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Identification of a new secretory factor, CCDC3/Favine, in adipocytes and endothelial cells

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ABSTRACT

The vascular system secretes many bioactive factors. In a gene chip database, we searched for novel genes with signal sequences that are specifically expressed in murine aorta, and focused on one gene previously named *CCDC3* (NCBI nucleotide entry NM_028804), and we designated as *Favine* (fat/vessel-derived secretory protein). Northern blot analysis revealed that *CCDC3* was expressed abundantly in the aorta and adipose tissues. The mRNA levels of *CCDC3* were higher in adipose tissues of obese *db/db* mice than control mice, and induced during differentiation of rat primary adipocytes. In differentiated adipocytes, *CCDC3* mRNA expression was enhanced by insulin and pioglitazone, a PPARgamma agonist, and suppressed by TNF-alpha, isoproterenol and norepinephrine. Transient expression experiments followed by N-terminal amino acid sequence analysis revealed secretion of CCDC3 protein into the culture medium, which was dose-dependently reduced by brefeldin A, an inhibitor of Golgi-mediated secretory pathway. When expressed in COS-7 cells, CCDC3 protein was post-transcriptionally modified with *N*-glycosylation, and formed a dimer complex. These results indicate that CCDC3 is a protein secreted by adipocytes and endothelial cells, and that its level is regulated both hormonally and nutritionally.

Introduction

Obesity is the most common nutritional disorder in industrial countries, and is associated with the metabolic syndromes and atherosclerotic diseases [1]. We have demonstrated that adipocytes play crucial roles in metabolic syndromes and vascular diseases [2]. Adipose tissue is not only a passive reservoir for energy storage but also produces and secretes a variety of bioactive molecules such as tumor necrosis factor (TNF)-α, leptin, resistin, and plasminogen activator inhibitor type 1 (PAI-1) [3]. We conceptualized these molecules secreted from adipocytes as 'adipocytokines' [4,5]. Leptin is a major adipocytokine, and its plasma levels correlate positively with the amount of adipose tissue and body mass index (BMI) [6]. Leptin regulates adipose tissue weight by affecting appetite and energy homeostasis [7], and leptin deficiency causes obesity and diabetes [8]. In the course of research on adipocytokines, we identified another major adipocytokine, adiponectin [9]. Plasma levels of adiponectin are decreased in obesity [10,11], and hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation and reduced blood flow in human subjects [12]. Adiponectin promotes glucose uptake and fatty acid oxidation in liver and skeletal muscle [13], and has potent anti-inflammatory and anti-atherogenic properties. [14–16]. These investigations indicate that dysregulated production of adipocytokines is associated with the pathophysiology of metabolic diseases and atherosclerosis.

The vascular system represents a highly active metabolic and endocrine organ producing a multitude of secretory molecules, including vasoactive peptide hormones, growth factors, and coagulation factors [17]. It thereby regulates the balance between vasoconstriction and vasodilation, and between coagulation and fibrinolysis. For example, endothelial cells are an important source of PAI-1, and considerable evidence links PAI-1 to myocardial infarction [18]. Thrombomodulin is also expressed in endothelial cells, plays a role as a protein C cofactor and has anticoagulant activity. Low soluble thrombomodulin is associated with increased risk of coronary artery disease [19]. While it is well known that endothelial function is impaired in obese individuals [20], the mechanism of this dysfunction is not completely clear.

Our major research goal is the identification of novel secretory factors from the vascular system and adipose tissue. The present study focused on novel genes with signal sequences at N-terminus that are specifically expressed in blood vessels and regulated by obesity. Here, we describe a previously undescribed gene, *CCDC3* (NM_028804), which fulfilled the above requirements. We

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demonstrate that CCDC3 is a secretory protein expressed in the vascular system and adipose tissues, and its mRNA expression level in adipose tissue is actively regulated in hormonal alternations and obesity.

Materials and methods

Cloning of CCDC3. Screening of the gene chip database of Daiichi Sankyo Co., Ltd. was carried out to identify novel secretory genes in blood vessels. Thirteen unidentified genes that were expressed in murine aorta with signal sequences, were extracted.

Antibodies, recombinant proteins, and other reagents. HRP-conjugated anti-HA antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). HRP-conjugated anti-His antibody was from Qiagen (Santa Clarita, CA), recombinant murine TNF-α from PeproTech (Rocky Hill, NJ), Nickel Sepharose from GE (Piscataway, NJ), WGA-agarose from Vector Laboratories (Burlingame, CA) and PNGaseF from New England Biolabs (Beverly, MA). Pioglitazone was a gift from Takeda Pharmaceutical Company (Osaka, Japan). Other reagents were purchased from Sigma–Aldrich (St. Louis, MO).

