The Expression of Porcine Adiponectin and Stearoyl Coenzyme a Desaturase Genes in Differentiating Adipocytes

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ABSTRACT : The gene expression of porcine adiponectin and stearoyl coenzyme A desaturase (SCD) was investigated in this study. The partial gene sequences for adiponectin and SCD were amplified by RT-PCR from subcutaneous adipose tissue and cloned by TA cloning techniques. Sequences of these genes were determined and found to be highly homologous to that of other species, suggesting similar function of these genes as in other species. The transcripts of these adipocyte-related genes in pig tissues were measured by Northern analysis. The transcripts for adiponectin and SCD were highly expressed in porcine subcutaneous adipose tissue; the transcripts for SCD were also barely detected in the liver, but the greatest concentrations were in the adipose tissue. In porcine stromal-vascular cells (S/V cells) cultured in vitro, transcripts for adiponectin and SCD increased gradually during adipocyte differentiation. The level of adipocyte adiponectin mRNA was associated with late adipocyte differentiation, indicating the gene may not be involved in adipocyte differentiation but has great importance in porcine adipocyte functions. The SCD transcripts were not detectable until 2 d after induction of adipocyte differentiation. It was highly expressed in differentiating porcine adipocytes (2 to 10 d after the induction of adipocyte differentiation), indicating a significant role of SCD in adipocytes. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 588-593)***

Key Words : Adiponectin, Stearoyl Coenzyme A Desaturase, Adipose Tissue, Pigs

INTRODUCTION

Fat deposition in pigs depends on two key events: the availability of excess energy from the diet and a cascade of molecular events that direct porcine adipocyte differentiation. In pigs, adipose tissue is the primary site for lipogenesis from glucose and for storage of lipids. Preadipocytes need to differentiate to adipocytes for fat deposition. Peroxisome proliferator-activated receptor γ (PPARγ) and adipocyte determination and differentiation-dependent factor 1 are important transcription factors that involve in initiating and enhancing adipocyte differentiation (Rosen et al., 2000; Gondret et al., 2001; Poisson et al., 2001). Recent research showed that adipose tissue was not only an energy storage site, but also an endocrine and paracrine tissue that secreted leptin, adiponectin and other factors into the blood circulation (Mohamed-Ali et al., 1998; Havel, 2002). Using microarray technique, Kim and Moon (2003) found that adipocyte-specific secretory factor and H-rev107 were expressed in rat adipose tissue. Kim et al. (2001) cloned lactate dehydrogenase from the adipose tissue of the Korean Cattle.

Adiponectin, also known as Acrp30, AdipoQ, apM1 and GBP28, was first cloned in 1995 from mouse (Scherer et al., 1995). Recent evidence showed that adiponectin was secreted by adipocytes and had significant roles in inhibiting gluconeogenesis (Combs et al., 2001) and increasing fatty acid oxidation in mice (Yamouchi et al., 2001). Stearoyl coenzyme A desaturase (SCD) is a rate-limiting enzyme catalyzing the formation of palmitoleate and oleate from palmitate and stearate. It inserted its functions through up-regulating enzymes in lipogenic pathways and down-regulating enzymes in lipid oxidation (Ntambi et al., 2002).

Although functions of adiponectin in rodents have been revealed, porcine adiponectin has not been reported. We conducted this experiment to clone porcine adiponectin and SCD genes and study the tissue distribution and expression of these genes in differentiating adipocytes.

MATERIALS AND METHODS

Animals

For cloning of gene fragments and tissue distribution studies, three crossbred pigs (*Sus domesticus*; sows were predominantly Landrace-Yorkshire crossbreds mated to a Duroc boar) at age 8 to 9 wk old were killed, with an electrocution combined with exsanguinations 2 h after feeding. The protocol was approved by the Experimental Animal Management and Use Committee at National Taiwan University. Heart, kidney cortex, liver, longissimus muscle, and subcutaneous adipose tissue were quickly dissected and frozen in liquid nitrogen with storage at -70°C until RNA extraction.

