Role of Dexras1 in brown and beige adipose tissues

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Directed by Professor Jae-woo Kim

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ABSTRACT

Role of Dexras1 in brown and beige adipose tissues

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While the global epidemic of obesity and its associated metabolic disease has greatly increased the needs to develop anti-obesity drugs, brown fat and beige fat have emerged as attractive targets of the drugs to promote weight loss. According to the recent studies, brown adipocytes and 'brown-like' adipocytes in white fat, beige adipocytes, that are generated by various stimuli (such as cold exposure and β3-adrenergic receptor agonists), uncouple mitochondrial respiratory chain and ATP synthesis and dissipate chemical energy as heat. Here, I demonstrated that Dexras1 negatively regulates brown adipose tissue (BAT) and beige adipose tissue thermogenesis. Dexras1, a small G protein of the Ras family, is markedly induced by dexamethasone and it is

expressed in many tissues, especially in white adipose tissue (WAT), and BAT. However, the only known role of it in WAT is that it is required for adipogenesis by glucocorticoid, and its function in BAT has scarcely been explored. In this study, I report that loss of Dexras1 promoted thermogenic activities during cold exposure in terms of maintaining mice body temperature and expression of thermogenic genes in BAT. Also, Dexras1 knockout mice showed increased thermogenic gene expression in subcutaneous WAT (scWAT), but not in epididymal WAT (epiWAT), in response to cold exposure, which is accompanied by developing more beige adipocytes in scWAT than wild type mice. Overexpression of Dexras1 in HIB1 cells, a brown fat cell line, suppressed uncoupling protein 1 (UCP1) expression which is a key thermogenic protein in BAT. When HIB1 cells were co-treated with a β3-adrenergic receptor agonist and dexamethasone to increase UCP1 and Dexras1 expression respectively, the UCP1 expression of the cells was offset compared to the cells with only β3-adrenergic stimulation. Furthermore, primary adipocytes from BAT and scWAT of Dexras1 knockout mice showed much higher *Ucp1* mRNA expression than those of wild type mice with or without β3-adrenergic stimulation. In addition, Dexras1 might affect the number and the cristae structure of BAT mitochondria because BAT of Dexras1-deleted mice had more and smaller mitochondria as well as ones with tighter cristae after cold exposure. In conclusion, these data suggest a novel role of Dexras1 in attenuating thermogenesis in brown and

beige adipose tissue upon the cold exposure and considering this, it can be expected that Dexras1 would be a therapeutic target for obesity and obesity-related disorder.

Key words: brown adipose tissue, beige adipose tissue, subcutaneous white adipose tissue, cold exposure, β-adrenergic receptor agonists, thermogenesis, UCP1, Dexras1

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I. INTRODUCTION

As the number of obese people has highly increased worldwide, the incidence of obesity-related metabolic diseases such as diabetes, cardiovascular diseases has significantly risen as well and the medical problem is getting more serious. To solve this situation, studies to develop anti-obesity drugs have been conducted for decades and a considerable number of the studies in recent years have revealed that brown and beige adipose tissue may have therapeutic potentials in the treatment of obesity.[1]

Adipocytes have been divided in two main types: white adipocytes and brown adipocytes. The major role of white adipocytes is to store chemical energy in the form of triglycerides. On the other hand, brown adipocytes are

packed with abundant mitochondria to generate heat (thermogenesis) mediated by the activated uncoupling protein 1 (UCP1), a protein inducing a proton leak across the inner mitochondrial membrane.[2]

In addition to brown fat cells, it was discovered that there is another kind of thermogenic adipocytes, beige adipocytes.[1,3] Beige adipocytes are induced in white adipose tissue (WAT) when it is stimulated by cold exposure or β3-adrenergic receptor agonists. Beige fat cells have the increased number of mitochondria compared to white fat cells, resulting in the color change of the cells from white to brown-like or beige. Also, they show comparable UCP1 expression to that of brown fat cells. This phenomenon that white adipocytes change similar to brown adipocytes, in other words, that beige adipocytes are generated, is called 'browning'.

Since the activation of brown or beige adipocytes can augment the energy expenditure through heat production, the mechanism is expected to be a promising target for obesity and metabolic syndrome treatment. However, it is not fully elucidated how the thermogenic activities of the cells occur yet.

Dexras1 is a Ras family small G protein, which is abundant in WAT and brown adipose tissue (BAT).[4] The expression of Dexras1 can be induced by dexamethasone, a synthetic glucocorticoid. In the previous studies, it was identified that Dexras1 is essential for the differentiation of white adipocytes on the basis of the observation that the body fat of Dexras1 knockout (KO) mice

was less than wild type (WT) mice when fed a normal or high fat diet. Dexras1 plays a role in adipogenesis by participating in insulin-like growth factor 1 (IGF-1) signaling, thereby affecting MAPK activation, which is essential for the progression of adipogenic signals in the cells.[5] Thus, it is likely that glucocorticoids regulates body fat composition and possibly fat behavior by means of Dexras1 as a mediator.

Dexras1 is also highly expressed in BAT; however, it remains unclear how Dexras1 works in BAT. In this study, I observed that BATs of Dexras1 KO mice were more reddish than those of WT mice. From this observation, I hypothesized that Dexras1 may have effects on activation of BAT as well as on the browning of WAT. To verify the hypothesis, I carried out a series of experiments including a cold exposure (4℃) of WT and Dexras1 KO mice to determine whether BATs of WT and KO mice act differently on the stimulation.[6,7,8]

According to the experimental results, a lack of Dexras1 enhances thermogenesis of BAT and subcutaneous WAT (scWAT) in mice in response to cold stress. Also, *Ucp1* expression was upregulated in primary BAT and scWAT cells from Dexras1 KO mice, consistent with the findings *in vivo*. On the contrary, when Dexras1 was overexpressed *in vitro*, UCP1 expression was diminished. Together, these data indicate a previously unknown role of Dexras1 that it suppresses thermogenic activities in brown and beige fat against cold

exposure. It suggests a possibility for Dexras1 to be a therapeutic target for obesity by regulating energy expenditure in adipose tissues.

II. MATERIALS AND METHODS

1. Cell culture and *in vitro* differentiation

The HIB1 preadipocytes were cultured and differentiated into adipocytes as described previously with slight modifications.[9] Briefly, preadipocyte HIB1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 8 μg/ml biotin, supplemented with 10% heat-inactivated calf serum at 37℃, in an atmosphere of 90% air and 10% CO_2. To induce differentiation, 2-day post-confluent HIB1 cells (designated day 0) were incubated in DMEM containing 10% fetal bovine serum (FBS), 0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μM dexamethasone, 20 nM insulin, 1 nM Triiodo-L-Thyronine (T3) and 125 μM indomethacin for 2 days. Cells were then cultured in DMEM containing 10% FBS, 20 nM insulin and 1 nM T3 for another 2 days, and refed every 2 to 3 days. Lipid accumulation in the cells was detected by oil red O staining on day 5, 6 or 7.

