C1q/Tumor Necrosis Factor-related Protein 11 (CTRP11), a Novel Adipose Stroma-derived Regulator of Adipogenesis*

Zhikui Wei, Marcus M. Seldin, Niranjana Natarajan, David C. Djemal, Jonathan M. Peterson, and G. William Wong

From the Department of Physiology and Center for Metabolism and Obesity Research, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Background: CTRP11 is a novel member of the C1q family with poorly defined function.
Results: CTRP11 inhibits 3T3-L1 adipocyte differentiation by inhibiting mitotic clonal expansion and adipogenic gene expression.
Conclusion: Adipose stroma-derived CTRP11 is a regulator of adipogenesis.
Significance: CTRP11 mediates potential paracrine cross-talk between adipocytes and cells of the stromal vascular compartment.

C1q/TNF-related proteins (CTRPs) are a family of secreted regulators of glucose and lipid metabolism. Here, we describe CTRP11, a novel and phylogenetically conserved member of the C1q family. Our studies revealed that white and brown adipose are major tissues that express CTRP11, and its expression is acutely regulated by changes in metabolic state. Within white adipose tissue, CTRP11 is primarily expressed by stromal vascular cells. As a secreted multimeric protein, CTRP11 forms disulfide-linked oligomers. Although the conserved N-terminal Cys-28 and Cys-32 are dispensable for the assembly of higher-order oligomeric structures, they are unexpectedly involved in modulating protein secretion. When co-expressed, CTRP11 forms heteromeric complexes with closely related CTRP10, CTRP13, and CRF (CTRP14) via the C-terminal globular domains, combinatorial associations that potentially generate functionally distinct complexes. Functional studies revealed a role for CTRP11 in regulating adipogenesis. Ectopic expression of CTRP11 or exposure to recombinant protein inhibited differentiation of 3T3-L1 adipocytes. The expression of peroxisome proliferator-activated receptor-γ and CAAT/enhancer binding protein-α, which drive the adipogenic gene program, was markedly suppressed by CTRP11. Impaired adipogenesis was caused by a CTRP11-mediated decrease in p42/44-MAPK signaling and inhibition of mitotic clonal expansion, a process essential for adipocyte differentiation in culture. These results implicate CTRP11 as a novel secreted regulator of adipogenesis and highlight the potential paracrine cross-talk between adipocytes and cells of the stromal vascular compartment in maintaining adipose tissue homeostasis.

The C1q protein family comprises over 30 secreted multimeric proteins, all of which share a signature C-terminal domain homologous to the globular domain of immune complement C1q (1). These include complement C1q (1), adiponectin (3), cerebellsins (4), multimerins (5), emilins (6), C1q/TNF-related proteins (CTRP) (7–12), CRF (13), C1qDC1 (also known as EEG1 or caprin-2) (14), otolin (15), and the nonfibrillar collagen VIII (16, 17) and collagen X (18, 19). These proteins play diverse roles in various tissue and organ systems (1).

Of the C1q family members, adiponectin and the CTRPs are considered important regulators of glucose and lipid metabolism. Adiponectin has been widely studied (20, 21); however, the metabolic function and regulation of CTRPs are only beginning to be elucidated (7–12, 22–27).

Although each of the CTRPs (CTRP1–15) has its unique tissue expression profile, they share common biochemical features (8, 9, 11, 12). They form trimers as their basic structural unit, driven by the globular C1q domain. Most CTRPs have one or more conserved Cys in their N termini that participate in intra- and inter-molecular disulfide bonding to enable the assembly of higher-order structures greater than a trimer. With the exception of CTRP4, these proteins have a variable length collagen domain, with its characteristic Gly-X-Y repeat, in the N terminus. Most secreted CTRPs circulate in plasma as potential hormones, with levels affected by metabolic states, sex, and genetic background of the animals.

Here we describe CTRP11 as a new and highly conserved member of the C1q family. We provide the first functional characterization of CTRP11 in the peripheral tissue and implicate it as a novel adipose stroma-derived regulator of adipogenesis.

MATERIALS AND METHODS

Antibodies and Reagents—Mouse anti-FLAG M2 monoclonal antibody was obtained from Sigma. Rat anti-HA (high affin-
Role of CTRP11 in Adipogenesis

Identification and Cloning of CTRP11—A search for adiponectin- and CTRP-like proteins in the NCBI GenBank databases identified a novel cDNA, which we designated CTRP11 or C1qTNF11. Based on genomic DNA sequence and overlapping EST clones, a PCR approach was used to clone the coding region of the Ctrp11 from a mouse testis cDNA library (Clontech). The primer pairs used were 5′-CGCTCGGGGTG-GCCATGGTGCTG-3′ and 5′-GGGGCAAGCAACATGAGAGTAAAGTG-3′. A 35-cycle PCR was carried out using Platinum PfX polymerase (Invitrogen) in the presence of 8% DMSO. The PCR product was agarose gel-purified and cloned into the pCR2.1 TOPO vector (Invitrogen). The entire cDNA insert was sequenced. The nucleotide sequence of mouse CTRP11 reported in this study has been deposited in the GenBank database under accession number DQ002403.

cDNA Constructs—C-terminal FLAG (DYKDDDDK) and HA (YPYDVPDYA) epitope-tagged CTRP11 were generated by PCR and cloned into pCR2.1 TOPO vector (Invitrogen). The entire insert was excised from the pCR2.1 TOPO vector and cloned into the EcoRI restriction site of mammalian expression vector pCDNA3.1 (Invitrogen). CDNs that encode C-terminal epitope-tagged truncated gCTRP10 (residue 1–12 and 145–287), gCTRP11 (residue 1–17 and 102–238), gCTRP13 (residue 1–22 and 124–255), and gCTRP14/CRF (residue 1–20 and 125–258) were chemically synthesized (Invitrogen) and subcloned into the EcoRI restriction site of pCDNA3.1. The overlapping PCR method was used to generate N-terminal Myc-tagged ERp44 in which the Myc epitope (EQKLISEEDL) was inserted immediately downstream of the signal peptide (residue 1–29). All constructs were verified by DNA sequencing. Mouse C-terminal HA-tagged adiponectin, CTRP1, CTRP2, CTRP3, CTRP5, CTRP6, CTRP9, CTRP10, CTRP13, CTRP14/CRF, CTRP10ΔCys, and CTRP13ΔCys constructs used in this study were described previously (8, 9, 11, 28).

