

The Role of DNA Methylation in Lens Development and Cataract Formation

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Abstract Epigenetics pertains to heritable alterations in genomic structural modifications without altering genomic DNA sequence. The studies of epigenetic mechanisms include DNA methylation, histone modifications, and microRNAs. DNA methylation may contribute to silencing gene expression which is a major mechanism of epigenetic gene regulation. DNA methylation regulatory mechanisms in lens development and pathogenesis of cataract represent exciting areas of research that have opened new avenues for association with aging and environment. This review addresses our current understanding of the major mechanisms and function of DNA methylation in lens development, age-related cataract, secondary cataract, and complicated cataract. By understanding the role of DNA methylation in the lens disease and development, it is expected to open up a new therapeutic approach to clinical treatment of cataract.

Keywords DNA methylation · Epigenetic · Lens development · Cataract

Introduction

Epigenetics, a branch of biology, pertains to the investigation of genomic structural modifications that regulate gene expression without altering the underlying genomic DNA sequence. The name is first used by Waddington (2012). There are three major manners for the regulation of

gene expression which includes DNA methylation, histone modifications, and microRNAs in the regulation mechanisms of epigenetics. DNA methylation is the most thoroughly studied epigenetic mark, because it is essential for normal development and plays a vital role in X-chromosome inactivation, imprinting, and transcriptional regulation (Kroeze et al. 2015). The process of DNA methylation is that the covalent attachment of a methyl (CH₃) group is added to the C5 position of a cytosine residue, forming 5-methylcytosine (5mC) (Kohli and Zhang 2013).

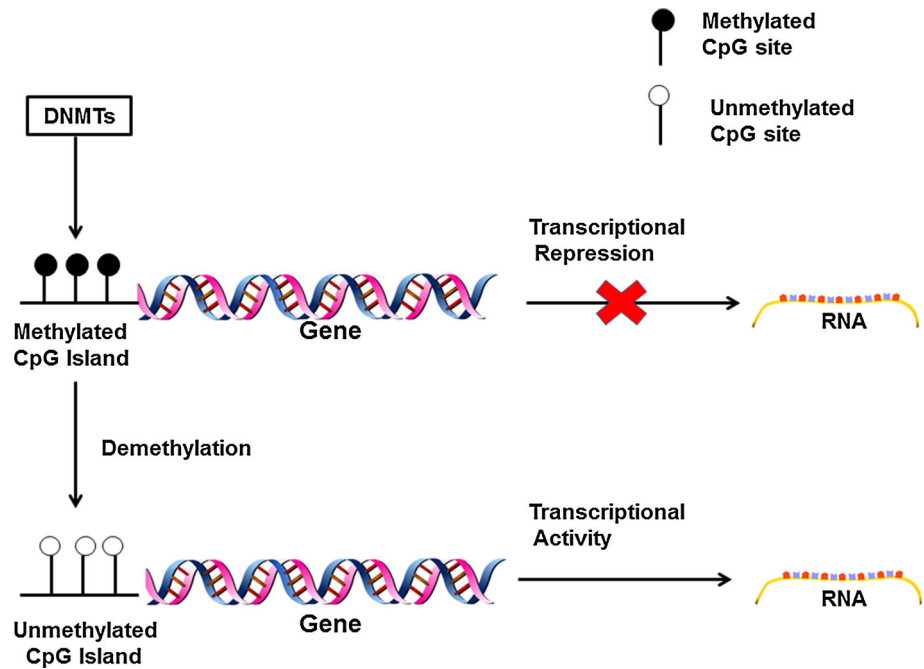
In the human genome, CpG dinucleotides are distributed asymmetrically. Thus, there are some GC-rich and -poor DNA regions. Tissue-specific pattern is used for modification of DNA methylation. For example, in some mammalian cells, cytosine residues in GC-rich regions (more than 50% GC in the region) are usually not methylated. But in the other cells, GC-rich regions are usually methylated which responded for dynamic and spatiotemporally expression of the genes. Those GC-rich regions are also called CpG islands (CGIs) and play an important role in regulation of gene expression, and they are often located in the promoters or the first exons around transcription start sites (TSSs) (Bird 2002).

