Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem polyadenylation sites

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A critical step in the synthesis of unsaturated fatty acids is catalysed by stearoyl-CoA desaturase (Scd). To determine the regulation of human Scd, we characterized the gene and its transcripts. Screening a human keratinocyte cDNA library and analysis of 3'-RACE (rapid amplification of cDNA ends) products from various tissues yielded a 5.2 kb cDNA encoding a 359 amino acid protein with a calculated molecular mass of 41.5 kDa. Analysis of 3'-RACE products suggested that alternative usage of polyadenylation sites generates two transcripts of 3.9 and 5.2 kb, a result consistent with Northern analysis. Southern analysis demonstrated the existence of two *SCD* loci in the human genome. Chromosomal mapping localized one locus to chromosome 10, and the second locus to chromosome 17. Characterization of genomic clones isolated from chromosome-specific libraries revealed that only the locus on chromosome 10 contained introns. Sequence analysis of the intron-less locus displayed

multiple nucleotide insertions and deletions, as well as in-frame stop codons. Reverse transcriptase-PCR analysis performed with primers specific to the intron-less locus failed to produce a PCR product from brain, liver and skin RNA, indicating that the locus on chromosome 17 is most likely a transcriptionally inactive, fully processed pseudogene. These results suggest strongly that there is one structural *SCD* gene in the human genome, and that it generates two transcripts by use of alternative polyadenylation sites. Although the primary sequence and intron-exon structure of *SCD* is phylogenetically conserved, divergence between rodent and human is seen in the number of *SCD* genes and in the generation of alternative transcripts, suggesting a species-specific component of *SCD* regulation and function.

Key words: enzymes, *Homo sapiens*, lipid metabolism, mono-saturated fatty acid, oleic acid.

INTRODUCTION

Stearoyl-CoA desaturase (Scd, EC 1.14.99.5) is an iron-containing enzyme that catalyses the oxidation of palmitoyl-CoA and stearoyl-CoA at the Δ^9 position to form the mono-unsaturated fatty acyl-CoA esters, palmitoleoyl-CoA and oleoyl-CoA, respectively [1]. The introduction of the *cis* double bond is catalysed by a set of microsomal electron-transport proteins composed sequentially of NADH-cytochrome *b5* reductase, cytochrome *b5* and the terminal Scd. Electrons flow from NADPH through NADH-cytochrome *b5* reductase to cytochrome *b5* with cytochrome *b5* as the direct electron donor to the desaturase [2]. The rate-limiting step in this reaction is at the desaturase, the complex regulation of which includes diet [3–6], hormones [7–12], temperature [13,14], metals [2,15], peroxisomal proliferators [16,17] and developmental processes [18].

Oleic acid, the principal product of Scd, is the major unsaturated fatty acid in lipid stores of human adipose tissue [19] and in the phospholipids of the red-blood-cell membrane [20]. The ratio of stearic acid to oleic acid has been implicated in the regulation of cell growth and differentiation through effects on cell-membrane fluidity [21] and signal transduction [22]. Increasing the ratio of stearic to oleic acids induces apoptosis in rat ventricular myocytes [23], whereas enrichment of oleate in low-density-lipoprotein particles confers resistance to oxidation [24]. Similarly, oleic acid is an endogenous anti-oxidant present in intestinal mucosa [25]. Furthermore, altered levels of Scd activity and/or mRNA levels correlate with senescence [26] and neoplasia [27,28].

The first mammalian Scd cDNA was isolated from rat liver and consists of a 4.9 kb sequence with a 1.0 kb open reading

frame encoding a 41.4 kDa protein [29]. The mRNA contains an unusually long 3'-untranslated region of 3.5 kb. Two Scd cDNAs, Scd-1 and Scd-2, were isolated subsequently from mouse 3T3-L1 adipocytes [30,31]. Like rat Scd, mouse Scd-1 and Scd-2, encoded by different genes, have identical transcript sizes of 4.9 kb and contain 3.5 and 3.6 kb 3'-untranslated regions respectively. Under normal conditions, mouse *SCD-1* is expressed in adipose tissue whereas *SCD-2* is expressed principally in the brain [31]. The genomic structures for all three genes are very similar, spanning approx. 15 kb and consisting of six exons and five introns [30–32]. To date, several other Scd cDNAs have been isolated from different species, including carp [14], hamster [33], sheep [34] and yeast [35]. Although differences in the molecular masses of these proteins have been reported, all share three regions containing highly conserved histidine motifs [36]. Site-directed mutagenesis revealed that the conserved histidine motifs are essential for the catalytic activity [36]. The postulated role for these histidine residues is to act as ligands for the iron atom that supports the electron-transfer reaction and thus the oxidation-reduction reaction for insertion of the double bond into fatty acids [36].

Despite the importance of lipid metabolism, particularly that of unsaturated fatty acids, in homeostasis of many organ systems, no studies have examined the structure and regulation of human Scd mRNA expression. Furthermore, the genomic organization of the human *SCD* gene has not been determined. Previous studies have resulted in the isolation of a 1.5 kb cDNA fragment from human liver, which was reported in the Genbank database (GenBank accession number, Y13647; no corresponding publication). Li and co-workers reported the isolation of a 0.76 kb partial cDNA from human adipose tissue by PCR

Abbreviations used: RACE, rapid amplification of cDNA ends; *SCD*, Scd, stearoyl-CoA desaturase gene and mRNA/cDNA/protein, respectively; RFLP, restriction fragment length polymorphism; RT, reverse transcriptase.

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analysis using primers derived from the rat cDNA sequence [28]. RNase protection assay indicated that increased Scd mRNA levels were associated with tumours derived from oesophagus, colon and liver [28].

