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Deep Sequencing Identifies *IDH1* R132S Mutation in Adult Medulloblastoma

Case Report

A 62-year-old woman presented with headaches in 2002. A magnetic resonance imaging scan showed a lateral right cerebellar hemisphere abnormality on fluid-attenuated inversion recovery, but no additional work-up was pursued at that time. In February 2007, she developed worsening headaches and dizziness; a repeat magnetic resonance imaging scan demonstrated an enlarged, gadolinium-enhancing mass composed of four distinct nodules (Fig 1A, arrow). Biopsy demonstrated a small round blue cell tumor, with lymphoma and small-cell lung cancer (SCLC) in the differential. No abnormalities were identified on a chest computed tomography scan. The patient's symptoms and brain imaging findings improved with corticosteroids, an outcome that is inconsistent with SCLC. A repeat biopsy was performed in July 2007. However, because of a postoperative course that was complicated by cerebellar swelling manifested by a new right pronator drift and increasing somnolence, posterior decompression was performed with complete resection of the tumor. The final pathologic diagnosis was medulloblastoma. Subsequent staging studies did not demonstrate additional disease or spinal spread. The patient was subsequently treated with adjuvant craniospinal irradiation. She received 36 Gy (relative biological effectiveness) to the whole cranium and spine with fractionated proton therapy, and the posterior fossa tumor bed was boosted to a total of 54 Gy (relative biological effectiveness). Proton therapy was used to eliminate additional irradiation of the anterior chest, abdomen, and pelvis and thus to minimize potential radiation-associated toxicity. The patient tolerated radiation therapy well; the majority of her symptoms during radiation were limited to fatigue and anorexia. Given her frail baseline performance status and the unclear benefit of chemotherapy in standard adult medulloblastoma with a gross total resection, chemotherapy was not administered.

Two tumor samples obtained in 2007 by biopsy (specimen M3) and gross total resection (specimen M13) were available for analysis. The patient did not receive any chemoradiation after the first biopsy (specimen M3). Morphologic evaluation revealed a classic medulloblastoma with prominent nodular architecture and no desmoplasia,

