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Cross-talk between interferon-gamma and interleukin-18 in melanogenesis

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ABSTRACT

Skin is the largest organ in our body and strategically placed to provide a metabolically active biological barrier against a range of noxious stressors. A lot of inflammatory cytokines, which are increased after ultraviolet (UV) irradiation produced by keratinocytes or other immunocytes, are closely related to pigmentary changes, including interleukin-18 (IL-18) and interferon-γ (IFN-γ). In this study, the effect of cross-talk between IL-18 and IFN-γ on melanogenesis was investigated. Treatment with IL-18 resulted in a dose-dependent increase of melanogenesis, while IFN-γ made an opposite effect. This influence of IL-18 and IFN-γ was mediated by regulations of microphthalmia-associated transcription factor (MITF) and its downstream enzymatic cascade expressions. Furthermore, IFN-γ inhibited basal and IL-18-induced melanogenesis. IFN-γ increased signal transducer and activator of transcription-1 (STAT-1) phosphorylation to play its position in regulating melanin pigmentation, and its inhibitory effect could be prevented by Janus Kinase 1 (JAK 1) inhibitor. IFN-γ could inhibit melanogenesis by decreasing melanocyte dendrite formation. In addition, IFN-γ inhibited the expressions of Rab Pases to suppress the mature and transport of melanosomes. IL-18 could rapidly induce Akt and PTEN phosphorylation and p65 expression in B16F10 cells. When treatment with IL-18 and IFN-γ together, the phosphorylation level of Protein Kinase B (Akt) and phosphatase and tensin homolog deleted on chromosome ten (PTEN) and expression of p65 NF-κB were inhibited, compared with treated with IL-18 only. Our studies indicated that IFN-γ could directly induce B16F10 cells apoptosis in vitro. Furthermore, we demonstrated that IFN-γ markedly up-regulated IL-18 binding protein (BP) production in normal human foreskin-derived epidermal keratinocytes in dose-dependent manner. UVB irradiation induced protease-activated receptor-2 (PAR-2) expression in NHEK, IFN-γ could inhibit this enhancement in a dose-dependent manner. These data suggest that IFN-γ plays a role in regulating inflammation- or UV-induced pigmentary changes, in direct/indirect manner.

1. Introduction

Skin is the largest organ in our body, which is located at the interface between external and internal environments, and so is strategically placed to provide not only a metabolically active biological barrier against a range of noxious stressors (ultraviolet radiation, mechanical, chemical and biological insults) but also to act as the periphery's 'sensing' system [1]. Epidermis and dermis are the two main parts of skin. Epidermal melanocytes, derived from the neural crest, are phenotypically prominent but histologically inconspicuous skin cells. Melanocytes are responsible for the pigmentation of skin and hair, and thereby contribute to the appearance of skin and provide photoprotection from damage by UV radiation [2]. They are also sensory and regulatory cells with computing capability, which exchange information between internal and external environment and then transform into organized regulatory network(s) for the maintenance of the cutaneous homeostasis [3,4]. For as much its local neuroendocrine functions, skin had been called 'our brain on the outside', and melanocytes been 'neurons of the skin' [5,6]. The classical neuroendocrine axes such as the cutaneous hypothalamicpituitary- adrenal axis (HPA) and hypothalamic-thyroid axis (HPT) exist in the skin, making the skin be an important peripheral neuroendocrine-immune organ [7–9]. In epidermis, melanocytes interact with keratinocytes to form epidermal melanin unit and their function can be regulated by keratinocytes.

UV light is a physical carcinogen and UV irradiation from sunlight has profound effects on the human skin. UV irradiation can increase the number of malanocytes, followed by synthesis of melanin, the

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dark pigment in melanocytes, which is synthesized by specific tyrosinase and tyrosinase-related enzymes. Furthermore, UV irradiation stimulates keratinocytes to release cytokines that promote melanogenesis in melanocytes residing in interfollicular epidermis [10]. In consequence, UV is often utilized in therapy for vitiligo, one of the skin depigmentation disorders [11,12]. However, UV irradiation also can cause sunburn, inflammation, cellular/tissue injury, cell death, skin cancer and so on [13]. Skin inflammation is mainly triggered by UVB with a wavelength range of 280–320 nm, which is the most important risk factor for skin cancer as well as suppression of the immune system [14–16]. In this process, keratinocytes play a central role in several responses to photo-damage after UVB exposure by releasing inflammatory cytokines such as IL-1, −6, −8, −10, and more recently IL-18 [17–19].

