AMP-activated Protein Kinase Contributes to UV- and H₂O₂-induced Apoptosis in Human Skin Keratinocytes*

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AMP-activated protein kinase or AMPK is an evolutionarily conserved sensor of cellular energy status, activated by a variety of cellular stresses that deplete ATP. However, the possible involvement of AMPK in UV- and H₂O₂-induced oxidative stresses that lead to skin aging or skin cancer has not been fully studied. We demonstrated for the first time that UV and H₂O₂ induce AMPK activation (Thr172 phosphorylation) in cultured human skin keratinocytes. UV and H₂O₂ also phosphorylate LKB1, an upstream signal of AMPK, in an epidermal growth factor receptor-dependent manner. Using compound C, a specific inhibitor of AMPK and AMPK-specific small interfering RNA knockdown as well as AMPK activator, we found that AMPK serves as a positive regulator for p38 and p53 (Ser15) phosphorylation induced by UV radiation and H₂O₂ to trigger pro-apoptotic effect of AMPK on UV- or H₂O₂-treated cells. Collectively, we conclude that AMPK contributes to UV-induced apoptosis in human skin keratinocytes and plays important roles in UV-induced signal transduction ultimately leading to skin photoaging and even skin cancer.

Ultraviolet (UV) spectrum is divided into UVC (200–280 nm), UVB (280–320 nm), and UVA (320–400 nm). UVB and UVA are of environmental significance and social concern, because UVC is filtered through the ozone layer. UV penetrates into the papillary area of the dermis (~0.2 mm) and induces DNA damages of residing keratinocytes and dendritic cells. They are perturbed both phenotypically and functionally undergoing apoptosis upon UV radiation (1, 2). Previous studies in human keratinocytes in vitro and in human skin in vivo have demonstrated that UV response comprises trans-activation of cell surface growth factor receptors, such as EGFR, and their attendant downstream signal transduction machinery such as MAPK and phosphatidylinositol 3-kinase/AKT (2–6). Although MAPK, including JNK and p38, is responsible for UV-induced apoptosis, other cellular signals such as PI3K/AKT (protein kinase B) serve as survival signals and reduce UV-induced widespread cell death. We also observed that AMPK serves as a negative regulator against UV-induced mTOR (mammalian target of rapamycin) activation in a TSC2-dependent manner. Inhibiting mTOR activity can induce AMPK activation. However, the question how AMPK is activated in cells remains to be answered.

AMPK is a heterotrimeric serine-threonine kinase that senses depletion of intracellular energy and responds by stimulating catabolic pathways that generate ATP (5, 6). One mechanism for sensing cellular energy levels involves allosteric activation of AMPK. Under conditions in which cellular energy demands are increased (such as enhanced cellular activities or cellular stresses) or when fuel availability is decreased (because of a reduced rate of glucose uptake), intracellular ATP is reduced and AMP levels rise. AMP then allosterically activates AMPK. In addition to allosteric activation, AMPK activity can be regulated by a mechanism involving covalent modification through the addition of a phosphogroup by other molecules such as LKB1 and CaMK or calmodulin-dependent protein kinase (5–10). A number of stimuli (11, 12) have been found that can induce AMPK activation. However, the question how UV radiation, the major cause of skin aging and skin cancer, activates AMPK remains unknown.

It is well established that the key function of AMPK is to regulate the energy balance within the cell. Once activated, AMPK phosphorylates downstream substrates, the overall effect of which is to switch off ATP consuming pathways (e.g.

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fatty acid synthesis and cholesterol synthesis) and to switch on catabolic pathways that generate ATP (e.g. fatty acid oxidation and glycolysis). Activation of AMPK requires phosphorylation of Thr172 in the activation loop of α subunit by upstream AMPK kinase (6, 13–15). AMPK activation also triggers a phosphorylation cascade that regulates the activity of various downstream targets, including transcription factors, enzymes, and other regulatory proteins, such as mTOR pathways (16), p53 (17), and p38 (18). However, the possible role of AMPK in UV-induced signal transduction and skin aging or cancer remains to be elucidated.

