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Role of Fgf receptor 2c in adipocyte hypertrophy in mesenteric white adipose tissue

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ABSTRACT

Fgf receptor 2c (*Fgfr2c*) was expressed in mature adipocytes of mouse white adipose tissue (WAT). To examine the role of *Fgfr2c* in mature adipocytes, we generated adipocyte-specific *Fgfr2* knockout (*Fgfr2* CKO) mice. The hypertrophy impairment of adipocytes in the mesenteric WAT but not in the subcutaneous WAT and decreased plasma free fatty acid (FFA) levels were observed in *Fgfr2* CKO mice. Although the expression of genes involved in adipocyte differentiation and lipid metabolism in the mesenteric WAT was essentially unchanged, the expression of *uncoupling protein 2* potentially involved in energy dissipation was significantly increased. Among potential Fgf ligands for Fgfr2c, *Fgf9* was preferentially expressed in the mesenteric WAT. The present findings indicate that Fgfr2c potentially activated by Fgf9 plays a role in the adipocyte hypertrophy in the mesenteric WAT and FFA metabolism and/or energy dissipation in the mesenteric WAT might be involved in the hypertrophy impairment.

1. Introduction

White adipose tissue plays crucial roles in energy homeostasis (Spiegelman and Flier, 1996). Obesity, the excessive development of white adipose tissue, is a risk factor for several diseases including type II diabetes, hypertension, cancer and atherosclerosis (Kopelman, 2000). Therefore, it is important to understand the molecular and cellular mechanisms by which white adipose tissue develops. The development of white adipose tissue involves the proliferation of preadipocytes and their subsequent differentiation into mature adipocytes, adipogenesis (Rosen and Spiegelman, 2000). Adipogenesis is one of the most intensively studied developmental processes. Several transcription factors are involved in adipogenesis. They include members of the CCAAT/enhancer binding protein (C/EBP) and peroxisome proliferator-activated receptor (PPAR) families (Rosen, 2005). The hypertrophy of mature adipocytes is also an important process for the development of white adipose tissue at postnatal stages. The hypertrophy caused by lipid accumulation results in an increase in the size of mature adipocytes. Mature adipocytes secrete a variety of signaling proteins, adipokines. Adipokines are involved in numerous physiological functions including food intake, energy metabolism and insulin sensitivity (Ailhaud, 2006). The hypertrophy of mature adipocytes is associated with dysregulated expression of adipokines, which causes metabolic disorders (Skurk et al., 2007). Therefore, it is important to elucidate the molecular mechanism underlying the regulation of the hypertrophy.

Fibroblast growth factors (Fgfs), secreted signaling proteins, play important roles in development and metabolism with multiple biological activities including cell proliferation and differentiation. The Fgf family comprises 22 members (Ornitz and Itoh, 2001; Itoh and Ornitz, 2004). *Fgf10*$^{-/-}$ mice die shortly after birth (Sekine et al., 1999). The embryonic development of white adipose tissue in these mice is greatly impaired (Sakaue et al., 2002). Analyses of *Fgf10*$^{-/-}$ white adipose tissue have indicated that Fgf10 plays crucial roles in the proliferation of preadipocytes and their subsequent differentiation into mature adipocytes at embryonic stages (Asaki et al., 2004; Konishi et al., 2006). An Fgf10 receptor is Fgf receptor 2b (Fgfr2b) that is preferentially expressed in preadipocytes (Yamasaki et al., 1999). These results indicate that Fgf10 plays roles in the embryonic development of white adipose tissue through the activation of Fgfr2b. There are two alternatively spliced variants of Fgfr2, Fgfr2b and Fgfr2c (Dell and Williams, 1992). Fgfr2c is a major variant. The two differ in their specificity for ligands and pattern of expression (Ornitz et al., 1996; Zhang et al., 2006; Orr-Urtreger

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et al., 1993). In contrast to Fgfr2b, Fgfr2c is not a receptor for Fgf10 (Zhang et al., 2006). We examined the expression of *Fgfr2c* in mouse white adipose tissue. *Fgfr2c* was preferentially expressed in mature adipocytes, indicating that Fgfr2c might play roles in mature adipocytes. However, as *Fgfr2*$^{-/-}$ mice died in the early embryonic stages, E10–E11 (Xu et al., 1998), the role of *Fgf2c* in mature adipocytes remains unclear. To examine the role of *Fgfr2c* in mature adipocytes, we generated adipocyte-specific *Fgfr2*$^{-/-}$ mice by disrupting *Fgfr2* selectively in mature adipocytes in postnatal stages. Here, we report that Fgfr2c plays a role in the hypertrophy of mature adipocytes in the mesenteric white adipose tissue at postnatal stages.

