Chapter

History and Genetics of Retinoblastoma

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Abstract

The history of retinoblastoma (RB) goes back to 1597 when Pieter Pawius of Amsterdam described a tumor that resembled retinoblastoma. “Fungus haematodes” was the first term used to describe retinoblastoma. Later, the American Ophthalmological Society approved the term retinoblastoma in 1926. The retinoblastoma protein is encoded by the RB1 gene located at 13q14. The functioning model of the tumor suppressor genes was first proposed by Alfred Knudson in the 1970s who precisely explained the hereditary mechanism of retinoblastoma. If both alleles of this gene are mutated, the protein is inactivated and this results in the development of retinoblastoma. One mutation can be either germline or somatic and the second one is always somatic. Differentiation between sporadic and germline retinoblastoma variants requires the identification of the RB1 germline status of the patient. This identification is important for assessing the risk of additional tumors in the same eye, the other eye, and the risk of secondary tumors. Thus, genetic testing is an important component of the management of all children diagnosed with retinoblastoma. In this chapter, we will go over the history, genetics, and counseling for patients with retinoblastoma.

Keywords: history, genetics, two-hit hypothesis, RB1 gene, tumor suppressor, epigenetics, Knudson, allele, germline, somatic

1. History

1.1 Introduction

Different tumors that resembled retinoblastoma were described in the past, the first of which was by Pieter Pawius of Amsterdam in 1597 [1]. The Dutch anatomist described a malignancy involving the left eye, temporal bone, and cranium [1]. Pawius described the tumor as “filled with substance like brain tissue mixed with thick blood and like crushed stone.” The crushed stone may have represented retinoblastoma classic calcifications. Then, in 1767, Hayes described another case of a child who had presented with the appearance “cat’s eyes in the dark” [2]. This was the first description of leukocoria in the scientific literature. In the last two centuries, retinoblastoma had different names. “Fungus haematodes” was the term used in the early 1800s. This term was used to describe a fungating vascular tumor that affected different parts of the body including the globe. Before the era of ophthalmoscopy, no one thought the tumor originated from the globe except
James Wardrop who had a different opinion. He believed “fungus haematodes” of the globe originated from the retina and should be recognized as a distinct entity [3]. Wardrop, who was a Scottish surgeon, reached this conclusion based on his dissections. However, at that time, Wardrop’s explanation and observation were not acknowledged. Wardrop was also the first surgeon to perform enucleation for retinoblastoma [3]. In 1854, Virchow suggested the term “glioma of the retina” [4]. Flexner and later Wintersteiner noticed tumor rosettes on histopathology which resembled the photoreceptors of the retina [5, 6]. Flexner proposed the name neuroepithelioma, and both thought that the photoreceptors were the origin of the tumor [5]. Finally, in 1922, Verhoeff coined the term retinoblastoma after he noticed the histologic similitude between the disease and embryonic retina [7]. Four years later, the American Ophthalmological Society decided to adopt the term retinoblastoma. In 1970, Tso described the appearance of fleurettes which represent advanced photoreceptor differentiation [8].

1.2 Retinoblastoma variants

Hirschberg categorized the disease per its growth into endophytum and exophytum [9]. In 1960, Schofield described few cases who had presented with hypopyon and no macroscopic evidence of retinoblastoma [10]. After enucleation, histological exam showed malignant cells and rosettes were found within the retina. He described the cases as having “diffuse infiltrating retinoblastoma” [10]. He gave the credit to Ashton who first used the term in 1958 after personal communication with him. Schofield stressed on including retinoblastoma as part of the differential diagnosis of hypopyon. In 1998, Grossniklaus was the first to use the name “anterior variant of diffuse retinoblastoma” [11]. He reported a case that was misdiagnosed as anterior uveitis. After the eye was enucleated, the tumor was found to be in the peripheral retina, iris, ciliary body, and anterior vitreous. In 1982, Bader et al. described three cases of trilateral retinoblastoma [12]. He found out retinoblastoma-like tumors in the suprasellar or parasellar region few months before the detection of intraocular retinoblastoma in each of the three cases. He concluded the association was more than a coincidence and having retinoblastoma gene may confer the risk of having other “ectopic” malignancies.

1.3 The history of genetics

In the 1800s, retinoblastoma was thought to be an autosomal dominant hereditary disease. Alfred Knudson, a cancer geneticist, had observed that patients with hereditary retinoblastoma developed multiple tumors in both eyes, while patients with the non-hereditary form had unilateral tumors. In 1971, Knudson proposed the two-hit hypothesis. It states that a second sporadic mutation should occur in patients with hereditary retinoblastoma before the development of retinoblastoma [13]. The other type described by Knudson was non-hereditary retinoblastoma. In non-hereditary retinoblastoma, two sporadic mutations in both alleles of retinal cells should take place. In 1986, RB gene was the first tumor suppressor gene to be identified in medical history.