Site-directed Mutagenesis—A PCR-based site-directed mutagenesis approach was used to mutate Cys-28 and Cys-32 to Ala using the high fidelity Pfu polymerase (Agilent Technologies). Successful mutagenesis was confirmed by DNA sequencing. The CTRP11 (C28A/C32A) mutant is designated CTRP11-HAΔCys and the CTRP14 (CRF; C28A/C32A) mutant is designated CTRP14-HAΔCys.

Isolation of Primary Adipocytes and Stromal Cells from Adipose Tissue—Primary adipocytes and stromal vascular cells (referred to as SVF or stromal vascular fraction) were isolated as previously described (9). Total RNA from stromal/vascular cells were extracted with TRI Reagent. Potential DNA contaminants were removed by RNase-free DNase I (Ambion).

Quantitative Real-time PCR—Quantitative real-time PCR was used to quantify the relative expression levels of CTRP11 mRNA in human and mouse multiple tissue cDNA panels (Clontech). Every mouse tissue was pooled from 200 to 1000 mice and every human tissue was pooled from 3 to 15 individuals. Thus, the expression of CTRP11 in each tissue represents an average value from the pooled cDNA. Other tissues profiled for CTRP11 expression include epididymal fat pads of 12-week-old leptin-deficient (ob/ob) mice and their wild-type lean controls, primary adipocytes, and stromal vascular cells, epididymal or ovarian fat pads from C57BL/6 male and female mice (8–10 weeks old), interscapular brown adipose tissue from C57BL/6 male mice, epididymal (visceral) and inguinal (subcutaneous) fat pads from C57BL/6 male mice fed ad libitum, overnight (16 h) fasted, and fasted/re-fed for 2 h. Primers used were: Ctrp11 forward, 5′-AGGCGCAATGTAAGACC-3′ and reverse, 5′-GGTTCATGATTGCAGGTTG-3′; 18 S rRNA forward, 5′-GCAATTTCCCATGAG-3′ and reverse, 5′-GGGCTCTCGAAACATCGAA-3′. The default PCR protocol was used on an Applied Biosystems Prism 7500 Sequence Detection System. cDNAs were synthesized from 2 μg of total RNA and 200 ng of random hexamers using SuperScript II RNase H-Reverse Transcriptase protocol (Invitrogen). For quantitative real-time PCR, samples were analyzed according to the protocol provided in SYBR® Green PCR Master Mix protocol (Applied Biosystems).

HEK 293 Cell Transfection—HEK 293 cells (GripTiteTM 293 cell line from Invitrogen) were cultured in DMEM containing 10% fetal bovine serum supplemented with 2 mM l-glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Transient transfection was performed using Lipofectamine 2000 reagent (Invitrogen). Sixteen h after transfection, medium was replaced by serum-free Opti-MEM I (Invitrogen), and cells were cultured for another 24–48 h before the conditioned medium was collected for Western blot analysis.

Recombinant CTRP11 Protein Production—Full-length recombinant CTRP11 and CTRP11ΔCys (C28A/C32A) mutant were produced and purified from transfected HEK 293 cells as previously described for other related CTRPs (22). The mammalian expression system ensures proper post-translational modifications (e.g. glycosylation and higher-order structure assembly) of the recombinant proteins. Purified proteins were dialyzed against...
20 mM Hepes buffer (pH 8.0) containing 135 mM NaCl in a 10-kDa cut-off Slide-A-Lyzer dialysis cassette (Pierce). Protein concentration was determined using a Coomassie Plus protein assay reagent (Thermo Scientific), and samples were aliquoted and stored at −80 °C. The purity of recombinant proteins was judged to be >95% by Coomassie-stained gel.
Co-immunoprecipitation and Western Blotting—The FLAG-tagged CTRP11 was co-transfected in different combinations with HA-tagged adiponectin or CTRPs into HEK 293 cells. Aliquots of the collected supernatants (250 μl) combined with 500 μl of IP buffer (150 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 1% Triton X-100) were subjected to co-immunoprecipitation using the EZView™ anti-FLAG affinity gel (Sigma). Samples were washed 3 times (10 min each) in PBS containing 0.1% Tween 20, developed in ECL reagent (Millipore) for 2–5 min, and subjected to the ECL glycoprotein detection protocol (29). The presence of a carbohydrate moiety was then detected by an EPSON scanner. The high magnification micrograph was loaded onto an AKTA FPLC and fractionated through a Superdex 200 HR 10/30 column (GE Healthcare) in PBS and collected in 0.5-ml fractions. The collected fractions were subjected to immunoblot analysis.

3T3-L1 Adipocyte Differentiation—Differentiation of 3T3-L1 preadipocytes was carried out as previously described using a mixture containing 3-isobutyl-1-methylxanthine, dexamethasone, and insulin (designated as MDI) (11). Two days before the induction of differentiation, 3T3-L1 cells were transfected with pCDNA3 control vector or vector encoding CTRP11, adiponectin, CTRP1, and CTRP10 using Lipofectamine 2000 (Invitrogen). Cells were differentiated for 8 days, and the amount of neutral lipid accumulation was quantified by Oil Red O staining. Total RNAs were isolated on a separate group of differentiated adipocytes, reverse transcribed, and subjected to real-time PCR analysis for adipogenic genes. Primers used in the real-time PCR analysis were: PPAR-γ forward, 5’-CCAGAGTCCTGCTGATCTGCG-3’ and reverse, 5’-GCCACCTCTTTGCTCTGCTC-3’; perilipin-1 forward, 5’-GGGACCTGTGAGTGTCTCCTC-3’ and reverse, 5’-GTATTTGGAAGCCGGAATCT-3’; perilipin-2 forward, 5’-GACCTGTGTCCTCCGGTTAT-3’ and reverse, 5’-CAACCGGAATTGTGGCCTC-3’; perilipin-4 forward, 5’-GTGCCAACAACCTCACAGATG-3’ and reverse, 5’-GGACATTCCTTTTGCCGATCAT-3’; adiponectin (Adpn) forward, 5’-ACTGCAACTACCATAGGCCCCAT-3’ and reverse, 5’-TGTCGACTGTTCCATTGATTCTCC-3’; FABP4 forward, 5’-ATCGGAAATATGGGAGTTTGG-3’ and reverse, 5’-GTCGCGGTGATTCTCATCGA-3’.

