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Isocitrate dehydrogenase 2 deficiency exacerbates dermis damage by ultraviolet-B via ΔNp63 downregulation

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ABSTRACT

Isocitrate dehydrogenase 2 (IDH2) is a key enzyme that maintains the balance of mitochondrial redox status by generating NADPH as a reducing factor, which is used to reduce oxidized antioxidant proteins and oxidized glutathione. Therefore, the role of IDH2 is crucial in organs that are easily influenced by reactive oxygen species (ROS) or mechanical damage. Humans are constantly exposed to ultraviolet (UV) radiation throughout their lifetime, which can cause various cutaneous diseases, such skin carcinoma, dermatitis, and sunburn. ROS play an important role in the initial step of these diseases; therefore, IDH2 deficient mice (*Idh2*$^{-/-}$) could be a useful model to investigate UV-mediated skin damage. When we exposed the dorsal skin of *Idh2*$^{-/-}$ mice to UVB, pyrimidine dimers and (6-4) photoproducts (6-4PPs), marker of photoproducts generated by UVB, were found in the dermis of the knockout mice. Increased collagen degradation, apoptosis, inflammation, and ROS levels in the dermis were also observed. These results indicated that UVB could reach the dermis by penetrating the epidermis. We then attempted to determine how the epidermis was breached, and observed a decrease in the expression level of ΔNp63, a major protein required for epidermis generation, in the *Idh2*$^{-/-}$ mice. The mito-TEMPO supplement significantly ameliorates UVB-induced damage in the skin of *Idh2*$^{-/-}$ mice. In the present study, we provided a role for IDH2 in protection against UVB-induced skin damage and a new connection between IDH2 and ΔNp63.

1. Introduction

Sunburn, immunosuppression, photoaging, erythema, skin carcinoma, and melanogenesis are diseases and conditions that can be induced by ultraviolet (UV) rays in sunlight. In particular, among the various UV rays, UVB (280–315 nm) radiation makes the largest contribution to many skin diseases because of its penetrative ability, energy state, and easy accessibility [1,2]. Although most UVB radiation is blocked by the epidermis and only reaches the shallow top region of the dermis, longer exposure to UVB can initiate inflammation and ROS generation mediated by lipid peroxidation [3]. In addition, if UV reaches the dermis, collagen fibers are degraded directly, without mediating other pathways, and forms wrinkles at the skin surface [4]. To minimize the damage from UV, more studies are required to understand its various mechanisms of action.

The development of the early epidermis is initiated by diverse proteins. Tumor protein 63 (TP63), also known as p63, is a member of the p53 family and has a distinct role in diverse pathways, such as skin development and regulation of the cell cycle. TP63 encodes for two main isoforms by alternative promoters (TAp63 and ΔNp63). The N-terminal truncated isoform ΔNp63 is involved in multiple functions during skin development and in adult stem/progenitor cell regulation. In contrast, TAp63 has been mostly restricted to its apoptotic function and more recently as the guardian of oocyte integrity [5,6]. ΔNp63 levels are highest in proliferative keratinocytes and these high levels are balanced with other proteins, such as Notch, p53, and p21 [7]. High levels of ΔNp63 induce the differentiation of stem cells to epidermal keratinocytes, and keratinocytes with moderate ΔNp63 levels maintain the proliferative capabilities of progenitor cells. Proliferation of keratinocytes at the epidermis is inhibited by Notch to avoid unnecessary overgrowth [8]. The balance between ΔNp63 and Notch provides the epidermis with sufficient thickness to protect the interior cells from the exterior environment.

Isocitrate dehydrogenases (IDHs) catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate, accompanied by reduction of $NAD(P)^+$ to NAD(P)H. IDH2 is found in the matrix of mitochondria and plays a major role in providing mitochondrial NADPH [9]. NADPH is required as a reducing equivalent for the regeneration of glutathione

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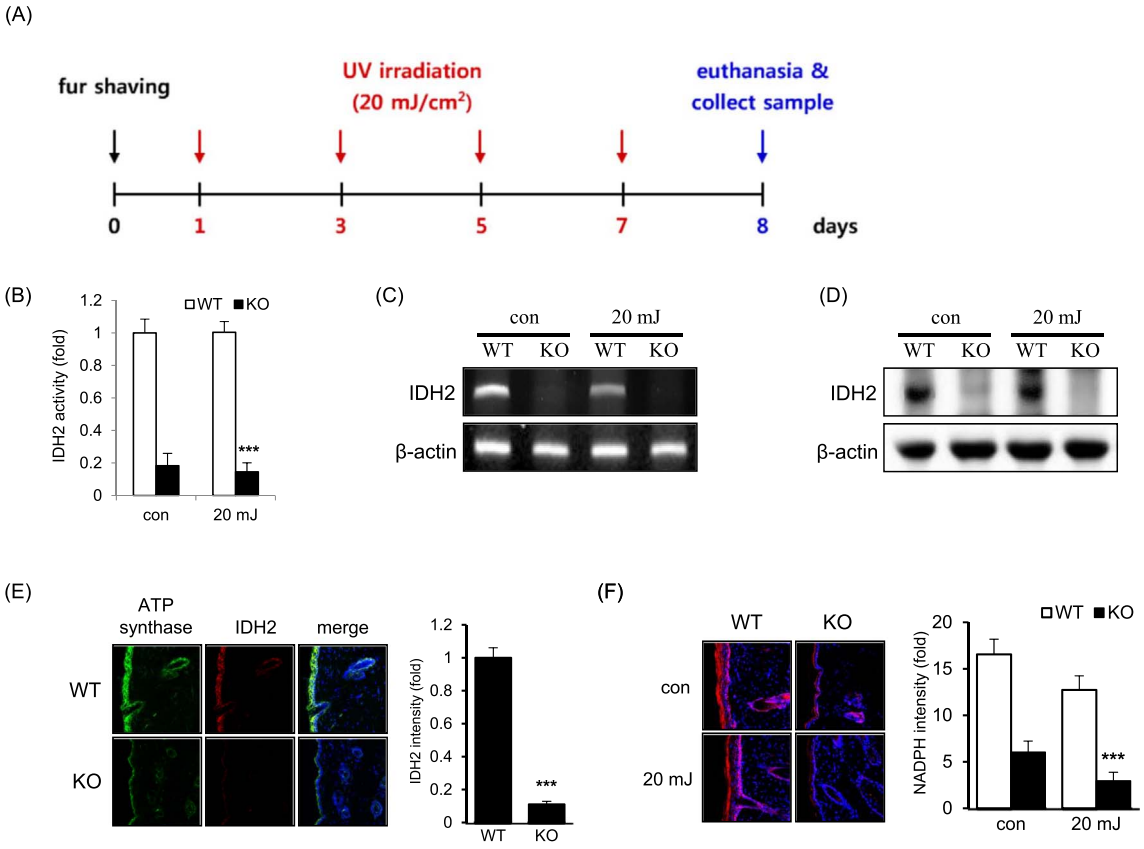

