• Review Article •

Biomarkers in retinoblastoma

Jie Sun[1], *Hui-Yu Xi*[1,2], *Qing Shao*[1], *Qing-Huai Liu*[1]

[1]Department of Ophthalmology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
[2]Department of Ophthalmology, Xuzhou First People's Hospital of Xuzhou Medical University, Xuzhou Eye Research Institute, Xuzhou 221002, Jiangsu Province, China
Co-first authors: Jie Sun and Hui-Yu Xi
Correspondence to: Qing Shao and Qing-Huai Liu. Department of Ophthalmology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China. 13913862499@139.com; liuqh@njmu.edu.cn
Received: 2019-03-19 Accepted: 2019-10-22

Abstract

● Retinoblastoma (RB) is the most common intraocular malignancy of childhood caused by inactivation of the *Rb* genes. The prognosis of RB is better with an earlier diagnosis. Many diagnostic approaches and appropriate clinical treatments have been developed to improve clinical outcomes. However, limitations exist when utilizing current methods. Recently, many studies have identified identify new RB biomarkers which can be used in diagnosis, as prognostic indicators and may contribute to understanding the pathogenesis of RB and help determine specific treatment strategies. This review focuses on recent advances in the discovery of RB biomarkers and discusses their clinical utility and challenges from areas such as epigenetics, proteomics and radiogenomics.

● **KEYWORDS:** retinoblastoma; biomarkers; epigenetics; proteomics; radiogenomics
DOI:10.18240/ijo.2020.02.18

Citation: Sun J, Xi HY, Shao Q, Liu QH. Biomarkers in retinoblastoma. *Int J Ophthalmol* 2020;13(2):325-341

INTRODUCTION

Retinoblastoma (RB) is the most common primary intraocular malignancy tumor in pediatric patients worldwide, accounting for 6% of cancers in children[1]. It affects children under the age of 5y with an incident in one of 15 000-20 000 live births[2]. RB has adverse effects on the quality of life of infants and it is estimated that almost 9000 newly diagnosed pediatric cases are reported every year. With the development of improved surveillance and knowledge and clinical techniques and treatments, the 5-year survival rate has greatly improved. Among the plenty of clinical treatment options for RB tumors, chemotherapy has become an important therapy to restore vision, including chemo-reduction and intra-arterial chemotherapy, which can reduce the rates of enucleation in RB cases[3-5]. To date, the clinical risk assessment of RB tumors has been largely determined by a combination of clinical and histopathological features[6]. But there are still a small percentage of RB cases that cannot be evaluated using these methods, and the impact of chemical drug resistance and side effects remains to be resolved. To improve therapeutic outcome, avoid enucleation, and prevent metastasis, there is an urgent need to further study the biology and molecular mechanisms underlying RB and identify the specific biomarkers that cause tumor progression. Therefore, the study of RB biomarkers would be helpful in determining the severity of this disease, early diagnosis and accurate prognosis assessment.

The occurrence of RB can be hereditary and non-hereditary, related to germline and somatic mutations in the tumor suppressor *Rb1* gene, respectively[7]. As a pediatric cancer that occurs either as a sporadic or an inherited disease, it is known that RB results from the biallelic inactivation of the *Rb1* gene, which is located at the 13q14 region of chromosome 13[8-9]. Evidence from the study of mechanism suggests that it confers unlimited proliferation potential to RB because the functional retinoblastoma protein (pRB) is essential for chromosomal stability[10-11]. An analysis of embryonic retinal cells identified differentiating cones as the cell of origin for RB. Studies have shown that in dissociated retinal cultures, *Rb1* knockdown induced the development, proliferation and malignancy of cone precursors and formed tumors in orthotopic xenografts with histologic features and protein expression profiles typical of differentiated human RB[12-13]. In a very small percentage of RB tumors, the *Rb1* gene has been inactivated by chromothripsis in chromosome 13[14]. Although most RB tumors show alteration in both *Rb1* alleles, it has been shown that a subset of early-onset, unilateral, malignant RB tumors do not have the mutations in the second *Rb1* allele. This RB subset is usually diagnosed in infants younger than 6mo, and is caused by the amplification of a known gene MYCN[15-16]. Although the critical role in the dysfunction of *Rb1* and *MYCN*

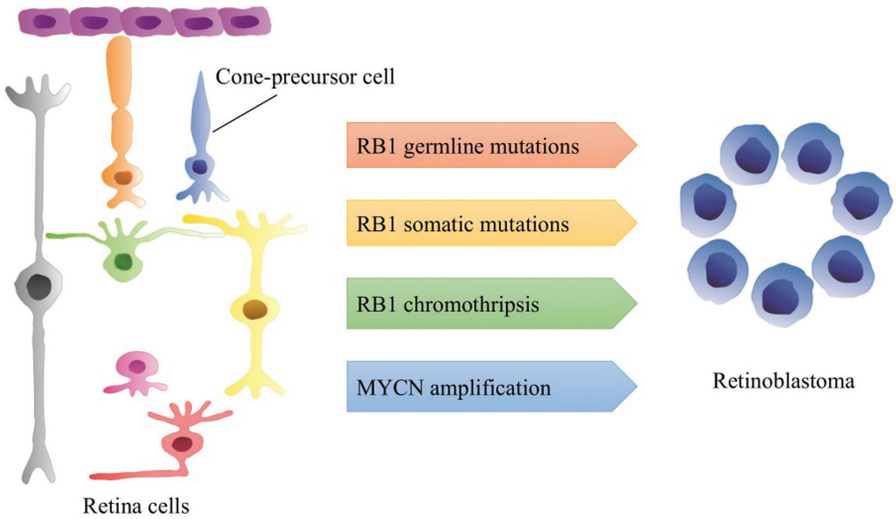

Figure 1 Pathogenesis of RB.

has been identified, current RB clinical treatments do not target these mutations specifically (Figure 1). Additionally, there are other identified oncogenes and suppressors. Some of them have become targets of action for the development of novel, efficient therapeutics. These genes that drive RB progression include: chromatic remodeling factors, MDM4, KZF14, DFR; transcription factor E2F3; and the tumor suppressor CDH11[17]. Although many studies have investigated the pathogenesis of RB, a thorough understanding of these mechanisms involving the cellular and molecular targets is lacking. Hence, research on the identification of RB biomarkers would be helpful to deepen our understanding of RB pathogenesis. Also, the new biomarkers could serve as potential indicators, leading to new therapeutics and help determine specific strategies for treatment.

Epigenetic Biomarkers in Retinoblastoma Aside from genetic mechanisms, epigenetic mechanisms play an important role in the progression of RB. It has been demonstrated that a variety of epigenetic alterations could act as potential biomarkers for RB pathogenesis. Studies have shown that *Rb1* is involved in the regulation of most major epigenetic alterations, including site-specific DNA methylation, histone modification, modification of microRNA (miRNA) and long non-coding RNA (lncRNA), and ATP-dependent chromatin remodeling[18-19]. It has been shown that inactivation of *Rb1* can lead to dysregulation of the tumor suppressor and oncogenic pathways through epigenetic mechanisms[20]. Moreover, the reprogramming of epigenomics is essential for tumorigenesis and provides a relatively new avenue for therapeutic targets against RB, as epigenetic modifications can be reversible[21-22]. Thus, epigenetic regulators should be integrated into approaches identifying new RB therapeutics.

DNA methylation biomarkers of retinoblastoma DNA methylation is one of the hallmark epigenetic events most studied in cancers[23]. DNA methylation involves the addition of a methyl group to the 5' carbon of a cytosine ring located 5' to a guanosine base in a CpG dinucleotide and is catalyzed by DNA methyltransferases (DNMTs). These CpGs are often clustered together and called CpG islands, the majority of which are found in the promoter region of genes. Hypermethylation of CpG islands in the promoter region leads to gene silencing through the inhibition of transcription or *via* recruitment of chromatin remodeling co-repressor complexes[24]. During RB tumorigenesis, the role of promoter methylation was first described when methylation of CpG islands (CpG 106) overlaps with the *Rb1* promoter and exon1[22]. It is now known that low expression of *Rb1* is associated with hypermethylation of the *Rb1* promoter[25]. In RB, aberrant DNA methylation has been found to be involved in many genes beyond *Rb1*, including *RASSF1A* (tumor suppressor related to microtubule stability), *MGMT* and *p16INK4A* (tumor suppressor) *etc*[26].

RASSF1A, the human Ras association domain family 1A gene, is located at 3p21.3 and encodes a predicted peptide of MW 39 000 and contains a Ras association domain. Loss of *RASSF1A* expression by promoter hypermethylation is a common epigenetic event in RB. A high frequency (82%, 56/68) of hypermethylation at the CpG sites of the *RASSF1A* promoter have been detected in RB carcinoma samples. The RB cell lines WERI-Rb-1 and Y79 carried a completely methylated *RASSF1A* promoter and did not express *RASSF1A*. Such epigenetic silencing was not detected among normal or nonmalignant retinal tissues, which indicates that aberrant promoter methylation of *RASSF1A*, may contribute to the pathogenesis of RB[27].

MGMT, the DNA-repair enzyme O6-methylguanine-DNA methyltransferase, is transcriptionally silenced by promoter hypermethylation in several human cancers[28]. Studies have

indicated that *MGMT* hypermethylation is related to poor differentiation. Hypermethylation of the MGMT promoter was found to be prominent among RB with poor tissue differentiation and was more frequently detected among patients with bilateral disease[29]. It has been determined that methylation of the *MGMT* promoter increases the sensitivity of glioma to alkylating agents[30]. In RB, alkylating agents such as carboplatin or cisplatin have been commonly used for treatment. The investigation of the effects of *MGMT* hypermethylation on the response to chemotherapy in RB is required. Therefore, silencing of *MGMT* is a poor prognostic factor in RB and may be a good predictive marker for chemotherapy when alkylating agents are used.

p16INK4A, which encodes a CDK inhibitor that prevents cell cycle progression, has been shown to play a crucial role in RB progression[31]. In fact, a high expression of *p16INK4A* in $Rb^{-/-}$ retina cells was proposed to prevent RB development by blocking transformation at the stage of the non-proliferative RB precursor lesion. In a recent study, evidence of an inherited *p16INK4A* downregulation has been shown in RB families, suggesting that this alteration might be a possible novel heritable susceptibility marker to RB. This study revealed a downregulation of *p16INK4A* in about half of the tumor and blood samples from RB patients. Furthermore, in most cases with *p16INK4A* downregulation at least one of the parents showed the same alteration in blood cells. More importantly, it was observed that *p16INK4A* downregulation seems to be due to the hypermethylation. The finding that the methylation of the *p16INK4A* promoter seems to be a germline and heritable alteration opens up the new possibility of monitoring high-risk families through simple blood sample analysis[32].

