expert reviews in molecular medicine

Retinoblastoma: from bench to bedside

Richard L. Hurwitz, Patricia Chévez-Barrios, Milton Boniuk, Murali Chintagumpala and Mary Y. Hurwitz

Retinoblastoma (Rb) is the most common primary ocular malignancy of children and is caused by a mutation in the gene *RB1*. Approximately 40% of cases are associated with one or more constitutional mutations, and are therefore heritable, whereas the other 60% are sporadic. Rb is exclusively found in young children. In some cases, Rb tumours metastasise to extraocular organs including bone, lung and brain. Although there is no effective treatment for metastatic disease, non-metastatic cases can be cured by removal of the eye (enucleation). Newer treatment strategies emphasise salvaging the affected eye whenever possible. Animal models of Rb have been developed with xenograft and transgenic techniques. Each model has both strengths and weaknesses for exploring the mechanisms of disease development and progression and the efficacy of new treatment strategies.

Retinoblastoma (Rb) is a malignant tumour originating from the retina and is the most common primary malignancy of the eye in children. Rb usually affects children under the age of 3 years (Ref. 1). In the USA, Rb affects 1 in every 15 000–20 000 live births (Refs 2, 3). Approximately one-quarter of the cases present bilaterally (i.e. affect both eyes) and have been attributed to autosomal dominant, heritable mutations in the *RB1* gene on chromosome 13 (Ref. 4). Most of the other three-quarters of cases have been attributed to sporadic acquired mutations in the same gene and present with unilateral disease, although a small percentage of unilateral tumours are also caused by constitutional mutations.

Rb has a metastatic potential manifested by invasion of either the optic nerve or the choroid that can result in spread to non-ocular sites. Although most children with Rb present without

All the authors are at Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.
Tel: +1 832 824 4260; Fax: +1 832 825 4038.

Richard L. Hurwitz (corresponding author), Associate Professor, Pediatrics.
E-mail: rhurwitz@bcm.tmc.edu
Patricia Chévez-Barrios, Assistant Professor, Pathology. E-mail: pchevez@bcm.tmc.edu
Milton Boniuk, Professor, Ophthalmology. E-mail: mboniuk@bcm.tmc.edu
Murali Chintagumpala, Associate Professor, Pediatrics. E-mail: ralic@bcm.tmc.edu
Mary Y. Hurwitz, Assistant Professor, Pediatrics. E-mail: mhurwitz@bcm.tmc.edu

Website of the Retinoblastoma Center at the Texas Children's Cancer Center:
http://www.txccc.org/diseases/retino/retino_main.html

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

metastases in the USA, this is not true in other parts of the world (Ref. 1). Patients who present without metastatic disease have a greater than 90% cure rate when treated by surgical removal of the eye (enucleation) (Ref. 3). Patients with metastatic disease have a poor prognosis and only a few individuals survive even after intensive chemotherapy (Ref. 1).

Although surgical intervention remains the standard therapy for Rb, enucleation results in loss of vision and can result in an unsatisfactory cosmetic appearance as the child grows older (Ref. 5). If the tumour is small enough, it can be eradicated with either cryotherapy or laser photocoagulation (or both) with preservation of the eye and useful vision (Refs 6, 7, 8). Recently, alternative therapeutic interventions including chemotherapy, chemoreduction, and/or radiotherapy have been used to salvage the affected eye. These approaches are particularly useful for more-advanced but still-contained Rb (Refs 9, 10, 11).

We review here current knowledge of the aetiology, pathology and treatment of Rb. We explore the different animal models of this disease and describe how they are used to understand disease progression and to develop new treatment strategies. Finally, we discuss questions that are currently under study in human clinical trials for the treatment of Rb.

The genetic basis for Rb

Frequency of Rb

Most Rbs appear sporadically; however, the disease can also be inherited (Ref. 12) and is usually transmitted as a typical mendelian autosomal dominant trait with high penetrance. Sporadic cases typically present with unilateral unifocal disease at a slightly later average age. Of all cases, about 60% are non-hereditary and unilateral, 15% are genetically determined and unilateral, and 25% are genetically determined and bilateral (Refs 13, 14).

Mutational events underlying Rb

The observation that most familial cases of Rb are bilateral and multifocal (i.e. tumours arise in more than one location) can be explained by a 'two-hit' model (Refs 13, 15). According to the model, as few as two stochastic mutational events are required for tumour initiation, the first of which can be inherited through the germline (in heritable cases) or can occur somatically in individual retinal cells (in non-heritable cases). The second event occurs somatically in both cases and leads to tumour formation from each doubly defective retinal cell.

The first evidence that supported an inherited mechanism for Rb development was the presence in some Rb patients of a microscopically visible deletion in one chromosome 13 allele in constitutional cells (i.e. cells from anywhere in the body – not specifically from the tumour) (Refs 16, 17, 18, 19). Although the size and location of these deletions varied between families, each deletion minimally encompassed chromosome 13, band q14 (Refs 20, 21). This chromosomal locus contains the *RB1* gene. In the 'two-hit' model, such deletions in the germline could act as the first hit and confer the risk of tumour formation as a result of a second event. The increasing resolution of cytogenetic technology and the development of DNA probes for loci in the immediate vicinity of the *RB1* gene locus have allowed the cloning of the *RB1* gene and helped define its function as a tumour suppressor.

Patients who do not have a gross deletion in chromosome 13 but who have bilateral or familial Rb might have submicroscopic mutations at the *RB1* locus similar to mutations that have been found in the tumour cells of patients with non-hereditary Rb. The second step of tumourigenesis in both hereditary and non-hereditary Rb involves somatic alteration of the normal allele at the *RB1* locus such that the mutant allele is unmasked. This is known as loss of heterozygosity or LOH (Fig. 1). Thus, the first mutation in this process, although it might seem to behave as an autosomal dominant trait in the family, is in fact a recessive defect in the individual retinal cell. Elimination of the chromosome containing the wild-type allele followed by reduplication of the remaining mutant chromosome might be another way by which the affected *RB1* locus becomes homozygous within the cell (Refs 4, 22, 23, 24).

Although the unmasking of predisposing mutations at the *RB1* locus occurs through similar mechanisms in sporadic and heritable Rb, only the latter carries the initial mutation in each cell. Patients with heritable disease also seem to be at greatly increased risk for the development of additional primary tumours, particularly osteogenic sarcoma during childhood and other solid tumours such as breast or bladder tumours later (Ref. 25). This high propensity is genetically determined by the predisposing *RB1* mutation

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

and approaches a lifelong risk of 20%. The notion of a pathogenetic association between retinoblastoma and osteogenic sarcoma (both rare tumours) was tested using restriction fragment length polymorphism (RFLP) analysis. The analysis indicated that osteosarcomas arising in patients with Rb had become homozygous specifically around the chromosomal region carrying the *RB1* locus on chromosome 13 (Ref. 26). Furthermore, these same chromosomal mechanisms eliciting LOH were also observed in both sporadic and familial osteogenic sarcomas, suggesting a genetic similarity in pathogenetic causality.

The examination of cases of bilateral Rb without a family history of Rb showed that disease more commonly arises subsequent to a new germline mutation in the paternal allele followed by somatic alteration or loss of the maternally derived wild-type allele (Refs 27, 28). This suggests that mutations in the *RB1* locus occur more often during spermatogenesis. Analyses of sporadic osteosarcomas also showed preferential mutation of the paternal allele (Ref. 29).

