Published in final edited form as:

Clin Experiment Ophthalmol. 2014 January ; 42(1): 33–52. doi:10.1111/ceo.12132.

The genomic landscape of retinoblastoma: a review

Brigitte L Thériault, PhD[1], **Helen Dimaras, PhD**[2,3,4], **Brenda L Gallie, MD**[1,2,3,5], and **Timothy W Corson, PhD**[6]

[1]The Campbell Family Cancer Research Institute, Ontario Cancer Institute, Princess Margaret Cancer Centre, Toronto, Ontario, Canada

[2]The Department of Ophthalmology & Vision Science, University of Toronto, Toronto, Ontario, Canada

[3]The Division of Visual Science, Toronto Western Research Institute, Toronto, Ontario, Canada

[4]Sick Kids Research Institute, Toronto, Ontario, Canada

[5]The Departments of Medical Biophysics and Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

[6]Eugene and Marilyn Glick Eye Institute, Departments of Ophthalmology and Biochemistry and Molecular Biology and Simon Cancer Center, Indiana University School of Medicine, Indianapolis, Indiana, USA

Abstract

Retinoblastoma is a paediatric ocular tumour that continues to reveal much about the genetic basis of cancer development. Study of genomic aberrations in retinoblastoma tumours has exposed important mechanisms of cancer development, and identified oncogenes and tumour suppressors that offer potential points of therapeutic intervention. The recent development of next-generation genomic technologies has allowed further refinement of the genomic landscape of retinoblastoma at high resolution. In a relatively short period of time, a wealth of genetic and epigenetic data has emerged on a small number of tumour samples. These data highlight the inherent molecular complexity of this cancer, despite the fact that most retinoblastomas are initiated by the inactivation of a single tumour suppressor gene. Here, we review the current understanding of the genomic, genetic and epigenetic changes in retinoblastoma, highlighting recent genome-wide analyses that have identified exciting candidate genes worthy of further validation as potential prognostic and therapeutic targets.

Keywords

retinoblastoma; cytogenetics; epigenetics; microRNAs; single nucleotide polymorphisms

Correspondence: Dr. Timothy W. Corson, Eugene and Marilyn Glick Eye Institute, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA, tcorson@iupui.edu.

Competing/conflicts of interest: BLG was part owner of Solutions by Sequence and Member of the Board of Retinoblastoma Solutions, which are now merged into Impact Genetics with BLG as Medical Director. All other authors declare that they have no conflicts of interest.

INTRODUCTION

Retinoblastoma – a genetic disease

Retinoblastoma is a paediatric eye tumour arising in the retina, representing the most common childhood intraocular malignancy.[1] Retinoblastoma was the first disease demonstrating a genetic basis for cancer development,[2] initiated by biallelic inactivation of the *RB1* gene.[3] More recently, as discussed later in this review, a second genetic form of retinoblastoma has been discovered: that initiated by amplification of the *MYCN* gene.

Retinoblastoma is either heritable or non-heritable. The heritable form can result in tumours affecting either one (unilateral, 60% of all cases) or both (bilateral) eyes, while the non-heritable form leads only to unilateral tumours. All bilateral retinoblastoma is heritable and tends to present at an earlier age, whereas unilateral retinoblastoma is heritable in only a small percentage (15%) of cases.[3–5] All heritable retinoblastoma results from biallelic *RB1* inactivation; the first *RB1* mutation (M1) is constitutional, while the second mutation (M2) occurs somatically in one or more retinal cells.[3] In a small proportion of cases, M1 occurs in one cell of the multicell embryo, resulting in mosaicism in the proband.[5] Most non-heritable retinoblastomas are caused by biallelic *RB1* loss where both *RB1* mutational events (M1 and M2) arise in a single somatic retinal cell. A small fraction of non-heritable retinoblastoma result from *MYCN* amplification with normal *RB1*, also arising somatically in a single retinal cell (see below). A predisposing constitutional *RB1* mutation leads to earlier age of presentation (15 months for bilateral vs. 27 months for unilateral in developed countries).[3]

With an incidence of 1 in 15,000 to 20,000 live births, translating to approximately 9,000 new cases every year worldwide,[3,6] the impact of retinoblastoma on health care systems continues after initial diagnosis and treatment. Constitutional mutation of the *RB1* gene predisposes individuals to second cancers later in life, such as lung, bladder, bone, skin and brain cancers.[7] The heritable nature and second cancer susceptibility associated with retinoblastoma translates into a need for life-long follow-up, such as genetic testing and counseling for families and offspring to determine heritable risk, and to monitor for and treat second cancers.

Discovery of a tumour suppressor and initial genomic profiling

Over 40 years ago, Knudson proposed that retinoblastoma was initiated by inactivation of a putative tumour suppressor gene.[1] His mathematical study of the discrepancy in the time to diagnosis between unilateral and bilateral patients led to the hypothesis that two mutational events are rate limiting for the development of retinoblastoma. This postulate was further refined by Comings in 1973 to suggest that mutation of two alleles of a single gene was the cause.[8] These studies informed the discovery of the first tumour suppressor gene, *RB1* on chromosome 13q14.[9–11] We later confirmed that both alleles of the *RB1* gene are indeed mutated in retinoblastoma.[12] Study of the benign, non-proliferative precursor lesion retinoma led us to discover that loss of function of the *RB1* gene can initiate retinoma, but is insufficient for the development of retinoblastoma.[13]

We postulated that additional genetic changes, termed M3-Mn in keeping with Knudson's nomenclature, are required for the progression of benign retinoma to malignant retinoblastoma.[13,14] Early genomic profiling through karyotype analyses and comparative genomic hybridization (CGH) studies indeed revealed that retinoblastomas also contained many genomic changes, including recurrent gains of chromosome 1q, 2p and 6p, and losses of chromosome 13q and 16q.[14] We and others went on to map specific regions of gains/losses to develop a genomic signature of putative M3-Mn events, subsequently identifying

oncogenes and tumour suppressors in these regions that could facilitate tumour progression.[15,16]

New genomic technologies, new horizons

These initial efforts in the genomic profiling of retinoblastomas led to an explosion in the study of the molecular pathogenesis of this cancer, but the importance of these findings translates beyond retinoblastoma, as many similar genomic changes have been identified in other cancers.[17–20] Recent advances in genomic (single nucleotide polymorphism [SNP] analysis and next-generation sequencing) and epigenetic (methylation and miRNA) analysis methodologies now allow us a "high-resolution" view of specific aberrations. These techniques open the door to enhanced understanding of retinoblastoma development and progression, moving towards potentially curative therapeutic interventions. However, a higher-resolution view has also uncovered an even more complex genomic landscape in individual retinoblastomas that requires careful validation.

This review summarizes our current understanding of the retinoblastoma genome. We highlight the candidates that have emerged as the most tangible therapeutic targets. We also examine in detail the emerging genome-wide expression, sequencing and epigenetic data that will contribute to a greater understanding of initiation and progression of retinoblastoma, and possibly offer even better targets for prevention and cure in the future (Figure 1).

RECURRENT GAINS AND LOSSES REVEAL CANDIDATE ONCOGENES AND TUMOUR SUPPRESSORS IN RETINOBLASTOMA

We have previously reviewed in detail[14] the first karyotypic, CGH and array CGH (aCGH) studies leading to the identification of minimal regions of gain (MRGs) and minimal regions of loss (MRL) frequent in retinoblastomas, including chromosome gains at 1q32, 2p24, 6p22, and losses at 13q and 16q22–24. New technology has subsequently validated these initial discoveries. Candidate oncogenes in the retinoblastoma genome include *MDM4* (also known as *MDMX*),[16,21] *KIF14*,[18] *MYCN*,[15] and *DEK* and *E2F3*,[22] plus a candidate tumour suppressor, *CDH11* (Table 1).[23] There is mounting evidence implicating these genes as drivers in retinoblastoma progression.

MDM4 – mouse double minute 4, human homolog (1q32.1)

MDM4 is a nuclear protein that binds through its transcriptional activation domain to inhibit p53 activity. It also binds and inhibits the activity of the E3 ubiquitin ligase MDM2, which mediates the degradation of p53.[24] Genomic amplification and overexpression of *MDM4* have been noted in multiple cancers, including glioblastoma, cutaneous melanoma, osteosarcoma, breast and colorectal carcinomas; these changes are more frequent in tumours with wild-type *TP53*.[25] Amplification and overexpression of *MDM4* has been observed in 65% of retinoblastomas in comparison to fetal retina,[21] and could explain inactivation of the p53 pathway without any genetic alteration of the *TP53* gene in retinoblastoma.[14]

Functional analyses of MDM4 have shown that in mice lacking *Rb1* and *Rbl1* (p107), MDM4 could promote tumorigenesis, and that treatment of retinoblastoma cell line xenografts with the small molecule nutlin-3, which targets the p53-MDM2/4 interaction, can reduce tumour growth.[21] Subconjunctival delivery of nutlin-3 in preclinical models of retinoblastoma demonstrated some efficacy in mediating p53-dependent cell death in retinoblastoma.[26] However recent evidence has shown that only a small number of retinoblastomas overexpress *MDM4* mRNA and protein vs. normal retinal tissues,[27] suggesting that other mechanisms of p53 pathway inactivation, such as loss of the $p14^{ARF}$

tumour suppressor protein expression could be responsible for progression of some retinoblastomas.[28]

KIF14 – Kinesin Family Member 14 (1q32.1)

KIF14 is a mitotic kinesin and molecular motor essential for the last stages of cytokinesis.[29,30] *KIF14* is overexpressed in over 50% of primary retinoblastomas. In retinoma lesions, gain of 1q32 is the most prevalent karyotypic abnormality following loss of the *RB1* gene. These findings point to *KIF14* gain as a possible M3 event.[13,18,19] Gene-specific analysis of retinomas via fluorescence *in-situ* hybridization (FISH) confirmed that *KIF14* gain was present in all retinomas studied, while gain of *MDM4* was present in a smaller proportion of cases. This highlights the significance of the *KIF14* oncogene as a potential driving event in the progression of retinoblastoma.[13] Genomic gain of 1q is seen in many other cancer types, including breast, lung, liver, papillary renal cell, esophageal, glioblastoma, ovarian cancers and meningiomas.[31] The *Kif14* locus was also gained in the SV40 large T antigen-induced model of retinoblastoma (TAg-RB),[32] and *KIF14* showed low-level amplification in a human retinoblastoma.[15]

KIF14 mRNA is overexpressed in retinoblastomas,[18,33] in TAg-RB, and in many other cancers.[17,19,20,32,34,35] Expression correlates with poor prognosis in breast,[17] lung,[19] and ovarian cancers.[20] Stable or transient knockdown of *KIF14* significantly reduces proliferation, migration and colony formation in established cancer cell lines *in vitro*,[19,20,36] pointing to a crucial role for KIF14 in tumour formation and progression, perhaps separate from its role in cytokinesis. KIF14 was recently shown to interact in a specific manner with Radil, a crucial mediator of Rap1a–mediated integrin inside-out signalling.[36] KIF14 controls the amount of Radil-Rap1a activity at the cell membrane to promote cell adhesion and migration, favouring metastatic progression in breast cancer cells. These studies demonstrate *KIF14* as an important oncogene promoting tumorigenesis in multiple cancers, offering opportunities for therapeutic disruption of specific cancer-causing protein interactions.[37]

There is still debate as to which 1q gene is important in retinoblastoma development. *MDM4* and *KIF14* are located within 4 Mbp of each other at chromosome 1q32.1. Via high-resolution quantitative multiplex PCR of five sequence-tagged sites spanning 1q25.3 – 1q41, we identified in retinoblastoma and breast tumours a 3.06 Mbp MRG spanning 1q31.3 – 1q32.1.[18] This region contained *KIF14*, but excluded *MDM4*. Via CGH, others have identified MRGs in retinoblastomas encompassing both candidate genes.[16,38,39] Two studies concluded that *MDM4* was the candidate gene within the MRG; although *KIF14* was located within the defined MRG, its genomic expression was not tested.[16,40] In any case, the fact that we and other groups have shown gene-specific gain of both *MDM4* and *KIF14* in retinomas and retinoblastomas[13,18,21,41] underscores the importance of both genes in this 1q region of gain in the pathogenesis of retinoblastoma.

