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A review of retinoblastoma on its discovery, genetic aspects, and treatments

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Abstract. Retinoblastoma, a critical early-childhood retinal cancer, poses significant challenges and opportunities for oncological research. This paper provides a comprehensive examination of Retinoblastoma, from its historical context and genetic underpinnings to contemporary diagnostic and therapeutic strategies. It delves into the pivotal role of the RB1 gene in the disease's hereditary and sporadic forms, underscoring the importance of genetic mutations and the potential of molecular genetics in advancing patient care. Through an analysis of current findings and treatment approaches, this study highlights the ongoing evolution in managing Retinoblastoma, emphasizing early detection, the exploration of genetic landscapes, and the development of targeted therapies. Future research directions are proposed, aiming at refining diagnostic accuracy and improving treatment outcomes, thereby offering hope for affected children and their families.

Keywords: Retinoblastoma, RB1 gene mutations, Molecular diagnostics in oncology

1. Introduction

Retinoblastoma is a primary malignancy of the retina that predominantly affects young children, often diagnosed before the age of five. Common symptoms include Leukocoria, which presents as a white pupillary reflex, Strabismus, or misaligned eyes. Additional symptoms include heterochromia (differences in iris color) and other vision problems. Epidemiologically, retinoblastoma affects approximately 1 in every 18,000 live births and accounts for about 3% of all childhood cancers [2]. This incidence rate remains relatively consistent across different populations, indicating limited influence from environmental factors. The disease is highly curable in the early stages but can become fatal if not treated promptly. The survival rate in developed countries exceeds 95% due to advancements in diagnostic and treatment techniques Many studies have been done on it, which have revealed its genetic factors and developed relatively efficient diagnoses and treatments. Nowadays, retinoblastoma serves as a model for understanding cancer genetics such as RB1 genes and p27 as well as corresponding therapeutics utilizing specific RNAs. This paper reviews the latest advances in the diagnosis and treatment of retinoblastoma from its historical findings, clinical features and genetic basis so as to generate a holistic view about the current understanding of retinoblastoma.

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2. Discovery and development of Retinoblastoma

2.1. Discovery of retinoblastoma

Retinoblastoma was first documented in 1597 by Petrus Pawius [1], a Dutch professor. Initially, these tumors were misinterpreted as a fungal infection called "fungus haematodes". In the 18th century, William Hayes reported similar cases [1], though the understanding of the disease remained primitive. The true nature of retinoblastoma began to unravel as James Wardrop, a British surgeon, identified the tumor's origin in the retina in the 19th century. He observed its tendency to affect young children and its potential to spread to other body parts, such as the optic nerve and brain [1].

The early treatment of retinoblastoma was enucleation, which is the surgical removal of the eye, but it was unpopular due to the lack of anesthesia. The development of the ophthalmoscope and the discovery of chloroform as an anesthetic made this procedure more feasible later. In addition, the introduction of X-ray treatment, documented by Verhoeff in 1921, offered another treatment for early retinoblastoma [1].

The term "retinoblastoma" itself was coined following contributions from several researchers. Rudolf Virchow suggested that the tumors might derive from neuroglia, a type of brain cell. Flexner and Wintersteiner identified characteristic rosettes in early-stage tumors, named Flexner–Wintersteiner rosettes, a hallmark of retinoblastoma. The resemblance of tumor cells to undifferentiated retinal cells, or "retinoblasts," was noted, and Jacob Knapp proposed a congenital origin for the disease [1].

Clinically, retinoblastoma presents primarily in children, often diagnosed before the age of five. Common symptoms include Leukocoria, which presents as a white pupillary reflex, Strabismus, or misaligned eyes. Additional symptoms include heterochromia (differences in iris color) and other vision problems. Epidemiologically, retinoblastoma affects approximately every 18,000 live births and accounts for about 3% of all childhood cancers [2]. This incidence rate remains relatively consistent across different populations, indicating limited influence from environmental factors. The disease is highly curable in the early stages but can become fatal if not treated promptly. The survival rate in developed countries exceeds 95% due to advancements in diagnostic and treatment techniques [2, 3].

