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REVIEW

Tumor heterogeneity in retinoblastoma: a literature review

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Abstract

Tumor heterogeneity, characterized by the presence of diverse cell populations within a tumor, is a key feature of the complex nature of cancer. This diversity arises from the emergence of cells with varying genomic, epigenetic, transcriptomic, and phenotypic profiles over the course of the disease. Host factors and the tumor microenvironment play crucial roles in driving both inter-patient and intra-patient heterogeneity. These diverse cell populations can exhibit different behaviors, such as varying rates of proliferation, responses to treatment, and potential for metastasis. Both inter-patient heterogeneity and intra-patient heterogeneity pose significant challenges to cancer therapeutics and management. In retinoblastoma, while heterogeneity at the clinical presentation level has been recognized for some time, recent attention has shifted towards understanding the underlying cellular heterogeneity. This review primarily focuses on retinoblastoma heterogeneity and its implications for therapeutic strategies and disease management, emphasizing the need for further research and exploration in this complex and challenging area.

Keywords Tumor heterogeneity · Cellular heterogeneity · Retinoblastoma · Cancer complexity · Tumor microenvironment

Abbreviations

AAV	Adeno-associated virus
AH	Aqueous humor
AI	Artificial intelligence
AML	Acute myeloid leukemia
BFB	Breakage fusion bridge cycle
CCSK	Clear cell sarcoma of the kidney
cfDNA	Cell-free DNA
CIN	Chromosomal instability
CNNs	Convolutional neural networks
CNS	Central nervous system
CP	Cone precursor
CPL	Cone precursor-like cells
CSC	Cancer stem cells
CTCs	Circulating tumor cells
ctDNA	Circulating tumor DNA
DSBs	Double strand breaks
EMT	Epithelial-to-mesenchymal transition
EVs	Extracellular vesicles
HR-MAS MRS	High-resolution magic-angle spinning magnetic resonance spectroscopy
HR-RB	High-risk RB
HRHF	High-risk histopathological features
ICRB	International Classification of Retinoblastoma
ITH	Intra-tumoral heterogeneity
LIME	Local Interpretable Model-agnostic Explanations
LR-RB	Low-risk RB
miRNAs	MicroRNAs
ML	Machine learning
MRI	Magnetic resonance imaging
ncRNAs	Non-coding RNAs
NHEJ	Non-homologous end joining
OXPHOS	Oxidative phosphorylation
pRB	Retinoblastoma protein
RB	Retinoblastoma
RFLP	Restriction fragment length polymorphism
RL-cells	Retinoma-like cells
scDNA-seq	Single-cell DNA sequencing

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SCNVs	Somatic copy number variations
scRNA-seq	Single-cell RNA sequencing
SHAP	Shapley additive explanations
SNAs	Single nucleotide alterations
SNVs	Single nucleotide variations
SYK	Spleen tyrosine kinase
tdEVs	Tumor-derived extracellular vesicles
TEPs	Tumor-educated platelets
tRB	Trilateral retinoblastoma
VH	Vitreous humor

1 Introduction

Cancer cell development is a product of the imbalance between the activity of oncogene and tumor suppressor gene function [1]. The growth of cancer cells is a dynamic evolutionary process resulting in the clonal expansion of cancer cells along with divergent tumor sub-cell populations [2]. This leads to the emergence of tumor heterogeneity that poses significant challenges in cancer treatment and affects disease prognosis, as it grants tumor cells considerable advantages for survival [3]. Clinicopathological representation of intra-tumor heterogeneity can be characterized by phenotypic [4], genotypic [4], immunophenotypic [5], metabolic [6–8], and epigenetic variations [9]. Various studies on different cancers have now established that tumor heterogeneity is a key challenge in the management of solid tumors [3, 10–13].

The eye is one of the five human sensory organs responsible for converting electromagnetic/light energy to electrical energy that senses to form a virtual image on the innermost layer of the eye, the retina [14]. The retina is composed of two main types of photoreceptor cells: rods, which make up the majority of the retina and are responsible for scotopic vision, and cone cells, which are primarily responsible for color vision [15, 16]. Retinoblastoma (RB) is a solid pediatric tumor arising from the retina of the eye [17]. RB arises from either primitive retinal stem cells or cone precursor cells, the photoreceptor responsible for color vision [14, 18]. It is a rare but most common cause of death due to intra-ocular cancer in children [19]. Most high-income nations like the USA (~ 300 cases per year) have lower RB incidences than low-income nations in the Asia Pacific and Africa. India has been reported to be on top of the list in RB incidence (~ 2000 cases per year) [20]. Early diagnosis of RB significantly affects the survival rates of the patients. Developed countries with higher chances of early diagnosis and timely treatment and care of RB show high survival rates [19–22]. However, in low- and middle-income countries, due to late diagnosis, lack of proper treatment, and management of RB, the survival rate is below 40% [23]. The five-year survival rate of RB patients is highly dependent on the health care and management system of a country and is approximately 99%, 89%, and 90% for high-income, upper and middle-income, and lower-middle-income countries, respectively [24]. In India, the five-year survival rate of children with RB has significantly increased to over 80% [25]. Diagnosis at the late stages of RB limits the treatment modality. However, the incorporation of neoadjuvant chemotherapy, enucleation, external beam orbital radiotherapy, and adjuvant chemotherapy have improved the chances of life salvage in RB patients. Local extension and/or distant metastasis of RB is a great challenge in the management of the disease and greatly affects the prognosis of RB patients [17, 26]. Additionally, even after all types of therapy, there is still a 6–45% chance of developing a new tumor or 0–45% chance of recurrence, depending on the type, method, and combination of therapies used [27]. The heterogeneity observed in RB in genetic, epigenetic, phenotypic, and cellular factors could account for the emergence of cells with invasive metastatic properties and the development of chemoresistance. This heterogeneous nature of RB tumors poses a great challenge in the management and prognosis of RB. Understanding heterogeneity (both intra and inter) in RB is crucial for disease management, clinical intervention, and patient survival. This review primarily focuses on the various types of heterogeneity observed in RB and their implications for treatment outcomes (Fig. 1). It will highlight future research prospects related to retinoblastoma heterogeneity and the potential for identifying early diagnostic and prognostic markers.

2 Molecular pathology of retinoblastoma and clinical presentation

RB, an intraocular tumor of infants or early childhood, is clinically presented by a white pupillary reflex, known as leukocoria (cat's eye reflex) (70–90%). Other conditions like strabismus (77%) (crossed or deviating eyes), proptosis (90%), decreased vision (in case of bilateral presentation), hypopyon (2%), red eye, and pain (5%) have also known to be associated [25, 26]. RB is initiated by biallelic mutations in the RB1 gene of cone cells at the retinal layer of eye [22]. Incidentally, RB is the first example of Knudson's "two-hit" hypothesis, which states that a tumor suppressor gene must acquire at least two mutations to transform a cell into a cancerous state. In the case of RB, the rate of acquiring these two mutations was determined to be approximately 2×10^{-7} per year [28]. Later, a restriction fragment length polymorphism (RFLP) study showed that deletion of chromosome band 13q14 is responsible for RB [29]. Loss of heterozygosity of chromosome 13q14 is associated with approximately 60–75% of RB cases [30–32].

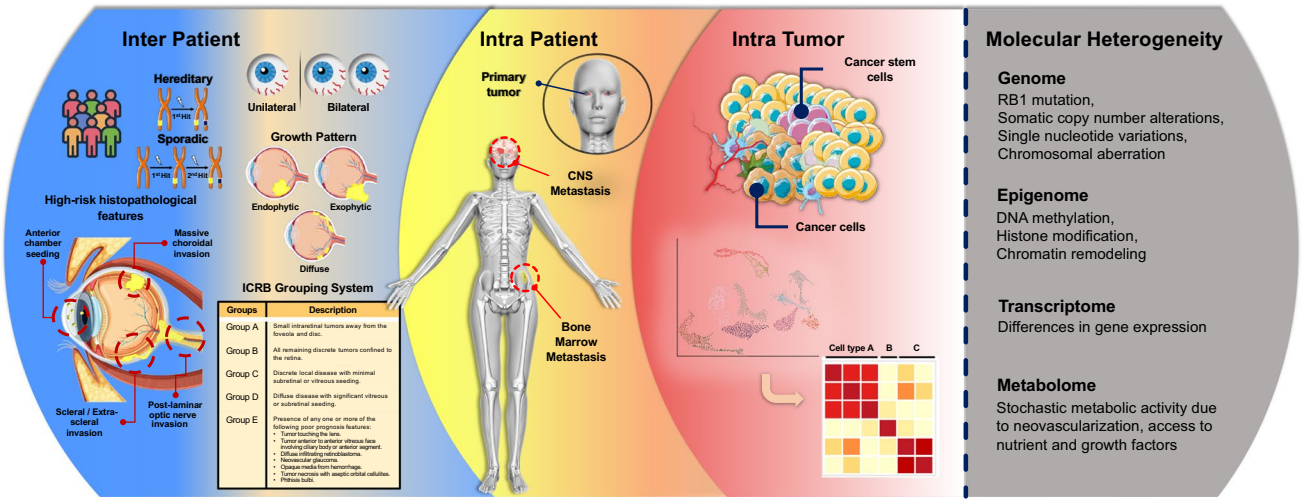

Fig. 1 Schematic representation of the different types of heterogeneity in retinoblastoma (RB): **Inter-patient heterogeneity** arises from differences in sporadic and hereditary RB cases and is further compounded by variations in high- and low-risk histopathologic features**,** which may be present even within the same ICRB grouping**,** thereby influencing treatment response and prognosis. **Intra-patient heterogeneity** reflects differences between primary and metastatic tumor sites, which may exhibit distinct tumorigenic characteristics. **Intra-tumor heterogeneity** is shaped by the presence of cancer stem cells and multiple potential cells of origin for RB**.** These variations are driven by **molecular heterogeneity**, encompassing diverse genetic, epigenetic, transcriptomic, and metabolomic alterations, which collectively contribute to heterogenous RB progression

RB can occur in both hereditary and non-hereditary forms. Most of the RB cases are non-hereditary (60%), arising due to somatic mutations (sporadic mutations) in the RB1 gene during the development of retina, and are clinically represented as unilateral. Among these non-hereditary tumors, 98% result from biallelic loss of the RB1 gene, while the remaining 2% arise from amplification of the MycN gene with the normal RB1 gene. Non-hereditary RB is typically diagnosed at a later age with a median of two years. The hereditary RB, which constitutes 40% of cases, clinically presents as bilateral (80%), unilateral (15%), and trilateral (5%) RBs. These hereditary RB cases are attributed to germline pathogenic mutations in the RB1 gene. All bilateral cases are hereditable, but most arise from de novo mutations in patients with unaffected parents with no mutation in the RB1 gene. Hereditary RB is diagnosed at an earlier age (median: one year) than non-hereditary (median: two year) RB[33]. Further, hereditary RB tumors are transmitted in an autosomal dominant fashion, and the patients with hereditary RB have a higher risk of secondary cancers like osteosarcomas, melanomas, and soft tissue sarcomas due to the accumulation of secondary mutations[18]. In addition, in the children affected by RB, cases of neuroectodermal brain tumor (also called trilateral RB, tRB) have also been reported[34]. The most common neuroectodermal tumor in tRb is pineoblastoma [35]. Neuroectodermal lesions occur in the brain due to shared embryologic origins of the retina and pineal gland, both derived from neuroectoderm [36]. Retinoblastoma arises from RB1 gene inactivation in photoreceptor precursors [37]. In tRB, germline RB1 mutations enable a second "hit" in neuroectodermal tissues, like the pineal gland, which retains photoreceptive traits from evolutionary history [38]. Interestingly, at least in fish and amphibians, pinealocytes predominantly exhibit retinal cone photoreceptor-like characteristics [39]. The expression of several cone opsins in the pineal gland and retina supports the idea that pinealocytes retain traces of cone-like cells. However, conclusive evidence for distinct "cone-type" and "rod-type" photoreceptors remains unavailable, with newer evidence suggesting ciliary and rhabdomeric precursor ancestry for the retina and pineal gland [38].

Trilateral RB shows a genotype–phenotype correlation, with ~90% of cases tied to germline RB1 mutations, often null, causing severe bilateral RB and pineoblastomas [35]. Identical RB1 mutations in retinal and pineal tumors support a genetic basis [36], but no mutation uniquely predicts tRB, implying stochastic or contextual factors. Early second hits may favor intracranial tumors [40], with mosaicism potentially modulating risk [41]. Advances in genomic and epigenetic profiling may further elucidate these associations.

Further, in RB patients, complications such as vitreous haze and floating opacities may occur when the internal limiting membrane of the retina ruptures during tumor growth, leading to vitreous seeding. In advanced RB cases, the tumor may infiltrate neighboring structures such as the uvea, optic nerve, choroid, sclera, and orbit representing local extension. This local extension poses a risk for distant metastasis to sites such as central nervous system (CNS), lymph nodes,

and bone marrow and in some cases to the liver, as well [42]. There is a degree of heterogeneity among RB patients, even in terms of clinical presentation and eye involvement.

3 RB1 gene structure and function

The human RB1 gene (phenotype MIM No. 180200/Gene MIM No. 614041) has a complex structure located on the largest acrocentric chromosome 13 (13q14.2). It has 27 exons and 26 introns with a core promotor, spanning over approximately a region of 296.69 bp. The coding sequence region has intersected with introns of variable size ranging from 80 bp (intron 15) to more than > 60 kb (intron 17) [43–46]. RB1 gene encode mRNA of approximately 4.7 kb and 928 amino acids and is a nuclear phosphoprotein of 110–114 kDa. RB protein (pRb) has a unique "A/B pocket domain" along with the N- and C- terminal domains. The "A/B pocket domain" carries hotspots for mutations and the box B domain has the most missense mutations (exon 19 and exon 21). In the A/B pocket, the presence of "L-X-C-X-E-binding cleft" provides pRb high-affinity interaction region for other cellular proteins (e.g., E2F, p53, CAPD3, etc.) [47]. pRb is one of the first tumor suppressors to be identified [48]. The hypo-phosphorylated pRb sequesters E2F1 transcription factor and restricts the cell cycle at G1/S state while in its hyperphosphorylated state (by Cdk4/cyclins D and Cdk2/Cyclin E) it promotes cell proliferation [49, 50]. Mutations in this binding pocket cleft ("L-X-C-X-E-binding cleft") destabilize the interaction of the pRb-E2F1 complex and leads to uncontrolled proliferation [51].

4 Heterogeneity in retinoblastoma

During RB progression, precursor cone cells acquire various genetic and non-genetic adaptations as their survival strategy. This results in a heterogeneous nature both at phenotypic and genotypic level [52]. In RB, the cone precursor cell of infants selectively acquires RB1 gene mutation due to proliferation and micro-environmental pressure. Subsequent cellular division of mutationally active cone precursor cells results in diversification, generating clonal variants with additional genotypic and non-genotypic alterations [22]. This generates intra-tumoral mosaicism in RB [14]. Somatic mosaicism has been well documented in RB [53–55]. The tumor heterogeneity can be broadly categorized at different levels of cellular and genomic organization as genomic (chromosomal and DNA), non-genomic (transcriptional, epigenetics), functional (proteome, metabolic and immunological, etc.), stemness, cell and micro-environmental heterogeneity [56]. In the following sections, we will discuss the different levels of heterogeneity present in RB (Fig. 1) and their impact on patient management and survival.

4.1 Heterogeneity due to mutations in RB1 gene

The RB1 gene is known to harbor various mutations and has several mutational hotspots. According to the RBGMdb online database, 932 mutations were identified in RB1 gene [57]. The deletion of exons 13–17 of the RB1 gene has been observed in many tumors including RB, breast cancer, and osteosarcoma [43]. While RB typically exhibits high penetrance (80–90%), the pathogenicity of RB1 gene mutations and penetrance of RB can vary significantly [58–60]. It is well established that individuals with the constitutional heterozygous RB1 ($RB1^{-/+}$), as seen in familial cases, are at an increased risk of a second somatic insult to the RB1 gene. Consequently, it is not surprising that 90% of individuals with heterozygous $RB1^{-/+}$ develop bilateral RB [61]. However, cases of unilateral multifocal RB are also reported in familial instances. For example, a p.V654L missense mutation in the exon 19 in the RB1 gene found in a Taiwanese familial RB has been shown to associated with the development of unilateral RB with only 36% penetrance [62]. Furthermore, it might be possible that the unilateral cases in familial RB arise from the generation of a distinct clone, different from bilateral cases, due to the randomness of second somatic mutational events on the wild-type allele. Additionally, the unique presence of certain mutations (including, but not limited to c.180_187 del, c.528 del, c.2035_2039 del, c.2299_2300 del, and c.1050 -2 A > T) and higher mutation rate found in bilateral RB might lead to its different disease progression behavior compared to unilateral RB [63].

Moreover, mutations in the RB1 gene are differentially represented in unilateral and bilateral RB, with bilateral cases exhibiting high number of mutational events compared to unilateral ones [59, 64, 65]. Irrespective of laterality, the type of genomic mutations in the RB1 gene among RB patients can vary widely, including insertion/deletion, missense mutations, point mutations, nonsense mutations, frameshift mutations, single nucleotide variations (SNVs), and splice variants, and potentially lead to generation of inter-tumoral heterogeneity seen in the patients [66]. The penetrance of each type of mutation might affect the disease outcome adversely and influence the tumor response to medications [64, 67, 68]. A study by Price EA et al. on RB1 gene mutation screening in 403 RB cases indicated that the substitution mutations were the most frequent (58.4%), while missense mutations were seen in only 3.5% of RB cases [69]. The A/B pocket domain was found to be the most common hotspot region in the RB1 gene, with a mutation rate of 58.6% (domain A at 37.1% and domain B at 27.1%) [69], consistent with earlier findings of 58.1% [64] and 40% [70]. Another study on 136 RB cases in the Turkish

children population revealed that the most pathogenic mutations on RB1 gene were indel and small genetic rearrangement mutations (78.9%), with missense mutations observed in only 1% of cases [59]. Although an extensive correlation between different mutation types or mutational load on RB1 gene and the severity of the RB disease or treatment response has not been established, evidence suggests that the different types of mutations in the RB1 gene can lead to a varying response to the therapy. For instance, Manukonda et al. identified seven novel SNV mutations in RB1 gene (six SNVs; c.653 T > G*, c.1172 C > C/A*, c.1649 T > G*, c.296G > A*, c.19_20 insG*, c.1050 - 6_1050 - 2 del* and one small mutation; c.784_787 del CGGAinsGAA CAGTTGTTC*) that were associated with tumor recurrence [71]. However, patients with mutations like c.25 dupA*, c.772_776 del, c.1981 C > T, and exon 7–17 deletion in RB1 gene responded well to chemotherapy and showed tumor regression [71]. Further, it was reported that the presence of germline RB1 p.V654L could result in unilateral RB with low penetrance [62]. Many low-penetrance familial mutations have been found in RB genes, potentially explaining the interpatient heterogeneity observed in RB progression, which is generally attributed to the partial function of the mutant RB1 [72–76]. Ongoing studies examining genetic variations in the RB1 gene among RB patients continue to reveal new mutations, underscoring the intricate and diverse nature of these mutations among patients worldwide [55, 56], further contributing to genetic heterogeneity. Interestingly, these novel mutations most frequently appear in the A/B pocket domain of the RB1 gene, suggesting this domain's sensitivity to mutations that contribute to RB development and heterogeneity [55].

4.2 Clinical and histopathological heterogeneity in retinoblastoma

Cavitary RB, a low-grade subtype occurring in 2–7% of tumors, can also contribute to interpatient heterogeneity [77, 78]. This is an uncommon subtype where ophthalmoscopically visible cavitary spaces can be seen at presentation (4%) or following systemic chemotherapy (3.2%) [77]. Further, the incidence of cavitary RB can vary according to sex, with males having a 1.2 times higher incidence than females, which may contribute to additional heterogeneity [79]. Additionally, it is more common in bilateral cases (72%) than in unilateral cases (28%), possibly because the multifocal structure of bilateral tumors makes them more likely to transform into the cavitary variant later on [77–79]. Cavitary RB was initially thought to be chemoresistant, given its poor response to intravenous chemotherapy, but subsequently shown to be stable without the need for aggressive adjuvant therapy [78, 79]. Yet close follow-up is required in case of any possible tumor advancement. Cavitary RB generally has a favorable prognosis [80, 81].

