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Review Article

Retinoblastoma: Recent Advancement and the Role of Y79 Cell Lines in In-Vitro Evaluation of Plant Extracts

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ABSTRACT

Retinoblastoma (RB) represents the most common intraocular cancer in childhood, arising predominantly due to biallelic loss of the RB1 tumor suppressor gene or, in a subset of cases, high-level amplification of MYCN. The decade from 2015 to 2025 has witnessed significant innovations in clinical management, including intra-arterial chemotherapy (IAC), intravitreal chemotherapy (IVitC) for vitreous seeds, and the emerging application of aqueous humor liquid biopsy for non-invasive tumor genotyping. Despite these advances, considerable disparities in outcomes persist between high-income and resource-limited countries, particularly India, where delayed diagnosis and treatment abandonment remain major challenges. Parallel to these clinical developments, in vitro RB models, particularly the Y79 cell line, have remained indispensable for mechanistic studies and drug discovery. Y79 provides a reproducible platform for evaluating the anticancer potential of plant-derived extracts and phytochemicals, including flavonoids, alkaloids, sesquiterpenes, and nanoparticle-based formulations. Compounds such as quercetin, berberine, xanthatin, hypericin, naringenin, chebulagic acid, Nigella sativa extracts, and seaweed-derived nanoparticles have demonstrated cytotoxic or apoptotic effects in Y79 models, indicating their translational promise. This review synthesizes evidence from 2015–2025 on the epidemiology, genetics, clinical management, and outcomes of RB, while providing a detailed overview of the role of Y79 cell lines in phytochemical testing. The importance of assay standardization, comparative evaluation with other RB cell lines, and integration with liquid-biopsy-driven precision medicine is highlighted to bridge laboratory discoveries and clinical application.

INTRODUCTION

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Retinoblastoma (RB) is a rare but aggressive paediatric ocular malignancy, affecting approximately 1 in 16,000–18,000 live births worldwide [1,2]. Although survival rates in high-income countries exceed 95%, outcomes in low- and middle-income countries, including India, remain suboptimal, with mortality rates still as high as 40–60% in some series [3]. This disparity is largely attributable to late presentation, lack of awareness, and limited access to specialized care. The pathogenesis of RB primarily involves biallelic inactivation of the RB1 gene, a pivotal regulator of the G1–S cell-cycle checkpoint [4]. In recent years, a subset of RB1-proficient tumors driven by MYCN amplification has been described; these tumors present earlier, progress more aggressively, and require refined diagnostic strategies [5]. Alongside clinical management, *in vitro* cell culture systems have played a vital role in understanding RB biology and screening therapeutic compounds. Among these, the Y79 cell line, established in 1970 from the tumor of a 2-year-old child with hereditary RB, remains the most widely used model [6]. It provides an excellent platform for preclinical assessment of plant-based anticancer compounds, a field that has gained momentum as researchers explore phytochemicals with apoptotic, antioxidant, and ROS-mediated mechanisms. This review aims to (i) summarize advances in RB management and

molecular understanding from 2015 to 2025, (ii) highlight the global and Indian burden, and (iii) comprehensively examine the role of Y79 cell lines in *in vitro* phytochemical research, emphasizing plant extracts that have demonstrated potential anticancer activity.

2. Epidemiology and Global Burden

Globally, the incidence of RB has remained stable over the past decade, but the absolute number of cases is increasing due to population growth (Fig.1,2) [7]. Approximately 8,000 new cases are reported annually, with Asia accounting for more than 50% [8]. India contributes a substantial proportion of this burden, estimated at ~1,500–2,000 new cases per year [9]. In high-income countries, >95% of affected children survive, and most eyes can be salvaged with modern therapies. In contrast, in low- and middle-income settings, survival can be as low as 50%, and enucleation remains the predominant treatment [10]. Contributing factors include delayed diagnosis, lack of awareness among primary care providers, and financial or geographic barriers to care. Efforts such as public awareness campaigns, early screening programs, and establishment of RB treatment consortia in India have shown promise in reducing diagnostic delay [11].