In this study, we found for the first time that UV and H₂O₂ induce AMPK activation and downstream ACC and PFK2 phosphorylation in cultured human skin keratinocytes and reactive oxygen species (ROS)-mediated EGFR trans-activation is involved in LKB1/AMPK activation. Using AMPK inhibitor (Compound C), AMPKα siRNA knockdown as well as AMPK activator AICAR, we found that AMPK is actively involved in UV- and H₂O₂-induced signal transduction and skin cell damage probably by positively regulating downstream p38, p53 activation, and inhibiting mTOR activation. Our study provides new insights into understanding the cellular and molecular mechanisms involved in UV-induced skin cell damage leading to skin aging and skin cancer.

EXPERIMENTAL PROCEDURES

UV Light Apparatus—As previously reported (19, 20), the UV-irradiation apparatus used in this study was a F36T12 EREVHO UV tubes. A Kodak film was mounted 4 cm in front of the plate. UV light of wavelength <290 nm. Irradiation intensity was monitored by a photontherapy radiometer (model P1, Fotodyne, Milwaukee, WI). Before UV irradiation, cells were washed two times with phosphate-buffered saline (PBS) and incubated in PBS for each well. Cells were then irradiated at the desired intensity without a plastic dish lid. After irradiation, cells were returned to incubation in fresh medium with treatments for various time points prior to harvest. Mouse skin dendritic cells (XS 106 cell line) were cultured in 10% fetal bovine serum in RPMI 1640 with granulocyte-macrophage colony-stimulating factor (Sigma).

Chemicals and Reagents—PD153035, AG1478, SB203580, and AMPK inhibitor (AMPKi, compound C) were from Calbiochem (San Diego, CA). EGFR (1005) antibody, goat anti-rabbit IgG-horseradish peroxidase, and goat anti-mouse IgG-horse-ridech (San Diego, CA). EGFR (1005) antibody, goat anti-rabbit IgG at appropriate dilutions and incubated at room temperature for 1 h. Antibody binding was detected using a enhanced chemiluminescence (ECL) Detection system from GE Healthcare following the manufacturer’s instructions and visualized by fluorescence detection.

Activity—The in vitro kinase assay was described previously (16, 21). Briefly, 20–40 μg of proteins were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking with 10% milk in Tris-buffered saline, membranes were incubated with specific antibodies in a dilution buffer (2% bovine serum albumin in Tris-buffered saline) overnight at 4 °C followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG at appropriate dilutions and incubated at room temperature for 1 h. Antibody binding was detected using a chemiluminescence (ECL) Detection system from GE Healthcare following the manufacturer’s instructions and visualized by fluorescence detection.

AMPKα RNA Interference Experiments—As described previously (22), siRNA for AMPKα1/α2 (sc-45312) was purchased from Santa Cruz Biotechnology, Santa Cruz, CA) and 30 μL of protein A/G-agarose beads at 4 °C for 3 h. Following the incubation, beads were washed 3 times with lysis buffer and 2 times with kinase buffer containing 25 mM HEPES, pH 7.4, 50 mM KCl, 20% glycerol, 10 mM MgCl₂, 4 mM MnCl₂, 1 mM dithiothreitol, 1 mM glycerophosphate, and 1 mM Na₃VO₄. After the final wash, beads were assayed for kinase activity against purified 4E-BP1 substrate by adding 4E-BP1 substrate (Santa Cruz Biotechnology) to the beads for 30 min at 30 °C. The reactions were terminated by boiling in the presence of 1× SDS sample buffer. The samples were subjected to SDS-PAGE, and phosphorylation of 4E-BP1 was detected by Western blotting using anti-phospho-4E-BP1 (Ser65) antibody.

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the final AMPKα siRNA concentration of 100 nM. AMPKα protein expression was determined by Western blot.

