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# Regulation of Proopiomelanocortin and Melanocortin 1 Receptor by UVB: Inhibitory Effect of Antioxidants

Yoko Funasaka*

Division of Dermatology, Department of Clinical Molecular Medicine, Faculty of Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan

Epidermal cells produce a panel of antioxidants as well as cytokines after UVB irradiation, which counteract reactive oxygen species, however, how these antioxidants might regulate melanogenesis is unclear. An important constituent of the cellular antioxidant buffering system which controls the redox state of proteins is thioredoxin (TRX), a 13-kD protein that catalyzes thiol-disulfide exchange reactions, regulates activation of transcription factors, and possesses several other biological functions similar to cytokines. TRX suppressed the UVB-induced production and secretion of α-melanocyte stimulating hormone (α-MSH) and of adrenocorticotropic hormone (ACTH), and also suppressed proopiomelanocortin (POMC) mRNA expression by normal human keratinocyte (KC)s. Further, L-cysteine, N-acetyl-cysteine, α-tocopheryl ferulate showed suppressive effect on UVB-induced POMC mRNA expression. However, TRX released from UVB-irradiated KCs stimulated melanogenesis by up-regulating MSH receptor expression and its binding activity in melanocyte (MC)s. UVB-induced KC derived cytokines such as IL1, IL6, and ET1 upregulated MSH-receptor binding ability as well as MC1-R mRNA expression in cultured normal human MCs. MC1-R has a tendency to be upregulated by UVB-induced KC-derived cytokines as well as by direct UVB irradiation. These results suggest that antioxidants such as TRX suppresses UVB induction of POMC, but in the case of MC1-R, this gene can be mainly in the trend of upregulation by UVB-induced KC-derived factors including TRX.

**Key words:** proopiomelanocortin, MSH, MC1-R, antioxidants, thioredoxin, UVB, melanogenesis, melanocyte

## INTRODUCTION

The melanotropin ( melanocyte-stimulating hormone, MSH ) and the MSH receptor ( MSH-R ) is known to play a key role in ultraviolet B ( UVB)-induced melanogenesis in mouse melanoma cell line [1]. However, the significance of MSH as a physiologic regulator of cutaneous pigmentation in human has been controversial because of signal transduction of MSH-R after being activated by MSH was masked by cyclic adenosine 3', 5'-monophosphate (cAMP) inducer such as cholera toxin

Correspondence:
e-mail:funasaka@med.kobe-u.ac.jp

(CT ) and IBMX which were often used in normal human melanocyte (MC) culture[2]. The another reason is that the number of MSH-R is very few in cultured MCs. We have previously shown that some cytokines and growth factors derived from UVB- irradiated KCs upregulate MSH-R binding ability and mRNA expression of melanocortin 1 receptor (MC1-R) [3]. Further, we have also found that one of antioxidants which are induced by UVB irradiation in KCs, thioredoxin (TRX), upregulates both MSH binding ability and mRNA expression of MC1-R [4]. This indicates that UVB acts on skin cells both in a direct and indirect manner, and not only cytokines but also antioxidants are involved in UVB- induced melanogenesis. Here we review the effect of UVB and antioxidants on the regulation of MSH and MC1-R expression in KCs and MCs.

## Regulation of MSH receptor / MC1-R expression

MSH-R is a G-protein-coupled receptor, which activates adenylyl cyclase, and therefore is expected to have sequence homology with other members of this large gene family. By RT/PCR and cDNA cloning techniques, the existence of these receptors in humans and rodents has been shown. Receptor binding studies have verified that human MCs express MC1-R[5,6].

According to the reports from two groups, Scatchard analyses have revealed that cultured normal human MCs possess only a few hundred binding sites for α-MSH on the cell surface, while B16 mouse melanoma cells show the binding sites up to ten thousand [7,8]. Although the $K_d$ value for this binding in normal human MCs is similar to that in the B16F1 cells, there are approximately ten times more binding sites on mouse melanoma cells [7]. It is interesting that α-MSH has been shown to increase cAMP levels in normal human MCs at much smaller levels than in mouse melanoma cells, consistent with the differences in receptor numbers [9]. This low level of cAMP activation as a result of the small numbers of α-MSH-Rs, may be insufficient for stimulus for cultured normal human MCs to proliferate or to induce melanogenesis [9].