TFFs, which are required to maintain and reconstitute epithelial integrity, have been indicated in the involvement of processes including oncogenic modification, tumor growth and metastasis. Previous studies have demonstrated that *TFF1* was the only TFF peptide expressed at detectable levels RB cells. *TFF3* is the only TFF family member expressed in healthy human retina. In contrast, *TFF2* is the only TFF peptide expressed in the murine retina. After determining the TFFs promoter methylation status in RB cell lines, the correlation of endogenous *TFF* expression with promoter methylation was revealed. Up-regulation of *TFF1* displayed low-methylation or high demethylation of certain CpG islands in the promoter region[33-34]. Many studies have observed that the *TFF1* knockdown generates lower viability of RB cells in many RB cell lines, which could lead to deceased proliferation rates and the induction of apoptosis by activating p53[35]. Similarly, all RB cell lines with low-expression of endogenous *TFF3* showed high density methylation of all CpG islands. *TFF3* executes a significant pro-apoptotic, anti-proliferative, and tumor suppressive effect in RB cells, suggesting it could be a starting point for future additive chemotherapeutic approaches to reduce RB tumor size[36].

Histone modification biomarkers of retinoblastoma DNA exists in the nucleus in the form of chromatin, which is usually composed by DNA, histones, no-histones and a small amount of DNA. The histones H2A, H2B, H3, H4, are the basic structural proteins of chromatin, and constitute a nucleosome by wrapping about 200 base pairs of DNA[37]. Post-translational modifications can be made at many sites on the N-terminal tail of histones, including methylation, acetylation, ubiquitination, and phosphorylation. All of these modifications can cause a change in the conformation of the DNA-histone complex, affecting the binding of various transcription factors to DNA and gene transcription. Among these, histone acetylation is essential for maintaining histone function and DNA transcription, which is accomplished by coordinated catalysis of histone acetyltransferase (HAT). Thus, the disequilibrium of histone acetylation will cause changes in the corresponding chromosomal structure and gene transcription levels, which affect cell cycle, differentiation and apoptosis, and ultimately lead to tumorigenesis. Methylation is also an important modification of histones, and occurs mostly on the lysine and arginine residues of H2, H3. Histone methylation is catalyzed by specific histone methyltransferases (HMTs) and this process can be modified in a regulatable dynamic way. Thus, even without the introduction of DNA mutations, the modification of histones can alter the expression of oncogenes or tumor suppressors in cancer[38]. Among these changes, aberrant expression of an HMT could be a feature of RB.

Enhancer of Zeste homolog 2 (EZH2), an HMT enzyme which catalyzes trimethylation at lysine 27 on histone (H3H3K27me3), is the first epigenetic enzyme to be dysregulated in human RB. It is selectively expressed in cultured human RB cell lines and RB tumor samples, but is not present in non-proliferating cells of the normal fetal and postnatal retina. The specific presence of EZH2 in RB tissue suggested the protein might be a novel therapeutic target. Moreover, the EZH2 protein could be used to aid in the detection of invasive RB cells. EZH2 could also be used as a marker to identify single RB cell invasion into the optic nerve, a site of invasion whose involvement is an indication for systemic chemotherapy following enucleation. Indeed, inhibitors of EZH2 have been shown to kill human RB cells by impairing intracellular ATP production but did not affect primary human fetal retinal pigment epithelium (RPE). Thus, the dysregulation of EZH2 is a feature of human RB and an EZH2 inhibitor is a starting point for the development of epigenetic therapies in RB.

Ubiquitin-like with PHD and ring finger domains 1 (UHRF1) is not only a critical epigenetic regulator for DNA methylation

and histone modification, but also acts as an important regulator of tumor cell proliferation and survival. UHRF1 is highly expressed in human RB tumors and human RB cell lines but there is no detectable expression in normal retina[39]. A recent study revealed that high UHRF1 expression in RB promotes tumor cell survival against genotoxic insults by enhancing DNA repair and consequently reducing apoptotic cell death. Stable knockdown of UHRF1 sensitized RB cells to chemotherapeutic drugs such as etoposide and camptothecin. X-ray repair cross complementing 4 (XRCC4) is a key mediator for the drug sensitivity upon UHRF1 depletion in RB cells[40]. Therefore, UHRF1 can serve as a potential biomarker and an efficient therapeutic target to improve the efficiency of current chemotherapy for RB treatment.

TFF1, which has been described above, has been regarded, not only as a differentially methylated gene in RB, but also as one of the up-regulated genes with an activating histone modification in primary RB[33].

Biomarkers from non-coding RNA regulation in retinoblastoma Regulation of RNA-levels has emerged as one of the key epigenetic regulators for transcriptional regulation and modification of chromatin structure *via* formation of an RNA scaffold for RNA-protein interactions. Non-coding RNA (ncRNA) mainly consists of long non-coding RNA (lncRNA) and microRNA (miRNA). It can also be divided into two categories based on their location, nuclear and cytoplasmic RNA, which to a large extent, determines their mode of action[41].

miRNAs are short non-coding RNAs of about 21-22 nucleotides. It is known that miRNA is processed from a hairpin precursor and binds to the 3′ UTR of target mRNAs in the cytoplasm. It can repress gene expression by further corporation with the RNA-induced silencing complex (RISC), which degrades mRNA and inhibits of translation[42]. It has been demonstrated that miRNAs can be detected as diagnostic biomarkers for many types of cancer and the expression of miRNAs can be changed by various cellular pathogenesis signals; this alteration plays an important role in various cancers including RB[43-44]. The impact of miRNAs in RB can be either as potential components of oncogenes or tumor suppressors. Hence, the use of miRNAs can make a difference as an accessible and non-invasive biomarker for diagnoses and new therapies[45]. Many studies characterizing miRNA expression profiles have been performed to identify differential expression of miRNAs in RB tissues and cells. We can figure out the expression of a subset of miRNAs is in a higher level in RB compared to the healthy retina, and there are still a lot of miRNAs are down-regulated in RB (Table 1)[46-72].

Among these highly expressed miRNAs in RB, many have been proved to play a vital role in regulating tumorigenesis and progression. miR-17 to 92, an important cluster, which was selected as oncomir-1, was shown to be related to tumor development. miR-17 to 92 can induce RB cell proliferation in the case of *Rb1* inactivation and many studies have revealed the regulation mechanisms of miR-17 to 92. Indeed, RB in the miR-17 to 92 overexpression mouse model was highly invasive, with tumor cells observed in the optic nerve[73]. Epithelial cell adhesion molecule (EpCAM), is a type I transmembrane glycoprotein, which plays a role in RB tumorigenesis. It has been shown that the inhibition of EpCAM efficiently reduced miR-17 to 92 inhibition[49]. Another notable factor is single transducer and activator of transcription 3 (STAT3), which is activated in many cancers, and has been shown to be activated in RB tissues. It has been shown that activation of STAT3 triggers various cellular events by regulating the expression of genes involved in cell cycle, tumor migration and angiogenesis. Thus, STAT3 can be regarded as a signaling hub and leads to the upregulation of many oncogenic miRNAs including miR-17 to 92 clusters in RB through a positive feedback loop of miR-17 to 92/STAT3. Also, it was shown that the inhibition of STAT3 in RB reduces proliferation and migration[74]. Furthermore, the inactivation of miR-17 to 92 can restrain RB formation and co-silencing of p53 and miR-17/20a reduced the survival of RB cells. Thus, the lethal interaction between *p53* and the *Rb1* mutation results in the high expression of miR-17 to 92. Overall, these findings identify miR-17 to 92, especially miR17 and miR-20a as important biomarkers for therapeutic treatment of RB[75]. As we have identified above, *TFF1* can affect *p53* expression by regulation of miRNAs. miR-18a has been reported to regulate the p53 pathway by targeting interferon regulatory factor 2 (IRF2)[76]. miR-18a, which is encoded by the miR-17 to 92 cluster and is upregulated in RB, can be downregulated by the forced overexpression of *TFF1* mentioned above[35]. Interestingly, the overexpression of miR-130 and miR-181 is involved in the upregulation of EpCAM, inhibition of miR-181c and miR-130 can change RB cell properties by inducing apoptosis and reducing viability and proliferation. The significant role of EpCAM-regulated miR-130 and miR-181 can be detected in RB progression and these target genes and miRNAs can serve as new possibilities to reduce carcinogenesis[49]. Another highly overexpressed miRNA in RB tissue is miR-21, which targets *PDCD4*, a tumor suppressor gene, to regulate RB progression and metastasis. Therefore, miR-21 could also serve as a therapeutic target for RB[48,77].

Studies performed in RB have also identified clusters of miRNAs which are down-regulated compared to normal retina. Several miRNAs have shown great influence on RB pathogenesis. The Let-7 family, the most studied down-regulated miRNA in RB, has been shown to repress the

Table 1 MicroRNAs in RB

Expression in RB	MicroRNA(s)	Target gene(s)
Upregulation		
Tissue	miR-17-92[46]	EpCAM/STAT3
	miR-21[47-48]	PTEN/PI3K/AKT/PDCD4
	miR-130b, miR-181c[49]	Caspase3
	miR-494, miR-513, miR-518c, miR-129, miR-198, miR-492, miR-498, miR-503[50]	
Cell lines	miR-21[47]	PDCD4
	miR-24[51]	p14ARF
	miR-181b, miR-125a-3p, miR-30c-2[52]	HIF, VEGF
	miR-10b, miR-29, miR-34a, miR-124, miR-135b, miR-142-5p, let-7c, let-7i, miR-31, miR-200a, miR-19b, miR-195, miR-222, miR-181c[48-49,53-54]	
Serum	miR-17, miR-18a, miR-20a, miR-103, miR-182, miR-183, miR-30a, miR-29, miR-101, miR-26a, miR-378, miR-494, miR-16[55]	
Downregulation		
Tissue	miR-let-7e[56]	HMGA1/HMGA2
	miR-101[57]	EZH2
	miR-433[58]	Notch1, PAX6
	miR-183[59]	LRP6
	miR-204[60]	CyclinD2/MMP-9
	miR-125b[61]	CDK6, CDC25A, LIN28A
	miR-25[61]	BCL2L1
	miR-124[62]	STAT3
	miR-145[63]	ADAM19
	miR-613[64]	E2F5
	miR-129-3p, miR-382, miR-504, miR-22, miR-129-5p, miR-532-5p, miR-486-3p, miR-21, miR-200c, miR-143, miR-497[54,65-68]	
Cell lines	miR-491-3p[52]	HIF, VEGF
	miR-26a[69]	Beclin 1
	miR-125a-5p[70]	TAZ-EGFR
	miR-204[60]	CyclinD2, MMP-9
	miR-183[59]	LRP6
	miR-22[71]	
Serum	miR-19, miR-92a, miR-10b, let-7a, let-7c, let-7e, miR-129, miR-21, miR-25, miR-198, miR-503, miR-217, miR-9, miR-320, miR-373, miR-498, miR-92a[55]	
Plasma	miR-320, miR-21, let-7e[72]	

RB: Retinoblastoma.

functionality of oncogenes of HMGA, C-Myc, and Ras family functionally. high-mobility group A (HMGA), which modulates transcription by affecting chromatin structure, is expressed in many tumors. HMGA1 and HMGA2 are the most meaningful types of HMGA[56]. In many RB samples, the high level of HMGA1 and 2 is accompanied by the down-regulation of Let-7. Thus, it can be suggested that there is a significant inverse correlation between HMGA1 and 2 and Let-7. The function of Let-7 as tumor suppressor can be regulated by HMGA 1 and 2[78]. There are other miRNAs that are down-regulated in RB tissues and cell lines. miR-183 targeting the low-density lipoprotein receptor related protein 6 (LRP6) can suppress RB cell viability, invasion and proliferation but was down-regulated compared to healthy retina[59]. As we mentioned above, EZH2 is highly expressed in RB, but miR-101, which is down-regulated, can inhibit the expression of EZH2. miR-101 can reduce cell growth and induce cell cycle arrest and apoptosis and functions as an important tumor suppressor[57]. In addition, the up-regulation of miR-204 in RB cells and tissues is propitious to the inhibition of RB cell proliferation and metastasis, both *in vitro* and *in vivo*. Thus, miR-204 can suppress the progression of RB; its critical regulative role for RB was identified by targeting the matrix metalloproteinases-9 (MMP9) and cyclin D2 genes[60].

