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GDF1 is a novel mediator of macrophage infiltration in brown adipose tissue of obese mice

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ABSTRACT

We previously demonstrated a marked upregulation in the bone morphogenic protein (BMP)/growth differentiation factor (GDF) family member, GDF5, which is capable of promoting brown adipogenesis, in brown adipose tissue (BAT) of obese mice. In this study, we identified other GDF family members, besides GDF5 that are responsive to different obesogenic signals in BAT using inborn and acquired obesity animal models. In BAT from leptin-deficient *ob/ob* mice, *GDF1* expression was preferentially downregulated, whereas the expression of several other genes in the BMP/GDF family, including GDF5, was upregulated. Moreover, in cultured brown adipocytes exposed to tunicamycin and hydrogen peroxide, at concentrations not affecting cellular viability, *GDF1* expression was significantly downregulated. Recombinant GDF1 failed to significantly alter brown adipogenesis, despite the promoted phosphorylation of Smad1/5/8 in cultured brown adipocytes, but accelerated Smad1/5/8 phosphorylation with a concomitant increase in the number of migrating cells during exposure in a manner sensitive to activin-like kinase inhibitors in macrophagic RAW264.7 cells. Similarly, accelerated migration was observed in murine peritoneal macrophages exposed to GDF1. These results indicate that obesity could lead to predominant downregulation of GDF1 expression in BAT, which can modulate cellular migration through a mechanism relevant to activation of the downstream Smad signaling pathway in adjacent macrophages.

1. Introduction

Brown adipose tissue (BAT) generates heat through mitochondrial uncoupling of lipid oxidation, whereas white adipose tissue (WAT) serves as a storage depot for excess energy [1,2]. Recent studies have demonstrated that adult humans have substantial amounts of functioning BAT [3–6]. Accordingly, BAT presents a potential therapeutic target to combat obesity and related metabolic diseases through a mechanism relevant to accelerated energy expenditure in humans. Brown fat-like adipocytes, which have been described as brite or beige cells with a feature expressing uncoupling protein-1 (UCP1), are sporadically found in WAT, whereas classical brown adipocytes reside in the interscapular and perirenal regions.

Several factors are implicated in the development of beige cells in WAT. Of these, activation of peroxisome proliferator-activated receptor-γ (PPARγ) facilitates transformation of white adipocytes into brown adipocytes [7,8], whereas fibroblast growth factor-21 was found to promote the conversion of white adipocytes to brown adipocytes [9]. Bone morphogenic protein (BMP)-7 accelerates brown adipocyte differentiation and thermogenesis [10], whereas BMP8B increases BAT thermogenesis through both central and peripheral mechanisms [11]. In addition to these well-known endogenous factors, we recently reported the importance of growth differentiation factor-5 (GDF5), which is a member of the BMP/GDF family, in brown adipogenesis [12]. In fact, transgenic mice overexpressing GDF5 in adipose tissues display increased systemic energy expenditure along with the appearance of beige cells in subcutaneous WAT [12]. Taken together, these findings indicate that particular BMP/GDF family members may be implicated in the development and maturation of brown adipocytes. Thus, these previous findings prompted us to comprehensively search for particular BMP/GDF family members responsive to obesity in BAT using leptin-deficient *ob/ob* mice as well as obese mice fed with a high fat diet (HFD) *in vivo*.

Abbreviations: ALK, activin-like kinase; BAT, brown adipose tissue; BMP, bone morphogenic protein; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle medium; ER, endoplasmic reticulum; GDF, growth differentiation factor; HFD, high fat diet; PCR, polymerase chain reaction; PPARγ, peroxisome proliferator-activated receptor-γ; UCP1, uncoupling protein-1; WAT, white adipose tissue; WT, wild-type

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2. Materials and methods

2.1. Materials

A brown adipocyte cell line derived from newborn wild-type (WT) mice was kindly provided by Dr. C.R. Kahn (Joslin Diabetes Center, Boston, MA, USA) [10]. Macrophagic RAW264.7 cells and adipocytic 3T3-L1 cells were obtained from ATCC (Manassas, VA, USA). Myoblastic C2C12 cells were provided by RIKEN (Tsukuba, Japan). Recombinant mouse GDF1 was purchased from R&D Systems (Minneapolis, MN, USA). Both LDN193189 and SB431542 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Anti-glyceraldehyde-3-phosphate dehydrogenase, anti-phospho Smad1/5/8, anti-phospho Smad2, and anti-phospho Akt were obtained from Cell Signaling Technology (Danvers, MA, USA). A Mouse GDF1 enzyme-linked immunosorbent assay (ELISA) Kit was obtained from Bluegene (Shanghai, China). Thunderbird SYBR qPCR Mix was supplied by Toyobo Co., Ltd. (Osaka, Japan) and specific primers for each gene for PCR were listed in Table 1. Diet-induced obesity (DIO) rodent purified diet with 60% energy from fat was obtained from Japan SLC (Shizuoka, Japan). Other chemicals used in this study were of the highest purity and commercially available.

2.2. Mice and ELISA

Male WT and *ob*/*ob* mice with a C57BL/6 background were obtained from Japan SLC (Shizuoka, Japan). The study was conducted in accordance with the guidelines of the Japanese Society for Pharmacology and was approved by the Committee for the Ethical Use of Experimental Animals of Kanazawa University. Blood was collected from the heart of mice under anesthesia using needles and syringe, followed by leaving on ice for 5 min and subsequent centrifugation at 4 °C for 5 min at 20,000*g* for collection of plasma. BAT was removed from animals after decapitation, followed by rinsing with PBS and subsequent homogenization in PBS. The suspension was sonicated and then centrifugation at 4 °C for 5 min at 5000*g* for collection of supernatant. GDF1 level was measured by ELISA according to manufacturer's protocols.