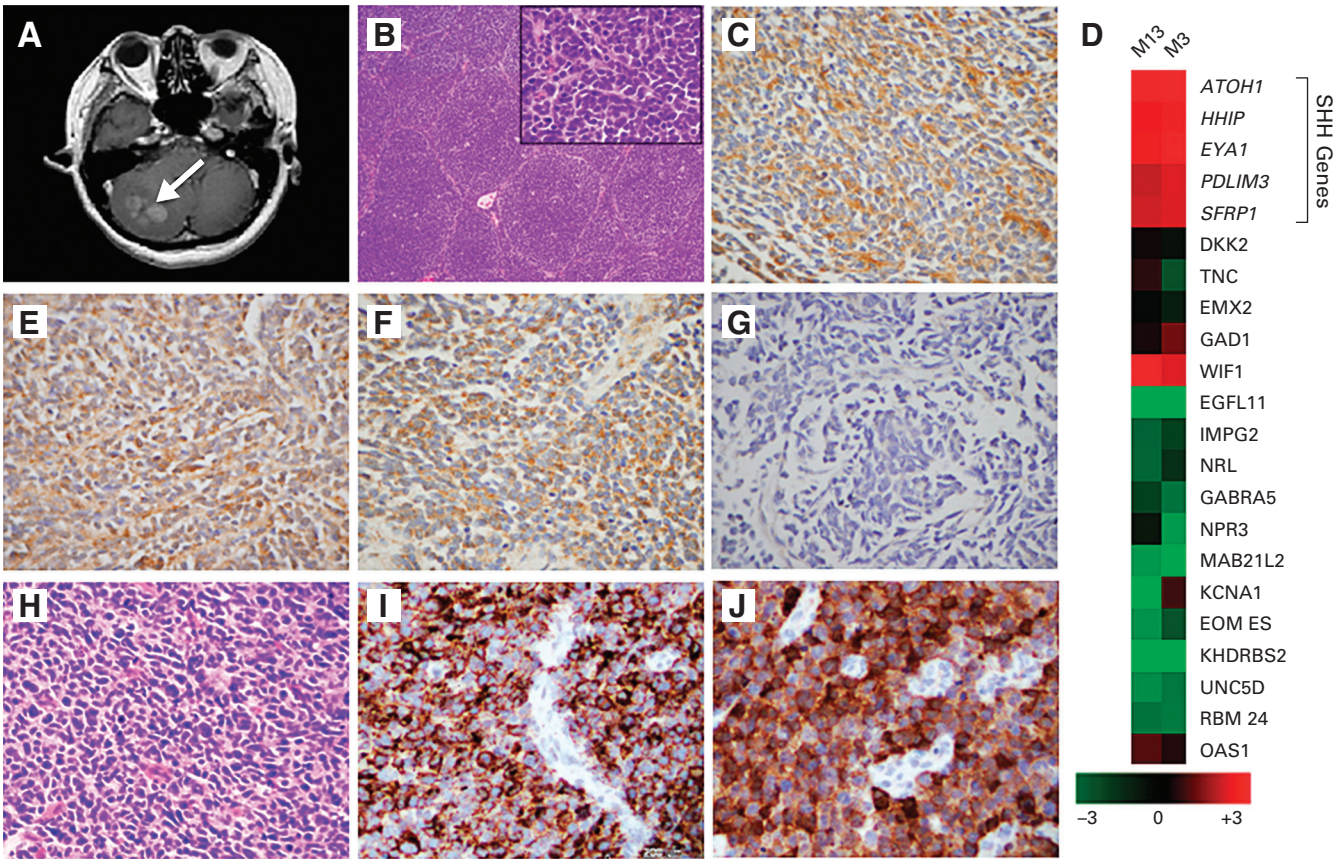

Fig 1.

