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Original Article

The roles of AMPK-mediated autophagy and mitochondrial autophagy in a mouse model of imiquimod-induced psoriasis

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Abstract: Background: Psoriasis is a systemic inflammatory disease characterized by epidermal hyperplasia and skin inflammatory infiltrates. Inactivation of AMPK has been shown to decrease autophagy, thereby inhibiting elimination of inflammatory factors and harmful substances, and aggravating psoriasis. However, the molecular mechanism through which AMPK affects psoriasis remains to be further explored. In this study, we investigated whether AMPK regulates autophagy through the ULK1/Atg7 signaling pathway and regulates mitochondrial autophagy through the PINK1/Parkin signaling pathway, thereby affecting a mouse model of psoriasis. Methods: Imiquimod was used to induce psoriasis-like lesions on the backs of mice. The severity of skin lesions in psoriatic mice was evaluated with the skin inflammation severity score, and epidermal thickness was measured on the basis of H&E staining. RT-PCR, western blotting and immunofluorescence staining were used to detect indicators of autophagy and mitochondrial autophagy. Results: AMPK activity was inhibited in the psoriasis mouse model, the autophagy-associated proteins ULK1/Atg7 were inhibited, and the mitochondrial autophagy proteins PINK1/Parkin were also decreased. Results indicated that autophagy and mitochondrial autophagy were inhibited in the mouse model. When AMPK signaling was upregulated, ULK1/Atg7 and PINK1/Parkin were upregulated, autophagy and mitochondrial autophagy increased, and skin lesions in the mouse model were alleviated. ULK1/Atg7 and PINK1/Parkin were down-regulated when AMPK signaling was downregulated, and psoriasis-like skin lesions were aggravated in mice. These results indicated that AMPK regulates autophagy through the ULK1/Atg7 signaling pathway and regulates mitochondrial autophagy through the PINK1/Parkin signaling pathway, thus affecting the prognosis of psoriasis in the mouse model. Conclusion: AMPK affects the prognosis of psoriasis in a mouse model by regulating autophagy and mitochondrial autophagy.

Keywords: AMPK, ULK1, autophagy, PINK1, mitochondrial autophagy, psoriasis

Introduction

Psoriasis is one of the most common chronic, relapsing, immune-mediated inflammatory skin diseases [1]. The main clinical symptoms of patients with psoriasis are sharply distinguished by silvery white scales, which can be seen on body parts such as elbows, the scalp, umbilicus, and lumbar area [2]. However, the pathogenesis of psoriasis is not fully understood. Other than hereditary predisposition and environmental factors, an abnormal immune response is regarded as the main cause of psoriasis and is closely involved in the disease [3, 4]. The prevalence of psoriasis is approximately 0.5-11.4% of adults and 0-1.4% of children worldwide [5, 6]. Although psoriasis itself is seldom life-threatening, people with psoriasis are at increased risk of comorbidities such as metabolic syndrome, cardiovascular disease, asthma, and inflammatory bowel disease [7].

Exploring the pathogenesis of psoriasis is helpful to identify effective targets for treatment.

Studies have shown that the AMP activated protein kinase (AMPK) signaling pathway is closely associated with psoriasis [3]. Moreover, psoriasis may be associated with AMPK signaling pathway mediated autophagy and mitochondrial autophagy [8].

AMPK, an enzymatic complex regulating energy metabolism, has a key role in regulating the immune homeostasis network. It is also involved in the molecular mechanisms underlying the pathophysiology of chronic inflammatory diseases. AMPK is consequently involved in the regulation of immune diseases such as atherosclerosis, psoriasis, joint inflammation, and inflammatory bowel disease [9]. Studies have shown that AMPK activity is inhibited in psoriasis [10]. AMPK is a key energy sensor that promotes autophagy by regulating cell metabolism, thereby maintaining energy homeostasis. Autophagy is a process through which components of the cell are degraded so that the essential activity and viability can be maintained in the presence of nutrient limitation [11]. During this process, AMPK can be activated by phosphorylation, and the activated AMPK regulates a variety of metabolic processes including autophagy. AMPK directly promotes autophagy by phosphorylation of autophagy-related proteins such as mTORC1 and ULK1, or indirectly promotes autophagy by affecting the expression of downstream autophagy-related genes. AMPK promotes autophagy by directly activating Ulk1 through phosphorylation of Ser 317 and Ser 777. The mechanism of regulation of Ulk1, an autophagy-initiating kinase, is central to understanding autophagy regulation. This study demonstrates a biochemical mechanism of Ulk1 activation by upstream signals and the functional importance of this regulation in autophagy induction. AMPK, though a coordinated cascade of phosphorylation, activates Ulk1 kinase. This coordinated phosphorylation is important for the role of Ulk1 in autophagy induction. ULK1 regulates autophagy by modulating the expression of Atg7, in a regulatory mode of autophagy [12, 13].

In addition, AMPK participates in mitochondrial autophagy by regulating the PTEN-induced kinase 1 (PINK1)/Parkin signaling pathway. PINK1 is a serine/threonine kinase active in mitochondria. A study on Parkinson has shown that the PINK1-Parkin signaling cascade is an important pathway mediating mitochondrial autophagy [14]. PINK1 is inserted into the intima and intermembranous spaces of mitochondria through a translocator that binds the mitochondrial outer membrane complex [14]. AMPKM KO mice show significant mitochondrial disease and accumulation of the autophagy protein P62 [15]. However, overexpression of AMPK promotes PINK1 accumulation in the mitochondrial outer membrane and the direct phosphorylation of Parkin at ser6580 by PINK1 [16], thus causing Parkin aggregation in the cytoplasm of damaged mitochondria and the subsequent recruitment of the autophagy receptor LC3, thereby leading to mitochondrial autophagy and the clearance of damaged mitochondria through autophagy [17, 18]. Parkin overexpression enhances mitochondrial autophagy, whereas Parkin deficiency leads to increased mitochondrial dysfunction [19].

