INTRODUCTION

Recent progress in medical science has provided new therapeutic approaches in various fields, however, challenges in the field of neurodegeneration remain unsolved. Disorders of the neural retina reduce the quality of vision, impair the quality of life, and create social and healthcare issues.1,2 One of the risk factors that promote retinal neurodegeneration in...
age-related macular degeneration is excessive light stimulation.3 Treatments that alleviate this detrimental influence may help to improve the prognosis of the affected patients. However, the underlying mechanisms are not fully understood.

Previous reports have shown that excessive light exposure causes accumulation of oxidative stress, which induces endoplasmic reticulum stress4,5 and DNA damage6 in the retina. The oxidative stress may be caused by dysregulated rhodopsin metabolism during the visual cycle.7,8 When the stress level and associated damage become extensive, the efficiency of the tissue repair systems become impaired, resulting in photoreceptor apoptosis and visual function impairment.9,10 Hence, methods to prevent retinal degeneration via blocking of light with protective glasses,11 and suppressing oxidative stress with antioxidants such as NAC12 and lutein,6 have been proposed. However, these treatments may not fully protect against damage; thus, alternative methods are anticipated.

The retina is a neural tissue that receives light stimuli and converts them into electrical signals by photoreceptors to enable vision.13 This tissue demands a great amount of energy,14-17 and multiple stress response mechanisms, such as unfolded protein response (UPR)4,5 and DNA repair6 which are involved in preserving retinal homeostasis during light stimulation, require energy in the form of adenosine triphosphate (ATP).18-22 Regulation of the energy balance may be a key factor involved in neuroprotection against retinal diseases as energy decompensation may fail to operate these stress response mechanisms. Similar stress response mechanisms are also involved in other neurological diseases such as Alzheimer’s disease.23 Thus, information on the actual ATP status during pathogenesis and the impact of the intervention on disease prognosis may serve to inform the development of a new therapeutic approach against neurodegenerative diseases.

One system involved in the regulation of cellular energy homeostasis is that of adenosine monophosphate (AMP)-activated protein kinase (AMPK).24,25 AMPK monitors changes in ATP levels and increases the rate of ATP-producing pathways in response to an increase in AMP/ADP relative to ATP. Thus, AMPK maintains the energy balance of demand and supply. AMPK is composed of three subunits, α, β, and γ, that respectively act as the protein kinase domain, carbohydrate-binding module, and cystathionine-β-synthase domains, which form the adenosine nucleotide-binding domain. Binding of AMP to the AMPKγ subunit causes an allosteric response in AMPK, and can greatly enhance liver kinase B1 (LKB1)-dependent phosphorylation at Thr172 of the AMPKα subunit, which is the major phosphorylation site of AMPK; as a result, AMPK becomes substantially activated.25-27 Furthermore, only the allosteric response increases AMPK activity to a lesser degree in the order of several-fold changes.24,28 The degree of allosteric activation is influenced by the ATP concentration.29,30

The AMPK activator, 5-aminooimidazole-4-carboxamide riboside (AICAR), is an adenosine analog that is taken up into cells by adenosine transporters and phosphorylated by adenosine kinase, thus generating the AMP-mimetic AICAR monophosphate.29,30 Similarly to cellular AMP, this monophosphate can bind to the AMPKγ subunit, and allosterically activate AMPK without affecting the ADP:ATP ratio or altering the oxygen uptake.13,24

In the current study, we analyzed whether AICAR can modulate energy metabolism during photostress and suppress the light-induced retinal degeneration in a mouse model. The results of this study will facilitate our understanding of the mechanisms underlying the photostress-induced retinal neuro-disorders and provide a proof of concept for new therapeutic approaches to prevent neural deterioration. Further, development of a concept that focuses exclusively on supplementing the energy supply required for tissue repair and compensation to allow for recovery from the condition, rather than regulating stress signals in an attempt to avoid tissue damage, will serve to inform the development of new neuroprotective strategies for therapies in various fields.

