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TNFSF14 inhibits melanogenesis via NF-kB signaling in melanocytes

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ABSTRACT

Melanin synthesis in melanocytes is affected by various cytokines. Here, we reported for the first time that tumor necrosis factor superfamily member 14 (TNFSF14) inhibits melanogenesis in the primary culture of human epidermal melanocytes. TNFSF14 is known to bind to its receptors herpes virus entry mediator (HVEM) and lymphotoxin β receptor (LTβR) for signal transduction, but TNFSF14-induced hypopigmentation was independent of HVEM and LTβR in melanocytes. To explore signaling in melanocytes treated with TNFSF14, we performed RNA-seq and found that nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) signaling is activated by TNFSF14. Further, we observed that inhibition of NF-kB effectively blocks the hypopigmentation induced by TNFSF14. We conclude that TNFSF14 inhibits melanogenesis in melanocytes via NF-κB signaling and could be applied in the treatment of cutaneous pigment disorders.

1. Introduction

Skin color is a result of melanin pigments that are synthesized within melanosomes, which are produced by melanocytes in the bottom layer of the epidermis. Several inflammatory mediators such as cytokines directly and/or indirectly affect melanin synthesis. Interleukin (IL)-4, IL-17α, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), and interferon-γ (IFN-γ) inhibit melanogenesis in melanocytes through triggering various molecular events [1–5]. Thus, skin inflammation is closely related to melanogenesis.

Tumor necrosis factor superfamily (TNFSF) member 14 (TNFSF14), which is also known to be homologous to lymphotoxin, exhibits inducible expression and competes with HSV glycoprotein D for binding to herpesvirus entry mediator (HVEM), a receptor expressed on T lymphocytes (LIGHT), and is a cytokine belonging to TNFSF. TNFSF14 is known to play an important role in diverse immunological processes. TNFSF14 leads to T cell activation through interaction with HVEM by enhancing T cell proliferation [6–8] and induces cytokine secretion through binding to lymphotoxin β receptor (LTβR) on mast cells [9]. TNFSF14 has also been implicated in various pathogenic conditions related to inflammatory responses such as tumors, type 2 diabetes, and atherosclerosis [10–12].

Recently, it was reported that TNFSF14 promotes skin fibrosis through the production of the innate cytokine thymic stromal lymphopoietin (TSLP) and TGF-β [13], suggesting that TNFSF14 could play an important role in skin inflammation. However, the effect of TNFSF14 on melanogenesis has not been investigated. Therefore, we studied the effect of TNFSF14 on melanocytes and demonstrated that in melanocytes, TNFSF14 induces hypopigmentation through activation of nuclear factor κ-light-chain-enhancer of activated B cell (NF-κB) signaling.

2. Materials and methods

2.1. Cell culture, transfection, and treatment

Normal human epidermal melanocytes prepared from the foreskins of three donors were purchased from Life Technologies (Carlsbad, CA, USA) and maintained in Medium 254 (Life Technologies) containing a human melanocyte growth supplement (Life Technologies) at 37 °C in an atmosphere containing 5% CO_2.

Melanocytes were treated with carrier-free recombinant human TNFSF14 protein (R&D Systems, Minneapolis, MN, USA) for the indicated times. Denatured TNFSF14 proteins were prepared by boiling them for 5 min at 95 °C. To inhibit NF-κB signaling, melanocytes were pretreated with InSolution™ NF-κB Activation Inhibitor (Calbiochem, San Diego, CA, USA) for 2 h before treatment with recombinant TNFSF14 protein.

Melanocytes were transfected with Silencer® Select siRNAs (Life Technologies) against *HVEM* (ID, s16700, and s16701) and *LTBR* (ID, s8326, and s8327) using RNAiMAX (Life Technologies) according to the manufacturer's instructions.

