Trichosanthes kirilowii extract enhances repair of UVB radiation-induced DNA damage by regulating BMAL1 and miR-142-3p in human keratinocytes

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Abstract. Ultraviolet B (UVB) radiation induces DNA damage, oxidative stress and inflammation, and suppresses the immune system in the skin, which collectively contribute to skin aging and carcinogenesis. The DNA damage response, including DNA repair, can be regulated by the circadian clock and microRNA (miRNA) expression. The aim of the present study was to evaluate the reparative action of *Trichosanthes kirilowii* extract (TKE) against UVB irradiation-induced DNA damage in human keratinocytes. TKE demonstrated low cytotoxicity in normal HaCaT keratinocytes at low doses (up to 100 μg/ml). The results of a comet assay revealed that TKE enhanced the repair of UVB-induced DNA damage. TKE significantly upregulated the expression of the core clock protein, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1 (BMAL1), and downregulated the expression of miRNA (miR)-142-3p, as demonstrated using western blotting and the reverse transcription-quantitative polymerase chain reaction. Furthermore, the suppression of miR-142-3p by a specific inhibitor positively correlated with the repair activity. Overall, the data obtained demonstrated that TKE enhanced the repair of UVB-induced DNA damage by regulating the expression of BMAL1 and miR-142-3p. Consequently, TKE can be considered a potential candidate for the treatment of skin diseases associated with UVB-induced damage.

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Abbreviations: TKE, *Trichosanthes kirilowii* extract; BMAL1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1; UVB, ultraviolet B radiation; Per, period; Cry, cryptochrome; miR-142-3p, microRNA 142-3p

Key words: Trichosanthes kirilowii, DNA damage repair, circadian rhythm, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1, microRNA-142-3p, keratinocytes

Introduction

Ultraviolet (UV) radiation leads to DNA damage, cell senescence and apoptosis. It also results in the formation of the two most common DNA lesions, cyclobutane pyrimidine dimers and 6-4 photoproducts. These lesions impede DNA replication and transcription, and are repaired by nucleotide excision repair (NER) (1,2). NER is critical in the repair of UV-induced DNA lesions and its actions involve the endonucleolytic cleavage of two phosphodiester bonds followed by excision of the damaged DNA. The excised oligonucleotide is replaced by DNA repair synthesis, and the continuity of the DNA strand is re-established by DNA ligase (3).

The circadian rhythm is responsible for regulating various physiological processes, including hormone production, temperature and sleep pattern. It is found in the majority of living organisms, including animals, plants and fungi. The mammalian circadian clock system is organized as a central oscillator. This system is controlled by the suprachiasmatic nucleus (SCN) and the peripheral oscillators (4-6). The core clock oscillator includes the two heterodimeric transcriptional factors, CLOCK and brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like protein-1 (BMAL1), which activates the transcription of their transcriptional repressors, Period (*Per*) and Cryptochrome (*Cry*) (5,6).

The DNA damage response pathways include DNA repair, damage checkpoints and cell cycle arrest. Current evidence indicates that these pathways are associated with the circadian clock (2,7,8). Furthermore, there is evidence that the normal circadian rhythm and gene expression levels in the skin are suppressed by UVB radiation, as has been demonstrated for the gene expression levels of *Bmal1, Per* and *Clock* in human keratinocytes (9,10).

MicroRNAs (miRNAs) are small non-coding RNA molecules, which are involved in the regulation of gene expression by binding to the 3'-untranslated regions (UTRs) of target mRNAs and thus interrupting protein translation (11-13). Numerous studies have shown that miRNAs are important in biological processes, including cell proliferation, apoptosis, development, metabolism and differentiation (14-17). Previous studies have implicated miRNAs in the modulation of the circadian clock, revealing that miRNA (miR)-219 regulates the length of the

circadian period, whereas miR-132 modulates *Per* gene transcription and protein stability (18,19). Although miR-142-3p is present in other cells, it is involved in the post-translational modulation of BMAL1 in mouse SCN, NIH3T3 and human 293ET cells (20,21); however, the molecular mechanisms by which miR-142-3p mediates the post-transcriptional regulation of BMAL1 in skin cells remain to be elucidated. Several studies have reported an association between UV radiation exposure and alterations in miRNA expression in keratinocytes (22,23). UVB radiation-exposed keratinocytes exhibit several specific miRNA response patterns (24).

