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Melanocyte Stimulating Hormone이 멜라닌 색소침착에 미치는 영향

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The Effects of Melanocyte Stimulating Hormone on Melanin Pigmentation

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멜라닌세포의 성장 및 기능조절에 관한 연구가 피부색소계에 대한 이해 및 기미의 병인연구 및 치료제개발에 필요하다. 멜라닌합성은 tyrosinase활성도, tyrosinase mRNA생성과 단백질합성, tyrosinase이 후과정에 작용하여 멜라닌세포 특성을 조절하는 tyrosinase related protein (TRP)-1, 2 등의 발현 및 최종산물인 멜라닌양에 의해 결정되며, 이 과정들이 피부에 존재하는 여러 물질들에 의하여 조절된다.

Melanocyte stimulating hormone (MSH)은 주로 뇌하수체 전엽에서 분비되나, 최근 자외선 조사나 염증시 각질형성세포에서 분비된다고 알려진 이후 피부에서 MSH작용에 대한 관심이 증가되고 있다. 사람에서 배양된 멜라닌세포에 대하여 MSH가 tyrosinase 활성도를 증가시켰으며 이는 tyrosinase 및 TRP-1, 2의 단백질양을 증가시켰으나 이들 효소의 mRNA 전사증가에는 영향을 미치지 않았다. MSH 수용체가 멜라닌세포 내에 발현되었으며 MSH가 이 수용체에 결합시 세포내 cAMP를 증가시켰다.

피부는 MSH 및 agouti signal protein (ASP) 등을 분비하므로, 이들이 멜라닌합성과정에 미치는 영향을 관찰하기 위하여 배양된 인체 멜라닌세포에 ASP 투여시 tyrosinase활성도 및 TRP-1양이 감소되었으며, MSH에 의한 cAMP 및 tyrosinase활성도 증가를 억제하였다.

자외선에 반응하여 피부가 멜라닌합성을 증가시키는 기전을 연구하기 위하여 배양된 멜라닌세포에 자외선 조사시 tyrosinase 활성도, tyrosinase, TRP-1 양이 감소되었으나, MSH 첨가로 tyrosinase 활성도, tyrosinase, TRP-1양이 정상으로 회복되거나 오히려 증가되었다.

이상의 결과를 근거로 피부색소계는 멜라닌세포와 다른 피부구성세포들간의 상호 작용에 의하여 조절됨을 알 수 있었다. 피부는 기저상태나 자외선 조사에 의해 MSH, ASP 등을 분비하여 파라크린작용으로 멜라닌합성을 조절한다. 이 과정에서 MSH가 중요한 역할을 할 것으로 사료된다.

Key words : MSH, Melanocytes
Melanogenesis, Pigmentation
Agouti signal protein

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INTRODUCTION

The variation in skin color between human ethnic groups, and the difference in the pigmentary response to stress, the incidence of pigmentary disorders such as vitiligo, albinism, melasma and melanoma have raised questions about how pigment system is regulated in the skin. Melanin is produced by melanocytes and human skin color is determined by the functional unit of the synthesis and distribution of melanin. Visible skin pigmentation depends on a wide variety of factors that influence melanocyte function at various levels. Final pigmentation is determined by factors that affect melanoblast development and migration in the developing embryo[1], melanocyte survival and proliferation in the skin[2], melanocyte function in response to environmental stimuli[3,4], and melanin granule distribution and subsequent processing by neighboring keratinocytes[5].