Animals. Male C57BL/6J mice, male *KK/Ay* mice, male *db/db* mice, and male *m+/m+* mice were purchased from Clea (Tokyo, Japan). Male LDL-receptor deficient mice were from Charles River (Kanagawa, Japan). Five-week-old-male LDL-receptor deficient mice were fed western diet (0.15% cholesterol, 15% unsalted butter) for 30 weeks. Six-week-old-male Apolipoprotein E-deficient (ApoE) knockout (KO) mice [21] were fed western diet (F2WTD; 0.15% cholesterol, 20% fat, Oriental Yeast, Suita, Japan) for 7 weeks. Eight-week-old-male C57BL/6J mice were fed high-fat diet (F2HFHSD; 30% fat, Oriental Yeast, Suita, Japan) for 7 weeks. The descending blood vessels (aorta and vein) and epididymal white adipose tissue (WAT) of each mouse were dissected out, washed with phosphate-buffered saline (PBS) and immediately frozen in liquid nitrogen. All experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of Osaka University.

Cell culture. COS-7 cells and HepG2 cells were purchased from ATCC. Human umbilical vein endothelial cells (HUVEC) and human aortic smooth muscle cells (HASMC) were from Kurabo (Osaka, Japan). Rat preadipocytes were obtained from Hokudo (Sapporo, Japan), and induced to differentiate by 5–12 days culture in the differentiation medium.

Establishments of CCDC3-stably expressing COS-7 cells. The full-length murine *CCDC3* cDNA with HA-tag or His-tag at the C terminal was inserted into a pPyCAGIP vector (a kind gift from Dr. Ian Chambers), and two types of stable COS-7 cell lines expressing murine CCDC3 with HA-tag (HA-CCDC3-COS cells) or His×6-tag (His-CCDC3-COS cells) were generated.

Isolation of stromal vascular fraction and mature adipocytes fraction. Fractionation of adipose tissue into mature adipocytes fraction (AdipF) and the stromal vascular fraction (SVF) pellet were performed as described previously [22].

Effects of various hormones on CCDC3 mRNA expression in cultured cells. Differentiated rat adipocytes on day-5 culture were treated with each reagent in serum-free Dulbecco's modified Eagle medium (DMEM) containing 0.1% bovine serum albumin (BSA) for 24 h. HUVEC were treated with TNF-α in MCDB 131 medium containing 10% FBS for 24 h, or treated with pioglitazone in MCDB 131 medium containing 10% FBS and 2 ng/ml basic Fibroblast Growth Factor (BioVision, Inc., USA) for 24 h. Cells were harvested for mRNA measurement.

RNA isolation and Northern blot analysis. Total RNA was extracted from each tissue or cell cultures using TRIzol (Invitrogen, Carlsbad, CA), RNA STAT-60 (Tel-Test, Friendswood, TX) or RNeasy (Qiagen) according to the instructions supplied by the manufacturer. Northern blot analysis was performed as described previously [23].

Quantitative real-time RT-PCR. Quantitative real-time RT-PCR was performed as described previously [22]. The sequences of primers used in real-time RT-PCR are listed in Supplementary Table 1.

BFA treatment. COS-7 cells stably expressing HA-tagged murine CCDC3 were cultured in 6-well plates. Cells were treated with brefeldin A (BFA) for 1 h, refreshed with new medium containing BFA, and maintained for another 6 h. Cell lysates and cultured media were harvested, followed by immunoprecipitation with anti-HA antibody-conjugated agarose for 4 h. Bound proteins were subjected to Western blotting with anti-HA antibody.

Multimerization of CCDC3. Culture medium and cell lysates were collected from His-CCDC3-COS cells. Cultured media were purified with Nickel Sepharose column and eluted proteins were suspended in the indicated sample buffer. For reducing conditions, the sample buffer contained 5% 2-mercaptoethanol and 10 mM dithiothreitol. For heat-denatured conditions, the samples were treated at 95 °C for 10 min. Next, 10 μg of cell lysates/lane were subjected to Western blotting. Non-transfected COS-7 cells were used as a negative control.

PNGase treatment. COS-7 cells were transfected with pPyCAGIP-CCDC3-HA. The cell lysates and cultured media were harvested 48 h later, and 10 ml of the medium was incubated with anti-HA antibody-conjugated agarose. Next, 10 μg of proteins from cell lysates and CCDC3 protein bound to agarose were treated with 1000 units of PNGaseF, followed by Western blotting using anti-HA antibody.

Lectin-binding experiments. COS-7 cells were transfected with pPyCAGIP-CCDC3-His. After 48 h, 1 ml of conditioned medium was incubated with 20 μl of WGA-agarose at 4 °C for 2 h. After washing with 60 mM phosphate buffer containing 300 mM NaCl, CCDC3 was eluted with phosphate buffer containing 0.5 M GlcNAc, 8 mM chitotriose and 0.05% CHAPS, 4 times sequentially (E1–E4) [24]. Agarose beads and eluted products were subjected to Western blotting using anti-His×6 antibody.

Statistical analysis. Values were expressed as means ± SEM. Differences between two groups were examined for statistical significance using the Student's *t*-test. A *P* value less than 0.05 was considered statistically significant.

