Unusual Clinical Behavior of a Very Late Retinoblastoma Relapse in a Patient with a Germline RB Mutation

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Abstract

Retinoblastoma is the most common ocular tumor of childhood with cure rates exceeding 95%. Patients with high-risk features typically relapse within 3 years of diagnosis. We report a patient with low-risk bilateral retinoblastoma who suffered systemic relapse after eight years. His disease at first relapse was chemosensitive without PET avidity or bone marrow disease following therapy. Six months later, he experienced an isolated CNS relapse and succumbed to refractory disease. "Oncoseq" exome sequencing confirmed the presence of germline RB mutation among all tissues as well as somatic changes which may provide insights into the biology of relapse and tumor.

Introduction:

Retinoblastoma is the most common pediatric intraocular malignancy, with an incidence of 1 per 15,000–20,000 live births [1]. Retinoblastoma is highly curable with survival rates exceeding 95% utilizing therapies including systemic chemotherapy, intra-arterial chemotherapy, and local therapies (e.g. focal laser), and in advanced cases, eye enucleation [1,2]. High-risk features for extra-ocular spread include tumor invasion of the post laminar optic nerve, choroid, anterior segment or sclera with relapses occurring with 30 months from diagnosis and 22-24 months from the time of enucleation [1,3, 4].

We report the case of a patient with bilateral retinoblastoma diagnosed at the age of two months, who suffered a widespread extraocular relapse 8 years following initial systemic chemotherapy and local therapy. Despite a dramatic response to his first cycle of retrieval chemotherapy, he ultimately succumbed to refractory CNS disease. Targeted exome sequencing of tissue from his original tumor and relapse tumor, revealed somatic mutations which may add to the understanding of tumor dormancy.

Case Report:

A two-month-old African American male presented with leukocoria and was diagnosed with group B (IIRC) bilateral retinoblastoma harboring a germline intronic *RB1* mutation (Table 1A). He received 6 cycles of systemic chemotherapy on the Children's Oncology Group (COG) ARET0331 protocol with carboplatin, vincristine, and focal laser therapy. Due to disease progression, his right eye was enucleated at the age of 12 months. The histopathology of the enucleated eye did not show high-risk features (e.g. choroid, anterior chamber, optic nerve or scleral involvement). He did not receive additional therapy post enucleation.

He remained in remission until 9 years of age when he presented with vague wrist pain. His physical exam demonstrated minimal soft tissue swelling of the wrist. His complete blood count (CBC) was normal and

a lactate dehydrogenase (LDH) level was 271 U/L. He was evaluated by rheumatology, diagnosed with idiopathic arthritis and started on naproxen therapy with pain relief.

Three months later, he developed worsening leg pain waking him from sleep. His physical exam showed a 2 cm left axillary lymph. His hemoglobin was 10.9 gm/dL, platelets 136,000/mm^3, LDH > 6000 U/L (undiluted) and uric acid 7.4 mg/dL (Figure 1A). Bone marrow evaluation demonstrated diffuse tumor infiltration by metastatic retinoblastoma confirmed by immunophenotyping and synatophysin staining. Cytogenetics demonstrated a complex abnormal karyotype including gains of chromosome 1q, 2p, 6p, and 13q32-34.

A PET/CT scan demonstrated diffuse bony FDG uptake as well as uptake in the liver, left axillary and infraclavicular regions (Figure 1D). A brain/orbits MRI did not show evidence of CNS disease. He was reclassified as stage 4A disease.

He began treatment per the COG ARET0321 protocol for metastatic retinoblastoma (Figure 1B) with the intention to ultimately proceed to stem cell transplant. His first treatment cycle was complicated by tumor lysis syndrome leading to acute renal failure requiring hemodialysis.

Tumor surveillance after the first cycle, demonstrated no evidence of bone marrow disease and a PET/CT showed no abnormal FDG uptake except for mild uptake in the left axillary node. His LDH which had peaked at 22,331 U/L, normalized to 275 U/L (Figure 1A). Following the third cycle of chemotherapy, he continued to have no evidence of residual tumor. Stem cell transplantation was postponed due to the development of cisplatin-induced thrombotic microangiopathy (TMA).

Eight months following his initial relapse, he developed left eye deviation. A MRI (Figure 1F) demonstrated a right cerebellar mass (confirmed to be retinoblastoma following a gross total resection) with spinal metastasis. Metastatic disease restaging did not demonstrate evidence of tumor outside of the CNS.

He received craniospinal proton radiation therapy (40 Gy) with daily carboplatin and weekly vincristine (Figure 1B) Due to worsening TMA, further systemic chemotherapy was stopped. He then received intra-Ommaya topotecan, intrathecal topotecan and metronomic chemotherapy with vinblastine and oral cyclophosphamide. After four weeks, he expired due to progressive disease. At autopsy, CNS involvement by retinoblastoma was confirmed without evidence of systemic or bone marrow disease.

Discussion:

In the management of retinoblastoma, identifying high-risk features is important, as a failure of treatment can lead to a 24% risk of metastasis. [5-9]. Conversely, relapse in low-risk patients is rare with five-year event free survival (EFS) of >95% [10].