2. Experimental animals

10-week-old male C57BL/6J WT and Dexras1 KO mice were housed maximum four per cage under 12-hour light 12-hour dark cycle at room temperature. For cold exposure experiment, WT and Dexras1 KO mice were kept at 4℃ for 7 hours when indicated.[7,8,10,11] Their body temperatures were

measured with a rectal probe (Kent Scientific Corporation). For dexamethasone treatment, mice were injected intraperitoneally with dexamethasone sodium phosphate (0.5 mg/kg, Santa Cruz Biotechnology) and sacrificed after 4 hours. Animal protocols were performed in accordance with National Institutes of Health guidelines and approved by the Johns Hopkins University Committee on Animal Care and the Committee on Animal Investigations of Yonsei University.

3. Primary brown and white adipocyte cultures

Stromal vascular fraction (SVF) cells were isolated from minced BAT or scWAT of 6-week-old WT and Dexras1 KO mice as previously described with slight modifications.[12,13] BAT SVF cells were maintained in DMEM/F12 containing 20% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37℃ in an atmosphere of 90% air and 10% CO_2. WAT SVF cells were maintained in DMEM/F12 containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. For brown and white adipocyte differentiation, confluent cells were incubated in DMEM/F12 containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 2 μg/ml dexamethasone, 5 μg insulin, 1 nM T3, 125 μM indomethacin and 0.5 μM rosiglitazone for 2 days. Cells were then cultured in DMEM/F12 containing 10% FBS, 5 μg insulin and 1 nM T3 for another 2 days, and refed every 2 to 3 days.

4. Transient transfection assay

Cells were transiently transfected using microliter volume electroporation of HIB1 preadipocytes with OneDrop MicroPorator MP-100 (Digital Bio) to maximize the transfection efficiency. The cells were trypsinized, washed with phosphate buffered saline (PBS), and resuspended in 10 μl of resuspension buffer R with 0.5 μg of plasmid at a concentration of 200,000 cells per pipette. The cells were then microporated at 1,650 V, with one 20-ms pulse. Following microporation, the cells were seeded in 35-mm cell culture dishes and placed at 37℃ in an atmosphere of 90% air and 10% CO_2.

5. Western blot analysis and antibodies

For protein analysis, cells were washed with PBS and lysed in a buffer containing 1% sodium dodecyl sulfate (SDS) and 60 mM Tris-Cl, pH 6.8, and tissues were lysed in PRO-PREP™ (iNtRON Biotechnology). The lysate was mixed briefly using a vortex, boiled for 10 minutes, and centrifuged at 13,000 g for 10 minutes at 4℃. Protein concentrations were assessed using the BCA assay kit (Pierce). Protein samples of equal amount were separated by SDS-PAGE and transferred to nitrocellulose membranes. Immunoblot analysis were performed using the following antibodies: polyclonal antibody against UCP1 (Abcam), FLAG (sigma), PKA, phospho-PKA, p38, phospho-p38, AMPK, phospho-AMPK, CREB, phospho-CREB (Cell Signaling), PGC1α and β–actin,

mouse monoclonal antibody against OPA1, MFN1 and DRP1 (Santa Cruz Biotechnology).

6. Gene expression analysis

Total RNA was isolated from cells or tissues using TRIzol (Invitrogen) according to the manufacturer's instructions. For quantitative RT-PCR, cDNA was synthesized from 5 μg of total RNA using random hexamer primers and SuperScript reverse transcriptase II (Invitrogen). PCR was performed using the following primers: Dexras1, 5′-GGC CGT TTC GAG GAT GCT TA-3′ , 5′-ACG TCT CCT GTG AGG ATA GAG A-3′ ; UCP1, 5′-GGA TTG GCC TCT ACG ACT CA-3′ , 5′-TGC CAC ACC TCC AGT CAT TA-3′ ; PGC1α, 5′-CCG AGA ATT CAT GGA GCA AT-3′ , 5′-TTT CTG TGG GTT TGG TGT GA-3′ ; Elovl3, 5′-ATG AAC TTT GGC GTC CAT TC-3′ , 5′-CTT TCT CCT GCC TCC AGA TG -3′ ; Dio2, 5′-GAT GCT CCC AAT TCC AGT GT-3′ , 5′-TGA ACC AAA GTT GAC CAC CA-3′ ; CIDEA, 5'-CTC GGC TGT CTC AAT GTC AA-3′ , 5′-GGA ACT GTC CCG TCA TCT GT-3′ ; OPA1, 5′-GGA AAG GAA CAC GAC GAC AT-3′ , 5′-CTT CCA AAG CAT TGT GCT GA-3′ ; MFN1, 5′-CCA AGC CCA ACA TCT TCA TT-3′ , 5′-AGC TTC CGA CGG ACT TAC AA-3′ ; DRP1, 5′-ATG CCT GTG GGC TAA TGA AC-3′ , 5′-AGT TGC CTG TTG TTG GTT CC-3′ ; rRNA, 5′-GCA GGT GTT TGA CAA CGG CAG-3′ , 5′- GAT GAT GGA GTG TGG CAC CGA -3′.

7. Transmission electron microscopy

Brown adipose tissues were excised into small pieces (<1 mm^3) and fixed with 2% glutaraldehyde in 0.1 M PBS buffer (pH 7.4) for 3 days.[14] Each specimen was postfixed in 1% osmium tetroxide for 2 hours before embedding in epon resin. Transmission electron microscopy was performed with a Camera-Megaview III (Soft imaging system-Germany) at 80 kV on ultra-thin sections (70 nm) and stained with uranyl acetate and counterstained with lead citrate.

8. Histology analysis

Mouse BAT, scWAT and epiWAT were fixed for 48 hours in 10% buffered formalin, followed by tissue processing, paraffin embedding, sectioning, and hematoxylin and eosin staining. For imaging, I used Nikon eclipse E800 microscope (Nikon Instruments Inc, Melville, NY) fitted with Spot digital camera (Diagnostic Instruments Inc, Sterling Heights, MI).

9. Statistical analysis

All results are expressed as mean ± s.d. Statistical comparisons of groups were made using an unpaired Student's t test.