2  MATERIALS AND METHODS

2.1  Animals and treatments

Male BALB/c mice (7-8 weeks old; CLEA Japan, Tokyo, Japan) were housed in an air-conditioned room maintained at 22 ± 2°C under a 12-hours dark/light cycle (light on from 8:00 to 20:00) with free access to a standard diet (CLEA Japan) and tap water. Mice were divided into groups and intraperitoneally treated either with the AMPK activator, AICAR (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 125 or 250 mg/kg body weight (BW) or with phosphate-buffered saline as a vehicle immediately before and after dark adaptation preceding the light exposure. Control mice not exposed to light were also treated with the vehicle. All animal experiments complied with the ARRIVE guidelines and were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research, the National Institutes of Health guide for the care, and the guidelines for the Animal Care Committee of Keio University (Approval Number, 08002).

2.2  Light exposure

The light exposure experiments were performed as described previously.5,6,11,12,31-34 Briefly, prior to light exposure, mice were dark-adapted by maintaining them in complete darkness for 12 hours. Their pupils were dilated with a mixture of 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P; Santen Pharmaceutical, Osaka, Japan) just before exposure to light. The mice were then exposed to 3000 lux from a white
fluorescent lamp (FHD100ECW; Panasonic, Osaka, Japan) for 1 hours, starting at 9:00 AM, in a dedicated exposure box maintained at 22 ± 2°C, containing stainless-steel mirrors on all walls and the floor (Tinker N, Kyoto, Japan). Following light exposure, the mice were returned to their cages and maintained under dim cyclic light (5 lux, 12 hours on/off) until they were euthanized at different sampling time points, according to the experimental protocol.

2.3 | Histological analyses

Mouse eyes were enucleated and fixed in 4% paraformaldehyde overnight at 4°C. After fixation, the eyes were embedded in OCT compound (Sakura Finetek Japan, Tokyo, Japan) and frozen, and the cryosections (6- to 8-μm thick), which included the optic nerve head to the most peripheral region of the retina, were analyzed.

The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay was performed using the ApopTag red apoptosis detection kit (Millipore, Bedford, MA, USA) according to the manufacturer’s protocol. The nuclei were counterstained with Cellstain-4',6-diamidino-2-phenylindole (DAPI) solution (2 μg/mL; Dojindo Molecular Technologies, Kumamoto, Japan). In each section, the TUNEL-positive cells and the cells of the outer nuclear layer (ONL) were counted and averaged.

To analyze the photoreceptor contents, sections were stained with rabbit anti-rhodopsin antibody (1:10 000; Cosmo Bio Co., Ltd., Tokyo, Japan) overnight at 4°C, followed by incubation with Alexa 488-conjugated goat anti-rabbit IgG (Invitrogen Japan, Tokyo, Japan) and subsequent counterstaining with DAPI solution (2 μg/mL; Invitrogen). Sections were examined under a microscope equipped with a digital camera (Olympus Co., Tokyo, Japan), and fluorescent images were obtained using a confocal microscope (TCS-SP5; Leica, Tokyo, Japan).