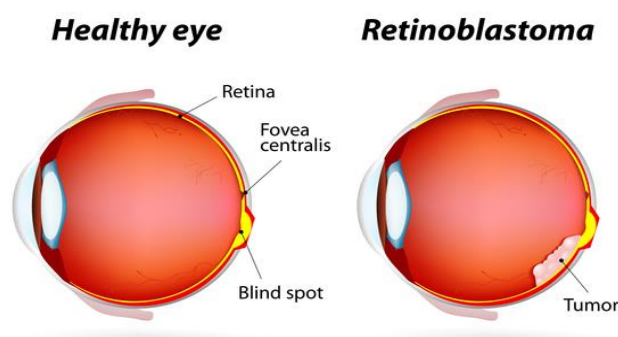


Fig.1: A clean anatomical illustration showing the retinal area and basic disease localization without clutter—perfect for general descriptions

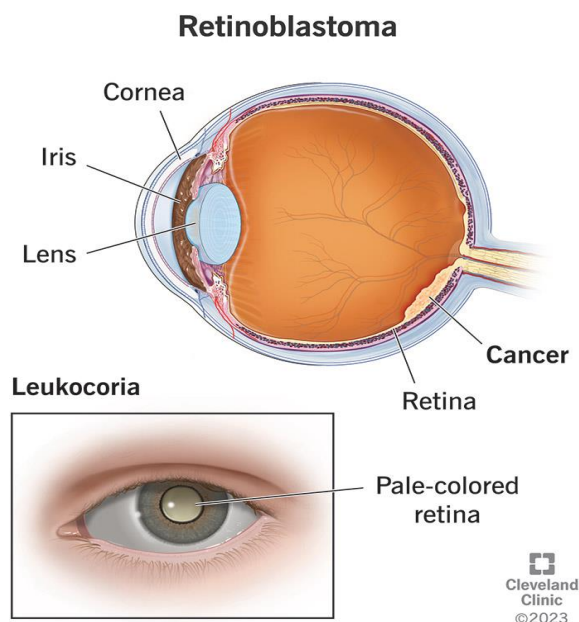


Fig.2: Clinical and Cross-Sectional Illustration (Cleveland Clinic)

A labelled cross-section of the eye showing the tumor in the retina and an external image demonstrating leukocoria (white pupil)

3. Molecular Pathogenesis

3.1 RB1 Mutations and the Two-Hit Hypothesis

The classic two-hit model proposed by Knudson remains central to RB biology. Germline carriers of RB1 mutations typically present with bilateral disease, whereas sporadic biallelic mutations lead to unilateral tumors [4].

3.2 MYCN-Amplified Subtype

Since 2015, attention has focused on MYCN-amplified, RB1-proficient tumors, representing 1–2% of RB cases. These tumors present in very young infants (<1 year) with unilateral, aggressive growth and poor differentiation [5,12]. MRI radiomics and gene expression profiling are being explored for accurate identification.

3.3 Epigenetics and Transcriptomics

Beyond genetic hits, epigenetic silencing of tumor suppressors, alterations in chromatin remodeling,

and dysregulated microRNAs contribute to tumorigenesis [13]. Transcriptomic profiling of Y79 and related RB cell lines has provided insight into therapy resistance mechanisms, including etoposide-resistant subclones [14].

4. Diagnosis and Imaging

Diagnosis continues to rely on ophthalmic examination under anesthesia, often supported by ultrasound and MRI for staging. CT scans are avoided to minimize radiation. Recent innovations include MRI-based radiomics, which can distinguish MYCN-amplified tumors from RB1-mutant cases [12]. Staging systems have been refined, with the International Intraocular Retinoblastoma Classification (IIRC) and the AJCC 8th edition widely used to guide therapy.

