Orchestrating epigenetic roles targeting ocular tumors

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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.

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However, the genome does not exist as a linear entity within actual cells. Inside the nucleus, the genome is organized in three-dimensional space 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. In order to adapt to various states of chromatin, several kinds of histone variants are incorporated in a nucleosome. These variants can finally affect the gene expression including transcriptional activation and deactivation. 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. Furthermore, since Dekker et al discovered the method of chromosome conformation capture (3C), C technology has enabled and significantly accelerated the exploration of chromosome remodeling. The long-range interaction between promoters and enhancers/repressors significantly influences gene transcription, and spatial interactions between these elements are integral to their function. 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. 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. 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. Furthermore, except for these DNMTs, some other chromatin binding proteins such as HP1 also play an important role in DNA methylation. DNMT3A and DNMT3B can interact with mHP1α and direct DNA methylation. In normal cells, CpG island methylation typically occurs during developmental phenomena, such as X-chromosome inactivation. Recent findings suggest that many tumor suppressor genes are methylated, thus leading to tumorigenesis.

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. In addition, RB1 has been reported to be involved in many other tumors. 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. 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. Another study also reported nine unilateral, sporadic RBs with hypermethylation in the 5’ region of the RB1 gene. 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, and promoter hypermethylation of the MGMT gene has been observed in lower stage RB patients. 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%). Interestingly, deletions of some of these tumor suppressor genes may drive RB. Moreover, high-density methylation of numerous other genes, such as TFF3, the apoptotic effector CASP8, the DNA repair gene MLH1, and the RB2/p130 gene, is involved in RB (Table 1).

The pathogenesis of uveal melanoma (UM) varies among patients. 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. Furthermore, hypermethylation of the hTERT promoter and the TRAIL receptors DcR1 and DcR2 was detected at a relatively high frequency in cases of UM. 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
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CXCR4 gene via demethylation. 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.

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, whereas hypermethylation of the \( CDKN2A \) gene promoter was demonstrated to have an effect on periocular sebaceous carcinoma and was associated with younger patient age. Methylation of the \( E\text{-}\text{cadherin} \) promoter region was detected in 72% of eyelid sebaceous gland carcinoma, and this effect could contribute to the reduced disease-free survival of patients. Furthermore, a study in \textit{Drosophila} suggested that the downregulation of Rbf due to DNA hypermethylation was associated with eye cancer, and a loss of methylation at the \( DNMT3L \) promoter was detected in ocular surface squamous neoplasia (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. 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 H3K27, lead to the repression of gene expression.

### Table 1 DNA methylation in ocular tumors

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

**Abbreviation:** RB, retinoblastoma.
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. 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. 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. Moreover, HDAC inhibitors, such as valproic acid, reverse the effect of BAP1 loss in UM and inhibit tumor metastasis (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. 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. The same phenomenon has been observed for miR-137 and miR-34b/c. Furthermore, the oncogene MITF is downregulated by overexpression of miR-137 in

<p>| Table 2 Histone modifications in ocular tumors |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Modification</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipsqueak and Lola</td>
<td>H3K9 deacetylation and H3K27 methylation</td>
<td>Tumorigenesis in the Drosophila eye</td>
<td>66</td>
</tr>
<tr>
<td>MHC2TA</td>
<td>Trimethylated H3K27me3</td>
<td>Uveal melanoma</td>
<td>76, 77</td>
</tr>
</tbody>
</table>

<p>| Table 3 Non-coding RNA in ocular tumors |</p>
<table>
<thead>
<tr>
<th>Non-coding RNA</th>
<th>Expression in tumor</th>
<th>Function</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7</td>
<td>Decreased</td>
<td>Repress oncopogenes</td>
<td>RB</td>
<td>89–92</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Decreased</td>
<td>Tumor suppressor</td>
<td>RB</td>
<td>83, 93</td>
</tr>
<tr>
<td>miR-24, 125b, 191, 181a and 423</td>
<td>Decreased</td>
<td>Tumor suppressor</td>
<td>RB</td>
<td>94</td>
</tr>
<tr>
<td>miR-124a</td>
<td>Decreased</td>
<td>Tumor suppressor</td>
<td>UM</td>
<td>100, 101</td>
</tr>
<tr>
<td>miR-137</td>
<td>Decreased</td>
<td>Down-regulate MITF</td>
<td>UM</td>
<td>102</td>
</tr>
<tr>
<td>miR-34b/c</td>
<td>Decreased</td>
<td>Down-regulate c-Met, p-Akt, and some cell cycle proteins</td>
<td>UM</td>
<td>103</td>
</tr>
<tr>
<td>miR-17–92 cluster</td>
<td>Over-expressed</td>
<td>Oncogenic miRNA</td>
<td>RB</td>
<td>80, 95</td>
</tr>
<tr>
<td>miR-181b</td>
<td>Over-expressed</td>
<td>Enhances angiogenesis</td>
<td>RB</td>
<td>98, 99</td>
</tr>
<tr>
<td>miR-20a, 125b, 146a, 155, 181a, and 223</td>
<td>Over-expressed</td>
<td>Oncogenic miRNAs</td>
<td>UM</td>
<td>104</td>
</tr>
<tr>
<td>miR-18a, -199a, -495, -549 and let-7b</td>
<td>Over-expressed</td>
<td>Oncogenic miRNAs</td>
<td>UM</td>
<td>105, 106</td>
</tr>
<tr>
<td>IncRNA BANC</td>
<td>Over-expressed</td>
<td>Promote RB cell proliferation, migration, and invasion</td>
<td>RB</td>
<td>85</td>
</tr>
<tr>
<td>LncRNA-ROR</td>
<td>Over-expressed</td>
<td>Promote TESC expression</td>
<td>UM</td>
<td>116</td>
</tr>
</tbody>
</table>

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

In recent years, IncRNA has been confirmed to facilitate common cancer hallmarks, such as replicative immortalty, resistance to cell death, and evasion of growth suppression.\textsuperscript{88} Various IncRNAs, including H19, PANDA, and IncRNA-ROR, are associated with tumorigenesis to varying degrees both in vitro and in vivo.\textsuperscript{36,107–115} However, to our knowledge, only a few studies have been conducted on ocular tumors to date. One such study suggested that IncRNA BANCR was over-expressed in RB tissues and cell lines. Patients with increased IncRNA BANCR expression exhibited poorer survival, and knocking down IncRNA BANCR expression significantly suppressed RB cell proliferation, migration, and invasion in vitro.\textsuperscript{93} Another study of UM was performed by our group. We recently found that IncRNA-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 metastasis\textsuperscript{116} (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.\textsuperscript{117} The HDAC inhibitors vorinostat (suberoylanilide hydroxamic acid) and romidepsin (FK228, depsipeptide) are effective for cutaneous T cell lymphoma.\textsuperscript{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.\textsuperscript{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;\textsuperscript{63} and HDAC inhibitors, such as valproic acid, may have therapeutic potential for inducing differentiation and prolonged dormancy of micrometastatic disease in UM.\textsuperscript{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.\textsuperscript{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.\textsuperscript{128,129} Differences in epigenetic marks, such as IncRNA 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 IncRNA 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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