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FGF-10 Is a Growth Factor for Preadipocytes in White Adipose Tissue

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FGF-10 is a mesenchymal factor affecting epithelial cells during pattern formation. However, the expression and physiological role of FGF-10 in adults remains to be elucidated. We examined the expression of FGF-10 mRNA in a variety of adult rat tissues, and found to be most abundant in white adipose tissue. In white adipose tissue, FGF-10 mRNA was expressed in preadipocytes but not in mature adipocytes. The expression in white adipose tissue during postnatal development was also examined. The expression level was low at postnatal day 10 (P10). However, FGF-10 mRNA was abundantly detected later on (P28 and P48) when white adipose tissue growth was stimulated. We also examined the activity of recombinant FGF-10 for primary rat preadipocytes. FGF-10 showed significant mitogenic activity for primary preadipocytes, but did not affect the differentiation of preadipocytes. The expression profile of FGF-10 mRNA and the activity of FGF-10 reported here indicate that FGF-10, a unique secreted factor produced in white adipose tissue, acts as a growth factor for preadipocytes in white adipose tissues.

The prototypic fibroblast growth factors (FGFs), FGF-1 (aFGF) and FGF-2 (bFGF), were isolated from brain and pituitary as mitogens for fibroblasts. FGFs are widely expressed in developing and adult tissues and are polypeptides with multiple biological activities involved in angiogenesis, mitogenesis, cellular differentiation and repair of tissue injury (1, 2, 3). The FGF family presently consists of 19 members, FGF-1 to FGF-19, sharing a conserved ~120 amino-acid residue core with ~30 to 60% amino acid identity (4, 9). These molecules also appear to play important roles in developing and/or adult tissues.

FGF-10 was originally identified from rat embryos by homology-based polymerase chain reaction (PCR). Rat FGF-10 cDNA encodes a protein of 215 amino acids with a hydrophobic amino terminus which serves as a signal sequence (5). FGF-10 initiates and maintains outgrowth of chick limb bud, and also induces branching morphogenesis in embryonic mouse lung. These results indicate that FGF-10 in embryos is a mesenchymal factor affecting epithelial cells during pattern formation (6, 7, 13, 14). However, the physiological role of FGF-10 in adults remains to be elucidated. We examined the expression of FGF-10 mRNA in a variety of adult rat tissues. We found that FGF-10 mRNA was most abundantly expressed in white adipose tissue among the adult tissues examined. Here, we report the expression profile of FGF-10 in white adipose tissue and its activity for primary rat preadipocytes.

MATERIALS AND METHODS

Preparation of RNA. RNA was prepared from rat tissues using an RNA extraction kit (Amersham Pharmacia Biotech).

Northern blotting analysis. Aliquots of RNAs were dissolved on a denaturing agarose gel (1%) containing formaldehyde and transferred to a nitrocellulose membrane in 20X SSC (1X SSC = 0.15 M NaCl/0.015 M sodium citrate) overnight. A ^{32}P-labeled rat FGF-10 cDNA (5) or leptin cDNA (11) was prepared with a random primer labeling kit (Amersham Pharmacia Biotech) with deoxycytidine 5′-[α-^{32} P]triphosphate (~110 TBq/mmol) (ICN Biochemicals Inc.). The membrane was incubated in hybridization solution containing the labeled probe as described (8) and analyzed with a radio-imaging analyzer (BAS 2000, Fuji Photo Film Co., Tokyo, Japan)

Preparation of mature adipocytes and stromal-vascular cells. Mature adipocytes and stromal-vascular cells were prepared from rat subcutaneous, epididymal and mesenteric white adipose tissues essentially according to the method of Ogawa et al. (11).

Preparation of recombinant FGF-10. The cDNA encoding a mature form of human FGF-10 (amino acid residues 40 to 208) (20) with a DNA fragment encoding an E tag (GAPVPYPDPLEPR) and a His_6 tag (HHHHHH) at the 3′ terminus of the coding region was con-

