α2 but Not α1 AMP-activated Protein Kinase Mediates Oxidative Stress-induced Inhibition of Retinal Pigment Epithelium Cell Phagocytosis of Photoreceptor Outer Segments*

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Oxidative stress causes retinal pigment epithelium (RPE) cell dysfunction and is a major risk factor leading to the development of dry-type age-related macular degeneration. Taking pharmacological and genetic approaches, we address the mechanisms by which sublethal oxidative stress inhibits RPE cell phagocytosis. Sublethal oxidative stress dose-dependently inhibited RPE cell phagocytosis of photoreceptor outer segments (POS) and activated AMP-activated protein kinase (AMPK) as determined by increased Thr172 and Ser79 phosphorylation of AMPKα and its substrate acetyl-CoA carboxylase, respectively. Similar to oxidative stress, 5-aminoimidazole-4-carboxamide riboside (AICAR), a pharmacological activator of AMPK, inhibited RPE cell phagocytosis of POS in a dose-dependent manner. Inhibition of RPE cell phagocytosis by AICAR was fully reversed by blockade of AICAR translocation into cells by dipyridamole or inhibition of AICAR conversion to ZMP by adenosine kinase inhibitor 5-iodotubercidin. In agreement, AICAR-induced activation of AMPK was abolished by preincubation with dipyridamole or 5-iodotubercidin. Knock-out experiments further revealed that α2 but not α1 AMPK was involved in RPE cell phagocytosis and that activation of α2 AMPK contributed to the inhibition of RPE cell phagocytosis by oxidative stress. Inhibition of RPE cell phagocytosis by activation of α2 AMPK was associated with a dramatic increase in acetyl-CoA carboxylase phosphorylation. In comparison, AMPK had no role in oxidative stress-induced breakdown of RPE barrier function. Taken together, reduction in POS load under oxidative stress might direct RPE cells to a self-protected status. Thus, activating AMPK could have therapeutic potential in treating dry macular degeneration.

The retinal pigment epithelium (RPE) is a monolayer of pigmented cells forming a part of the blood-retina barrier (1). Basal membrane of the RPE is in contact with Bruch membrane, whereas the apical membrane faces the photoreceptor outer segments. The close structural interactions of RPE cells with the outer retina indicate that the major functions of the RPE layer are to maintain the survival and normal functioning of photoreceptors by controlling nutrients/waste products exchange (2), phagocytosing shed outer segments (3), shuttling retinoids to synthesize visual pigments (4), and producing trophic factors necessary for photoreceptor survival. Failure of any one of these functions can result in degeneration of the retina, loss of visual function, and blindness.

Age-related macular degeneration (AMD) is an idiopathic retinal degenerative disease that predominates in the elderly in the Western world as a cause of irreversible, profound vision loss (5, 6). The pathogenic mechanisms whereby environmental factors contribute to the development of AMD remain elusive. However, growing evidence indicates that oxidative stress injury to the RPE plays an important role in the etiology of AMD. The RPE is at high risk for oxidative injury due to its location in a highly oxygenated environment, its high levels of light exposure, and generation of reactive oxygen species during POS phagocytosis (7–9). Most studies have focused on oxidative injury-induced death of RPE (10, 11), a very late stage of dry AMD. In comparison, in the early stage of AMD development, oxidative insult induces a set of profound physiological responses in RPE leading to dysfunction without initiation of cell death (12). However, minimal data are available regarding the effects of sublethal oxidative injury on RPE functions such as phagocytosis and blood-retinal barrier as well as their mechanisms of action.