1.4 The treatment history

Wardrop advocated enucleation in 1809 as the only treatment [3]. However, Wardrop’s patients failed to survive despite enucleation. Von Graefe suggested the optic nerve should be excised during enucleation. This resulted in better survival rates. One of the major turn points in the history of retinoblastoma was
introduction of ophthalmoscope, which allowed earlier detection of retinoblas-
toma. Retinoblastoma was a fatal disease, so the primary was aim to save life of
affected patients. The first radiation treatment for retinoblastoma was reported, in
1903, by Hilgartner in Texas [14]. Hilgartner reported the survival of the patient
but not the eye following a series of X-rays. Verhoeff, in 1921, used radiotherapy to
cure retinoblastoma [15]. However, 60 years following radiation therapy, Verhoeff’s
patient developed lid basal cell carcinoma and recurrence of retinoblastoma [16].
In 1930, Moore used surgically inserted radioactive radon seed to cure retinoblas-
toma [17]. Weve introduced the use of diathermy for treating retinoblastoma [18].
However, it resulted in major complications including large chorioretinal scarring
and scleral thinning. Then, Stahlard established plaque brachytherapy [19]. External
beam radiotherapy (EBRT) was used and resulted in improvement in eye survival
rates [20]. However, EBRT cause second cancers in patients with germline muta-
tions by causing the second hit described by Knudson [21, 22].

Schwickerath used xenon arc photocoagulation to treat retinoblastoma [23].
Harvey Lincoff developed cryotherapy for the treatment of small retinoblastoma
tumors [24]. In 1953, Kupfer was the first to use chemotherapy, nitrogen mustard,
along with radiotherapy for retinoblastoma [25]. To decrease systemic side effects
of chemotherapy, the idea of local chemotherapy came up. Kaneko used intra-
arterial chemotherapy for retinoblastoma, which resulted in substantially higher
drug concentration in the eye [26, 27]. Retinoblastoma mortality rate decreased
substantially from 100 to 2% in the last 50 years [25]. Indeed, retinoblastoma is a
story of success and there is still more to come.

2. Genetics of retinoblastoma

2.1 Introduction

Retinoblastoma (RB) is the most common intraocular malignancy in children
affecting 1 in 18,000 live births which occurs as a result of biallelic inactivation of
RB1 gene [28]. Hereditary RB is due to heterozygous germline mutation in one copy
of the RB1 gene, hence is inherited as an autosomal dominant trait. In this form, all
body cells have a dysfunctional RB1 allele and, thus, are vulnerable to neoplasia.
The non-hereditary form of RB is consequent to somatic mutations, which is known
to affect both RB1 alleles in retinal cells [29].

In these patients, RB development requires a second, somatic, mutation in the
same cells that renders the other allele nonfunctional. The cumulative incidence
rates of non-ocular tumors reach up to 90% at 30 years in patients who were
exposed to radiation vs. 68% at 32 years in patients without radiation exposure [30].

The aim of this chapter is to provide a valuable summary of retinoblastoma
genetics that is essential for genetic counseling and estimation of short-term (mul-
tifocal and bilateral ocular tumors) and long-term (secondary tumors) risks with an
overall improvement of healthcare planning and management of our patients.

2.2 The RB1 gene and protein function

The RB1 gene located on the long arm of chromosome 13 (13q14) is a negative
regulator element in the cell cycle process and was the first tumor suppressor gene
identified [31]. This gene codes for the RB protein which has multiple cellular func-
tions; it prevents the dividing cells from uncontrollable cycles in the mitosis stage
and has a role in genomic stability, apoptosis, and differentiation [32]. Inactivation
of the RB protein is usually caused by deletions and nonsense mutations [33].
2.3 Inheritance of retinoblastoma

Retinoblastoma is an autosomal dominant inherited disease. Thus, there is a 50% risk of inheriting a germline mutation for each child born to a patient with a germline RB1 mutation. However, 90% of the children with a germline RB1 mutation will develop retinoblastoma with an overall risk of having a child with hereditary retinoblastoma of 45%. The remaining 10% of children with the mutated RB1 gene will be unaffected carriers [34].

More than 900 mutations have been identified in the RB1 gene. Different types of mutations have been identified in RB1 gene; deletions and gene rearrangements represent majority of mutations (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=RB1). Additionally, de novo mutation is considered to be present in most children with heritable retinoblastoma, as a positive family history is elicited in only 10% of all affected children, which is then transmissible in subsequent generations. In these children, in whom a germline mutation is present with a negative family history, 30% have bilateral disease and 60% develop unilateral RB [35]. A germline RB1 mutation is present in 15% of children diagnosed with a unilateral RB [34].