Trichosanthes kirilowii is a traditional medicine used in East Asia for treating patients with diabetes, cancer-associated symptoms, coughing and breast abscesses (25,26). However, the effects of *T. kirilowii* on skin cells and its effect on DNA damage repair remain to be fully elucidated. Therefore, the aim of the present study was to investigate the effects of *T. kirilowii* extract (TKE) against UVB-induced DNA damage in skin cells. In addition, the involvement of miR-142-3p and BMAL1 in the TKE-mediated repair of UVB-induced DNA damage was investigated.

Materials and methods

Preparation of TKE. The *T. kirilowii* plant material used in the present study was purchased from Jeong-woo-dang Oriental Medicine Market (Seoul, Korea). To prepare the TKE, the powdered *T. kirilowii* (50 g) was extracted for 3 h with 70% ethanol (1 liter) at room temperature (20-25˚C) while stirring. The supernatant was collected by filtration, and the ethanol was removed using a rotary vacuum evaporator.

Cell culture conditions. The HaCaT cells were obtained from Cell Line Service GmbH (Eppelheim, Germany) and were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (all HyClone; GE Healthcare Life Sciences, Logan, UT, USA) in a humidified atmosphere of 5% CO_2 at 37˚C.

UVB exposure and subsequent TKE treatment. The HaCaT cells were incubated in serum-free medium for 24 h in culture plates, washed twice in phosphate-buffered saline (PBS), exposed to the desired doses (12.5 mJ/cm^2) of UVB radiation under a thin layer of PBS, and then immediately incubated with serum-free medium containing various concentrations of TKE (25, 50, 100 and 200 μg/ml) for 24 h in in a humidified atmosphere of 5% CO_2 at 37˚C.

Measurement of cell viability using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The HaCaT cells were cultured in 96-well plates (1x10^4 cells/well) for 24 h in complete and serum-free media, sequentially. The cells were exposed to UVB radiation and then incubated with serum-free medium containing various concentrations (25-200 μg/ml) of TKE. Following culture for 24 h, the cell viability was determined using the MTT assay. Briefly, to each well, 20 μl of MTT solution (5 mg/ml) was added and the plate was incubated for an additional 4 h at 37˚C. The formazan product, which formed was solubilized in 100 μl of isopropanol, and the absorbance was measured at 560 nm.

Western blot analysis. The cells were rinsed twice with ice-cold PBS, following which cell lysates were prepared using radioimmunoprecipitation assay lysis buffer, comprising 150 mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 50 mM Tris-hydrochloride (pH 8.0) containing a protease inhibitor cocktail. The lysate protein concentrations were determined using bicinchoninic acid reagent. The cell lysate samples (40 μg) were then separated using 8% SDS-polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride membranes. The membranes were blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) in 1X TBS Tween-20 buffer for 20 min at room temperature. The membranes were incubated with anti-human BMAL1 (1:3,000; cat. no. SC-8550; Abcam, Cambridge, MA, USA) or anti-human actin (1:1,000; cat. no. SC-1615-R; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C overnight, followed by incubation with a horseradish peroxidase-conjugated secondary antibody (1:10,000; cat. no. SC-2768; Santa Cruz Biotechnology, Inc.) at room temperature for 1 h. Finally, the protein-antibody conjugates on the membranes were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Comet assay (single-cell gel electrophoresis). Following UVB radiation, the cells were scraped and embedded in low melting point agarose on CometSlides™ (Trevigen, Gaithersburg, MD, USA) at 4˚C for 10 min. The slides were then incubated with lysis buffer at 4˚C for 1 h, followed by immersion in an alkaline unwinding solution for 30 min at room temperature (20-25˚C). The slides were electrophoresed at 50 V, rinsed with distilled water and 70% ethanol, and then stained with SYBR®-Green (Trevigen) for 10 min. The DNA damage was visualized using a fluorescent microscope (IX71; Olympus Corporation, Tokyo Japan). These data were analyzed with analySIS LS Starter version 2.2 (Olympus Soft Imaging Solutions GmbH, Münster, Germany).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from the cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The RNA concentration was quantified using a UV spectrophotometer, and cDNA was synthesized from 1 μg total RNA. The cDNA (250 ng) was then subjected to a PCR using EmeraldAmp® GT PCR master mix (Takara Bio, Inc., Otsu, Japan). The primers (10 pmol) used for PCR analysis were synthesized by Bioneer Corporation (Daejeon, Korea). The cycling conditions were as follows: 30 cycles at 94˚C for 30 sec, 60˚C for 1 min, and 72˚C for 30 sec. The primer sequences used were as follows: BMAL1, forward 5'-AAGGATGGCTGTTCAGCACA-3' and reverse 5'-CAAAAATCCATGGCTGCCC-3'; and β-actin, forward 5'-ACACTGTGCCCATCTACG-GGGG-3' and reverse 5'-ATGATGGAGTTGAAGGTAGTTTCGTGGAT-3'. The PCR products were run on 2% agarose gels containing ethidium bromide.