IL-18, a member of the IL-1 family of cytokines, was initially identified as an IFN-γ-inducing factor. IL-18 acts on the immune system to increase IFN-γ production from Th1 and NK cells, to augment NK cell cytotoxicity, and to activate Th1 cell proliferation [11,20]. After treatment with pro-inflammatory mediators, IL-18 can be secreted by keratinocytes and plays a vital role in the epidermis [21]. It is a pleiotropic pro-inflammatory cytokine and often found in chronic inflammatory skin conditions, as well as other autoimmune inflammatory diseases [22]. Our previous study demonstrated that IL-18 augmented growth ability of human primary melanocytes by PTEN inactivation through the Akt/NF-κB pathway [23]. Inflammatory cytokines are closely related to pigmentary changes. It was reported that IFN-γ was highly expressed in the lesions of vitiligo patients. UVB irradiation can induce the secretion of IFN-γ in keratinocytes or macrophages. Furthermore, IFN-γ blockade abolished macrophage-enhanced melanoma growth and survival [24]. IFN-γ signaling impedes maturation of the key organelle melanosome by concerted regulation of pigmentation genes [25]. IL-18BP is an endogenous antagonist with high neutralizing capacity that inhibits the action of IL-18 by preventing interaction with its cell surface receptors [26,27]. IFN-γ is so far the only described robust inducer of IL-18BP expression thereby acting in particular on diverse non-leukocytic cell types, among others colon carcinoma cells, HaCaT keratinocyte [28,29], fibroblast-like synovial cells [30], and HepG2 cells [31].

Though it has been reported that increased IFN-γ played a direct role in the pathogenesis of vitiligo by inducing melanocyte death [32], the indirect role of IFN-γ through the cross-talk with other cytokines has not been elucidated. The IFN-γ–mediated hypopigmentation cross-talk in delicately balancing skin pigmentation and its implications in disease pathophysiology have drawn researchers' attention. The purpose of this work was to estimate the indirect effect of IFN-γ, by the cross-talk with IL-18, on melanogenesis, and the potential mechanisms.

2. Materials and Methods

2.1. Reagents

Recombinant interleukin-18 (IL-18) was from Prospec-Tany TechnoGene Ltd. (Israel). IFN-γ, Dimethylsulfoxide (DMSO), L-3,4-dihydroxyphenylalanine (L-DOPA), 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), melanin, β-actin primary antibody, and horseradish peroxidase-conjugated secondary antibody were purchased from Sigma-Aldrich (USA). TYR and TRP-1 antibodies were from Santa Cruz Biotechnology (USA). MITF, TRP-2, Rab 7, Rab 27a, and Rab 17 antibodies were from Abcam Technology (UK). Antibodies against p-p38, phosphorylated cAMP response element binding protein (p-CREB), p-STAT-1, t-STAT-1, p-Akt, p-PTEN, p65, Bcl-2, Bax and PAR-2 were from Cell Signaling Technology (USA). The monoclonal *anti*-IFN-γ antibody (Ab), IL-18, IL-18BP and IFN-γ ELISA Kit were purchased from R&D systems (USA). ProLong gold anti-fade reagent with DAPI were from Life Technologies Corporation (USA), enhanced BCA protein assay kit, phenylmethylsulfonyl fluoride (PMSF) and cell lysis buffer for Western and IP, Nuclear and Cytoplasmic Protein Extraction Kit and One Step TUNEL Apoptosis Assay Kit were from Beyotime Institute of Biotechnology (China).