RB demonstrates clinical heterogeneity also due to variations in its growth patterns, which may be endophytic, exophytic, mixed, or diffuse infiltrating. Studies from developing countries have reported a greater prevalence of endophytic tumors (45–61%) compared to exophytic tumors (33–40%), with many cases displaying a mixed pattern (21%). In contrast, studies from developed countries indicate a predominance of exophytic tumors (62%) over endophytic tumors (31%) [82–84]. These differences may stem from regional disparities or variation in the age of presentation in developed versus developing countries. Regardless of the frequency, this growth pattern variability, in turn, results in distinct clinical presentations, diagnostic challenges, and treatment strategies. Endophytic tumors grow inwards into the vitreous cavity, presenting as a yellow-white mass with an increased risk of vitreous seeding. In such cases, in addition to control of the main retinal tumor with standard treatment strategies, intravitreal chemotherapy may be needed for the control of vitreous seeds [85]. In contrast, exophytic tumors grow outward into the subretinal space, frequently leading to retinal detachment and an elevated risk of choroid invasion. This can result in secondary glaucoma and potential extraocular tumor spread [86]. In such cases, based on extent of the tumor, local tumor control is achieved with intravenous/intra-arterial chemotherapy or plaque radiotherapy. Enucleation is preferred in eyes with choroidal invasion, while eyes with extraocular tumor extension require multimodal treatment. In some cases, the tumors exhibit features of both endophytic and exophytic, i.e., a mixed growth pattern, again increasing clinical heterogeneity and necessitating specific treatment strategies. Chemotherapy, either systemic or intra-arterial, in addition to appropriate adjuvant focal treatment, is required in eyes with mixed growth pattern. Advanced tumors necessitate enucleation [87]. Diffuse infiltrating RB are usually rare and account for about 1–2% of cases worldwide. It lacks discrete mass and infiltrates the entire retina, causing diffuse retinal thickening without classic calcification. It is usually associated with pseudohypopyon or pseudovitritis that simulates inflammatory conditions and hinders diagnosis. Most eyes with diffuse infiltrating RB require enucleation [88]. This diversity of growth patterns, such as endophytic, exophytic, mixed, and diffuse infiltrating, highlights the clinical heterogeneity of RB and its influence on treatment decision-making.

Heterogeneity exists in the histopathological features of retinoblastoma as well. Based on the histopathological features, RB is classified into low-risk (LR-RB) and high-risk (HR-RB) forms. LR-RB is confined to the retina without high-risk histopathological features (HRHF), whereas HR-RB is associated with HRHF, including massive choroidal invasion (≥ 3 mm), post-laminar optic nerve

invasion, residual tumor at the transected optic nerve margin, anterior segment invasion (affecting the iris, ciliary body, or trabecular meshwork), and extrascleral extension or orbital involvement. However, the histopathological criteria for the definition of HR-RB vary significantly due to diagnostic variability in different regions. Inconsistent histopathological definitions—such as the threshold for massive choroidal invasion or the grading of anaplasia—contribute to discrepancies in risk stratification. Additionally, inter-observer variability among pathologists complicates standardization, as highlighted in multicenter studies [89]. Regional heterogeneity in HRHF prevalence further contribute to these differences. For instance, massive choroidal invasion (≥ 3 mm), a widely recognized HR feature, is most prevalent in Asia (31%) compared to Europe (13%) and North America (19%). Similarly, post-laminar optic nerve invasion—a key indicator of extraocular spread—is significantly higher in Asia (27%) than in Australia (0%). Transected optic nerve margins, associated with poor prognosis, is most frequently observed in South America (11%) but are absent in Europe. Additionally, less definitive HRHF, such as iris invasion (10% in Asia vs. 3% in South America) and trabecular meshwork invasion (6% in Asia vs. < 1% in South America), also shows regional variation [24, 90]. Racial heterogeneity in HRHF has also been reported. In a study of 1426 patients who underwent primary enucleation for RB, it was noted that massive choroidal infiltration was significantly higher in Asians (30%) and Hispanics (26%) compared to Caucasians (15%). Similarly, post-laminar optic nerve infiltration was higher in Asians (28%) and Hispanics (20%) compared to Caucasians (11%) [91].

Clinically, LR-RB often presents with leukocoria or strabismus and may belong to groups A to E of the International Classification of Retinoblastoma (ICRB) [92]. In contrast, eyes with HR-RB are likely to present with secondary glaucoma and iris neovascularization, and mostly belong to groups D and E [90]. Group D is characterized by tumors with diffuse subretinal or vitreous seeding, indicating intraocular dissemination, while group E includes cases with severe complications such as neovascular glaucoma, massive vitreous hemorrhage, or complete retinal detachment. However, some cases within groups D and E may still be considered low-risk, when there is no evidence of HRHF [90, 93].

The presence of HRHF significantly elevates the risk of systemic metastasis, and prophylactic adjuvant chemotherapy is recommended in such cases [89].The survival rates of patients with untreated HR-RB are lower than those with LR-RB, while adjuvant treatment significantly improves the survival. However, despite adjuvant treatment, racial heterogeneity has been reported with survival in patients with HRHF [91, 94, 95]. The cause of regional and racial heterogeneity in survival and histopathological features of eyes with RB needs further exploration.

4.3 Genomic heterogeneity in retinoblastoma beyond the RB1 gene

In most RB cases, RB1 gene mutation (biallelic loss or loss of heterozygosity) is a prerequisite for RB initiation; however, other somatic mutations or oncogene expression drive the RB oncogenesis [96]. During embryonic development, retinal cells are subjected to micro-environmental stress and hypoxic conditions, which can result in retinal precursor cells with $RB1^{-/-}$ that could undergo tumorigenesis by accumulating other genetic alteration [97]. By multi-omics analysis of RB cells isolated from patients, Liu et al. demonstrated the presence of two molecular subtypes of RB. Subtype 1, the most heritable type, showed fewer genetic alterations and higher expression of cone cell markers with differentiated features. Subtype 2 exhibited recurrent genetic alterations along with MYCN amplification, less expression of cone cell markers with dedifferentiated features, and increased tumor heterogeneity [98]. Moreover, subtype 2, displaying a greater array of genetic alternation including MYCN other than RB1 inactivation were more prone to metastasize [98]. This reinforces the idea that for malignant transformation, benign retinal lesions and retinoma require additional mutations in tumor suppressors or oncogene overexpression in addition to the initiating biallelic RB1 mutations [99]. The overexpression of MYCN oncogene provides a significant advantage in RB tumorigenesis and can independently drive the initiation of RB. Notably, $RB1^{-/-}/MYCN^{A}$ tumors with lower amplification (2–9 copies) of MYCN were diagnosed at a later age (> 38 months), while $RB1^{+/+}/MYCN^{A}$ with high amplification (over 29 copies) of MYCN was diagnosed at a very early age and exhibited highly aggressive features [22, 58, 100–102]. Cases of RB having many copies of MYCN, along with the loss of one copy of 13q, have also been reported [100]. It is hypothesized that $RB1^{+/+}/MYCN^{A}$ tumors originate from early retinal precursor cells, while $RB^{-/-}/MYCN^{A}$ tumors develop from cone photoreceptor precursor cells [103]. Moreover, $RB1^{-/-}$ tumor cells express higher levels of cone photoreceptor markers such as RxRγ and TRβ2, suggesting their origin from cone precursor cells in retina [22]. Apart from MYCN, overexpression of several genes like EZH2, OTX2, KIF14, E2 F3, and others has been observed in RB, indicating their role in the progression and tumor development. Another significant non-RB1 driver gene in RB is the CREBBP gene. CREB-binding protein acts as a tumor suppressor and acetyltransferases and regulates gene transcription by histone acetylation [104]. In RB, less than five percent tumors represent mutations in CREBBP gene [105]. In addition, "driver genes" like CREBBP, MHS3, ADRID1A,

CDH11, and others have been identified to be involved in the transformation of retinal precursor cells to RB [33, 53, 106]. Somatic copy number variations (SCNVs) in several genes like RAB23, DEK, NUP153, TTRAP, MYCN MLH3, WT1, PAX6, and GATA5 (gain in copy number) and CHFR, TP73, IGSF4, CREBBP, BCOR, and TSC21D1 (loss in copy number) are also reported to contribute to RB pathogenesis [107, 108]. These genetic alterations, including mutations, single nucleotide variants (SNVs), somatic copy number alterations (SCNAs), chromosomal aberrations, and events like chromothripsis, create a heterogenic population of cells during the tumorigenesis process[109–111].

In RB, the different gene alterations, along with the foundational RB1 mutation, could also result in inter-eye tumor heterogeneity. Winter et al. documented two case reports of bilateral RB wherein they identified inter-eye tumor heterogeneity in two patients [112]. In patient 1, the two tumors differed in chromosome 13q, 1q, and 6p copy number alteration, with a heterozygous loss of 13q in the left tumor. The left tumor that showed orbital recurrence also had additional gains in MYCN, 15q, and 22q11.1, along with a loss in 9p21. In patient 2, the pathogenic mutation BCOR was found only in the left eye [112]. These findings reveal the presence of both intra-tumoral heterogeneity and interorbital tumor heterogeneity. Further, heterogeneity might arise due to the phenomenon of recurrent mutation where multiple copies of the same allele may co-segregate during cell division and produce variants in the cell population, which is also seen in RB [113–115]. One such recurrent mutation in RB is a non-RB1 gene mutation, BCOR (BCL 6 corepressor) mutation [116]. The gene BCOR encodes BCL6 corepressor, which is involved in transcriptional regulation and is highly expressed in the human retina [113, 117]. BCOR has been found to be mutated in 10% of RB patients [105].

To summarize, several non-RB1 gene alterations (E2F, OTX2, E2Z2, MDM4, KIF14, SOX2, Survivin, etc.) have been well documented in RB cases [33, 118]. These alterations occur in varying percentages of RB patients, representing an additional source of genetic heterogeneity. The most common non-RB1 genes altered in RB with their frequencies are summarized in Table 1.

4.4 Heterogeneity due to chromosomal aberrations

Chromosomal aberrations are structural (deletion, insertion, translocation, and reversion) or numerical changes (aneuploidy or polyploidy) in chromosomes. It creates genomic instability and may lead to cancer progression [119]. Chromosomal instability (CIN) is one of the chromosomal aberrations that drive cancer heterogeneity [120]. In RB, RB1 gene inactivation also leads to chromosomal instability due to defects in chromosomal segregation [121]. Oliveros and Yunis have demonstrated the chromosomal evolution in RB tumors and showed chromosomal rearrangement during RB development [122]. They reported early and late chromosomal rearrangements during the cell divisions in RB cells. The early chromosome arrangements consists of + lq, + 6p, − 13/del(13q), − 16/del(16q), and − 17/del(17p) and has a frequency of 70% to 100% in tumor cells while the late chromosomal rearrangement was − 8, − 17/del(17p), − 22, + 3/+ 3q, − 4, − 19, + lq, + 7/+ 7q, − 14, and + 21 and showed lower frequency [122]. Their data indicated the importance of early chromosomal rearrangements in RB development and indicated that low-frequency chromosomal rearrangements could favor the generation of tumor heterogeneity. In addition, a phenomenon called "chromothripsis" has been reported in RB and is suggested to cause CIN and contribute to heterogeneity. Chromothripsis is a catastrophic event involving multiple double strand breaks (DSBs) in the chromosome, which are subsequently repaired by a mutation-prone repair mechanism, non-homologous end joining (NHEJ) resulting in CIN [108, 123]. The massive and complex chromosomal rearrangement due to chromothripsis enhances the breakage fusion bridge cycle (BFB) and is likely to cause alterations in gene functions and translocation of genes in the subsequent clonal cells and thus promotes heterogeneity [123].

4.5 Transcriptional heterogeneity in retinoblastoma

Inter-patient and intra-tumoral heterogeneity (ITH) is one of the hallmarks of each cancer type and several factors such as genetic, epigenetic, and microenvironmental factors drive it. Each cell type within a growing solid tumor is subjected to distinct microenvironmental stresses, potentially leading to the emergence of cell types or clusters that differ from their original parent cells. Cellular adaptability to these stresses is established by the alterations in the gene function either at the transcriptional or post transcriptional level. The transcriptional heterogeneity among RB patients was highlighted through gene expression profiling of 21 RB patients, revealing the existence of two distinct groups based on their invasiveness and cell of origin [124]. Group 1 appeared to originate from retinal progenitor cells with invasive phenotype, while group 2 seemed to derive from photoreceptor cone cells [124]. Another study, using 76 patients, identified two clusters with variation in photoreceptorness and the presences of SCNVs, but suggested these clusters might be continuous rather them dichotomous [125]. Nevertheless, along with a reanalysis of transcriptomic data from 55 patients, these studies reinforced the evidence of transcriptional heterogeneity among patients. In these analyses, some patients were excluded due to suspected contamination with normal retinal cells and tumor-infiltrating immune cells. However, these samples may also reflect heterogeneity

Table 1 Heterogeneity in genomic and chromosomal alterations in retinoblastoma patients

Gene	Gene name	Frequency in RB patients (%)	Genomic alteration	Association of gene with RB metastasis, recurrence or chemoresistance	References
ARID1A	Adrenoceptor alpha 1 A	3.6	Somatic mutation	Mutation leads to aggressive phenotype of RB tumor	[102, 281]
ARLTS1	ADP ribosylation factor like GTPase 11	11	Loss at 13q13.2-q22.3	Not determined for retinoblastoma	[282]
BCOR	BCL6 corepressor	13	Mutations or loss; nonsense variants that result in truncated protein	Mutation is associated with relapse and poor prognosis for metastasis free survival. Mutation promotes RB progression and can be targeted for chemotherapy	[102, 116]
CDH11	Cadherin 11	58	Loss at 16q21	Loss of expression correlated with optic nerve invasion in a murine transgenic model of retinoblastoma with functional, retinal progenitor-specific inactivation of p107, pRb, and p53 proteins	[283]
CDH13	Cadherin 13	12	Loss at 16q24.2	Not determined for retinoblastoma	[284]
CHFR	Checkpoint with forkhead and ring finger domains	16	Loss at 12q24.33	Not determined for retinoblastoma	[107]
CREBBP	CREB binding protein	2 (genomic loss) 2.8 (SNP and indels)	Loss at 16p13.3; NM_001079846 (c.T4308G: p.C1436 W); NM_001079846 (c.6629_6631 del: p.2210_2211 del)	Downregulation may enhance the metastatic nature; recurrent mutations observed in RB and might be associated with recurrence	[33, 102, 106]
CYLD	CYLD lysine 63 deubiquitinase	11	Loss at 16q12.1-q21	Not determined for retinoblastoma	[282]
DEK	DEK proto-oncogene	40–54	Gain at 6p22.3	Associated with malignant progression in RB	[118, 282]
E2F3	E2F transcription factor 3	70	Gain at 6p22.3	High E2F3 expression may enhance the RB metastasis; low expression may sensitize the RB cells for chemotherapy	[285]
ETS1	ETS proto-oncogene 1, transcription factor	11	Loss at 11q24.3	Not determined for retinoblastoma	[282]
GATA5	GATA binding protein 5	25	Loss at 20q13.33	Not determined for retinoblastoma	[107]
IGSF4	Immunoglobulin superfamily member 4/ Cell adhesion molecule 1	8	Loss at 11q23	Not determined for retinoblastoma	[107]
KIF14	Kinesin family member 14	50	Gain at 1q32.1	Low expression may sensitize the RB cells for chemotherapy	[285, 286]

Table 1 (continued)

Gene	Gene name	Frequency in RB patients (%)	Genomic alteration	Association of gene with RB metastasis, recurrence or chemoresistance	References
MCL1	Myeloid cell leukemia sequence 1	22	Gain at 1q12-q25.3	Not determined for retinoblastoma	[282]
MDM2	MDM2 proto-oncogene		rs2279744G > T; rs2279744 TG > GG	Correlated with tumorigenesis by negative regulation of tumor suppressor protein p53; MDM2 inhibitor in combination to gene therapy could offer a great target for retinoblastoma treatment	[118, 287]
MDM4	MDM4 regulator of p53	65	Gain at 1q32.1; rs4252668 T > C; rs116197192G > A	Overexpression of MDM4 enhance cell proliferation and delays DNA damage-induced apoptosis	[118, 287]
miR- 106b~25	MicroRNA 106b~25	15	Gain of 5qG2–3 (4/32)	Promotes metastasis (migration and invasion)	[118, 140, 288]
miR- 17~92	MicroRNA 17~92	15	Gain at 13q32	Promotes proliferation and development of tumor with Rb1 and Rbl mutations; associated with brain metastasis and might be a potential target for chemotherapy	[118, 140, 289]
MLH3	MutL homolog 3	25	Gain at 14q24.3	MLH3 mutations contribute RB progression and low expression might lead to metastasis and also sensitize to chemotherapy for RB cells	[118]
MUC1	Mucin 1	22	Gain at 1q12-q25.3	Not determined for retinoblastoma	[282]
MYCN	MYCN proto-oncogene, bHLH transcription factor	13–34 (gain) 3–30 (amplification)	Gain or Amplification at 2p24.3	High copy number results in metastasis and increased chemoresistance and is associated with poor prognosis and recurrence of RB	[101, 118]
NTHL1	Nth-like DNA glycosylase 1	12	c.268 C > T (p. Gln90*)	Overexpression leads to metastasis	[102]
PAX6	Paired box 6	16	Gain at 11p13	Expression is associated with an increased proliferation and a decreased apoptosis; might promote metastasis	[118, 290]
RPTOR	Regulatory associated protein of MTOR complex 1	3.6	Somatic mutation	Not determined for retinoblastoma	[102]
SHC1	SHC (source homology 2 domain containing) transformation protein 1	22	Gain at 1q12-q25.3	Not determined for retinoblastoma	[282]
TNXB	Tenascin XB	41.6	Gain at 6p21.3	Not determined for retinoblastoma	[107]

Table 1 (continued)

Gene	Gene name	Frequency in RB patients (%)	Genomic alteration	Association of gene with RB metastasis, recurrence or chemoresistance	References
TP53	Tumor protein p53	8	Loss at 17p13.1	Mutation is associated with highly aggressive growth and significantly associated with choroid invasion and brain metastasis; expression is associated with high-risk features but no clinical correlation is available for recurrence	[118, 234, 291]
TP73	Tumor protein p73	8	Loss at 1p36	Not determined for retinoblastoma	[107]
TSC2	TSC complex subunit 2	2.4	CNV at 16p13.3	Inactivation leads to sensitization of RB cells towards cell death	[102, 292]
WT1	WT1 transcription factor	25	Gain at 11p13	Not determined for retinoblastoma	[107]

caused by different cell population within the tumor and should have been considered. The presence of two subclusters of RB patients was further supported by Affymetrix analysis of 59 patients [98]. Interestingly, the degree of photoreceptor differentiation and the presence of SCNVs, as observed in previous studies, were two key features distinguishing the subtypes. Additionally, subtype 2 differed from subtype 1 with higher expression of stemness markers and neuronal/ganglion markers. Furthermore, another study identified an MYCN-driven cluster within subtype 2, which further increased the degree of inter-patient heterogeneity and suggested three major clustering (clusters A, B, and C) of RB patients [126]. Clusters B and C, even though showing similar transcriptional profile, differ in the molecular circuitry. Apart from heterogeneity seen among patients, earlier analyses indicated the presence of ITH due to a mix of differentiated and undifferentiated cells within the same tumor [127]. Recent advantage in the technology with the advent of single-cell RNA sequencing (scRNA-seq) has enabled a more in-depth and complex understanding of ITH [128]. Using the 10 × genomics platform, Collin et al. revealed the presence of various clusters of cells at different cell-cycle stages in RB tumors, as determined by the expression of cell-cycle progression genes. Interestingly, by pseudo-time analysis, they identified a unique cluster of cone cells in their G2/M phase as an origin for RB [129]. Wong et al., using spatial transcriptomics (10X Visium) on RB samples, identified ten transcriptional distinct cluster cells with a composition of proliferating cells in different cell cycle (G2/M, and S phage) markers suggesting a transcriptionally heterogeneous population. Specifically, cluster 9 was unique among them with a high cone score suggesting a cone cell origin for this cluster [130]. Intra-tumor heterogeneity in RB tumors was further supported by single cell RNA sequencing of 14,739 cells from two RB tumors with endophytic growth. Here, the RB tumors also appeared to consist of multiple transcriptionally distinct clusters, primarily comprising two major cell types: cone cells and RB cells. The RB cells consisted of three subtypes, and the UBE2C gene was identified as the "pivot" gene in RB cells. Further analysis revealed that UBE2C amplification could drive cone progenitor cells to undergo a proliferative stage in RB tumors, thereby generating heterogeneity by acting as a switch between different cell types [131]. Similarly, Wu et al. carried out scRNA-seq analysis of seven RB samples to delineate and understand the heterogeneous nature of RB. They profiled 69,820 RB cells and identified 17 transcriptionally distinct clusters, primarily consisting of less proliferative cone precursor (CP)-like cells and proliferative MKI67 + CP cells. The RB tumor also contained small percentages of other cell types, such as glial cells, rod-like cells, and cone-like cells, which were considered normal cells as no copy number changes were identified in Chr1q, Chr6p, or Chr16q. This study further

highlighted the presence of a heterogeneous cell population in RB tumors, showing cells at various stages of malignant transformation. For example, MKI67 + CP cells with a high proliferative nature and high copy number variations represented the malignant cell state. Cone-like cells with retention of several visual-related functions, represented the normal cone cells, while CP-like cells with mixed phenotypes represented intermediate stage in the malignant transformation. Further, it should be noted that tumor-associated macrophages may contribute to a part of transcriptional heterogeneity by altering pathways in the associated tumor cells [132]. Liu et al. applied scRNA-seq to four RB samples (from two intraocular and two extraocular patients) and analyzed 128,454 cells. Interestingly, they found a unique set of cells specific to extraocular tumors, further suggesting inter and intra-transcriptional heterogeneity. Using expression patterns of highly variable genes they found 24 clusters of 9 different cell types. Tumor-type heterogeneity was also observed based on the expression profiles of cell-type markers. Specifically, intraocular RB tumors had primarily cones or cone-like cells, rods or rod-like cells, and retinoma-like cells, whereas extraocular RB tumors consisted mainly of microglia and rod precursor-like cells [133]. The three major cell types—cone precursor-like cells (CPL), MKI67 + photoreceptor-decreased cells (MKI67 + PhrD cells), and retinoma-like cells (RL-cells)—were equally represented in both intraocular and extraocular tumors but exhibited further cellular and molecular heterogeneity. Notably, the higher expression of SOX4 in the MKI67 + PhrD cells of extra-orbital tumors suggests a role for this population in local extension [133]. These studies consistently support a transcriptionally heterogeneous population in RB tumors, including a unique subset of cells with the potential to drive malignancy and recurrence.