Results

Selection of CCDC3 gene

Tissue specificity for the mRNA expression of 13 candidate genes, extracted by gene chip analysis, was examined by quantitative real-time PCR. Among the candidates, three genes were expressed in the aorta and adipose tissues, and their expression levels were altered in adipose tissues of obese mice. In this study, we focused on one gene. This gene was first cloned by the National Institutes of Health Mammalian Gene Collection, and was previously named *CCDC3* (*c*oiled-coil *d*omain *c*ontaining 3), (NCBI nucleotide entry NM_028804) for its coiled-coil domain motif [25]. However, it has not been characterized. The murine gene for *CCDC3* encodes a 273-amino acid protein (NCBI protein entry NP_083080) with a calculated molecular mass of 32 kDa. Database analysis revealed that the amino acid sequence of CCDC3 is highly conserved among species (Fig. 1A).

Expression profiles of CCDC3

Next, the expression of *CCDC3* was investigated in various mouse tissues (Fig. 1B). Northern blot analysis revealed that *CCDC3*

A

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Mus musculus          MPLPLLLAALCLAASPAPARACQLPSEWRPLSEGCRAELAETIVYAKVLA
Rattus norvegicus     MPLPLLLAALCLAVSPAPARACQLPSEWRPLSEGCRAELAETIVYAKVLA
Homo sapiens          MLRQLLLAALCLAGPPAPARACQLPSEWRPLSEGCRAELAETIVYARVLA
Danio rerio           -----MHLALLLTLGFGFAFACQLPSEWRPLSEGCRAELAEIIVYAKVLA
Xenopus tropicalis    ---MKTLIFLWLASLLGLLKGCQLPHDWRPQTEACRAELVETVVFAKVLA
                             * *          ****  ***  * ***** *  * * ***

Mus musculus          LHPEVPG--LYNYLPWQYQA-GEGGLFYSAEVEMLCDQAWGSMLEVPAGS
Rattus norvegicus     LHPEVPG--LYNYLPWHYQA-GEGGLFYSAEVEMLCDQAWGSMLEVPAGS
Homo sapiens          LHPEAPG--LYNHLPWQYHA-GQGGLFYSAEVEMLCDQAWGSMLEVPAGS
Danio rerio           IHDEPYASSLYNYLPYQKHERAEDALLYAAEVELLCDQAWGSMLEVPAGS
Xenopus tropicalis    LHKDSYS--VYNYLPWQYNS----DLFFSAEIELLCDQAWGSMLEVPAGS
                       *        ** **          *   ** * ****************

Mus musculus          RLNLTGLGYFSCHSHTVVQDYSYFFFVRMDENYNLLPHGVNFQDAIFPDT
Rattus norvegicus     RLNLTGLGYFSCHSHTVVQDYSYFFFVRMDENYNLLPHGVNFQDAIFPDT
Homo sapiens          RLNLTGLGYFSCHSHTVVQDYSYFFFLRMDENYNLLPHGVNFQDAIFPDT
Danio rerio           RLNLTGLGYFSCQSHTVMENNSYIFFLRMDENYNILPHGVNFQDAIFPDT
Xenopus tropicalis    RLNVTGLGYFPCHSYTVMENNSYYFFLRMDENYNLLPHGVNFQDPIFPDT
                      *** ****** * * **    ** ** ******* ********* *****

Mus musculus          QENRRMFSSLFQFANCSQGQQLTTFSSDWEVQEDNRLMCSSVQKALFEEP
Rattus norvegicus     QENRRMFSSLFQFANCSQGQQLTTFSSDWEIQEDNRLMCSSVQKALFEEE
Homo sapiens          QENRRMFSSLFQFSNCSQGQQLATFSSDWEIQEDSRLMCSSVQKALFEEE
Danio rerio           LENRRLFSSLFQFSNCSGGQTMHNFNSDWESQEDSRLLCSSMQKVLFEEE
Xenopus tropicalis    PENHRMFASLFQFSNCSPGIEPQVYTPDWDAQEDHRLLCSTVQKALLEEE
                       ** * * ***** *** *        **  *** ** **  ** * ***

Mus musculus          DHVKKLQQKVATLEKRNRQLRERVKKVKRSLRQARKNSRHLELVNQKLNP
Rattus norvegicus     DHVKKLQQKVATLEKRNRQLRERVKKVKRSLRQARKNSRHLELVNQKLNE
Homo sapiens          DHVKKLQQKVATLEKRNRQLRERVKKVKRSLRQARKKGRHLELANQKLSE
Danio rerio           ERVGQLEERVAELERKNQQLKERVGKLRHSLRKTRKGLRRAERERQEFHE
Xenopus tropicalis    ERVKNLSQKVRMLEKANGHLREKVKNLKRSLRQATIENKEALQAKCLNDK
                        *  *   *  **  *  * * *     ***

