Eyes on DNA methylation: current evidence for DNA methylation in ocular development and disease

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Received: 6 December 2011 / Accepted: 5 March 2012 / Published online: 29 March 2012
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Abstract Epigenetic modulation of chromatin states constitutes a vital component of the cellular repertoire of transcriptional regulatory mechanisms. The development of new technologies capable of generating genome-wide maps of chromatin modifications has re-energized the field. We are now poised to determine how species- and tissue-specific patterns of DNA methylation, in concert with other chromatin modifications, function to establish and maintain cell- and tissue-specific patterns of gene expression during normal development, cellular differentiation, and disease. This review addresses our current understanding of the major mechanisms and function of DNA methylation in vertebrates with a historical perspective and an emphasis on what is known about DNA methylation in eye development and disease.

Keywords DNA methylation · Transcriptional regulation · Ocular cancer · Retina · Lens · Müller glia

Introduction

Research over the past half century has revealed the importance of transcriptional regulatory genes and, by extension,

regulation of transcription in the genetics of eye development and disease. However, our understanding of the contribution of epigenetics in these same processes has lagged, particularly when it comes to the visual system. Epigenetics refers to changes in chromatin structure that permanently affect gene expression in a cell or tissue without altering the actual nucleotide sequence. DNA methylation, along with the covalent modifications of histone tails by acetylation and methylation, is a major mechanism of epigenetic gene regulation. Non-coding RNAs (e.g., *Xist*) also contribute to epigenetic gene regulation by mediating genomic imprinting and heterochromatin formation through recruitment of chromatin-modifying complexes [1–3]. What distinguishes epigenetic gene regulation from simple transcriptional activation or repression is that epigenetic marks within the chromatin structure are maintained in daughter cells following mitosis. Further, epigenetic modifications can be influenced by environment [4], diet [5–7], and aging [8, 9]. This has raised interest in understanding the potential contribution of epigenetics to the etiology of multifactorial and late onset diseases such as glaucoma, age-related macular degeneration, and cataract.

Historical perspectives on DNA methylation

DNA methylation was initially discovered in the 1960's as a mechanism contributing to the strain specificity of bacteriophage infections of *E. coli* [10]. It was soon apparent that nucleic acid methylation was not unique to prokaryotes, but was also present in eukaryotes [11]. Early interest in the role of DNA methylation was stimulated by findings that various mutagens, including methylmethane sulfonate and dimethylnitrosourea, altered methylation patterns. It was initially thought that the link between DNA methylation and cancer reflected defects in DNA repair, such as defective repair of mutagen-

This work supported in part by NIH-R01EY012792.

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induced methylation and/or from a loss of normal patterns of DNA methylation through overzealous DNA repair [12]. Research to decipher the connection between gene silencing and methylation was subsequently fueled by the finding that transformed cell lines undergo de novo methylation, resulting in permanent gene silencing [13].

Despite extensive research on the role of DNA methylation in cancer, progress in understanding its role in normal development and cellular differentiation has been comparatively slow. A role for DNA methylation in regulating differentiation and in reprogramming somatic nuclei to pluripotency was postulated shortly after the discovery of DNA methylation [14]. Although intriguing, this proposal was made in the absence of clear evidence for methylation or demethylation in eukaryotes. It was several years before methyltransferase activity was detected in eukaryotic cells [15, 16]. The availability of genome databases and development of new technologies capable of generating genome-wide maps of chromatin modifications has re-energized the field.

Patterns of cytosine methylation

In animal cells, DNA methylation occurs almost exclusively on cytosine residues located within CpG dinucleotides, where the “p” stands for the phosphodiester bond linking the adjacent cytosine and guanine nucleotides. Non-CpG methylation is well documented in plants [17, 18] and more recently, CpHpG methylation (where H is A, T, or C) has been detected in embryonic stem cells [19, 20]. Non-CpG methylation in vertebrates appears to be restricted to trinucleotide repeat regions. In vertebrates, overall methylation of CpG dinucleotides across the genome is estimated to be 70–80 %. Most of the genome has a lower than expected frequency of CpG dinucleotides, in part because of spontaneous deamination of methylated cytosine to thymine [21]. In contrast, genomic regions containing higher than expected levels of C+G bases and CpG dinucleotides, known as CpG islands, are typically associated with promoter regions and may extend into the coding and intronic regions [22]. Cytosine methylation varies inversely with CpG density, such that CpG islands are typically hypomethylated, creating a chromatin environment permissive for transcription (Fig. 1a).

