Acta Physiologica Sinica, April 25, 2020, 72(2): 139–147
DOI: 10.13294/j.aps.2020.0018 http://www.actaps.com.cn

Original Article

Chronic stress induces fur color change from dark to brown by decreasing follicle melanocytes and tyrosinase activity in female C57BL/6 mice

SHEN Xiao-Liang[1,#], LIU Yun-Zi[1,#], GONG Hong[1,#], ZHANG Yi[1], WU Teng-Yun[1], XIA Min[2], JIANG Chun-Lei[1,*]

[1]*Department of Stress Medicine, Faculty of Psychology, Naval Medical University, Shanghai 200043, China;* [2]*State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China*

Abstract: Increasing evidence suggests that stress may induce changes in hair color, with the underlying mechanism incompletely understood. In this study, female C57BL/6 mice subjected to electric foot shock combined with restraint stress were used to build chronic stress mouse model. The melanin contents and tyrosinase activity were measured in mouse skin and B16F10 melanoma cells. The enzyme-linked immunosorbent assay (ELISA) was used to determine the content of tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) in the mouse skin. The content of nuclear factor κB (NFκB)/p65 subunit in mouse skins was valued by immunofluorescence staining. The results demonstrated that under chronic stress, the fur color turned from dark to brown in C57BL/6 mice due to the decrease of follicle melanocytes and tyrosinase activity in C57BL/6 mouse skin. Simultaneously, inflammatory responses in skins were detected as shown by increased NFκB activity and TNF-α expression in stressed mouse skin. In cultured B16F10 melanoma cells, TNF-α reduced the melanogenesis and tyrosinase activity in a dose-dependent manner. These findings indicate that chronic stress induces fur color change by decreasing follicle melanocytes and tyrosinase activity in female C57BL/6 mice, and TNF-α may play an important role in stress-induced hair color change.

Key words: chronic stress; fur color; melanocyte; melanin; tyrosinase activity; inflammation

慢性应激通过减少毛囊黑色素细胞和酪氨酸酶活性导致雌性C57小鼠毛发颜色变浅

申晓亮[1,#]，刘韵资[1,#]，龚 洪[1,#]，张 懿[1]，吴腾云[1]，夏 敏[2]，蒋春雷[1,*]

[1]海军军医大学心理系军事应激医学教研室，上海 200043；[2]中国科学院上海生命科学研究院生物化学与细胞生物学研究所，细胞生物学国家重点实验室，上海 200031

摘 要：越来越多的证据表明压力可能会导致头发颜色发生变化，但其潜在机制尚不完全清楚。本研究采用雌性C57BL/6小鼠脚底电刺激结合束缚来建立慢性应激小鼠模型，并用比色法检测小鼠皮肤和B16F10黑色素瘤细胞中黑色素含量和酪氨酸酶活性；通过酶联免疫吸附实验(ELISA)测定小鼠皮肤中肿瘤坏死因子α (tumor necrosis factor α, TNF-α)、白细胞介素1β (interleukin-1β, IL-1β)和白细胞介素6 (interleukin-6, IL-6)含量；通过免疫荧光染色评估小鼠皮肤中核因子κB (nuclear factor κB, NFκB)/p65亚基的含量。结果显示：C57BL/6小鼠在慢性应激下由于皮肤中的毛囊黑色素细胞和酪氨酸酶活性降低，其毛皮颜色从暗色变为棕色。同时，慢性应激小鼠皮肤炎症反应增加，表现为皮肤中NFκB活性和TNF-α表达增加。在体外，TNF-α以剂量依赖性方式降低B16F10黑色素瘤细胞中黑色素生成和酪氨酸酶活性。以上结果表明，慢性应激通过降低雌性C57BL/6小鼠的毛囊黑色素细胞和酪氨酸酶活性来诱导皮毛颜色改变，而TNF-α可能在应激诱导的毛色改变中起重要作用。

Received 2019-08-25 Accepted 2020-02-27

This work was supported by grants from the Natural Science Foundation of Shanghai Municipality, China (No. 17ZR1437800) and the National Natural Science Foundation of China (No. 31871171, 31900827).

[#]These authors contributed equally to this work.

[*]Corresponding author. Tel: +86-21-81871135; E-mail: cljiang@vip.163.com

关键词：慢性应激；毛发颜色；黑色素细胞；黑色素；酪氨酸酶活性；炎症
中图分类号：Q45

Stress is inevitable in the daily life. As a shock organ, skin can response to stress. In mice, ultraviolet type B (UVB) irradiation and wounding can induce direct migration from follicular melanocyte stem cells (McSC) to epidermis in a melanocortin 1 receptor (Mc1r) signaling dependent manner [1]. So far, several literatures have reported the phenomenon of rapidly graying after stress [2, 3]. However, there is no experimental evidence so far.

Hair cycle contains three dynamic phases: anagen, catagen and telogen, periodically [2–4]. Anagen is the main phase of hair shaft formation and pigmentation. Melanin components (eumelanin or/and phenomelanin) are synthesized in melanosomes of melanocytes under catalysis of tyrosinase. The primary distinguishing feature of follicular melanogenesis is the tight coupling of hair follicle melanogenesis to the hair growth cycle [5]. Normal hair pigmentation results from a fine tuned concerted interactions between the cells of the entire dermal papilla in the anagen hair follicle [6, 7]. Dysfunction of any component that essential for pigmentation may induce fading hair, such as down-regulation of tyrosinase activity, reduction of melanosomes and melanocytes [5, 7].