Here we report the cloning and full-length sequencing of the primary transcript of human *SCD*. Our data show, for the first time, that human *SCD* is expressed as two mRNA species (3.9 and 5.2 kb), which are generated by alternative usage of two polyadenylation signals. We describe the mapping of two *SCD* loci, localized to chromosomes 10 and 17, and the discovery that the *SCD* locus on chromosome 17 is a fully processed, transcriptionally inactive pseudogene. We report for the first time the genomic structure of the human *SCD* gene on chromosome 10, which spans 24 kb and is composed of six exons and five introns with an intron–exon organization highly conserved with rat and mouse *SCD* genes. Significantly, both gene number and transcriptional regulation differ markedly between rodent and human, suggesting a species-specific component of regulation and function of unsaturated fatty acid metabolism.

EXPERIMENTAL

Screening of the keratinocyte cDNA library

A human-foreskin keratinocyte cDNA library (Clontech, La Jolla, CA, U.S.A.) was screened using a partial PCR-generated Scd cDNA from human surgical scalp biopsies that was subcloned into pBlueScript (Stratagene, La Jolla, CA, U.S.A.). The probe was labelled with [α - 32 P]dCTP using a random-primer labelling kit from Amersham Pharmacia (Arlington Heights, IL, U.S.A.) according to supplier's instructions. Plaque lift filters were hybridized overnight in hybridization buffer containing $6 \times \text{SSC}$ [where $1 \times \text{SSC}$ is $0.15 \text{ M NaCl}/0.015 \text{ M}$ sodium citrate (pH 7.0)], $5 \times \text{Denhardt's}$, 50% formamide, 0.5% SDS, 0.1 mg/ml sonicated salmon sperm, at 42°C . The filters were washed twice at room temperature for 20 min in $2 \times \text{SSC}/0.1\%$ SDS buffer, and twice in $1 \times \text{SSC}/0.1\%$ SDS buffer at 65°C for 1 h.

3'-RNA end-region determination by 3'-RACE (rapid amplification of cDNA ends)

The 3'-untranslated sequence was obtained by 3'-RACE reaction using a Marathon cDNA amplification kit with Advantage KlenTaq Polymerase Mix according to the manufacturer's instructions (Clontech). In essence, the first cDNA strand was synthesized from poly(A)⁺ RNA of human brain and liver (Clontech), adipose (InVitrogen, Carlsbad, CA, U.S.A.) and human 'scalp hair-plugs' (discarded material from hair-follicle-transplant surgery) employing a 3'-RACE general adaptor primer 1 (AP1) supplied by the manufacturer (Clontech). The 3'-RACE gene-specific primer was 5'-GCTGATCCTCATAATCCCGA-CGTGGC-3' (nucleotides 737–763, see Figure 2, below). The PCR amplification was performed under stringent conditions including: (i) denaturing at 94°C for 1 min; (ii) five cycles of 94°C for 30 s and 72°C for 4 min; (iii) five cycles of 94°C for 30 s and 70°C for 4 min; and (iv) 25 cycles of 94°C for 30 s and 68°C for 4 min in a total volume of $50 \mu\text{l}$. After the cycling was completed, the PCR products were analysed on a 1% agarose gel. The PCR products were subcloned in TA vector pCR2.1 (InVitrogen), and the nucleotide sequences of the cDNA clones were determined.

RNA isolation and Northern-blot analysis

Total RNA was isolated from human scalp hair-plugs using the RNA STAT-60 (Tel-Test 'B', Friendswood, TX, U.S.A.). The

total RNA was resolved by electrophoresis on a 6% (w/v) formaldehyde/ 1.0% (w/v) agarose gel, and transferred on to nylon filters (Hybond-N, Amersham Pharmacia). Hybridization with ^{32}P -labelled probes was carried out at 68°C in ExpressHyb hybridization solution (Clontech) plus 0.1% $\mu\text{g/ml}$ salmon sperm DNA for 1 h. Stringent washes were performed in $2 \times \text{SSC}/1.0\%$ SDS at 65°C for 1 h and blots exposed to BioMax-MR films with an intensifying screen at -80°C . Hybridization of the Northern blots of poly(A)⁺ RNA from various human tissues (MTN blot, Clontech) was performed according to the procedure recommended by the manufacturer.

Southern-blot analysis

Human genomic DNA ($10 \mu\text{g/lane}$; Clontech) was digested with individual restriction enzymes as indicated, electrophoresed on a 0.8% agarose gel, and then transferred on to a nylon membrane (Hybond-N). The membrane was hybridized for 1 h at 68°C in ExpressHyb hybridization solution and 0.1% $\mu\text{g/ml}$ salmon sperm DNA and then washed with $0.1 \times \text{SSC}/0.1\%$ SDS at 65°C for 1 h.

Genomic-library screening

Human genomic-DNA library derived from whole-blood DNA in λ DASH vector (Stratagene) and chromosome-specific libraries LA10NS01 (chromosome 10, *EcoRI*), LA17NS03 (chromosome 17, *EcoRI*), LL10NS01 (chromosome 17, *HindIII*), and LL17NS02 (chromosome 17, *HindIII*; American Type Culture Collection, Rockville, MD, U.S.A.) were screened with ^{32}P -labelled probes containing the entire Scd cDNA transcript. Hybridization, washing and autoradiography were performed as described above for Southern-blotting analysis.

Chromosome mapping of the *SCD* gene

A panel of mono-chromosomal somatic-cell hybrid clones was used for the assignment of the human *SCD* gene locus (Quantum Biotechnologies Inc., Montreal, Canada). The blot was prepared with the *EcoRI* restriction enzyme. Conditions for the hybridization and washing were the same as for the Southern blotting. Hybridization signals were detected by exposure to X-ray film at -80°C for 4 days.