**Cell Viability Assay (MTT Dye Assay)—**Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (20). Briefly, cells were collected and seeded in 96-well plates at a density of $2 \times 10^5$ cells/cm$^2$. Different seeding densities were optimized at the beginning of the experiments (data not shown). After incubation for 24 h, cells were exposed to fresh medium containing reagents at 37 °C. After incubation for up to 24 h, 20 µl of MTT tetrazolium (Sigma) salt dissolved in Hank’s balanced salt solution at a concentration of 5 mg/ml was added to each well and incubated in a CO$_2$ incubator for 4 h. The medium was aspirated from each well and 150 µl of dimethyl sulfoxide (Sigma) was added to dissolve formazan crystals and the absorbance of each well was obtained using a Dynatech MR5000 plate reader at a test wavelength of 490 nm with a reference wavelength of 630 nm.

**Assessment of the Percentage of Apoptotic Cells—**To detect apoptotic cells (20), cells were stained with DNA binding dye Hoechst 33342 (Sigma). After the cells were exposed to UV and the test compounds for the allotted time periods, they were fixed with 4% formaldehyde in PBS for 10 min at 4 °C, and then washed with PBS. To stain the nuclei, cells were incubated for 20 min with 20 µg/ml of Hoechst 33342. After washing with PBS, the cells were observed under a fluorescence microscope (Zeiss Axiophoto 2, Carl Zeiss, Germany). Cells exhibiting condensed chromatin and fragmented nuclei were scored as apoptotic cells. A minimum of 200 cells were scored from each sample.

**Measurement of Keratinocytes Mitochondrial Membrane Potential—**HaCaT cell mitochondrial membrane potential ($\Delta \Psi_m$) was assessed with fluorescent probe...
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**RESULTS**

**UV Radiation and \( H_2O_2 \) Induces AMPK Activation in Cultured Human Skin Keratinocytes**—To investigate the role of AMPK in UV signaling, we first tested whether UV or \( H_2O_2 \) induces AMPK activation using cultured human skin keratinocytes (HaCaT cells). The results showed that UV radiation induces AMPKα phosphorylation in a dose- and time-dependent manner (Fig. 1, A–D). Furthermore, as expected, AMPK activation by 5-aminoimidazole-4-carboxamide-1β,4-ribonofuranoside (AICAR) also induces AMPK activation in a dose- (Fig. 1, E and F) and time (Fig. 1, K and L)–dependent manner. AMPKα–specific siRNA down-regulates AMPKα expression and largely inhibits UV- and \( H_2O_2 \)-induced AMPK activation (Fig. 1, M and N). UV also induces AMPK activation in cultured human skin fibroblasts (Fig. 1O) and dendritic cells (X5 106 cell line) (Fig. 1P).

**ROS-mediated EGFR Activation Is Involved in UV-induced LKB1/AMPK Activation**—The data above show that both UV and \( H_2O_2 \) induce AMPK activation (AMPKα phosphorylation at Thr\(^{172} \)) in HaCaT cells. However, the cellular signals involved in this AMPK activation are not fully studied. Previous studies using human skin keratinocytes and dendritic cells have revealed a critical role of EGFR in UV-induced cellular signals (3, 26), studies also show a critical role of LKB1 for AMPK activation. As expected, in this study, we observed that both UV and \( H_2O_2 \) induce EGFR activation in HaCaT cells (Fig. 2, A and B). Interestingly, UV also induces LKB1 phosphorylation in a time- and dose-dependent manner in HaCaT cells (Fig. 2, C and D). EGFR inhibitor PD 153035 and AG 1478 inhibit both LKB1/AMPK activation (Fig. 2E). Together, these data further confirm the key role of EGFR activation, EGFR knock-out is shown in Fig. 2G, UV induces LKB1/AMPK phosphorylation of signal but not in EGFR knock-out HaCaT cells. Furthermore, the induction of AMPK is also inhibited by pretreatment with the antioxidant NAC and pyrrolidine dithiocarbamate (Fig. 2H) in UV-treated HaCaT cells. Antioxidant NAC and EGFR inhibitor PD 153035 also reduce \( H_2O_2 \)-induced LKB1/AMPK activation (Fig. 2I). As expected, UV- and \( H_2O_2 \)-induced ROS production is inhibited by NAC pretreatment (Fig. 2J). Collectively, our data suggest that ROS-mediated EGFR trans-activation is involved in UV-induced LKB1/AMPK activation.