4.6 Epigenetic heterogeneity in retinoblastoma

Epigenetic changes are non-genetic alterations in the genome influenced by the microenvironment. It includes DNA methylation, chromatin remodeling, histone modification, and non-coding RNA-mediated gene silencing [134]. These modifications control cellular functions like cell division and proliferation, migration, metabolism, and immunity, by altering gene expression [135], hence responsible for the clonal variations and heterogeneity in cancer, including RB [9, 135–137]. The functional loss of the RB1 gene is a major culprit for RB. The RB1 gene is subject to epigenetic regulation [138] and, in turn, epigenetically regulates several other driver genes [139]. Though the majority of RB tumors arise due to biallelic loss of the RB1 gene, however, in $RB^{+/-}$ tumor and $RB^{+/+}$ tumor, epigenetic reprogramming of the RB1 gene also contributes to the tumorigenesis process [140]. These include DNA modifications, non-coding RNA (ncRNA)-mediated regulation, and chromatin modulations [113, 121]. Hypermethylation of promoter and exon 1 overlapping CpG Island (CpG106) of the RB1 gene by DNMT family enzymes is one of the mechanisms of the RB1 gene dysregulation. Differential overexpression of certain DNMTs (DNMT1, DNMT3A, and DNMT3B) in differentially differentiated RB tumors has been reported [141, 142]. These findings indicated that heterogeneous dysregulation of the RB1 gene might lead to the generation of inter and intra-tumor heterogeneity in RB [141]. Furthermore, the RB 1 gene is also involved in the epigenetic regulation of other oncogenes like spleen tyrosine kinase (SYK) or tumor suppressors like BCOR, RASSF1A, and MGMT. The proto-oncogene SYK is predominantly expressed in hematopoietic cells and regulates the immunomodulatory signaling [143] and is also essential for tumor cell survival and proliferation [116, 144, 145]. However, its role in retinal development has not been established. Moreover, its upregulation in metastatic RB upon the RB1 loss indicated that this gene is epigenetically regulated by the RB1 gene [116]. In RB ($RB^{-/-}$), the SYK gene promoter is activated by increased histone activating modification of H3K4Me3 and K3 K9/14 Ac and unchanged modification of repressive histone marker (H3K9Me), leading to more access of RNA Pol II at SYK promoter [116]. Another histone modifier EZH2 protein which acts as histone methyl transferases modifies to H3K27 to K3 K27 me2/3 is crucial for the proliferation, differentiation, and EMT of cancer cells [146]. These modifications inactivate the target gene in RB cells [146]. However, EZH2 has also been shown to have a dual nature depending on its localized expression pattern [147]. Upregulated expression of EZH2 has also been observed in RB tumors with differential expression patterns depending on the stage, differentiation, and metastatic nature of RB tumor [148, 149]. The expression of EZH2 is epigenetically regulated by E2F family proteins, which are downstream to pRB [148, 150]. EZH2 also forms a complex with RB1 through recruitment by E2F2 and has been observed to mediate the silencing of repetitive DNA sequences via H3K27 me3 deposition. The inability of RB1 mutants to form a complex with EZH2 leads to the dispersion of H3K27 me3 and the expression of repetitive elements, which can contribute to tumorigenesis [151]. Interestingly, EZH2 suppresses RB1 expression by modulating H3K27ac enrichment at the RB1 enhancer. Notably, EZH2 expression inversely correlates with RB1 levels, and inhibiting EZH2 methylation activity increases RB1 expression, accompanied by enhanced H3K27ac enrichment at the RB1 enhancer [152].

BCOR mutation, a recurrent mutation seen in a few cases of RB (13%) [116], may alter the expression pattern of certain genes involved in cell proliferation and differentiation by modulating methylation of H3K4 and H3K64 [115, 153]. Truncated BCOR expression has also been reported

in RB [105, 116]. Similarly, the heterogeneous promotor hypermethylation of several other tumor suppressor genes like MSH6 (50%) [107], CD44 (42%) [107], PAX5A (42%) [107], GATA5 (25%) [107], RASSF1A (59–82%), and MGMT (15–35%) has also been reported in RB cases (Table 2) [154–156]. Most recently, based on the level of CpG methylation in RB tumors, patients were grouped into three distinct clusters, highlighting the role of DNA methylation in contributing to underlying heterogeneity[126]. Moreover, the role of pRB in the development of epigenetically derived heterogeneity cannot be ignored. pRB interacts with HATs, HDACs, and chromatin remodelers, such as brahma homolog 1 (BRG1), through its pocket domain (LXCXE binding motif). This interaction regulates the expression of genes involved in cell division and proliferation, like E2F. In RB, the loss of pRB function leads to significant alterations in gene expression patterns [157]. Besides DNA methylation and histone modifications, other well-known epigenetic modifications such as ubiquitination and sumoylation at histone proteins are also observed in RB and other cancers [137, 158–162]. ncRNAs, RNAs that are not translated to proteins, are also important epigenetic modifiers controlling cellular and epigenome homeostasis [163, 164]. They can be of housekeeping (rRNA, tRNA, snRNA, snoRNA, etc.) or regularity nature (miRNA, siRNA, piRNA, lncRNA, etc.) [165]. Several lnc-RNAs like BDNF-AS and MT1JP [166], LINC00202 [167], CANT1 [168], and GAU1 [169] have been shown to be heterogeneously expressed among patients and can be employed as potential prognostic biomarkers for RB [166, 170]. MicroRNAs (miRNAs) are the most widely studied nc-RNAs and are reported to modulate about 60% of protein-coding genes in the human genome [171]. miRNA- 98 (miR- 98) has been shown to control RB growth and metastasis through IGF1R/k-Ras/Raf/MEK/ERK signaling pathway [172]. Although RB tumor usually has lower expression of miR- 98 as compared to normal retina, heterogeneity can be seen in its expression among the RB patients. Importantly, the RB patients with low expression of miR- 98 showed poor prognosis as compared to those with high expression [172]. Similarly, the expression

Table 2 Heterogeneity in epigenetic alteration in retinoblastoma patients

Gene	Gene name	Alteration (%)	Kind of alterations	Association of gene with RB metastasis, recurrence, or chemoresistance	References
APC2	APC regulator of WNT signaling pathway	70	Hypermethylation	Mutation leads to malignant transformation and metastasis; downregulation might have positive chemotherapeutic response on RB cells	[33, 293]
CD44	CD44 molecule (Indian blood group)	42	Hypermethylation	High expression is associated with enhanced metastasis and induces chemoresistance and stem cell differentiations	[107, 294]
CDKN2 A	Cyclin-dependent kinase inhibitor 2 A	55	Hypermethylation	Correlated with poor differentiation and metastatic progression	[295]
GATA5	GATA binding protein 5	25	Hypermethylation	Not determined for retinoblastoma	[107]
GSTP1	Glutathione S-transferase pi 1	8	Hypermethylation	Not determined for retinoblastoma	[107]
MGMT	O- 6-Methylguanine-DNA methyltransferase	35–58	Hypermethylation	Downregulation by promoter methylation leads to metastasis	[107, 296]
MLH1	MutL homolog 1	67	Hypermethylation	Not determined for retinoblastoma	[297]
MSH6	MutS homolog 6	50	Hypermethylation	Not determined for retinoblastoma	[107]
PAX5	Paired box 5	41.6	Hypermethylation	Not determined for retinoblastoma	[107]
RASSF1 A	Ras association domain family member 1	59–80	Hypermethylation	Silencing of RASSF1 A by promoter hyper-methylation might results in enhanced metastasis; inactivation can dysregulate the RAS, Hippo, Wnt, and other tumor-related signaling pathways, which potentially results in drug resistance	[118, 298]
SYK	Spleen tyrosine kinase	93–100	Hypomethylation	Not determined for retinoblastoma	[116, 239]
TP53	Tumor protein p53	8	Hypermethylation	Not determined for retinoblastoma	[107]
VHL	Von Hippel-Lindau	8	Hypermethylation	Downregulation of VHL causes increases in sensitivity to chemotherapy (vincristine)	[107, 299]

Table updated from previously published data [118]

of miR- 101 seems to be inversely proportional to EZH2 expression and varies among RB patients with high EZH2 and low miR- 101, which showed invasive phenotype [173]. Several miRNA studies have demonstrated the diverse role of miRNA in RB pathogenesis [137, 174] and have a potential role in generating the heterogeneity in RB and hence can be targeted for therapeutic applications [137, 163, 175–177].

4.7 Metabolic heterogeneity in retinoblastoma

The developing tumor cells acquire not only genetic alterations but also selectively favor specific metabolic pathways that have advantages for their fitness and survival [178]. These adaptive metabolic adjustments in dividing tumor cells also selectively adopt specific metabolic processes during the metastasis cascade events [179, 180]. In solid tumors, the tumor microenvironment, the altered cell metabolism, vascularization and oxygenation, and cellular communications promote the intra-tumoral metabolic heterogeneity and can induce cellular plasticity [181, 182]. There are various reports documenting intra-tumoral metabolic heterogeneity in different tumors [10, 53, 99–101, 103, 113]. The functional loss of the pRB1 (RB1 protein) in the mutant lung cancer cell (k-Ras$^{-/-}$) has been reported to modulate cell metabolism via uplifting glucose metabolic genes [183]. However, Babu et al. demonstrated that RB tumors with pRB1 loss lack HK1 (hexokinase 1), resulting in a metabolic switch to mitochondrial-mediated oxidative phosphorylation (OXPHOS) for ATP generation and exhibit elevated fatty acid oxidation [184]. Moreover, upon pRB1 overexpression, there was a decrease in mitochondrial respiration, suggesting that RB tumors with varying functional RB1 protein levels might exhibit different metabolic profiles. Although not directly observed in RB tumors, a haplo-insufficient mouse model of the RB (RB1$^{+/-}$) gene demonstrated that a partial deficiency of the RB1 gene could lead to metabolic alterations, including increased fatty acid oxidation and heightened insulin sensitivity [185]. Gulati et al. performed serum metabolomics from RB patients and demonstrated differential metabolite patterns in unilateral and bilateral RB patients. This study revealed variations in metabolic profiles among healthy, unilateral, and bilateral RB patients. Importantly, lactate, a byproduct of anaerobic glycolysis, was explicitly found in the serum of unilateral RB, while various amino acids such as proline, arginine, methionine, and threonine, usually derived from citric acid intermediates, were specifically found in the serum of bilateral RB. Although this was a serum-based study, it demonstrated metabolic heterogeneity in RB tumors, with distinct glycolysis and citric acid cycle regulation in unilateral and bilateral RB tumor [186]. Distinct metabolic profiles for unilateral and bilateral RB patients were further supported by another study that analyzed metabolites in neonatal blood spots collected from RB patients. In unilateral RB, they found a unique enrichment of hippuric acid and glutamine, indicative of the tyrosine metabolism pathway. Conversely, in bilateral RB, there was an enrichment of arachidonic acid and N-acetylneuraminic acid, suggesting enhanced linoleic acid metabolism, amino sugar metabolism, and bile acid biosynthesis [187]. Kohe et al. utilized high-resolution magic-angle spinning magnetic resonance spectroscopy (HR-MAS MRS) on enucleated RB patients (48 unilateral and 4 bilateral) and identified three distinct metabolic subgroups in RB. Levels of taurine, hypotaurine, phosphocholine, creatine, and lipid could discriminate different subgroups of RB. Group 1 had elevated lipid levels and low levels of taurine, hypotaurine and creatine, while group 3 had lower lipid levels but high levels of taurine and creatine. Group 2 was characterized by the highest levels of hypotaurine and hypocholine. These observations provide further evidence of metabolic heterogeneity in RB [188]. The retinoblastoma protein (pRB) has also been involved in various metabolic signaling pathways in different other cell types like p53 [189–191], Wnt [192–194], Ras/Mek/Erk [172, 195, 196], Notch [197–199], Hippo/YAP [200, 201], and UBE2T/STAT3 [202]. The multifaceted role of RB protein (pRB) in metabolism indicates its potential role in generating metabolic heterogeneity in RB tumors. Furthermore, the activation of different metabolic pathways in RB cells due to RB1 loss or dysfunctional RB protein might influence the generation of cellular plasticity in the RB tumor and, hence responsible for generating a metabolically heterogeneous RB [203]. Moreover, metabolic dynamics due to stochastic influences in cancer tissue can be deciphered using single-cell metabolomics and would be an ideal approach to understand the metabolic heterogeneity in RB and other cancers [204, 205].

5 Role of stem cells in retinoblastoma heterogeneity

In most cancers, a certain subset of cells tends to exhibit extensive proliferation and self-renewal capacity referred to as cancer stem cells (CSC) or cancer stem-like cells [206]. RB tumors also contain a cancer stem cell population. Using the first knockout mouse model of RB, four different retinal cell populations, namely, retinal stem cells, proliferating progenitor cells, newly post mitotic cells, or differentiated cells capable of reentering the cell-cycle, have been suggested to be the cell of origin of RB [207]. However, a study conducted in a mouse model with double inactivation of RB/p107 showed that intrinsically death-resistant precursors could give rise to RB tumor by overcoming differentiation-induced growth arrest [208]. Subsequent studies demonstrated cone precursor cells as the cell of origin for

RB in humans [209–214]. While early studies in murine models suggested multiple potential cells of origin for RB, human studies have increasingly pointed to cone precursor cells as the likely origin. However, heterogeneity within RB tumors remains an area of active investigation. Kapatai et al. identified two distinct retinoblastoma subtypes—one expressing multiple retinal cell markers with characteristic chromosomal alterations and another resembling cone photoreceptors with high metabolic activity and proliferation [124]. In contrast, Kooi et al. proposed a tumor progression model, suggesting that RB tumors originate as smaller, more differentiated lesions with fewer genomic alterations and evolve into larger, less differentiated, more proliferative, and genomically unstable tumors [125]. While both studies used human samples, the former suggests intrinsic molecular subtypes, whereas the latter supports a dynamic progression model, underscoring the complexity of RB heterogeneity. These findings highlight ongoing debate regarding the precise cell of origin in retinoblastoma, with evidence varying between murine models and human studies. The discrepancies underscore species-specific differences in tumor initiation, emphasizing the need for further investigation using advanced lineage-tracing models and human-derived systems to resolve these inconsistencies. Like other cancers, in RB, CSCs could lead to the generation of ITH. pRB protein has been shown to control the pluripotency of cells and loss of the RB gene has been shown to favor the iPSCs induction by controlling Sox2 and Oct4 [215]; whether loss of RB1 itself favors CSC-like properties is unclear. However, several studies suggested the presence of CSC-like cells in RB. Murali et al. screened RB tumor cells with putative stem cell markers (ABCG2, CD44, and CXCR4) and retinal cell markers (CD90 and CD133). They identified two distinct cell subpopulations; population 1 contained $CD44^{+}$ high and was mostly negative for CD133, CXCR4, and CD90, while population 2 was positive for all the markers studied, including CD44, CD133, CXCR4, and CD90. Population 1 showed higher expression of retinal progenitor markers like Syntaxin 1 A and PROX1 than population 2, suggesting a tumor hierarchy [216]. Similar to primary tumors, the Y79 cells, a commonly used human RB cell line, exhibited two distinct population based on the expression of CD133, although these cells did not express CD44 at all. Evidence for tumor hierarchy was also found here, with $CD133^{lo}$ cells which had high expression of embryonic stem cells genes and progenitor genes, being able to differentiate into $CD133^{hi}$ cells [217]. These studies also highlight and support the presence of heterogeneous cell populations and heterogeneity among proposed stem cell populations. Another indication of CSC-like cells presence in RB tumors comes from the frequently observed amplification or overexpression of OTX2 in primary RB tumors and cell lines [218]. Homeodomain transcription factor, OTX2, is important for the formation of photoreceptor cells. Higher expression of OTX2 may contribute to the gain of proliferation and stem cell-like properties to the RB tumor cells and can induce transdifferentiation of the retinal cells via Crx, Nrl, and Wnt signaling pathway [219]. The presence of CSC population in RB tumor is also supported by the study done by Seigel et al. that identified the expression of various stem cell markers (musashi- 1 and 2, prominin1 (CD133), sialomucin (CD164), PAX6, nestin, neuroD1, jagged1 and 2, noggin, smoothened, frizzled2, numb, patched, NCAM-1, Notch4, reelin, paxillin, VISX1, leukemia inhibitory factor, HESX1, MCM2, and NET1) in both cell line (Y79 and WERI-RB1) and human RB tumors [220]. Further, the expression of ATP binding cassette ABCG2, a stem cell marker, and minichromosomal maintenance 2 protein (MCM2), a neuronal stem cell marker by a subpopulation of RB tumors, points towards the presence of CSC-like cells in RB tumor [221, 222]. More recently, scRNA-seq study on RB tumors also demonstrated the presence of a subset cell population in RB tumors having varying degrees of stemness markers expression [98]. All these studies supported the presence of subpopulation of cells in RB tumor with stem cell-like features and their contribution to the RB tumor heterogeneity.