Reverse transcriptase (RT)-PCR analyses

Total RNA ($5 \mu\text{g}$) isolated from human scalp hair-plug or $5 \mu\text{g}$ of total RNA from human brain or liver (Clontech) were reverse transcribed in a random-primed reaction with an RT-PCR kit (Stratagene). The Scd primers used were as follows: a 5' primer (sense, position 263–283) and a 3' primer (antisense, position 526–545). The pseudo-*SCD* gene primers used were: a 5' primer (sense, position 1605–1625) and a 3' primer (antisense, position 1801–1821). PCR amplifications were carried out for 25 cycles, each cycle consisting of 30 s at 94°C , 30 s at 55°C and 40 s at 72°C for both primer pairs. The PCR products were separated by electrophoresis on a 1.3% agarose gel and visualized by ethidium bromide staining. The expected sizes of the PCR fragments were 283 bp for Scd and 217 bp for pseudo-*SCD* gene.

DNA sequencing

The DNA sequences in both directions were determined by the dideoxy chain-termination method using Sequenase version 2.0 (U.S. Biochemical Corp., Cleveland, OH, U.S.A.) or by Yale Sequencing Facility (W. M. Keck Biotechnology Laboratory,

Yale University, New Haven, CT, U.S.A.) with an ABI automatic sequencer model 372 (Applied Biosystems, Foster, CA, U.S.A.).

RESULTS

Cloning of a skin cDNA encoding Scd from a human-foreskin keratinocyte cDNA library

To generate a probe for screening the keratinocyte library, we first amplified a 580 bp PCR product from human scalp hair-plug total RNA using primers derived from human adipose Scd (forward, nucleotides 20–40; reverse, nucleotides 621–641 [28]). The amplified fragment was subcloned and its identity was confirmed by nucleotide-sequence analysis. By screening approx. 1×10^6 plaques, a total of eight overlapping clones were isolated, subcloned and subjected to DNA sequencing. Sequence analysis revealed that all of the clones were partial clones. From two overlapping clones, 34 and 21 (Figure 1), 2005 bp were sequenced, which contained the complete open reading frame flanked by 235 bp of 5'-untranslated sequence and 690 bp of 3'-untranslated sequence (nucleotides 1–2005 in Figure 2). Neither a conserved polyadenylation signal nor a poly(A)⁺ tail were found in the 3'-untranslated region, suggesting that the cDNA sequence was incomplete.

Use of 3'-RACE to isolate the full-length human Scd transcript(s)

To obtain full-length cDNA for human *SCD*, we applied 3'-RACE to poly(A)⁺ RNA isolated from human scalp hair-plugs, adipose tissue, brain and liver using a Marathon cDNA Amplification Kit. The sense primer was designed from the sequence of λ clone 21 (position 737–763 bp in Figure 2). 3'-RACE was

carried out by Advantage cDNA Polymerase Mix, which possesses 3'-5' exonuclease activity to ensure that DNA fidelity is maintained during the amplification process. Two amplification products of ≈ 3.2 and ≈ 4.4 kb were amplified specifically from both liver and brain (Figure 1), suggesting the presence of multiple poly(A)⁺ tails. A 2.5 kb PCR product was amplified from both skin and adipose mRNAs (Figure 1). All PCR products from liver, brain, adipose and skin were subcloned, and completely sequenced in both directions.

The cDNA sequences from all PCR fragments amplified from four different tissues and the λ clones isolated from the human keratinocyte λ cDNA library were all identical in the areas of overlap. We also performed 5'-RACE on liver, brain, adipose and skin mRNA using the general adaptor primer 1 (AP1) and an antisense primer, which complemented the 3'-RACE gene-specific primer. Sequencing analysis revealed that all of the cloned sequences are identical to each other and to λ clone 34 (see Figure 1). These data indicate that there is one open reading frame for human Scd mRNA transcripts from all four tissues examined under the conditions used (see the Experimental section). The complete nucleotide cDNA of human Scd is depicted in Figure 2. It is composed of 5221 bp: 235 bp in the 5'-untranslated sequence, 1080 bp in the open reading frame encoding 359 amino acids, and 3906 bp in the 3'-untranslated region containing two consensus polyadenylation signals (AATAAA) and a poly(A)⁺ tail 14 bp downstream of the most 3' polyadenylation signal. The size of the cDNA sequence corresponded well with that of the 3.2 kb PCR fragment amplified from liver or brain if the internal polyadenylation signal was used. Sequence of the 3.2 kb fragment from both liver and brain revealed that it did contain a poly(A)⁺ tail, which began 18 bp

Figure 1 Isolation of cDNA clones encoding human Scd

λ Clones isolated from human keratinocyte cDNA library are indicated as clones 34 and 21. All other clones are 3'-RACE PCR products. A restriction-endonuclease map of the full-length Scd cDNA is indicated in the lower panel of the Figure (5'–3' reading left to right). Solid black bars denote open reading frames with start and stop codons indicated by arrowheads. The open boxes, hatched boxes and A(n) indicate untranslated regions, polyadenylation signals and tails, respectively. Restriction sites are as follows: B, *Bgl*II (nucleotides 2990, 4181); H, *Hind*III (nucleotides 1834, 3486); P, *Pst*I (nucleotides 255, 321, 4308); RI, *Eco*RI (nucleotide 4846); RV, *Eco*RV (nucleotides 262, 1052). Probes used for Northern blots are indicated as probes A (clone 21) and B.