**AMPK Is Involved in UV- and \( H_2O_2 \)-induced p38 Activation**—Because previous studies have suggested that p38 MAPK is a downstream signal of AMPK upon various stimuli (18, 27, 28), and UV induces p38 activation (24), next we tested the possible role of AMPK in UV- and \( H_2O_2 \)-induced p38 activation.
demonstrated in Fig. 3, A and B, compound C (AMPKi), an AMPK inhibitor, largely impairs the activation of p38 MAPK upon UV radiation, whereas SB203580, a p38 inhibitor, does not affect AMPK activation. Furthermore, AMPK also positively regulates H2O2-induced p38 activation, because AMPKi inhibits H2O2-induced p38 activation, whereas AICAR, an AMPK activator, enhances it (Fig. 3, C and D). AICAR alone also induces p38 activation, which is reversed by compound C (Fig. 3, E and F). To further confirm the role of AMPK in UV-induced apoptosis, HaCaT cells with or without AMPKα siRNA were treated with UV radiation (25 mJ/cm²) or H2O2 (250 μM) for 1 h, p-p38 and T-p38 were detected by Western blot (G). *, p < 0.05 versus untreated group. #, p < 0.05 versus same time point as the UV-treated group. Data are presented as the mean ± S.E. for three independent experiments.
**FIGURE 5.** UV and H₂O₂ induce phosphorylation of ACC and PFK2. HaCaT cells were treated with different doses of UV (5, 15 and 25 mJ/cm²) and cultured for 120 min (A) or treated with 25 mJ/cm² of UV and cultured for the indicated time points (0, 30, 60, and 120 min) (C). p-ACC (Ser79) and β-actin were detected by Western blot. ACC phosphorylation was quantified (B and D). HaCaT cells were also treated with different doses of H₂O₂ (50, 125, and 250 μM) and cultured for 120 min (E) or treated with 250 μM H₂O₂ and cultured for the indicated time points (G). p-ACC (Ser79) and β-actin were detected by Western blot. ACC phosphorylation was quantified (F and H). HaCaT cells were pre-treated with AMPK activator AICAR (2 mM) or inhibitor compound C (AMPKi, 10 μM) for 1 h, followed by UV (25 mJ/cm²) (I and J) or H₂O₂ (250 μM) (K and L) for different time points (30, 60, and 120 min), p-ACC (Ser79) and β-actin were detected by Western blot. HaCaT cells were also treated with UV and H₂O₂ for the indicated time points, p-PFK2 (Ser466) and β-actin were detected by Western blot (M). HaCaT cells were pre-treated with AMPK inhibitor compound C or AICAR for 1 h, followed by UV radiation, p-PFK2 and β-actin were detected by Western blot (N). HaCaT cells were treated with compound C for 1 h, followed by AMPK activator AICAR (2 mM) for different time points (30, 60, 90, and 120 min), p-PFK2 (Ser466) and β-actin were detected by Western blot (O). *, p < 0.05 versus untreated group. **, p < 0.05 versus the same time point as the UV-treated group. Data are presented as the mean ± S.E. for three independent experiments.
induced p38 activation, AMPKα siRNA was used. As demonstrated in Fig. 3G, activation of p38 in response to UV or H$_2$O$_2$ is inhibited in AMPKα siRNA-treated HaCaT cells. Collectively, our data suggest that AMPK is involved in UV- and H$_2$O$_2$-induced p38 activation.