In addition to these upregulated and downregulated miRNAs in RB, there are a number of miRNAs which have variable expression in RB cell lines and primary RB samples. Among these, the miR-34 family is important in RB tumorigenesis and progression because they act as tumor suppressors by inducing cell cycle arrest and the inhibition of apoptosis[79]. The miR-34 family, including miR-34a and miR-34b, is related to an important tumor suppression pathway, the p53 pathway,

which transcriptionally activates the miR-34 family. miR-34a has been shown to be a tumor suppressor by targeting HMGB1 mRNA, which suppresses the translation of HMGB1. As we mentioned above, HMGB1 promotes tumor formation, invasion and migration and it can also inhibit apoptosis and induce drug resistance by regulating autophagy[80]. Thus, miR-34a acts as autophagy inhibitor and promotes the cytotoxicity of chemotherapy drugs. miR-34b is another member of the miR-34 family, which is upregulated in RB cell lines and is regulated by posttranscriptional regulation of gene expression[81-82]. In addition to this effect on the p53 pathway, miR-34 can also mediate the downregulation of p14ARF to abrogate p53-mediated surveillance[51]. Furthermore, other studies have emphasized the potential role of miRNAs as important biomarkers of RB. It was shown that miR-302, Let-7e and miR-21 were downregulated in the plasma of RB patients compared to normal people[72]. Similarly, various serum miRNAs are changed in serum samples[55]. Thus, miRNAs levels can be used as new biomarkers for diagnosis and prognosis.

LncRNA is a class of non-coding RNA longer than 200 nucleotides. Unlike miRNA, lncRNA can be folded into secondary structures to form more versatile and flexible interactions between RNA and protein. The vital roles of lncRNA in tumor initiation and progression has been previously shown[83]. In recent years, many studies have demonstrated that lncRNA are dysregulated in RB and have characterized the roles of lncRNA in RB[84]. Also, lncRNA can compete with miRNAs by binding to the same regions of miRNAs, resulting in the downregulation of target mRNAs. The important roles of lncRNA in RB indicate that they can be critical potential diagnostic and prognostic biomarkers. In RB cell lines, it has been shown that the lncRNA colon cancer-associated transcript 1 (CCAT1) is upregulated and has an effect on RB cell migration and proliferation by negative regulation of miR-218-5p[85]. The lncRNA actin filament-associated protein 1 antisense RNA1 (ATAP1-AS1) is elevated in RB cell lines and tissues and is related to tumor size and invasion. Moreover, the high expression of AFAP1-AS1 is an independent adverse prognostic factor for RB patients. These studies suggest that both CCAT1 and AFAP1-AS1 could serve as potential biomarkers and therapeutic targets for RB[86]. Another oncogenic lncRNA is brain-derived neurotropic factor antisense (BDNF-AS), which is downregulated in RB tumors and cells. When upregulated, BDNF-AS can suppress the cell-cycle transition and results in the inhibition of RB progression. Therefore, the low expression of BDNF-AS can be used as a prognostic biomarker[87]. Maternally expressed gene 3 (MEG3), a lncRNA, is downregulated in RB tissues and its level is negatively related to nodal and distant metastasis. Moreover, the tumor suppressive role of MEG3 is regulated by negatively affecting the activity of the Wnt/β-catenin pathway. Thus, it can also be a biomarker for prognosis and a target for molecular therapeutics[88]. Interesting, lncRNA are also closely correlated with miRNAs to regulate RB biological characteristics. For instance, the 3′-UTR of the lncRNA differentiation antagonizing non-protein coding RNA (DANCR) and the mRNA of MMP9 can be targeted by miR-34c and miR-613; DANCR is upregulated in RB tissues and cells[89]. Conversely, the lncRNA H19 is downregulated in RB and it inhibits RB cell proliferation and induces cell apoptosis and cell cycle arrest. These inhibitions of RB are due to the counteraction of the function of the miR-17 to 92 cluster by binding to each other. Through this binding, H19 represses the activation of STAT3 induced by miR-17-92 and induces p21 expression which is, conversely, suppressed by the miR-17 to 92 cluster[90]. In summary, lncRNA are prospective biomarkers for RB progression and are also therapeutic targets.

Biomarkers from Proteomic Analysis in Retinoblastoma

Although a number of potential biomarkers have been reported, the development in the field of proteomics enables the profiling of proteins for the identification of reliable biomarkers or specific therapeutic targets in RB. Proteomics is a systematic study for probing the expression of total proteins in cells and tissues and the analysis of proteomics is an accurate, high-throughput strategy for the identification of proteins[91]. At first, the methods for detecting the RB proteome were gel-based approaches, such as two-dimensional (2-DE) electrophoresis and mass spectrometry (MS), but these gel-based approaches can only detect a small portion of the total proteins[92]. In recent years, the arrival of proteomics technology has provided a solid foundation for the better understanding of RB pathogenesis by global detection and quantitation of proteins. These high-throughput, gel-free proteomics technologies such as iTRAQ, ESI-MS/MS, and LC-MS/MS, combined with bioinformatics analysis can identify thousands of proteins for proteomic profiling in RB. iTRAQ (isobaric tags for relative and absolute quantitation) is a method of labeling amines in peptides; the peptides are then subjected to fractionation by bRPLC. The use of ESI-MS/MS and LC-MS/MS can then be used to identify and quantitate protein[93]. Lastly, the identified proteins are submitted to bioinformatics analysis and annotations. Overall, these strategies can detect the comprehensive proteomic signature as well as potential biomarkers in RB.

Biomarkers from proteomic profiling in retinoblastoma tissues Mallikarjuna *et al*[91] reported the proteome of fresh RB tissues using a 2-DE-MS/MS. This study revealed that twenty-seven proteins were differentially expressed in RB compared to normal retina, including 11 downregulated and 16 upregulated proteins. The detection of mRNA levels of

some of the identified proteins and immunohistochemistry were consistent with the proteomic data. Among these, proteins including CRABP2, peroxiredoxin 6, apolipoprotein A1, and recoverin were significantly more expressed in RB with invasion. Recently, with the advent of proteomics technology, more studies have been performed to investigate the proteomic profiling in RB tissue. The study performed by Danta *et al*[94] using high- resolution MS between RB and normal retina, quantified 3587 proteins overall and identified 899 differentially expressed proteins; 402 were upregulated and 497 were downregulated. This study corroborated the previously reported overexpressed proteins in RB, including spleen tyrosine kinase (SYK), vimentin (VIM) and stathmin1 (STMN1). Beyond that, novel proteins were identified in RB; this study validated RACGAP1, CHGA and AHSG IGF2BP1 by IHC, which were not previously reported. Among these, the functional validation of insulin growth factor 2 mRNA binding protein 1 (IGF2BP1) has been demonstrated suggesting that it plays a vital role in RB cell proliferation and migration.

In addition to these studies in general RB tissues, the comparative proteomes in both human papilloma virus (HPV) positive and negative RB patients were conducted because the important role of HPV in abolishing pRB protein function was identified[95]. The significant association between high risk HPV subtypes and sporadic RB was revealed over the past decade[96]. Thus, this study aimed to detect potential RB-specific prospective targets and to clarify the possible role of HPV, which could help us understand the mechanism of RB progression. The analysis of this study revealed that in HPV positive RB, β-catenin (CTNNB1) was the most important regulated signaling pathway compared to HPV negative RB and suggested that glial fibrillary acidic protein (GFAP), retinol-binding protein 3 precursor (RBP3), and apolipoprotein A1 (APOA1) could act as potential targets and biomarkers for research on the therapy and support of disease mechanisms.

Cellular retinoic acid binding proteins (CRABPs), the members of the fatty acid binding family of proteins, have aberrant expression in RB compared to normal retina[97]. Through the studies mentioned above, it was observed that CRABP1 was significantly downregulated but CRABP2 was overexpressed in RB. CRABP1 slowed down the proliferation of RB cells through retinoic acid reporter (RAR)-mediated transcription programs, but CRABP2 was involved in RB cell proliferation *via* the activation of the PPARβ/δ and FABP5 proteins[98-99].

Alpha-crystallin-A (CRYAA), a major lens structural protein, acts as an anti-apoptotic factor by restricting the product of cytochrome C from the mitochondria, which could inhibit apoptosis in tumor cells. The increased expression of CRYAA was detected in RB tissues compared to healthy retina and this overexpression was correlated with the apoptotic index[95]. Thus, CRYAA may have a positive role in preventing apoptosis of RB cells and the understanding of that mechanism could help us target CRYAA-expressing RB cells with specific anticancer drugs[100].

APOA1, which transports cholesterol from peripheral tissues to the liver for excretion, was overexpressed in an RB cohort compared to healthy retina. APOA1 is reported to directly promote tumor survival through kinase activation in recurrent head and neck squamous cell carcinoma, but the elucidation of its precise role in RB requires more studies to elucidate[91].

VIM is a cytoskeletal intermediate filament protein located in mesenchymal cells and is expressed in astrocytes and Müller cells in healthy retina to preserve cell integrity and resist stress. VIM was overexpressed in RB tissues, especially in HPV positive tissues compared to negative RB tissues. The overexpression of VIM was accompanied by an elevation of sex-determining region Y box 2 (SOX-2) and vascular endothelial growth factor (VEGF) in high-risk factor (HRF) RB, which partially elucidated the aggressiveness of HRF RB[101]. The important role of VIM as a marker in the epithelial mesenchymal transition (EMT) could provide evidence of a connection between VIM overexpression and tumor progression, including RB cell invasiveness and poor prognosis[20].