The *RB1* gene

In 1986, Dryja and colleagues isolated the *RB1* gene, determined the genomic organisation of the

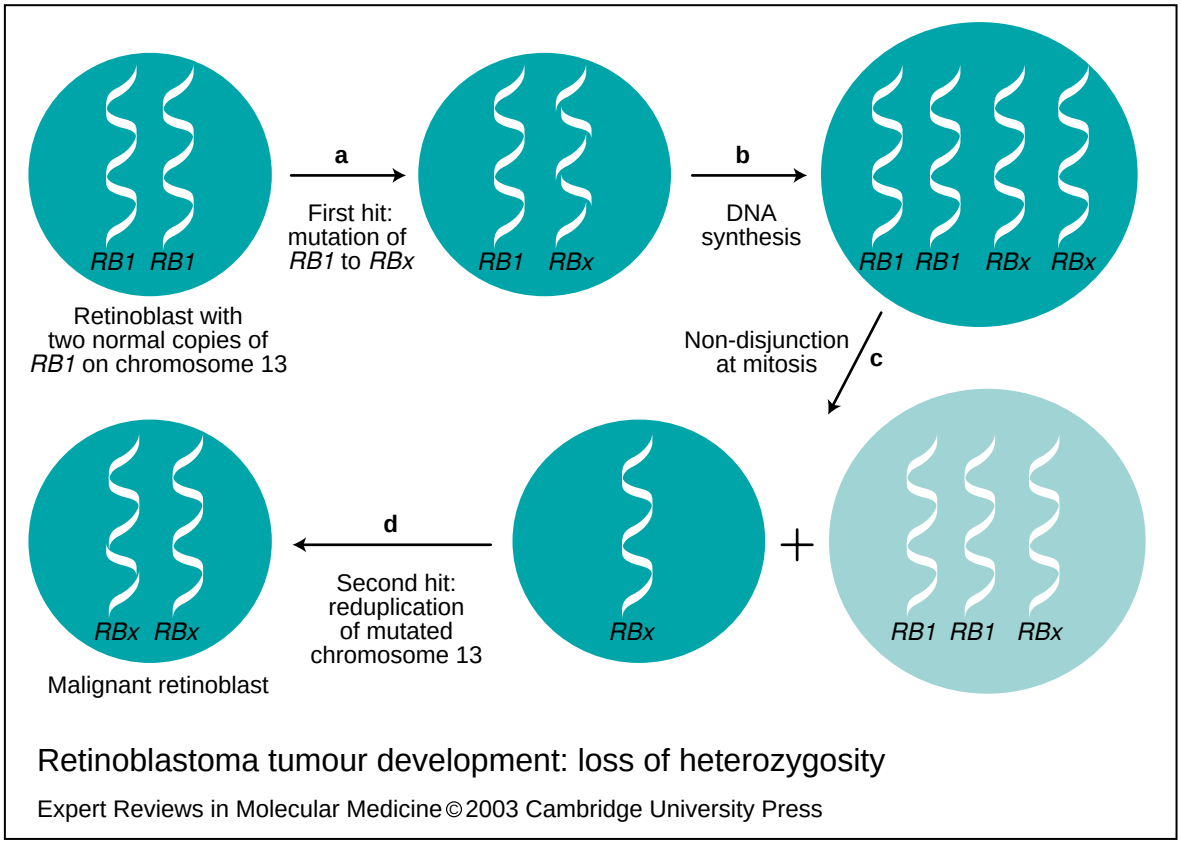


Figure 1. Retinoblastoma tumour development: loss of heterozygosity. (a) 'First hit': an *RB1* (retinoblastoma gene) mutation (*RBx*) on chromosome 13q14 results in a heterozygous retinoblast (only chromosome 13 is represented in the figure). (b) This non-malignant heterozygous cell undergoes DNA synthesis as it prepares to enter mitosis. (c) During mitosis, a non-disjunction event occurs, resulting in a daughter cell with only a single copy of chromosome 13 containing *RBx*. (d) Chromosome 13 reduplicates, resulting in a cell homozygous for the *RBx* mutation. After this 'second hit' the cell has lost RB protein function and has malignant potential. Cells containing one chromosome 13 with a mutated *RB1* gene or cells containing a reduplicated chromosome 13 have been observed. Other possible mechanisms of LOH (not shown) include somatic recombination with subsequent segregation resulting in homozygosity at all loci distal to the recombination site, deletion of a segment of chromosome 13 including 13q14, localised gene conversion in the neighbourhood of the *RB1* locus, and a point mutation in the other, normal *RB1* allele **(fig001rhh)**.

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

180 kb locus (comprising 27 exons) and analysed the expression of its 4.7 kb mRNA transcript in tumour and normal tissues (Ref. 30). Introduction of the wild-type gene into Rb and osteosarcoma cell lines using recombinant retroviral vector transfer resulted in a partial reversal of the tumourigenic phenotype (Refs 31, 32). Further characterisation of the complete *RB1* genomic sequence (Ref. 33) allowed a rigorous cataloguing of the different mutations affecting the gene in Rb tumours. To date, over 200 disease-causing mutations have been identified in the *RB1* genes of patients (Refs 30, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43).

RB1 is expressed as a 4.7 kb transcript in normal human and rat tissues including brain, kidney, ovary, spleen, liver, placenta and retina (Ref. 44). The expressed protein contains 928 amino acids and has an estimated molecular mass of 110 kDa. Although the number of different types of tumours that occur as a result of inherited mutations of the *RB1* locus is small, the broad tissue expression and species conservation of this gene suggest a common and potentially important role in the growth or differentiation of many cell types.

Investigations of *RB1* gene alterations at both the DNA and the RNA level have cumulatively revealed a strong correlative relationship between the lack of *RB1* gene product and the appearance of Rb tumours. In addition to osteogenic sarcomas, other tumour types might also contain mutations involving the *RB1* gene. Molecular analyses of small-cell lung carcinomas have revealed structural abnormalities within the *RB1* gene in approximately 15% of cases (Ref. 45). LOH for chromosome 13 has been detected in about 25% of breast cancers and related breast-cancer-derived cell lines (Refs 46, 47). By contrast, a detailed analysis of the effects of chromosome 13 mutations in tumours clearly shows that not all tumours are either a direct or an indirect result of LOH of the *RB1* locus (Ref. 48). The cumulative data suggest that only subsets of tumours might share a common pathogenetic mechanism that results from unmasking mutations affecting the tumour-suppressing function of *RB1*.

RB protein and the cell cycle

The RB protein is localised primarily in the cell nucleus (Ref. 49). Post-translational phosphorylation of the RB protein in quiescent cells overrides growth suppression and allows cell division to occur (Ref. 50). The RB protein also has a role in the regulation of the cell cycle of actively dividing cells. The unphosphorylated RB protein (p110RB) has been demonstrated to bind to E2F1, a transcription factor and a cell-cycle regulator, during the G1 phase of the cell cycle. The RB–E2F1 complex masks the E2F1 transactivation domain and inhibits surrounding enhancer elements, thereby causing transcription of E2F1-regulated genes to cease. The RB protein accomplishes this by physically associating with a histone deacetylase (HDAC1). This recruitment of the deacetylase to the E2F1-regulating domain by RB allows the deactylation of histones, thereby modulating the local structure of chromatin (Refs 51, 52). Phosphorylation of the RB protein at the G1–S boundary results in the release of these transcriptional factors, allowing E2F1 and the enhancers to become positive transcriptional elements. Additional cell-cycle-specific kinases become activated and facilitate the progression of the cells through G2 and M phases. At the completion of the cell cycle, phosphatases dephosphorylate the RB protein, allowing the protein to again sequester E2F1 and form an inactive complex.