The “2-hit” hypothesis, which was first proposed by Knudson, described two complementary mutations that are essential for the development of hereditary and non-hereditary forms of retinoblastoma. The first “hit,” or mutation, in heritable retinoblastoma is a germline mutation affecting all body cells. The second mutation is a somatic mutation occurring in many retinoblasts with subsequent multifocal or bilateral lesion. On the other hand, the first and second mutations in non-heritable retinoblastoma occur somatically in a single retinoblast presenting as a unilateral and unifocal retinoblastoma [36].

2.4 Epigenetics of RB

Epigenetics is the study of heritable changes occurring in gene activity and expression of a specific phenotype. These changes do not cause alterations in the DNA sequence, external and/or environmental factors might affect cellular and physiological phenotypic traits [37].

RB1 gene has been linked to the regulation of numerous epigenetic processes. These processes include DNA methylation, histone modification, and microRNA regulation [38–43]. In addition, dysfunction of RB1 gene causes deregulations in many tumor suppressor pathways. Tumorigenesis requires this epigenetic deregulation against which new therapeutic options can be invented. Retinoblastoma was the first tumor discovered to be showing the actions of epigenetics on the pathogenesis of cancer [44].

2.4.1 MicroRNAs in RB

MicroRNAs are small, conserved, single-stranded, and non-coding RNA that comprise 1–5% of the human genome and are involved in regulating at least 30% of protein-coding genes [45–49]. MicroRNAs play an essential role in the regulation of gene expression governing various cellular and metabolic pathways [50–56]. MicroRNAs’ deregulation has been linked to the development of RB and other human diseases [57–59]. Thus, microRNA studies on RB have offered novel understandings of the disease mechanisms. Messenger RNA (mRNA), a large family of RNA molecules that convey genetic information from DNA to ribosome, was also studied in cases of RB. A three-fold increase was noted in mRNA levels of ACVR1C/ALK7 in retinoblastomas invading the optic nerve. This suggests that ACVR1C/SMAD2 pathway has a function in promoting invasion and growth of RB [60].
2.4.2 DNA methylation

DNA methylation involves the addition of methyl groups to the DNA molecule. This process can change the DNA segment activity without altering its sequence. When this segment is located in a gene promoter, DNA methylation usually acts to block gene transcription. The role for promoter methylation in retinoblastoma development was discovered when there was methylation of a CpG island (CpG 106) that overlapped the RB1 promoter [61]. This has resulted in a decreased gene expression confirming the epigenetic factor in retinoblastoma tumorigenesis [44, 62–64].

Methylation of DNA segment was also reported in tumor suppressor genes beyond RB1. These genes include RASSF1A (RAS-associated domain family 1A) that was methylated in 59% of tumors analyzed and adenomatous polyposis coli (APC) in 6% [65]. Furthermore, hypermethylation of O6-methylguanine-DNA methyltransferase (MGMT) was detected in 15% of RB tumors. This was associated with advanced-stage RB suggesting that the presence of methylated MGMT is a poor prognostic indicator [66].

2.5 Features of heritable RB

2.5.1 13q deletion syndrome

Children with this syndrome may present with characteristic dysmorphic features, developmental delay, and intellectual disability. Interstitial chromosome deletion or translocation of region 13q14 was found in approximately 6% of patients with RB [67]. The larger the size of chromosomal deletion, the more severe the associations. Dysmorphic facial features include high and broad forehead, short nose, prominent philtrum, and a thick everted lower lip [67]. Karyotype or chromosomal microarray is usually performed to detect chromosomal deletions, translocations, and copy number alteration [68].

2.5.2 Trilateral RB

Trilateral retinoblastoma indicates the concomitant presence of a heritable retinoblastoma and a midline tumor or a pineoblastoma [12, 69]. Around 5–13% of patients with RB develop trilateral retinoblastoma [70]. Therefore, in children with heritable retinoblastoma, a brain magnetic resonance imaging with gadolinium contrast every 6 months is recommended until the age of 5 years [71].

2.5.3 Secondary malignant neoplasms

As patients with heritable RB age, the risk of non-ocular malignancies significantly increases. These tumors include osteosarcoma, soft tissue sarcoma, epithelial cancers, and melanoma. It has been suggested that a greater risk for second cancers occurs in patients with familial RB compared with those with a de novo RB1 mutation [72]. External beam radiation therapy for patients with heritable disease has a further increased risk of developing second malignancies.

2.5.4 Low-penetrant retinoblastoma

In a typical “null” germline mutation, there is a 90% chance that patient will develop RB. In a few families, however, the penetrance is far less than 90% with subsequent reduced expressivity and an increased proportion of unilateral RB. Some patients will be carriers with no tumors [73]. These low-penetrant
mutations are usually missense mutations. Moreover, a low level of RB protein production due to mutations in the promoter region can occur without total absence of the protein [33].