AMP-activated protein kinase (AMPK) is a metabolic-sensing Ser/Thr kinase expressed in all cell types examined to date (13, 14). AMPK exists as a heterotrimer consisting of a catalytic α subunit and regulatory β and γ subunits (15). The catalytic subunit of AMPKα has two major isoforms, α1 and α2. The α1 isoform is primarily cytoplasmic, whereas α2 is predominantly nuclear and plays a role in transcriptional regulation (16–18). AMPK is activated by energy deficiency to coordinate a switch from ATP-consuming pathways to catabolic pathways to produce a positive energy balance. AMP activates AMPK via an allosteric effect, by stimulation of Thr172 phosphorylation in the activation domain through an upstream kinase LKB1 (19), and by inhibition of Thr172 dephosphorylation by protein phosphatases. All three effects of AMP on AMPK are antagonized by

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The abbreviations used are: RPE, retinal pigment epithelium; ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide riboside; AMD, age-related macular degeneration; AMPK, AMP-activated protein kinase; POS, photoreceptor outer segment; TER, transepithelial electrical resistance; PBS, phosphate-buffered saline; DPY, dipyridamole; FITC, fluorescein isothiocyanate; siRNA, small interfering RNA; IODO, 5-iodotubercidin.
ATP, so AMPK monitors any small changes in the cellular AMP:ATP ratio (20). AMPK is also activated by a variety of cellular stresses that deplete ATP such as glucose deprivation (21, 22), ischemia (23), and oxidative stress (24). This suggests that AMPK, in addition being a key regulator of physiological energy dynamics, may affect RPE cell function under oxidative stress conditions.

Sensing ATP levels may be important in the cell response to oxidative stress, but the consequences of persistent activation of AMPK that have compromised energy supplies are unknown. In an attempt to return cells to homeostasis, numerous damage-repairing processes that consume ATP are activated (25). Overactivation of these pathways during oxidative stress can lead to energy failure and cell dysfunction (26). At present, it is unclear whether AMPK in RPE cells is activated by sublethal oxidative stress. If so, whether its activation would be protective or damaging? In the present study we utilize an in vitro sublethal oxidative stress model to delineate changes in AMPK activity incurred during environmental stress and to determine the effects on RPE cell phagocytosis and barrier integrity. AMPK activation by oxidative insult and by a pharmacological activator reduces RPE cell phagocytosis, whereas no effect of AMPK on oxidative stress-induced breakdown of RPE barrier function is detected. α2-isoform mediates oxidative stress inhibition of phagocytosis. These data suggest that AMPK activation during oxidative stress might switch RPE cells to a self-protected status.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium/F-12 (1:1) medium and fetal bovine serum were purchased from Invitrogen. 5-Aminoimidazole-4-carboxamide riboside (AICAR), FITC-labeled dextran (70 kDa), and anti-β-actin were from Sigma. Control siRNA and validated siRNAs targeting AMPKα1 (sense 5-GGUUGGCAACAAUGAUAUGt-3) and AMPKα2 (sense 5-GGUUUCUUGAAAAACACGCUTt-3) were purchased from Applied Biosystems. Enhanced chemiluminescence reagents were from GE Healthcare. Human retinal pigment epithelium cell line ARPE19 was from ATCC (Rockville, MD). Anti-Thr(P)172 AMPK, anti- Ser(P)79 ACC, and anti-AMPKα antibodies were purchased from Cell Signaling Technology (Beverly, MA). Antibodies against AMPKα1 and AMPKα2 were obtained from Bethyl Laboratories (Montgomery, TX). Tissue culture-treated polycarbonate transwells (12 mm in outer diameter and 0.4 μm in pore size) were purchased from Costar (Corning, NY).

RPE Cell Culture and siRNA Transfection—Human RPE cell line ARPE19 was obtained from ATCC, and the cells were cultured in 1:1 of Dulbecco’s modified Eagle’s medium/F-12 with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were grown at 37 °C in a humidified atmosphere of 95% air, 5% CO2. The cells were passed every 3–4 days by digestion with 0.05% trypsin, 0.02% EDTA. 10 × 10^5 cells per 10-cm dish were seeded for 24 h and transfected with control siRNA, and validated siRNAs targeting human AMPKα1 and AMPKα2 (Ambion) were diluted in Opti-MEM1 at a concentration of 25 nM using Lipofectamine 2000 (Invitrogen). 8 h after transfection, medium was exchanged with fresh complete medium, and cells were cultivated overnight before being re-seeded for experiments.