MYCN - v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (2p24.3)

MYCN encodes N-Myc, a basic helix-loop-helix protein (bHLH) that binds with other bHLH proteins, acting as a transcription factor to control the expression of cell cycle genes that promote proliferation.[42] It is frequently amplified in tumours of neuroectodermal origin, including neuroblastoma, retinoblastoma, glioblastoma, medulloblastoma, rhabdomyosarcoma and small cell lung carcinoma,[42–49] and is associated with poor prognosis in neuroblastoma.[50] *MYCN* is also amplified in the archetypal retinoblastoma cell line Y79,[51] and has been reported amplified in approximately 3% of primary retinoblastomas.[14,15]

Through an international collaboration with four other clinical *RB1* testing centres, we have recently discovered a subset of retinoblastomas that possess a wild type *RB1* gene and a full-length, functional pRb protein ($RB1^{+/+}$, as evidenced by presence of hypo- and hyperphosphorylated forms of pRb that bind to E2F1), but demonstrate high-level amplification of the *MYCN* gene (28 to 121 copies; $MYCN^{A}$).[52] These $RB1^{+/+}$, $MYCN^{A}$ tumours, which represent approximately 1% of all retinoblastomas, have a relatively stable genome by aCGH, apart from *MYCN* amplification. The frequency of copy number alterations was significantly reduced in $RB1^{+/+}$, $MYCN^{A}$ tumours vs. $RB1^{-/-}$ or $RB1^{+/-}$ tumours. The minimal amplicon was found in two primary tumours to contain only the *MYCN* gene, in contrast to previous studies in $RB1^{-/-}$ retinoblastoma and neuroblastoma tumours where *MYCN* co-amplified with genes *NAG* and *DDX1*.[53–55]

Of 15 $RB1^{+/+}MYCN^{A}$ tumours evaluated, three showed unusual changes at chromosome 17q (17q21.3-qter or 17q24.3-qter gain), while two tumours showed 11q loss. Both regions are commonly altered in neuroblastoma, but rare in $RB1^{-/-}$ retinoblastoma. Other changes included gains at 14q and 18q, and losses at 11p.[52] These unilateral, $MYCN^{A}$ tumours possess histologic features similar to neuroblastoma (large prominent nucleoli) and present with large, invasive tumours at a young age (4 to 5 months) compared to *RB1*-inactivated, unilateral, non-familial retinoblastoma (27 months).[3] This new subset of retinoblastoma challenges the dogma that this cancer is always initiated by the loss of both copies of the *RB1* gene. These findings have immediate clinical impact, as patients with $RB1^{+/+}MYCN^{A}$ tumours have *MYCN* amplification only in the tumour cells. These children have no special risk for retinoblastoma or second cancers later in life, and may benefit from future *MYCN*-directed therapies to save vision.

DEK - oncogene, DNA binding (6p22.3), and E2F3 – E2F transcription factor 3 (6p22.3)

Genes for DEK, a chromatin remodelling factor and histone chaperone protein, and E2F3, a pRb-regulated transcription factor crucial in cell cycle control, are located within a small region on chromosome 6p22.3. Genes on 6p are frequently gained (54% by CGH) and overexpressed in retinoblastomas, manifest as an isochromosome 6p (i(6p)).[14] However, spectral karyotyping and multicolour banding analyses identified novel 6p rearrangements and recurrent translocations in many retinoblastoma cell lines, pointing to additional mechanisms of gain for the short arm of chromosome 6p and activation of the *DEK* and *E2F3* oncogenes.[56]

Although frequently gained and overexpressed together,[57] there are instances where gain of only one gene is present. In some retinomas, gene-specific gain of *DEK* is present while *E2F3* remains 2-copy,[13] while primary retinoblastomas show a higher proportion of tumours with *E2F3* gain (70%) than gain of *DEK* (40%);[15] this ratio is similar in the TAg-RB mouse model.[32] Furthermore some evidence points to transcriptional regulation of *DEK* by *E2F3*, adding a level of complexity to the regulation of expression of these oncogenes in cancer.[58] Both *DEK* and *E2F3* have important oncogenic roles in multiple cancers. *DEK* was first discovered in acute myeloid leukemia (AML) as a fusion gene with nucleoporin (*NUP214*) in 1% of the leukemic cells,[59] and is overexpressed in multiple cancers including melanoma, hepatocellular carcinomas, brain tumours, and breast cancers.[14,60] Furthermore, *DEK* overexpression may confer stem cell-like properties on cancer cells that facilitate tumour progression and chemoresistance,[61] demonstrating its importance as a tumour-initiating oncogene. *E2F3* is gained and overexpressed in bladder, prostate, lung and breast cancers,[14,62,63] and siRNA-mediated knockdown of *E2F3* in bladder, prostate and breast cancer cells significantly reduced their proliferative capacity. These results point to inactivation of E2F3 as an attractive therapeutic target in multiple cancers.[62,63]

CDH11 – Cadherin 11 (16q21)

CDH11 (also called osteoblast cadherin) encodes a type II classical cadherin, an integral membrane protein that mediates calcium-dependent cell-cell adhesion that is involved in bone development and maintenance. Loss of *CDH11* is common in osteosarcoma, and correlates with tumour invasion and metastasis.[64,65] *CDH11* has also been identified as a candidate tumour suppressor gene in invasive ductal and lobular breast carcinomas[66] and is involved in invasive gliomas.[67] The *CDH11* promoter is methylated and effectively silenced in many different cancer cell lines, demonstrating its role in tumorigenesis.[68] Furthermore, hypermethylation of the *CDH11* promoter was identified in metastatic cell lines derived from melanomas and head and neck cancers in comparison to their respective primary tumours, strengthening the postulate that loss of CDH11 expression is important in metastatic progression.[69] We identified a hotspot loss of chromosome 16q, and narrowed the MRL to the *CDH11* gene, lost in 58% of retinoblastomas tested. We also studied expression of *Cdh11* in the TAg-RB murine model, and demonstrated that many TAg-RB tumours exhibited loss of *Cdh11*.[23,32]

Subsequently, Laurie et al. documented that loss of *Cdh11* expression correlated with optic nerve invasion in a murine transgenic model of retinoblastoma with functional, retinal progenitor-specific inactivation of p107, pRb and p53 proteins.[70] By crossing the TAg-RB mouse with a *Cdh11*-null mouse, we showed that tumour formation was significantly reduced, and in the tumours that did form, cell proliferation was increased while apoptotic marker expression greatly decreased.[71] These results clearly indicate a tumour suppressive role for *Cdh11* in retinoblastoma development and progression, at least in mice. However, in addition to loss of 16q22, Gratias et al. Identified, by conventional and matrix CGH, loss of heterozygosity at 16q24, an MRL encompassing the potential tumour suppressor *CDH13*.[72] Loss of this region also associated with intraocular seeding, implicating *CDH13*, perhaps in addition to *CDH11*, as an important tumour suppressor in retinoblastoma.[72]

EMERGING TARGETS FROM GENOMIC AND EXPRESSION ARRAY ANALYSES

Genomic analyses

Since our previous review,[14] high-resolution aCGH and SNP array analyses of retinoblastomas have revealed novel regions of genomic imbalance pointing to new target genes (Table 2; Figure 1). One study profiled tumour from 10 bilateral and 8 unilateral patients, 2 who had retinoma. In addition to the characteristic genomic changes such as gain at 1q, 2p, and 6p, and losses at 13q and 16q, there were gains in two small regions of chromosome 9 (9q22.2 and 9q33.1) and loss at 11q24.3 in retinoblastomas, along with gains at 6p, 5p and 5q in retinomas.[41] The MRGs defined in this study encompassed larger chromosomal regions than were previously characterized (1q12 – 25.3, 6p25.3 – 11.1 and 16q12.1 – 21), but also excluded previously characterized regions, such as 1q32.1. Thus additional candidate genes were identified on 1q such as *MUC1*, a membrane-bound protein overexpressed in many epithelial cancers that confers resistance to apoptosis, *MCL1*, a member of the Bcl-2 family of anti-apoptotic proteins (previously shown to be overexpressed in retinoblastoma[40]), and *SHC1*, a signalling adapter molecule that mediates the transforming activity of oncogenic tyrosine kinases, and also identified as a candidate target gene from a previous study.[16]

On chromosome 11, the novel candidate *ETS1*, a well-known transcription factor involved in proliferation, senescence and tumorigenesis was also identified. On chromosome 13q, a recently characterized tumour suppressor gene, *ARLTS1*, was identified as being within the MRL.[73] It encodes a Ras family pro-apoptotic protein, and its loss of function (through

deletion, SNPs and methylation) has been demonstrated in multiple cancers including breast, lung and ovarian cancers.[41]

In addition to loss of *CDH11* on chromosome 16q, the MRL encompassed the tumour suppressor gene *RBL2* encoding the RB family member p130. *RBL2* was previously identified as an important tumour suppressor gene involved in the progression of human[13,74] and mouse retinoblastomas.[75] One study found high frequency (close to 60%) loss of 16q22 specifically encompassing the *RBL2* gene in 19 primary retinoblastomas, further implicating this gene as an important player in the progression of retinoblastoma.[76] Sampieri and colleagues evaluated chromosomal gains common between retinoma and retinoblastoma that included the oncogenes *DEK* and *E2F3*, in agreement with previous findings.[13] Novel gains of 5p and 5q were also found in both retinoma and retinoblastoma tissues; potential candidate genes identified within these regions included the oncogene *SKP2* (p45; 5p13) known to be overexpressed in many cancers and involved in the ubiquitin-mediated degradation of p27, and *BIRC1* (on 5q13.2), an inhibitor of apoptosis that suppresses caspase activity.[41]

Using a whole genome sampling array (WGSA), Ganguly et al. also identified novel regions of gain/loss in 25 unilateral retinoblastomas compared to their matched normal tissues.[38] Novel regions of gain included chromosomes 1q44, 3p25. 11q14, 11q25, 14q23, 15q21, 16p13, 17p11, 19q13 and 20q13, while regions of loss included 6p22, 7p21 and 21q2. On chromosome 1q, *MDM4* and *GAC1*, genes involved in the regulation of p53 activity were identified. *SMYD3* was identified as a novel target, located on 1q44. It is a histone methyltransferase that regulates the transcriptional activity of the RNA polymerase complex. Another novel gain on chromosome 14q23 identified the target gene *CEP170*, a protein involved in centriole architecture that gives rise to chromosomal abnormalities during mitosis. *SIX1* and *SIX4*, homeobox proteins, were also identified as novel target genes within this region. Along with *CEP170*, these genes are all associated with bilateral anophthalmia, further pointing to the potential importance of developmental genes in the formation and progression of retinoblastoma.[38] To our knowledge, further expression and functional analyses of these genes in retinoblastoma have not yet been reported.

A recent study by Livide et al. identified a number of novel targets in retinoblastomas.[77] Using a methylation specific multiplex ligation probe assay (MS-MLPA), they analysed a total of 39 genes in 12 tumours with corresponding normal retinal tissues, and found alterations in 25 genes.[77] These included gains in *TNXB* (6p21), an anti-adhesion extracellular matrix glycoprotein involved in tumour progression, and deletions in *TP53*, *CDH11*, *GATA5*, *CHFR*, *TP73*, *IGSF4* and *BRCA2*, as well as changes in the methylation status of a number of additional genes (see below).

Microarray expression studies

Surprisingly few studies have taken a comprehensive look at differential gene expression patterns in retinoblastomas (Table 2). Chakraborty et al. conducted a comparative microarray analysis between 10 retinoblastomas and 3 adult retina samples. They identified deregulated genes in functional classes including the insulin and JAK/STAT signalling pathways, axon guidance, extracellular matrix–receptor interactions, proteasome, sugar metabolism, ribosomes, cell adhesion molecules, and tight junction complexes.[78] Confirmatory semi-quantitative RT-PCR analysis validated insulin signalling pathway genes (*PIK3CA*, *AKT1*, *FRAP1* and *RPS6KB1*) as significantly upregulated in tumours vs. normal tissues, suggesting that the PI3K/AKT/mTOR/S6K1 signalling pathway is dysregulated in retinoblastoma. Other upregulated genes included *CDC25A*, a cell cycle progression gene, and *ERBB3*, involved in cancer development and progression.

LATS2, a serine-threonine kinase and tumour suppressor, and *CHFR*, a mitotic checkpoint pathway gene, were found downregulated in retinoblastomas. Regional biases of gene expression were also found, where gene expression changes mapped to particular chromosomal regions, including clusters of upregulated genes mapping to chromosomes 16 and 17, and clusters of downregulated genes mapping to chromosome 1. Interestingly, none of the previously validated retinoblastoma candidate genes on chromosomes 1 and 16 (*KIF14*, *MDM4*, *CDH11*)[14] were identified as differentially expressed in this study, perhaps due to the small sample size or use of adult retina as comparator. Nonetheless, these data encourage future testing of known pathway inhibitors for retinoblastoma treatment.

A recent study undertook a microarray expression comparison between matched normal retina and retinoblastoma tissues of 6 patients.[79] Increased expression was seen for 1116 genes, and 837 genes showed decreased expression in tumours vs. normal retina. These genes fell into functional groups including cell cycle regulation, cell death, DNA replication, recombination and repair, cellular growth and proliferation, and cellular assembly and organization. Among these groups, the DNA damage response pathway genes were most differentially expressed and included previously identified players such as breast cancer associated genes *BRCA1* and *2*, *AHR* and *ATM* signalling genes, and G_2/M DNA damage checkpoint regulation genes, polo-like kinase (*PLK1*), *E2F*s, and checkpoint kinase 1 (*CHK1*). The identified kinases are of particular interest, as there are drugs targeting these kinases that could be tested for therapeutic relevance.[79] Interestingly, the authors also found overexpression of cone-cell-specific markers in retinoblastomas, supporting previous reports of a cone progenitor cell of origin for retinoblastoma,[80] or that cone differentiation is a “default” pathway in failed retinal differentiation.

SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH DEVELOPMENT OF RETINOBLASTOMAS

Since *TP53* is rarely mutated in retinoblastoma,[81] other mechanisms of p53 inactivation in these tumours have been discovered, including the genomic gain and overexpression of key inhibitors of p53 activity, *MDM2* and *MDM4* (see above). *MDM2* was the first modifier gene identified in retinoblastoma (Table 3), when Castera et al. identified a T>G transversion SNP at nucleotide 309 in the *MDM2* promoter (rs2279744) to be highly associated with the incidence of bilateral and unilateral retinoblastoma in *RB1* mutation carrier families.[82] This allele confers enhanced transcription of mRNA leading to overexpression and accumulation of the MDM2 protein, effectively abrogating the function of the p53 protein.[82]

The p.Arg72Pro substitution in p53 protein (c.215G>C,) decreases the ability of p53 to induce apoptosis, in essence causing functional inactivation.[83] In development of retinoblastoma, a significant association of the Pro/Pro variant of p.Arg72Pro has been documented, while only a weak negative association was seen with MDM2–309.[84]

MDM4, another key regulator of p53 activity found to be gained and overexpressed in retinoblastomas (see above) is also a genetic modifier in retinoblastoma. Genotype studies of 104 retinoblastoma patients found that both the *MDM2* rs2279744G (vs T) and *MDM4* rs4252668C (vs T) SNPs were present at a higher frequency in control patients, while *MDM2* rs2279744TG and GG genotypes, and the *MDM4* rs116197192G allele were present at high frequency in retinoblastoma patients and associated with poor survival.[85]

To elucidate the relationship between *MDM2/4* SNPs and gene/protein expression, McEvoy and colleagues performed sequencing and expression analysis for *MDM2/4* in 44 retinoblastomas and 3 orthotopic xenografts derived from primary tumours, in comparison

to normal blood and retina tissues. The *MDM2*-309 and *MDM4*-7 (rs1563828C>T) SNPs did not correlate with elevated gene expression in retinoblastomas.[86] However, the orthotopic xenografts demonstrated high levels of MDM4 protein associated with the *MDM4* SNP 34091C>A. This variant was first documented in ovarian cancer to cause high overexpression of *MDM4* due to loss of regulation by miR-191.[87] The orthotopic xenografts had the 34091AA genotype, and this genotype was associated with high levels of expression of the MDM4 protein while maintaining mRNA levels similar to those in fetal retina. These results suggest that SNPs may enforce functional changes in tumour suppressive pathways to promote tumorigenesis in retinoblastoma.

The first whole genome sequencing (WGS) study of retinoblastomas was recently presented (Table 3). Zhang et al. characterized the genetic and epigenetic alterations of 4 primary retinoblastomas along with matched normal tissues. They validated 668 somatic sequence mutations and 40 structural variations, with on average 167 mutations per case, but only 11 genes were found to harbour mutations leading to amino acid changes.[88] Their calculated mutation rate was 15-fold lower than in other tumour types, except for AML. The only structural variations that were identified were loss *RB1* and gain of *MYCN*, suggesting very few genomic changes are present after loss of the *RB1* gene.[88] These results diverge with many other reports demonstrating genomic instability and presence of characterized genomic alterations following loss of *RB1* (see above).[89] The 11 genes containing somatic mutations were further sequenced in 46 retinoblastomas. *BCOR*, a transcriptional corepressor involved in *BCL6* repression, was the only gene that showed recurrent mutation in 13% (6 out of 46) of cases.[88] As *BCOR* mutations are recurrent in AML patients, and it is expressed in the developing retina, this gene may be an important player in some retinoblastomas.[89]

EPIGENETIC CHARACTERIZATION OF RETINOBLASTOMAS

Methylation analyses of retinoblastomas

Methylation of the *RB1* promoter was first demonstrated in 1989 by Greger et al., who identified CpG 106, an island overlapping the promoter and exon 1, to be methylated in some retinoblastomas,[90] thus silencing gene expression. Since then, multiple CpG islands within the *RB1* promoter and gene have been identified and characterized in retinoblastomas, demonstrating an epigenetic component to *RB1* inactivation and subsequent development of retinoblastoma.[91] Methylation of the *RB1* promoter is the causative M1 in 8% of unilateral non-germline tumours.[4]

Aberrant methylation of additional genes has also been shown in retinoblastomas (Table 4). *RASSF1A*, a tumour suppressor involved in microtubule stability, is inactivated by promoter hypermethylation in anywhere from 59 to 80% of retinoblastomas in comparison to normal retinal tissues.[92–94] It is inactivated by methylation in multiple cancers.[94] *MGMT*, encoding an O^6-alkylguanine-DNA alkyltransferase, was also found hypermethylated, but in a smaller proportion of retinoblastomas (58% and 35% in two studies).[77,92,95]

$p16^{INK4A}$ (*CDKN2*) has long been implicated as a tumour suppressor in retinoblastoma development. Recently, Indovina and colleagues studied $p16^{INK4A}$ expression and promoter methylation in a cohort of retinoblastomas along with peripheral blood from both patients and their parents.[96] Fifty-five percent of retinoblastoma patients showed a downregulation of $p16^{INK4A}$ expression in blood. In over half of these, one of the parents possessed the same downregulation of $p16^{INK4A}$ in their blood cells. Interestingly, methylation analysis of the *CDKN2* promoter in this cohort revealed that patients and parents harbouring the same alteration showed promoter hypermethylation, suggesting that this alteration could be heritable, and therefore could become a novel susceptibility marker for these patients.[96]

Additionally, these results begin to provide a basis for the investigation of demethylating agents for therapeutic interventions.[2,96]

Livide et al. recently identified a novel set of hypermethylated genes in multiple retinoblastomas, in addition to confirmation of hypermethylation in previously identified genes *MGMT*, *RB1* and *CDKN2*.[77] Hypermethylation was found in 7 novel genes, including *MSH6* (50%), a post-replication DNA repair mismatch gene commonly mutated in cancer; *CD44* (43%), a cell surface glycoprotein involved in cell-cell and cell-matrix interactions; *PAX5* (42%), a member of the paired box family of transcription factors involved in developmental processes that is deregulated in lymphomas; *GATA5* (25%), a transcription factor involved in cardiac smooth muscle cell diversity; *TP53* (8%); *VHL* (8%), a tumour suppressor involved in the predisposition to Von Hippel-Lindau syndrome via the ubiquitin-mediated degradation of HIF1; and *GSTP1* (8%), a glutathione *S*-transferase enzyme playing a role in susceptibility to many diseases including cancer.[77] Although these studies were done on microdissected tumour and matched normal retina, it remains possible that observed methylation “changes” reflect the methylation status of the undefined retinoblastoma cell of origin.

Zhang et al. conducted a chromatin immunoprecipitation-on-chip and methylation analysis of 4 primary retinoblastomas and one orthotopic xenograft.[88] They identified a total of 104 genes that were differentially expressed and which also exhibited correlative histone modifications in retinoblastomas when compared to normal tissues. Only 15 of these genes have been identified as known cancer genes.[88] Upregulated genes with activating histone modifications included *TFF1*, a secreted gastrointestinal mucosa protein overexpressed in some digestive tumours and breast cancers; *SYK*, a novel proto-oncogene involved in breast cancer; and *MCM5*, important in DNA replication and cell cycle regulation. Downregulated genes with associated inactivating histone modifications included *CTNND1*, a catenin involved in cell-cell adhesion; *SOX2*, involved in embryonic development and a cause of syndromic microphthalmia; and *ADAMTS18*, thought to act as a tumour suppressor. The authors validated the proto-oncogene *SYK*, a druggable kinase.[88] This kinase has no documented role in the developing retina, but has importance in several haematological malignancies.[88,91] The *SYK* promoter showed high activating histone and RNA polymerase binding activity, and real-time RT-PCR and immunohistochemical analysis of primary tumours and xenografts demonstrated high SYK expression. Treatment of retinoblastoma cell lines and animals with established xenografts with anti-*SYK* shRNA or a small-molecule SYK inhibitor reduced tumour growth both *in vitro* and *in vivo*, suggesting a potential new therapeutic target for retinoblastoma.

Differential microRNA expression in retinoblastomas

MicroRNAs (miRNAs) are a large class of small non-coding RNAs that regulate gene expression by targeting mRNAs to either inhibit transcription or destabilize the transcript, effectively downregulating protein expression. miRNAs have been implicated in many crucial cellular pathways in normal and cancer cells, and their role in retinoblastomas has only recently been examined (Table 5 and Table 6).

We were the first to profile miRNA expression in retinoblastoma by microarray,[97] and showed substantial downregulation of let-7b in three tumours versus normal retina, as well as decreased let-7c, miR-24, miR-125b, miR-191, miR-181a and miR-423. No miRNA was uniformly overexpressed in these samples. Downregulation of let-7b was confirmed in a larger cohort by qPCR, and putative let-7b target genes *CDC25A* and *BCL7A* were upregulated in tumours. Importantly, this overexpression was abrogated by exogenous let-7b. Mu et al. recently confirmed that the let-7 family was highly expressed in retinal tissues, with significantly decreased expression in 39% of primary tumours tested.[98]

Furthermore, a significant inverse association between let-7 expression and expression of high mobility group proteins *HMGA1* and *HMGA2* was found, indicating that let-7 may be acting as a tumour suppressor in retinoblastoma. let-7 has been documented to regulate the expression of *HMGA2*.[91]

Subsequent studies identified additional miRNAs involved in the progression of retinoblastoma (Table 5). One microarray analysis of human retinoblastoma tissues identified 13 highly expressed miRNAs compared to normal retina, including miR-373, previously identified as a putative "oncomiR" in testicular germ cell tumours.[91,99] Another tumour suppressor miRNA, miR-34a, was identified as differentially expressed in retinoblastomas and cell lines.[100] Loss of miR-34a has been shown in various cancers, including brain, breast, colorectal, lung, pancreatic, and prostate.[91] miR-34a demonstrated variable expression in two primary retinoblastomas and two cell lines, and treatment of Y79 and WERI-Rb1 cells with miR-34a decreased cell growth and increased apoptosis; this effect was improved with topotecan co-treatment, suggesting that miR-34a acts as a tumour suppressor in retinoblastoma cells, mediating proliferation and chemotherapeutic resistance.[100]

Conkrite and colleagues demonstrated that the miR-17~92 cluster, one of the first "oncomiR" clusters to be characterized, is a potential therapeutic target in retinoblastoma.[101] By studying a murine model of retinoblastoma initiated by mutations in *Rb1* and *Rbl1*, they identified through aCGH a focal amplification in 14qE (syntenic to human 13q32) which contains the miR-17~92 cluster. One mouse tumour exhibited a 14 Mbp gain at 5qG2–3, containing the miR-17~92 paralog, miR106b~25.[101] miR-17~92 has been implicated in tumorigenesis, as it promotes proliferation, inhibits differentiation, and increases angiogenesis in lymphomas.[91] However, out of 32 human retinoblastomas, only one demonstrated low-level gain of the human miR-17~92 locus, but 4 tumours exhibited low-level gain of miR106b~25, thus suggesting relevance of this cluster to both murine and human diseases. Interestingly, microarray analyses and deep sequencing (RNA-Seq) of both murine and human retinoblastomas revealed high expression of miR-17~92. Overexpression of miR-17~92 in mice was by itself insufficient for tumour formation, however deletion of both *Rb1* and *Rbl1* and overexpression of miR-17~92 accelerated retinoblastoma development, with presence of frequent brain metastases.[101] miR-17~92 increased the proliferative capacity of pRb/p107-deficient cells by suppressing $p21^{Cip1}$ expression, thereby promoting proliferation and development of retinoblastoma.

Nittner et al. extended these findings to human cell lines, investigating whether miR-17~92 could promote survival in human cells with inactivated *RB1*. In RBL15, WERI-Rb1 and Y79 retinoblastoma cell lines, inhibition of miR-17~92 suppressed tumour formation in xenografts. Co-silencing of miR-17/20a and p53 was cooperative in decreasing the viability of human retinoblastoma cells.[102] Together, these studies identify miR-17~92 as an interesting therapeutic target worthy of further study.

