Sriwijaya Journal of Ophthalmology
Journal Homepage: https://sriwijayaopthalmology.com/index.php/sjo

Molecular Genetics Mutations of Retinoblastoma

Mutia Arnisa Putri[1*]

[1]Department of Opthalmology, Faculty of Medicine, Universitas Sriwijaya/Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

ARTICLE INFO

Keywords:
Retinoblastoma
RB1 gene
Bilateral
Unilateral
MYCN oncogen

Corresponding author:
Mutia Arnisa Putri

E-mail address:
mutiaarnisa@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/sjo.v4i2.50

ABSTRACT

Retinoblastoma is a cancer that arises because both copies of the *RB1* gene that normally suppresses retinoblastoma are lost from a developing retinal cell in fetuses, babies, and young children. Retinoblastoma is the prototype genetic cancer in one or both eyes of young children, most retinoblastomas are initiated by bial¬lelic mutation of the retinoblastoma tumor suppressor gene, *RB1*, in a developing retinal cell. All those with bilateral retinoblastoma have heri¬table cancer, although 95% have not inherited the *RB1* mutation. Non¬heritable retinoblastoma is always unilateral, with 98% caused by loss of both *RB1* alleles from the tumor, whereas 2% have normal *RB1* in tumors initiated by amplification of the *MYCN* oncogene. A rare subset of retinoblastoma is initiated by somatic amplification of the *MYCN* oncogene in a predisposing retinal cell.[1] The retinoblastoma protein (pRB), encoded by *RB1*, is an important transcription factor.[2]

1. Introduction

Retinoblastoma is a cancer that arises because both copies of the *RB1* gene that normally suppresses retinoblastoma are lost from a developing retinal cell in fetuses, babies, and young children. Retinoblastoma can affect one (unilateral) or both eyes (bilateral).[1] Retinoblastoma is the most common childhood intraocular ma¬lignancy that affects one or both eyes.[2] Because of the strong links between clinical care and genetic causation, retinoblastoma is considered the prototype of heritable cancers.[3] The ratio of unilateral:bilateral disease is 60:40 with familial disease occurring in ~4-5% or less of patients. The average annual incidence of retinoblastoma was 5.8 per million children under the age of 10 years and 10.9 per million under 5 years of age.[6] There is a trend for worsening survival with increasing age at diagnosis through 2 years of age but no statistically significant difference in survival between children with unilateral and bilateral disease.4 Worldwide, about 8000 children are newly diagnosed with retinoblastoma ev¬ery year (1/16,000 live births), but most have no access to knowl¬edge of the important role genetics plays in many aspects of retino¬blastoma.

Retinoblastoma (RB) is a rare embryonic neoplasm of retinal origin and the most common intraocular tumour in children, with 3% incidence of all paediatric tumours and a frequency averaging 1:20.000 live born in different populations. It has good prognosis if diagnosed early but it is life-threatening when diagnosed late.5 In 98% of patients, retinoblastoma is caused by bi-allelic inactivation of the *RB1* tumor suppressor gene on chromosome 13q14. This *RB1* inactivation can be somatic (tumor-specific), due to a germline mutation or deletion accompanied by somatic inactivation of the remaining allele, or can arise during early embryogenesis leading to post-zygotic mosacism.

Similar to many pediatric neoplasms, the overall somatic mutation burden in retinoblastomas is very low, and only a small subset of retinoblastomas have been identified to harbor additional recurrent mutations involving the BCOR and CREBBP transcriptional regulatory genes. Approximately 2% of retinoblastomas do not harbor *RB1* alterations, and instead are driven by focal high-level amplification of the *MYCN* oncogene.6 We analyzed the *RB1* gene for mutations within the exons and splice junctions in patients with retinoblastoma in order to determine the range of mutations.[3]

Retinoblastoma and Gene Mutations

The location of the predisposing gene on chromosome 13q was suggested by observations made during the early 1960s that large deletions involving chromosome 13q14 were associated with retinoblastoma and other abnormalities. No one knows what really causes the genomic damage to the *RB1* gene, but retinoblastoma arises at a constant rate in all races irrespective of local environment.[1] Both, hereditary and nonhereditary retinoblastomas are nearly always initiated by mutation of the same tumor suppressor gene, the *RB1* gene.[2]

***RB1* gene**

The *RB1* gene resides on chromosome 13q14 and encodes the retinoblastoma protein (pRB), an important regulator of cell division cycle in most cell types, and the first tumor suppressor gene discovered. Normally, cell division is inhibited by hypo¬phosphorylated pRB binding to E2F molecules and blocking their transactivation of *RB1*, E2F, and other promoters of molecules that support cell division. To resume cell division, cyclin-dependent kinases re-phosphorylate pRB, activating promoters of key proteins important in cell division. Loss of pRB therefore allows uncontrolled cell division. In many cell types, loss of the *RB1* gene is compensated by increased expression of other related proteins.[1]

