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Human fibroblasts treated with hydrogen peroxide stimulate human melanoblast proliferation and melanocyte differentiation, but inhibit melanocyte proliferation in serum-free co-culture system

Tomohisa Hirobe[a,*], Tatako Shibata[b], Kiyoshi Sato[b]

[a] Fukushima Project Headquarters, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan
[b] Shiseido Global Innovation Center, 2-2-1 Hayabuchi, Tsuzuki-ku, Yokohama 224-8558, Japan

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ABSTRACT

Background: Oxidative stress caused by hydrogen peroxide (H_2O_2) elicits harmful effects on human melanocytes such as DNA damage and cell death. On the contrary, H_2O_2 is known to possess beneficial effects on melanocytes. However, mechanisms of the beneficial effects of H_2O_2 on melanocytes have not been fully understood, especially the indirect effects on melanocyte proliferation and differentiation from cells constituting surrounding tissue environment such as fibroblasts.
Objective: The aim of this study was to clarify whether H_2O_2-treated human fibroblasts affect the proliferation and differentiation of human melanocytes using serum-free co-culture system.
Methods: Epidermal melanoblasts and melanocytes were co-cultured with H_2O_2-treated or control fibroblasts in serum-free culture media. The effects of H_2O_2-treated fibroblasts were detected by changes in the proliferation and differentiation of melanoblasts/melanocytes.
Results: H_2O_2-treated fibroblasts stimulated the proliferation of melanoblasts and the differentiation, melanogenesis, and dendritogenesis of melanocytes, but inhibited the proliferation of melanocytes. In the melanocytes co-cultured with H_2O_2-treated fibroblasts, the expression of tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and KIT was increased, whereas TYRP2 and microphthalmia-associated transcription factor showed no change.
Conclusion: These results suggest that H_2O_2-treated fibroblasts can produce and release some mitogenic and melanogenic factors toward melanoblasts in addition to some proliferation-inhibiting factors toward melanocytes. The stimulation of melanocyte differentiation seems to be performed through the upregulation of TYR, TYRP1, and KIT.

1. Introduction

Mammalian melanocytes characterized by pigment-producing organelles (melanosomes) and dendrites are located mainly in the epidermis, dermis, and hair follicles of the skin. Differentiated melanocytes in the epidermis migrate into hair follicles and colonize hair bulb melanocytes where mature melanosomes are transferred to surrounding keratinocytes and finally pigmented hairs are completed. During skin development, surrounding tissue environment plays an important role in the regulation of melanocyte proliferation and differentiation [1]. To induce the differentiation of melanocytes, many pigmentation-related proteins including tyrosinase (TYR), TYR-related protein-1 (TYRP1), TYRP2 (dopachrome tautomerase; DCT), KIT, and microphthalmia-assosiated transcription factor (MITF) are required [1]

Keratinocytes that constitute the epidermis and hair follicles control the proliferation and differentiation of melanocytes in the epidermis and hair follicles [2–4]. Numerous keratinocyte-derived factors such as endothelin-1 (ET-1), ET-2, ET-3, stem cell factor [SCF, or steel factor (SLF), KIT ligand (KITL)], hepatocyte growth factor (HGF), granulocyte-macrophage colony-stimulating factor (GMCSF), and leukemia inhibitory factor (LIF) have been reported to promote the proliferation and differentiation of melanocytes [2–4]. Fibroblasts in the dermis and dermal papilla also control the proliferation and differentiation of melanocytes in the epidermis, dermis, and hair follicles through paracrine factors including basic

* Corresponding author.
E-mail address: hirobe.tomohisa@qst.go.jp (T. Hirobe).

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fibroblast growth factor (bFGF or FGF2) [5], acidic FGF (or FGF1) [5], transforming growth factor-β1 [6], keratinocyte growth factor [6,7], SCF [7], HGF [7,8], interleukin (IL)-1α [6], IL-1β [6], dickkopf-related protein 1 [9], and neuregulin-1 [10].

Of environmental factors that regulate the proliferation and differentiation of melanocytes, ultraviolet radiation (UV) is one of the most important factors for humans and animals [2]. UV generates reactive oxygen species (ROS) that elicit harmful effects on melanocytes such as DNA damage and cell death [11]. Hydrogen peroxide (H_2O_2) is a major mediator of oxidative damage in cells [11]. The oxidative stress caused by H_2O_2 greatly affects melanocyte function and results in depigmentation disorder such as vitiligo [12]. On the contrary, H_2O_2 possesses beneficial effects on melanocyte function [13,14], namely lower concentration (<0.3 mM) of H_2O_2 stimulates TYR activity and melanin synthesis in melanocytes, while higher concentration (>0.3 mM) reduces TYR activity and melanin synthesis. However, it has not been clarified whether the major sources of H_2O_2 after UV exposures are melanocytes or cells constituting surrounding tissue environment. Recent study shows that keratinocytes are major source of H_2O_2 produced and released in the skin [15]. Moreover, in co-culture system of melanocytes and keratinocytes, lower concentration of H_2O_2 stimulates melanin synthesis and melanosome transfers from melanocytes to keratinocytes [14]. However, it is not known whether H_2O_2-treated fibroblasts can affect melanocyte function in a similar way. The dermis-derived factors are thought to be transferred to the epidermis and lead to the stimulation of epidermal melanoblast proliferation and melanocyte differentiation, dendritogenesis, and melanogenesis. Heparanase-induced loss of heparan sulfate chains at the dermal-epidermal junction may promote the transfer of dermis-derived factors [16]. Thus, it is possible that dermal factors including H_2O_2 can affect the function