At present, the understanding of psoriasis is not comprehensive, and effective targets for psoriasis treatment remain to be further explored. The purpose of this study was to investigate the relationship between the AMPK signaling cascade and autophagy and mitochondrial autophagy in a mouse model of psoriasis. By activating and inhibiting AMPK signaling, the expression of the associated proteins in ULK1/Atg7 and PINK1/Parkin signaling pathways and their effects on psoriasis were examined to explore the role and molecular mechanism of AMPK in psoriasis.

Materials and methods

Study design and experimental groups

Experimental animals: All experiments received approval from the Institute of Animal Care Committee of Zhangjiagang Traditional Chinese Medicine Hospital (Zhangjiagang, China) and were conducted according to the guidelines on the care and use of animals of the National Institutes of Health. SPF female BALB/c mice 6-8 weeks of age and weighing approximately 20 g were provided by the Shanghai JSJ Experimental Animal Co., Ltd. The mice were housed under constant temperature and relative humidity, and regular light/dark cycles. Food and water were readily available.

Establishment of psoriasis model mice and experimental groups: The mice were divided into five groups: a control group, Imiquimod (IMQ) group, IMQ + vehicle group, IMQ + AICAR group, and IMQ + compound C (CC) group. The hair was shaved on the backs of all mice with a hair shaving machine, thus, exposing a skin area of approximately 2 cm×3 cm. In the control group, petroleum jelly was applied to the shaved areas on the backs of mice once per day for 8 consecutive days. In the IMQ group, 62.5 mg 5% IMQ (Aldara 3M Health Limited, UK) cream was applied to the shaved areas on the backs of mice once per day for 8 consecutive days [18, 19]. In the IMQ + AICAR group, the AMPK agonist AICAR (Cayman, USA, 100 mg/kg) was intraperitoneally injected once per day for 8 consecutive days [20]. In the IMQ + CC group, the AMPK inhibitor CC (Cayman, USA, 10 mg/kg) was intraperitoneally injected once per day for 8 consecutive days. In the IMQ + vehicle group, 10% DMSO was intraperitoneally injected once per day for 8 consecutive days.

Tissue collection and sectioning

After anesthesia, a skin area 2 cm in diameter was collected from the experimental area on the backs of the mice. Some skin tissues were soaked in 4% paraformaldehyde at 4°C and used as frozen sections. The rest of the tissue samples were promptly frozen and stored at -80°C until further use [21]. All procedures for tissue excision and selection were performed by two pathologists who were blinded to the experimental conditions.

Scoring the severity of skin inflammation

An objective scoring system was developed according to the clinical psoriasis area and severity index (PASI) to assess the severity of back skin inflammation. Infected skin areas were not included in the total score. Erythema, scaling, and thickening were rated independently on a scale of 0 to 4 as follows; 0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked. The cumulative score (erythema plus scaling plus thickening) served as a measure of the PASI of inflammation (scale 0-12) [22].

Hematoxylin and eosin (H&E) staining

Frozen sections were washed in deionized water and stained with hematoxylin (Baso, China) for 20 min, then washed in running water for 15 min; soaked in 1% hydrochloric acid ethanol; washed in ammonia water for 1 minute, and then rinsed in running water. After washing, samples were soaked in 50% ethanol, 70% ethanol, and 80% ethanol for 3 min, then stained with 0.5% eosin (Baso, China) ethanol solution for 3 min. The samples were soaked in 95% ethanol and absolute ethanol for 3 min, and then in equal amounts of xylene ethanol, xylene 1, and xylene 2 sequentially for 5 min. The samples were then sealed with neutral resin.

Western blot analysis

Western blot analysis was as previously described [23]. Skin tissue was homogenized in tissue protein extraction reagent with protease inhibitor cocktail (CWBIO, China) and incubated on ice for 20 min. The samples were centrifuged at 13000×g at 4°C for 20 min, the supernatant was collected, and the bicinchoninic acid (BCA) method and a Pierce TM BCA protein detection kit (Thermo Fisher, USA) were used to determine the protein concentration. Protein loading buffer (Beyotime, China) was added, and samples were boiled at 100°C for 8 min. Equivalent amounts of extracted proteins were loaded and electrophoresed with a TGX Stain-Free FastCast Acrylamide Kit (BIO-RAD, USA), then transferred to PVDF membranes (Millipore, USA). QuickBlock™ Western (Beyotime, China) was used to block the PVDF membranes for 30 min at room temperature. The primary antibodies rabbit anti-phospho-AMPK (CST, USA), rabbit anti-AMPK (CST, USA), rabbit anti-phospho-ULK1 (CST, USA), rabbit anti-ULK1 (CST, USA), rabbit anti-LC3B (CST, USA), rabbit anti-Atg7 (CST, USA), rabbit anti-p62 (CST, USA), rabbit anti-PINK1 (Abcam, UK), rabbit anti-Parkin (CST, USA), and rabbit anti-GAPDH (Sigma, USA) were incubated in a refrigerator at 4°C on a shaking table overnight. The membranes were then incubated with secondary antibodies, including goat anti-rabbit IgG-HRP (Invitrogen, USA), for 1 h at room temperature. Immunoblots were finally probed with Immobilon® Western Chemiluminescent HRP Substrate (Millipore, USA) and visualized with an imaging system (GE Healthcare Bio-Sciences AB, Sweden). All data were analyzed by ImageJ software (National Institutes of Health, United States).

