Epigenetic Modifications Compromise Mitochondrial DNA Quality Control in the Development of Diabetic Retinopathy

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PURPOSE. Diabetes causes dysfunction in the retinal mitochondria and increases base mismatches in their DNA (mtDNA). The enzyme responsible for repairing the base mismatches, MutL homolog 1 (Mlh1), is compromised. Diabetes also favors many epigenetic modifications and activates DNA methylation machinery, and Mlh1 has a CpG-rich promoter. Our aim is to identify the molecular mechanism responsible for impaired mtDNA mismatch repair in the pathogenesis of diabetic retinopathy.

METHODS. Human retinal endothelial cells, incubated in 20 mM glucose, were analyzed for mitochondrial localization of Mlh1 by an immunofluorescence technique, Mlh1 promoter DNA methylation by the methylated DNA capture method, and the binding of Dnmt1 and transcriptional factor Sp1 by chromatin immunoprecipitation. The results were confirmed in retinal microvessels from streptozotocin-induced diabetic mice, with or without Dnmt inhibitors, and from human donors with diabetic retinopathy.

RESULTS. Compared with cells in 5 mM glucose, high glucose decreased Mlh1 mitochondrial localization, and its promoter DNA was hypermethylated with increased Dnmt-I binding and decreased Sp1 binding. Dnmt inhibitors attenuated Mlh1 promoter hypermethylation and prevented a decrease in its gene transcripts and an increase in mtDNA mismatches. The administration of Dnmt inhibitors in mice ameliorated a diabetes-induced increase in Mlh1 promoter hypermethylation and a decrease in its gene transcripts. Similar decreases in Mlh1 gene transcripts and its promoter DNA hypermethylation were observed in human donors.

CONCLUSIONS. Thus, as a result of the epigenetic modifications of the Mlh1 promoter, its transcription is decreased, and decreased mitochondrial accumulation fails to repair mtDNA mismatches. Therapies targeted to halt DNA methylation have the potential to prevent/halt mtDNA damage and the development of diabetic retinopathy.

Keywords: diabetic retinopathy, DNA methylation, epigenetics, mitochondria, mismatch repair, mutL homolog 1
The expression of a gene can also be regulated by epigenetic modifications, and these modifications are now implicated in many chronic diseases, including cancer and diabetes.14–16 Diabetes alters the epigenetic machinery in the retina and its vasculature, and several genes implicated in the development of diabetic retinopathy are epigenetically modified.15,17,18 Methylation of cytosine in the cytosine-guanosine rich region (CpG island) of a gene promoter is generally associated with decreased transcriptional factor binding and suppression of its expression,19,20 and in diabetes, the machinery critical in maintaining the DNA methylation status is activated.17

Mlh1 promoter is a TATA-less promoter with multiple CpG islands,21 and Sp1 is considered as one of the transcription factors important in its transcription.22 Increased Mlh1 promoter DNA methylation and its transcriptional suppression is implicated in many chronic diseases associated with mtDNA heteroplasmy.21,23,24 How diabetes decreases Mlh1 in the retina microvasculature is not clear. The goal of this study is to investigate the molecular mechanism responsible for the impaired mismatch repair mechanism in the pathogenesis of diabetic retinopathy. DNA methylation status of the Mlh1 promoter was evaluated in human retinal endothelial cells (HRECs) using the pharmacological and molecular inhibitors of DNA methyltransferases (Dnmts). In vitro results were confirmed in an in vivo model using retinal microvessels from streptozotocin-induced diabetic mice, with or without the administration of Dnmt inhibitors. To transition into the human diseases, the key parameters of Mlh1 transcription were confirmed in the retinal microvessels from human donors with documented diabetic retinopathy.