Oil Red O Staining and Quantification—Oil Red O staining was carried out as previously described (30). Conditioned media were aspirated from the 3T3-L1 adipocytes, and cell monolayers were washed three times with PBS then fixed for 2 min with 3.7% formaldehyde. 0.5% Stock Oil Red O prepared in isopropyl alcohol was diluted with water (3:2), filtered through a 0.45-μm filter, and incubated with the fixed cells for 1 h at room temperature. Cell monolayers were then washed with water and photographed. The pictures of the wells were taken by an EPSON scanner. The high magnification micrograph was taken by an Olympus IX50 microscope. Oil Red O stain that bound to neutral lipids within adipocytes was extracted in isopropyl alcohol and the absorbance was measured on a plate reader (BioTek, model SynergyMx) at 490–510 nm. To quantify glycerol content in the conditioned medium of adipocytes, medium (2 days old) was harvested from adipocytes on day 8 of differentiation under basal conditions (without β3-adrenergic stimulation). Glycerol levels were measured using a TG/glycerol kit from WAKO according to the manufacturer’s instructions.

DNA Content Measurement in Adipocytes—Cellular DNA content was extracted using the TRI Reagent kit according to the manufacturer’s instructions. The DNA content was quantified using a fluorometer. The amount of DNA was determined by comparing the fluorescence of the sample to a standard curve. The results were expressed as the percentage of DNA content in the differentiated adipocytes compared to that of the control cells.

**FIGURE 1. Identification of CTRP11.** A, the deduced protein sequence of CTRP11 consists of four domains: a signal peptide (SP) for secretion, an N-terminal domain (green line), collagen domain (blue line), and C-terminal globular domain (red line) are indicated. Arrows indicate the conserved residues found in all C1q/TFN superfamily members (33). Amino acid numberings are indicated on the left.
the manufacturer’s instruction. Adipocytes on day 3 of differ-
entiation were used for DNA extraction. The DNA concentra-
tion was then measured by OD_{260/280} on a Nanodrop 1000
spectrophotometer (Thermo Scientific).

Sequence Alignment and Phylogenetic Tree Analysis of
CTRP11—The orthologous genes that encode the CTRP11
protein from multiple vertebrate species were extracted from
the National Center for Biotechnology Information (NCBI)
database or the Ensembl database. In some cases, we retrieved
CTRP11 orthologs by querying a particular vertebrate genome
using the CTRP11 protein sequence. Multiple sequence align-
ments were performed using the ClustalW program (31).

### TABLE 1

Percent amino acid identities of CTRP11 from different vertebrate
species

| Species                      | Amino acid identity to mouse CTRP11 |
|------------------------------|-------------------------------------|
|                              | Full-length | C1q domain |
| Mouse (M. musculus)          | 100         | 100         |
| Human (H. sapiens)           | 96          | 100         |
| Dog (C. familiaris)          | 97          | 100         |
| Elephant (L. africana)       | 96          | 100         |
| Mouse lemur (M. murinus)     | 95          | 100         |
| Armadillo (D. novemcinctus)  | 94          | 100         |
| Cattle (Bos taurus)          | ?           | 100         |
| Opossum (M. domestica)       | 93          | 100         |
| Horse (E. caballus)          | 94          | 100         |
| Cat (Felis catus)            | ?           | 100         |
| European rabbit (O. cuniculus)| 96          | 100         |
| Tree squirrel (Spermophilus tridecemlineatus) | ? | 100 |
| Anole lizard (A. carolinensis)| 77          | 94          |
| Frog (X. laevis)             | 73          | 91          |
| Zebrafish (D. rerio)         | 72          | 87          |

Sequences used in alignment included: human (Homo sapiens;
GenBank accession number NP_001008224), mouse (Mus
musculus; AAY21935), dog (Canine familiaris; XP_851062),
pig (Sus scrofa; XP_003126157), elephant (Loxodonta africana;
Ensembl accession number ENSLAFP00000014689), horse
(Equis caballus; XP_001492004), rabbit (Oryctolagus cunicu-
lus; XP_002711149), armadillo (Dasypus novemcinctus; ENSD-
NOP00000013845), opossum (Monodelphis domestica; XP_
001364371), anole lizard (Anolis carolinensis; XP_003223236),
frog (Xenopus laevis; NP_001090380), and zebrafish (Danio
rerio; XP_695928). The phylogenetic tree was generated using
neighbor-joining (NJ) method and MEGA program version 4
(32) based on ClustalW alignments of the C1q domain of input
protein sequences.

### RESULTS

Identification of CTRP11 cDNA and Analysis of Its Gene—In
a search for proteins homologous to adiponectin and CTRPs,
we identified a novel and highly conserved paralog that we des-
ignated as C1q/TNF-related protein-11 (CTRP11) (Fig. 1A). A
PCR approach was used to clone the coding region of the
Ctrp11 from mouse testis. The deduced amino acid sequence of
CTRP11 consists of four distinct domains: a signal peptide, a
short N-terminal region with two conserved cysteine residues,
a collagen domain with 14 Gly-X-Y repeats, and a C-terminal
globular domain homologous to the C1q domain of immune
complement protein C1q (Fig. 1A). Mouse Ctrp11 is ~4.3 kb in
size, consists of two exons, and is located on chromosome 5F1
(Fig. 1B). Human CTRP11 is ~4.8 kb in size, consists of two
exons, and is located on chromosome 12q13.12 (Fig. 1B).

![FIGURE 2. Expression of CTRP11 in mouse and human tissues.](image)
Unlike most CTRPs (7), the globular C1q domain of CTRP11 is encoded by two exons instead of a single exon. Phylogenetic analysis revealed that CTRP11 is modestly related to most members of the C1q family (Fig. 1), sharing 23–38% amino acid identity at the C1q domain. However, three of the family members, CTRP10, CTRP13, and CTRP14 (also known as CRF), are closely related to CTRP11 and form a separate clade on the cladogram. They share 80–88% amino acid identity with CTRP11 at the C1q domain.