GFAP is another cytoskeletal intermediate filament protein which was overexpressed in RB. Similar to VIM, GFAP can act as biomarker for activated Müller and astrocyte cells, but GFAP is only expressed in Müller cells after retinal injury[102]. Thus, although GFAP has been identified as a serum biomarker for glioblastoma multiforme and was recently overexpressed in RB, it is still questionable to consider GFAP as a biomarker in RB as elevated GFAP expression could be detected as a specific response to retinal injury. However, GFAP is considered to be a marker in RB glia cells, which enhance RB cell proliferation and induce RB cell apoptosis. Hence, the precise function of GFAP requires further studies.

RBP3, a glycoprotein synthesized in photoreceptors, was significantly downregulated in RB. It was shown that RBP3 is in charge of transferring retinoids between the RPE and the outer segment (OS) of photoreceptors. Further, the reduction of RBP3 resulted in impaired RAR activity and photoreceptor preservation, a condition which may lead to the proliferation of RB cells and impairment or loss of photoreceptors.

Besides these proteins discussed above, the analysis of the network and pathways of proteins demonstrates that most proteins have interactions with signaling molecules like Akt, myc, and hsp90. Both Akt, a serine threonine protein, and the transcriptional regulator myc are related to the regulation of cell development, survival, and death, and both of them are deregulated oncoproteins in many cancers. Akt is involved

in tumor suppression by MDM2-mediated proteasomal degradation. Also, the elevated expression of Akt1 and other vital factors of the PI3K/Akt/mTOR pathway have been detected in unilateral RB tumors, which suggest the possibility of these regulators as potential biomarkers and therapeutic targets for RB[103].

The proteomic analysis of the vitreous humor in RB tumors has also been conducted. Because the vitreous humor is associated with the size of RB tumors in the eye, the vitreous humor is an auspicious source to study specific protein targets in RB. The alterations in the retina will lead to changes of both proteomic and biochemical properties in RB. Most importantly, the vitreous humor has a greater advantage for studying biomarkers in RB by acting as a medium[104-105] for many aspects, such as lower risk of tumor seeding when collecting samples and direct delivery of chemotherapeutic drugs. Thus, this study conducted proteomic analysis of vitreous fluid to explore novel therapeutic targets and biomarkers in RB by using iTRAQ labelling coupled with ESI-MS/MS and bioinformatics analysis[106]. This analysis showed 431 differentially expressed proteins, including 362 upregulated and 69 downregulated proteins that were detected. Several proteins like MMP2, TNC, and CRABP1 were highlighted for use as potential therapeutic targets in RB. In addition, p38MAPK and Akt signaling were revealed as important regulated pathways in RB through network and pathway analysis.

MMP2 protein is a zine-dependent enzyme which was significantly overexpressed in RB than controls; the elevated MMP2 levels were closely associated with RB cell differentiation and optic nerve invasion in RB.

Tenascin (TNC), another extracellular matrix glycoprotein, was upregulated in RB. Though the precise role of TNC in RB is still unknown, it has been reported that TNC-C could act as modulator for tumor cell growth and migration. Also, the correlation between high levels of TNC and poor prognosis has been reported for many other cancers[107].

CTNNB1 was identified as a hub protein, which could connect proteins. In the dataset of this study, all detected proteins had an interaction with upregulated CTNNB1, suggesting the vital role of CTNNB1 in the associated pathways.

SOX2, a member of high-mobility group transcription factors family, has a key role in embryotic development and cell differentiation[108]. Interestingly, recent studies demonstrate that SOX2 is overexpressed in lung, liver, stomach and breast cancers, and is associated with tumor development, invasion and metastasis[109]. It has been reported that the expression of SOX2 is increased in RB tissues and is correlated with the degree of RB differentiation and optic nerve invasion. SOX2 gene is highly expressed in the peripheral blood of children with RB. The level of SOX2 expression was significantly higher in the peripheral blood of the poorly differentiated group compared with the well-differentiated group[110]. However, further research is needed to clarity the specific molecular mechanism of SOX2. To summarize, SOX2 has an important role in the incidence and development of RB, and SOX2 gene expression may be a novel target biomarker for the early diagnosis and treatment of RB.

Membrane proteomic biomarkers of retinoblastoma

Recently, the role of membrane proteins involved in ion transport and signal transduction has been demonstrated. Since these membrane proteins can act as secretory molecules, this makes them alterative biomarkers for tumor diagnosis and prognosis[106]. Of the total proteome, 30% are membrane proteins including peripheral and integral proteins. Integral membrane proteins contain transmembrane domains which go through the lipid bilayer but peripheral proteins interact with the integral membrane without traversing the lipid bilayer. Peripheral proteins are much easier to extract due to the hydrophobic nature of integral membrane proteins[111-113]. In this study, the researchers used iTRAQ but used high-resolution MS to extract and quantitate the membrane proteins in RB primary tumors and normal retina[114].

Based on the membrane proteome analysis, 3122 proteins were detected, among which 663 proteins were dysregulated, including 282 upregulated and 381 downregulated in RB tissues compared to healthy retina. The bioinformatics analysis illustrated that most proteins were related to RB cell growth, communication and transport. Furthermore, the comparison between the membrane proteomic data and the proteomic profiling detected on whole RB proteome and vitreous humor from RB patients showed that 86 proteins were accordant in these three studies, with 314 proteins that were specifically dysregulated in the membrane protein data, suggesting that these proteins could be novel in RB. The common proteins which was present both in the membrane proteome and the vitreous humor proteome suggested that these proteins could be used as biomarkers for the diagnosis and prognosis of invasive RB. When forced to identify molecular functions, the laminB1 (LMNB1) and transferrin receptor (TFRC) showed significant positive expression in RB tissues by immunohistochemistry compared to normal retina, which indicated that both could be novel biomarkers and therapeutic targets for RB.

LMNB1, an important member of the lamin protein family, is usually located in the membrane and matrix of the nuclear membrane. It plays a vital role in cellular functions, like gene expression and nuclear stability[115]. LMNB1's role in cancer and especially in RB is largely unexplored and its role in cell progression will be elucidated by future studies.

TFRC is a glycosylated protein, which can bind to diferric transferrin (TF) and forms a complex at acidic pH, releasing

the ferric ion. It has been reported that the overexpression of TFRC could lead to the disruption of iron homeostasis, which can induce cancer progression. The overexpression of TFRC and TF have been detected in RB, indicating the disruption of iron homeostasis[116]. However, further studies will need to be conducted to reveal the potential mechanism of TFRC in RB.

Proteins in aqueous humor and serum of retinoblastoma

Early diagnosis of RB is essential for reducing mortality. Current research focuses on safe and non-invasive assessment modalities that can diagnose disease accurately. Ideally, such molecular assays would be applicable to non-invasively obtained body fluids such as aqueous humor and blood.

Lactate dehydrogenase (LDH) is a conventional RB marker. Studies have shown that the presence of RB is significantly associated with the aqueous humor LDH level. The LDH activity in the aqueous humor and serum did not show any correlation with each other[117]. There was no correlation between aqueous humor LDH levels and the following clinical features such as sex, family history, bilaterality, prior treatment, presentation age, enucleation age, and metastasis[118]. In conclusion, detecting LDH levels in aqueous humor can be a useful adjunct to the clinical diagnosis of RB.

Survivin is a bifunctional inhibitor of apoptosis protein that has been implicated in protection from apoptosis and regulation of mitosis. It is overexpressed in most human neoplasms and has the potential to be used as a tumor biochemical marker[119]. Recently, it was reported that coexistence of survivin and heat shock protein 90 probably plays an important role in cellular proliferation in RB[120]. The concentration of survivin in serum and aqueous humor was significantly high in RB. Additionally, there was a positive and significant correlation between aqueous humor survivin and RB staging (P=0.000) and optic nerve affection (P=0.003). The specificity of survivin in both serum and aqueous humor was >90%. However, the sensitivity of serum survivin (100%) was much higher than that of aqueous humor (48%)[121]. Thus, measurement of survivin might become a molecular marker for the diagnosis and follow-up of RB patients.

Transforming growth factor beta (TGF-β) is a cytokine that can act as both a tumor-suppressor and a tumor-promoter, depending on the cellular state and environment[122]. TGF-β1 is overexpressed in many tumors and thought to contribute to tumor progression, invasion and metastasis[123]. Shehata *et al*[124] found that TGF-β1 were significantly higher in RB patients in aqueous humor and serum samples than the corresponding control group. And there is a positive significant correlation between mean aqueous humor concentration of TGF-β1 protein and poor differentiation of RB (r=0.69, P=0.001).

Radiogenomics Biomarkers in Retinoblastoma

Radiogenomics, also called imaging genomics, is a rapidly evolving term that can be used to identify the association between genetic variation and imaging features. The radiogenomics term was created by Andreassen *et al*[125] in 2002. In the past 20y, genomic data such as DNA microarrays, RNA and DNA-seq has gradually emerged. It allows radiogenomics to not only be used as vital images for diagnosing diseases, but also to establish new correlations with cellular genomics. Based on these studies, many researchers have used this to identify imaging biomarkers for diagnosis, especially for noninvasive genotyping. Given the promising results, these imaging features could be a substitution for normal genomics. Thus, radiogenomic biomarkers could have potential roles both in prognosis prediction and precise treatments of patients.

Since RB cannot be diagnosed by histopathologic and biopsy-based genotyping, it is necessary to use noninvasive genotyping of RB. Magnetic resonance (MR) imaging is an essential and standard method for diagnosis and examination of the extent of RB[126]. Radiogenomics provides the imaging-genomic associations in both diagnosis and further clinical prognosis and decision in RB.

In the study of Jeasen *et al*[127], they explored two different ways to identify MR imaging features as biomarkers for utilitarian gene pathways and RB progress. One of the two ways was the assessment of the predefined photoreceptor-related gene expression signature, called photoreceptorness. As we have described above, pRB protein depletion induced RB cells to differentiate from cone precursors. RB without photoreceptorness became poorly differentiated morphologic characteristics in advanced stages of RB and loss of this photoreceptorness gene expression was related to drug sensitivity *in vivo*[128-129]. From this study, MR features suggested that this photoreceptorness could act as an indicator for advanced stages of RB. RB with photoreceptorness loss showed larger eye size, the equal enhancement of the choroid beneath the tumor, multisite lesions and a tumor filling the entire eye. Thus, these radiogenomic biomarkers could be predictors of prognosis and assist in RB treatment. The second way to identify MR imaging features as biomarkers was the analysis of the association between MR images and gene expression profiles. The image-genomic correlation could be detected using 10 MR features and 1336 dysregulated genes. It revealed that subretinal seeding in MR images showed an association with RNA processing and multifocal lesions, and was associated with low expression of cilia development genes, which could restrain RB cell growth[130-131]. Thus, these results indicate that radiogenomic biomarkers could be helpful for predicting tumor process and treatment effects. Moreover, among these imaging-genomic correlations, two individual genes were relevant to RB. RB with smaller size

was correlated with PAX2 overexpression, which could affect development of the optic stalk and the RPE[132]. The other gene *MYCN*, whose amplification could drive RB as we introduced above, showed an association with subretinal seeding. From the results of MR images, a defined radiophenotype of RB was detected, including a combination of plaque shaped, diffuse growth and multifocal lesions. This newly distinct imaging phenotype was related to the downregulation of KAL1 and the upregulation of SERTAD3[133]. The dysregulations of both KA1 and SERTAD3 were significantly related to shape, diffuse growth and multifocality. The stimulation of SERTAD3 to E2F may cause this more aggressive radiophenotype in RB. However, the correlation between RB and KAL1 is still unknown and requires more research.