At our institution over the past 25 years, 4 additional patients with high-risk features, developed extraocular metastatic retinoblastoma within one year of diagnosis (Table 1B). An observational study involving 519 patients, noted that 402 patients with low-risk features did not develop metastatic disease [9], which makes this case unique as he had low risk disease at presentation.

The marked LDH level in our patient (Figure 1A) reflected the increased proliferative index of his tumor. LDH is an oxoreductase that is important in generating ATP under states of hypoxia. The rapid proliferation of the tumor likely contributed to its chemotherapy sensitivity and the development of tumor lysis syndrome and acute renal failure during the first cycle of retrieval chemotherapy and achieved a near complete remission (minimal residual FDG uptake in an axillary lymph node) (Figure 1B, D&E).

To characterize the genomic landscape and tumor progression in this patient, targeted sequencing was performed on the eye (September, 2009), bone marrow (February, 2018), and brain (October, 2018) (Table 1A). The patient's normal DNA from the eye tissue revealed a germline pathogenic variant in the RB1 tumor suppressor gene, and in all three tumors biallelic inactivation of RB1, acquired by somatic inactivation of the remaining allele through copy-neutral LOH of RB1. While the initiation of tumorigenesis in retinoblastoma begins with RB1 inactivation, additional genetic alterations may be required for continued tumor growth. A set of high-confidence somatic mutations and copy number changes were present in each of the three tumor

samples [Figure 1C, Table 1A] Similar to many pediatric neoplasms including retinoblastomas [11,12], the overall somatic mutation burden in these tumors was low and all the mutations were non-recurrent with unknown functional impact. Aside from RB1, recurrent gene mutations are rare in retinoblastoma [12,13]. Copy number analysis of the primary cancer, biopsied in 2009, demonstrated copy neutral LOH of 13q and losses on 3q. Two metastatic samples, biopsied in 2018, shared these events in the primary cancer, with additional gains on chromosomes 1q, 2p, 6p, 12, and 15. The brain biopsy showed further gains of chromosome 19 and copy loss on 17p (Figure 1C). This is consistent with prior reports of several highly recurrent copy number alterations in retinoblastoma, such as gain of 1q, 2p, 6p, and loss of 13q and 16q [12,13].

Cancers may recur after decades of latency. This phenomenon may be due to an acquired ability of cancer cells, possibly brought on by the stress of chemotherapy, to manipulate the tumor microenvironment and evade the immune system, metastasizing to sanctuary tissues and enter a state of cellular senescence where they are metabolically active, but do not proliferate until stimulated to "reawaken" and divide [14-17]. This may explain the late relapse in our patient.

Of the mutations found [Table 1A], *CYP21A2, CD276, FANC2D* , are associated with PolyPhen scores predicting that they may be pathogenic. Interestingly, these three have been previously reported in tumor migration, invasion, and cell cycle control [18-26], and may have played a role in this patient's tumor dormancy.

The observed mutational changes support the notion that due to the low burden of somatic mutation, the mechanism of tumorigenesis/evasion may be related to another mechanism, however, the observed mutations should not be dismissed and their role in tumor dormancy should be further investigated.

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Supplement:

METHODS

Patient enrollment

Sequencing of clinical samples was performed under our Institutional Review Board (IRB)–approved studies at the University of Michigan (Michigan Oncology Sequencing Protocol, MI-ONCOSEQ, IRB # HUM00046018, HUM00067928, HUM00056496).

Integrative Clinical Sequencing

Two archival formalin-fixed paraffin-embedded (FFPE) samples and a frozen bone marrow sample were obtained for sequencing. A section of FFPE blocks were cut for evaluation, and remaining portions of each

specimen were retained for nucleic acid extraction. Hematoxylin and eosin (H&E)-stained sections were reviewed by a board certified pathologist to identify areas with highest tumor content and an area of normal tissue. Tumor genomic DNA and total RNA from the frozen bone marrow were extracted using the AllPrep DNA/RNA/miRNA kit (QIAGEN). DNA from FFPE samples was extracted using the DNeasy Blood and Tissue Kit (QIAGEN), and total RNA was extracted using the miRNeasy FFPE kit (QIAGEN). RNA integrity was measured on an Agilent 2100 Bioanalyzer (Agilent Technologies).

Integrative clinical sequencing was performed in a Clinical Laboratory Improvement Amendments (CLIA) compliant sequencing lab as described before (Refs 4-5). Paired-end target-captured exome libraries from tumor and normal samples, and tumor transcriptome libraries were sequenced using the Illumina HiSeq2500. Aligned exome and transcriptome sequences were analyzed to detect somatic mutations, copy-number alterations, gene fusions, and gene expression as described before (Ref 4).

Figure legends:

Figure 1: Panel A: LDH trend following his first relapse. Normal LDH levels were recorded at his previous annual visits (9/15/16 and 11/13/17). At the time of first relapse (2/5/18), the LDH level was >6000U/L (undiluted) which reached a maxiumum level of 22,331 U/L and subsequently normalized after recovery following his first cycle of chemotherapy using cisplatin, cyclophospamide, vincristine and etoposide.

