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Communication

Molecular cloning and expression analysis of a new gene for short-chain dehydrogenase/reductase 9

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We report here the cloning and characterization of a novel human short-chain dehydrogenases/reductase gene *SCDR9*, isolated from a human liver cDNA library, and mapped to 4q22.1 by browsing the UCSC genomic database. *SCDR9* containing an ORF with a length of 900 bp, encoding a protein with a signal peptide sequence and an adh_short domain. GFP localization shows SCDR9 protein concentrated in some site of the cytoplasm, but not in the ER. Expression pattern in eighteen tissues revealed that *SCDR9* is expressed highly in liver. Soluble recombinant protein was successfully purified from *Escherichia coli* using pET28A(+) expression vector. Our data provides important information for further study of the function of the *SCDR9* gene and its products.

Keywords: short-chain dehydrogenase/reductase, liver, clone

INTRODUCTION

The short-chain dehydrogenase/reductase (SDR) superfamily comprises a large group of functionally diversed proteins expressed in prokaryotes and eukaryotes spanning from bacteria to mammals and presently contains at least 3000 members. It was initially termed "insect-type alcohol dehydrogenase family", but later renamed to "short-chain dehydrogenase/reductase family", indicating the shorter polypeptide chain length of SDR enzymes compared with the classical zinc-containing alcohol dehydrogenases of the "medium-chain dehydrogenase/reductase superfamily" (MDR). The average chain-length of typical SDR enzymes was initially determined to be about 250 amino-acid residues (Persson *et al.*, 1991; Jornvall *et al.*, 1995). However, since the number of available SDR structures increased, a considerable variation of this parameter is now evident due to the occurrence of large multidomain proteins with an integrated SDR domain (e.g. 17β-hydroxysteroid dehydrogenases 4) and proteins with additional N- or C-terminal hydrophobic parts, considered to be responsible for membrane attachment to lipid membranes (e.g. of the endoplasmic reticulum) (Oppermann *et al.*, 1997). By a functional assignment scheme based on specific sequence motifs and coenzyme specificity, the SCDR superfamily can be divided into five families: classical, extended, intermediate, divergent, and complex (Persson *et al.*, 2003). Although residue identity between different forms usually does not exceed 15–20%, their three-dimensional structures are very similar (Oppermann *et al.*, 2003). Most SCDR enzymes have a conserved

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Abbreviations: AKR, aldo-keto reductase; BLAT, BLAST-like alignment tool; DHRS8, dehydrogenase/reductase (SDR family) member 8; EST, expressed sequence tag; 17β- HSD, 17β-hydroxysteroid dehydrogenases; IPTG, isopropyl β-D-thiogalactosidae; SCDR, short-chain dehydrogenase/reductase; ORF, open reading frame.

TGxxxGxG coenzyme (NAD(P)$^+$ or NAD(P)H) binding region at the N-terminus and a specific YXXXK motif functions as the active site (Jornvall *et al.,* 1995). SDR proteins mediate the metabolism of a wide range of substrates including steroids, flavonoids, retinoids, aldehydes, ketones, sugars, and polycyclic aromatic hydrocarbons, and thus may serve to modulate intercellular and intracellular signaling pathways.