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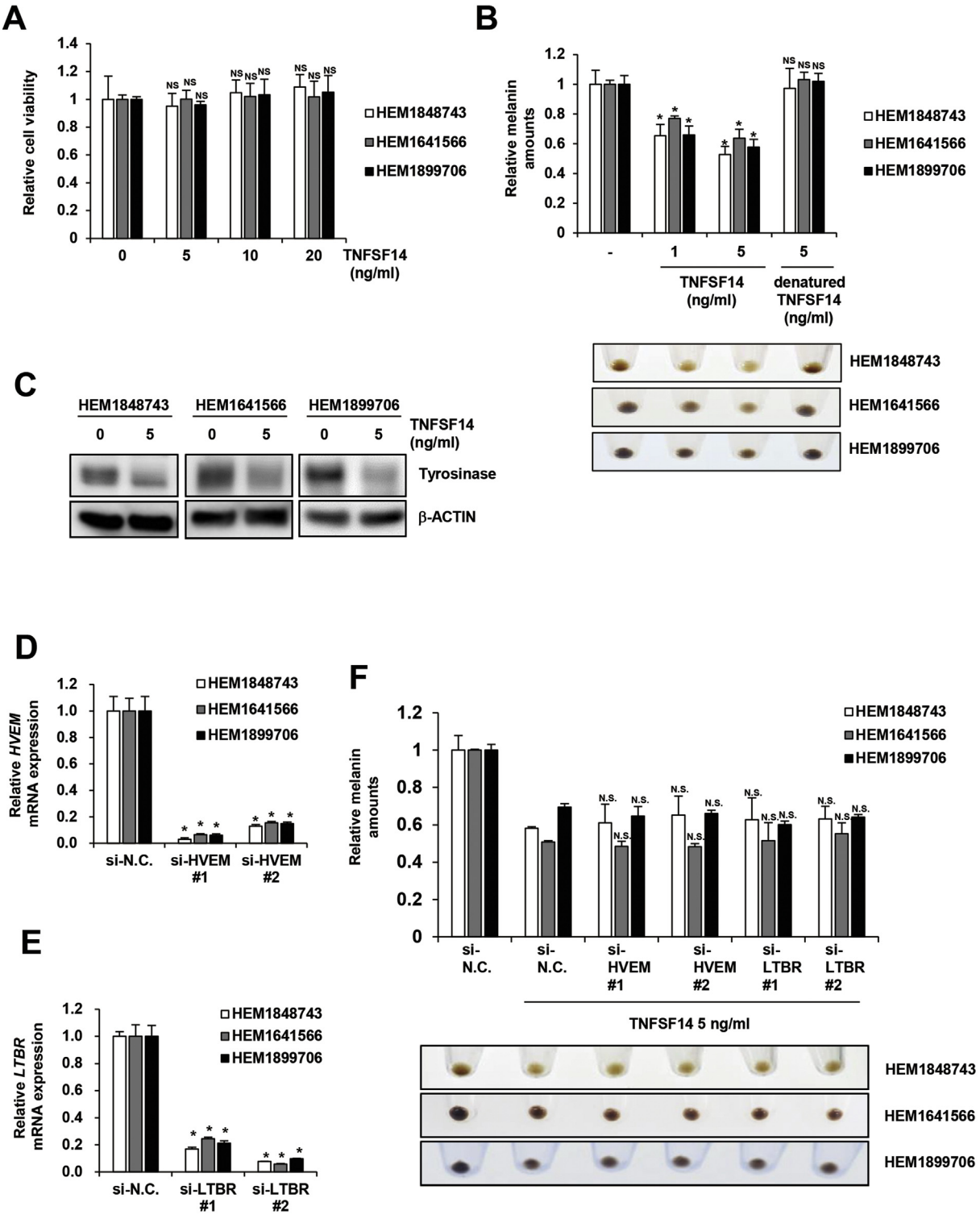

Fig. 1. Treatment of melanocytes with recombinant TNFSF14 inhibits melanogenesis. (A) Human primary melanocytes were treated with recombinant human TNFSF14 at the indicated concentration for 4 days. Cell proliferation was measured by the WST-1 assay. The data are presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$ vs. non-treated melanocytes. (B) Human primary melanocytes were treated with recombinant TNFSF14 at the indicated concentrations for 4 days. The relative melanin content was determined. The data are presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$. Representative images of cell pellets (2×10^4 cells) are shown. (C) Expression levels of tyrosinase and β-actin proteins extracted from human primary melanocytes treated with recombinant TNFSF14 at 5 ng/ml were examined by western blotting. (D) Melanocytes were transfected with si-HVEM or si-N.C. After 2 days, *HVEM* mRNA expression was examined by performing RT-qPCR. The data are presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$ vs si-N.C. (D) Melanocytes were transfected with si-LTBR or si-N.C. After 2 days, *LTBR* mRNA expression was examined by performing RT-qPCR. The data are presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$ vs si-N.C. (E) Melanocytes were transfected with si-HVEM, si-LTBR, or si-N.C. before 1 day of treatment with recombinant TNFSF14. After incubation with recombinant TNFSF14 at 5 ng/ml for 4 days, the relative melanin content was determined. Representative images of cell pellets (2×10^4 cells) are shown. The data are presented as the mean ± SD of three independent experiments. $^{*}P < 0.05$ vs si-N.C. treated with TNFSF14.