2. Materials and methods

2.1. Expression of genes in adipocytes and stromal-vascular cells examined by reverse transcription-polymerase chain reaction

Mature adipocytes and stromal-vascular cells were prepared from mouse subcutaneous and mesenteric white adipose tissues essentially according to the method of Ogawa et al. (1995). Total RNA was extracted from the cells using an RNeasy mini kit (Qiagen). cDNA was synthesized from the RNA as a template in a reaction mixture containing moloney murine leukemia virus reverse transcriptase (Gibco BRL) and a random hexadeoxynucleotide primer (Takara, Japan). The cDNA was amplified by polymerase chain reaction (PCR) with Taq DNA polymerase (Wako, Japan) and primers specific for indicated genes. The nucleotide sequences of primers used are shown in Table 1.

2.2. Mutant mice

All mice were maintained in a mixed strain background and housed in a temperature-controlled environment with 12-h light/dark cycles. The generation of *Fgfr2*$^{flox/flox}$ mice and *aP2-Cre-ER*$^{T2(tg/0)}$ mice has been described previously (Yu et al., 2003; Imai et al., 2001). *Fgfr2*$^{flox/flox}$ mice were mated with *aP2-Cre-ER*$^{T2(tg/0)}$ mice to obtain *aP2-Cre-ER*$^{T2(tg/0)}$/*Fgfr2*$^{flox/+}$ mice. Then the *aP2-Cre-ER*$^{T2(tg/0)}$/*Fgfr2*$^{flox/+}$ mice were mated with *Fgfr2*$^{flox/flox}$ mice to obtain *aP2-Cre-ER*$^{T2(tg/0)}$/*Fgfr2*$^{flox/flox}$ mice and *aP2-Cre-ER*$^{T2(0/0)}$/*Fgfr2*$^{flox/flox}$ littermates. Male *aP2-Cre-ER*$^{T2(tg/0)}$/*Fgfr2*$^{flox/flox}$ mice and male *aP2-Cre-ER*$^{T2(0/0)}$/*Fgfr2*$^{flox/flox}$ littermates were injected intraperitoneally with tamoxifen (Sigma) at a dosage of 0.1 mg/(g day) for 5 consecutive days biweekly from 8 to 18 weeks of age. Tamoxifen was dissolved in autoclaved corn oil (Sigma) at a concentration of 25 mg/ml by sonication for 45 min. All mice were individually housed and weighed biweekly from 8 to 18 weeks of age.

2.3. Genotyping of mice

For genotyping the *Fgfr2*$^{flox/flox}$ mice, PCR was performed with genomic DNA using primers, sense (5′-ATAGGAGCAACAGGCGG-3′) and antisense (5′-TGCAAGAGGCGACCAGTCAG-3′), yielding 207 and 142 bp fragments as described (Yu et al., 2003). For genotyping the *aP2-cre-ER*$^{T2(tg/0)}$ mice, PCR was performed with genomic DNA using primers, sense (5′-ATGTCCAATTTACTGACCG-3′) and antisense (5′-CGCCGCATAACCAGTGAAAC-3′), yielding a 350 bp fragment.

2.4. Detection of Fgfr2 recombination in mice

The recombination of *Fgfr2* was detected by PCR analysis of genomic DNA purified from indicated tissues or cells from control and *Fgfr2* CKO mice. Primers P1 (5′-ATAGGAGCAACAGGCGG-3′) and P3 (5′-CATAGCACAGGCCAGGTTG-3′) produced a 471 bp fragment indicative of Cre-mediated *Fgfr2* recombination (Fig. 2A).

The efficiency of *Fgfr2* recombination was assessed by PCR analysis of genomic DNA of mature adipocytes isolated from control and *Fgfr2* CKO mice. Primers P1 (5′-ATAGGAGCAACAGGCGG-3′) and P2 (5′-TGCAAGAGGCGACCAGTCAG-3′) produced a 207 bp fragment of the floxed region of *Fgfr2* without recombination (Fig. 2A). Primers P4 (5′-GTGGCTCACAACCATCCGTAATG-3′) and P5 (5′-CACTCCTGGCAAGAGCTCAATTT-3′) produced a 350 bp fragment indicative of the reference (Fig. 2A). The PCR products within the linear range of amplification were separated by electrophoresis on a 1.5% agarose gel, visualized by ethidium bromide staining and quantified with NIH Image software. After correction for the reference levels, the efficiency of *Fgfr2* recombination was assessed relative to control littermates.

2.5. Histological analysis

Mesenteric and subcutaneous white adipose tissues of 18-week-old control and *Fgfr2* CKO mice were fixed in Bouin's fixative, dehydrated, embedded in paraffin and sectioned. Sections (6 μm) were stained with hematoxylin and eosin and examined by light microscopy. Images of adipose tissue sections were captured and adipocyte sizes were measured for at least 150 cells per mice with NIH Image software.