6 Management of retinoblastoma and challenges due to RB heterogeneity

Retinoblastoma is potentially curable, and the prognosis of the disease is highly dependent on early diagnosis and accurate therapy. However, the management of RB is complex and challenging due to the heterogenic nature of the tumor and requires a multidisciplinary approach to treatment [223]. Recent advancements in treatment modalities, like selective intra-arterial chemotherapy (SIAC), intravitreal chemotherapy, and intracameral chemotherapy, have critically improved the chances of globe salvage and vision preservation [14, 223, 224]. However, local extra-ocular extension and metastasis after chemotherapy remain a great challenge in the treatment of advanced RB tumors. Tumor heterogeneity is one of the major causes of chemoresistance and recurrence [225, 226]. This is primarily because not all tumor cells behave similarly or respond equally to chemotherapy. In RB, the presence of certain mutations, such as 6p gain, invariably correlates with a poor response to therapy [227]. Further presence of BCOR mutations is associated with poor prognosis in those patients [98]. Tumor heterogeneity either spatial (within or/and between the primary tumor and metastasis) or temporal (heterogeneity due to polyclonal properties of tumor that evolved over a period of time) is the key challenge in the treatment and management of any cancer [2]. Despite extraordinary advances in treatment modality either in chemotherapy or focal therapy, new tumors develop

in 6–45% of patients. Additionally, the recurrence rate varies from 0 to 45% depending on the treatment approach such as systemic, intravitreal, and intra-arterial chemotherapy or combination of all [27]. These observations suggest that in RB, a subset of cells either evade treatment or do not respond to therapy. The presence of CSC-like cells which are extremely difficult to detect and hardly targetable by chemotherapy [136, 206, 228] could partly explain RB recurrence. It is well established that even a single CSC can lead to relapse and metastasis[229, 230]. Indeed, in a group of patients with stemness features, poor prognosis was observed [98]. Further, heterogeneous expression of epigenetic factors (such as EZH2, BCOR), long non-coding RNAs (BDNF-AS, MT1JP, and LINC00202) and miRNAs (miR- 98, miR- 101) among patients contributes to varying degree of prognosis [98, 170, 173].

Apart from genetic heterogeneity, variations in clinical and histopathological features across widely used RB grouping systems can also impact its management. RB is clinically classified into groups A through E, with group A representing early-stage disease and group E indicating advanced-stage tumors. Although this classification widely used as a clinical framework for treatment decision-making, however, it does not fully encapsulate the biological complexity and heterogeneity of RB [89]. Tumors within the same clinical group can exhibit widely varying degrees of aggressiveness and metastatic potential, complicating risk stratification. For instance, approximately 17% of group D RB cases display high-risk pathological features upon post-enucleation histological analysis, while some group E tumors, despite their advanced clinical staging, demonstrate low-risk characteristics. This discrepancy highlights the inherent limitations of current staging paradigms in accurately predicting tumor behavior and underscores the necessity for complementary molecular and imaging-based biomarkers to refine prognostication and therapeutic decision-making [231].

Management of group A–C RB typically involves focal therapies such as laser photocoagulation, cryotherapy, and brachytherapy, which are frequently administered in conjunction with systemic or intra-arterial chemotherapy to optimize tumor control while preserving visual function [95, 232]. However, group D RB management typically involves systemic chemotherapy aimed at tumor reduction, with enucleation considered when there is inadequate response to chemotherapy or when vision preservation is unlikely [233]. Group E RB with high-propensity for high-risk features is managed with enucleation, often followed by adjuvant chemotherapy in those with HRHF to prevent systemic metastasis. In contrast, eyes without HRHF following enucleation display a 99% 2-year event-free survival without chemotherapy, indicating minimal risk of metastasis [95, 224]. A fundamental clinical challenge arises from the inability to identify HRHFs before enucleation. Since these pathological determinants are crucial for assessing metastatic risk, their post-surgical identification complicates prognostic evaluation and treatment planning [89]. Such HRHFs in RB have been correlated with molecular markers linked to chemoresistance, impacting treatment efficacy and prognosis. For instance, a retrospective study found that there was an elevated p53 expression, a recognized poor prognostic indicator, in 35% of cases with choroidal invasion, an established HRHF. Although not statistically significant, it highlighted the interplay between histopathologic aggression and molecular dysregulation [234]. Similarly, chromosome 6p amplification has been associated with aggressive histopathologic features in RB, with 30.4% of enucleated eyes exhibiting high-risk characteristics, suggesting its potential role as a biomarker for tumor aggression and chemoresistance [227]. Likewise, increased $p16^{INK4a}$ expression has been observed in undifferentiated tumors and Homer-Wright rosettes, indicating a role in tumor plasticity and treatment resistance [235], while elevated miR- 181a levels have been associated with epithelial-to-mesenchymal transition (EMT) and chemotherapy resistance [236]. Identifying these molecular markers alongside histopathologic assessment could refine risk stratification, guide personalized treatment strategies, and improve prognostic accuracy in RB management.

However, the classification of RB into high-risk and low-risk categories remains predominantly dependent on post-enucleation histopathological assessment. This reliance on enucleated specimens presents a significant limitation for patients undergoing eye-preserving therapies, as the absence of tumor tissue precludes precise risk stratification and individualized therapeutic planning. Emerging liquid biopsy techniques offer promising solutions to this challenge. This underscores a critical need for pre-enucleation molecular and imaging biomarkers to facilitate early risk stratification, accurately delineate tumor behavior, and refine therapeutic decision-making in RB. Non-invasive biomarkers capable of assessing metastatic potential and treatment response before enucleation could significantly enhance prognostic evaluation, enabling more precise risk-adapted treatment strategies and ultimately improving clinical outcomes.

The analysis of cell-free DNA (cfDNA) obtained from aqueous humor (AH) or blood represents a minimally invasive strategy for tumor genomic characterization in RB [237–239]. These approaches have enabled the detection of genetic and epigenetic alterations resembling those in respective RB tumor tissue, supporting their potential as surrogate tumor biopsies in RB [238, 239]. In particular, highly recurrent SCNAs such as gain of 1q, 2p, 6p, loss of 13q, 16q, and focal MYCN amplification could be detected in AH-derived cfDNA [238]. Furthermore, Berry and her team, through a five-year follow-up study utilizing

AH-derived cfDNA analysis, identified chromosome 6p gain and focal MycN gain as poor prognostic markers in RB. They also proposed AH cfDNA tumor fraction as a determinant of treatment response, advocating for the suitability of AH as a companion diagnostic for RB [240]. In the coming years, AH may be incorporated as a routine diagnostic tool, at least in developed countries.

Additionally, radiogenomics—an integrative approach combining imaging data with genomic profiling—has demonstrated potential in refining risk stratification and guiding personalized treatment strategies. Recent studies indicate that specific imaging features may correlate with underlying genetic alterations, further enhancing the predictive accuracy of non-invasive diagnostics [241].

Further integration of basic research findings into clinical practices may improve the management of the disease. Apart from other SCNVs, BCOR has been found to be mutated in a subset of RB cases and has been correlated with poor prognosis. This mutation can be detected in cfDNA isolated from the aqueous humor (AH) of RB patients [113]. BCOR is frequently mutated in various malignancies, including acute myeloid leukemia (AML), clear cell sarcoma of the kidney (CCSK), high-grade endometrial stromal sarcomas, and myelodysplastic syndrome, where its alterations are linked to aggressive tumor phenotypes and therapy resistance [242–245]. Given BCOR's association with poor prognosis and recurrence in RB, its detection in AH-derived cfDNA may serve as a foundation for novel prognostic markers and targeted therapeutic strategies in RB [102, 116]. Interestingly, the DNA methyltransferase inhibitor decitabine has shown 57% complete remission in BCOR-mutant myelodysplastic syndrome and demonstrated efficacy in RB cell lines [245, 246]. Exploring its combination with chemotherapy for BCOR-mutant RB, identified via AH cfDNA biopsy, could offer valuable therapeutic insights.

Additionally, basic research has indicated that oncolytic virotherapy may be a promising strategy for RB treatment, leveraging genetically engineered adenoviruses to selectively target and lyse malignant cells. A particularly compelling approach utilizes the E2F1 promoter to drive virus replication [247]. In RB, inactivation of the RB1 gene results in pRb dysfunction, leading to aberrant E2F1 activity. By exploiting this tumor-specific deregulation, adenoviruses engineered to replicate under E2F1 promoter control can selectively proliferate in RB cells while sparing normal cells with intact pRb function [247, 248]. This targeted viral replication induces oncolysis and potentiates anti-tumor immune responses, positioning oncolytic adenoviruses as a novel therapeutic avenue with the potential to enhance RB treatment efficacy while minimizing off-target effects.

7 Current research challenges in exploring retinoblastoma tumor heterogeneity

Despite significant advancements in elucidating the complexity of RB tumor heterogeneity, several persistent challenges continue to hinder progress in this field. These obstacles span biological, technical, and clinical domains, affecting both fundamental research efforts and their translational applications.

As mentioned previously, limited access to tumor tissue presents a critical barrier to advancing RB heterogeneity research, as enucleation is typically reserved for advanced-stage disease [249]. Moreover, in cases of secondary enucleation, tumor calcification presents a significant obstacle to assessing heterogeneity, potentially obscuring crucial insights into the mechanisms underlying chemoresistance. Calcifications are a hallmark of RB, often complicating histopathological evaluations by obscuring detailed tissue architecture, thereby making it challenging to accurately assess intra-tumoral heterogeneity [250]. Liquid biopsy methodologies utilizing aqueous humor or blood-derived cfDNA provide a promising alternative for tumor characterization. However, these approaches remain constrained by technical challenges, including the limited yield of tumor-derived cfDNA, the potential for non-tumor DNA contamination, and the need for improved sensitivity and specificity in detecting intra-tumoral variations [251].

The dynamic clonal evolution of RB tumors presents another major challenge, particularly in the context of therapeutic resistance [252]. Subclonal populations within the tumor can accumulate genetic and epigenetic alterations that facilitate evasion of chemotherapeutic agents, thereby diminishing treatment efficacy [253]. Despite growing interest in the mechanisms driving these adaptive changes, a comprehensive understanding remains elusive, limiting the development of targeted strategies to mitigate resistance and improve clinical outcomes [252, 254]. Single-cell techniques, including single-cell RNA sequencing (scRNA-seq), single-cell DNA sequencing (scDNA-seq), single-cell proteomics, and single-cell epigenetics, have been widely used to examine intra-tumor heterogeneity. These approaches are advantageous in dissecting cellular composition and molecular characteristics, providing deeper insights into cellular heterogeneity [254, 255].Additionally, scTrio-seq, a single-cell triple-omics sequencing technique that simultaneously analyzes CNVs, DNA methylation, and transcriptome [256], could further enhance our understanding of the heterogeneity and complexity of RB.

The availability of robust preclinical models also remains a significant hurdle. While retinoblastoma cell lines such as RB116, Y79, and WERI-Rb1, genetically

engineered mouse models (GEMMs), and patient-derived organoids have yielded valuable insights into RB pathobiology, they fail to fully recapitulate the heterogeneity observed in human tumors. Disparities in tumor microenvironmental factors, immune interactions, and evolutionary trajectories between these models and actual patient tumors present substantial barriers to translating preclinical findings into effective clinical interventions [257].

Further progress in RB heterogeneity research necessitates the integration of multi-omics datasets encompassing genomics, transcriptomics, epigenomics, and proteomics. However, the sheer complexity of analyzing and interpreting such vast datasets, particularly given the limited availability of tumor biopsy material, presents a formidable challenge. Standardized computational frameworks for harmonizing these diverse data modalities are still under development, posing obstacles to deriving clinically meaningful insights.

Ultimately, the overarching objective of investigating RB tumor heterogeneity is to facilitate precision oncology approaches. However, translating these findings into clinical practice remains fraught with difficulties, largely due to the lack of validated biomarkers for risk stratification and treatment response monitoring. Ethical considerations surrounding experimental biopsies in pediatric patients further complicate efforts to implement personalized therapeutic strategies tailored to tumor heterogeneity.

Addressing these multifaceted challenges requires a concerted interdisciplinary effort, leveraging advancements in liquid biopsy technologies, patient-derived organoid models, single-cell sequencing, single-cell multi-omics, and computational bioinformatics. Overcoming these barriers will be pivotal in refining risk stratification, predicting therapeutic responses, and developing novel targeted interventions that account for the heterogeneous nature of RB tumors.

8 Conclusion and future directions

Cancer cell heterogeneity presents a significant challenge in the management of any cancer. The presence of inter- and intra-tumor heterogeneity has been recognized in RB. Studies employing omics techniques, such as high throughput RNA-seq analysis, WES, and WGS, have revealed genomic and transcriptomic heterogeneity. scRNA-seq based studies have been instrumental in identifying the presence of distinct cell populations based on the cell cycle status and the expression profiles, suggesting a heterogeneous population within a tumor. Further, studies using cell surface markers analysis, functional studies and scRNA-seq analysis have

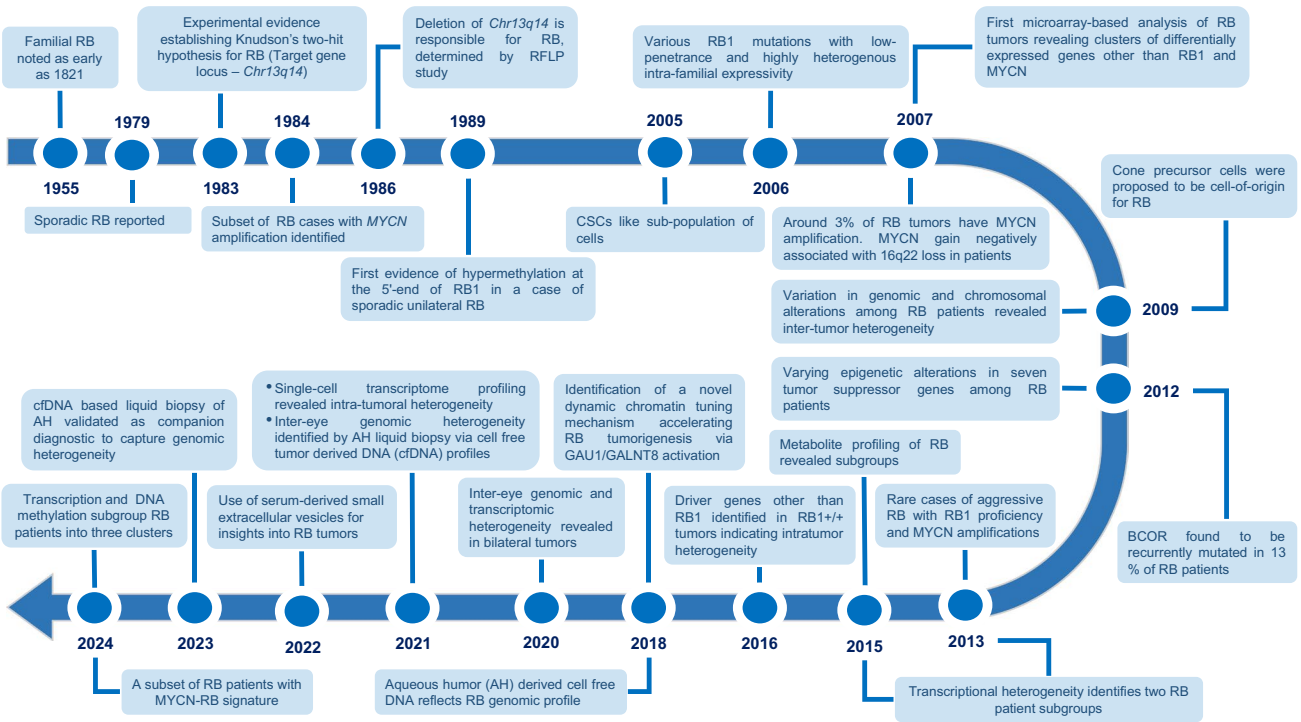

Fig. 2 Chronological overview of pivotal discoveries that expanded our knowledge of tumor heterogeneity in retinoblastoma (RB): Key milestones encompass genetic, epigenetic, and transcriptomic alterations, the identification of MYCN-amplified RB subsets, and the involvement of cancer stem-like cells. Advancements in molecular profiling, single-cell transcriptomics, and liquid biopsy methodologies underscore the evolving landscape of RB heterogeneity

supported the presence of CSC-like subpopulation in RB tumors. Figure 2 illustrates the chronological progression of studies exploring tumor heterogeneity in retinoblastoma Most insights into tumor inter- and intra-heterogeneity are derived from analyzing tumor tissue after enucleation. However, since it is not feasible to acquire this information in real-time during RB management due to the lack of opportunities for tumor biopsy, translating these findings into actionable research remains challenging. A promising alternative to traditional biopsies is the minimally invasive approach of liquid biopsy [258]. Liquid biopsy allows for the detection of diverse analytes, including metabolites, proteins, RNA, circulating tumor cells (CTCs), cell-free circulating nucleic acids—particularly circulating tumor DNA (ctDNA)—tumor-derived extracellular vesicles (tdEVs), tumor-educated platelets (TEPs), and autoantibodies. These components make liquid biopsy a valuable tool for biomarker evaluation and the exploration of tumor heterogeneity in cancer patients [259]. Importantly, liquid biopsy enables longitudinal monitoring of genomic and molecular changes, providing real-time insights throughout the course of treatment [239, 258, 260, 261]. In RB, liquid biopsies derived from blood, aqueous humor (AH), and vitreous humor (VH) have shown significant potential as sources of biomarkers [258–260, 262, 263]. Metabolomic analysis of VH successfully distinguished advanced RB patients from non-advanced and control groups, highlighting its applicability in understanding inter-patient heterogeneity [264]. Similarly, serum metabolomic analysis from RB patients demonstrated its utility in distinguishing between unilateral and bilateral cases, as well as between invasive and non-invasive disease [186, 265]. Such insights could guide critical decisions, such as enucleation versus globe-salvage strategies. While such samples may not completely fill the lacunae regarding our knowledge of intra tumor heterogeneity, especially in multifocal tumors; liquid biopsy can partially address inter-patient tumor heterogeneity by predicting tumor subtypes and assessing disease severity prior to enucleation.

To further enhance RB management, it is essential to detect patient-specific genetic and epigenetic modifications, which can help design more targeted and effective treatment strategies. Modifications such as SNVs, CNVs, and specific promoter methylation contribute significantly to RB tumor heterogeneity and are associated with metastasis risk and therapy resistance (discussed in Sects. 4.3 and 4.6) [113]. In RB, genomic analysis of cfDNA extracted from AH liquid biopsy samples have consistently revealed SCNV and SNVs profiles that mirror those of primary tumors [260, 266]. Notably, genetic alterations detected in AH-derived cfDNA/ctDNA have demonstrated strong correlations with treatment responses, underscoring their potential as predictive biomarkers for therapeutic outcomes [240]. Further exploration of these approaches is warranted to better understand their applicability in reflecting inter-tumor heterogeneity and to advance personalized RB management strategies.

Analyzing transcriptomic heterogeneity in tumor tissue through liquid biopsy remains challenging, but recent advancements have demonstrated the potential of transcriptomic profiling of biofluid derived extracellular vesicles (EVs). EVs are lipid bilayer vesicles secreted by all cells, including tumor cells, and they carry the contents of their parent cells. In colorectal cancer, transcriptomic profiling of plasma-derived EVs has enabled accurate annotation of the cancer-specific transcriptome and molecular subtypes, providing valuable insights into the tumor's transcriptomic landscape [267]. Therefore, analyzing RNA content within EVs offers a promising tool for exploring transcriptomic alterations and identifying biomarkers that reflect tumor molecular heterogeneity. In RB, serum-derived EVs have also been studied to investigate tumor-specific RNA and treatment-resistant pathways, comparing samples from complete remission with those from resistant cases [268, 269]. However, further analysis is required to determine whether EVs RNA evaluation can be used for subtyping Rb tumors.

In addition, artificial intelligence (AI) and machine learning (ML) have emerged as pivotal tools in the study of intraocular tumors, particularly in the diagnosis and prognostication of RB, and should be tested to accurately identify clinical heterogeneity. The use of AI and ML could also be helpful in addressing diagnostic variability. AI-driven fundus imaging, employing deep learning models such as convolutional neural networks (CNNs), has demonstrated remarkable accuracy in detecting RB-specific features, including leukocoria, tumor masses, and vascular irregularities, despite the complexities introduced by tumor heterogeneity [270]. Multiple studies have substantiated AI's diagnostic efficacy, with Lima et al. reporting a pooled sensitivity of 98.2% and specificity of 98.5% across diverse tumor subtypes [271]. Additional studies by Kaliki et al., Vempuluru et al., and Aldughayfiq et al. have further validated AI's robustness in RB classification by utilizing Local Interpretable Model-agnostic Explanations (LIME) and SHAPley Additive exPlanations (SHAP) to highlight heterogeneous features like tumor margins and calcification, demonstrating high sensitivity and specificity across varied populations and tumor subtypes [272–274].