In response to stressors from environment or within the body, hypothalamic-pituitary-adrenal (HPA) axis and sympathetic-adrenomedullary system play the most important roles [8, 9]. In addition, chronic stress is usually associated with inflammation [9]. Although appropriate neuroendocrine responses can help the body fight with outside changes, hypercorrections can cause endocrine disorders or even diseases. It has been proved that stress can induce many kinds of diseases, such as depression, non-alcohol fatty steatohepatitis and cardiovascular diseases [10–13]. Recent studies have found that there is also a corticotropin releasing hormone/proopiomelanocortin (CRH/POMC) system in skin that plays the similar role as HPA axis [14, 15]. Here we investigate the relationship between chronic stress and fur color change, and explore the mechanisms behind this phenomenon.

1 MATERIALS AND METHODS

1.1 *Animals*

In our experiments, twenty-two female C57BL/6 wild type mice (8 weeks old; Sino-British SIPPR/BK Lab Animal Ltd., Shanghai, China) were used. After 7 days of adaptation to aquaculture environment, mice were randomly assigned into two groups (control and stress; n = 11, respectively). All animals were maintained on a 12-hour light/12-hour dark cycle, with food and water freely available. The temperature of the colony room was maintained at 22–23 °C. Animal protocols were approved by the Animal Care and Use Committee of the Naval Medical University.

1.2 *Stress protocol*

Mice in stress group suffered electric foot shock and restraint stress every day for 90 days. For electric foot shock, mouse in stress group was placed into a box with a floor composed of stainless grids, and a scrambled electric shock was delivered through the floor grids by a PST-001 AC stimulator (StarMedical, Tokyo, Japan). An interval timer was connected to the stimulator to allow shocks for 7.5-second periods every 2 min, the intensity of which was 25 V. Electric foot shock was applied at 10:00 am and lasted for 15 min [16, 17]. For restraint stress, mouse was individually put into a 50 mL, conical centrifugal tube with multiple punctures that allowed for a close fit to mouse. Restraint stress was applied at 19:00 pm and lasted for 2 h [18]. During the same period, mice in control (placed in the electric shock box for 15 min without any stimulation) and stress groups were kept isolated from each other to avoid any acoustic or olfactory communication between two groups.

1.3 *Sample harvest*

After 90 days, all mice were fasted overnight, and then anaesthetized using 10% chloral hydrate. Depilatory paste was used for skin depilation. After being cleaned thoroughly with phosphate-buffered saline (PBS), dorsal total skin was harvested from the body. Subcutaneous fat was scrapped off with scalpels. Skins excised from mice were fixed overnight in 4% paraformaldehyde for paraffin embedding or kept in −80 °C for biochemical analysis.

1.4 *Immunohistochemistry*

After being deparaffinized in increasing concentrations of ethanol and xylene, tissue slides were pretreated with 3% H_2O_2 (Sinopharm, China) for 10 min, followed

by antigen retrieval for 3 min through high pressure. Then slides were blocked by 10% goat serum (Maixin, Fuzhou, Fujian, China) at 37 °C for 30 min. Next, mouse HMB45 antibody [19] (1:100; Abcam, USA) was applied overnight at 4 °C followed by anti-rabbit horseradish peroxidase-conjugated antibody (Gene Create, Wuhan, Hubei, China) at 37 °C for 30 min. Immunoperoxidase staining was developed by using a DAB chromagen (Gene Create, Wuhan, Hubei, China) and counterstained with hematoxylin (Gene Create, Wuhan, Hubei, China).

For analysis, secondary scoring method was used. Chiefly, the percentage of positive cells (PPC) was measured by Image Pro Plus 6.0 software. The standard for scoring was: for PPC < 25%, scores were marked as 1; 25%–50%, 2; 50%–75%, 3; > 75%, 4. Then pigmenting intensity was evaluated. The standard for scoring was: for negative, scores were marked as 0; canary, 1; claybank, 2; sepia, 3. The multiplication of the two items was confirmed as the secondary scoring. For measurement, three random hair follicles in each section were selected.

1.5 *Immunofluorescence*

After being deparaffinized in increasing concentrations of ethanol and xylene, tissue slides were pretreated with 3% H_2O_2 (Sinopharm, China) for 10 min, followed by antigen retrieval for 3 min through high pressure. Then slides were blocked by 10% goat serum (Maixin, Fuzhou, Fujian, China) at 37 °C for 30 min. Next, tissue sections were incubated with anti-nuclear factor κB (NFκB)/p65 rabbit polyclonal IgG antibody (1:100; Abcam, USA) overnight at 4 °C, followed by biotinylated goat anti-rabbit IgG antibody (Gene Create, Wuhan, Hubei, China) at 37 °C for 60 min away from light. Sections were counterstained with DAPI (Beyotime, Nanjing, Jiangsu, China) for 20 min away from light.

1.6 *Measurement of cytokines*

50 mg skin samples stored in −80 °C were homogenized in RIPA lysis buffer with 1% phenylmethylsulfonyl fluoride (PMSF; Beyotime, Nanjing, Jiangsu, China) using a Tissue-Tearor (Jingxin, Shanghai, China) for 2 min at 4 °C. The concentration of protein was determined by BCA (Beyotime, Nanjing, Jiangsu, China) method, and absorbance was detected at 562 nm. The measurements of cytokines and melanocytes were conducted using an enzyme-linked immunosorbent assay (ELISA) kit (ExCell, Shanghai, China). All those assays were performed according to the manufacturer's instructions. Absorbance was measured at 450 nm. All values were normalized to protein concentrations.