2.4 | Immunoblot analyses

The eyes were enucleated, and retinas were isolated and placed in a lysis buffer that included a protease inhibitor cocktail (Complete, EDTA-free; Roche, Mannheim, Germany) and phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich, St. Louis, MO). The lysate was treated with Laemmli’s sample buffer and separated by 10% SDS-polyacrylamide gel electrophoresis. The proteins were electrically transferred to a polyvinylidene fluoride membrane (Immobilon-P; Millipore) in a Trans-Blot SD Cell (Bio-Rad Laboratories, Hercules, CA, USA). After the transfer, the membrane was blocked with 5% skim milk in Tris Buffered Saline-T (TBS-T) or TNB blocking buffer (0.1 M Tris-HCl pH 7.5, 0.15 M NaCl, 0.5% TSA Blocking Reagent [Perkin-Elmer Life Sciences, Waltham, MA, USA]), then incubated overnight at 4°C with rabbit anti-rhodopsin polyclonal antibody (1:100 000; LSL, Osaka, Japan), rabbit anti-cytochrome c oxidase subunit 4 (COX4) polyclonal antibody (1:1 000; Abcam, Cambridge, UK), mouse anti-α-tubulin antibody (1:1000; Sigma-Aldrich), or anti-GAPDH antibody (1:100 000; Sigma-Aldrich). The membrane was then incubated with horseradish peroxidase-conjugated secondary antibody. Finally, the signals were detected using the enhanced chemiluminescence system (ECL Blotting Analysis System; Amersham, Arlington Heights, IL, USA), measured with the ImageJ program, and normalized to α-tubulin or GAPDH.

2.5 | Real-time PCR

Total RNA was isolated from the retina with TRIzole (Invitrogen) and reverse-transcribed using the High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. The mRNA levels of Cox4i1, Prkaa1, and Prkaa2 were normalized to that of Gapdh. The respective forward and reverse primer sequences of the murine genes were: Cox4i1, 5′-CGACTGGAGCAGCTTTCCC-3′ and 5′-CTGTTATCTCTGCGCAAGC-3′; Prkaa1, 5′-CAGGACCCTCTACATCATC-3′ and 5′-TTCTTTCTTCTGGTTACCC-3′; Prkaa2, 5′-ATGCCCAAGTGAAGCTAG-3′ and 5′-ATAATTGGCCATCCAGAC-3′; and Gapdh, 5′-AACATTGGGCCCCATCTTCA-3′ and 5′-GATGACCCCTTCTGCTTCC-3′. Real-time PCRs were performed using the StepOnePlus Real-Time PCR system (Applied Biosystems), and the gene expression was quantified using the 2−ΔΔCT method.

2.6 | Electroretinogram (ERG) recordings

Mice were dark-adapted for a minimum of 12 hours and then placed under dim-red illumination before conducting the ERGs as previously reported. Mice were anesthetized with intraperitoneal injection of combined anesthetics (midazolam 4 mg/kg BW [Sandoz Japan, Tokyo, Japan], medetomidine 0.75 mg/kg BW [Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan], and butorphanol tartrate 5 mg/kg BW [Meiji Seika Pharma Co., Ltd., Tokyo, Japan]) and kept on a heating pad throughout the experiment. Mouse pupils...
were dilated using a single drop of a tropicamide and phenylephrine mixture (Mydrin-P; Santen Pharmaceutical). The ground and reference electrodes were then placed on the tail and in the mouth, respectively, while the active gold wire electrodes were placed on the cornea. Recordings were made using a PowerLab System 2/25 (AD Instruments, New South Wales, Australia). Full-field scotopic ERGs were measured in response to a flash stimulus at intensities ranging from −2.1 to 2.9 log cd s/m². The responses were differentially amplified and filtered through a digital bandpass filter ranging from 0.3 to 450 Hz. Each stimulus was delivered using a commercial stimulator (Ganzfeld System SG-2002; LKC Technologies, Inc., Gaithersburg, MD). The a-wave amplitude was measured from the baseline to the trough, whereas the b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave. The implicit times of the a- and b-waves were measured from the onset of the stimulus to the peak of each wave. The peak points were automatically indicated by the system and confirmed by the examiner.