Covalent modification of methyl cytosine into 5-hydroxymethylcytosine (5-hmC) is another recently identified DNA modification that appears to be abundant in embryonic stem cells and the brain [23, 24]. Conversion of 5-mC to 5-hmC is catalyzed by methylcytosine dioxygenase, which is part of the family of 3-oxoglutarate- and Fe(II)-dependent TET oncogenes [25]. ES cells have a high density of 5-hmC in promoter and enhancer regions, particularly those associated with H3K4me1 and H3K27ac histone modifications and binding sites for pluripotency-regulating transcription factors (e.g.,

NANOG, OCT4) [26, 27]. This suggests that 5-hmC may be a new marker of active gene regulatory regions (Fig. 1d). Interestingly, bisulfite conversion, the widely used “gold standard” method for analysis of DNA methylation, does not distinguish between 5-mC and 5-hmC [28]. Thus, there is need for caution in interpreting the results of bisulfite sequencing data and new methods will need to be developed to understand the biological significance of this novel chromatin modification.

CpG islands and gene regulation

Approximately 70 % of genes in a vertebrate genome are associated with CpG islands, with the largest single group of CpG island-containing genes consisting of the so-called “housekeeping” genes [22]. These genes are constitutively expressed in all cell types, consistent with the association between CpG island hypomethylation and transcriptional activation. However, not all CpG island-containing genes are ubiquitously expressed. Using a rigorous criteria for identification of CpG islands (>500 bp long, G+C content >50 %, a CpG observed/CpG expected ratio of ≥ 0.6 and located within 2,000 bp of the transcription start site) reduces the number of identified CpG island-containing genes in the human genome to ~35 % [29]. Interestingly, analysis of gene ontology terms associated with the 17,820 identified genes revealed high representation of the gene ontology terms development, morphogenesis, synaptogenesis, and neurogenesis. Many CpG island-containing genes associated with neurogenesis also show tissue-restricted and developmentally restricted patterns of expression and genome-wide analysis has revealed dynamic changes in methylation patterns during development [30].

Cell-type-specific analysis has revealed that regulation of transcription by methylation is more complex than a simple on/off switch of hypo or hyper methylation. Modulating promoter activity does not require total CpG methylation and partial methylation can increase as well as decrease promoter activity. For example, our analysis of the effects of DNA methylation on activity of the *EphA5* gene showed that in vitro methylation of 10 of 98 CpG dinucleotides within a 2,549 bp promoter construct could reduce activity by 20 %, whereas methylation of a non-overlapping set of 18/98 CpG dinucleotides increased promoter activity by 35 % [31]. Dynamic differences in methylation also occur in CpG dinucleotides located on the “shores” of CpG islands [32]. Analysis of multiple tissue types showed that over 30 % of differentially methylated CpG are located between 0 and 500 bp from a CpG island compared to <10 % located within CpG islands themselves [33] (Fig. 1d). Finally, many sites that show tissue-specific variation in methylation during development also have variable methylation during differentiation of induced pluripotent stem cells and in cancer [32, 33].

Fig. 1 Effects of DNA methylation and histone modifications in transcription of CpG island-containing genes. **a** Chromatin with unmethylated cytosine residues (open circles) within CpG islands and adjacent shores is associated with trimethylation of histone H3 on lysine 4 ($H3K4me3$) and transcriptional activation. **b** Chromatin with hypermethylated cytosine residues within CpG islands and shores (black circles) is associated with H3K9 trimethylation ($H3K9me3$) and

transcriptional silencing. **c** Methylation of cytosine residues located in the shores of the CpG island is sufficient for transcriptional repression, even when the CpG island is hypomethylated. Histone modifications associated with methylation in CpG shores have not been determined. **d** Hydroxymethylcytosine residues (H) are associated with H3K4 methylation ($H3K4me1$) and H3K27 acetylation and transcriptional activation typical of an open chromatin structure

DNA methyltransferase genes

Establishment of open and closed chromatin structure is regulated by both DNA methylation and histone modification and DNA methylation is inversely correlated with methylation of lysine 4 on histone H3 ($H3K4$) and positively correlated with $H3K9$ methylation [34] (Fig 1a, b). This is mediated, in part, through differential recruitment of de novo DNA methyltransferases (DNMTs) and histone remodeling complexes to the chromatin [35]. This section presents a brief overview of the major players involved in modulating DNA methylation of chromatin. Readers interested in more details regarding the molecular mechanisms underlying interactions between histone-modifying complexes and DNA methylation are referred to several excellent reviews [36–38].