2.2. Influence of genetic factors on retinoblastoma

The modern understanding of retinoblastoma's genetics was revolutionized in 1971 by Alfred G Knudson's "two-hit hypothesis". It proposed the need for two genetic mutations in the development of retinoblastoma and categorized retinoblastoma into familial form (inherited) and sporadic (non-inherited) forms. "Two-hit hypothesis" then leads to identifying the RB1 gene on chromosome 13. In 1986, Thaddeus P. Dryja, an American ophthalmologist, conducted an experiment to identify relative genes that impact retinoblastoma. He discovered three cloned DNA fragments, one of which, H3-8, was absent in some retinoblastoma tumors. This fragment was used to find neighboring DNA sequences. Among these, a highly conserved sequence was used to screen a retinal cDNA library, identifying a transcript (clone p4.7R) expressed in various tissues but not in retinoblastoma. Along with cloning and sequencing, this finding confirmed the site as the RB1 gene. Later, RB1 was further identified as the tumor-suppressing gene [4].

Besides the RB1, retinoblastoma also shows patterns in other gene expressions. Based on the comparison of gene profiling between retinoblastoma cells and normal retina, cone-associated genes such as Retinoid X receptor gamma (RXRγ) and thyroid hormone receptor beta 2 (TRβ2) are predominantly expressed in retinoblastoma [5]. Interestingly, this high expression of cone-associated genes always relates to less genomic instability. Later research further finds the different extents in cone-associated gene expression between the bilateral retinoblastoma and the unilateral retinoblastoma. Bilateral retinoblastoma is associated with the enriched expression, whereas unilateral one shows a reduced signal. The different expressions of cone-associated genes correspond to two facts: loss of cone-associated gene expression correlates to advanced retinoblastoma, and bilateral retinoblastoma usually develops earlier than unilateral one [6].

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3. Mutations in the RB1 and its influences

Based on Knudson's "two-hit" hypothesis, later studies found that in hereditary retinoblastoma, the transmission follows an autosomal dominant pattern, where a mutation in the RB1 gene is present in every cell, including germ cells. This mutation can be inherited from an affected parent or arise de novo in parental germ cells or early embryonic development. It is notable due to high penetrance, which means individuals carrying the mutation have an increased risk of developing the disease. Conversely, the non-hereditary, or sporadic, form results from somatic mutations in retinal cells, occurring post-conception and not inherited from parents [7]. These mutations are confined to the somatic cells of the retina and do not affect germ cells; thus, they are not passed to offspring. Sporadic retinoblastoma typically manifests as unilateral, affecting only one eye, and is unifocal, representing a single initiation site of tumorigenesis. The absence of a family history of the disease is common in sporadic cases.

Accompanying the identification of the RB1 gene is pinpointing its location on chromosome 13. Familial retinoblastoma is closely linked to mutations in the RB1 gene on chromosome 13q14.2. The inactivation of the RB1 gene in retinoblastoma leads to some extent but minimal genomic instability due to specific cellular mechanisms. Genomic studies have identified other genes that might associated with retinoblastoma, including frequent gains in chromosomes 1q, 2p, and 6p and loss in 16q. These alterations involve various genes like MDM4, KIF14, and MYCN [8]. Notably, 1q gain and 16q loss are linked to increased genomic instability and are more common in late-diagnosed, non-hereditary cases. High amplification of MYCN is a significant mark, particularly in tumors without RB1 mutation [8].

As mentioned above, cloning the RB1 gene contributed to understanding retinoblastoma. The process of cloning the RB1 gene began with isolating DNA containing the gene and then inserting it into a cloning vector. This vector was then introduced into host cells, typically bacteria or yeast, in a process known as transformation. The transformed cells were cultured, and those containing the RB1 gene were selected and screened. The final step involved amplifying and analyzing the cloned gene, enabling more profound insights into its function and role in retinoblastoma. Gene cloning advanced the understanding of retinoblastoma and set the stage for discovering other tumor suppressor genes [5].

