

Orchestrating epigenetic roles targeting ocular tumors

Xuyang Wen*

Linna Lu*

He Zhang

Xianqun Fan

Department of Ophthalmology,
Ninth People's Hospital, Shanghai Jiao
Tong University School of Medicine,
Shanghai, People's Republic of China

*These authors contributed equally
to this work

Abstract: Epigenetics is currently one of the most promising areas of study in the field of biomedical research. Scientists have dedicated their efforts to studying epigenetic mechanisms in cancer for centuries. Additionally, the field has expanded from simply studying DNA methylation to other areas, such as histone modification, non-coding RNA, histone variation, nucleosome location, and chromosome remodeling. In ocular tumors, a large amount of epigenetic exploration has expanded from single genes to the genome-wide level. Most importantly, because epigenetic changes are reversible, several epigenetic drugs have been developed for the treatment of cancer. Herein, we review the current understanding of epigenetic mechanisms in ocular tumors, including but not limited to retinoblastoma and uveal melanoma. Furthermore, the development of new pharmacological strategies is summarized.

Keywords: ocular tumors, epigenetics, retinoblastoma, uveal melanoma, epigenetic drugs

Introduction

Since Gregor Johann Mendel formulated the basic concepts of heredity, giving rise to the basic understanding of genetics, the mystery of genetics has been constantly explored for over a century. From the DNA double helix to base sequence, scientists opened a door into the micro world. A multitude of virulent genes were identified, and an increasing number of drugs have been developed to cure genetic diseases. The molecular underpinnings of terminal diseases, such as cancer, have been unveiled, and gene therapy has gradually become a reality.¹⁻³

However, with the broadening of genetic research, scientists found that phenotypic variations could not be identified for nucleotide sequence variations in every case, and it was noted that some genes could interact with their environment to yield a phenotype. The word “epigenetics” was subsequently coined by Conrad Waddington in 1940. With the development of this field of study, the word epigenetics now refers specifically to the study of meiotically heritable changes in gene expression that occur without changes in the DNA sequence.⁴

Epigenetic regulation includes several phenomena, such as DNA methylation, histone modifications, alterations in non-coding RNA, histone variation, nucleosome location, and chromosome remodeling. These changes can work together or individually to affect gene expression which is involved in a wide variety of pathologies, including cancer.⁵

Among these phenomena, DNA methylation, histone modifications, and alterations in non-coding RNA are the regulations on linear DNA level. Quantity of research has been carried out to explore the relationship between ocular tumors and these variations.⁶⁻⁸ These findings are explained in further detail below.

Correspondence: He Zhang; Xianqun Fan
Department of Ophthalmology, Ninth
People's Hospital, Shanghai Jiao Tong
University School of Medicine, Shanghai,
200025, People's Republic of China
Email zhanghe@sjtu.edu.cn;
drfanxianqun@126.com



However, the genome does not exist as a linear entity within actual cells. Inside the nucleus, the genome is organized in three-dimensional space⁹ and many regulations are carried out in this pattern, such as histone variation, nucleosome location, and chromosome remodeling. As the basic components of a nucleosome, histones are essential to chromatin structure and function.¹⁰ In order to adapt to various states of chromatin, several kinds of histone variants are incorporated in a nucleosome.^{11–13} These variants can finally affect the gene expression including transcriptional activation and deactivation.¹⁴ Nucleosome location is another critical factor for transcriptional regulation and DNA repair. The acquisition of transcription information depends on the location of the nucleosome in DNA, and the stability or removal of nucleosome positioning may be important factors influencing gene transcription regulation.^{15–17} Furthermore, since Dekker et al discovered the method of chromosome conformation capture (3C),¹⁸ C technology has enabled and significantly accelerated the exploration of chromosome remodeling.^{19–22} The long-range interaction between promoters and enhancers/repressors significantly influences gene transcription, and spatial interactions between these elements are integral to their function.^{23,24} However, these regulatory models have not been reported in ocular tumors to date, and this area may be of great interest for future studies. These phenomena are not explained in further detail below.

Ocular tumors are a malignant disease that can seriously affect an individual's health and quality of life. The prognosis typically ranges from facial deformity and vision loss to death. Similar to other types of tumors, the occurrence and development of ocular tumors has been primarily attributed to direct damage to specific genes.^{25,26} However, with the discovery of disease-related epigenetic mechanisms, epigenetic disruptions have been increasingly found to affect tumorigenesis.