1.7 *Cell culture*

B16F10 mouse melanoma cells (ATCC, USA) were cultured in 1640 medium (Gibco, USA) containing 10% fetal calf serum (Gibco, USA) and 1% penicillin/streptomycin (Beyotime, Nanjing, Jiangsu, China) at 37 °C under 5% CO_2. Depending on different requirements, 1.0×10^4 or 2.0×10^3 cells/well were seeded into 6-well or 96-well culture plates (For measurements of melanin content and tyrosinase activity, 6-well plates were used; For cell proliferation assay, 96-well plates were used) for 12 h of pre-incubation. Then, original medium was changed by medium with different concentrations of tumor necrosis factor-α (TNF-α) (Details were described in Fig. 4; PEPROTECH, USA) and incubated for 48 h, with a medium changing after 24 h.

1.8 *Measurement of melanin contents*

A method reported previously was used for melanin measurement [20]. For skins' melanin assay, 200 mg skin samples stored in −80 °C were homogenized in 200 μL extraction buffer (0.1 mol/L sodium phosphate buffer, pH 6.8, 1% Triton X-100, 1 mmol/L PMSF, 10 μg/mL aprotinin and 10 μg/mL leupeptin) using a Tissue-Tearor (Jingxin, Shanghai, China) for 2 min at 4 °C. While for B16F10 cells, after 48 h of incubation, culture medium was removed and cells were washed twice with 1 mL of PBS followed by digestion with 0.25% trypsin for 3 min. Then cells were removed into a 1.5 mL centrifuge tube and centrifuged at 1 000 r/min for 5 min at 37 °C. After being washed by PBS, 200 μL extraction buffer described above was added into the tube. After vortex shock for 30 s, all tubes were incubated on ice for 30 min. Then, samples from both skin and B16F10 melanoma cells were centrifuged at 16 000 *g* for 5 min at 4 °C. Supernatants were saved for protein quantification and tyrosinase activity determination. For precipitates, after being washed by 200 μL ethanol/ether (1:1), 200 μL NaOH (1 mol/L) dissolved in dimethyl sulfoxide (DMSO) were added and incubated at 60 °C for 30 min. Melanin content in extract was determined by absorption at 490 nm with a micro plate spectrophotometer. The value was normalized to protein concentration in the supernatant. Standard was provided by GenMed (Shanghai, China).

1.9 *Measurement of tyrosinase activity*

For proteins harvested form melanin measurement, the concentrations were determined by Bradford (Beyo-

time, Nanjing, Jiangsu, China) method and then adjusted to 50 μg/100 μL with extraction buffer. 100 μL proteins were added to a 96-well micro plate with 100 μL 1% *L*-3, 4-dihydroxyphenylalanine (*L*-DOPA; Sigma, USA), a substrate of tyrosinase, and incubated for 1 h at 37 °C. Tyrosinase activity was determined by absorbance changes at 460 nm, which are associated with dopachrome formation [21].

1.10 *Toxicity test*

After 48 h of incubation, 10 μL CCK8 reagents (Dojindo, Japan) per well was added into the 96-wells plates. Cells were incubated for another 3 h, and then absorbance at 450 nm was detected. Results were expressed as fold-change of control.

1.11 *Statistical analysis*

Statistical analysis was performed with SPSS 17.0 (SPSS Science, Chicago, IL, USA). For statistical analysis between two experimental groups, Student's *t*-test was used. For statistical analysis among multi-experimental groups, one-way analysis of variance was used, followed by *post hoc* Student-Newman-Keuls test. Statistical difference was defined at $P < 0.05$ for all comparisons.

2 RESULTS

2.1 Chronic stress induces fur color change and decreases melanin synthesis in mouse skin

To investigate whether chronic stress is linked to fur color change, a 90-day stress protocol for C57BL/6 mice was made in order to lead to chronic stress status. At the end of the time, fur color of mice suffering chronic stress turned from dark to brown, from head to buttock, while fur color in control group still remained dark (Fig. 1*A*).

As hair color reflects the quantity of melanin in hair shaft, melanin content in mouse skins were then detected. Stress mice exhibited remarkable reduction of melanin content compared to control ones ($P = 0.043$) (Fig. 1*B*).

2.2 Chronic stress decreases follicle melanocytes and tyrosinase activity in mouse skin

To investigate causes of melanin reduction, firstly, melanocytes in hair follicles were labeled through immunohistochemistry method using anti-melanoma antibody (HMB45). Analysis results showed that melanocytes in stressed mouse hair follicles decreased remarkably compared to melanocytes in control ones ($P < 0.01$) (Fig. 2*B*, *C*). In addition, after depilation, it