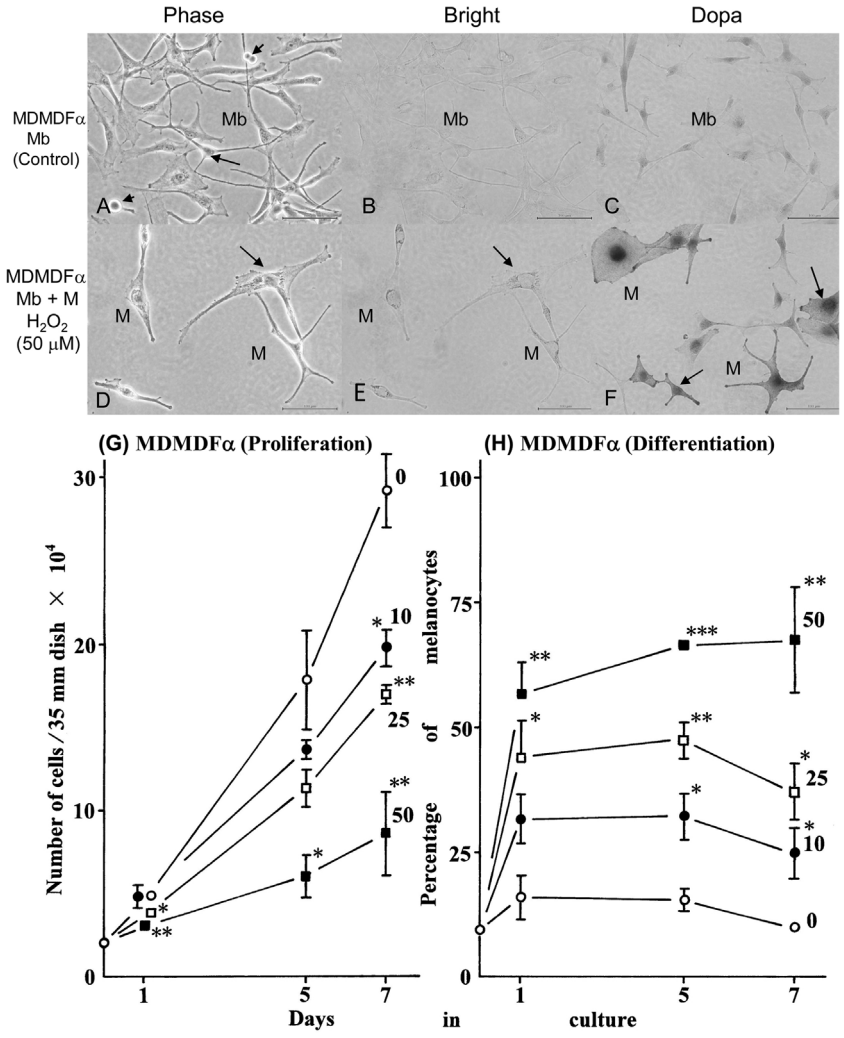

Fig. 1. Effects of H_2O_2 on the proliferation of human epidermal melanoblasts. Melanoblasts were cultured in MDMDFα for 7 days. Control (A, B, C) cultures and 50 μM H_2O_2 (D, E, F)-treated cultures. Numerous melanoblasts were observed in the control (A) culture, whereas fewer melanoblasts were observed in the H_2O_2 (D)-treated culture. However, H_2O_2 (E) stimulated the differentiation of melanocytes more greatly compared with the control (B) culture. Dopa reaction reveals that H_2O_2 (F) increased the number of dopa-positive melanocytes compared with the control (C) culture. Moreover, H_2O_2 increased dendritogenesis and cell expansion of melanocytes. Phase, phase-contrast microscopy (A, D); Bright, bright-field microscopy (B, C, E, F); Dopa, dopa reaction; Mb, melanoblasts; M, melanocyte; MDMDFα, human melanoblast-proliferation medium. Short arrows indicate mitoses of melanoblasts. Long arrows indicate differentiated melanocytes. Scale bar, 100 μm.

Kinetics of melanoblast proliferation (G) and melanocyte differentiation (H). Melanoblasts were treated with H_2O_2 at concentrations of 0 (Control, ○), 10 (●), 25 (□), and 50 (■) μM. The number of melanoblasts decreased by H_2O_2 at all concentrations tested (G). In contrast, the percentage of melanocytes in the melanoblast-melanocyte population was increased by H_2O_2 at all concentrations tested (H). The number of cells was counted at 1, 5, and 7 days and the percentage of melanocytes in the melanoblast-melanocyte population was calculated. The data are averages of results from three experiments. Each experiment was performed with different cultures. Bars indicate standard errors of the mean (SEM) and are shown only when they are larger than symbols. Statistically significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.