Immunofluorescence staining

The procedure was as previously described [24]. The frozen tissue sections were immersed

in 1% acetone fixative solution for 4-5 μm, fixed at room temperature for 5 min, dried, and washed with PBS three times for 3 min. Then samples were incubated in 3% H_2O_2-methanol solution at room temperature for 10 min, soaked in PBS three times and incubated with goat serum at room temperature for 20 minutes. The primary antibodies rabbit anti-TOMM20 (Abcam, UK) and mouse anti-LC3B (Sant Cruz, USA) were added and incubated at 37°C in a wet box for 2 h. After samples were washed in PBS three times, the secondary antibodies goat anti-rabbit (Jackson ImmunoResearch, USA) and goat anti-mouse (Jackson ImmunoResearch, USA) were added and incubated in the dark at 37°C for 1 h. The samples were soaked and washed with PBS three times. DAPI (Key GEN, China) dye solution was then added and incubated at room temperature away from light for 5 min. Samples were treated with anti-fluorescence quenching solution ((Key GEN, China)) and observed under a fluorescence microscope (Olympus, Japan).

Real time-PCR

Total RNA was isolated from lesional skin tissues with TRIzol Reagent (Invitrogen, USA. According to the protocol provided by the manufacturer (Thermo Fisher, USA), complementary DNA was synthesized with 1 μg of total RNA. Real-time PCR was then performed with a QuantStudio™ Dx Real-Time PCR Instrument (Life Technologies Corporation, USA) with a PowerUp™SYBRTM Green Master Mix kit (Thermo Fisher, USA). The reaction was as follows: the template was denatured at 95°C for 2 min, and this was followed by 40 cycles of amplification (95°C for 15 s, 60°C for 15 s, and 72°C for 1 min). Three replicate wells were analyzed for each sample. The expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as the internal reference for each sample, and the relative mRNA expression levels of the target genes were calculated through relative quantification. The forward and reverse primer sequences of each gene are shown in **Figure 5A**.

Statistical analyses

All data are presented as mean ± standard deviation (SD). GraphPad Prism 8.0 software was used for all statistical analyses. One-way ANOVA for multiple comparisons and Student-Newman-Keuls post hoc test were used to determine the differences among groups; $P< 0.05$ was considered to indicate a significant difference.

Results

Effects of AMPK on IMQ-induced skin inflammation in the psoriasis mouse model

We first assessed whether IMQ induced skin inflammation and examined the effect of AMPK on skin inflammation in the psoriasis mouse model. IMQ induced epidermal thickening, erythema, and severe keratosis on the back skin in mice. No significant changes were observed on the skin in control mice (**Figure 1A**). H&E staining was used to evaluate the effects of AMPK signaling on the skin lesions in mice (**Figure 1F**, **1G**). Compared with the skin thickness of 24.83±1.169 μm in the control group, the skin thicknesses in the IMQ group and IMQ + vehicle group were 79.67±4.457 μm and 79±2.19 μm, respectively. The skin thicknesses in the IMQ + AICAR and IMQ + CC groups were 53.33±11.38 μm and 89.08±4.82 μm, respectively. These results suggested that AMPK signaling is involved in the regulation of skin inflammation, and overexpression of AMPK decreases skin thickness, in the psoriasis mouse model.

Effects of AMPK modulation on erythema, scaling, thickening, and PASI scores

Erythema, scaling, and thickening appeared on the skin of mice in the IMQ group and the IMQ + vehicle group, and the erythema score, scaling score, thickening score, and PASI score were significantly higher than those in the control group. Compared with the IMQ + vehicle group, the IMQ + AICAR group had higher scores, to different degrees. Compared with the IMQ + vehicle group, there was no significant difference in the scaling score of the IMQ + CC group, although the erythema, thickening and PASI scores were significantly higher (**Figure 1B-E**).

Effects of AMPK on autophagy-associated proteins in the psoriasis mouse model

p-AMPK expression was lower in the IMQ group and the IMQ + vehicle group than the control group. WB showed that p-AMPK expression was higher in the IMQ + AICAR group than the IMQ + vehicle group, and significantly lower in

