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Somatic *DICER1* mutations in adult-onset pulmonary blastoma

To the Editor:

Several rare lung tumours morphologically mimic embryonal structures of the developing human lung. Historically, these blastomatous tumours were described under the umbrella term of pulmonary blastoma. Subsequently, distinct entities were recognised, such as childhood pleuropulmonary blastoma (PPB) [1] (International Classification of Diseases for Oncology (ICD-O-3) code 8973/3). Later, adult-onset pulmonary blastoma was separated into well-differentiated fetal adenocarcinoma (WDFA) (ICD 8333/3) and pulmonary blastoma (ICD 8972/3) [2]. Pulmonary blastoma is a biphasic epithelial and mesenchymal malignancy, whereas PPB is purely sarcomatous and WDFA is characterised by a monophasic immature epithelium. Since 1988, the childhood PPB has received particular attention because of 1) its unique developmental progression from relatively indolent neonatal-onset lung cysts, to aggressive cystic-solid and solid sarcomas by age 72 months; 2) PPB's status heralding a newly recognised familial tumour predisposition syndrome and 3) its strong association with both germ-line and somatic *DICER1* mutations, which is not only true for PPB, but also for many other tumours in pleiotropic predisposition syndrome (now referred to as DICER1 syndrome). Until recently, neither WDFA nor pulmonary blastoma had been observed in families manifesting DICER1 syndrome [3]. However, in 2015, we identified a second somatic *DICER1* RNase IIIb mutation [4] in a WDFA that arose in a 16-year-old germ-line *DICER1* mutation carrier [5]. In addition to *DICER1* mutations in PPB and WDFA, somatic *CTNNB1* mutations (encoding β-catenin) appear to characterise WDFA and pulmonary blastoma [6], and are far less frequent in PPB [7, 8]. In contrast, *TP53* mutations are found in both PPB [8] and pulmonary blastoma [9], but not WDFA [9].

Given the original pathological grouping of WDFA, PPB and pulmonary blastoma and the existence of partially-overlapping molecular abnormalities in these lesions, as described above, we questioned whether pulmonary blastoma might also be characterised by *DICER1* mutations. We therefore analysed *DICER1* status in one infant and two adult pulmonary blastomas by Sanger sequencing and/or targeted capture followed by next-generation sequencing.

The study was approved by the Institutional Review Board of the Faculty of Medicine of McGill University (A12-M117-11A) and was performed with full informed patient or parental consent. Tumours were reviewed by pathologists at the referring institutions and by central reference pathologists (D. Bouron-Dal Soglio and V-H. Nguyen). We obtained peripheral blood DNA from cases 1 (adult onset) and 3 (infant onset); formalin-fixed paraffin embedded (FFPE) tumour tissue from all cases; FFPE non-tumourous lung tissue from case 2 (adult onset); and snap-frozen tumour tissue from case 3.

Sanger sequencing and/or Fluidigm Access Array-based next-generation sequencing (Fluidigm, San Francisco, CA, USA) was used to screen for coding mutations and mutations located near the exon–intron

boundaries in tumour genomic DNA (gDNA), as described previously [10]. Constitutional DNA was subsequently used to determine the germ-line or somatic origin of the identified mutations by PCR amplification of the region of interest followed by Sanger sequencing. DNA and RNA were extracted from FFPE samples as previously described [11].

We PCR amplified exon 3 of *CTNNB1* using the following three primer pairs and a previously-described Touchdown PCR programme [12]: *CTNNB1*_1_F: 5′-ATGGAACCAGACAGAAAAGCG-3′ and *CTNNB1*_1_R: 5′CAGGATTGCCTTTACCACTCA-3′; *CTNNB1*_2_F: 5′TTTGATGGAGTTGGACATGG-3′ and *CTNNB1*_2_R: 5′CAGGACTTGGGAGGTATCCA-3′; *CTNNB1*_3_F: 5′TTTGATGGAGTTGGACATGG-3′ and *CTNNB1*_3_R: 5′GAAGGACTGAGAAAATCCCTGTT-3′. Primer pairs were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) and UCSC *in silico* PCR software (http://genome.ucsc.edu/cgi-bin/hgPcr?command=start) was used to ensure yield of a single product. Sequencing was performed by the McGill University and Genome Quebec Innovation Centre (MUGQIC) using conventional Sanger sequencing methods. Sequences were analysed visually using Lasergene Version 10 (DNASTAR; Madison, WI, USA).