III. RESULT

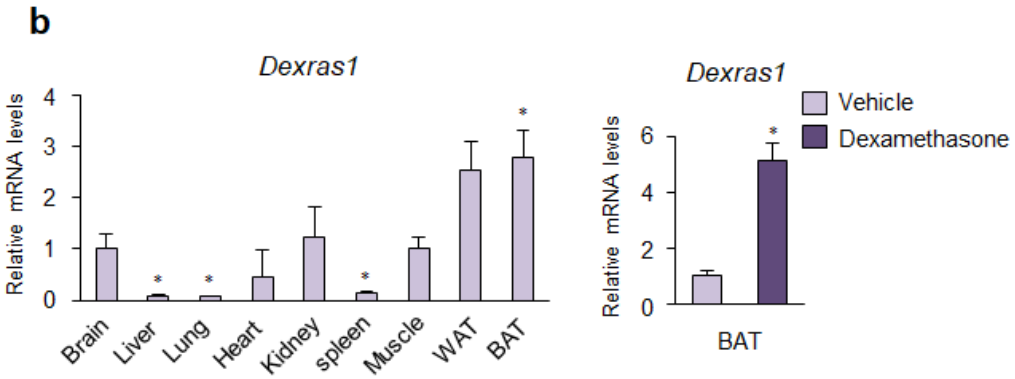
1. Effects of cold exposure on thermogenic activities in Dexras1 KO and WT mice

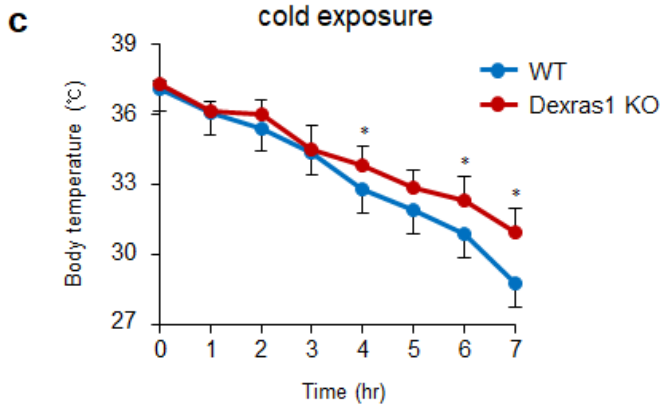
A. Dexras1 KO mice are more thermostable to cold than WT mice

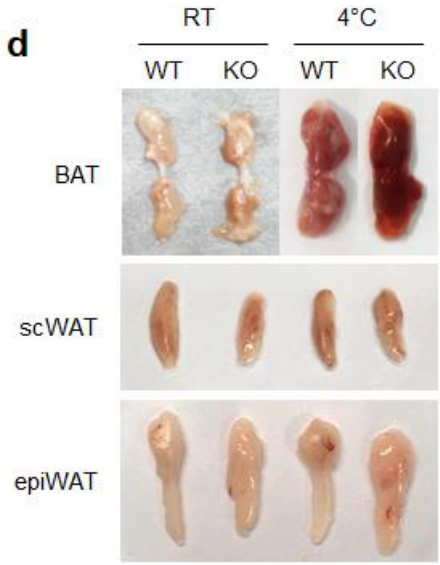
The Dexras1 KO and WT mice used in this experiment were grossly normal and had no difference in body weight (Fig. 1a). In WT mice, Dexras1 is highly expressed particularly in WAT and BAT (Fig. 1b). When WT mice were injected with dexamethasone intraperitoneally, the expression of Dexras1 in BAT was significantly increased.

To assess whether the ablation of Dexras1 affects thermogenesis, Dexras1 KO and WT mice were exposed to cold (4℃) for 7 hours.[7,8,10,11] During cold exposure, the average body temperature of Dexras1 KO mice dropped more slowly than that of WT mice (Fig. 1c), suggesting that Dexras1 negatively regulates thermogenesis. When comparing the color of fat tissues after cold stimulation, BAT of Dexras1 KO mice was more reddish than that of WT mice (Fig. 1d). In the case of WAT, there was no distinctive difference in the colors of scWAT and epididymal WAT (epiWAT) between the cold-exposed groups. The size of lipid droplets in BAT and scWAT was smaller in the cold-exposed KO mice than WT mice (Fig. 1e). On the other hand, Dexras1 KO and WT mice had

no difference in the lipid deposition of epiWAT after cold exposure. Together, these data indicate that Dexras1 KO mice are more thermostable upon a cold exposure than WT mice, and that Dexras1 may also play a role in browning of scWAT.