Overall, the advantages of radiogenomics are considerable for genotyping RB because it is a noninvasive test and can be used to monitor all disease stages. However, there are limitations of RB radiogenomics, such as the limited generalizability and the difference in spatial resolution.

The Other Genetic Biomarkers in Retinoblastoma Because overall survival and vision melioration depend on the severity of disease, early diagnosis and accurate prognostic evaluation are important. Recently, investigations have identified other genetic biomarkers such as gene polymorphism, which can affect disease progression. These genetic biomarkers can not only help us to effectively understand RB pathogenesis, but also induce the development of new therapeutic methods.

Biomarkers from gene single-nucleotide polymorphism of retinoblastoma The TP53 gene is well known to be the most frequently mutated gene in human cancer. In addition to mutations, there are >20 different coding region single nucleotide polymorphisms (SNPs) in the TP53 gene. Several of these SNPs are known to alter p53 pathway function[134]. It has been suggested that the p53 pathway targets pRB for degradation and in physiological conditions controls the cell cycle and apoptosis in retinal cone precursor cells, from which the RB cell lineage originates[135]. The Arg to Pro change in codon 72 (also known as p.Arg72Pro, rs1042522 G>C) is the most frequently studied functional SNP in p53. Previous studies have shown that p53 rs1042522 modulates susceptibility to hereditary RB in an Italian population[136]. However, no association was found between RB cancer risk and the rs1042522 genotype in a Chinese population. Interesting, the heterozygous genotype of p53 rs1042522 is significantly associated with decreased RB invasion in a Chinese Han population. Furthermore, subgroup analyses supported the association between the p53 rs1042522 GC genotype and RB invasion among patients with a lag time greater than 1mo or without pre-enucleation treatment. Therefore, these findings may be useful in the near future for the promotion of prevention strategies guided by the patient's genotype because the p53 rs1042522 genotype and clinical characteristics (lag time and pre-enucleation treatment) were associated with a reduced risk of RB invasion[137].

The MDM2 gene is an important negative regulator of the p53 suppressor gene, promoting the degradation of p53 through its E3 ubiquitin ligase activity[138]. The polymorphism in MDM2 most widely studied is rs2279744 (also known as MDM2 SNP T309G), which is a T>G transversion SNP on human chromosome 12. An increased risk of RB was found for the MDM2 rs2279744 polymorphism. Also, this SNP could increase the affinity of the transcriptional activator Sp1 which increases the expression of MDM2 DNA and protein, ultimately leading to the attenuation of the p53 pathway. These results determined that the MDM2 rs2279744 polymorphism may be a risk factor for the development of RB. Likewise, it has also been shown that the MDM2 rs937283 polymorphism was significantly associated with decreased risk of RB. The MDM2 rs937283 SNP, known as G2164A, leads to an A to G base change at the nucleotide 2164 in the promoter region of MDM2 gene[139].

The MDM4 gene (also known as MDMX), located on chromosome 1q32, is described as an MDM2 homologue with high structural similarity which has been shown to promote the proteasome-mediated degradation of p53 and negatively regulate the p53 pathway. It was observed that MDM4 is overexpressed in RB compared with fetal retina[140]. Recent studies have reported that both the AA genotype and the A allele at MDM4 rs11801299 were related to an increased risk of developing RB as well as tumor invasion and poor pathological differentiation. Further, the G allele at rs1380576 reduced the risk of developing RB, and was associated with low tumor aggressiveness. In addition, the G allele at rs11801299 reflected poor prognosis of RB patients[141].

Another meaningful SNP is rs4938723 T>C, which is located in the promoter region in the mir-34b/c gene and alters mir-34b/c gene expression. The mir-34b/c inhibits p53 antagonists and pro-apoptotic proteins and it is a part of the p53 pathway. A recent study has implied that a germline *Rb1* mutation and the SNP rs4938723CC of the mir-34b/c gene can speed RB genesis and patients with these mutations could have earlier diagnosis. Thus, the mir-34b/c SNP rs4938723 T>C may act as a potential biomarker for hereditary RB[82]. Though the interaction of SNPs can provide novel markers for RB prognosis, diagnosis, and treatment, further studies need to be done.

Genetic biomarkers in high-risk retinoblastoma Besides biomarkers for RB diagnosis, prognosis, and therapy, genetic biomarkers to define high-risk RB are also essential to reduce mortality. Nowadays, the calculation of RB clinical

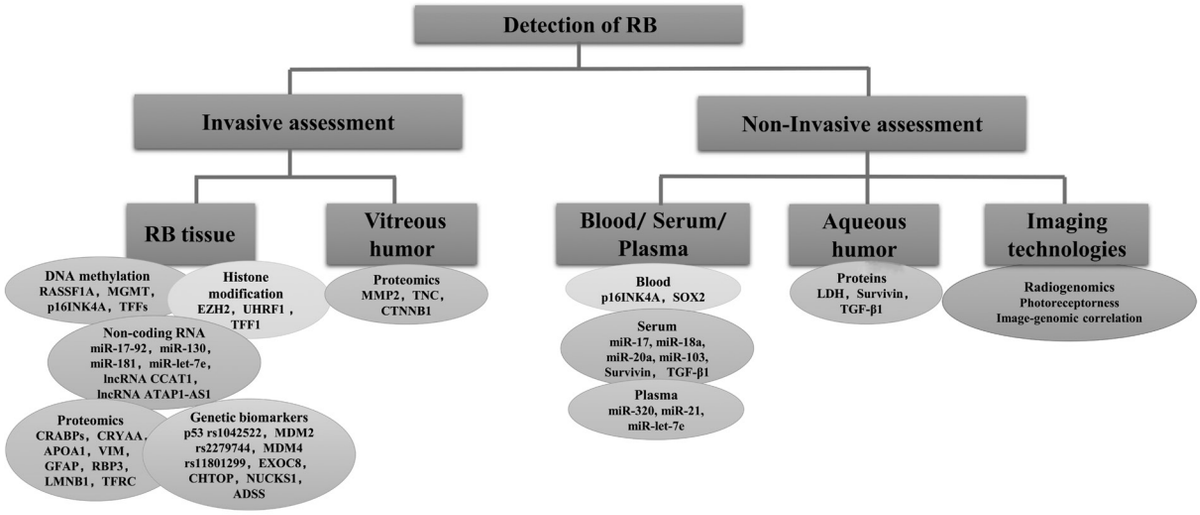

Figure 2 Detection of RB.

risk is mainly determined by histopathologic features combined with clinical traits. These features, called high-risk histopathologic features (HRFF), are detected in most RB cases[142]. However, there is a small portion of RB patients that do not have these typical features even after progressing to metastasis and death[143]. Thus, it is important to discover additional biomarkers to predict high risk RB and the effect of chemotherapy. Cellular anaplasia, which is different from cellular differentiation, indicated high-risk RB and predicted the RB metastases even without HRRFs[6]. Furthermore, as we all know, gene profiles could assist researchers in identifying mechanisms devoted to anaplastic severity and define genetic markers of anaplastic grades. Hudson *et al*[144] conducted gene profiles to characterize anaplastic grades in RB, and showed that different anaplastic grades have various gene expressions. Especially in severe anaplasia, photoreceptor and nucleoporin expression were highly dysregulated, including downregulation of photoreceptor genes and markedly increased nucleoporin expression. A limited gene set consisting of EXOC8, CHTOP, NUCKS1, and ADSS accurately separated severe anaplasia from all other samples, which means this gene set could predict severe anaplasia. Overall, these results contribute to identification of biomarkers for high-risk RB.

DISCUSSION

RB, one of the most common primary pediatric malignancies, usually develops from the immature cells of the retina. In recent years, the morbidity and mortality of RB have greatly decreased, due to accurate early diagnosis and prognosis. A better understanding of RB disease progress facilitates the identification of biomarkers for diagnosis and treatment. RB biomarkers are rapidly being discovered through investigations of processes involved in RB carcinogenesis. Previous studies have identified many promising RB biomarkers, such as epigenetic alterations. Generally, epigenetic alterations result from genetic deregulation and interact with each other in RB pathogenesis. Though these epigenetic and genetic biomarkers have emerged as important diagnostic approaches, more studies are needed for validation, better diagnoses and therapies in RB. Apart from epigenetics, the emergence of other research techniques enables us to detect other biomarkers in RB. For example, both proteomic and radiogenomics approaches were used to identify biomarkers, which implies that these approaches could play an important role in diagnosis and prognosis. Because tumor biopsy is not typically used to diagnose RB, the utilization of radiogenomics and the vitreous proteome provides a significant opportunity to reveal the biology of the RB tumor. However, it should be mentioned that the translation of proteomic and radiogenomics to therapeutic regimens has not been implanted, primarily because targeted RB therapies need further development. Furthermore, appropriate disease and treatment stratification is essential to improve the survival rate of RB patients. Hence, the identification of RB genetic markers from different anaplasia grades could be helpful in distinguishing high-risk RB.

As with all diseases, the identification of biomarkers could greatly facilitate the detection and diagnosis of RB. There are two general ways in which a detection of RB can be made: by using either an invasive or non-invasive procedure. Histopathological analysis of excised tissue is the primary invasive procedure. Non-invasive assessment modalities of RB may be broadly divided into aqueous humor, blood biomarker assessments and imaging-based technologies (Figure 2). Non-invasive biomarkers can not only diagnosis of at-risk patients, but also asymptomatic screening, monitoring disease recurrence and response to treatment. However, prior to the development of clinically applicable blood-based assays, confirmatory studies are needed to further modify and validate the proposed biomarkers.