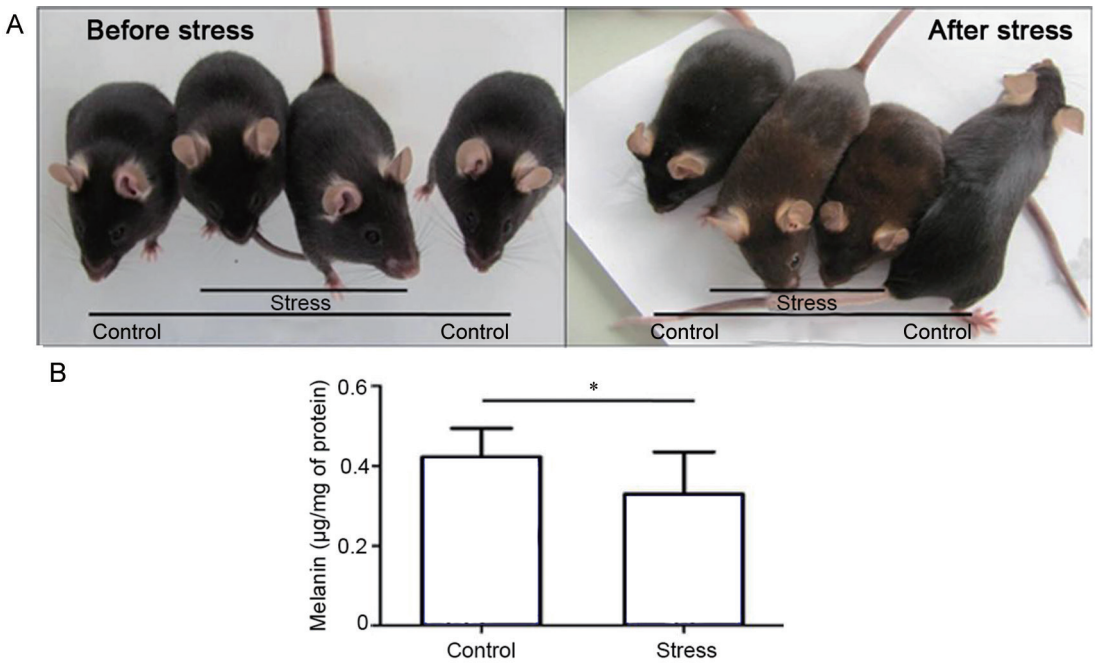

Fig. 1. Chronic stress induces fur color change and decreases melanin synthesis in mouse skin. *A*: The left photo was taken before mice in stress group suffering 90 days of chronic stress, while the right one was after mice in stress group suffering the treatment. In both pictures, the middle two mice are from stress group, while the ambilaterals are from control group. *B*: Melanin quantity in mouse skins decreased after 90 days of chronic stress. Error bars represent the SD from independent samples ($n = 11$). $^{*}P < 0.05$.