Thus, positive and negative regulation of transcription, and therefore cell proliferation, are linked to the phosphorylation cycle of the RB protein. In tumours in which the RB protein is mutated or absent, these intracellular transcriptional elements are dissociated, thus allowing uncontrolled progression through the cell cycle. Such behaviour results in unchecked cell proliferation consistent with a malignant phenotype. The *RB1* gene therefore encodes a tumour suppressor such that, when normal function is lost in both alleles, cell division becomes uncontrolled and tumour growth is initiated (Fig. 1).

The viral oncoproteins of polyomaviruses (SV40), adenoviruses (Ad-2 and Ad-5) and papillomaviruses (HPV-16) have also been shown to complex with the RB protein (Refs 53, 54, 55). Because one function of viral oncoproteins is to allow the creation of a cellular environment that is permissive for DNA synthesis, one of their modes of action might involve sequestration of the antiproliferative unphosphorylated RB protein. Releasing the cell from its negative regulation by RB might allow the cell to enter S phase and synthesise DNA. Taken together, the data support a model in which the

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

unphosphorylated form of RB is the species that is active in growth suppression.

Clinical presentation of Rb

Most cases of Rb in the USA are diagnosed while the tumour remains intraocular, without local invasion or distant metastases. However, in developing countries, the diagnosis is frequently made only after an enlarged eye or gross orbital extension is apparent, and local invasion is common.

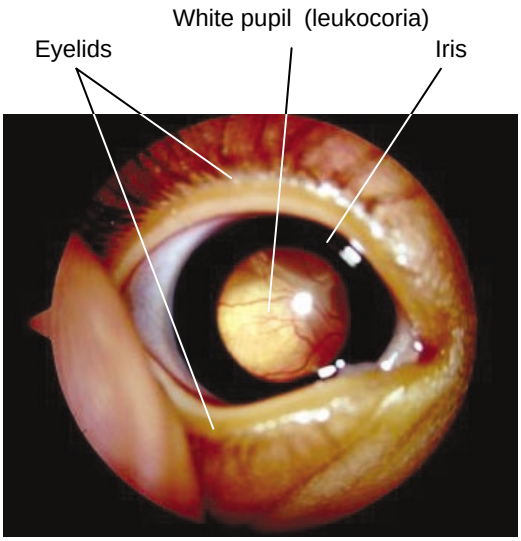
The signs and symptoms of an intraocular tumour depend on its size and position. The most common presenting sign is leukocoria (the presence of a white pupillary reflection) of one or both eyes (Fig. 2). Leukocoria is apparent when the tumour is large or has caused a total retinal detachment, leading to a retrolental (behind the lens) mass that is visible through the pupil. If vitreous haemorrhage occurs because of bleeding from the vessels in the retinoblastoma, the pupil might appear to have a dark reflex instead of the white reflex (Refs 56, 57). The second most common presenting sign is strabismus (crossed eyes). In addition, loss of central vision because of a tumour in the macula (the area of the retina that provides central vision) may disrupt the fusional reflex (the innate ability of both eyes to focus on a single image) and cause the affected eye to drift. Other, less common presenting signs include cataract, differences in pupil size (anisocoria) or iris colour (heterochromia), or bulging of one or both eyes (proptosis).

Diagnosis

Most commonly, a parent or relative of an affected child notes an abnormality of the eye that prompts physician evaluation. Early detection of the tumour benefits the patient by increasing the chance of being able to salvage the affected eye. Detection involves a paediatrician looking for leukocoria with an ophthalmoscope. The gross appearance of a creamy pink to snow-white mass projecting into the vitreous humour during the ophthalmoscopic examination (Fig. 2) might suggest Rb; however, associated findings of retinal detachment, vitreous haemorrhage, or opaque media often make inspection difficult. Pupillary dilation and examination with the patient under anaesthesia are essential to evaluate the retina fully. Ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI) of the orbit and brain are commonly used to confirm diagnosis and to detect ectopic disease. When the tumour is in an advanced stage, distinguishing vitreous seeding from multifocal tumours can be difficult; this distinction has important ramifications for prognosis for the patient and for genetic counselling for the family.

Pathology

Primary Rb tumours originate in the sensory retina and occupy the retina and vitreous cavity. The tumour is usually white-grey with a chalky appearance, a soft, friable or crumbly consistency, and presents with bright white speckles corresponding to internal calcifications. The gross features of Rb depend on the growth pattern of the tumour (Refs 56, 58, 59). Some of these patterns correlate with clinical presentations



Photograph of an eye from a patient with retinoblastoma

Published in Expert Reviews in Molecular Medicine by Cambridge University Press 2003

Figure 2. Photograph of an eye from a patient with retinoblastoma. The most common presenting sign of retinoblastoma is leukocoria (a lack of normal red reflex of the eye). Leukocoria is manifested by a retrolental white mass that is visible through the pupil and occurs when the tumour is large or has caused total retinal detachment. For a diagrammatic section through the eye see fig001rdb in Ref. 108. Image reproduced from Ref. 1, with permission from Lippincott Williams & Wilkins (© Copyright 2002, Lippincott Williams & Wilkins) **(fig002rhh)**.

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

and differences in biological behaviour, especially as they relate to intraocular and extraocular types of tumour spread.

The endophytic growth pattern is represented by tumours arising from the retina and growing into the vitreous cavity (Fig. 3a). These tumours tend to entirely fill the cavity and produce floating tumour spheres called vitreous seeds. If a tumour is left untreated, it eventually invades the anterior portion of the eye, reaching the aqueous venous channels and the conjunctiva. From there, the tumour can permeate the lymphatic vessels and metastasise to regional lymph nodes (Refs 56, 58, 60, 61, 62).

Exophytic tumours grow from the retina into the subretinal space and often cause serous detachments of the retina (Fig. 3b). These tumours can invade the choroid through Bruch's membrane, which lies on the inner side of the choroid (Refs 56, 58, 63, 64). Mixed endophytic and exophytic tumour growth is the most common pattern (Refs 56, 58) (Fig. 3c).

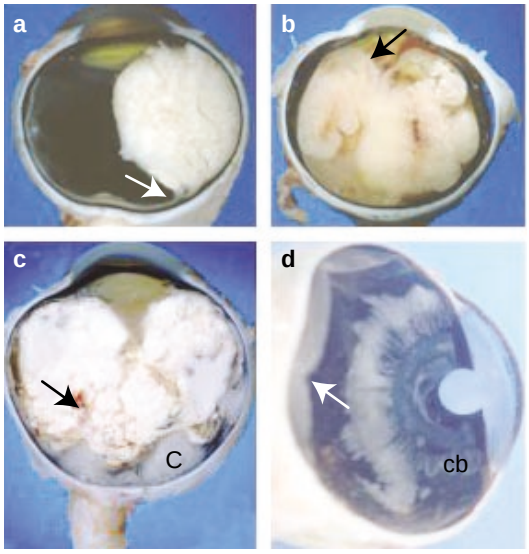
Histology

Histological examination of an affected eye in Rb shows one or more tumours with large areas of necrosis and multifocal calcifications replacing portions of the retina. Small hyperchromatic cells with a high nucleus to cytoplasm ratio form the majority of the tumour. The tumour cells are mitotically active but frequently exhibit apoptosis, which results in the release of DNA from cell nuclei (Refs 56, 58, 59). The released DNA forms deposits on the basement membranes of the blood vessels, lens (capsule), retina (internal limiting membrane) and choroid (Bruch's membrane), a finding unique in an ocular tumour (Ref. 65).

