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Retinoblastoma. Fifty Years of Progress. The LXXI Edward Jackson Memorial Lecture

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Abstract

Purpose—To review the progress made in understanding the genetic basis, molecular pathology, and treatment of retinoblastoma since the previous Jackson lecture on the topic was published 50 years ago.

Design—Perspective based on personal experience and the literature.

Methods—The literature regarding retinoblastoma was reviewed since 1963. Advances in understanding the biology and treatment of retinoblastoma provided context through the author's clinical, pathological and research experiences.

Results—Retinoblastoma was first identified in the 1500s and defined as a unique clinicopathologic entity in 1809. Until the mid-1900s, knowledge advanced sporadically, with technological developments of ophthalmoscopy and light microscopy, and with the introduction of surgical enucleation, chemotherapy and radiation therapy. During the last 50 years, research and treatment have progressed at an unprecedented rate due to innovations in molecular biology and the development of targeted therapies. During this time period, the retinoblastoma gene was discovered; techniques for genetic testing for retinoblastoma were developed; and plaque brachytherapy, chemoreduction, intraarterial chemotherapy, and intraocular injections of chemotherapeutic agents were successfully introduced.

Conclusions—Nearly all patients with retinoblastoma in developed countries can now be cured of their primary cancer- a remarkable achievement for a childhood cancer that once was uniformly fatal. Much of this success is owed to deciphering the role of the Rb gene, and the benefits of targeted therapies, such as chemoreduction with consolidation as well as intra-arterial and intravitreal chemotherapies. Going forward, the main challenge will be ensuring that access to care is available for all children, particularly those in developing countries.

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Introduction

It is an honor to deliver the LXXI Edward Jackson Memorial Lecture. Although Dr. Jackson is probably best known for the Jackson cross cylinder, he profoundly influenced the entire course of modern ophthalmology. Doctor Jackson was a major initiator of the predecessor society of the American Academy of Ophthalmology, founder of the American Board of Ophthalmology and the founding editor of the American Journal of Ophthalmology.¹ In addition to being an educator and clinician, Dr. Jackson was known for his grace and kindness¹, qualities that serve as an example for today's ophthalmologists.

When Dunphy delivered the XX Jackson Memorial Lecture in 1963², he called his lecture "The Story of Retinoblastoma". In this lecture, he recounted the history of retinoblastoma, from the 1500s to the mid 1960s. Many advances have been made in the fifty years since then; for instance, the Knudson two-hit hypothesis, cloning the retinoblastoma gene, chemoreduction therapy, and intra-arterial chemotherapy, to list just a few. Dunphy divided the history of retinoblastoma into four general periods: the prehistologic, histologic, enucleation, and irradiation/chemotherapy periods.² The retinoblastoma story revolved around numerous personalities, including ophthalmologists, pathologists and researchers. It is time for an update of that story. I propose that there have been three additional periods since Dunphy's lecture: 1) the period of molecular biology; 2) the period of targeted therapy; 3) and the period of global health awareness. As in Dunphy's time, the current era is made possible because of the contributions of ophthalmologists, pathologists and researchers. Although there have been tremendous advances in understanding the biology and treatment of retinoblastoma, a major challenge persists today: that of ensuring access to care in many parts of the world.

History

In addition to Dunphy's lecture², Albert has provided an historic review of retinoblastoma.³ Summarizing briefly, Pawius of Amsterdam is credited as the first to recognize retinoblastoma in an autopsy report of a young child published in 1597.⁴ Wardrop of Edinburgh established retinoblastoma as a distinct entity in 1809 and advocated enucleation as preferred treatment.⁵ Steven at the New York Hospital is believed to have reported the first case in the American literature in 1818.⁶ In those days, retinoblastoma was known as *fungus haematodes*, a term coined by Hey of Leeds, England.⁵ In 1854, the famous German pathologist, Virchow, introduced the term *glioma of the retina*.⁷ Hirshberg later divided these gliomas into the *exophytum* and *endophytum* types⁸, known today as *exophytic* and *endophytic* growth patterns. In 1891, Flexner of Johns Hopkins described the histologic finding of cellular rosettes in the tumor⁹; in 1897, Wintersteiner of Vienna, who was apparently unaware of Flexner's paper, described the structure's lumen, a component which now permits subclassification as the *Flexner-Wintersteiner* rosette.¹⁰ This clear-center rosette is due to a recapitulation of the external limiting membrane of the retina. The central portion of the *Homer Wright* rosette, which can be found in retinoblastoma as well, is filled with neuropil. This feature can be seen in medulloblastoma and neuroblastoma, thus it lacks the specificity that the *Flexner-Wintersteiner* rosette has for retinoblastoma.³ Robin and Langenbeck noted in the early 1800s that the tumor microscopically arose in the retina.^{11;12}