2.7 | Measurement of oxygen consumption rate (OCR)

Oxygen consumption rates were measured using an XF-24 Extracellular Flux Analyzer (Seahorse Biosciences), according to the manufacturer’s instructions and previous reports. Briefly, the eyes were enucleated, and the retinas were placed in ice-cold medium and dissected. The neural retinas were punched out using a 1 mm diameter biopsy puncher (Miltex Instrument, Integra LifeScience, NJ) and placed in the dedicated wells of the analyzer and covered with Islet Fluxpak mesh inserts. The measurements were performed under basal conditions and following the addition of the mitochondrial inhibitors from the XF Cell Mito Stress Kit (Seahorse Biosciences) at the following concentrations: oligomycin at 2.5 μM (only for 661W cells; see below), (2-fluorophenyl)-6-[(2-fluorophenyl)amino]-1,2,5-oxadiazolo[3,4-e]pyrazin-5-yl) amine (BAM15) at 8 μM, and rotenone/antimycin A at 2 μM. Since a previous study recommended to avoid using oligomycin for retinal punches due to cytotoxic effects, we did not use it. For experiments with the 661W photoreceptor cell line (see below), the cells were plated at 10 000 cells per well, and the experiments were performed 2 days afterwards, according to the manufacturer’s protocol of the Mito Stress Kit (Seahorse Biosciences) and a previous report.

2.8 | Measurement of cytochrome c oxidase (CcO) activity

Either the eyes were enucleated and the retinas were isolated, or 661W cells (see below) were snap-frozen with liquid nitrogen. The samples were placed in lysis buffer provided in the kit before measuring the CcO activity using the Complex IV Rodent Enzyme Activity Microplate Assay Kit (Abcam) according to the manufacturer’s instructions. The luminescent signals were measured using the Cytation 5 system (BioTek, Winooski, VT, USA).

2.9 | Measurement of ATP levels

Next, the eyes were enucleated and the retinas were isolated, or 661W cells (see below) were snap-frozen with liquid nitrogen. The samples were placed in lysis buffer before measuring the ATP content using the ATP Bioluminescence Assay Kit CLSII (Sigma-Aldrich). The luminescent signals were determined using the Cytation 5 system (BioTek).

2.10 | Cell culture

The 661W cell line (a kind gift from Dr. Muayyad R. Al-Ubaidi, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA) was maintained in Dulbecco’s Modified Eagle Medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere of 5% CO₂. AMPK knockdown was performed 24 hours before each assay using siRNA for random control or Prkka1 and Prkka2 (Thermo Fisher, MA) and Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher) according to the manufacturer’s protocol.

2.11 | Statistical analysis

Results were expressed as means ± standard deviations save for those of OCR which were expressed as mean ± standard errors. The values were processed for statistical analyses using one-way ANOVA with Tukey’s post hoc test for comparison among three or more groups or two-tailed Student’s t-tests for comparison between two groups, after checking for normality using SPSS Statistics 24 (IBM, Armonk, NY, USA) software. Differences were considered statistically significant at P < .05.

3 | RESULTS

3.1 | Photoreceptor death and rhodopsin loss after light exposure were reduced by AICAR treatment

To elucidate the effects of AICAR on the light-induced retinal degeneration, we analyzed the number of apoptotic cells
using the TUNEL assay on day 2 following light exposure, when a clear change in photoreceptor apoptosis has been previously reported. Apoptosis was induced only in the ONL containing the photoreceptors (Figure 1A). However, the number of apoptotic cells was significantly reduced by AICAR treatment (Figure 1A,B).

We also analyzed the content of rhodopsin, the visual pigment of the photoreceptor cells. Rhodopsin protein levels were reduced by light exposure on day 2, however, this reduction was attenuated by AICAR treatment (Figure 1C).

Morphological changes of the retina were not significant at this time point (Figure 1D).

### 3.2 | AICAR suppressed photostress-induced photoreceptor loss and morphological changes

Next, we evaluated the retinal morphology on day 4 after light exposure with or without AICAR treatment using immunohistochemistry for rhodopsin (Figure 2A). This is the time point when the retinal morphological changes were evident, as reported previously. The light exposure induced significant thinning of the photoreceptor cell layer; however, these changes were substantially alleviated and the thickness was preserved by AICAR treatment at a dose of 250 mg/kg BW (Figure 2A,B). In addition, the length of the rod photoreceptor outer segments (OSs), where rhodopsin is concentrated, was reduced by light exposure; however, this OS shortening was substantially attenuated by AICAR at the dose of 250 mg/kg BW (Figure 2A,C).