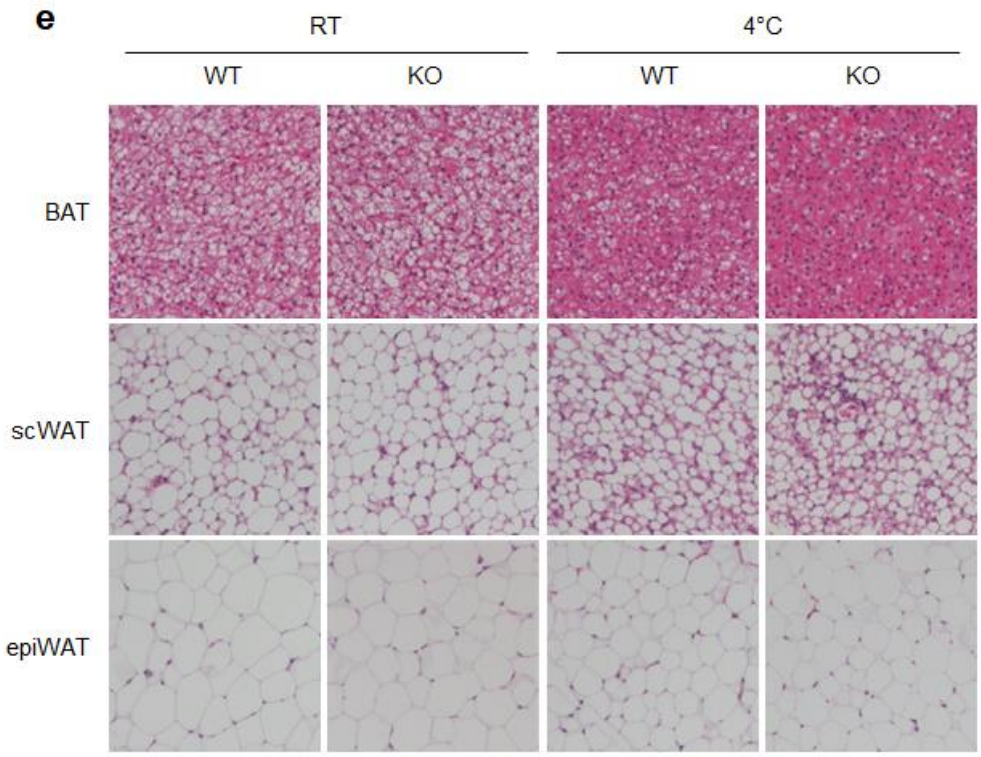


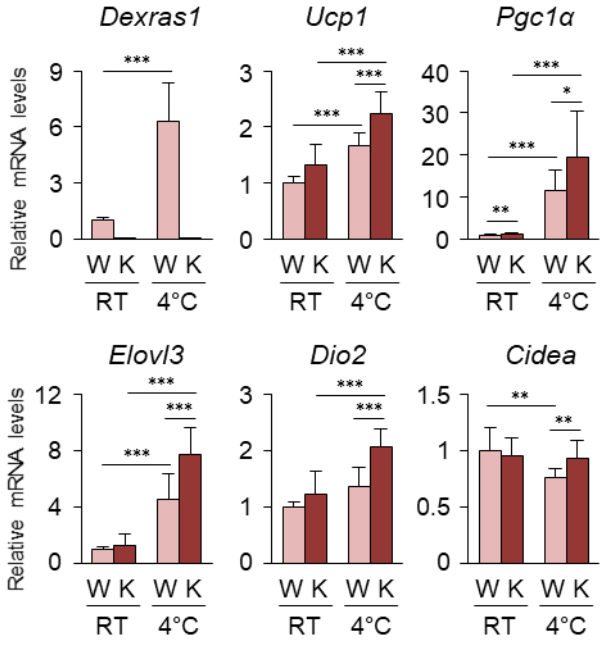
Figure 1. Phenotypic differences between Dexras1 KO and WT mice in response to cold exposure. (a) 10-week-old male C57/BL6 WT and Dexras1 KO mice (left). The average body weight of 10-week-old male WT and KO mice (right). n=6 per group. (b) Relative mRNA levels of *Dexras1* in each tissue from WT mice (left). Dexamethasone induced *Dexras1* expression in BAT (right). WT mice were injected with dexamethasone (0.5 mg/kg) intraperitoneally and sacrificed after 4 hours. (c) The average body temperatures of 10-week-old male WT and KO mice exposed to cold (4℃) at the indicated time points. Student's test, $*p < 0.05$, n=4 per group. (d and e) Representative photographs (d) and H&E staining (e) of BAT (top), scWAT (middle) and epiWAT (bottom) from WT and KO mice at room temperature (RT) or 4℃ for 7 hours.

B. Increased thermogenic gene expression of BAT and scWAT in Dexras1 KO mice

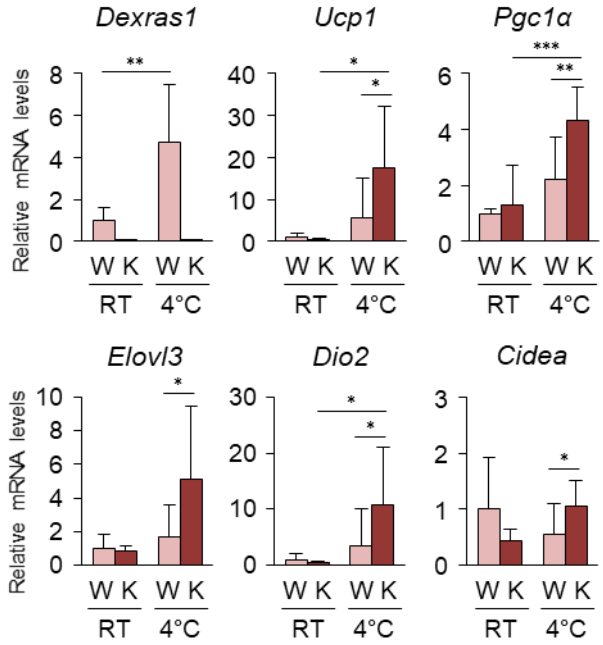
Because Dexras1 KO showed the attenuation of thermogenesis, it is expected that the expression of thermogenic genes have changed. The analysis of the mRNA expression showed that the representative thermogenic genes (*Ucp1, Pgc1α, Elovl3, Dio2* and *Cidea*) were more expressed in BAT and scWAT of Dexras1 KO mice than WT mice in response to cold (Fig. 2a).[11,13] Interestingly, the expression of *Dexras1* in BAT and scWAT was remarkably elevated when cold stimulation was given. The expression levels of UCP1 proteins in BAT and scWAT were also higher in Dexras1 KO mice than WT mice after cold experience, consistent with the mRNA expression patterns (Fig. 2b). In addition, the expression levels of signaling molecules that are known as upstream proteins of UCP1 were analyzed. In BAT, the phosphorylation of protein kinase A (PKA), p38 mitogen-activated protein kinase (p38) and AMP-activated protein kinase (AMPK) was noticeably increased in Dexras1 KO mice with cold stress. Whereas, in scWAT, there was no significant difference in the phosphorylation of PKA, p38 or AMPK between the cold-exposed groups. To summarize, these results demonstrate that the loss of Dexras1 promotes the thermogenic gene expression in BAT and scWAT against cold.