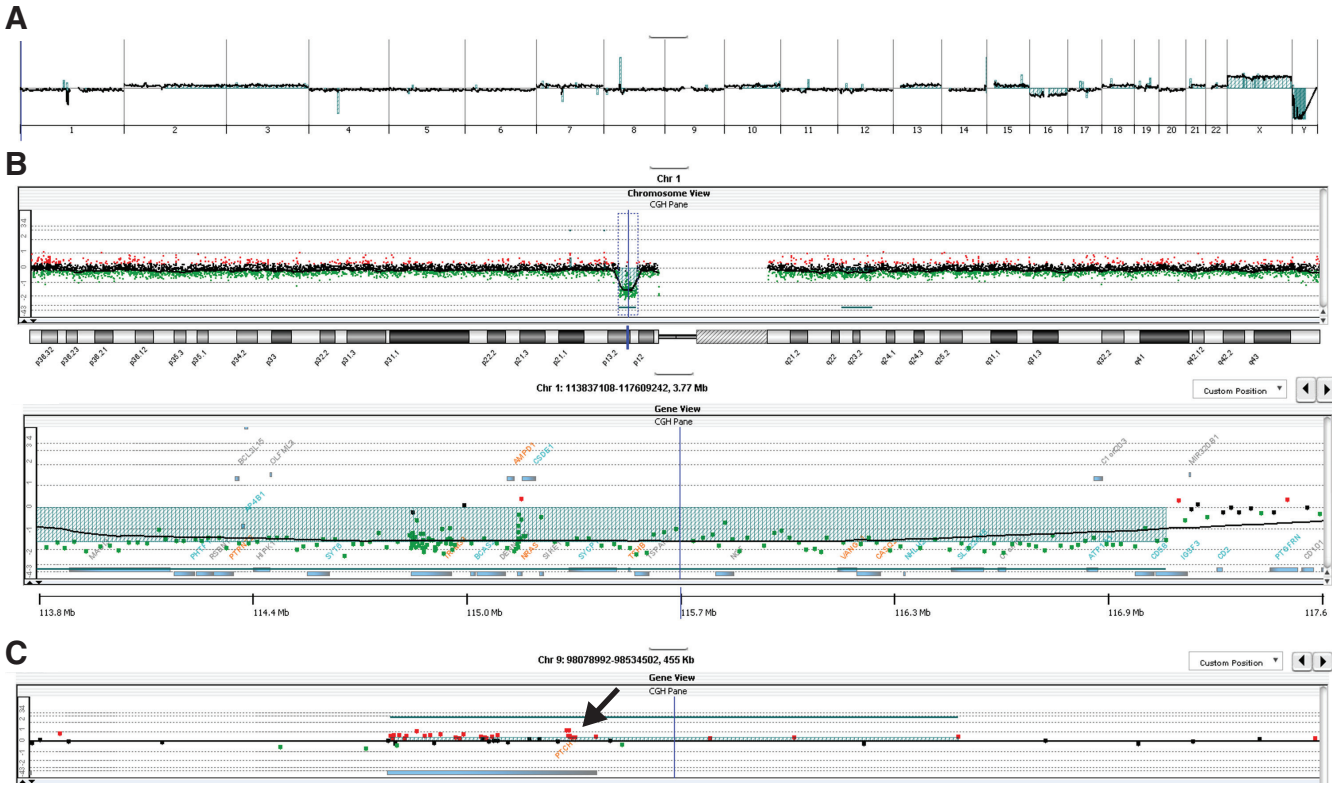

Fig 2.

anaplastic, or large-cell features (Fig 1B). The tumor infiltrated the subarachnoid space and cerebellar cortex in the usual manner for medulloblastoma. Tumor cells were strongly positive for synaptophysin, a marker of neural origin, but not for glial fibrillary acidic protein. Because the clinical presentation of this tumor was unusual, we sought to classify the tumor and to seek novel genetic mutations or rearrangements that might explain its clinical behavior.

To subclassify the medulloblastoma, we used a consensus panel of immunohistochemical markers.[1] The tumor was positive for GAB1, DKK1, and Kv1.1, typical for sonic hedgehog (SHH), Wnt, and group 4, respectively (Figs 1C, 1E, and 1F, respectively), but negative for GLI1, CTNNB1, and NPR3 (Fig 1G). To explore the expression profile of the tumor, we performed gene expression profiling using the NanoString platform (NanoString Technologies, Seattle, WA). RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tissue using a Qiagen RNeasy FFPE kit (Valencia, CA), and a total of 250 ng of RNA was analyzed for each sample. A custom code set including 22 medulloblastoma-specific subtyping gene probes and three housekeeping genes was synthesized by NanoString Technologies following previously defined protocols.[2] Expression analysis was conducted using the nCounter Gene Expression system (NanoString Technologies). Protocol recommendations outlined by NanoString Technologies were followed with respect to sample preparation, detection, and scanning. Medulloblastoma subclass assignment was performed as suggested previously.[2] NanoString analyses showed a clear upregulation of the SHH-associated genes *ATOH1*, *HHIP*, *EYA1*, *PDLIM3* and *SFRP1* (Fig 1D).

Subtypes of medulloblastoma are characterized by distinct chromosomal rearrangements and amplifications.[3,4] We performed array comparative genomic hybridization (aCGH) on FFPE tumor as described previously.[5] aCGH data have been deposited in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) and are accessible under accession number GSE41990. The tumor showed minimal changes, including a loss of chromosome 16 (Fig 2A), which has been seen predominantly in group 3 medulloblastomas and rarely in SHH tumors, and a small homozygous deletion in 1p13.2 region (Fig 2B; Chr, chromosome; CGH, comparative genomic hybridization). Of the genes in this locus, only *NGF* seems potentially relevant, given that its *Trk* receptors have been implicated in the biology of medulloblastoma. However, we did not observe other large chromosomal aberrations that are typical for medulloblastoma, or focal gains or losses such as amplification of *GLI2*, *MYCN*, *MYC*, *CCND2*, or deletion of *PTCH1*, which is characteristic of SHH medulloblastomas (Fig 2C, arrow), *PTEN*, or *TP53*. Overall, the copy number profile of this tumor is unique from that of pediatric medulloblastoma.

We performed deep sequencing to gain deeper insight into the genomic alterations of this tumor. We analyzed 10 ng of genomic DNA from M3 and M13 samples using focused deep amplicon sequencing with the AmpliSeq Cancer panel (Life Technologies, Gaithersburg, MD), covering 739 mutations in 46 genes.[6] Indexed amplicon libraries were pooled for emulsion polymerase chain reaction and sequencing on the Ion Torrent PGM platform (Life Technologies). Average base pair coverage was more than 500× for each case. Variant calling was performed using the Torrent Variant Caller v2.2 (Life Technologies) against the hg19 reference human genome. Both M3 and M13 samples contained a heterozygous *IDH1* CGT > AGT R132S mutation (Fig 3A), but no other cancer gene mutations. Variant frequency for M3 and M13 samples was 18.21% and 28.11%, with 280×