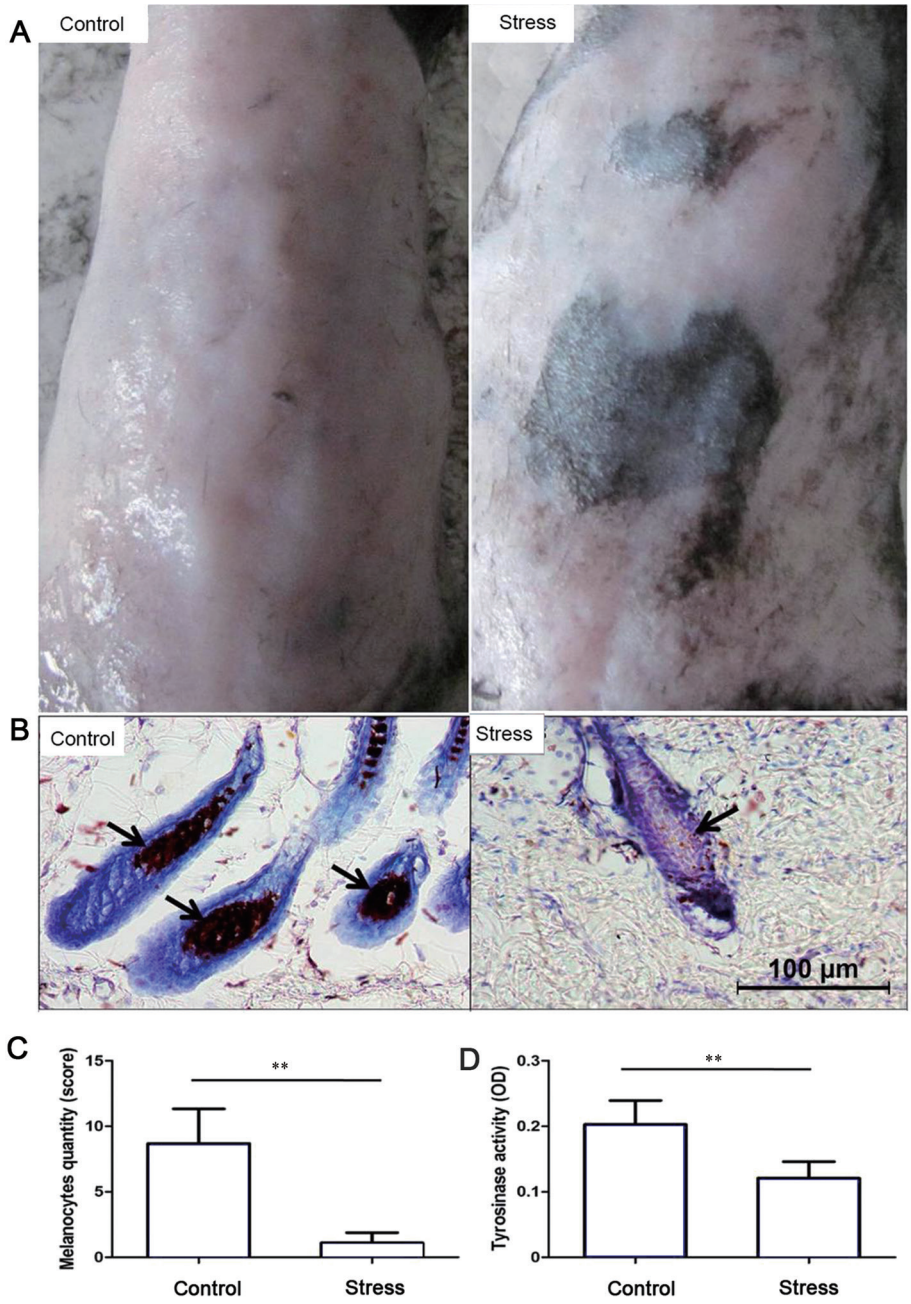

Fig. 2. Chronic stress decreases follicle melanocytes quantity and tyrosinase activity in mouse skin. *A*: In stressed mice, epidermis turned from white to dark after 90 days of treatment. *B*: The labeling of melanocytes in hair follicles by using immunohistochemistry method with HMB45 antibody. Both photos were taken after mice in stress group suffering 90 days of treatment. *C*: The statistical analysis of immunohistochemistry result. Error bars represent the SD from independent samples ($n = 11$). $^{**}P < 0.01$. *D*: Tyrosinase activity in mouse skin decreased after chronic stress. OD reflects the quantity of dopachrome, the production under catalysis of tyrosinase. Error bars represent the SD from independent samples ($n = 11$). $^{**}P < 0.01$. Scale bar, 100 μm.

could be found that stressed mouse skin showed staccato dark in an irregular shape (Fig. 2*A*), a region where is white in normal mice. These results indicate that chronic stress can induce melanocytes reduction in fur follicles.

As the key enzyme, tyrosinase activity can affect melanogenesis in melanocytes. Thus, tyrosinase activity in mouse skin was then detected. The result showed that tyrosinase activity decreased significantly in chronic stress mice compared with that in control ones ($P < 0.01$) (Fig. 2*D*). These facts showed that descendent tyrosinase activity is also a causative factor for melanogenesis reduction.

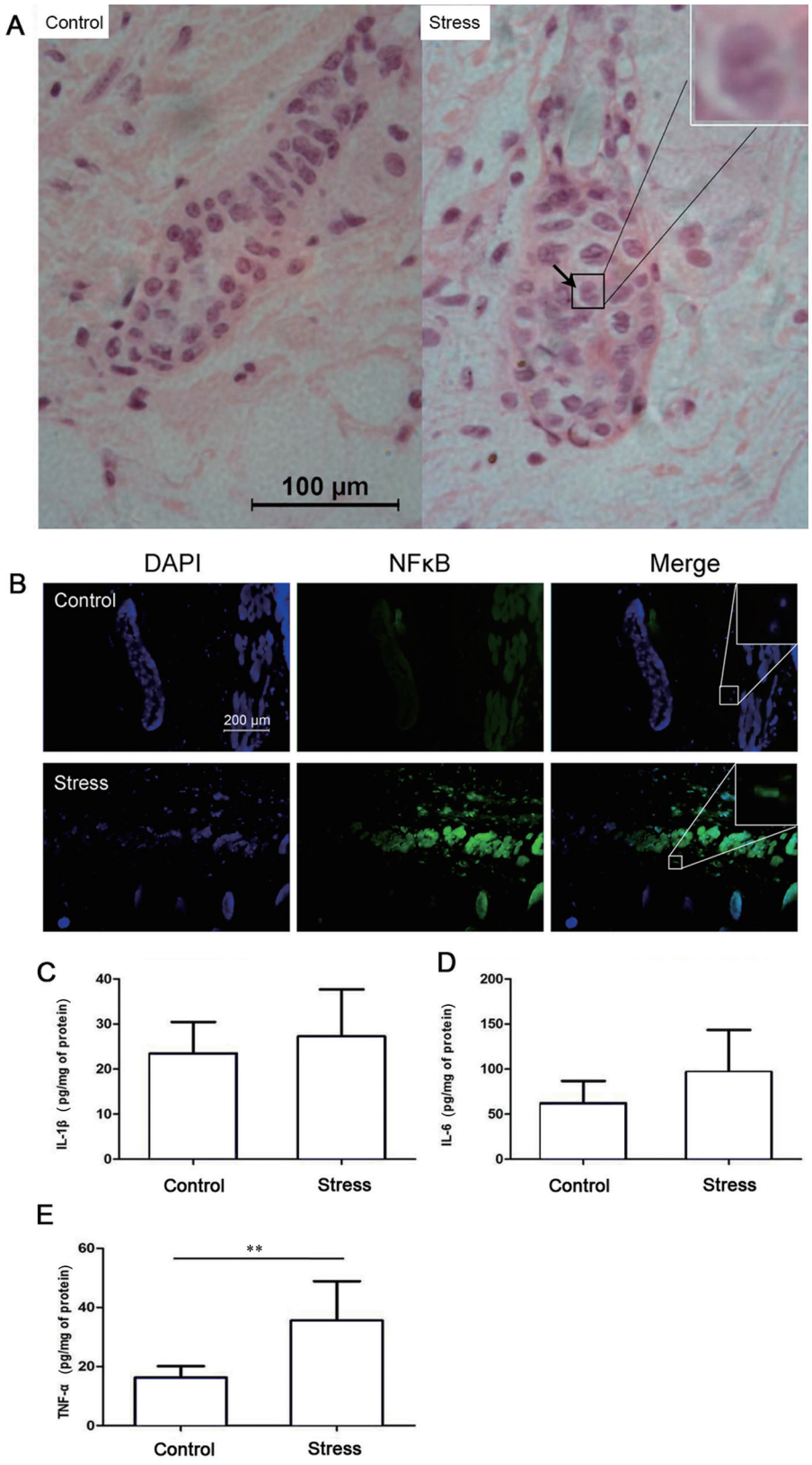

Fig. 3. Chronic stress induces inflammatory responses in mouse skin. *A*: Representative photomicrographs of mouse skin by HE staining. Arrows indicate neutrophils. Scale bar, 100 μm. *B*: Intracellular location of NFκB/p65 subunit in mouse skins using immunofluorescence method. The overlaps of DAPI and NFκB/p65 reflect the activation of NFκB signal pathway. Scale bar, 200 μm. *C*–*E*: In skins of stressed mice, TNF-α quantity increased significantly with an increase tendency of IL-1β and IL-6. Error bars represent the SD from independent samples ($n = 11$). $^{**}P < 0.01$.

