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The profile of gene expression during bovine adipogenesis: Cloning and expression of type XII collagen isoforms*

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ABSTRACT

To isolate candidate genes involved in bovine adipocyte differentiation, we have constructed a subtraction library from a clonal bovine intramuscular preadipocyte (BIP) cell line using PCR-subtraction. We have isolated a set of subtracted cDNA fragments whose respective mRNA levels are up regulated during adipogenesis. Two cDNA clones were determined to be type XII collagen alpha-1. From the expression analysis of type XII collagen, the XIIA-2 isoform was mainly expressed in differentiated BIP cells and adipose tissues. These results suggest that type XII collagen may be associated with adipocyte differentiation and adipose formation in cattle and is a potentially useful marker for adipogenesis.

KEY WORDS: adipogenesis, BIP cell, bovine, PCR-subtraction

INTRODUCTION

Adipose tissue plays an important role in energy homeostasis, through the accumulation of excess energy in adipose cells. Moreover, adipocytes are an

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important endocrine cell that secretes various physiologically active molecules. Adipocyte differentiation begins during development and adipose tissue is formed in various parts of the body before birth. However, intramuscular adipose tissue differentiates in the final phase of pre-partum adipose tissue development and after birth. Japanese Black cattle are able to deposit large amounts of intramuscular fat (marbling). We have established a bovine intramuscular preadipocyte clonal cell line (BIP cell) derived from *M. longissimus thoracis* of Japanese Black cattle (Aso et al., 1995). BIP cells can differentiate into mature adipocytes accompanied by the cytoplasmic accumulation of lipid droplets. This cell line serves as a useful model for the study of adipose tissue metabolism and adipocyte differentiation mechanisms in ruminants (Nakajima et al., 1998; Inoue-Murayama et al., 2000; Tkasuga et al., 2001; Miyashita et al., 2002; Tahara et al., 2004).

We have identified the genes expressed during adipocyte differentiation using suppression subtractive hybridization with BIP cells. We isolated cDNA fragments, the transcriptional activity of which increases during adipocyte differentiation. Accordingly, we have found that the expression of type XII collagen was induced during adipogenic differentiation. In the present experiment, we have measured the gene expression of type XII collagen at each differentiation stage in BIP cells, in addition to several bovine tissues, in order to clarify its role in ruminant adipogenesis.

MATERIAL AND METHODS

Cell culture and adipocyte differentiation

BIP cells were routinely cultured in DMEM containing 10% FBS and passaged every 3 days. In order to produce mature adipocytes, confluent BIP cells were shifted to an adipogenic medium (50 ng/ml insulin, 0.25 μM dexamethasone, 5 mM octanoate, 10 mM acetate and 10% FBS in DMEM).

Subtraction and cDNA cloning

Total RNA samples were extracted from BIP cells and several tissues from a Japanese Black Cattle using TRIZOL reagent. Suppression subtractive hybridization was done using a PCR-select cDNA subtraction kit (Clonetech). Subtracted plasmid DNA was sequenced by the dye termination method.

Expression of collagen XII

We determined the protein and gene expression of type XII collagen by immunofluoresence staining, Western blot analysis, Northern blot analysis and RT-PCR amplification.

RESULTS

Subtraction and cDNA cloning

In this study, a total of 620 clones were sequenced, and their homology was searched with the registered sequences in the GenBank. Of these clones, 442 (71.3%), were identified as homologous with the previously known genes and 124 (20.0%) showed homology with bovine EST clones. The remaining 54 clones (8.7%) did not show homology with reported sequences and were supposed to be novel genes. We selected one of the clones, designated clone 41, because its mRNA transcription level was clearly elevated during differentiation. We screened the lambda ZAP II-BIP S4 cDNA library of differentiating BIP cells using the radiolabeled clone 41 fragment as a probe and isolated 2 clones. Each clone was highly homologous with human and mouse type XII collagen alpha 1. These data show that clone 41-10 has a deletion between nucleotides 2,039 and 3,185 of clone 41-9. However the deduced amino acid sequence of the carboxy-terminal of clone 41-10 is 50 residues longer than that of clone 41-9.

Detection of type XII collagen in BIP cells

During adipogenic differentiation, the protein expression of type XII collagen was analysed by immunostaining and Western blotting using BIP cells. Non-fibrous structures of type XII collagen were clearly observed on the differentiated BIP cells. On the other hand, type XII collagen was not detected in unstimulated BIP cells. Type XII collagen was detected at low levels after 2 days and underwent a rapid increase during adipogenesis.

Expression of type XII collagen splicing isoforms in BIP cells

mRNA expression of type XII collagen splicing isoforms in BIP cells was examined by Northern blot analysis. All splicing isoforms (XIIA-1, XIIA-2, XIIB-1 and XIIB-2) existed and were up-regulated during the adipogenic differentiation of BIP cells. The transcripts of XIIA splicing isoforms (XIIA-1 and XIIA-2) were elevated about 4 times during adipose conversion, and the XIIA-2 splicing isoform was highly expressed in differentiated BIP cells.

RT-PCR was performed to analyse the type of collagen XII splicing isoform expressed in BIP cells during adipogenic differentiation. XIIA and XII-2 type splicing isoforms were induced by adipogenic stimulation at 2 days and have increased expression during differentiation. These data suggest that the splicing isoform in differentiated BIP cells is mainly an XIIA-2 type.

Tissue distribution of collagen XII

Nested PCR was used to analyse the distribution of type XII collagen splicing isoforms expressed in bovine tissues. XII-2 type was expressed in all tissues

investigated: type XII collagen seems to be produced in a large number of tissues. XII-1 type was detected only in adipose tissues and was particularly expressed in subcutaneous fat. The expression of the XIIA type was greatest in subcutaneous and intraperitoneal fat, but this isoform was expressed at low levels in the other perinephrial and mesenteric fat, and was detected in skeletal muscles, lung and kidney, and was very low in brain, heart and liver. XIIB type was expressed in adipose tissues, skeletal muscles and lung. These results show that bovine adipose tissues produce a type XII collagen, but splicing isoforms were different in different locations.

DISCUSSION

Results presented herein are the first to demonstrate that type XII collagen is closely related to adipogenic differentiation *in vitro* and *in vivo*, and suggest that the XIIA-2 isoform was mainly expressed during adipogenesis in BIP cells. As XIIA was also mainly expressed in bovine adipose tissues, these results suggest that the expression of XIIA-2 is a feature of bovine adipocytes.

The XIIA isoform has both a collagen and a proteoglycan role; proteoglycans are major components of the ECM and participate in histogenesis and functional differentiation. As adipocytes have twin roles of energy storage and organizing energy supply, its size has to change continuously. In addition, adipocytes require a more flexible ECM because adipose tissue also serves as a shock absorbent tissue. In order to fulfil these roles, we consider that the restructure of the ECM of the cell surface is required during adipogenic differentiation. This study provides new understanding on the role of type XII collagen in adipogenesis.

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