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사람 모유두세포에서 코르티코트로핀분비인자에 의한 모발성장관련사이토카인의 발현 조절

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Corticotropin-Releasing Factor Down-Regulates Hair Growth-Related Cytokines in Cultured Human Dermal Papilla Cells

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요 약: 코르티코트로핀분비인자(Corticotropin-releasing factor)는 스트레스 반응에 관여하는 호르몬으로, 최근 스트레스가 탈모와 같은 피부질환에 영향을 미친다는 보고들이 많아지고 있다. 보고에 따르면, 사람 모낭 배양에서 코르티코트로핀분비인자는 길이생장을 억제하며, 모낭의 조기퇴행을 유도하고 모기질각질형성세포(hair matrix keratinocyte)의 세포사멸을 촉진시킨다. 본 연구에서는 코르티코트로핀분비인자가 모발성장과 모주기 조절에 핵심적으로 역할하는 모유두세포에 미치는 영향에 대해 알아보고자 했다. 시상하부-뇌하수체-부신축의 주요 스트레스호르몬들인 코르티코트로핀분비인자, 부신피질자극호르몬, 그리고 코르티솔을 사람 모유두세포에 처리하였다. 흥미롭게도, 코르티코트로핀분비인자가 모발성장과 관련된 사이토카인(KGF, Wnt5a, TGFβ-2, Nexin)의 발현을 변화시키는 것을 관찰하였으며, 세포 내 cAMP의 수준을 증가시켰고, 수용체의 발현을 억제시켰다. 이러한 변화는 수용체의 길항제인 antalarmin과 astressin2B, 또는 PKA 억제제의 전처리로 인해 막을 수 있었다. 코르티코트로핀분비인자는 cAMP/PKA경로를 통해 POMC의 발현을 유도하는데, 사람 모유두세포에서도 이 호르몬의 처리가 POMC mRNA의 발현을 증가시키는 것을 확인할 수 있었으나 부신피질자극호르몬의 변화는 western blot으로는 확인할 수 없었다. 이러한 결과들을 바탕으로, 코르티코트로핀분비인자가 그 수용체를 통해 사람 모유두세포 내 모발성장 관련 사이토카인의 발현을 조절함을 확인하였으며, 이는 코르티코트로핀분비인자의 수용체 길항제가 스트레스성 탈모환자를 위한 치료제 혹은 화장품 소재로써 활용될 수 있음을 보여준다.

Abstract: Corticotropin-releasing factor (CRF) is involved in the stress response and there is increasing evidence that stress influences skin disease such as hair loss. In cultured human hair follicles, CRF inhibits hair shaft elongation, induces premature regression and promotes the apoptosis of hair matrix keratinocytes. We investigated whether CRF influences the dermal papilla cells (DPC) that play pivotal roles in hair growth and cycling. Human DPCs were treated with CRF, adrenocorticotropic hormone (ACTH) and cortisol, key stress hormones along the hypothalamic-pituitary -adrenal

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(HPA) axis for 1-24 h. Interestingly, CRF modulated the expression of cytokines related to hair growth (KGF, Wnt5a, TGF β-2, Nexin) and increased cAMP production in cultured DPCs. CRF receptors were down-regulated by negative feedback systems. Pretreatment of CRF receptor antagonists or protein kinase A (PKA) inhibitor prevented the CRF-induced modulation. Since the CRF induces proopiomelanocortin (POMC) expression through the cAMP/PKA pathway, we analyzed POMC mRNA. CRF stimulated POMC expression in cultured human DPCs, yet we were unable to detect ACTH levels by western blot. These results indicate that CRF operates within DPCs through CRF receptors along the classical CRF signaling pathway and CRF receptor antagonists could serve as potential therapeutic and cosmetic agents for stress-induced hair loss.

Keywords: *CRF, cytokine, dermal papilla cell, hair loss, stress*

1. Introduction

Corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH) and glucocorticoid, central components of the HPA axis are activated by psychological stress[1]. These neurohormones also play an important role in regulation of local stress response in peripheral tissues including skin[2,3]. Human hair follicles display a fully functional HPA axis and establish classical regulatory feedback loops[4,5]. In addition, human melanocytes were stimulated by CRF and ACTH to secrete cortisol via CRF receptors[6].

CRF, a 41-amino acid peptide is a main trigger of the HPA axis and it plays a central role in the activation of hormonal and neuronal signaling cascade in vertebrates as a response to stress[7-9]. CRF interacts with membrane receptors, CRF1 receptor and CRF2 receptor, that belong to the class B subtype of G protein coupled receptors (GPCR)[10,11].