2.3 Chronic stress induces inflammatory responses in mouse skin

In previous studies, literatures have reported the mechanism of UVB-induced McSCs migration. This partially clarified the melanocytes diminution induced by chronic stress (Details are stated in discussion). Here, we investigated the mechanism of chronic stress-induced tyrosinase activity decrease. Based on the connections between chronic stress and inflammation, we detected inflammatory responses in mouse skin. Hematoxy-Eosin (HE) staining showed there were a few inflammatory cells in stress group, but no inflammatory cell was found in control group (Fig. 3*A*). Then, immunofluorescence method labeled by NFκB/p65 primary antibody was used for measurement of NFκB signaling pathway state (activated or not). The results showed that NFκB signaling pathway was activated in skins of stressed mice (Fig. 3*B*).

Then, pro-inflammatory cytokines in mouse skins including interleukin (IL)-1β, IL-6 and TNF-α, which have been proved playing important roles in the pathogenic process of chronic stress-related diseases [22–24], were detected. IL-1β and IL-6 showed increasing tendency but without statistical significance, however, TNF-α increased significantly in stressed mice compared with that in control ones (P = 0.367, 0.064 and < 0.002, respectively) (Fig. 3*C*–*E*). These results suggested that TNF-α existing in mouse skin may play an important role in chronic stress-induced tyrosinase activity decrease.

2.4 TNF-α decreases melanogenesis and tyrosinase activity in B16F10 melanoma cells

In order to identify the effects of TNF-α on melanogenesis and tyrosinase, B16F10 melanoma cells, which originate from C57BL/6 mice, were used for study. Firstly, cytotoxicity of TNF-α on B16F10 melanoma cells were detected. Data showed that different concentrations of TNF-α (10^{-12} to 10^{-8} mol/L) had no significant cytotoxicity on B16F10 melanoma cells (P = 0.847, 0.909 0.971, 0.956, and 0.128, compared with control, respectively) (Fig. 4*A*).

Next, the effects of TNF-α on the melanogenesis and tyrosinase activity were detected. Results showed that melanin quantity and tyrosinase activity descended significantly in TNF-α treated groups compared with those in control ones in a dose dependent manner (for tyrosinase, P value of melanin was 0.041, 0.001, and < 0.000 1, respectively; for melanin, P value was 0.866, < 0.001, and < 0.000 1, respectively) (Fig. 4*B*, *C*).

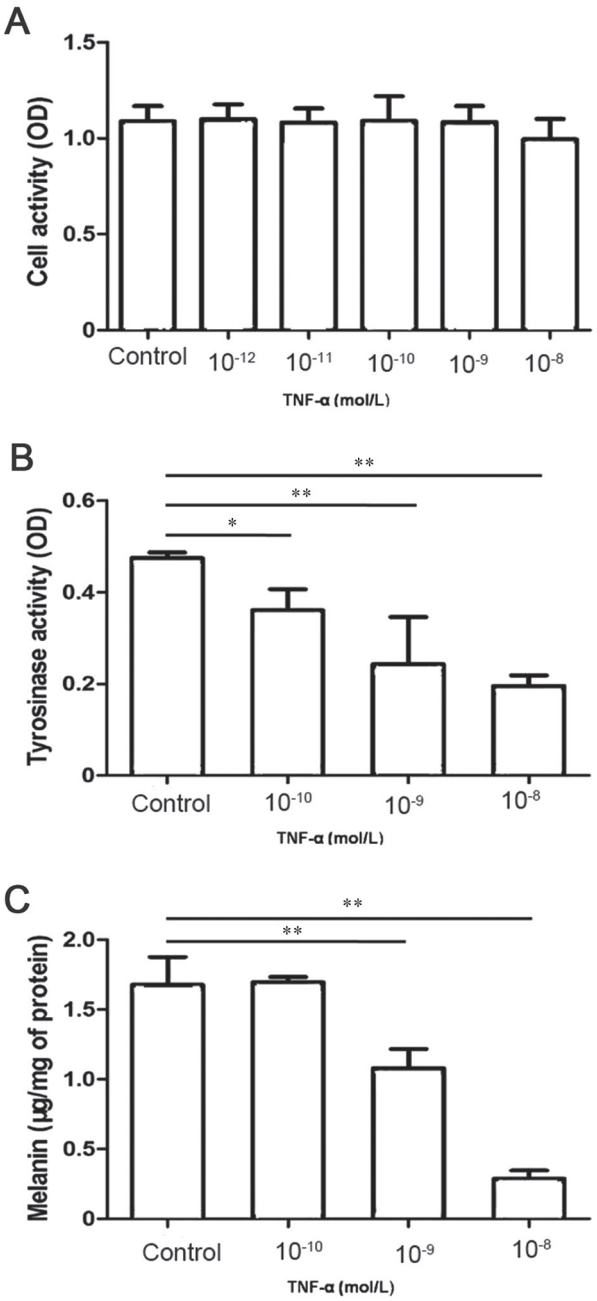

Fig. 4. TNF-α decreases melanogenesis and tyrosinase activity in B16F10 melanoma cells. *A*: TNF-α has no significant toxicity on B16F10 melanoma cells. Cells were treated for 48 h with different doses of TNF-α ranging from 10^{-12} to 10^{-8} mol/L. OD represents the absorbance at 450 nm, which can reflect the activity of cells. The results are expressed as fold-change of control. Error bars represent the SD from independent samples (n = 6). *B*–*C*: Dose-dependent inhibitions of TNF-α on tyrosinase activity (*B*) and melanogenesis (*C*) in B16F10 melanoma cells. Cells were treated for 48 h with different doses of TNF-α ranging from 10^{-10} to 10^{-8} mol/L. Error bars represent the SD from three independent samples (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$.