Despite these advancements, significant challenges persist, particularly in leveraging AI for prognostication. While Vempuluru et al. demonstrated high accuracy in classifying RB tumors into ICRB groups to suggest severity (e.g., group E as advanced), predicting metastatic potential and treatment response remains inherently complex due to the underlying biological heterogeneity of RB [273]. Moreover, the dynamic nature of tumor progression, including events such as vitreous seeding, limits the predictive power of current

AI-based models, necessitating longitudinal data integration to enhance prognostic accuracy [271, 273]. Furthermore, the scarcity of high-quality, standardized imaging datasets and interpatient variability in tumor phenotypes constrain the generalizability of AI-driven models. Addressing these limitations will require a multimodal diagnostic approach, integrating AI with magnetic resonance imaging (MRI), genomic profiling, and liquid biopsy-based biomarkers to refine risk stratification and improve therapeutic decision-making. Such advancements have the potential to bridge the gap between computational analytics and personalized medicine, ultimately advancing the precision and efficacy of RB management.

Gene therapies also represent a promising frontier in RB treatment, with suicide gene therapy, oncolytic adenoviruses, and adeno-associated virus (AAV)-mediated approaches demonstrating considerable therapeutic potential. However, the inherent heterogeneity of RB tumors characterized by distinct genetic alterations within subpopulations can contribute to tumor recurrence and metastasis and poses a significant challenge to treatment efficacy and resistance mechanisms [4, 96].

One of the widely investigated approaches, HSV-TK/ganciclovir suicide gene therapy has shown promise in selectively inducing tumor cell apoptosis while preserving healthy tissues by phosphorylation of the prodrug ganciclovir into its active form, which then incorporates into the DNA of the dividing tumor cells, leading to DNA chain termination and subsequent cell death. Preclinical studies confirm tumor suppression, though complete eradication remains elusive, with autophagy inhibition implicated as a key mechanism of action [275, 276]. Similarly, oncolytic virotherapy utilizing VCN-01 exploits dysregulated RB1 signaling to enable selective viral replication within tumor cells. Preclinical studies in mice and juvenile rabbits and early-phase clinical trials have demonstrated its efficacy against chemoresistant RB while maintaining a favorable safety profile with minimal systemic viral exposure and immune activation in treated patients [247].

Recombinant AAV2 (rAAV2)-mediated RB1 gene restoration has also emerged as a viable strategy, significantly inhibiting tumor growth in preclinical models [277]. However, the persistence of additional oncogenic mutations underscores the need for broader therapeutic interventions. Given that RB1 restoration alone may be insufficient, future research should focus on multigenic gene editing and combinatorial therapeutic approaches that integrate gene therapy with chemotherapy and immunotherapy to more effectively address RB tumor heterogeneity and enhance overall treatment outcomes [33].

Despite significant advancements, key questions regarding the molecular determinants of intra-tumoral heterogeneity in RB remain unanswered, particularly in the context of tumor evolution and therapeutic resistance [278]. Future research should prioritize single cell sequencing and spatial transcriptomics [279] to delineate tumor microenvironmental interactions and clonal dynamics that contribute to differential treatment responses. Furthermore, the integration of high-resolution multi-omics approaches with radiogenomic and computational modeling [280] could refine biomarker discovery, enhance risk stratification, and facilitate the development of precision therapeutic interventions tailored to heterogeneous RB subtypes. However, further validation through large-scale studies is necessary to standardize these methodologies and ensure their clinical applicability.

A key challenge remains targeting the heterogeneous tumor population. Research focused on identifying nodes specific to tumor-type-specific clusters and developing node-specific treatment could be beneficial. Inter-patient and inter-eye genomic heterogeneity has been observed and shown to affect their response to treatment as well as the extent of malignancy. RB tumors with certain genomic alternation persist all the available treatments, leading to recurrence. This underscores the importance of developing new therapies targeting specific gene alterations alongside chemotherapy, similar to approaches in other tumors. Targeted therapy for CSCs and specific tumor subpopulations may offer a more effective way of treating RB patients.

Given that RB tumor heterogeneity profoundly influences treatment response, including the development of chemoresistance, a deeper understanding of its molecular and epigenetic landscape is essential. Leveraging liquid biopsy-based studies alongside advancements in AI-driven analytics and gene therapy holds immense potential for unraveling this heterogeneity and translating these insights into clinical practice. Such an approach could help identify patients for treatment stratification, improve therapeutic response, and enhance diagnostic and prognostic precision, ultimately optimizing outcomes for RB patients.

9 Limitations of the study

The authors acknowledge that this review is inherently constrained by the scope and quality of available literature. Furthermore, the evolving nature of research in this field necessitates continuous reassessment as new data emerges.

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Declarations

Competing interests The authors declare no competing interests.

References

1. Lee, E. Y. H. P., & Muller, W. J. (2010). Oncogenes and tumor suppressor genes. *Cold Spring Harbor Perspectives in Biology, 2*, a003236–a003236. https://doi.org/10.1101/cshperspect.a003236
2. Zhu, L., Jiang, M., Wang, H., et al. (2021). A narrative review of tumor heterogeneity and challenges to tumor drug therapy. *Annual Translational Medicine, 9*, 1351. https://doi.org/10.21037/atm-21-1948
3. El-Sayes, N., Vito, A., & Mossman, K. (2021). Tumor heterogeneity: A great barrier in the age of cancer immunotherapy. *Cancers (Basel), 13*, 806. https://doi.org/10.3390/cancers13040806
4. Biswas, A., & De, S. (2021). Drivers of dynamic intratumor heterogeneity and phenotypic plasticity. *American Journal of Physiology-Cell Physiology, 320*, C750–C760. https://doi.org/10.1152/ajpcell.00575.2020
5. Zelezníková, T., & Babusíková, O. (2006). The impact of cell heterogeneity and immunophenotypic changes on monitoring minimal residual disease in acute myeloid leukemia. *Neoplasma, 53*, 500–506.
6. Patra, S., Elahi, N., Armorer, A., et al. (2021). Mechanisms governing metabolic heterogeneity in breast cancer and other tumors. *Frontiers in Oncology, 11*, 700629. https://doi.org/10.3389/fonc.2021.700629
7. Sengupta, D., & Pratx, G. (2016). Imaging metabolic heterogeneity in cancer. *Molecular Cancer, 15*, 4. https://doi.org/10.1186/s12943-015-0481-3
8. Moldogazieva, N. T., Mokhosoev, I. M., & Terentiev, A. A. (2020). Metabolic heterogeneity of cancer cells: An interplay between HIF-1, GLUTs, and AMPK. *Cancers (Basel), 12*, 862. https://doi.org/10.3390/cancers12040862
9. Guo, M., Peng, Y., Gao, A., et al. (2019). Epigenetic heterogeneity in cancer. *Biomarker Research, 7*, 23. https://doi.org/10.1186/s40364-019-0174-y
10. Fisher, R., Pusztai, L., & Swanton, C. (2013). Cancer heterogeneity: Implications for targeted therapeutics. *British Journal of Cancer, 108*, 479–485. https://doi.org/10.1038/bjc.2012.581
11. Cyll, K., Ersvær, E., Vlatkovic, L., et al. (2017). Tumour heterogeneity poses a significant challenge to cancer biomarker research. *British Journal of Cancer, 117*, 367–375. https://doi.org/10.1038/bjc.2017.171
12. Heppner, G. H., & Miller, B. E. (1983). Tumor heterogeneity: Biological implications and therapeutic consequences. *CANCER AND METASTASIS REVIEW, 2*, 5–23. https://doi.org/10.1007/BF00046903
13. Janku, F. (2014). Tumor heterogeneity in the clinic: Is it a real problem? *Therapeutic Advances Medicine Oncology, 6*, 43–51. https://doi.org/10.1177/1758834013517414
14. Byroju, V. V., Nadukkandy, A. S., Cordani, M., & Kumar, L. D. (2023). Retinoblastoma: Present scenario and future challenges. *Cell Communication and Signaling, 21*, 226. https://doi.org/10.1186/s12964-023-01223-z
15. Kawamura, S., & Tachibanaki, S. (2008). Rod and cone photoreceptors: Molecular basis of the difference in their physiology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 150*, 369–377. https://doi.org/10.1016/j.cbpa.2008.04.600
16. Molday, R. S., & Moritz, O. L. (2015). Photoreceptors at a glance. *Journal of Cell Science, 128*, 4039–4045. https://doi.org/10.1242/jcs.175687
17. Gupta, A. K., & Meena, J. P. (2020). A narrative review of retinoblastoma and recent advances in its management. *Pediatric Medicine, 3*, 20. https://doi.org/10.21037/pm-20-79
18. Mendoza, P. R., Grossniklaus, H. E. (2015). The biology of retinoblastoma. *Progress in Molecular Biology and Translational Science*. Elsevier, pp. 503–516.
19. Jain, M., Rojanaporn, D., Chawla, B., et al. (2019). Retinoblastoma in Asia. *Eye (London, England), 33*, 87–96. https://doi.org/10.1038/s41433-018-0244-7
20. Finger, P. T., & Tomar, A. S. (2022). Retinoblastoma outcomes: A global perspective. *The Lancet Global Health, 10*, e307–e308. https://doi.org/10.1016/S2214-109X(21)00598-2
21. Gupta, N., Pandey, A., Dimri, K., & Prinja, S. (2020). Epidemiological profile of retinoblastoma in North India: Implications for primary care and family physicians. *J Family Med Prim Care, 9*, 2843. https://doi.org/10.4103/JFMPC.JFMPC_265_20
22. Dimaras, H., Corson, T. W., Cobrinik, D., et al. (2015). Retinoblastoma. *Nature Reviews Disease Primers, 1*, 1–23. https://doi.org/10.1038/nrdp.2015.21
23. Burges, M., Qaddoumi, I., Brennan, R. C., et al. (2020). Assessment of retinoblastoma capacity in the Middle East, North Africa, and West Asia Region. *JCO Global Oncology*, pp. 1531–1539. https://doi.org/10.1200/GO.20.00321
24. Fabian, I. D., Abdallah, E., Abdullahi, S. U., et al. (2022). The Global Retinoblastoma Outcome Study: A prospective, cluster-based analysis of 4064 patients from 149 countries. *The Lancet Global Health, 10*, e1128–e1140. https://doi.org/10.1016/S2214-109X(22)00250-9
25. Kaliki, S., Patel, A., Iram, S., et al. (2019). Retinoblastoma in India: Clinical presentation and outcome in 1,457 patients (2,074 Eyes). *Retina, 39*, 379–391. https://doi.org/10.1097/IAE.0000000000001962
26. Kakarala, C. L., Raval, V. R., Mallu, A., et al. (2023). Metastatic retinoblastoma at presentation: Clinical presentation, treatment,

and outcomes. *Oman Journal Ophthalmology, 16*, 524–528. https://doi.org/10.4103/ojo.ojo_176_22
27. Berry, J. L., Kogachi, K., Murphree, A. L., et al. (2019). A review of recurrent retinoblastoma: Children's Hospital Los Angeles Classification and Treatment Guidelines. *International Ophthalmology Clinics, 59*, 65–75. https://doi.org/10.1097/IIO.0000000000000269
28. Knudson, A. G. (1971). Mutation and cancer: Statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America, 68*, 820–823. https://doi.org/10.1073/pnas.68.4.820
29. Friend, S. H., Bernards, R., Rogelj, S., et al. (1986). A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature, 323*, 643–646. https://doi.org/10.1038/323643a0
30. Choy, K. W., Pang, C. P., Yu, C. B. O., et al. (2002). Loss of heterozygosity and mutations are the major mechanisms of RB1 gene inactivation in Chinese with sporadic retinoblastoma. *Human Mutation, 20*, 408. https://doi.org/10.1002/humu.9077
31. Zhang, X. L., Fu, W. L., Zhao, H. X., et al. (2005). Molecular studies of loss of heterozygosity in Chinese sporadic retinoblastoma patients. *Clinica Chimica Acta, 358*, 75–80. https://doi.org/10.1016/j.cccn.2005.02.013
32. Ramprasad, V. L., Madhavan, J., Murugan, S., et al. (2007). Retinoblastoma in India: Microsatellite analysis and its application in genetic counseling. *Molecular Diagnosis & Therapy, 11*, 63–70. https://doi.org/10.1007/BF03256223
33. Marković, L., Bukovac, A., Varošanec, A. M., et al. (2023). Genetics in ophthalmology: Molecular blueprints of retinoblastoma. *Human Genomics, 17*, 82. https://doi.org/10.1186/s40246-023-00529-w
34. O'Brien, J. M. (2001). Retinoblastoma: Clinical presentation and the role of neuroimaging. *AJNR American Journal Neuroradiology, 22*, 427–429. https://pmc.ncbi.nlm.nih.gov/articles/PMC7976830/
35. Kivelä, T. (1999). Trilateral retinoblastoma: A meta-analysis of hereditary retinoblastoma associated with primary ectopic intracranial retinoblastoma. *Journal of Clinical Oncology, 17*, 1829–1837. https://doi.org/10.1200/JCO.1999.17.6.1829
36. Bader, J. L., Miller, R. W., Meadows, A. T., et al. (1980). Trilateral retinoblastoma. *The Lancet, 316*, 582–583. https://doi.org/10.1016/S0140-6736(80)92009-7
37. Dyer, M. A., & Bremner, R. (2005). The search for the retinoblastoma cell of origin. *Nature Reviews Cancer, 5*, 91–101. https://doi.org/10.1038/nrc1545
38. Mano, H., & Fukada, Y. (2007). A median third eye: Pineal gland retraces evolution of vertebrate photoreceptive organs†. *Photochemistry and Photobiology, 83*, 11–18. https://doi.org/10.1562/2006-02-24-IR-813
39. Ekström, P., & Meissl, H. (2003). Evolution of photosensory pineal organs in new light: The fate of neuroendocrine photoreceptors. *Philosophical Transactions of the Royal Society B: Biological Sciences, 358*, 1679. https://doi.org/10.1098/RSTB.2003.1303
40. Paulino, A. C. (1999). Trilateral retinoblastoma is the location of the intracranial tumor important? https://doi.org/10.1002/(SICI)1097-0142(19990701)86:1
41. Rushlow, D., Piovesan, B., Zhang, K., et al. (2009). Detection of mosaic RB1 mutations in families with retinoblastoma. *Human Mutation, 30*, 842–851. https://doi.org/10.1002/HUMU.20940
42. Finger, P. T., Harbour, J. W., & Karcioglu, Z. A. (2002). Risk factors for metastasis in retinoblastoma. *Survey of Ophthalmology, 47*, 1–16. https://doi.org/10.1016/S0039-6257(01)00279-X
43. Hong, F. D., Huang, H. J., To, H., et al. (1989). Structure of the human retinoblastoma gene. *Proceedings of the National Academy of Sciences, 86*, 5502–5506. https://doi.org/10.1073/pnas.86.14.5502
44. Verma, R. S., Ramesh, K. H., Samonte, R. V., & Conte, R. A. (1996). Mapping the homolog of the human Rb1 gene to Chromosome 14 of higher primates. *Mammalian Genome, 7*, 591–592. https://doi.org/10.1007/s003359900175
45. Castéra, L., Dehainault, C., Michaux, D., et al. (2013). Fine mapping of whole RB1 gene deletions in retinoblastoma patients confirms PCDH8 as a candidate gene for psychomotor delay. *European Journal of Human Genetics, 21*, 460–464. https://doi.org/10.1038/ejhg.2012.186
46. Yao, Y., Gu, X., Xu, X., et al. (2022). Novel insights into RB1 mutation. *Cancer Letters, 547*, 215870. https://doi.org/10.1016/j.canlet.2022.215870
47. Dick, F. A., & Rubin, S. M. (2013). Molecular mechanisms underlying RB protein function. *Nature Reviews Molecular Cell Biology, 14*, 297–306. https://doi.org/10.1038/nrm3567
48. Singh ,N., Frolov, M., Dyson, N. (2013). Retinoblastoma protein (pRB). *Encyclopedia of Biological Chemistry.* Elsevier, pp. 104–107.
49. Yun, J., Li, Y., Xu, C.-T., & Pan, B.-R. (2011). Epidemiology and Rb1 gene of retinoblastoma. *International Journal of Ophthalmology, 4*, 103–109. https://doi.org/10.3980/j.issn.2222-3959.2011.01.24
50. Engel, B. E., Cress, W. D., & Santiago-Cardona, P. G. (2015). The retinoblastoma protein: A master tumor suppressor acts as a link between cell cycle and cell adhesion. *Cell Health Cytoskelet, 7*, 1–10. https://doi.org/10.2147/CHC.S28079
51. Henley, S. A., & Dick, F. A. (2012). The retinoblastoma family of proteins and their regulatory functions in the mammalian cell division cycle. *Cell Division, 7*, 10. https://doi.org/10.1186/1747-1028-7-10
52. Shlyakhtina, Y., Moran, K. L., & Portal, M. M. (2021). Genetic and non-genetic mechanisms underlying cancer evolution. *Cancers (Basel), 13*, 1380. https://doi.org/10.3390/cancers13061380
53. Rathore, S., Verma, A., Ratna, R., et al. (2023). Retinoblastoma: A review of the molecular basis of tumor development and its clinical correlation in shaping future targeted treatment strategies. *Indian Journal of Ophthalmology, 71*, 2662–2676. https://doi.org/10.4103/IJO.IJO_3172_22
54. Amitrano, S., Marozza, A., Somma, S., et al. (2015). Next generation sequencing in sporadic retinoblastoma patients reveals somatic mosaicism. *European Journal of Human Genetics, 23*, 1523–1530. https://doi.org/10.1038/ejhg.2015.6
55. Gargallo, P., Oltra, S., Balaguer, J., et al. (2021). Retinoblastoma and mosaic 13q deletion: A case report. *Int J Retina Vitreous, 7*, 50. https://doi.org/10.1186/s40942-021-00321-9
56. Proietto, M., Crippa, M., Damiani, C., et al. (2023). Tumor heterogeneity: Preclinical models, emerging technologies, and future applications. *Frontiers in Oncology, 13*, 1164535. https://doi.org/10.3389/fonc.2023.1164535
57. Valverde, J. R., Alonso, J., Palacios, I., & Pestaña, Á. (2005). RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. *BMC Genetics, 6*, 53. https://doi.org/10.1186/1471-2156-6-53
58. Lohmann, D. R., Gallie, B. L. (1993). Retinoblastoma. In M. P. Adam, J. Feldman, G. M. Mirzaa, et al. (Eds.), *GeneReviews®*. pp. 1993–2025. University of Washington, Seattle, Seattle (WA), from https://www.ncbi.nlm.nih.gov/books/NBK1452/
59. Akdeniz Odemis, D., Kebudi, R., Bayramova, J., et al. (2023). RB1 gene mutations and genetic spectrum in retinoblastoma cases. *Medicine, 102*, e35068. https://doi.org/10.1097/MD.0000000000035068