Some Rb tumours are poorly differentiated, whereas others exhibit a degree of differentiation represented by formation of highly characteristic rosettes (termed Flexner-Wintersteiner rosettes), which are composed of tall cuboidal cells that circumscribe an apical lumen (Refs 66, 67). The cells in these rosettes have apical projections that are derived from photoreceptors of the cone lineage, which supports the idea that Rb tumours arise from undifferentiated retinal cells that differentiate into photoreceptors (Refs 68, 69, 70, 71, 72). About 6% of tumours show benign photoreceptor differentiation into groups of cells with short cytoplasmic processes, abundant cytoplasm, and small round nuclei characteristic of photoreceptors. These groups of cells resemble a bouquet of flowers and are called 'fleurettes' (Refs 73, 74, 75). Neither significant mitotic activity nor necrosis is observed within the fleurettes (Refs 73, 74, 75).

Metastasis and recurrence

If left untreated, Rb usually fills the eye and completely destroys the internal architecture of the globe. The most common route of spread is by invasion through the optic nerve. Once in the



Growth patterns of retinoblastoma

Published in Expert Reviews in Molecular Medicine by Cambridge University Press 2003

Figure 3. Growth patterns of retinoblastoma. (a) Gross photograph of an eye with an endophytic growth pattern of retinoblastoma. The tumour mass is growing from the retina (arrow) into the vitreous cavity. (b) Gross photograph of an eye with an exophytic growth pattern of retinoblastoma. The tumour is growing from the retina (arrow) into the subretinal space, with associated retinal detachment. (c) Gross photograph of an eye with a mixed growth pattern (the most frequent type of retinoblastoma). The tumour grows into both the vitreous cavity and the subretinal space, with the retina (arrow) entrapped in the middle. The tumour has massively invaded the choroid (C). (d) Gross photograph of an eye with a diffuse retinoblastoma. There is no well-formed mass; instead there are white seeds of tumour cells along the retina (arrow) and ciliary body (cb). Figure reproduced from Ref. 1, with permission from Lippincott Williams & Wilkins (© Copyright 2002, Lippincott Williams & Wilkins) **(fig003 rhh)**.

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

nerve, the tumour cells spread directly along the nerve fibre bundles towards the optic chiasm, or infiltrates through the pia (the vascular membrane of the brain and spinal cord) into the subarachnoid space. From the subarachnoid space, Rb can metastasise to the cerebrospinal fluid (CSF), brain and spine. The second major route of spread is into the orbit from massive involvement of the choroid either via scleral canals (areas within the sclera where ciliary vessels, nerves and vortex veins enter or exit the eye) or by direct extension through the sclera (Ref. 76). Extraocular extension generally occurs within six months if intraocular tumours are left untreated. Extraocular extension dramatically increases the risk of haematogenous and lymphatic spread to the soft tissues and bones (Refs 56, 59).

Histologically, Rb metastases appear less differentiated than intraocular tumours. Rosettes are rarely encountered and fleurettes have never been described. When well-differentiated extraocular tumours appear outside of the orbit, a differential diagnosis of a primary primitive neuroectodermal tumour must be considered.

Metastatic disease is still associated with a poor prognosis. Most clinical findings are not useful in predicting the occurrence of metastasis in children with Rb, although histopathological data provide a fair estimate of its risk. Multivariate statistical analysis has suggested that the most important prognostic indicator for the development of metastasis is the presence of tumour in the optic nerve, and invasion of the choroid by tumour increases the possibility for haematogenous spread (Refs 57, 60, 61, 63, 76, 77, 78, 79, 80).

Treatment of Rb

The management of Rb is multifaceted. The diagnosis and treatment of patients involves a team approach requiring paediatric oncologists, ophthalmologists and radiologists skilled in the treatment of patients with Rb. Child psychologists, social workers, nurses and genetic counsellors who can support families dealing with the difficulties of caring for a child who not only has cancer but might also lose an eye and vision, also have an important team role. The goals of treatment are, most importantly, to save the child's life and, second, to salvage the eye and/or vision. Therapy is tailored to each case based on the overall situation, including the presence of metastatic disease, risks for second cancers, overall health of the patient, laterality of the disease, size and location of the tumour(s), and visual prognosis.

Several medical and surgical options exist for the treatment of Rb (Ref. 81). Currently, primary treatment options include enucleation, laser photocoagulation, cryotherapy, thermotherapy, chemothermotherapy, external beam or plaque radiotherapy, intravenous chemoreduction, systemic chemotherapy for possible metastatic disease, and orbital exenteration (a surgical procedure that removes the eye, all other intraorbital contents, and the eyelids) (Ref. 1). As radiotherapy and systemic chemotherapy can result in substantial toxicity to the child, alternative modalities are being tested to salvage affected eyes without later life-threatening consequences. These alternative approaches include subconjunctival chemoreduction and gene therapy (see below).

Future directions

Despite advances in understanding the aetiology of Rb and in its treatment, many questions remain. If the Rb protein is expressed in most human cells, why are mutations in the *RB1* gene mainly associated with primary malignancies of the retina and bone? Why is the disease found only in human children and not in the young of other species? Why do Rb tumours have such a uniquely characteristic route of tissue invasion that leads to metastases? Can metastatic disease be treated or prevented? Is there an option that will allow tumours to be treated without causing secondary malignancies but will still salvage the eye? The remainder of this article summarises available animal models of Rb and concludes with a summary of novel therapeutic strategies for the treatment of Rb.

Animal models of Rb

The ideal model of Rb would be an animal with the naturally occurring disease; however, Rb is unique to humans. Attempts to develop animal models have provided several model systems, each with distinct advantages but also with limitations (Ref. 82). Most animal models are xenograft models that are created by injecting human Rb tumour cells into the anterior chamber (AC), subretinal space or vitreous body of immunodeficient mice or rats. Injection of tumour cells into the AC is favoured because of the accessibility of the site for both injection and follow-up observation (Ref. 83).

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

Xenograft models: AC or subretinal injections

Injection of the human Rb cell line Y79 into the ACs of nude mice (a homozygous mutant with a severe defect in cellular immunity) resulted in the invasion of the optic nerve and brain; in contrast, injection into immunologically normal littermates resulted in little tumour growth (Ref. 84). These studies did not clarify whether the tumour spread by non-specific extension or by specific migration through the optic nerve to the central nervous system as is characteristic of human Rb. Tumour involvement of the AC is a late occurrence in human disease and the physiological environment of the AC differs significantly from that of the vitreous cavity in which naturally occurring tumours form (Refs 85, 86). This AC xenograft model therefore has limited utility for the study of metastatic behaviour.

An animal model in which Rb cells were injected into the subretinal space produced tumours that more closely resemble human Rb in location (Refs 87, 88). However, injections into the subretinal space disrupt the choroid and the retina, and therefore use of this model to study invasiveness and progression of Rb is limited.

Xenograft models: intravitreous injections

Xenograft models of Rb have been developed using intravitreous injections of two different human Rb cell lines into mice (Ref. 89). The characteristics of the tumours formed were reproducibly different. Tumours formed when WERI-Rb cells were injected resembled localised, non-metastatic human Rb. Tumours formed when Y79 cells were injected exhibited the histopathological characteristics of aggressive human Rb; they invaded the optic nerve and brain and metastasised to the contralateral optic nerve, probably resulting in metastasis to other distant sites.