2.6. Expression of genes in mesenteric white adipose tissue examined by semi-quantitative reverse transcription-polymerase chain reaction

Total RNA was prepared from mesenteric white adipose tissue of 18-week-old control and *Fgfr2* CKO mice using an RNeasy mini kit (Qiagen). cDNA was synthesized as described above. Portions of the reaction mixture were subjected to PCR (within the linear range of amplification) with Taq DNA polymerase (Wako, Japan) and primers specific for mouse *Leptin*, *peroxisome proliferators-activated receptor gamma 2* (*PPARγ2*), *uncoupling protein 2* (*UCP2*), *glucose transporter 1* (*GLUT1*), *glucose transporter 4* (*GLUT4*), *lipoprotein lipase* (*LPL*), *hormone-sensitive lipase* (*HSL*), *fatty acid synthase* (*FAS*), *acetyl-CoA carboylase 1* (*ACC1*) and *18s rRNA*. The nucleotide sequences of primers used are shown in Table 1. The PCR products were separated by electrophoresis on a 1.5–2% agarose gel, visualized by ethidium bromide staining, and quantified with NIH Image software. After correction for *18S rRNA* levels, the abundance of each specific mRNA was expressed as the fold change relative to control littermates. Values are the mean ± S.E.M. obtained from more than four pairs of littermates.

2.7. Blood parameter analyses and rectal temperature measurement

Blood samples were obtained from 18-week-old control and *Fgfr2* CKO mice. Blood glucose was measured by a blood glucose test meter (Glutest R, Sanwa-Kagaku, Japan). Plasma concentrations of triglycerides, cholesterol, and free fatty acids (FFAs) were measured using triglyceride E-Test, cholesterol E-test and NEFA C-Test kits (WAKO, Japan), respectively. The rectal temperature of 17-week-old control and *Fgfr2* CKO mice was measured by a digital thermometer, KN-91 (Natsume, Japan).

Table 1
PCR primer sequences used

Gene	Sense primer	Antisense primer
Fgfr2	5′-CGCCCACAATGAGGTGGTTA-3′	5′-TCACCACCATGCAGGCGATT-3′
Fgfr2b	5′-GGGATAAATAGCTCCAATGC-3′	5′-TCACAGGCGCTTGCTGTTTG-3′
Fgfr2c	5′-GGTGTTAACACCACGGACAA-3′	5′-CTCACAGGCGCTGGCAGAACT-3′
Leptin	5′-TGCTCCAGCAGCTGCAAGGTGCAAG-3′	5′-TCAGCATTCAGGGCTAACATCCAACTGTT-3′
UCP2	5′-GCATTGCAGATCTCATCACT-3′	5′-CCTTGGTGTAGAACTGTTTG-3′
PPARγ2	5′-CCCAGAGCATGGTGCCTT-3′	5′-GGCATCTCTGTGTCAACCATGGT-3′
Glut1	5′-ATGGATCCCAGCAGCAAGAA-3′	5′-GACTTGCCCAGTTTGGAGAA-3′
Glut4	5′-AAAACAAGATGCCGTCGGGT-3′	5′-CCTGATGTTAGCCCTGAGTA-3′
LPL	5′-GGAGTTTGGCTCCAGAGTTT-3′	5′-TAGCCAGCTGACACTGGATA-3′
HSL	5′-CAGGGCAAAGAAGGATCGAA-3′	5′-GTGTGCCACACCCAACAGTT-3′
FAS	5′-CCATGGAGGAGGTGGTGATA-3′	5′-CGTCTCGGGATCTCTGCTAA-3′
ACC1	5′-CACATGAGATCCAGCATGTC-3′	5′-TTCTGGGAGTTTCGGGTTCT-3′
Fgf4	5′-CCCTATTTGCTCTCGCTACT-3′	5′-CTCGTCGGTAAAGAAAGGCA
Fgf5	5′-AGAATGAGCCTGTCCTTGCT-3′	5′-CTTGGAATCTCTCCCTGAAC-3′
Fgf6	5′-GAGGCTGTTCATCACTATGT-3′	5′-CCGTTCTACCGTGGAGATCT-3′
Fgf8	5′-CCCAACAGGTAACTGTTCAG-3′	5′-GGCAATTAGCTTCCCCTTCT-3′
Fgf9	5′-GTCCTCTGATGGCTCCCTTA-3′	5′-AGACACTGTCTTTGTCAGCTT-3′
Fgf17	5′-AAGAAGTCTCTCCAGCGATG-3′	5′-TGGCCTCCCTGACTACGTTT-3′
Fgf18	5′-TGCCTGTGTGTTTACACTTTCTA-3′	5′-TGGTGAAGCCCACATACCAA-3′
Fgf20	5′-CCATGGCTCCCTTGACCGAA-3′	5′-GGCTCTAGATTCATCAAGTG-3′
18S	5′-CTTAGAGGGACAAGTGCA-3′	5′-ACGCTGAGCCAGTCAGTGTA-3′