Histologic resemblance of the tumor to undifferentiated embryonic retina prompted the famous American ophthalmic pathologist, Verhoeff, to name the tumor *retinoblastoma*, a term that was adopted by the American Ophthalmological Society in 1926.¹³ Cases of spontaneously regressed retinoblastoma in phthisical eyes began to appear in the literature in the early 1900s.³ Gallie and colleagues described a clinically benign variant of retinoblastoma they called *retinoma* in 1982.¹⁴ The following year, Margo and colleagues at the Armed Forces Institute of Pathology reported the histologic features of this benign variant that they called *retinocytoma*.¹⁵ Trilateral retinoblastoma was described in the early 1980s.^{16;17} This entity includes bilateral retinoblastoma and a third primary intracranial tumor^{16;18}, which may be a primitive neuroectodermal tumor, pinealblastoma or ependymblastoma.¹⁹ Another unusual variant of retinoblastoma is anterior diffuse retinoblastoma.^{20;21} This tumor arises in the far peripheral retina as a small, inconspicuous intraretinal mass and seeds the anterior chamber via the aqueous causing a pseudohypopyon.

Clinical Features

Retinoblastoma, the most common intraocular malignancy in childhood, comprises 4% of all pediatric cancers.²² The incidence of retinoblastoma is approximately 1 in 20,000 live births²³ and there are about 200-300 new cases of retinoblastoma in the United States each year.²² In more populated countries, such as India and China, there are about 1,000 new cases of retinoblastoma annually.²² Leukocoria is the most common presenting sign (60%) followed by strabismus (20%).²³ Tumor growth patterns include *endophytic* (Figure 1), in which the tumor grows into the vitreous; *exophytic* (Figure 2), in which the tumor grows under the retina toward the choroid; *mixed* (both endophytic and exophytic); and *diffuse infiltrating* which forms a greyish plaque on the retinal surface.²³⁻²⁵ A particular variant of the diffuse growth pattern is *anterior diffuse* (Figure 3). As mentioned above, a small primary tumor arises in the peripheral retina in this variant then seeds the anterior chamber via the aqueous.²⁶

The clinical differential diagnosis of retinoblastoma includes Coats disease, persistent fetal vasculature, vitreous hemorrhage, ocular toxocariasis, familial exudative vitreoretinopathy, rhegmatogenous retinal detachment, coloboma, astrocytic hamartoma, combined hamartoma of the retina/retinal pigment epithelium and endogenous endophthalmitis²⁷ (Table 1). The benign variant of retinoblastoma, retinocytoma, may clinically appear as regressed retinoblastoma with associated calcification (Figure 4).

The Reese-Ellsworth Classification of clinical stage of the disease was published in 1963²⁸ and has now been largely supplanted by the International Classification (Table 2), which was developed by a team of retinoblastoma experts in Paris in 2003 including Murphree²⁹, Desjardins, Gallie, Shields, Shields, and others.. Retinoblastoma spreads by three pathways: anteriorly via the vitreous/subretinal space into the anterior segment; posteriorly via the optic nerve into the cranial cavity; externally via the choroid to the systemic circulation or through the sclera into the orbit.²³ Prognostic risk factors for dissemination include optic nerve and/or orbital invasion^{30;31} and massive choroidal invasion.³²⁻³⁴

Second primary tumors in hereditary retinoblastoma survivors represent a greater risk of death than the retinoblastoma itself.^{35;36} The cumulative risk for these tumors is roughly 1% per year, reaching 50% at 50 years.³⁶ Radiotherapy, especially to the site of retinoblastoma before age 1, greatly increases the chances of a second tumor.^{37;38} These tumors are mainly sarcomas, in particular, leiomyosarcoma³⁹ in the field of radiation and osteosarcoma outside of the field.

Pathology

Retinoblastoma is derived from cells of neuroectodermal origin from the inner layer of the optic cup. The tumor may grow toward the vitreous and/or subretinal space forming a multilobulated white mass with associated vitreous or subretinal seeds. In these cases, the tumor grows in sleeves and cuffs around central vessels from which it derives its nutrition. When the tumor outgrows its vascular supply 90-110µm away from the central vascular channel, it undergoes ischemic necrosis and may contain areas of dystrophic calcification, thus yielding a grossly visible chalky or cottage cheese appearance.^{40;41} The tumor may also grow diffusely within the retina and derive its nutrition from the retinal vasculature. Vitreous and subretinal seeds of retinoblastoma are avascular and contain areas of necrosis in the center of the seed away from the vitreous or subretinal fluid. Cytologically, retinoblastoma is made up of small, round, blue cells. Retinoblastoma may exhibit differentiation which becomes less evident with age.⁴²⁻⁴⁴ Signs of differentiation, going from least to most differentiated, include Homer Wright or Flexner-Wintersteiner rosettes and fleurettes (Figure 5).⁴⁰ Fleurettes are particularly abundant in retinocytoma, which also contain areas of calcification. The two most important histologic prognostic factors for distant metastases are optic nerve invasion beyond the lamina cribrosa and “massive” choroidal invasion measuring 3mm or greater in diameter in which the tumor comes in contact with the sclera.^{40;45} The approximate survival rates for children with retinoblastoma extending into the prelaminar, laminar, post-laminar optic nerve and surgical margin of resection are >90%, 85%, 60% and 35%, respectively.⁴⁶⁻⁴⁸ The overall survival rate when there is massive choroidal invasion is approximately 70%.⁴⁹

