

Research Paper

Novel approaches to vitiligo treatment via modulation of mTOR and NF- κ B pathways in human skin melanocytes

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Abstract

Vitiligo is a skin depigmentation disorder with an increasing prevalence. Among recognized mechanisms is the oxidative stress that affects melanocytes which are responsible for skin pigmentation. Studies have shown that high concentration of hydrogen peroxide, or H₂O₂, induces apoptotic activities. Few studies have been done with lower doses of H₂O₂. Using human skin melanocytes, we investigated the effect of moderate concentration of H₂O₂ on melanocyte dendrites. Confocal data show that H₂O₂ at 250 μ M induces loss of dendrites, as indicated by cytoskeletal proteins. α -melanocyte stimulating hormone or α -MSH pretreatment protects against H₂O₂-induced loss of dendrites, while α -MSH alone enhances dendrites. PI3K/AKT inhibitor LY294002 and mTORC1 inhibitor Rapamycin inhibit α -MSH-induced dendrites. In this study, we also investigated the effect of TNF α on cultured human skin melanocytes, since TNF α plays important roles in vitiligo. Confocal data demonstrate that TNF α induces NF κ B activation. Western blot analysis shows that TNF α induces I κ B phosphorylation and degradation. Interestingly, α -MSH does not have any effect of TNF α -induced I κ B degradation and NF- κ B activation. α -MSH, however, activates mTORC1 pathway. TNF α induces p38 but not AMPK α activation. Collectively, our data suggest that modulation of mTOR and NF- κ B pathways may be a novel approach for better clinical management of vitiligo.

Key words: α -MSH, mTORC1, TNF α , vitiligo

Introduction

Vitiligo is a depigmentation disorder with an estimated worldwide prevalence from 0.5 to 2% [1, 2]. Vitiligo is not life threatening, but affects people cosmetically and even emotionally as a result of a negative social stigma. With the increasing prevalence of this skin disorder, more and more people are interested in understanding the cellular and molecular mechanism in hopes of overcoming and better yet preventing this disorder.

Vitiligo occurs when functioning melanocytes

disappear from the epidermis and the production of pigments decreases, leaving white patches on the skin. Vitiligo is the result of complex interactions of biochemical, environmental and immunological events in a permissive genetic milieu, while the precise mechanism of vitiligo pathogenesis has remained elusive [3, 4]. It has been recognized that this unique type of skin disorder is associated with melanocyte and possibly other skin cell apoptosis [5, 6]. Melanocyte death is related to reactive oxygen

species, cytokines[7, 8], and more complicatedly, autoimmunity[3, 9-13].

Reactive oxygen species or ROS, has been shown to be related to a variety of human diseases including vitiligo[12]. Hydrogen peroxide or H_2O_2 , is one of the ROS that induces apoptosis. Excess ROS has been documented in active vitiligo skin. The removal of H_2O_2 by antioxidants has been proven to be beneficial to patients with vitiligo[14]. And minocycline, an antibiotic possessing antioxidant activity, has been shown to protect melanocytes against H_2O_2 -induced apoptosis in vitro and may be used for the treatment in the early stages of vitiligo[15].

Melanocytes are responsible for melanin production and transport to surrounding keratinocytes. Melanin transfer takes place at the junction between dendrites of melanocytes and membrane of keratinocytes. Dendrites play critical roles of melanosomal transfer in melanocytes[16, 17]. In addition to apoptosis of melanocytes, the damage of dendrites may also affect melanin transport leading to vitiligo. Our preliminary study showed that oxidative stress such as H_2O_2 treatment induces loss of dendrites. However, the mechanism of the loss of dendrites remains to be unraveled.

In addition to oxidative stress, there also is evidence for altered immunological processes in vitiligo, particularly in chronic and progressive conditions. While both innate and adaptive immunities are proposed to be involved as a primary event or as a consequence in vitiligo, there is an interplay between ROS and the immune system in the pathogenesis of vitiligo. There is evidence linking oxidative stress and autoimmunity to vitiligo pathogenesis, supporting the notion of a convergent terminal pathway of oxidative stress-autoimmunity-mediated melanocyte loss in vitiligo[12, 18, 19].

α -MSH activates putative cell surface receptor MC1R and stimulates melanogenesis and proliferation of human melanocytes. α -MSH also promotes human melanocyte survival by inhibiting UV-induced apoptosis[15, 20]. Studies predict that the survival effect of α -MSH is caused by reduction of UV-induced DNA damage and contributes to the prevention of melanoma[20-22]. Our preliminary studies have shown that H_2O_2 induces loss of dendrites in human melanocytes, and α -MSH protects this loss of dendrites. However, the molecular mechanism of this process remains unknown.

PI3K/AKT and mTOR pathway has been suggested to be associated with cell survival in response to UV radiation and oxidative stress[23]. Growth factors protect against UV and oxidative stress-induced apoptosis via activation of AKT and

mTOR pathways[24-26]. While indirect data suggest that α -MSH-stimulated melanogenesis through the activation of MEK/ERK or PI3K/AKT[27], the question of whether α -MSH protects against oxidative stress-induced cell damage either apoptosis or loss of dendrites via AKT/mTOR pathway activation remains to be addressed.