60. Harbour, J. W. (2001). Molecular basis of low-penetrance retinoblastoma. *Archives of Ophthalmology, 119*, 1699. https://doi.org/10.1001/archopht.119.11.1699
61. Leiderman, Y. I., Kiss, S., & Mukai, S. (2007). Molecular genetics of RB1 ——The retinoblastoma gene. *Semin Ophthalmol, 22*, 247–254. https://doi.org/10.1080/08820530701745165
62. Hung, C.-C., Lin, S.-Y., Lee, C.-N., et al. (2011). Low penetrance of retinoblastoma for p.V654L mutation of the RB1 gene. *BMC Medicine Genetics, 12*, 76. https://doi.org/10.1186/1471-2350-12-76
63. Li, L., Li, H., Zhang, J., et al. (2022). Five novel RB1 gene mutations and genotype–phenotype correlations in Chinese children with retinoblastoma. *International Ophthalmology, 42*, 3421–3430. https://doi.org/10.1007/s10792-022-02341-2
64. Tomar, S., Sethi, R., Sundar, G., et al. (2017). Mutation spectrum of RB1 mutations in retinoblastoma cases from Singapore with implications for genetic management and counselling. *PLoS ONE, 12*, e0178776. https://doi.org/10.1371/journal.pone.0178776
65. Lan, X., Xu, W., Tang, X., et al. (2020). Spectrum of RB1 germline mutations and clinical features in unrelated Chinese patients with retinoblastoma. *Frontiers in Genetics, 11*, 142. https://doi.org/10.3389/fgene.2020.00142
66. Salk, J. J., Fox, E. J., & Loeb, L. A. (2010). Mutational heterogeneity in human cancers: Origin and consequences. *Annual Review of Pathology: Mechanisms of Disease, 5*, 51–75. https://doi.org/10.1146/annurev-pathol-121808-102113
67. Knudsen, E. S., & Knudsen, K. E. (2008). Tailoring to RB: Tumour suppressor status and therapeutic response. *Nature Reviews Cancer, 8*, 714–724. https://doi.org/10.1038/nrc2401
68. Ali, M. J., Parsam, V. L., Honavar, S. G., et al. (2010). RB1 gene mutations in retinoblastoma and its clinical correlation. *Saudi Journal of Ophthalmology, 24*, 119–123. https://doi.org/10.1016/j.sjopt.2010.05.003
69. Price, E. A., Price, K., Kolkiewicz, K., et al. (2014). Spectrum of RB1 mutations identified in 403 retinoblastoma patients. *Journal of Medical Genetics, 51*, 208–214. https://doi.org/10.1136/jmedgenet-2013-101821
70. Sagi, M., Frenkel, A., Eilat, A., et al. (2015). Genetic screening in patients with retinoblastoma in Israel. *Familial Cancer, 14*, 471–480. https://doi.org/10.1007/s10689-015-9794-z
71. Manukonda, R., Pujar, A., Ramappa, G., et al. (2022). Identification of novel RB1 genetic variants in retinoblastoma patients and their impact on clinical outcome. *Ophthalmic Genetics, 43*, 64–72. https://doi.org/10.1080/13816810.2021.1989602
72. Lohmann, D. R., Brandt, B., Höpping, W., et al. (1994). Distinct RB1 gene mutations with low penetrance in hereditary retinoblastoma. *Human Genetics, 94*, 349–354. https://doi.org/10.1007/BF00201591
73. Onadim, Z., Hogg, A., Baird, P. N., & Cowell, J. K. (1992). Oncogenic point mutations in exon 20 of the RB1 gene in families showing incomplete penetrance and mild expression of the retinoblastoma phenotype. *Proc Natl Acad Sci U S A, 89*, 6177–6181. https://doi.org/10.1073/pnas.89.13.6177
74. Bremner, R., Du, D. C., Connolly-Wilson, M. J., et al. (1997). Deletion of RB exons 24 and 25 causes low-penetrance retinoblastoma. *American Journal of Human Genetics, 61*, 556–570. https://doi.org/10.1086/515499
75. Richter, S., Vandezande, K., Chen, N., et al. (2003). Sensitive and efficient detection of RB1 gene mutations enhances care for families with retinoblastoma. *American Journal of Human Genetics, 72*, 253–269. https://doi.org/10.1086/345651
76. Alekseeva, E. A., Babenko, O. V., Kozlova, V. M., et al. (2021). Parental origin of the RB1 gene mutations in families with low penetrance hereditary retinoblastoma. *Cancers (Basel), 13*, 5068. https://doi.org/10.3390/cancers13205068
77. Chaudhry, S., Onadim, Z., Sagoo, M. S., & Reddy, M. A. (2018). The recognition of cavitary retinoblastoma tumors: Implications for management and genetic analysis. *Retina, 38*, 782–787. https://doi.org/10.1097/IAE.0000000000001597
78. Rojanaporn, D., Kaliki, S., Bianciotto, C. G., et al. (2012). Intravenous chemoreduction or intra-arterial chemotherapy for cavitary retinoblastoma: Long-term results. *Archives of Ophthalmology, 130*, 585–590. https://doi.org/10.1001/ARCHOPHTHALMOL.2011.2825
79. Raval, V., & Kaliki, S. (2022). Cavitary retinoblastoma: A review of literature. *Survey of Ophthalmology, 67*, 723–728. https://doi.org/10.1016/J.SURVOPHTHAL.2021.10.002
80. Zheng, Y., Froehler, M. T., Friedman, D. L., & Daniels, A. B. (2021). Intra-arterial chemotherapy as primary treatment for cavitary retinoblastoma: Excellent response in eight tumors. *Ophthalmol Retina, 5*, 479–485. https://doi.org/10.1016/J.ORET.2020.08.011
81. Azarcon, C., Wells, J. R., Hubbard, G. B. I. (2023). Regression of cavitary retinoblastoma after intra-arterial chemotherapy and transpupillary thermotherapy. *Digital Journal of Ophthalmology, 29*. https://doi.org/10.5693/DJO.01.2022.01.001_VIDEO2
82. Nawaiseh, I., Al-Hussaini, M., Alhamwi, A., et al. (2015). The impact of growth patterns of retinoblastoma (endophytic, exophytic, and mixed patterns). *Turk Patoloji Derg, 31*, 45–50. https://doi.org/10.5146/TJPATH.2014.01278
83. Palazzi, M., Abramson, D. H., & Ellsworth, R. M. (1990). Endophytic vs exophytic unilateral retinoblastoma: Is there any real difference? *Journal of Pediatric Ophthalmology and Strabismus, 27*, 255–258. https://doi.org/10.3928/0191-3913-19900901-10
84. Surakiatchanukul, W. T., Tjoumakaris, S. I., Jabbour, P. M., & Shields, C. L. (2020). Comparison of primary intra-arterial chemotherapy for retinoblastoma based on exophytic versus endophytic growth pattern. *Investigative Ophthalmology & Visual Science, 61*, 2810–2810.
85. Pandey, A. N. (2014). Retinoblastoma: An overview. *Saudi Journal of Ophthalmology, 28*, 310–315. https://doi.org/10.1016/J.SJOPT.2013.11.001>
86. Grossniklaus, H. E. (2014). Retinoblastoma. Fifty years of progress. The LXXI Edward Jackson Memorial Lecture. *Am J Ophthalmol, 158*, 875. https://doi.org/10.1016/J.AJO.2014.07.025
87. Review of Retinoblastoma - American Academy of Ophthalmology. https://www.aao.org/education/disease-review/review-of-retinoblastoma. Accessed 20 Mar 2025.
88. Jijelava, K. P., & Grossniklaus, H. E. (2013). Diffuse anterior retinoblastoma: A review. *Saudi Journal of Ophthalmology, 27*, 135. https://doi.org/10.1016/J.SJOPT.2013.06.006
89. Kaliki, S., Shields, C. L., Cassoux, N., et al. (2022). Defining high-risk retinoblastoma: A multicenter global survey. *JAMA Ophthalmol, 140*, 30–36. https://doi.org/10.1001/JAMAOPHTHALMOL.2021.4732
90. Kaliki, S., Vempuluru, V. S., Bakal, K. R., et al. (2024). High-risk histopathological features of retinoblastoma following primary enucleation: A global study of 1426 patients from 5 continents. *Retina*. https://doi.org/10.1097/IAE.0000000000004250
91. Kaliki, S., Vempuluru, V. S., Ahmad, A., et al. (2024). Impact of race on the outcomes of retinoblastoma treated with primary enucleation: A global study of 1426 patients. *Clinical and Experimental Ophthalmology, 0*, 1–14. https://doi.org/10.1111/CEO.14488
92. Fabian, I. D., Reddy, A., & Sagoo, M. S. (2018). Classification and staging of retinoblastoma. *Community Eye Health, 31*, 11.
93. Kaliki, S., Shields, C. L., Rojanaporn, D., et al. (2013). High-risk retinoblastoma based on International Classification of Retinoblastoma: Analysis of 519 enucleated eyes. *Ophthalmology, 120*, 997–1003. https://doi.org/10.1016/J.OPHTHA.2012.10.044

94. Singh, U., Katoch, D., Kaur, S., et al. (2017). Retinoblastoma: A sixteen-year review of the presentation, treatment, and outcome from a tertiary care institute in Northern India. *Ocul Oncol Pathol, 4*, 23. https://doi.org/10.1159/000477408
95. Retinoblastoma Treatment (PDQ®) - NCI. https://www.cancer.gov/types/retinoblastoma/hp/retinoblastoma-treatment-pdq#_15. Accessed 20 Mar 2025.
96. Stenfelt, S., Blixt, M. K. E., All-Ericsson, C., et al. (2017). Heterogeneity in retinoblastoma: A tale of molecules and models. *Clinical and Translational Medicine, 6*, 42. https://doi.org/10.1186/s40169-017-0173-2
97. Field, M. G., Kuznetsoff, J. N., Zhang, M. G., et al. (2022). RB1 loss triggers dependence on ESRRG in retinoblastoma. *Science Advances, 8*, eabm8466. https://doi.org/10.1126/sciadv.abm8466
98. Liu, J., Ottaviani, D., Sefta, M., et al. (2021). A high-risk retinoblastoma subtype with stemness features, dedifferentiated cone states and neuronal/ganglion cell gene expression. *Nature Communications, 12*, 5578. https://doi.org/10.1038/s41467-021-25792-0
99. Sampieri, K., Mencarelli, M. A., Carmela Epistolato, M., et al. (2008). Genomic differences between retinoma and retinoblastoma. *Acta Oncol (Madr), 47*, 1483–1492. https://doi.org/10.1080/02841860802342382
100. Rushlow, D. E., Mol, B. M., Kennett, J. Y., et al. (2013). Characterisation of retinoblastomas without RB1 mutations: Genomic, gene expression, and clinical studies. *The lancet Oncology, 14*, 327–334. https://doi.org/10.1016/S1470-2045(13)70045-7
101. Zugbi, S., Ganiewich, D., Bhattacharyya, A., et al. (2020). Clinical, genomic, and pharmacological study of MYCN-amplified RB1 wild-type metastatic retinoblastoma. *Cancers (Basel), 12*, 2714. https://doi.org/10.3390/cancers12092714
102. Francis, J. H., Richards, A. L., Mandelker, D. L., et al. (2021). Molecular changes in retinoblastoma beyond RB1: Findings from next-generation sequencing. *Cancers (Basel), 13*, 1–12. https://doi.org/10.3390/CANCERS13010149
103. Roohollahi, K., De Jong, Y., Van Mil, S. E., et al. (2022). High-level MYCN-amplified RB1-proficient retinoblastoma tumors retain distinct molecular signatures. *Ophthalmology Science, 2*, 100188. https://doi.org/10.1016/j.xops.2022.100188
104. Zhu, Y., Wang, Z., Li, Y., et al. (2023). The role of CREBBP/EP300 and its therapeutic implications in hematological malignancies. *Cancers (Basel), 15*, 1219. https://doi.org/10.3390/cancers15041219
105. Kooi, I. E., Mol, B. M., Massink, M. P. G., et al. (2016). Somatic genomic alterations in retinoblastoma beyond RB1 are rare and limited to copy number changes. *Science and Reports, 6*, 25264. https://doi.org/10.1038/srep25264
106. Kooi, I. E., Mol, B. M., Massink, M. P. G., et al. (2016). A meta-analysis of retinoblastoma copy numbers refines the list of possible driver genes involved in tumor progression. *PLoS ONE, 11*, e0153323. https://doi.org/10.1371/journal.pone.0153323
107. Livide, G., Epistolato, M. C., Amenduni, M., et al. (2012). Epigenetic and copy number variation analysis in retinoblastoma by MS-MLPA. *Pathology & Oncology Research, 18*, 703–712. https://doi.org/10.1007/s12253-012-9498-8
108. McEvoy, J., Nagahawatte, P., Finkelstein, D., et al. (2014). RB1 gene inactivation by chromothripsis in human retinoblastoma. *Oncotarget, 5*, 438–450. https://doi.org/10.18632/oncotarget.1686
109. Bakhoum, S. F., & Landau, D. A. (2017). Chromosomal instability as a driver of tumor heterogeneity and evolution. *Cold Spring Harbor Perspectives in Medicine, 7*, a029611. https://doi.org/10.1101/cshperspect.a029611
110. Comaills, V., & Castellano-Pozo, M. (2023). Chromosomal instability in genome evolution: From cancer to macroevolution. *Biology (Basel), 12*, 671. https://doi.org/10.3390/biology12050671
111. Hattori, A., Okamura, K., Terada, Y., et al. (2019). Transient multifocal genomic crisis creating chromothriptic and non-chromothriptic rearrangements in prezygotic testicular germ cells. *BMC Medical Genomics, 12*, 77. https://doi.org/10.1186/s12920-019-0526-3
112. Winter, U., Ganiewich, D., Ottaviani, D., et al. (2020). Genomic and transcriptomic tumor heterogeneity in bilateral retinoblastoma. *JAMA Ophthalmol, 138*, 569. https://doi.org/10.1001/jamaophthalmol.2020.0427
113. Francis, J. H., Richards, A. L., Mandelker, D. L., et al. (2021). Molecular changes in retinoblastoma beyond RB1: Findings from next-generation sequencing. *Cancers (Basel), 13*, 149. https://doi.org/10.3390/cancers13010149
114. Wakeley, J., Fan, W.-T.L., Koch, E., & Sunyaev, S. (2023). Recurrent mutation in the ancestry of a rare variant. *Genetics, 224*, iyad049. https://doi.org/10.1093/genetics/iyad049
115. Murphree, A. L., & Triche, T. J. (2012). An epigenomic mechanism in retinoblastoma: The end of the story? *Genome Med, 4*, 15. https://doi.org/10.1186/gm314
116. Zhang, J., Benavente, C. A., McEvoy, J., et al. (2012). A novel retinoblastoma therapy from genomic and epigenetic analyses. *Nature, 481*, 329–334. https://doi.org/10.1038/nature10733
117. Zhou, Y., Wojcik, A., Sanders, V. R., et al. (2018). Ocular findings in a patient with oculofaciocardiodental (OFCD) syndrome and a novel BCOR pathogenic variant. *International Ophthalmology, 38*, 2677–2682. https://doi.org/10.1007/s10792-017-0754-5
118. Thériault, B. L., Dimaras, H., Gallie, B. L., & Corson, T. W. (2014). The genomic landscape of retinoblastoma: A review. *Clinical and Experimental Ophthalmology, 42*, 33–52. https://doi.org/10.1111/ceo.12132
119. Thompson, S. L., & Compton, D. A. (2011). Chromosomes and cancer cells. *Chromosome Research, 19*, 433–444. https://doi.org/10.1007/s10577-010-9179-y
120. Thompson, S. L., Bakhoum, S. F., & Compton, D. A. (2010). Mechanisms of chromosomal instability. *Current Biology, 20*, R285–R295. https://doi.org/10.1016/j.cub.2010.01.034
121. McEvoy, J. D., & Dyer, M. A. (2015). Genetic and epigenetic discoveries in human retinoblastoma. *Critical Reviews in Oncogenesis, 20*, 217–225. https://doi.org/10.1615/critrevoncog.2015013711
122. Oliveros, O., & Yunis, E. (1995). Chromosome evolution in retinoblastoma. *Cancer Genetics and Cytogenetics, 82*, 155–160. https://doi.org/10.1016/0165-4608(94)00212-T
123. Rode, A., Maass, K. K., Willmund, K. V., et al. (2016). Chromothripsis in cancer cells: An update. *International Journal of Cancer, 138*, 2322–2333. https://doi.org/10.1002/ijc.29888
124. Kapatai, G., Brundler, M. A., Jenkinson, H., et al. (2013). Gene expression profiling identifies different sub-types of retinoblastoma. *British Journal of Cancer, 109*, 512–525. https://doi.org/10.1038/BJC.2013.283
125. Kooi, I. E., Mol, B. M., Moll, A. C., et al. (2015). Loss of photoreceptorness and gain of genomic alterations in retinoblastoma reveal tumor progression. *eBioMedicine, 2*, 660–670. https://doi.org/10.1016/J.EBIOM.2015.06.022
126. Ryl, T., Afanasyeva, E., Hartmann, T., et al. (2024). A MYCN-driven de-differentiation profile identifies a subgroup of aggressive retinoblastoma. *Communication Biology, 7*, 919. https://doi.org/10.1038/S42003-024-06596-6
127. Eagle, R. C. (2009). High-risk features and tumor differentiation in retinoblastoma: A retrospective histopathologic study. *Archives of Pathology and Laboratory Medicine, 133*, 1203–1209. https://doi.org/10.5858/133.8.1203
128. Jovic, D., Liang, X., Zeng, H., et al. (2022). Single-cell RNA sequencing technologies and applications: A brief overview. *Clinical and Translational Medicine, 12*, e694. https://doi.org/10.1002/CTM2.694

129. Collin, J., Queen, R., Zerti, D., et al. (2021). Dissecting the transcriptional and chromatin accessibility heterogeneity of proliferating cone precursors in human retinoblastoma tumors by single cell sequencing—Opening pathways to new therapeutic strategies? *Investigative Opthalmology & Visual Science, 62*, 18. https://doi.org/10.1167/iovs.62.6.18
130. Wong, R., Wang, L., Urrutia-Cabrera, D., et al. (2023). Using spatial transcriptomics to study retinoblastoma. *Invest Ophthalmology Visual Science, 64*, 1265. https://iovs.arvojournals.org/article.aspx?articleid=2786540
131. Yang, J., Li, Y., Han, Y., et al. (2021). Single-cell transcriptome profiling reveals intratumoural heterogeneity and malignant progression in retinoblastoma. *Cell Death & Disease, 12*, 1100. https://doi.org/10.1038/s41419-021-04390-4
132. Wu, C., Yang, J., Xiao, W., et al. (2022). Single-cell characterization of malignant phenotypes and microenvironment alteration in retinoblastoma. *Cell Death & Disease, 13*, 438. https://doi.org/10.1038/s41419-022-04904-8
133. Liu, Y., Hu, W., Xie, Y., et al. (2024). Single-cell transcriptomics enable the characterization of local extension in retinoblastoma. *Commun Biol, 7*, 11. https://doi.org/10.1038/s42003-023-05732-y
134. Chen, J. F., & Yan, Q. (2021). The roles of epigenetics in cancer progression and metastasis. *The Biochemical Journal, 478*, 3373–3393. https://doi.org/10.1042/BCJ20210084
135. Govarthanan, K., Kumar Gupta, P., Zipporahe, B., et al. (2021). Epigenetic regulation−The guardian of cellular homeostasis and lineage commitment. *BIOCELL, 45*, 501–515. https://doi.org/10.32604/biocell.2021.014441
136. Baylin, S. B., & Jones, P. A. (2016). Epigenetic determinants of cancer. *Cold Spring Harbor Perspectives in Biology, 8*, a019505. https://doi.org/10.1101/cshperspect.a019505
137. Benavente, C. A., & Dyer, M. A. (2015). Genetics and epigenetics of human retinoblastoma. *Annual Review of Pathology: Mechanisms of Disease, 10*, 547–562. https://doi.org/10.1146/annurev-pathol-012414-040259
138. Price, E. A., Kolkiewicz, K., Patel, R., et al. (2018). Detection and reporting of RB1 promoter hypermethylation in diagnostic screening. *Ophthalmic Genetics, 39*, 526–531. https://doi.org/10.1080/13816810.2018.1479432
139. Karmakar, A., Ahamad Khan, Md. M., Kumari, N., et al. (2022). Identification of epigenetically modified hub genes and altered pathways associated with retinoblastoma. *Front Cell Dev Biol, 10*, 743224. https://doi.org/10.3389/fcell.2022.743224
140. Conkrite, K., Sundby, M., Mukai, S., et al. (2011). miR-17~92 cooperates with RB pathway mutations to promote retinoblastoma. *Genes & Development, 25*, 1734–1745. https://doi.org/10.1101/GAD.17027411
141. Lee, C., & Kim, J. (2022). Genome maintenance in retinoblastoma: Implications for therapeutic vulnerabilities (Review). *Oncology Letters, 23*, 192. https://doi.org/10.3892/ol.2022.13312
142. Qu, Y., Mu, G., Wu, Y., et al. (2010). Overexpression of DNA methyltransferases 1, 3a, and 3b significantly correlates with retinoblastoma tumorigenesis. *American Journal of Clinical Pathology, 134*, 826–834. https://doi.org/10.1309/AJCPHGQ69FXDFWII
143. Krisenko, M. O., & Geahlen, R. L. (2015). Calling in SYK: SYK's dual role as a tumor promoter and tumor suppressor in cancer. *Biochimica et Biophysica Acta, 1853*, 254–263. https://doi.org/10.1016/j.bbamcr.2014.10.022
144. Yang, Y.-L., Zhou, H., Yue, L.-R., & Shen, H. (2020). Spleen tyrosine kinase expression and its correlation with necrosis and high-risk histopathologic features in retinoblastoma. *Chinese Medical Journal (Engl), 133*, 1752–1753. https://doi.org/10.1097/CM9.0000000000000889
145. Brennan, R. C., Wilson, M. W., Dyer, M. A., & Bahrami, A. (2012). SYK expression in metastatic retinoblastoma. *Journal of Clinical Oncology, 30*, e13544–e13544. https://doi.org/10.1200/jco.2012.30.15_suppl.e13544
146. Gan, L., Yang, Y., Li, Q., et al. (2018). Epigenetic regulation of cancer progression by EZH2: From biological insights to therapeutic potential. *Biomark Res, 6*, 1–10. https://doi.org/10.1186/S40364-018-0122-2/FIGURES/2
147. Sun, S., Yu, F., Xu, D., et al. (2022). EZH2, a prominent orchestrator of genetic and epigenetic regulation of solid tumor microenvironment and immunotherapy. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1877*, 188700. https://doi.org/10.1016/j.bbcan.2022.188700
148. Lin, Z., Tang, L., Chen, S., et al. (2023). EZH2 expression in retinoblastoma: A potential therapeutic target. *Ophthalmic Research, 66*, 1014–1019. https://doi.org/10.1159/000531530
149. Khan, M., Walters, L. L., Li, Q., et al. (2015). Characterization and pharmacologic targeting of EZH2, a fetal retinal protein and epigenetic regulator, in human retinoblastoma. *Laboratory Investigation, 95*, 1278–1290. https://doi.org/10.1038/labinvest.2015.104
150. Peng, Y., Bui, C. H., Zhang, X. J., et al. (2023). The role of EZH2 in ocular diseases: A narrative review. *Epigenomics, 15*, 557–570. https://doi.org/10.2217/epi-2023-0147
151. Ishak, C. A., Marshall, A. E., Passos, D. T., et al. (2016). An RB-EZH2 complex mediates silencing of repetitive DNA sequences. *Molecular Cell, 64*, 1074–1087. https://doi.org/10.1016/J.MOLCEL.2016.10.021
152. Yang, R., Fei, L., Xue, Y., et al. (2025). Increasing RB1 expression by targeting EZH2 in triple-negative breast cancer. *Journal of Cellular and Molecular Medicine, 29*, e70384. https://doi.org/10.1111/JCMM.70384
153. Astolfi, A., Fiore, M., Melchionda, F., et al. (2019). BCOR involvement in cancer. *Epigenomics, 11*, 835–855. https://doi.org/10.2217/epi-2018-0195
154. Choy, K. W., Lee, T. C., Cheung, K. F., et al. (2005). Clinical implications of promoter hypermethylation in RASSF1A and MGMT in retinoblastoma. *Neoplasia, 7*, 200–206. https://doi.org/10.1593/neo.04565
155. Harada, K., Toyooka, S., Maitra, A., et al. (2002). Aberrant promoter methylation and silencing of the RASSF1A gene in pediatric tumors and cell lines. *Oncogene, 21*, 4345–4349. https://doi.org/10.1038/sj.onc.1205446
156. Choy, K. W., Pang, C. P., To, K. F., et al. (2002). Impaired expression and promotor hypermethylation of O6-methylguanine-DNA methyltransferase in retinoblastoma tissues. *Investigative Ophthalmology & Visual Science, 43*, 1344–1349.
157. Sachdeva, U. M., & O'Brien, J. M. (2012). Understanding pRb: Toward the necessary development of targeted treatments for retinoblastoma. *The Journal of Clinical Investigation, 122*, 425–434. https://doi.org/10.1172/JCI57114
158. Zhou, L., Ng, D.S.-C., Yam, J. C., et al. (2022). Post-translational modifications on the retinoblastoma protein. *Journal of Biomedical Science, 29*, 33. https://doi.org/10.1186/s12929-022-00818-x
159. Meng, F., Qian, J., Yue, H., et al. (2016). SUMOylation of Rb enhances its binding with CDK2 and phosphorylation at early G1 phase. *Cell Cycle, 15*, 1724–1732. https://doi.org/10.1080/15384101.2016.1182267
160. Chen, X., Chen, S., Jiang, Z., et al. (2021). Ubiquitination-related miRNA–mRNA interaction is a potential mechanism in

