Acta Neuropathologica (2020) 139:1115–1118
https://doi.org/10.1007/s00401-020-02139-5

CORRESPONDENCE

Pineoblastoma is uniquely tolerant of mutually exclusive loss of *DICER1*, *DROSHA* or *DGCR8*

Leanne de Kock[1] · **Barbara Rivera**[2,4,5] · **William D. Foulkes**[3,4,5,6]

Received: 20 January 2020 / Revised: 19 February 2020 / Accepted: 20 February 2020 / Published online: 2 March 2020

In 2012, we reported a single case of a child with a pineoblastoma and a loss-of-function (LOF) germline *DICER1* pathogenic variant [9]. Unlike all previously studied *DICER1*-related tumours, the second hit in the pineoblastoma was complete loss of the wild-type chromosome 14q (chr14q). Blood-derived cDNA analysis confirmed the absence of RNA product from the mutant allele. We then showed that this was not a unique case—in follow-up studies, all five pineoblastomas with *DICER1* mutations for which tumour tissue was available also exhibited LOF *DICER1* pathogenic variants paired with either another LOF variant or loss of heterozygosity (LOH) [1, 4], and in each case, we were able to show a complete absence of DICER1 protein by immunohistochemistry [1].

Three recent papers in *Acta Neuropathologica* confirm our earlier observations [4, 5, 7]. Combined, of the 41 pineoblastomas found to have alterations in or involving *DICER1*, 22 exhibited biallelic *DICER1* inactivation.

In DICER1 syndrome, the predisposing alteration is most often LOF in nature. The second somatic events in DICER1 lesions are predominantly missense alterations involving one of five amino acid residues in the RNase IIIb domain (Fig. 1) [2]. These so-called "hotspot" mutations have been demonstrated to interfere with catalytic cleavage of one strand of a microRNA hairpin precursor, resulting in altered microRNA expression patterns that are hypothesized to underlie tumour formation. To answer the question of whether the mechanism of *DICER1* alteration in pineoblastoma is truly distinct from other DICER1 syndrome-related tumours, we used a catalogue of all *DICER1* alterations reported in the literature as of December 2019 (updated from [2]) to compare the prevalence of LOH and biallelic inactivation in DICER1 syndrome tumours.

Of 668 tumours found to bear one or more *DICER1* alterations (whether or not DICER1 syndrome related), 59 (9%) had LOH of the wild-type allele, focal deletions of *DICER1*, or homozygous loss of chr14q [i.e. complete loss of at least one *DICER1* allele (hereafter, allele loss)] [Online Resource (OR) 1 and Fig. 1]. The tumours bearing allele loss were comprised of nine different types and prevalence of allele loss in each tumour type ranged from 2 to 67%. Allele loss was most prevalent in pineoblastomas (33/49, 67%), followed by *DICER1*-related central nervous system (CNS) sarcomas (8/35, 23%) (OR1); the prevalence of allele loss in each of these tumour types was statistically significantly greater compared to that in other tumour types combined, Fisher's exact test p values < 0.0001 (OR2A–B).

Among the 350 tumours known to have two *DICER1* alterations, 22 (6%) had an RNase IIIb hotspot mutation coupled with LOH; whereas 269 (77%) bore a hotspot mutation coupled with a LOF alteration—the latter being the classic pattern of mutations observed in DICER1 syndrome (Fig. 1 and OR3). In total, 32/350 (9%) tumours had two hits that truncated or removed the protein, 24 of which exhibited

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00401-020-02139-5) contains supplementary material, which is available to authorized users.

✉ William D. Foulkes
william.foulkes@mcgill.ca

1 Harry Perkins Institute of Medical Research, QEII Medical Centre, Centre for Medical Research, University of Western Australia, Perth, WA, Australia