Figure 2 The cDNA and deduced amino acid sequences of human Scd

The numbering of nucleotides is shown on the left. Amino acids are given by their single-letter code, and are numbered on the right. Sequence used to design the primer for 3'-RACE is underlined. The three histidine-containing motifs are double underlined. Polyadenylation signals are boxed (AATAAA). Possible RNA destabilization sequences are dash boxed (ATTTA). This sequence has been submitted to the GenBank database with accession no. AF097514.

downstream from the internal polyadenylation signal; this suggests that the 3.2 kb transcript originated from alternative poly(A)⁺ signal usage. The 2.5 kb PCR fragment amplified from skin and adipose tissue did not possess a poly(A)⁺ tract or a polyadenylation signal at its 3' terminus despite its isolation from oligo-dT primed cDNA. This suggests that the 2.5 kb fragments resulted from non-poly(A)⁺ tail priming of mRNA. To generate a complete 3'-untranslated sequence from skin mRNA, a nested primer was synthesized from the 3' end of the 2.5 kb PCR fragment (nucleotides 2521–2538 in Figure 2) and was used for a second round of 3'-RACE on skin mRNA. A 2.7 kb PCR fragment was amplified (Figure 1), and sequence analysis indicated that it had a sequence identical with the remainder of the human Scd cDNA sequences.

A 1.5 kb cDNA fragment was isolated previously from human liver and reported in the GenBank database. It contains the entire coding region of Scd plus an upstream region of 208 bp and a downstream 3'-untranslated region of 157 bp ending with 26 bp of As. However, no classic polyadenylation signals were found in the 3'-untranslated region, indicating an incomplete cDNA sequence. The sequence of liver cDNA reported in the

database differs from the sequence reported here at a number of locations: eight nucleotides in the coding region (resulting in six variant amino acids in the liver Scd cDNA: Ser-25 → Pro, Arg-26 → Gly, Leu-224 → Met, Thr-320 → Asn, Cys-326 → Trp and Ala-333 → Thr); three positions in the 5'-untranslated region; and 17 positions in the 3'-untranslated region. At no time were we able to isolate a clone representing the liver cDNA deposited previously in the database. We also compared the partial adipose Scd cDNA sequence (which differs from the liver cDNA sequence) with our Scd cDNA sequence. Differences (15 bp) were found in the areas of overlap resulting in a total of six amino acid changes in the adipose Scd cDNA, which include: Leu-5 → Met, Asp-8 → Glu, Ser-25 → Pro, Arg-26 → Gly, Leu-224 → Met and Phe-237 → Cys.

A GenBank database search using Blast [37] revealed that our Scd cDNA sequence was highly homologous with those of mouse Scd-1 and Scd-2. The nucleotide and deduced amino acid sequences of human Scd were compared with those of mouse Scds. Within the open reading frame, the nucleotide identity between the human Scd sequence and mouse Scd-1 and Scd-2 were 85 and 82 %, respectively. Two of the histidine-containing

Figure 3 Northern-blot analysis of *Scd* expression in human tissues

Human multiple-tissue Northern blots (Clontech) containing a minimum of 2 μ g of poly(A)⁺ RNA per lane and total RNA (25 μ g) from human scalp were hybridized with ³²P-labelled probes. (A) Probe A (clone 21, see Figure 1). (B) Blots were stripped and re-probed with ³²P-labelled PCR-generated probe B (see Figure 1). β -Actin probe (β -actin) indicates equal loading of mRNA. Tissues in the multiple-tissue blot are indicated as follows: lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; and lane 8, pancreas. Lane 9 is human scalp hair-plug total RNA. RNA molecular-mass markers (kb) are indicated on the left. The blots were exposed for 24 h at -80°C .

regions (amino acids 120–126 and 157–171, Figure 2) of human *Scd* were 100% identical with those of *Scd*-1 and *Scd*-2. The third histidine-containing region (amino acids 294–308, Figure 2) was 87% identical to both *Scd*-1 and *Scd*-2, suggesting the importance of those regions. Furthermore, the spacing of these histidine regions is conserved between human and mouse *Scds*. The 5'-untranslated region of human *Scd* was also highly homologous to mouse *Scd*-1 and *Scd*-2, showing 70 and 81% nucleotide identities, respectively. In addition, like mouse *Scd*-1 and *Scd*-2, human *Scd* has a long 3'-untranslated region; this region shows a relatively low sequence identity with that of mouse *Scd*-1 (68%) and *Scd*-2 (68%).

Northern-blot analysis and characterization of the 3'-untranslated region of the *Scd* transcripts

To confirm the size of full-length transcript(s) obtained with 3'-RACE and to determine the pattern of human *Scd* expression, Northern-blot analysis of poly(A)⁺ mRNA from multiple human tissues (MTN blot) and total RNA from human scalp hair-plugs was performed. Hybridization with probe A (clone 21, Figure 1; nucleotides 283–2005, Figure 2) and probe B (located at the most 3' end of the *Scd* cDNA sequence, Figure 1; nucleotides 4390–5190, Figure 2) indicated that the two *Scd* transcripts of 3.9 and 5.2 kb arise from differential usage of two distinct polyadenylation signals that are separated by 1.2 kb in all the tissues examined (Figure 3). Indeed, probe A, which is located 5' to the proximal polyadenylation signal, hybridized with both the 3.9 and the 5.2 kb transcripts (Figure 3A) whereas probe B, located 3' to the internal polyadenylation signal, hybridized only with the 5.2 kb species (Figure 3B). There were other hybridization bands observed in liver tissue with probe B (Figure 3B, lane 5). Equal loading of mRNAs in the multiple-tissue blot was confirmed by re-probing for human β -actin (Figure 3A, lower panel). *Scd* appeared to be expressed ubiquitously, suggesting a fundamental role for the protein. However, the overall level of *Scd* transcripts differed among tissues, with high levels in brain and liver (Figure 3A, lanes 2 and 5, respectively), intermediate levels in heart and lung (Figure 3A, lanes 1 and 4, respectively) and low levels in placenta, skeletal muscle, kidney and pancreas

Figure 4 Southern-blot analysis of the human *SCD* loci

Sample of human genomic DNA (10 μ g) digested with restriction endonucleases *Bgl*II, *Eco*RI, *Eco*RV, *Hind*III and *Pst*I were electrophoresed, blotted and hybridized sequentially with three different probes; p1 (left), p2 (middle) and p3 (right). Locations of probes with respect to the *SCD* gene are indicated in Figure 8. Sizes in kb are indicated on the left.