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**FIGURE 1** AICAR reduced photoreceptor death and visual pigment loss after light exposure. TUNEL assaying showing that apoptotic cells (pink) were only observed in the ONL, the photoreceptor cell layer. Images on day 2 after light exposure with or without AICAR treatments. Nuclei were counterstained with DAPI (blue, A). Ratio of the numbers of TUNEL-positive cells to ONL cells in a section indicating that AICAR attenuated apoptotic cell death (B) Immunoblot analyses showed that protein levels of retinal rhodopsin were reduced 2 days after light exposure; however, AICAR treatments attenuated this reduction (C) The ONL thickness in the retinal section was not changed by light exposure with or without AICAR treatment two days after light exposure (D) LE, light exposure; ONL, outer nuclear layer; veh, vehicle treatment. Data are represented as mean ± standard deviation. n for LE (−) with vehicle treatment, LE (+) with vehicle treatment, LE (+) with AICAR treatment (125 mg/kg BW), LE (+) with AICAR treatment (250 mg/kg BW); 4, 7, 6, 7 (B), 8 for all (C), 4, 7, 6, 7 (D). Scale bar, 100 μm. **P < .01, n.s. not significant

**FIGURE 2** AICAR suppressed photostress-induced photoreceptor loss and morphological changes. Immunohistochemistry using antibodies against rhodopsin (green) 4 days after light exposure with or without AICAR treatments. Nuclei were counterstained with DAPI (blue; A). ONL thickness (B) and OS length (C) were reduced by light exposure, however, the light-induced thinning and shortening were suppressed by AICAR. LE, light exposure; ONL, outer nuclear layer; OS, outer segment; veh, vehicle treatment. Data are represented as mean ± standard deviation. n for LE (−) with vehicle treatment, LE (+) with vehicle treatment, LE (+) with AICAR treatment (125 mg/kg BW), LE (+) with AICAR treatment (250 mg/kg BW); 7, 8, 6, 8. Scale bar, 50 μm. **P < .01, n.s. not significant
3.3 | AICAR protected against light-induced visual impairment

To evaluate the protective effect of AICAR on light-induced visual impairment, we measured scotopic ERGs of light-exposed mice treated with or without AICAR, as well as control mice not exposed to light and vehicle-treated mice. On day 2 following light exposure, when photoreceptor cells became apoptotic (Figure 1), both a- and b-wave amplitudes decreased, thus, the visual function was impaired in vehicle-treated mice compared with mice not exposed to light; however, AICAR treatment significantly suppressed this impairment in all stimulation conditions (Figure 3A,B). On day 4, when morphological changes were evident (Figure 2), the light-induced impairment continued to be suppressed by AICAR at 250 mg/kg BW, ie, the higher dose in the current study (Figure 3C,D). The a-wave represents the photoreceptor function, indicating that a protective effect of AICAR was evident at least on the photoreceptor function. The protective effect on the b-wave can also be explained by the effect of AICAR on photoreceptor functions as the b-wave indicates subsequent neural signaling to the photoreceptors. Implicit times were not changed, consistent with previous reports.6,12,32 Since significant effects were always obtained at the dose of 250 mg/kg BW, the following experiments were performed using this higher dose.