Conservation of CTRP11 throughout Vertebrate Evolution—Sequence alignment revealed striking conservation of CTRP11 throughout vertebrate evolution (Fig. 1D and Table 1). Mouse CTRP11 and its corresponding human, dog, elephant, lemur, armadillo, cattle, opossum, horse, cat, rabbit, and squirrel orthologs share >93% amino acid identity over the full-length protein and a striking 100% amino acid identity in their presumed functional C-terminal globular domain. Distantly related vertebrate species such as the anole lizard (a reptile), frog (an amphibian), and zebrafish (a teleost) share an impressive 94, 91, and 87% amino acid identity, respectively, to the mouse CTRP11 in their globular C1q domain (Table 1). Such a remarkable degree of conservation over 250 million years of evolution (since the emergence of teleost) implies an essential and highly conserved function for CTRP11. Structure-based alignment of adiponectin, complement C1q, and TNF family members (TNF-α, TNF-β, and CD40L) indicates multiple highly conserved residues important for the packing of the hydrophobic core of the protomer (33). These conserved residues shared by the C1q and TNF-α superfamily of proteins are also conserved in CTRP11 (Fig. 1D, arrows).

Adipose as a Major Tissue That Expresses Ctrp11 mRNA—A survey of 17 adult mouse tissues and four developmental stages of the mouse embryo using quantitative real-time PCR showed that testis and adipose tissue expressed the highest levels of Ctrp11 transcript (Fig. 2A). Other tissues, such as the brain, kidney, and placenta, expressed lower levels of the transcript. Interestingly, the Ctrp11 transcript is also expressed highly during the early stage (embryonic day 11) of mouse development, indicating a potential role in development. In humans, CTRP11 is also expressed at the highest levels in testis and adipose tissue (Fig. 2B). In contrast to mouse, human skeletal muscle and kidney also expressed CTRP11 (Fig. 2B). None of the immune cells expressed appreciable amounts of CTRP11 transcript.

Stromal Vascular Cells and Brown Adipose Tissue Express Ctrp11—In addition to adipocytes, adipose tissue contains endothelial, fibroblastic, and immune cells. Collectively, these nonadipocytic cells are referred to as the SVF. We sought to examine whether Ctrp11 is expressed by adipocytes or SVF
within the adipose tissue. Quantitative real-time PCR revealed that Ctrp11 is expressed predominantly by SVF rather than adipocytes (Fig. 3A). Unlike adiponectin, Ctrp11 is not induced by adipogenesis (Fig. 3B), seen by comparing pre-adipocytes with mature adipocytes. Of the different adipose depots, brown adipose tissue (BAT) expresses a higher level of Ctrp11 transcript than subcutaneous (inguinal) and visceral (epididymal) white adipose tissue (Fig. 3C).

Expression of Ctrp11 mRNA Is Regulated by Metabolic State—Expression of several CTRPs is responsive to acute changes in metabolic state induced by fasting and re-feeding, indicative of their involvement in energy homeostasis (12, 22). Similarly, expression of Ctrp11 was significantly up-regulated in re-fed compared with fasted mice (Fig. 3D), suggestive of its role in energy metabolism. However, the expression of Ctrp11 was unchanged in two mouse models of obesity (leptin-deficient ob/ob and diet-induced obese) representing chronic excess caloric intake (Fig. 3, E and F).

Sexually Dimorphic Expression Patterns of Ctrp11—Several of the CTRPs (e.g. Ctrp5 and Ctrp13) exhibit sexually dimorphic expression patterns, with female mice expressing higher levels of the transcript compared with male mice (9, 11). Likewise, we observed that females express ~2.5-fold higher levels of Ctrp11 mRNA in their adipose SVF relative to male mice (Fig. 3G).

CRC11 Is a Secreted Glycoprotein—When expressed in heterologous HEK 293 cells, secreted CTRP11 protein was readily detected in the conditioned medium (Fig. 4A). The predicted molecular mass of mature CTRP11 is 23 kDa. However, the apparent molecular mass of the secreted CTRP11 on immunoblot is ~29 kDa, suggesting the presence of post-translational modification. Peptide-N-glycosidase treatment of CTRP11 did not result in mobility shift of the protein on immunoblot, indicating the absence of N-linked glycans (Fig. 4B, left panel), consistent with the lack of a consensus Asn residue needed for the attachment of an N-linked carbohydrate. However, using a more general method to detect the presence of the sugar moiety, we showed that mammalian cell-produced CTRP11 indeed contains carbohydrate moiety (Fig. 4B, right panel). The nature of the glycans is unknown; however, CTRP11 contains a potential lysine residue (Lys-57) in its collagen domain that conforms to the pentapeptide motif GXKGE, known to be glycosylated in other CTRPs (8, 9).

Role of the Conserved N-terminal Cys-28 and Cys-32 in Protein Secretion—On the nonreducing immunoblot, CTRP11 existed predominantly as dimers and trimers (Fig. 4C, lanes 1 and 3). Two of the N-terminal Cys residues (Cys-28 and Cys-32) are highly conserved from zebrafish to humans (Fig. 1D). Mutations of the two conserved Cys residues abrogated the formation of intermolecular disulfide linkage (Fig. 4C, lane 5). The assembly of trimeric and hexameric complexes of wild-type CTRP11 was further confirmed by gel filtration chromatographic analysis (Fig. 4D). Surprisingly, the assembly of higher-order multimeric complexes was largely unaffected in the
C28A/C32A double mutant (Fig. 4D) despite involvement of the cysteine residues in intermolecular disulfide bridges (Fig. 4C, compare lanes 3 and 5). Unexpectedly, Cys-28 and Cys-32 appeared to be involved in modulating protein secretion; mutations led to a striking enhancement in the amount of CTRP11 secreted from transfected HEK 293 cells compared with wild-type protein (Fig. 4, E and F). This is analogous to adiponectin in which the conserved N-terminal Cys-39 regulates protein secretion by forming a disulfide linkage with ERp44, an endoplasmic reticulum chaperone (34). However, unlike adiponectin, both FLAG-tagged wild-type CTRP11 and the mutant (C28A/C32A) physically interacted with Myc-tagged ERp44 equally well when co-expressed in HEK 293 cells (data not shown), suggesting that increased protein secretion of the Cys mutant is independent of ERp44.