(Figure 3A, lanes 3, 6, 7 and 8, respectively). The skin sample (Figure 3A, lane 9) cannot be compared directly here for overall level of *Scd* transcripts since it represents total RNA; although, on multiple-tissue blots of total RNA, skin was found to express intermediate levels of *Scd* transcripts (results not shown). It must be noted, in addition, that the relative concentrations of the two transcripts differed among tissues, with the 5.2 kb transcript being most abundant in brain and the 3.9 kb transcript more abundantly expressed in heart and skin.

Genomic analysis of the human *SCD* gene

Multiple genes encoding *Scds* have been isolated and characterized in the mouse and rat genomes [30–32]. As a first step in elucidating the human genomic structure of the *SCD* gene(s) in the human genome, we performed a Southern-blot analysis of genomic DNA digested by *Bgl*II, *Eco*RI, *Eco*RV, *Hind*III and *Pst*I and hybridized sequentially with three ³²P-labelled probes; p1, p2 and p3 derived from human *Scd* cDNA (Figure 4, left, middle and right panels, respectively). Probe p1, a 283 bp fragment, corresponds to nucleotides 263–545; p2, a 206 bp fragment, corresponds to nucleotides 677–882, and probe p3 is a 801 bp fragment representing the most 3' end of the *Scd* cDNA sequence (nucleotides 4390–5190) and harbouring an *Eco*RI restriction-enzyme site at bp 4847 (the locations of the probes are depicted in Figure 8B, see below). All three probes yielded two hybridization bands in all restriction-endonuclease digests with the exception of probe p3 hybridizing to three bands in the *Eco*RI enzyme digest (Figure 4, right panel, *Eco*RI). This difference is due to the presence of an *Eco*RI site in the *Scd* cDNA sequence and thus accounts for two fragments of 2.5 and 4.0 kb generated by *Eco*RI digestion. These results indicated that there are two *SCD* loci in the human genome and suggested

Figure 5 Chromosome mapping of *SCD* loci

Southern-blot analysis performed on DNA derived from somatic-cell hybrids containing individual human chromosomes is depicted in lanes 1–22, X and Y in a mouse or hamster background using probes p1 (upper panel) and p3 (lower panel). Probes p1 and p3 were the same as in Figure 4. Genomic DNAs from human, mouse and hamster are also indicated; mix indicates a mixture of mouse and human genomic DNA. DNA size markers (in kb) are indicated on the left.

that the three bands detected by p3 in *EcoRI* digest highlight an *EcoRI* restriction fragment length polymorphism (RFLP) in one of the loci.

Chromosome mapping of *SCD* genes using a chromosome-specific Southern blot

We next attempted to determine the chromosomal location of human *SCD* loci using *EcoRI*-digested DNA from a human–rodent mono-chromosomal cell hybrid panel with probes p1 and p3 (Figure 5). The 5.6 kb band identified from total human genomic DNA with p1 (Figure 5, upper panel; human, compare with Figure 4, left panel, *EcoRI*) was present only in the lane that contained human chromosome 10 genomic DNA (cell line 001AH; Figure 5, upper panel, lane 10). The 8.8 kb hybridization band identified in total human genomic DNA with p1 (Figure 5, upper panel; human, compare with Figure 4, left panel, *EcoRI*) was located in a lane containing chromosome 17 genomic DNA (cell line A71AR; Figure 5, upper panel, lane 17). When the blot was stripped and rehybridized with probe 3, two hybridization bands of 2.5 and 4.0 kb identified in total genomic DNA (Figure 5, lower panel; human, compare with Figure 4, right panel, *EcoRI*), were localized on chromosome 10 (Figure 5, lower panel, lane 10). Probe p3, specific to the 3' end of the *Scd* cDNA sequence, hybridized to the same 8.8 kb *EcoRI* fragment as p1, which is specific to the beginning of the *Scd* coding sequence, suggesting the *EcoRI* site at bp 4846 is absent in the *SCD* locus on chromosome 17 (Figure 5, lower panel, lane 17). This RFLP (*EcoRI*) indicates that the cDNA is derived from the locus on chromosome 10.

Genomic characterization of the *SCD* loci on chromosomes 10 and 17

To characterize the genomic structures of the two *SCD* loci localized on chromosomes 10 and 17, we first used PCR to