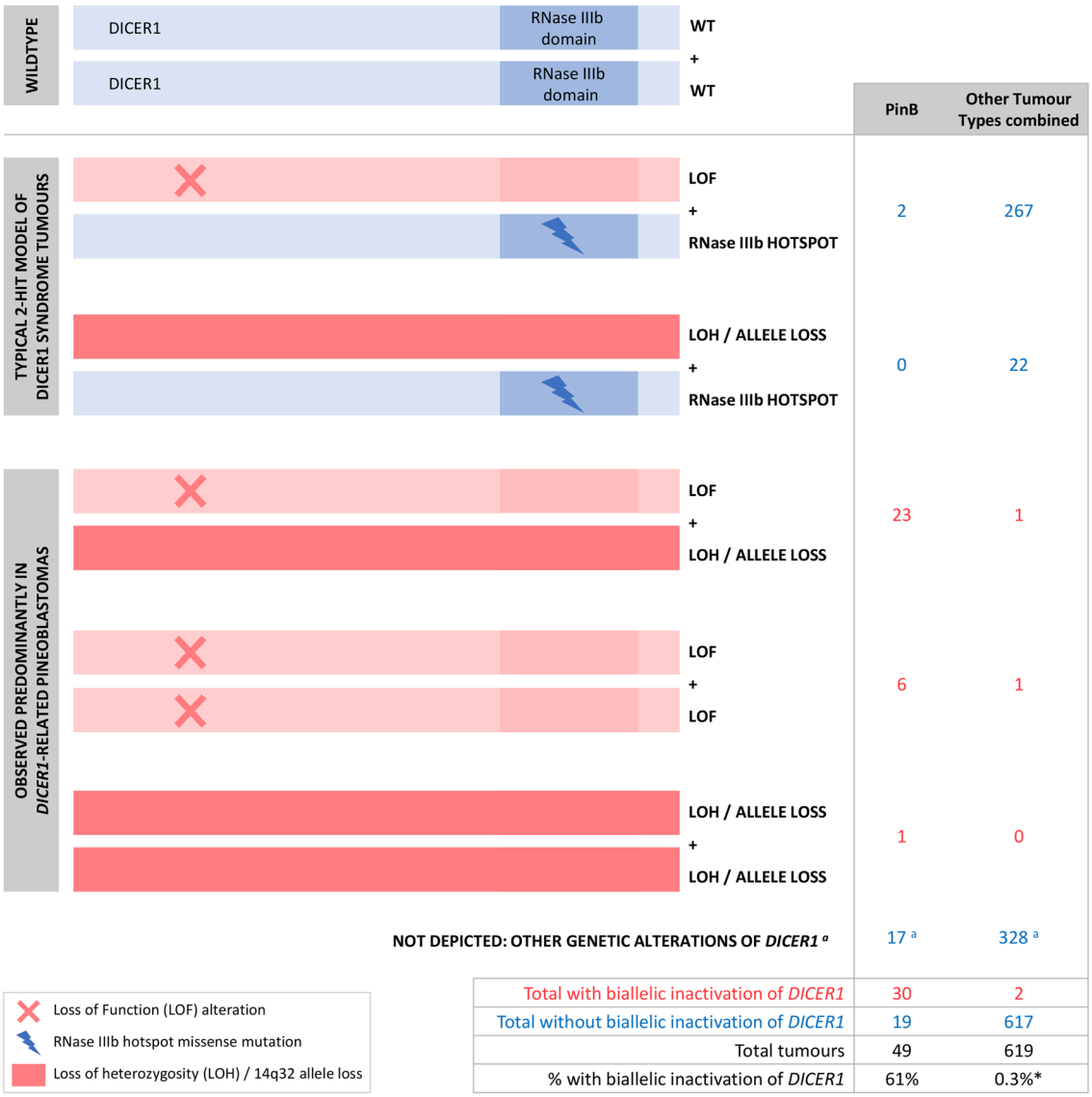
2 Program in Molecular Mechanisms and Experimental Therapy in Oncology (Oncobell), IDIBELL, Hospitalet de Llobregat, Barcelona, Spain

3 Department of Human Genetics, McGill University, Montreal, QC, Canada

4 Gerald Bronfman Department of Oncology, McGill University, Montreal, QC, Canada

5 Cancer Axis, Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, 3755 Côte-Sainte-Catherine Road, Montreal, QC H3T 1E2, Canada