GENES RELATED TO HUMAN MELANIN PIGMENTATION

Tyrosinase is a critical enzyme to produce melanin from amino acid, tyrosine. The reaction cascade to make melanin is generally referred to as the Raper-Mason scheme and involves a complex series of oxidation and rearrangements to make pigmented biopolymer. Other two tyrosinase related gene families, tyrosinase related protein (TRP)-1 and -2 which upregulate the black/brown eumelanin synthesis rather than yellow / red pheomelanin also play an important role to determine the final skin color[6]. In mice, approximately 150 genes at 60 loci are known to influence eye, skin or hair color. About 50 of these genes are primarily pigmentary genes and the rests alter pigmentation by pleotropic mechanisms[7]. The major pigmentary genes largely behave in a typical Mendelian fashion and produce discrete effects such as white-spotting, albinism, yellow, black or brown hair color. In human, relatively small number of gene pairs are acting additively without dominance on the continuous worldwide variation in human skin color from very light pink to virtually black. This indicates the polygenic inheritance of human skin color. Several gene loci of greatest importance in melanogenesis of mice are as follows; *c* locus which encodes tyrosinase; *b* locus for TRP-1; *a* locus for agouti signal protein; *e* (extension) locus for melanocyte stimulating hormone (MSH) receptor; *sl* (slaty) locus for TRP-2. Alleles at the *a* locus regulate the ratio of pheomelanin to eumelanin within the pelage and determine the melanogenesis by way of the tissue environment such as MSH[8]. MSH which is encoded by the *e* locus binds its receptor and elevates intracellular cAMP and finally induces eumelanogenesis in the melanocytes[9].

SIGNAL TRANSDUCTION PATHWAYS RELATED TO MELANIN PIGMENTATION

The melanocytes can be altered by a wide variety of factors which are produced by melanocyte itself (autocrine), local (paracrine) or systemic (endocrine) environment. Over the past two decades, the culture of human melanocytes has increased enormous understanding on how the proliferation and differentiation of melanocytes are regulated via receptors and signal transduction[2,10-14]. It became possible to identify some molecular controls of melanocyte proliferation and differentiation in terms of second messengers and transcription factors because considerable knowledge had been derived from other cellular systems. Melanocytes transduce the signals of protein kinase C stimulators (phorbol ester, endothelin-1), cAMP/protein kinase A stimulators (MSH, cholera toxin, forskolin, isobutylmethylxanthine), receptor tyrosine kinase activators (basic fibroblast growth factor (bFGF), mast cell growth factor, hepatocyte growth factor)[15]. Those factors are produced in the skin and mediate their own signals by themselves or in combination with other factors to induce proliferation / death, differentiation, dendricity/morphology, migration, matrix protein production, cytokine production, immune/inflammatory response and pigmentation[16]. In this article, the pigmentary response of human epidermal melanocytes to MSH will be discussed.

Synthesis of MSH

MSH is the best known hormone that is involved in regulating mammalian pigmentation. Its effect is at two levels; eliciting an increase in the proliferative rate of melanocytes and a stimulation in the production of melanins. The human pituitary gland produces significant quantities of proopiomelanocortin (POMC) which has potential melano-

tropic activity. POMC is mostly formed in the anterior and intermediary lobes of pituitary gland. POMC is cleaved into α, β, γ-MSH, ACTH (adrenocrticotropic hormone), CLIP (corticotrophin-like protein; ACTH1-17), β, γ-LPH (lipotropin), α, β, γ-endorphin and enkephalin by different prohormone convertases[17]. PC (prohormone convertase)-1 cleaves POMC into ACTH, β-LPH and PC-2 cleaves ACTH into small fragment such as ACTH1-17, desacetyl α-MSH (Fig. 1). POMC peptides are subjected to post-translational modifications and some of these modifications are regulated by neurotransmitters. Among those, dopamine, gamma-aminobutyric acid (GABA), opioids, and histamine are capable of modifying POMC peptides[18]. The cellular origin of MSH is currently an area of active interest (Table 1). The production and cleavage of POMC could occur other than in pituitary gland, such as many other parts of the brain, adrenal gland, placenta, lymphocytes of the thymus and spleen. Interestingly, immunoreactive α-MSH peptide was found in the skin of human and other mammals by radioimmuno assay[19] and immunohistochemical stain[20]. POMC gene expression was also noted in normal skin and it was modulated by various cytokines. Recently, α-MSH is produced in the cultured keratinocytes in response to ultraviolet irradiation, interleukin-1, and phobol myristate stimulation[21]. This finding suggests the possibility that α-MSH may act as paracrine factors derived from adjacent keratinocytes.