The International Retinoblastoma Staging Working Group (IRSWG) has provided guidelines for handling eyes enucleated for retinoblastoma, including preparation of the pupil-optic nerve section and breadloafing the calottes to evaluate for choroidal invasion.⁴⁵ It is estimated that approximately 20% of eyes enucleated with retinoblastoma in the United States will exhibit high risk features.^{50;51} Clinical predictors of high risk histologic features include older patient age, time from diagnosis to enucleation, hyphema, pseudohypopyon, staphyloma, orbital cellulitis and elevated intraocular pressure.^{52;53} Histologic examination of enucleated eyes after chemoreduction therapy for retinoblastoma may show Type 1 (cottage cheese), Type 2 (fish flesh), Type 3 (combination of Types 1 and 2) or Type 4 (complete) regression, or histologically intact, presumably viable tumor cells.⁵⁴⁻⁵⁶ The Type 2 regression pattern may result in retinocytoma-like clinical appearance; the tumor may have the same histological features. It is not surprising that regressed tumors have the same histological appearance of retinocytoma, since well differentiated tumors are presumably relatively resistant to chemotherapy as cells are not cycling.⁵⁶ Tumors treated by intraarterial chemotherapy (IAC) exhibit similar regression patterns.⁵⁷ Treatment failures are

due to persistence of vitreous seeds, subretinal seeds, intraretinal tumor or tumor that is unresponsive to chemotherapy.⁵⁸

Molecular Biology

The information in this section represents our current understanding of the molecular mechanisms and pathways involved in development, extraocular extension and metastasis of retinoblastoma based on evidence available at this time; some of this information will likely prove to be incorrect or an oversimplification of these mechanisms. The understanding of these mechanisms will change as experimental investigations will further clarify these molecular pathways in the future.

Genetics and Molecular Mechanisms

The most far reaching advances in retinoblastoma since Dunphy's lecture² have been in understanding the genetics and molecular origins of the tumor and mechanisms of progression. These fields of endeavor have provided major breakthroughs in deciphering the pathogenesis of cancer. It had long been known that retinoblastoma could be sporadic or heritable⁵⁹ when Knudson at the MD Anderson Cancer Center developed his now celebrated "two-hit" hypothesis.⁶⁰ Knudson had graphed the percent of undiagnosed cases versus age at diagnosis and found two distinct curves, one linear and the other exponential.⁶⁰ The linear curve corresponded to patients who had bilateral tumors and theoretically inherited one copy of a mutated gene (germline mutation) and as their retina developed, acquired the second mutation (second "hit"). The exponential curve corresponded to children who had unilateral tumors and acquired both mutations in their developing retina. The heritable variety of retinoblastoma may be explained by the inheritance of a germline mutation in combination with an acquired mutation in the developing retina, whereas the acquired variety of retinoblastoma develops after mutations in both alleles of the retinoblastoma gene arise in the developing retina (Figure 6). Individuals who harbored a germline mutation in all cells were predisposed to develop a variety of other malignancies.⁶¹ As the field progressed and laboratory techniques improved, investigators were able to localize the retinoblastoma gene (RB1) to chromosome 13q14.2.⁶² In 1984, Dryja's laboratory cloned the RB1 gene⁶³, a landmark achievement as this was the first human tumor suppressor gene cloned. The gene was subsequently sequenced.⁶⁴ The RB1 gene was found to be large (approximately 200kb) and had 27 dispersed exons.⁶⁵ Two structurally related retinoblastoma-like genes (RBL1, RBL2) have since been identified. RBL1 and RBL2 are located at chromosomes 20q11.2 and 16q12.2, respectively.^{66;67} Investigators have recently found that retinoblastoma may arise after MYC mutation in the absence of RB mutations.⁶⁸ These tumors appear to be diagnosed at an earlier age than those with RB mutations. It became evident that although mutations of both alleles of the RB1 gene were necessary in most cases, they were not sufficient for phenotypic expression.^{69;70} Dimaris and colleagues found that loss of both copies of RB1 may lead to retinoma (retinocytoma) and progressive genomic instability in retinoma then leads to retinoblastoma.⁷¹ Gallie's laboratory found that additional mutational events ("three hits") were required for progression to a fully a malignant tumor.⁷²