DNA methylation is catalyzed and maintained by DNA methyltransferases (DNMTs) (Jin and Robertson 2013). Two main types of methyltransferase activity have been found in mammals: a de novo activity and a maintenance activity. DNMT3a and DNMT3b have been identified as de novo methyltransferases. They can methylate cytosine at CpG dinucleotides on both strands. DNMT1, the principal DNA methyltransferase in mammalian cells, acts to restore methylated cytosines at CpGs on the newly duplicated strands (Okano et al. 1998; Robert et al. 2003; Wang et al. 2016a). A schematic diagram showed the basic process for the gene expression regulated by DNA methylation (Fig. 1).

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Fig. 1 The schematic diagram for the basic process of DNA methylation regulated the gene expression



Human lens development involves some vital gene expression (Cvekl and Ashery-Padan 2014). Many gene expressions for development can be regulated by DNA methylation (Cvekl and Duncan 2007). Environmental exposures and diet also alter DNA methylation patterning (Green and Marsit 2015). As a result, DNA methylation modifications may be particularly relevant in the pathogenesis of complex age-related diseases and lens development with known environmental risk factors. Cataract, an age-related disease, includes a lot of types which can be affected by environment and age. In this review, we focus on the role of DNA methylation in lens development and the several types of cataracts [age-related cataract (ARC), secondary cataract, and complicated cataract].

Lens Development

During the past half-century, researches have revealed the importance of transcriptional regulatory genes in the genetics of eye development and disease. However, a few researches focused on DNA methylation in the development of lens. DNA methylation profiling was performed in human lens epithelial cells (LECs) and lens fiber cells for γ -crystallin, which encodes a structural protein in the lens, to evaluate the role of epigenetic regulation in the development of lens. A clear inverse correlation between γ -crystallin expression and methylation of the promoter region had been found. During the lens development, the younger lens fiber cells cover the older cells. Due to

different patterns of DNA methylation in the promoter of γ -crystallin, the consequence is the creation of γ -crystallin gradient across the whole lens, which correlates inversely with the water gradient and thereby prevents optical aberration (Peek et al. 1991). Using an in vitro differentiation system, a research showed that there is a plethora of positive and negative factor interactions in the proximal γ -crystallin promoter. During lens development, the balance between these factors shifts toward repression. The pattern of expression of the silencing factor is the major reason for repression of the gene. Another probable cause is lack of demethylation (Klok et al. 1998). The other research showed that the status of γ -crystallin promoter lost the methylation after the LECs differentiate to lens fiber cells when used fibroblast growth factor (bFGF) to mediate rat lens fiber cell differentiation (Dirks et al. 1996).

Evidence suggests that Uhrf1 interacts with Dnmt1 in zebrafish lens (Bostick et al. 2007; Bestor 2000). During the development of zebrafish lens, there are defects in lens development and maintenance during the knockdown of Uhrf1 and Dnmt1 (Tittle et al. 2011). A study suggested dynamic and spatiotemporally restricted patterns of de novo DNA methyltransferase gene expression during zebrafish lens development. For example, the *dnmt4/dnmt7* expressed before 36 hpf and *dnmt6/dnmt8* expressed at 48 hpf which played a vital role for maintenance of the continuously proliferating LECs (Seritrakul and Gross 2014). A brief summary of those studies is included in Table 1.

Table 1 DNA methylation in lens development

Gene	Study population	Tissue	Effect/significance	References
γ -crystallin (γ A– γ F)	Wistar rats	Lens epithelial cells	Demethylation increased mRNA and protein levels of γ -crystallin	Klok et al. (1998)
γ D-crystallin	Wistar rats	Lens	Demethylation increased mRNA and protein levels of γ D-crystallin	Kohli and Zhang (2013)
γ B-crystallin γ D-crystallin	Wistar rats	Lens epithelial cells	Methylation suppress promotor activity of γ B-crystallin and γ D-crystallin	Kroeze et al. (2015)
dnmt3–dnmt8	Zebrafish	Lens	Mediate DNA methylation	Palsamy et al. (2012)
Dnmt1	Zebrafish	Lens	Abnormal lens morphology	Palsamy et al. (2014a)

Age-Related Cataract (ARC)

DNA methylation profiling of α A-crystallin (CRYAA) gene was investigated in human LECs, which encodes a major structural protein in the lens, to evaluate the role of epigenetic modification at the promoter in the formation of age-related nuclear cataract. CRYAA gene mRNA and protein levels are both downregulated due to the hypermethylation at the CpG island of CRYAA promoter (Zhou et al. 2012b).