DNA methylation in ocular tumors

DNA methylation represents one of the earliest identified epigenetic modification pathways. DNA methylation typically occurs at the 5' end of the cytosine within CpG dinucleotides and leads to gene silencing. CpG sites are methylated by DNMTs, a family of enzymes with three main members: DNMT1, DNMT3A, and DNMT3B.⁶ DNMT1 is a persistent DNA methyltransferase that maintains existing methylation patterns following DNA replication. DNMT3A and DNMT3B primarily target previously unmethylated CpGs and participate in the regulation of cell growth and differentiation.²⁷ Furthermore, except for these DNMTs, some other chromatin

binding proteins such as HP1 also play an important role in DNA methylation.²⁸ DNMT3A and DNMT3B can interact with mHP1 α and direct DNA methylation.²⁹ In normal cells, CpG island methylation typically occurs during developmental phenomena, such as X-chromosome inactivation.³⁰ Recent findings suggest that many tumor suppressor genes are methylated, thus leading to tumorigenesis.^{31–33}

RB1 inactivation is the primary cause of retinoblastoma (RB); its inactivation is typically caused by loss-of-function mutations and the most recent study showed that RB function might be sexually dimorphic.^{34,35} In addition, *RB1* has been reported to be involved in many other tumors.³⁶ However, many differential gene expression profiles of RB tumors in comparison with normal retinas have recently been characterized, and many of these differences are caused by epigenetic changes.^{37–39} A previous study reported five unilateral RB patients with no mutation in the *RB1* gene. The 5' end of the *RB1* gene, including its promoter region and exon 1, exhibits hypermethylation in these patients.⁴⁰ Another study also reported nine unilateral, sporadic RBs with hypermethylation in the 5' region of the *RB1* gene.⁴¹ Numerous other hypermethylated genes were recently found to be involved in the pathogenesis of RB. Hypermethylation of the promoter region of the *RASSF1A* gene has been detected in 82% to 89% of RB cases,^{42–44} and promoter hypermethylation of the *MGMT* gene has been observed in lower stage RB patients.^{43,45} Another study investigated the methylation status of 25 tumor suppressor genes in 12 RB tumors compared with corresponding normal retinas. Hypermethylation of several cancer-related genes was detected: *MGMT* (58%), *NEUROG1* (52%), *MSH6* (50%), *CD44* (42%), *PAX5* (42%), and *GATA5* (25%).^{42,46} Interestingly, deletions of some of these tumor suppressor genes may drive RB.⁴⁶ Moreover, high-density methylation of numerous other genes, such as *TFE3*,⁴⁷ the apoptotic effector *CASP8*,^{48,49} the DNA repair gene *MLH1*,⁵⁰ *APC-2*,³¹ and the *RB2/p130* gene, is involved in RB^{51,52} (Table 1).

The pathogenesis of uveal melanoma (UM) varies among patients.⁵³ *RASSF1A* and *MGMT* appear to have similar effects in UM and RB. A positive correlation was noted between *RASSF1A* or *MGMT* promoter methylation and the development of UM.^{54–57} Furthermore, hypermethylation of the *hTERT* promoter and the TRAIL receptors *DcR1* and *DcR2* was detected at a relatively high frequency in cases of UM.^{58,59} Another study demonstrated that *CXCR4* and *CCR7* expression in UM enabled directional migration of these tumor cells to the liver, and that the demethylating agent 5-aza-2'-deoxycytidine (5-Aza) upregulates the repressed