The function of RB has been described by Harbour.⁷³ The protein (Rb), composed of 928 amino acids, is a nuclear phosphoprotein that contains an N-terminal region, a central pocket

domain with A and B boxes, and a C terminus.⁷⁴ The pocket is formed by interactions of the A and B boxes and tumor forming mutations disrupt this pocket.^{75;76} Rb binds to the chromatin remodeling proteins HDAC and BRG1 via the LxCxE binding site and to E2F transcription factors via its E2F binding site.^{73;77-79} Rb is multifunctional: it suppresses transcription via histone deacetylases (HDACs) and BRG; it also regulates the cell cycle via E2F. Phosphorylated Rb binds directly to E2F and blocks the E2F transactivation domain.^{80;81} When Rb is dephosphorylated via p16, chromatin remodeling proteins (HDAC, BRG1), which bind to Rb, alter chromatin structure and prevent access to transcription thus leading to exit from the cell cycle.⁸² When Rb is phosphorylated, HDAC and BRG1 are released, thus allowing progression through the cell cycle by de-repression of cyclins due to sequential Rb phosphorylation involving cyclins and cyclin dependent kinases (CDKs). Under stress and excess proliferation, phosphorylation of Ser567 changes the tertiary structure of Rb, releasing E2Fs and leading to apoptosis. Thus, Rb plays a central role in the fate of a cell either through exit of the cell cycle or apoptosis.⁷³ Mutations in Rb and/or inhibition of Rb by oncoproteins produced by tumor causing viruses (SV40, adenovirus, HPV) abrogate Rb's ability to mediate exit from the cell cycle or apoptosis and lead to uncontrolled cell division, a hallmark of cancer. Perturbations in genes such as BRCA2 on chromosome 13, CDKN2 on chromosome 9, and TP53/BRCA1 on chromosome 17 co-operate with alterations in Rb by promoting tumor progression.⁸³ Breast cancer related genes BRCA1 and BRCA2 are involved with DNA damage-response pathways and in addition to viral oncoproteins may directly alter the function of Rb.⁸³ Loss of p16/INK or p16/INK promoter hypermethylation may alter cell cycle exit. Additionally, alterations in the phosphorylated retinoblastoma protein (pRb) phosphorylation/inactivation by CDK4/cyclin D1 dysregulates Rb's ability to promote cell cycle exit and TP53 loss facilitates cell survival.⁸³ These other events, or "third hits", appear to be necessary for human retinoblastoma to progress. RB1/pRb inactivation results in loss of its ability to regulate exit from the cell cycle and up-regulates ZEB protein, a transcriptional repressor of E-cadherin, a determinant of epithelial to mesenchymal transformation which allows for transmigration and invasion, two other hallmarks of cancer.⁸³ The biologic events that lead to retinoblastoma formation are summarized in Figure 7.

Molecular Pathology of Retinoblastoma

Several studies have attempted to define the cellular origin of retinoblastoma. One study suggested that human retinoblastoma exhibits properties of a *cone* precursor cell.⁸⁴ Other investigations have implicated multiple cell types, including horizontal, amacrine, Müller glial precursors, and photoreceptors, thus suggesting the existence of a progenitor cell or retinal precursor cell (RPC) that differentiates to multiple cell types.⁸⁵⁻⁸⁸ Gene expression profiling (GEP) of mRNA and comparative genomic hybridization (CGH) of DNA were utilized recently to evaluate 21 retinoblastomas.⁸⁹ Results of this initial study showed different patterns of retinal gene expression that when analyzed by unsupervised hierarchical clustering, followed by principle component analysis to categorize the retinoblastomas into three groups.⁸⁹ Group 1 retinoblastomas expressed genes found in early retinal development, thus suggesting a RPC origin. Group 2 retinoblastomas expressed genes found after cone cell development, consistent with a cone cell origin. Group 3 retinoblastomas expressed both rod and cone genes, similar to normal retinal samples. When compared with

histology and cytogenetics, Group 1 tumors had increased frequencies of post-laminar optic nerve invasion and choroidal invasion compared with the other groups. Group 2 tumors exhibited an increased frequency of photoreceptor differentiation based on the extent of Flexner-Wintersteiner rosettes.⁸⁹ Cytogenetic alterations most frequently associated with adverse clinical features (gain of 1q or 6p and loss of 16q) were more frequent in Group 1 than the other groups.⁸⁹ This implies that retinoblastoma may be stratified by gene expression profile with regards to clinical behavior and outcome. This classification system is based on cellular origin - RPCs or cone precursor cells (Figure 8). It is hypothesized that retinoblastomas that arise from RPCs exhibit a higher anaplasia grade than those that arise from cone precursor cells. Our laboratory has recently studied the association of tumor anaplasia (an apparent manifestation of the gene expression profile) and the development of metastases and survival among 125 patients who underwent enucleation for retinoblastoma (Figure 9). We found that there was a significant correlation between grade of anaplasia and survival (Figure 10). Further retinoblastoma GEP studies are needed to validate the initial study.⁸⁹