Immunohistochemistry (IHC) for β-catenin and DICER1 was performed on deparaffinised 4-µm tissue sections using a Ventana automated slide scanner (Ventana, Inc., Tucson, AZ, USA), following the manufacturer's instructions. The anti-DICER antibody ab14601 (Abcam, Cambridge, MA, USA) was used at a 1:50 dilution and the rabbit monoclonal anti-β-catenin antibody (Epitomics, Burlingame, CA, USA) was used at a 1:100 dilution.

We acquired three cases of pulmonary blastoma (figure 1a–i): The first was diagnosed following a history of dyspnoea in a 29-year-old female of Austrian, Lebanese, English and Jewish descent. The patient underwent a gross surgical resection, but within 3 weeks, recurrent disease was detected on computed tomography (CT) in the lower right anterior chest, centred in the pleural space. Eight cycles of combination chemotherapy with cisplatin, doxorubicin and cyclophosphamide was implemented. Despite treatment, on CT, her chest mass appeared bi-lobar with one region having decreased in size, while another had enlarged. No evidence of metastatic disease was detected. At the time of writing, additional surgical resection was under consideration. There is no family history of DICER1 syndrome-like features. The second pulmonary blastoma was diagnosed in a 25-year-old female from Catalonia, Spain, who presented with cough and haemoptysis [13]. Information on treatment and follow-up have been described by Bosch–Barrera *et al.* [13]. The third pulmonary blastoma occurred in a 3-month-old child from the Basque region of Spain, who had tachypnoea and minor respiratory distress. The tumour was fully resected and the patient was treated with four cycles of combination chemotherapy consisting of carboplatin and etoposide phosphate, which was well-tolerated. Her family history is unremarkable.

The entire coding region of *DICER1* was screened for mutations in tumour gDNA. We identified a predicted-truncating somatic *DICER1* mutation (c.1668_1668delC; p.I557Sfs*5) and a typical RNase IIIb somatic hotspot *DICER1* mutation (c.5125G>A; p.D1709N) in case 1 (figure 1j–l). Similarly, a nonsense somatic mutation (c.1232C>A, p.S411*) and a RNase IIIb somatic hotspot mutation (c.5425G>A; p.G1809R) were identified in case 2 (figure 1m–o). Screening of constitutional DNA confirmed the somatic origin of the identified mutations. No likely pathogenic *DICER1* mutations were found in the third (infant) pulmonary blastoma case. We also screened the three patients' tumours for exon 3 *CTNNB1* mutations and identified a somatic mutation in case 1 (c.110C>T, p.S37F) and case 2 (c.98C>G, p.S33C) (figure 1l and o). In accordance, the two adult tumours exhibited multifocal aberrant epithelial nuclear/cytoplasmic β-catenin positivity on IHC staining (figure 1g and h). Aberrant cytoplasmic β-catenin positivity was also observed in mesenchymal cells of case 1 (data not shown).

Pulmonary blastoma is a rare subtype of sarcomatoid carcinoma and exhibits a biphasic histological pattern consisting of malignant mesenchyme and epithelium, which resemble fetal lung [2]. The *DICER1* RNase IIIb hotspot missense mutation identified in each of the adult-onset pulmonary blastomas is typical of somatic mutations identified in other DICER1-associated tumours. Both hotspot mutations have been shown to interfere with the production by DICER1 of 5p miRNAs consequent to the substitution of a key metal-ion binding amino acid within the catalytic RNase IIIb domain (in the case of p.D1709N), or close proximity to such a site (in the case of p.G1809R). Furthermore, the presence of a truncating *DICER1* mutation coupled with the RNase IIIb hit supports the two-hit model of tumourigenesis observed in other DICER1-related lesions. The identification of these characteristic *DICER1* mutations in two cases strongly suggests that adult-onset pulmonary blastoma is associated with somatic, but not germ-line mutations in *DICER1*. Some pulmonary blastomas (and WDFAs) contain morular structures consisting of squamoid nests with optically clear nuclei (figure 1e, g and h). Sekine *et al.* [6] noted that these morular formations are associated with the presence of somatic *CTNNB1* mutations. Consistent with these prior observations, cases 1 and 2 contained both morules and a *CTNNB1* mutation. Case 3 lacked both phenomena. The identified p.S37F and p.S33C *CTNNB1* mutations have been previously reported to occur in lung cancers and result in the substitution of a serine residue at a glycogen synthase kinase (GSK)-3β phosphorylation

