Mitochondrial Superoxide Anion Overproduction in Tet-mev-1 Transgenic Mice Accelerates Age-Dependent Corneal Cell Dysfunctions

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PURPOSE. The Tet-mev-1 mouse expressing a mitochondrial complex-II mutated SDHC\textsuperscript{V69E} gene controlled by a tetracycline (Tet)-On/Off system can overproduce O$_2^-$ and is a versatile whole-animal model for studying mitochondrial oxidative stress. Here we report a series of age-dependent variations in corneal epithelium, endothelium, and parenchymal cells of the Tet-mev-1 mice relative to wild-type C57BL/6j mice.

METHODS. Measurements of (1) mitochondrial electron transport enzyme activities; (2) O$_2^-$ production; (3) carbonylated protein, and 8-hydroxydeoxyguanosine (8-OHdG) levels as markers of oxidative stress; (4) pathologic analyses under optical and electron microscopy; (5) hematoxylin-eosin or toluidine-blue staining; and (6) immunohistochemistry with an anti-\beta-catenin antibody were performed in the eye, especially the cornea.

RESULTS. Complex II-III activity was decreased by electron leakage between complex II and CoQ. This resulted in increased age-dependent intracellular oxidative stress in the eye of Tet-mev-1 mice. Corneal epithelialization was delayed in Tet-mev-1 mice after 20% ethanol treatment, as the number of cells and mitotic cells decreased in the corneal epithelium of Tet-mev-1 mice compared with that of wild type. The age-dependent decrease in cell number accelerated in the corneal endothelium cells. Moreover, it was suggested that the corneal thickness was decreased by thinning of parenchymal cells with age in Tet-mev-1 mice.

CONCLUSIONS. These results suggest that mitochondrial oxidative stress with electron transport chain dysfunction can influence pathogenesis and progression of age-related corneal diseases, as well as generalized corneal aging acceleration. (Invest Ophthal Vis Sci. 2012;53:5780–5787) DOI:10.1167/iows.12-9573

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Supported by the grant-in-aid for Scientific Research Young Scientists (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Tokai University School of Medicine Research Aid.

Submitted for publication January 25, 2012; revised May 30, 2012; accepted July 1, 2012.

Disclosure: H. Onouchi, None; T. Ishii, None; M. Miyazawa, None; Y. Uchino, None; K. Yasuda, None; P.S. Hartman, None; K. Kawai, None; K. Tsubota, None; N. Ishii, None

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It has recently become apparent that oxidative stress can contribute to eye disease, including corneal disease such as dry eye\textsuperscript{1–3} and retinal disease such as age-related macular degeneration.\textsuperscript{4} Most intracellular oxidative stress originates in the form of superoxide anion (O$_2^-$) generated from the mitochondrial electron transport system.\textsuperscript{5} The nematode mev-1 mutant of Caenorhabditis elegans has a mutation at the 71st position changing glycine to glutamate (G71E) in the mitochondrial complex-II cytochrome b large (SDHC) subunit\textsuperscript{6} and produces excessive O$_2^-$ from mitochondria.\textsuperscript{7} The mev-1 mutant has proven extremely useful for the study of endogenous oxidative stress and its effects on aging, apoptosis, and mutagenesis.\textsuperscript{1,8–10} We have constructed a transgenic mouse cell line (SDHC E69 cells) with a mutation at the 69th position from valine to glutamate (V69E) that mimics C. elegans mev-1 mutant.\textsuperscript{11} As expected, complex-II enzyme activity was diminished, and mitochondrial O$_2^-$ were elevated, which resulted in intracellular oxidative damage. The resultant oxidative stress induced excessive apoptosis and frequently transformed the cells that escaped apoptosis.\textsuperscript{11} We have also constructed a transgenic fly that has a mev-1-like dominant negative SdhC resulting in increasing oxidative stress and reduced lifespan.\textsuperscript{12}

We then created a modified tetracycline (Tet)-On/Off system that enables the transgene to be expressed at lower levels such that they more reasonably approximate endogenous expression.\textsuperscript{13} A Tet-mev-1 conditional transgenic mouse with a point mutation (V69E) in mitochondrial complex-II SDHC subunit was then constructed. The Tet-mev-1 mouse can overproduce O$_2^-$ from mitochondria, and the amount of O$_2^-$ production from electron transport ratio or leakage can be experimentally controlled by modulating doxycycline dosage.\textsuperscript{13} Tet-mev-1 mouse holds the promise of serving as a suitable model to clarify the influence of excessive mitochondrial O$_2^-$ on eye diseases, including corneal diseases such as dry eye and keratoconus, and retinal disease such as age-related macular degeneration. Given this, we focused on the eye in Tet-mev-1 mouse to explore the relationship between intracellular oxidative stress by excessive mitochondrial O$_2^-$ production and eye diseases, especially in the cornea. In this study, eye disease phenomena, especially corneal disease phenotypes, were analyzed at 3 months and greater.