It has been shown that proopiomelanocortin (POMC)-derived peptides are present in the skin and further, UVB stimulates production and secretion of α-MSH and ACTH in KCs and MCs [10,11]. Furthermore, the injection of human volunteers with MSH or ACTH or with superpotent analogue of MSH caused maximal darkening in sun-exposed areas of the skin, suggesting a synergistic interaction between MSH and UV radiation. Such an interaction has been studied in mouse melanoma cells where UV is thought to act by increasing the binding of MSH to its receptor. Considering few binding sites on ordinary cultured human MCs, up-regulation of MSH-R might play an essential role in inducing the effects of melanotropins on human MCs.

UVB-induced KC derived cytokines such as IL1, IL6, and ET1 upregulated MSH-R binding ability as well as MC1-R mRNA expression in cultured normal human MCs [3]. Further, another sets of cytokines induced by UVB in KCs such as IL8, IL10, GM-CSF, TGFβ1, and bFGF upregulated MSH-R binding ability as well as MC1-R mRNA

expression (Funasaka et al, manuscript in preparation). Interestingly, IL2 and IL4 which are not induced by UVB-irradiated KCs did not enhance MSH-R binding ability (our observation). These results indicate that MC1-R has a tendency to be upregulated by UVB-induced KC-derived cytokines as well as by direct UVB irradiation.

## Regulation of POMC

The POMC gene product is a 31-kDa prohormone that is post-translationally cleaved to generate adrenocorticotropins ( ACTH ), lipotropins ( LPH ), endorphins and three isotypes ($\alpha$, $\beta$, $\gamma$) of MSH; $\alpha$ -MSH being the biologically most active form for MCs. POMC gene induction is known to be regulated by cortitotropic hormone (CRH), IL1, $Ca^{++}$, cAMP inducers, or UVB.

The induction of POMC mRNA expression in KCs by UVB was suppressed by L-cysteine, N-acetyl-cysteine, $\alpha$-tocopheryl ferulate, and thioredoxin (TRX) [12] which indicates that UVB-induced upregulation of POMC gene expression might be negatively regulated by intracellular glutathione level.

## Biological effect of TRX

Thioredoxin (TRX) is a small multifunctional protein with a redox-active disulfide/dithiol motif within a conserved active site sequence -Cys-Gly-Pro-Cys-. Increasing evidence has indicated that cellular redox status modulates various aspects of cellular events, including proliferation and apoptosis. Human TRX was initially discovered as an adult T cell leukemia-derived factor (ADF) produced by human T-cell lymphotropic virus type I-infected T cells, which up-regulates the interleukin (IL)2 receptor $\alpha$-chain and IL2. Subsequently, TRX has been shown to regulate various intracellular molecules via thiol redox control involving transcription factors such as nuclear factor (NF)-$\kappa$B, activator protein 1 (AP-1), myb, redox factor 1, and mitogen-activated kinase. TRX has been reported to induce AP-1 through *de novo* transcription of *c-fos* and *c-jun*, and TRX enhances the DNA-binding activity of Jun and Fos. TRX is also a stress-inducible protein whose expression is enhanced by various types of stress, e.g. viral infection, exposure to UV, X-ray irradiation, and hydrogen peroxide ($H_2O_2$). Furthermore TRX is a scavenger of reactive oxygen intermediates (ROI), and recombinant TRX (rTRX) protects against cytotoxicity in which the generation of ROI seems to be involved. The sum of these data suggests that TRX plays a number of important biological roles in the intracellular and in the extracellular compartments.

We have previously reported that TRX expression and releasae in KCs were enhanced by UVB irradaition, and released TRX protects KCs from cell death in an autocrine manner [12] through upregulating MC1-R expression on KCs which results in enhancement of MSH signaling and cell protection. Although TRX upregulates MC1-R expression in both MCs and KCs, this peptide inhibits UVB- induced POMC expression. These results suggest that KC-derived TRX regulates the expression of POMC and its receptor in a different manner. The interpretation of the sum of these results in UVB-induced melanogenesis should need further

experimental studies.

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