**AMPK Positively Regulates UV- and H$_2$O$_2$-induced Phosphorylation of p53**—p53 tumor suppressor exerts anti-proliferative effects, including growth arrest, apoptosis, and cell senescence, in response to UV radiation (29). Its activation form (both phosphorylation and acetylation) is regulated by a number of proteins such as ATM (30), Chk1/2 (30), M2M, p300 (31), and SIRT1 (15, 32). However, whether AMPK also affects UV-induced p53 activation is unknown. As shown in Fig. 4, UV- and H$_2$O$_2$-induced phosphorylation of p53 is blocked by EGFR inhibitors (PD and AG) and antioxidant NAC, suggesting that the upstream components of AMPK regulate p53 activation. Furthermore, AMPK inhibitor compound C (AMPKi) and p38 inhibitor SB 203580 inhibit UV-induced phosphorylation p53 (Fig. 4, C and D), suggesting the positive regulatory role of AMPK activation in UV-induced p53 phosphorylation, which is consistent with recent studies demonstrating that AMPK directly phosphorylates p53 at a specific site (33). To further confirm this notion, siRNA-mediated AMPKα knockdown was used. As shown in Fig. 4E, phosphorylation of p53 in AMPKα knockdown cells is impaired, compared with control siRNA-treated cells. Collectively, our data suggest that AMPK positively regulates UV- and H$_2$O$_2$-induced phosphorylation of p53.

**UV Radiation Induces ACC and PFK2 Phosphorylation in an AMPK-dependent Manner**—AMPK was originally perceived as a regulator of cellular energy balance that acts in a cell-autonomous manner. AMPK activation in UV-induced apoptosis

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AMPK Negatively Regulates UV-induced mTOR Activation in TSC2-dependent Manner—AMPK has been shown to serve as a negative signal against mTOR activation in a number of cell models by phosphorylating activation of TSC2 leading to mTOR inhibition (5, 8). Our previous studies have demonstrated that UV activates mTOR in cultured human skin keratinocytes. However, the possible role of AMPK in UV-induced mTOR activation is not fully elucidated. In this study, as demonstrated in Fig. 6, A–D, AMPK inhibitor enhances both UV- and H2O2-induced S6K and 4E-BP1 phosphorylation (indicators of mTOR activation), whereas AICAR inhibits them. Furthermore, UV and H2O2 induce S6K activation in wild-type but not in TSC2 knock-out (where S6K is overactivated by H2O2) (Fig. 6). To further confirm the effects of AMPK on UV-induced mTOR activation, AMPK knock-down was used. As demonstrated in Fig. 6, I and J, AMPKα knock-down enhances UV- and H2O2-induced S6K phosphorylation. Furthermore, compound c also enhances UV-induced mTOR phosphorylation at Ser2448 (Fig. 6K) and mTOR activity (Fig. 6L), whereas AICAR inhibits them and AMPK siRNA enhances UV-induced mTOR activity (Fig. 6, M and N).

AMPK Activation Contributes to UV-induced Skin Cell Damage—Because published studies have demonstrated the critical role of AMPK activation in cell death or apoptosis (33), we next examined the functional results of AMPK activation in UV-treated cells. As shown in Fig. 7, A–D, although AMPK inhibitor compound C inhibits UV-induced ACC phosphorylation, AICAR, AMPK activator, enhances it (Fig. 5, I–L). UV and H2O2 also induce PKF2 phosphorylation in a time- and dose-dependent manner. Furthermore, whereas AMPK inhibitor compound C inhibits UV-induced ACC phosphorylation, AICAR, AMPK activator, enhances it (Fig. 5, I–L). UV and H2O2 also induce PKF2 phosphorylation (Fig. 5M), which is largely impaired by AMPK inhibitor pretreatment, but is enhanced by AICAR pretreatment (Fig. 5N). AICAR alone also induces PKF phosphorylation, which is blocked by the AMPK inhibitor (Fig. 5O). These results suggest that AMPK activation might also be involved in metabolic regulation in skin keratinocytes in response to UV radiation.

restore the energy state of the cell by acutely and chronically enhancing processes that generate ATP, such as fatty acid oxidation by phosphorylation and inhibiting acetyl-CoA carboxylase or ACC (6). AMPK also phosphorylates and activates PFK2 to restore ATP under anaerobic conditions (34). We next investigated whether UV radiation also activates those two key enzymes. As indicated in Fig. 5, A–H, both UV and H2O2 induce ACC phosphorylation in a time- and dose-dependent manner. Furthermore, whereas AMPK inhibitor compound C inhibits UV-induced ACC phosphorylation, AICAR, AMPK activator, enhances it (Fig. 5, I–L). UV and H2O2 also induce PKF2 phosphorylation (Fig. 5M), which is largely impaired by AMPK inhibitor pretreatment, but is enhanced by AICAR pretreatment (Fig. 5N). AICAR alone also induces PKF phosphorylation, which is blocked by the AMPK inhibitor (Fig. 5O). These results suggest that AMPK activation might also be involved in metabolic regulation in skin keratinocytes in response to UV radiation.