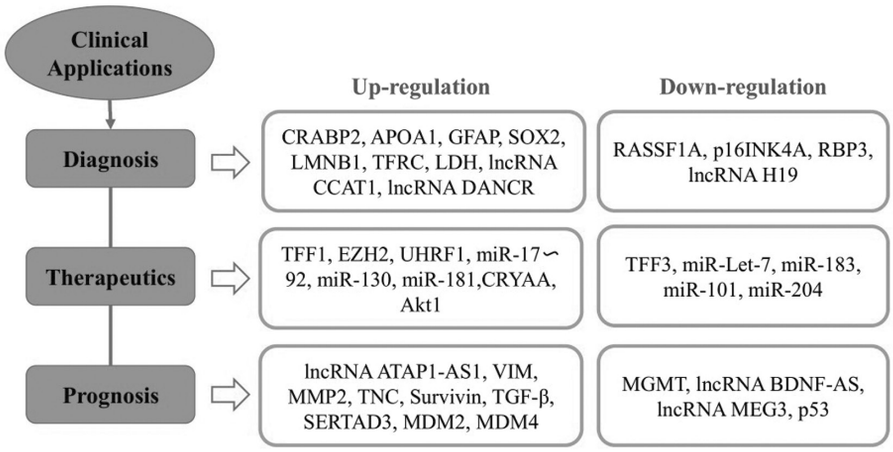

Figure 3 Clinical applications of biomarkers in RB.

In summary, this review introduces a multitude of RB biomarkers identified from different areas. So far, the data indicate that these biomarkers warrant further investigation and could not only play critical roles in RB biological processes, but also will help to correlate and confirm the diagnosis, identify therapeutic targets or serve as a prognostic indicator of treatment (Figure 3).

ACKNOWLEDGEMENTS

Foundations: Supported by the National Natural Science Foundation of China (No.81672673); the National Key Research and Development Program of China (No.2017YFA0104101).

Conflicts of Interest: Sun J, None; **Xi HY,** None; **Shao Q,** None; **Liu QH,** None.

REFERENCES

1 Dimaras H, Corson TW, Cobrinik D, White A, Zhao JY, Munier FL, Abramson DH, Shields CL, Chantada GL, Njuguna F, Gallie BL. Retinoblastoma. *Nat Rev Dis Primers* 2015;1:15021.

2 Kivelä T. The epidemiological challenge of the most frequent eye cancer: retinoblastoma, an issue of birth and death. *Br J Ophthalmol* 2009;93(9):1129-1131.

3 Hahn SM, Kim HS, Kim DJ, Lee SC, Lyu CJ, Han JW. Favorable outcome of alternate systemic and intra-arterial chemotherapy for retinoblastoma. *Pediatr Hematol Oncol* 2016;33(1):74-82.

4 Shields CL, Say EA, Pointdujour-Lim R, Cao C, Jabbour PM, Shields JA. Rescue intra-arterial chemotherapy following retinoblastoma recurrence after initial intra-arterial chemotherapy. *J Fr Ophtalmol* 2015;38(6):542-549.

5 Shields CL, Lally SE, Leahey AM, Jabbour PM, Caywood EH, Schwendeman R, Shields JA. Targeted retinoblastoma management: when to use intravenous, intra-arterial, periocular, and intravitreal chemotherapy. *Curr Opin Ophthalmol* 2014;25(5):374-385.

6 Mendoza PR, Specht CS, Hubbard GB, Wells JR, Lynn MJ, Zhang Q, Kong J, Grossniklaus HE. Histopathologic grading of anaplasia in retinoblastoma. *Am J Ophthalmol* 2015;159(4):764-776.

7 Aerts I, Lumbroso-Le Rouic L, Gauthier-Villars M, Brisse H, Doz F, Desjardins L. Retinoblastoma. *Orphanet J Rare Dis* 2006;1:31.

8 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(6089):643-646.

9 Knudsen ES, Sexton CR, Mayhew CN. Role of the retinoblastoma tumor suppressor in the maintenance of genome integrity. *Curr Mol Med* 2006;6(7):749-757.

10 Indovina P, Pentimalli F, Casini N, Vocca I, Giordano A. RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. *Oncotarget* 2015;6(20):17873-17890.

11 Zheng L, Lee WH. Retinoblastoma tumor suppressor and genome stability. *Adv Cancer Res* 2002;85:13-50.

12 Bremner R, Sage J. Cancer: The origin of human retinoblastoma. *Nature* 2014;514(7522):312-313.

13 Xu XL, Singh HP, Wang L, Qi DL, Poulos BK, Abramson DH, Jhanwar SC, Cobrinik D. Rb suppresses human cone-precursor-derived retinoblastoma tumours. *Nature* 2014;514(7522):385-388.

14 McEvoy J, Nagahawatte P, Finkelstein D, Richards-Yutz J, Valentine M, Ma J, Mullighan C, Song GC, Chen X, Wilson M, Brennan R, Pounds S, Becksfort J, Huether R, Lu C, Fulton RS, Fulton LL, Hong X, Dooling DJ, Ochoa K, Mardis ER, Wilson RK, Easton J, Zhang JH, Downing JR, Ganguly A, Dyer MA. RB1 gene inactivation by chromothripsis in human retinoblastoma. *Oncotarget* 2014;5(2):438-450.

15 Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Thériault BL, Prigoda-Lee NL, Spencer C, Dimaras H, Corson TW, Pang RE, 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* 2013;14(4):327-334.

16 Hsieh AL, Walton ZE, Altman BJ, Stine ZE, Dang CV. MYC and metabolism on the path to cancer. *Semin Cell Dev Biol* 2015;43:11-21.

17 Thériault BL, Dimaras H, Gallie BL, Corson TW. The genomic landscape of retinoblastoma: a review. *Clin Exp Ophthalmol* 2014;42(1):33-52.

18 Wilting RH, Dannenberg JH. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. *Drug Resist Updat* 2012;15(1-2):21-38.

19 Reis AH, Vargas FR, Lemos B. More epigenetic hits than meets the eye: microRNAs and genes associated with the tumorigenesis of retinoblastoma. *Front Genet* 2012;3:284.

20 Zhang J, Benavente CA, McEvoy J, *et al*. A novel retinoblastoma therapy from genomic and epigenetic analyses. *Nature* 2012;481(7381): 329-334.

21 Patel DJ, Wang Z. Readout of epigenetic modifications. *Annu Rev Biochem* 2013;82:81-118.

22 Greger V, Passarge E, Höpping W, Messmer E, Horsthemke B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Hum Genet* 1989;83(2):155-158.

23 Cantone I, Fisher AG. Epigenetic programming and reprogramming during development. *Nat Struct Mol Biol* 2013;20(3):282-289.

24 Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005;6(8):597-610.

25 Ohtani-Fujita N, Fujita T, Aoike A, Osifchin NE, Robbins PD, Sakai T. CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. *Oncogene* 1993;8(4):1063-1067.

26 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(3):703-712.

27 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(3):200-206.

28 Esteller M, Gaidano G, Goodman SN, Zagonel V, Capello D, Botto B, Rossi D, Gloghini A, Vitolo U, Carbone A, Baylin SB, Herman JG. Hypermethylation of the DNA repair gene O(6)-methylguanine DNA methyltransferase and survival of patients with diffuse large B-cell lymphoma. *J Natl Cancer Inst* 2002;94(1):26-32.

29 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(5):1344-1349.

30 Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* 2000;343(19):1350-1354.

31 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(10):1363-1372.

32 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(1):143-150.

33 Philippeit C, Busch M, Dünker N. Epigenetic control of trefoil factor family (TFF) peptide expression in human retinoblastoma cell lines. *Cell Physiol Biochem* 2014;34(3):1001-1014.

34 Weise A, Dünker N. High trefoil factor 1 (TFF1) expression in human retinoblastoma cells correlates with low growth kinetics, increased cyclin-dependent kinase (CDK) inhibitor levels and a selective down-regulation of CDK6. *Histochem Cell Biol* 2013;139(2):323-338.

35 Busch M, Große-Kreul J, Wirtz JJ, Beier M, Stephan H, Royer-Pokora B, Metz K, Dünker N. Reduction of the tumorigenic potential of human retinoblastoma cell lines by TFF1 overexpression involves p53/caspase signaling and miR-18a regulation. *Int J Cancer* 2017;141(3):549-560.

36 Große-Kreul J, Busch M, Winter C, Pikos S, Stephan H, Dünker N. Forced trefoil factor family peptide 3 (TFF3) expression reduces growth, viability, and tumorigenicity of human retinoblastoma cell lines. *PLoS One* 2016;11(9):e0163025.

37 Biel M, Wascholowski V, Giannis A. Epigenetics—an epicenter of gene regulation: histones and histone-modifying enzymes. *Angew Chem Int Ed Engl* 2005;44(21):3186-3216.

38 Rivera C, Gurard-Levin ZA, Almouzni G, Loyola A. Histone lysine methylation and chromatin replication. *Biochim Biophys Acta* 2014;1839(12):1433-1439.

39 Kan G, He H, Zhao Q, Li X, Li M, Yang H, Kim JK. Functional dissection of the role of UHRF1 in the regulation of retinoblastoma methylome. *Oncotarget* 2017;8(24):39497-39511.

40 He H, Lee C, Kim JK. UHRF1 depletion sensitizes retinoblastoma cells to chemotherapeutic drugs via downregulation of XRCC4. *Cell Death Dis* 2018;9(2):164.

41 Dorn GW 2nd, Matkovich SJ. Menage a Trois: intimate relationship among a microRNA, long noncoding RNA, and mRNA. *Circ Res* 2014;114(9):1362-1365.

42 Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. *Nat Rev Genet* 2007;8(2):93-103.

43 de Carvalho IN, de Freitas RM, Vargas FR. Translating microRNAs into biomarkers: What is new for pediatric cancer? *Med Oncol* 2016;33(5):49.

44 Keshavarzi M, Sorayayi S, Jafar Rezaei M, Mohammadi M, Ghaderi A, Rostamzadeh A, Masoudifar A, Mirzaei H. MicroRNAs-based imaging techniques in cancer diagnosis and therapy. *J Cell Biochem* 2017;118(12):4121-4128.

45 Golabchi K, Soleimani-Jelodar R, Aghadoost N, Momeni F, Moridikia A, Nahand JS, Masoudifar A, Razmjoo H, Mirzaei H. MicroRNAs in retinoblastoma: Potential diagnostic and therapeutic biomarkers. *J Cell Physiol* 2018;233(4):3016-3023.

46 Kandalam MM, Beta M, Maheswari UK, Swaminathan S, Krishnakumar S. Oncogenic microRNA 17-92 cluster is regulated by epithelial cell adhesion molecule and could be a potential therapeutic target in retinoblastoma. *Mol Vis* 2012;18:2279-2287.

47 Gui F, Hong Z, You Z, Wu H, Zhang Y. MiR-21 inhibitor suppressed the progression of retinoblastoma via the modulation of PTEN/PI3K/AKT pathway. *Cell Biol Int* 2016;40(12):1294-1302.