a

BAT



scWAT



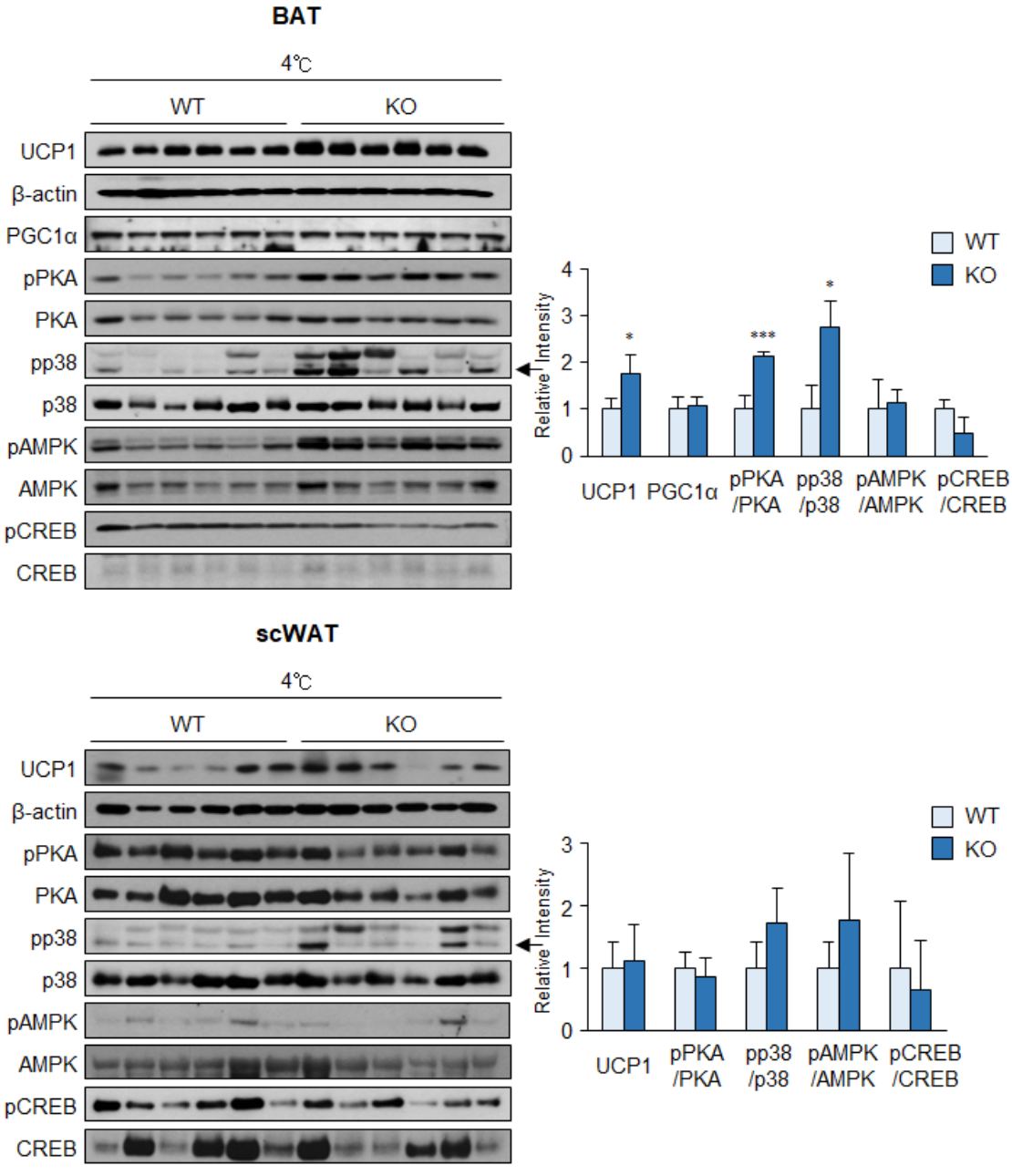


Figure 2. Thermogenic gene expression in BAT and scWAT of Dexras1 KO and WT mice after cold stimulation. (a) Relative mRNA levels of *Dexras1* and brown- and/or beige-selective thermogenic genes in BAT (top) and scWAT (bottom) of WT (W) and KO (K) mice at RT or 4℃ for 7 hours. Student's test, *p < 0.05, **p <0.01, ***p < 0.001, n=2-3 per RT group, or n=5-6 per 4℃ group. (b) Western blot analysis for UCP1 and its upstream proteins in BAT (top) and scWAT (bottom) of WT and KO mice at 4℃ for 7 hours (left). Relative intensity of protein bands are expressed as means in bar graphs (right). β-actin is used as a loading control.

2. Augmentation of *Ucp1* expression in primary BAT and scWAT cells from Dexras1 KO mice

Next, I compared the *Ucp1* expression in primary fat cells from Dexras1 KO and WT mice.[12,13] In the case of primary BAT cells, the cells from Dexras1 KO mice showed much higher *Ucp1* mRNA expression than those from WT mice on day 7 of differentiation (Fig. 3a). Also, the expression of *Ucp1* was greatly induced in the primary scWAT cells lacking Dexras1, when stimulated with CL 316243, a β3-adrenergic agonist, as well as in normal conditions on day 7 (Fig. 3b). To sum up, *Ucp1* expression was elevated in both the primary brown and subcutaneous fat cells from Dexras1 KO mice than WT mice, suggesting that Dexras1 negatively regulates UCP1 expression upon β3-adrenergic stimuli, probably by affecting the upstream signaling pathway. It cannot be ruled out a possibility that Dexras1 also affects brown or beige adipogenesis.

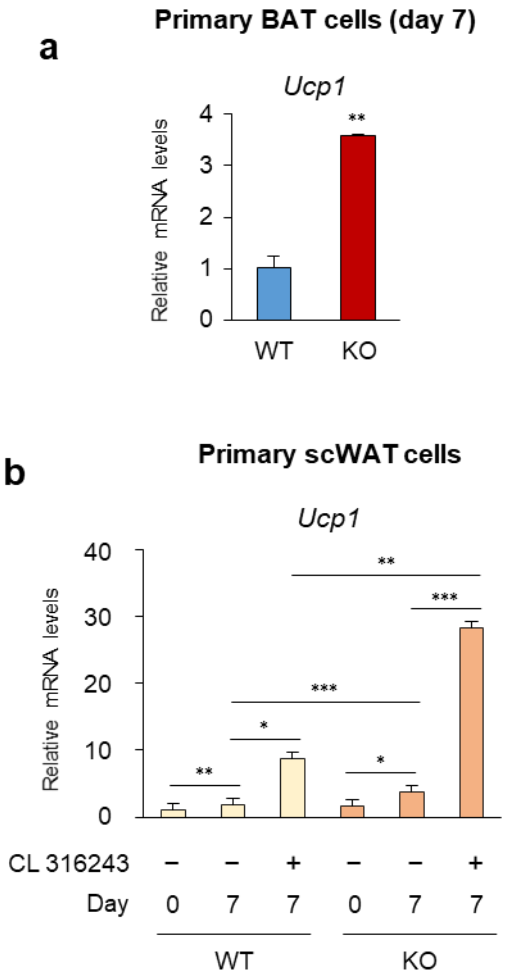


Figure 3. *Ucp1* expression of primary adipocytes from BAT and scWAT of Dexras1 KO and WT mice. (a) Relative mRNA levels of *Ucp1* in primary BAT cells from WT and KO mice 7 days after induction of differentiation. (b) Relative mRNA levels of *Ucp1* in primary scWAT cells from WT and KO mice on day 0 or 7 with or without CL 316243 treatment, a β3-adrenergic agonist (1 μM, 4 hours). Student's test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3. Suppressed UCP1 expression in Dexras1-overexpressed or dexamethasone-treated HIB1 cells

To investigate how UCP1 expression is affected when there is a large amount of Dexras1, I overexpressed pcDNA or Dexras1-FLAG in HIB1 cells, a brown fat cell line.[9] During the differentiation of HIB1 cells, the basal expression levels of *Dexras1* and *Ucp1* increase with time, especially on day 6 and 7 (Fig. 4a and 4b).

When pcDNA or Dexras1-FLAG were overexpressed in HIB1 cells, both the control and the Dexras1-overexpressed groups normally differentiated and there was no remarkable difference in the degree of differentiation between the two groups (Fig. 5a). In terms of gene expression, *Dexras1* was induced about 2.4 times more in the Dexras1-overexpressed cells while *Ucp1* expression was reduced by 37% compared to the control group on day 5 (Fig. 5b). Likewise, UCP1 expression at the protein level was considerably reduced in the Dexras1-overexpressed HIB1 cells on day 5 (Fig. 5c). Additionally, it was observed that the phosphorylation of CREB was suppressed in the Dexras1-overexpressed cells, but the phosphorylation of the other UCP1 upstream proteins such as PKA and AMPK was not influenced.

In order to examine the effect of dexamethasone, which is well known to remarkably induce the expression of Dexras1[4], on the UCP expression, I treated dexamethasone on HIB1 cells on day 7. This experiment would exclude

the Dexras1 effect on the cell differentiation and only show the effect on the UCP expression. In addition, CL 316243 was treated on HIB1 cells solely or with dexamethasone on day 7 to activate UCP1 expression. As expected, the expression of *Ucp1* was raised in the cells treated with CL 316243 (Fig. 5d). However, importantly, the co-treatment with CL 316243 and dexamethasone did attenuate the rise of *Ucp1* expression in HIB1 cells. In other words, the increased Dexras1 by dexamethasone treatment inhibited the upregulation of *Ucp1* expression by CL 316243 in the cells.

Put together, these experimental results reveal that Dexras1 represses the UCP1 expression in HIB1 cells *in vitro*.