An important hydroxysteroid dehydrogenases family is the 17β-hydroxysteroid dehydrogenase family which takes action at position 17 and modulates the biological potency of estrogens and androgens. Until now, there are ten members identified in human. All members belong to the SDR superfamily except for 17β-hydroxysteroid dehydrogenase 5, which belongs to the AKR family (Mindnich *et al.,* 2004). Many severe diseases are reported due to their abnormality. For example, mutations in *hsd17B3* gene were found in pseudohermaphroditism (Mendonca *et al.,* 1999); mutations in *HSD17B4* lead to severe phenotypes including hypotonia, facial dysmorphism, psychomotor delay, neonatal seizures, neuronal migration defects and demyelination, and patients usually die during their first year of life (van Grunsven *et al.,* 1998). In addition, in a number of multifactorial diseases like cancer and neuronal diseases, androgens and estrogens seem to play an important role. In colon cancer tissue or colon cancer cell lines, a lower oxidative activity (lower conversion of estradiol to estrone) was observed (Oduwole *et al.,* 2002).This activity could be due to 17β-HSD 2, 4, 8, and 10. Several prostate cancer cell lines express multiple forms of 17β-HSDs, whereas HSD17B2 seems to be either lost or downregulated (Castagnetta *et al.,* 1997). In studies of Alzheimer's disease 17βHSD 10 was found to bind to the β-amyloid peptide and to convert steroid hormones (He *et al.,* 1999). It was suggested that high levels of the enzyme might disrupt steroid hormone homeostasis in synapses and contribute to synapse loss in the hippocampus of the mouse model of Alzheimer's disease (He *et al.,* 2002). The identification and subsequent functional characterization of new short-chain dehydrogenases/reductases may help to identify novel targets for drug development.

We have isolated a novel human cDNA for a short-chain dehydrogenase/reductase from a human adult liver cDNA library. Its deduced protein has a typical SDR domain, and it is highly similar to human dehydrogenase/reductase member 8 (78%). We name the gene human short-chain dehydrogenase/reductase 9 (SCDR9). Here, we report the cloning, mRNA tissue distribution, together with subcellular location, prokaryotic expression and purification of the protein encoded by the human *SCDR9* gene.

MATERIALS AND METHODS

Molecular cloning and bioinformatics analysis. TBLASTN GenBank searches with the reported coding sequence of human dehydrogenase/reductase member 8 (GenBank Accession No. NM_016245) allowed us to identify two EST (AV658935, AV648633) which were shown to have no homology to known genes. These two ESTs were used to screen human dbEST (Altschul *et al.,* 1997), and the identified human ESTs (Accession No. AV658935, AV648633, AV645328, AV682467) were assembled into a contig which contains an open reading frame of 900 nucleotides encoding a protein of 300 amino acids. To verify the sequence of the contig, primers of SCDR9-up (5′-tacacaaggactgaaccagaagg-3′) and SCDR9-down (5′-atcatgcatacatctctggctgg-3′) were designed and synthesized (Bio-Asia biotech Co. Ltd). PCR amplification was performed with these primers and a human liver cDNA library used as template. The PCR conditions were: denaturing at 94°C for 45 s, annealing at 53°C for 45 s, elongating at 72°C for 1 min. The PCR product was cloned into pMD18-T vector (Clontech) and subjected to sequencing. The cDNA sequence of *SCDR9* was used for genomic searching for chromosome localization at http://genome.ucsc.edu. The following tools were used for analysis of the ***SCDR9-encode protein: SMART for domain searching (http://smart.embl-heidelbeg.de) and the OMIGA software for molecular mass prediction. The cellular localization of the protein was predicted by WoLF PSORT (http://wolfpsort.seq.cbrc.jp/). Other analyses were performed by the GeneDoc program.

Expression pattern analysis. The expression pattern of *SCDR9* gene was determined by PCR amplification of cDNA of 18 tissues, including uterus, thymus, spleen, colon, bone marrow, prostate, pancreas, liver, placenta, brain, skeletal muscle, bladder, stomach, kidney, ovary, lung, testis and heart (BioChain Institute, Inc. USA). The forward primer sequence of *SCDR9* was 5′-cgacagagcatattggttctgtgg -3′, and the reverse primer was 5′-ccaatacaggccataatcttgtgct-3′. β-Actin-specific forward primer was 5′-ctcgtgctactctctctttc-3′ and the reverse primer was 5′-catgtctcgatcccacttaac-3′. There are 514 base pairs between the two primers of *SCDR9*. About 29 cycles (for β actin) and 28 cycles (for *SCDR9*) of amplification (1 min at 94°C, 1 min at 59°C and 1 min at 72°C) were performed using *Taq* DNA polymerase. The PCR products were then electrophoresed on 1% agarose.