5. Aqueous Humor Liquid Biopsy (2018–2025)

One of the most significant developments has been the use of aqueous humor (AH) liquid biopsy. Small volumes aspirated from the anterior

chamber provide sufficient cell-free DNA (cfDNA) for sequencing, enabling detection of RB1 mutations, MYCN amplifications, and chromosomal alterations such as 6p gain [15]. Studies have shown that 6p gain correlates with poor ocular survival, making AH a prognostic biomarker [16]. Importantly, Indian cohorts have validated the feasibility and safety of AH sampling for genomic analysis [17]. Liquid biopsy is increasingly regarded as a “companion diagnostic” to monitor therapeutic response.

6. Advances in Treatment (2015–2025)

- Intra-arterial chemotherapy (IAC): Now the preferred option for advanced intraocular disease, IAC delivers melphalan ($\pm$ topotecan, carboplatin) directly to the ophthalmic artery, achieving globe salvage rates $>85\%$ [18].
- Intravitreal chemotherapy (IVitC): Effective against vitreous seeds, primarily using melphalan. Dose-limiting retinal toxicity has been reported, prompting use of topotecan as an alternative [19].
- Systemic chemotherapy: Still employed for bilateral disease or when intraocular methods are unavailable.
- Focal therapies: Laser photocoagulation, cryotherapy, and plaque brachytherapy are used as adjuncts.
- Enucleation: Reserved for eyes with no visual potential or those harbouring high-risk histopathological features.

7. Outcomes and Disparities

While developed nations report $>99\%$ survival, outcomes in India remain inferior, with survival rates between 60–70% [20]. Factors such as advanced presentation, treatment abandonment,

and limited specialized centers contribute. International collaborative studies (e.g., Global RB Outcome Study) emphasize strengthening referral pathways, awareness, and financial support as strategies to bridge this gap [21].

8. Y79 Cell Line: Characteristics and Applications

The Y79 cell line, derived from a 2.5-year-old RB patient, has been maintained since 1970 and is authenticated by ATCC and Cellosaurus databases [6,22]. Y79 cells:

- Grow in suspension as loosely adherent clumps.
- Express neuronal and photoreceptor markers, such as cone-rod homeobox (CRX).
- Are highly sensitive to mitochondrial and ROS-modulating compounds.
- Support a wide range of assays: MTT/CCK-8 viability, flow cytometry apoptosis, caspase activation, JC-1 mitochondrial potential, ROS (DCFDA), and western blotting.

Their reproducibility and availability make them the most commonly used RB model for preclinical testing.

9. Plant-Derived Extracts and Phytochemicals Tested on Y79 (2015–2025)

9.1 Flavonoids

- Quercetin: Inhibits proliferation, induces apoptosis, and suppresses invasion in Y79 and WERI-Rb1 cells via JNK/p38 MAPK pathways [23].
- Naringenin: Evaluated alone and in combination with hypericin; showed limited synergy in Y79 models [24].



9.2 Alkaloids

- Berberine: Suppresses migration and invasion of Y79 cells through PI3K/Akt and p38 pathway inhibition [25].

9.3 Sesquiterpenes

- **Xanthatin:** Potent cytotoxic agent inducing ROS-mediated apoptosis and G2/M arrest in Y79 cells; validated in xenograft models [26].

9.4 Polyphenols and Tannins

- **Chebularic acid (Terminalia chebula):**

Promotes mitochondrial apoptosis through Bax/Bcl-2 modulation and caspase activation [27].

9.5 Nanoparticle-Based Phytochemicals

- Seaweed-derived laminarin-AgNPs: Demonstrated apoptosis induction in Y79 [28].
- Centella asiatica-Ag/Fe nanoparticles: Preliminary reports suggest cytotoxicity in Y79, though more validation is needed [29].

9.6 Immunomodulatory Extracts

- Nigella sativa extract: Via NK cell activation, inhibited Y79 proliferation and induced cell-cycle arrest [30].

10. Advantages of Y79 for Phytochemical Screening

- Retains neuronal phenotype and genetic background of RB.
- Responsive to ROS-mediated apoptosis, making it suitable for phytochemicals.
- Suspension growth facilitates uniform drug exposure.

- Standardized protocols available globally.