MATERIALS AND METHODS

Mice

The Tet-mev-1 transgenic mice, derived in a C57BL/6 background, were generated as previously described.\textsuperscript{13} Genotyping was performed by PCR and Southern blot analysis.\textsuperscript{13} All animal experiments were performed in compliance with the ARVO Statement for the Use of Investigative Ophthalmology & Visual Science, August 2012, Vol. 53, No. 9 Copyright 2012 The Association for Research in Vision and Ophthalmology, Inc.
Animals in Ophthalmic and Vision Research. The mice were continuously exposed to doxycycline (Dox; 2 mg/mL with 0.2% saccharine in drinking water; Sigma-Aldrich Japan, Tokyo, Japan) throughout both prenatal and postnatal development.\textsuperscript{13}

**Western Blot Analysis**

Proteins extracted from the eye tissues of 3-month-old Jet-meer-1 and wild-type C57BL/6j mice (the combined sample of two eyes from a mouse was used as one sample) were examined by Western blots using rabbit antibodies to SDHC and glyceraldehyde 3-phosphate dehydrogenase (GAPDH).\textsuperscript{14} The mouse SDHC 23-59 peptide antibody was made by MBL company (Nagoya, Japan; 1:3000 dilution). Anti-GAPDH antibody was purchased from Cell Signaling Technology Inc. (Tokyo, Japan).\textsuperscript{15}


For protein isolation from the mitochondrial fraction, eye tissues (the combined sample of four eyes from two mice was used as one sample) were homogenized (10% wt/vol) in isolation buffer (210 mM mannitol, 70 mM sucrose, 0.1 mM EDTA, and 5 mM Tris-HCl [pH 7.4]) with a Teflon homogenizer. Mitochondria were isolated by differential centrifugation and suspended in Tris-EDTA buffer (0.1 mM Tris-EDTA [pH 7.4]). The solubilized mitochondrial proteins were used for the measurements of complex I–II and II–III activities. The activities of NADH-cytochrome c oxidoreductase (complex I–III) and succinate-cytochrome c oxidoreductase (complex II–III) were measured as previously described.\textsuperscript{15} Complex I–III activity was assayed by measuring NADH-sensitive NADH-cytochrome c reductase activity at 37°C in 200 μL 0.1 M Tris-SO\textsubscript{4} buffer (pH 7.4), containing 0.32 mg cytochrome c, and 1 mM sodium cyanate. Complex II–III activity was assayed by measuring malonate-sensitive succinate-cytochrome c reductase activity. The reference cuvette contained 20 μL of 20% sodium malonate solution.\textsuperscript{13}

**Measurement of Superoxide Anion (O$_{2}^{−}$) Accumulation in Mitochondria**

The mouse eye tissue's intact mitochondria extract (the combined sample of two eyes from a mouse was used as one sample) was prepared by the same protocol as used to measure Complex I–III and II–III activities. O$_{2}^{−}$ was measured using the chemiluminescent probe 2-methyl-16-p-methoxyphenylimidazopyrazinone (ATTO). Mitochondria were homogenized (10% wt/vol) in isolation buffer (210 mM mannitol, 70 mM sucrose, 0.1 mM EDTA, and 5 mM Tris-HCl [pH 7.4]) with a Teflon homogenizer. Mitochondria were isolated by differential centrifugation and suspended in Tris-EDTA buffer (0.1 mM Tris-EDTA [pH 7.4]). The solubilized mitochondrial proteins were used for the measurements of complex I–II and II–III activities. The activities of NADH-cytochrome c oxidoreductase (complex I–III) and succinate-cytochrome c oxidoreductase (complex II–III) were measured as previously described.\textsuperscript{15} Complex I–III activity was assayed by measuring NADH-sensitive NADH-cytochrome c reductase activity at 37°C in 200 μL 0.1 M Tris-SO\textsubscript{4} buffer (pH 7.4), containing 0.32 mg cytochrome c, and 1 mM sodium cyanate. Complex II–III activity was assayed by measuring malonate-sensitive succinate-cytochrome c reductase activity. The reference cuvette contained 20 μL of 20% sodium malonate solution.\textsuperscript{13}