Bioinformatic analysis. The target gene prediction database Targetscan (version 6.2; targetscan.org) was used to search for predicted BMAL-1-targeted miRNAs.

RT-quantitative PCR (RT-qPCR) analysis. The RNA (1 μg) was reverse-transcribed using the Mir-X™ miRNA First-Strand Synthesis kit (Clontech Laboratories, Inc., Mountain View, CA, USA) to generate cDNA. The LightCycler® 480 SYBR-Green I Master (Roche Diagnostics, Inc., Indianapolis, IN, USA) was used for the RT-qPCR procedure, according to the manufacturer's protocol to quantify the miRNA transcript levels. The appropriate quantification cycle (Cq) value was determined using the automatic baseline determination feature. The reaction solution contained 10 μl 2X Master Mix, 1 μl miR-specific primer for human miR-142-3p, 1 μl universal primer, cDNA (100 ng) and nuclease-free water to a total volume of 20 μl. The sequence of the hsa-miR-142-3p primer was as follows: 5'-TGTAGTTTCCTACTTTATGGA-3'. U6 was used for miRNA level normalization. The U6 primer was as follows: Forward, 5'-CCUCGUGCCGUUCCAGGUAGUU-3' and reverse, 5'-CUACCUGAUGAACGGCAGGUU-3'. DNA was amplified using 45 cycles of denaturation for 10 sec at 95˚C and annealing for 10 sec at 60˚C. Each sample was assessed in triplicate. The relative expression of the miRNA was quantified using Lightcycler® 480 II software (Roche Diagnostics Inc., Indianapolis, IN, USA) and the $2^{-\Delta\Delta Cq}$ method (27).

Transient transfection with synthetic miRNA mimics and inhibitors. The mimic and inhibitor oligonucleotides for miR-142-3p and the negative controls were synthesized by Genolution Pharmaceutical (Seoul, Korea). Prior to miRNA transfection, the HaCaT cell culture medium was replaced with serum-reduced medium of opti-minimal essential medium (MEM) I (Gibco; Thermo Fisher Scientific, Inc.). The transfection was performed using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The HaCaT cells were incubated with the oligonucleotides/lipofectamine mixture for 6 h, following which the opti-MEM I medium was replaced with the growth medium. The cells were harvested 24 h following transfection.

Statistical analysis. Statistical analysis was performed using Student's t-test and was based on at least three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference. The GraphPad Prism 5 program (GraphPad Software, Inc., La Jolla, CA, USA) was used to evaluate statistical significance.