Retinoblastoma cell lines have also been probed to assess the biological implications of some differentially expressed miRNAs. Jo et al. conducted an expression analysis of two retinoblastoma cell lines with different growth properties.[103] The authors found that the miRNA expression pattern of SNUOT-Rb1 cells (overexpressing miR-10b, miR-29a, and let-7c), which are adherent and demonstrate rapid growth, is completely different from the miRNA expression pattern of Y79 cells (overexpressing miR-34a, miR-124, and miR-135b), which are non-adherent and slower growing. Bioinformatics analysis of these differentially expressed miRNAs showed direct relation to gene expression control of biological processes such as cell adhesion, proliferation, death and division,[103] suggesting targets that could be validated in clinical samples.

For example, a target for miR-10b is the tumour suppressor *HOXD10*. Its transcriptional inhibition leads to activation of Rho kinase activation and tumour cell invasion.[104] Using a novel functional linkage method of analysing publicly available STRING protein-protein interaction data, Li et al. identified a set of 53 differentially expressed miRNAs in retinoblastomas in comparison to normal retinal tissue. This study confirmed previously identified miRNAs, such as let-7a, let-7c, miR-20a, miR-124, and miR-125, but also identified novel miRNAs such as miR-21, −155 and −301.[105]

Expression analysis of 12 retinoblastomas identified several other novel miRNAs that may play a role in tumorigenesis. Through microarray analysis, Martin et al. identified 41 differentially miRNAs as compared to normal retinal tissues.[106] They went on to validate five of these (miRs-129-3p, −129-5p, −382, −504, and −22) as highly downregulated in 12 primary tumours, two cell lines and two mouse retinoblastomas. Many of these identified miRNAs have demonstrated roles in other cancers. For instance, the most downregulated miRNA, miR-129, is involved in cell cycle regulation by inhibiting the cell cycle regulatory genes *CDK4* and *CDK6*, and is silenced in gastric, esophageal and colorectal cancers.[106,107] Another interesting target, miR-382, is involved in repressing *MYC* transcription. In osteosarcoma, loss of miR-382 leads to upregulation of c-Myc, in turn activating the miR-17~92 cluster involved in human and mouse retinoblastomas (see above).[101,102] However, when miRNA expression levels were associated with clinical variables, no significant correlations could be found with optic nerve invasion or intraocular neovascularization, warranting the analysis of a greater patient cohort.

Finally, one recent study performed an *in silico* analysis of the miRNA expression profile of 14 late-stage retinoblastoma patient serum samples with publicly available miRNA expression data on retinoblastomas, to identify miRNA and gene targets that could be used as potential serum biomarkers.[108] A total of 33 miRNAs, including 25 upregulated and eight downregulated miRNAs in both serum and retinoblastoma tumours were identified. Real-time PCR of an additional 20 retinoblastoma serum samples validated a total of five miRNAs, three upregulated (miR-17, miR-18a and miR-20a) and two downregulated (miR-19b, and miR-92a-1). Although the first study of its kind, these results postulate exciting candidate miRNAs for development into serum biomarkers, and will pave the way for other studies to help define predictive biomarkers for retinoblastoma patients.

IMPACT OF THE RETINOBLASTOMA GENOMIC LANDSCAPE ON CLINICAL PRACTICE

As evidenced by the number of studies presented, the genomic landscape of retinoblastoma is becoming increasingly complex. However, most of the studies reviewed here are based on a small number of clinical samples, and therefore the data needs to be functionally validated in cell-based and animal models, as well as clinically validated in larger patient cohorts before being included in standard of care recommendations. That said, ophthalmologists should be aware of other genomic changes beyond *RB1* mutation with the distinct potential to affect therapeutic decisions and long-term surveillance and care:

1. MYCN status

Retinoblastoma tumours can now be subdivided into two molecular classes: $RB1^{-/-}$, and $RB1^{+/+}$, $MYCN^{A}$. This latter subset of retinoblastoma tumours has a wild-type, functional *RB1* gene, but high level amplification of the *MYCN* gene present only in the tumour cells. This means that these children have no special risk for retinoblastoma or second cancers later in life, and may not need life-long surveillance. These tumours are highly aggressive, are histologically different, and arise earlier with a very young age of presentation compared

to *RB1*$^{-/-}$ retinoblastomas. Because these tumours are highly aggressive, enucleation is likely the optimal therapy. If a *MYCN* retinoblastoma is confirmed molecularly, any disseminated disease might be treatable with future therapies that target *MYCN*. Currently, this changes genetic counseling for survivors with this type of tumour, as they carry no additional risk for tumours in the other eye, second cancers, and no risk of affecting the next generation.

2. Candidate genes as surveillance markers and therapeutic targets

Gene-specific copy number gains or losses, and/or changes in gene expression, such as *KIF14*, *MDM4*, *MYCN*, *DEK*, *E2F3*, *CDH11*, *miR-17~92*, and *SYK* have demonstrated importance in retinoblastomas via multiple lines of evidence (Table 1). These genes have demonstrated functional importance in cell lines, animal models and patient tumours, and may be associated with progression and/or poor outcomes. These genes could be developed into markers that would facilitate surveillance of tumour recurrence or metastasis (as shown for *RB1*[109], *KIF14* and *E2F3*[15]), as well as being the target of new therapies to treat recurring retinoblastomas.

CONCLUSIONS

The study of retinoblastoma has revealed guiding principles of the molecular initiation and progression of cancer. Similarly, ongoing studies via higher resolution genomic technologies will continue to facilitate our exploration into the molecular intricacies of this model cancer, with the hopes of refining causative molecular pathogenic pathways and offering opportunities for therapy. Next-generation sequencing (NGS) in particular holds the promise of read depth that could possibly identify mechanisms of convergent evolution of gene mutations and gene copy number changes. This type of technology has the potential to reveal the identity of initiating vs. progression changes, facilitating diagnosis and therapeutic management. While NGS is being used as a tool for mutation discovery, its current sensitivity and accuracy for identifying *RB1* mutations is still not published, let alone clinically validated. Although NGS technologies promise to be more affordable for clinical mutation testing for retinoblastoma, detection of all kinds of mutation has yet to be demonstrated.

Although *RB1* loss is the causative genetic alteration underlying most retinoblastoma development, it is becoming increasingly evident that other genetic, genomic, epigenetic and gene expression events are also necessary for tumour development (Figure 1). Complementing and complicating these studies, the discovery of *MYCN*^{A} *RB1*$^{+/+}$ retinoblastoma opens new avenues for comparing the molecular progression of this subtype with “classic” retinoblastoma.

Integrating the results from different approaches will contribute to a greater understanding of this cancer. Especially, pan-”omics” approaches incorporating whole-genome sequencing, epigenetics, expression, proteomics and even metabolomics on large numbers of tumours will help define crucial molecular events in retinoblastoma (Figure 2). However, some of these technologies have already begun to open the door to a wealth of genetic, expression, and epigenetic data, revealing an inherent molecular complexity for this disease. Careful scrutiny, compilation of multiple data sources, functional validation in animal and cell culture models, and most important, clinical studies, promise to define clinically relevant associations that can impact care. Importantly, it is noteworthy that most of the studies reviewed here focus on small numbers of tumours with very little associated clinical information. The study of larger cohorts of patient tumours and their clinical data is crucial. Given the wide distribution of retinoblastoma cases worldwide, multi-centre, international collaboration is a necessity to enable robust, powerful studies.

The concept of precision medicine has been applied to clinical retinoblastoma management for more than 20 years. The discovery of the genetic nature of retinoblastoma decades ago led to the development of genetic testing of patients and families at risk, ushering in the concept of individualized medicine to guide patient management, treatments and outcomes, now often across generations of a family. In many respects, the framework is already in place to evolve such individualized testing to incorporate new genetic and genomic technologies into the standard of care, providing refined information on disease severity, molecular pathophysiology, and prognosis for responses to treatments. Only then will we gain a clear insight into the important and targetable tumorigenic pathways of retinoblastoma, many of which will be applicable to other malignancies, as has already been repeatedly demonstrated. Most importantly, individualized testing and analysis will also provide prospective validation of the emerging candidates enabling therapeutic strategies with improved outcomes for retinoblastoma patients.

Acknowledgments

We thank Cynthia VandenHoven for assistance with the clinical images. Related work in the laboratory of TWC is supported by an Alcon Research Institute Young Investigator Grant, a Pediatric Ophthalmology Career Starter Research Grant from the Knights Templar Eye Foundation, and by the Indiana Clinical and Translational Sciences Institute through NIH NCATS CTSA Grant TR000006.

Funding sources: Related work in the laboratory of TWC is supported by an Alcon Research Institute Young Investigator Grant, a Pediatric Ophthalmology Career Starter Research Grant from the Knights Templar Eye Foundation and by the Indiana Clinical and Translational Sciences Institute through NIH NCATS CTSA Grant TR000006.

REFERENCES

1. Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA. 1971; 68:820–823. [PubMed: 5279523]
2. Saxena P, Kaur J. Differential expression of genes in retinoblastoma. Clin Chim Acta. 2011; 412:2015–2021. [PubMed: 20951689]
3. Dimaras H, Kimani K, Dimba EA, Gronsdahl P, White A, Chan HS, Gallie BL. Retinoblastoma. Lancet. 2012; 379:1436–1446. [PubMed: 22414599]
4. Richter S, Vandezande K, Chen N, Zhang K, Sutherland J, Anderson J, Han L, Panton R, Branco P, Gallie B. Sensitive and efficient detection of *RB1* gene mutations enhances care for families with retinoblastoma. Am J Hum Genet. 2003; 72:253–269. [PubMed: 12541220]
5. Rushlow D, Piovesan B, Zhang K, Prigoda-Lee NL, Marchong MN, Clark RD, Gallie BL. Detection of mosaic *RB1* mutations in families with retinoblastoma. Hum Mut. 2009; 30:842–851. [PubMed: 19280657]
6. Kivela T. The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. Brit J Ophthalmol. 2009; 93:1129–1131. [PubMed: 19704035]
7. Eng C, Li FP, Abramson DH, Ellsworth RM, Wong FL, Goldman MB, Seddon J, Tarbell N, Boice JD Jr. Mortality from second tumors among long-term survivors of retinoblastoma. J Natl Cancer Inst. 1993; 85:1121–1128. [PubMed: 8320741]
8. Comings DE. A general theory of carcinogenesis. Proc Natl Acad Sci USA. 1973; 70:3324–3328. [PubMed: 4202843]
9. Dryja TP, Friend S, Weinberg RA. Genetic sequences that predispose to retinoblastoma and osteosarcoma. Symp Fund Cancer Res. 1986; 39:115–119.
10. Lee WH, Shew JY, Hong FD, Sery TW, Donoso LA, Young LJ, Bookstein R, Lee EY. The retinoblastoma susceptibility gene encodes a nuclear phosphoprotein associated with DNA binding activity. Nature. 1987; 329:642–645. [PubMed: 3657987]
11. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature. 1986; 323:643–646. [PubMed: 2877398]