Cell Viability Assay—ARPE19 (1.5 × 10^4/well) cells were seeded in 96-well flat-bottom micro-culture plates for 4 days, treated with 0.5 mM hydrogen peroxide for 30 min, washed twice with medium, and then continued culture for 24 h. Untreated control cells were handled in a similar fashion without hydrogen peroxide. Viable cells were then determined by the addition of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) for 4 h following the manufacturer’s instructions (Roche Applied Science).

Isolation and FITC Labeling of POS—Fresh bovine eyes were obtained from local slaughterhouse. POS were isolated by discontinuous sucrose density (25–60%) centrifugation (27) and stored in 20 mM Tris acetate, pH 7.2, 10 mM glucose, 2 mM MgCl2, 0.1 mM NaCl, and 2.5% sucrose at −80 °C. Before use, POS were thawed, spun down, and suspended in 900 μl of serum-free medium. POS were dyed by the addition of 100 μl of 1 mM sodium bicarbonate, pH 9.0, and 10 μl of 10 mg/ml FITC (Molecular Probes) for 1 h at room temperature in the dark (28, 29). POS were washed 3 times with serum-free medium at 7000 rpm for 5 min and re-suspended in growth medium at the concentration of 10 × 10^7 particles/ml. POS preparation was positive for immunostaining with anti-rhodopsin antibody.

POS Feeding and Quantification of Phagocytosis—RPE cells were placed in an individual well of a 24-well tissue culture plate (1 × 10^5/well) and grown for 4 days to reach complete confluence. Each well of confluent RPE cells was layered with 300 μl of Dulbecco’s modified Eagle’s medium/F-12 containing 10% serum and 5 × 10^6 POS particles and was incubated at 37 °C for the indicated lengths of time. After POS challenge, the cells were washed 3 times by vigorous agitation with PBS containing CaCl2 and MgSO4 to remove unbound POS, detached using 0.05% trypsin, 0.02% EDTA, washed twice with PBS, and then re-suspended in 0.5 ml of PBS for the flow cytometric assay. FITC-labeled POS uptake was measured using a fluorescence-activated cell sorter (FACScan; BD Biosciences). Cells were analyzed with 488-nm excitation and a 530 nm band-pass filter in the emission path. Untreated control cells were handled in a similar fashion with the negative control to set the gate in each experiment. Each flow cytometry run consisted of 5000 scattering events. A logarithmic scale of relative fluorescence intensity was used, and POS phagocytosis was calculated by subtracting the autofluorescence from the mean fluorescence of cells challenged with FITC-POS. The data are presented as the mean of three independent experiments.

For signal transduction pathway inhibition, cells were pretreated for 1 h with 2 mM AICAR only or for 30 min of exposure to 0.5 mM hydrogen peroxide. After treatment, hydrogen peroxide was washed out. FITC-labeled POS was then added to the cells and incubated for another 4 h. Flow cytometry for analysis of phagocytosis was performed as above.

Cell Extraction and Immunoblotting—Confluent ARPE19 cells were stimulated with the chemicals as described in the legends of Figs. 1, B and C, 2C, and 4 in media with 0.5% FBS. After treatment, cells were washed twice with cold PBS containing 2 mM NaF and 2 mM vanadate and lysed in modified radioimmune precipitation lysis buffer (150 mM NaCl, 1% Tri-
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A

![Graph showing the regulation of phagocytosis by α2 AMPK](image1.png)

B

![Graph showing the phosphorylation of AMPKα](image2.png)

C

![Graph showing the time course of elevation of Thr(P)172](image3.png)