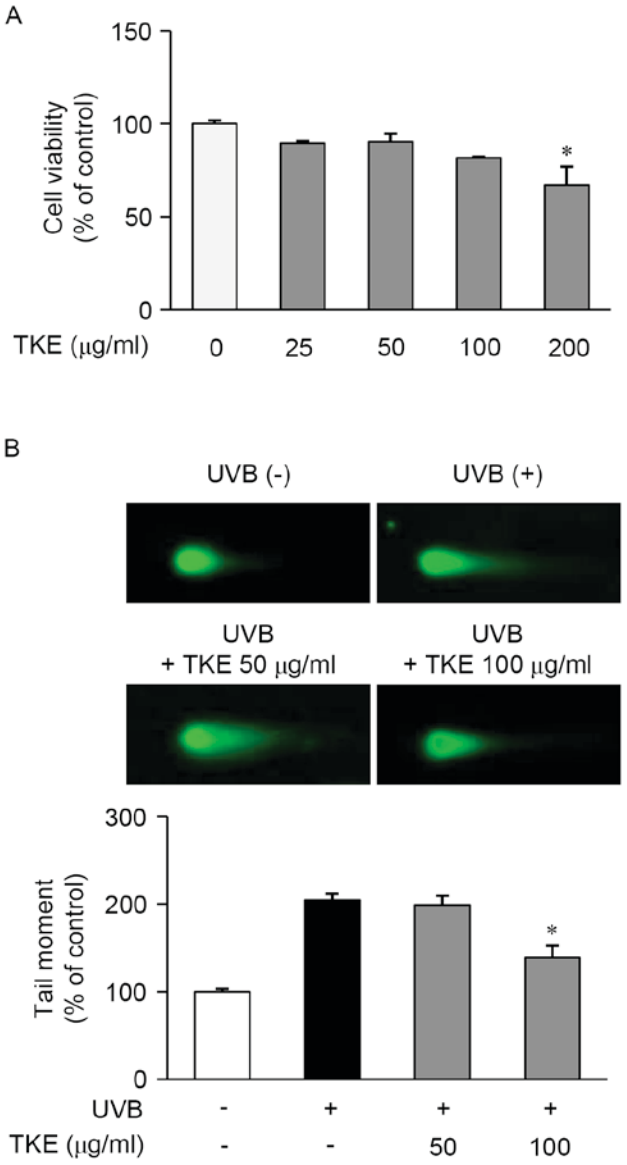

Figure 1. Effects of TKE on DNA repair in UVB radiation-exposed HaCaT cells. (A) Cell viability of TKE-treated cells was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Results are presented as the mean ± standard deviation of three independent experiments. (B) HaCaT cells were exposed to UVB radiation (12.5 mJ/cm^2) with or without TKE treatment (50 and 100 μg/ml). UVB-induced DNA damage was determined using a comet assay. Comet tails were measured in each cell under a microscope and expressed as the mean ± standard deviation (μm) of at least 30 cells in each treatment group. *$P<0.05$, compared with the UVB-exposed control. TKE, ethanol extract of *Trichosanthes kirilowii*; UVB, ultraviolet B.

Results

TKE promotes the repair of UVB-mediated DNA damage in HaCaT cells. DNA damage is caused by UV radiation. Keratinocytes are affected by UV radiation in the outermost layer of skin. HaCaT cells are a spontaneously immortal keratinocyte cell line, which has been widely used in investigations involving the skin. The present study first examined the effect of TKE at concentrations of 25, 50, 100 and 200 μg/ml on the viability of HaCaT cells treated for 24 h using an MTT assay. As shown in Fig. 1A, TKE at a concentration of 200 μg/ml, but not at concentrations of ≤100 μg/ml, was significantly cytotoxic and, based on these results; concentrations of 50-100 μg/ml were selected for use in the subsequent experiments.

The reparative and protective effects of 8 h treatment with TKE against DNA damage induced by UVB radiation were determined in HaCaT cells using a comet assay. The exposure of HaCaT cells to UVB radiation (12.5 mJ/cm^2) induced extensive DNA damage, as reflected in the difference in tail lengths between the comets of the cells exposed to UVB radiation and those not exposed (Fig. 1B). However, treatment of the UVB-exposed cells with TKE (100 μg/ml) reduced the DNA damage or fragmentation, compared with that in the untreated UVB-exposed cells (Fig. 1B). In addition, the preliminary

