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cDNA Cloning and Expression of a Novel Adipose Specific Collagen-like Factor, apM1 (Adipose Most Abundant Gene Transcript 1)

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We isolated a novel adipose-specific gene, apM1, the transcript of which is the most abundant in the mRNA population from human adipose tissue. Northern blotting revealed that the human apM1 gene transcript is exclusively expressed in adipose tissue. The apM1 gene encodes a 244 amino acid open reading frame containing a putative signal sequence and G-X-Y repeats (66 amino acids) followed by a cluster of aromatic residues near the C terminus having high local similarity with collagens X and VIII and complement factor C1q. Thus, apM1 is likely to be a novel collagen-like secretory protein exclusively produced by adipose tissue.

Adipose tissue serves as an energy reservoir by means of fat storage. Increasing evidence however, suggests that adipose tissue produces and secretes several proteins. These proteins are thought to regulate adipose tissue function and to affect the metabolism of the whole body (1–4). We initiated a systematic analysis of active genes in this tissue by constructing a 3′-directed cDNA library, in which the mRNA population is faithfully reflected (5). By collecting sequence information by analysing random clones, we obtained an expression profile that reflects actively transcribed genes in this tissue and discovered novel genes that are specifically expressed in this tissue.

A partial cDNA sequence, GS3109, the transcripts of which were most abundant was studied further. It had no corresponding entry in GenBank nor in dbEST, and it was specifically expressed at high levels in the adipose tissue. Here we describe the sequence of the full-length cDNA, apM1, which is a novel adipose-specific collagen-like factor.

MATERIALS AND METHODS

Materials. Abdominal subcutaneous and visceral fat tissues were collected from two female patients at surgery for myoma uteri. Informed consent was obtained from each of them. Both donors were of normal weight and had no history of metabolic or endocrinological disorders. From 3 grams of pooled adipose tissues, about 0.5 micrograms of poly(A)$^+$ RNA was prepared using a Quick prep mRNA purification kit according to the manufacturer's protocol (Pharmacia, Uppsala, Sweden).

Library construction and sequencing. Construction of the 3′-directed cDNA library and transformation into *Escherichia coli* were as described (6). Briefly, cDNA was synthesized using a pUC19-based vector primer, digested by the dam-sensitive four-base cutter MboI, circularized by linkage to the other terminus of the vector and transformed into *E.coli.* The transformant colonies were randomly selected and cultured in 96-well plates. The inserted cDNAs amplified with flanking primers, were cycle sequenced.

Northern blots. Total RNA (10 micrograms) from the adipose or other tissues (purchased from CLONETECH) were denatured in formamide and formaldehyde at 65°C for 5 min and separated by electrophoresis in agarose gels containing formaldehyde (7). RNA was blotted onto Hybond N nylon membranes (Amersham), and hybridized with a [$^{\alpha\text{-}32}$P]dCTP-labelled cDNA probe using a random priming kit (Amersham). The membranes were washed in 2× SSC-0.1%SDS, then in 0.1× SSC-0.1%SDS at 65°C for 15 min each and visualized by autoradiography with Kodak XR5 film at −80°C with an intensifying screen.

Cloning of a full-length cDNA. A full length cDNA library was constructed using vector primed cDNA (8). Briefly, the vector-primed cDNA was blunted at the 5′terminus with T_4 polymerase, circularized with T4 ligase and transformed into

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E.coli DH5. About 5000 transformants of this library were screened with ^{32}P-labeled apM1 cDNA (9). From among 20 positive clones, the longest cDNA insert was sequenced. The insert of this clone was amplified using Vent polymerase (New England Biolabs, Beverly, MA), using kinated flanking primers (SK:5′CGCTCTAGAACTAGTGGATC3′, T7:5′GTAATACGACTCACTATAGGGC3′), by means of 30 cycles of 96°C for 30 sec, 60°C for 60 sec, 72°C for 120 sec, followed by 72°C for 10 min. The amplified product was purified by means of Sephacryl S400 column chromatography, ligated, sheared by sonication and subcloned into M13. DNA from the transformant phages was purified and analysed by means of cycle sequencing using dye primer M13 (Perkin Elmer Applied Biosystems Division, Foster City, CA). The reaction products were sequenced using an autosequencer 373A and assembled using the DNASIS program (Hitachi software, Tokyo, Japan).

RESULTS

The GS3109 was the most abundant transcript in the expression profiles of adipose tissue (5). We isolated a full length cDNA corresponding to GS3109, and sequenced the longest clone. This clone was 4517 bp long and had a 244-amino-acid open reading frame (Figure 1), followed by a long 3′-untranslated region. (The nucleotide sequence of the apM1 has been deposited in DDBJ under accession number D45371).

A potential Kozak sequence was present with an adenosine residue three bases upstream of the ATG (10). Amino-acid sequencing revealed a putative signal sequence at the amino-terminus and no hydrophobic regions compatible with a transmembrane domain, indicating that the product of this gene is a secretory protein. Short collagen-like motif G-X-Y repeats were located immediately downstream of a short N-terminal non-collagenous sequence (11). A database search using the BLASTp program (12) showed significant sequence similarity with collagen X, VIII and complement protein C1q within the C-terminal region. Figure 1 shows the results of the best alignment.