**Measurement of Carbonylated Protein Contents**

Proteins from the mouse eye tissue's intracellular membrane's fraction (the combined sample of two eyes from a mouse was used as one sample) were isolated by differential centrifugation and suspended in Tris-EDTA buffer (50 mM Tris-EDTA [pH 7.4] and 5 mM EDTA). Carbonylated protein measurements were performed by 2, 4-dinitrophenylhydrazine (DNPH) antibodies as described.\textsuperscript{13} The concentration of intracellular membrane-fraction proteins, which were treated with DNPH as described,\textsuperscript{16} were determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL). The plates were treated with anti-DNPH antibody in PBS containing 0.1% BSA, 0.1% NaN\textsubscript{3}, 1 mM MgCl\textsubscript{2}, anti-rabbit IgG antibody in PBS containing 0.05% Tween-20, and then the o-phenylenediamine (OPD)H$_{2}$O\textsubscript{2} solution (OPD; Sigma-Aldrich Japan). Finally, 1 M H$_{2}$SO\textsubscript{4} was added in the plates after a 15-minute incubation at room temperature, and the peroxidase activity in the plates was measured at 492 nm on SpectraMax 250 (Molecular Devices Co., Sunnyvale, CA).

**Immunohistochemistry**

The enucleated mouse eyes were immediately in Tissue-Tek optimal cutting temperature (OCT) compound (Sakura Finetek, Tokyo, Japan) and frozen in dry ice–ethanol for immunohistochemical or hematoxylin and eosin staining. For fluorescent labeling, frozen sections (8 μm) were fixed in ice-cold acetone, blocked with goat serum, and stained with mouse antibody to 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Nikken SEIL, Shizuoka, Japan), pre-conjugated with anti-mouse Ig Alexa Fluor 488 (Molecular Probes, Eugene, OR). For the immunostaining of 8-OHdG, images with positive reactions were merged with images from a laser microscope (LSM META510; Zeiss, Oberkochen, Germany).

**Corneal Epithelial Surgery**

The animals were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg). One filter paper (0.9 mm diameter) soaked with 20% ethanol was placed on the central cornea for 1 minute before performing a circular injury (1.5 mm diameter) in the center of the cornea.\textsuperscript{17} The injury was performed with a 27-gauge needle and a spatula under a dissection microscope as described by Escario et al.\textsuperscript{17} The cornea was stained with 1% sodium fluorescein as described by Turpie et al. and photographed using hand slit-lamp microscopy (Kowa, Tokyo, Japan) at the same time every day.\textsuperscript{18}

**Electron Microscopy**

For transmission electron microscopy (TEM), tissues were fixed in 2.5% glutaraldehyde and then postfixed in 1% OsO\textsubscript{4}. The fixed tissues were dehydrated and embedded in Quetol 812 and sectioned with a diamond knife. Quetol 812 sections (2 μm) were counterstained with toluidine blue and examined with a light microscope. Ultrathin sections (2 μm) were stained in 2% uranyl acetate and lead citrate and examined with an electron microscope (JEM-1200EX; JEOL, Tokyo, Japan).

**Flat-Mount Preparations and Digital Images**

The enucleated eyes were sectioned at the equator, and the anterior half, including the lens and vitreous, was discarded. The retinas were carefully peeled from the eyeball and optic nerve by using specialized scissors and forceps under a biomicroscope (SPZ-50FTM; Carton Optical Industries, Ltd., Burlingame, CA). Briefly, flat mounts were fixed by cold methanol for 30 minutes. After that, the flat mounts were stained with mouse monoclonal antibody to β-catenin (BD Japan, Tokyo, Japan) and were stained by avidin-biotinylated enzyme complex. Flat mounts were examined with a fluorescence microscope (BZ-9000; KEYENCE Japan, Osaka, Japan). Fluorescein images of β-catenin were analyzed after normalization to the background, and the degree of fluorescence of the cytoplasm of each cell was calculated from average pixel intensities by using software that accompanied the microscope.

**The Number of Corneal Endothelial Cells**

The flat mounts of corneal endothelial cells were photographed by fluorescence microscope as described above, on multiple areas, mainly the center. The number of corneal endothelial cells was counted in the
The counts were indicated as mean-value analyses.