CRF signal transduction through CRF receptors is linked to the activation of adenylate cyclase (AC) or phospholipase C (PLC)[12]. The main signaling pathway of CRF is increased of intracellular cAMP from activation of AC stimulates protein kinase A (PKA). PKA phosphorylates a number of downstream targets in the cytosol and CREB in the nucleus, inducing transcription of certain genes[13]. In human skin, CRF1 receptor is the major receptor and it is expressed in epidermal and dermal compartments while CRF2 receptor is detected predominantly in dermal parts[5]. Furthermore, CRF receptors participate in the regulation of skin cell proliferation, differentiation and apoptosis[14-16].

So far, there are several evidences that severe psycho-emotional stress may significantly influences hair growth and causes the onset of alopecia areata[17-20]. CRF inhibited hair shaft elongation and promoted the apoptosis of hair matrix keratinocytes in *ex vivo* culture of human hair follicles[4]. Intriguingly, CRF receptor antagonists induced hair re-growth in alopecic CRF over-expressing mice[21]. Although these interesting data strongly suggested that CRF may influence human hair growth, we lack data about the influence of CRF on DPCs, specialized mesenchymal cells in hair follicles that plays important role in hair formation.

Based on these previous facts, we hypothesized that CRF might influence the regulation of cytokines expression in human DPCs. Then, we found that CRF down-regulated hair growth-related cytokines through CRF receptor in DPCs.

2. Materials and Methods

2.1. Cell culture and treatment

Human follicle dermal papilla cells (HFDPCs) were purchased from PromoCell (Heidelberg, Germany) and maintained in follicle dermal papilla cell growth medium and supplement kit (PromoCell, Heidelberg, Germany). For cell stimulations, human DPCs were plated at 3.5×10^5 cells per 100 mm dish for one day before stimulation and medium was refreshed with or without synthetic human CRF, ACTH (American Peptide Company, Sunnyvale, CA) or cortisol (Sigma, St. Louis, MO) at a concen-

tration of 10^{-7} M - 10^{-9} M. Treatment was carried out at 37 ℃ for the indicated times and cells were collected separately for the assays. Antalarmin, astressin, astressin-2B (Sigma, St. Louis, MO) or PKA inhibitor (myristoylated 14-22 amide, Calbiochem) were added to the plate 20 min before the addition of CRF.

2.2. RT-PCR

The total RNA was extracted from the human DPCs using Easy-spin total RNA extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacture's protocol. Then, cDNA was synthesized from 2 μg of total RNA using a reverse transcriptase, oligo dT and dNTPs. The cDNA was amplified in a PCR reaction containing 10 pM of specific primers: 5'-CCA CAC AAG ACC TGG TCT ACA TC-3'and 5'-GTC TGC ACG GTC TTG AAC TG-3'to amplify Wnt5a mRNA, 5'-AGG GTA CAA TGC CAA CTT CTG -3'and 5'-GGT TCT AAA TCT TGG GAC ACG-3'to amplify TGFβ-2 mRNA, 5'-GAT CCT GCC AAC TTT GCT CTA C-3'and 5'-GGC TGG AAC AGT TCA CAT TTG-3'to amplify KGF mRNA, 5'-CTC GTC AAC GCA GTG TAT TTC-3'and 5'-CAG CAT TGG CAC TTG ATA GG-3'to amplify Nexin mRNA, 5'-CTA CGG CGG TTT CAT GAT CT-3'and 5'-CCC TCA CTC GCC CTT CTT G-3'to amplify proopiomelanocortin (POMC) mRNA, and 5'-GCG TGA CAT TAA GGA GAA GC-3'and 5'-AGG AAG GAA GGC TGG AAG A-3'to amplify Actin mRNA. PCR products was separated on 3% NuSieve agarose gel and visualized by ethidium bromide staining. The expression of each gene was normalized to that of Actin by band densitometry analysis using ImageJ 1.48 software (National Institutes of Health, Bethesda, MD).

2.3. Western blot analysis

Proteins were extracted from cells using PRO-PREP protein extraction solution (Intron Biotechnology, Seongnam, Korea). Proteins were separated on 12% SDS-PAGE gels and transferred to PVDF membranes. After blocking with Tris buffered saline, 0.05% Triton X-100 and 5% milk, the membranes were incubated with primary goat anti-human KGF (Santa Cruz Biotechnology Inc., USA), TGFβ-2, Wnt5a (Abcam, Cambridge, UK), CRF1/2 receptors, CRF1 receptor and ACTH (Santa Cruz Biotechnology Inc., USA) antibodies followed by incubation with horseradish peroxidase conjugated goat anti-goat IgG (Santa Cruz Biotechnology Inc., Santa Cruz, CA). The target proteins were visualized with Immobilon Western Chemiluminescent HRP Substrate (Millipore Corporation, Billerica, MA).