2.3. Culturing of brown adipocytes, white adipocytes and myoblasts, Oil Red O staining, and determination of cellular viability

Brown pre-adipocyte cell lines were derived from newborn wild-type (WT) mice [10]. Brown pre-adipocytes were cultured in a differentiation induction cocktail (20 nM insulin, 1 nM triiodothyronine, 0.125 mM indomethacin, 0.5 μM dexamethasone,

Table 1
List of primers used for qPCR in this study.

Genes	Upstream (5'-3')	Downstream (5'-3')
Acvr1	TGCTAATGATGATGGCTTTCC	TTCACAGTGGTCCTCGTTCC
Acvrl1	GGGCCTTTTGATGCTGTCG	TGGCAGAATGGTCTCTTGCAG
Acvr1b	CCCCCTTGTTGTCCTCCT	GGCCCCATCTGTCTCACA
Acvr1c	GTCTGGCTCACCTGCACAT	CAGCTATGGCACAAGTGTCAC
Acvr2a	GCGTTCGCCGTCTTTCTTATC	GTTGGTTCTGTCTCTTTCCCAAT
Acvr2b	ACCCCCAGGTGTACTTCTG	CATGGCCGTAGGGAGGTTTC
Amh	CCACACCTCTCTCCACTGGTA	GGCACAAAGGTTCAGGGGG
ap2	GATGCCTTTGTGGGAACCT	CTGTCGTCTGCGGTGATTT
Arg1	GAACACGGCAGTGGCTTTAAC	TGCTTAGCTCTGTCTGCTTTGC
BMP1	TTGTACGCGAGAACATACAGC	CTGAGTCGGGTCCTTTGGC
BMP2	GGGACCCGCTGTCTTCTAGT	TCAACTCAAATTCGCTGAGGAC
BMP3	ACTCCGTGAGACTGAGCCAA	CCTGTCATAGAGCCACAGCATA
BMP4	TTCCTGGTAACCGAATGCTGA	CCTGAATCTCGGCGACTTTTT
BMP5	TTACTTAGGGGTATTGTGGGCT	CCGTCTCTCATGGTTCCGTAG
BMP6	AGAAGCGGGAGATGCAAAAGG	GACAGGGCGTTGTAGAGATCC
BMP7	ACGGACAGGGCTTCTCCTAC	ATGGTGGTATCGAGGGTGGAA
Bmpr1a	AATGCAAGGATTCACCGAAAGCCC	ACAGCCATGGAAATGAGCACAACC
Bmpr1b	CCCTCGGCCCAAGATCCTA	CAACAGGCATTCCAGAGTCATC
Bmpr2	AGCAATCGCCCATCGAGACTTGAA	TTCTGGAGGCATATAGCGCTTGGT
Cebpa	CAAGAACAGCAACGAGTACCG	GTCACTCGTCAACTCCAGCAC
Cebpb	CAAGTTCCGCAGGGTGCT	CCAAGAAGACGGTGGACAA
GDF1	TTCTGCCAGGGCACGTGCG	GGAGCAGCTGCGTGCATGAG
GDF2	CGCAGCCTTAACCTCAGC	GTTGGAGGCAGGCGTAGA
GDF3	ATGCAGCCTTATCAACGGCTT	AGGCGCTTTCTCTAATCCCAG
GDF5	ATCGGACTGTTCAACCTTTCAG	GCACTCTTATCAAGGGTTAGGTC
GDF6	TGCACGTGAACTTCAAGGAGCTGGGCT	TCATCAGCGTCTGGATGATGGCGTGGT
GDF7	GAGGGCGTTTGCGACTTTC	CTGCTTGTAGACCACGTTGTT
Id1	CGACTACATCAGGGACCTGCA	GAACACATGCCGCCTCGG
Inha	ATGCACAGGACCTCTGAACC	GGATGGCCGGAATACATAAG
Inhba	GGAGAACGGGTATGTGGAGA	TGGTCCTGGTTCTGTTAGCC
Inhbb	CCTGAGTGAATGCACACCAC	CGAGTCCAGTTTCGCCTAGT
Lefty1	TGTGTGTGCTCTTTGCTTCC	GGGGATTCTGTCCTTGGTTT
Mcp1	TCCCAATGAGTAGGCTGGAG	AAGTGCTTGAGGTGGTTGTG
Nodal	CCATGCCTACATCCAGAGCCTGC	TGGTGTTCCAGGAGGACCCTGCC
Pparg	TCAGCTCTGTGGACCTCTCC	ACCCTTGCATCCTTCACAAG
Ppargc1a	GTCAACAGCAAAAGCCACAA	TCTGGGGTCAGAGGAAGAGA
Prdm16	GACATTCCAATCCCACCAGA	CACCTCTGTATCCGTCAGCA
Retnla	TCAGCAATCCCATGGCGTATAA	TCATCAGTATTCACTGGGACCATCA
Tgfb1	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
Tgfb2	TCGACATGGATCAGTTTATGCG	CCCTGGTACTGTTGTAGATGGA
Tgfb3	CCTGGCCCTGCTGAACTTG	TTGATGTGGCCGAAGTCCAAC
Tgfbr1	TGTGCACCATCTTCAAAAACA	ACCAAGGCCAGCTGACTG
Tnfa	GTAGCCCACGTCGTAGCAAAC	CTGGCACCACTAGTTGGTTGTC
Ucp1	TACCAAGCTGTGCGATGTCCA	GCACACAAACATGATGACGTTCC
36b4	GAGGAATCAGATGAGGATATGGGA	GAGGAATCAGATGAGGATATGGGA