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structed in a transfer vector DNA, pAcGP67 A (PharMingen). Recombinant baculovirus containing the FGF-10 cDNA with the tag sequences was obtained by cotransfection of Sf9 cells with the recombinant pAcGP67 A and a linearized BaculoGold Baculovirus DNA (PharMingen). High Five insect cells (approximately $2X10^6$ cells/ml) were infected with the resultant recombinant baculovirus and incubated at 27 °C for 65 h in serum-free medium EX-CELL 400 (JRH Biosciences). The culture medium was dialyzed against phosphate-buffered saline (PBS), and applied to a column of Ni-NTA agarose (QIAGEN) in PBS containing 20 mM imidazole and 0.5 M NaCl. After washing the column with PBS containing 20 mM imidazole and 0.5 M NaCl, recombinant FGF-10 was eluted from the column with PBS containing 250 mM imidazole, 0.5 M NaCl and 50 μg/ml bovine serum albumin (BSA), and applied to a column of Sephadex G-25 (Amersham Pharmacia Biotech) in PBS containing 250 μg/ml BSA.

Mitogenic activity assay. Preadipocytes were prepared from rat epididymal adipose tissue essentially according to the method of Mitchell et al. (23). Primary rat preadipocytes were plated onto 96-well plates ($3.0X10^3$ cells/well) and cultured in medium 199 with 10% fetal calf serum. After 1 day in culture at 37 °C in an atmosphere of 5% CO_2, the cells were cultured in medium 199 with no fetal calf serum. After 20 h, the cells were cultured in medium 199 with 0.1% fetal calf serum and recombinant FGF-10 (0 to 25 ng/ml). After 3 days, cell proliferation was examined by determination of cell density using a WST-1 cell counting kit (Wako, Osaka, Japan).

RESULTS AND DISCUSSION

Expression of FGF-10 mRNA in adult rat tissues. We examined the expression of FGF-10 mRNA in a variety of adult rat tissues including brain, heart, lung, liver, kidney, small intestine, spleen, pancreas, thymus, stomach, muscle, testis and epididymal white adipose tissue by PCR using specific primers. FGF-10 mRNA was significantly detected in the adipose tissue and lung, but not in other tissues. FGF-10 mRNA was much more abundant in epididymal white adipose tissue than in lung (data not shown). We also examined the expression of FGF-10 mRNA in adult rat tissues including brain, heart, lung, liver, kidney, small intestine and epididymal white adipose tissue by Northern blotting analysis. The integrity of RNA was confirmed by electrophoresis on a denaturing agarose gel containing formaldehyde (data not shown). The labeled probe hybridized to an mRNA of ~4.5 kb in epididymal white adipose tissue and lung (Fig. 1). FGF-10 mRNA in the adipose tissue was much more abundant than in lung. These results are consistent with the PCR results.

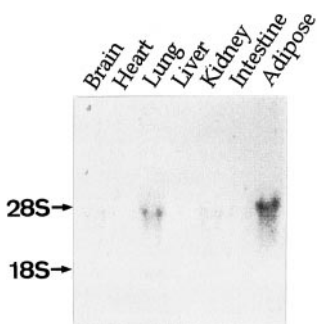

FIG. 1. Expression of FGF-10 mRNA in rat adult tissues. Aliquots of RNAs (10 μg) were electrophoresed on a denaturing agarose gel (1%) containing formaldehyde and transferred onto a nitrocellulose membrane. Hybridization was performed with a ^{32}P-labeled rat FGF-10 cDNA probe. The positions of 28S and 18S RNAs are indicated. Lanes Brain, Heart, Lung, liver, Kidney, Intestine, and Adipose indicate RNA from brain, heart, lung, liver, kidney, small intestine, and epididymal white adipose tissue, respectively.

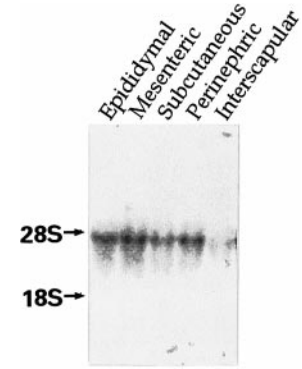

FIG. 2. Expression of FGF-10 mRNA in rat adipose tissues. Aliquots of RNAs (10 μg) were electrophoresed on a denaturing agarose gel (1%) containing formaldehyde and transferred onto a nitrocellulose membrane. Hybridization was performed with a ^{32}P-labeled rat FGF-10 cDNA probe. The positions of 28S and 18S RNAs are indicated. Lanes Epididymal, Mesenteric, Subcutaneous, Perinephric, Interscapular, and Retroperitoneal indicate RNA from epididymal, mesenteric, subcutaneous, and perinephric white adipose tissues, and interscapular brown adipose tissue, respectively.

