Chapter 42 – Retinoblastoma

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Retinoblastoma is an uncommon malignant ocular tumor of childhood, occurring in 1 : 18 000 live births.[] Late diagnosis globally results in up to 70% mortality; where optimal therapy is accessible, more than 95% of children are cured. An integrated team approach of clinical specialists (ophthalmologists, pediatric oncologists and radiotherapists, nurses, geneticists) with imaging specialists, child life (play) specialists, parents, and others is an effective way to manage retinoblastoma. National guidelines can bring the whole health team up to developed standards and set the stage for audits, studies, and clinical trials to continuously evolve better care and outcomes.[9] The tumor(s) arises from embryonic retinal cells so the majority of cases occur under the age of 4 years. Primary treatments include enucleation and chemotherapy with laser and cryotherapy. Patients with a constitutional mutation of the RB1 tumor suppressor gene are at increased life-long risk of developing other cancers, which is increased with exposure to radiation (Figs 42.1 and 42.2).[3,4] Therefore, radiation is no longer a primary therapy to save an eye, and screening for extraocular and trilateral retinoblastoma is performed with MRI and ultrasound, not CT scan.

The study of retinoblastoma has been seminal in the understanding of cancer in general. Studies of retinoblastoma have revealed that hereditary and non-hereditary tumors are initiated by the loss of both alleles of the tumor suppressor gene, RB1,[5,6] The existence of specific genes that act to suppress cancer was predicted from clinical studies of retinoblastoma.[7,8] The RB1 gene was the first tumor suppressor gene to be cloned,[5] and has been found to have a critical role in many types of cancer.

Fig. 42.1 (A) Family tree: mother was cured of bilateral retinoblastoma by enucleation of one eye and external beam radiation of the other eye. Forty-two years later, she developed metastatic hemangiosarcoma in the path of the exit beam of radiation (red*). Both children were delivered at 36 weeks’ gestation to facilitate early treatment of tumors and developed bilateral tumors. Mother and both children carry a germline RB1 mutation (M1, deletion of ATTTC starting at bp 778, reading to a STOP, 9 codons away) that results in no pRB when the normal RB1 allele is lost (M2) from a developing retinal cell, initiating a tumor. (B) RetCam® images: prior to treatment, right eye (IIRC group A, more than 1.5 mm from optic disk) of the boy at 3 months, showing two tumors; stable right eye of boy age 4 years after laser, two cycles of CEV (carboplatin, etoposide, and vincristine) with cyclosporin A chemotherapy, and more laser treatments. (C) RetCam® images: prior to treatment, left eye (IIRC group B, tumor less than 3 mm from fovea) of the girl at 2 months; laser scar and new tumor above nerve at 4 months of age; recurrence in original scar extending toward fovea, with tumor vascularization showing on fluorescein angiography; flat scars at age 2.5 years after laser, two cycles of CEV with cyclosporin A chemotherapy to control recurrence threatening vision chemotherapy and more laser.
(Images by Leslie MacKee, Cynthia VandenHoven and Carmelina Trimboli.)
Pathogenesis of retinoblastoma

Heritable and non-heritable retinoblastoma

All children with retinoblastoma tumors in both eyes (bilateral) have an RB1 gene mutation on one of their chromosomes (13) that predisposes them to develop retinal tumors in infancy and other cancers throughout life (see Figs. 42.1 and 42.2). While 90% have no family history of retinoblastoma and are the first affected in their family with a new germ line mutation, 50% of their offspring will inherit the mutant RB1 gene and develop tumors. Most children without a family history with retinoblastoma in only one eye have normal constitutional RB1 alleles, but the eye tumor(s) loses both functional alleles, similar to hereditary tumors. Fifteen percent of persons who had unilateral retinoblastoma have constitutional RB1 mutations that can be transmitted to their offspring. Molecular and clinical genetics is an integral part of the management of all families affected by retinoblastoma.

Loss of both RB1 alleles induces retinoblastoma

The observation that the children with bilateral retinoblastoma tend to be diagnosed at a younger age than those with non-heritable retinoblastoma led to Knudson's prediction that two mutational events were required to initiate retinoblastoma tumors. His analysis suggested that in the presence of a predisposing constitutional mutation a single second mutation in one developing retinal cell initiated tumor development (heritable retinoblastoma), but both alleles were mutated in the single developing retinal cell in non-heritable unilateral retinoblastoma. The two events could be mutations of both alleles of a gene that would “suppress” tumor formation in the retina. The chance of losing the second RB1 allele from developing retinal cells with only one normal RB1 allele is sufficiently high that multiple tumors are common in hereditary retinoblastoma (see Fig. 42.1). However, it is virtually impossible for children without constitutional RB1 mutations to lose both alleles from several retinal cells so they develop only one, unilateral tumor (Fig. 42.3), and tend to be diagnosed at an older age than children with hereditary retinoblastoma.