Autoimmunity remains a complex issue, and vitiligo specific antigens are yet to be identified. Studies thus far have shown that UV radiation induces generation of reactive oxidative species and cytokines, which may have deleterious effects on melanocytes and may partially account for an increased rate of vitiligo[28]. Cytokines such as tumor necrosis factor (TNF)-alpha or TNF α , a paracrine inhibitor of melanocytes, play critical roles in the pathogenesis of several autoimmune diseases including vitiligo[29]. Anti-TNF α has been tested for the treatment of vitiligo[30-32]. And yet, the cellular and molecular mechanisms of the actions of cytokines have not been thoroughly studied in cultured human melanocytes.

Given that H_2O_2 induces cell damages, either apoptosis or loss of dendrites or both, oxidative stress may be associated with vitiligo, and cytokines such as TNF α play important roles in vitiligo, we undertook this study to investigate the effect of H_2O_2 on melanocyte dendrites, to study cell signaling pathway leading to α -MSH's protective activity, to investigate the effect of cytokines on melanocytes, and to study the cell signaling pathways of cytokine actions. We, for the first time, found that oxidative stress reduces dendrites and α -MSH protects against oxidative stress-induced loss of dendrites via activation of mTORC1 pathway. We also, for the first time, found that TNF α , but not IL1 β , activates NF κ B pathway and α -MSH does not crosstalk with TNF α cell signaling pathway. Our novel findings support the notion that modulation of mTORC1 pathway and NF- κ B pathway may offer better clinical management of vitiligo.

Materials and Methods

Cell Culture

Primary human skin melanocytes were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in 254 medium (Invitrogen, Carlsbad, CA) with growth factor supplements and Penicillin/Streptomycin (1:100, Sigma-Aldrich, St. Louis, MO) as described previously[15, 20]. Cells were cultured in a CO₂ incubator at 37°C. For confocal microscopy, cells were seeded in eight well chamber slides and grew to eighty percent of confluence for treatment. For

Western blot analysis, cells were cultured in six well slides and grew to ninety percent confluence for treatment.

Reagents and Antibodies

Recombinant human TNF α and IL1 β were purchased from R & D Systems (Minneapolis, MN). α -MSH was from Sigma-Aldrich (St. Louis, MO). LY294002 and Rapamycin were from EMD/Calbiochem (San Diego, CA). Rabbit Anti-p65, Rabbit anti-I κ B, Rabbit-anti α -tubulin were purchased from Santa-Cruz Biotechnology (Santa-Cruz, CA). Rabbit anti-phosphor I κ B, rabbit anti-phosphor p38, and rabbit anti-phosphor-S6 ribosomal protein were purchased from Cell Signaling Technology (Beverly, MA). Mouse anti- β -actin was purchased from Sigma-Aldrich (St. Louis, MO). Alexa Fluor 488 goat anti-mouse IgG or anti-rabbit IgG, Alexa Fluor 594 goat anti-mouse IgG or anti-rabbit IgG, and Alexa Fluor 680 goat anti-rabbit IgG were from Life Technologies (Grand Island, NY). IRDye Goat anti-mouse and anti-rabbit secondary antibodies were from LI-COR (Lincoln, NE).

Confocal microscopy

As previously reported[33], cells were cultured in eight well chamber slides to 90% confluence and treated with various reagents and fixed with 4% formaldehyde in PBS for 10 min. After PBS wash, the cells were permeabilized with cold methanol for 15 min. After PBS wash, cells were blocked with goat serum for 30 min. After PBS wash, cells were stained with various antibodies for 1 hour. After PBS wash, cells were stained with Alexa fluor labeled secondary antibodies for 1 hour. After PBS wash, cells were stained with Hoechst dye for 15 min. After PBS wash, slides were disassembled, covered by anti-fade (Invitrogen, Carlsbad, CA), and slides were sealed for confocal microscopic observation (Carl Zeiss LSM 700). Images were captured by Zen 2009 Light Edition and exported to Photoshop and processed and assembled in Adobe Illustrator CS6.

Quantification of dendricity

Images of cultured melanocytes were captured under confocal microscope, with one cell under one microscope field. The length of the dendrite was measured using a ruler. A total of six cells were used for quantification of dendricity.

Western blot analysis

As previously reported[34], cells were cultured in six-well plates to 80% confluence. Cells were treated with various reagents. Cells were collected at different time points by scraping them into 120 μ l of RIPA cell lysis buffer (50 mM Tris-HCL, pH 7.4, 150

mM NaCl, 1% NP40, 1 mM EDTA, 0.25% sodium deoxycholate, with 1 mM NaF, 10 μ M Na₃VO₄, 1mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail) in a microfuge tube. Cell lysates were incubated in 4°C for 30 min on a shaker. After centrifugation at 12000rpm for 10 min, supernatant was collected. Twenty micrograms of proteins were denatured in 5x SDS-PAGE sample buffer for 5 min at 95°C. The proteins were separated by 10% SDS-PAGE and transferred onto PVDF membrane (EMD/Millipore, Bedford, MA). Nonspecific binding was blocked with 10% dry milk in TBS for 1 h at room temperature. After blocking, membranes were incubated with specific antibodies in dilution buffer (2% BSA in TBS) overnight at 4°C on the shaker. The blots were incubated with secondary antibodies at 1:10000 dilution for 1 h. Antibody binding was detected using Odyssey Infrared Imaging System (LI-COR, Lincoln, NE) following manufacturer's instructions. The images were exported to and processed in Photoshop and assembled in Adobe Illustrator CS6.