Table 1. α-MSH prodection in different cell types.

Tissue/Cell source	Stimulus
Pituitay gland	IL-1,2, dopamin, CRF*
Brain	
Adrenal medulla	
Placenta	
Deratinocyte	phorbol ester, IL-1, UVB
Melanocyte	phorbol ester, IL-1, UVB
Langerhans cell	
Fibroblast	
Endothelial cell	
Monocyte	Lipopolysaccharide
Macrophage	
Mast cell	

*CRF, corticotropin-releasing factor

MSH receptor

The identification of the murine extension locus as being the gene for the melanocortin 1-receptor (MC1-R) has opened up opportunities to obtain a genetic handle on the study of cutaneous pigmentation. The human MC1-R gene, encoding for a seven transmembrane G-protein coupled receptor of 317 amino acids, was cloned in 1992. At the same time, on the basis of Southern hybridization of human genomic DNA at low stringency to a human MC1-R probe, Mountjoy et al.[22] cloned total five members of the melanocortin receptor gene family. According to the order in which they were cloned, the five receptors are named MC1-R, MC2-R, MC3-R, MC4-R, and MC5-R and they showed different tissue distribution and altered affinities for the various melanotropic peptides (Table 2)[23]. MC1-R mRNA is

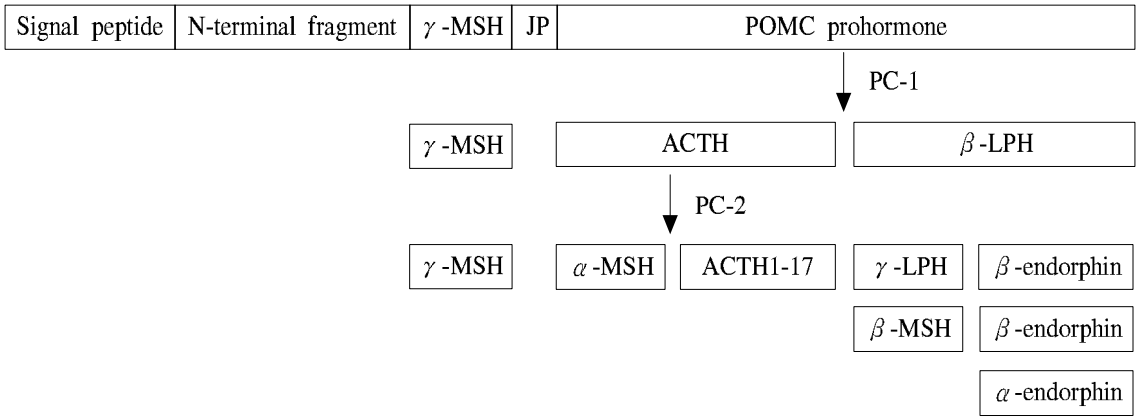

Fig. 1. Structure of the POMC precursor and its derived peptides. MSH, melanocyte stimulating hormone; JP, joining peptide; POMC, proopiomelanocortin; ACTH, adrenocorticotropic hormone; LPH, lipotropin.

expressed nearly exclusively in melanocytes and has been shown to have equal affinity for α-MSH and ACTH[9]. Upon binding to MC1-R, melanotropins activate adenylate cyclase and increase the intracellular cAMP concentration and protein kinase A activity. These changes in the second messengers produce a variety of cellular responses including proliferation and pigmentation of melanocytes[2]. Naturally occurring variants of the MC1-R were discovered to encode constitutively active receptors responsible for dark coat colors in the *sombre* and *tobacco* mice[24]. Data on the human system showed that MC1-R variant alleles are higher in people with fair skin and/or red hair. A certain variant occurs at a higher frequency in patients with melanoma than in controls[25]. Concomitant treatment of interferon-α, β, γ increased the action of MSH in melanoma cells and this suggested that interferon increased the MC1-R sensitivity to MSH[26]. Ultraviolet (UV) irradiation also increased the MC1-R sensitivity in melanoma cells[27]. MSH itself increased the expression of MC1-R mRNA in normal human melanocytes. This effect was evident within 4 h, reached maximum level in 6 h, and declined to basal level after 24 h of treatment 9 . Endothelin-1 also increased the MC1-R mRNA in cultured melanocytes and subsequently induced higher cAMP formation (Im S, unpublished data).