amplify the introns from genomic DNA of human–rodent somatic cell hybrids containing chromosomes 10 and 17 with human-specific primers derived from various regions of the cDNA sequence. To our surprise, reactions performed on chromosome 17 genomic DNA with different cDNA primers consistently yielded one PCR product migrating at the predicted mobility of the cDNA. These results suggested that the *SCD* locus on chromosome 17 contained no intervening sequences (results not shown). In contrast, PCR fragments ranging in size from 0.7 to 4.0 kb were amplified from chromosome 10 genomic DNA with appropriate primers, suggesting that the *SCD* locus on chromosome 10 contains introns (results not shown). To further characterize the *SCD* gene locus on chromosome 17, we screened two endonuclease-digested chromosome 17-specific phage libraries (*EcoRI* and *HindIII*) with ³²P-labelled cDNA probes consisting of the entire *Scd* transcript. A total of 10 independent clones were isolated from the *HindIII*-digested library and no positive clones were isolated from the *EcoRI*-digested library. Based on PCR analysis with different regions of cDNA and restriction-enzyme mapping, we divided the 10 independent clones into two groups, each consisting of five identical clones. One clone from each group, clone 4 and clone 14, were selected for complete DNA sequencing analysis. Clone 4, which was 3.0 kb in length, contained an uninterrupted linear sequence similar to human *Scd* cDNA consisting of a 1437 bp 5'-untranslated region, a recognizable complete coding region and a 525 bp 3'-untranslated region. The overlapping areas of nucleotide sequence of clone 4 were compared with human *Scd* cDNA sequence: the comparison of nucleotides 1203–2521 of clone 4 and nucleotides 1–1317 of *Scd* cDNA is shown in Figure 6. As compared with *Scd* cDNA, clone 4 contained multiple genetic lesions, including substitution, deletion and insertion sites that resulted in two premature in-frame stop codons. The nucleotide identity was 96% between clone 4 and *Scd* cDNA sequence in the overlapping region. Clone 14, 5.6 kb in length, is distinguished from clone 4 by a *HindIII* restriction-enzyme site (nucleotides

Figure 6 Sequence of ψ SCD compared with Scd cDNA

The upper line represents the sequence of ψ SCD. The lower line represents the sequence of the Scd cDNA. Vertical lines correspond to nucleotide identity between the ψ SCD and Scd cDNA. Deletion and insertions are shown by the dots (...). Alignment begins with nucleotide 1 and ends with 1318 of the Scd cDNA sequence. The start (ATG) and stop (TGA) codons of Scd cDNA are boxed. The two in-frame stop codons (TGA) of ψ SCD are dash boxed. The nucleotide sequences used to design the specific primers (forward and reverse orientations) for Scd and ψ SCD are underlined and double underlined, respectively.

Figure 7 RT-PCR using total RNA isolated from human tissues with Scd- and ψ SCD-specific primers

Following incubation with (+) or without (–) RT, cDNA was amplified by PCR using primers specific to either Scd (A) or ψ SCD (B). Each PCR reaction (20 μ l) was loaded on a 1.2% agarose gel, electrophoresed and stained with ethidium bromide. B, brain; L, liver; S, human scalp hair-plug. In addition to the RT-PCR reactions, positive-control PCR reactions with Scd- and ψ SCD-specific primers were carried out on 0.01 μ g of Scd cDNA (A) and 0.01 μ g of ψ SCD (B) respectively. M, DNA markers (100 bp ladders), indicated on the right.

1834–1839, Figure 2) and contained an uninterrupted linear sequence that shared 95 % nucleotide-sequence identity with the human Scd cDNA sequence from bp 1834 to 5219 (results not shown). Furthermore, these results suggest that clones 4 and 14 represent a processed pseudogene for human SCD (ψ SCD) and that the structural human SCD genomic locus is localized on chromosome 10.

RT-PCR using ψ SCD- and Scd-specific primers

In order to determine whether ψ SCD is transcriptionally active, RT-PCR was performed on human brain, liver and skin RNAs. PCR primers were designed to specifically amplify either ψ SCD or Scd. The nucleotide sequences of the primers and the points of divergence are shown in Figure 6. Figure 7 shows a representative result of an RT-PCR reaction using these primers and RNAs from brain, liver and skin. The expected 283 bp PCR fragment was amplified from human brain, liver, and skin RNAs using Scd-specific primers (Figure 7A). No amplification products were visualized in the RT-PCR reaction containing the primers specific for ψ SCD (Figure 7B). To demonstrate whether the primers were able to amplify a correct-size band under the

Figure 8 Genomic organization of the human *SCD* gene

(A) Overlapping genomic λ phage clones (clones 19 and 4 from *EcoRI* and *HindIII*-digested chromosome-10 specific libraries) and clones 1C, 13 and 3A (human genomic library) that encode the human *SCD* gene are aligned relative to the exon–intron organization of the gene, below. (B) Relative locations of the *SCD* exons within the gene. Exons are depicted schematically as boxes: black boxes indicate a coding region; open boxes represent non-translated regions. Exon numbers are indicated above the boxes. Restriction endonucleases *EcoRI* (E) and *HindIII* (H) are indicated by vertical lines. Probes (p1, p2, and p3) used for the genomic Southern-blot analysis in Figures 4 and 5 are indicated above the boxes. The black and white portions in the cDNA indicate the coding and 5'- or 3'-untranslated regions, respectively. The three histidine-containing regions are indicated by striped boxes.

conditions used, we performed PCR using 0.01 μ g of *Scd* cDNA (Figure 7A, *SCD*) or ψ *SCD* DNA (Figure 7B, ψ *SCD*). Negative controls were incubated without RT to discount the possibility of genomic DNA contamination. No amplification products were found. The results suggest that ψ *SCD* either is transcriptionally inactive or produces a highly unrelated transcript.