In vitro binding studies have suggested that a specific difference in the membrane protein structure of the cells might partially explain the observed difference in metastatic behaviour of the two animal models. Adherent cell lines derived from monkey choroid, rat C_6 glioma or human Hs 683 glioma (all derived from tissues that are similar in origin to the target tissues of invasive Rb) were found to bind Y79 cells rapidly. Specificity was suggested since Y79 cells did not bind to human embryonic kidney cells. Trypsin abolished binding, suggesting protein on the surface of Y79 cells is involved in the cell–cell interaction. Treatment with neuraminidase had no effect. WERI-Rb cells did not bind to choroidal, glioma or embryonic kidney cell lines under the same experimental conditions. Understanding the nature of these cell–cell interactions might aid in understanding the biochemical events related to metastasis of Rb. Furthermore, this simple in vitro binding assay might predict which patients with Rb are prone to develop metastatic disease. Finally, this in vitro model might provide a simple assay to test for drugs that prevent Rb from invading the choroid and optic nerve.

Transgenic models

Several transgenic models of Rb have been developed (recently reviewed in Ref. 90) that might be useful for studying the molecular events responsible for tumour evolution following *RB1* inactivation in different tissues. Each of the successful transgenic models has been created using viral oncogene products, most notably the SV40 T antigen, to bind and interrupt the function of the endogenous tumour suppressor proteins RB or p53. Several viral oncogene constructs used to create transgenic mice included eye-specific promoters [e.g. interphotoreceptor retinoid-binding protein (IRBP), opsin and crystallin] (Refs 91, 92, 93, 94, 95), although the most frequently used construct for therapeutic studies included the promoter for the human luteinising protein beta subunit (Refs 82, 96). The tumours formed in the eyes of these transgenic mice resemble human Rb in many histopathological characteristics; however, they often lack the photoreceptor rosette phenotype seen in the naturally occurring human disease and there is a higher frequency of tumours developing in the brain and other extraocular locations.

A transgenic model generated by inactivating both alleles of the *RB1* gene has not been established since the homozygous $RB1^{-/-}$ genotype is incompatible with embryonic development. The heterozygous $RB1^{+/-}$ genotype (seen in children with inherited Rb) results only in extra-ocular tumour formation in the mouse (Refs 97, 98, 99).

Novel therapeutic modalities

Subconjunctival chemoreduction for intraocular Rb

To avoid the toxicity of systemically administered chemotherapy, local delivery of these agents to

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

achieve chemoreduction for intraocular Rb is being assessed. Studies in animal models have shown that carboplatin (which has anti-neoplastic activity) penetrates the sclera, allowing effective dosages to be achieved within the vitreous cavity (Refs 100, 101, 102). Subconjunctival injections of carboplatin in humans as either a primary or a secondary treatment have achieved tumour regression within three to four weeks, but the response might not be long-term (Ref. 103). Further investigations will determine the efficacy of local application of carboplatin chemotherapy.

Gene therapy

An alternative approach to local chemoreduction is gene therapy. One form of gene therapy, termed suicide gene therapy (Fig. 4), employs the delivery of the Herpes simplex thymidine kinase gene by a replication-defective adenoviral vector injected directly into the tumour. Ganciclovir, a nucleoside analogue, is then administered intravenously. The tumour cells express the thymidine kinase that phosphorylates the ganciclovir. The resulting nucleotide analogue is a potent inhibitor of DNA synthesis and causes the death of the dividing tumour cells. Non-dividing cells are unaffected (Ref. 104).

The safety of suicide gene therapy has been demonstrated in patients with brain tumours (Ref. 105). Effective reduction of Rb tumours in a mouse model of the disease has also been demonstrated (Ref. 106). An approved Phase I clinical trial is currently testing the safety of this approach for children with Rb at the Texas Children's Cancer Center (http://www.tccc.tch.tmc.edu/diseases/retino/retino_main.html). Although it is too early to draw conclusions, patients treated to date have shown both a reduction in tumour size and elimination of vitreous seeds without any significant toxicity (Ref. 1).

International cooperative studies

Because Rb is uncommon, classical clinical trials to address basic diagnostic and therapeutic questions are difficult to perform. An Rb study group formed by the American College of Surgeons Oncology Group (ACoSOG) (http://surgery.ucdmc.ucdavis.edu/surgonc/ACSOG.html) initiated a study to determine whether cyclosporine can enhance the effects of chemoreduction in patients with localised Rb. The Children's Oncology Group (http://www.childrensoncologygroup.org/) is examining the effectiveness of systemic chemotherapy in preventing distant metastases in patients with locally invasive Rb (Ref. 107).

Concluding remarks

Rb is caused by mutations in the *RB1* gene. The disease occurs sporadically or it can be heritable. Rb occurs only in human children. When the disease presents before metastatic spread, the cure rate is greater than 90%. Clinical research focuses on developing therapeutic options that preserve the eye and vision without long-term toxicity.

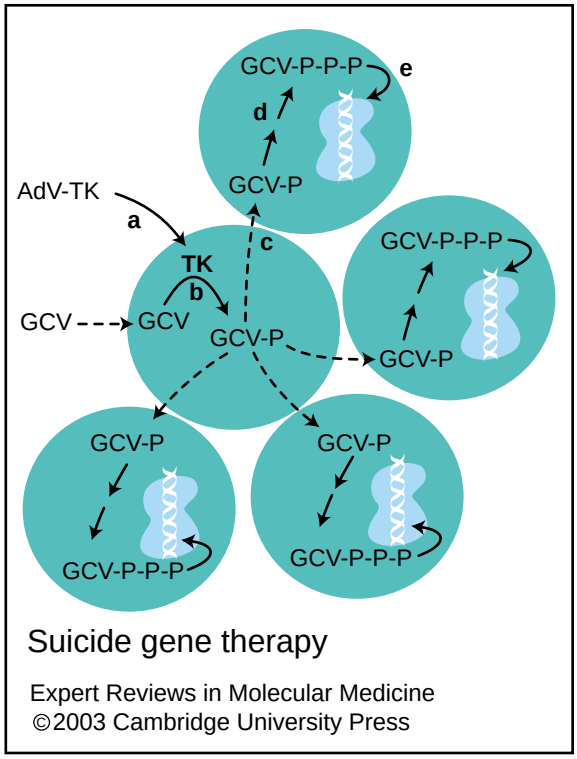


Figure 4. Suicide gene therapy. (a) An adenoviral vector (AdV) delivers the Herpes simplex thymidine kinase (TK) gene to its target tumour cell. (b) The expressed TK phosphorylates the antiviral drug ganciclovir (GCV), a nucleoside analogue, to its nucleotide (GCV-P), a biochemical reaction that mammalian kinases cannot achieve. (c) GCV-P can be transported to adjacent cells through gap junctions where (d) mammalian kinases can add two additional phosphates producing the trinucleotide (GCV-P-P-P). (e) GCV-P-P-P can be incorporated into DNA; however, DNA polymerases cannot replicate DNA containing GCV-P-P-P. Mitosis is interrupted and the cell dies. Because GCV-P can be transported to adjacent cells, not every cell needs to be transduced by the viral vector in order to be killed – the so-called bystander effect **(fig004rhh)**.

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

Several recently developed animal models of Rb will facilitate our understanding of the molecular mechanisms involved in tumour formation and allow the development of new therapeutic options to treat local disease and to prevent metastases.