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DISCUSSION

As well as the numerous metabolic targets of AMPK for which it is best known, AMPK has many other downstream effectors. Recent studies have revealed that AMPK inhibits cell growth and proliferation, and also positively regulates apoptosis (33). These findings are not surprising, given that cell growth, DNA replication, and mitosis are all major consumers of ATP, and also that the upstream regulator of AMPK, LKB1, is known to be a tumor suppressor. In this study, we discovered that UV and H2O2 induce AMPK activation in cultured skin cells (Fig. 1). ROS-mediated EGFR trans-activation is involved in UV-induced LKB1/AMPK activation (Fig. 2). We also observed that AMPK activation contributes to UV- and H2O2-induced skin cell damage (Fig. 7) probably by positively regulating p38 (Fig. 3) and p53 phosphorylation (Fig. 4) and inhibiting the mTOR pathway (Fig. 6). Collectively, we propose cell signaling pathways involved in UV-induced AMPK activation, as depicted in Fig. 8.

Although we found that UV-induced AMPK activation consistently occurs in three different cell types (Fig. 1), our results seem inconsistent with what are mentioned in a recent study (35), which showed that UV radiation reduces AMPK activity (Thr172 phosphorylation) via down-regulation of LKB1 expression. This may be due to the difference of tested time points between previous work and our study. Instead of time course (15, 30, 60, 120, 180, 240, and 480 min as we tested in this study) (Figs. 1, C and D, and 2C), a previous study reported the data collected at only 4-h time points (35). We show here that AMPK phosphorylation begins at 0.5–1 h and lasts for at least 4–6 h after 25 ml/cm² of UV radiation (Fig. 2C). Also we are unable to see LKB1 expression change even at 4–6 h after UV radiation (Fig. 2C). Instead, we see clearly LKB1 phosphorylation after UV radiation at earlier time points in HaCaT cells (Fig. 2, C–E), and this LKB1 phosphorylation lasts for about 4 h (Fig. 2, C and D). Thus, LKB1 phosphorylation might serve as an upstream activator of AMPK phosphorylation induced by UV. We propose that UV/LKB1/AMPK phosphorylation is mediated by UV-induced ROS because ROS is known to be a strong AMPK activator as shown in a number of studies (36–38), and more importantly, in our studies, antioxidants such as NAC and pyrrolidine dithiocarbamate inhibit UV-induced AMPK phosphorylation (Fig. 2H). Another explanation is that the dose of UV used in our study is slightly different from a previous study (35) where as high as 37.5 ml/cm² of UV was used, which might cause some nonspecific effects. Interestingly, in our study, at 4 h, UV-induced AMPK phosphorylation is most pronounced at 25 ml/cm² (Fig. 2D). However, even at a very high dose of UV (35 and 45 ml/cm²), we can still see enhanced AMPK/LKB1 phosphorylation (rather than decrease) (Fig. 2D).

LKB1 phosphorylates and activates the catalytic α-subunit of AMPK at its T-loop residue Thr172 in a cell-free system (7, 39). AMPK is minimally activated in cells that lack or have decreased LKB1 expression and LKB1 forms a heterotrimeric complex, with regulatory proteins termed STRAD and MO25, which are required for its activation and cytosolic targeting (40). Thus, LKB1 plays a crucial role in activating AMPK. In this study, we found that UV (Fig. 2, C and F) and H2O2 (Fig. 2G) induce LKB1 phosphorylation. However, because of limitation of experimental materials, we can only presume that LKB1 is upstream for AMPK activation upon UV and ROS stimulation. Further experiments using LKB1 deficiency cells are necessary to confirm our hypothesis. Furthermore, the question whether LKB1 activation plays other important roles in UV-induced skin cell damage remains to be further addressed.