3.4 | AICAR promoted energy production under conditions of photostress

Next, we measured the OCR, which represents mitochondrial respiration, to analyze the influence of light exposure on energy metabolism. In retinal punches obtained immediately after light exposure, basal OCR levels were decreased in the retinas from light-exposed and vehicle-treated mice compared with those from mice not exposed to light (Figure 4). AICAR treatment at 250 mg/kg BW significantly increased OCR levels compared with vehicle treatment (Figure 4A). The increase in OCR was associated with an increase in ATP levels (Figure 4B). These results suggest that AICAR can promote energy production under conditions of photostress.
light (Figure 4A,B). However, immediately following light exposure, the levels were higher and preserved in those treated with AICAR compared with the light-exposed and vehicle-treated mice (Figure 4A,B). This improved effect was not preserved 48 hours after light exposure (Figure 4C,D). Under these conditions, the extracellular acidification rates, representing glycolysis and measured by flux analyzer, did not differ among the experimental groups (data not shown). Moreover, the mRNA levels of Cox4i1 which encodes cytochrome c oxidase (COX, also called CcO) subunit 4 isoform 1, (Figure 4E) and protein levels of COX subunit 4 (COX4) (Figure 4F) were not affected by light exposure or AICAR treatment. By contrast, the CcO activity was reduced in the retinas derived from light-exposed and vehicle-treated mice, whereas this reduction was attenuated by AICAR treatment immediately following light exposure (Figure 4G); while this activity was seen to decrease by 48 hours post-exposure (Figure 4H).

The retinal ATP levels in light-exposed and vehicle-treated mice increased immediately after light exposure...
compared with non-light-exposed mice; however, the ATP levels began to decline by 24 hours and became significantly lower than non-light-exposed mice after 48 hours (Figure 4I). By contrast, AICAR treatment preserved the higher ATP levels after 24 hours, compared with those of light-exposed and vehicle-treated mice, or those of non-light-exposed mice, whereas AICAR treatment did not show effects after 48 hours (Figure 4I).

3.5 | AMPK regulated CcO activity in the mitochondrial respiratory system

To assess whether AMPK modulation directly affects the mitochondria energy metabolism, we used the 661W photoreceptor-derived cell line. After confirming with real-time PCRs that AMPK was knocked down by a combination of AMPKα1 and AMPKα2 siRNAs (AMPKα1, reduction by 13%; AMPKα2, reduction by 36%; data not shown), we found that OCR at baseline was reduced (Figure 5A,B). The levels of OCR remained significantly lower after an ATP synthase inhibitor, oligomycin, was added, and a similar trend was observed after using the mitochondrial protonophore uncoupler, BAM15.

The CcO activity was also decreased by AMPK knockdown (KD) (Figure 5C), indicating that AMPK affected the enzyme’s activity. Simultaneously, AMPK KD reduced the ATP levels in the cell line (Figure 5D). Taken together, AMPK activated the CcO and secured ATP levels. Under the same conditions, Cox4i1 mRNA (Figure 5E) and COX4 protein (Figure 5F) levels were not changed.

4 | DISCUSSION

We demonstrated that light-induced visual function impairment was suppressed by the AMPK activator, AICAR. Photostress increased the apoptosis of photoreceptors, decreased rhodopsin protein levels, and subsequently led to photoreceptor loss and OS shortening. However, these changes were all prevented by AICAR treatment after light exposure. Mitochondrial respiratory functions were impaired from an early stage after light exposure due to reduced mitochondrial enzyme activity that ultimately led to reduced ATP levels in the retina. The impact of AMPK in preserving mitochondrial enzyme activity and ATP levels was confirmed in a photoreceptor-derived cell line.

The mechanisms of how light exposure causes visual function impairment have been explained by photoreceptor cell loss through light-induced oxidative stress, and stress response signals, including the renin-angiotensin system. The visual impairment observed in ERGs on day 4 after light exposure was consistent with the histological data showing a reduction in the numbers of photoreceptor cells in the current study. In addition, we demonstrated that the substantial visual impairment was already obvious 2 days after light exposure. At this time point, some of the photoreceptor cells were affected by light exposure and showed apoptotic changes; however, the ONL thickness representing the photoreceptor cell number was comparable to that in control mice. Instead, the levels of rhodopsin, a functional visual pigment, were reduced. Thus, visual impairment at this earlier time point may be due to the reduction in the functional molecule, rhodopsin, rather than photoreceptor cell loss. This is similar to previous reports describing visual impairment due to rhodopsin protein reduction and not to photoreceptor apoptosis in retinal inflammation. OS shortening 4 days after light exposure was consistent with the reduction in rhodopsin protein levels, given that rhodopsin knockout mice show OS shortening in a dose-dependent manner; homozygous knockout mice display more shortening than heterozygous knockout mice, and overexpression of rhodopsin elongates the OS. Importantly, ATP levels were initially elevated by photostress; however, this increase did not retained. Therefore, the

