UCSF

UC San Francisco Previously Published Works

Title

Recurrent KBTBD4 small in-frame insertions and absence of DROSHA deletion or DICER1 mutation differentiate pineal parenchymal tumor of intermediate differentiation (PPTID) from pineoblastoma.

Permalink

<https://escholarship.org/uc/item/4494z38w>

Journal

Acta neuropathologica, 137(5)

ISSN

0001-6322

Authors

Lee, Julieann C
Mazor, Tali
Lao, Richard
et al.

Publication Date

2019-05-01

DOI

10.1007/s00401-019-01990-5

Peer reviewed

Recurrent *KBTBD4* small in-frame insertions and absence of *DROSHA* deletion or *DICER1* mutation differentiate pineal parenchymal tumor of intermediate differentiation (PPTID) from pineoblastoma

Julieann C. Lee¹ · Tali Mazor^{2,8} · Richard Lao³ · Eunice Wan³ · Alpha B. Diallo⁴ · Nicholas S. Hill⁴ · Naina Thangaraj⁴ · Katherine Wendelsdorf⁴ · David Samuel⁵ · Cassie N. Kline⁶ · Anuradha Banerjee⁶ · Kurtis Auguste² · Corey Raffel² · Nalin Gupta² · Mitchel Berger² · David R. Raleigh^{2,7} · Anny Shai² · Joanna J. Phillips^{1,2} · Andrew W. Bollen¹ · Tarik Tihan¹ · Arie Perry^{1,2} · Joseph Costello² · David A. Solomon¹

Received: 8 February 2019 / Revised: 10 March 2019 / Accepted: 11 March 2019 / Published online: 14 March 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

The pineal body is a small endocrine gland in the midline of the brain that secretes melatonin to modulate circadian rhythms. A group of primary tumors which arise from the pineal gland termed pineal parenchymal tumors are classified as pineocytoma (grade I), pineal parenchymal tumor of intermediate differentiation (PPTID; grade II or III), or pineoblastoma (grade IV). Most pineoblastomas arise in children, whereas pineocytomas and PPTIDs typically occur later in life. Pineocytomas are associated with favorable prognosis, with 5-year survival exceeding 90% following gross total resection. In contrast, pineoblastomas are

embryonal tumors with a propensity for cerebrospinal dissemination and poor outcome despite resection, craniospinal radiation, and systemic chemotherapy [3]. PPTIDs are morphologically heterogeneous with intermediate histologic features and variable clinical outcomes [3, 5]. Recently, pineoblastomas were identified to harbor frequent mutations of the *DICER1* gene or homozygous deletion of the *DROSHA* gene that both encode microRNA-processing enzymes [2, 6, 7]. However, the genetic alterations responsible for driving PPTID and pineocytoma are unknown.

To investigate the molecular pathogenesis of pineal parenchymal tumors, we performed whole-exome sequencing on genomic DNA extracted from tumor and matched normal tissue from eight patients (Fig. 1a) as described in the Supplementary Methods (Online Resource 1). The clinical features and outcomes of this patient cohort are presented in Supplementary Table 1 (Online Resource 2). Imaging features are shown in Supplementary Fig. 1 (Online Resource 1). The four pineoblastomas were primitive small round blue cell tumors with frequent mitoses, karyorrhectic debris, and foci of necrosis [Supplementary Fig. 2 (Online Resource 1)]. In contrast, the three PPTIDs were histologically characterized by sheets of tumor cells with uniform round nuclei containing delicate chromatin and contained only occasional mitotic figures [Supplementary Fig. 3 (Online Resource 1)]. The one pineocytoma contained numerous pineocytomatous rosettes and lacked appreciable mitotic activity [Supplementary Fig. 4 (Online Resource 1)].

Among the four pineoblastomas, two harbored somatic mutations in *DICER1* and the other two harbored focal homozygous deletions of *DROSHA* on chromosome 5p13 [Fig. 1c, d; Supplementary Figs. 5–7 (Online Resource 1)]. Pineoblastoma PPT #1 contained a somatic *DICER1* frameshift mutation (p.V1080 fs) on one allele and a hotspot

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00401-019-01990-5>) contains supplementary material, which is available to authorized users.