In addi¬tion to loss of *RB1*, specific alterations in copy number of other genes are common in *RB1*-/- retinoblastoma. There are gains (4–10 copies) in oncogenes *MDM4, KIF14 (1q32), MYCN* (2p24), *DEK*, and *E2F3* (6p22) and loss of the tumor suppressor gene *CDH11* (16q22-24). Other less common genomic alterations in retinoblastoma tumors include differential expression of specific microRNAs, recurrent single nucleotide variants/insertion-de¬letions in the genes BCOR and *CREBBP*,39 and upregulation of spleen tyrosine kinase (*SYK*).[1]

RB1 inactivation has been implicated in more than 97% of all RB cases with mutations in this gene being undetectable in the remaining case. Recent reports suggest that other genes may play a role in either driving tumor initiation or progression. It has been postulated that probable candidate genes may be located in chromosomal regions with recurrent and losses observed in RB tumors. Rushlow et al provided evidence that retinoblastoma could also be caused by *MYCN* oncogene amplification and predicted that 18% of cases who are diagnosed with non-familial unilateral RB before the age of 6 months would harbour only *MYCN* amplification and no *RB1* mutations. They also quoted another 1.5% of unilateral non-familial RB whose pathogenesis could not be explained as they harboured normal *RB1* and *MYCN* genes.[5]

As soon as both copies of *RB1* are inactivated, the immature retinal transitional cells become genetically unstable and uncontrollably proliferative. During this stage, the proliferation is countered by the senescence protein p16INK4A. If the proliferating cells respond to the action of p16INK4A, the tumor stops its proliferation and gets arrested as retinoma. On the other hand, if the genetic instability in the abnormal retinal cell takes the upper hand (increase in KIF14 and E2F3 levels), it counters and overtakes the cellular senescence and become malignant. Normal or slightly lower expression of P27 and p57 may not be able to counter the proliferative capability of the very high expressing oncogenes (KIF14 and E2F3). By nullifying the activity of p53 induced cell death (overexpressing MDM2), the retinal tumor cells escape cell death and progress.[7]

pRB Structure

pRB is divided into four regions, the N terminus,

the A and B domains separated by a spacer region, and the C terminus. The disruption of growth suppression by pRB requires the A and B domains as well as portions of the C-terminal domain. The nuclear c-Abl protein binds to the C terminus of pRB, promoting proliferation. The C terminus is required for growth suppression along with the A and B domains. The A/B region of pRB binds to the LXCXE (Leucine-X-Cysteine-X-Glutamic acid, where X is any amino acid) epitope of the viral oncoproteins.[2]

Table 1. Some of the proteins that bind pRB, selected to show the diversity of functional classes that interact with pRB

Fuction	**pRB-binding proteins**
CDK Inhibitor	P21
Chromatin modulator	BRG1
Corepresessor	RBBP8, RBBP4, RBBP1, HBP1
Cyclin	CCNDI1,2,3, CCNA1, CCNB1, CCNC
Deacetylase	SIRT1
E3 ligase	SKP2, APC/C
Histon deacetylase	HDAC1,2,3
Histon demethylase	RBP2
Methyltransferase	DNMT1
Molecular chaperone	HSP75
Nuclear matrix component	Lamin A, P84
Phosphatase	PP-1a2
Pol I transcription factor	UBF
Pol II transcription factor	TAFII250
Pol II transcription factor	TFIIIB
Regulator of p53 stability	MDM2, MDM4
Replication licensing factor	MCM7
Ser/Thr kinase	CDK1, CDK2
Signaling molecule	Raf-1
Transcription factor	ATF-2, c-Jun, c-Myc, N-Myc, C/EBP, E2F1-3, Elf-1, hBRM, ID2, MyoD, Myogenin, SPI1, SP1, Pax-3, PHox, Chx10, RIZ
Tumor suppressor	Prohibitin, BRCA 1
Tyrosine kinase	c-Abl
Viral oncoprotein	E1A, E7, Tag
Viral transcription factor	EBNA-5, HCMV IE2

***MYCN*-Amplified Retinoblastoma**

Approximately 1% of unilateral tumors are not initiated by mutation in *RB1*, but by amplification of the *MYCN* oncogene. N-Myc, encoded by the *MYCN* gene, is a transcription factor and chromatin regulator homologous to the commonly activated oncogene, c-Myc. *MYCN* amplification was first detected in the childhood nervous system tumor neuroblastoma, in which it is associated with a poor prognosis. *MYCN* genomic gain or amplification in retinoblastoma can occur as a secondary event after *RB1*mutation, but it is overrepresented in unilateral retinoblastoma.[2]