Culture of porcine stromal vascular cells

Porcine S/V cells were isolated from 5 to 7 d old crossbred pigs. The pigs were killed by captive bolt pistol coupled with exsanguination. Adipose tissues were taken

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Table 1. Characteristics of the porcine-isolated DNA sequences

Genes[1]	Primers[2]	Source of primers	Size, bp	Nucleotide Homology, %	
ADN	S 5'-CAGGTCGTGATGGCAGAGAT-3'	Dog	399	Dog=90% (AF417206)	(U00968)
	A 5'-AGGTGAAGAGCATAGCCTTG-3'			Rat=82% (NM144744)	(L16995)
SCD	S 5`-GAAGAAGACATCCGTCCTGA-3`	Mouse	418	Mouse=83% (AF509570)	(X52773)
	A 5`-CGTGAGAGAAGAAGAAGCCA-3'			Rat=82% (AF509569)	(M84817)
18S (AF102857)	S 5'-CTCGATGCTCTTAGCTGAGT-3'	Human	649	Human=100% (M10098)	(M10098)
	A 5'-CTAGTTAGCATGCCGAGAGT-3'			Mouse=100% (V01270)	(X00686)

[1] GenBank accession number is indicated parenthetically. [2] S: sense; A: antisense.

from the pigs and the stromal vascular (S/V) cells were isolated and cultured following the procedure stated by Ding et al. (1999, 2002). The cell number was determined by using a hemocytometer with trypan blue staining and the cells were plated at a density of $6\times10^4/cm^2$. Three plates per stage were used for isolation of RNA. Cells were incubated in DMEM/F12+10% fetal bovine serum at 37°C under 5% CO_2 in air for 24 h to allow attachment and proliferation of the cells. After 24 h, the medium was removed and replaced by the differentiation medium {DMEM/F12 with differentiation factors [100 nM bovine insulin (I-5500; Sigma Chemical Co., St. Louis, MO), 50 ng hydrocortisone/ml (H-4001; Sigma Chemical) and 10 μg transferrin/ml (T-1408; Sigma Chemical)]}. Culture conditions followed the procedures described by Suryawan and Hu (1993) and Ding et al. (2002).

Extraction of RNA

Total RNA was extracted by the guanidinium-phenol-chloroform extraction method (Chomzinski and Sarki, 1987). The quality of the RNA was monitored by examination of the 18S and 28S ribosomal RNA bands after electrophoresis. The RNA was quantified by spectrophotometry at 260 nm and stored at -70°C. The extraction procedures have been described by McNeel and Mersman (1999).

Cloning of the porcine gene fragments

Two μg of adipose tissue total RNA from a crossbred pig was reverse transcribed (RT) at 42°C with a SuperScript II kit (Invitrogen, Carsbad, CA). The transcribed single strand DNA was amplified by PCR for 36 cycles, using pairs of sense and antisense primers (Table 1). The conditions for PCR were denaturation at 94°C for 30 s (4 min in the first cycle), annealing for 30 s and extension at 72°C for 2 min (12 min in the last cycle). The annealing temperatures for adiponectin and SCD1 were 58 and 60°C, respectively. The PCR product for each gene was separated by gel electrophoresis and purified by gel extraction and cloned into a pGEM-TEasy vector (Promega, Madison, WI). Sequences of these PCR gene fragments were determined and homologies of these fragments with gene fragments from other species were compared.

Northern analysis

Total RNA (20 μg of each sample) was electrophoresed and transferred to nylon membranes. The membrane was prehybridized at 42°C in UltraHyb (Ambion, Austin, TX) for 30 min and then the denatured cDNA probe (95°C for 10 min) was added at a concentration of 1 pM of each cDNA/ml, to hybridize with the targeted mRNA transcript overnight at 42°C. The cDNA probe was labeled with BrightStar™ BioDetect Nonisotopic Detection Kit (Ambion, Austin, TX). Following hybridization, the membrane was washed twice in 2 X-SSC (300 mM NaCl, 30 mM sodium citrate, at pH 7.0) containing 0.1% SDS at room temperature for 5 min. The membrane was then washed twice in 0.2 X-SSC containing 0.1% SDS for 15 min at 42°C. All membranes were hybridized at the same time with a single probe so comparisons could be made between tissues and different differentiation stages. The washing and color development procedure followed the manufacture's suggestion (Ambion, Austin, TX). The signals were detected by exposing X-ray film with the membranes for 2 h and quantified by color intensity. Each transcript was normalized to the value for the 18S ribosomal RNA in that sample. The same membranes were stripped with 0.1% SDS at 100°C for 5 min and hybridized with different probes to determine transcript concentrations.