CTRP11 and CTRP10 Form Heteromeric Complexes—C1q family members are known to form heteromeric complexes (8, 9, 11, 12). When co-expressed in mammalian cells, epitope-tagged CTRP11 co-immunoprecipitated with the closely related CTRP10, CTRP13, and CTRP14 (CRF) from the conditioned medium of transfected cells (Fig. 5A). The heteromeric association is specific; CTRP11 did not co-immunoprecipitate with other related C1q family members when co-expressed (Fig. 5A). Heteromeric complexes formed during biosynthesis; mixing of supernatant containing individually expressed protein did not result in co-immunoprecipitation (Fig. 5B). The conserved N-terminal Cys residues are not required for heteromeric complex assembly; mutations of these Cys residues (Cys-28 and Cys-32 in CTRP11, Cys-29 and Cys-33 in CTRP10, Cys-28 and Cys-32 in CTRP13, Cys-28 and Cys-32 in CTRP14) did not abrogate their physical association when co-expressed (Fig. 6, A and B), consistent with our previous study implicating the role of the C-terminal globular domain in homo- and heteromeric complex formation (8, 9). Indeed, co-expressing just the globular C1q domains of CTRP10, CTRP11, and CTRP13 was sufficient for the formation of heteromeric complexes (Fig. 6, C and D).
Role of CTRP11 in Adipogenesis

Ectopic Expression of CTRP11 Suppresses 3T3-L1 Adipocyte Differentiation—Because CTRP11 is expressed by stromal vascular cells within the adipose compartment, we explored whether this secreted protein plays a role in regulating adipocyte differentiation. When ectopically expressed, CTRP11 significantly inhibited 3T3-L1 preadipocyte differentiation compared with cells expressing vector control, as revealed by a marked reduction in Oil Red O staining of neutral lipids within the lipid droplets of adipocytes (Fig. 7A). Quantification revealed a 60% decrease in neutral lipid accumulation in CTRP11-expressing adipocytes relative to cells expressing vector control (Fig. 7B, left panel). The reduced accumulation of intracellular neutral lipids also correlated with a 5-fold reduction in glycerol levels found in the supernatant of CTRP11-expressing adipocytes compared with control cells (Fig. 7B, right panel). Glycerol release reflects nonstimulated basal lipolysis. Suppression of adipogenesis by CTRP11 is specific; ectopic expression of adiponectin, CTRP1, and CTRP10, three related members of the C1q protein family, has little or no effect on neutral lipid (triglyceride) accumulation in differentiated adipocytes (data not shown). Quantitative real-time PCR analysis indicated that the expression of PPAR-γ, a major transcription factor controlling adipogenesis (36), was greatly reduced in CTRP11-expressing 3T3-L1 cells (Fig. 7C). Accordingly, a number of adipocyte-specific genes, such as adiponectin and FABP4, and genes involved in lipid droplet metabolism and formation, including perilipin-1 (Plin1), perilipin-2 (Plin2), and perilipin-4 (Plin4), were also significantly reduced in CTRP11-expressing adipocytes (Fig. 7C). At the protein level, both C/EBP-α and PPAR-γ expression, two major transcription factors driving the expression of adipogenic genes (36–38), were greatly reduced (Fig. 7, D and E). Protein markers for mature adipocytes, including adiponectin (intracellular and secreted) and lipin-1, were substantially down-regulated in adipocytes, the greater the amount of glycerol released from non-stimulated lipolysis. Expression levels were normalized to β-tubulin levels. *, p < 0.05; **, p < 0.01; #, p < 0.001.

FIGURE 7. Ectopic expression of CTRP11 suppresses 3T3-L1 adipocyte differentiation. A, Oil Red O staining of adipocytes transfected with control vector (pCDNA3.1) or vector expressing CTRP11 2 days before (day −2) the induction of differentiation. Images were taken on day 8 of adipocyte differentiation. Top panels are photographs of adipocyte staining, and bottom panels are micrographs of adipocyte staining at ×10 magnification. Ctrl, control. B, quantification of Oil Red O staining of adipocytes, and glycerol released from the adipocytes expressing control vector or CTRP11 (n = 3). C, quantitative real-time PCR analyses of markers in adipocytes expressing control vector or CTRP11 (n = 6). The relative expression levels were normalized to 18 S rRNA levels. adiponectin; FABP4, fatty acid-binding protein 4; Plin1, perilipin-1; Plin2, perilipin-2; Plin4, perilipin-4. D, Western blot analyses of markers in adipocytes expressing control vector or CTRP11. Intracellular β-tubulin serves as the loading control. E, quantification of adipocyte markers as shown in panel D (n = 3). The relative expression levels were normalized to β-tubulin levels. *, p < 0.05; **, p < 0.01; #, p < 0.001.

FIGURE 6. Globular C1q domain mediates heteromorphic complex formation. A, domain structure of CTRP10, CTRP11, and CTRP13. Only the Cys residues located in the N-terminal region (black ball-and-stick) were mutated, whereas Cys residues located in the globular C1q domain (gray ball-and-stick) were spared. B, conditioned media containing individually expressed or co-expressed proteins were subjected to immunoprecipitation (IP) followed by immunoblot (IB) analysis with the anti-HA antibody (top panel). The middle and bottom panels indicate the presence of input proteins in the supernatants. C, truncation constructs encoding only the signal peptide (SP) and globular C1q domain of CTRP10, CTRP11, and CTRP13. D, conditioned media containing individually expressed or co-expressed proteins were subjected to immunoprecipitation followed by immunoblot analysis with the anti-HA antibody (top panel). The middle and bottom panels indicate the presence of input proteins in the supernatants.
CTRP11-expressing adipocytes compared with control cells (Fig. 7, D and E). These data indicate that CTRP11-expressing 3T3-L1 cells have reduced adipogenesis, leading to reduced intracellular neutral lipid accumulation, glycerol release (basal lipolysis), and expression of adipogenic genes and mature adipocyte markers.

**Conditioned Medium Containing CTRP11 Suppresses 3T3-L1 Adipocyte Differentiation**—To verify the paracrine effect of stromal vascular cell-derived CTRP11 on adipocyte differentiation, we collected serum-free conditioned media from control vector- or CTRP11-transfected HEK 293 cells. Control or CTRP11-containing media were given to 3T3-L1 preadipocytes when they reached 100% confluence (day 0), and fresh media containing CTRP11 or control were replaced 2 days after (day 0), followed by adipocyte differentiation protocol (see “Materials and Methods”). Conditioned medium containing CTRP11 robustly inhibited the adipogenesis program of 3T3-L1 cells relative to control medium, reflected by a marked reduction (80%) of intracellular neutral lipid accumulation (Fig. 8, A and B) and glycerol released into the supernatant (derived from basal lipolysis) (Fig. 8B, right panel). At the mRNA level, medium containing CTRP11 suppressed PPAR-γ, adipogenic and lipid droplet biogenesis genes (Fig. 8C). At the protein level, significant suppression of expression of adipogenic proteins and markers by CTRP11-containing medium was also observed (Fig. 8, D and E). Of these, adiponectin, an adipocyte-specific protein, showed the most dramatic reduction (Fig. 8, D and E). The observed differences were greater for intracellular adiponectin compared with adiponectin secreted into the conditioned medium. Intracellular fraction of adiponectin reflects synthesis and secretion, whereas the secreted protein accumulates over time (day 7–8 conditioned medium). Together, these results confirm that CTRP11 functions in a paracrine fashion to inhibit adipocyte differentiation.