E2F Protein Families in Cell Cycle Progression

The E2F-binding site (TTTSSCGC, where S is C or G) has been identified in promoters of genes encoding S-phase-regulatory proteins such as DNA polymerase α, thymidylate synthase (TS), proliferating cell nuclear antigen (PCNA), and ribonucleotide

reductase (RR), and cell-cycle progression genes such as those encoding cyclin A, cyclin E, CDK1 and B-Myb. The E2F1 and *RB1* genes are also regulated by E2F activity, suggesting the existence of an autoregulatory loop in the RB/E2F pathway. pRB mutants that bind free E2F but do not repress transcription are unable to block S-phase entry.[2]

2. Discussion

The retinoblastoma gene (*RB1*) gene located on chromosome 13q14, consists of 27 exons. A wide spectrum of heterogeneous *RB1* gene variants that includes – single nucleotide variations (SNVs), small insertions/deletions (InDels) and structural variations (SVs) had been reported in RB patients. Some of the variants such as nonsense and frameshift are associated with bilateral RB, while other types have unilateral RB or milder phenotypic expression8. It is a tumor suppressor gene and in it's absence, chromosomal aberrations accumulate leading to tumor initiation, progression, and ultimately metastasis. About one third of RB tumours are hereditary and bilateral, with a median age of one year at diagnosis. It is caused by an *RB1* constitutional mutation (M1) on one allele followed by a somatic *RB1* mutation, on the other allele (M2), leading to loss of function of the RB protein and initiation of tumor.[5]

Retinoblastoma is initiated when the remaining normal allele is inactivated (M2) in a developing retinal cell carrying M1. Sporadic unilateral retinoblastoma occurs in children with normal *RB1* constitutional alleles, who develop rare somatic mutations of both alleles (M1/M2) in a developing retinal cellIn heritable retinoblastoma (also called germline retinoblas¬toma), the first *RB1* allele is mutated (M1) in nearly all cells, in¬cluding germline reproductive cells, whereas the second allele is mutated (M2) in the retinal cells that become cancer, usually in both eyes. The most common M2 event is loss of the normal *RB1* allele and duplication of the mutated M1 allele, in that 2 copies of the mutated *RB1* gene remain; a mutational event referred to as loss of heterozygosity (LOH). Of nonheritable retinoblastoma, 98% have both *RB1* M1 and M2 events within a retinal cell. In the remaining 2%, retinoblas¬toma is induced by somatic amplification of the *MYCN* oncogene, in the presence of normal *RB1* genes.[1]

Amplification of the *MYCN* oncogene has been reported in a small subset (~2-5%) of retinoblastomas, typically those with wild-type *RB1* alleles. Such *MYCN* amplified, *RB1* wild-type retinoblastomas are reported to have aggressive histologic features and young age at diagnosis compared with sporadic retinoblastoma harboring somatic *RB1* inactivation.6 Accurate identification of *RB1* pathogenic variants in a reduced time is very important for diagnosis, confirmation, genetic counseling, risk assessment, and carrier screening of RB patients and their family members.[8]

3. Conclusion

RB mainly affected children under five years and both sexes are equally affected. Unilaterality was predominant. The precise identification of the RB1 mutations in each family with RB has been predicted to enhance the quality of clinical management of the affected patient and relatives at risk. Retinoblastomas with *RB1* inactivation and additional pathogenic alterations have an association with higher histologic grade, anaplasia, and higher pathologic stage, all of which are known predictors of more aggressive disease.

4. References

1. Soliman SE, Racher H, Zhang C, MacDonald H, Gallie BL. Genetics and molecular diagnostics in retinoblastoma - An Update. Asia-Pacific J Ophthalmol. 2017; 6(2): 197–207.
2. Goodrich DW, Lee WH. The molecular genetics of retinoblastoma. Cancer Surv. 1990; 9(3): 529–54.
3. Kiran VS, Kannabiran C, Chakravarthi K, Vemuganti GK. Mutational Screening of the RB1 Gene in Indian Patients with Retinoblastoma Reveals Eight Novel and Several Recurrent Mutations. 2003; 652(May):16–21.

4. Margo CE, Harman LE, Mulla ZD. Retinoblastoma. 1985.
5. Ibrahim NO, Saleem M, Eltayeb E, Mekki S, Elnaim EG, Idris AB, et al. Molecular Genetic Analysis of RB1 gene among Sudanese children with Retinoblastoma. bioRxiv. 2019.
6. Afshar AR, Pekmezci M, Bloomer MM, Cadenas J, Stevers M, Banerjee A, et al. Next-generation sequencing of retinoblastoma identifies pathogenic alterations beyond RB1 inactivation that correlate with aggressive histopathologic features. 2019.
7. Jagadeesan M, Khetan V, Mallipatna A. Genetic perspective of retinoblastoma: From present to future. Indian J Ophthalmol. 2016; 64(5): 332–6.
8. Devarajan B, Prakash L, Kannan TR, Abraham AA, Kim U. Targeted next generation sequencing of RB1 gene for the molecular diagnosis of Retinoblastoma. 2015; 1–10.