Retinoblastoma protein (pRb) is the product of the RB1 gene, which regulates cell cycle progression from the G1 phase to the S phase. It does this primarily by inhibiting E2F transcription factors, key players in cell cycle progression and DNA synthesis. Moreover, pRb's functionality extends beyond simple inhibition of cell cycle progression. It involves various cellular processes, including DNA damage repair, apoptosis, and differentiation. The protein's ability to bind to different cellular proteins enables it to influence a wide range of cellular functions, underscoring its importance in maintaining cellular integrity. Mutations in the RB1 gene, leading to dysfunctional or absent pRb, result in the loss of these critical regulatory functions, contributing to the development of retinoblastoma [9].

Mutations in the RB1 gene are diverse, ranging from point mutations, deletions, and insertions to more subtle epigenetic modifications. Each type of mutation impacts the function of the retinoblastoma protein (pRb) uniquely, contributing to the disease's clinical variability.

RB1 gene spans 180 kb and contains 27 exons. The recent advancements in genomic technologies have enabled a more detailed and comprehensive analysis of RB1 gene mutations, which contributes to understanding the full spectrum of genetic alterations in retinoblastoma. Currently, around 2500 pathogenic variants have been discovered in the RB1 gene, with more than 500 being somatic or germline mutations leading to RB1 inactivation. Most mutations prevent the gene from producing functional proteins, causing unregulated cell division. These mutations are distributed mainly within exons 1–25, with 85% being single nucleotide variants or insertion-deletions. The mutation spectrum includes nonsense mutations, out-of-frame exon skipping, splice site variants, chromosomal rearrangements, large exonic deletions, and promoter region methylation. Studies have identified mutational hotspots and recurrent mutations, with a significant percentage occurring in the regions coding the pocket domains of the RB1 protein. The CpG islands across several exons are also notable mutational hotspots. High-throughput techniques have identified novel pathogenic variants, contributing to a better understanding of the RB1 gene's role in retinoblastoma [10].

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Although the onset of retinoblastoma primarily results from mutations in the RB1 tumor suppressor gene of both alleles, besides RB1, other genes also contribute to retinoblastoma. For example, the MYCN gene plays a significant role, often in cases exclusive of RB1 mutations. MYCN amplification, found in less than 2% of cases, marks a rare, aggressive non-hereditary RB subset. Karyotype and comparative genomic hybridization analyses reveal that somatic copy number alterations (SCNAs) are a common tumorigenesis mechanism in RB. Recurrent alterations include gains of chromosomes 1q, 2p, 6p, and losses of 13q and 16q14, with 6p gain correlating with lower ocular salvage rates. Key oncogenes and tumor suppressor genes show copy number changes, such as MDM4, KIF14, MYCN, DEK, E2F3, and CDH11. Other genetic alterations include OTX2 amplification and BCOR mutations [10, 11].

The biochemistry and cell biology of wild-type and mutant gene products in retinoblastoma, particularly focusing on the retinoblastoma protein (pRb), offer insights into the disease's molecular mechanisms. pRb, encoded by the RB1 gene, plays a critical role in various cellular processes, including cell cycle regulation, apoptosis, and DNA repair.

In the cell, pRb interacts with numerous proteins, primarily regulating the cell cycle by controlling the transition from the G1 phase to the S phase. Its interaction with E2F transcription factors is particularly notable. In its active (hypophosphorylated) state, pRb binds to these factors, preventing the transcription of genes necessary for DNA synthesis and cell cycle progression. This function of pRb as a "gatekeeper" is crucial in preventing uncontrolled cell division and tumorigenesis.

However, mutations in the RB1 gene lead to the production of dysfunctional pRb, disrupting its role in cell cycle control. This disruption is characterized by the failure to adequately inhibit E2F transcription factors, resulting in uncontrolled progression to the S phase and consequent unregulated cell proliferation. The mechanisms by which RB1 mutations lead to the loss of cell cycle regulation are crucial to understanding the development of retinoblastoma [5, 6, 8].

Beyond cell cycle regulation, pRb is also implicated in apoptosis and DNA repair processes. The protein influences apoptotic pathways and is involved in maintaining genomic integrity by participating in DNA damage response pathways. The loss or alteration of these functions in mutant pRb contributes to the oncogenic process in retinoblastoma. Understanding these roles is essential for comprehending the broader impact of RB1 mutations [5, 6, 8].