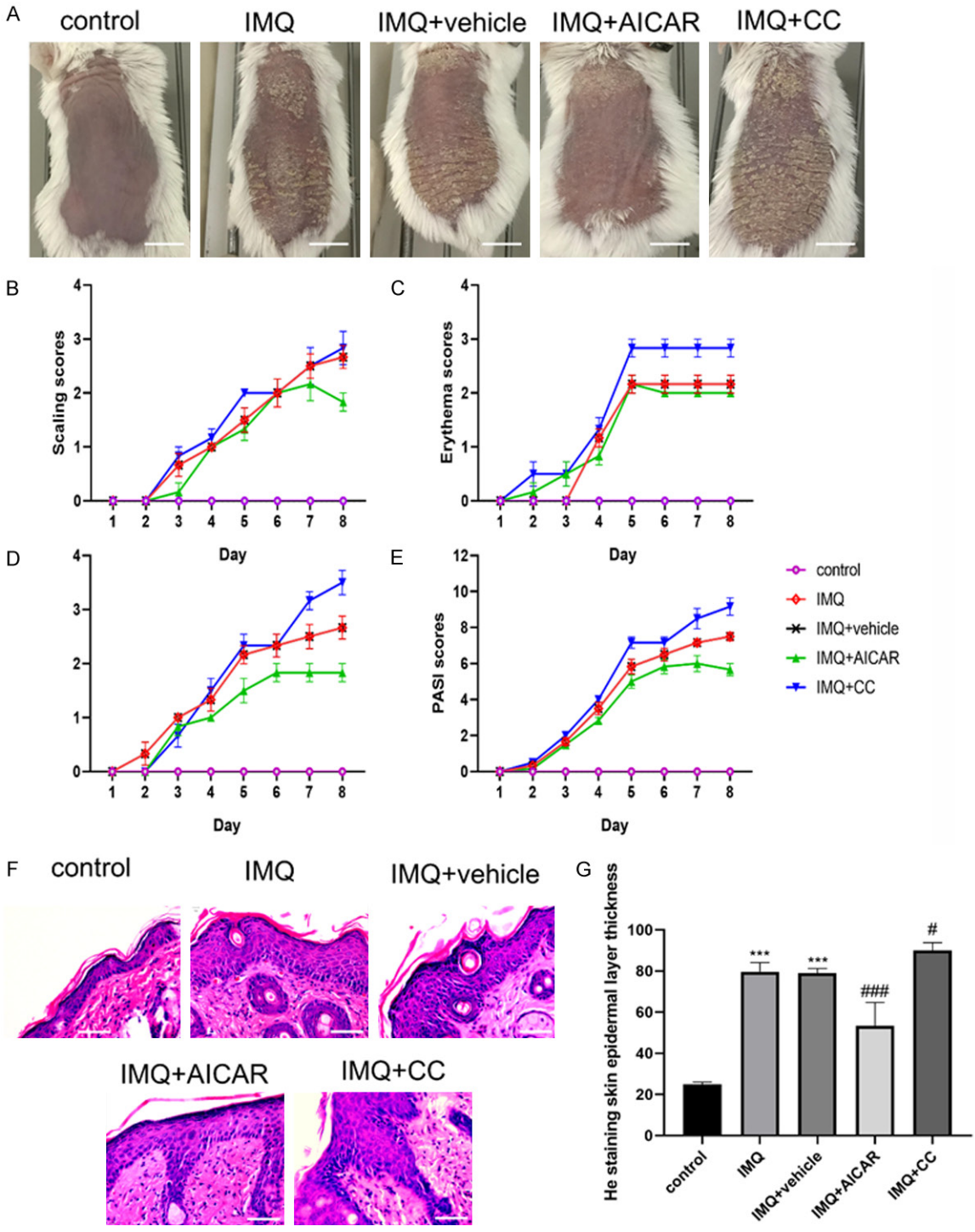

Figure 1. IMQ-induced skin inflammation in mice is similar to the psoriasis phenotype. BALB/c mice in the control group were treated with petroleum jelly, and those in the IMQ group, the IMQ + AICAR group, the IMQ + vehicle group, and the IMQ + CC group had IMQ applied to the back skin daily. Phenotypic manifestations in the dorsal skin in mice after 8 days of treatment (A). Scaly back skin on a daily scale of 0 to 4 for scaling scores, erythema scores, and thickness scores (B-D). The cumulative score (erythema plus scaling plus thickness) is depicted (E). HE staining was used to evaluate the skin thickness on the dorsal surfaces in mice (F, G). Statistical analysis was conducted with one-way ANOVA, then with a Student-Newman-Keuls post hoc test. The scale bar of (A) is 1 cm. The scale bar of (F) is 50 μm, the magnification is 400×. There were n=6 animals per group. Data are expressed as the mean ± SD. ***P<0.001, compared with the control group. $^{\#}$P<0.05, $^{\#\#\#}$P<0.001, compared with the IMQ + vehicle group.