FIGURE 1. Sublethal oxidative stress inhibits RPE cell phagocytosis and activates AMPK. A, inhibition of RPE phagocytosis by hydrogen peroxide. Confluent cells were treated with various concentrations of hydrogen peroxide for 30 min, washed out, and incubated with 5 × 10⁶ POS in 300 μl of growth medium for 4 h without the presence of hydrogen peroxide. Phagocytosis was determined by the flow cytometer. Data shown were the mean ± S.D. of three experiments. B and C, time course of elevation of Thr(P)172 (pThr172) AMPKα and Ser(P)79 (pS79) ACC after oxidative stress. Western blot analysis (IB) was performed on lysates derived from treated or untreated RPE cells at various times to detect phosphorylated AMPKα (B) or ACC (C). Total AMPKα and ACC were served as loading controls. Shown are one immunoblot and densitometric quantitation of three experiments. p < 0.05 (*) and p < 0.001 (***)) versus control.

RESULTS

Inhibition of Phagocytosis of POS by Hydrogen Peroxide—To address the effect of oxidative stress on RPE cell phagocytosis, cells were treated with up to 0.5 mM hydrogen peroxide for 30 min in growth medium and then were cultivated in fresh complete medium. This sublethal oxidative stress condition did not cause RPE cell death but dose-dependently inhibited RPE cell phagocytosis of POS (Fig. 1A and data not shown). Thr172 phosphorylation of catalytic subunit AMPKα is required for AMPKα activation, and activated AMPKα can phosphorylate Ser79 of acetyl-CoA carboxylase (ACC), one of the AMPK substrates. Oxidative stress with 0.5 mM hydrogen peroxide dramatically activated AMPKα, reached the peak within 15 min, and declined 1 h later (Fig. 1B) as evidenced by increased phos-
AICAR led to an increase in Ser\(^{79}\) phosphorylation of ACC (Fig. 2C), demonstrating that AICAR activates AMPK signaling in RPE cells. Preincubation with DPY or IODO did abolish AICAR-induced AMPK activation (Fig. 2C), demonstrating that AMPK\(\alpha\) activation is well correlated with inhibition of RPE cell phagocytosis by AICAR.

**Regulation of Phagocytosis by AMPK\(\alpha_2\) but Not by AMPK\(\alpha_1\)—**

To confirm if AMPK\(\alpha\) is of functional relevance in RPE cell phagocytosis and evaluate possible AMPK isoform-specific activity, we used RNA interference technology to knock down AMPK\(\alpha\). Validated siRNA constructs complementary to AMPK\(\alpha_1\) or \(\alpha_2\) (Applied Biosystems) were transfected into ARPE19 cells. AMPK\(\alpha\) expression was measured 72 h post-transfection by Western blot analysis using isoform-specific antibodies. \(\alpha_1\) and \(\alpha_2\) AMPK siRNAs selectively suppressed AMPK\(\alpha_1\) and AMPK\(\alpha_2\) protein, respectively. Optical density analysis of the data from three independent experiments revealed that AMPK\(\alpha_1\) and AMPK\(\alpha_2\) proteins were reduced to 6 and 1% of respective controls (Fig. 3A). AMPK\(\alpha_1\) repression caused a 37% compensational increase of AMPK\(\alpha_2\) protein. Combined treatment with both \(\alpha_1\) and \(\alpha_2\) AMPK siRNAs led to 66 and 92% reduction of AMPK\(\alpha_1\) and AMPK\(\alpha_2\) protein, respectively. AMPK\(\alpha_1\) siRNA had no effect on POS phagocytosis. AMPK\(\alpha_2\) siRNA, however, significantly inhibited the phagocytosis of POS by 40%. Combined knockdown of both \(\alpha_1\) and \(\alpha_2\) did not make further reduction in RPE cell phagocytosis (Fig. 3B). These observations revealed that RPE cell phagocytosis depends, at least in part, on AMPK\(\alpha_2\) but not AMPK\(\alpha_1\) in normal conditions. Under stress conditions, oxidative stress-induced inhibition of RPE cell phagocytosis was not effected by silencing AMPK\(\alpha_1\), as POS phagocytosis by RPE cells transfected with control siRNA and AMPK\(\alpha_1\) siRNA was inhibited to a similar extent by hydrogen peroxide treatment (Fig. 3C). However, knockdown of AMPK\(\alpha_2\) reversed the inhibition of RPE cell phagocytosis induced by hydrogen peroxide, suggesting that oxidative stress-induced activation of AMPK\(\alpha_2\) mediated the inhibition of RPE cell phagocytosis.