Our work showed a role for DNA methylation in regulation of *MGMT* expression in LECs of ARC. Hypermethylation at the promoter region of *MGMT* led to decreased transcript. The expression of *MGMT* due to DNA methylation regulates the expression of the gene and may be involved in the development of ARC (Li et al. 2014). In another study, we showed that altered *OGG1* mRNA and protein expression were correlated with hypermethylation of a CpG island of *OGG1* in lens cortex of ARC. The hypermethylation change of *OGG1* gene in the susceptibility to oxidative stress-induced cortical ARC is warranted to further study (Wang et al. 2015). We also investigated the epigenetic modification in *WRN* gene. We found that the low expression of *WRN* linked to a hypermethylation in the gene promoter might contribute to the ARC formation (Zhu et al. 2015a). Recently, we focused on the relationship between epigenetic modification and the expression of *ERCC6* gene. We found DNA methylation at the special locus of *ERCC6* promotor which plays the vital role in regulation of the gene. The low expression of *ERCC6* contributes to the weak ability for repairing the DNA damage in the LECs which induce by the UVB (Wang et al. 2016a). Then, we investigate the expression profile of DNA methylation and transcriptional repression-associated genes in LECs of ARC. The expression of five genes increased in LECs of ARCs when compared with the controls. Our data showed a global perspective on expression of DNA methylation and transcriptional repression-associated genes which may be the reason that

responded for downregulation of some genes in LECs (Wang et al. 2016b).

Klotho is an anti-aging gene whose expression levels closely correlated with age. In the different age groups, a study showed that the methylation status of the *Klotho* promoter increased with age which reduces the expression of the *Klotho* gene. Thus, DNA methylation modification of the gene may be involved in the ARC formation, and these findings may give the insight to develop targeted therapies (Jin et al. 2015).

Valproic acid (VPA), a drug used for epilepsy treatment, can induce the formation of bilateral congenital cataracts (Hanhart et al. 2010). The promoter of *Keap1* gene lost the methylation in LECs after treatment with VPA. The expression of *Dnmt1*, *Dnmt3a*, and *Dnmt3b*, passive DNA demethylation pathway enzymes, were active (Palsamy et al. 2014c). *Keap1* is a negative regulatory protein which can repress the *Nrf2* antioxidant protein expression contributed to the various cataractogenic stressors. Those findings might be responsible for cataract formation. In the sodium selenite-induced cataract model, the demethylated *Keap1* promoter contributes to overexpression of the gene. Overexpression of *Keap1* protein suppresses the role of *Nrf2* for antioxidant protection and leading to the cataract formation (Palsamy et al. 2014b). DNA methylation of *Keap1* promotor also was studied in cataractous lenses of different ages. There were significantly decreased levels of DNA methylated in the *Keap1* promoter in the older age group, which decreased activity of *Nrf2* and led to the failure of the antioxidant system (Gao et al. 2015).

Methylglyoxal (MGO) is reactive aldehydes in abnormal cell which can be induced by Hyperglycemia. It can alter the functions and conformation of lens proteins by crystalline aggregations which are a key contributor to lens opacity. The methylation level of the promoter of *Keap1* downregulates during the HLEs treatment with MGO. It contributes to *Nrf2*-dependent antioxidant system failure, leading to the altered cellular redox balance and cataract formation (Palsamy et al. 2014a). Table 2 shows the brief summary of those studies.

Secondary Cataract and Complicated Cataract

The incidence for secondary nuclear cataracts after pars plana vitrectomy (PPV) is about 80% in 2 years. In a study, DNA methylation status of α A-crystallin (CRYAA) gene promoter was evaluated in the nuclear cataract after PPV. The expression of CRYAA is decreased by hypermethylation at the gene promoter. But the methylation rates do not differ between the C3F8 tamponade group and SiO tamponade group after PPV (Zhu et al. 2015b). In a research about high myopia-induced dark nuclear cataract, hypermethylation of CpG islands in the CRYAA promoter was much greater in high-myopic dark nuclear cataract than in ARC group. This is consisted with the gene lower transcription in the high-myopic dark nuclear cataract (Zhu et al. 2013).