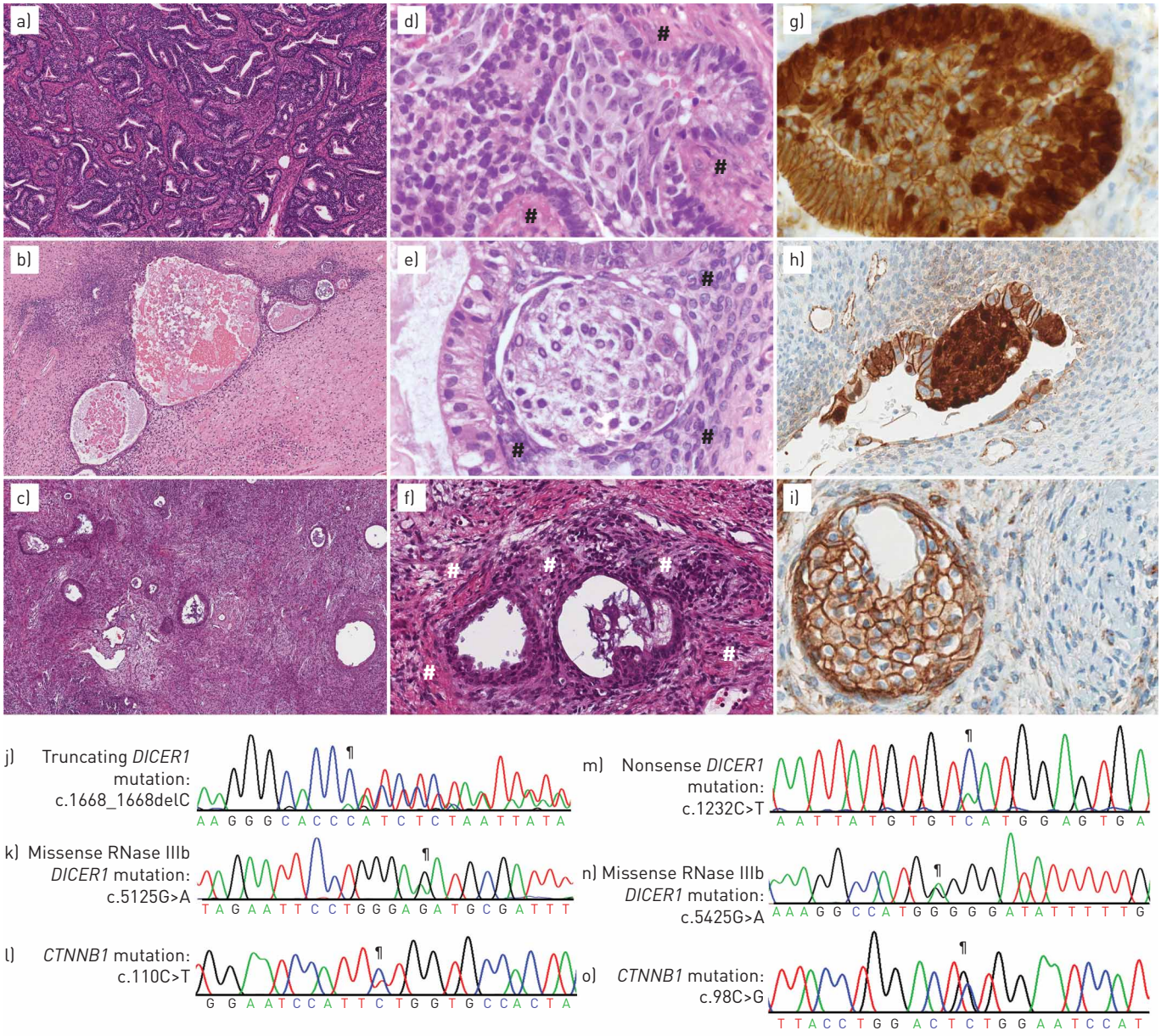

FIGURE 1 a–i) Haematoxylin and eosin staining. a–f) The epithelial glandular formations of pulmonary blastoma populate a stroma displaying variable cellularity (case 1: a and d (pre-chemotherapy); case 2: b and e (post-chemoradiotherapy); case 3: c and f (pre-chemotherapy)). Stromal compartments are shown (#) in d–f. g–i) β-catenin immunohistochemical staining: g and h) The epithelium of the two adult-onset pulmonary blastomas (case 1 (g) and case 2 (h)) displayed mostly membranous, but also multifocal cytoplasmic and nuclear immunohistochemical positivity for β-catenin. h) A morular formation exhibiting a nest of epithelial cells with optically clear nuclei. i) The epithelium of the infant pulmonary blastoma (case 3) displayed membranous β-catenin positivity. j–l) Chromatograms showing somatic mutations identified in case 1 as marked (¶): j) the predicted-truncating *DICER1* mutation, c.1668_1668delC; k) *DICER1* RNase IIIb mutation, c.5125G>A; and l) the *CTNNB1* c.110C>T mutation. m–o) Somatic mutations identified in case 2: m) the nonsense *DICER1* mutation, c.1232C>T; n) *DICER1* RNase IIIb mutation, c.5425G>A; and o) the *CTNNB1* mutation, c.98C>G. The wild-type sequence is provided below each chromatogram.

site, which stabilises β-catenin and constitutively activates the WNT signalling pathway [6]. The relative importance of *DICER1* mutations compared with activation of the WNT-pathway in the causation of pulmonary blastoma is unknown.

This is the first report of *DICER1* mutations in pulmonary blastoma. A single WDFA has been found to harbour a germ-line *DICER1* mutation [5] coupled with a somatic RNase IIIb hotspot mutation [4]. Prior to this study, PPB, pulmonary blastoma and WDFA were distinguished on morphological, immunohistochemical and molecular grounds. Our findings suggest that despite certain morphological distinctions, these diseases may have some overlapping molecular profiles that support the older pathological rubrics in which they were grouped. The similarities and differences should be explored further. New molecular data have shown that all

three tumours possess either germ-line or somatic mutations in *DICER1*, and these mutations are likely to be deleterious and functionally important. We conclude that pulmonary blastoma presenting later in life can be associated with somatic *DICER1* mutations. Further studies will be required to determine the generalisability of our findings, but it seems likely that *DICER1* mutations will be found to be important drivers of both pulmonary blastoma and WDFA, as well as PPB.