Function of the retinoblastoma protein

The product of the RB1 gene (pRB) is a 110 kDa phosphoprotein that interacts with many proteins in the regulation of the cell cycle, differentiation, and control of genomic stability. DNA tumor viruses that induce cancer, such as human papilloma virus, do so in part by binding to pRB through the “pocket” region of pRB.
in the RR for leukemia.\[11\] Although pRB is key to all cycling cells, its function in development is highly tissue-specific. A subset of developing retinal cells may be uniquely dependent on pRB in order to differentiate terminally into adult, functioning retina. Loss of pRB promotes genomic changes and instability, leading to further mutations in oncogenes and other tumor suppressor genes that result in a retinal tumor.\[12,13\]

**Spectrum of RB1 mutations**

The majority of RB1 mutations are unique to each family, and are distributed throughout the RB1 gene with no real hot spots.\[9\] Sensitive mutation identification requires determination of the copy number of each exon and the gene promoter to reveal large deletions and duplications, sequencing for point mutations, examination of the mRNA to confirm or detect intronic mutations altering exon splicing, and assay for the methylation status of the promoter in tumor samples (Fig. 42.4). Application of these techniques, combined with a retinoblastoma-specific focused expertise in interpreting the data, identifies over 95% of the RB1 mutations\[9,14\] (see Figs 42.1–42.4).

**Other manifestations of RB1 mutant alleles**

Mutation of RB1 also predisposes to benign retinal tumors, retinoma,\[15\] ectopic intracranial retinoblastoma (trilateral retinoblastoma),\[16,17\] and second non-ocular malignancies.\[18,19\]

**Retinoma**

A retinoma is a non-malignant manifestation of the RB1 mutation.\[15\] Three features characterize these non-progressive lesions: an elevated grey retinal mass, calcification, and surrounding retinal pigment epithelium (RPE) proliferation and pigmentation (Fig. 42.5). These features are also seen after radiation treatment for retinoblastoma. If documented in childhood, which is very rare, retinoma is a quiescent tumor that has not progressed to malignancy. Occasional cases occur where a retinoma progresses to active retinoblastoma. However, retinoma commonly underlies active retinoblastoma and can be discovered on pathologic examination of an enucleated eye.\[12\] A distinctive feature is fleurette formation and absence of proliferative markers. Both RB1 alleles are mutant in the retinoma and genomic instability is detectable, which progresses in degree and number of genes involved in the adjacent highly proliferative retinoblastoma.\[12\] Discovery of retinoma on retinal examination of a relative of a patient with retinoblastoma indicates that they carry the RB1 mutant allele (see Fig. 42.5).
The most accurate way to predict who in a family will develop retinoblastoma is to test them for the precise RB1 gene mutant allele found in the proband. In the absence of precise knowledge of the RB1 gene mutant allele in tumor or blood, the empiric risk for the relatives of retinoblastoma patients to be affected can be estimated. Offspring of patients with a family history of retinoblastoma or bilateral tumors have a 50% risk of inheriting the mutant allele and a 45% risk of developing retinoblastoma, due to incomplete penetrance. When two affected children are born to apparently normal parents, one parent must be carrying but not expressing the mutant allele. Hence, there is also a 45% risk that any subsequent child born will develop retinoblastoma. The risk that other relatives have inherited the mutant allele depends on the number of intervening “apparently normal” individuals, each of which have a 10% chance of carrying but not expressing the mutant allele. The risk falls by a factor of 0.1 for each intervening unaffected generation. When two affected children have a risk of developing retinoblastoma or bilateral tumors have a 50% risk of inheriting the mutant allele and a 45% risk of developing retinoblastoma, due to incomplete penetrance. When two affected children are born to apparently normal parents, one parent must be carrying but not expressing the mutant allele. Hence, there is also a 45% risk that any subsequent child born will develop retinoblastoma. The risk that other relatives have inherited the mutant allele depends on the number of intervening “apparently normal” individuals, each of which have a 10% chance of carrying but not expressing the mutant allele. The risk falls by a factor of 0.1 for each intervening unaffected generation.

Timely and sensitive molecular diagnosis of RB1 mutations has a strong positive effect on quality of outcomes: early treatment of retinoblastoma achieves lower risks and better health outcomes, allows families to make informed family-planning decisions, and costs less than conventional surveillance. The savings when at risk children avoid repeated examinations substantially exceeds the one-time cost of molecular testing. Moreover, health care savings continue to accrue as succeeding generations avoid the unnecessary examinations and often do not need molecular analysis because their parents do not carry the family’s mutant allele.

The RB1 mutations usually result in unstable or absent protein. Such mutations show high penetrance (> 95% of offspring affected) and expressivity (average of seven tumors per child). More uncommon RB1 mutations cause lower penetrance and expressivity. In “in frame” deletions or insertions that result in a stable but defective pRB, promoter mutations that result in a reduced amount of otherwise normal protein, and splice mutations that may be additionally altered by unlinked “modifier genes.”