Catalase Enzyme Activity Assay

Corneas were pulverized to a fine powder in liquid nitrogen and were added to extraction buffer (0.1 M potassium phosphate [pH 7.7] and 0.2% Triton X-100). Samples were centrifuged at 14,000 g at 4°C for 15 minutes. Protein concentrations were measured by a BCA protein assay kit (Pierce). Catalase enzyme activity was measured according to the manufacturer’s protocol (Amplex Red Catalase Kit; Molecular Probes). At the end of incubation, fluorescence intensity was measured with a laser-based scanning system (BIO-TEK FL600; BioTek Instruments, Inc., Winooski, VT) with excitation at 530 nm and emission at 590 nm.

Statistical Analyses

All data are presented as means ± SD, and the differences were analyzed with Student’s t test. P values < 0.05 were considered statistically significant.

RESULTS

SDHC Protein Levels and Complex I–III and Complex II–III Activities in the Eye

The protein levels of SDHC, one of the mitochondrial electron transport chain complex-II subunits, were examined in eyes of 3-month-old, wild-type C57BL/6j mice and Tet-mev-1 mice continuously given Dox. The Tet-mev-1 mice had significantly increased SDHC protein levels with the mev-1–type mutated SDHC (SDHC^{V69E}) expressed by the transgene in their eyes compared with those of wild-type C57BL/6j mice (Fig. 1A). This confirmed that the Tet-On/Off system functioned in eyes.

The activities of complex I–III were experimentally identical in the eyes of wild-type C57BL/6j and Tet-mev-1 mice. In contrast, complex II–III activity influenced by SDHC^{V69E} in Tet-mev-1 mice was reduced by almost 40% compared with wild-type mice (Fig. 1B).

Figure 1. Biological and biochemical functional changes caused by the SDHC^{V69E} mutation in the eye. (A) Quantitative alterations of SDHC/GAPDH protein levels measured by Western blot analysis. The experiment was performed using 3-month-old wild-type C57BL/6j and Tet-mev-1 mice. Results are expressed as mean ± SD; n = 4 in each group; *P < 0.01 versus wild-type C57BL/6j mice. (B) The activities of complex I–III and complex II–III in the eye. The experiment was conducted on 12-month-old wild-type C57BL/6j and Tet-mev-1 mice. Results are expressed as mean ± SD; n = 4 in each group; *P = 0.01 versus wild-type C57BL/6j mice.

Figure 2. Accumulation of intracellular oxidative damage in the eye. (A) The accumulation of superoxide anion (O_2^-) in mitochondria in the eye in 3-month-old (hatched boxes) and 12-month-old (black boxes) wild-type C57BL/6j and Tet-mev-1 mice. Results are expressed as mean ± SD; n = 6 in each group; *P < 0.01 versus wild-type C57BL/6j mice. (B) The accumulation of carbonylated proteins in the eye of 6- to 12-month-old wild-type C57BL/6j and Tet-mev-1 mice. Results are expressed as mean ± SD; n = 6 in each group; *P = 0.05 versus wild-type C57BL/6j mice. (C) Immunohistochemistry of 8-OHdG in the corneal epithelium. The experiment was conducted using 3- and 6-month-old wild-type C57BL/6j and Tet-mev-1 mice. Red: nucleus. Green: 8-OHdG.
Oxidative Damages in Eye

The measurements of oxidative damage in the cells of the eye were performed to investigate if oxidative stress and damage were also present in these tissues. It has been reported that superoxide anion \( \text{O}_2^- \) is generated in mitochondria because of the inappropriate single-electron reduction of diatomic oxygen. The eyes in 3-month-old Tet-mev-1 mice had significantly increased \( \text{O}_2^- \) levels relative to the wild-type C57BL/6j mice at the same age (Fig. 2A). At 12 months of age, \( \text{O}_2^- \) levels in the wild-type C57BL/6j mice had increased, while levels in Tet-mev-1 mice were even higher (Fig. 2A). These results support the notion that electron transport dysfunction increased the mitochondrial \( \text{O}_2^- \) levels in the eyes of Tet-mev-1 mice with the SDHC\textsuperscript{V69E} mutation, when induced by Dox, relative to wild-type C57BL/6j mice. Moreover, this finding was age-dependent.

The accumulation of carbonylated proteins, a marker of oxidized proteins, was tested in the eye. The accumulation of carbonylated proteins was increased in Tet-mev-1 mice (Fig. 2B). At 12 months of age, \text{O}_2^- levels in the wild-type C57BL/6j mice had increased, while levels in Tet-mev-1 mice were even higher (Fig. 2A). These results support the notion that electron transport dysfunction increased the mitochondrial \( \text{O}_2^- \) levels in the eyes of Tet-mev-1 mice with the SDHC\textsuperscript{V69E} mutation, when induced by Dox, relative to wild-type C57BL/6j mice. Moreover, this finding was age dependent.