Establishment and maintenance of DNA methylation patterns in the mammalian genome rely on the three major DNA methyltransferases: DNMT1, DNMT3a, and DNMT3b. DNMT1 is a maintenance methyltransferase that functions during DNA replication to re-methylate newly synthesized strands of DNA and facilitates cellular inheritance of epigenetic modifications [39]. Embryonic stem cells (ES) lacking *Dnmt1* are viable, but have only about 1/3 of the normal CpG methylation levels [40] and *Dnmt1* is necessary and sufficient for maintaining the methylation signatures of imprinted genes in the zygote [41]. Mice lacking DNMT1 die in mid-gestation and show genome-wide reductions in DNA methylation, with

growth retardation, increased cell death, and a failure of vascular development, although eye development has not been characterized [42]. Conditional knockout of *Dnmt1* in the forebrain in mice results in defects in the formation and organization of the barrel cortex, indicating a role for DNA methylation in the formation of sensory maps [43].

DNMT3a and DNMT3b are de novo methyltransferases that can initiate methylation of previously unmethylated DNA [44]. DNMT3a is not required for embryonic and fetal development and homozygous *Dnmt3a* knockout mice appear normal at birth, although they subsequently die around the fourth postnatal week [45]. Loss of *Dnmt3b* is embryonic lethal in mice and results in a variety of developmental defects, most notably in the neural tube [45]. Lack of DNMT3b in mouse embryonic fibroblasts results in genome-wide DNA hypomethylation, chromosomal breaks, aneuploidy, and polyploidy, suggesting that DNMT3b contributes to maintaining methylation patterns and to genome stability [46]. In somatic cells, DNMT3b has been proposed to maintain the patterns of DNA methylation that silence germ line-specific genes [47]. Mutations in DNMT3b have been identified in some patients with ICF-1 (immunodeficiency-centromeric instability-facial anomalies syndrome 1) and is associated with increased areas of hypomethylation in their genomes [48]. The facial dysmorphism in ICF patients includes abnormal epicanthal folds and telcanthus. Interestingly, analysis of gene expression changes in lymphoblastoid cells from ICF patients showed upregulation

of multiple genes associated with eye development (e.g., *Lhx2*, *Six3*, *Ascl1*, *Isl2*, *Eomes*, *EphB1*, *Sema3b*, *Robo1*) [49].

In addition to the three canonical DNMTs, *Dnmt3l* (DNA methyltransferase 3-like) encodes a structurally related protein that lacks catalytic activity [50]. DNMT3L interacts directly with H3K4 and recruits DNMT3a to the nucleosome, providing a link between histone modifications and de novo DNA methylation [51, 52]. Both DNMT3L and DNMT3a are essential for establishing methylation patterns associated with imprinted genes in germ line cells [53].

DNMT2 was first identified as a DNA methyltransferase based on sequence similarity, but it lacks the N-terminal regulatory region typical of other DNMTs [54]. DNMT2 has minimal ability to methylate DNA and instead methylates tRNA [55]. Because of these findings, *Dnmt2* was recently re-named *Trdm1* (tRNA aspartic acid methyltransferase 1). Homozygous mice lacking catalytic domains of DNMT2 are homozygous viable and fertile, with no obvious morphological defects [55]. In contrast, morpholino knockdown of *dnmt2* in zebrafish embryos results in defects in multiple tissues including the eye, without detectable reduction in global DNA methylation [56]. Overexpression of a nuclear-targeted *dnmt2* failed to rescue defects in the morphants, whereas overexpression of *dnmt2* containing a nuclear export signal kept the protein in the cytoplasm and effected rescue with high efficiency [56].

Methyl CpG-binding proteins

Methylated DNA is bound by methyl Cp-binding protein 2 (MeCp2) and the methyl-binding domain proteins, MBD1, MBD2, and MBD3 [57, 58]. MeCp2 is capable of binding to a single CpG dinucleotide [59]. During replication, the transcriptional repression domain (TRD) of MeCp2 interacts with DNMT1, recruiting it for methylation of cytosine residues on the newly replicated DNA strand [60]. In non-replicating cells, the TRD functions independently as a transcriptional repressor to interpret the methylation signature [61]. MeCp2 also represses indirectly by recruiting co-repressors such as histone deacetylases (HDACs) and SIN3a [62], which remodel the histones and reinforce the chromatin compaction associated with gene silencing. Mutations in *MeCp2* have been implicated in the etiology of Rett syndrome, a neurodevelopmental disorder that primarily affects females, beginning in their second year [63]. Despite apparently normal development in the first year and a half, patients with Rett syndrome progressively lose their ability to speak and show severe changes in motor and neurological functions that are often misdiagnosed as autism or multiple sclerosis. In the visual system, patients have substantial refractive errors, but reports on changes in visual function are contradictory [64–66]. However, mice lacking MeCp2 showed defects in the maturation and strengthening of retino-