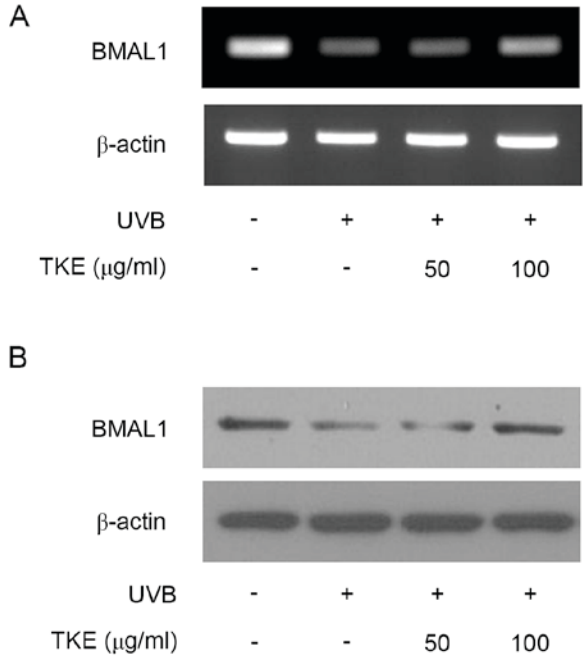

Figure 2. Effects of TKE on mRNA and protein expression of BMAL1. Levels of BMAL1 in HaCaT cells were determined using (A) reverse transcription-polymerase chain reaction and (B) western blot analysis. β-actin and actin were used as internal controls. Results were obtained from at least three independent experiments. Data were analyzed using analySIS LS Starter. TKE, ethanol extract of *Trichosanthes kirilowii*; BMAL1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1.

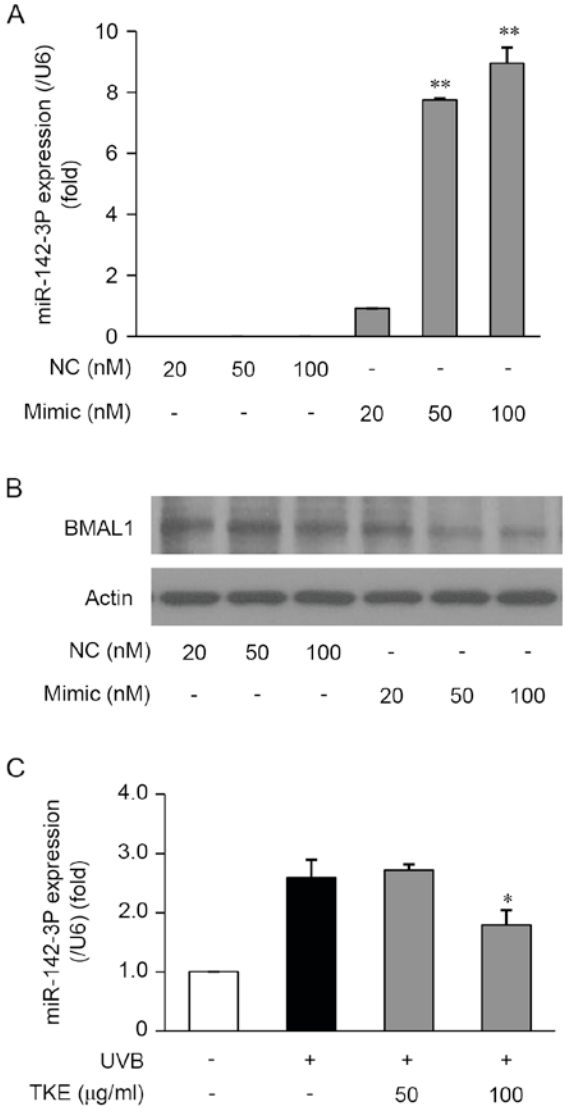

Figure 3. Effects of TKE on expression of BMAL1 are mediated by the down-regulation of miR-142-3p. (A) Cultured HaCaT cells were transfected with 20, 50 and 100 nM miR-142-3p mimic or miR-142-3p mimic NC. The expression of miR-142-3p was examined using RT-qPCR analysis. **P<0.01, compared with NC. (B) Western blot analysis was used to measure the expression of BMAL1 in transfected HaCaT cells. (C) HaCaT cells were exposed to UVB radiation with or without TKE treatment. Expression levels of miR-142-3p were determined using RT-qPCR; *P<0.05, compared with UVB-exposed control. TKE, ethanol extract of *Trichosanthes kirilowii*; BMAL1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1; miR, microRNA; UVB, ultraviolet B; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; NC, negative control.

experiments indicated that TKE protected the HaCaT cells from UVB-induced DNA damage (data not shown). These findings demonstrated that TKE may be involved in DNA damage repair and may also protect against UVB-induced DNA damage.