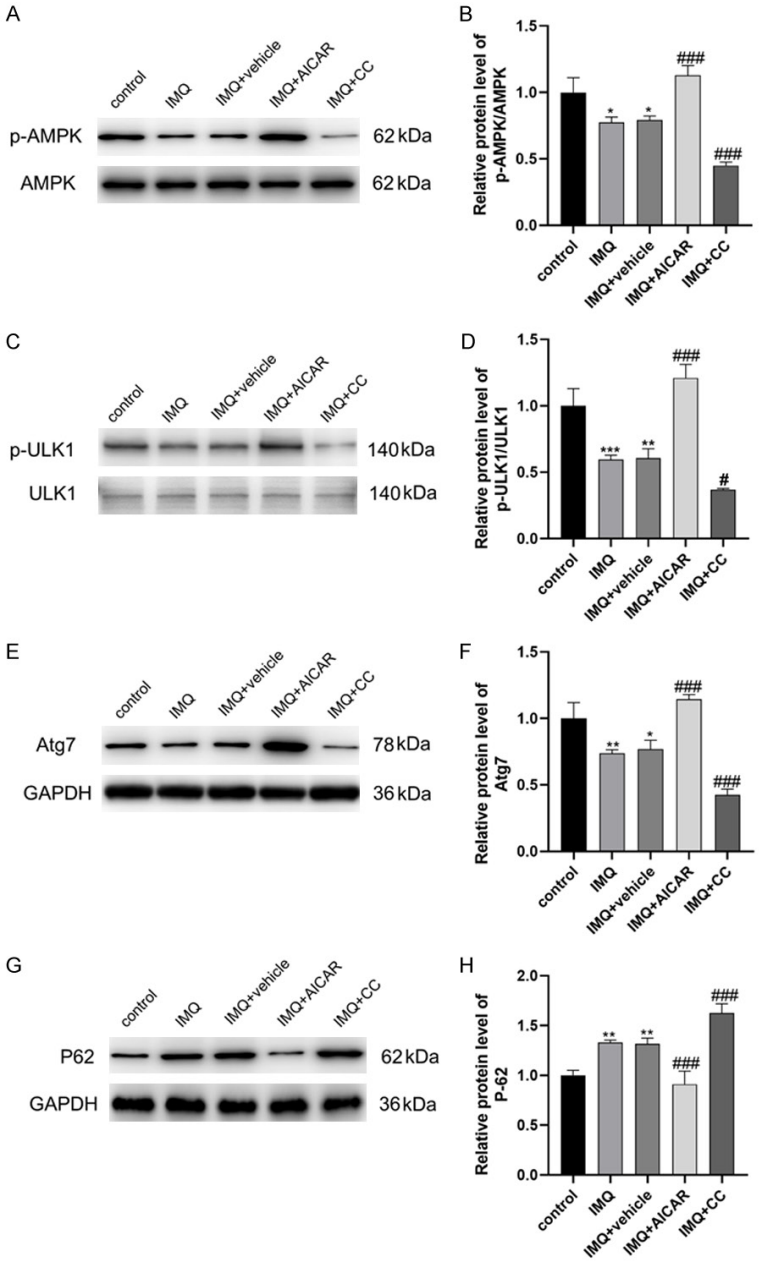

Figure 2. Expression levels of autophagy-associated proteins in mice after AMPK modulation. The protein-expression levels of AMPK and p-AMPK (A, B), ULK1 and p-ULK1 (C, D), Atg7 (E, F), and P62 (G, H) were assessed to determine the effects of AMPK modulation on back skin injury in a psoriasis mouse model. Statistical analysis was conducted with one-way ANOVA, then with a Student-Newman-Keuls post hoc test. There were n=6 animals per group. Data are expressed as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001, compared with the control group. $^{\#}$P<0.05, $^{\#\#\#}$P<0.001, compared with the IMQ + vehicle group.

the IMQ + CC group than the IMQ + vehicle group. No significant differences in AMPK levels were observed (**Figure 2A**, **2B**). The expression trend of p-ULK1 was approximately the same as that of p-AMPK, and the total amount of ULK protein did not significantly change, thus, indicating that AMPK and ULK1 function through phosphorylation in the psoriasis mouse model (**Figure 2C**, **2D**). Atg7 was also regulated by AMPK, and Atg7 was significantly diminished in the psoriasis mouse model. After AMPK agonist treatment, the Atg7 expression was greater than that in the IMQ + vehicle group. After AMPK inhibitor treatment, Atg7 was lower in the IMQ + vehicle group than the control group (**Figure 2E**, **2F**). The trend in P62 was approximately opposite from that of Atg7 (**Figure 2G**, **2H**).

Effects of AMPK on mitophagy-associated proteins in the psoriasis mouse model

We detected mitochondrial autophagy-associated proteins PINK1 and Parkin by WB. The expression of PINK1 and Parkin was lower in the IMQ group and the IMQ + vehicle group than the control group, and mitochondrial autophagy was inhibited. After AMPK agonist treatment, the expression of PINK1 and Parkin was up-regulated relative to that in the IMQ + vehicle group. After AMPK inhibitor treatment, the expression of PINK1 and Parkin was significantly lower than that in the IMQ + vehicle group (**Figure 3A-D**). These results indicated that the PINK1/Parkin signaling pathway was regulated by AMPK signaling in the psoriasis mouse model. We found that there was almost no LC3B in the mitochondrial outer membrane in mice in the IMQ group and the IMQ + vehicle group. LC3B was significantly higher in the IMQ + AICAR group than the IMQ +