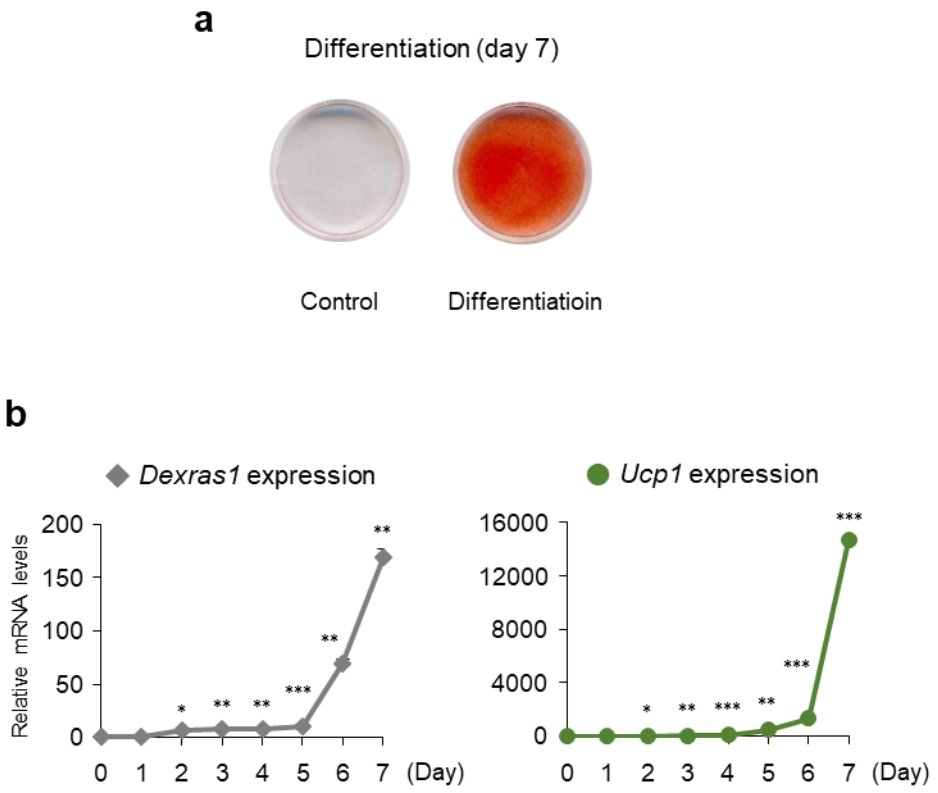


Figure 4. Basal gene expression of *Dexras1* and *Ucp1* in HIB1 cells. (a) Oil red O (ORO) staining of the control (left) and the differentiated (right) groups of HIB1 cells on day 7. (b) Relative mRNA levels of *Dexras1* (left) and *Ucp1* (right) during HIB1 cells differentiation. Student's test, *p < 0.05, **p <0.01, ***p < 0.001.

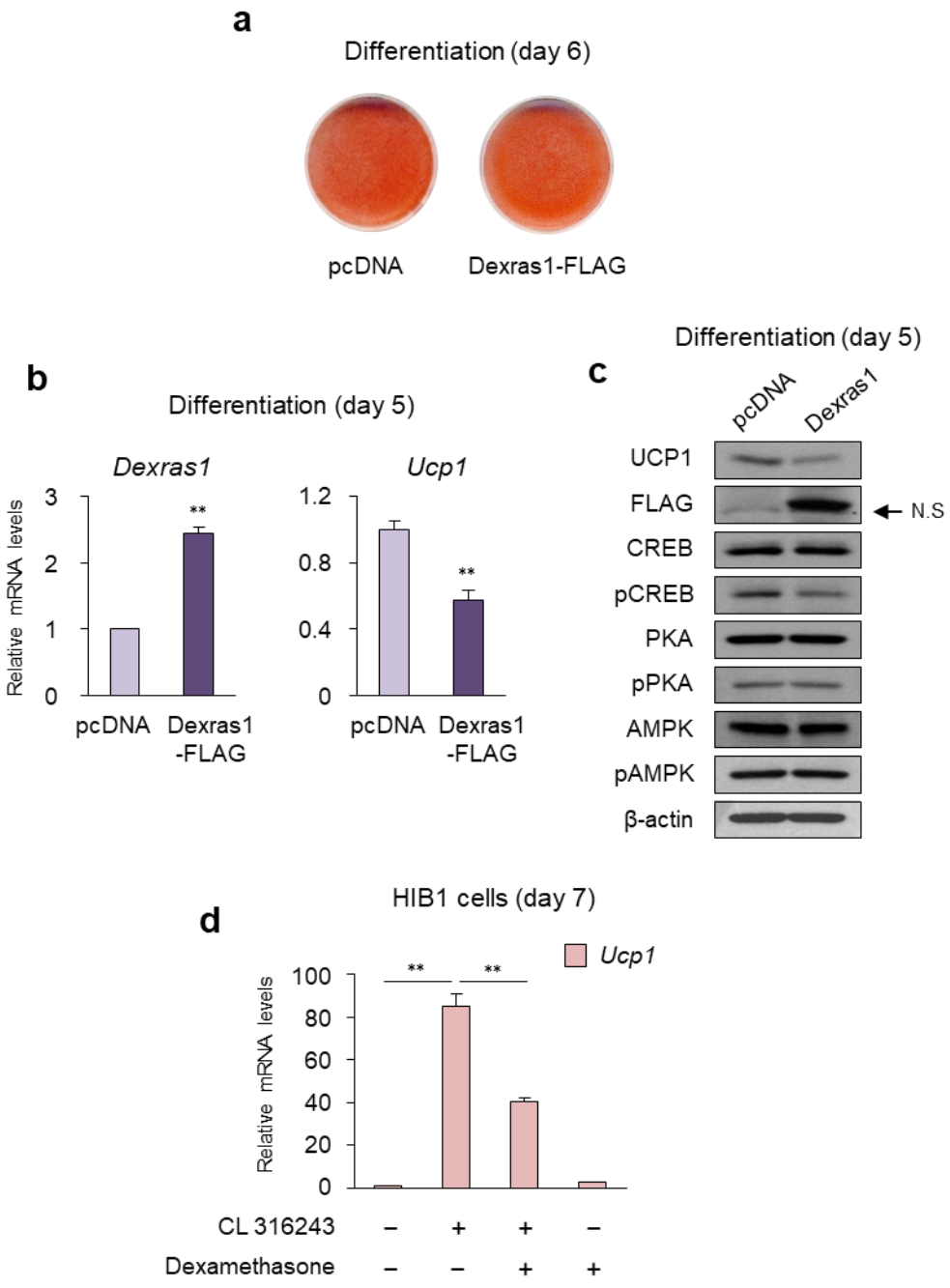
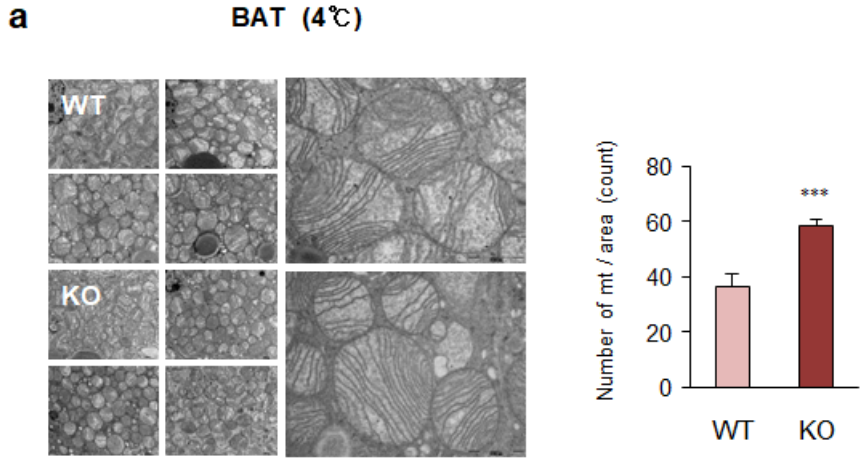