To analyze the tissue distribution of the apM1 gene transcript, the cDNA probe was hybridized to a northern blot of RNAs from several human tissues. A 4.5 kb apM1 mRNA was detected in adipose tissue (Figure 2), but not in skeletal muscle, small intestine, placenta, uterus, ovary, kidney, liver, lung, brain or heart. Thus we concluded that this gene is expressed only in the adipose tissue. Our results of body mapping (5, 6, 13–15) in which active genes are listed in over 35 human tissues, support this view (Okubo, pers. commun).

DISCUSSION

A novel adipose-specific gene, apM1 is described. The deduced amino acid sequence showed

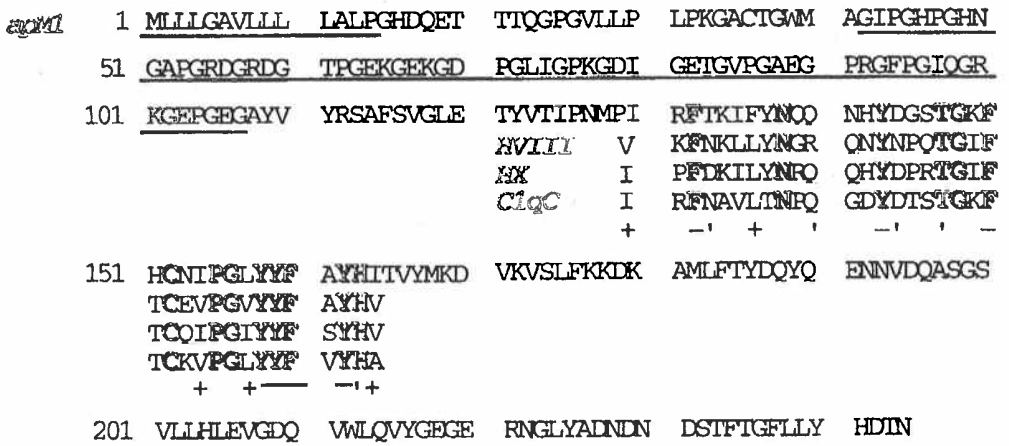

FIG. 1. Deduced amino acid sequence of apM1 (244a.a.). The first underline indicates a signal sequence and the second, a region encoding Gly-X-Y triplets (G-X-Y repeats). In the C-terminal non-collagenous region, the sequence is aligned with human VIII collagen (*HVIII*), human X collagen (*HX*), and C chain of human C1q (*C1qC*). Identical residues are indicated in open-face type, conserved hydrophobic residues as (+), conserved hydrophilic/H-bonding residues as (′) and conserved aromatic residues as (−). (The nucleotide sequence of the apM1 has been deposited in DDBJ under Accession No. D45371.)

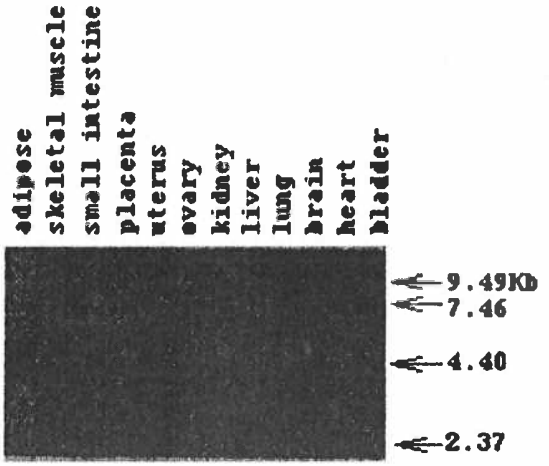

FIG. 2. Adipose-specific expression of apM1. Tissue distribution of apM1 as tested by Northern blotting. Total RNA from adipose tissue and various tissues was resolved by electrophoresis and hybridized with an apM1 cDNA probe. The apM1 detected a 4.5Kb RNA in adipose tissue but not in other tissues.

that this gene codes for a 244-amino-acid polypeptide with a putative signal sequence. This protein possesses short collagen-like G-X-Y repeats, immediately downstream of a short N-terminal non-collagenous sequence, which is a hallmark of all collagens and collagen-like molecules such as complement protein C1q (16), mannan binding protein (MBP) (17), lung surfactant proteins SP-A (18), SP-D (19), macrophage scavenger receptor (20), and conglutinin. MBP, SP-A, SP-D and conglutinin are C-type lectins (21) and form oligomeric complexes through the collagen-like domain and bind to carbohydrate ligands at the C-terminal end of their polypeptide chains. In C1q, the C-terminal domain is also involved in binding with the Fc regions of immunoglobulin (16).

The role of the apM1 protein is not clear at present. The collagen-like domains of apM1 is a little shorter than other collagen-like domains. However, it has a cysteine residue within the short non-collagenous segment preceding the collagen-like domain and shows significant sequence similarity with the C-terminal domains of collagen X, VIII and complement factor C1q, including a cluster of aromatic residues involved in trimerization (22). The apM1 protein is therefore likely to form complexes through the collagen-like domains. Further studies will be needed to understand the function of apM1, and to identify a specific ligand which may bind at the C-terminal portion of this protein.

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