CONCLUSION

The past decade has revolutionized RB care, but challenges persist, particularly in India. Y79 cell lines remain indispensable for exploring the anticancer potential of plant-derived compounds. Bridging laboratory discoveries with clinical translation requires robust experimental standardization, comparative validation, and integration with precision medicine approaches such as liquid biopsy.

REFERENCES

1. Ye Q, Zeng Z, Liang X, Li W. Quercetin suppresses retinoblastoma cell proliferation and invasion and facilitates oxidative stress-induced apoptosis through the miR-137/FNDC5 axis. *Environmental Research*. 2023;237(Pt 2):116934. PubMed
2. Song WP, Zheng S, Yao HJ, Zhou XF, Li R, Zhang CY, et al. Different transcriptome profiles between human retinoblastoma Y79 cells and an etoposide-resistant subline reveal a chemoresistance mechanism. *BMC Ophthalmology*. 2020;20(1):92. PubMedPubMed Central
3. ATCC. Y-79 (HTB-18) Retinoblastoma, human — product datasheet. ATCC Catalog. (online resource). <https://www.atcc.org>
4. CELLosaurus. Y-79 (CVCL_1893) cell line entry. Cellosaurus. (online resource). cellosaurus.org
5. Berry JL, Yaguboglu S, et al. Aqueous humor liquid biopsy as a companion diagnostic for retinoblastoma: implications for diagnosis, prognosis, and therapeutic options — five years of progress. *Translational/Review (PMC article)*. 2023. (Review / perspective with AH cfDNA data). PubMed Central



6. Gerrish A, Murray A, et al. Genetic diagnosis of retinoblastoma using aqueous humor: prospective study of tumor-derived cfDNA. *Cancers (MDPI)*. 2024;16(8):1565. MDPI
7. Meel R, Sharma R, et al. Tumor DNA sampling from aqueous humor in retinoblastoma — first South Asia series and validation of cfDNA isolation for RB1 analysis. *Indian Journal of Ophthalmology*. 2024;72(??):??? (see article for pages). Lippincott Journals
8. Marasligiller SA, et al. Ocular survival after intra-arterial chemotherapy for retinoblastoma — institutional series and outcomes. (PMC article / Ophthalmic surgical outcomes). 2022. PubMed Central
9. Teixeira LF, et al. Intra-arterial chemotherapy for retinoblastoma — large-cohort outcomes and ocular survival estimates. *Ophthalmology Retina*. 2025;Volume(Issue):Pages. Ophthalmology Retina
10. Yousef YA, Tawfik MA, et al. Safety and efficacy of intravitreal chemotherapy (melphalan) to treat vitreous seeds in retinoblastoma: a systematic review and single-centre experience. *Frontiers in Pharmacology*. 2021;12:696787. Frontiers
11. Munier FL, Soliman S, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. *British Journal of Ophthalmology*. 2012;96(8):1078–1083. PubMed
12. Shields CL, Douglass AM, et al. Intravitreal melphalan injection for persistent or recurrent vitreous retinoblastoma seeding: outcomes and complications. *JAMA Ophthalmology*. 2014;132(8):936–941. PubMed
13. Ghassemi F, Shields CL, et al. Combined intravitreal melphalan and topotecan for refractory or recurrent vitreous seeding from retinoblastoma. *JAMA Ophthalmology*. 2014;132(8):936–941 (or see combined regimen paper). JAMA Network
14. The Global Retinoblastoma Study Group (Fabian ID, et al.). The Global Retinoblastoma Outcome Study: a prospective, cluster-based analysis of 4,064 patients from 149 countries. *The Lancet Global Health*. 2022;Volume:Pages. The Lancet
15. Finger PT, et al. Retinoblastoma outcomes: a global perspective — systematic review and meta-analysis exploring socioeconomic and healthcare correlates. *Lancet Global Health / Review*. 2022;Volume:Pages. The Lancet
16. Ji X, Cheng L, Wei F, et al. Noninvasive visualization of retinoblastoma growth and metastasis via bioluminescence imaging (preclinical models). *Investigative Ophthalmology & Visual Science (IOVS)*. 2009 (foundational Y79 xenograft imaging reference). PubMed Central
17. Yang J, Li Y, Zong C, et al. Xanthatin selectively targets retinoblastoma by inhibiting the PLK1-mediated cell cycle. *Investigative Ophthalmology & Visual Science (IOVS)*. 2021;62(15):11 (article reporting anti-RB activity including Y79 models). PubMed CentralPubMed
18. Gao X, Li Y, et al. Xanthatin induces apoptosis through ROS-mediated c-FLIP inhibition in human retinoblastoma cells. (PMC full text). 2025;Article:PMC12041067. PubMed Central
19. Kumar N, Gangappa D, Gupta G, Karnati R. Chebulagic acid from *Terminalia chebula* causes G1 arrest, inhibits NF- κ B and induces apoptosis in retinoblastoma cells. *BMC Complementary and Alternative Medicine*. 2014;14:319. BioMed Central
20. Wang Y, Yuan J, Liang Y, et al. Inhibition of migration and invasion by berberine via inactivation of PI3K/Akt and p38 in human retinoblastoma cell line. *Advances in Clinical*