TKE modulates mRNA and protein expression levels of BMAL1 in UVB-irradiated HaCaT cells. The results of the present study demonstrated that TKE exhibited reparative effects on UVB-induced DNA damage (Fig. 1B). Several reports have indicated that the NER DNA repair system is dependent on the circadian rhythm (28-30). To further investigate the molecular mechanism by which TKE modulates DNA damage repair, the present study examined changes in the expression of BMAL1, which is important in the circadian rhythm (9,10). The UVB radiation decreased the mRNA and protein expression levels of BMAL1, compared with levels in the unstimulated control cells, which was consistent with a previous report using normal human keratinocytes (10). However, TKE (100 μg/ml) treatment markedly increased mRNA and protein the expression of BMAL1 (Fig. 2A and B). Overall, TKE treatment affected the expression levels of BMAL1, suggesting that specific cellular response mechanisms may be involved in TKE-mediated DNA damage repair in keratinocytes.

TKE upregulates the expression of BMAL1 via the inhibition of miR-142-3p. It has been reported that miRNAs are closely associated with the regulation of DNA damage in addition to circadian rhythms (31). To determine the potential regulatory role of BMAL1-targeted specific miRNAs in target gene transcription, the present study used TargetScan to predict BMAL1-targeted specific miRNAs in the HaCaT cells. It was found that miR-142-3p showed a high level of interaction with the 3'-UTR of BMAL1 and a high probability of regulating the expression of BMAL1. A previous study demonstrated that miR-142-3p directly targeted BMAL1 3'-UTRs and regulated the mRNA and protein levels of BMAL1 in human 293ET cells (21). To investigate whether miR-142-3p is involved in the regulation of BMAL1 in human keratinocytes, the HaCaT cells were transfected for 24 h with the miR-142-3p mimic and a mimic control. The results of the subsequent RT-qPCR analysis demonstrated that the miR-142-3p mimic significantly increased the expression of miR-142-3p in the HaCaT