These findings demonstrated the connection between inflammation and decreased melanogenesis and tyrosinase activity.

3 DISCUSSION

Though almost all people don't deny stress can induce hair color change, based on their life experience or personal feeling, there is no experimental evidence so far. The key findings of this present study showed that chronic stress can induce fur color change in female C57BL/6 mice by decreasing melanocytes in hair follicles and tyrosinase activity mediated by TNF-α (Fig. 5). There are many studies on stress-related diseases and mechanisms, but to our knowledge, this is the first report proposing that chronic stress is a contributing factor to fur color change.

Recent report showed wounding and UVB irradiation can induce direct migration of follicular McSCs to the epidermis before their initial cell division [1]. On the one hand, this can potentially deplete the follicular pool of these cells; On the other hand, these immigrant McSCs can differentiate into melanocytes in epidermis and synthesize melanin, and results in the pigmentation of epidermis [1]. This study showed after chronic stress, melanocytes in hair follicles decreased significantly accompanied with the darkening of mouse skin. Based on the theory mentioned above, we presume the decrease of follicle melanocytes may be induced by the migration of follicular McSCs to epidermis, which should have differentiated into melanocytes in hair follicles in appropriate moment.

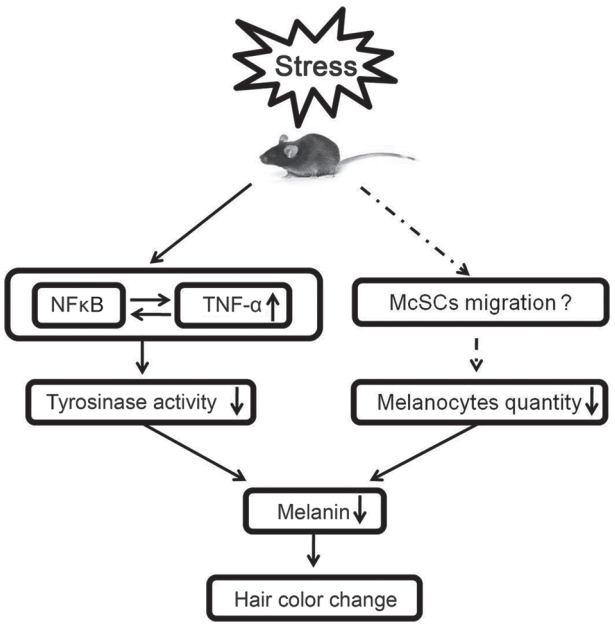

Fig. 5. Graphical summary. McSCs: melanocyte stem cells.

Previous studies have demonstrated that TNF-α can suppress melanogenesis in human melanocytes [25]. Here, we proved that TNF-α had the similar effect on mouse cells, which is accordant with the result in human study. Thus, the present study, from a certain extent, has a reference meaning for understanding the relationship between chronic stress and hair color in human beings. In this study, just a few inflammatory cells were found in brown hair mouse skin, which isn't enough to cause serious skin inflammatory response. Abundant inflammatory factors in the skin may transport through blood from other part of the body [9]. In summary, these findings indicate that inflammation may play an important role in stress-induced hair color change and infer the potential insight into the prevention and treatment of this disease.

Previous studies have shown that female rats secrete more stress hormones, including arginine vasopressin (AVP), adreno-corticotropin hormone (ACTH), prolactin and corticosterone in response to stress [26]. The protein composition of hair shafts also have been modified in response to stress-induced perifollicular neurogenic inflammation [27]. One of the major stress-associated neurohormones in man, prolactin, differentially regulates a subset of hair follicle associated keratins and keratin-associated proteins in human scalp hair follicles [28]. Therefore, it is conceivable that the fur color change visible in response to stress may also have been brought about by neuroendocrine stress mediators, such as prolactin.

However, other mechanisms may also be involved in mouse fur color change, such as the transport barriers of melanin and melanosomes, McSCs decrease and hormone levels. These provide us directions for the following research.

* * *

ACKNOWLEDGMENTS: We thank Gang WANG at State Key Laboratory of Cell Biology (Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences) for the generous gift of B16F10 melanoma cells.