12. Dunn JM, Phillips RA, Becker AJ, Gallie BL. Identification of germline and somatic mutations affecting the retinoblastoma gene. Science. 1988; 241:1797–1800. [PubMed: 3175621]
13. Dimaras H, Khetan V, Halliday W, Orlic M, Prigoda NL, Piovesan B, Marrano P, Corson TW, Eagle RC Jr. Squire JA, Gallie BL. Loss of *RB1* induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. Hum Mol Genet. 2008; 17:1363–1372. [PubMed: 18211953]
14. Corson TW, Gallie BL. One hit, two hits, three hits, more? Genomic changes in the development of retinoblastoma. Genes Chromosomes Cancer. 2007; 46:617–634. [PubMed: 17437278]
15. Bowles E, Corson TW, Bayani J, Squire JA, Wong N, Lai PB, Gallie BL. Profiling genomic copy number changes in retinoblastoma beyond loss of *RB1*. Genes Chromosomes Cancer. 2007; 46:118–129. [PubMed: 17099872]
16. Zielinski B, Gratias S, Toedt G, Mendrzyk F, Stange DE, Radlwimmer B, Lohmann DR, Lichter P. Detection of chromosomal imbalances in retinoblastoma by matrix-based comparative genomic hybridization. Genes Chromosomes Cancer. 2005; 43:294–301. [PubMed: 15834944]
17. Corson TW, Gallie BL. *KIF14* mRNA expression is a predictor of grade and outcome in breast cancer. Int J Cancer. 2006; 119:1088–1094. [PubMed: 16570270]
18. Corson TW, Huang A, Tsao MS, Gallie BL. *KIF14* is a candidate oncogene in the 1q minimal region of genomic gain in multiple cancers. Oncogene. 2005; 24:4741–4753. [PubMed: 15897902]
19. Corson TW, Zhu CQ, Lau SK, Shepherd FA, Tsao MS, Gallie BL. *KIF14* messenger RNA expression is independently prognostic for outcome in lung cancer. Clin Cancer Res. 2007; 13:3229–3234. [PubMed: 17545527]
20. Theriault BL, Pajovic S, Bernardini MQ, Shaw PA, Gallie BL. Kinesin family member 14: an independent prognostic marker and potential therapeutic target for ovarian cancer. Int J Cancer. 2012; 130:1844–1854. [PubMed: 21618518]
21. Laurie NA, Donovan SL, Shih CS, Zhang J, Mills N, Fuller C, Teunisse A, Lam S, Ramos Y, Mohan A, Johnson D, Wilson M, Rodriguez-Galindo C, Quarto M, Francoz S, Mendrysa SM, Guy RK, Marine JC, Jochemsen AG, Dyer MA. Inactivation of the p53 pathway in retinoblastoma. Nature. 2006; 444:61–66. [PubMed: 17080083]
22. Orlic M, Spencer CE, Wang L, Gallie BL. Expression analysis of 6p22 genomic gain in retinoblastoma. Genes Chromosomes Cancer. 2006; 45:72–82. [PubMed: 16180235]
23. Marchong MN, Chen D, Corson TW, Lee C, Harmandayan M, Bowles E, Chen N, Gallie BL. Minimal 16q genomic loss implicates cadherin-11 in retinoblastoma. Mol Cancer Res. 2004; 2:495–503. [PubMed: 15383628]
24. Marine JC, Jochemsen AG. Mdmx and Mdm2: brothers in arms? Cell Cycle. 2004; 3:900–904. [PubMed: 15254433]
25. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer. 2012; 13:83–96. [PubMed: 23303139]
26. Laurie NA, Shih CS, Dyer MA. Targeting MDM2 and MDMX in retinoblastoma. Curr Cancer Drug Targets. 2007; 7:689–695. [PubMed: 18045074]
27. Guo Y, Pajovic S, Gallie BL. Expression of p14ARF, MDM2, and MDM4 in human retinoblastoma. Biochem Biophys Res Commun. 2008; 375:1–5. [PubMed: 18644346]
28. To KH, Pajovic S, Gallie BL, Theriault BL. Regulation of p14ARF expression by miR-24: a potential mechanism compromising the p53 response during retinoblastoma development. BMC Cancer. 2012; 12:69. [PubMed: 22336108]
29. Carleton M, Mao M, Biery M, Warrener P, Kim S, Buser C, Marshall CG, Fernandes C, Annis J, Linsley PS. RNA interference-mediated silencing of mitotic kinesin KIF14 disrupts cell cycle progression and induces cytokinesis failure. Mol Cell Biol. 2006; 26:3853–3863. [PubMed: 16648480]
30. Gruneberg U, Neef R, Li X, Chan EH, Chalamalasetty RB, Nigg EA, Barr FA. KIF14 and citron kinase act together to promote efficient cytokinesis. J Cell Biol. 2006; 172:363–372. [PubMed: 16431929]
31. Thériault BL, Corson TW. KIF14 (kinesin family member 14). Atlas Genet Cytogenet Oncol Haematol. 2012; 16:695–699.

32. Pajovic S, Corson TW, Spencer C, Dimaras H, Orlic-Milacic M, Marchong MN, To KH, Theriault B, Auspitz M, Gallie BL. The TAg-RB murine retinoblastoma cell of origin has immunohistochemical features of differentiated Muller glia with progenitor properties. Invest Ophthalmol Vis Sci. 2011; 52:7618–7624. [PubMed: 21862643]
33. Madhavan J, Mitra M, Mallikarjuna K, Pranav O, Srinivasan R, Nagpal A, Venkatesan P, Kumaramanickavel G. KIF14 and E2F3 mRNA expression in human retinoblastoma and its phenotype association. Mol Vis. 2009; 15:235–240. [PubMed: 19190782]
34. Markowski J, Oczko-Wojciechowska M, Gierek T, Jarzab M, Paluch J, Kowalska M, Wygoda Z, Pfeifer A, Tyszkiewicz T, Jarzab B, Niedzielska I, Borgiel-Marek H. Gene expression profile analysis in laryngeal cancer by high-density oligonucleotide microarrays. J Physiol Pharmacol. 2009; 60(Suppl 1):57–63. [PubMed: 19609014]
35. Markowski J, Tyszkiewicz T, Jarzab M, Oczko-Wojciechowska M, Gierek T, Witkowska M, Paluch J, Kowalska M, Wygoda Z, Lange D, Jarzab B. Metal-proteinase ADAM12, kinesin 14 and checkpoint suppressor 1 as new molecular markers of laryngeal carcinoma. Eur Arch Otorhinolaryngol. 2009; 266:1501–1507. [PubMed: 19609547]
36. Ahmed SM, Theriault BL, Uppalapati M, Chiu CW, Gallie BL, Sidhu SS, Angers S. KIF14 negatively regulates Rap1a-Radil signaling during breast cancer progression. J Cell Biol. 2012; 199:951–967. [PubMed: 23209302]
37. Basavarajappa HD, Corson TW. KIF14 as an oncogene in retinoblastoma: a target for novel therapeutics? Future Med Chem. 2012; 4:2149–2152. [PubMed: 23190103]
38. Ganguly A, Nichols KE, Grant G, Rappaport E, Shields C. Molecular karyotype of sporadic unilateral retinoblastoma tumors. Retina. 2009; 29:1002–1012. [PubMed: 19491728]
39. Mairal A, Pinglier E, Gilbert E, Peter M, Validire P, Desjardins L, Doz F, Aurias A, Couturier J. Detection of chromosome imbalances in retinoblastoma by parallel karyotype and CGH analyses. Genes Chromosomes Cancer. 2000; 28:370–379. [PubMed: 10862045]
40. Gratias S, Schuler A, Hitpass LK, Stephan H, Rieder H, Schneider S, Horsthemke B, Lohmann DR. Genomic gains on chromosome 1q in retinoblastoma: consequences on gene expression and association with clinical manifestation. Int J Cancer. 2005; 116:555–563. [PubMed: 15825178]
41. Sampieri K, Amenduni M, Papa FT, Katzaki E, Mencarelli MA, Marozza A, Epistolato MC, Toti P, Lazzi S, Bruttini M, De Filippis R, De Francesco S, Longo I, Meloni I, Mari F, Acquaviva A, Hadjistilianou T, Renieri A, Ariani F. Array comparative genomic hybridization in retinoma and retinoblastoma tissues. Cancer Sci. 2009; 100:465–471. [PubMed: 19183342]
42. Woo CW, Tan F, Cassano H, Lee J, Lee KC, Thiele CJ. Use of RNA interference to elucidate the effect of MYCN on cell cycle in neuroblastoma. Ped Blood Cancer. 2008; 50:208–212.
43. Barr FG, Duan F, Smith LM, Gustafson D, Pitts M, Hammond S, Gastier-Foster JM. Genomic and clinical analyses of 2p24 and 12q13-q14 amplification in alveolar rhabdomyosarcoma: a report from the Children's Oncology Group. Genes Chromosomes Cancer. 2009; 48:661–672. [PubMed: 19422036]
44. Bayani J, Zielenska M, Marrano P, Kwan Ng Y, Taylor MD, Jay V, Rutka JT, Squire JA. Molecular cytogenetic analysis of medulloblastomas and supratentorial primitive neuroectodermal tumors by using conventional banding, comparative genomic hybridization, and spectral karyotyping. J Neurosurg. 2000; 93:437–448. [PubMed: 10969942]
45. Hui AB, Lo KW, Yin XL, Poon WS, Ng HK. Detection of multiple gene amplifications in glioblastoma multiforme using array-based comparative genomic hybridization. Lab Invest. 2001; 81:717–723. [PubMed: 11351043]
46. Nau MM, Brooks BJ Jr, Carney DN, Gazdar AF, Battey JF, Sausville EA, Minna JD. Human small-cell lung cancers show amplification and expression of the N-myc gene. Proc Natl Acad Sci USA. 1986; 83:1092–1096. [PubMed: 2869482]
47. Dietzsch E, Lukeis RE, Vrazas V, Hasthorpe S, Garson OM. Characterization of homogeneously staining regions in a small cell lung cancer cell line, using in situ hybridization with an *MYCN* probe. Genes Chromosomes Cancer. 1994; 10:213–216. [PubMed: 7522047]
48. Salido M, Arriola E, Carracedo A, Canadas I, Rovira A, Espinet B, Rojo F, Arumi M, Serrano S, Albanell J, Sole F. Cytogenetic characterization of NCI-H69 and NCI-H69AR small cell lung

cancer cell lines by spectral karyotyping. Cancer Genet Cytogenet. 2009; 191:97–101. [PubMed: 19446745]

49. Fix A, Lucchesi C, Ribeiro A, Lequin D, Pierron G, Schleiermacher G, Delattre O, Janoueix-Lerosey I. Characterization of amplicons in neuroblastoma: high-resolution mapping using DNA microarrays, relationship with outcome, and identification of overexpressed genes. Genes Chromosomes Cancer. 2008; 47:819–834. [PubMed: 18553563]
50. Moreau LA, McGrady P, London WB, Shimada H, Cohn SL, Maris JM, Diller L, Look AT, George RE. Does *MYCN* amplification manifested as homogeneously staining regions at diagnosis predict a worse outcome in children with neuroblastoma? A Children's Oncology Group study. Clin Cancer Res. 2006; 12:5693–5697. [PubMed: 17020972]
51. Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J. Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature. 1983; 305:245–248. [PubMed: 6888561]
52. Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Theriault BL, Prigoda-Lee NL, Spencer C, Dimaras H, Corson TW, Pang R, Massey C, Godbout R, Jiang Z, Zacksenhaus E, Paton K, Moll AC, Houdayer C, Raizis A, Halliday W, Lam WL, Boutros PC, Lohmann D, Dorsman JC, Gallie BL. Characterisation of retinoblastomas without *RB1* mutations: genomic, gene expression, and clinical studies. Lancet Oncol.
53. Godbout R, Packer M, Bie W. Overexpression of a DEAD box protein (DDX1) in neuroblastoma and retinoblastoma cell lines. J Biol Chem. 1998; 273:21161–21168. [PubMed: 9694872]
54. Godbout R, Squire J. Amplification of a DEAD box protein gene in retinoblastoma cell lines. Proc Natl Acad Sci USA. 1993; 90:7578–7582. [PubMed: 7689221]
55. Weber A, Imisch P, Bergmann E, Christiansen H. Coamplification of *DDX1* correlates with an improved survival probability in children with *MYCN*-amplified human neuroblastoma. J Clin Oncol. 2004; 22:2681–2690. [PubMed: 15226335]
56. Paderova J, Orlic-Milacic M, Yoshimoto M, da Cunha Santos G, Gallie B, Squire JA. Novel 6p rearrangements and recurrent translocation breakpoints in retinoblastoma cell lines identified by spectral karyotyping and mBAND analyses. Cancer Genet Cytogenet. 2007; 179:102–111. [PubMed: 18036396]
57. Grasemann C, Gratias S, Stephan H, Schuler A, Schramm A, Klein-Hitpass L, Rieder H, Schneider S, Kappes F, Eggert A, Lohmann DR. Gains and overexpression identify *DEK* and *E2F3* as targets of chromosome 6p gains in retinoblastoma. Oncogene. 2005; 24:6441–6449. [PubMed: 16007192]
58. Carro MS, Spiga FM, Quarto M, Di Ninni V, Volorio S, Alcalay M, Muller H. DEK expression is controlled by E2F and deregulated in diverse tumor types. Cell Cycle. 2006; 5:1202–1207. [PubMed: 16721057]
59. von Lindern M, Fornerod M, van Baal S, Jaegle M, de Wit T, Buijs A, Grosveld G. The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. Mol Cell Biol. 1992; 12:1687–1697. [PubMed: 1549122]
60. Privette Vinnedge LM, Ho SM, Wikenheiser-Brokamp KA, Wells SI. The DEK oncogene is a target of steroid hormone receptor signaling in breast cancer. PloS One. 2012; 7:e46985. [PubMed: 23071688]
61. Privette Vinnedge LM, Kappes F, Nassar N, Wells SI. Stacking the DEK: From chromatin topology to cancer stem cells. Cell Cycle. 2012; 12
62. Vimala K, Sundarraj S, Sujitha MV, Kannan S. Curtailing overexpression of E2F3 in breast cancer using siRNA (E2F3)-based gene silencing. Arch Med Res. 2012; 43:415–422. [PubMed: 22960857]
63. Olsson AY, Feber A, Edwards S, Te Poele R, Giddings I, Merson S, Cooper CS. Role of E2F3 expression in modulating cellular proliferation rate in human bladder and prostate cancer cells. Oncogene. 2007; 26:1028–1037. [PubMed: 16909110]
64. Kashima T, Nakamura K, Kawaguchi J, Takanashi M, Ishida T, Aburatani H, Kudo A, Fukayama M, Grigoriadis AE. Overexpression of cadherins suppresses pulmonary metastasis of osteosarcoma in vivo. Int J Cancer. 2003; 104:147–154. [PubMed: 12569568]

