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Epigenetics of retinoblastoma: beyond the two-hit model

Epigenética do retinoblastoma: além do modelo dos dois eventos

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The early discovery that biallelic inactivation of Retinoblastoma Gene (RB1) can initiate tumor formation established one of the foundational concepts of modern cancer genetics and placed retinoblastoma (RB) in the spotlight of cancer biology[1]. The disease provided the experimental foundation for the tumor-suppressor gene concept and contributed substantially to the formulation of Knudson's two-hit hypothesis. In germline cases, tumorigenesis begins with functional loss of both alleles of RB1, which encodes the retinoblastoma protein (pRB), a central regulator of cell-cycle progression and transcription[2].

A small subset of (less than 10%) RBs are characterized by MYCN amplification that may arise without the canonical biallelic inactivation of RB1[3]. MYCN is a transcription factor that regulates a broad network of genes involved in: cell-cycle progression, ribosome and protein synthesis, cellular metabolism, growth, differentiation, and apoptosis. Recent methylation and transcriptomic studies have identified a MYCN-driven molecular subgroup characterized by DNA hypomethylation. This finding suggests that MYCN could be associated with a broader transcriptional and epigenetic state that promotes dedifferentiation.(3) Importantly, MYCN-driven biology is not restricted to RB1-proficient tumors. MYCN amplification tends to occur in very young children and are frequently unilateral and clinically aggressive[4].

Modern literature still supports the loss of both alleles of RB1 to be the key event in RB, and while we are not abandoning Knudson's model; the new evidence showing other epigenetic events may contribute to progression and phenotypical behavior of this neoplasm drives science to factor in that other epigenetic events are part of this story[2]. Whole-genome studies have demonstrated that some RBs contain remarkably few additional recurrent coding alterations after RB1 inactivation suggesting that epigenetic deregulation can provide critical mechanisms contributing to tumorigenesis[5,6].

Epigenetics is particularly relevant in RB because pRB is itself an important regulator of chromatin biology. Beyond its canonical interaction with E2F transcription factors, pRB participates in chromatin regulation through interactions with proteins involved in nucleosome organization, histone mo-

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dification, DNA methylation, and non-coding RNA networks. Loss of pRB can be viewed not simply as the removal of a cell-cycle checkpoint but as an event capable of reorganizing the transcriptional and chromatin landscape of the developing retinal cell[7].

The emerging epigenetic model is not a replacement of the two-hit hypothesis but an extension of it because RB1 loss may create a permissive state that activates oncogenic pathways, and silence tumor-suppressive programs.

DNA methylation is among the best characterized epigenetic abnormalities in RB and MYCN is involved in this process. Epigenetic silencing of RB1 itself was demonstrated as early as 1989, when hypermethylation of the 5′ region of the gene was identified in a sporadic unilateral RB[8].

Subsequent studies established that methylation of the RB1 promoter represents a mechanism of RB1 inactivation in a subset of tumors, particularly unilateral sporadic disease. This finding was an early demonstration that epigenetic silencing can contribute to RB1 inactivation, expanding the molecular mechanisms through which RB1 function may be lost[9].

The methylome of RB extends well beyond RB1. RASSF1A acts as a crucial tumor suppressor gene involved in cellular signaling and cytoskeletal regulation. RASSF1A promoter hypermethylation has been reported in approximately 59%–82% of retinoblastomas across different studies[10]. Treatment of RB cell lines with the DNA-demethylating agent 5-aza-2′-deoxycytidine restored RASSF1A expression, providing experimental evidence linking methylation with transcriptional silencing[10].

Similarly, methylation of MGMT, a DNA-repair gene, has been reported in a subset of RBs. Although these observations are biologically compelling, differences in sample size, methodology, tumor composition, and historical staging systems have produced variable methylation frequencies across studies. Consequently, individual methylation markers should not yet be regarded as universally validated clinical biomarkers[7,10].

Histone modifications and chromatin remodeling determine the accessibility of regulatory DNA and therefore influence whether genes are transcriptionally active or repressed. Loss of pRB is relevant in this context because pRB interacts with multiple