Effect of MSH on melanin pigmentation of human skin

Injection of the melanotropins such as α-MSH and β-MSH to human subjects elicited a darkening of the skin[28]. This phenomenon resulted from elevated melanogenesis within epidermal melanocytes and increased transport of melanosome within keratinocytes. Injection of high doses of ACTH also induced cutaneous hyperpigmentation in human subject[29]. The influence of the exogenous melanotropins was most evident in dark-skinned individuals and hyperpigmentation was mostly pronounced in the sunexposed areas. Bolognia et al[30] reported that in guinea pig, topical administration of β-MSH along with UVR elicits a significantly greater darkening of the skin than either agent alone. Addison's disease increased pigmentation of skin and mucous membrane owing to excess deposits of melanin by the elevated levels of either β-LPH or ACTH or both. Nelson's syndrome, the subset of patients with Cushing's disease caused the elevated blood levels of ACTH and β-LPH and intense cutaneous hyperpigmentation[23].

Condition for MSH experiment on cultured human melanocytes

Despite the well-documented darkening of human subjects following injections of melanotropins, there is debate over whether α-MSH has the ability to elevate melanogenic activity in human melanocytes *in vitro*. Several groups of investigators have examined the direct effects of melanotropins on cultured human melanocytes. Some found no response to α-MSH, whereas others noted that α-MSH caused a significant increase in intracellular cAMP without appreciable stimulation of tyrosinase activity and melanin formation[31,32]. Those investigators routinely used the cAMP inducers, such as cholera toxin, in the melanocyte growth media. In the presence of these factors human melanocytes

Table 2. Cellular distribution, ligand specificity, and funciton of different melanocortin receptors.

Isotype	Cell Source	Specific Ligand	Function
MC1-R	Melanocyte	α-MSH, ACTH	Pigmentation
MC2-R	Adrenal cortex, adipocyte	α,β,γ-MSH, ACTH	Steroidogenesis
MC3-R	Hypothalamus, limbic system, placenta, gut	α,β,γ-MSH, ACTH	Unknown
MC4-R	Hypothalmus, laimbic system, cortex, brain stem	α,β,γ-MSH, ACTH, β-LPH	Feeding regulation, thermoregulation
MC5-R	Exocrine glands, muscle, brain, adipocyte	ACTH	Regulation of function of exocrine gland

The expression and function of MC1-R in keratinocyte, fibroblast, endothelial cell, monocyte, macrophage, and mast cell need further detalied investigation.

failed to respond to melanotropins. It was shown that α-MSH was the only melanogenic but not mitogenic for human melanocytes maintained in a medium devoid of any melanocyte-specific mitogen. Others reported that α-MSH was mitogenic but not melanogenic for these cells. However recently, normal human melanocytes maintained in a medium containing phorbol myristate acetate and bFGF without bovine pituitary extract and any cAMP enhancers, both showed mitogenic and melanogenic response to the α-MSH[33].

Binding characteristics of MSH to MC1-R

Primary normal human melanocytes expressed mRNA for the MC1-R[9]. From the binding assay of different melanotropins to the MC1-R, IC_{50} values ranged with the following order of potency: α-MSH, ACTH > β-MSH > γ-MSH in affinity for the MC1-R. All of the above peptides resulted in a dose-dependent increase in cAMP levels with the following order of potency: α-MSH, ACTH > β-MSH > γ-MSH. The binding affinity of these peptides to the MC1-R correlated directly with the increase in cAMP and the melanogenic and mitogenic potential to these peptides.