geniculate synapses associated with eye opening and vision-dependent remodeling [67]. This observation is consistent with reported defects in synaptic maturation and plasticity in other portions of the central nervous system associated with loss of MeCp2 [68, 69].

MBD1–3 were discovered based on homology to the methyl-binding domains of MeCp2 [70]. All three MBDs share significant homology within their N-terminal DNA-binding domains, with MBD4 showing the highest sequence similarity to MeCp2. Outside of the MBDs, they are significantly different, with highest sequence similarity between MBD2 and MBD3 (~70 % amino acid identity) [71]. MBD1–3 are expressed broadly in all mouse tissues examined, although MBD1 and MBD2 transcript levels are lower in ES cells [72].

MBD1 contains several cysteine-rich CxxC domains that share homology to motifs found in DNA methyltransferase and the trithorax-related gene, HRX [73]. The CxxC domain functions as a transcriptional repressor independent of histone deacetylase activity [74]. MBD1 recognizes TCGCA and TGGCA sequences most efficiently via its MBD; the CxxC domain can recognize a single methylated CpG, but shows no sequence specificity [75]. MBD2 can bind methylated DNA containing a single methylated site, although the efficacy of repression is directly correlated to methylation density [76]. In the genome, MBD2 is stably associated with heavily methylated DNA and recruits a co-repressor complex known as MeCp1 to genomic sites containing 12 or more methylated CpG sites [77]. MBD2/MeCp1 complexes also contain histone deacetylase activity and can be immunoprecipitated by antibodies against HDAC1 and HDAC2 [77]. MBD3 lacks the ability to bind to methylated DNA, but forms a component of the NuRD complex, a large multiunit protein complex containing histone deacetylase and nucleosome remodeling activities [78, 79]. The NuRD-MBD3 complex is subsequently recruited to methylated DNA through interactions with MBD2 [80].

Expression and function of DNA methyltransferases in the eye

In the mouse retina, *Dnmt1* is most highly expressed during development with mRNA levels highest at embryonic day 11.5 and showing a progressive decrease with age [81]. DNMT1 protein is detected in most post-mitotic neurons in the inner retina, with strongest immunoreactivity in retinal ganglion cells and amacrine, and lower levels in bipolar and horizontal cells and cone photoreceptors [81]. Consistent with a role in retinal development, morpholino knockdown of *dnmt1* in zebrafish embryogenesis results in defects in retinal lamination, loss of melanin pigmentation, a significant down-regulation of interphotoreceptor retinoid-binding protein (*irbp/rbp3*) expression and defects in the

retinal pigmented epithelium (RPE) and photoreceptor differentiation [82].

Dnmt3a is expressed broadly in the embryo at E8.5 and E9.5, whereas *Dnmt3b* expression is specifically enriched in the neural tube and eyes [45]. *Dnmt3a* and *3b* mRNAs and proteins are both highly expressed in the developing optic cup but retinal expression progressively decreases with age [81]. In the postnatal retina, immunoreactivity for DNMT3a and 3b is most prominent in the ganglion cell layer and inner nuclear layer (in amacrine and horizontal cells), with labeling also present in cone photoreceptors and pigmented epithelium [81]. In contrast, a previous study showed broad immunoreactivity for DNMT3b, but not DNMT1 or DNMT3a in the human retina [83]. The basis for this difference is not known. Ocular phenotypes of mouse knockouts of *Dnmt3a* or *Dnmt3b* have not yet been described. However, morpholino knockdown of *dnmt3*, the zebrafish ortholog of the mammalian *Dnmt3b*, results in multiple defects in embryos, most notably a reduction in the size of the brain and eye [84]. Compared to *dnmt1* morphants, knockdown of *dnmt3b* resulted in a more severe disorganization of the retinal laminations and a less differentiated retinal phenotype. Although *irbp* expression was downregulated in *dnmt1* and *dnmt3* morphants, only *dnmt3* morphants lacked expression of other markers of photoreceptor and amacrine cells including *crx*, *neurod*, and *ascl1a*. Intriguingly, both *dnmt1* and *dnmt3* knockdown resulted in defects in RPE differentiation, with *dnmt1* morphants lacking RPE on the dorsal side and *dnmt3* morphants lacking RPE on the ventral side of the optic cup [82, 84]. These findings support a potential role for DNA methylation in patterning the developing eyecup, as well as in regulating differentiation of the RPE and photoreceptors.