2.6 Genetic testing

2.6.1 Clinical context

Heritability of retinoblastoma is confirmed if a proband with retinoblastoma has a family history of retinoblastoma. In the absence of family history of RB, genetic testing is required to identify heterozygous germline RB1 pathogenic variants. This will allow early diagnosis and identification of potential carriers of the heritable RB1 mutations which eventually will improve disease management and family counseling.

The following staging system has been proposed to facilitate description of one’s genetic risk of possessing germline RB1 pathogenic variant: [74, 75]

| HX                      | Unknown or insufficient evidence of a constitutional (germline) RB1 pathogenic variant |
|-------------------------|----------------------------------------------------------------------------------------|
| H0                      | Normal RB1 alleles in blood tested with demonstrated high-sensitivity assays           |
| H0*                     | Normal RB1 in blood with <1% residual risk for mosaicism                                |
| H1                      | Bilateral retinoblastoma, trilateral retinoblastoma (retinoblastoma with intracranial CNS midline embryonic tumor), family history of retinoblastoma, or RB1 pathogenic variant identified in blood |

2.6.2 Single-gene testing

For bilateral, unilateral familial, and unilateral multifocal retinoblastoma, peripheral blood DNA should be tested initially using sequence analysis and gene-targeted deletion/duplication analysis of RB1. If blood testing did not reveal any mutation, molecular analysis of tumor DNA (if either eye was enucleated) should subsequently be done. Mosaicism can be assumed if germline mutation was not found in this group of patients [76].

For unilateral unifocal non-familial retinoblastoma, the chance of carrying RB1 germline mutation is 15%. And, with the high sensitivity of RB1 mutation detection techniques which reach up to (95%), testing blood from unilateral patients, extracted at the time of the first examination under anesthesia may confirm the diagnosis of germline RB1 mutation. If blood DNA testing did not reveal any mutation, molecular analysis of tumor DNA (if the eye was enucleated) should subsequently be done. If pathogenic variants were detected in the tissue, the blood DNA should be re-tested looking for these specific variants or any large rearrangements within the RB1 gene. If tumor tissue was unavailable, a negative blood result will reduce the chance of possessing a mutation to less than 1%. With such very low risk of RB development, the proband needs no additional exam under anesthesia and frequent examination in the clinic is sufficient. The same conclusion also applies to the offspring.

It has been previously reported by Rushlow et al. that retinoblastoma is not exclusively caused by mutations in RB1 gene; mutations in MYCN oncogene has been predicted to be responsible for 18% of cases diagnosed with non-familial unilateral RB in children less than 6 months of age. They reported group of patients who harbored mutations in MYCN amplification and no RB1 mutations. An additional 1.5% of patients with unilateral non-familial RB were found to have normal RB1 and MYCN genes [77].
2.6.3 Chromosomal microarray (CMA)

Chromosomal microarray analysis (CMA) is a technology used for the detection of clinically significant microdeletions or duplications and genetic rearrangement (including RB1), with a high sensitivity for submicroscopic aberrations [78]. It can be utilized in retinoblastoma patients with dysmorphic features, developmental delay, and/or other congenital anomalies [79, 80].

2.7 Genetic counseling

Genetic testing and counseling are essential parts of RB disease management; they help delineate heritable RB for non-heritable ones. The increased knowledge of molecular basis of RB allowed for better understanding and management of the disease. Genetic evaluation and counseling should be done in collaboration with a geneticist and genetic counselor [81]. The aim of the geneticist is to implement the proper test to detect the underlying disease causing mutation and communicate with the genetic counselor who will educate patients and their families about their condition in general, discuss anticipated risk of developing subsequent tumors, and construct the appropriate screening plan for the affected individual and their relatives.

At first encounter of a patient and family of retinoblastoma, detailed family history should be obtained and family pedigree should be drawn with special attention to relatives at risk. Then, genetic testing is offered after discussion about purpose, possible outcome, and limitations. Specimens are then collected and sent to a specialized laboratory for analysis. Another counseling session is arranged to convey and interpret the reported results. Accordingly, additional testing and counseling should be offered to the relatives at risk.

2.7.1 Prenatal screening

In the presence of family history of retinoblastoma and when a specific RB1 mutation is detected, a pregnancy at risk can be tested by chorionic villus sampling or amniocentesis. Prenatal testing and preimplantation genetic diagnosis are indicated as approximately 30% of newborns with an RB1 mutation will harbor a vision-threatening tumor [82, 83].