2.4. Immunocytochemistry

Cells were cultured at a concentration of 3.5 × 10^5 cells per well in 6-well plate. After 24 h, fresh medium supplemented with CRF at a concentration of 10^{-7} M - 10^{-9} M was added. After 3 days, cells were fixed with 4% formaldehyde in PBS for 15 min, washed with PBS and blocked with 5% BSA in PBS for 1 h. The slides were subsequently incubated with CRF1/2 receptors (sc-1757, Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a 1 : 50 dilution or CRF1 receptor (sc-12381, Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a 1:50 dilution in 5% BSA in PBS overnight at 4 ℃. Cells were then labeled with rabbit anti-goat IgG (H + L) conjugated with the green fluorescent dye CF^{TM}488A (Biotium Inc., Hayward, CA) for 2 h. Then immediately mounted and visualized with fluorescence microscopy.

2.5. cAMP assay

We measured cAMP concentration in cultured human DPCs using a Parameter cAMP kit (R&D systems, Minnneapolis, MN). Human DPCs were incubated (5 × 10^5 cells/mL) for 30 min at 37 ℃ in DPC culture media containing 0.5 mM of the potent phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (Sigma, St. Louis, MO). Then 10^{-7} M of CRF, antalarmin and astressin 2B were added to DPC culture media containing 0.5 mM of IBMX and cells were incubated with the ligand for 24 h at 37 ℃. Right after following stimulation, cells were washed with cold PBS and then lysed with lysis buffer. The reaction was processed according to the

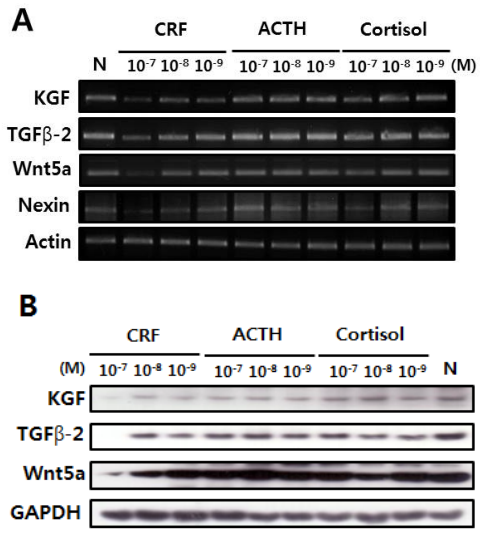

Figure 1. CRF modulates expression of cytokines related to hair growth in cultured DPCs.
Human DPCs were treated with CRF, ACTH or cortisol (10^{-7} M, 10^{-8} M, 10^{-9} M) for 24 h. (A) mRNA expression of hair growth-related cytokines was measured by RT-PCR. (B) Protein expression of hair growth-related cytokines was measured by Western blot.

manufacturer's protocol. A Bio-Rad microplate reader was used to measure concentrations of cAMP and the concentration was recalculated from the standard curve.

3. Results

3.1. CRF suppresses the expression of hair growth–related cytokines in human DPCs

We investigated whether CRF, ACTH and cortisol are involved in regulation of KGF, TGFβ-2, Wnt5a and Nexin, known as hair growth-related cytokines in human DPCs. Human DPCs were treated with CRF, ACTH and cortisol at a concentration of 10^{-7} M - 10^{-9} M for 24 h and the mRNA and protein expression levels of these cytokines were analyzed. In CRF-treated human DPCs, the expression of mRNAs of KGF, TGFβ-2, Wnt5a and Nexin were decreased in concentration dependent manner (Figure 1A) and this suppression was confirmed by reduced protein levels of these cytokines (Figure 1B). The expression of the cytokines was reduced similarly with less extent by CRF in shorter periods of treatments such as 1 or 6 h (data not shown). Besides the noticeable suppressive effect of CRF, the downstream members of HPA axis, ACTH and cortisol showed little or no effect on the synthesis of mRNAs and proteins of these cytokines in human DPCs.