DNA methylation in ocular cancers

Given the role of DNA methylation in tumorigenesis, it is not surprising that changes in DNA methylation are associated with altered gene expression in ocular tumors. In retinoblastoma, the most common childhood tumor of the retina [85], methylation-sensitive PCR of promoter methylation showed that the tumor suppressor gene, RAS-associated domain family 1A (*RASSF1A*) was silenced by DNA hypermethylation in 82 % of tumors, but not in adjacent normal retinal tissues [86]. O6-methylguanine-DNA methyltransferase (*MGMT*), a methyltransferase gene involved in DNA repair, is hypermethylated in a subset of retinoblastoma primary tumors and is associated with earlier disease onset (before age 2) [87]. DNMT1, 3a, and 3b are frequently over-expressed in retinoblastoma and expression levels are positively correlated with pathogenesis [83]. However, increased CpG methylation in retinoblastoma does not simply reflect a generalized hypermethylation, as multiple genes that are silenced by hypermethylation in other cancers

(e.g., *p14^{RF}*, *p15^{INK4b}*, *p16^{INK4a}*, or *VHL*) remain hypomethylated in retinoblastoma tumors [87].

Uveal melanoma is a highly aggressive tumor arising from the pigmented cells of the uvea, including the iris, ciliary body, and choroid [88]. Gene silencing by DNA methylation has been proposed to contribute to the pathophysiology of uveal melanoma, but reports of methylation patterns are contradictory. *RASSF1a* was found to be hypermethylated in 50 % of archival frozen tumor specimens and in 91 % of uveal melanoma cell lines [89]. In contrast, in 23 uveal melanoma biopsies, only telomerase (*hTERT*) showed high frequency of hypermethylation (52 %), whereas six additional genes known to be hypermethylated in cutaneous melanoma, including *RASSF1*, were methylated in less than 15 % of tumor samples [90]. Methodological differences may account for some of the variability in these results; however, determining the role of methylation in the etiology and metastasis of uveal melanoma will require additional research.

DNA methylation in regulation of ocular and retinal gene expression

The body of published research on regulation of ocular gene expression by DNA methylation is small when compared to the vast literature related to methylation in cancer, embryonic stem cells, and other tissues. The earliest studies used DNA digestion by methyl-sensitive restriction endonucleases followed by Southern blot analysis to identify correlations between methylation status and gene expression in ocular vs. non-ocular tissues. Subsequently, bisulfite sequencing enabled identification of all methylated CpG dinucleotides in a region of interest. However, both of these approaches require a priori identification of genes of interest and are relatively slow and labor intensive.

An early study of the role of DNA methylation in regulating ocular genes tested if tissue-specific hyper- or hypomethylation could be responsible for differential expression of chicken delta crystalline (*Cryd*) in ocular (lens, embryonic neural retina, and RPE) vs. extra ocular (liver, kidney) tissues [91]. Despite some variation in methylation status of the internal cytosine of the CCGG recognition site of HpaII in different tissues, there was no correlation between methylation and mRNA expression. Likewise, lentoid bodies generated from embryonic neural retina showed no correlation between *Cryd* methylation and mRNA expression [92]. Analysis of a GC rich promoter domain in the gene encoding retinal fatty acid-binding protein (*Fabp5*) also showed no methylation in retina, where the gene is highly expressed, and in liver, where no transcripts were detectable [93]. However, a role for DNA methylation in lens development was recently demonstrated in zebrafish, where morpholino knockdown of *dnmt1* resulted

in lens dismorphia, ruptures of the lens capsule and unraveling of lens fibers.

Ten years later, a similar approach found a developmental and tissue-specific decrease in methylation in the promoter of the interphotoreceptor retinoid-binding protein (*Rbp31*) gene that correlated with upregulation of mRNA expression during retinal development [94, 95]. A more recent study also implicated DNA methylation in regulating *irbp/rbp31* in zebrafish retinas, but showed downregulation of expression following morpholino knockdown of *dnmt1*, despite a general loss of genome-wide methylation [82]. Nevertheless, defects in cone photoreceptor morphology were also observed following *dnmt1* knockdown in zebrafish [96]. This is consistent with preferential detection of DNMT1 protein in cones vs. rods in the mouse retina [81]. Together, results support a role for DNA methylation in photoreceptor development, but suggest that the relationship between methylation and regulation of photoreceptor-specific gene expression may have species-specific differences.