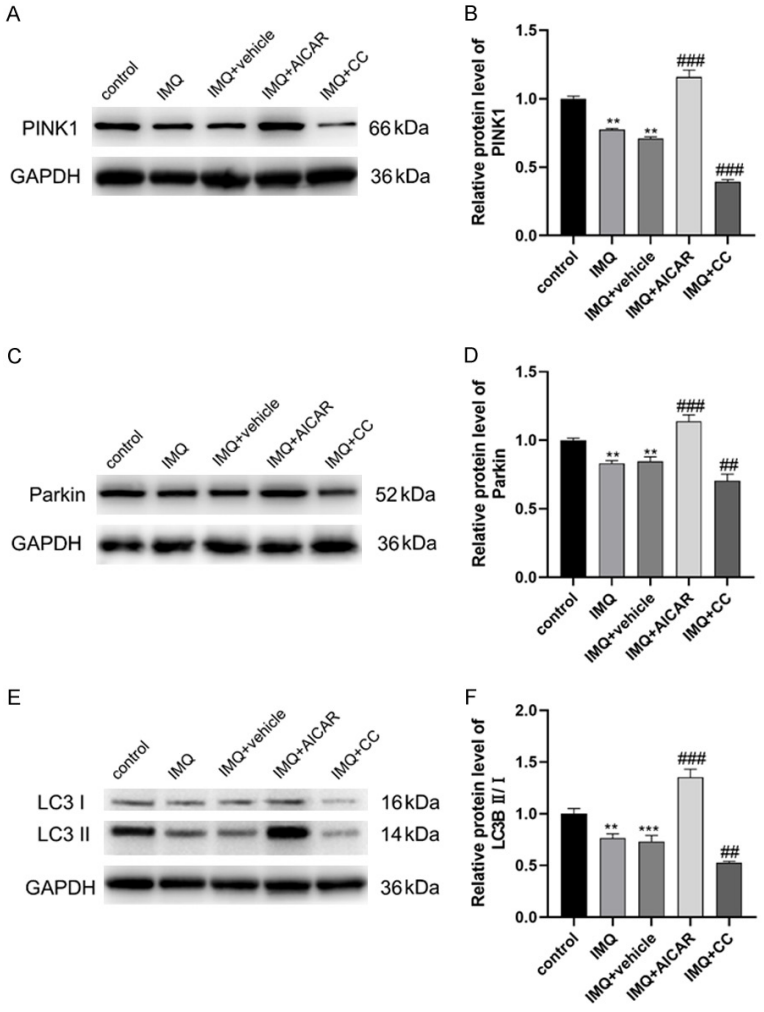

Figure 3. Expression levels of mitophagy-associated proteins in mice after AMPK modulation. The protein-expression levels of PINK1 (A, B), Parkin (C, D), and LC3 (E, F) were evaluated to determine the effect of AMPK modulation on autophagy in a psoriasis mouse model. Statistical analysis was conducted with one-way ANOVA, then with a Student-Newman-Keuls post hoc test. There were n=6 animals per group. Data are expressed as the mean ± SD. $^{**}P<0.01$, $^{***}P<0.001$, compared with the control group. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, compared with the IMQ + vehicle group.

vehicle group, whereas LC3B was inhibited in the IMQ + CC group compared with the IMQ + vehicle group (**Figure 4**). These findings were consistent with the results for LC3B protein (**Figure 3E**, **3F**).

Effects of AMPK modulation on mRNA expression of Atg7, P62, Parkin, and PINK1

The expression of Atg7 (**Figure 5B**), P62 (**Figure 5C**), PINK1 (**Figure 5D**), and Parkin (**Figure 5E**) in the control group, IMQ group, IMQ + vehicle group, IMQ + AICAR group, and IMQ + CC group were assessed by real-time PCR. The level of ATG7 mRNA in the IMQ group and the IMQ + vehicle group was lower than that in the control group. ATG7 mRNA levels were significantly higher in the IMQ + AICAR group, and lower in the IMQ + CC group, than in the IMQ + vehicle group. The mRNA expression trend for Parkin was approximately the same as that for ATG7. PINK1 and P62 showed opposite trends, and their mRNA levels increased in the IMQ and the IMQ + vehicle groups. The level in the IMQ + AICAR group was higher, and that in the IMQ + CC group was lower, than that of the IMQ + vehicle group. The sequences of PCR primers are shown in **Figure 5A**.

Discussion

IMQ is an effective immune activator. Large doses of local repeated application to the back skin of mice cause erythema, peeling, and keratinocyte hyperplasia with acanthosis, all which are consistent with the conditions of psoriasis (**Figure 1**). Therefore, the IMQ-induced mouse model has been widely used in the study of psoriasis. The pathogenesis of psoriasis is complex. Previous studies have shown that typical psoriasis is characterized by epidermal and dermal specific immunity and infiltration of inflammatory cells. These reactions increase inflammation in the skin layer, stimulate the proliferation and abnormal differentiation of keratinocytes, and damage the skin [25].

Autophagy clears inflammatory cells in the body and plays an important role in the progression of psoriasis. Many studies have shown that AMPK inhibits the cellular inflammatory response by increasing the level of cyclic adenosine phosphate, whereas the level of cAMP in psoriatic epidermis in patients is lower than that in uninfected individuals [26], in agree-