REFERENCES

1 Chou WC, Takeo M, Rabbani P, Hu H, Lee W, Chung YR, Carucci J, Overbeek P, Ito M. Direct migration of follicular melanocyte stem cells to the epidermis after wounding or

UVB irradiation is dependent on Mc1r signaling. Nat Med 2013; 19(7): 924–929.
2 Tan SP, Weller RB. Sudden whitening of the hair in an 82-year-old woman: the 'overnight greying' phenomenon. Clin Exp Dermatol 2012; 37(4): 458–459.
3 Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. Curr Biol 2009; 19(3): R132–R142.
4 Skellett AM, Millington GW, Levell NJ. Sudden whitening of the hair: an historical fiction? J R Soc Med 2008; 101(12): 574–576.
5 Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. Exp Gerontol 2001; 36(1): 29–54.
6 Schallreuter KU, Salem MM, Hasse S, Rokos H. The redox--biochemistry of human hair pigmentation. Pigment Cell Melanoma Res 2011; 24(1): 51–62.
7 Slominski A, Wortsman J, Plonka PM, Schallreuter KU, Paus R, Tobin DJ. Hair follicle pigmentation. J Invest Dermatol 2005; 124(1): 13–21.
8 Dhabhar FS, Miller AH, Mcewen BS, Spencer RL. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. J Immunol 1995; 154(10): 5511–5527.
9 Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiol Rev 2000; 80(3): 979–1020.
10 Checkley S. The neuroendocrinology of depression and chronic stress. Br Med Bull 1996; 52(3): 597–617.
11 Specchia G, Falcone C, Traversi E, La Rovere MT, Guasti L, De Micheli G, Ardissino D, De Servi S. Mental stress as a provocative test in patients with various clinical syndromes of coronary heart disease. Circulation 1991; 83(4 Suppl): I108–I114.
12 Katz RJ, Roth KA, Carroll BJ. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci Biobehav Rev 1981; 5(2): 247–251.
13 Liu YZ, Chen JK, Zhang Y, Wang X, Qu S, Jiang CL. Chronic stress induces steatohepatitis while decreases visceral fat mass in mice. BMC Gastroenterol 2014; 14: 106.
14 Ito N, Ito T, Kromminga A, Bettermann A, Takigawa M, Kees F, Straub RH, Paus R. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. FASEB J 2005; 19(10): 1332–1334.
15 Arck PC, Handjiski B, Hagen E, Joachim R, Klapp BF, Paus R. Indications for a 'brain-hair follicle axis (BHA)': inhibition of keratinocyte proliferation and up-regulation of keratinocyte apoptosis in telogen hair follicles by stress and substance P. FASEB J 2001; 15(13): 2536–2538.
16 Blanc G, Herve D, Simon H, Lisoprawski A, Glowinski J, Tassin JP. Response to stress of mesocortico-frontal dopaminergic neurones in rats after long-term isolation. Nature 1980; 284(5753): 265–267.
17 Knepel W, Nutto D, Hertting G. Evidence for inhibition by beta-endorphin of vasopressin release during foot shock-induced stress in the rat. Neuroendocrinology 1982; 34(5): 353–356.
18 Yamamoto K, Takeshita K, Shimokawa T, Yi H, Isobe K, Loskutoff DJ, Saito H. Plasminogen activator inhibitor-1 is a major stress-regulated gene: implications for stress-induced thrombosis in aged individuals. Proc Natl Acad Sci U S A 2002; 99(2): 890–895.
19 Xu H, Delling M, Li L, Dong X, Clapham DE. Activating mutation in a mucolipin transient receptor potential channel leads to melanocyte loss in varitint-waddler mice. Proc Natl Acad Sci U S A 2007; 104(46): 18321–18326.
20 Manga P, Orlow SJ. Inverse correlation between pink-eyed dilution protein expression and induction of melanogenesis by bafilomycin A1. Pigment Cell Res 2001; 14(5): 362–367.
21 Horibe I, Satoh Y, Shiota Y, Kumagai A, Horike N, Takemori H, Uesato S, Sugie S, Obata K, Kawahara H, Nagaoka Y. Induction of melanogenesis by 4'-O-methylated flavonoids in B16F10 melanoma cells. J Nat Med 2013; 67(4): 705–710.
22 Lee CW, Ko HH, Lin CC, Chai CY, Chen WT, Yen FL. Artocarpin attenuates ultraviolet B-induced skin damage in hairless mice by antioxidant and anti-inflammatory effect. Food Chem Toxicol 2013; 60: 123–129.
23 Campanini MZ, Pinho-Ribeiro FA, Ivan AL, Ferreira VS, Vilela FM, Vicentini FT, Martinez RM, Zarpelon AC, Fonseca MJ, Faria TJ, Baracat MM, Verri WJ, Georgetti SR, Casagrande R. Efficacy of topical formulations containing Pimenta pseudocaryophyllus extract against UVB-induced oxidative stress and inflammation in hairless mice. J Photochem Photobiol B 2013; 127: 153–160.
24 Hirotsu C, Rydlewski M, Araujo MS, Tufik S, Andersen ML. Sleep loss and cytokines levels in an experimental model of psoriasis. PLoS One 2012; 7(11): e51183.
25 Swope V B, Abdel-Malek Z, Kassem LM, Nordlund JJ. Interleukins 1 alpha and 6 and tumor necrosis factor-alpha are paracrine inhibitors of human melanocyte proliferation and melanogenesis. J Invest Dermatol 1991; 96(2): 180–185.
26 ter Horst JP, de Kloet ER, Schachinger H, Oitzl MS. Relevance of stress and female sex hormones for emotion and cognition. Cell Mol Neurobiol 2012; 32(5): 725–735.
27 Paus R, Arck P. Neuroendocrine perspectives in alopecia areata: does stress play a role? J Invest Dermatol 2009; 129(6): 1324–1326.
28 Ramot Y, Biro T, Tiede S, Toth BI, Langan EA, Sugawara K, Foitzik K, Ingber A, Goffin V, Langbein L, Paus R. Prolactin--a novel neuroendocrine regulator of human keratin expression *in situ*. FASEB J 2010; 24(6): 1768–1779.