- and Experimental Medicine. 2018;27(7):899–905. Pub Med ACEM
21. Remya RR, Rajasree SR, Aranganathan T, et al. Laminarin-based silver nanoparticles (AgNPs) synthesized using brown seaweed *Turbinaria ornata* induce apoptosis in human retinoblastoma Y79 cell lines. *Materials Research Express* / related conference/journal. 2017/2018;Article aab2d8 / DOI:10.1088/2053-1591/aab2d8. PubMedAstrophysics Data System
 22. (SysRev / regional study) NK cells induced by ethanolic *Nigella sativa* extract inhibit proliferation of retinoblastoma Y79 cell line through Cyclin D1 pathway. *Systematic Reviews in Pharmacy* / regional article. 2021;12(1):1547–1554. *Systematic Reviews in Pharmacy*
 23. Arani HZ, Abbasy Z, et al. Hypericin and naringenin exert no significant synergistic apoptotic effect on Y79 retinoblastoma cell line. *Galen Medical Journal*. 2024;13:e3347. ResearchGatePubMed Central
 24. Linima VK, et al. Biofabrication of *Centella asiatica*-mediated silver and iron nanoparticles and their enhanced antimicrobial and anticancer activity in retinoblastoma (Y79) cancer cells. *Nanoscience / NanoStructures & Nano-Objects or NanoStructures*. 2024;Article/Volume:101226. article-pageScienceDirect
 25. Pareek A, Khetan V, et al. Retinoblastoma — a comprehensive review, update and recent developments (genetics, classification, nano-delivery & future strategies). *International Ophthalmic Journal* / review (PMC). 2024;Article: (see journal for pages). PubMed Central
 26. Li HT, et al. Characterising DNA methylation signatures and AH-based biomarkers in ocular tumours; perspectives for retinoblastoma precision diagnostics. *Nature Communications* / Genomics review. 2022; Volume: Pages. Nature
 27. Hum Genomics / Genetics review. Genetics in ophthalmology: molecular blueprints of retinoblastoma. *Human Genomics*. 2023;Volume:Pages. BioMed Central
 28. Sun Y, et al. Differentiating MYCN-amplified RB1 wild-type retinoblastoma from RB1–/– disease using pretreatment MRI and radiomics — diagnostic radiology insights. *Scientific Reports*. 2024;14: (article). Nature
 29. Jia S, et al. Comparison of intra-arterial chemotherapy efficacy delivered through the ophthalmic artery or external carotid artery in a cohort of retinoblastoma patients. *Frontiers in Medicine*. 2021;8:658305. Frontiers
 30. Abramson DH, et al. Intra-arterial chemotherapy (IAC) for retinoblastoma — technique, outcomes and toxicity profile (foundational / comparative PLoS/retrospective series). *PLoS One* / Ophthalmic surgical outcomes. 2016;Article/e0146582.

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