3.2. CRF receptors are down–regulated by prolonged exposure to CRF in human DPCs

We examined the expression of CRF receptors in cultured human DPCs and showed that human DPCs were stained with antibodies against CRF1/2 receptors or CRF1 receptors. Cells were exposed to CRF at a concentration of 10^{-7} M - 10^{-9} M for 1 h, 6 h, 24 h and 72 h. While there were no detectable changes in immunofluorescence of CRF receptors by CRF treatment up to 24 h (data not shown), the green signals in the cytosol were reduced after treatment with CRF for 72 h in a concentration-dependent manner (Figure 2A, B). The down-regulation of CRF receptors expression by CRF was abolished by pre-treatment of astressin and a non-selective CRF receptor antagonist (Figure 2C).

3.3 CRF modulates the cytokine levels through CRF receptors along the classical CRF signaling pathways in human DPCs

To elucidate the mechanism of CRF signaling pathway, we pre-treated CRF receptor antagonists or PKA inhibitor before CRF treatment and analyzed the mRNA and protein expression levels of cytokines. The reduction of cytokine expression, both in mRNA and protein levels, in CRF-treated human DPCs was prevented by pre-treatment of cells with astressin or PKA inhibitor (Figure 3A, B). To find out which receptor is actively participates in CRF signaling in human DPCs, cells were pre-exposed to specific antagonists against CRF1 and CRF2 receptors, antalarmin and astressin2B respectively. As shown in Figure 3C, reduced RNA levels of cytokines by CRF-treatment were recovered by both antarlamin and astressin2B. We conducted cAMP assay to investigate whether CRF activated adenylate cyclase via

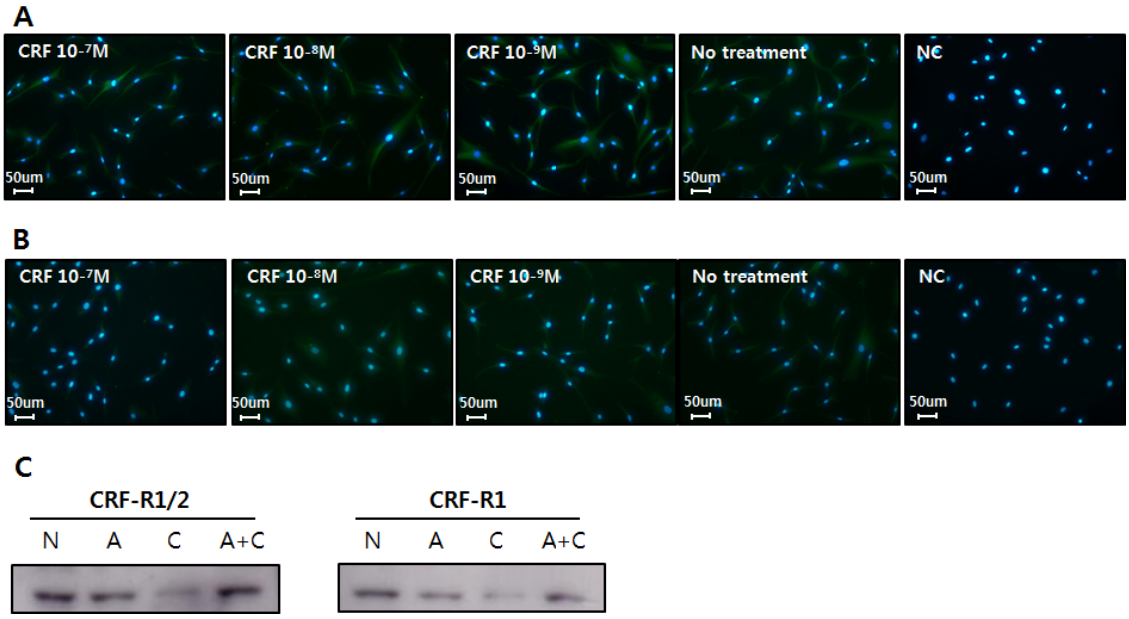

Figure 2. CRF receptors are down-regulated by negative feedback mechanism in cultured DPCs.
Human DPCs were treated with CRF (10^{-7} M, 10^{-8} M, 10^{-9} M) for 72 h. (A-B) Immunofluorescence (green) of CRF1/2 receptor and CRF1 receptor were detected in cultured human DPCs, respectively. Blue fluorescence show nuclei stained with DAPI. Primary antibody-omitted group was used as background controls (NC). (C) Negative feedback regulation of CRF receptors by CRF was evaluated by Western blot. N: No treatment, A: astressin treatment, C: CRF treatment, A+C: astressin and CRF treatment.