Structural organization of the human *SCD* gene

Having established that the locus on chromosome 17 was a processed pseudogene, we next set out to determine the genomic structure of the functional *SCD* gene on chromosome 10. We first screened two endonuclease-digested chromosome 10-specific phage libraries (*EcoRI* and *HindIII*) with the same probes used for the chromosome 17-specific library. Three identical λ clones, clones 19, 20 and 21, were isolated from the *EcoRI*-digested chromosome 10-specific phage library. Clone 19 was selected for detailed analysis by restriction-endonuclease mapping, Southern-blotting and nucleotide sequencing. One clone, clone 4, was isolated from a *HindIII*-digested library. Clone 19, which is 5.9 kb in length, contains approx. 20% of the 5'-coding region of *SCD*, including approx. 3.7 kb of 5'-untranslated region/promoter region and 2.2 kb of nucleotide sequence extending in the 3' direction (Figure 8). Clone 4, 5.9 kb in length, contained the central region of the *SCD* open reading frame. To identify the remaining *SCD* coding sequence, we screened a human genomic library with the same probes used for the

chromosome-specific libraries. A total of 10 independent λ genomic clones were isolated and characterized. Restriction-enzyme mapping revealed that none of the clones represented the ψ *SCD* gene. The genomic clones overlapped with clone 19, clone 4 and with each other to cover the rest of coding region of the *SCD* locus. Clones 1C, 13 and 3A, which contained inserts of 11, 11.5 and 7.7 kb in size respectively, were chosen for detailed analysis (Figure 8A). The total genomic DNA sequence contained within these clones was approx. 24 kb and the restriction-enzyme maps of *EcoRI* and *HindIII* is shown in Figure 8(B). The localization of exon–intron boundaries and the size of the introns were determined either by DNA sequencing or estimated by agarose-gel electrophoresis of PCR-generated DNA fragments using primers complementary to the flanking exons and the genomic λ clone as templates. The organization of the human *SCD* gene is very similar to that reported for the mouse *SCD-1* and *SCD-2* and the rat *SCD* [30–32] consisting of six exons and five introns. The sequence of the exons in the gene was identical to that obtained for the cDNA, indicating that the genomic clones encoded the human *SCD*.

Table 1 shows the sizes of introns and the intron–exon splice-junction sequences in the coding region of the *SCD* gene. As shown, exon sizes range from 131 (3rd exon) to 4106 bp (6th exon) and they are interrupted by five introns varying in size from 0.7 to 4.0 kb. The exon–intron boundaries conform to typical splice-donor and acceptor-consensus sequences [38]. Each splice-donor site begins with an invariant GT dinucleotide,

Table 1 Exon–intron boundaries of the human *SCD* gene

Lower-case letters indicate intron sequence, upper — case letters indicate exon sequence.

Exon		Exon–intron boundaries		
Number	Position in cDNA	5'-Donor site	Intron size (bp)	3'-Acceptor site
1	1–262	GACGATgtagtt	700	ttccagATCTCT
2	263–545	TTTGGGgtaagc	4000	tgccagGGGTAT
3	546–676	TTCCAGgtaaga	2000	ccccagAATGAT
4	677–882	GAGGAGgtgagt	2000	atgtagGATCTA
5	883–1115	CTGTGGgtaagt	3500	tttcagGTGAGG
6	1116–5221			

whereas each splice-acceptor site ends with an invariant AG dinucleotide. The human *SCD* gene intron splice phasing is type 0 (the intron occurs between codons) for introns 1 and 3, type 1 (intron interrupts bases 1 and 2 of the codon) for introns 2 and 5, and type 2 (intron interrupts bases 2 and 3 of the codon) for intron 4.

DISCUSSION

There is a growing list of genes reported to encode Scd in different species such as carp [14], mouse [30,31], rat [32], sheep [34] and yeast [35]. However, little information is available concerning human Scd with respect to tissue expression, transcript regulation and genomic structure. This report is the first to document the isolation of the full-length Scd cDNA and to characterize the *SCD* gene.

The cDNA sequence reported here represents a full-length transcript because (i) Northern-blot analysis using a probe from a common region of the cDNAs recognized two mRNAs of about 3.9 and 5.2 kb, the sizes of which correlate well with the cDNA clones, (ii) two different polyadenylation sites were found in the Scd cDNA sequence corresponding well to the sizes of these two transcripts, and (iii) a 15 bp poly(A)⁺ tail was identified at the 3' terminus of the cDNA. We have also demonstrated that the 3.9 and 5.2 kb Scd transcripts arise as a consequence of the two different polyadenylation signals, indicating that the two differentially expressed transcripts encode the same 41.5 kDa polypeptide. The function of alternative polyadenylation sites in the 3'-untranslated region is not clear; however, since *SCD* responds to many stimuli including nutrient intake, hormones and temperature, this dynamic response may require additional transcriptional control, such as alternative polyadenylation usage. The alternative transcripts may differ in stability or translatability, thus allowing rapid and efficient response to changes in cellular environment [39]. Indeed three AUUUA sequences were found within the 3'-untranslated region, including one in the 3.9 kb transcript and two in the 5.2 kb transcript. This sequence has been identified as the 'mRNA instability' signal that is involved in mRNA decay [40,41]. Alternative poly(A)⁺ signal usage and tissue-specific distribution of mRNA isoforms is a regulatory mechanism utilized by the cationic amino acid transporter [42] and eIF-2 α genes [43], both of which require rapid response to cell growth. Similarly, Scd transcripts may increase transiently in rapidly growing cells in order to maintain fluidity of expanding cellular membranes. Indeed, hepatic Scd mRNA increases when clofibrate is administered to mice, a treatment that causes rapid proliferation of organelles and plasma membrane [16]. Considering the fact that the ratio of 3.9 and 5.2 kb Scd transcripts varies dramatically in different human