Fig. 1. Characterization of isocitrate dehydrogenase 2 (IDH2) knockout mice. (A) Experimental protocol of UVB irradiation of mice. The shaved dorsal region of each mouse was irradiated using an UV illuminator once every two days for 7 days. (B) IDH2 activity in the skin from wild-type (WT) (*Idh2*$^{+/+}$) and knockout (KO) mice (*Idh2*$^{-/-}$). (C) Immunoblotting analysis of IDH2 and (D) Reverse transcription PCR (RT-PCR) analysis of *Idh2* gene expression of skin from WT and *Idh2*$^{-/-}$ mice. β-Actin was used as an internal control. (E) Immunofluorescence staining for IDH2 (red) and ATP synthase (green) in skin tissues from WT and *Idh2*$^{-/-}$ mice. Histograms represent the quantification of fluorescence intensity. (F) Intracellular NADPH was determined by immunofluorescence using fluorochrome-conjugated anti-NADPH antibodies. Histograms represent the quantification of fluorescence intensity. In (B), (E), and (F), the results are shown as the mean ± SD (n = 3–6 mice). ***$p < 0.001$ between the two genotypes indicated.

(GSH) by glutathione reductase and antioxidant proteins, such as thioredoxin and glutaredoxin, which are essential components of the mitochondrial antioxidant system [10]. Therefore, the role of IDH2 is critical in reactive oxygen species (ROS)-associated diseases. Previously, we showed that hypertrophy occurred in the hearts of *Idh2*$^{-/-}$ mice, and in *Idh2*-silenced H9c2 cardiomyocytes, decreased generation of NADPH induced higher ROS levels, apoptosis, senescence, and drug sensitivity [11,12]. IDH2 is important in organs that consume massive amounts of ATP via their abundant mitochondria, such as the heart and muscle, or in organs that need the capacity to handle ROS produced by exterior stresses, such as the skin. In the present study, we investigated the role of IDH2 in protection against UVB-induced skin damage and identified a new connection between IDH2 and ΔNp63.

2. Materials and methods

2.1. Materials

β-NADPH, β-NADP$^+$, ethylenediaminetetraacetic acid (EDTA), and xylenol orange were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Primary antibodies were purchased from Cell signaling (Beverly, MA, USA), Santa Cruz Biotechnology (Santa Cruz, CA, USA), and Abcam (Cambridge, MA, USA), and fluorescence-conjugated secondary antibodies were purchased from Molecular Probes (Eugene, OR, USA).

2.2. Animal preparation

Experiments were performed using 8-week-old male C57BL/6 mice with different genotypes, including wild type (WT) *Idh2*$^{+/+}$ and knockout (KO) *Idh2*$^{-/-}$ mice generated by breeding, as described previously [11]. Animals were housed in temperature and humidity-controlled cages with a 12 h light/dark cycle, and were allowed free access to chow and water. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Kyungpook National University. The dorsal fur of mice was shaved completely with a hair trimmer after anesthesia using mixture of xylazine hydrochloride and ketamine. The shaved dorsal region of each mouse was irradiated using an UV illuminator once every two days for 7 days (four times in total, with a total UV dosage was 80 mJ/cm^2; the UV dosage was measured using UV meter). A filtered UV illuminator that can conduct UVB only was used. The day after the final UV irradiation, mice were sacrificed and the obtained skin was stored in a freezer for subsequent experiments. Some mice were treated with 2-(2,2,6,6-tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl) triphenylphosphonium chloride (mito-TEMPO, 5 mg/kg body weight; Sigma, St. Louis, MO), via intraperitoneal (i.p.) injection once daily during UV irradiation.

2.3. RNA isolation and reverse transcription (RT)-PCR

To extract mRNA, a 1 cm × 1 cm sized whole skin piece was treated with 500 μL of TRIzol reagent and incubated for 30 min at room temperature. RNA was reverse transcribed to cDNA using a First-strand