6 Cancer Research Program, Research Institute of the McGill University Health Centre, Montréal, QC, Canada



	PinB	Other Tumour Types combined
WT + WT		
LOF + RNase IIIb HOTSPOT	2	267
LOH / ALLELE LOSS + RNase IIIb HOTSPOT	0	22
LOF + LOH / ALLELE LOSS	23	1
LOF + LOF	6	1
LOH / ALLELE LOSS + LOH / ALLELE LOSS	1	0
NOT DEPICTED: OTHER GENETIC ALTERATIONS OF *DICER1* [a]	17 [a]	328 [a]
Total with biallelic inactivation of *DICER1*	30	2
Total without biallelic inactivation of *DICER1*	19	617
Total tumours	49	619
% with biallelic inactivation of *DICER1*	61%	0.3%*

Fig. 1 Graphic representation of the different combinations of genetic events involving *DICER1* observed in tumours. In the table, the total number of pineoblastomas bearing the depicted biallelic genetic events is indicated, along with that for all other tumour types combined. Superscript a: please refer to Online Resource 1 for a breakdown of the other genetic alterations that are not depicted here. Asterisk: *P* value for the difference between these two percentages is 8.18E−37 (Online Resource 2C)

LOH coupled with a LOF *DICER1* alteration (Fig. 1, OR1 and OR3). DICER1 function was thus majorly compromised or completely abrogated in these 32 tumours, which included 30 pineoblastomas, 1 Wilms tumour [6], and 1 breast carcinoma [12] (see OR2C–D for statistics). There are a further 318 tumours in which only 1 *DICER1* alteration was identified, 256 of which were RNase IIIb hotpot mutation positive [2]. It remains possible that additional allele loss events have gone unrecognized in this set of tumours [10].

The evidence presented herein demonstrates that *DICER1*-related pineoblastomas predominantly exhibit complete *DICER1* loss (30/49 cases, 61%; OR1), often bearing one LOF alteration (dispersed throughout the gene; OR4) coupled with LOH (23/30 cases; Fig. 1) [1, 3–5, 7, 9, 11]. Systematic analysis of the allele fractions contributing to the observed LOH was not performed in most of the published studies. Such analysis was performed by Pugh and colleagues on a set of 15 pleuropulmonary blastomas bearing *DICER1* alterations [8]: 9/15 were copy quiet and 6/15 exhibited copy number gains or losses or copy-neutral LOH, notably, with retention or duplication of the RNase IIIb hotspot mutant allele. These findings suggest that allelic imbalance events in pleuropulmonary blastoma favour the retention of the allele bearing the RNase IIIb hotspot mutation.

Further analyses are needed to determine whether this is also the case for other DICER1 syndrome lesions.

Biallelic inactivation of two other microRNA processing genes, *DROSHA* and *DGCR8*, have been identified in 31 and 6 pineoblastomas, respectively, which too perturb microRNA production [3, 5, 7, 11] (OR5; the prevalence of biallelic inactivation of *DROSHA* in pineoblastoma is statistically significantly greater than in Wilms tumour, a tumour type that frequently features microRNA biogenesis gene mutations, Fisher's exact test p value < 0.00001; The numerical difference between biallelic inactivation of *DGCR8* in pineoblastoma versus Wilms tumour is of borderline significance ($p = 0.0103$)). The pineoblastoma cell-of-origin thus appears uniquely capable of tolerating complete or near-complete absence of microRNAs generated through the canonical pathway.

DICER1-related CNS sarcomas also exhibit increased prevalence of LOH of *DICER1* (typically coupled with an RNase IIIb hotspot mutation)—of note is that only 5/13 reported cases with available matched-normal DNA occurred in the presence of a germline *DICER1* alteration, meaning that most cases are wholly post-zygotic in origin. LOH is significantly more prevalent in tumours bearing biallelic confirmed somatic *DICER1* alterations than in tumours from patients with germline predisposing alterations ($p = 1.6E{-}06$; OR6), possibly explaining the increased prevalence of LOH in *DICER1*-related CNS sarcomas, and, in contrast to pineoblastoma, suggesting an intolerance of their normal cell-of-origin to near-complete loss of microRNA biogenesis. Thus, the order of *DICER1* hits, their provenance, and the tissue of origin all appear to contribute to the likelihood of LOH being the second event in DICER1 syndrome tumours.