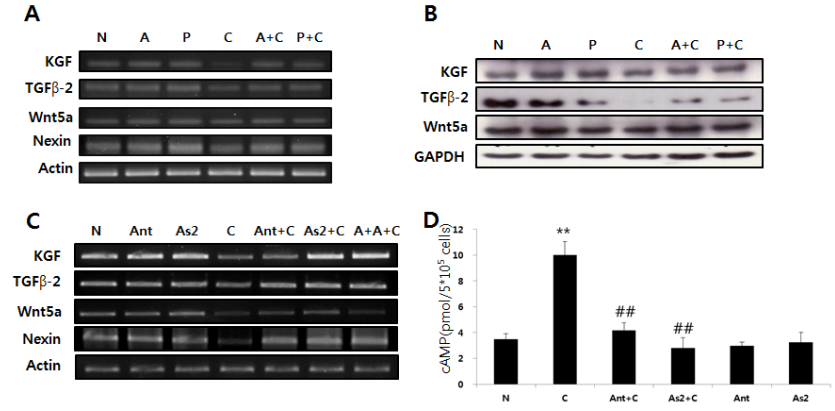

Figure 3. CRF modulates hair growth-related cytokines through CRF receptors along the classical CRF signaling pathway in DPCs.
Human DPCs were pre-treated with CRF antagonists or PKA inhibitor and then treated with CRF at 10^{-7} M concentration for 24 h. (A, C) mRNA expression of hair growth-related cytokines was measured by RT-PCR. (B) Protein expression of hair growth-related cytokines was measured by Western blot. (D) Intracellular cAMP levels were measured by specific assay kit. $^{**}p < 0.01$ *vs.* no treatment control, $^{\#\#}p < 0.01$ *vs.* CRF treatment group. N: No treatment, A: astressin treatment, P: PKA inhibitor treatment, C: CRF treatment, A+C: astressin and CRF treatment, P+C: PKA inhibitor and CRF treatment, Ant: antalarmin treatment, As2: astressin2B treatment, Ant+C: antalarmin and CRF treatment, As2+C: astressin2B and CRF treatment, A+A+C: antalarmin, astressin2B and CRF treatment.

interaction with CRF receptor in human DPCs. CRF stimulated intracellular cAMP production and CRF receptor antagonists interrupted cAMP production (Figure 3D).

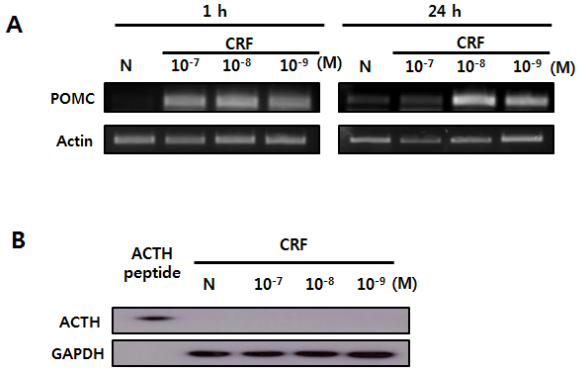

Figure 4. CRF induces POMC mRNA expression in cultured human DPCs. Human DPCs were treated with CRF (10^{-7} M, 10^{-8} M, 10^{-9} M) for 1-24 h. (A) mRNA expression of POMC was measured by RT-PCR. (B) Protein expression of ACTH was measured by Western blot.

3.4. CRF induced POMC mRNA expression in cultured human DPCs

Since the CRF induces POMC expression through the cAMP/PKA pathway, we analyzed POMC mRNA and POMC-derived ACTH peptides. Significant up-regulation of POMC mRNA levels in cultured human DPCs was observed from 1 h after CRF stimulation at a concentration of 10^{-7} M - 10^{-9} M (Figure 4A). The expression of POMC mRNA was decreased to basal levels after 24 h of treatment with 10^{-7} M CRF. As shown in Figure 4B, ACTH peptide was not detected in both non-treated human DPC and CRF-treated human DPCs.