Table I DNA methylation in ocular tumors

Gene	The percentage of patients with hypermethylation	Function	Disease	References
<i>RB1</i>	All of five patients analyzed	Tumor suppressor	Retinoblastoma	40
<i>RB1</i>	All of nine patients analyzed	Tumor suppressor	Retinoblastoma	41
<i>RASSF1A</i>	82%–89%	RAS-associated domain family	Retinoblastoma	42–44
			Uveal melanoma	54–57
<i>MGMT</i>	15%–58%	MGMT	Retinoblastoma	43, 45
			Uveal melanoma	54–57
<i>NEUROG1</i>	52%	Neurogenin	Retinoblastoma	42, 46
<i>MSH6</i>	50%	DNA mismatch repair MutS family	Retinoblastoma	
<i>CD44</i>	42%	Cell-surface glycoprotein	Retinoblastoma	
<i>PAX5</i>	42%	Transcription factor	Retinoblastoma	
<i>GATA5</i>	25%	Transcription factor	Retinoblastoma	
<i>TFF3</i>	All of eight RB cell lines	Trefoil factor	Retinoblastoma	47
<i>CASP-8</i>	Two RB cell lines	Apoptotic effector	Retinoblastoma	48, 49
<i>MLH1</i>	67%	DNA repair gene	Retinoblastoma	50
<i>APC-2</i>	70%	APC	Retinoblastoma	50
<i>RB2/p130</i>	40%	Retinoblastoma-related protein	Retinoblastoma	51, 52
<i>TERT</i>	52%	Ribonucleoprotein polymerase	Uveal melanoma	58, 59
<i>DcR1</i> and <i>DcR2</i>	91%–97%	Receptors for TRAIL	Uveal melanoma	58, 59
<i>CXCR4</i> and <i>CCR7</i>	/	Hypermethylation of this gene can inhibit metastasis	Uveal melanoma	60–62
<i>p16/INK4a</i>	A part in <i>Chlamydomophila psittaci</i> -negative cases	Stabilizer of the tumor suppressor protein	Marginal zone lymphoma of the ocular adnexa	64
<i>CDKN2A</i>	46%	Stabilizer of the tumor suppressor protein	Periocular sebaceous carcinoma	32
<i>E-cadherin</i>	72%	Component of the cell–cell adhesion complex	Eyelid sebaceous gland carcinoma	65
<i>DNMT3L</i>	Loss of methylation	DNA (cytosine-5-)-methyltransferase 3-like	Ocular surface squamous neoplasia	33

Abbreviation: RB, retinoblastoma.

CXCR4 gene via demethylation.^{60–62} Another recent study reported that 5-Aza causes significant decreases in growth, invasion, and clonogenicity in UM. In addition, 5-Aza decreased the number of metastases from the eye to the lung in a murine xenograft model.⁶³

In addition to these two main malignant ocular tumors, DNA methylation has been reported in other ocular tumors. Methylation of the *p16/INK4a* gene promoter was noted in marginal zone lymphoma of the ocular adnexa,⁶⁴ whereas hypermethylation of the *CDKN2A* gene promoter was demonstrated to have an effect on periocular sebaceous carcinoma and was associated with younger patient age.³² Methylation of the *E-cadherin* promoter region was detected in 72% of eyelid sebaceous gland carcinoma, and this effect could contribute to the reduced disease-free survival of patients.⁶⁵ Furthermore, a study in *Drosophila* suggested that the downregulation of Rbf due to DNA hypermethylation was associated with eye cancer,⁶⁶ and a loss of methylation at the *DNMT3L* promoter was detected in ocular surface squamous neoplasia³³ (Table 1).

Histone modifications in ocular tumors

Multiple post-translational modifications have been noted on histones. The enzymes involved in such modifications include HAT, HDAC, HMT, HDM, ubiquitin ligase, deubiquitinase, kinase, and phosphatase.^{7,67} Genome-wide studies have demonstrated that these modifications in specific regions can lead to the activation or repression of gene expression. For example, the post-translational modifications of histones, including monomethylation of H4K20 and H2BK5; trimethylation of H3K4, H3K36 and H3K79; and acetylation of H3K9 and H3K14, activate gene expression. In contrast, dimethylation of H3K9 and trimethylation of H3K9 and H3K27, lead to the repression of gene expression.^{68–70} Similar to DNA methylation, many chromatin binding proteins can also affect histone modifications. HP1 can specifically recognize and bind to methylated histone H3K9 which leads to epigenetic silencing.^{71,72} In addition, HP1 proteins have been demonstrated to harbor a wide variety of modifications such as phosphorylation, acetylation, ubiquitination, and so on.^{73,74}

Table 2 Histone modifications in ocular tumors

Gene	Modification	Disease	References
<i>Pipsqueak</i> and <i>Lola</i>	H3K9 deacetylation and H3K27 methylation	Tumorigenesis in the <i>Drosophila</i> eye	66
<i>MHC2TA</i>	Trimethylated H3K27me3	Uveal melanoma	76, 77