Acknowledgements and funding

We gratefully acknowledge the Foundation for Research, the Retina Research Foundation, the National Cancer Institute, the General Clinical Research Center at Texas Children's Hospital, the Davenport Foundation, and Texas Children's Hospital for financial support. We thank our peer reviewers Dr Sharon E. Plon and Dr Richard A. Lewis for their constructive comments on the article.

References

1 Hurwitz, R.L. et al. (2002) Retinoblastoma. In Principles and Practice of Pediatric Oncology (4th edn), (Pizzo, P.A. and Poplack, D.G., eds), pp. 825-846, Lippincott, Williams and Wilkins, Philadelphia, USA
2 Devesa, S.S. (1975) The incidence of retinoblastoma. Am J Ophthalmol 80, 263-265, PubMed: 75222705
3 Tamboli, A, Podgor, M.J. and Horm, J.W. (1990) The incidence of retinoblastoma in the United States: 1974 through 1985. Arch Ophthalmol 108, 128-132, PubMed: 90121053
4 Cavenee, W.K. et al. (1983) Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305, 779-784, PubMed: 84039786
5 Byrne, J. et al. (1995) Survival after retinoblastoma: long-term consequences and family history of cancer. Med Pediatr Oncol 24, 160-165, PubMed: 95139877
6 Shields, J.A. (1994) The expanding role of laser photocoagulation for intraocular tumors. The 1993 H. Christian Zweng Memorial Lecture. Retina 14, 310-322, PubMed: 95116739
7 Shields, J.A. et al. (1989) The role of cryotherapy in the management of retinoblastoma. Am J Ophthalmol 108, 260-264, PubMed: 89371891
8 Shields, J.A., Shields, C.L. and De Potter, P. (1994) Clinical management of retinoblastoma. Curr Opin Ophthalmol 5, 83-88, PubMed: 94910446
9 Murphree, A.L. et al. (1996) Chemotherapy plus local treatment in the management of intraocular retinoblastoma. Arch Ophthalmol 114, 1348-1356, PubMed: 97062277
10 Lueder, G.T. and Goyal, R. (1996) Visual function after laser hyperthermia and chemotherapy for macular retinoblastoma. Am J Ophthalmol 121, 582-584, PubMed: 96196207
11 Chan, H.S. et al. (1994) Multidrug resistance. Clinical opportunities in diagnosis and circumvention. Hematol Oncol Clin North Am 8, 383-410, PubMed: 94314740
12 Schappert-Kimmijser, J., Hemmes, G.D. and Nijland, R. (1966) The heredity of retinoblastoma. Ophthalmologica 151, 197-213, PubMed: 67016161
13 Knudson, A.G., Jr. (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68, 820-823, PubMed: 71153958
14 Bonaiti-Pellie, C. and Briard-Guillemot, M.L. (1981) Segregation analysis in hereditary retinoblastoma. Hum Genet 57, 411-419, PubMed: 82029276
15 Hethcote, H.W. and Knudson, A.G., Jr. (1978) Model for the incidence of embryonal cancers: application to retinoblastoma. Proc Natl Acad Sci U S A 75, 2453-2457, PubMed: 78226384
16 Lele, K.P, Penrose, L.S. and Stallard, H.B. (1963) Chromosome deletion in a case of retinoblastoma. Ann Hum Genet 27, 171
17 Chaum, E. et al. (1984) Cytogenetic analysis of retinoblastoma: evidence for multifocal origin and in vivo gene amplification. Cytogenet Cell Genet 38, 82-91, PubMed: 84284390
18 Turleau, C. et al. (1985) Cytogenetic forms of retinoblastoma: their incidence in a survey of 66 patients. Cancer Genet Cytogenet 16, 321-334, PubMed: 85151736
19 Squire, J., Gallie, B.L. and Phillips, R.A. (1985) A detailed analysis of chromosomal changes in heritable and non-heritable retinoblastoma. Hum Genet 70, 291-301, PubMed: 85259629
20 Francke, U. (1976) Retinoblastoma and chromosome 13. Cytogenet Cell Genet 16, 131-134, PubMed: 77025155
21 Ward, P. et al. (1984) Location of the retinoblastoma susceptibility gene(s) and the human esterase D locus. J Med Genet 21, 92-95, PubMed: 84188365
22 Cavenee, W. et al. (1984) Isolation and regional localization of DNA segments revealing polymorphic loci from human chromosome 13. Am J Hum Genet 36, 10-24, PubMed: 84125334
23 Dryja, T.P. et al. (1984) Homozygosity of chromosome 13 in retinoblastoma. N Engl J Med 310, 550-553, PubMed: 84117420
24 Godbout, R. et al. (1983) Somatic inactivation of genes on chromosome 13 is a common event in

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

retinoblastoma. Nature 304, 451-453, PubMed: 83271496
25 Eng, C. et al. (1993) Mortality from second tumors among long-term survivors of retinoblastoma. J Natl Cancer Inst 85, 1121-1128, PubMed: 93308746
26 Hansen, M.F. et al. (1985) Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predisposition. Proc Natl Acad Sci U S A 82, 6216-6220, PubMed: 85298323
27 Dryja, T.P. et al. (1989) Parental origin of mutations of the retinoblastoma gene. Nature 339, 556-558, PubMed: 89281720
28 Zhu, X.P. et al. (1989) Preferential germline mutation of the paternal allele in retinoblastoma. Nature 340, 312-313, PubMed: 89314156
29 Toguchida, J. et al. (1989) Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 338, 156-158, PubMed: 89143788
30 Dryja, T.P., Friend, S. and Weinberg, R.A. (1986) Genetic sequences that predispose to retinoblastoma and osteosarcoma. Symp Fundam Cancer Res 39, 115-119, PubMed: 88098807
31 Huang, H.J. et al. (1988) Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells. Science 242, 1563-1566, PubMed: 89072738
32 Takahashi, R. et al. (1991) The retinoblastoma gene functions as a growth and tumor suppressor in human bladder carcinoma cells. Proc Natl Acad Sci U S A 88, 5257-5261, PubMed: 91271368
33 Bookstein, R. et al. (1988) Human retinoblastoma susceptibility gene: genomic organization and analysis of heterozygous intragenic deletion mutants. Proc Natl Acad Sci U S A 85, 2210-2214, PubMed: 88176914
34 Harbour, J.W. (1998) Overview of RB gene mutations in patients with retinoblastoma. Implications for clinical genetic screening. Ophthalmology 105, 1442-1447, PubMed: 98375486
35 Friend, S.H. et al. (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323, 643-646, PubMed: 87039336
36 Fung, Y.K. et al. (1987) Structural evidence for the authenticity of the human retinoblastoma gene. Science 236, 1657-1661, PubMed: 87263349
37 Dunn, J.M. et al. (1989) Mutations in the RB1 gene and their effects on transcription. Mol Cell Biol 9, 4596-4604, PubMed: 90097870
38 Yandell, D.W. et al. (1989) Oncogenic point mutations in the human retinoblastoma gene: their application to genetic counseling. N Engl J Med 321, 1689-1695, PubMed: 90081757
39 Lohmann, D.R. et al. (1996) The spectrum of RB1 germ-line mutations in hereditary retinoblastoma. Am J Hum Genet 58, 940-949, PubMed: 96216145
40 Yilmaz, S., Horsthemke, B. and Lohmann, D.R. (1998) Twelve novel RB1 gene mutations in patients with hereditary retinoblastoma. Mutations in brief no. 206. Online. Hum Mutat 12, 434, PubMed: 20133869
41 Blanquet, V. et al. (1995) Spectrum of germline mutations in the RB1 gene: a study of 232 patients with hereditary and non hereditary retinoblastoma. Hum Mol Genet 4, 383-388, PubMed: 95315934
42 Cowell, J.K., Jaju, R. and Kempski, H. (1994) Isolation and characterisation of a panel of cosmids which allows unequivocal identification of chromosome deletions involving the RB1 gene using fluorescence in situ hybridisation. J Med Genet 31, 334-337, PubMed: 94351681
43 Szijan, I. et al. (1995) Identification of RB1 germline mutations in Argentinian families with sporadic bilateral retinoblastoma. J Med Genet 32, 475-479, PubMed: 95395826
44 Lee, W.H. et al. (1987) Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 235, 1394-1399, PubMed: 87149066
45 Harbour, J.W. et al. (1988) Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science 241, 353-357, PubMed: 88264450
46 T'Ang, A. et al. (1988) Structural rearrangement of the retinoblastoma gene in human breast carcinoma. Science 242, 263-266, PubMed: 89019355
47 Bookstein, R. et al. (1989) Human retinoblastoma gene: long-range mapping and analysis of its deletion in a breast cancer cell line. Mol Cell Biol 9, 1628-1634, PubMed: 89261791
48 Seizinger, B.R. et al. (1991) Report of the committee on chromosome and gene loss in human neoplasia. Cytogenet Cell Genet 58, 1080
49 Lee, W.H. et al. (1987) The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity. Nature 329, 642-645, PubMed: 88014238
50 Buchkovich, K., Duffy, L.A. and Harlow, E. (1989) The retinoblastoma protein is phosphorylated