4. Discussion

In recent years, the problems of hair loss have been increasing in women and young people. To these groups, various types of stresses can be one of the important factors that cause hair loss[5,22-24]. Unlike androgenetic alopecia, there is limited knowledge about the relationship between stress and hair loss. Oxidative damage and stress hormones may be candidates for the mediators of stress. Recently, several investigators reported the role of oxidative damage and stress hormones causing hair loss in *in vivo* models and cultured hair follicles of mouse and human[24,25]. To understand more about the stress-induced hair loss, it is important to investigate the effects of these factors in cellular levels. We found that there were no noticeable changes in the levels of anagenic markers of DPCs after hydrogen peroxide treatment (data not shown) besides the damaging effect of hydrogen peroxide in cultured human DPCs. It can be postulated that the primary target of oxidative damage in hair follicles could be origin of keratinocyte such as matrix cells and outer root sheath cells. We also evaluated the effect of CRF on human DPCs. We demonstrated that activation of CRF receptors; CRF caused down-regulation of hair growth-related cytokines at the transcriptional level as well as the translational level in cultured human DPCs. To our knowledge, this is the first report to show operation of CRF via interaction of CRF receptors in cultured human DPCs.

In our present study, we showed that several cytokines such as KGF, Wnt5a, Nexin, and TGFβ-2 in CRF-treated human DPCs were expressed at lower levels than that in non-treated human DPCs. Interestingly, there was no significant regulation of cytokines by treatment with ACTH and cortisol. DPCs are population of specialized fibroblasts that has an essential function in the control of hair growth and hair cycle[26]. Therefore, secretory factors of DPCs affect not only the cells producing them, but also the surrounding cells. Recombinant KGF stimulates hair growth in nude mice by stimulating follicular proliferation and the skin of KGF knockout mice develops a matted appearance [27,28]. In the anagen phase, DPC showed the highest staining levels of Wnt5a protein, yet adenovirus-mediated Wnt5a significantly inhibited hair shaft growth[29]. Wnt5a is expressed in the developing dermal condensate and a target of Sonic hedgehog in hair follicle morphogenesis[30]. These reports suggest that Wnt5a is a dynamic factor in the hair cycle. TGFβs also have been shown to both positive and suppressive effects on hair growth. TGFβ-2 are specifically expressed by cultured human DPCs and inhibition of TGFβ-2 signal impaired hair folliculogenesis in an human DPC transplantation animal model[31] while TGFβ-2 also induced premature catagen[32]. In DPCs

of anagen follicles, Nexin is accumulated[33]. Altogether, these reports suggest that CRF induces hair loss partially via modulation of hair growth-related cytokines.

Both CRF1 and CRF2 receptor proteins are expressed and successfully worked in human DPCs as evidence is shown that prevention of the CRF-induced modulation of cytokines and intracellular cAMP production by CRF receptor antagonists. Surprisingly, it was found that the levels of CRF receptors were reduced by prolonged exposure to CRF. This phenomenon is consistent with previous findings and can be explained as a negative feedback mechanism of G protein coupled receptor[34,35].

The signal transduction pathway activated through CRF receptors is consistent with stimulation of cAMP production, subsequent activation of PKA and calcium-activated pathway by phospholipase C[3]. In this study, CRF promoted cAMP production and down-regulated cytokines and modulation of CRF is abolished by CRF antagonist. These result indicated that DPCs have a classical CRF signaling pathway. Fascinatingly, POMC expression was increased in human DPCs from 1 h after CRF treatment, yet the intracellular ACTH by CRF stimulation was unable to detect in cultured human DPC by western blot. Since POMC mRNA expression was induced by CRH, further investigations should attempt to clarify whether human DPCs show a fully functional peripheral HPA system. Outer root sheath (ORS) cells and DPCs are reported to support each other in hair follicles regarding cell proliferation, differentiation and migration[36, 37]. Therefore, further research into the co-culture of ORS cells and DPCs and effect of CRF on ORS cells are required for better understanding of the complex dynamics of hair follicles. In addition, it is necessary to confirm whether CRF activates Ca^{2+} signaling pathway in human DPCs.

Consequently, our current data could serve as the basis for the effect of CRF in cultured human DPCs and also suggest that CRF receptor antagonists could be possible therapeutic agents for stress-induced hair loss through inhibition of CRF-induced catagen transition.

