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Short communication

Histamine receptor 2-mediated growth-differentiation factor-15 expression is involved in histamine-induced melanogenesis

Hye Ja Lee[a], Mi Kyung Park[a], Eun Ji Lee[a], You Lee Kim[a], Hyun Ji Kim[a], Joon Hee Kang[a], Hwan Mook Kim[b], Ai Young Lee[c], Chang Hoon Lee[a,*]

[a] College of Pharmacy, Dongguk University-Seoul, Goyang 410-820, Republic of Korea
[b] College of Pharmacy, Gacheon University, Incheon 406-799, Republic of Korea
[c] Department of Dermatology, College of Medicine, Dongguk University, Ilsan Hospital, Goyang 410-820, Republic of Korea

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ABSTRACT

Vitiligo is a progressive depigmenting disorder. Histamine has been shown to induce melanogenesis via histamine receptor 2, suggesting the possibility of histamine as a repigmenting agent for the treatment of vitiligo. However, the role and signaling mechanism of histamine are still unclear in melanogenesis, especially in relation to growth-differentiation factor-15, which is a protein belonging to transforming growth factor beta and found to be overexpressed in metastatic or malignant melanoma. We found that histamine induces growth-differentiation factor-15 in melanoma cell lines such as SK-MEL-2, B16F10, and melan-a cells. Therefore, in the present study, the role of growth-differentiation factor-15 in histamine-induced melanogenesis was investigated using gene silencing or overexpression of growth-differentiation factor-15 and histamine related compounds such as histamine, amthamine, and cimetidine. Gene silencing of growth-differentiation factor-15 suppressed histamine-induced proliferation, melanin production, tyrosinase activity, and chemotactic migration of SK-MEL-2 cells. Histamine-induced expression of tyrosinase, tyrosinase-related protein 1, and tyrosinase-related protein 2 was also suppressed by growth-differentiation factor-15 gene silencing. On the other hand, overexpression of growth-differentiation factor-15 using a plasmid containing growth-differentiation factor-15 in SK-MEL-2 cells increased melanin production and chemotactic migration. Amthamine induced expression of growth-differentiation factor-15 in a time and concentration dependent manner. Amthamine-induced expression of growth-differentiation factor-15 was suppressed by cimetidine.

Our results suggest that growth-differentiation factor-15 is a new player in histamine-induced melanogenesis, which can help researchers to extend the knowledge of the role of the transforming growth factor beta family in melanogenesis and in skin pigment disorders such as vitiligo.

1. Introduction

Skin pigmentation disorder may result from problems in melanocyte proliferation, differentiation, melanogenesis, migration or dendricity (Yamaguchi and Hearing, 2009). In melanocytes, melanins are synthesized within melanosomes which contain three major pigment enzymes: tyrosinase, tyrosinase-related protein (TRP-1), and TRP-2 (Park et al., 2009). The expression of these proteins is regulated transcriptionally by the microphthalmia-associated transcription factor (MITF). MITF is a unique, important transcription factor involved in all aspects of melanocyte survival and function (Tachibana et al., 1996).

Skin hypopigmentation may result from a reduction of melanocytes or from an inability of melanocytes to produce melanin or properly transport melanosomes (Plensdorf and Martinez, 2009). In particular, vitiligo represents a progressive depigmenting disorder characterized by the destruction of functional melanocytes from the epidermis through a combination of multiple processes influencing melanocyte function and survival (Birlea et al., 2009). Repigmention of vitiligo requires proliferation, differentiation, migration or increases in dendricity of melanocytes (Yamaguchi et al., 2007). Recently, several amines including histamine (HA) and serotonin have been reported to be involved in melanogenesis and showed a possibility of beneficial effects on vitiligo mediated by histamine receptor 2 (HR2) or serotonin receptor 2, respectively (Kim and Lee, 2010; Lee et al., 2011).

Growth-differentiation factor 15 (GDF-15) is a protein belonging to the transforming growth factor beta (TGF-β) superfamily that plays a role in the regulation of inflammatory and apoptotic

* Corresponding author. Tel.: +82 31 961 5213; fax: +82 31 961 5206.
E-mail addresses: uatheone@dongguk.edu, uatheone@gmail.com (C.H. Lee).

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pathways in injured tissues and during disease processes (Zimmers et al., 2005). Although GDF-15 has been reported to exhibit both tumorigenic and antitumorigenic activities, recent reports show that GDF-15 is overexpressed in malignant melanoma and is correlated with the tumorigenicity of melanoma cells (Boyle et al., 2009). However, the role of GDF-15 in melanogenesis is not reported at all.

In this paper, we found that GDF-15 expression is induced by HA via HR2 and discovered that GDF-15 is involved in HA-induced melanogenesis.

2. Materials and methods

2.1. Reagents

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from WelGENE Inc. (Daegu, South Korea). Histamine (HA), HA agonist and antagonist were purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals were of standard analytical grade. Antibodies against tyrosinase (TYR), tyrosinase-related protein (TRP-1), TRP-2, and GDF-15 were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) and antibodies to phospho-extracellular signal-regulated kinase (ERK), ERK, phospho-c-Jun N-terminal kinase (JNK), JNK and β-actin were from Cell Signaling Technology (Beverly, MA, USA).