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

during specific phases of the cell cycle. Cell 58, 1097-1105, PubMed: 89376561
51 Magnaghi-Jaulin, L. et al. (1998) Retinoblastoma protein represses transcription by recruiting a histone deacetylase. Nature 391, 601-605, PubMed: 98127658
52 Brehm, A. et al. (1998) Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature 391, 597-601, PubMed: 98127657
53 Whyte, P. et al. (1988) Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 334, 124-129, PubMed: 88261565
54 DeCaprio, J.A. et al. (1988) SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. Cell 54, 275-283, PubMed: 88270506
55 Dyson, N. et al. (1989) The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243, 934-937, PubMed: 89146136
56 McLean, I.W. et al. (1994) Tumors of the retina. In Tumors of the Eye and Ocular Adnexa (McLean, I.W. et al., eds), pp. 101-130, Armed Forces Institute of Pathology, Washington D.C.
57 Margo, C.E. and Zimmerman, L.E. (1983) Retinoblastoma: the accuracy of clinical diagnosis in children treated by enucleation. J Pediatr Ophthalmol Strabismus 20, 227-229, PubMed: 84064131
58 Spencer, W.H. (1975) Optic nerve extension of intraocular neoplasms. Am J Ophthalmol 80, 465-471, PubMed: 76016536
59 Zimmerman, L.E., Font, R.L. and Ts'o, M.O. (1972) Application of electron microscopy to histopathologic diagnosis. Trans Am Acad Ophthalmol Otolaryngol 76, 101-107, PubMed: 72179860
60 Karcioglu, Z.A. et al. (1997) Workup for metastatic retinoblastoma. A review of 261 patients. Ophthalmology 104, 307-312, PubMed: 97205130
61 Tosi, P. et al. (1989) Histopathological evaluation for the prognosis of retinoblastoma. Ophthalmic Paediatr Genet 10, 173-177, PubMed: 90067526
62 Croxatto, J.O., Fernandez Meijide, R. and Malbran, E.S. (1983) Retinoblastoma masquerading as ocular inflammation. Ophthalmologica 186, 48-53, PubMed: 83116256
63 Kopelman, J.E., McLean, I.W. and Rosenberg, S.H. (1987) Multivariate analysis of risk factors for metastasis in retinoblastoma treated by enucleation. Ophthalmology 94, 371-377, PubMed: 87230127
64 Donaldson, S.S. and Smith, L.M. (1989) Retinoblastoma: biology, presentation, and current management. Oncology (Huntingt) 3, 45-51; discussion 51-42, PubMed: 90352047
65 Datta, B.N. (1974) DNA coating of blood vessels in retinoblastomas. Am J Clin Pathol 62, 94-96, PubMed: 74267189
66 Wintersteiner, H. (1897) Das neuroepithelioma retinae. In Ein Anatomische und Klinische Studie (Doeticke, F., ed.), p. 14, Dentisae
67 Ts'o, M.O., Fine, B.S. and Zimmerman, L.E. (1969) The Flexner-Wintersteiner rosettes in retinoblastoma. Arch Pathol 88, 664-671, PubMed: 70056971
68 Tajima, Y. et al. (1994) Cytodiagnostic clues to primary retinoblastoma based on cytologic and histologic correlates of 39 enucleated eyes. Acta Cytol 38, 151-157, PubMed: 94196969
69 Kivela, T. (1986) Neuron-specific enolase in retinoblastoma. An immunohistochemical study. Acta Ophthalmol (Copenh) 64, 19-25, PubMed: 86182769
70 Donoso, L.A., Folberg, R. and Arbizo, V. (1985) Retinal S antigen and retinoblastoma. A monoclonal antibody histopathologic study. Arch Ophthalmol 103, 855-857, PubMed: 85225035
71 Bogenmann, E., Lochrie, M.A. and Simon, M.I. (1988) Cone cell-specific genes expressed in retinoblastoma. Science 240, 76-78, PubMed: 88178076
72 Hurwitz, R.L. et al. (1990) Expression of the functional cone phototransduction cascade in retinoblastoma. J Clin Invest 85, 1872-1878, PubMed: 90270419
73 Ts'o, M.O., Zimmerman, L.E. and Fine, B.S. (1970) The nature of retinoblastoma. I. Photoreceptor differentiation: a clinical and histopathologic study. Am J Ophthalmol 69, 339-349, PubMed: 70136919
74 Ts'o, M.O., Fine, B.S. and Zimmerman, L.E. (1970) The nature of retinoblastoma. II. Photoreceptor differentiation: an electron microscopic study. Am J Ophthalmol 69, 350-359, PubMed: 70136920
75 Ts'o, M.O. et al. (1970) A cause of radioresistance in retinoblastoma: photoreceptor differentiation. Trans Am Acad Ophthalmol Otolaryngol 74, 959-969, PubMed: 71002865
76 Khelfaoui, F. et al. (1996) Histopathologic risk factors in retinoblastoma: a retrospective study of 172 patients treated in a single institution. Cancer