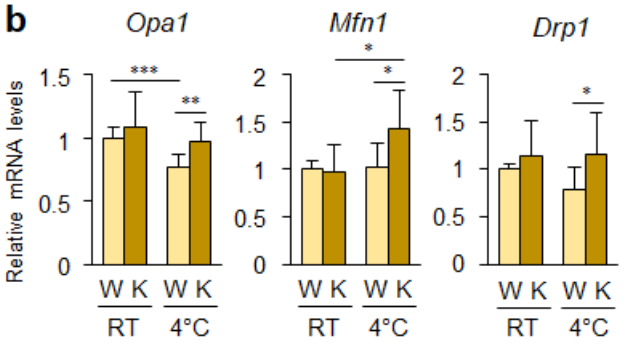
Figure 5. Gene expression of Dexras1-overexpressed or dexamethasone-treated HIB1 cells. (a) ORO staining of HIB1 cells overexpressing pcDNA (left) or Dexras1-FLAG (right) on day 6. (b) Relative mRNA levels of *Dexras1* (left) and *Ucp1* (right) in pcDNA- or Dexras1-overexpressed HIB1 cells on day 5. (c) Western blotting against UCP1 and its upstream proteins in HIB1 cells overexpressing pcDNA (left) or Dexras1-FLAG (right) on day 5. (d) Relative mRNA levels of *Ucp1* in HIB1 cells treated with 1 μM CL 316243 and/or 0.5 μM dexamethasone for 4 hours on day 7. Student's test, **p <0.01.

4. Enhanced BAT mitochondrial dynamics in Dexras1 KO mice more than WT mice in response to cold exposure

Next, I examined BAT mitochondria of Dexras1 KO and WT mice, which had been exposed to cold (4℃) for 7 hours, using transmission electron microscope (TEM) (Fig. 6a).[14] As a result, it was found that the mitochondria in BAT of Dexras1 KO mice were more and smaller than the control group after cold. Moreover, the cristae of the BAT mitochondria were tighter in the KO mice group. On the basis of these findings, I formed a hypothesis that Dexras1 deficiency may have an influence on mitochondrial fusion/fission or cristae structure in BAT when stimulated with cold. To test the hypothesis, I analyzed the expression of mitochondrial dynamics and/or structure-related genes, including OPA1, MFN1 and DRP1[15]: OPA1 is a mitochondrial protein which regulates mitochondrial inner membrane fusion and cristae structure. MFN1 proteins mediate mitochondrial outer membrane fusion. DRP1 is a fundamental protein for mitochondrial fission (Fig. 6b). According to the analysis, the mRNA levels of *Opa1*, *Mfn1* and *Drp1* were higher in BAT of Dexras1 KO mice than WT mice after cold exposure. When comparing the RT groups with the 4℃ groups, WT mice showed a significant decrease in mRNA expression or no meaningful change after cold stimulation. On the other hand, in the case of KO mice, the decline in gene expression was smaller than WT mice, or rather the mRNA expression levels were raised after cold stress. At the protein levels, OPA1 and DRP1 were more induced in cold-exposed KO mice than WT mice (Fig. 6c). In conclusion, these findings imply that the ablation of Dexras1

enhances BAT mitochondrial fusion/fission and makes the inner mitochondrial membrane tighter in response to cold exposure.





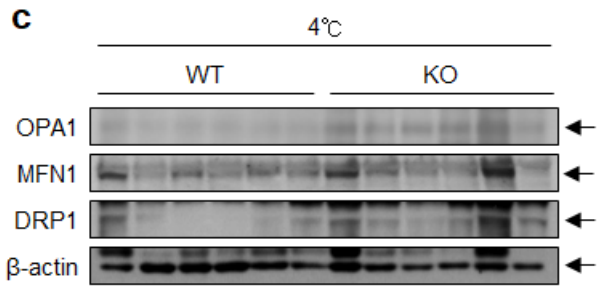


Figure 6. Transmission electron microscope images and gene expression of BAT mitochondria of Dexras1 KO and WT mice after cold exposure. (a) Transmission electron microscope (TEM) analysis of BAT mitochondria in cold exposed WT (above) and Dexras1 KO (below) mice for 7 hours (left). Magnification, x 12,000 (left) and x 30,000 (right), n=3 per group. The average number of BAT mitochondria per area of 8 TEM images (x 12,000) taken from 3 mice per group (right). (b) Relative mRNA levels of mitochondrial fusion/fission genes, *Opa*1, *Mfn1* and *Drp1* in BAT of WT (W) and KO (K) mice at 4℃ for 7 hours. n=3 per RT group or n=5 per 4℃ group. (c) Western blot analysis for OPA1, MFN1 and DRP1 proteins in BAT of cold-exposed WT and KO mice. Student's test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

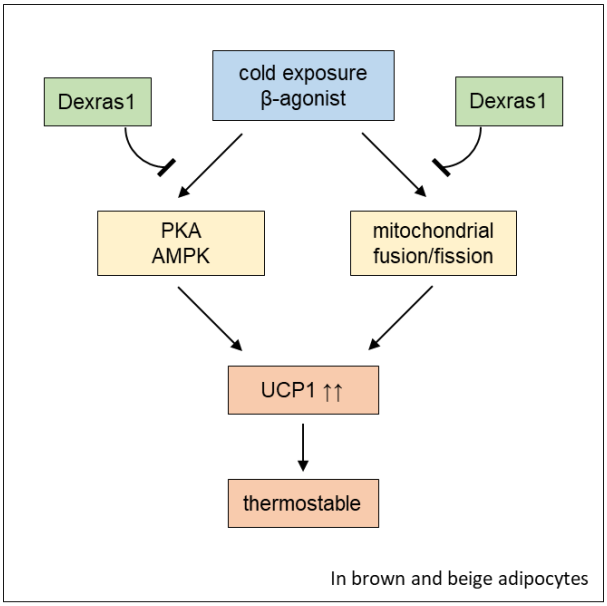


Figure 7. A schematic model of Dexras1 functions in brown and beige adipocytes. When cold or β-agonist stimulation is given, Dexras1 downregulates UCP1 expression in brown and beige fat cells by inhibiting the phosphorylation of PKA or AMPK, or by suppressing mitochondrial fusion/fission at least in BAT.