Mus musculus          KLGASSAQQHINALGREPVRAPYLHG-----
Rattus norvegicus     KLGGPGAQQHVNALGREPERAPYRHG-----
Homo sapiens          KLAAGALPHIN---ARGPVRPPYLRG-----
Danio rerio           RLKTAERHAGHHLNAIPPQHSPSQHRYTHGL
Xenopus tropicalis    ETGGKDLKTGNHKKVLARAPKKPTGNRLD--
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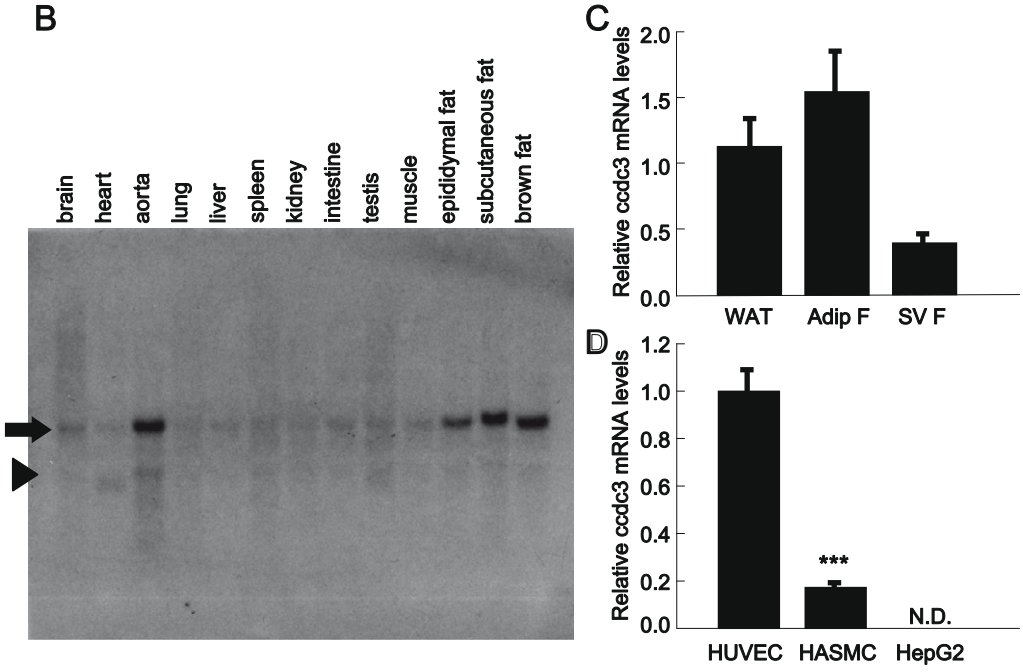

Fig. 1. Deduced amino acid sequence and the mRNA expression profiles of *CCDC3*. (A) Deduced amino acid sequence of mouse CCDC3 (273 a.a.). Signal peptides are underlined, and coiled-coil domain is boxed. Automated amino acid homology between species using CLUSTALW analysis. (B) Tissue distribution of *CCDC3*. Total RNA was extracted from the indicated tissues of 11-week-old C57BL/6J mice, and subjected to Northern blot analysis with probes specific for *CCDC3*. Arrow indicates the major transcript of *CCDC3*. Extra bands (arrowhead) were not detected with another probe (data not shown). (C) *CCDC3* mRNA expression levels in the adipocytes fraction (AdipF), stromal vascular fraction (SVF), and epididymal white adipose tissue (WAT) of 6-week-old male C57BL/6J mice ($n = 3$). (D) *CCDC3* mRNA expression levels in human umbilical vein endothelial cells (HUVEC), human aortic smooth muscle cells (HASMC) and human hepatocellular carcinoma (HepG2) cells ($n = 5$). The mRNA levels were measured by quantitative real-time PCR and normalized to 36B4 or cyclophilin mRNA level. Data in (C) and (D) are means ± SEM. $^{***}P < 0.001$.

was expressed mainly in the aorta and adipose tissues. To further investigate *CCDC3* expression in adipose tissues, adipose tissues were fractionated into mature adipocyte fraction (AdipF), and stromal vascular fraction (SVF). *CCDC3* mRNA levels were higher in AdipF than SVF (Fig. 1C). We also determine *CCDC3* expression level in human cultured cells derived from vascular systems. *CCDC3* mRNA levels were significantly higher in HUVEC than HASMC and HepG2 cells (Fig. 1D). These results suggest that *CCDC3* is expressed mainly in mature adipocytes in adipose tissues, and in endothelial cells in the vascular system.

Regulation of CCDC3 mRNA expression in adipose cells and tissues

The *CCDC3* mRNA expression level increased during differentiation of rat primary white adipocytes (Fig. 2A). Furthermore, insulin or pioglitazone, a PPARγ agonist, induced *CCDC3* mRNA expression in differentiated white and brown adipocytes (Fig. 2B and C), while norepinephrine and beta-adrenergic receptor agonist, isoproterenol, and TNF-α (data not shown) suppressed *CCDC3* mRNA expression (Fig. 2D and E). We also compared *CCDC3* mRNA levels in control and obese diabetic mice including *db/db* mice and high-fat-and-high-sucrose diet-fed mice. *CCDC3* mRNA expression levels were increased in both obese mice models (Fig. 2F and G). These results indicate that *CCDC3* expression is hormonally- and nutritionally-regulated in adipose cells and tissues.