![Figure 5](image-url)
light-exposed retina may have initially generated more ATP to support the self-repair systems, such as UPR and DNA repair. However, ATP levels decreased afterwards, which most likely caused insufficient energy supply for repair responses and, without therapeutic interventions, affected photoreceptor survival (Figure 6). By contrast, the AMPK activator, AICAR, continuously supplied ATP through preservation of CcO activity, and the successful operation of the self-repair systems may have protected the photoreceptors and the visual function. Photostress without treatment caused energy decompensation, while AICAR contributed to the compensation of the energy demand under stress.

Under the current protocol for AICAR treatment, the ATP supply did not last 48 hours after light exposure. This was consistent with the results that CcO activity and OCR were also reduced after 48 hours. The efficacy of AICAR was declined by this time point; nonetheless, photoreceptor cells were substantially protected both morphologically and functionally. Thus, the critical time window for supplying ATP to promote cellular survival in the current model was from immediately to 24 hours after light exposure, and a timely ATP supply protected the photoreceptor cells. We show a proof of concept in the current study, and further validations, such as whether additional treatments enhance the protective effect, would be a topic for further studies that would apply the concept to a new therapeutic approach.

CcO, the complex IV enzyme of the respiratory system is made up of multiple subunits that couples the transfer of electrons from cytochrome c to molecular oxygen and contributes to a proton electrochemical gradient across the inner mitochondrial membrane which is used for ATP synthesis. This enzyme can directly affect the OCR and eventually increases the transmembrane difference of the proton electrochemical potential which is utilized to synthesize ATP. Hence, the proposed mechanism involved in these pathological and therapeutic actions is as follows (Figure 6). CcO activity was reduced after light exposure, suggesting that the reduction was one of the influencers of photostress, and therefore, the increase in ATP production after light exposure was only transient. Photostress-induced CcO inactivation may occur either indirectly through AMPK inactivation, or directly. AMPK was indispensable for CcO activity as shown in vitro, and the activity was induced by AICAR in vivo, suggesting that AICAR increased CcO activity through AMPK activation. Although further studies are required, the knowledge of AMPK functions in energy supply pathways, at least in part, through CcO activity may help to develop new future therapeutic approaches.

Moreover, ATP levels are reported to be reduced in Alzheimer’s disease, and the effect of AMPK activity on the generation and degradation of pathogenic ß-amyloid protein has been analyzed. It would be interesting to establish whether AMPK activation can regulate ATP levels to compensate and normalize these pathological conditions as well. The concept of ATP compensation to preserve homeostasis...
even under disease stress could become a common concept of neuroprotective therapies in various fields.

AICAR treatment preserved ATP synthesis by retaining 
CCo activity in the retina exposed to photostress, ultimately
leading to improved survival of photoreceptors under 
photostress conditions. Previous reports have primarily
investigated therapeutic approaches to suppress stress
signals; however, the current study has provided proof of
concept on the impact and how to provide cells with sufficient
energy needed to recover cellular homeostasis and to survive
in the presence of cellular stress. Therefore, the therapeutic
approach proposed in the current study may open a new era
of treatment strategies for neurodegeneration, hence, further
studies are warranted.

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CONFLICT OF INTEREST
None.

AUTHOR CONTRIBUTIONS
H. Kawashima performed research and analyzed data, Y.
Ozawa designed research and wrote the paper, E. Toda, K.
Homma, and H. Osada assisted with research, T. Narimatsu,
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