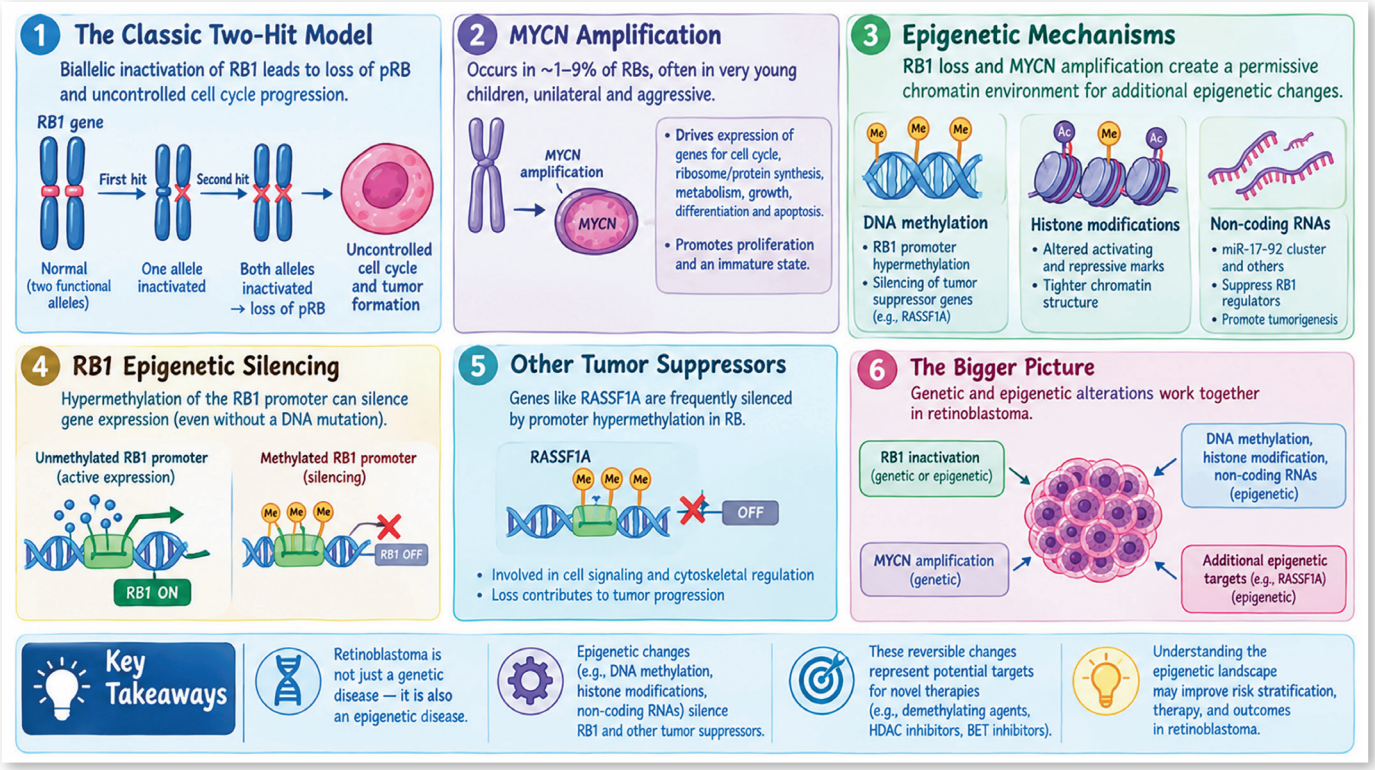


Figure 1. Schematic depiction of genetic and epigenetic pathways of retinoblastoma (RB).

chromatin-associated proteins and contributes to transcriptionally repressive chromatin conditions impacting proliferation, differentiation, apoptosis, and cellular identity[7].

Integrative genomic and epigenomic analysis identified increased SYK expression in RB despite the absence of recurrent genetic alterations in the gene. Functional experiments demonstrated that SYK contributed to tumor-cell survival[5]. Epigenomic analysis can reveal cancer-driving pathways activated through altered chromatin states.

Non-coding RNAs add another layer of regulatory complexity to RB biology. MicroRNAs, long non-coding RNAs, and circular RNAs can modulate transcription, mRNA stability, translation, chromatin organization, and signaling pathways, potentially contributing to the phenotypic consequences of RB1 loss[11].

It seems the relationship between RB1 and epigenetics is a central feature of RB biology. The conventional interpretation is that RB1 loss removes a brake on E2F-dependent cell-cycle progression. A broader view recognizes that pRB also functions as a chromatin regulator, interacting with chromatin--remodeling complexes, histone-modifying enzymes, DNA methylation, and regulation of non-coding RNA networks[10]. Thus, when RB1 is inactivated, the resulting phenotype may extend beyond uncontrolled proliferation to encompass widespread transcriptional and chromatin remodeling[7].

The next phase of retinoblastoma research should move beyond cataloguing epigenetic differences toward establishing causality[9,10]. First, studies should integrate DNA methylation with chromatin accessibility, histone modifications, transcription-factor binding, and gene expression rather than examining individual epigenetic marks in isolation. Second, single-cell approaches may clarify the variation of epigenetic states across tumor populations and progression. Third, comparisons with appropriate developmental retinal controls are essential because the tumor arises in a rapidly changing developmental environment. Finally, functional experiments are required to distinguish epigenetic drivers from molecular consequences of tumor formation.

The central challenge for the field is therefore to determine which epigenetic abnormalities are causal, which are adaptive, and which are merely consequences of tumor development. Resolving this distinction may identify biomarkers and therapeutic vulnerabilities while minimizing the risks associated with manipulating the epigenome in children. The future of retinoblastoma research will therefore not lie in choosing between genetics and epigenetics, but in understanding how RB1 loss reshapes the developmental and epigenetic landscape of the retinal cell. The two-hit model remains the foundation; epigenetics helps us understand what follows.

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