IV. DISCUSSION

This study demonstrates that Dexras1 inhibits thermogenesis of brown fat and subcutaneous fat when mice are exposed to cold. If cold or β-agonist stimulation is given, β-adrenergic receptors on the brown fat and subcutaneous fat cells are activated, followed by the activation of the downstream signaling pathways including PKA signaling.[1] And then, it leads to the expression of UCP1 and thermogenesis in the fat cells. When Dexras1 KO mice were cold exposed, UCP1 expression in BAT and scWAT was elevated more than WT mice. Also, the phosphorylation of PKA and AMPK in BAT was more increased in KO mice. This indicates that Dexras1 acts as a negative regulator in the expression of UCP1, and it is involved in PKA and AMPK signaling during the process. In addition, BAT mitochondrial fusion/fission might be promoted in the cold-stimulated Dexas1 KO mice compared to WT mice as evidenced by mitochondrial protein analysis. It is known that the increased BAT mitochondrial dynamics gives rise to the enhanced sensitivity of mitochondria to free fatty acids, resulting in the activation of UCP1 as well as thermogenesis.[16] That is, the improved BAT mitochondrial fusion/fission in KO mice may also be a reason for the higher UCP1 expression in KO mice. For these reasons, the body temperatures of Dexras1 KO mice can be better maintained during cold exposure and, in other words, Dexras1 KO mice can be

more thermostable to cold than WT mice.

Dexras1 is a small GTPase and has been suggested to inhibit receptor-G protein signaling, resulting in suppression of adenylyl cyclase *in vitro*.[17,18] In this study, it was identified that phosphorylation of PKA and p38, the downstream molecules of adenylyl cyclase in BAT thermogenesis, was enhanced in Dexras1 KO mice when cold-exposed. Given the previous reports, Dexras1 might repress activation of adenylyl cyclase in BAT by interfering β3-adrenergic receptor-G protein signaling. Thus, it is needed to verify this hypothesis and elucidate the signaling pathway.

It has been reported that the expression of UCP1 in BAT is reduced by glucocorticoid treatment.[19] In addition, this study showed that dexamethasone highly induced Dexras1 expression in BAT (Fig. 1b). When guessing the link between the two phenomena, the raised expression of Dexras1 in BAT by glucocorticoid may contribute to, or may be a cause of the decrease of UCP1 expression in BAT. Interestingly, Dexras1 expression was significantly elevated in BAT and scWAT when WT mice were stimulated with cold. This might be a buffering effect that modulates the rate at which the thermogenic activities caused by cold exposure occur, or another kind of response. It is also possible that Dexras1 is involved in the fine control of adaptive thermogenesis during cold exposure, in response to glucocorticoid. Further research is needed to determine the cause of this phenomenon.

In conclusion, lack of Dexras1 promotes thermogenesis in brown and beige adipose tissue in response to cold through PKA and p38 signaling axis. These results suggest that Dexras1 would be an effective target for a new anti-obesity drug development.

V. CONCLUSION

1. Loss of Dexras1 enhances thermogenesis in BAT and scWAT of mice against cold.
2. Primary BAT and scWAT cells from Dexras1 KO mice show the higher expression of *Ucp1*.
3. Dexras1 overexpression in HIB1 cells represses UCP1 expression.
4. BAT mitochondrial fusion/fission is more activated in Dexras1 KO mice in response to cold exposure.

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ABSTRACT (IN KOREAN)

갈색지방 및 베이지지방에서 Dexras1의 역할 규명

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김다은

비만 및 비만으로 인한 대사질환이 전세계적으로 증가함에 따라 비만치료제 개발의 필요성이 크게 높아지고 있다. 그에 따라 갈색지방 및 베이지지방이 체중감량 약물의 매력적인 타깃으로 대두되고 있다. 최근 연구들에 따르면, 갈색지방세포와 다양한 자극에 의해 발생하는 백색지방 내 '갈색유사' 지방세포, 즉 베이지지방세포가 미

토콘드리아의 전자전달계를 uncoupling 하여 화학에너지를 열에너지 형태로 발산한다.

본 연구는 Dexras1 가 갈색지방 및 베이지지방의 열 발생을 음성적으로 조절함을 밝히고 있다. Dexras1 은 Ras family 의 small G protein 중 하나로, 덱사메타존에 의해 그 발현이 크게 증가되며 체내 여러 조직, 특히 백색지방과 갈색지방에서 많이 발현된다. 하지만 갈색지방에서의 기능은 거의 연구된 바가 없으며 백색지방에서의 역할로는 당질코르티코이드에 의한 지방세포분화에 필요하다는 것만 알려져 있다.

본 연구자는 마우스가 추위 자극에 노출될 경우 체온 유지 및 갈색지방 내 발열 유전자의 발현에 관한 측면에서 Dexras1 의 결손이 발열활동을 촉진시킴을 발견하였다. 또한 Dexras1 KO 마우스는 추위에 노출됐을 때 WT 마우스보다 피하지방에서 발열 유전자들이 더 많이 발현됐으며, 베이지지방세포가 더 많이 발생한 것으로 나타났다. 하지만 내장지방에서는 이 같은 변화가 관찰되지 않았다.

갈색지방 세포주의 하나인 HIB1 세포에서 Dexras1 을 강화하였을 때 갈색지방의 주요 발열 단백질인 UCP1 의 발현이 억제되었다. 또한 HIB1 세포에 각각 UCP1 과 Dexras1 발현을 증가시키는 CL 316243 과 덱사메타존을 함께 처리하자 CL 316243 만 처리한 세포에 비해

UCP1 의 발현 증가가 상쇄되는 것으로 나타났다.

이에 더하여, Dexras1 KO 마우스의 갈색지방과 피하지방에서 분리한 일차 지방세포들은 WT 마우스의 세포보다 더 높은 *Ucp1* mRNA 발현을 보였다.

또한 추위에 노출된 Dexras1 KO 마우스의 갈색지방은 WT 마우스의 갈색지방보다 미토콘드리아의 개수가 더 많고 그 크기가 더 작았으며 미토콘드리아의 크리스테 역시 더 촘촘하였다. 이를 고려할 때 Dexras1 이 갈색지방 내 미토콘드리아의 개수나 크리스테 구조에 영향을 줄 수 있을 것으로 생각된다.

결론적으로, 본 연구의 실험결과들은 Dexras1 이 추위에 대한 반응으로 발생하는 갈색지방 및 베이지지방 내 발열작용을 감소시킨다는 Dexras1 의 새로운 역할을 제시한다. 또한 이를 바탕으로 Dexras1 이 비만 및 비만관련 질병 치료에 있어 타깃이 될 수 있을 것으로 기대된다.

핵심되는 말: 갈색지방, 베이지지방, 피하지방, 추위 노출, β-adrenergic receptor agonists, 열 발생, UCP1, Dexras1