Preferential expression of FGF-10 mRNA in white adipose tissue. There are two types of adipose tissues, white and brown. Their physiological roles are quite different. White adipose tissue stores energy whereas brown adipose tissue dissipates energy. White adipose tissue plays a crucial role in energy balance. It is a major energy reservoir that stores triacylglycerol in periods of energy excess and mobilizes triacylglycerol during energy deprivation (16, 17). In contrast, brown adipose tissue is a specialized tissue of mammals for facultative thermogenesis. Its physiological significance has been recognized in newborns when the decrease in environmental temperature at birth requires an adaptive increase in heat production (21). Brown adipose tissue is also speculated to normally function to prevent obesity (22).

We also examined the expression of FGF-10 mRNA in a variety of adipose tissues including epididymal, mesenteric, subcutaneous, and perinephric white adipose tissues, and interscapular brown adipose tissue. FGF-10 mRNA was abundantly expressed in all white adipose tissues examined (Fig. 2). However, the expression level of FGF-10 mRNA in interscapular brown adipose tissue was much lower than that in white adipose tissue. This result indicates that FGF-10 mRNA in adults is preferentially expressed in white adipose tissue.

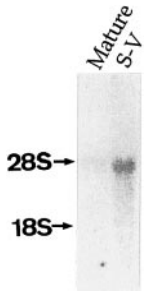

FIG. 3. Expression of FGF-10 mRNA in mature adipocytes and stromal-vascular cells of the subcutaneous white adipose tissue. Aliquots of RNAs (8 μg) were electrophoresed on a denaturing agarose gel (1%) containing formaldehyde and transferred onto a nitrocellulose membrane. Hybridization was performed with a ^{32}P-labeled rat FGF-10 cDNA probe. The positions of 28S and 18S RNAs are indicated. Lanes Mature and S-V indicate RNA from mature adipocytes and stromal-vascular cells of subcutaneous white adipose tissue, respectively.

White adipose tissue is composed of mature adipocytes and stromal-vascular cells (16). To determine the cellular localization of FGF-10 mRNA expression in white adipose tissue, we prepared mature adipocytes and stromal-vascular cells from subcutaneous adipose tissue. Leptin mRNA, which produces a hormone inducing satiety and increasing energy expenditure, was preferentially expressed in mature adipocytes but not stromal-vascular cells of white adipose tissue (11). We also examined the expression of leptin mRNA in these fractions by Northern blotting analysis. Leptin mRNA was preferentially detected in mature adipocytes but not stromal-vascular cells (data not shown), indicating that the mature adipocytes and stromal-vascular cells were properly prepared. In contrast to leptin mRNA, FGF-10 mRNA was preferentially detected in stromal-vascular cells but not in mature adipocytes (Fig. 3). In epididymal and mesenteric adipose tissues, FGF-10 mRNA was also preferentially detected in stromal-vascular cells (data not shown). Although stromal-vascular cells of white adipose tissues are potentially heterogeneous, over 90% of stromal-vascular cells differentiate into mature adipocytes (19). FGF-10 mRNA was also detected in 3T3-L1 preadipocytes (W. Ogawa et al., unpublished observation). Therefore, we conclude that FGF-10 mRNA is preferentially expressed in preadipocytes of white adipose tissue.

Expression of FGF-10 mRNA in rat adipose tissue during postnatal development. Rat white adipose tissue cannot be detected macroscopically during embryonic life and at birth. The white adipose tissue growth was greatly stimulated during postnatal development. The suckling-weaning transition is a physiological situation during which marked nutritional changes occur spontaneously (12). White adipose tissue growth changes very little during the suckling, but increases markedly after weaning (12). We examined the expression level of FGF-10 mRNA in subcutaneous and mesentric white adipose tissues at suckling (postnatal day (P) 10) and after weaning (P28 and P49). The expression of FGF-10 mRNA in white adipose tissue was low at P10. However, FGF-10 mRNA was abundantly expressed at P28 and P49 (Fig. 4). FGFs are local signaling molecules which act on proximal cells (1). Therefore, FGF-10 in white adipose tissue should act on cells within the tissue. These results indicate that FGF-10 is involved in white adipose tissue growth.