p38 is involved in inflammation, cell growth and differentiation, cell cycle, and cell death (41, 42). Recent studies have indicated that AMPK serves as an upstream signal for p38 activa-
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by increasing recruitment of p38 MAPK to TAB1. And yet, other studies have yielded different conclusions (27, 28, 43). Our studies using AMPK inhibitor and AMPKα siRNA knockdown have demonstrated that inhibition of AMPK impairs p38 activation in response to UV or H\(_2\)O\(_2\) (Fig. 3, A–D and G). We conclude that AMPK may serve as a positive regulator for p38 activation upon UV radiation, which might contribute to the pro-apoptotic effect of AMPK on UV-treated cells (Fig. 7G).

AMPK activation causes a G1/S phase cell cycle arrest in different cell lines, and this is associated with accumulation of tumor suppressor p53 and cyclin-dependent kinase inhibitors p21 and p27, which act downstream of p53 (17, 44–46). This process involves phosphorylation of p53 at Ser\(^{15}\) (17, 44–46), although it has not been shown that there is a direct phosphorylation. In this study, as demonstrated in Fig. 4, AMPK activation serves as a positive regulator for UV-induced p53 phosphorylation (Ser\(^{15}\)). However, as seen in Fig. 4, C–E, UV- and H\(_2\)O\(_2\)-induced p53 phosphorylation is reduced (but not largely impaired nor totally blocked) as a result of AMPK inhibition, which suggest that UV-induced p53 phosphorylation is not solely dependent on AMPK activation, and AMPK may be one of many molecules that phosphorylate p53 upon UV radiation. We propose that this positive regulatory effect of AMPK on p53 phosphorylation might contribute to its pro-apoptotic effect (Fig. 7H).

AMPK activation may also inhibit cell growth and survival because of its general effects on biosynthesis, including the ability to inhibit fatty acid synthesis and the mTORC1 pathway (48). AKT/PKB phosphorylates TSC2 at sites that inhibit its Rheb-GAP activity, and this might have the opposite effect. Our results suggest that UV radiation induces the activation of AMPK in specific cell lines, and this AMPK activation serves as a positive regulator for UV-induced p53 phosphorylation. In this study, as demonstrated in Fig. 4, AMPK may serve as a positive regulator for p38 phosphorylation and activation of TSC2 explains the inhibitory effect of AMPK in UV-induced mTOR activation. In this study, using AMPK inhibitor and specific siRNA knockdown as well as AMPK activator AICAR and TSC2-deficient cells, we found that AMPK serves as a negative feedback signal against UV-induced mTOR activation (Fig. 6), which may also be involved in the pro-apoptotic effects of AMPK (Fig. 7A).

AMPK stimulates catabolic pathways that generate ATP, including the fatty acid oxidation pathway. Most fatty acids are oxidized in mitochondria, and their entry into the organelle is blocked by malonyl-CoA, which inhibits carnitine palmitoyltransferase-1, an enzyme required for the transport of fatty acids across the inner mitochondrial membrane. Malonyl-CoA is produced by one of the target enzymes for AMPK, acetyl-CoA carboxylase, which exists as two isozymes, ACC1 (α) and ACC2 (β) (5). Both are phosphorylated and inactivated by AMPK. In this study, we also discovered that UV and H\(_2\)O\(_2\) induce ACC phosphorylation and inactivation in an AMPK-dependent manner (Fig. 5), which might be responsible for reduced fat accumulation or adipogenesis (49). Furthermore, we also found that PFK2, the key protein of glycolysis is also phosphorylated upon UV radiation in an AMPK-dependent manner (Fig. 5). Based on these observations, together with the critical role of AMPK in energy balancing and metabolism, we propose that the metabolic system might be impaired after UV radiation in human skin cells, which might be another critical mechanism responsible for skin cell damage. The fat accumulation and energy storage in human skin cells might also be impaired (as the possible consequence of AMPK-dependent ACC phosphorylation in keratinocytes). Thus, this study provides novel insights into understanding the cellular mechanisms involved in UV-induced skin aging or skin cancer.

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