Amniocentesis can be used to screen the fetus for carrying RB1 gene using the above-mentioned methods. If an RB1 pathogenic variant was identified, then fetal ultrasonography may be used to identify intraocular tumor as small as 2–3 mm in size [84]. If tumors are present, preterm delivery to allow early treatment may be indicated. Alternatively, early term delivery (i.e., 36–38 weeks gestation) can be induced even if no intraocular tumor can be detected as this was found to improve the visual outcome and reduce the need for invasive tests and therapies in the postnatal period [83, 85].

Prenatal testing and management may raise several ethical issues especially if it involved termination of pregnancy. A generally accepted approach is to discuss all options with the parents and leave them to decide on further steps.

2.7.2 Genetic screening and counseling after birth

Genetic testing should be performed carefully especially when the propand’s blood is found negative for pathogenic mutations. It remains possible that large genetic rearrangement is being missed or they are mosaicism for RB1 mutation.

Carriers of RB1 pathogenic variants should be frequently examined for development of new tumors whether under anesthesia or in the outpatient setting until they
reach 9 years of age and the frequency of follow-ups should be reduced thereafter. For relatives who do not carry the mutation, no additional monitoring is required [86]. However, proband’s offspring will always be at risk of developing RB and be tested for the RB1 pathogenic mutation identified and proper counseling and clinical monitoring should be implemented. Additionally, all retinoblastoma survivors should have life-long surveillance for other lethal secondary tumors [21, 87, 88].

Conflict of interest

We do not have any financial interest to declare.

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References

[1] Pawius P. Observatio XXIII. Tumor oculorum. In: Observationes Anatomicae Selectiores Appended to: Bartholinus T. Historiarum Anatomicarum Rarioorum, Centuria III & IV. Copenhagen: Denmark Petrus Morsing; 1657

[2] Hayes R. The Case of a diseased eye communicated to Mr. William Hunter by Mr. Hayes, Surgeon. In: Medical Observations and Inquiries. By a Society of Physicians in London. Vol. 3. London: William Johnston; 1767. p. 120

[3] Wardrop J. Observations on the Fungus Haematodes or Soft Cancer. Edinburgh: George Ramsay and Co; 2013. p. 1809

[4] Virchow R. Die Krankhaften Geschwulste. Vol. Vol. 2. Berlin: August Hirschwald; 1864. pp. 151-169

[5] Flexner S. A peculiar glioma (neuroepithelioma?) of the retina. Bulletin of the Johns Hopkins Hospital. 1891;2:115-119

[6] Wintersteiner H. Das Neuroepithelioma Retinae. Eine Anatomische Und Klinische Studie. Vienna: Franz Deuticke; 1897

[7] Verhoeff FH, Jackson E. Minutes of the proceedings, 62nd annual meeting. Transactions of the American Ophthalmological Society. 1926;24:38-43

[8] Tso MOM, Zimmerman LE, Fine BS. The nature of retinoblastoma: I. photoreceptor differentiation. A clinical and histopathologic study. American Journal of Ophthalmology. 1970;69:339-349

[9] Hirschberg J. Die Markschwann die Natzhaut. Berlin: Hirschwald; 1869

[10] Schofield PB. Diffuse infiltrating retinoblastoma. The British Journal of Ophthalmology. 1960;44:35-41

[11] Grossniklaus HE, Dhaliwal RS, Martin DF. Diffuse anterior retinoblastoma. Retina. 1998;18(3):238-241

[12] Bader JL, Meadows AT, Zimmerman LE, Rorke LB, Voute PA, Champion LA, et al. Bilateral retinoblastoma with ectopic intracranial retinoblastoma: Trilateral retinoblastoma. Cancer Genetics and Cytogenetics. 1982;5(3):203-213

[13] Knudson AG Jr. Mutation and cancer: A statistical study of retinoblastoma. Proceedings of the National Academy of Sciences of the United States of America. 1971;68:820-823

[14] Hilgartner HL. Report of a case of double glioma treated by x-ray. Texas Med Journal. 1903;18:322-332

[15] Verhoeff FH. Retinoblastoma successfully treated with x-rays: Normal vision retained after thirty-four years. Second report on a case. Archives of Ophthalmology. 1952;48:720-722

[16] Albert DM, McGhee CNJ, Seddon JM, Weichselbaum RR. Development of additional primary tumors after 62 years in the first patient with retinoblastoma cured by radiation therapy. American Journal of Ophthalmology. 1984;97:189-196

[17] Moore RF, Stallard HB, Milner JG. Retinal gliomata treated by radon seeds. The British Journal of Ophthalmology. 1931;15:673-696

[18] Weve HJM. On diathermy in ophthalmic practice. Transactions of the Ophthalmological Societies of the United Kingdom. 1939;15:43-80