A study performed in 25 human cancer cell lines revealed that the global loss of monoacetylation and trimethylation of histone H4 is a common hallmark of human tumor cells.⁷⁵ With regard to eye cancer, by studying tumorigenesis in the *Drosophila* eye, it was noted that deacetylated H3K9 and methylated H3K27 of *Pipsqueak* and *Lola* contributed to the tumor phenotype.⁶⁶ Another study reported that the HMT EZH2 could act on *MHC2TA* promoter IV (CIITA-PIV) chromatin, resulting in high levels of trimethylated histone H3K27me3. This modification leads to the silencing of *MHC2TA* in UM cells and ultimately causes tumorigenesis.^{76,77} Moreover, HDAC inhibitors, such as valproic acid, reverse the effect of *BAP1* loss in UM and inhibit tumor metastasis^{78,79} (Table 2).

Non-coding RNA in ocular tumors

Non-coding RNA refers to RNA that does not code for proteins. These RNAs are transcribed from the genome and directly exercise their biological function at the RNA level. According to the length of non-coding RNA, these RNAs are divided into three types: RNA length less than 50 bp, such as microRNA (miRNA) and small interfering RNA; RNA with lengths of greater than 200 bp, such as long non-coding RNA (lncRNA); and other RNAs with a length between 50 bp and 500 bp, such as ribosomal RNA and transfer RNA. Previous studies of non-coding RNA have primarily focused on miRNAs, which can result in gene silencing via

translational repression or target mRNA degradation. These studies reported that miRNAs are associated with several human diseases, including ocular tumors.^{80–84} Recently, lncRNA has been receiving increasing amounts of attention. Mounting evidence suggests that lncRNA plays an important role in epigenetic regulation and affects cell proliferation and differentiation.^{85–88}

Reduced expression levels of *let-7*, which can dramatically repress oncogenes, such as *HMG2A*, *c-Myc*, and members of the *Ras* family, have been observed in RB cases.^{89–92} Other studies have reported that miR-34a is a tumor suppressor miRNA in RB.^{83,93} Furthermore, miR-24, 125b, 191, 181a, and 423 are also decreased in RB.⁹⁴ Regarding oncogenic miRNA, the miR-17~92 cluster is over-expressed in primary RB tumors and cell lines.^{80,95} miR-17~92 is a target of *E2F*, and loss of *RB1* may lead to increased expression of miR-17~92 through depressed *E2F* activity.^{96,97} Moreover, a recent report has suggested that hypoxia-induced miR-181b enhances angiogenesis of RB cells by targeting *PDCD10* and *GATA6*^{98,99} (Table 3).

Several studies of miRNAs have also been conducted in UM. As for tumor suppressor miRNAs, miR-124a is significantly downregulated, and its re-expression in UM cell lines dramatically decreases cell proliferation, migration, and invasion.^{100,101} The same phenomenon has been observed for miR-137¹⁰² and miR-34b/c.¹⁰³ Furthermore, the oncogene *MITF* is downregulated by overexpression of miR-137 in

Table 3 Non-coding RNA in ocular tumors

Non-coding RNA	Expression in tumor	Function	Disease	References
<i>let-7</i>	Decreased	Repress oncogenes	RB	89–92
miR-34a	Decreased	Tumor suppressor	RB	83, 93
miR-24, 125b, 191, 181a and 423	Decreased	Tumor suppressor	RB	94
miR-124a	Decreased	Tumor suppressor	UM	100, 101
miR-137	Decreased	Down-regulate <i>MITF</i>	UM	102
miR-34b/c	Decreased	Down-regulate <i>c-Met</i> , <i>p-Akt</i> , and some cell cycle proteins	UM	103
miR-17-92 cluster	Over-expressed	Oncogenic miRNA	RB	80, 95
miR-181b	Hypoxia-induced	Enhances angiogenesis	RB	98, 99
miR-20a, 125b, 146a, 155, 181a, and 223	Over-expressed	Oncogenic miRNAs	UM	104
miR-18a, -199a, -495, -549 and <i>let-7b</i>	Over-expressed	Oncogenic miRNAs	UM	105, 106
lncRNA <i>BANCR</i>	Over-expressed	Promote RB cell proliferation, migration, and invasion	RB	85
lncRNA- <i>ROR</i>	Over-expressed	Promote <i>TESC</i> expression	UM	116

Abbreviations: RB, retinoblastoma; miRNA, microRNA; UM, uveal melanoma.