**CTRP11 Has No Effect on Terminal Differentiation of Adipocytes**—Strong inhibition of adipocyte differentiation was observed when 3T3-L1 cells were incubated with CTRP11-containing medium on day −2 and day 0, suggesting that CTRP11 acts on the early stage of differentiation to regulate adipogenesis. To address whether CTRP11 also regulates the late stage of differentiation, CTRP11-containing medium was given to 3T3-L1 cells on day 3 of differentiation. As shown in Fig. 9A, CTRP11 treatment, if given after cells have already undergone 2 days of differentiation, had no effect on neutral lipid accumulation, glycerol release, or adipogenic gene and marker expression in differentiating adipocytes (Fig. 9). These results suggest that CTRP11 acts at the early stage to inhibit adipocyte differentiation.

![FIGURE 8. Conditioned media containing CTRP11 suppresses 3T3-L1 adipocyte differentiation.](image-url)
Purified Recombinant CTRP11 Suppresses 3T3-L1 Adipocyte Differentiation—To further confirm and provide a quantitative evaluation of the inhibitory action of CTRP11 on adipocyte differentiation, we treated cells with purified recombinant CTRP11 protein. As shown in Fig. 10, when 3T3-L1 pre-adipocytes were treated with 5 μg/ml of purified CTRP11 at day -2 and day 0 of differentiation, adipogenesis was strikingly suppressed (Fig. 10, A and B). The CTRP11Cys mutant (C28A/C32A) inhibited adipogenesis to the same extent as the wild-type protein (Fig. 10, A and B), consistent with the role of Cys-28 and Cys-32 in modulating protein secretion and not the assembly of higher-order oligomeric structures.

CTRP11 Inhibits p44/42 MAPK Signaling and Mitotic Expansion of 3T3-L1 Cells—We examined the specific process targeted by CTRP11 in the early stage of 3T3-L1 differentiation. It is known that growth-arrested 3T3-L1 cells undergo additional rounds of cell division upon induction of differentiation by hormonal signals during the onset of adipogenesis. This mitotic clonal expansion is essential for the induction of adipogenesis (39). Inhibition of p42/44 MAPK signaling with MAPK kinase inhibitor U-0126 suppresses mitotic clonal expansion and adipogenesis (39). We explored whether CTRP11 inhibits adipogenesis by inhibiting mitotic clonal expansion of 3T3-L1 cells. Indeed, ectopic expression of CTRP11 diminished the differentiation mixture MDI-induced p44/42 MAPK activation in 3T3-L1 cells compared with controls (Fig. 11A). When DNA content was quantified as a read-out for cell proliferation, MDI induced a 2-fold increase in cellular DNA content in cells expressing vector control, indicative of mitotic clonal expansion (Fig. 11B). However, 3T3-L1 cells expressing CTRP11 failed to undergo MDI-induced mitotic clonal expansion as judged by the lack of increase in cellular DNA content. Similarly, differentiating cells incubated with conditioned medium containing recombinant CTRP11 also showed reduced p44/42 MAPK activation and failed to undergo MDI-induced mitotic clonal expansion needed for differentiation to proceed (Fig. 11, C and D). Together, our results indicate that CTRP11 suppresses adipogenesis in culture via inhibition of mitotic clonal expansion.

DISCUSSION

We provided evidence here that CTRP11 is a novel adipose stroma-derived secretory protein of the C1q family. It shares multiple common biochemical features with the other CTRPs we have recently characterized (7–9, 11, 12). These include similar domain structure and post-translational modifications like glycosylation and the assembly of higher-order oligomeric structures.

CTRP11 stands out among proteins of the C1q family, exhibiting striking conservation throughout vertebrate evolution.

FIGURE 9. CTRP11 has no effect on the late stage of 3T3-L1 adipocyte differentiation. A, Oil Red O staining of adipocytes incubated with control conditioned media or conditioned media containing CTRP11 on day 3. Images were taken on day 8 of adipocyte differentiation. Top panels are photographs of adipocyte staining, and bottom panels are micrographs of adipocyte staining at ×10 magnification. Ctrl, control. B, quantification of Oil Red O staining of adipocytes, and glycerol released from the adipocytes incubated with control or conditioned media containing CTRP11 (n = 3). C, quantitative real-time PCR analyses of adipocyte markers in adipocytes incubated with control or media containing CTRP11 (n = 6). The relative expression levels were normalized to 18S rRNA levels. Adpn, adiponectin; FABP4, fatty acid-binding protein 4; Plin1, perilipin-1; Plin2, perilipin-2; Plin4, perilipin-4. D, Western blot analyses of markers in adipocytes treated with control or media containing CTRP11. Intracellular β-tubulin serves as the loading control. E, quantification of markers in adipocytes as shown in panel D (n = 3). The relative expression levels were normalized to β-tubulin levels.
Role of CTRP11 in Adipogenesis

Orthologous CTRP11 found in human, mouse, dog, elephant, lemur, armadillo, cattle, opossum, horse, cat, rabbit, and squirrel share >93% amino acid identity over the full-length protein and 100% amino acid identity in their presumed functional globular C1q domain (constituting 60% of the protein). Distantly related species such as Xenopus and zebrafish also share an impressive 91 and 87% amino acid identity, respectively, at the globular domain with mouse CTRP11, suggesting an essential and conserved function of CTRP11.

