

The Broiling Soup of Retinoblastoma Genetics

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Abstract

Right from the time, two hit hypothesis of retinoblastoma was described to the present advancements in the field of genetics involving retinoblastoma; there has been a plethora of information available. The retinocytoma-retinoblastoma phenotype is attributed to the mutations occurring in RB gene locus; is a well known fact. Recently there has been add on researches on the same. The role of epigenetics and the role of genomic imprinting (selective expression of a gene) is new. This article tries to summarize the overwhelming information available in the field of genetics in retinoblastoma. It tries to clear the basic concepts about the hereditary retinoblastoma and it's various aspects. Also an attempt has been made to make the concept of maternal imprinting more clear in the article.

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Introduction

Retinoblastoma is the most common intraocular malignancy in childhood¹ with an incidence of 1 in 16000 live births.² It is a prototype of hereditary cancers and the paradigm of the “two hit hypothesis” by Alfred Knudson,1971.³ Cavaneer proved knudson's work and confirmed the recessive nature of the disease.⁴ In 1986, the gene locus of retinoblastoma was identified to be on chromosome 13. This made RB gene to be the first tumor suppressor gene to be cloned.⁵ The knowledge about the genetics involved in the disease has been growing since then by leaps and bounds. Targeted gene therapy for patients at risk and prenatal diagnosis in the familial cases will totally change today's perspective of retinoblastoma. Therefore its important to update our genetic and phenotypic understanding of the disease along with the clinical understanding.

Basic Concepts

RB1 gene is a tumor suppressor gene whose encoded protein represses the cell cycle progression at the G1 checkpoint. Broadly the mutations of the RB1 gene can be classified into germline mutations and somatic mutations (Table 1) (Figure 2).⁶ The term familial retinoblastoma is used only for those with a positive family history; therefore, germline/hereditary retinoblastoma is not synonymous with familial retinoblastoma. Rather, only 6%-10% of hereditary cases are familial (Figure 1).

Inheritance Pattern and Phenotype in Retinoblastoma

The offspring of the parent with germline mutation inherits a heterozygous state of the RB allele (RB+/RB-). This causes a high risk of development of the retinoblastoma as the probability of second hit is 90-95%. This results in loss of heterozygosity and leads to tumor development. A parent with germline RB1 mutation has a 50% chance of passing it to offspring. Therefore, the inheritance of the high risk genotype (RB+/RB-) is similar to autosomal

dominant diseases. However, for the disease to manifest phenotypically; biallelic inactivation in any one of the retinoblast is necessary.

Shields JA,⁷ reported the risk of retinoblastoma in familial and sporadic cases. In a patient with positive family

Germline mutations	Somatic Mutations
<ul style="list-style-type: none"> • Mutations in germ cells. 	<ul style="list-style-type: none"> • Mutations in any tissue other Than germ cells e.g. retinal Precursor cells.
<ul style="list-style-type: none"> • Heritable form Transferred to 50% of the offspring as the(RB+/-) trait 	<ul style="list-style-type: none"> • Never inherited as somatic cells Are not passed to the offspring
<ul style="list-style-type: none"> • 45% 	<ul style="list-style-type: none"> • 53%
<ul style="list-style-type: none"> • All cells of the offsprings carry an inactive allele of RBI from the parent and need one more "hit" • Early presentation (12 months). Multifocal and bilateral tumors. • Susceptible to secounday tumor Development (risk 25%) 	<ul style="list-style-type: none"> • First "hit" during Embryogenesis or preimplantaion and second hit occurs in the same retinoblast leter in life • Late Presentations (1-2 years of age) Single, Unilateral tumor. • No risk of secondary cancers