**METHODS**

**Retinal Endothelial Cells**

Human retinal endothelial cells, obtained from Cell Systems Corporation (cat. no. ACBRI 181, Cell Systems Corp., Kirkland, WA, USA), were cultured in Dulbecco’s modified Eagle medium (DMEM) supplemented with 12% heat-inactivated fetal bovine serum, 20 µg/ml endothelial cell growth supplement, and 1% each insulin, transferrin, selenium, glutamax, and antibiotic/antimitotic.25 Cells from the fifth to eighth passage were incubated in normal D-glucose (5 mM [NG]) or high D-glucose (20 mM [HG]) for 96 hours in the presence or absence of a Dnmt inhibitor, 5-Aza-2’-deoxycytidine (Aza, 1 µM; cat. no. A5656, Sigma-Aldrich Corp., St. Louis, MO, USA), L-glucose (20 mM), instead of 20 mM D-glucose, was used as the osmotic/metabolic control.25–27

A group of cells from the fifth to sixth passage were transfected with Dnmt1-siRNA (cat. no. SC-35204, Santa Cruz Biotechnology, Santa Cruz, CA, USA) using transfection reagent (cat. no. SC-29528, Santa Cruz Biotechnology). As a control, parallel incubations were run using nontargeting scrambled RNA or reagent alone.26 Transfection efficiency was evaluated by quantifying Dnmt1 gene transcripts and protein expression and, as reported earlier, was about 40%.26 Each incubation condition had HRECs from the same batch and the same passage.

**Mice**

Two days after establishment of streptozotocin-induced diabetes in C57BL/6 mice (7–8 weeks old, either sex),28 they were anesthetized by intraperitoneal injection of ketamine/xylazine (100 mg/kg ketanest and 12 mg/kg xylazine). Dnmt1-siRNA (ID: M-8205624, Thermo Fisher Scientific, Waltham, MA, USA), 2 µg in 2 µL nuclease-free water and mixed with Invivofectamine (cat. no. IVF 3001, Invitrogen, Carlsbad, CA, USA), was injected intravitreally in the right eye under a dissecting microscope. The left eye was injected with a negative control siRNA (SC, cat. no. 12935-300, Invitrogen).28 Five weeks after the administration of siRNA, the mice were killed, and their retinas were quickly isolated. Controls included age-matched normal mice and diabetic mice without any siRNA administration. Intravitreal administration of Dnmt1-siRNA reduced the Dnmt1 gene transcripts in the retinal microvessels by 35% to 45%. The treatment of animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the Wayne State University’s Institutional Animal Care and Use Committee.

**Human Donors**

Eye globes from human donors with documented diabetic retinopathy (40–75 years age, 14–41 years of diabetes) and age-matched nondiabetic donors enucleated within 6 to 8 hours of death were obtained from the Eversight Eye Bank (Ann Arbor, MI, USA). The retina were isolated, and the microvessels were prepared by hypotonic shock method.29

**Mitochondrial Localization**

Mitochondrial localization of Mlh1 was performed by immunofluorescence technique using an Mlh1 antibody (cat. no. ab92312, Abcam, Cambridge, MA, USA; 1:100 dilution). CoxIV (cat. no. ab53985, Abcam; 1:100 dilution) was used as a mitochondrial marker. Secondary antibodies against Mlh1 and CoxIV included AlexaFluor 488 (green)–conjugated antirabbit (cat. no. A11008, Molecular Probes-Life Technologies, Grand Island, NE, USA; 1:500 dilution) and Texas red (red)–conjugated antimouse (cat. no. TI-2000, Vector Laboratories, Burlingame, CA, USA; 1:500 dilution), respectively. After mounting the coverslips in 4’6-diamidino-2-phenylindole (DAPI)-containing (blue) Vectashield (Vector Laboratories) mounting medium, they were captured in ZEISS 40X (Carl Zeiss, Inc., Chicago, IL, USA) objective magnification with the Apotome module, using 408 nm, 488 nm, and 562 nm wavelengths. The captured images were calibrated with the ZEISS proinbuilt software package and modules. The random regions of interest were made in the outer nuclear regions, and using the colocalization software module, the Pearson’s correlation coefficient was determined.30 The intensity profile was determined by line region of interest across the cell, and the fluorescence intensity of blue (DAPI), green (Mlh1), and red (CoxIV) channels were plotted.

The mitochondria were isolated using a mitochondria isolation kit from Thermo Fisher (Wilmington, DE, USA; cat. no. 89874).31 After digesting the homogenate with the kit reagents, the mitochondrial rich fraction was obtained by differential centrifugation at 700g for 10 minutes followed by centrifugation at 3000g for 15 minutes. The mitochondrial pellet, after rinsing, was suspended in PBS.