48 Shen FM, Mo MH, Chen L, An SJ, Tan XH, Fu YB, Rezaei K, Wang Z, Zhang L, Fu SW. MicroRNA-21 down-regulates Rb1 expression by targeting PDCD4 in retinoblastoma. *J Cancer* 2014;5(9):804-812.

49 Beta M, Khetan V, Chatterjee N, Suganeswari G, Rishi P, Biswas J, Krishnakumar S. EpCAM knockdown alters microRNA expression in retinoblastoma—functional implication of EpCAM regulated miRNA in tumor progression. *PLoS One* 2014;9(12):e114800.

50 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 Syst* 2009;25(1):13-20.

51 To KH, Pajovic S, Gallie BL, Thériault BL. Regulation of p14ARF expression by miR-24: a potential mechanism compromising the p53 response during retinoblastoma development. *BMC Cancer* 2012;12:69.

52 Xu X, Jia R, Zhou Y, Song X, Wang J, Qian G, Ge S, Fan X. Microarray-based analysis: identification of hypoxia-regulated microRNAs in retinoblastoma cells. *Int J Oncol* 2011;38(5):1385-1393.

53 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 Pediatr Hematol Oncol* 2011;33(7):529-533.

54 Montoya V, Fan H, Bryar PJ, Weinstein JL, Mets MB, Feng G, Martin J, Martin A, Jiang H, Laurie NA. Novel miRNA-31 and miRNA-200a-mediated regulation of retinoblastoma proliferation. *PLoS One* 2015;10(9):e0138366.

55 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.

56 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(4):493-502.

57 Lei Q, Shen F, Wu J, Zhang W, Wang J, Zhang L. MiR-101, downregulated in retinoblastoma, functions as a tumor suppressor in human retinoblastoma cells by targeting EZH2. *Oncol Rep* 2014;32(1):261-269.

58 Li XH, Yang L, Shuai TJ, Piao TH, Wang R. MiR-433 inhibits retinoblastoma malignancy by suppressing Notch1 and PAX6 expression. *Biomed Pharmacother* 2016;82:247-255.

59 Wang J, Wang X, Li Z, Liu H, Teng Y. MicroRNA-183 suppresses retinoblastoma cell growth, invasion and migration by targeting LRP6. *FEBS J* 2014;281(5):1355-1365.

60 Wu X, Zeng Y, Wu S, Zhong J, Wang Y, Xu J. MiR-204, down-regulated in retinoblastoma, regulates proliferation and invasion of human retinoblastoma cells by targeting CyclinD2 and MMP-9. *FEBS Lett* 2015;589(5):645-650.

61 Yang Y, Mei Q. MiRNA signature identification of retinoblastoma and the correlations between differentially expressed miRNAs during retinoblastoma progression. *Mol Vis* 2015;21:1307-1317.

62 Qu K, Lin T, Pang Q, Liu T, Wang Z, Tai M, Meng F, Zhang J, Wan Y, Mao P, Dong X, Liu C, Niu W, Dong S. Extracellular miRNA-21 as a novel biomarker in glioma: Evidence from meta-analysis, clinical validation and experimental investigations. *Oncotarget* 2016;7(23):33994-34010.

63 Sun Z, Zhang A, Jiang T, Du Z, Che C, Wang F. MiR-145 suppressed human retinoblastoma cell proliferation and invasion by targeting ADAM19. *Int J Clin Exp Pathol* 2015;8(11):14521-14527.

64 Zhang Y, Zhu X, Zhu X, Wu Y, Liu Y, Yao B, Huang Z. MiR-613 suppresses retinoblastoma cell proliferation, invasion, and tumor formation by targeting E2F5. *Tumour Biol* 2017;39(3):1010428317691674.

65 Martin A, Jones A, Bryar PJ, Mets M, Weinstein J, Zhang G, Laurie NA. MicroRNAs-449a and -449b exhibit tumor suppressive effects in retinoblastoma. *Biochem Biophys Res Commun* 2013;440(4):599-603.

66 Venkatesan N, Deepa PR, Khetan V, Krishnakumar S. Computational and *in vitro* investigation of miRNA-gene regulations in retinoblastoma pathogenesis: miRNA mimics strategy. *Bioinform Biol Insights* 2015;9:89-101.

67 Wang LL, Hu HF, Feng YQ. Suppressive effect of microRNA-143 in retinoblastoma. *Int J Ophthalmol* 2016;9(11):1584-1590.

68 Li J, Zhang Y, Wang X, Zhao R. MicroRNA-497 overexpression decreases proliferation, migration and invasion of human retinoblastoma cells via targeting vascular endothelial growth factor A. *Oncol Lett* 2017;13(6):5021-5027.

69 Li M, Chen XM, Wang DM, Gan L, Qiao Y. Effects of miR-26a on the expression of Beclin 1 in retinoblastoma cells. *Genet Mol Res* 2016;15(2).

70 Zhang Y, Xue C, Zhu X, Zhu X, Xian H, Huang Z. Suppression of microRNA-125a-5p upregulates the TAZ-EGFR signaling pathway and promotes retinoblastoma proliferation. *Cell Signal* 2016;28(8):850-860.

71 Sreenivasan S, Thirumalai K, Danda R, Krishnakumar S. Effect of curcumin on miRNA expression in human Y79 retinoblastoma cells. *Curr Eye Res* 2012;37(5):421-428.

72 Liu SS, Wang YS, Sun YF, Miao LX, Wang J, Li YS, Liu HY, Liu QL. Plasma microRNA-320, microRNA-let-7e and microRNA-21 as novel potential biomarkers for the detection of retinoblastoma. *Biomed Rep* 2014;2(3):424-428.

73 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(16):1734-1745.

74 Jo DH, Kim JH, Cho CS, Cho YL, Jun HO, Yu YS, Min JK, Kim JH. STAT3 inhibition suppresses proliferation of retinoblastoma through down-regulation of positive feedback loop of STAT3/miR-17-92 clusters. *Oncotarget* 2014;5(22):11513-11525.

75 Nittner D, Lambertz I, Clermont F, Mestdagh P, Köhler 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. *Nat Cell Biol* 2012;14(9):958-965.

76 Chen YJ, Wu H, Zhu JM, Li XD, Luo SW, Dong L, Liu TT, Shen XZ. MicroRNA-18a modulates P53 expression by targeting IRF2 in gastric cancer patients. *J Gastroenterol Hepatol* 2016;31(1):155-163.

77 Ding Y, Wu M, Liu J, Wu C, Huang R, Zhu R, Fei J. Seed-targeting anti-miR-21 inhibiting malignant progression of retinoblastoma and analysis of their phosphorylation signaling pathways. *Exp Eye Res* 2014;122:1-8.

78 Singh MK, Singh L, Sen S, Pushker N, Sharma A, Ahamad FC, Chawla B, Kashyap S. Role of high-mobility group protein A isoforms and their clinicopathologic significance in primary retinoblastoma. *Appl Immunohistochem Mol Morphol* 2017;25(4):244-250.

79 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(10):4542-4551.

80 Liu K, Huang J, Xie M, Yu Y, Zhu S, Kang R, Cao LZ, Tang D, Duan X. MIR34A regulates autophagy and apoptosis by targeting HMGB1 in the retinoblastoma cell. *Autophagy* 2014;10(3):442-452.

81 Lodygin D, Tarasov V, Epanchintsev A, Berking C, Knyazeva T, Körner H, Knyazev P, Diebold J, Hermeking H. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. *Cell Cycle* 2008;7(16):2591-2600.

82 Carvalho IN, Reis AH, Dos Santos AC, Vargas FR. A polymorphism in mir-34b/c as a potential biomarker for early onset of hereditary retinoblastoma. *Cancer Biomark* 2017;18(3):313-317.

83 Kung JT, Colognori D, Lee JT. Long noncoding RNAs: past, present, and future. *Genetics* 2013;193(3):651-669.

84 Crea F, Clermont PL, Parolia A, Wang Y, Helgason CD. The non-coding transcriptome as a dynamic regulator of cancer metastasis. *Cancer Metastasis Rev* 2014;33(1):1-16.

85 Zhang H, Zhong J, Bian Z, Fang X, Peng Y, Hu Y. Long non-coding RNA CCAT1 promotes human retinoblastoma SO-RB50 and Y79 cells through negative regulation of miR-218-5p. *Biomed Pharmacother* 2017;87:683-691.

86 Hao F, Mou Y, Zhang L, Wang S, Yang Y. LncRNA AFAP1-AS1 is a prognostic biomarker and serves as oncogenic role in retinoblastoma. *Biosci Rep* 2018;38(3):BSR20180384.

87 Shang W, Yang Y, Zhang J, Wu Q. Long noncoding RNA BDNF-AS is a potential biomarker and regulates cancer development in human retinoblastoma. *Biochem Biophys Res Commun* 2018;497(4):1142-1148.

88 Gao Y, Lu X. Decreased expression of MEG3 contributes to retinoblastoma progression and affects retinoblastoma cell growth by regulating the activity of Wnt/β-catenin pathway. *Tumour Biol* 2016;37(2):1461-1469.

89 Wang JX, Yang Y, Li K. Long noncoding RNA DANCR aggravates retinoblastoma through miR-34c and miR-613 by targeting MMP-9. *J Cell Physiol* 2018;233(10):6986-6995.

90 Zhang A, Shang W, Nie Q, Li T, Li S. Long non-coding RNA H19 suppresses retinoblastoma progression via counteracting miR-17-92 cluster. *J Cell Biochem* 2018;119(4):3497-3509.

91 Mallikarjuna K, Sundaram CS, Sharma Y, Deepa PR, Khetan V, Gopal L, Biswas J, Sharma T, Krishnakumar S. Comparative proteomic analysis of differentially expressed proteins in primary retinoblastoma tumors. *Proteomics Clin Appl* 2010;4(4):449-463.

92 Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlet-Jones M, He F, Jacobson A, Pappin DJ. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics* 2004;3(12):1154-1169.

93 Wiese S, Reidegeld KA, Meyer HE, Warscheid B. Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research. *Proteomics* 2007;7(3):340-350.

94 Danda R, Ganapathy K, Sathe G, Madugundu AK, Ramachandran S, Krishnan UM, Khetan V, Rishi P, Keshava Prasad TS, Pandey A, Krishnakumar S, Gowda H, Elchuri SV. Proteomic profiling of retinoblastoma by high resolution mass spectrometry. *Clin Proteomics* 2016;13:29.

95 Naru J, Aggarwal R, Mohanty AK, Singh U, Bansal D, Kakkar N, Agnihotri N. Identification of differentially expressed proteins in retinoblastoma tumors using mass spectrometry-based comparative proteomic approach. *J Proteomics* 2017;159:77-91.

96 Antoneli CB, Ribeiro KB, Sredni ST, Arias VE, Andreoli MA, de Camargo B, Sobrinho JS, Prado JC, Soares FA, Villa LL. Low prevalence of HPV in Brazilian children with retinoblastoma. *J Med Virol* 2011;83(1):115-118.