The degree of corneal epithelialization after exposure to 20% ethanol was also tested. The rate of re-epithelialization in 3-month-old Tet-mev-1 mice was significantly slower than in age-matched wild-type C57BL/6j mice (Fig. 4A). In 12-month-old mice, the re-epithelialization process was significantly slower in both mice groups and was also delayed in the Tet-mev-1 mice relative to wild-type C57BL/6j mice (Fig. 4A). These results suggest that the ability to reform epithelium declines as animals age and this ability is compromised by the oxidative stress inherent in Tet-mev-1 mice. Moreover, the corneal fluorescein staining, indicating keratitis, was observed in Tet-mev-1 mouse after epithelialization (Fig. 4B).

Figure 4C shows the electron microscope images of basal epithelial cells in the cornea. Abnormalities in the nucleus and membranes were observed in basal epithelial cells of 12-month-old Tet-mev-1 mice but not wild-type mice. This was anticipated, given the increased oxidative stress in Tet-mev-1 mice. Collectively, these results suggest that the oxidative stress from mitochondria accelerates aging in the epithelium.
Tet-mev-1

6 in the group of 3- and 12-month-old mice and wild-type mice at 3 months of age. Conversely, 12-month-old mice treated with 20% doxycycline showed the abnormal nuclear membrane structures. This led to increased superoxide anion (O2·−) production, which is likely caused by the chronic oxidative stress inherent in Tet-mev-1 mice.

**Corneal Endothelium**

Figure 5A shows electron micrographs of corneal endothelial cells and Descemet’s membrane. At 12 months of age, Descemet’s membrane in Tet-mev-1 mice was thick relative to wild-type C57BL/6j mice.

Collectively, these results suggest that the mitochondrial oxidative stress led to decreases in the number of endothelial cells of Tet-mev-1 mice, thus accelerating the normal course of events. Of interest, the electron microscopy images suggest that the increase in thickness of Descemet’s membrane could be caused by the chronic oxidative stress inherent in Tet-mev-1 mice.

**DISCUSSION**

Mitochondria play an indispensable role in the generation of adenosine triphosphate (ATP) in aerobic eukaryotes. Almost paradoxically, mitochondria are also the primary source of intracellular oxidative stress, owing to endogenously generated reactive oxygen species (ROS) that result from the combination with free electrons with molecular oxygen. Once generated, ROS can readily attack a wide variety of cellular entities, resulting in cellular, tissue, and organ damage that ultimately compromises organismal viability.21–24 Recent research has focused on the influence of ROS on eyes diseases1,4,25; however, a specific role and the molecular mechanisms by which ROS effect ocular dysfunctions (e.g., dry eyes, glaucoma, age-related macular degeneration) remain elusive.

In this report, we investigated corneal dysfunctions in Tet-mev-1 mice with a Tet-On/Off system using doxycycline to induce gene expression of a mutant mitochondrial complex-II SDHC gene expression. The Tet-mev-1 mouse has a mitochondrial electron transport chain dysfunction that results from a complex-II SDHC

**Corneal Stroma**

Figure 6A shows micrographs of corneal cross-sections of 6-, 8-, 10-, and 33-month-old wild-type C57BL/6j and Tet-mev-1 mice. Corneal thickness was significantly reduced in the 10-month-old Tet-mev-1 mice relative to the wild-type C57BL/6j mice, especially in the corneal stroma (Fig. 6A, 6B). These data suggest that oxidative stress from mitochondria might cause corneal thinning with decreasing number of corneal stromal cells.

**Figure 4.** Corneal epithelialization and epithelial basal cells. (A) Corneal epithelialization in 3-month-old (left chart) and 12-month-old (right chart) C57BL/6j and Tet-mev-1 mice. Results show the recovered days in corneal epithelialization; n = 5 in 3-month-old and n = 4 in 12-month-old wild-type C57BL/6j mice; n = 6 in the group of 3- and 12-month-old Tet-mev-1 mice; *P = 0.032, **P = 0.067 versus wild-type C57BL/6j mice. (B) Images of corneal re-epithelialization state in 3-month-old wild-type C57BL/6j and Tet-mev-1 mice treated with 20% ethanol exposure. (C) Electron micrographs of epithelial basal cells in cornea of 3- and 12-month-old wild-type C57BL/6j and Tet-mev-1 mice. Red arrows show the abnormal nuclear membrane structures.