**Western Blotting**

Western blotting was performed in the isolated mitochondrial fraction32 using Mlh1 and CoxIV antibodies (ab92312 and ab53985; Abcam) at 1:1000 dilution each.

**Plasmid and Constructs**

Mlh1 promoter (−2077 to −617) was amplified and cloned into the promoter-less pGL3-Basic-IREs plasmid (plasmid no. 64784,
Addgene, Watertown, MA, USA). Deletion promoter fragments containing 934bp (−1137 to −2050) and 843bp (−1235 to −2077) of the Mlh1 promoter were generated by PCR. The primers used to clone these promoter fragments containing BamHI and HindIII are listed in Table 1. The pGL3-control vector (cat. no. E1741, Promega, Madison, WI, USA) was used as a control vector.

Transient transfection was performed in the HRECs using TurboFect transfection reagent (cat. no. R0534, Thermo Fisher) according to the manufacturer’s instructions. Briefly, 1 μg of DNA in 100 μL of serum-free Dulbecco’s modified Eagle’s medium was mixed with 2 μL of transfection reagent and incubated at room temperature for 20 minutes. After rinsing and incubating the cells in Dulbecco’s modified Eagle’s medium overnight, they were incubated for 96 hours in

<table>
<thead>
<tr>
<th>Restriction Endonuclease</th>
<th>Position</th>
<th>Primers With 5' RE-Recognition Sites</th>
</tr>
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<tbody>
<tr>
<td>HindIII</td>
<td>617-636</td>
<td>5'-TCTAAGCTTTGGACTTTGTTGCCCCTAC-3'</td>
</tr>
<tr>
<td>BamHI</td>
<td>2070-2058</td>
<td>5'-CAGGGATCCCGCCGGAGCTATTGA-3'</td>
</tr>
<tr>
<td>HindIII</td>
<td>1137-1156</td>
<td>5'-CAGAGCTTACCACCGGATAAAGACCAGG-3'</td>
</tr>
<tr>
<td>BamHI</td>
<td>2070-2050</td>
<td>5'-ACCGGATCCGGGACGTATTGAATTGGACAG-3'</td>
</tr>
<tr>
<td>HindIII</td>
<td>1235-1254</td>
<td>5'-CGGAGCTTAGCTGTTGCTGATATTCC-3'</td>
</tr>
<tr>
<td>BamHI</td>
<td>2077-2057</td>
<td>5'-CAGGGATCCCTTCCAGGCGACATTGAT-3'</td>
</tr>
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Luciferase Activity

Luciferase activity was determined using Pierce Firefly Luciferase Glow Assay Kit (cat. no. 16176, Thermo Fisher). Briefly, after washing the cells with 1X Dulbecco’s phosphate-buffered saline buffer and lysing with 1X cell lysis buffer for 15 minutes, 10 to 20 μL cell lysate was transferred to a black opaque 96-well plate containing 50-μL working solution. The samples were incubated for 10 minutes at room temperature, and the light output was measured at 613 nm. The results were normalized with the value obtained from the cell lysate of pGL3-control vector transfected cells. Each luciferase assay was performed in triplicate, and all transfection experiments were repeated three or more times.

Quantification of Methylated Cytosin

The levels of 5-methyl cytosine (5mC) were quantified in the total genomic DNA by a MethyLamp Methylated DNA capture kit (cat. no. P-1015, EPIGENTEK, Farmingdale, NY, USA) using Mlh1 promoter primers (Table 2).

<table>
<thead>
<tr>
<th>Region</th>
<th>Position</th>
<th>Primer</th>
<th>Number of CpG</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>−659 to −522</td>
<td>Forward: CTTGGCTTCGGTTAAAAGGC</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: GGGGCTTGGGCTGAGAGG</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>−383 to −261</td>
<td>Forward: AGTATTTTCGCTAGCCCTCG</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: GCGTTATTTGGGTGGTGAAGG</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>−246 to −122</td>
<td>Forward: GTCTATCCACATCTCGGCCGA</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: CTCGCTGAGGTGATCTGCG</td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>−13 to +72</td>
<td>Forward: CCCCGAGCTTCCAAAAAGCAG</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: CTGCCGCTACCCTAGAAGGA</td>
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tal groups was analyzed using one-way ANOVA. A \( P \) value < 0.05 was considered as statistically significant.

