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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 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 detectible 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 somaticmutations 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.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Mutant *RB1* is commonly detectable in the plasma of children with newly diagnosed retinoblastoma. Few cases of pseudoretinoblastoma have been similarly studied.

WHAT THIS STUDY ADDS

⇒ No mutant *RB1* cell free DNA (cfDNA) was detected in a diverse group of pseudoretinoblastomas.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Analysis of *RB1* cfDNA in plasma may be useful in the non-invasive differential diagnosis of retinoblastoma. Additional research is needed to determine the true sensitivity and specificity of cfDNA for the diagnosis of retinoblastoma.

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.[2] 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 status[11] 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 haematopoiesis are filtered out in the results. Children with

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Table 1 Detection of plasma *RB1* cell-free DNA (cfDNA) in pseudoretinoblastoma lesions

Diagnosis	No of cfDNA cases	# or Number positive for *RB1*
Coats disease	15	0
PHPV/PFV	2	0
Retinal detachment	3	0
Vitreous haemorrhage	2	0
Iris pigmentation	2	0
Embryonal rhabdomyosarcoma	1	0
Alveolar rhabdomyosarcoma	1	0
Medulloepithelioma	1	0
Small round blue cell tumour/Iris	1	0
Retinoma	2	0
Hamartoma RPE/astrocytic	1/1	0
Coloboma	1	0
Melanoma/choroid (child)	1	0
Iris cyst	2	0
Nevus/choroid (child)	1	0
Cataract (child)	1	0
FEVR	2	0

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.

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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.

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