65. Kawaguchi J, Takeshita S, Kashima T, Imai T, Machinami R, Kudo A. Expression and function of the splice variant of the human cadherin-11 gene in subordination to intact cadherin-11. J Bone Mineral Res. 1999; 14:764–775.
66. Roylance R, Gorman P, Papior T, Wan YL, Ives M, Watson JE, Collins C, Wortham N, Langford C, Fiegler H, Carter N, Gillett C, Sasieni P, Pinder S, Hanby A, Tomlinson I. A comprehensive study of chromosome 16q in invasive ductal and lobular breast carcinoma using array CGH. Oncogene. 2006; 25:6544–653. [PubMed: 16702952]
67. Delic S, Lottmann N, Jetschke K, Reifenberger G, Riemenschneider MJ. Identification and functional validation of CDH11, PCSK6 and SH3GL3 as novel glioma invasion-associated candidate genes. Neuropathol Appl Neurobiol. 2012; 38:201–212. [PubMed: 21722156]
68. Li L, Ying J, Li H, Zhang Y, Shu X, Fan Y, Tan J, Cao Y, Tsao SW, Srivastava G, Chan AT, Tao Q. The human cadherin 11 is a pro-apoptotic tumor suppressor modulating cell stemness through Wnt/beta-catenin signaling and silenced in common carcinomas. Oncogene. 2012; 31:3901–3912. [PubMed: 22139084]
69. Carmona FJ, Villanueva A, Vidal A, Munoz C, Puertas S, Penin RM, Goma M, Lujambio A, Piulats JM, Mesia R, Sanchez-Cespedes M, Manos M, Condom E, Eccles SA, Esteller M. Epigenetic disruption of cadherin-11 in human cancer metastasis. J Pathol. 2012; 228:230–240. [PubMed: 22374749]
70. Laurie N, Mohan A, McEvoy J, Reed D, Zhang J, Schweers B, Ajioka I, Valentine V, Johnson D, Ellison D, Dyer MA. Changes in retinoblastoma cell adhesion associated with optic nerve invasion. Mol Cell Biol. 2009; 29:6268–6282. [PubMed: 19786571]
71. Marchong MN, Yurkowski C, Ma C, Spencer C, Pajovic S, Gallie BL. Cdh11 acts as a tumor suppressor in a murine retinoblastoma model by facilitating tumor cell death. PLoS Genet. 2010; 6:e1000923. [PubMed: 20421947]
72. Gratias S, Rieder H, Ullmann R, Klein-Hitpass L, Schneider S, Boloni R, Kappler M, Lohmann DR. Allelic loss in a minimal region on chromosome 16q24 is associated with vitreous seeding of retinoblastoma. Cancer Res. 2007; 67:408–416. [PubMed: 17210724]
73. Yendamuri S, Trapasso F, Calin GA. ARLTS1 - a novel tumor suppressor gene. Cancer Lett. 2008; 264:11–20. [PubMed: 18375053]
74. Tosi GM, Trimarchi C, Macaluso M, La Sala D, Ciccodicola A, Lazzi S, Massaro-Giordano M, Caporossi A, Giordano A, Cinti C. Genetic and epigenetic alterations of RB2/p130 tumor suppressor gene in human sporadic retinoblastoma: implications for pathogenesis and therapeutic approach. Oncogene. 2005; 24:5827–5836. [PubMed: 16007224]
75. MacPherson D, Sage J, Kim T, Ho D, McLaughlin ME, Jacks T. Cell type-specific effects of Rb deletion in the murine retina. Genes Dev. 2004; 18:1681–1694. [PubMed: 15231717]
76. Priya K, Jada SR, Quah BL, Quah TC, Lai PS. High incidence of allelic loss at 16q12.2 region spanning RBL2/p130 gene in retinoblastoma. Cancer Biol Ther. 2009; 8:714–717. [PubMed: 19252413]
77. Livide G, Epistolato MC, Amenduni M, Disciglio V, Marozza A, Mencarelli MA, Toti P, Lazzi S, Hadjistilianou T, De Francesco S, D'Ambrosio A, Renieri A, Ariani F. Epigenetic and copy number variation analysis in retinoblastoma by MS-MLPA. Pathol Oncol Res. 2012; 18:703–712. [PubMed: 22278416]
78. Chakraborty S, Khare S, Dorairaj SK, Prabhakaran VC, Prakash DR, Kumar A. Identification of genes associated with tumorigenesis of retinoblastoma by microarray analysis. Genomics. 2007; 90:344–353. [PubMed: 17604597]
79. Ganguly A, Shields CL. Differential gene expression profile of retinoblastoma compared to normal retina. Mol Vis. 2010; 16:1292–1303. [PubMed: 20664703]
80. Xu XL, Fang Y, Lee TC, Forrest D, Gregory-Evans C, Almeida D, Liu A, Jhanwar SC, Abramson DH, Cobrinik D. Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. Cell. 2009; 137:1018–1031. [PubMed: 19524506]
81. Kato MV, Shimizu T, Ishizaki K, Kaneko A, Yandell DW, Toguchida J, Sasaki MS. Loss of heterozygosity on chromosome 17 and mutation of the p53 gene in retinoblastoma. Cancer Lett. 1996; 106:75–82. [PubMed: 8827049]

82. Castera L, Sabbagh A, Dehainault C, Michaux D, Mansuet-Lupo A, Patillon B, Lamar E, Aerts I, Lumbroso-Le Rouic L, Couturier J, Stoppa-Lyonnet D, Gauthier-Villars M, Houdayer C. MDM2 as a modifier gene in retinoblastoma. J Natl Cancer Inst. 2010; 102:1805–1808. [PubMed: 21051655]
83. Dumont P, Leu JI, Della Pietra AC 3rd. George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nature Genet. 2003; 33:357–365. [PubMed: 12567188]
84. Epistolato MC, Disciglio V, Livide G, Berchialla P, Mencarelli MA, Marozza A, Amenduni M, Hadjistilianou T, De Francesco S, Acquaviva A, Toti P, Cetta F, Ariani F, De Marchi M, Renieri A, Giachino D. p53 Arg72Pro and MDM2 309 SNPs in hereditary retinoblastoma. J Hum Genet. 2011; 56:685–686. [PubMed: 21814224]
85. de Oliveira Reis AH, de Carvalho IN, de Sousa Damasceno PB, Ferman SE, Lucena E, Lopez-Camelo JS, Seuanez HN, Vargas FR. Influence of MDM2 and MDM4 on development and survival in hereditary retinoblastoma. Ped Blood Cancer. 2012; 59:39–43.
86. McEvoy J, Ulyanov A, Brennan R, Wu G, Pounds S, Zhang J, Dyer MA. Analysis of MDM2 and MDM4 single nucleotide polymorphisms, mRNA splicing and protein expression in retinoblastoma. PloS One. 2012; 7:e42739. [PubMed: 22916154]
87. Wynendaele J, Bohnke A, Leucci E, Nielsen SJ, Lambertz I, Hammer S, Sbrzesny N, Kubitza D, Wolf A, Gradhand E, Balschun K, Braicu I, Sehouli J, Darb-Esfahani S, Denkert C, Thomssen C, Hauptmann S, Lund A, Marine JC, Bartel F. An illegitimate microRNA target site within the 3' UTR of MDM4 affects ovarian cancer progression and chemosensitivity. Cancer Res. 2010; 70:9641–9649. [PubMed: 21084273]
88. Zhang J, Benavente CA, McEvoy J, Flores-Otero J, Ding L, Chen X, Ulyanov A, Wu G, Wilson M, Wang J, Brennan R, Rusch M, Manning AL, Ma J, Easton J, Shurtleff S, Mullighan C, Pounds S, Mukatira S, Gupta P, Neale G, Zhao D, Lu C, Fulton RS, Fulton LL, Hong X, Dooling DJ, Ochoa K, Naeve C, Dyson NJ, Mardis ER, Bahrami A, Ellison D, Wilson RK, Downing JR, Dyer MA. A novel retinoblastoma therapy from genomic and epigenetic analyses. Nature. 2012; 481:329–334. [PubMed: 22237022]
89. Temming P, Corson TW, Lohmann DR. Retinoblastoma tumorigenesis: genetic and epigenetic changes walk hand in hand. Future Oncol. 2012; 8:525–528. [PubMed: 22646767]
90. Greger V, Passarge E, Hopping W, Messmer E, Horsthemke B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet. 1989; 83:155–158. [PubMed: 2550354]
91. Reis AH, Vargas FR, Lemos B. More epigenetic hits than meets the eye: microRNAs and genes associated with the tumorigenesis of retinoblastoma. Frontiers Genet. 2012; 3:284.
92. Choy KW, Lee TC, Cheung KF, Fan DS, Lo KW, Beaverson KL, Abramson DH, Lam DS, Yu CB, Pang CP. Clinical implications of promoter hypermethylation in *RASSF1A* and *MGMT* in retinoblastoma. Neoplasia. 2005; 7:200–206. [PubMed: 15799820]
93. Harada K, Toyooka S, Maitra A, Maruyama R, Toyooka KO, Timmons CF, Tomlinson GE, Mastrangelo D, Hay RJ, Minna JD, Gazdar AF. Aberrant promoter methylation and silencing of the *RASSF1A* gene in pediatric tumors and cell lines. Oncogene. 2002; 21:4345–4349. [PubMed: 12082624]
94. Liu R, Gao L, Lu GX, Tang LS, Zhu XH, Wang J. [Methylation status of RASSF1A and DAPK promoter in retinoblastoma]. [Zhonghua yan ke za zhi] Chinese J Ophthalmol. 2009; 45:631–635.
95. Choy KW, Pang CP, To KF, Yu CB, Ng JS, Lam DS. Impaired expression and promotor hypermethylation of O6-methylguanine-DNA methyltransferase in retinoblastoma tissues. Invest Ophthalmol Vis Sci. 2002; 43:1344–1349. [PubMed: 11980845]
96. Indovina P, Acquaviva A, De Falco G, Rizzo V, Onnis A, Luzzi A, Giorgi F, Hadjistilianou T, Toti P, Tomei V, Pentimalli F, Carugi A, Giordano A. Downregulation and aberrant promoter methylation of p16INK4A: a possible novel heritable susceptibility marker to retinoblastoma. J Cell Physiol. 2010; 223:143–150. [PubMed: 20039270]
97. Huang JC, Babak T, Corson TW, Chua G, Khan S, Gallie BL, Hughes TR, Blencowe BJ, Frey BJ, Morris QD. Using expression profiling data to identify human microRNA targets. Nat Methods. 2007; 4:1045–1049. [PubMed: 18026111]

98. Mu G, Liu H, Zhou F, Xu X, Jiang H, Wang Y, Qu Y. Correlation of overexpression of HMGA1 and HMGA2 with poor tumor differentiation, invasion, and proliferation associated with let-7 down-regulation in retinoblastomas. Hum Pathol. 2010; 41:493–502. [PubMed: 20004941]

99. Zhao JJ, Yang J, Lin J, Yao N, Zhu Y, Zheng J, Xu J, Cheng JQ, Lin JY, Ma X. Identification of miRNAs associated with tumorigenesis of retinoblastoma by miRNA microarray analysis. Childs Nerv Sys. 2009; 25:13–20.

100. Dalgard CL, Gonzalez M, deNiro JE, O'Brien JM. Differential microRNA-34a expression and tumor suppressor function in retinoblastoma cells. Invest Ophthalmol Vis Sci. 2009; 50:4542–4551. [PubMed: 19443717]

101. Conkrite K, Sundby M, Mukai S, Thomson JM, Mu D, Hammond SM, MacPherson D. miR-17~92 cooperates with RB pathway mutations to promote retinoblastoma. Genes Dev. 2011; 25:1734–1745. [PubMed: 21816922]

102. Nittner D, Lambertz I, Clermont F, Mestdagh P, Kohler C, Nielsen SJ, Jochemsen A, Speleman F, Vandesompele J, Dyer MA, Schramm A, Schulte JH, Marine JC. Synthetic lethality between Rb, p53 and Dicer or miR-17-92 in retinal progenitors suppresses retinoblastoma formation. Nature Cell Biol. 2012; 14:958–965. [PubMed: 22864477]

103. Jo DH, Kim JH, Park WY, Kim KW, Yu YS, Kim JH. Differential profiles of microRNAs in retinoblastoma cell lines of different proliferation and adherence patterns. J Ped Hematol Oncol. 2011; 33:529–533.