RESULTS

The accumulation of Mlh1 in the mitochondria was significantly lower, and the overlap of Mlh1 and CoxIV intensity peaks was also compromised in the cells exposed to high glucose when compared to the cells in normal glucose. The accompanying figure shows lower Pearson's correlation in the cells exposed to high glucose when compared with the cells in normal glucose, further confirming decreased Mlh1-CoxIV interactions in high glucose. However, the use of 20 mM L-glucose, instead of 20 mM D-glucose, had no effect on Mlh1 accumulation in the mitochondria (Fig. 1a). Glucose-induced decrease in mitochondrial localization of Mlh1 was further confirmed by western-blotting Mlh1 in the isolated mitochondria, and as shown in Figure 1b, compared to cells in normal glucose, the mitochondria isolated from the cells in high glucose had significantly lower Mlh1 expression.

DNA methylation suppresses gene expression, and in diabetes the machinery responsible for maintaining DNA methylation status is activated.\(^{17,33}\) Because \( Mlb1 \) has a CpG-rich promoter,\(^{21}\) to investigate the role of epigenetics in \( Mlb1 \) suppression, 5mC levels were quantified at its promoter. Figure 2a shows about a threefold increase in 5mC levels at the \( Mlb1 \) promoter in the cells exposed to high glucose when compared with the cells in normal glucose. This increase in 5mC was accompanied by a fivefold increase in the binding of Dnmt1, the only member of the Dnmt family elevated in the retinal capillaries in hyperglycemic milieu\(^{17}\) (Fig. 2b). In the same cell preparations, the binding of the transcriptional factor Sp1 and \( Mlb1 \) gene transcripts were decreased by more than 50% (Figs. 2c, 2d). The incubation of cells in 20 mM L-glucose, instead of D-glucose, had no effect on either 5mC or the binding of Dnmt1 or Sp1 at the \( Mlb1 \) promoter.

To confirm the role of DNA hypermethylation in the decreased expression of \( Mlb1 \) in hyperglycemic milieu, the cells manipulated for Dnmt function were analyzed. The inhibition of Dnmts by Aza attenuated a glucose-induced
In the cells, but not scrambled RNA transfected cells, were also
Differential distribution of 5mC in these four CpG-rich regions
significantly different from those in normal glucose (Fig. 5a).
5mC levels in high glucose, but the values were not
with the cells in normal glucose, and R1 had slightly higher
levels in the cells exposed to high glucose when compared
(Table 1). Regions R3 and R4 had significantly higher 5mC
levels at the
promoter by methylated DNA immunoprecipitation method, (b, c) Dnmt1 and Sp1 binding by ChIP technique,
and (d) Mlh1 gene transcripts by qPCR. Each measurement was made in duplicate in three to four samples per group. The values obtained from
cells in NG are considered as 1. NG and HG = 5 mM and 20 mM glucose, respectively; D-si and SC = cells transfected with Dnmt1-siRNA and control
negative RNA, respectively, and incubated in HG; Aza = 1 μM Aza-2'-deoxyxytidine. *P < 0.05 versus NG and #P < 0.05 versus HG.

In the same cell preparations, the inhibition of Dnmts by
either Dnmt1-siRNA or by Aza attenuated an increase in base
mismatches in the D-loop region experienced by the cells in
high-glucose conditions, further supporting the role DNA
methylation in Mlh1 transcriptional regulation (Fig. 3).

To further investigate the role of DNA methylation, the 5' region of the human Mlh1 promoter (from −2077 to −617) was
subcloned into a luciferase reporter plasmid, pGL3-basic vector. Transient transfection of HRECs with this luciferase
reporter plasmid had a strong promoter activity in pGL-
vector. Transient transfection of HRECs with this luciferase
subcloned into a luciferase reporter plasmid, pGL3-basic

The Mlh1 promoter is a CpG-rich promoter, and to identify the
methylated sites, four regions (R) of its proximal promoter
(−659 to 72; R1–R4) with 8 to 16 CpG counts were analyzed
(Table 1). Regions R3 and R4 had significantly higher 5mC
levels in the cells exposed to high glucose when compared
with the cells in normal glucose, and R1 had slightly higher
5mC levels in high glucose, but the values were not
significantly different from those in normal glucose (Fig. 5a).