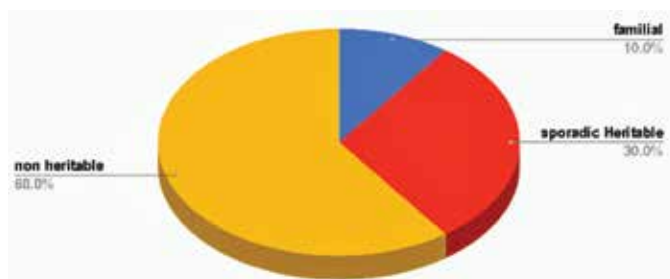


Figure 1: Pie chart depicting percentage distribution based on inheritance

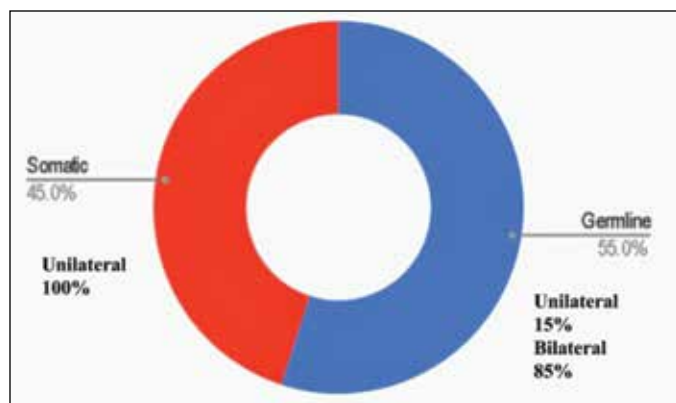


Figure 2: Percentage distribution of somatic and germline mutations in retinoblastoma

Category	Features ¹¹
Familial (10%)	<ul style="list-style-type: none"> • RBI gene is inherited from an affected parent. • All cells of the body contain RBI gene mutation • Heritable
Sporadic Heritable (30%)	<ul style="list-style-type: none"> • The parent is unaffected (negative family history) • Parents develops a new germline mutation and transmits it to the offspring • All cells of the offspring contain the mutation • Heritable
Non Heritable (60%)	<ul style="list-style-type: none"> • Biallelic inactivation in a single retinoblast after conception. • Non heritable

histor, 40% of the siblings and 40% of the offspring are at risk of developing retinoblastoma. In sporadic cases with unilateral retinoblastoma; the risk in siblings and offspring is 1% and 8% respectively. In sporadic bilateral cases the risk in siblings and offspring in 6% and 40% respectively (Table 2).

Tumor Initiation and Progression

The two hits are imperative for development of the tumor. The first hit / mutation (M1) is the germline or somatic mutations like deletions, nonsense mutations, missense mutations, frameshift, splicing mutations and rarely by large rearrangements of the chromosome⁸. The second hit (M2) can occur with any of the former mechanisms or viral inactivation but it mostly occurs by mechanisms causing loss of heterozygosity (LOH). It is an integral process of tumor initiation and accounts for genesis of 50-70% of recessive trait tumors like retinoblastoma. Uniparental disomy, chromosomal nondisjunction, mitotic recombination are some mechanisms leading to LOH.

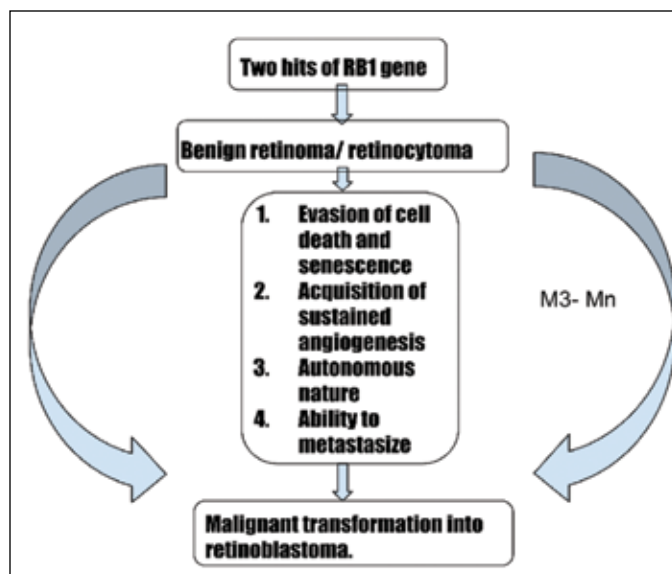


Figure 3: Flowchart depicting the journey from retinocytoma to retinoblastoma

Retinocytoma Vs Retinoblastoma (Phenotypic Selection)

The mutations and the gene involved in both the retinocytoma (benign) and retinoblastoma (malignant) are similar; yet what determines the benign expression of tumor in a subset of patients needs to be understood. Biallelic RB1 inactivation imparts limitless replicative potential to the retinal precursor cells. This leads to a preneoplastic stage and the cells get arrested in a stage of retinoma/retinocytoma. The conversion to the malignant retinoblastoma depends on the rate of acquisition of further necessary "hits" (M3, M4...Mn) (Figure 3).⁹

Demaris et al¹⁰ examined eyes enucleated for RB for underlying retinoma and showed the first evidence of progression of senescent retinoma to retinoblastoma. A senescence protein (p16INK4A) is capable of arresting the proliferative cells at the G1 phase of the cell cycle. This protein is overexpressed during early stages of the retinoma and its depletion leads to progression to retinoblastoma. Some cells manage to escape the senescence and clonally progress to retinoblastoma.¹¹ Coevolution of MED4/RB1 gene locus proves to be protective against large deletions of the RB1 gene locus. Hence MED4 can be considered an essential survival gene (survival gene hypothesis)

Concept of Penetrance in Retinoblastoma

Penetrance of a genetic mutation tells about the percentage of individuals with the mutation manifesting the disease. Penetrance is said to be complete if all the individuals who have the disease causing mutation have clinical symptoms. In incomplete penetrance, some individuals do not express the disease (carriers) (Table 3).