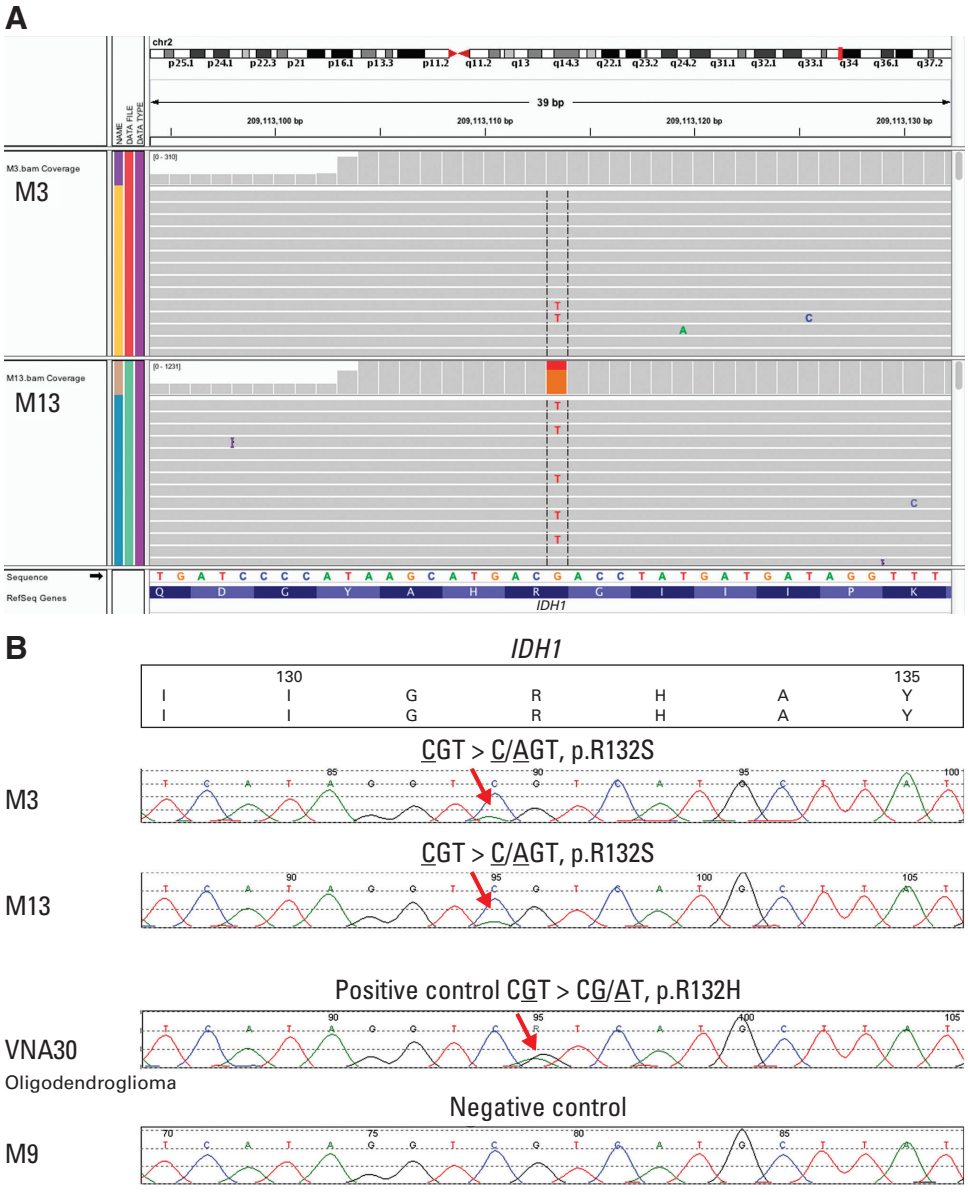

Fig 3.

and 1,110× coverage, respectively ($P = 1 \times 10^{-10}$ and 2.5×10^{-7}, respectively). No other variants were flagged in the Catalogue of Somatic Mutations in Cancer database. To confirm the *IDH1* mutation, we performed bidirectional focused Sanger sequencing of *IDH1* and *IDH2*, as described previously.[7] Analysis of DNA tracings was carried out using Mutation Surveyor version 3.2 (Softgenetics, State College, PA) and confirmed a heterozygous CGTAGT mutation in both patient's samples (Fig 3B).