Targeted Therapy

Chemoreduction and Consolidation

The other major advances in retinoblastoma since Dunphy's lecture are related to targeted therapy. The goal of treatment of retinoblastoma is to ensure elimination of the tumor while minimizing collateral injury to other tissues. Without diminishing the chances of cure, preservation of the eye and vision is an important priority. The cure rate of enucleation alone in unilateral (non-heritable) cases without extraocular disease is 85% to 90%.^{32;90} Adjuvant chemotherapy is utilized for eyes with optic nerve invasion posterior to the lamina cribrosa or massive choroidal invasion.⁹¹ These treatments include VDC (vincristine, cyclophosphamide, doxorubicin or idorubicin), VCE (vincristine, carboplatin, etoposide) (Figure 11) or hybrid regimens.^{90;92} A conservative, eye salvaging approach is recommended for unilateral cases with early stage disease, especially in young children who may have heritable retinoblastoma and later develop a tumor in the contralateral eye.^{90;92} Patients with bilateral (heritable) retinoblastoma should undergo chemoreduction with a combination of vincristine, carboplatin and etoposide.⁹³⁻⁹⁷ For early stage tumors (International Classification Group B), a two agent regimen including vincristine and carboplatin is effective.⁹⁴⁻⁹⁶ For later stage tumors with vitreous seeds (Groups C and D) a three agent regimen is utilized. Consolidation may be used alone in Group A tumors or in combination with chemoreduction in Groups C-D. Consolidation includes cryotherapy for small anterior tumors, thermotherapy/laser photocoagulation for posterior tumors and brachytherapy for larger tumors.⁹² The reasons for treatment failures are vitreous seeds of tumor, subretinal seeds of tumor, intraretinal tumor and tumor resistance.⁵⁸ The first three types of failure are due to an inability of drug reach tumor (e.g. diffusion); the fourth type of failure is mainly due to well differentiated, non-cycling tumor cells that are resistant to treatment modalities dependent on cell division.

Periocular injections of carboplatin have been used for the treatment of vitreous seeds. This approach has largely been abandoned due to lack of success⁹⁸ and toxicity to tissues of the

visual pathway such as the optic nerve.⁹⁹ Some variants of Groups C and D eyes with retinoblastoma appear to respond better to chemoreduction than other forms. For instance, posterior central tumors without subretinal seeds respond better than peripheral tumors.¹⁰⁰ Extraocular retinoblastoma may occur locally, regionally or distantly.⁹² Local, isolated orbital retinoblastoma comprises 60% to 70% of recurrent/metastatic retinoblastoma with CNS involvement and distant metastases making up the remainder of these cases.¹⁰¹ Treatment of orbital disease at the time of diagnosis includes radiation and chemotherapy, usually with vincristine, cyclophosphamide, doxorubicin, platinum- and epipodophyllotoxin based regimens.¹⁰² This is followed by enucleation and consolidation with orbital irradiation.⁹² A similar treatment is recommended for patients with retinoblastoma at the margin of optic nerve resection. Preauricular and cervical lymph nodes should be evaluated and irradiated if retinoblastoma is present.⁹⁰ Treatment for patients with intracranial dissemination includes platinum-based chemotherapy, CNS-directed therapy and possibly irradiation to the CNS and spinal axis.⁹² For patients with distant metastasis, high dose chemotherapy and autologous stem cell rescue have been utilized.⁹²

Intra-arterial Chemotherapy (IAC)

Intra-arterial chemotherapy (IAC) was developed in Japan by Kaneko as an eye-preserving therapy for patients who had retinoblastoma with vitreous seeds.¹⁰³⁻¹⁰⁵ In the United States, IAC was initially used by Abramson.¹⁰⁶⁻¹⁰⁹ IAC has been usually employed used for Group D and Group E retinoblastomas and involves trans-femoral catheterization with a balloon catheter which is advanced under fluoroscopy into the origin of the ophthalmic artery. Once secured, melphalan, topotecan and/or carboplatin are infused.¹⁰⁸ This procedure is often repeated several times over a period of weeks to months. Gobin has reported an ocular event free survival at two years of approximately 70% for all eyes, 80% for eyes without prior treatment, and 60% for eyes that had previous treatment failures.¹⁰⁹ IAC for retinoblastoma is now performed in a number of centers in the United States¹¹⁰⁻¹¹⁴ and around the world.¹¹⁵⁻¹²³ Complications include eyelid erythema/edema, emboli to the retina/choroid with occlusive vasculopathy, vitreous hemorrhage and cerebral vasoconstriction.^{111;113;124;125} Some argue that systemic chemoreduction is protective against retinoblastoma metastases and/or third tumors (pinealblastoma) whereas localized IAC does not provide that benefit.¹²⁶ Another controversy is that IAC may mask histologic high risk features in eyes with extensive retinoblastoma that eventually undergo enucleation.¹²⁷ Additionally, IAC is performed in major cancer centers by skilled interventional radiologists/neurosurgeons with technique and skill levels that can vary. Nonetheless, impressive globe salvaging results have been reported in some advanced group retinoblastoma eyes. IAC currently appears to be a reasonable option for select patients.