Regulation of CCDC3 mRNA expression in vessels

Similar to adipocytes, pioglitazone promoted, and TNF-α suppressed *CCDC3* mRNA expression in HUVEC (Fig. 3A and B), whereas insulin had no effect (data not shown). The *CCDC3* mRNA expression levels were not significantly different in the aortas of the control, obese mouse models including *KK/Ay* and *db/db*, and atherosclerotic models, including LDLR-KO and ApoE-KO mice (Fig. 3C–E).

Secretion of CCDC3 protein through Golgi pathways

Based on the deduced amino acid sequence, CCDC3 contains a putative signal peptide for secretion, and this peptide should be cleaved before secretion (Fig. 1A underlined). To confirm this, COS-7 cells were transiently transfected with a plasmid encoding CCDC3 with C-terminal HA-tag. The expressed CCDC3 protein was detected by anti-HA antibody in cell lysates and culture medium as a 37-kDa protein (data not shown). Next, soluble secreted CCDC3 was immunopurified from cultured media of CCDC3-expressing COS-7 cells, and the excised bands were subjected to automated Edman degradation amino acid sequence analysis. The actual N-terminal sequence of CCDC3 corresponds to that predicted by signal-P (Fig. 1A), indicating that the signal sequence is actually cleaved in the course of secretion of CCDC3. Treatment with BFA, a reversible inhibitor of Golgi-mediated secretion, reduced the secretion of CCDC3 by COS-7 cells stably expressing