Discussion

Transcriptome and whole genome analyses have revealed mRNA, copy number, and mutation profiles, allowing subclassification of pediatric medulloblastomas. Current treatment of medulloblastoma is associated with significant morbidity, including infertility and growth problems,[8] endocrine abnormalities,[9] decline in cognition and intellect,[10-12] and secondary malignancies.[13] Therefore, significant effort has focused on the identification of tumor subcategories that would allow clinicians to predict behavior, manage optimal care,[14-16] and develop targeted therapy.[17] Pediatric medulloblastomas can be subclassified into at least four major groups: SHH, Wnt, group 3 (Group C, photoreceptor/GABAergic, high *MYC* amplification), and group 4 (Group D, neuronal/glutamatergic, low *MYC* amplification, *CDK6*/*MYCN* amplification), with groups 3 and 4 being more common.[1]

IDH1/2 mutations have not been observed in malignancies of the pediatric CNS. Yan et al[18] investigated 55 cases of medulloblastoma specifically for *IDH1/2* mutations. Parsons et al[19] analyzed 22 medulloblastomas. Similarly, Jones et al[20] did not observe *IDH1/2* mutations in their cohort of 125 matched patients with tumor-normal medulloblastoma using deep sequencing. Pugh et al[3] analyzed 92 patients using whole-exome hybrid capture and deep sequencing and documented a single case involving a 13-year-old

boy with Wnt medulloblastoma and an *IDH1* R132C mutation. Results of medulloblastoma reports demonstrate that medulloblastomas show relatively low mutation rates.[3,19,20] Most data about adult medulloblastoma are derived from small and retrospective cohorts. Tumors are located laterally and desmoplastic histology is relatively common. Late relapses, 5 or even 10 years after diagnosis, are more common in adults.[21] Molecularly, three main groups were identified in adult medulloblastoma: SHH tumors, Wnt tumors, and non-SHH/non-Wnt tumors. SHH tumors are the most common, Wnt and non-SHH/non-Wnt are mostly group 4, whereas group 3 tumors are rare in adults.[22] Wnt and group 4 medulloblastomas have significantly worse outcome in adults. Adult SHH tumors with loss of 10q or 17p, gain of 2 or 17q, and/or *GLI2* amplification have worse prognosis than pediatric tumors with the same rearrangements.[23] Increasing evidence suggests that adult medulloblastomas are distinctly different from pediatric tumors,[23] with more balanced genomes, and they require a different clinical stratification,[24] also suggesting the importance of point mutations rather than rearrangements in tumorigenesis.

The presence of a hotspot *IDH1* mutation in an SHH medulloblastoma suggests that the mutation rate or baseline genes that are involved in the tumorigenesis of adult medulloblastomas might be different than those of tumors occurring in the pediatric population. Our patient case showed few copy number changes and mutations, confirming that adult medulloblastoma does not show unusually high levels of mutations or copy number changes compared with pediatric tumors.

Our findings suggest that mutations in the *IDH1* gene may be an independent event in the medulloblastoma mutagenesis cascade. Given that *IDH1* mutations are often associated with less aggressive behavior and favorable outcome in brain tumors in adults, this might explain the older age at presentation and slow growth, both features that are unusual for medulloblastoma. Furthermore, we also identified another case of adult medulloblastoma (Fig 1H) that was synaptophysin positive with an *IDH1* R132H mutation according to immunohistochemistry (Figs 1I and 1J, respectively), which suggests that *IDH1* mutations could be a more common phenomenon in medulloblastoma.

Our report raises several important issues. Additional studies are necessary to establish the frequency of *IDH1/2* mutations in medulloblastoma and their prognostic relevance. The presence of an *IDH1* mutation in SHH medulloblastoma and Wnt medulloblastoma,[3] which are presumed to have different cells of origin, raises an important question about tumor-initiating events and whether an *IDH1* mutation alters the behavior of SHH or Wnt tumors. Although classified as an SHH tumor, our medulloblastoma showed the variable protein expression profile of SHH, Wnt, and group 4 medulloblastoma by immunohistochemistry, and a chromosomal loss that is typical of group 3 medulloblastoma by aCGH. Medulloblastomas might have a distinctly different mutation spectrum in adults, despite their histologic, imaging, and even major molecular similarities. Therefore, careful correlation of sequenced samples with clinical information is paramount in the discovery of driver mutations and potential therapeutic targets.

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