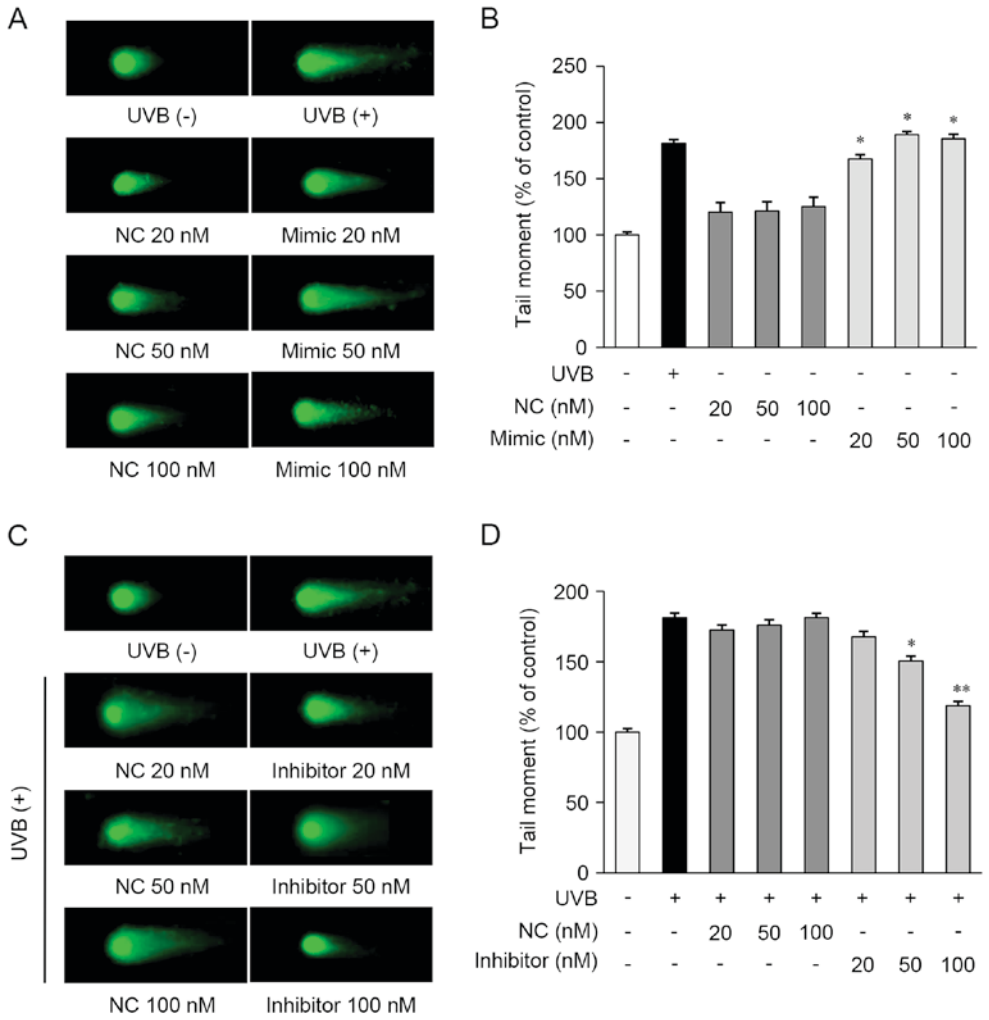

Figure 4. Effects of miR-142-3p on UVB-induced DNA damage in HaCaT cells. UVB-induced DNA damage was determined using a comet assay. (A) Representative immunofluorescence images and (B) graph of the results. The comet tails of each cell were measured using a microscope and expressed as the mean ± standard deviation of at least 30 cells in each treatment group. *P<0.05, compared with non-UVB-exposed control. Cultured HaCaT cells were pre-treated with miR-142-3p inhibitor and miR-142-3p inhibitor NC and then exposed to UVB radiation. Cells were harvested 8 h after exposure. (C) Representative immunofluorescence images and (D) graph of the results. Comet tails of each cell were measured using a microscope and expressed as the mean ± standard deviation of at least 30 cells in each treatment group. *P<0.05 and **P<0.01, compared with the UVB-exposed control. These data were analyzed with analySIS LS Starter. TKE, ethanol extract of *Trichosanthes kirilowii*; BMAL1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1; miR, microRNA; UVB, ultraviolet B; NC, negative control.

cells, compared with that in cells transfected with the mimic control (Fig. 3A). Furthermore, the results of western blot analysis demonstrated that the miR-142-3p mimic markedly inhibited the protein expression of BMAL1 (Fig. 3B). The results of the RT-PCR analysis also revealed that the mimic suppressed the mRNA expression levels of BMAL1 (data not shown). Taken together, the downregulation of the mRNA and protein expression of BMAL1 in HaCaT cells suggested that miR-142-3p may be involved in the molecular mechanisms underlying the repair of and protection against UVB-induced DNA damage.

Based on the above findings, the present study examined the effects of TKE on miRNA expression levels in the HaCaT cells. To investigate whether the expression of BMAL1 is regulated by TKE through the modulation of miR-142-3p, RT-qPCR analysis was performed in the HaCaT cells treated with or without TKE. In the HaCaT cells exposed to UVB radiation (12.5 mJ/cm^2) for 24 h, the expression levels of miR-142-3p were increased, however, in UVB-exposed cells treated with TKE (100 μg/ml) for 24 h, a decrease in the expression of miR-142-3p was observed (Fig. 3C). Taken together, these observations suggested that TKE-mediated DNA damage repair in HaCaT cells may be correlated with suppression of the expression of miR-142-3p.

miR-142-3p suppresses the repair of UVB-induced DNA damage. To examine the role of miRNAs in the repair of UVB-induced DNA damage, a comet assay was performed in the HaCaT cells. The cells were transfected with the miR-142-3p mimic, mimic control, miR-142-3p inhibitor, or inhibitor control. As shown in Fig. 4A and B, the miR-142-3p mimic-transfected cells exhibited increased DNA damage, compared with the cells not exposed to UVB. However, the mimic negative control showed no observable effects in the cells.

The present study then determined whether the miR-142-3p inhibitor affected the repair of UVB-induced DNA damage in HaCaT cells and found that it significantly decreased the

expression of miR-142-3p in HaCaT cells, compared with control cells transfected with the inhibitor control (data not shown). The miR-142-3p inhibitor-transfected cells exhibited reduced DNA damage, compared with the UVB-exposed cells (Fig. 4C and D). These results indicated that miR-142-3p inhibited the repair of UVB-induced DNA damage in HaCaT cells. Taken together, these results suggested that TKE enhanced the repair of UVB-induced DNA damage by regulating the expression of miR-142-3p and BMAL1.