Intravitreal Injections

Although for many years ophthalmic surgeons were strongly advised to avoid needle passes through the eye wall into the vitreous in children with retinoblastoma for fear of tumor seeding¹²⁸, various investigators have attempted intravitreal injections of chemotherapeutic agents beginning in the early 1960s.¹²⁹⁻¹³¹ Ghassemi and Shields reported using intravitreal injections of melphalan¹³² and combined melphalan/topotecan¹³³ to treat vitreous seeds of retinoblastoma. Munier of Switzerland reported a technique of intra-vitreous injection of

melphalan for refractory or recurrent retinoblastoma tumor seeds using a 32 gauge needle on a tuberculin syringe and freezing the needle with a cryoprobe as it is withdrawn from the eye to prevent tumor seeding along the needle track.^{134;135} Using this technique, he observed no tumor seeding in 135 cases. Transient vitreous hemorrhage occurred in only 3 patients.¹³⁴ Suzuki and Kaneko in Japan reported successful injections in 237 eyes of 227 patients with only one case of extra-ocular tumor spread.¹³⁶ Novel injection techniques are being developed to deliver chemotherapeutic agents locally to retinoblastoma. As these techniques are perfected, it is anticipated that they will be used more widely.

Emerging Technologies

Developments in targeted killing of retinoblastoma using gene therapy and nanotechnology have paralleled progress in the field of intravitreal injection. Subramanian and colleagues in Houston have used intravitreal injections of an adenovirus vector containing herpes simplex thymidine kinase gene in one eye of eight patients with bilateral retinoblastoma and vitreous seeds.¹³⁷ The patients were then given intravenous ganciclovir and one of the eight patients achieved sustained resolution of the vitreous seeds 38 months after therapy.¹³⁷ In addition to gene therapy, sustained drug release platforms, some of which have implications for retinoblastoma therapy, are being developed for targeted intraocular drug delivery.¹³⁸ One of the methods is a sustained release episcleral explant.¹³⁹ My laboratory has been investigating periocular injection of sustained release nanoparticles containing carboplatin¹⁴⁰ and intravitreal topotecan in a rabbit model of retinoblastoma that develops vitreous seeds.¹⁴¹ We have been able to achieve therapeutic levels of intravitreal topotecan with successful elimination of the vitreous seeds in our model (Figure 12). We are also able to inject topotecan into the suprachoroidal space of our model using microneedles (Figure 13). Results are currently being evaluated. Suprachoroidal injection potentially allows a route of targeted drug delivery with minimal risk of extraocular spread of tumor, as the vitreous and subretinal spaces are not entered.

Global Health

Although mortality from retinoblastoma in developed countries is approximately 3%, mortality from the primary tumor in developing countries approaches 60% in many areas.¹⁴² Over the past century, enucleation (1900s), external beam radiation (1920s), photocoagulation (1950s), cryotherapy (1970s), chemoreduction (1990s), and chemoreduction/consolidation (2000s) have sequentially improved survival in children with retinoblastoma to 97% in developed countries.¹⁴³ In contrast, in developing countries survival rates range from 79% in upper middle income communities to 40% in lower income regions (Figure 14).¹⁴⁴ Despite the great advances in molecular biology and treatment for retinoblastoma that have occurred during the past 50 years, access to care is a major barrier to successful therapy in many parts of the world. This is the great remaining challenge in retinoblastoma. Gallie conducted a World Retinoblastoma Survey through an Internet Database and found that the mean age at diagnosis and median follow-up for retinoblastoma patients were for Asia, North American and Europe were 22 months/1.2 years, 12 months/3 years and 9 months/5 years, respectively.¹⁴² This implies that access to care is a major problem in Asia, especially considering that there are about 300 new cases of retinoblastoma

in North America and about 3,000 new cases in Asia each year.¹⁴² Approximately 92% of the world's children have minimal access to care for retinoblastoma.¹⁴² Although there are relevant social, cultural and political issues, it is troubling that many children are dying from a curable disease. A group of retinoblastoma researchers and clinicians from 10 countries met in 2007 to address this issue.¹⁴⁵ Strategies for early diagnosis in developing countries were discussed and global initiatives by non-governmental organizations were presented.¹⁴⁵ Educational outreach, awareness campaigns, twinning with institutions in developed countries, and national programs are currently addressing access to care for retinoblastoma patients in developing countries.¹⁴⁵ The internet and telemedicine are being used as tools to implement these initiatives. Our current challenge is not technological, but rather social: ensuring that children who live in under developed regions of the world can have access to appropriate medical care.