Panel B: Summary of the timeline of clinical clinical events and medical management.

Panel C: Copy number assessment was done by targeted exome sequencing of matched tumor and normal samples with chromosomal changes at the different stages of relapse compared to the initial tumor. The most notable copy number aberration was uniparental disomy (UPD) of chromosome 13. Germline RB1 mutation in combination with UPD 13 resulted in biallelic inactivation of RB1. On array CGH, there was aneuploidy with additional chromosomal alterations during disease progression.

Panel D: Abnormal FDG uptake on PET/CT scan demonstrating diffuse bony involvement and uptake in liver, left axillary and infraclavicular regions.

Panel E: After one cycle of chemotherapy, demonstrating no abnormal FDG uptake except for minimal left axillary uptake.

Panel F: 3 x 3.2 x 2.5 cm mass lesion in the posteromedial aspect of the right cerebellar hemisphere with associated edema and mass effect

Table 1a: Epigenetic mutations observed at initial diagnosis and relapses with their corresponding PolyPhen-2 score. Tumor sequencing using "OncoSeq" for whole exome sequencing (WES) and transcriptome (RNA seq) sequencing revealed among all three samples (eye, bone marrow, brain metastases), the germline mutation in the RB1 gene (NM_000321.2: c.1961-2A>G) involving chromosome 13, intron 19. The WES did not demonstrate any driver fusions or mutations among the three samples but acquired somatic mutations of uncertain significance.

Table 1b: Summary of the past twenty-five-year experience for relapsed extraocular retinoblastoma at Children's Hospital of Michigan.

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Table 1a

Genetic Mutations at Diagnosis and Relapse with their corresponding PolyPhen Score

	Gene Involved	Chromosomal Location (GRCh37)	Mutation	PolyPhen-2 Score
Germline	RB1	chr13:49033822	NM_000321.2: c.1961-2A>G	
Eye	FANCD2	chr3:1010547	NM_033084.4: c.1829T>G, p.Val610Gly	0.996
	NR3C1	chr5:142689683	NM_000176.2: c.1447C>G, p.Gln483Glu	0.989
	SKP2	chr5:36163803	NM_005983.3: c.337T>C, p.Cys113Arg	0.799
Bone Marrow	CYP21A2	chr6:31973557	NM_000500.7: c.92T>C, p.Leu31Pro	0.990
	CD276	chr15:73996733	NM_025240.2: c.635_636delTGinsAT, p.Leu212His	0.995
Brian	PRSS3	chr9:33750687	NM_007343.3: c.173C>G, p.Ala58Gly	0.000
	FANCD2	chr3:10105477	NM_033084.4: c.1829T>G, p.Val610Gly	0.996
	PKHD1	chr6:51712632	NM_138694.3: c.8048G>A, p.Gly2683Asp	0.128
	TAF1L	chr9:32631644	NM_153809.2: c.3934G>A, p.Val1312Ile	Unable to Predict

Table 1b: Children's Hospital of Michigan Relapsed Retinoblastoma Over The Past 25 Years

Patient	Age at Diagnosis	Initial Therapy	Disease	Histopathology Features of Enucleated Eye(s)	Time to relapse from diagnosis	Site of relapse	Outcome
1	10 months	Carboplatin/Etoposide/ Thermo laser/Enucleation	Bilateral Retinoblastoma	Exophytic tumor involving the retina and extending to both sides of the iris and ciliary body including the peripheral angles of the anterior chamber, Schlemm's canal and the posterior chamber. No tumor involving the optic nerve, choroid or sclera.	2 years	Bone Marrow	Alive post SCT
2	3 years	Carboplatin/Cyclophosphamide/ Vincristine/Enucleation	Unilateral Retinoblastoma	Involvement of the anterior chamber as well as extension into the optic nerve	3 months	Bone Marrow	DOD
3	2 years	Carboplatin/Etoposide/ Enucleation/	Unilateral Retinoblastoma	Predominantly endophytic growth with extensive tumor filling the entire vitreous cavity with complete retinal detachment. There is prelaminar invasion of the optic nerve with no involvement of the anterior chamber.	8 months	Systemic + Orbit	DOD
4	2 years	Carboplatin/Etoposide/ Vincristine/Cyclophosphamide/ Enucleation	Bilateral Retinoblastoma	Left eye: Exophytic and endophytic tumors involving the retina with retinal detachment. Tumor extends into the optic nerve and into the vitreous. No evidence of invasion of the choroid or anterior segment. Right eye: Exophytic and endophytic tumor involving the optic nerve with retinal detachment. There is invasion into the choroid and destruction of the ciliary body.	1 year	Orbital only	Alive post salvage chemo + radiotherapy
Current patient	2 months	Carboplatin/Vincristine/ Thermo Laser/Enucleation	Bilateral Retinoblastoma	No involvement of the anterior chamber, choroid or optic nerve	8 years	Systemic	DOD