Statistical analysis

Statistical comparisons were performed using an unpaired Student's *t* test in Microsoft Excel.

Results

H₂O₂ induces apoptosis and loss of dendrites in cultured human skin melanocytes

To test the effect of H₂O₂ on melanocytes, we first cultured human melanocytes in eight well chamber slides and treated with a different doses of H₂O₂ (ranging from 10 μ M to 500 μ M). The results showed that as expected, melanocytes died 24 hours later at high concentrations, as previously reported[15]. This is one of the causes of the loss of melanocytes leading to vitiligo[15, 35]. Existing data have indicated that dendrites of melanocytes play critical roles in melanin transfer from melanocytes to surrounding keratinocytes. The loss or reduction of dendrites may also affect the capability of melanin transport. Interestingly, we found that, at the concentration of 250 μ M, melanocytes lose dendrites within four hours of the treatment, as shown in Fig. 1 A and B. α -melanocyte stimulating hormone or α -MSH, has been shown to have protective effect on melanocytes[20]. To test whether α -MSH could protect melanocytes from the loss of dendrites under H₂O₂ treatment, we treated the cells with α -MSH first for one hour and then H₂O₂ for two hours. We found that melanocytes pre-treated with α -MSH maintained the integrity of dendrites, while H₂O₂ alone induced loss of dendrites (Fig. 1C, 1D). We also observed that

α -MSH induced S6 phosphorylation in melanocytes (Fig. 1C). S6, a ribosomal protein, downstream of mTORC1, plays an important role in protein

synthesis, thus is critical for cell survival and maintaining dendrites of melanocytes in response to oxidative stress.