Effects of MSH on melanogensis of cultured human melanocytes

Human melanocyte strains established from donors with different skin types responded to α-MSH or ACTH with dose-dependent increases in proliferation and tyrosinase activity[33,34]. The minimal effective dose for either melanotropin is 0.1 nM, and their effects are evident in neonatal, as well as adult foreskin melanocyte derived from Caucasian, African American or Korean. The mitogenic effect increased incrementally with continuous treatment with 100 nM α-MSH for up to 8 days, whereas the stimulatory effect on tyrosinase activity became maximal after 4 days and did not increase further with prolonged treatment of this hormone. The molecular mechanisms by which α-MSH elicit their synergistic effects of melanocyte proliferation are possibly through the phosphorylation of ERK2 in combination with bFGF and endothelin-1[35,36]. Treatment with α-MSH resulted in marked morphologic alterations of human melanocytes, most evident as increased dendricity. Electron microscopic studies revealed the presence of more melanized melanosornes and more intense dopa reactivity in the trans-Golgi area, coated vesicles, and melanosomes in α-MSH-treated melanocytes compared with untreated melanocytes. Western blot analysis showed that α-MSH also increased the amounts of tyrosinase and TRP-1, 2. Northern blot analysis of these same cells showed that α-MSH did not significantly alter the levels of mRNA transcripts for the above three melanogenic proteins[33].

Interaction of MSH with agouti signal protein

The other hormone known to regulate mammalian pigmentation is the agouti signal protein (ASP), the product of the a locus[37]. In mice, ASP mRNA is produced by cells in the dermal papillae. Its protein product acts on melanocytes in the overlying hair follicle but not on adjacent follicles, demonstrating the paracrine nature of ASP action. In mice, levels of ASP mRNA correlate with both the visual appearance of coat color and pheomelanin/ eumelanin ratio. Many of the pigmentary effects of increased ASP expression are overcome by administration of α-MSH or by activating mutations of the MC1-R, which suggests that ASP functions as an α-MSH antagonist. ASP in normal murine melanocytes inhibited expression of tyrosinase, TRP-1, 2 in concert with the reduction of steady-state mRNA levels for these protein[38]. A human homolog for the murine ASP gene has been cloned and its product has been characterized. The human homolog of ASP is expressed at levels detectable by Northern hybridization in heart, ovaries, and testes, and at levels detectable by RT-PCR in many tissues including newborn foreskin and adipose tissue[39]. Treatment of human melanocytes with 1-10 nM recombinant human ASP blocked the stimulatory effects of α-MSH on cAMP formation and tyrosinase activity. In the absence of exogenous α-MSH, ASP inhibited basal levels of tyrosinase activity and reduced the level of immunoreactive TRP-1 without significantly altering the level of immunoreactive tyrosinase. In addition, ASP blocked the melanogenic effects of forskolin or dibutyryl cAMP, agents that act downstream to the MC1-R[40]. These results demonstrate that the functional relationship between the agouti and MC1-R gene products is similar in mice and humans and suggest a potential physiologic role

for ASP in regulation of human pigmentation (Fig. 2).

Interaction of MSH with ultraviolet B radiation

A hallmark of sun exposure is the increased melanin synthesis by cutaneous melanocytes which protects against photodamage and photocarcinogenesis. Some discrepancies exist in the effects of ultraviolet B (UVB) irradiation between human skin and cultured melanocytes[3,4,41,42]. Irradiation of human skin with UVB light increases the number of functioning melanocytes and stimulates melanin synthesis *in vivo*. The studies on the effects of UV radiation on human melanocytes *in vitro* showed that these cells responded with growth arrest, decreased survival, and increased melanogenesis. Interestingly, by dissecting the UV responses of melanocyte, in the absence of any cAMP enhancer in the culture medium, UVB-irradiated melanocytes failed to mount a melanogenic response after a single or multiple irradiations with a sublethal dose (5 or 15 mJ/cm^2) of UVB[34]. The activity as well as the level of tyrosinase were both decreased following UVB treatment in the absence of cAMP enhacers. However, by adding the α-MSH in the culture media, UVB-induced inhibition of tyrosinase activity recovered to normal level or even increased higher than basal activity, suggesting that α-MSH may act as a transducer in inducing the protective melanin pigment in human skin. This is further evidenced by the reports that irradiation of human keratinocytes with UVB light stimulates the synthesis and release of α-MSH and ACTH (Fig. 2)[21].