Discussion

T. kirilowii has been used in the treatment of diabetes, respiratory diseases, and cancer-related symptoms (25,26). The extracts and active components of *T. kirilowii* have been reported to exert anticancer activities (32). However, the effects of *T. kirilowii* on skin cells and its effect on DNA damage repair have not been reported previously. In the present study, the reparative effect of TKE was shown on UVB-induced DNA damage in HaCaT keratinocytes, which was likely mediated by regulation of the circadian clock and miRNA expression.

DNA damage is caused by UV radiation. The DNA damage response includes the DNA repair system, NER. The mechanism underlying NER has been shown to involve the circadian rhythm. Several reports have indicated that the circadian oscillations of NER activity are associated with that of the protein level of xeroderma pigmentosum group A (XPA) (2,7,8,28). XPA is crucial in DNA damage recognition and has a regulatory function on the circadian clock. XPA is positively regulated by CLOCK and BMAL1, and negatively regulated by CRY and PER (29). BMAL1 is closely associated with time-dependent UV sensitivity and the efficiency of DNA repair (30). These findings are consistent with a previous report that BMAL1-silenced cells showed markedly reduced DNA repair responses (33) and another study, which reported that BMAL1 regulates the proportion of cells in the S-phase of the cell cycle, which are sensitive to DNA damage (30). In the present study, DNA damage was measured in human keratinocytes (HaCaT cells) using a comet assay. The comet tail lengths of cells treated with UVB followed by 100 μg/ml TKE showed a significant decrease compared to that of the UVB-treated controls. TKE exerted reparative effects against UVB-induced DNA damage (Fig. 1A). In addition, TKE upregulated the UVB-reduced expression of BMAL1 (Fig. 2). Therefore, these results suggested that TKE regulated the expression of BMAL1, which repaired keratinocytes and protected them from UVB-induced damage. These events appear to be associated with the BMAL1-modulated DNA repair system.

The NER system is regulated by miRNA-mediated gene regulation. Several miRNAs have been implicated in the DNA repair pathway. The upregulation of miR-192, miR-890 and miR-744-2p inhibits NER in cancer cells (31). In the present study, the expression levels of miR-142-3p were significantly decreased in the TKE-treated HaCaT cells exposed to UVB, compared with the unexposed cells (Fig. 3C). Several previous studies have reported that miR-142-3p is expressed in the spleen, thymus and hematopoietic cells (33-35). It has also been reported that miR-142-3p functions as a tumor suppressor by targeting numerous tumor-associated genes (36,37). miR-142-3p has been shown to be expressed at significantly higher levels in cells from patients with psoriasis and atopic dermatitis, compared with normal cells (38,39). In addition, miR-142-3p was found to be upregulated in patients with systemic sclerosis (40). From these previous findings, it was hypothesized that miR-142-3p may be involved in other skin diseases.

Previous studies have demonstrated that miRNAs are important regulators of the circadian clock. miR-142-3p directly targets BMAL1 3'-UTRs and regulates the mRNA and protein levels of BMAL1 in mouse SCN, NIH3T3 and human 293ET cells (20,21). In the present study, the miR-142-3p mimic markedly inhibited the protein expression of BMAL1 in human keratinocytes (Fig. 3B). The miR-142-3p mimic increased comet tail length, compared with that of control cells without UVB exposure (Fig. 4A and B). The miR-142-3p inhibitor suppressed the repair of UVB-induced DNA damage in the HaCaT cells by its regulation of potential target genes, including *bmal1* (Fig. 4C and D). These findings provide a novel basis for the correlation between the regulation of BMAL1 and the suppression of miR-142-3p by TKE.

Although the present study focused on the repair mechanism of DNA damage by BMAL1 and miR-142-3p, this may constitute only one of numerous mechanisms, which may include regulation by other clock factors within the circadian clock gene or other miRNAs. Further investigations are necessary to identify the active component in TKE and to determine the detailed mechanism involved in the BMAL1-mediated repair of UVB-induced DNA damage. In conclusion, the results of the present study provided evidence of the beneficial effects of TKE in the repair of UVB-induced DNA damage in HaCaT cells. These findings may have important implications for the treatment of various diseases caused by UVB-induced photodamage, including photoaging and sunburn.

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