[19] Stallard HB. The treatment of retinoblastoma. Ophthalmologica. 1966;151:214-230
[20] Reese AB, Ellsworth RM. The evaluation and current concept of retinoblastoma therapy. Transactions - American Academy of Ophthalmology and Otolaryngology. 1963;67:164-172

[21] Fletcher O, Easton D, Anderson K, Gilham C, Jay M, Peto J. Lifetime risks of common cancers among retinoblastoma survivors. Journal of the National Cancer Institute. 2004;96:357-363

[22] Kleinerman RA, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, et al. Risk of new cancers after radiotherapy in long-term survivors of retinoblastoma: An extended follow-up. Journal of Clinical Oncology. 2005;23:2272-2279

[23] Meyer-Schwickerath G. Lichtkoagulation. Albrecht von Graefe's Archiv für Ophthalmologie. 1954;156:2-34

[24] Lincoff H, McLean J, Long R. The cryosurgical treatment of intraocular tumors. American Journal of Ophthalmology. 1967;63:389-399

[25] MacCarthy A, Birch JM, Draper GJ, Hungerford JL, Kingston JE, Kroll ME, et al. Retinoblastoma: Treatment and survival in Great Britain 1963 to 2002. The British Journal of Ophthalmology. 2009;93:38-39

[26] Yamane T, Kaneko A, Mohri M. The technique of ophthalmic arterial infusion therapy for patients with intraocular retinoblastoma. International Journal of Clinical Oncology. 2004;9:69-73

[27] Taich P, Requejo F, Asprea M, Sgroi M, Gobin P, Abramson DH, et al. Topotecan delivery to the optic nerve after ophthalmic artery chemosurgery. PLoS Ōne. 2016;11:e0151343

[28] Kivela T. The epidemiological challenge of the most frequent eye cancer: Retinoblastoma, an issue of birth and death. The British Journal of Ophthalmology. 2009;93:1129-1131

[29] Lee V, Hungerford JL, Bunce C, Ahmed F, Kingston JE, Plowman PN. Globe conserving treatment of the only eye in bilateral retinoblastoma. The British Journal of Ophthalmology. 2003;87:1374-1380

[30] Abramson DH, Ellsworth RM, Kitchin FD, Tung G. Second nonocular tumors in retinoblastoma survivors. Are they radiation-induced? Ophthalmology. 1984;91:1351-1355

[31] Hong FD, Huang HJ, To H, et al. Structure of the human retinoblastoma gene. Proceedings of the National Academy of Sciences of the United States of America. 1989;86:5502-5506

[32] Korenjak M, Anderssen E, Ramaswamy S, et al. RBF binding to both canonical E2F targets and noncanonical targets depends on functional dE2F/dDP complexes. Molecular and Cellular Biology. 2012;32:4375-4387

[33] Valverde JR, Alonso J, Palacios I, et al. RB1 gene mutation up-date, a meta-analysis based on 932 reported mutations available in a searchable database. BMC Genetics. 2005;6:53

[34] Gallie B, Erraguntla V, Heon E, et al. Retinoblastoma. In: Taylor D, Hoyt C, editors. Pediatric Ophthalmology and Strabismus. 3rd ed. London: Elsevier; 2004. pp. 486-504

[35] Draper GJ, Sanders BM, Brownbill PA, et al. Patterns of risk of hereditary retinoblastoma and applications to genetic counselling. British Journal of Cancer. 1992;66:211-219

[36] Knudson AG Jr. Mutation and cancer: Statistical study of retinoblastoma. Proceedings of the National Academy of Sciences of the United States of America. 1971;68:820-823
[37] Dupont C, Armant DR, Brenner CA. Epigenetics: Definition, mechanisms and clinical perspective. Seminars in Reproductive Medicine. 2009;27(5):351-357

[38] Chi P, Allis CD, Wang GG. Covalent histone modifications—Miswritten, misinterpreted and mis-erased in human cancers. Nature Reviews. Cancer. 2010;10:457-469

[39] Lu J, Ruhf M-L, Perrimon N, Leder P. A genome wideRNA interference screen identifies putative chromatin regulators essential for E2F repression. Proceedings of the National Academy of Sciences of the United States of America. 2007;104:9381-9386

[40] Benetti R, Gonzalo S, Jaco I, Muñoz P, Gonzalez S, et al. A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. Nature Structural & Molecular Biology. 2008;15:998

[41] Wen H, Andrejka L, Ashton J, Karess R, Lipsick JS. Epigenetic regulation of gene expression by Drosophila Myb and E2F2-RBF via the Myb-MuvB/dREAM complex. Genes & Development. 2008;22:601-614

[42] Bourgo RJ, Siddiqui H, Fox S, Solomon D, Sansam CG, et al. SWI/SNF deficiency results in aberrant chromatin organization, mitotic failure, and diminished proliferative capacity. Molecular Biology of the Cell. 2009;20:3192-3199