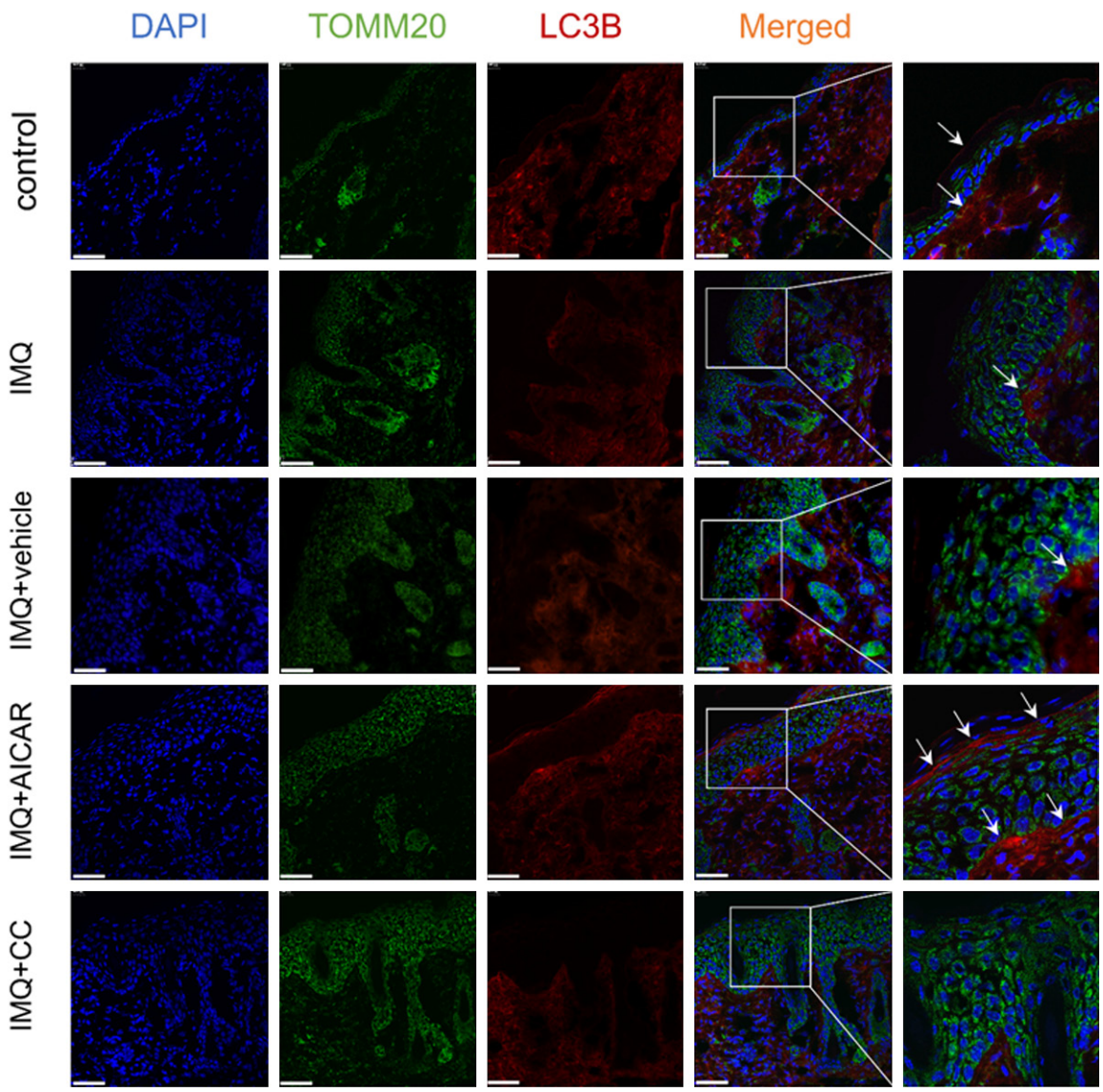

Figure 4. The effect of AMPK modulation on mitochondrial autophagy was evaluated by double IF staining of the mitochondrial outer membrane in psoriasis-like skin tissue. Green represents the mitochondrial outer membrane marker TOMM20, and red represents LC3B. The scale bar of **Figure 4** is 50 μm, the magnification is 400×. There were n=6 animals per group.

ment with our findings. We found that AMPK signaling was diminished in the skin tissues of psoriatic mice. The AMPK signaling agonist AICAR decreased the PASI scores and smoothened the skin on the backs of mice, and histologic examination suggested a decrease in epidermal thickness. However, the AMPK inhibitor CC showed opposite results after treatment and increased the severity of psoriatic skin. These results suggested that AMPK signaling is involved in the regulation of IMQ-induced psoriasis. AMPK is an effective autophagy regulator [27]. AMPK phosphorylation activates the ULK1 kinase and induces autophagy [19]. Autophagy is regulated by activation of the ULK1/Atg7 signaling pathways [28]. The autophagy-associated protein Atg7 plays an important role in autophagy. A study of Atg7 knockout mice has shown that Atg7 is involved in autophagy and plays an important role in epidermal keratinization [29]. Our study found that the expression of components of the AMPK-ULk1/Atg7 signaling pathway decreased in psoriatic mice, thus, indicating inhibition of autophagy in psoriatic mice. After APMK stimulation, ULK1/Atg7 activity and autophagy signaling were also

A

Genes	Primers	Length of target fragment, bp
GAPDH	F:5'-GTGTTCCTACCCCCAATGTG-3' R:5'-CATCGAAGGTGGAAGAGTGG-3'	114
Atg7	F:5'-GTTCGCCCCCTTTAATAGTGC -3' R:5'-TGAACTCCAACGTCAAGCGG-3'	161
P62	F:5'- TGAAGGCTATTACAGCCAGAGTCAA -3' R:5'-CCTTCAGTGATGGCCTGGT-3'	121
PINK1	F:5'-TTCTTCCGCCAGTCGGTAG -3' R:5'-CTGCTTCTCCTCGATCAGCC-3'	141
Parkin	F:5'-GAGGTCCAGCAGTTAAACCCA -3' R:5'-CACACTGAACTCGGAGCTTTC-3'	96