UM,¹⁰² whereas *c-Met*, *p-Akt*, and some cell cycle proteins are downregulated by miR-34b/c.¹⁰³ Regarding oncogenic miRNAs, five miRNAs, including miR-20a and -125b, affect tumor metastasis in UM patients.¹⁰⁴ Moreover, miR-18a, -199a, -495, -549, and let-7b are also overexpressed in UM^{105,106} (Table 3).

In recent years, lncRNA has been confirmed to facilitate common cancer hallmarks, such as replicative immortality, resistance to cell death, and evasion of growth suppression.⁸⁸ Various lncRNAs, including *H19*, *PANDA*, and *lncRNA-ROR*, are associated with tumorigenesis to varying degrees both in vitro and in vivo.^{36,107–115} However, to our knowledge, only a few studies have been conducted on ocular tumors to date. One such study suggested that *lncRNA BANCR* was over-expressed in RB tissues and cell lines. Patients with increased *lncRNA BANCR* expression exhibited poorer survival, and knocking down *lncRNA BANCR* expression significantly suppressed RB cell proliferation, migration, and invasion in vitro.⁸⁵ Another study of UM was performed by our group. We recently found that *lncRNA-ROR* played an important role in ocular melanoma. *lncRNA-ROR* occupied and activated the *TESC* promoter by repelling the histone G9A methyltransferase and promoting the release of histone H3K9 methylation. Suppression of *ROR* in tumors resulted in silencing of *TESC* expression followed by restoration of G9A-mediated histone H3K9 methylation in the *TESC* promoter, which significantly reduced tumor growth and metastasis¹¹⁶ (Table 3).

Epigenetic drugs for ocular tumor treatment

One of the most important reasons for the focus on epigenetics in recent years is that epimutations are reversible in contrast with genetic mutations, which are difficult to completely restore. Epigenetic drugs can restore the normal epigenetic landscape in cancer cells by several modes, such as inhibiting enzymes of the epigenetic machinery. Currently, several epigenetic drugs have been approved by the US Food and Drug Administration (FDA) for the treatment of cancer. The DNMT inhibitors Vidaza (5-aza-cytidine) and decitabine (5-Aza) are approved for use in patients. The former is effective for the treatment of myelodysplastic syndrome and acute non-lymphocytic leukemia, whereas the latter is the improved version that exhibits enhanced efficacy in a variety of malignancies.¹¹⁷ The HDAC inhibitors vorinostat (suberoylanilide hydroxamic acid) and romidepsin (FK228, depsipeptide) are effective for cutaneous T cell lymphoma.^{118,119} In addition to these epigenetic drugs approved by the FDA, even more

drugs are currently undergoing clinical trials or are at the stage of laboratory research.^{120–125}

In ocular tumors, DNMT and HDAC inhibitors also play an anticancer role. In addition, 5-Aza decreased the number of metastases from the eye to the lung in a murine xenograft model;⁶³ and HDAC inhibitors, such as valproic acid, may have therapeutic potential for inducing differentiation and prolonged dormancy of micrometastatic disease in UM.^{78,79} Regarding RB, the proto-oncogene *SYK* is upregulated in cases of RB and is required for tumor cell survival. Targeting *SYK* with the small-molecule inhibitor BAY61-3606 or R406 could remarkably induce RB tumor cell death in vitro and in vivo.^{126,127}

Future prospects

Due to technological developments and high-throughput technologies, the study of epigenetic processes is currently possible at a much broader level than only single genes.^{128,129} Differences in epigenetic marks, such as lncRNA and histone modifications, between cancer cells and normal cells can be easily explored through microarray analysis, chromatin immunoprecipitation sequencing, and RNA sequencing. With the popularization of techniques, such as C technology, the study of chromosome remodeling will continue to attract the attention of researchers. Although lncRNA and chromosome remodeling have already become an interesting area of study for mechanisms of tumorigenesis, research in this area in ocular tumors remains minimal. Additional studies should be conducted to enable a better understanding of such epigenetic mechanisms in ocular tumors.

Finally, in view of the use of epigenetic drugs for cancer treatment, the identification of optimal doses for single and combined therapies requires careful analysis. Furthermore, because most of the epigenetic drugs are nonspecific, they may be a “double-edged sword” and cause unpredictable side effects. Thus, it is necessary for doctors to design personalized treatment programs. Hopefully, more accurate personalized treatments that are free of side effects can be identified to cure malignant tumors in the future.

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