Differential Effects of \(\alpha_1\)AMPK and \(\alpha_2\)AMPK on Oxidative Stress-induced AMPK Signaling—**

To address the underlying signaling events that might explain the isoform-specific effect on RPE cell phagocytosis, measurement of AMPK signaling was performed. We first differentiated the contribution of \(\alpha\)-isoforms to the level of total phosphorylated AMPK\(\alpha\), which detects both AMPK\(\alpha_1\) and AMPK\(\alpha_2\) phosphorylated at Thr\(^{172}\). Hydrogen peroxide increased Thr\(^{172}\) phosphorylation of

![FIGURE 2. Inhibition of RPE cell phagocytosis by activation of AMPK.](image-url)
AMPKα, and application of siRNA targeted to AMPKα1 reduced total phosphorylated AMPKα to a very low level (Fig. 4A). In comparison, AMPKα2 siRNA very weakly repressed hydrogen peroxide-induced Thr172 phosphorylation of total AMPKα, indicating that α1 AMPK is the dominant isoform in RPE cells.

One of AMPK essential functions is to conserve ATP consumption by suppressing fatty acid synthesis pathway through phosphorylation of ACC. The Ser79 phosphorylation of ACC in AMPKα siRNA-treated cells was examined to determine relative contribution of α isoforms. In contrast to Thr172 phosphorylation of AMPKα, AMPKα1 siRNA did not significantly affect hydrogen peroxide-stimulated Ser79 phosphorylation of ACC (Fig. 4B). However, hydrogen peroxide-induced increase in Ser79 phosphorylation of ACC was markedly reduced in AMPKα2-repressed cells. These observations indicated that AMPKα2 is the major kinase isoform regulating phosphorylation of ACC in response to oxidative stress in RPE cells.

No Role of AMPK in Oxidative Stress-induced Breakdown of RPE Monolayer—Integrity of the RPE monolayer was determined by measuring TER. RPE cells grown in transwells for 3 days were switched to 1% FBS-containing medium, and progression of TER was monitored daily up to 1 week. TER reading showed that resistance reached to a plateau in 3 days and that no further increase was detected up to 1 week culture (data not shown). The TER values of ARPE19 monolayer averaged 51.8 ohms/cm². To evaluate the effect of oxidative stress on monolayer integrity, the TER was measured in ARPE19 cells exposed to 1 mM hydrogen peroxide as a function of time up to 6 h. Treatment with hydrogen peroxide caused a significant decrease in TER versus untreated control at 3 h (Fig. 5A). Further exposure reduced TER to 23% of control. AMPK knockdown by siRNA did not affect the TER reading, and the decrease in TER by hydrogen peroxide was also not altered by AMPK knockdown (Fig. 5A), suggesting that AMPK is not involved in regulating oxidative stress-induced breakdown of RPE monolayer.

To confirm no role of AMPK in oxidative stress-induced monolayer breakdown observed by TER measurement, transepithelial flux assays were performed. Dextran flux from apical to basolateral side was increased after hydrogen peroxide treatment (Fig. 5B). There was a ~70% increase in fluorescence after 6 h of exposure. Similar to the TER measurement, knockdown of AMPKα1 and α2 by siRNA did not alter the flux rate of dextran through the ARPE19 monolayer before and after hydrogen peroxide treatment, confirming that AMPK plays no role in regulating breakdown of RPE monolayer by oxidative stress.