97 Daxecker F, Daxenbichler G, Marth C. Cellular retinoic acid binding protein (CRABP) in retinoblastoma. *Ophthalmic Paediatr Genet* 1987;8(1):47-48.

98 Vreeland AC, Levi L, Zhang W, Berry DC, Noy N. Cellular retinoic acid-binding protein 2 inhibits tumor growth by two distinct mechanisms. *J Biol Chem* 2014;289(49):34065-34073.

99 Persaud SD, Lin YW, Wu CY, Kagechika H, Wei LN. Cellular retinoic acid binding protein I mediates rapid non-canonical activation of ERK1/2 by all-trans retinoic acid. *Cell Signal* 2013;25(1):19-25.

100 Mao YW, Liu JP, Xiang H, Li DW. Human alphaA- and alphaB-crystallins bind to Bax and Bcl-X(S) to sequester their translocation during staurosporine-induced apoptosis. *Cell Death Differ* 2004;11(5):512-526.

101 Garcia JR, Gombos DS, Prospero CM, Ganapathy A, Penland RL, Chévez-Barrios P. Expression of angiogenic factors in invasive retinoblastoma tumors is associated with increase in tumor cells expressing stem cell marker Sox2. *Arch Pathol Lab Med* 2015;139(12):1531-1538.

102 Lupien C, Brenner M, Guérin SL, Salesse C. Expression of glial fibrillary acidic protein in primary cultures of human Müller cells. *Exp Eye Res* 2004;79(3):423-429.

103 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(3):344-353.

104 Angi M, Kalirai H, Coupland SE, Damato BE, Semeraro F, Romano MR. Proteomic analyses of the vitreous humour. *Mediators Inflamm* 2012;2012:148039.

105 Ulrich JN, Spannagl M, Kampik A, Gandorfer A. Components of the fibrinolytic system in the vitreous body in patients with vitreoretinal disorders. *Clin Exp Ophthalmol* 2008;36(5):431-436.

106 Naru J, Aggarwal R, Singh U, Mohanty AK, Bansal D, Mangat N, Kakkar N, Agnihotri N. Proteomic analysis of differentially expressed proteins in vitreous humor of patients with retinoblastoma using iTRAQ-coupled ESI-MS/MS approach. *Tumour Biol* 2016;37(10):13915-13926.

107 Hancox RA, Allen MD, Holliday DL, Edwards DR, Pennington CJ, Guttery DS, Shaw JA, Walker RA, Pringle JH, Jones JL. Tumour-associated tenascin-C isoforms promote breast cancer cell invasion and growth by matrix metalloproteinase-dependent and independent mechanisms. *Breast Cancer Res* 2009;11(2):R24.

108 Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. *Cell Stem Cell* 2013;12(1):15-30.

109 Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, Liu F, Que J, Lan X. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. *Cell Signal* 2013;25(5):1264-1271.

110 Tong B, Zeng J, Wu Y, Xiong W. Enhanced SOX2 expression in retinoblastoma tissues and peripheral blood is associated with the clinicopathological characteristics of the disease. *Oncol Lett* 2015;9(3):1244-1248.

111 Wu CC, Yates JR 3rd. The application of mass spectrometry to membrane proteomics. *Nat Biotechnol* 2003;21(3):262-267.

112 Polisetty RV, Gautam P, Sharma R, Harsha HC, Nair SC, Gupta MK, Uppin MS, Challa S, Puligopu AK, Ankathi P, Purohit AK, Chandak GR, Pandey A, Sirdeshmukh R. LC-MS/MS analysis of differentially expressed glioblastoma membrane proteome reveals altered calcium signaling and other protein groups of regulatory functions. *Mol Cell Proteomics* 2012;11(6):M111.013565.

113 Tan S, Tan HT, Chung MC. Membrane proteins and membrane proteomics. *Proteomics* 2008;8(19):3924-3932.

114 Danda R, Ganapathy K, Sathe G, Madugundu AK, Krishnan UM, Khetan V, Rishi P, Gowda H, Pandey A, Subramanian K, Prasad TSK, Elchuri SV. Membrane proteome of invasive retinoblastoma: differential proteins and biomarkers. *Proteomics Clin Appl* 2018;12(5):e1700101.

115 Dittmer TA, Misteli T. The lamin protein family. *Genome Biol* 2011;12(5):222.

116 Aisen P, Wessling-Resnick M, Leibold EA. Iron metabolism. *Curr Opin Chem Biol* 1999;3(2):200-206.

117 Abramson DH, Piro PA, Ellsworth RM, Kitchin FD, McDonald M. Lactate dehydrogenase levels and isozyme patterns. Measurements in the aqueous humor and serum of retinoblastoma patients. *Arch Ophthalmol* 1979;97(5):870-871.

118 Piro PA Jr, Abramson DH, Ellsworth RM, Kitchin D. Aqueous humor lactate dehydrogenase in retinoblastoma patients. Clinicopathologic correlations. *Arch Ophthalmol* 1978;96(10):1823-1825.

119 Andersen MH, Svane IM, Becker JC, Straten PT. The universal character of the tumor-associated antigen survivin. *Clin Cancer Res* 2007;13(20):5991-5994.

120 Jiang LB, Liu XQ, Li B, He XJ, Jin YL, Li LQ, Gao F, Wang NL. Heat shock proteins and survivin: relationship and effects on proliferation index of retinoblastoma cells. *Histol Histopathol* 2008;23(7):827-831.

121 Shehata HH, Abou Ghalia AH, Elsayed EK, Ziko OO, Mohamed SS. Detection of survivin protein in aqueous humor and serum of retinoblastoma patients and its clinical significance. *Clin Biochem* 2010;43(4-5):362-366.

122 Hill JJ, Tremblay TL, Cantin C, O'Connor-McCourt M, Kelly JF, Lenferink AE. Glycoproteomic analysis of two mouse mammary cell lines during transforming growth factor (TGF)-beta induced epithelial to mesenchymal transition. *Proteome Sci* 2009;7:2.

123 Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 1995;268(5215):1336-1338.

124 Shehata HH, Abou Ghalia AH, Elsayed EK, Ahmed Said AM, Mahmoud SS. Clinical significance of high levels of survivin and transforming growth factor beta-1 proteins in aqueous humor and serum of retinoblastoma patients. *J AAPOS* 2016;20(5):444.e1-444.e9.

125 Andreassen CN, Alsner J, Overgaard J. Does variability in normal tissue reactions after radiotherapy have a genetic basis—where and how to look for it? *Radiother Oncol* 2002;64(2):131-140.

126 De Jong MC, van der Meer FJ, Göricke SL, Brisse HJ, Galluzzi P, Maeder P, Sirin S, De Francesco S, Sastre-Garau X, Metz KA, Cerase A, Noij DP, van der Valk P, Moll AC, Castelijns JA, de Graaf P, European Retinoblastoma Imaging Collaboration. Diagnostic accuracy of intraocular tumor size measured with MR imaging in the prediction of postlaminar optic nerve invasion and massive choroidal invasion of retinoblastoma. *Radiology* 2016;279(3):817-826.

127 Jansen RW, de Jong MC, Kooi IE, Sirin S, Göricke S, Brisse HJ, Maeder P, Galluzzi P, van der Valk P, Cloos J, Eekhout I, Castelijns JA, Moll AC, Dorsman JC, de Graaf P. MR imaging features of retinoblastoma: association with gene expression profiles. *Radiology* 2018;288(2):506-515.

128 Kooi IE, Mol BM, Moll AC, van der Valk P, de Jong MC, de Graaf P, van Mil SE, Schouten-van Meeteren AY, Meijers-Heijboer H, Kaspers GL, Te Riele H, Cloos J, Dorsman JC. Loss of photoreceptorness and gain of genomic alterations in retinoblastoma reveal tumor progression. *EBioMedicine* 2015;2(7):660-670.

129 Kapatai G, Brundler MA, Jenkinson H, Kearns P, Parulekar M, Peet AC, McConville CM. Gene expression profiling identifies different sub-types of retinoblastoma. *Br J Cancer* 2013;109(2):512-525.

130 Koh CM, Sabò A, Guccione E. Targeting MYC in cancer therapy: RNA processing offers new opportunities. *Bioessays* 2016;38(3): 266-275.

131 Cao M, Zhong Q. Cilia in autophagy and cancer. *Cilia* 2015;5:4.

132 de Graaf P, Knol DL, Moll AC, Imhof SM, Schouten-van Meeteren AY, Castelijns JA. Eye size in retinoblastoma: MR imaging measurements in normal and affected eyes. *Radiology* 2007;244(1):273-280.

133 Darwish H, Cho JM, Loignon M, Alaoui-Jamali MA. Overexpression of SERTAD3, a putative oncogene located within the 19q13 amplicon, induces E2F activity and promotes tumor growth. *Oncogene* 2007;26(29):4319-4328.

134 Barnoud T, Parris JLD, Murphy ME. Common genetic variants in the TP53 pathway and their impact on cancer. *J Mol Cell Biol* 2019;11(7):578-585.

135 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(6):1018-1031.

136 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(9):685-686.

137 Chen R, Liu S, Ye H, Li J, Du Y, Chen L, Liu X, Ding Y, Li Q, Mao Y, Ai S, Zhang P, Ma W, Yang H. Association of p53 rs1042522, MDM2 rs2279744, and p21 rs1801270 polymorphisms with retinoblastoma risk and invasion in a Chinese population. *Sci Rep* 2015;5:13300.

138 Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004; 119(5):591-602.

139 Cao Q, Wang Y, Song X, Yang W. Association between MDM2 rs2279744, MDM2 rs937283, and p21 rs1801270 polymorphisms and retinoblastoma susceptibility. *Medicine (Baltimore)* 2018;97(49): e13547.

140 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(7115):61-66.

141 Yu F, Jiang Z, Song A. Association of rs11801299 and rs1380576 polymorphisms at MDM4 with risk, clinicopathological features and prognosis in patients with retinoblastoma. *Cancer Epidemiol* 2019;58:153-159.

142 Shields CL, Shields JA, Baez K, Cater JR, De Potter P. Optic nerve invasion of retinoblastoma. Metastatic potential and clinical risk factors. *Cancer* 1994;73(3):692-698.

143 Mabtum ED, Bonanomi MT, Lima PP, Almeida MT. Orbital retinoblastoma: case report. *Arq Bras Oftalmol* 2013;76(4):247-249.

144 Hudson LE, Mendoza P, Hudson WH, Ziesel A, Hubbard GB 3rd, Wells J, Dwivedi B, Kowalski J, Seby S, Patel V, Geisert E, Specht C, Grossniklaus HE. Distinct gene expression profiles define anaplastic grade in retinoblastoma. *Am J Pathol* 2018;188(10):2328-2338.