[43] Gonzalo S, Blasco MA. Role of Rb family in the epigenetic definition of chromatin. Cell Cycle. 2005;4:752-755

[44] Zhang J, Benavente CA, McEvoy J, Flores-Otero J, Ding L, et al. A novel retinoblastoma therapy from genomic and epigenetic analyses. Nature. 2012;481:329-334

[45] Rajewsky N. L(ou)sy miRNA targets? Nature Structural & Molecular Biology. 2006;13:754-755

[46] Rajewsky N. microRNA target predictions in animals. Nature Genetics. 2006;38(Suppl):S8-S13

[47] Liu W, Mao SY, Zhu WY. Impact of tiny miRNAs on cancers. World Journal of Gastroenterology. 2007;13:497-502

[48] Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RH, Cuppen E. Phylogenetic shadowing and computational identification of human microRNA genes. Cell. 2005;120:21-24

[49] Stanczyk J, Pedrioli DM, Brentano F, Sanchez-Pernaute O, Kolling C, Gay RE, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis and Rheumatism. 2008;58:1001-1009

[50] Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metabolism. 2006;3:87-98

[51] Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with ‘antagomirs’. Nature. 2005;438:685-689

[52] Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature. 2005;436:214-220

[53] Naguibneva I, Ameyar-Zazoua M, Polesskaya A, Ait-Si-Ali S, Groisman R, Souidi M, et al. The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. Nature Cell Biology. 2006;8:278-284

[54] Garzon R, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R, et al. MicroRNA fingerprints during human
mega-karyocytopoiesis. Proceedings of the National Academy of Sciences of the United States of America. 2006;103:5078-5083

[55] Monticelli S, Ansel KM, Xiao C, Socci ND, Krichevsky AM, Thai TH, et al. MicroRNA profiling of the murine hematopoietic system. Genome Biology. 2005;6:R71

[56] Ambros V. The functions of animal microRNAs. Nature. 2004;431:350-355

[57] Li BQ, Zhang J, Huang T, Zhang L, Cai YD. Identification of retinoblastoma related genes with shortest path in a protein-protein interaction network. Biochimie. 2012;94:1910-1917

[58] Dalgard CL, Gonzalez M, deNiro JE, O’Brien JM. Differential microRNA-34a expression and tumor suppressor function in retinoblastoma cells. Investigative Ophthalmology and Visual Science. 2009;50:4542-4551

[59] Jo DH, Kim JH, Park WY, Kim KW, Yu YS. Differential profiles of microRNAs in retinoblastoma cell lines of different proliferation and adherence patterns. Journal of Pediatric Hematology/Oncology. 2011;33:529-533

[60] Asnaghi L, White DT, Key N, Choi J, Mahale A, Alkatan H, et al. ACVR1C/SMAD2 signaling promotes invasion and growth in retinoblastoma. Oncogene. 2019;38(12):2056-2075

[61] Greger V, Passarge E, Höpping W, Messmer E, Horsthemke B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Human Genetics. 1989;83:155-158

[62] Ohtani-Fujita N, Dryja TP, Rapaport JM, Fujita T, Matsumura S, et al. Hypermethylation in the retinoblastoma gene is associated with unilateral, sporadic retinoblastoma. Cancer Genetics and Cytogenetics. 1997;98:43-49

[63] Richter S, Vandendaele K, Chen N, Zhang K, Sutherland J, et al. Sensitive and efficient detection of RB1 gene mutations enhances care for families with retinoblastoma. American Journal of Human Genetics. 2003;72:253-269

[64] Livide G, Epistolato MC, Amenduni M, Disciglio V, Marozza A, et al. Epigenetic and copy number variation analysis in retinoblastoma by MS-MLPA. Pathology Oncology Research. 2012;18:703-712

[65] Harada K, Toyooka S, Maitra A, Maruyama R, Toyooka KO, et al. Aberrantpromotermethylation and silencing of the RASSF1A gene in pediatric tumors and cell lines. Oncogene. 2002;21:4345-4349

[66] Choy KW, Lee TC, Cheung KF, Fan DS, Lo KW, et al. Clinical implications of promoter hypermethylation in RASSF1A and MGMT in retinoblastoma. Neoplasia. 2005;7:200-206

[67] Bojinova RI, Schorderet DF, Addor MC, et al. Further delineation of the facial 13q14 deletion syndrome in 13 retinoblastoma patients. Ophthalmic Genetics. 2001;22:11-18

[68] Baud O, Cormier-Daire V, Lyonnet S, et al. Dysmorphic phenotype and neurological impairment in 22 retinoblastoma patients with constitutional cytogenetic 13q deletion. Clinical Genetics. 1999;55:478-482