Anterius corneae, which can lead to delayed corneal epithelialization.

In the eye of Tet-mev-1 mouse model, complex II–III activity was decreased when the mutant SDHC was induced by doxycycline treatment. As a consequence, mitochondrial superoxide anion (O2·−) production, which is likely caused by the electron leakage between complex II and ubiquinone, was increased. This led to increased levels of carbonylated protein and 8-OHdG nucleotide levels in the eye as Tet-mev-1 mice grew older.

In the corneal epithelium of Tet-mev-1 mice, the proliferation of epithelial basal cells was decreased, resulting in delayed epithelialization relative to wild-type C57BL/6j mice, particularly as animals aged (Figs. 3, 4). It has recently been reported that keratitis and delayed epithelialization might be caused by hyperglycemia and high oxidative stress conditions.26 We anticipate that the molecular mechanisms of keratitis and delayed epithelialization with hyperglycemia and oxidative stress...
will be clarified by future analyses of Tet-mev-1 mice. Moreover, it has been reported that hydrogen and N-acetyl-L-cysteine prevent oxidative stress–induced angiogenesis in a mouse corneal alkali-burn model.\textsuperscript{27} We believe that Tet-mev-1 mouse analyses can help clarify more details of these pathogeneses.

In the corneal endothelium of Tet-mev-1 mice, it was confirmed that the age-dependent decrease in cell number was accelerated compared with wild-type C57BL/6j mice. Of interest, increase in thickness of Descemet’s membrane was present in Tet-mev-1 mice relative to wild-type C57BL/6j mice. In general, Descemet’s membrane increases with age. The thickened Descemet’s membrane of Tet-mev-1 mice might be caused by chronic mitochondrial oxidative stress. Recently, the relationship between Fuchs’ corneal dystrophy (FCD) and oxidative stress was clarified by Jurkunas and colleagues.\textsuperscript{28} Both decrease of corneal endothelial cells and increase in thickness of Descemet’s membrane observed in FCD are consistent with our data in Tet-mev-1 mice. Therefore, we anticipate that the molecular mechanisms of FCD pathogenesis with oxidative stress might be clarified by analyses of Tet-mev-1 mice in the future.

Finally, thinning of the cornea with age in Tet-mev-1 mice was precociously caused compared with wild-type C57BL/6j mice. We also observed increasing catalase activity in Tet-mev-1 mice.

\textbf{Figure 5.} Corneal endothelial phenotypes. (A) Immunohistochemistry of corneal endothelial whole-mount preparations visualizing β-catenin in 3-, 12-, and 24-month-old wild-type C57BL/6j and Tet-mev-1 mice. (B) Number of corneal endothelial cells in wild-type C57BL/6j and Tet-mev-1 mice. The number of corneal endothelial cells was counted in photo area (16.3 mm\textsuperscript{2}); n = 5 in each group. (C) Electron micrographs of corneal endothelial cells and Descemet’s membrane in 3- and 12-month-old wild-type C57BL/6j and Tet-mev-1 mice. Red scale bars show the Descemet’s membrane thickness. (D) Descemet’s membrane. (E) Corneal endothelial cells.
mice as already reported in human keratoconus (data not shown). These results suggest that the pathogenesis of corneal thinning was due to the decreasing number of corneal stromal cells by excessive mitochondrial oxidative stress with increasing catalase activity.

In conclusion, SDHC V69E mutation causes an electron leakage between complex II and ubiquinone in the electron transport chain. This results in mitochondrial superoxide anion overproduction in Tet-mev-1 mice. We have previously reported that the mitochondrial oxidative stress can induce apoptosis induction through the cytochrome c release and cell cycle arrest with p21 activation in the cell. These cellular functional changes might cause the corneal cell dysfunctions, resulting in accelerated decreasing endothelial cells with age, thinning of parenchymal cells, and delayed corneal epithelialization. Moreover, it is expected that the oxidative stress could also cause the organic dysfunctions leading to chronic inflammation and hyperglycemia in individuals, which might result in not only thickened Descemet’s membrane with increasing collagen IV in the cornea but also retinal dysfunctions such as age-related macular degeneration and glaucoma. In the future, we expect that more details in these pathogeneses with oxidative stress may be clarified by analyses of Tet-mev-1 mice.

Acknowledgments

The authors thank Akira Akatsuka, Education and Research Support Center, Tokai University School of Medicine, for obtaining the corneal electron microscopy images.

References