Differential distribution of 5mC in these four CpG-rich regions
was further confirmed by quantifying the binding of Dnmt1; as
shown in Figure 5b, in high-glucose conditions, R3 had a more
than fourfold increase in Dnmt1 binding compared to about
threefold in R4 and less than twofold in R1, further confirming
the importance of the R3 region. In the same samples, the
values obtained from the control IgG were almost negligible.

Regulation of Dnmt1 by siRNA or by Aza in a high-glucose
medium, in addition to preventing a decrease in Mlh1 gene
transcripts, also attenuated an increase in 5mC and Dnmt1
binding at the Mlh1 promoter and prevented a decrease in Sp1
binding, further confirming the role of DNA methylation in the
transcriptional regulation of Mlh1 (Fig. 2).

Consistent with the in vitro model, Mlh1 gene transcripts
were significantly decreased in retinal microvessels from
diabetic mice when compared with normal mice (Fig. 6a).
Furthermore, diabetic mice had a twofold increase in 5mC
levels at the Mlh1 promoter (Fig. 6b). To further confirm the
role of DNA methylation in Mlh1 transcriptional regulation,
retinas from mice receiving intraperitoneal administration of
Aza or intravitreal administration of Dnmt1-siRNA were
analyzed. The inhibition of Dnmt by either its siRNA or by its
pharmacologic inhibitor significantly attenuated diabetes-in-
duced alterations in the gene transcripts of Mlh1 and protected
the hypermethylation of its promoter. Compared to Dnmt1-
siRNA, Aza administration had a better effect in ameliorating
diabetes-induced alterations in the gene transcripts of Mlh1 and protected
the hypermethylation of its promoter. Compared with the experimental models, MLH1 gene expression and its promoter DNA
methylation were determined in the retinal microvessels from
human donors with diabetic retinopathy. Consistent with our
results from the experimental models, MLH1 expression was decreased by ~50% and that of DNMT1 was increased by
threefold in the retinal microvessels from donors with diabetic retinopathy when compared with age-matched nondiabetic donors (Fig. 7a). As seen in our in vitro model, the methylation analysis of MLH1 promoter also showed differential DNA methylation in R1 to R4; although R1 and R2 had similar 5mC levels in diabetic retinopathy and nondiabetic donors, the 5mC levels were more than sixfold higher in R3 and about fourfold higher in the R4 regions in diabetic retinopathy donors when compared with nondiabetic donors. Similarly, in R3 and R4 regions, Dnmt1 binding was also increased by about sevenfold and threefold, respectively. However, although R1 had similar 5mC levels in diabetic retinopathy and nondiabetic donors, Dnmt1 binding was threefold higher in diabetic retinopathy donors. However, normal rabbit IgG values obtained from the same samples were <1% compared to those obtained from antibodies against 5mC or Dnmt1 (Figs. 7b, 7c).

DISCUSSION
Recent studies have shown that mitochondrial dysfunction plays a major role in the development of diabetic retinopathy. Mitochondria are the only other subcellular structures with their own DNA; each cell has multiple mitochondria, and each mitochondria has several copies of DNA. The mutation and substitution rate of mtDNA is significantly higher than that of nuclear genes, but each cell can also have a population of distinct mtDNA genomes; mismatches in mtDNA, without apparent functional consequences, are routinely observed in normal subjects. The MMR system corrects the postreplication errors and maintains genetic stability, and an efficient MMR system also recognizes sequence variants in mtDNA and cuts them. Our previous study has shown that in diabetic retinopathy, the number of sequence variants is increased in the mtDNA, and the expression of Mlh1 is also decreased. Furthermore, mtDNA is hypermethylated, and the inhibition of Dnmts also ameliorates sequence variants in the mtDNA. Here we show that the mitochondrial accumulation of Mlh1, which helps fix the mismatches made during DNA replication, is decreased in diabetes. The promoter is hypermethylated, and the activity of