Genomic Imprinting In Retinoblastoma (New Concept)

Genomic imprinting is an epigenetic phenomenon that causes

Table 3: Classification of Retinoblastoma based on penetrance

High Penetrance	Very common
	<ul style="list-style-type: none"> • Penetrance is 90%. If a patient with retinoblastoma transmits the mutation to 50% of its offspring, 45% of them will develop retinoblastoma • Involves nonsense mutations causing complete truncation of proteins.
Low Penetrance	<ul style="list-style-type: none"> • Benign forms (retinoma) and unilateral are more common • Missense mutations involving partial inactivation of the protein, late onset of the mutations in less proliferative stages

genes to be expressed in a parent of origin specific manner. Recent data has revealed that the tumor suppressor gene RB1 on chromosome 13 is preferentially expressed from the mother. The paternal chromosome on the contrary possesses a weaker promoter compared to that of the maternal chromosome. Therefore there is a strong evolutionary selection for maternal inhibition of cell proliferation.¹²

Loss of maternal imprinting represents a novel mechanism for RB1 pathway inactivation.¹³

It can therefore be hypothesized that when the mutated RB gene comes from the father, the penetrance is low, as the excessive cell proliferation is taken care of by the imprinted maternal RB gene. But maternally inherited mutated RB gene leads to high penetrance as the paternal RB gene is evolutionary weak. Further genetic studies are however needed to prove this hypothesis.

Mosaicism

When RB1 mutation occurs after the one cell stage division has progressed, only a fraction of cells express the RB+/- trait. Such individuals are labelled a mosaic. Blood DNA testing may give a false negative test report in parental mosaicism and declare the future sibling of the affected patient to be falsely risk free. Allele specific PCR is more specific in the diagnosis of mosaicism.

Epigenetics And Retinoblastoma (Another Frontier)

“EPI” means above and therefore epigenetics means (above genetics) designates events which modify gene expression in a heritable way without inducing any structural changes.¹⁴ These changes are hypermethylation, microRNA regulation, histone modification and ATP dependent chromatin reorganisation. The role of epigenetics in retinoblastoma was suggested by Zhang et al.¹⁵ These epigenome modifications can be modulated by chemicals, nutrition, environmental pollution and aging.

Therefore, rather than being a single causation, retinoblastoma consists of a “genomic landscape”.¹⁶ It ranges from RB1 inactivation, multiple genetic hits, imprinting and various epigenetic mechanisms that impact the malignant potential to the tumor and is responsible for the variable phenotypic presentation.

Retinoblastoma Without RB1 Gene Inactivation

Rushlow et al found 2.7% of retinoblastoma arise without any RB1 mutation. 52% of these cases were found to have an amplification of MYCN.¹⁷ These mutations lead to unilateral retinoblastoma with aggressive nature. It is often missed due to less calcifications and is not heritable.

13 q Deletion Syndrome

Nearly 5%–6% of all Retinoblastoma are associated with deletion of chromosome 13.¹⁸ Children with 13q deletion syndrome have characteristic features like a dysmorphic facies (thick anteverted earlobes, broad forehead, prominent philtrum, short nose and a thick everted lower lip), psychomotor retardation, cardiac and brain anomalies and ocular malformations like microphthalmos, coloboma, and congenital cataract.¹⁹ Identifying these unique features in a child helps in reducing the financial burden associated with the routine DNA sequencing. Also screening of systemic anomalies assumes an important role once the 13 q syndrome is confirmed.

Trilateral Retinoblastoma

Bader et al. reported bilateral RB with pineoblastoma in 1980 and termed it as “trilateral RB”.²⁰ Recently pineoblastoma is referred to as “primary neuroectodermal tumor” or PNET. The incidence is 5%–13%, and it is particularly high in familial RBs.²¹ Therefore cases of familial retinoblastoma should be screened with an MRI to rule out neuroblastoma, the prognosis however is dismal.

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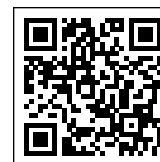
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