**DISCUSSION**

AMPK activation by sublethal oxidative stress results in the inhibition of RPE cell phagocytosis. This inference is based on the following observations 1) Treatment of RPE cells with hydrogen peroxide led to reduction in POS uptake and to an activation of AMPK. 2) Treatment with pharmacological AMPK activator, AICAR, attenuated the RPE cell phagocytosis, which depends on AMPK activation. 3) The inhibition of RPE cell phagocytosis was due to the activation of α2 AMPK as evident from the studies with AMPK siRNA experiments. 4) The reduction in RPE cell phagocytosis might be mediated by α2 AMPK-ACC pathway.

The clearance of POS occurs throughout the lifespan of RPE cells and is important for the maintenance of retinal integrity and function of the RPE monolayer. The RPE environment favors the generation of reactive oxygen species. RPE generate reactive oxygen species from phagocytosis (8, 9), lipid peroxidation from phagocytosed POS (7), and intense light irradiation (31). High oxygen consumption in the macular area adds a further oxidative stress burden to RPE cells (32). With aging, chronic oxidative stress causes RPE cell dysfunctions that are believed to be central in the development of AMD. Thus, it is of considerable interest to understand how sublethal oxidative stress affects RPE function. We used an *in vitro* oxidative stress model to address sublethal oxidative stress effects on the major...
functions of RPE cells, phagocytosis, and barrier integrity. With 30 min of exposure, hydrogen peroxide dose-dependently inhibited the capacity of RPE cells to phagocytose POS (Fig. 1A). Because of higher cell density in assaying RPE cell barrier integrity, 1 mM hydrogen peroxide was required to cause significant breakdown of RPE monolayer as determined by TER reading and dextran flux from apical to basolateral side (Fig. 5). In parallel, sublethal oxidative stress quickly activated the AMPK pathway, demonstrated by the phosphorylation of Thr\(^{172}\) and Ser\(^{79}\) of AMPK and ACC, respectively (Fig. 1, B and C). Does AMPK activation mediate oxidative stress-induced inhibition of RPE phagocytosis and monolayer breakdown? A pharmacological approach was first used to address this question. As a pharmacological activator of AMPK, AICAR has been used extensively to study physiological roles of AMPK (33, 34). In our study, similar to hydrogen peroxide, AICAR did reduce RPE cell phagocytosis in a dose-dependent manner (Fig. 2A) and mediated its effect via activation of AMPK (Fig. 2, B and C), which is supported by pharmacological blockade of intracellular translocation and conversion to ZMP. Treatment of dipyridamole and iodotubericidin, inhibitors of AICAR translocation and conversion to ZMP, respectively, abrogated AICAR-induced activation of AMPK (Fig. 2C) and inhibition of RPE cell phagocytosis (Fig. 2B). Dipyridamole alone enhanced RPE cell phagocytosis of POS. The molecular mechanisms remain to be addressed.

To further evaluate the role of AMPK in oxidative stress-induced inhibition of phagocytosis, AMPK was knocked down by siRNAs against AMPK. Knockdown of \(\alpha_2\) AMPK, but not \(\alpha_1\) AMPK, by isoform-specific AMPK siRNA resulted in inhibition of the reduction of RPE cell phagocytosis by oxidative stress (Fig. 3C). This evidence points strongly toward \(\alpha_2\) AMPK activation being the major cause of phagocytosis inhibition by oxidative stress. In comparison, oxidative stress-induced breakdown of RPE monolayer integrity was not altered by AMPK knockdown, suggesting that AMPK selectively mediates oxidative stress-induced inhibition of RPE cell phagocytosis.