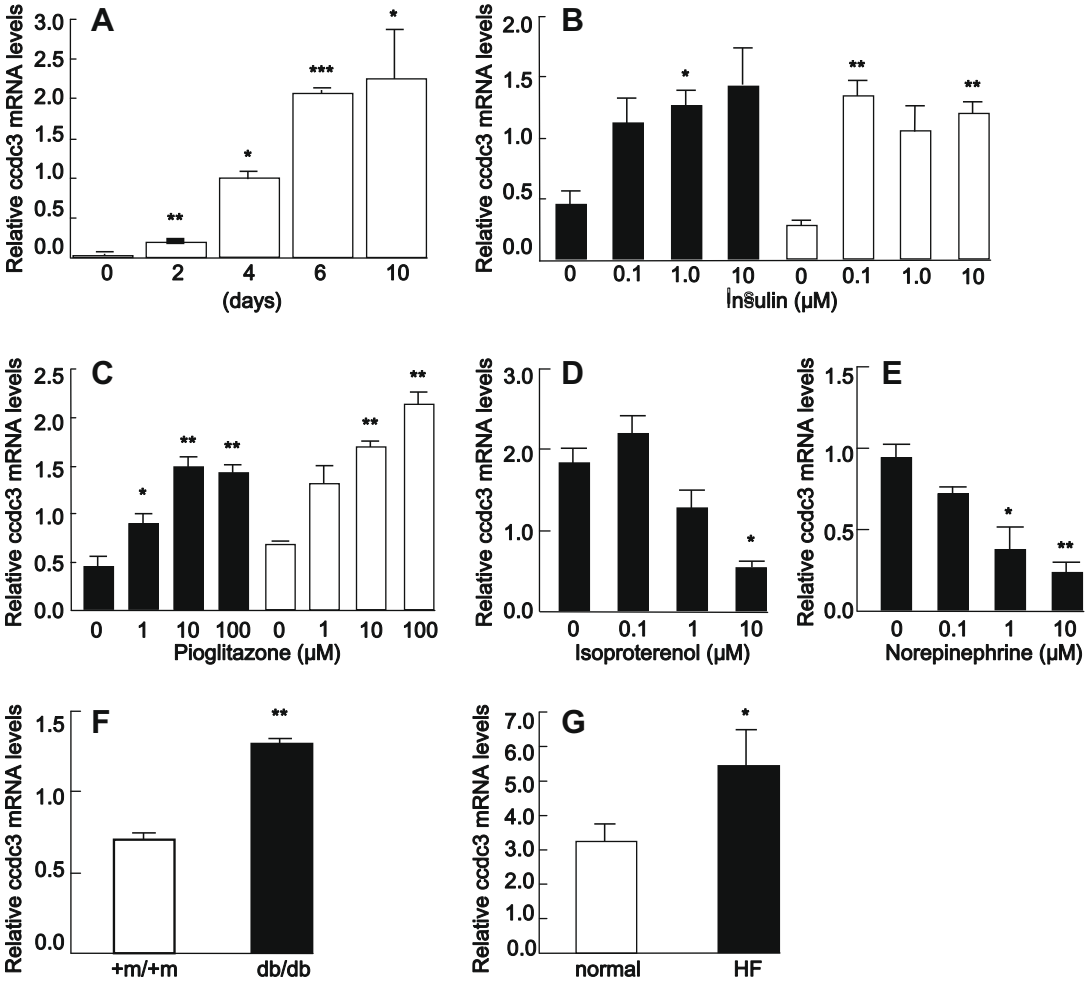

Fig. 2. Regulation of *CCDC3* mRNA expression levels in adipocytes cells and tissues. (A) *CCDC3* mRNA expression during adipocyte differentiation. Rat primary white adipocytes were harvested at the indicated days after induction of differentiation ($n = 3$). (B–E) Regulation of *CCDC3* mRNA expression by insulin, pioglitazone, isoproterenol, or norepinephrine. Differentiated rat white adipocytes (open bars) and brown adipocytes (closed bars) were stimulated with the indicated concentrations of insulin (B), pioglitazone (C), or isoproterenol (D) and norepinephrine (E) for 24 h ($n = 3$). (F) *CCDC3* mRNA expression in the epididymal white adipose tissue (WAT) of 8-week-old male *m+/m+* mice ($n = 5$) and 8-week-old male *db/db* diabetic mice ($n = 5$). (G) mRNA expression of *CCDC3* in epididymal white adipose tissues of normal chow-fed (normal) and high-fat diet-fed (HF) mice. High-fat diet was provided for 7 weeks ($n = 4$). The *CCDC3* mRNA expression level was measured by quantitative real-time PCR and normalized to *36B4* or *β-actin* level. Data are means ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

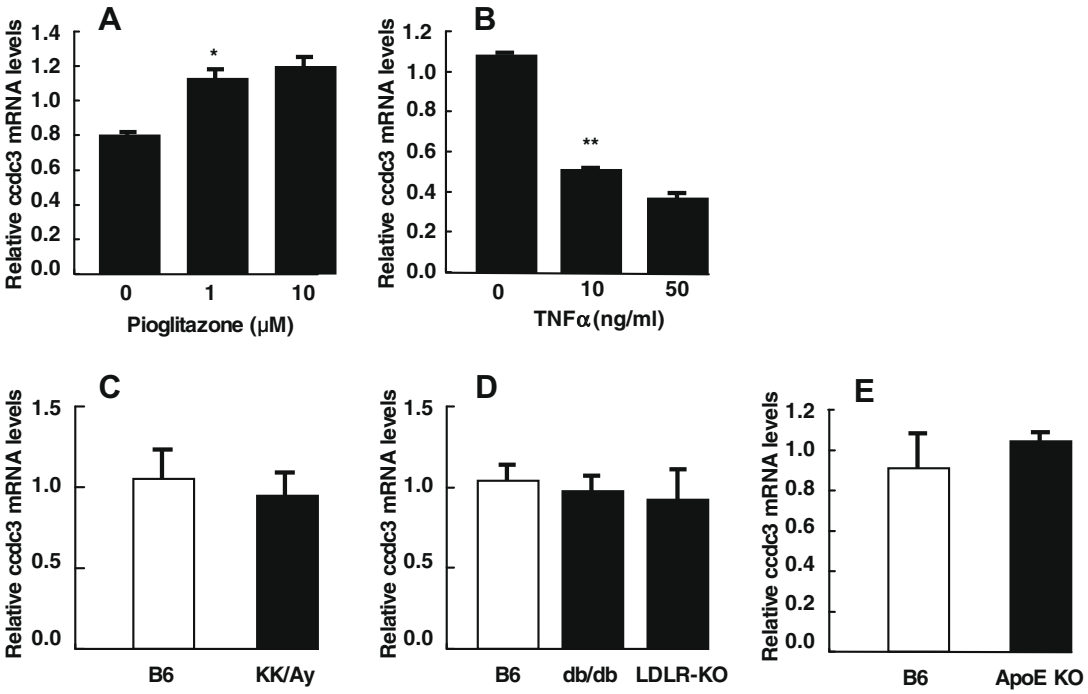

Fig. 3. Regulation of *CCDC3* mRNA expression level in blood vessels. (A, B) Regulation of *CCDC3* mRNA expression in HUVEC by the indicated concentrations of pioglitazone (A) and TNF-α (B) for 24 h ($n = 4$). (C) *CCDC3* mRNA expression in blood vessels (aorta and vein) of 6-week-old male C57BL/6J mice ($n = 5$) and 6-week-old male *KK/Ay* obese mice ($n = 5$). (D) *CCDC3* mRNA expression in blood vessels (aorta and vein) of 6 week-old male C57BL/6J mice ($n = 5$), 8-week-old male *db/db* diabetic mice ($n = 5$), and 34-week-old male LDL-receptor deficient mice ($n = 3$). (E) *CCDC3* mRNA expression in aortas of control C57B6/J (B6) ($n = 4$) and ApoE-KO mice ($n = 3$). *CCDC3* mRNA expression level was measured by quantitative real-time PCR. Data are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$.

