RIN2* overexpression in tumors, including both low-grade glioma and glioblastoma (Fig. 4B). The overexpressed RIN2 might exert its oncogenic signaling via activating Rab5.

The oncogenic mechanisms for RIN2::BRAF need further functional evaluation. Several aspects of the chimeric fusion protein could contribute to the de-regulation of BRAF signaling: (1) the auto-inhibitory domains of BRAF (CR1 and CR2) are lost due to rearrangement (Fig. 3A); (2) the N-terminal RIN2 contains an SH2 domain, a known dimerization domain, that could lead to dimerization of the RIN2::BRAF, resulting in constitutive kinase activation independent of upstream regulation; and (3) the chimeric fusion protein may possess different expression level or cellular localization than the wild-type BRAF.

Although pilocytic astrocytoma is typically curable by surgical resection, rare locations or relapsed tumors may be targeted by kinase inhibitors. Tumors with *BRAF* V600E (class I mutation) may be sensitive to vemurafenib, dabrafenib, or encorafenib. Tumors with *BRAF* fusion (class II mutation) could be targeted by dimer disrupter TAK-580 [12]. A subgroup of pilocytic astrocytoma may carry non-*BRAF* tyrosine kinase rearrangement including *NTRK*, *ROS1*, or *FGFR1*. These tumors can be treated with specific kinase inhibitors, i.e., entrectinib and larotrectinib for *NTRK* rearrangement, taletrectinib and crizotinib for *ROS1* rearrangement, and erdafitinib for *FGFR1* rearrangement.

In summary, we describe the first case of *RIN2::BRAF* rearrangement in a rare adult pilocytic astrocytoma of the septum pellucidum. Its oncogenic mechanism and therapeutic implication are discussed.

Author contribution Liu followed up patient and prepared the manuscript; Dai and Dai made pathological analysis; Zhu, Chen, and Chen prepared the clinical data Chen, Shi, and Xiao made DNA and RNA NGS analysis; Dong analyzed data and revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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