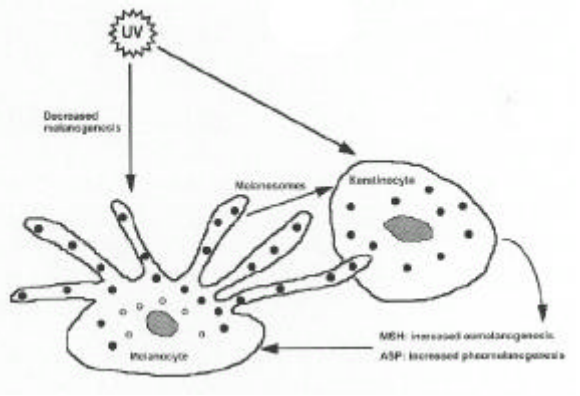

Fig. 2. A new proposal for the regulation of melanogenesis by MSH, ASP, and UV light. MSH, melanocyte stimulating hormone; ASP.

Roles of MSH in other systems

α-MSH is speculated to modulate cutaneous inflammatory reactions[43]. α-MSH, a potent antagonist to IL-1, both block the sensitization and elicitation phases of contact hypersensitivity. α-MSH antagonizes the effects of the proinflammatory cytokines such as IL-1α, IL-1β, IL-6, and tumor necrosis factor-α. α-MSH downregulates the production of interferon-γ in lymphocytes stimulated by mitogen or antigen. In contrast, α-MSH increases the antiinflammatory cytokine, IL-10[17,18,44]. Additionally, α-MSH might exert its immunomodulatory effects by downregulating the expression of MHC class I antigens on monocytes and keratinocytes while not affecting the expression of MHC class II antigens and intercellular adhesion molecule 1. The effect of α-MSH on cells residing in the dermis has not been fully investigated (Table 2). Dermal α-MSH may be derived from the epidermis, dermal fibroblasts, or inflammatory cells infiltrating the dermis such as monocytes or lymphocytes. POMC peptides are known to have lipolytic activity and upregulate the synthesis of collagenase/MMP-1 in human fibroblasts. POMC peptides also have neurotropic actions including increased neuronal survival, neurite growth, protein synthesis, and nerve regeneration. In combination with the immunomodulatory effects of α-MSH, these actions may provide some evidences that α-MSH may contribute the neurogenic inflammation and wound healing[18].

PERSPECTIVES

Significant progress has been made over the last decade in our understanding of the regulation of the melanogenesis via action of melanotropic peptides. It is becoming clear in many mammals how variation in the coat color can occur. The application of all this knowledge to human pigmentation remains to be accomplished. Does MSH act as a key player or it just contribute a minor role in human pigmentation? What are the actual roles of MSH *in vivo* after UV irradiation or internal hormonal changes, in the pathogenesis of certain pigmentary disorders? This will be an interesting area of investigation for the dermatologists. While many

significant advances have been made in the peptide chemistry of the melanotropins, surprisingly no high-affinity antagonists of the melanotropin receptors have been identified. The development of selective antagonists to different melanotropin receptor isotypes in addition to agouti product will be useful for the analysis of melanotropin receptor functions *in vivo* and will provide new therapeutic modalities to some pigmentary disorders which are related to the function of melanotropins. Further investigation will also be focused on how downstream pathway after MC1-R binding controls the melanogenesis and other related proteins.

CONCLUSIONS

1. Human keratinocytes synthesize and secrete α-MSH in response to UV light and other mitogen stimulation.
2. Human melanocytes express MC1-R and transduce α-MSH signal by increasing cAMP level upon α-MSH binding.
3. α-MSH increases the proliferation and melanogenesis in cultured human melanocytes.
4. ASP blocks the melanogenic effects of α-MSH.
5. α-MSH plays a key role in increasing the tyrosinase activity after UV light.

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