CCDC3 dose-dependently (Fig. 4A, left), but increased CCDC3 levels within the cells (Fig. 4A, right). These data indicate that CCDC3 is secreted from cells through the ER-Golgi pathway.

Multimerization and glycosylated modification of CCDC3 protein

For further characterization of CCDC3 protein, we generated COS-7 cells stably expressing mouse CCDC3 with C-terminal His-tag. When CCDC3-expressing COS-7 cells were subjected to Western blotting without reduction, both intracellular and secreted CCDC3 protein migrated at 75 kDa, approximately twice the size of CCDC3 monomer (Fig. 4B lanes 4, 5, 8, 9; arrowhead). On the other hand, under reducing conditions without heating, CCDC3 proteins migrated at both 75 and 37 kDa (Fig. 4B lanes 3 and 7), and under reducing and heat-denatured conditions, CCDC3 migrated at 37-kDa (Fig. 4B lanes 2 and 6; arrow), indicating that CCDC3 forms a dimeric structure sensitive to reducing agent.

Since computer analysis predicted that CCDC3 had two consensus *N*-glycosylation sites, we investigated glycosylated modification of CCDC3. CCDC3 proteins were immunopurified from cell lysates or culture medium of COS-7 cells transiently transfected with CCDC3-HA, and then treated with peptide: *N*-glycosidase F (PNGaseF). Treatment with PNGaseF resulted in a faster migration of the expressed CCDC3 than that without treatment (Fig. 4C, lanes 2, 4 vs lanes 1, 3), suggesting that CCDC3 protein is *N*-glycosylated. Next, we tested the binding between CCDC3 and wheat germ agglutinin (WGA), a high-affinity lectin for *N*-acetylglucosamine and sialic acid residues [26]. Culture media of COS-7 cells transfected with His-tagged CCDC3 were treated with WGA-agarose. His-tagged CCDC3 bound to WGA was eluted with a cocktail of *N*-acetylglucosamine (GlcNac) and chitotriose (GlcNac trimer), common competitive ligands of WGA [27]. These competitors effectively eluted CCDC3 from WGA lectin (Fig. 4B, lanes 1–4). These results indicate that CCDC3 is an *N*-glycosylated protein with affinity to WGA.

Discussion

This paper describes the identification and characterization of CCDC3. CCDC3 is a secretory protein with glycosylated modification. *CCDC3* mRNA is mainly expressed in vascular endothelial cells and mature adipocytes.

The mRNA levels of *CCDC3* increased during differentiation of adipocytes, and were higher in adipose tissue of obese than control mice. Among the drugs tested, pioglitazone, a promoter of adipocyte differentiation, increased, while TNF-α, isoproterenol and norepinephrine suppressed *CCDC3* mRNA expression.

The results showed *CCDC3* gene expression in vascular endothelial cells. These cells produce secretory factors including vWF, endothelin, and PAI-1, all of which are related to the pathophysiology of atherosclerosis [28]. On the other hand, the *CCDC3* mRNA levels in vessels were almost similar in mouse models of atherosclerosis, obesity and diabetes, suggesting that the regulatory mechanisms of *CCDC3* are different between adipose tissues and vascular systems. The significance of *CCDC3* expression in the vasculature awaits further clarification.

CCDC3 protein contains putative coiled-coil motif in its COOH-terminal domain, and this motif is conserved among species (Fig. 1A). The coiled-coil motif is found in many proteins, such as skeletal and motor proteins, and is involved in molecular recognition systems and protein refolding processes [29]. One known function of coiled-coil motif is recognition of other molecules. For example, tetranectin has both carbohydrate recognition domain and coiled-coil domain, and relative orientation between them is important for binding to carbohydrates [30]. The coiled-coil domains of Angiopoietin-like (Angptl) 3 and Angptl 4 interact with lipoprotein lipase and inhibit its activity by blocking enzyme dimerization [31]. Another function of the coiled-coil motif is oligomerization of secretory proteins such as thrombospondin [32], tetranectin [30], and angptl [33]. In the present study, monomeric conversion of CCDC3 required both heat and reducing agent.