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Reference

1. P. C. Arck, A. Slominski, T. C. Theoharides, E. M. Peters, and R. Paus, Neuroimmunology of stress: skin takes center stage, *J. Invest. Dermatol.,* **126**(8), 1697 (2006).
2. V. A. Botchkarev, Stress and the hair follicle: exploring the connections, *Am. J. Pathol*., **162**(3), 709 (2003).
3. A. Slominski, J. Wortsman, T. Luger, R. Paus, and S. Solomon, Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress, *Physiol. Rev*., **80**(3), 979 (2000).
4. N. Ito, T. Ito, A. Kromminga, A. Bettermann, M. Takigawa, F. Kees, R. H. Straub, and R. Paus, Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol, *FASEB J*., **19**(10), 1332 (2005).
5. E. M. Peters, P. C. Arck, and R. Paus, Hair growth inhibition by psychoemotional stress: a mouse model for neural mechanisms in hair growth control, *Exp. Dermatol*., **15**(1), 1 (2006).
6. A. Slominski, B. Zbytek, A. Szczesniewski, I. Semak, J. Kaminski, T. Sweatman, and J. Wortsman, CRH stimulation of corticosteroids production in melanocytes is mediated by ACTH, *Am. J. Physiol. Endocrinol. Metab*., **288**(4), E701 (2005).
7. G. Aguilera, Corticotropin releasing hormone, receptor regulation and the stress response, *Trends Endocrinol. Metab*., **9**(8), 329 (1998).
8. A. Slominski, A. Pisarchik, D. J. Tobin, J. E. Mazurkiewicz, and J. Wortsman, Differential expression of a cutaneous corticotropin-releasing hormone system, *Endocrinology*, **145**(2), 941 (2004).
9. M. H. Perrin and W. W. Vale, Corticotropin releas-

ing factor receptors and their ligand family, *Ann. N. Y. Acad. Sci*., **885**, 312 (1999).
10. D. K. Grammatopoulos and G. P. Chrousos, Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists, *Trends Endocrinol. Metab*., **13**(10), 436 (2002).
11. E. W. Hillhouse, H. Randeva, G. Ladds, and D. Grammatopoulos, Corticotropin-releasing hormone receptors, *Biochem. Soc. Trans*., **30**(4), 428 (2002).
12. A. Slominski, J. Wortsman, A. Pisarchik, B. Zbytek, E. A. Linton, J. E. Mazurkiewicz, and E. T. Wei, Cutaneous expression of corticotropin-releasing hormone (CRH), urocortin, and CRH receptors, *FASEB J*., **15**(10), 1678 (2001).
13. R. L. Hauger, V. Risbrough, R. H. Oakley, J. A. Olivares-Reyes, and F. M. Dautzenberg, Role of CRF receptor signaling in stress vulnerability, anxiety, and depression, *Ann. N. Y. Acad. Sci*., **1179**, 120 (2009).
14. K. W. Carlson, S. S. Nawy, E. T. Wei, W. Sade´e, V. A. Filov, V. V. Rezsova, A. Slominski, and J. M. Quillan, Inhibition of mouse melanoma cell proliferation by corticotropin-releasing hormone and its analogs, *Anticancer Res*., **21**(2A), 1173 (2001).
15. M. E. Quevedo, A. Slominski, W. Pinto, E. Wei, and J. Wortsman, Pleiotropic effects of corticotropin releasing hormone on normal human skin keratinocytes, *In Vitro Cell Dev. Biol. Anim*., **37**(1), 50 (2001).
16. B. Zbytek and A. T. Slominski, Corticotropin-releasing hormone induces keratinocyte differentiation in the adult human epidermis, *J. Cell Physiol*., **203**(1), 118 (2005).
17. M. K. Hordinsky and M. E. Ericson, Relationship between follicular nerve supply and alopecia, *Dermatol. Clin*., **14**(4), 651 (1996).
18. A. Katsarou-Katsari, L. K. Singh, and T. C. Theoharides, Alopecia areata and affected skin CRH receptor upregulation induced by acute emotional stress, *Dermatology*, **203**(2), 157 (2001).
19. L. V. Spencer and J. P. Callen, Hair loss in systemic disease, *Dermatol. Clin*., **5**(3), 565 (1987).
20. Y. Gauthier, [Stress and skin: experimental approach], *Pathol. Biol. (Paris)*, **44**(10), 882 (1996).
21. L. Wang, M. Million, J. Rivier, C. Rivier, N. Craft, M. P. Stenzel-Poore, and Y. Tache´, CRF receptor antagonist astressin-B reverses and prevents alopecia in CRF over-expressing mice, *PLoS One*, **6**(2), e16377 (2011).
22. R. R. Driskell, C. Clavel, M. Rendl, and F. M. Watt, Hair follicle dermal papilla cells at a glance, *J. Cell Sci*., **124**(Pt 8), 1179 (2011).
23. P. C. Arck, B. Handjiski, E. M. Peters, A. S. Peter, E. Hagen, A. Fischer, B. F. Klapp, and R. Paus, Stress inhibits hair growth in mice by induction of premature catagen development and deleterious perifollicular inflammatory events via neuropeptide substance P-dependent pathways, *Am. J. Pathol*., **162**(3), 803 (2003).
24. N. Liu, L. H. Wang, L. L. Guo, G. Q. Wang, X. P. Zhou, Y. Jiang, J. Shang, K. Murao, J. W. Chen, W. Q. Fu, and G. X. Zhang, Chronic restraint stress inhibits hair growth via substance P mediated by reactive oxygen species in mice, *PLoS One*, **8**(4), e61574 (2013).
25. Z. Lu, T. W. Fischer, S. Hasse, K. Sugawara, Y. Kamenisch, S. Krengel, W. Funk, M. Berneburg, and R. Paus, Profiling the response of human hair follicles to ultraviolet radiation, *J. Invest. Dermatol*., **129**(7), 1790 (2009).
26. S. J. Choi, A. R. Cho, S. J. Jo, S. T. Hwang, K. H. Kim, and O. S. Kwon, Effects of glucocorticoid on human dermal papilla cells *in vitro*, *J. Steroid. Biochem. Mol. Biol*., **135**, 24 (2013).
27. L. Guo, L. Degenstein, and E. Fuchs, Keratinocyte growth factor is required for hair development but not for wound healing, *Genes Dev*., **10**(2), 165 (1996).
28. D. M. Danilenko, B. D. Ring, D. Yanagihara, W. Benson, B. Wiemann, C. O. Starnes, and G. F. Pierce, Keratinocyte growth factor is an important endogenous mediator of hair follicle growth, devel-