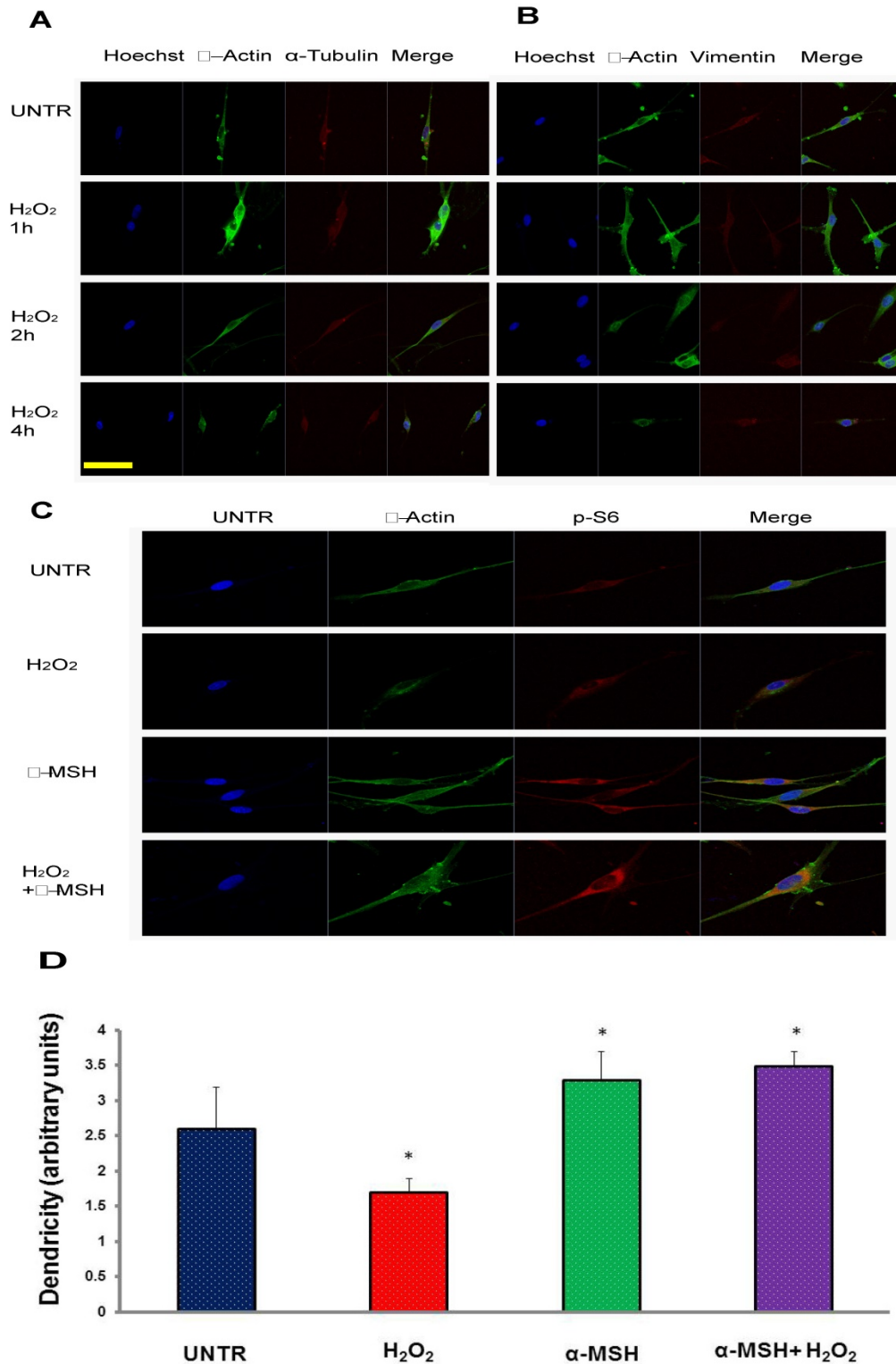


Figure 1. H₂O₂ induces loss of dendrites and α -MSH protects against it in cultured human skin melanocytes. Cultured human skin melanocytes in eight well chamber slides were treated with 250 μ M of H₂O₂ and fixed and stained with Hoechst for nucleus, stained with anti β -actin and α -tubulin as shown in (A), anti β -actin and vimentin as shown in (B) for cytoskeletal proteins. And cells were pretreated with α -MSH (10⁻⁸M) for 1 hour and then treated with H₂O₂ for two hours. The cells were then fixed and stained with Hoechst for nucleus, stained with anti β -actin for cytoskeletal actin and stained with anti-phosphor-ribosomal protein S6 antibody for S6 phosphorylation as shown in (C). Dendricity was quantified based on β -actin staining in C, mean \pm SD, *p<0.05 (D). Scale bar=50 μ m.

mTORC1 plays important roles in maintaining dendrites under oxidative stress

To investigate the cellular signaling pathways that are important in dendrites, we treated cells with H_2O_2 , α -MSH alone, or a combination. Confocal data showed that α -MSH slightly induced AKT phosphorylation (Fig. 2A), however, remarkably induced S6 phosphorylation (Fig. 1C). To confirm this observation, we performed Western blot analysis. Cells were cultured in six well plates and treated with α -MSH and cell lysates were collected at different

time points after treatment. The data showed that α -MSH significantly induced S6 phosphorylation in a time dependent manner (Fig. 2B). Confocal data also showed that LY294002, an inhibitor of PI3K/AKT slightly reduced α -MSH-induced S6 phosphorylation, and Rapamycin, an inhibitor of mTORC1, almost completely abolished α -MSH-induced S6 activation (Fig. 2C). These data suggest that mTORC1 plays an important role in α -MSH-induced S6 phosphorylation which may bypass PI3K/AKT and mTORC2.