These findings illustrate that although allele loss and biallelic inactivation of *DICER1* are infrequent mechanisms of alteration in DICER1 syndrome-related tumours, they do occur with increased prevalence in certain tumour types, particularly pineoblastsoma. An appreciable fraction of pineoblastomas are driven by microRNA processing alterations that cause global microRNA dysregulation. The apparent tolerance of many pineoblastomas for biallelic inactivation of one of the microRNA biogenesis genes presents a unique therapeutic opportunity that could be investigated with the use of a synthetic lethal screen of isogenic pineoblastoma cell lines with and without engineered microRNA biogenesis gene knockouts.

Acknowledgements We thank Drs Anthony Liu, Annie Huang, Bryan Li, David Jones, and Elke Pfaff for providing additional data and clarifications.

Funding WDF was funded by the Canadian Institutes of Health Research, Foundation Grant FDN-148390. BR received the support of a fellowship (LCF/BQ/PI19/11690009) from "laCaixa" Foundation (ID100010434) and from the Mia Neri Foundation and Cassa di Sovvenzioni e Risparmio fra il Personale della Banca d'Italia.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

References

1. 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. https://doi.org/10.1007/s00401-014-1318-7
2. de Kock L, Wu MK, Foulkes WD (2019) Ten years of DICER1 mutations: provenance, distribution, and associated phenotypes. Hum Mutat 40:1939–1953. https://doi.org/10.1002/humu.23877
3. Lee JC, Mazor T, Lao R, Wan E, Diallo AB, Hill NS et al (2019) Recurrent KBTBD4 small in-frame insertions and absence of DROSHA deletion or DICER1 mutation differentiate pineal parenchymal tumor of intermediate differentiation (PPTID) from pineoblastoma. Acta Neuropathol 137:851–854. https://doi.org/10.1007/s00401-019-01990-5
4. Li BK, Vasiljevic A, Dufour C, Yao F, Ho BLB, Lu M et al (2019) Pineoblastoma segregates into molecular sub-groups with distinct clinico-pathologic features: a Rare Brain Tumor Consortium registry study. Acta Neuropathol 139:223–241. https://doi.org/10.1007/s00401-019-02111-y
5. Liu APY, Gudenas B, Lin T, Orr BA, Jr Klimo P, Kumar R et al (2019) Risk-adapted therapy and biological heterogeneity in pineoblastoma: integrated clinico-pathological analysis from the prospective, multi-center SJMB03 and SJYC07 trials. Acta Neuropathol 139:259–271. https://doi.org/10.1007/s00401-019-02106-9
6. Palculict TB, Ruteshouser EC, Fan Y, Wang W, Strong L, Huff V (2016) Identification of germline DICER1 mutations and loss of heterozygosity in familial Wilms tumour. J Med Genet 53:385–388. https://doi.org/10.1136/jmedgenet-2015-103311
7. Pfaff E, Aichmuller C, Sill M, Stichel D, Snuderl M, Karajannis MA et al (2019) Molecular subgrouping of primary pineal parenchymal tumors reveals distinct subtypes correlated with clinical parameters and genetic alterations. Acta Neuropathol 25:1–5. https://doi.org/10.1007/s00401-019-02101-0
8. Pugh TJ, Yu W, Yang J, Field AL, Ambrogio L, Carter SL et al (2014) Exome sequencing of pleuropulmonary blastoma reveals frequent biallelic loss of TP53 and two hits in DICER1 resulting in retention of 5p-derived miRNA hairpin loop sequences. Oncogene 33:5295–5302. https://doi.org/10.1038/onc.2014.150
9. 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. https://doi.org/10.1136/jmedgenet-2012-100898
10. Sakaguchi M, Nakano Y, Honda-Kitahara M, Kinoshita M, Tanaka S, Oishi M et al (2019) Two cases of primary supratentorial intracranial rhabdomyosarcoma with DICER1 mutation which may belong to a "spindle cell sarcoma with rhabdomyosarcoma-like feature, DICER1 mutant". Brain Tumor Pathol 36:174–182. https://doi.org/10.1007/s10014-019-00352-z
11. Snuderl M, Kannan K, Aminova O, Dolgalev I, Heguy A, Faustin A et al (2015) Mb-17 * novel candidate oncogenic drivers in pineoblastoma. Neuro-Oncology 17:iii23. https://doi.org/10.1093/neuonc/nov061.93

12. Yates LR, Gerstung M, Knappskog S, Desmedt C, Gundem G, Van Loo P et al (2015) Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 21:751–759. https://doi.org/10.1038/nm.3886