Summary

In summary, retinoblastoma is a remarkable success story. This cancer was virtually uniformly fatal 200 years ago and is now uniformly curable if detected early. Most children with retinoblastoma in the United States retain their eyes, and many enjoy useful vision. In the 400 years prior to Dunphy's lecture², progress was slow and sporadic, tied to key innovations like the ophthalmoscope, microscopic diagnosis, surgical enucleation and radiation therapy. In the 50 years since his lecture, major advances have been made at an accelerated rate, including understanding the molecular biology of the tumor and targeted, globe-sparing therapy. It is notable that these achievements were made by individual ophthalmologists, pathologists and oncologists, often in the absence of clinical trials. I wish to thank the American Journal of Ophthalmology and American Academy of Ophthalmology for giving me the great honor and privilege of delivering the LXXI Edward Jackson Memorial Lecture.

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Figure 1.

Endophytic retinoblastoma. Left. Endophytic retinoblastoma extends from the retina into the vitreous, thus resulting in a white mass associated with tumor shedding into the vitreous (i.e. seeds). Right. The primary tumor is vascularized (red) whereas the vitreous seeds (blue) are avascular.

Figure 2.

Exophytic retinoblastoma. Left. Exophytic retinoblastoma extends from the retina into the subretinal space and clinically appears as a white to slightly yellow subretinal mass. Right. The primary tumor is vascularized (red) and subretinal seeds (blue) are avascular.

Figure 3.

Anterior diffuse retinoblastoma. Left. Anterior diffuse retinoblastoma results in a white pseudohypopyon. Right. The primary tumor arises as a small mass in the area of the ora serrate and seeds into the aqueous in front of the anterior hyaloid, spreads through the pupil, and accumulates in the peripheral iris.

Figure 4. Retinocytoma. This patient has two calcified peripheral retinal lesion that represent retinocytoma, once confused with spontaneously regressed retinoblastoma. The patient's daughter developed retinoblastoma. (courtesy of G. Baker Hubbard III MD)

Figure 5. Retinoblastoma pathology. Upper left. Retinoblastomas have a gross white, fleshy to cottage-cheese appearance. Upper right. Retinoblastoma is a small round blue cell tumor, forming sheets of cells. Lower left. Retinoblastoma may exhibit rosette formation, including Homer Wright rosettes with neuropil in the lumen (arrows), or Flexner-Wintersteiner

rosettes with a clear lumen (arrowheads). Lower right. Retinoblastoma may contain well differentiated areas, exhibiting cells with small nuclei, rather abundant cytoplasm, and fleurettes with photoreceptor outer segments in the lumen. (upper right, lower left and lower right, hematoxylin and eosin, 25X)

Figure 6.

Retinoblastoma inheritance, Heritable retinoblastoma requires two mutations: one that is inherited in the patient's germline and the other in the developing retina. These tumors are usually bilateral, multiple, and occur at an early age. Non-heritable (acquired) retinoblastoma also requires two mutations, although both of these mutations occur in the developing retina. These tumors are usually single, unilateral, and occur at a later age.

Figure 7.

Rb in progression to retinoblastoma. 1. Retinoblastoma (Rb) recruits proteins such as HDAC and BRG1 to E2F sites resulting in chromatin structure alterations and prevention of access to transcriptional machinery. 2. Phosphorylation by CDKs release HDAC and BRG1, thus releasing histone from DNA, allowing access to transcriptional machinery and resulting in cell division. Mutations in both alleles of Rb result in uncontrolled proliferation/retinocytoma formation, and further events including P16Ink4a, P14/Arf, or BRCA2 mutations, SV40 interactions with Rb, or Zeb1 activation by inactivated pRb allow progression to retinoblastoma. 3. The cell cycle may be exited under normal conditions when Rb is hypophosphorylated via MITF induction of p16, thus allowing HDAC and BRG1 to rebind to Rb via E2F sites and suppress transcription. 4. Alternative cell cycle exit occurs under stress or hyperproliferation; Rb is hyperphosphorylated, releases E2f and undergoes Tp53 mediated apoptosis, which may be suppressed by BRCA1 mutations. Thus, Rb mutations initiate the process and retinoblastoma results through progressive mutational events involving chromosome 9, 13 and 17.

Figure 8.

Retinoblastoma cell of origin. Gene expression profiling (GEP) of retinoblastoma has resulted in two general phenotypes supporting a primitive retinal precursor cell (RPC) origin of retinoblastoma. This cell type is present in the outer nuclear zone of the developing retina. If mutational events occur early, before further differentiation of the RPC, group 1 retinoblastoma occurs; if the mutational event occurs later, group 2 retinoblastoma occurs. Group 1 retinoblastoma expresses RPC genes and group 2 retinoblastoma expresses cone genes. Group 1 retinoblastoma has a more primitive phenotype and group 2 retinoblastoma may exhibit photoreceptor differentiation, including rosette and fleurette formation.