Statistical analysis

The data for developmental patterns of transcripts in the differentiating S/V cells in vitro were analyzed using an ANOVA procedure and Duncan's new multiple range test was used to evaluate the differences among means.

RESULTS AND DISCUSSION

Porcine gene fragments

The RT-PCR product for porcine adiponectin gene was generated from porcine adipose tissue RNA. It was 399 bp in length and sequence analysis showed that the sequence of cloned porcine adiponectin cDNA fragment was highly homologous compared with that of mouse (Sherer et al., 1995), rat (GenBank NM144744) and dog (GenBank AF417206). The homology between pig and mouse, rat, and dog was 83, 82 and 90%, respectively. The porcine

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Pig ADN   (    1) caggt----------------------------------------------
Mouse     (    1) ....c----------------------------------------------
Rat       (    1) .....tggatggcaggcatcccaggatatcctggtcacaatgggataccg
Canis     (    1) ....c----------------------------------------------

Pig       (    6) ---cgtgatggcagagatggcgtccctggcgagaagggtgagaaaggaga
Mouse     (    6) ---...................act.....a........a...........
Rat       (   51) ggc.................act.....a........a.....g.....
Canis     (    6) ---...................ac......t..a................

Pig       (   53) tacaggtcttactggtcctaagggtgacactggggaatctggagtgactg
Mouse     (   53) .g.......ct..............g..a..a..tgt....a....a.
Rat       (  101) cg.....g..ct..............c.a..a..tg.....a....a.
Canis     (   53) .c........gt.....................t...a.......a....

Pig       (  103) gggttgaaggtccccgaggtttcccaggaatcccgggcagaaaaggagaa
Mouse     (  103) .a.c......g..a..g..c.....c....c...t.....g.......g
Rat       (  151) .a.cg.....g..a..g..c..t..c....c...t.....g.......g
Canis     (  103) ...................c..t.......c...a..a..g........

Pig       (  153) cctggagaaagcgcctatgtctaccgttcagcattcagtgtgggcctgga
Mouse     (  153) .........gc...t.....g..t..c.....g...........g.....
Rat       (  201) ..c......gc...t..ca.g..t.ac...........c..a..g.....
Canis     (  153) .....g.....t........ac.......g..............gt....

Pig       (  203) gactcgggtcactgtccctaacatgcccattcgctttaccaagatcttct
Mouse     (  203) ...c..c........t..c..tg.a..............t..........
Rat       (  251) ...c..c...........c..tg.t..............t..........
Canis     (  203) ..gc...a..........c..tg.t.................a.......

Pig       (  253) acaatcagcaaaaccactatgatgtcaccactggcaaattccactgcaac
Mouse     (  253) ....c..a..g..t..t.....c.g..g.........g...t........
Rat       (  301) ....c..a..g..t..t.....c.g..g......................
Canis     (  253) ......t............c....g.........a.....t........

Pig       (  303) attcctgggctgtactacttctccttccacatcacggtctacttgaagga
Mouse     (  303) .....g..a..c...........t.a............g...a....a..
Rat       (  351) .....g.....c.....t..t....a............g...a.......
Canis     (  303) ..........................a.........a..............