FIGURE 3. Regulation of DNA methylation and base mismatches. DNA from HRECs was amplified using semi-qPCR for the D-loop and digested with mismatch-specific surveyor endonuclease. The samples were analyzed on a 2% agarose gel. The parent amplicon band intensity was quantified by densitometry, and the values obtained from cells in NG was considered as 100%. Data are represented as mean ± SD, and each measurement was made in duplicate in three to five samples in each group. Mismatches and the parent amplicon band intensity from (a) Dnmt1-siRNA transfected HRECs and (b) Aza-treated HRECs. NG and HG = HRECs in 5 mM and 20 mM glucose, respectively; Dsi and SC = cells transfected with Dnmt1-siRNA and negative control RNA, respectively; Aza = 1 μM 5-Aza-2'-deoxyxytidine. *P < 0.05 compared with NG; #P < 0.05 compared with HG.

FIGURE 4. Effect of inhibition of Dnmts on Mlh1 promoter activity. Mlh1 promoter was amplified and cloned into the promoter less pGL3-Basic-IREs plasmid, and deletion promoter fragments generated were used for the transfection of HRECs. The promoter activity was determined by a Pierce Firefly Luciferase Glow Assay Kit, and intensity was measured at 613 nm. pGL3-control vectors transfected cells were used as control. The values are represented as mean ± SD as three or more assays, each performed in duplicate. NG and HG = HRECs in 5 mM and 20 mM glucose, respectively; Aza = 1 μM 5-Aza-2'-deoxyxytidine; L-Glu = 20 mM L-Glucose. *P < 0.05 compared to NG and #P < 0.05 compared to HG.
Mitochondria are the major source of ROS production, and ROS leakage during oxidative phosphorylation can react with cellular components including DNA. The mutation rate of mtDNA is 10-fold to 50-fold higher than that of nuclear DNA. Although both the mutant and wildtype copies can coexist in mtDNA, an increase in heteroplasmy decreases the energy production. Our previous work has shown that although both Mlh1 and Msh2 are decreased in the retina in diabetes, the overexpression of Mlh1 ameliorates a diabetes-induced increase in mtDNA mismatches, but that of Msh2 fails to provide any benefit. To recognize and correct the biosynthetic errors formed during DNA replication or the mispaired bases generated in DNA recombination, the localization of MMR enzymes in the mitochondria is essential. The results presented here clearly demonstrate that the localization of Mlh1 in the mitochondria is also decreased in hyperglycemic milieu, further supporting its role in mtDNA mismatch repair.

Diabetes activates DNA methylation machinery in the retina and its vasculature, and along with the mtDNA, DNA methylation status of many genes associated with mitochondrial homeostasis is also altered. Our previous work has shown a positive correlation between mtDNA methylation and an increase in base mismatches. The methylation of CpG in the promoter of a gene is generally associated with impaired transcriptional activity. This is accompanied by decreased binding of the transcriptional factor Sp1. Furthermore, pharmacological or molecular inhibition of Dnmts reduces a glucose-induced decrease in Sp1 binding, and maintains its promoter activity. This is accompanied by decreased binding of the transcriptional factor Sp1.

Mlh1 promoter methylation also shows differential distribution of 5mC in four regions of the CpG-rich proximal promoter region (−659 to 72). Among the four regions, R3, a short region of ~100 bp just upstream of the start site, has the highest levels of 5mC and increased Dnmt1 binding, suggesting an irregular methylation pattern in the Mlh1 promoter region.
result of the conversion of cytosine to thymine, and the deamination rate of 5mC is considerably higher than that of cytosine or guanosine to adenine. A lack of protective histones in mtDNA, and its close proximity to the ROS-producing electron transport chain, also make mtDNA prone to mutations. Hypermethylation of mtDNA in diabetes further makes it more susceptible to mutation. A decreased translocation of Mlh1, the main enzyme responsible for fixing up the mismatches, inside the mitochondria in diabetes and the hypermethylation of its promoter further adds insult to the already damaged mtDNA, compromising mitochondrial homeostasis. Thus, strategies to regulate of DNA methylation (or its deamination) have the potential to protect mitochondrial homeostasis by preventing base mismatches and impeding the development of diabetic retinopathy.

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**References**