the progression of retinoblastoma. *Investigative Opthalmology & Visual Science, 62*, 3. https://doi.org/10.1167/iovs.62.10.3
161. MacDonald, J. I., & Dick, F. A. (2012). Posttranslational modifications of the retinoblastoma tumor suppressor protein as determinants of function. *Genes & Cancer, 3*, 619–633. https://doi.org/10.1177/1947601912473305
162. Gibney, E. R., & Nolan, C. M. (2010). Epigenetics and gene expression. *Heredity (Edinb), 105*, 4–13. https://doi.org/10.1038/hdy.2010.54
163. Wei, J.-W., Huang, K., Yang, C., & Kang, C.-S. (2017). Non-coding RNAs as regulators in epigenetics. *Oncology Reports, 37*, 3–9. https://doi.org/10.3892/or.2016.5236
164. Williams, B. P., & Gehring, M. (2020). Principles of epigenetic homeostasis shared between flowering plants and mammals. *Trends in Genetics, 36*, 751–763. https://doi.org/10.1016/j.tig.2020.06.019
165. Zhang, P., Wu, W., Chen, Q., & Chen, M. (2019). Non-coding RNAs and their integrated networks. *Journal of Integrative Bioinformatics, 16*, 20190027. https://doi.org/10.1515/jib-2019-0027
166. Wu, J., Qian, D., & Sun, X. (2020). Long noncoding RNAs as potential biomarkers in retinoblastoma: A systematic review and meta-Analysis. *Cancer Cell International, 20*, 1–12. https://doi.org/10.1186/S12935-020-01281-0/TABLES/3
167. Yan, G., Su, Y., Ma, Z., et al. (2019). Long noncoding RNA LINC00202 promotes tumor progression by sponging miR-3619-5p in retinoblastoma. *Cell Structure and Function, 44*, 51–60. https://doi.org/10.1247/csf.18033
168. Ni, H., Chai, P., Yu, J., et al. (2020). LncRNA CANT1 suppresses retinoblastoma progression by repellinghistone methyltransferase in PI3Kγ promoter. *Cell Death & Disease, 11*, 306. https://doi.org/10.1038/s41419-020-2524-y
169. Chai, P., Jia, R., Jia, R., et al. (2018). Dynamic chromosomal tuning of a novel GAU1 lncing driver at chr12p13.32 accelerates tumorigenesis. *Nucleic Acids Research, 46*, 6041–6056. https://doi.org/10.1093/nar/gky366
170. Nasrolahi, A., Azizidoost, S., Radoszkiewicz, K., et al. (2023). Long non-coding RNAs involved in retinoblastoma. *Journal of Cancer Research and Clinical Oncology, 149*, 401–421. https://doi.org/10.1007/s00432-022-04398-z
171. Friedman, R. C., Farh, K.K.-H., Burge, C. B., & Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research, 19*, 92–105. https://doi.org/10.1101/gr.082701.108
172. Guo, L., Bai, Y., Ji, S., & Ma, H. (2019). MicroRNA-98 suppresses cell growth and invasion of retinoblastoma via targeting the IGF1R/k-Ras/Raf/MEK/ERK signaling pathway. *International Journal of Oncology*. https://doi.org/10.3892/ijo.2019.4689
173. Lei, Q., Shen, F., Wu, J., et al. (2014). MiR-101, downregulated in retinoblastoma, functions as a tumor suppressor in human retinoblastoma cells by targeting EZH2. *Oncology Reports, 32*, 261–269. https://doi.org/10.3892/or.2014.3167
174. Karla, T.-H., Matilde, R.-C., & Meraz-Ríos, M. A. (2023). Epigenetic and posttranscriptional regulation in retinoblastoma. *Advances in Bioscience and Biotechnology, 14*, 190–209. https://doi.org/10.4236/abb.2023.144013
175. Plousiou, M., & Vannini, I. (2019). Non-coding RNAs in retinoblastoma. *Frontiers Genetics, 10*, 1155. https://doi.org/10.3389/fgene.2019.01155
176. Golabchi, K., Soleimani-Jelodar, R., Aghadoost, N., et al. (2018). MicroRNAs in retinoblastoma: Potential diagnostic and therapeutic biomarkers. *Journal of Cellular Physiology, 233*, 3016–3023. https://doi.org/10.1002/jcp.26070
177. Doghish, A. S., Moustafa, H. A. M., Elballal, M. S., et al. (2023). miRNAs as potential game-changers in retinoblastoma: Future clinical and medicinal uses. *Pathology, Research and Practice, 247*, 154537. https://doi.org/10.1016/J.PRP.2023.154537
178. Boroughs, L. K., & DeBerardinis, R. J. (2015). Metabolic pathways promoting cancer cell survival and growth. *Nature Cell Biology, 17*, 351–359. https://doi.org/10.1038/ncb3124
179. Hammoudi, N., Ahmed, K. B. R., Garcia-Prieto, C., & Huang, P. (2011). Metabolic alterations in cancer cells and therapeutic implications. *Chinese Journal of Cancer, 30*, 508–525. https://doi.org/10.5732/cjc.011.10267
180. Bergers, G., & Fendt, S.-M. (2021). The metabolism of cancer cells during metastasis. *Nature Reviews Cancer, 21*, 162–180. https://doi.org/10.1038/s41568-020-00320-2
181. Hass, R., von der Ohe, J., & Ungefroren, H. (2020). Impact of the tumor microenvironment on tumor heterogeneity and consequences for cancer cell plasticity and stemness. *Cancers (Basel), 12*, 3716. https://doi.org/10.3390/cancers12123716
182. Bar-Hai, N., & Ishay-Ronen, D. (2022). Engaging plasticity: Differentiation therapy in solid tumors. *Frontiers in Pharmacology, 13*, 944773. https://doi.org/10.3389/fphar.2022.944773
183. Conroy, L. (2020). *The retinoblastoma protein mediates metabolic reprogramming in lung cancer. University of Louisville*. Electronic Theses and Dissertations. Paper 3405. https://doi.org/10.18297/etd/3405
184. Suresh Babu, V., Dudeja, G., Sa, D., et al. (2022). Lack of retinoblastoma protein shifts tumor metabolism from glycolysis to OXPHOS and allows the use of alternate fuels. *Cells, 11*, 3182. https://doi.org/10.3390/cells11203182
185. Petrov, P. D., Ribot, J., Palou, A., & Luisa Bonet, M. (2015). Improved metabolic regulation is associated with retinoblastoma protein gene haploinsufficiency in mice. *American Journal of Physiology-Endocrinology and Metabolism, 308*, E172–E183. https://doi.org/10.1152/ajpendo.00308.2014
186. Gulati, K., Manukonda, R., Kairamkonda, M., et al. (2023). Serum metabolomics of retinoblastoma: Assessing the differential serum metabolic signatures of unilateral and bilateral patients. *ACS Omega, 8*, 48233–48250. https://doi.org/10.1021/acsomega.3c07424
187. Yan, Q., He, D., Walker, D. I., et al. (2023). The neonatal blood spot metabolome in retinoblastoma. *EJC Paediatric Oncology, 2*, 100123. https://doi.org/10.1016/j.ejcped.2023.100123
188. Kohe, S., Brundler, M.-A., Jenkinson, H., et al. (2015). Metabolite profiling in retinoblastoma identifies novel clinicopathological subgroups. *British Journal of Cancer, 113*, 1216–1224. https://doi.org/10.1038/bjc.2015.318
189. DiCiommo, D., Gallie, B. L., & Bremner, R. (2000). Retinoblastoma: The disease, gene and protein provide critical leads to understand cancer. *Seminars in Cancer Biology, 10*, 255–269. https://doi.org/10.1006/scbi.2000.0326
190. Laurie, N. A., Donovan, S. L., Shih, C.-S., et al. (2006). Inactivation of the p53 pathway in retinoblastoma. *Nature, 444*, 61–66. https://doi.org/10.1038/nature05194
191. Engeland, K. (2022). Cell cycle regulation: P53–p21-RB signaling. *Cell Death and Differentiation, 29*, 946–960. https://doi.org/10.1038/s41418-022-00988-z
192. Fu, C., Wang, S., Jin, L., et al. (2021). CircTET1 inhibits retinoblastoma progression via targeting miR-492 and miR-494-3p through Wnt/β-catenin signaling pathway. *Current Eye Research, 46*, 978–987. https://doi.org/10.1080/02713683.2020.1843685
193. Leach, L. L., Buchholz, D. E., Nadar, V. P., et al. (2015). Canonical/ -catenin Wnt pathway activation improves retinal pigmented

epithelium derivation from human embryonic stem cells. *Investigative Ophthalmology & Visual Science, 56*, 1002–1013. https://doi.org/10.1167/iovs.14-15835
194. Tell, S., Yi, H., Jockovich, M.-E., et al. (2006). The Wnt signaling pathway has tumor suppressor properties in retinoblastoma. *Biochemical and Biophysical Research Communications, 349*, 261–269. https://doi.org/10.1016/j.bbrc.2006.08.044
195. Lee, K. Y., Ladha, M. H., McMahon, C., & Ewen, M. E. (1999). The retinoblastoma protein is linked to the activation of Ras. *Molecular and Cellular Biology, 19*, 7724–7732. https://doi.org/10.1128/MCB.19.11.7724
196. Liu, H., & Zhou, M. (2017). Antitumor effect of Quercetin on Y79 retinoblastoma cells via activation of JNK and p38 MAPK pathways. *BMC Complementary and Alternative Medicine, 17*, 531. https://doi.org/10.1186/s12906-017-2023-6
197. Asnaghi, L., Tripathy, A., Yang, Q., et al. (2016). Targeting Notch signaling as a novel therapy for retinoblastoma. *Oncotarget, 7*, 70028–70044. https://doi.org/10.18632/oncotarget.12142
198. Xiao, W., Chen, X., & He, M. (2014). Inhibition of the Jagged/Notch pathway inhibits retinoblastoma cell proliferation via suppressing the PI3K/Akt, Src, p38MAPK and Wnt/β-catenin signaling pathways. *Molecular Medicine Reports, 10*, 453–458. https://doi.org/10.3892/mmr.2014.2213
199. Yang, Q., Tripathy, A., Yu, W., et al. (2017). Hypoxia inhibits growth, proliferation, and increases response to chemotherapy in retinoblastoma cells. *Experimental Eye Research, 162*, 48–61. https://doi.org/10.1016/j.exer.2017.07.001
200. Zhao, N., Zhou, L., Lu, Q., et al. (2022). SOX2 maintains the stemness of retinoblastoma stem-like cells through Hippo/YAP signaling pathway. *Experimental Eye Research, 214*, 108887. https://doi.org/10.1016/j.exer.2021.108887
201. Nicolay, B. N., Bayarmagnai, B., Moon, N. S., et al. (2010). Combined inactivation of pRB and Hippo pathways induces dedifferentiation in the Drosophila retina. *PLoS Genetics, 6*, e1000918. https://doi.org/10.1371/journal.pgen.1000918
202. Xu, N., Cui, Y., Shi, H., et al. (2022). UBE2T/stat3 signaling promotes the proliferation and tumorigenesis in retinoblastoma. *Investigative Opthalmology & Visual Science, 63*, 20. https://doi.org/10.1167/iovs.63.9.20
203. Venkadakrishnan, V. B., Yamada, Y., Weng, K., et al. (2023). Significance of RB loss in unlocking phenotypic plasticity in advanced cancers. *Molecular Cancer Research, 21*, 497–510. https://doi.org/10.1158/1541-7786.MCR-23-0045
204. Wei, D., Xu, M., Wang, Z., & Tong, J. (2021). The development of single-cell metabolism and its role in studying cancer emergent properties. *Frontiers in Oncology, 11*, 814085. https://doi.org/10.3389/fonc.2021.814085
205. Evers, T. M. J., Hochane, M., Tans, S. J., et al. (2019). Deciphering metabolic heterogeneity by single-cell analysis. *Analytical Chemistry, 91*, 13314–13323. https://doi.org/10.1021/acs.analchem.9b02410
206. Nguyen, L. V., Vanner, R., Dirks, P., & Eaves, C. J. (2012). Cancer stem cells: An evolving concept. *Nature Reviews Cancer, 12*, 133–143. https://doi.org/10.1038/nrc3184
207. Zhang, J., Schweers, B., & Dyer, M. A. (2004). The first knockout mouse model of retinoblastoma. *Cell Cycle, 3*, 952–959.
208. Chen, D., Livne-bar, I., Vanderluit, J. L., et al. (2004). Cell-specific effects of RB or RB/p107 loss on retinal development implicate an intrinsically death-resistant cell-of-origin in retinoblastoma. *Cancer Cell, 5*, 539–551. https://doi.org/10.1016/j.ccr.2004.05.025
209. Mastrangelo, D., Senior Scientist Research, Loré, C., et al. (2012). The epigenetic origin of retinoblastoma. *European Ophthalmic Review, 06*, 130. https://doi.org/10.17925/EOR.2012.06.02.130
210. Bremner, R., & Sage, J. (2014). The origin of human retinoblastoma. *Nature, 514*, 313. https://doi.org/10.1038/nature13748
211. (2014). Cone precursor cells are the cell-of-origin in retinoblastoma. *Cancer Discovery, 4*, 1254. https://doi.org/10.1158/2159-8290.CD-RW2014-207
212. Xu, X. L., Fang, Y., Lee, T. C., et al. (2009). Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. *Cell, 137*, 1018–1031. https://doi.org/10.1016/j.cell.2009.03.051
213. Villanueva, M. T. (2014). Establishing the origin of retinoblastoma. *Nature Reviews Cancer, 14*, 707. https://doi.org/10.1038/nrc3849
214. Xu, X. L., Singh, H. P., Wang, L., et al. (2014). Rb suppresses human cone-precursor-derived retinoblastoma tumours. *Nature, 514*, 385–388. https://doi.org/10.1038/nature13813
215. Kohno, S., Kitajima, S., Sasaki, N., & Takahashi, C. (2016). Retinoblastoma tumor suppressor functions shared by stem cell and cancer cell strategies. *World J Stem Cells, 8*, 170–184. https://doi.org/10.4252/wjsc.v8.i4.170
216. Balla, M. M. S., Vemuganti, G. K., Kannabiran, C., et al. (2009). Phenotypic characterization of retinoblastoma for the presence of putative cancer stem-like cell markers by flow cytometry. *Investigative Opthalmology & Visual Science, 50*, 1506. https://doi.org/10.1167/iovs.08-2356
217. Nair, R. M., Balla MMs, Khan I, et al. (2017). In vitro characterization of CD133lo cancer stem cells in Retinoblastoma Y79 cell line. *BMC Cancer, 17*, 779. https://doi.org/10.1186/s12885-017-3750-2
218. Li, J., Di, C., Jing, J., et al. (2015). OTX2 is a therapeutic target for retinoblastoma and may function as a common factor between C-MYC, CRX, and phosphorylated RB pathways. *International Journal of Oncology, 47*, 1703–1710. https://doi.org/10.3892/ijo.2015.3179
219. Xiong, Y., Ji, H., You, Z., et al. (2019). Otx2 enhances transdifferentiation of Müller cells-derived retinal stem cells into photoreceptor-like cells. *Journal of Cellular and Molecular Medicine, 23*, 943–953. https://doi.org/10.1111/jcmm.13995
220. Seigel, G. M., Hackam, A. S., Ganguly, A., et al. (2007). Human embryonic and neuronal stem cell markers in retinoblastoma. *Molecular Vision, 13*, 823–832.
221. Mohan, A., Kandalam, M., Ramkumar, H. L., et al. (2006). Stem cell markers: ABCG2 and MCM2 expression in retinoblastoma. *British Journal of Ophthalmology, 90*, 889–893. https://doi.org/10.1136/bjo.2005.089219
222. Seigel, G. M., Campbell, L. M., Narayan, M., & Gonzalez-Fernandez, F. (2005). Cancer stem cell characteristics in retinoblastoma. *Molecular Vision, 11*, 729–737.
223. Chawla, B., & Singh, R. (2017). Recent advances and challenges in the management of retinoblastoma. *Indian Journal of Ophthalmology, 65*, 133. https://doi.org/10.4103/ijo.IJO_883_16
224. Schaiquevich, P., Francis, J. H., Cancela, M. B., et al. (2022). Treatment of retinoblastoma: What is the latest and what is the future. *Frontiers in Oncology, 12*, 822330. https://doi.org/10.3389/fonc.2022.822330
225. Maleki, E. H., Bahrami, A. R., & Matin, M. M. (2024). Cancer cell cycle heterogeneity as a critical determinant of therapeutic resistance. *Genes Dis, 11*, 189–204. https://doi.org/10.1016/j.gendis.2022.11.025
226. Anand, U., Dey, A., Chandel, A. K. S., et al. (2023). Cancer chemotherapy and beyond: Current status, drug candidates,