Human RPE cells expressed both \(\alpha_1\) and \(\alpha_2\) isoforms with dominance of \(\alpha_1\) AMPK protein (data not shown). \(\alpha_1\) AMPK was the most prominent isoform phosphorylated on Thr\(^{172}\) in response to oxidative stress as the result of a more abundant protein expression and/or as a result of a higher sensitivity for phosphorylation by the upstream kinases (Fig. 4A). However, \(\alpha_2\)-, but not \(\alpha_1\)-isoform, conveyed the effects of oxidative stress-induced inhibition of RPE cell phagocytosis. The effect of \(\alpha_2\)-isoform knock-out on oxidative stress-in-
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duced RPE cell phagocytosis was associated with a dramatic decrease in AMPK signaling, as determined by a significant reduction of ACC phosphorylation (Fig. 4B). Thus, the α2 rather than α1 AMPK is the dominant enzyme-controlling ACC pathway during oxidative stress. It is interesting to note that knock-out of the α1 isoform led to a 40% increase in protein content of the α2 isoform, whereas knock-out of the α2 isoform did not alter α1-AMPK expression (Fig. 3A). The up-regulation of one α-isofom when the other is missing indicates a compensatory counteraction to restore AMPK activity. Therefore, the increase of α2-protein in α1-knock-down RPE cells supports the concept that the α2-AMPK activity in general is the most important contributor of AMPK signaling under these conditions. Oxidative stress activates both α1 and α2 AMPK. Why is oxidative stress-induced inhibition of RPE cell phagocytosis selectively regulated by α2 AMPK? Right now we do not have an explanation for this preferential regulation. One of the possible explanations could be that the differential localization of the α1 (cytosol) and α2 (nuclear) isoforms of the catalytic subunits of AMPK contributes to the isoform-selective regulation of RPE cell phagocytosis in response to oxidative stress. It is also possible that in RPE cells, the α2 isoform-specific effect is attributed to the selective regulation of ACC pathway by α2 AMPK. To date, more than 10 direct AMPK targets have been identified (13). ACC are the key enzymes controlling metabolism of fatty acid. Phosphorylation of ACC by AMPK results in inhibition of fatty acid biosynthesis and enhancement of β-oxidation. In RPE cells, oxidative stress-induced Ser79 phosphorylation of ACC was mainly carried out by α2 AMPK, raising the possibility that ACC might mediate the inhibitory effects of AMPK on phagocytosis. However, these remain to be further clarified.

Activation of AMPK has been shown to cause death or attenuate growth in cancer cells (35, 36), suggesting AMPK as an efficient growth inhibitor and apoptosis inducer. On the other hand, AMPK has a protective effect on stress-injured cells in heart ischemia and reperfusion injury models (37, 38). These studies presented AMPK as a protective agent. It could be speculated that in actively dividing cancer cells, inhibition of ATP-consuming processes by AMPK may be less compatible with their survival, whereas in non-dividing cells, where the protective effects of AMPK have been observed under acute stress, the shutdown of ATP-consuming pathways may show a protective effect. What is the significance of reduced phagocytosis under oxidative stress?

Continued RPE phagocytosis of POS is a further major insult to stressed RPE cells. Reduction of RPE cell phagocytosis by α2 AMPK activation could protect oxidative-stressed RPE cells from further damage by decreasing phototoxicity caused by the oxidized spent outer segments.

In conclusion, knock-out of the α2 isoform of AMPK abolished oxidative stress-induced inhibition of RPE cell phagocytosis, whereas knock-out of the α1 isoform had no effect in this respect. We also showed that the α2 isoform delivered the vast majority of AMPK signaling in term of activation of ACC pathway during oxidative stress stimulation. In contrast, oxidative stress-induced breakdown of RPE barrier function was essentially unaffected by knock-out of both α isoforms of AMPK. The data presented above indicate that the α2 isoform of AMPK is the preferable target when designing new drugs aimed at protecting oxidative-stressed RPE cells due to the importance of this isoform in oxidative stress-induced inhibition of phagocytosis.

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