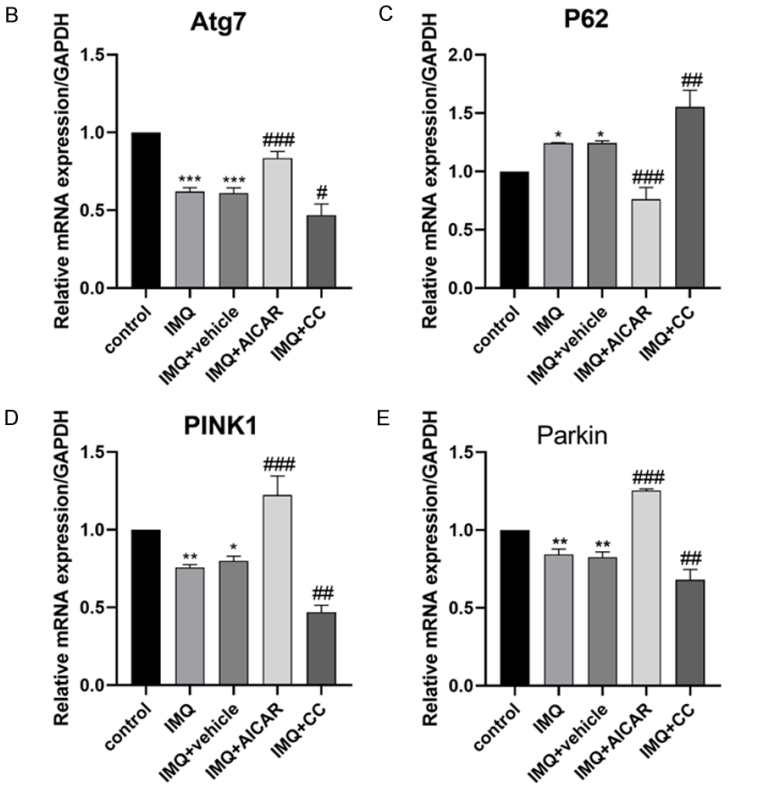

Figure 5. Effects of AMPK modulation on the mRNA expression of autophagy and mitochondrially associated proteins. The sequences of the forward and reverse primers for Atg7, P62, PINK1, and Parkin (A). The mRNA expression levels of Atg7 (B), P62 (C), PINK1 (D), and Parkin (E) in the skin after back injury in the psoriasis mouse model. Statistical analysis was conducted with one-way ANOVA, then with a Student-Newman-Keuls post hoc test. There were n=6 animals per group. Data are expressed as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001, compared with the control group. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, compared with the IMQ + vehicle group.

enhanced. The psoriasis symptoms in the mice also improved significantly. Inhibition of AMPK consequently inhibits the expression of autophagic proteins in this signaling pathway. Our results demonstrate that AMPK-ULK1/Atg7 signaling affects the development of psoriasis by regulating autophagy (**Figure 2**). Autophagy eliminates inflammatory factors in the body. In healthy keratinocytes, autophagy decreases epidermal P62 levels, thereby preventing epidermal inflammation [30]. The increased expression of P62 in mice with psoriasis may be due to increased skin keratinocytes and Toll-like receptor signaling pathway activation, thus leading to excessive cell proliferation and inflammation [31, 32]. Upregulation of AMPK signaling enhanced autophagy associated signals such as ULK1 and Atg7, decreased P62, and alleviated skin inflammation. LC3 is often used as an autophagy marker indicating the number of autophagosomes. proLC3, a component of the autophagosome membrane, is synthesized in the cytoplasm and transformed into LC3-I by Atg4B; subsequently, it enters autophagosomes. After Atg7 catalysis, LC3-I combines with phosphatidylethanolamine, thus forming LC3-II through ubiquitination modification [33]. Through the detection of autophagy signals on the mitochondrial outer membrane, we found that LC3-II was almost entirely absent in the mitochondrial outer membrane in the psoriasis mouse model; consequently, AMPK was activated, and LC3-II in the mitochondrial outer membrane was also significantly increased (**Figure 4**). These findings suggest that AMPK signaling is involved in the regulation of mitochondrial autophagy in mice with psoriasis.

AMPK can also induce the fragmentation of damaged mitochondria and promote the trans-

location of autophagy into damaged mitochondria, thereby regulating mitochondrial autophagy [34]. AMPK has been shown to participate in mitochondrial autophagy by regulating PINK1/Parkin signaling in degenerative neuropathy and heart failure [16]. PINK1/Parkin, an important pathway mediating mitochondrial autophagy, can improve the efficiency of mitochondrial autophagy [35]. The activation of the PINK1/Parkin pathway promotes the ubiquitination of mitochondrial outer membrane protein and further triggers the translocation of ubiquitin binding receptors to mitochondria, thus completing mitochondrial initiation [36]. To investigate whether AMPK signaling might be involved in the regulation of psoriasis in mice through the modulation of mitochondrial autophagy by PINK1/Parkin, we further examined the changes in PINK1 and Parkin after excitation and inhibition of AMPK. We found that after psoriasis, mitochondrial autophagy was inhibited, and PINK1/Parkin signaling decreased. When AMPK signaling was activated, PINK1/Parkin signaling increased, mitochondrial autophagy was enhanced, LC3-II in the mitochondrial outer membrane significantly increased, and the skin symptoms in mice were alleviated (**Figure 3**). Inhibition of AMPK signaling blocked the mitochondrial autophagy signal. These results suggest that the PINK1/Parkin signaling pathway is at least partially regulated by AMPK signaling. PINK1 recruits autophagy regulators to mitochondria, which subsequently initiate mitochondrial autophagy, and it regulates Parkin-mediated mitochondrial autophagy in mammals [37, 38], in agreement with our results.

The mechanisms of autophagy and mitochondrial autophagy in the regulation of psoriasis are very complex. According to our study, autophagy signals (ULK1 and Atg7) and mitochondrial autophagy signals (Pink1 and Parkin) are inhibited in the skin tissues of psoriasis mice. Autophagy signals are reduced, the functions of inflammatory factors and other harmful substances are inhibited, and inflammatory symptoms in the skin are aggravated. We found that in psoriasis, upregulation of AMPK, an important molecule in immune regulation, results in ULK1/Atg7 signaling pathway phosphorylation and promotes autophagy as well as mitochondrial autophagy through the PINK1/Parkin pathway, thereby inhibiting inflammation and affecting the development of psoriasis. Inhibition of AMPK signaling increases skin inflammation in psoriasis.

Conclusions

By studying an Imiquimod (IMQ)-induced psoriasis mouse model, we found that inhibition of AMPK signaling inhibited autophagy and mitochondrial autophagy, thereby aggravating the severity of psoriasis in mice. Up-regulation of AMPK signaling promoted autophagy and mitochondrial autophagy, thus playing a protective role in psoriatic mice. These results suggest that AMPK may be a possible target for psoriasis prevention and control.

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Disclosure of conflict of interest

None.

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