associated risks and progress in targeted therapeutics. *Genes Dis, 10*, 1367–1401. https://doi.org/10.1016/j.gendis.2022.02.007
227. Xu, L., Polski, A., Prabakar, R. K., et al. (2020). Chromosome 6p Amplification in Aqueous Humor Cell-Free DNA Is a Prognostic Biomarker for Retinoblastoma Ocular Survival. *Molecular Cancer Research, 18*, 1166–1175. https://doi.org/10.1158/1541-7786.MCR-19-1262
228. Sadida, H. Q., Abdulla, A., Al, M. S., et al. (2024). Epigenetic modifications: Key players in cancer heterogeneity and drug resistance. *Transl Oncol, 39*, 101821. https://doi.org/10.1016/j.tranon.2023.101821
229. Marzagalli, M., Fontana, F., Raimondi, M., & Limonta, P. (2021). Cancer stem cells-key players in tumor relapse. *Cancers (Basel), 13*, 376. https://doi.org/10.3390/cancers13030376
230. Yu, Z., Pestell, T. G., Lisanti, M. P., & Pestell, R. G. (2012). Cancer stem cells. *International Journal of Biochemistry & Cell Biology, 44*, 2144–2151. https://doi.org/10.1016/j.biocel.2012.08.022
231. Sari, N. M., Hadiputri, R., Kuntorini, M. S., et al. (2021). High-risk histopathologic features of retinoblastoma treated at a tertiary hospital in West Java, Indonesia. *Ocul Oncol Pathol, 7*, 353–360. https://doi.org/10.1159/000517100
232. Chintagumpala, M., Chevez-Barrios, P., Paysse, E. A., et al. (2007). Retinoblastoma: Review of current management. *Oncologist, 12*. https://doi.org/10.1634/theoncologist.12-10-1237
233. Fabian, I. D., Stacey, A. W., Johnson, K. C., et al. (2018). Primary enucleation for group D retinoblastoma in the era of systemic and targeted chemotherapy: The price of retaining an eye. *British Journal of Ophthalmology, 102*, 265–271. https://doi.org/10.1136/BJOPHTHALMOL-2017-310624
234. Seema, R., Parul, S., Nita, K., & Kamlesh. (2014). High-risk histomorphological features in retinoblastoma and their association with p53 expression: An Indian experience. *Indian Journal of Ophthalmology, 62*, 1069–1071. https://doi.org/10.4103/0301-4738.146747
235. Liu, Y., Zhong, X., Wan, S., et al. (2014). p16INK4a expression in retinoblastoma: A marker of differentiation grade. *Diagnostic Pathology, 9*, 180. https://doi.org/10.1186/s13000-014-0180-1
236. Suresh Babu, V., Bisht, A., Mallipatna, A., et al. (2022). Enhanced epithelial-to-mesenchymal transition and chemoresistance in advanced retinoblastoma tumors is driven by miR-181a. *Cancers (Basel), 14*. https://doi.org/10.3390/cancers14205124
237. Abramson, D. H. (2022). Cell free DNA (cfDNA) in the blood of retinoblastoma patients The Robert M. Ellsworth Lecture. *Ophthalmic Genetics, 43*, 731–735. https://doi.org/10.1080/13816810.2021.2004433
238. Berry, J. L., Xu, L., Kooi, I., et al. (2018). Genomic cfDNA analysis of aqueous humor in retinoblastoma predicts eye salvage: The surrogate tumor biopsy for retinoblastoma. *Molecular Cancer Research, 16*, 1701–1712. https://doi.org/10.1158/1541-7786.MCR-18-0369
239. Li, H. T., Xu, L., Weisenberger, D. J., et al. (2022). Characterizing DNA methylation signatures of retinoblastoma using aqueous humor liquid biopsy. *Nature Communications, 13*. https://doi.org/10.1038/S41467-022-33248-2
240. Berry, J. L., Pike, S., Shah, R., et al. (2024). Aqueous humor liquid biopsy as a companion diagnostic for retinoblastoma: Implications for diagnosis, prognosis, and therapeutic options: Five years of progress. *American Journal of Ophthalmology, 263*, 188–205. https://doi.org/10.1016/J.AJO.2023.11.020
241. Jansen, R. W., Roohollahi, K., Uner, O. E., et al. (2024). Correlation of gene expression with magnetic resonance imaging features of retinoblastoma: A multi-center radiogenomics validation study. *European Radiology, 34*, 863–872. https://doi.org/10.1007/S00330-023-10054-Y/FIGURES/4
242. Roy, A., Kumar, V., Zorman, B., et al. (2015). Recurrent internal tandem duplications of BCOR in clear cell sarcoma of the kidney. *Nature Communications, 6*, 1–7. https://doi.org/10.1038/ncomms9891
243. Fayoumi, A. (2025). BCOR abnormalities in endometrial stromal sarcoma. *Gynecologica Oncology Report, 57*. https://doi.org/10.1016/J.GORE.2024.101672
244. Hu, D., Shen, K., Guo, Y. S., et al. (2024). The clinical implications of BCOR mutations in a large cohort of acute myeloid leukemia patients: A 5-year single-center retrospective study. *Leukemia and Lymphoma, 65*. https://doi.org/10.1080/10428194.2024.2387730
245. Li, X., Xu, F., Zhang, Z., et al. (2021). Dynamics of epigenetic regulator gene BCOR mutation and response predictive value for hypomethylating agents in patients with myelodysplastic syndrome. *Clinical Epigenetics, 13*. https://doi.org/10.1186/S13148-021-01157-8
246. Gherardini, L., Sharma, A., Taranta, M., Cinti, C. (2024). *Epigenetic reprogramming by decitabine in retinoblastoma*. https://doi.org/10.20944/PREPRINTS202404.1746.V1
247. Pascual-Pasto, G., Bazan-Peregrino, M., Olaciregui, N. G., et al. (2019). Therapeutic targeting of the RB1 pathway in retinoblastoma with the oncolytic adenovirus VCN-01. *Science Translational Medicine, 11*. https://doi.org/10.1126/SCITRANSLMED.AAT9321/SUPPL_FILE/AAT9321_TABLE_S6.XLSX
248. Koch, J., Schober, S. J., Hindupur, S. V., et al. (2022). Targeting the retinoblastoma/E2F repressive complex by CDK4/6 inhibitors amplifies oncolytic potency of an oncolytic adenovirus. *Nature Communications, 13*, 1–18. https://doi.org/10.1038/s41467-022-32087-5
249. Berry, J. L., Xu, L., Murphree, A. L., et al. (2017). Potential of aqueous humor as a surrogate tumor biopsy for retinoblastoma. *JAMA Ophthalmol, 135*, 1221–1230. https://doi.org/10.1001/JAMAOPHTHALMOL.2017.4097
250. Galluzzi, P., Hadjistilianou, T., Cerase, A., et al. (2009). Is CT still useful in the study protocol of retinoblastoma? *AJNR. American Journal of Neuroradiology, 30*, 1760–1765. https://doi.org/10.3174/AJNR.A1716
251. Kothari, P., Marass, F., Yang, J. L., et al. (2020). Cell-free DNA profiling in retinoblastoma patients with advanced intraocular disease: An MSKCC experience. *Cancer Medicine, 9*, 6093–6101. https://doi.org/10.1002/CAM4.3144
252. Haeno, H., Iwasa, Y., & Michor, F. (2007). The evolution of two mutations during clonal expansion. *Genetics, 177*, 2209–2221. https://doi.org/10.1534/GENETICS.107.078915
253. Liu, A., Gao, Y., Wang, Q., et al. (2023). The heterogeneity and clonal evolution analysis of the advanced prostate cancer with castration resistance. *Journal of Translational Medicine, 21*, 1–13. https://doi.org/10.1186/S12967-023-04320-2/FIGURES/7
254. Liang, A., Kong, Y., Chen, Z., et al. (2023). Advancements and applications of single-cell multi-omics techniques in cancer research: Unveiling heterogeneity and paving the way for precision therapeutics. *Biochemistry and Biophysical Reports, 37*. https://doi.org/10.1016/J.BBREP.2023.101589
255. Pan, D., & Jia, D. (2021). Application of single-cell multi-omics in dissecting cancer cell plasticity and tumor heterogeneity. *Frontiers Molecular Bioscience, 8,* 757024. https://doi.org/10.3389/fmolb.2021.757024
256. Hou, Y., Guo, H., Cao, C., et al. (2016). Single-cell triple omics sequencing reveals genetic, epigenetic, and transcriptomic heterogeneity in hepatocellular carcinomas. *Cell Research, 26*. https://doi.org/10.1038/cr.2016.23

257. Sen, R., Thathapudi, N. C., Sharma, D., et al. (2022). Tumor models of retinoblastoma: In Vivo, Ex Vivo, and In Vitro Models. *Handbook of Animal Models and its Uses in Cancer Research*, pp. 1–25. https://doi.org/10.1007/978-981-19-1282-5_30-1
258. Ghose, N., & Kaliki, S. (2022). Liquid biopsy in retinoblastoma: A review. *Semin Ophthalmol, 37*, 813–819. https://doi.org/10.1080/08820538.2022.2078165
259. Soda, N., Clack, K., & Shiddiky, M. J. A. (2022). Recent advances in liquid biopsy technologies for cancer biomarker detection. *Sensors & Diagnostics, 1*, 343–375. https://doi.org/10.1039/D2SD00010E
260. Berry, J. L., Xu, L., Polski, A., et al. (2020). Aqueous humor is superior to blood as a liquid biopsy for retinoblastoma. *Ophthalmology, 127*, 552–554. https://doi.org/10.1016/J.OPHTHA.2019.10.026
261. Xu, L., Kim, M. E., Polski, A., et al. (2021). Establishing the clinical utility of ctDNA analysis for diagnosis, prognosis, and treatment monitoring of retinoblastoma: The aqueous humor liquid biopsy. *Cancers (Basel), 13*, 1–15. https://doi.org/10.3390/CANCERS13061282
262. Marrugo-Ramírez, J., Mir, M., & Samitier, J. (2018). Blood-based cancer biomarkers in liquid biopsy: A promising non-invasive alternative to tissue biopsy. *International Journal of Molecular Sciences, 19*, 2877. https://doi.org/10.3390/IJMS19102877
263. Duque, G., Manterola, C., Otzen, T., et al. (2022). Cancer biomarkers in liquid biopsy for early detection of breast cancer: A systematic review. *Clinical Medicine Insights Oncology, 16*. https://doi.org/10.1177/11795549221134831
264. Babu, V. S., Mallipatna, A., Deepak, S. A., et al. (2022). Integrated analysis of cancer tissue and vitreous humor from retinoblastoma eyes reveals unique tumor-specific metabolic and cellular pathways in advanced and non-advanced tumors. *Cells, 11*. https://doi.org/10.3390/CELLS11101668
265. Gulati, K., Poluri, K. M., Kaliki, S. (2024). Assessing the metabolic variations of invasive and noninvasive unilateral retinoblastoma patients. *ACS Omega, 9*. https://doi.org/10.1021/ACSOMEGA.4C06014
266. Di Sario, G., Rossella, V., Famulari, E. S., et al. (2023). Enhancing clinical potential of liquid biopsy through a multi-omic approach: A systematic review. *Frontiers Genetics, 14*. https://doi.org/10.3389/FGENE.2023.1152470
267. Bahrambeigi, V., Lee, J. J., Branchi, V., et al. (2024). Transcriptomic profiling of plasma extracellular vesicles enables reliable annotation of the cancer-specific transcriptome and molecular subtype. *Cancer Research, 84*, 1719–1732. https://doi.org/10.1158/0008-5472.CAN-23-4070
268. Manukonda, R., Yenuganti, V. R., Nagar, N., et al. (2022). Comprehensive analysis of serum small extracellular vesicles-derived coding and non-coding RNAs from retinoblastoma patients for identifying regulatory interactions. *Cancers (Basel), 14*. https://doi.org/10.3390/CANCERS14174179
269. Manukonda, R., Jakati, S., Attem, J., et al. (2023). Identifying treatment resistance related pathways by analyzing serum extracellular vesicles of patients with resistant versus regressed retinoblastoma. *Investment Ophthalmology Visual Science, 64*. https://doi.org/10.1167/IOVS.64.11.26
270. Farabi Maleki, S., Yousefi, M., Hajiesmailpoor, Z., et al. (2024). Role of artificial intelligence in ocular tumors: A systematic review. *Journal of Clinical Oncology, 42*, e15070–e15070. https://doi.org/10.1200/JCO.2024.42.16_SUPPL.E15070
271. Lima, R. V., Arruda, M. P., Muniz, M. C. R., et al. (2024). Artificial intelligence methods in diagnosis of retinoblastoma based on fundus imaging: A systematic review and meta-analysis. *Graefe's Archive for Clinical and Experimental Ophthalmology, 263*, 547–553. https://doi.org/10.1007/S00417-024-06643-2/METRICS
272. Kaliki, S., Vempuluru, V., Ghose, N., et al. (2023). Artificial intelligence and machine learning in ocular oncology: Retinoblastoma. *Indian Journal of Ophthalmology, 71*, 424–430. https://doi.org/10.4103/IJO.IJO_1393_22
273. Vempuluru, V. S., Viriyala, R., & Ayyagari, V. (2024). Artificial intelligence and machine learning in ocular oncology, retinoblastoma (ArMOR): Experience with a multiracial cohort. *Cancers, 16*, 3516. https://doi.org/10.3390/CANCERS16203516
274. Aldughayfiq, B., Ashfaq, F., Jhanjhi, N. Z., & Humayun, M. (2023). Explainable AI for retinoblastoma diagnosis: Interpreting deep learning models with LIME and SHAP. *Diagnostics, 13*, 1932. https://doi.org/10.3390/DIAGNOSTICS13111932
275. Hurwitz, M. Y., Marcus, K. T., Chévez-Barrios, P., et al. (2004). *Suicide gene therapy for treatment of retinoblastoma in a murine model* (vol. 10, pp. 441–448). https://home.liebertpub.com/hum. https://doi.org/10.1089/10430349950018887
276. HSV-TK/GCV can induce cytotoxicity of retinoblastoma cells through autophagy inhibition by activating MAPK/ERK. https://www.spandidos-publications.com/10.3892/or.2018.6454. Accessed 20 Mar 2025.
277. Shi, H., He, X., Yang, Z., et al. (2023). The use of rAAV2-RB1–mediated gene therapy in retinoblastoma. *Investigative Ophthalmology & Visual Science, 64*, 31–31. https://doi.org/10.1167/IOVS.64.15.31
278. Marusyk, A., & Polyak, K. (2010). Tumor heterogeneity: Causes and consequences. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1805*, 105–117. https://doi.org/10.1016/j.bbcan.2009.11.002
279. Smit, M. M., Feller, K. J., You, L., et al. (2022). Spatially annotated single cell sequencing for unraveling intratumor heterogeneity. *Front Bioeng Biotechnol, 10*, 829509. https://doi.org/10.3389/FBIOE.2022.829509/BIBTEX
280. Saxena, S., Jena, B., & Gupta, N. (2022). Role of artificial intelligence in radiogenomics for cancers in the era of precision medicine. *Cancers, 14*, 2860. https://doi.org/10.3390/CANCERS14122860
281. Kaewkhaw, R., & Rojanaporn, D. (2020). Retinoblastoma: Etiology, modeling, and treatment. *Cancers (Basel), 12*, 2304. https://doi.org/10.3390/cancers12082304
282. Sampieri, K., Amenduni, M., Papa, F. T., et al. (2009). Array comparative genomic hybridization in retinoma and retinoblastoma tissues. *Cancer Science, 100*, 465–471. https://doi.org/10.1111/J.1349-7006.2008.01070.X
283. Marchong, M. N., Yurkowski, C., Ma, C., et al. (2010). Cdh11 acts as a tumor suppressor in a murine retinoblastoma model by facilitating tumor cell death. *PLoS Genetiscs, 6*. https://doi.org/10.1371/JOURNAL.PGEN.1000923
284. Gratias, S., Rieder, H., Ullmann, R., et al. (2007). Allelic loss in a minimal region on chromosome 16q24 is associated with vitreous seeding of retinoblastoma. *Cancer Research, 67*, 408–416. https://doi.org/10.1158/0008-5472.CAN-06-1317
285. KIF14 and E2F3 mRNA expression in human retinoblastoma and its phenotype association - PubMed. https://pubmed.ncbi.nlm.nih.gov/19190782/. Accessed 26 Aug 2024.
286. Madhavan, J., Coral, K., Mallikarjuna, K., et al. (2007). High expression of KIF14 in retinoblastoma: Association with older age at diagnosis. *Investigative Ophthalmology & Visual Science, 48*, 4901–4906. https://doi.org/10.1167/IOVS.07-0063
287. Qi, D. L., & Cobrinik, D. (2017). MDM2 but not MDM4 promotes retinoblastoma cell proliferation through p53-independent regulation of MYCN translation. *Oncogene, 36*, 1760–1769. https://doi.org/10.1038/ONC.2016.350

288. Bu, W., Wang, Y., & Min, X. (2018). MicroRNA-106b promotes the proliferation, migration and invasion of retinoblastoma cells by inhibiting the expression of ZBTB4 protein. *Experimental and Therapeutic Medicine, 16*, 4537–4545. https://doi.org/10.3892/ETM.2018.6811
289. Subramanian, N., Kanwar, J. R., Kanwar, R. K., & Krishnakumar, S. (2015). Blocking the maturation of OncomiRNAs using primiRNA-17~92 aptamer in retinoblastoma. *Nucleic Acid Therapeutics, 25*, 47–52. https://doi.org/10.1089/NAT.2014.0507
290. Meng, B. O., Wang, Y., & Bin, L. I. (2014). Suppression of PAX6 promotes cell proliferation and inhibits apoptosis in human retinoblastoma cells. *International Journal of Molecular Medicine, 34*, 399–408. https://doi.org/10.3892/IJMM.2014.1812
291. Zugbi, S., Aschero, R., Ganiewich, D., et al. (2023). Establishment and comprehensive characterization of a novel preclinical platform of metastatic retinoblastoma for therapeutic developments. *Investment Ophthalmology Visual Science, 64*. https://doi.org/10.1167/IOVS.64.15.27
292. Searle, J. S., Li, B., & Du, W. (2010). Targeting Rb mutant cancers by inactivating TSC2. *Oncotarget, 1*, 228–232. https://doi.org/10.18632/ONCOTARGET.130
293. Beta, M., Chitipothu, S., Khetan, V., et al. (2015). Hypermethylation of adenomatosis polyposis coli-2 and its tumor suppressor role in retinoblastoma. *Current Eye Research, 40*, 719–728. https://doi.org/10.3109/02713683.2014.954673
294. Soebagjo, H., Komaratih, E., Fatmariyanti, S., et al. (2019). Expression of MMP-14 and CD44 associated with proliferation of retinoblastoma cells. *Medicine Science | International Medical Journal, 1*. https://doi.org/10.5455/MEDSCIENCE.2018.07.8958
295. Indovina, P., Acquaviva, A., De Falco, G., et al. (2010). Down-regulation and aberrant promoter methylation of p16INK4A: A possible novel heritable susceptibility marker to retinoblastoma. *Journal of Cellular Physiology, 223*, 143–150. https://doi.org/10.1002/JCP.22019
296. Zhou, M., Tang, J., Fan, J., et al. (2024). Recent progress in retinoblastoma: Pathogenesis, presentation, diagnosis and management. *Asia Pacific Journal Ophthalmology (Philadelphia), 13*. https://doi.org/10.1016/J.APJO.2024.100058
297. Choy, K. W., Pang, C. P., Fan, D. S. P., et al. (2004). Microsatellite instability and MLH1 promoter methylation in human retinoblastoma. *Investigative Ophthalmology & Visual Science, 45*, 3404–3409. https://doi.org/10.1167/IOVS.03-1273
298. García-Gutiérrez, L., McKenna, S., Kolch, W., Matallanas, D. (2020). RASSF1A tumour suppressor: Target the network for effective cancer therapy. *Cancers (Basel), 12*. https://doi.org/10.3390/CANCERS12010229
299. Li, C., Zhao, J., Sun, W. (2020). microRNA-222-mediated VHL downregulation facilitates retinoblastoma chemoresistance by increasing HIF1α expression. *Investment Ophthalmology Visual Science, 61*. https://doi.org/10.1167/IOVS.61.10.9