Cell-free RB1 DNA not detected in the blood of pseudoretinoblastoma patients

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ABSTRACT

Cell-free DNA (cfDNA) is commonly found in the blood (plasma) of patients with cancer. When analysing cfDNA for a specific cancer-causing mutation, it is referred to as ctDNA. RB1 ctDNA is commonly present in the blood of retinoblastoma patients. We examined RB1 ctDNA from blood of 40 children with retinoblastoma to look alike lesions (‘pseudoretinoblastoma’) to determine if any RB1 abnormalities could be identified.

Objectives Because retinoblastoma diagnosis is usually made with the indirect ophthalmoscope without biopsy clinical errors continue to occur worldwide. Because cf RB1 is detectable in plasma of children with retinoblastoma, we wondered if it was present in the blood of pseudoretinoblastomas with the hope of ultimately developing a blood based test to aid clinicians in the diagnosis of retinoblastoma. The goal of this project was to see if circulating plasma RB1 cfDNA could be detected in the blood of patients with pseudoretinoblastoma.

Methods and analysis Plasma cfDNA for circulating RB1 cfDNA was assayed with MSKCC’s next generation sequencing, N.Y. State Approved assay called ACCESS to evaluate somatic mutations in 40 patients with pseudoretinoblastoma.

Results No plasma cfDNA RB1 was detected in the blood (plasma) of 40 patients with pseudoretinoblastoma.

Conclusion Plasma cfDNA RB1 is commonly detectible in retinoblastoma patients but not in patients with a diverse group of pseudoretinoblastomas.

INTRODUCTION

Cell-free DNA (cfDNA) is now a clinical tool used to aid the diagnosis, prognosis and management of diverse cancers.1 We have demonstrated that RB1 cfDNA is commonly detected in the plasma of retinoblastoma patients before treatment and in the one patient studied the level increased after a month without treatment.5 Following enucleation or intraarterial chemotherapy, it becomes undetectable.2 3

The diagnosis of retinoblastoma is usually made with the indirect ophthalmoscope and sometimes aided with ultrasound and MRI.4 Biopsy is not done because of the concern for spreading the cancer. A diverse group of intraocular lesions that resemble retinoblastoma (but are not cancer) simulate retinoblastoma and accurate diagnosis at times is very difficult and errors continue to be made resulting in inappropriate surgery and even removal of eyes in children who do not have cancer.5–7 In addition, RB1 abnormalities have been reported in a variety of other conditions including rhabdomyosarcoma.8

Tumour-specific plasma cfDNA is now being used in adult and paediatric solid cancers for diagnosis, differential diagnosis, prognosis, monitoring of response and detection of tumour mutations. We have shown that RB1 fragments (ctDNA) are commonly (60%–80%) present in the blood (plasma) of children with newly diagnosed retinoblastoma.2 9 10 The purpose of this study was to see if RB1 fragments are detectible in the plasma of patients with pseudoretinoblastoma.

METHODS

The cfDNA was analysed with hybridisation capture and next-generation sequencing in blood (plasma) using MSKCC’s analysis of circulating cfDNA to evaluate somatic status11 in 40 patients with diverse pseudoretinoblastoma lesions. This technique interrogates 129 established cancer mutations and because the buffy coat is simultaneously analysed germline defects and clonal haematoipoiesis are filtered out in the results. Children with
pseudoretinoblastoma like lesions that had been referred to our centre for a possible diagnosis of retinoblastoma were included.

**Patient involvement**

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

**RESULTS**

Table 1 depicts/shows that for 40 patients with pseudoretinoblastoma, plasma *RB1* cfDNA was not detectable in any patient.

**DISCUSSION**

The diagnosis of retinoblastoma is usually made with the indirect ophthalmoscope and often ultrasound and MRI are performed. There are more than 30 conditions that have been described that mimic the appearance of retinoblastoma and they are referred to as ‘pseudoretinoblastomas.’ The most common ‘pseudoretinoblastomas’ are Coats’ disease, persistent fetal vasculature syndrome, retinal detachment, ocular toxocariasis and other infectious, genetic and even traumatic lesions (table 1).1

Despite the assistance of ultrasound and MRI, the differential diagnosis of retinoblastoma remains a challenge and errors are still made with children’s eyes receiving inappropriate surgery including vitrectomy, retinal detachment repair and enucleation.5–7 In addition, some eyes with retinoblastoma are not enucleated because the clinician misdiagnosed them as benign conditions. The true, modern incidence of these errors is not known because few centres are eager to publish on their errors in diagnosis, but recent publications suggest an inappropriate enucleation rate in some centres of 11%–24%.5–7

MSKCC developed a next generation sequencing assay for 129 cancer-related genetic abnormalities and one of these genes is *RB1*. All exons of *RB1* are interrogated.