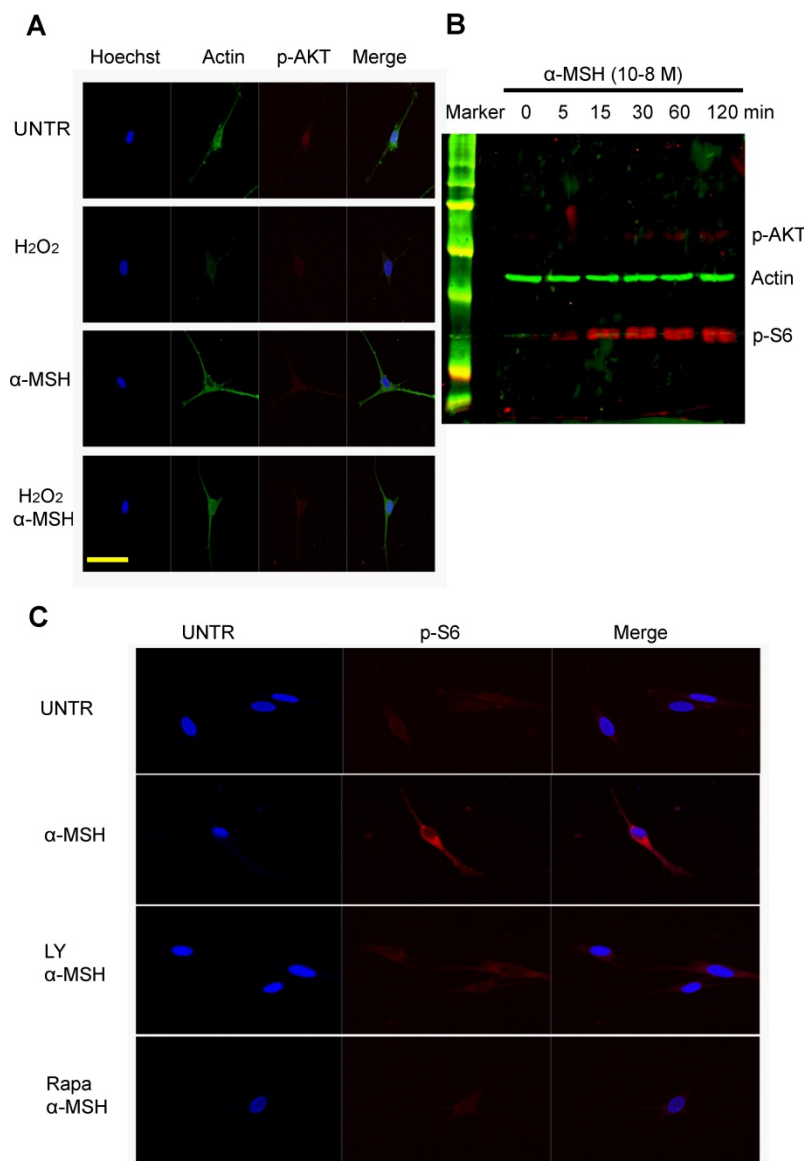


Figure 2. α -MSH induces mTORC1 as measured as S6 phosphorylation but moderately AKT activation in cultured human skin melanocytes. Cells were cultured in eight well slides and pretreated with or without α -MSH for 1 hour and then treated with H_2O_2 for two hours. Cells were then fixed and stained with Hoechst for nucleus, stained with anti β -actin for cytoskeletal proteins and anti-phosphor-AKT for AKT activation as shown in (A). Cells were cultured in six well plates and treated with α -MSH (10^{-8} M). Cell lysates were collected at different time points post treatment for Western blot analysis, probed by anti-phosphor AKT and anti-phosphor S6 for mTORC1 activation, by anti- β -actin as loading control, as shown in (B). And cells were pretreated with PI3K/AKT inhibitor LY294002 (10μ M), or mTORC1 inhibitor Rapamycin (10μ M) for 1h, and then treated with α -MSH (10^{-8} M) for 1h. Cells were fixed and stained with Hoechst for nucleus and anti-phosphor-S6 for S6 activation as shown in (C). Scale bar= 50μ m.

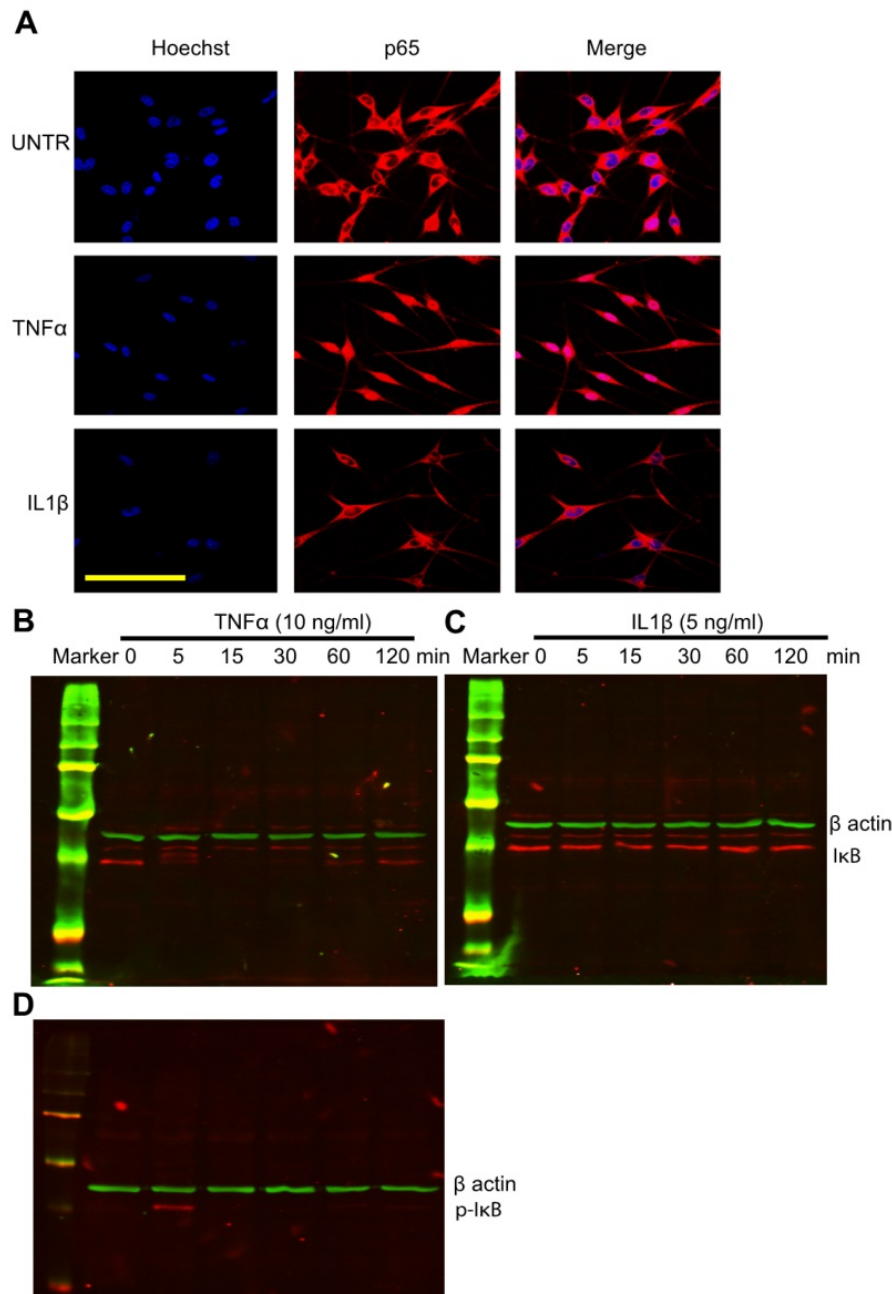


Figure 3. TNF α but not IL1 β induces NF κ B activation and TNF α induces I κ B phosphorylation and degradation in cultured human skin melanocytes. Cells were cultured in eight well chamber slides and treated with TNF α (10ng/ml) or IL1 β (5ng/ml) at different time points. Cells were fixed with 4% formaldehyde in PBS and stained with anti-p65, then observed by confocal microscopy as shown in A. Cells were cultured in six well plates and treated with TNF α (10ng/ml) or IL1 β (5ng/ml) at different time points. Cells were collected for SDS-PAGE/Western blot analysis using anti-I κ B for I κ B degradation by TNF α as shown in (B) or IL1 β as shown in (C), or using anti-phosphor-I κ B for I κ B phosphorylation by TNF α as shown in (D). β -actin was probed as loading control. Scale bar=50 μ m.