Both white and brown adipose tissues express CTRP11. The functional relevance of CTRP11 in brown adipose tissue is unclear. Within white adipose tissue, CTRP11 is primarily produced by cells of the stromal vascular fraction and not by adipocytes within white adipose tissue. Thus, endothelial cells, fibroblasts, immune cells (e.g. macrophages), and preadipocytes are among the potential cell types that express CTRP11. In the obese state, large numbers of macrophages are known to infiltrate the adipose tissue (40, 41). Notably, the suppression of adipogenesis by CTRP11 occurs within the first 2–3 days of 3T3-L1 cell differentiation in culture; no inhibition of adipogenesis was observed if 3T3-L1 cells were treated with CTRP11 on day 3 of differentiation. In growth-arrested 3T3-L1 cells, hormonal signals provided by MDI differentiation mixture induces additional rounds of cell division, a mitotic clonal expansion process essential for the induction of adipogenesis in culture (39). Differentiation of preadipocytes entails sequential and ordered induction of C/EBPs and PPAR-γ, transcription factors that orchestrate the program of adipogenesis (38, 43). Failure to undergo mitotic clonal expansion prevents implementation of the adipogenic program (39, 44). Inhibition of p42/44-MAPK signaling with MAPK kinase inhibitor U-0126 also suppresses mitotic expansion and adipogenesis (39). We showed that the ectopic expression of CTRP11 or treatment of cells with CTRP11 decreased MDI-induced p44/42 MAPK activation and suppressed mitotic clonal expansion of differentiating 3T3-L1 preadipocytes. Our results suggest that the inhibition of PPAR-γ and C/EBP-α expression and adipogenesis by CTRP11 is mechanistically linked to the inhibition of MDI-induced mitotic clonal expansion in cultured 3T3-L1 cells.

Unlike other tissues or organs, adipose tissue seems to have a vast ability to expand, as seen in many striking gain- and loss-of-function mouse models (45–48), as well as in morbidly obese humans (49, 50). The mechanism controlling the size of fat mass is not well understood. Among the secreted factors, insulin and bone morphogenetic proteins play a positive or instructive role in governing fat mass, either by directly regulating adipocyte function (e.g. promoting triglyceride storage) as in the case of insulin (51–53) or by promoting the differentiation of mesenchymal precursor cells along the adipocytic lineage (54, 55). In contrast, secreted proteins of the Wnt family negatively regulate adipocyte differentiation (56–58). The cleaved ectodomain of an epidermal growth factor-like repeat-containing transmembrane protein, Pref-1, derived from preadipocytes, is also a potent negative regulator of fat cell differentiation (59–61). In this context, our study suggests that CTRP11, a secreted protein of the C1q family, represents a potential negative regulator of fat mass through its action on adipocyte differentiation.

What roles CTRP11 may play in other tissues such as the brain, testis, and kidney that also express Ctip11 transcript are not clear and speculative at best. While current work was ongoing, Iijima et al. (62) described C1q-like protein 4 (C1ql4), a protein identical to CTRP11 that is expressed in the central nervous system. The authors did not examine the expression of
The expression of BA13 is highly restricted to the brain (64, 65), in striking contrast to the expression of CTRP11 by a variety of tissues in addition to the central nervous system. Although published studies (64, 65) and the available database in NCBI indicate that BA13 is not expressed in human and mouse adipose tissue, it still remains to be determined whether pre-adipocytes express BA13 and whether it mediates the suppressive effect of CTRP11 on adipocyte differentiation. Alternatively, CTRP11 may bind to a different receptor in the peripheral tissue to exert its biological function.

In summary, our study establishes a potentially novel paracrine pathway by which CTRP11 secreted from stromal vascular cells negatively regulates adipocyte differentiation. Although the secreted endogenous regulators of adipogenesis within the adipose compartment in vivo remain poorly defined, CTRP11 derived from the stromal vascular compartment may mediate paracrine cross-talk necessary to maintain adipose tissue homeostasis.

REFERENCES
1. Kishore, U., Gaboriaud, C., Waters, P., Shrive, A. K., Greenbough, T. J., Reid, K. B., Sim, R. B., and Arlaud, G. J. (2004) C1q and tumor necrosis factor superfamily. Modularity and versatility. Trends Immunol. 25, 551–561
2. Kishore, U., and Reid, K. B. (2000) C1q, structure, function, and receptors. Immunopharmacology 49, 159–170
3. Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H. F. (1995) A novel serum protein similar to C1q produced exclusively in adipocytes. J. Biol. Chem. 270, 26746–26749
4. Yuzaki, M. (2008) Cbln and C1q family proteins. New transneuronal cytokines. Cell. Mol. Life Sci. 65, 1698–1705
5. Hayward, C. P., and Kelton, J. G. (1995) Multimerin. Curr. Opin. Hematol. 2, 339–344
6. Colombatti, A., Doliana, R., Bot, S., Canton, A., Mongiat, M., Mungiguerra, G., Paron-Cilli, S., and Spesotto, P. (2000) The EMILIN protein...
Role of CTRP11 in Adipogenesis

family. Matrix Biol. 19, 289 – 301
7. Wong, G. W., Wang, I., Hug, C., Tsao, T. S., and Lodish, H. F. (2004) A family of Acr30/adiponectin structural and functional paralogs. Proc. Natl. Acad. Sci. U.S.A. 101, 10303 – 10307
8. Wong, G. W., Krawczyk, S. A., Kitidis-Mitrokokas, C., Ge, G., Spooner, E., Hug, C., Gimeno, R., and Lodish, H. F. (2009) Identification and characterization of CTRP9, a novel secreted glycoprotein, from adipose tissue that reduces serum glucose in mice and forms heterotrimers with adiponectin. FASEB J. 23, 241 – 258
9. Wong, G. W., Krawczyk, S. A., Kitidis-Mitrokokas, C., Revett, T., Gimeno, R., and Lodish, H. F. (2008) Molecular, biochemical and functional characterizations of C1q/TNF-related family members. Adipose tissue-selective expression patterns, regulation by PPAR-γ agonist, cytokine-mediated oligomerizations, combinatorial associations and metabolic functions. Biochem. J. 416, 161 – 177
10. Wei, Z., Peterson, J. M., Lei, X., Cebotaru, L., Wolfgang, M. J., Baldeviano, J. C., and Wong, G. W. (2011) Metabolic regulation by CTRP11 in Adipogenesis. J. Biol. Chem. 286, 15652 – 15665
11. Wei, Z., Peterson, J. M., and Wong, G. W. (2011) Cloning and sequencing of a gene expressed during terminal differentiation that encodes a novel inhibitor of growth. J. Biol. Chem. 279, 1916 – 1921
12. Wölfing, B., Buechler, C., Falk, W., Gross, P., Neumeier, M., Schölmerich, J., and Schäffler, A. (2008) Effects of the new C1q/TNF-related protein (CTRP-3) “carteconnon” on the adipogenic secretory adipokine. Obesity 16, 1481 – 1486
13. Kopp, A., Bala, M., Buechler, C., Falk, W., Gross, P., Neumeier, M., Schölmerich, J., and Schäffler, A. (2010) C1q/TNF-related protein-3 represents a novel and endogenous lipopolysaccharide antagonist of the adipocyte Endoclinin 151, 5267 – 5278
14. Turer, A. T., and Scherer, P. E. (2012) Adiponectin. Mechanistic insights into the brain involved in motor function. Brain Res. Mol. Brain Res. 63, 233 – 240
15. Tontonoz, P., Otto, T. C., and Lane, M. D. (2003) Mitotic clonal expansion. A synchronous process required for adipogenesis. J. Clin. Invest. 112, 1821 – 1830
16. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W., Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112, 1796 – 1808
17. Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, I., Nichols, A., Ross, I. S., Tartaglia, L. A., and Chen, H. (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. 112, 1821 – 1830
18. Kimmel, A. R., Brasaemle, D. L., McAndrews-Hill, M., Sztalryd, C., and Londos, C. (2010) Adoption of PERILIN as a unifying nomenclature for the mammalian PPAR-family of intracellular lipid storage droplet proteins. J. Lipid Res. 51, 468 – 471
Role of CTRP11 in Adipogenesis