In this study, we assayed for cfDNA of *RB1* and in all cases no *RB1* fragments were detectable. These assays were done simultaneously with true retinoblastoma samples (which did detect *RB1* abnormalities) and occasionally other, non-*RB1* abnormalities giving us additional confidence that the assay was accurate. This suggests that finding *RB1* cfDNA in the blood of a child with suspected retinoblastoma is not a pseudoretinoblastoma. Whether this test can be used as an aid in the differential diagnosis of retinoblastoma will depend on additional analyses of sensitivity and specificity in a larger cohort of both retinoblastoma and pseudoretinoblastoma eyes, but neither this study nor any prior published studies have demonstrated *RB1* abnormalities in pseudoretinoblastomas.

**Contributors**

Conceptualisation, DHA and JHF; Data curation, DHA, MR and JHF; Formal analysis, DHA, DM, MR and JHF; Funding acquisition, DHA and JHF; Investigation, DHA, LJ, MFB and JHF; Methodology, DHA, DM, ARB, LJ and JHF; Project administration, DHA, ARB and JHF; Resources, DM and ARB; Software, DM and MFB; Supervision, DM and ARB; Validation, DM, ARB and MFB; Visualisation, DHA and JHF; Writing—original draft, DHA, MR and JHF; Writing—review and editing, DHA, DM, ARB, LJ, MFB, MR and JHF. All authors have read and agreed to the published version of the manuscript. Guarantor, DHA.

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**Competing interests**

LJ is a consultant or advisor board member for Apexigen, Astra-Zeneca, Bristol-Myers Squibb/Celgene, Day One, Fennec, QED and Roche. MFB declares research funding from Grail; personal fees from Roche and PetDX; and a provisional patent for systems and methods for detecting cancer via cfDNA screening (PCT/US2019/027548).

**Patient and public involvement**

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication**

Not applicable.

**Ethics approval**

This study involves human participants and was approved by an Ethics Committee or Institutional Board. Informed consent was obtained from all subjects (or their legal guardian) using MSKCC Institutional Review Board Number 12-245. Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**Data availability statement**

Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as online supplemental information.

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REFERENCES
1 Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. *N Engl J Med* 2018;379:1754–65.
2 Abramson DH, Mandelker D, Francis JH, et al. Retrospective evaluation of somatic alterations in cell-free DNA from blood in retinoblastoma. *Ophthalmol Sci* 2021;1:100015–7.
3 Francis JH, Gobin YP, Brannon AR, et al. *RB1* Circulating Tumor DNA in the Blood of Patients with Unilateral Retinoblastoma: Before and after Intra-arterial Chemotherapy. *Ophthalmol Sci* 2021;1:100042.
4 Dimaras H, Corson TW, Cobrinik D, et al. Retinoblastoma. *Nat Rev Dis Primers* 2015;1:15021.
5 Kaliki S, Taneja S, Palkonda VAR. Inadvertent intraocular surgery in children with unsuspected retinoblastoma: a study of 14 cases. *Retina* 2019;39:1794–801.
6 Yang Y, Sun T, Cao B, et al. Pseudoretinoblastoma of 9 enucleated eyes simulating retinoblastoma in 70 enucleated eyes. *Int J Clin Exp Pathol* 2017;10:9475–81.
7 Chuah CT, Lim MCC, Seah LL, et al. Pseudoretinoblastoma in enucleated eyes of Asian patients. *Singapore Med J* 2006;47:617–20.
8 Zhu B, Davie JK. New insights into signalling-pathway alterations in rhabdomyosarcoma. *Br J Cancer* 2015;112:227–31.
9 Kothari P, Marass F, Yang JL, et al. Cell-Free DNA profiling in retinoblastoma patients with advanced intraocular disease: an MSKCC experience. *Cancer Med* 2020;9:6093–101.
10 Abramson DH. Cell free DNA (cfDNA) in the blood of retinoblastoma patients the Robert M. Ellsworth lecture. *Ophthalmic Genet* 2022;12:1–5.
11 Rose Brannon A, Jayakumaran G, Diosdado M, et al. Enhanced specificity of clinical high-sensitivity tumor mutation profiling in cell-free DNA via paired normal sequencing using MSK-ACCESS. *Nat Commun* 2021;12.