TNF α but not IL1 β , induces NF- κ B p65 translocation from the cytoplasm to the nucleus in human skin melanocytes

Accumulating data have shown that cytokines, especially TNF α , play an important role in vitiligo. It has been suggested that anti-TNF α antibody could be used to treat vitiligo[7]. However, the molecular mechanism of TNF α in vitiligo remains unclear. To study whether TNF α activates NF- κ B (with subunits

of p65 and p50) in human skin melanocytes, cells were treated with TNF α at various time points (0, 5, 15, 30 min, 1 and 2 hour) and fixed and stained with anti-p65 antibody. The confocal microscopy data showed that NF- κ B subunit p65 translocated from the cytoplasm to the nucleus in a time-dependent manner. Nuclear staining was most visible at 30 min after treatment (Fig. 3A). The results demonstrate that TNF α activates NF- κ B in cultured human melanocytes. To our knowledge, this is the first report of TNF α -induced

NF- κ B activation in cultured human skin melanocytes. Interestingly, however, IL1 β does not have any effect on p65 translocation, since in human skin keratinocytes IL1 β does activate NF- κ B. To further investigate the mechanism of NF- κ B activation, cells were treated with TNF α at different time points (0, 5, 15, 30 min, 1 and 2 hour). Collected cell lysates were subject to SDS-PAGE Western blot analysis. The results showed that TNF α induced I κ B degradation in a time-dependent manner. I κ B disappeared 5 min after TNF α treatment, remained invisible, and reappeared 30 min post-TNF α treatment (Fig. 3B). There is a band above I κ B which might be the phosphorylated form of I κ B. It is equally interesting but expected that IL1 β did not induce I κ B degradation (Fig. 3C), since IL1 β did not induce p65 translocation in melanocytes (Fig. 3A). To further investigate the activation and degradation of I κ B, cells were treated with TNF α at different time points (0, 5, 15, 30 min, 1 and 2 hour). Cell lysates were subject to

SDS-PAGE and Western blot by anti-phosphor I κ B. The results showed that TNF α induced phosphorylation of I κ B dramatically at 5 min (Fig. 3D), which was corresponding to the degradation of I κ B (Fig. 3A).

α -MSH does not inhibit TNF α -induced NF- κ B activation in cultured melanocytes

Existing data from other cells indicate that α -MSH inhibits TNF α -induced NF- κ B activation[36, 37]. To study whether α -MSH has any effect on TNF α -induced NF- κ B activation in cultured human skin melanocytes, cells were treated with α -MSH 1 hour prior to TNF α treatment. Western blot analysis showed that α -MSH pre-treatment did not affect TNF α -induced I κ B degradation (Fig. 4A), and NF- κ B p65 translocation (data not shown). These results are different from previous publication in keratinocyte[37]. It is likely that melanocytes are respond to α -MSH and TNF α differently from keratinocytes. And our data showed that α -MSH induced mTORC1 activation as indicated by phosphorylation of S6 ribosomal protein (Fig. 1C, Fig. 2B, 2C, Fig. 4B).

TNF α induces p38 but not AMPK α activation in culture human skin melanocytes

To further study the effect of TNF α on apoptotic pathways, cells were treated with TNF α at different time points (0, 5, 15, 30 min, 1 and 2 hour). Cell lysates were collected for SDS-PAGE and Western blot analysis. The results showed that TNF α induced p38 phosphorylation in a time dependent manner. p38 phosphorylation started a few minutes after treatment and peaked at about 15 minutes and the activity returned to basal level in one hour (Fig. 4B). AMPK α activation has been shown to be related apoptosis and mTOR pathways. Our data did not indicate that TNF α induced AMPK α activation in cultured human skin melanocytes (data not shown).

Discussion

Vitiligo is a common pigmentary skin disorder, characterized by the appearance of white macules on the skin, mucosal or hair that may spread over the entire body skin. Depigmentation arises from the loss of functioning melanocytes[38]. Treatment is often a tough challenge and involves a wide