Pig       (  353) tgtgaaggtcagcctctacaagaaggacaaggctatgctcttcacct
Mouse     (  353) .........g.......t...............cg.t..........
Rat       (  401) ......a..g.......t...............cg.t..........
Canis     (  353) ...c.....................a......................
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Figure 1. Porcine adiponectin sequence. Partial cDNA sequence for the pig adiponectin (AND). This fragment was obtained from porcine adipose tissues. The boldface type sequences are primer sequences for the RT-PCR. The dots indicate the same nucleotide. The homology between pig and mouse (Sherer et al., 1995), rat (GenBank NM144744) and dog (GenBank AF417206) was 83, 82 and 90%, respectively. The porcine sequence equilibrated to mouse sequence from 148 to 447 nt (Sherer et al., 1995).

sequence equilibrated to mouse sequence from 148 to 447 nt (Sherer et al., 1995; Figure 1). The high homology of the porcine adiponectin compared with the sequences from other species indicating a similar function of this gene in pigs as in other species. Adiponectin has been found to decrease the basal blood glucose level through increasing insulin sensitivity (Combs et al., 2001; Yamauchi et al., 2001) and to increase utilization of plasma free fatty acids through increasing expression of genes involved in fatty acid oxidation in the mouse (Fruebis et al., 2001).

The RT-PCR product for porcine SCD gene was generated from porcine adipose tissue RNA. It was 418 bp in length and sequence analysis showed that the sequence of cloned porcine SCD cDNA fragment was highly homologous compared with SCD1 gene of pig (GenBank Z97186), mouse (GenBank AF509570) and rat (GenBank

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Pig SCD    (    1) gaagaagacatccgtcctgaaatgaaagatgacatctatgacccaaccta
Mouse      (    1) ............................a..t..tc.c.....c.....
Rat        (    1) ........................g...a..t...c.c.....c.g...

Pig SCD    (   51) ccaggataaggagggcccaaggcccaagcttgaatatgtttggagaaaca
Mouse      (   51) t......g.......a..ccc.........g..g..c..c.....g....
Rat        (   51) .......g.......g..ccc.........g..g..c..c.....g....

Pig SCD    (  101) tcatcctcatgagtctgctacacttgggagccctgtatgggatcatattg
Mouse      (  101) ....t......gtc.....g..........g......c.........c..
Rat        (  101) ...........gcc.....g...g............c.......c.c..

Pig SCD    (  151) atccccacctgcaagatatacaccttgctctgggcgtttgcctactatct
Mouse      (  151) g.t...t........c.c.......gc....tc.g.a..tt......ca.
Rat        (  151) ......t...c....g.c................g.a.attt.....c..

Pig SCD1   (  201) gctgagtgctgtgggtgtcacggcaggagctcaccgcctgtggagtcacc
Mouse      (  201) .acc..c...c....ca....a..c..g.....t.....c.....c...a
Rat        (  201) ta.c.....cc....ca....a..c..g.....t...........c...a

Pig SCD    (  251) gaacttacaaagctcgactgcccctgcgggtcttcctgatcattgccaac
Mouse      (  251) ..........g..a..g............a.......t............
Rat        (  251) .g........g.....g.........a..a.......c............

Pig SCD    (  301) acgatggcattccagaatgacgtttatgaatgggcccgagatcaccgtgc
Mouse      (  301) ..c.....g..............g..c....................c..
Rat        (  301) ..c.....g........c..t..g..............g........c..

Pig SCD    (  351) ccaccacaagttttcagaaacagatgctgatccccacaattcccgacgtg
Mouse      (  351) ............c.........c.c..c..c..t...........c....
Rat        (  351) ............c..t..g...c.c..c..c..t.....c.....c....

Pig SCD    (  401) gcttcttcttctctcacg
Mouse      (  401) .................
Rat        (  401) ....t............
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Figure 2. Porcine SCD sequence. Partial cDNA sequence for the pig stearoyl coenzyme A desaturase (SCD). This fragment was obtained from porcine adipose tissues. The boldface type sequences are primer sequences for the RT-PCR. The dots indicate the same nucleotide. The sequence of cloned porcine SCD cDNA fragment was highly homologous compared with SCD1 gene of mouse (GenBank AF509570) and rat (GenBank AF509569). The homology between pig, mouse and rat was 83 and 82%, respectively. The porcine sequence equilibrated to mouse sequence from 249 to 667 nt (AF 509570).