Figure 9. Grading anaplasia in retinoblastoma anaplasia. Upper left. *No anaplasia* (retinoctyoma) exhibits small, compact nuclei, abundant eosinophilic cytoplasm, and photoreceptor differentiation. Upper right. *Mild anaplasia* is characterized by tumor cells with round,

hyperchromatic nuclei, increased nuclear to cytoplasmic ratios, and a moderate amount of eosinophilic cytoplasm. Lower left. *Moderate anaplasia* is characterized by cells with larger nuclei, high nuclear to cytoplasmic ratios, and scanty eosinophilic cytoplasm. Lower right. *Severe anaplasia* is characterized by cells with larger, hyperchromatic nuclei, nuclear molding/angulation, and virtually no cytoplasm. (hematoxylin and eosin, 100X)

Figure 10.

Retinoblastoma survival based on anaplasia grade. This Kaplan-Meier plot no deaths or metastases associated with mildly anaplastic tumors, an approximate 97% 10 year survival/no metastases in patients with moderately anaplastic tumors, and an approximate 75% 10 year survival/no metastases in patients with severely anaplastic tumors ($P < 0.01$).

Figure 11.

Chemotherapeutic agents used in treating retinoblastoma. In general, there are four classes of chemotherapeutic agents used to treat retinoblastoma. *Alkylating agents*, such as melphalan, cause DNA intrastrand linking and cross-linking, resulting in damaged DNA that is not transcribed. *Topoisomerase inhibitors*, such as etoposide and topotecan, work by capping free double strands of DNA caused topoisomerase cleavage. The capped, damaged DNA is unable to be repaired and is degraded via apoptosis. *Platinum based antineoplastic agents*, such as carboplatin, bind DNA bases, thus resulting in kinked DNA; this damaged DNA is then degraded via apoptosis. *Vinca alkylloids*, such as vincristine, bind tubulin dimers, thus blocking mitotic spindle formation and inhibiting mitosis. Typically, combinations of chemotherapeutic agents with different mechanisms of action are used to treat retinoblastoma via systemic chemoreduction, such as Vincristine, Etoposide, and Carboplatin (VEC) therapy, which is given over 5 to 6 cycles.

Figure 12.

Intravitreal injection of topotecan in rabbit model of retinoblastoma. Upper left. There are vitreous seeds of retinoblastoma (*) in the rabbit model. Upper right. The 32 gauge 4 mm needle (arrow) is being inserted at the pars plana. Lower left. The needle (arrow) is inserted

to its hub and topotecan is injected into the vitreous. Lower right. After 3 weekly injections of 20µg of topotecan, vitreous seeds have disappeared, the retina/optic nerve appear normal and there is no spread of extraocular tumor.

Figure 13.

Suprachoroidal injection of fluorospheres. Upper left. Comparison of microneedle (left) with standard 30 gauge needle; note that the microneedle is the same length as the bevel of the 30 gauge needle. Upper right. India ink injected over ora seratta migrates posteriorly in

suprachoroidal space over 30 seconds. Lower left. 10 micron diameter pink fluorospheres in hub of 1 cc syringe with microneedle inserted into suprachoroidal space in a rabbit. Lower right. After injection, a volume of 200 microliters of fluorospheres are in the suprachoroidal space and not in the hub of the needle.

Figure 14.

Regional 5-year survival of children with retinoblastoma. Five-year survival rates for children with retinoblastoma vary among countries by their socioeconomic rank, ranging from nearly 97% in the United States to 40% in low income developing countries. Modified from Canturk et al ¹⁴⁴

Table 1

<u>Differential Diagnosis for Retinoblastoma</u>	
Coats disease	40%
persistent fetal vasculature (PFV)	28%
vitreous hemorrhage	5%
ocular toxocariasis	4%
familial exudative vitreoretinopathy (FEVR)	3%
rhegmatogenous retinal detachment	3%
coloboma	3%
astrocytic hamartoma	2%
combined hamartoma of retina/rpe	2%
endogenous endophthalmitis	2%

approximate percentages of occurrence are shown

modified from Shields and colleagues²⁷

Table 2

International Classification of Retinoblastoma			
Group	Subgroup	General	Specific Features
A	A	small tumor	retinoblastoma 3mm in size
B	B	larger tumor	retinoblastoma > 3mm in size or
		macula	macular retinoblastoma 3mm to fovea
		juxtapapillary	juxtapapillary retinoblastoma location 1.5 mm to disc
		subretinal fluid	clear subretinal fluid 3mm from margin
		focal seeds	retinoblastoma with
C	C1		subretinal seeds 3mm from retinoblastoma
	C2		vitreous seeds 3mm from retinoblastoma
	C3		both subretinal and vitreous seeds 3mm from
		diffuse seeds	retinoblastoma with
D	D1		subretinal seeds >3mm from retinoblastoma
	D2		vitreous seeds >3mm from retinoblastoma
	D3		both subretinal and vitreous seeds >3mm from
E	E	extensive retinoblastoma	extensive retinoblastoma occupying >50% globe or
			neovascular glaucoma
			opaque media from hemorrhage in ac, vitreous or sr space
			invasion of postlaminar optic nerve, choroid, sclera, orbit, ac

mm=millimeters; ac=anterior chamber

modified from Shields and Shields²²