✉ David A. Solomon
david.solomon@ucsf.edu

- ¹ Division of Neuropathology, Department of Pathology, University of California, San Francisco, CA, USA
- ² Department of Neurological Surgery, University of California, San Francisco, CA, USA
- ³ Institute for Human Genetics, University of California, San Francisco, CA, USA
- ⁴ DNAnexus Inc., xVantage Group, Mountain View, CA, USA
- ⁵ Department of Hematology/Oncology, Valley Children's Hospital, Madera, CA, USA
- ⁶ Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of California, San Francisco, CA, USA
- ⁷ Department of Radiation Oncology, University of California, San Francisco, CA, USA
- ⁸ Present Address: Knowledge Systems Group, Dana Farber Cancer Institute, Boston, MA, USA

Fig. 1 Pineoblastoma is characterized by mutually exclusive *DICER1* mutation and *DROSHA* homozygous deletion and absence of *KBTBD4* mutation, whereas pineal parenchymal tumor of intermediate differentiation (PPTID) is characterized by a recurrent *KBTBD4* small in-frame insertion and absence of *DICER1* mutation or *DROSHA* deletion. **a** Clinicopathologic features and identified genetic alterations in the eight pineal parenchymal tumors. **b** Diagram of human *KBTBD4* protein with the location of the recurrent p.R313delinsPRR small in-frame insertion identified in the three PPTIDs. UniProt Q9NVX7, RefSeq NM_018095. **c** Pre-operative magnetic resonance imaging and tumor histology for patient PPT #1 with pineoblastoma harboring *DICER1* mutation. **d** Pre-operative magnetic resonance imaging and tumor histology for patient PPT #3 with pineoblastoma harboring *DROSHA* homozygous deletion. **e** Pre-operative magnetic resonance imaging and tumor histology for patient PPT #5 with PPTID harboring *KBTBD4* small in-frame insertion

missense mutation within the C-terminal Ribonuclease III domain on the other allele (p.E1813D) that has been recurrently found in pleuropulmonary blastomas, cystic nephromas, Sertoli–Leydig cell tumors, and other neoplasms known to be driven by *DICER1* mutation (Catalog Of Somatic Mutations In Cancer database). Pineoblastoma PPT #2 contained a somatic *DICER1* frameshift mutation (p.D1734 fs) with elimination of the remaining wild-type allele due to loss of chromosome 14q. The *DICER1* mutations were somatic in both PPT #1 and PPT #2, with no evidence of constitutional mosaicism in either patient (variant allele frequency in normal samples of 0%). Other than *DICER1*, no other genes harbored recurrent somatic nonsynonymous mutations among the four pineoblastomas [Supplementary Tables 2 and 3 (Online Resource 2)].

In contrast, none of the three PPTID harbored *DICER1* mutation or *DROSHA* deletion. Instead, the three PPTID were all found to harbor the identical somatic small in-frame insertion (p.R313delinsPRR) in the *KBTBD4* gene [Fig. 1b, e, and Supplementary Fig. 8 (Online Resource 1)], which encodes Kelch repeat- and BTB domain-containing protein 4 that is reported to regulate the Cullin3-based E3 ubiquitin ligase complex [1]. Similar small in-frame insertions in *KBTBD4* at codons 308–313 within a Kelch repeat domain were recently identified in a subset of Group 3 and Group 4 medulloblastomas that lacked other known genetic drivers such as *MYC* or *MYCN* amplification, *GFI1B* rearrangement, or *SNCAIP* duplication/*PRDM6* rearrangement [4]. Other than medulloblastoma, no other human tumor types have been identified to harbor recurrent *KBTBD4* mutations, and the functional mechanism by which these *KBTBD4* mutations drive tumorigenesis in PPTID and medulloblastomas is uncertain at present. Other than *KBTBD4*, no other genes harbored recurrent somatic nonsynonymous mutations among the three PPTID [Supplementary Tables 2 and 3 (Online Resource 2)].

The single case of pineocytoma lacked deletion of *DROSHA* and mutation of *DICER1* and *KBTBD4*. A small

number of somatic nonsynonymous mutations were identified of uncertain significance [Supplementary Tables 2 and 3 (Online Resource 2)], but identification of a recurrent genetic driver in pineocytoma awaits assessment of additional tumor samples.