104. Bourguignon LY, Wong G, Earle C, Krueger K, Spevak CC. Hyaluronan-CD44 interaction promotes c-Src-mediated twist signaling, microRNA-10b expression, and RhoA/RhoC up-regulation, leading to Rho-kinase-associated cytoskeleton activation and breast tumor cell invasion. J Biol Chem. 2010; 285:36721–36735. [PubMed: 20843787]

105. Li BQ, Zhang J, Huang T, Zhang L, Cai YD. Identification of retinoblastoma related genes with shortest path in a protein-protein interaction network. Biochimie. 2012; 94:1910–1917. [PubMed: 22627383]

106. Martin J, Bryar P, Mets M, Weinstein J, Jones A, Martin A, Vanin EF, Scholtens D, Costa FF, Soares MB, Laurie NA. Differentially expressed miRNAs in retinoblastoma. Gene. 2013; 512:294–299. [PubMed: 23103829]

107. Wu J, Qian J, Li C, Kwok L, Cheng F, Liu P, Perdomo C, Kotton D, Vaziri C, Anderlind C, Spira A, Cardoso WV, Lu J. miR-129 regulates cell proliferation by downregulating Cdk6 expression. Cell Cycle. 2010; 9:1809–1818. [PubMed: 20404570]

108. Beta M, Venkatesan N, Vasudevan M, Vetrivel U, Khetan V, Krishnakumar S. Identification and insilico analysis of retinoblastoma serum microRNA profile and gene targets towards prediction of novel serum biomarkers. Bioinform Biol Insights. 2013; 7:21–34. [PubMed: 23400111]

109. Dimaras H, Rushlow D, Halliday W, Doyle JJ, Babyn P, Abella EM, Williams J, Heon E, Gallie BL, Chan HS. Using *RB1* mutations to assess minimal residual disease in metastatic retinoblastoma. Transl Res. 2010; 156:91–97. [PubMed: 20627193]

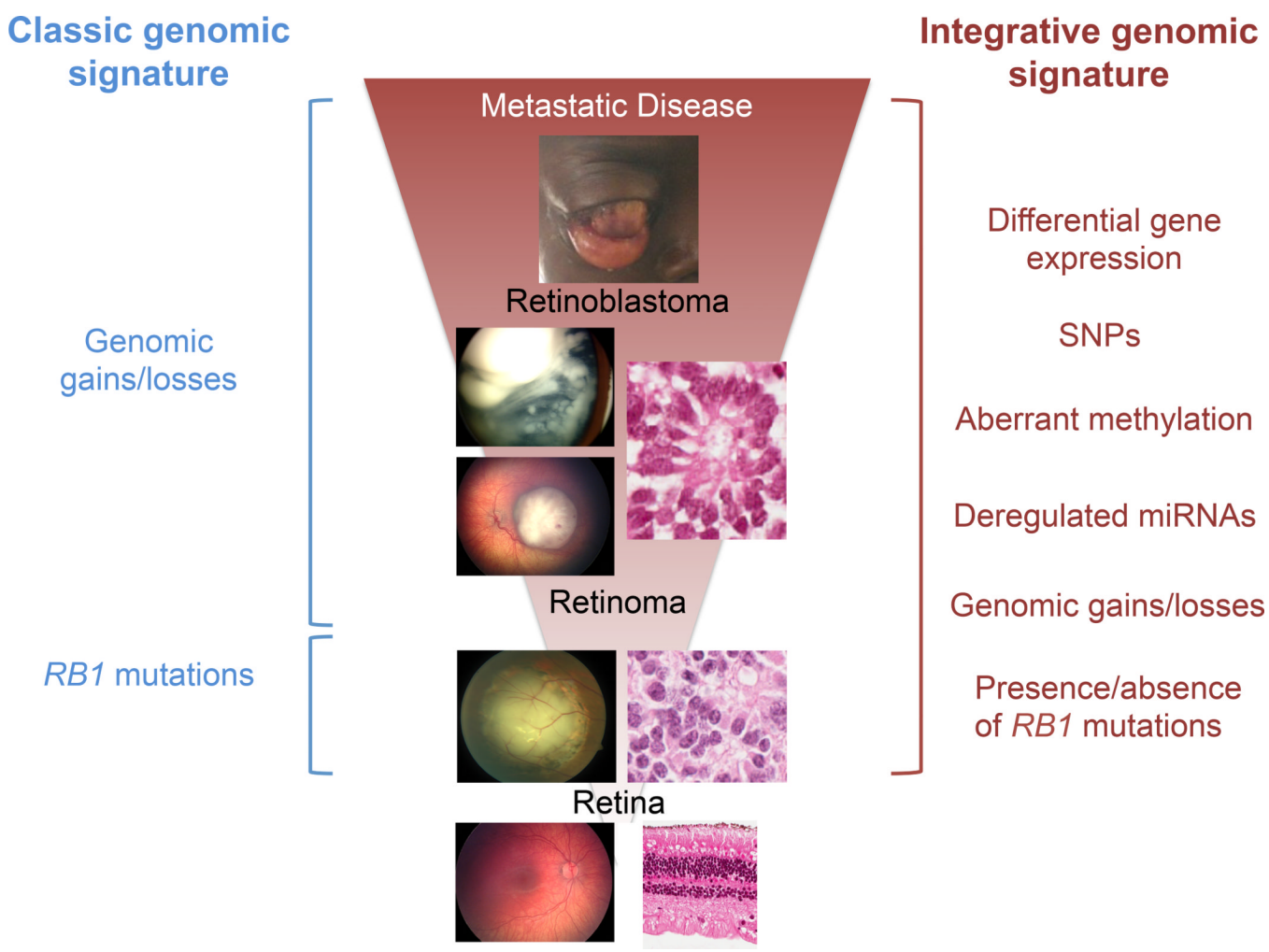

Figure 1.
Towards a broadened genomic landscape of retinoblastoma. miRNAs, microRNAs; SNPs, single nucleotide polymorphisms. All patients consented to have their clinical images used for education and research.

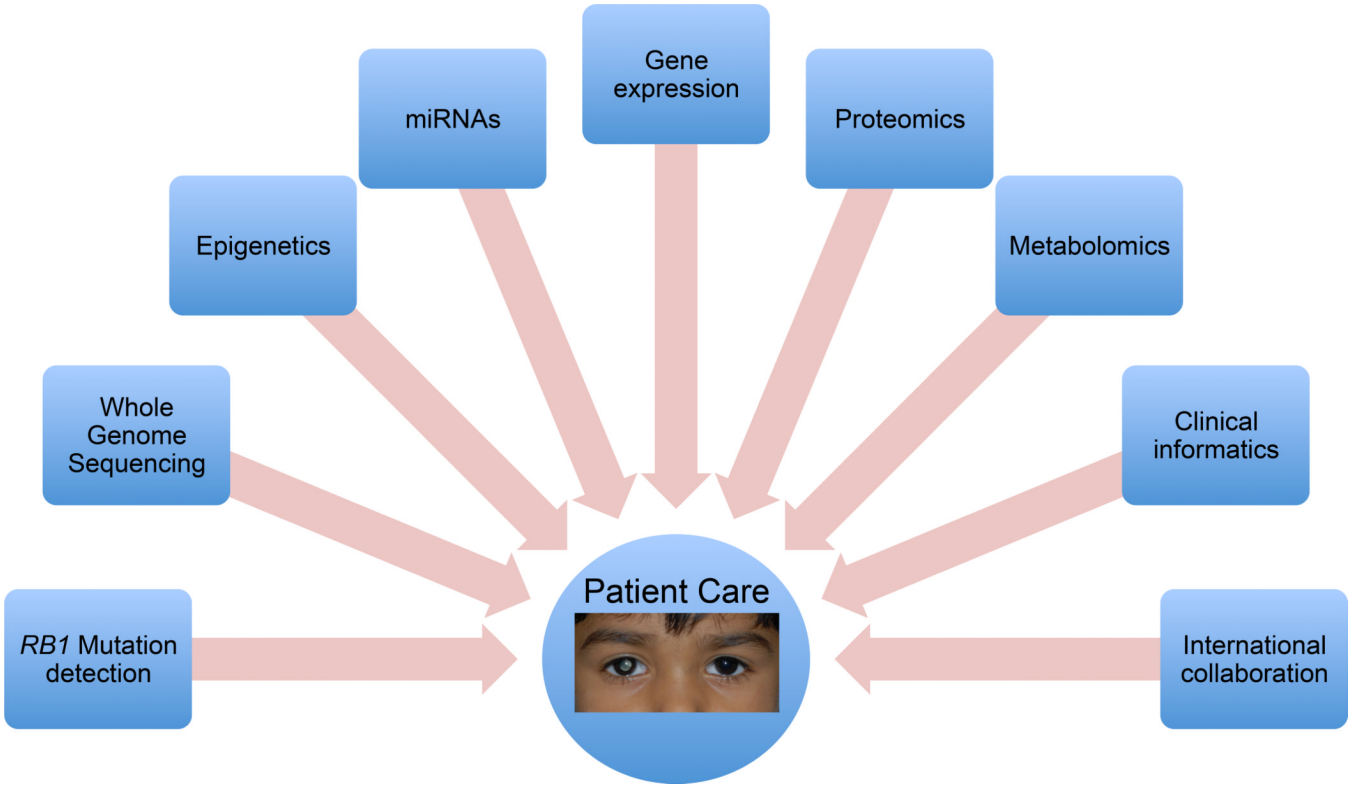

Figure 2.
The future of retinoblastoma management in a post-genomic era.

Table 1

Candidate oncogenes and tumour suppressor genes in retinoblastoma. Included genes have multiple lines of evidence (4 or more) supporting involvement

Gene name	Symbol	Gene type	Cytoband of minimal region	Change	Frequency of change (%)	Evidence	Reference
Kinesin family member 14	*KIF14*	oncogene	1q32.1	genomic gain	50	G, Ex, C, A	13, 15, 16, 18–21, 32, 33, 36, 38, 39, 41
Mouse double minute 4, human homolog	*MDM4*	oncogene	1q32.1	genomic gain	65	G, M, Ex, C,	13, 16, 18, 21, 25, 40, 41, 85–87
v-myc myelocytomatosis viral related oncogene, neuroblastoma derived	*MYCN*	oncogene	2p24.3	genomic gain or amplification	13 – 34 (gain) 3 – 30 (amplification)	G, Ex, C, A	14, 15, 32, 51, 52
DEK oncogene, DNA binding	*DEK*	oncogene	6p22.3	genomic gain	40 – 54	G, Ex, C, A	13–15, 56, 57
E2F transcription factor 3	*E2F3*	oncogene	6p22.3	genomic gain	70	G, Ex, C, A	14, 15, 33, 56, 57
Cadherin 11	*CDH11*	Tumour suppressor	16q21	genomic loss	58	G, Ex, C, A	23, 32, 70, 71
miR-17~92 cluster		oncogene	13q32	genomic gain	15	G, Ex, Ep, C, A	101,102
spleen tyrosine kinase	*SYK*	oncogene	9q22	Overexpression	NA	Ex, Ep, C, A	88

NA, not available; G, genomic studies (aCGH, MS-MPLA, NGS); M, mutational studies (SNP); Ex, expression studies (RT-PCR, real-time QPCR, microarray, protein in tumours and/or cell lines); Ep, epigenetic studies (methylation, miRNA), C, cell-based assays; A, animal studies (transgenic, xenograft)

Clin Experiment Ophthalmol. Author manuscript; available in PMC 2015 January 01.