2.2. Cell Culture

Murine B16F10 melanoma cell line was obtained from CAS (Chinese Academy of Sciences, China). The B16F10 cells were grown in DMEM medium (GIBCO, USA) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO, USA) in a humidified atmosphere with 5% CO_2 at 37 °C.

The studies on human material were approved by local ethic committee. Normal human foreskin-derived epidermal melanocytes (NHEM) were derived from young male adult foreskins (ethnic Han/aged 18 to 22 years) obtained at circumcision following standard protocols [33]. Briefly, foreskins were cut into strips and digested with 0.25% trypsin at 4 °C for 20 h. Epidermis was separated from dermis. The NHEM suspension was filtered and cells were washed twice at 1500 rpm for 5 min prior to resuspension in Medium 254 (containing the HMGS) plus antibiotics (100 μg/ml penicillin/streptomycin). NHEM were grown in a humidified atmosphere with 5% CO_2 at 37 °C.

Normal human foreskin-derived epidermal keratinocytes (NHEK) were derived from young male adult foreskins (ethnic Han/aged 18 to 22 years) obtained at circumcision following standard protocols. Briefly, foreskins were cut into strips and digested with 0.25% trypsin at 4 °C for 20 h. Epidermis was separated from dermis. The NHEK suspension was filtered and cells were washed twice at 1500 rpm for 5 min prior to resuspension in Medium 254 supplemented with Human Keratinocyte Growth Supplement (HKGS, Invitrogen) plus antibiotics (100 μg/ml penicillin/streptomycin), 4 mM glutamine and Ca^{2+} (0.07 mM) in a humidified atmosphere containing 5% CO_2 at 37 °C, as previously described [34,35].

2.3. Tyrosinase Activity and Melanin Contents Assay

TYR activity, as the dopa oxidase here, was measured by the rate of L-DOPA oxidation as reported [36]. B16F10 cells were treated with IL-18 or IFN-γ, washed with ice-cold PBS, lysed by incubation in cell lysis buffer (P0013) [containing 1 mM PMSF (ST506)] at 4 °C for 20 min, and then lysates were centrifuged at 14,000 rpm for 15 min to obtain the supernatant for activity assay and the pellet for melanin contents assay. Protein concentrations were determined by BCA kit with bovine serum albumin (BSA) as a standard. 100 μl of supernatant containing the same 30 μg total proteins was added to each well in 96-well plate, and then mixed with 100 μl 0.1% L-DOPA in 0.1 M PBS (pH 6.8) (M/V). After incubation at 37 °C for 0.5 h, the dopachrome was monitored by measuring the absorbance at 475 nm.

Total melanin in the cell pellet was dissolved in 100 μl of 1 N NaOH/10% DMSO for 1 h at 80 °C, and solubilized melanin was measured at 405 nm. Melanin content was calculated as a percent of the control.

2.4. Western Blot Analysis

The total cellular protein suspension was obtained as the method mentioned above. And the nuclear and cytoplasmic proteins were obtained as the protocol described in the product specifications (P0028). In brief, B16F10 cells were treated with IL-18 or IFN-γ, washed with ice-cold PBS, lysed by incubation in cell lysis buffer A (P0028-1) [containing 1 mM PMSF (ST506)] at 4 °C for 15 min, and then added buffer B (P0028–2) at 4 °C for 1 min, lysates were centrifuged at 14,000 rpm for 10 min to obtain the supernatant (cytoplasmic proteins). Added nuclear lysis buffer (P0028-3) [containing 1 mM PMSF (ST506)] into the precipitation and vortex for 30s, then in ice for 2 min. Made this operating cycle for 30 min and centrifuged at 14,000 rpm for 10 min to obtain the supernatant (nuclear proteins). Western blot was performed as described previously [37]. The primary antibodies used were MITF (ab140606), TYR (C-19) (SC7833), TRP-1 (SC10443), TRP-2 (ab74073), p-p38 (CST 4631), p-CREB(CST 9198), p-STAT-1(CST 9177), t-STAT-1 (CST 9175), Rab7