43. Farmer, S. R. (2006) Transcriptional control of adipocyte formation. Cell Metab. 4, 263–273
44. Tang, Q. Q., Otto, T. C., and Lane, M. D. (2003) CCAAT/enhancer-binding protein β is required for mitotic clonal expansion during adipogenesis. Proc. Natl. Acad. Sci. U.S.A. 100, 850–855
45. Ingalls, A. M., Dickie, M. M., and Snell, G. D. (1950) Obese, a new mutation in the house mouse. J. Hered. 41, 317–318
46. Hummel, K. P., Dickie, M. M., and Coleman, D. L. (1966) Diabetes, a new mutation in the mouse. Science 153, 1127–1128
47. Kim, J. Y., van de Wall, E., Laplante, M., Azzara, A., Trujillo, M. E., Hofmann, S. M., Schraw, T., Durand, J. L., Li, H., Li, G., Jelicks, L. A., Mehler, M. F., Hui, D. Y., Deshaies, Y., Shulman, G. I., Schwartz, G. J., and Scherer, P. E. (2007) Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J. Clin. Invest. 117, 2621–2637
48. Kusminski, C. M., Holland, W. L., Sun, K., Park, J., Spurgin, S. B., Lin, Y., Askew, G. R., Simcox, J. A., McClain, D. A., Li, C., and Scherer, P. E. (2012) MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. Nat. Med. 18, 1539–1549
49. Farooqi, I. S., Yeo, G. S., Keogh, J. M., Aminian, S., Jebb, S. A., Butler, G., Cheetham, T., and O’Rahilly, S. (2000) Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. J. Clin. Invest. 106, 271–279
50. Barsh, G. S., Farooqi, I. S., and O’Rahilly, S. (2000) Genetics of body-weight regulation. Nature 404, 644–651
51. Lewis, G. F., Carpentier, A., Adeli, K., and Giacca, A. (2002) Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr. Rev. 23, 201–229
52. Gries, F. A., and Steinke, J. (1967) Comparative effects of insulin on adipose tissue segments and isolated fat cells of rat and man. J. Clin. Invest. 46, 1413–1421
53. Ball, E. G., and Cooper, O. (1960) Studies on the metabolism of adipose tissue. III. The response to insulin by different types of adipose tissue segments and isolated fat cells of rat and man. J. Clin. Invest. 41, 2621–2637
54. Huang, H., Song, T. J., Li, X., Hu, L., He, Q., Liu, M., Lane, M. D., and Tang, Q. Q. (2009) BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc. Natl. Acad. Sci. U.S.A. 106, 12670–12675
55. Ross, S. E., Hemati, N., Longo, K. A., Bennett, C. N., Lucas, P. C., Erickson, R. L., and MacDougald, O. A. (2000) Inhibition of adipogenesis by Wnt signaling. Science 289, 950–953
56. Wright, W. S., Longo, K. A., Dolinsky, V. W., Gerin, I., Kang, S., Bennett, C. N., Chiang, S. H., Prestwich, T. C., Gress, C., Burant, C. F., Suslic, V. S., and MacDougald, O. A. (2012) Wnt10b inhibits obesity in ob/ob and agouti mice. Diabetes 56, 295–303
57. Cawthorn, W. P., Bree, A. J., Yao, Y., Du, B., Hemati, N., Martinez-Santibáñez, G., and MacDougald, O. A. (2012) Wnt6, Wnt10a and Wnt10b inhibit adipogenesis and stimulate osteoblastogenesis through a β-catenin-dependent mechanism. Bone 50, 477–489
58. Smas, C. M., and Sul, H. S. (1993) Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. Cell 73, 725–734
59. Smas, C. M., Chen, L., and Sul, H. S. (1997) Cleavage of membrane-associated pref-1 generates a soluble inhibitor of adipocyte differentiation. Mol. Cell. Biol. 17, 977–988
60. Lee, K., Villena, J. A., Moon, Y. S., Kim, K. H., Lee, S., Kang, C., and Sul, H. S. (2003) Inhibition of adipogenesis and development of glucose intolerance by soluble preadipocyte factor-1 (Pref-1). J. Clin. Invest. 111, 453–461
61. Iijima, T., Miura, E., Watanabe, M., and Yuzaki, M. (2010) Distinct expression of C1q-like family mRNAs in mouse brain and biochemical characterization of their encoded proteins. Eur. J. Neurosci. 31, 1606–1615
62. Bolliger, M. F., Martinelli, D. C., and Südhof, T. C. (2011) The cell-adhesion G protein-coupled receptor BA13 is a high-affinity receptor for C1q-like proteins. Proc. Natl. Acad. Sci. U.S.A. 108, 2534–2539
63. Shiratsuchi, T., Nishimori, H., Ichise, H., Nakamura, Y., and Tokino, T. (1997) Cloning and characterization of BA12 and BA13, novel genes homologous to brain-specific angiogenesis inhibitor 1 (BAI1). Cytogenet. Cell Genet. 79, 103–108
64. Kee, H. J., Ahn, K. Y., Choi, K. C., Won Song, J., Heo, T., Jung, S., Kim, J. K., Bae, C. S., and Kim, K. K. (2004) Expression of brain-specific angiogenesis inhibitor 3 (BAI3) in normal brain and implications for BA13-induced brain angiogenesis and malignant glioma. FEBS Lett. 569, 307–316