In summary, we have identified that pineoblastoma is characterized by mutually exclusive *DICER1* mutation or *DROSHA* homozygous deletion and absence of *KBTBD4* mutations. In contrast, PPTID is characterized by recurrent *KBTBD4* small in-frame insertions and absence of *DICER1* mutation or *DROSHA* homozygous deletion. Further studies are warranted to confirm these findings in a larger cohort of pineal parenchymal tumors. Nonetheless, these findings indicate a fundamental role for deregulation of microRNA processing in the pathogenesis of pineoblastoma, whereas PPTID appear to arise independently of disruption of microRNA-processing genes. The recurrent *KBTBD4* mutation in PPTID identified here will likely be useful in helping to diagnostically distinguish this tumor entity from pineoblastoma. Future studies will determine the potential prognostic significance of *DICER1* mutation versus *DROSHA* homozygous deletion in pineoblastoma, and also the oncogenic mechanism by which *KBTBD4* mutation promotes tumorigenesis of the pineal gland. The *KBTBD4* mutations identified in PPTID in this study, as well as those found in medulloblastomas [4], are all heterozygous mutations that cluster at a hotspot within one of the Kelch repeat domains. This genetic pattern of heterozygous variants that cluster at a mutational hotspot within a functional domain is strongly suggestive that these are activating, gain-of-function mutations, as opposed to inactivating, loss-of-function events. Thus, *KBTBD4* is likely to function as an oncogene, rather than a tumor suppressor gene, in PPTID and medulloblastoma.

Acknowledgements This study was supported by the NIH Director's Early Independence Award (DP5 OD021403) and a Career Development Award from the UCSF Brain Tumor SPORE grant (NIH P50 CA097257) to D.A.S. We thank the UCSF Brain Tumor Tissue Bank (supported by NIH P50 CA097257) for supplying key samples.

Data availability Scanned image files of the H&E-stained slides from the eight pineal parenchymal tumors from which representative images are presented are available for downloading and viewing at the following link: https://figshare.com/projects/Pineal_parenchymal_tumor/59621. Sequencing data files are available from the authors upon request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests related to this study.

Ethical approval This study was approved by the Committee on Human Research of the University of California, San Francisco, with a waiver of patient consent.

References

1. Canning P, Cooper CD, Krojer T, Murray JW, Pike AC, Chai-kwad A et al (2013) Structural basis for Cul3 protein assembly with the BTB-Kelch family of E3 ubiquitin ligases. *J Biol Chem* 288:7803–7814
2. de Kock L, Sabbaghian N, Druker H, Weber E, Hamel N, Miller S et al (2014) Germ-line and somatic DICER1 mutations in pineoblastoma. *Acta Neuropathol* 128:583–595
3. Fauchon F, Jouvét A, Paquis P, Saint-Pierre G, Mottolese C, Ben Hassel M et al (2000) Parenchymal pineal tumors: a clinicopathological study of 76 cases. *Int J Radiat Oncol Biol Phys* 46:959–968
4. Northcott PA, Buchhalter I, Morrissy AS, Hovestadt V, Weischenfeldt J, Ehrenberger T et al (2017) The whole-genome landscape of medulloblastoma subtypes. *Nature* 547:311–317
5. Raleigh DR, Solomon DA, Lloyd SA, Lazar A, Garcia MA, Sneed PK et al (2017) Histopathologic review of pineal parenchymal tumors identifies novel morphologic subtypes and prognostic factors for outcome. *Neuro Oncol* 19:78–88
6. Sabbaghian N, Hamel N, Srivastava A, Albrecht S, Priest JR, Foulkes WD (2012) Germline DICER1 mutation and associated loss of heterozygosity in a pineoblastoma. *J Med Genet* 49:417–419
7. Snuderl M, Kannan K, Pfaff E, Wang S, Stafford JM, Serrano J et al (2018) Recurrent homozygous deletion of DROSHA and microduplication of PDE4DIP in pineoblastoma. *Nat Commun* 9:2868

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.