Prokaryotic expression and purification. Recombinant SCDR9 protein was expressed in *Escherichia coli* in two expression vectors: pGEX-6P-1 (GST-tagged) and pET28A(+)(His-tagged). The SCDR9 coding sequence was generated by PCR amplification using *Pfu* polymerase (Stratagene Clon-

ing Systems, a Jolla, CA, USA). The primers used to generate the sequence were: pGEX-6P-1-SCDR9-up (5′-cgcgaattcatgaacatcatcctag-3′) containing an *Eco*RI restriction site and pGEX-6P-1-SCDR9-down (5′-cggctcgagtcatttcattttgatt-3′) containing an *Xho*I restriction site, and pET28A-SCDR9-up (5′-cgcgaattcatgaacatcatccta-3′) containing an *Eco*RI restriction site and pET28A-SCDR9-down (5′-cggctcgagtcatttcatttga-3′) containing an *Xho*I restriction site. The PCR products were digested with *Eco*RI and *Xho*I, and ligated into the *Eco*RI/*Xho*I restriction sites of the pGEX-6p-1 vector and pET28A(+) vector. The sequence of the construct was verified by sequencing. The correctly inserted vectors were transformed into *E. coli* strain BL21(DE3) and induced for SCDR9 with IPTG for 4 h. Recombinant SCDR9 protein was purified on Ni-NTA Sepharose flow according to manual handbook, respectively. The samples were analyzed by SDS/PAGE.

Subcellular localization. HeLa cells (American Type Culture Collection) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. PCR products of the complete *SCDR9* ORF with *Eco*RI and *Xho*I linker were ligated with the *Eco*RI/*Xho*I sites of pEGFP-N1 and pEGFP-C1 green fluorescence expression vector (Clontech). The PCR primers were: PEGFPN1-SCDR9-up: 5′-ggctcgagatgaacatcatcctag-3′, PEGFPN1-SCDR9-down: 5′-gcgaattcgtttcattttgattttg-3, and PEGFPC1-SCDR9-up: 5′-ggctcgagctatgaacatcatcc-3′, PEGFPC1-SCDR9-down: 5′-ccgaattctcatttcattttgattttg-3′. After 48 h, transfected cells were fixed with 3.7% formaldehyde in PBS for 10 min at room temperature. After fixation, the cells were stained with endoplasmic reticulum-specific fluorescence markers Concanavalin A/Texas Red® conjugate (Molecular Probes, USA), respectively. Fluorescence was viewed with fluorescence optics (Leica DM R2, Germany) and a Leica TCS-NT laser scanning microscope system.

RESULTS

Cloning and sequence analysis of human *SCDR9* gene

PCR product was amplified in human liver cDNA library, cloned into pMD18-T vector (TaKaRa Co. Ltd) and subjected to sequencing. The sequencing result was consistent with the contig, and this gene was subsequently submitted to GenBank with the Accession No. AY268355 and named short chain dehydrogenase/reductase 9 (SCDR9). The assembled cDNA of *SCDR9* is 1040 bp in length, containing an ORF from 15 to 915 nt. The ORF encodes a protein of 300 amino acids with a predicted molecular mass of 33.66 kDa using OMIGA software. It is the ninth short-chain dehydrogenase/reductase reported, so we named it short-chain dehydrogenase/reductase 9 (Fig. 1). The human *SCDR9* gene spans about 17 kb in the human genome and contains two conserved motifs which all SCDR family members have, one is the TGXGXXXG motif related to NAD(P)(H) binding, the other is the YXXXK motif related to its catalytic activity (Jornvall *et al.*, 1995). It is composed of seven exons and six introns, all of the exon–intron junctions are consistent with the AG-GT rule. *SCDR9* is mapped to chromosome 4q22.1 by browsing the UCSC genomic database (http://genome.ucsc.edu). Result of searching in the SMART database showed that SCDR9 protein contains a signal peptide sequence and adh_short domain (37 to 204). Searches of homology in international protein databases of NCBI using Blast service (T) with the deduced protein sequence revealed that human SCDR9 shares a high degree of homology (78%) with dehydrogenase/reductase (SDR family) member 8 (DHRS8, also recognized as 17-β-HSD11). Another surprising finding was that genes *SCDR9* and *DHRS8* are located together in the same chromosome loci in hu-