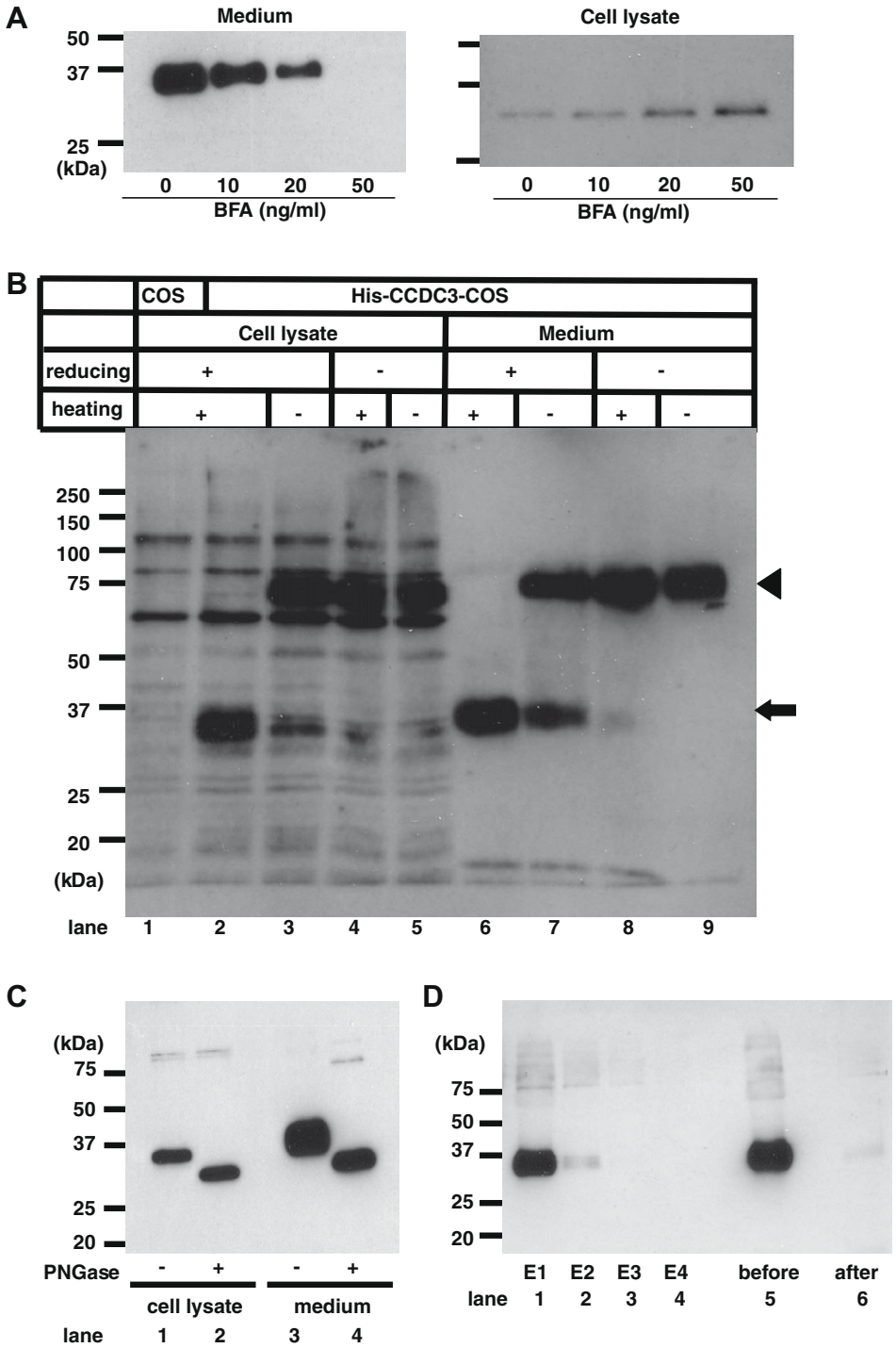

Fig. 4. Characterization of CCDC3 protein. (A) Effect of BFA on secretion of CCDC3. HA-CCDC3-COS cells were treated with the indicated concentrations of BFA for 6 h. The culture media (left) and cell lysates (right) were subjected to Western blotting with anti-HA antibody. (B) Multimerization of CCDC3 protein. His-CCDC3-COS cells were incubated for 2 days. Cell lysate and culture medium were treated with heat denaturation and reduced conditions, followed by Western blotting with anti-His×6 antibody. Arrow: monomer of CCDC3, arrowhead: dimer of CCDC3. (C) Analysis of glycosylated modification of murine CCDC3. HA-CCDC3-COS cells were treated with or without PNGase, and culture media or cell lysates were subjected to Western blotting with anti-HA antibody. (D) Affinity of CCDC3 to WGA. Culture media of His-CCDC3-COS cells were incubated with WGA-agarose, and bound CCDC3 was eluted sequentially 4 times (E1–E4) using the competitive ligand of WGA. Aliquots of agarose before and after elution, and eluted fractions were subjected to Western blotting with anti-His×6 antibody.

Therefore, dimerization of CCDC3 may be attributed to both disulfide bonds and coiled-coil domain.

In summary, the present study identified CCDC3 as a secretory factor from fat tissues and vascular systems, and that its expression is hormonally and nutritionally regulated in adipose cells. Based on these characteristics, we propose Favine (*fat*/*v*essel-derived secretory prote*in*) as a name for the protein coded by *CCDC3* gene. Although the existence and regulation of this protein in plasma remains to be elucidated, CCDC3/Favine protein secreted from adipocytes and vascular cells may play a role in the pathophysiology of obesity and atherosclerosis in auto-paracrine and/or endocrine manner.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.12.142.

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