[69] Jurkiewicz E, Pakula-Kosciesza I, Rutynowska O, et al. Trilateral retinoblastoma: An institutional experience and review of the literature. Child’s Nervous System. 2010;26:129-132

[70] Kivela T. Trilateral retinoblastoma: A meta-analysis of hereditary
retinoblastoma associated with primary ectopic intracranial retinoblastoma. Journal of Clinical Oncology. 1999;17:1829-1837

[71] Singh AD, Shields CL, Shields JA. New insights into trilateral retinoblastoma. Cancer. 1999;86:3-5

[72] Kleinerman RA, Yu CL, Little MP, et al. Variation of second cancer risk by family history of retinoblastoma among long-term survivors. Journal of Clinical Oncology. 2012;30:950-957

[73] Ahmad NN, Melo MB, Singh AD, Donoso LA, Shields JA. A possible hot spot in exon 21 of the retinoblastoma gene predisposing to a low penetrant retinoblastoma phenotype? Ophthalmic Genetics. 1999;20:225-231

[74] Mallipatna A, Gallie BL, Chévez-Barrios P, Lumbroso-Le Rouic L, Chantada GL, Doz F, et al. Retinoblastoma. In: Amin MB, Edge SB, Greene FL, editors. AJCC Cancer Staging Manual. 8th ed. New York, NY: Springer; 2017. pp. 819-831

[75] Soliman SE, Racher H, Zhang C, MacDonald H, Gallie BL. Genetics and molecular diagnostics in retinoblastoma—an update. Asia-Pacific Journal of Ophthalmology. 2017a;6:197-207

[76] Sippel KC, Fraioli RE, Smith GD, Schalkoff ME, Sutherland J, Gallie BL, et al. Frequency of somatic and germ-line mosaicism in retinoblastoma: Implications for genetic counseling. American Journal of Human Genetics. 1998;62(3):610-619

[77] Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Thériault BL, et al. Characterisation of retinoblastomas without RB1 mutations: Genomic, gene expression, and clinical studies. The Lancet Oncology. 2013;14:327-334

[78] Batzir NA, Shohat M, Maya I. Chromosomal microarray analysis (CMA) a clinical diagnostic tool in the prenatal and postnatal settings. Pediatric Endocrinology Reviews. 2015 Sep;13(1):448-454

[79] Mitter D, Ullmann R, Muradyan A, Klein-Hitpass L, Kanber D, Ounap K, et al. Genotype-phenotype correlations in patients with retinoblastoma and interstitial 13q deletions. European Journal of Human Genetics. 2011;19:947-958

[80] Castéra L, Dehainault C, Michaux D, Lumbroso-Le Rouic L, Aerts I, Doz F, et al. Fine mapping of whole RB1 gene deletions in retinoblastoma patients confirms PCDH8 as a candidate gene for psychomotor delay. European Journal of Human Genetics. 2013;21:460-464

[81] Dhar SU, Chintagumpala M, Noll C, Chévez-Barrios P, Paysse EA, Plon SE. Outcomes of integrating genetics in management of patients with retinoblastoma. Archives of Ophthalmology. 2011;129(11):1428-1434

[82] Canadian Retinoblastoma Society. National Retinoblastoma Strategy Canadian Guidelines for Care: Stratégie Thérapeutique du Rétinoblastome Guide Clinique Canadien [Internet]. 2009. Accessed: 2-20-19

[83] Soliman SE, Dimaras H, Khetan V, Gardiner JA, Chan HS, Héon E, et al. Prenatal versus postnatal screening for familial retinoblastoma. Ophthalmology. 2016;123:2610-2617

[84] Paquette LB, Miller D, Jackson HA, Lee T, Randolph L, Murphree AL, et al. In utero detection of retinoblastoma with fetal magnetic resonance and ultrasound: Initial experience. AJR Reports. 2012;2(1):55-62. DOI: 10.1055/s-0032-1316465

[85] Soliman S, Kletke S, Roelofs K, VandenHoven C, McKeen L, Gallie B. Precision laser therapy for
retinoblastoma. Expert Review of Ophthalmology. 2018;13:149-159

[86] Valenzuela AA, Chan HSL, Gallie BL. A language for retinoblastoma: Guidelines through standard operating procedures. In: Reynolds JD, editor. Pediatric Retina. New York: Spinger; 2011. pp. 205-234

[87] Temming P, Arendt M, Viehmann A, et al. How eye-preserving therapy affects long-term overall survival in heritable retinoblastoma survivors. Journal of Clinical Oncology. 2016;34:3183-3188

[88] Abramson DH, Mendelsohn ME, Servodidio CA, Tretter T, Gombos DS. Familial retinoblastoma: Where and when? Acta Ophthalmologica Scandinavica. 1998;76(3):334-338