2.2. Cell culture

Human melanoma SK-MEL-2 cells and mouse melanoma B16F10 cells were purchased from Korean Cell Line Bank (KCLB). Cells were grown and maintained at 37 °C in an atmosphere of 95% air, 5% CO_2 in DMEM supplemented to a final concentration of 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin.

Melan-a melanocytes were grown and maintained at 37 °C in an atmosphere of 95% air, 5% CO_2 in Roswell Park Memorial Institute medium (RPMI)-1640 supplemented to a final concentration of 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 200 nM phorbol 12-myristate 13-acetate. Cells were passaged every 3 days with a maximal passage number of 33. Confluent monolayers of melanocytes were harvested with a mixture of 0.05% trypsin and 0.53 mM ethylenediaminetetraacetic acid (EDTA) (Bibco BRL, Grand Island, NY).

2.3. Melanin measurement

The melanin content of the cultured melanoma was determined as described previously (Chang and Chen, 2012). For cellular melanin measurement, the cells were seeded into a 6-well plate at an appropriate density. After 24 h of cultivation, the cells were treated with various concentrations of HA for 48 h. The harvested cells were washed twice with phosphate buffered saline (PBS), resuspended in 1 N sodium hydroxide (NaOH) containing 10% dimethyl sulfoxide (DMSO) and then heated at 80 °C for 1 h. The absorbance of extracted melanin was read at 405 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader.

2.4. Tyrosinase assay

Cellular tyrosinase assay was determined as described previously (Chang and Chen, 2012). Cellular extracts were prepared as follows. 90 μl of each lysate was placed in a well of a 96-well plate, and 10 μl of 10 mM L-DOPA was then added. Control wells contained 90 μl of lysis buffer and 10 μl of 10 mM L-DOPA. After incubation at 37 °C, absorbance was measured every 10 min for at least 1 h at 475 nm using an ELISA reader.

2.5. Western blot analysis

After incubation, the cells were collected and washed twice with cold PBS. The cells were lysed in lysis buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% triton X-100, 2 mM EDTA, 1% DOC (deoxycholic acid), 0.1% sodium dodecyl sulfate (SDS), 1 mM $NaVO_3$, 10 mM NaF, 1 mM dithiothreitol (DTT)] and centrifuged to yield whole-cell lysates. Protein concentration was measured using the Bradford method. Aliquots of the lysates (20–30 μg of protein) were separated on a 4–12% SDS-polyacrylamide gel and transferred onto a polyvinylidene fluoride (PVDF) membrane (Invitrogen, Carlsbad, CA, USA) with glycine transfer buffer [192 mM glycine, 25 mM Tris–HCl (pH 8.8), 10% MeOH (v/v)]. After blocking the nonspecific site with 3% non-fat dry milk, the membrane was incubated with a specific primary antibody in 3% bovine serum albumin (BSA) at 4 °C overnight. The membrane was further incubated for 60 min with a peroxidase-conjugated secondary antibody (1:5000, Santa Cruz, CA, USA) at room temperature. Immunoactive proteins were detected using the PowerOpti-ECL Western blotting detection reagent (Animal Genetics Inc., Gyeonggi, Korea)

2.6. Chemotactic migration (chemotaxis) assay

Migration assays were performed using multiwell chambers (Neuroprobe Inc., Gaithersburg, MD) coated with 10 μg/ml fibronectin as a chemoattractant (Im et al., 2012). Briefly, the cells were suspended in DMEM at 1×10^6 cells/ml, and a 25 μl aliquot of this suspension was placed into the upper well of a chamber. Next, the aliquot was separated by an 8 μm polyhydrocarbon filter from the sample-containing lower well. After incubation for 5 h at 37 °C, the nonmigrated cells on the upper surface of the membrane were scrapped off, and the migrated cells on the lower surface were stained by Diff-quick, which were subsequently counted under four randomly chosen high power fields (400×).

2.7. GDF-15 gene silencing by small interfering RNA

A small interfering RNA (siRNA) duplex targeting human GDF-15, 5′-ACA UGC ACG CGC AGA UCA ATT-3′ and 5′-UUG AUC UGC GCG UGC AUG UTT-3′ (Samchully Pharm. Co., Seoul, Korea) was introduced into the cells using Lipofectamine 2000 (Invitrogen), according to manufacturer's instructions. The cells were then cultured with or without HA (10 μM). Universal negative siRNA (Invitrogen, Carlsbad, CA) was employed as a negative control.

2.8. Statistical analysis

Data are expressed as mean ± s.d. of at least three independent experiments performed in triplicate. $P < 0.05$ was considered statistically significant.

3. Results

3.1. GDF-15 is involved in HA-induced cell proliferation, melanogenesis and chemotactic migration

To examine the mediator of HA in HA-induced melanogenesis, we first confirmed the effects of HA on melanogenesis using SK-MEL-2 cells. When SK-MEL-2 cells were cultured with 2% FBS, their proliferation was extremely slow and exhibited less melanin pigmentation leading to amelanotic phenotypes (Mallick et al., 2002). HA treatment gradually increased cell proliferation, melanin synthesis and chemotactic migration (Supplementary Fig. 1A–C). In addition, after HA treatment, the expression of TYR, TRP-1, TRP-2 and GDF-15 was increased compared to the control (Fig. 1A).