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

77, 1206-1213, PubMed: 96232477
77 Messmer, E.P. et al. (1991) Risk factors for metastases in patients with retinoblastoma. Ophthalmology 98, 136-141, PubMed: 91179691
78 Shields, C.L. et al. (1993) Choroidal invasion of retinoblastoma: metastatic potential and clinical risk factors. Br J Ophthalmol 77, 544-548, PubMed: 94032076
79 Shields, C.L. et al. (1994) Optic nerve invasion of retinoblastoma. Metastatic potential and clinical risk factors. Cancer 73, 692-698, PubMed: 94130246
80 Chantada, G.L. et al. (1999) Retinoblastoma with low risk for extraocular relapse. Ophthalmic Genet 20, 133-140, PubMed: 99451194
81 Shields, J.A. (1992) Misconceptions and techniques in the management of retinoblastoma. The 1992 Paul Henkind Memorial Lecture. Retina 12, 320-330, PubMed: 93134161
82 Howes, K.A. et al. (1994) Photoreceptor cell tumors in transgenic mice. Invest Ophthalmol Vis Sci 35, 342-351, PubMed: 94156616
83 Madreperla, S.A. et al. (1991) Intraocular tumor suppression of retinoblastoma gene-reconstituted retinoblastoma cells. Cancer Res 51, 6381-6384, PubMed: 92034773
84 Gallie, B.L. et al. (1977) Heterotransplantation of retinoblastoma into the athymic 'nude' mouse. Invest Ophthalmol Vis Sci 16, 256-259, PubMed: 77140306
85 Tripathi, R.C. et al. (2000) Aqueous humor. In Fundamentals and Principles of Ophthalmology (Weingeist, T.A., Liesegang, T.J.and Grand, M.G., eds), pp. 325-328, The Foundation of the American Academy of Ophthalmology, USA
86 Tripathi, R.C. et al. (2000) Vitreous. In Fundamentals and Principles of Ophthalmology (Weingeist, T.A., Liesegang, T.J. and Grand, M.G., eds), pp. 338-343, The Foundation of the American Academy of Ophthalmology, USA
87 Rowe, S.G., Lee, W.H. and Madreperla, S. (1992) Subretinal and vitreous growth of human retinoblastoma cells in the mouse eye. Invest Ophthalmol Vis Sci 33, 875
88 del Cerro, M. et al. (1993) Transplantation of Y79 cells into rat eyes: an in vivo model of human retinoblastomas. Invest Ophthalmol Vis Sci 34, 3336-3346, PubMed: 94041941
89 Chévez-Barrios, P. et al. (2000) Metastatic and nonmetastatic models of retinoblastoma. Am J Pathol 157, 1405-1412, PubMed: 20476224
90 Mills, M.D., Windle, J.J. and Albert, D.M. (1999) Retinoblastoma in transgenic mice: models of hereditary retinoblastoma. Surv Ophthalmol 43, 508-518, PubMed: 99343182
91 Baehr, W. et al. (1988) Isolation and analysis of the mouse opsin gene. FEBS Lett 238, 253-256, PubMed: 89005694
92 Borst, D.E. and Nickerson, J.M. (1988) The isolation of a gene encoding interphotoreceptor retinoid-binding protein. Exp Eye Res 47, 825-838, PubMed: 89107463
93 Fong, S.L. et al. (1990) Characterization and comparative structural features of the gene for human interstitial retinol-binding protein. J Biol Chem 265, 3648-3653, PubMed: 90154038
94 Nathans, J. and Hogness, D.S. (1984) Isolation and nucleotide sequence of the gene encoding human rhodopsin. Proc Natl Acad Sci U S A 81, 4851-4855, PubMed: 84272729
95 al-Ubaidi, M.R. et al. (1992) Bilateral retinal and brain tumors in transgenic mice expressing simian virus 40 large T antigen under control of the human interphotoreceptor retinoid-binding protein promoter. J Cell Biol 119, 1681-1687, PubMed: 93107167
96 Albert, D.M. et al. (1994) Transgenic models of retinoblastoma: what they tell us about its cause and treatment. Trans Am Ophthalmol Soc 92, 385-400, PubMed: 95193163
97 Clarke, A.R. et al. (1992) Requirement for a functional Rb-1 gene in murine development. Nature 359, 328-330, PubMed: 93024878
98 Jacks, T. et al. (1992) Effects of an Rb mutation in the mouse. Nature 359, 295-300, PubMed: 93024873
99 Lee, E.Y. et al. (1992) Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. Nature 359, 288-294, PubMed: 93024872
100 Mendelsohn, M.E. et al. (1998) Intraocular concentrations of chemotherapeutic agents after systemic or local administration. Arch Ophthalmol 116, 1209-1212, PubMed: 98418453
101 Murray, T.G. et al. (1997) Subconjunctival carboplatin therapy and cryotherapy in the treatment of transgenic murine retinoblastoma. Arch Ophthalmol 115, 1286-1290, PubMed: 97478380
102 Harbour, J.W. et al. (1996) Local carboplatin therapy in transgenic murine retinoblastoma. Invest Ophthalmol Vis Sci 37, 1892-1898, PubMed: 96335097
103 Abramson, D.H., Frank, C.M. and Dunkel, I.J. (1999) A phase I/II study of subconjunctival carboplatin for intraocular retinoblastoma. Ophthalmology 106, 1947-1950, PubMed: 99447079

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003

104 Chen, S.H. et al. (1994) Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer in vivo. Proc Natl Acad Sci U S A 91, 3054-3057, PubMed: 94211800
105 Trask, T.W. et al. (2000) Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. Mol Ther 1, 195-203, PubMed: 20397457
106 Hurwitz, M.Y. et al. (1999) Suicide gene therapy for treatment of retinoblastoma in a murine model. Hum Gene Ther 10, 441-448, PubMed: 99156141
107 Honavar, S.G. et al. (2002) Postenucleation adjuvant therapy in high-risk retinoblastoma. Arch Ophthalmol 120, 923-931, PubMed: 22091744
108 Ying Qian and M. Reza Dana (2001) Molecular mechanisms of immunity in corneal allotransplantation and xenotransplantation. Exp. Rev. Mol. Med. 16 July, http://www.expertreviews.org/01003246h.htm

Further reading, resources and contacts

A recent article by Kolch and colleagues published in *Expert Reviews in Molecular Medicine* includes a discussion of Rb function in the context of Raf kinase signalling:

Walter Kolch, Ashwin Kotwaliwale, Keith Vass and Petra Janosch (2002) The role of Raf kinases in malignant transformation. Exp. Rev. Mol. Med. 25 April, http://www.expertreviews.org/02004386h.htm

The Retinoblastoma Center at theTexas Children's Cancer Center provides treatment for retinoblastoma patients from around the world, as well as conducting basic and clinical research into the disease. A Phase I clinical trial evaluating gene therapy as a treatment for Rb is open for patient accrual at the Center:

http://www.tccc.tch.tmc.edu/diseases/retino/retino_main.html

The National Retinoblastoma Research and Support Foundation (USA) and The Retinoblastoma Society (UK) provide patient information and support:

http://www.djo.harvard.edu/meei/PI/RB/NRRSF.html
http://webspace.dial.pipex.com/rbinfo/

The Children's Oncology Group (COG) grew out of the merger of four US paediatric cancer research organisations, and its primary objective is to conduct clinical trials of new therapies for children's cancer:

http://www.childrensoncologygroup.org/

The American College of Surgeons Oncology Group (ACoSOG) is a new cooperative group sponsored by the National Cancer Institute (NCI) with a focus on conducting trials in surgical oncology:

http://surgery.ucdmc.ucdavis.edu/surgonc/ACSOG.html

Features associated with this article

Figures

Figure 1. Retinoblastoma tumour development: loss of heterozygosity (fig001rhh).
Figure 2. Photograph of an eye from a patient with retinoblastoma (fig002rhh).
Figure 3. Growth patterns of retinoblastoma (fig003rhh).
Figure 4. Suicide gene therapy (fig004rhh).

Citation details for this article

Richard L. Hurwitz, Patricia Chévez-Barrios, Milton Boniuk, Murali Chintagumpala and Mary Y. Hurwitz (2003) Retinoblastoma: from bench to bedside. Exp. Rev. Mol. Med. 7 January, DOI: 10.1017/S1462399403005520

Accession information: DOI: 10.1017/S1462399403005520; 7 January 2003