The proposal that genes regulating development of ocular tissues are regulated by differential methylation has been further supported by some recent studies. Analysis of methylation patterns in the whole mouse embryos using methylated DNA immunoprecipitation with microarray analysis (MeDip-on-Chip) identified several genes expressed in the eye [e.g., complexin 4 (*Cplx4*), a gene encoding a synaptic protein expressed in retina and brain and alpha crystalline-A (*Cryaa*), a key gene for lens development] as differentially methylated during development. Both genes are hypermethylated in the epiblast around the time of implantation, but are unmethylated specifically in retina and lens respectively in adult mice [97].

Our work has shown a role for DNA methylation in regulation of *EphA5* receptor expression in the mouse retina [31]. *EphA5* is a member of the Ephrin receptors (*Eph*) family of tyrosine kinase receptors that regulate multiple patterning events during embryonic development, including the formation of topographical maps in the visual and other sensory systems throughout the CNS [98, 99]. Using bisulfite sequencing of DNA isolated from whole retinas, we found near complete hypomethylation in the proximal *EphA5* promoter that did not change from embryogenesis to adults [31]. However, a more detailed analysis of DNA isolated from the nasal or temporal retinas of neonatal mice revealed partial promoter methylation of CpG sites near the transcription start site in a fraction of clones. Methylation was increased in the nasal retina, where *EphA5* expression is lowest, compared to the temporal retina where *EphA5* expression is highest. However, these studies were performed on DNA samples from full thickness retinal explants, and additional analysis using DNA from purified cell populations are needed before we can attribute the differences in methylation to a particular cell type. We are continuing to pursue the significance of low-level methylation in modulating *EphA5* promoter activity to determine how

this may contribute to the nasal/temporal gradient of expression in the retina in vivo.

DNA methylation and stem cells

Mechanisms regulating genome demethylation are of great interest, as re-setting the epigenome is critical for pluripotency. Recent studies have shown that induced pluripotent stem cells (iPSCs) can retain characteristics of the cells of origin, predisposing them to re-differentiate along their original lineage [100]. Methylation appears to contribute to this cellular memory, as fully dedifferentiated iPSCs can acquire methylation patterns comparable to embryonic stem cells over time in culture [101]. Pharmacological demethylation can be achieved in vitro using 5-azacytidine (5-Aza) and 5-aza-2'-deoxycytidine (Decitabine), which is used therapeutically for demethylation in some cancers [102, 103]. This may have utility in further enhancing pluripotency.

Our lab is one of several investigating the stem-cell potential of Müller glia in the mammalian retina [104–109]. Although Müller glia are the source of stem cells capable of regenerating all types of retinal neurons in the retinas of teleost fish [110, 111], their regenerative capacity in the retinas of mammals is severely attenuated. We have demonstrated that several genes (e.g., *EphA5*, *EphB1*) that are silenced by hypermethylation in conditionally immortalized Müller glia (ImM10 cell line) in vitro, can be upregulated by in vitro demethylation with AzadC [31]. The significance of hypermethylation of this Müller glia cell line and whether similar patterns of methylation are present in other Müller cell lines or in vivo remains to be determined

Conclusions

One of the hurdles in cataloging and interpreting the scope and significance of changes in DNA methylation has been the lack of cell-specific and genome-wide data. Until recently, analysis of methylation patterns has focused on specific genes in cultured cells (particularly tumor derived or immortalized cells) or whole tissues. This approach relies on the assumptions that the researchers have predicted the relevant genes and that methylation patterns are tissue specific, rather than cell-type specific. Since most tissues are composed of multiple cell types, methylation variations that are restricted to a subset of cells within the tissue would be difficult to detect. The use of genome-wide methylation analysis, combined with cell sorting to obtain purified cell populations offers us the unique opportunity to generate comprehensive databases of chromatin modifications of specific cell populations, at distinct developmental stages in both healthy and diseased tissues. Over the next several years, continued use of high content and high-resolution strategies to determine the genome-wide changes in

DNA methylation signatures has the potential to reveal new roles for DNA methylation and other epigenetic mechanisms in controlling gene expression in the visual system during development, aging, and disease.

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