tissues, we speculate that alternative usage of two different polyadenylation sites in Scd could be a mechanism for regulating Scd activity in different tissues. In contrast, the rat Scd, mouse Scd-1 and mouse Scd-2 cDNAs all have a single functional polyadenylation signal sequence (AATAAA), approx. 24–30 nucleotides from the poly(A)⁺ track [30–32]. It would appear that the first polyadenylation signal found in the human primary transcript is absent in the rat and mouse. This difference may be related to the fact that the mouse has at least two structural *SCD* genes whereas humans only have one structural *SCD* gene. Thus despite conservation of a long 3'-untranslated region in human and rodent, different mechanisms of transcriptional regulation have evolved. The species-specific number of *SCD* genes and differences in transcriptional regulation suggest that rodent and human may utilize oleic acid in functions that are unique to each species. In support of this, Scd mRNA, as detected by *in situ* hybridization, is expressed in overlapping as well as unique patterns in rodent and human skin (results not shown), an organ that shows several species-specific adaptations. Relevant to lipid metabolism is the species-specific composition of sebum, the secretory product that covers the surface of the skin [44]. Indeed, it is the sebum-secreting sebaceous gland that shows differential expression of Scd mRNA in rodent and human (results not shown).

Because the sequences reported here diverge from the sequences reported earlier for human liver and adipose tissues, it is important to address the possibility that multiple Scd isoforms are encoded by multiple genes. We found that all PCR fragments amplified from 3'-RACE analysis in four different human tissues or the original λ cDNA clones isolated from human-foreskin keratinocyte cDNA library yielded consistently one single open reading frame. Under no conditions were we able to detect sequences with the magnitude of changes seen in the previously identified liver or adipose sequences. We thus conclude that the sequence reported in Figure 2 is the major product of the *SCD* gene. Further support for this conclusion was provided by Northern-blot analysis. Because no detectable hybridization bands other than the 3.9 and 5.2 kb transcripts were observed with probes covering almost the entire Scd transcript, this implied that there could not be alternative spliced isoforms in the human tissues examined.

To further test the possibility of multiple *SCD* genes, we undertook genomic analysis. Southern blotting of human genomic DNA digested with different restriction endonucleases demonstrated clearly the presence of only two loci in the human genome (Figure 4). This conclusion was further substantiated by chromosome mapping of a mono-chromosomal somatic-cell-hybrid Southern blot that mapped the two loci to chromosomes 10 and 17 (Figure 5). During the course of elucidating the genomic structures of each locus, we discovered that the *SCD* locus on chromosome 17 is a processed pseudogene (Figure 6). The pseudogene sequence, in comparison with the corresponding mRNA, appeared to accumulate 30 random point mutations and three nucleotide insertions in the coding region, including a C $\rightarrow$ T and a C $\rightarrow$ A mutation at bp 1815 and 1817, which creates a predicted in-frame stop codon in the middle of the first histidine cluster motif. In addition, primers that amplify selectively the pseudogene failed to produce a PCR product in any tissue tested in the RT-PCR experiments, indicating that this gene appears to be transcriptionally inactive (Figure 7). Although we cannot rule out transcription of the *SCD* pseudogene in any other tissues, we can predict that if a protein is encoded from such a transcript, it would be significantly truncated, containing none of the histidine-consensus motifs and, in all probability, therefore functionally inactive. These results, taken together, led us to conclude that

there is only one structural *SCD* gene and that it is on chromosome 10. The localization of the structural *SCD* gene on human chromosome 10 was further confirmed by the isolation of several individual genomic clones from the two chromosome 10-specific libraries. In addition, the two mouse genes *SCD-1* and *SCD-2* co-localize to chromosome 19 [45], which has a syntenic region on human chromosome 10. In light of our results, we believe that the disparate sequences reported for human *Scd* between laboratories are not due to the presence of different isoforms generated by alternative splicing or by different genes.

As a first step towards studying the mechanism of *SCD* gene regulation, we have analysed the genomic organization of the gene which encodes the human *Scd* protein. To our knowledge, this is the first report describing the organization of the human *SCD* gene. The gene spanning 24 kb is organized into six exons and five introns, with coding sequence distributed in all of the exons. As expected, differences were found in length among the introns in the genes of human and mouse. The human *SCD* exon structure, in terms of size, looks surprisingly similar to that of mouse *SCD-1* and *SCD-2* and rat *SCD*. Particularly, the intron-exon boundaries in the human gene correspond exactly to the locations of those in the mouse *SCD-1*. Although the functional significance of this conservation is unknown, it may reflect conserved protein-domain organization, since exon structure may, in many cases, represent functional domains of proteins [38]. Previous primary structure analysis of seven desaturase sequences isolated from different species predicted that all would generate hydrophobic proteins each containing two transmembrane domains and three histidine-containing regions localized to the cytoplasmic face of the endoplasmic reticulum [36]. The conserved histidine-containing regions have a consistent distance with respect to these membrane-spanning domains in all the sequences compared [36]. Comparison of the genomic structure with the proposed protein structural domains of human *SCD* reveal an absolute correlation between the regions encoding the three histidine-containing regions (exons 3, 4 and 6, respectively; Figure 8). The locations of the intron-exon boundaries in the corresponding part of the gene suggest a close relationship between exon boundaries and protein domains. Further work will be required on this aspect of the *SCD* gene and on its relevance for regulating the structural and catalytic properties of the enzyme.

In summary, we have isolated and characterized the human *SCD* gene and shown conclusively that a single gene encodes the two *Scd* transcripts. Genomic mapping localized this gene to human chromosome 10. In addition, we have identified a fully processed pseudo-*SCD* gene localized to chromosome 17 that is transcriptionally inactive. The successful cloning of the full-length cDNA encoding human *Scd* will allow for a more comprehensive investigation of the transcriptional regulation of *SCD* than was possible previously. Moreover, the present studies provide a molecular framework for future studies on the mechanism involved in the regulation of this gene and will allow investigation into the role of oleic acid in conserved as well as species-specific lipid metabolism.

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