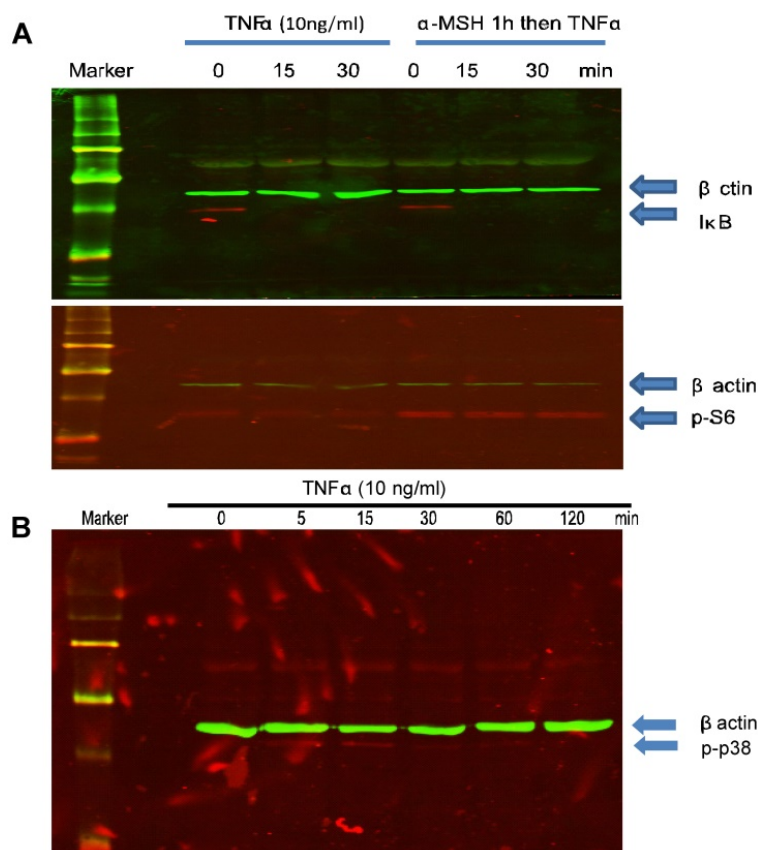


Figure 4. α -MSH does not inhibit TNF α -induced I κ B degradation but induces mTORC1 activation and TNF α induces p38 activation in cultured human skin melanocytes. Cells were cultured in six well plates, pre-treated with or without α -MSH (10^{-8} M) for 1 hour and treated with TNF α (10ng/ml) at different time points. Cells were collected for SDS-PAGE/Western blot analysis using anti-I κ B or phosphor-S6 as shown in (A). Cells were cultured in six well plates and treated with TNF α (10ng/ml) at different time points. Cells were collected for SDS-PAGE/Western blot analysis using anti-phosphor p38 as shown in (B). β -actin was probed as a loading control.

range of therapies[39] including UV radiation, laser[40-42], transplant[43, 44], and complementary medicine[45-47]. The most efficient therapy remains autologous skin graft, performed successfully daily in some of the dermatology clinics.

While the cellular and molecular mechanisms of vitiligo are yet to be fully elucidated, in the field of dermatology, three dominant theories regarding the etiology of vitiligo, namely, reactive oxygen species, cytokines and autoimmunity, have been proposed and supported by experimental and clinical data. However, due to the complexity of this disease, and the likely interconnections of those three possibilities, the studies on vitiligo remain an unparalleled challenge.

It has been proposed that melanocytes are under oxidative stress in vitiligo with a variety of causes including UV radiation and overproduction of cytokines. H_2O_2 levels are elevated locally in the skin of vitiligo patients. Increased ROS contributes to melanocyte apoptosis and the development of cutaneous diseases or disorders via autoimmunity[12]. However, mechanisms and inter-relationships between ROS and autoimmunity are unknown. Most recent data demonstrate that ER protein calreticulin or CRT exposure via H_2O_2 -induced oxidative stress plays a significant role in melanocyte apoptosis, suggesting a relationship between apoptosis and immune reactions during melanocyte destruction[48, 49].

In addition to apoptosis, we found in this study that H_2O_2 induced the loss of dendrites in melanocytes within hours of treatment (Fig. 1, 2). Dendrites are critically important in melanin transfer. The loss of dendrites would result in the reduced melanin levels in keratinocytes that account for the skin pigmentation. We found that α -MSH, that has been used in the treatment of vitiligo[50] protects against H_2O_2 -induced loss of dendrites in melanocytes (Fig. 1). This novel discovery supports the notion that α -MSH could be one of the options for the management of vitiligo.

α -MSH plays important roles in melanocyte growth and melanin production via cell surface receptor activation[51]. α -MSH has been also identified as a potent anti-inflammatory in various tissues including the skin. We found that α -MSH activates S6 ribosomal protein and enhances dendrites of melanocytes (Fig. 1) and protects against H_2O_2 -induced loss of dendrites. To further understand the molecular mechanism through which MSH protects against H_2O_2 -induced loss of dendrites, we investigated mammalian target of rapamycin or mTOR pathways that are related to protein synthesis, metabolism, cell survival and other cellular activities.

mTOR signaling pathway couples energy and nutrient abundance to the execution of cell growth and division. Mammalian TOR complex 1 (mTORC1) and mTORC2 exert their actions by regulating other important kinases, such as S6 kinase (S6K) and AKT[52] respectively, or collectively. mTORC1 triggers cell growth and proliferation by promoting protein synthesis and metabolism, and by reducing autophagy or enhancing survival[53]. Our data suggest that α -MSH-induced activation of mTORC1 pathway, inhibited by rapamycin (Fig. 2), help maintain dendrites of melanocytes under oxidative stress (Fig. 1, Fig. 2).

Studies have shown that levels of H_2O_2 and IL-6, a pro-inflammatory cytokine and a key factor in the pathogenesis of autoimmune diseases are elevated in vitiligo lesions, suggesting that H_2O_2 -induced overexpression of IL-6 by melanocytes via p38 and NF- κ B pathways may be a molecular linkage for the oxidative stress and inflammatory/autoimmune reactions in vitiligo and may provide a novel target for the treatment of vitiligo[54, 55]. However, the mechanism through which cytokines affect pigmentation and dendrites is not fully understood[56] and warrants further investigation.