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We identified two somatic *DICER1* mutations in each of two adult pulmonary blastomas, implicating *DICER1* in causation http://ow.ly/10aM9V

Leanne de Kock[1,2], Ismaël Bah[3], Joan Brunet[4], Harriet Druker[5], Itziar Astigarraga[6,7], Joaquim Bosch-Barrera[8], Dorothée Bouron-Dal Soglio[9], Van-Hung Nguyen[10], David Malkin[11], John R. Priest[12] and William D. Foulkes[1,2,13,14]
[1]Dept of Human Genetics, McGill University, Montreal, QC, Canada. [2]Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, Montreal, QC, Canada. [3]Dept of Pathology, McGill University Health Centre, Montreal, QC, Canada. [4]Dept of Medical Oncology, Hereditary Cancer Program, Catalan Institute of Oncology, Hospital Josep Trueta, Girona, Spain. [5]Division of Genetic Counselling and Haematology/Oncology, The Hospital for Sick Children; Dept of Molecular Genetics, The University of Toronto, Toronto, ON, Canada. [6]Servicio de Pediatria, Bio Cruces Health Research Institute, Hospital Universitario Cruces, Barakaldo, Bizkaia, Spain. [7]Departamento de Pediatria, Universidad del Pais Vasco UPV/EHU, Spain. [8]Dept of Medical Oncology, Catalan Institute of Oncology, Doctor Josep Trueta University Hospital, Girona, Spain. [9]Dept of Pathology, CHU-Sainte Justine and University of Montreal, Montréal, QC, Canada. [10]Division of Pediatric Pathology, The Montreal Children's Hospital, McGill University Health Centre, Montreal, QC, Canada. [11]Division of Haematology/Oncology, The Hospital for Sick Children; Depts of Pediatrics and Medical Biophysics, The University of Toronto, Toronto, ON, Canada. [12]Minneapolis, Minnesota, USA. [13]Dept of Medical Genetics, Research Institute of the McGill University Health Centre, Montreal, QC, Canada. [14]Program in Cancer Genetics, Department of Oncology and Human Genetics, McGill University, Montreal, QC, Canada.

Correspondence: William D. Foulkes, Dept of Medical Genetics, Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, 3755 Cote St. Catherine Road, Montreal, QC, Canada, H3T 1E2. E-mail: william.foulkes@mcgill.ca

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