White adipose tissue growth was accompanied by progressive enlargement (differentiation) as well as by increases in the number (proliferation) of adipocytes. Proliferation in white adipose tissues is greatly stimulated after weaning (~P20). However, adipose size is mostly increased from later postnatal development stages (~P40) (10). FGF-10 preferentially binds to the FGF receptor-2b (15). We examined the expression of FGFR-2b mRNA in mature adipocytes and stromal-vascular cells. FGFR-2b mRNA was preferentially detected in stromal-vascular cells but not mature adipocytes (data not shown). Therefore, FGF-10 is expected to be involved in the proliferation of preadipocytes.

Preparation of recombinant FGF-10. To examine the effect of FGF-10 on preadipocytes, we prepared recombinant FGF-10 from the culture medium of High Five insect cells infected with recombinant baculovirus containing FGF-10 cDNA. Purified recombinant FGF-10 was analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions. A major ~25 kDa protein was detected (data not shown). The observed molecular mass was larger than the calculated molecular mass of recombinant FGF-10 (22,563 Da), possibly due to glycosylation at the putative position 196 (Asn).

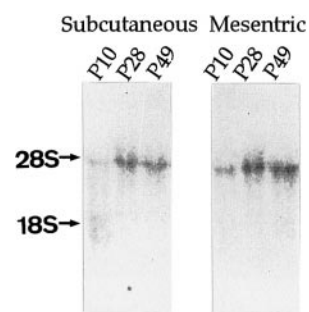

FIG. 4. Expression of FGF-10 mRNA in white adipose tissues during postnatal development. Aliquots of RNAs (10 μg) were electrophoresed on a denaturing agarose gel (1%) containing formaldehyde and transferred onto a nitrocellulose membrane. Hybridization was performed with a ^{32}P-labeled rat FGF-10 cDNA probe. The positions of 28S and 18S RNAs are indicated. Lanes P10, P28 and P49 indicate RNA from subcutaneous and mesenteric white adipose tissues at postnatal days 10, 28 and 49, respectively.

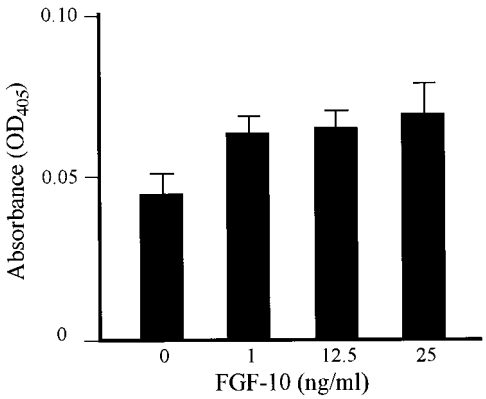

FIG. 5. Mitogenic activity of recombinant FGF-10 for primary rat preadipocytes. Mitogenic activity of recombinant FGF-10 was determined using primary rat preadipocytes from the epididymal white adipose tissue as described under "Materials and Methods".

Mitogenic activity of recombinant FGF-10 for primary preadipocytes. Primary preadipocytes were prepared from rat epididymal white adipose tissue. The mitogenic activity of recombinant FGF-10 for primary preadipocytes was examined by measuring cell density. FGF-10 showed significant mitogenic activity for primary preadipocytes even at 1 ng/ml (Fig. 5). We also examined the effect of FGF-10 on the differentiation of preadipocytes induced by isobutylmethylxanthine, dexamethason and insulin. However, FGF-10 (10 or 200 ng/ml) did not affect the differentiation (data not shown).

Adipocyte proliferation and differentiation are expected to be modulated by secreted growth factors. Tumor necrosis factor-α, which is produced in mature adipocytes, inhibits adipocyte differentiation and is a candidate mediator of insulin resistance in obesity. Pref-1, a member of the epidermal growth factor (EGF)-like family, is expressed in 3T3-L1 preadipocytes. Pref-1 strongly inhibits 3T3-L1 preadipocyte differentiation and has no mitogenic activity for 3T3-L1 preadipocytes. The expression of pref-1 mRNA is abolished during differentiation of 3T3-L1 preadipocytes to adipocytes. However, pref-1 mRNA was not detected in white adipose tissue (18). Thus, the physiological significance of pref-1 remains to be elucidated. In contrast, FGF-10 is preferentially expressed in preadipocytes of white adipose tissue and has mitogenic activity for primary preadipocytes but does not affect the differentiation. The present results indicate that FGF-10, a unique secreted factor produced in white adipose tissue, acts as a growth factor for preadipocytes.

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