TNF α has been suggested to play important roles in vitiligo. Anti-TNF α antibody which has been proposed and used, albeit in only one case in vivo, appears promising[7]. And yet, thus far, no reports have been published on the cellular signaling pathways of cytokine actions in human skin melanocytes. We, for the first time, have shown that TNF α , but not IL1 β , induces NF- κ B pathway activation through I κ B phosphorylation and degradation (Fig. 3). We have provided insights into the understanding of the molecular actions of cytokines in human skin melanocytes. Our data also support the notion and TNF α antibody could be used in human skin in vivo in vitiligo patients.

It has previously been shown in skin cell keratinocytes and melanocytes/melanoma cells that α -MSH inhibits TNF α -stimulated NF- κ B activity[37]. α -MSH has an anti-inflammatory action on dermal fibroblast signaling by inhibiting the pro-inflammatory activity of TNF α in vitro[55]. Interestingly, we found in this study that in human skin melanocytes, α -MSH activates mTORC1 pathway, and does not cross talk with TNF α -induced NF- κ B pathway (Fig. 4), which is also a novel observation. Since α -MSH plays such an important role in melanocytes, crosstalk with other pathways would introduce more complications to the complex cellular responses.

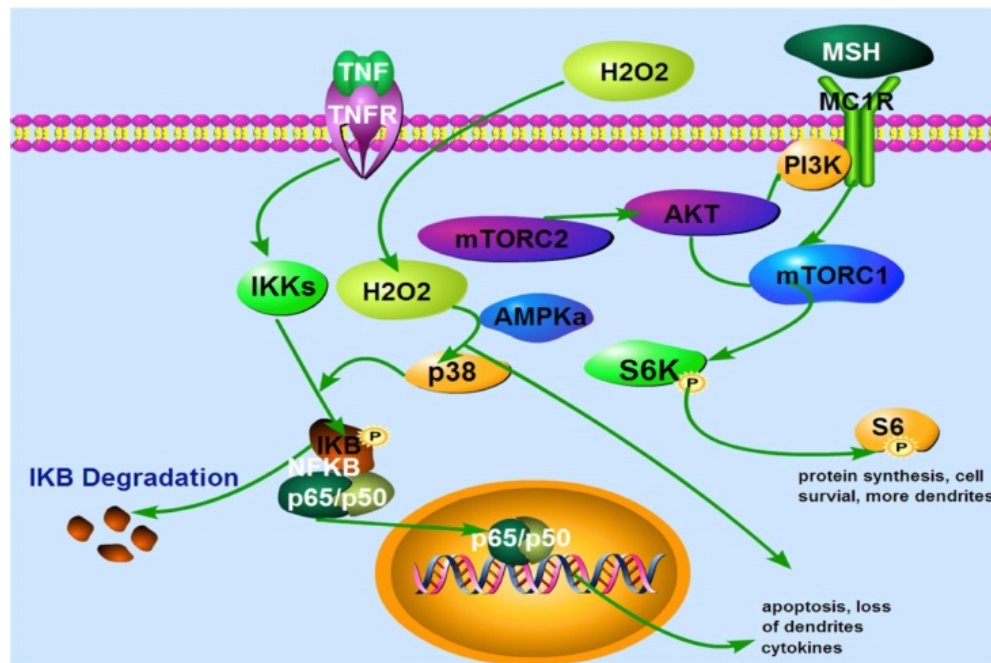


Figure 5. A proposed model that α -MSH activates mTORC1 and protects against H_2O_2 -Induced loss of dendrites in human skin melanocytes. Extracellular H_2O_2 activates p38 and NF κ B leading to apoptosis and loss of dendrites. α -MSH activates mTORC1 pathway via cell surface receptor MC1R which is blocked by rapamycin, leading to protein synthesis, cell survival and more dendrites. TNF α activates cell surface receptor which induces I κ B phosphorylation and degradation. This results in translocation of NF- κ B subunits p65 and p50 from cytoplasm to nucleus, and expression of more cytokines, apoptosis, and loss of dendrites. TNF α may also induce intracellular H_2O_2 production that activates NF- κ B pathway.

In conclusion, our data demonstrate for the first time that oxidative stress reduces dendrites of melanocytes. α -MSH protects against H_2O_2 -induced loss of dendrites via activation of mTORC1 pathway. Our data also for the first time show that TNF α induces NF- κ B activation via I κ B phosphorylation and degradation. α -MSH, does not inhibit TNF α -induced NF- κ B activation in cultured human skin melanocytes (Fig. 5). Collectively, our data suggest that modulation of mTORC1 and NF- κ B pathway may offer better approaches for clinical management of vitiligo.

Abbreviations

mTOR: mammalian target of rapamycin; MSH: melanocyte stimulating hormone;

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Author contributions

All authors contributed significantly to this research project and the preparation of the manuscript. JW, AX, JD and YW conceived and

designed the experiments and analyzed the data. FL cultured the cells and JW treated the cells and ran Western blot and confocal microscopy. JW and YW wrote the draft of the manuscript. WZ and HL were involved in the revision process. All authors revised the manuscript and approved the final version for publication. All authors are accountable for all aspects pertinent to the project including accuracy and integrity.

Competing Interests

The authors have declared that no competing interest exists.

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