AF509569). The homology between pig, mouse, and rat was 83 and 82%, respectively. The porcine sequence equilibrated to mouse sequence from 249 to 667 nt (AF 509570, Figure 2). The high homology of the porcine SCD compared with the sequences from other species indicates a similar function of this gene in pigs as in other species. In mice, SCD1 is a rate-limiting enzyme for the synthesis of palmit leate and oleate from palmitate and stearate (Kim and Ntambi, 1999). It also inserts its functions through up-regulating enzymes in lipogenic pathways and down-regulating enzymes of lipid oxidation (Ntambi et al., 2002).

Tissue distribution of porcine genes

The adiponectin transcript was highly expressed in the adipose tissue of pigs (Figure 3). It was not detectable in the heart, kidney, liver, and longissimus muscle. The result confirms that adiponectin expressed highly in adipose tissue as in other species (Scherer et al., 1995). The result that porcine adipose tissue expresses high adiponectin suggests an important role of the gene in pigs as in other species.

The SCD transcript was highly expressed in porcine adipose tissue and to a much lesser extent, in the liver (Figure 3). It was not detectable in the heart, kidney and longissimus muscle. The tissue distribution of porcine SCD was similar to what was reported in mice (Ntambi et al., 1988) and genetically selected pigs (Smith et al., 1999). Ntambi et al. (1988) indicated that the tissue distribution of mouse SCD was affected by feeding program and dietary fatty acid (FA) composition. Feeding starved mice increased the expression of SCD in both liver and adipose tissues.

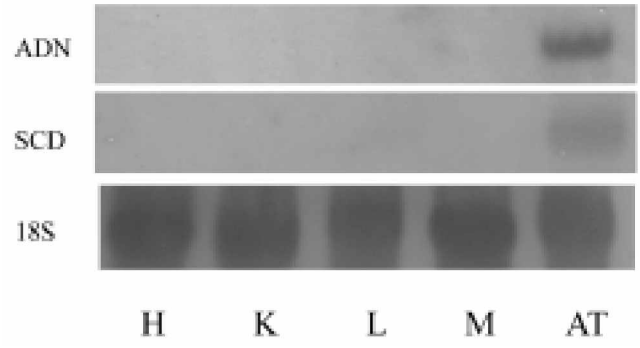

Figure 3. Tissue distribution of ADN and SCD. Tissue distribution of adiponectin (ADN) and stearoyl coenzyme A desaturase (SCD) transcripts. Total RNA was isolated from heart (H), kidney (K), liver (L), longissimus muscle (M) and subcutaneous adipose tissue (AT), obtained from 8 to 9 week-old pigs (n=3). Twenty μg of total RNA was electrophoresed and transferred to nylon membranes. The membranes were hybridized with porcine cDNA probes for various gene fragments. Transcripts for ADN and SCD, and 18S ribosomal RNA (18S) were measured. The figure represents the typical expression level of three animals. T The size of the primary transcript detected in porcine total RNA samples by each gene fragment was ADN=3.3 kb, SCD=4.6 kb and 18S=1.9 kb.

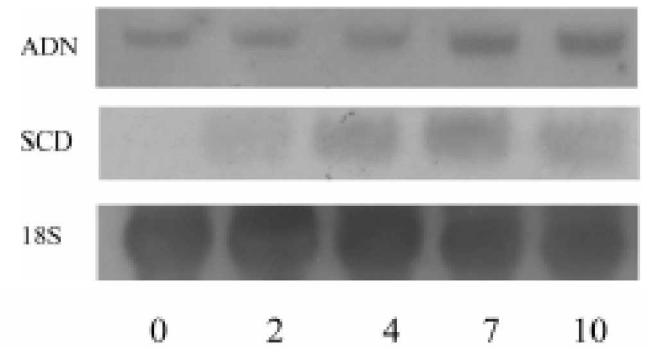

Figure 4. Ontogeny of ADN and SCD. The expression of adiponectin (ADN) and stearoyl coenzyme A desaturase (SCD) mRNA in differentiating porcine adipocytes. The S/V cells were isolated from 5- to 7 day-old pigs and plated in medium containing DMEM/F12 and 10% fetal bovine serum. After 24 h, serum containing medium was removed and cells were grown in serum-free medium containing DMEM/F12 and insulin, hydrocortisone, and transferring to stimulate differentiation. The time of addition of differentiation factors is indicated as 0 d. Plates were removed at 0, 2, 4, 7 and 10 days of differentiation. The total RNA from each stage (20 g) was electrophoresed and transferred to a nylon membrane. The membranes were hybridized with cDNA probes for AND and SCD. The data represent the mean of 3 experiments, each using S/V cells from a different pig. Data were analyzed with ANOVA and Duncan's new multiple range test was used to evaluate the differences among means.