Table 2

Emerging targets from recent genomic and expression analyses of retinoblastoma

Study Type	Gene name	Symbol	Minimal chromosomal region	Study type	Change	Primary tumours studied (#)	Frequency of change (%)	Involved in cancer	Reference
Genomic	glioma amplified on chromosome 1 protein	*GAC1*	1q32	WGSA	genomic gain	25	NA	Y	38
	SET and MYND domain containing 3	*SMYD3*	1q44		genomic gain	25		Y	
	centrosomal protein 170kDa	*CEP170*	14q23		genomic gain	25		N	
	SIX homeobox 1	*SIX1*	14q23		genomic gain	25	NA	N	
	SIX homeobox 4	*SIX4*	14q23		genomic gain	25		N	
	mucin 1	*MUC1*	1q12-q25.3	aCGH	genomic gain	18	22	Y	41
	myeloid cell leukemia sequence 1	*MCL1*	1q12-q25.3		genomic gain	18	22	Y	
	SHC (Src homology 2 domain containing) transforming protein 1	*SHC1*	1q12-q25.3		genomic gain	18	22	Y	
	S-phase kinase-associated protein 2, E3 ubiquitin protein ligase	*SKP2*	5p15.33		genomic gain	1 retinoma/retinoblastoma		Y	
	nucleotide-binding oligomerization domain, leucine rich repeat and BIR domain containing 1	*BIRC1*	5q13.2		genomic gain	1 retinoma/retinoblastoma		Y	
	v-ets erythroblastosis virus E26 oncogene homolog 1	*ETS1*	11q24.3		genomic loss	18	11	Y	
	ADP-ribosylation factor-like tumor suppressor protein 1	*ARLTS1*	13q13.2-q22.3		genomic loss	18	11	Y	
	cylindromatosis (turban tumor syndrome)	*CYLD*	16q12.1-q21		genomic loss	18	11	Y	

Study Type	Gene name	Symbol	Minimal chromosomal region	Study type	Change	Primary tumours studied (#)	Frequency of change (%)	Involved in cancer	Reference
	tenascin XB	*TNXB*	6p21.3		genomic gain	12	41	Y	
	paired box 6	*PAX6*	11p13		genomic gain	12	16	Y	
	Wilms tumor 1	*WT1*	11p13		genomic gain	12	25	Y	
	mutL homolog 3	*MLH3*	14q24.3		genomic gain	12	25	Y	
	tumor protein 73	*TP73*	1p36		genomic loss	12	8	Y	
	cell adhesion molecule 1	*IGSF4*	11q23	MS-MLPA	genomic loss	12	8	Y	77
	checkpoint with forkhead and ring finger domains, E3 ubiquitin protein ligase	*CHFR*	12q24.33		genomic loss	12	16	Y	
	cadherin 13	*CDH13*	16q24.2		genomic loss	12	8	Y	
	tumor protein 53	*TP53*	17p13.1		genomic loss	12	8	Y	
	GATA binding protein 5	*GATA5*	20q13.33		genomic loss	12	8	Y	
Gene expression	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	*PI3KCA*			upregulated	10	NA	Y	
	v-akt murine thymoma viral oncogene homolog 1	*AKT1*			upregulated	10	NA	Y	
	FK506 binding protein 12-rapamycin associatedprotein	*FRAP1*		Microarray	upregulated	10	NA	Y	78
	ribosomal protein S6 kinase, 70kDa, polypeptide 1	*RPS6KB1*			upregulated	10	NA	Y	
	cell division cycle 25 homolog A	*CDC25A*			upregulated	10	NA	Y	
	chromosome 17 open	*C17orf75*			upregulated	10	NA	N	

Clin Experiment Ophthalmol. Author manuscript; available in PMC 2015 January 01.

Study Type	Gene name	Symbol	Minimal chromosomal region	Study type	Change	Primary tumours studied (#)	Frequency of change (%)	Involved in cancer	Reference
	reading frame 75								
	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3	*ERBB3*			upregulated	10	NA	Y	
	LATS, large tumor suppressor, homolog 2	*LATS2*			downregulated	10	NA	Y	
	checkpoint with forkhead and ring finger domains, E3 ubiquitin protein ligase	*CHFR*			downregulated	10	NA	Y	
	breast cancer 1, early onset	*BRCA1*		microarray	differential functional gene group expression between tumour/normal	6	NA	Y	79
	breast cancer 2, early onset	*BRCA2*				6	NA	Y	
	aryl hydrocarbon receptor	*AHR*				6	NA	Y	
	ataxia telangiectasia mutated	*ATM*				6	NA	Y	
	polo-like kinase	*PLK*				6	NA	Y	
	E2F transcription factor	*E2F*				6	NA	Y	
	checkpoint kinase 1	*CHK1*				6	NA	Y	

NA, not available; WGSA, whole genome sampling array; aCGH, array comparative genomic hybridization; MS-MLPA, methylation-specific-multiplex ligation probe assay

Table 3

SNPs and gene mutations associated with retinoblastoma

Gene name	Symbol	SNP/ mutation	RefSeq gene	Positon	Nucleotide change	Amino acid change(s)	Effect of mutation	Risk	Odds Ratio	P value	Number of patients studied	Reference
mouse double minute 2	*MDM2*	2279744	NG_016708	5610	T>G	intronic	enhancement of mRNA expression	associated with incidence of retinoblastoma	4	0.001	336	82
mouse double minute 2	*MDM2*	2279744	NG_016708	5610	T>G	intronic	enhancement of mRNA expression	associated with incidence of retinoblastoma	0.6	0.04	104	85
mouse double minute 4	*MDM4*	11619719 2	NG_029367.1	26877	G>A	D153G	missense mutation in predicted casein kinase II ligation site	associated with incidence of retinoblastoma	5.41	0.001	104	85
mouse double minute 4	*MDM4*	4245739	NG_029367.1	38336	C>A	intronic	protein stabilization, insensitive to miR-191 mRNA inhibition	associated with retinoblastoma xenografts	NA	NA	44 (plus 3 orthotopic xenografts)	86
tumor protein 53	*TP53*	1042522	NM_000546.5	417	G>C	R72P	decreased induction of apoptosis	P/P variant associated with incidence of retinoblastoma	3.58	0.002	111	84
BCL6 corepressor	*BCOR*	NA	NA	NA	NA	N216-fs, E1290-fs, K1322-fs, A1434-fs, L1515-fs	premature truncation of protein	mutations, including 1 deletion, present in 6/46 tumours (13%)	NA	NA	46	88

NA, not available

Table 4

Differentially methylated genes in retinoblastoma

Gene name	Symbol	Methylation status in tumours	Assay	Frequency (%)	Reference
Ras association (RalGDS/AF-6) domain family member 1	*RASSF1A*	hypermethylation	MSP	82	92
	RASSF1A	hypermethylation	MSP	59	93
	RASSF1A	hypermethylation	MSP	60	94
O-6-methylguanine-DNA methyltransferase	*MGMT*	hypermethylation	MS-MLPA	58	77
	MGMT	hypermethylation	MSP	35	92
	MGMT	hypermethylation	MSP	35	95
cyclin-dependent kinase inhibitor 2A	*p16INK4A*	hypermethylation	MSP	91	96
mutS homolog 6	*MSH6*	hypermethylation	MS-MLPA	50	77
CD44 antigen	*CD44*	hypermethylation	MS-MLPA	43	77
paired box 5	*PAX5*	hypermethylation	MS-MLPA	42	77
GATA binding protein 5	*GATA5*	hypermethylation	MS-MLPA	25	77
tumor protein 53	*TP53*	hypermethylation	MS-MLPA	8	77
von Hippel-Lindau tumor suppressor	*VHL*	hypermethylation	MS-MLPA	8	77
glutathione S-transferase pi 1	*GSTP1*	hypermethylation	MS-MLPA	8	77
trefoil factor 1	*TFF1*	hypomethylation	Human methylation BeadChip	NA	88
Spleen tyrosine kinase	*SYK*	hypomethylation	Human methylation BeadChip	NA	88
minichromosome maintenance complex component 5	*MCM5*	hypomethylation	Human methylation BeadChip	NA	88
catenin (cadherin-associated protein), delta 1	*CTNND1*	hypermethylation	Human methylation BeadChip	NA	88
sex determining region Y	*SRY*	hypermethylation	Human methylation BeadChip	NA	88
SRY (sex determining region Y)-box 2	*SOX2*	hypermethylation	Human methylation BeadChip	NA	88
ADAM metallopeptidase with thrombospondin type 1 motif, 18	*ADAMTS18*	hypermethylation	Human methylation BeadChip	NA	88

MSP, methylation-specific PCR; MS-MLPA, methylation-specific-multiplex ligation probe assay; NA, not available

Table 5

Differentially expressed miRNAs in retinoblastoma

miRNAs	Expression in tumours	Method	Primary tumours studied (#)	Frequency of change (%)	Reference
let-7b, let-7c, miR-24, miR-125b, miR-191, miR 181a, miR-423	downregulated	microarray and qPCR	3	100	97
let-7 family	downregulated	RT-PCR	44	39	98
miR-494, let-7e, miR-513-1, miR-513-2, miR-518c, miR-129-1, miR-129-2, miR-198, miR-492, miR-498, miR-320, miR-503, miR-373	upregulated	microarray	9	100	99
miR-34a	variable	qPCR	2 (plus 2 cell lines)	NA	100
miR-17~92 and miR-106b~25 (paralog), let-7a, let-7f, miR-2, miR-7, miR-9, miR-16, miR-17a, miR-20a, miR-25, miR-26a, miR-30b, miR-30d, miR-92a, miR-93a, miR-96, miR-99b, miR-101, miR-103, miR-106b, miR-124, miR-143, miR-148b, miR-181a, miR-183, miR-216a, miR-217, miR-378, miR-1246	upregulated	microarray	32	15	101
let-7a, let-7b, let-7c, miR-10a, miR-10b, miR-20a, miR-21, miR-28, miR-29b, miR 30a-3p, miR-30b, miR-30c, miR-30d, miR-99a, miR-99b, miR-100, miR-103, miR-107, miR-124a, miR-125a, miR-125b, miR-133a, miR-136, miR-141, miR-145, miR-146a, miR-155, miR-181a, miR-181b, miR-182, miR-183, miR-190, miR-191, miR-206, miR-210, miR-222, miR-301, miR-302a, miR-302b, miR-320, miR-330, miR-335, miR-342, miR-368, miR-373, miR-380-5p, miR-382, miR-423, miR-433, miR-451, miR-452, miR-491	differentially expressed	computational	NA	NA	105
miR-129-3p, miR-382, miR-504, miR-22, miR-874, miR-139-3p, miR-758, miR-655, miR-129-5p, miR-200a, miR-370, miR-485-5p, miR-193a-5p, miR-330-5p, miR-429, miR-889, miR-499-5p, miR-342-5p, miR-448, miR-200b, miR-196b, miR-518f, miR-34c-5p	downregulated	microarray	12	100	106
miR-138, miR-155, miR-106b, miR-216a, miR-217, miR-20b, miR-17, miR-106a, miR-25, miR-652, miR-301b, miR-886-5p, miR-93, miR-34a, miR-18a, miR-449a, miR-449b, miR-224	upregulated	microarray	12	100	106
miR-1305, miR-424, miR-532-3p, miR-663b, miR-633, miR-194, miR-299-3p, miR-142-5p, miR-144, miR-93, miR-545, miR-374a, miR-374b, miR-665, miR-146b-5p, miR-194, miR-892b, miR-32, miR-501-5p, miR-513c, miR-513b	upregulated	microarray	14 serum samples	100	108

miRNAs	Expression in tumours	Method	Primary tumours studied (#)	Frequency of change (%)	Reference
let-7a, let-7d, let-7c, let-7f, miR-98, miR-let-7b, miR-1254, let-7e, miR-122, miR-221, miR-1299, miR-198-2, miR-486-3p, miR-375, miR-1260, miR-1287, miR-720, miR-124, miR-133a, miR-379, miR-129, miR-328, miR-335, miR-1228	downregulated	microarray	14 serum samples	100	108
miR-199a-3p, miR-99a, miR-125b, miR-214, miR-10b, miR-29b, miR-100, miR-224, miR-505, miR-29a, miR-363, miR-10a, miR-137, let-7c, miR-193a-3p, miR-374a, miR-130a, miR-29c, miR-335, miR-181a, miR-28-5p, miR-376a	upregulated	microarray	SNUOT-Rb1 cell line	NA	103
miR-124, miR-142-3p, miR-34a, miR-135b, miR-96, miR-142-5p, miR-183, miR-338-3p, miR-193b, let-7i, miR-182, miR-149, miR-let-7g, miR-34c-5p, miR-132, miR-34b	upregulated	microarray	Y79 cell line	NA	103

NA, not available

Table 6

Validated miRNAs and associated target genes in retinoblastoma

miRNA	Expression in tumours	Primary tumours studied (#)	Frequency of change (%)	Target genes	Target gene expression	Correlation with outcome/cellular function	Reference
let-7b	downregulated	3	100	*CDC25A* *BCL7A*	upregulated	NA	97
let-7 family	downregulated	44	39	*HGMA1* *HGMA2*	upregulated	poor tumour differentiation, invasion and proliferation	98
miR-34a	variable	2 (plus 2 cell lines)	NA	*CCND1*, *CCNE2*, *CDK4 E2F3*, *EMP1*, *MDMX*, *SIRT1*, *MLLT3*, *SLC30A3*, *CNTN2*, *PLCG1*, *ACTR1A*, *CDC25A*, *EFNB1*, *KCNH2*	downregulated in response to miR-34a induction	NA	100
miR-17~92 and miR-106b~25 (paralog)	upregulated	32	15	*CDKN1A* (p21Cip1)	downregulated in response to miR-17~92 inhibition	inhibition of miR-17~92 decreased cell line proliferation and tumour formation in mice	101
miR-129-3p, miR-129-5p, miR-382, miR-504, miR-22	downregulated	12 (plus 2 cell lines and mouse tumours)	100	*CDK4* and *CDK6* (miR-129); *MYC* (miR-382); *TP53* (miR-504); *HDAC4* and *MYCP* (miR-22)	NA	no significant correlation of miRNA expression and optic nerve invasion or intraocular neovascularization	106
miR-17, miR-18a, miR 20a	upregulated	20 serum samples	NA	via consensus regulatory network analysis: *PCNA*, *CDKN25A*, *CDC25A*, *KIF15*, *ERBB BAX*, *RAF1*, *MAP3K5*	NA	signature of advanced stagetumours	108
miR-19b, miR-92a	downregulated						

NA, not available