A study was performed in the diabetic cataractous lenses to evaluate epigenetic regulation of Keap1 gene and its potential role in the development of diabetic cataract. Demethylation of the CpG islands in the Keap1 promoter was observed, which activates the expression of Keap1 gene (Palsamy et al. 2012). Pseudoexfoliation syndrome (PEX) is involved in the abnormal production, deposition, and turnover of pseudoexfoliation material (PXM) in connective tissue and skin all over the body. In the eye disease, it includes secondary cataract. There are white, flake-like PXM deposited anteriorly on lens capsule. Evidences have showed that lysyl oxidase-like 1 (LOXL1) gene is a component of PXM localized around fibrous protein aggregates on the lens capsule surface. In the Uighur population, PEX gene promoter displays the hypermethylation in the LECs of cataracts when compared to the control ARC group. The study showed DNA methylation maybe played an important role in the formation of the type cataract (Ye et al. 2015).

Posterior capsule opacification (PCO) is a common complication of modern cataract surgery, especial for children's surgery. The pathogenesis for the disease is residual LECs undergo an epithelial-to-mesenchymal transition (EMT), followed by enhanced proliferation, migration, and collagen deposition after the cataract surgery. Using DNMT inhibitor to treat the HLECs, the crucial cellular events in PCO pathogenesis were suppressed in vitro. The exact mechanism needs to be elucidated in future. Zebularine may become a new therapeutic approach for the treatment of PCO (Zhou et al. 2012a). The DNA methylation studies of secondary cataract and complicated cataract in ocular diseases are summarized in Table 3.

In Summary

Although the complete spectrum of DNA methylation modifications needs to be more fully explored in the future, it is clear that altered DNA methylation is an important contributor to lens development and cataract. These diseases, including lens development, have contributions from environmental risk factors. Alterations in DNA methylation may indeed be the mechanistic link between environmental exposures and gene expression changes that set the stage for the development or progression of disease. Potentially disease-relevant DNA methylation has been identified in the diseases. However, a comprehensively characterized DNA methylation for the disease remains elusive. Although DNMTs inhibitors have proven particularly effective in cancer, those drugs have not yet been applied for human ophthalmic diseases including cataract. In the future, more thorough studies need to focus on the early biomarkers and novel therapeutic targets for the diseases.

Table 2 DNA methylation in ARC

Gene	Study population	Tissue	Effect/significance	References
CRYAA	Human	Lens epithelial cells	Reduce the expression of CRYAA	Palsamy et al. (2014b)
MGMT	Human	Lens epithelial cells	Reduce the expression of MGMT	Palsamy et al. (2014c)
WRN	Human	Lens epithelial cells	Reduce the expression of WRN	Robert et al. (2003)
OGG1	Human	Lens epithelial cells	Reduce the expression of OGG1	Peek et al. (1991)
ERCC6	Human	Lens epithelial cells	Reduce the expression of ERCC6	Green and Marsit (2015)
Klotho	Human	Lens epithelial cells	Reduce the expression of Klotho	Tittle et al. (2011)
Keap1	Human	Lens epithelial cells	Upregulate the expression of Keap1	Wang et al. (2015)
Keap1	Human	Lens epithelial cells	Upregulate the expression of Keap1	Wang et al. (2016a)
	Rat	Lens epithelial cells		
Keap1	Human	Lens epithelial cells	Upregulate the expression of Keap1	Wang et al. (2016b)
Keap1	Human	Lens epithelial cells	Upregulate the expression of Keap1	Ye et al. (2015)

Table 3 DNA methylation in secondary cataract and complicated cataract

Gene	Study population	Tissue	Effect/significance	References
CRYAA	Human	Lens epithelial cells	Reduce the expression of CRYAA	Zhou et al. (2012a)
Keap1	Human	Lens epithelial cells	Upregulate the expression of Keap1	Zhu et al. (2013)
LOXL1	Human	Lens epithelial cells	Reduce the expression of LOXL1	Zhu et al. (2015a)

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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