whereas feeding the mice diets with unsaturated FA. SCD mRNA expressed only in adipose tissues (Ntambi et al., 1988). In current study, all the pigs were killed at 2 h after feeding, the SCD gene should express at high levels. We found that adipose tissues expressed much greater SCD mRNA than that in the liver, indicating differences of the expression of SCD in pigs as in mice (Ntambi et al., 1988).

Porcine gene expression in differentiating adipocytes

The porcine adipocyte differentiation system, tested by several groups (Suryawan and Hu, 1993; Ding et al., 1999), was used to test the effect of adipocyte differentiation on the expression of adiponectin and SCD. The porcine preadipocytes expressed adiponectin transcript in a low level and the level of adiponectin expression increased as the differentiation proceeded. It increased four fold during the 10 days after the induction of adipocyte differentiation (Table 2). The expression pattern of adiponectin in differentiating adipocytes was similar to that of PPAR (Ding et al., 1999), indicating both transcripts are highly associated with adipocyte differentiation. In human differentiating adipocytes, the adiponectin was expressed highly 4 d after induction of adipocyte differentiation (Kappes and Loffler, 2000). In 3T3-L1 preadipocytes, adipocyte differentiation was associated with high level expression of adiponectin mRNA and secretion of adiponectin protein (Scherer et al., 1995; Bogan and Lodish, 1999). However, the adiponectin expression in 3T3-L1 adipocytes was down regulated by insulin and dexamethasone (Fasshauer et al., 2002). Therefore, other factors besides these two hormones are hypothesized to increase adiponectin expression during the differentiation of adipocytes.

The SCD transcript was not detectable in porcine S/V cells before induction of adipocyte differentiation. Induction of the expression of SCD occurred between 0 and 2 days after adipocyte differentiation was induced (Figure 4). The expression level of porcine SCD mRNA increased as the adipocyte differentiation proceeded (Table 2). This result indicates that the SCD expression is either correlated to adipocyte differentiation or the addition of insulin, hydrocortisone, and transferrin. The expression of SCD transcript was high in well-differentiated porcine adipocytes similar to that of 3T3-L1 mouse differentiated adipocytes (Ntambi et al., 1988). Smith et al. (1999) demonstrated that the expression of SCD mRNA was increased as the pigs

Table 2. The expression of adiponectin (ADN) and stearoyl coenzyme A desaturase (SCD) mRNA in differentiating porcine adipocytes

Genes*	0 d	2 d	4 d	7 d	10 d
ADN	11.18±11.17^{b}	13.86±13.85^{b}	26.44±19.11^{b}	57.65±8.59^{a}	60.41±18.25^{a}
SCD	0±0^{d}	24.76±4.15^{c}	60.3±8.22^{b}	88.89±5.9^{a}	59.86±4.5^{b}

*All the data were normalized by the value of 18S ribosomal RNA transcript.

$^{a-d}$ Means on the same row without common superscripts differ significantly (n=3).

increased adipose deposition. They also found that genetically obese pigs expressed greater level of SCD mRNA than the leaner pigs. In mice, SCD has been found to be associated with the adipose fat deposition and SCD-knockout mice accumulated much less fat in their adipose tissues (Ntambi et al., 2002). This evidence shows that SCD gene is important for adipocyte fat deposition in both mice and pigs. Since porcine SCD gene sequence is highly homologous with that of rodents, the function of porcine SCD in adipose fat metabolism and deposition should be similar to that of rodents.

Taken together, porcine adiponectin and SCD genes are highly expressed in adipose tissue and they are associated with adipocyte differentiation. The functions of these genes in adipocyte differentiation need to be further investigated.

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