The biochemistry and cell biology of pRb, both in its wild-type and mutant forms, reveal its complex roles in cellular functioning. These roles extend from cell cycle regulation to apoptosis and DNA repair, highlighting the multifaceted nature of pRb's function in cellular health and disease. However, besides causing retinoblastoma, RB1 also associates with other etiological factors.

4. Influence of molecular genetics and genomics on retinoblastoma

The advancements in molecular genetics and genomics have significantly influenced the diagnosis of retinoblastoma. Regarding biomarkers, invasive and non-invasive methods identify markers like APOA1, CDKN2A, CRABPs, and others. Aberrant splicing in retinoblastoma includes exon exclusion and mutually exclusive exons, affecting genes like ABCA4, DAZAP1, MDM4, Dab1, and RB1 [11].

Metabolomic and integrated omics studies have uncovered unique retinoblastoma biomarkers, highlighted altered gene-metabolite associations, and revealed proteins like HISTH2B2E, PATJ, and UBE2V1 differentially expressed in retinoblastoma. Genes related to the immune system, epigenetic regulation, and mitochondrial TCA cycle show significant dysregulation in retinoblastoma patients [12].

Next-generation sequencing (NGS) technologies have identified roles of non-coding RNAs, such as miRNAs and long non-coding RNAs (lncRNAs). These RNAs, including BANCR, AFAP1-AS1, and NEAT1, regulate retinoblastoma progression and metastasis [12, 13]. Differential expression of miRNAs is also observed, with some miRNAs downregulated, and others upregulated, reflecting their potential role in the disease's development and progression. In addition, the identification of biomarkers for early diagnosis and prognosis and labeled genes such as miR17a, miR18a, miR-20a and miR-103 show upregulation from patients [12]. Finding biomarkers may shed light on other options for diagnosis and treatments [13].

Emerging therapies such as immunotherapy, targeted therapies, and oncolytic viruses show promise in reducing reliance on cytotoxic agents, potentially improving survival and preserving ocular function. These advances signify a shift towards more effective, less toxic treatment strategies for retinoblastoma.

Current treatments for retinoblastoma primarily involve enucleation, high-dose chemotherapy, stem cell transplantation, and localized radiotherapy. Enucleation remains a primary approach in lower-income regions due to late diagnoses and resource limitations. Proton-beam radiation therapy, a recent advancement, is effective but financially out of reach for many. In addition, for cases with CNS metastasis, the prognosis is poor despite intensive therapy. New approaches like targeted drug delivery directly to the CNS through intrathecal and intravitreal injections are being researched [4, 13].

5. Conclusion

This paper comprehensively examines Retinoblastoma, from its historical context and genetic underpinnings to contemporary diagnostic and therapeutic strategies. The future directions for retinoblastoma research and management are multifaceted. Firstly, there is a need to enhance early detection methods, particularly in resource-limited countries where late presentations are common. This includes the development of non-invasive, cost-effective screening tools that could be widely implemented. Secondly, a deeper exploration into the genetic and epigenetic landscape of retinoblastoma. The role of non-coding RNAs, additional somatic mutations, and modifier genes in the disease's etiology and progression needs to be more deeply elucidated. Advances in targeted therapies infer a promising direction for less invasive and more precise treatment modalities. The exploration of novel therapeutic agents that can selectively induce apoptosis in retinoblastoma cells without harming the surrounding healthy tissue is crucial. The potential for immunotherapy, including oncolytic viruses and immune checkpoint inhibitors, should be further investigated to assess their efficacy and safety in pediatric populations.

Regarding treatment delivery, innovative approaches such as intravitreal and intrathecal injections that may potentially improve outcomes for patients with advanced or metastatic disease might be a worthful investigation. Developing such targeted drug delivery systems could also mitigate the adverse effects of systemic chemotherapy.

Retinoblastoma research stands at a point where the integration of genomic, molecular, and clinical data is required. Further clinical research adopting a multidisciplinary approach encompassing genetic counseling, advanced molecular diagnostics, targeted therapeutics, and supportive care will be crucial in improving patient outcomes and quality of life.

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