opment, and differentiation. Normalization of the nu/nu follicular differentiation defect and amelioration of chemotherapy-induced alopecia, *Am. J. Pathol*., **147**(1), 145 (1995).
29. Y. Xing, W. Xu, K. Yang, X. Lian, and T. Yang, Immunolocalization of Wnt5a during the hair cycle and its role in hair shaft growth in mice, *Acta. Histochem*., **113**(6), 608 (2011).
30. S. Reddy, T. Andl, A. Bagasra, M. M. Lu, D. J. Epstein, E. E. Morrisey, and S. E. Millar, Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis, *Mech. Dev*., **107**(1-2), 69 (2001).
31. K. Inoue, N. Aoi, Y. Yamauchi, T. Sato, H. Suga, H. Eto, H. Kato, Y. Tabata, and K. Yoshimura, TGF-beta is specifically expressed in human dermal papilla cells and modulates hair folliculogenesis, *J. Cell Mol. Med*., **13**(11-12), 4643 (2009).
32. K. Foitzik, T. Spexard, M. Nakamura, U. Halsner, and R. Paus, Towards dissecting the pathogenesis of retinoid-induced hair loss: all-trans retinoic acid induces premature hair follicle regression (catagen) by upregulation of transforming growth factor-beta2 in the dermal papilla, *J. Invest. Dermatol*., **124**(6), 1119 (2005).
33. D. W. Yu, T. Yang, T. Sonoda, K. Gaffney, P. J. Jensen, T. Dooley, S. Ledbetter, I. M. Freedberg, R. Lavker, and T. T. Sun, Message of nexin 1, a serine protease inhibitor, is accumulated in the follicular papilla during anagen of the hair cycle, *J. Cell Sci*., **108**(Pt 12), 3867 (1995).
34. A. Gidon, M. M. Al-Bataineh, F. G. Jean-Alphonse, H. P. Stevenson, T. Watanabe, C. Louet, A. Khatri, G. Calero, N. M. Pastor-Soler, T. J. Gardella, and J. P. Vilardaga, Endosomal GPCR signaling turned off by negative feedback actions of PKA and v-ATPase, *Nat. Chem. Biol*., **10**(9), 707 (2014).
35. Y. Ni, J. Sinnett-Smith, S. H. Young, and E. Rozengurt, PKD1 mediates negative feedback of PI3K/Akt activation in response to G protein-coupled receptors, *PLoS One*, **8**(9), e73149 (2013).
36. A. Limat, T. Hunziker, E. R. Waelti, S. P. Inaebnit, U. Wiesmann, and L. R. Braathen, Soluble factors from human hair papilla cells and dermal fibroblasts dramatically increase the clonal growth of outer root sheath cells, *Arch. Dermatol. Res*., **285**(4), 205 (1993).
37. T. Fujie, S. Katoh, H. Oura, Y. Urano, and S. Arase, The chemotactic effect of a dermal papilla cell-derived factor on outer root sheath cells, *J. Dermatol. Sci*., **25**(3), 206 (2001).