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  1 GGA CAG AGC AAA GCC ATG AAC ATC ATC CTA GAA ATC CTT CTG CTT CTG ATC ACC ATC ATC TAC TCC TAC TTG GAG TCG TTG GTG AAG TTT
                    M   N   I   I   L   E   I   L   L   L   L   I   T   I   I   Y   S   Y   L   E   S   L   V   K   F
 91 TTC ATT CCT CAG AGG AGA AAA TCT GTG GCT GGG GAG ATT GTT CTC ATT ACT GGA GCT GGG CAT GGA ATA GGC AGG CAG ACT ACT TAT GAA
    F   I   P   Q   R   R   K   S   V   A   G   E   I   V   L   I   T   G   A   G   X   G   I   G     R   Q   T   T   Y   E
                                                                    T   G   X   X   X   G   X   G
181 TTT GCA AAA CGA CAG AGC ATA TTG GTT CTG TGG GAT ATT AAT AAG CGC GGT GTG GAG GAA ACT GCA GCT GAG TGC CGA AAA CTA GGC GTC
    F   A   K   R   Q   S   I   L   V   L   W   D   I   N   K   R   G   V   E   E   T   A   A   E   C   R   K   L   G   V
271 ACT GCG CAT GCG TAT GTG GTA GAC TGC AGC AAC AGA GAA GAG ATC TAT CGC TCT CTA AAT CAG GTG AAG AAA GAA GTG GGT GAT GTA ACA
    T   A   H   A   Y   V   V   D   C   S   N   R   E   E   I   Y   R   S   L   N   Q   V   K   K   E   V   G   D   V   T
361 ATC GTG GTG AAT AAT GCT GGG ACA GTA TAT CCA GCC GAT CTT CTC AGC ACC AAG GAT GAA GAG ATT ACC AAG ACA TTT GAG GTC AAC ATC
    I   V   V   N   N   A   G   T   V   Y   P   A   D   L   L   S   T   K   D   E   E   I   T   K   T   F   E   V   N   I
451 CTA GGA CAT TTT TGG ATC ACA AAA GCA CTT CTT CCA TCG ATG ATG GAG AGA AAT CAT GGC CAC ATC GTC ACA GTG GCT TCA GTG TGC GGC
    L   G   H   F   W   I   T   K   A   L   L   P   S   M   M   E   R   N   H   G   H   I   V   T   V   A   S   V   C   G
541 CAC GAA GGG ATT CCT TAC CTC ATC CCA TAT TGT TCC AGC AAA TTT GCC GCT GTT GGC TTT CAC AGA GGT CTG ACA TCA GAA CTT CAG GCC
    H   E   G   I   P   Y   L   I   P   Y   C   S   S   K   F   A   A   V   G   F   H   R   G   L   T   S   E   L   Q   A
                                    Y   X   X   X   K
631 TTG GGA AAA ACT GGT ATC AAA ACC TCA TGT CTC TGC CCA GTT TTT GTG AAT ACT GGG TTC ACC AAA AAT CCA AGC ACA AGA TTA TGG CCT
    L   G   K   T   G   I   K   T   S   C   L   C   P   V   F   V   N   T   G   F   T   K   N   P   S   T   R   L   W   P
721 GTA TTG GAG ACA GAT GAA GTC GTA AGA AGT CTG ATA GAT GGA ATA CTT ACC AAT AAG AAA ATG ATT TTT GTT CCA TCG TAT ATC AAT ATC
    V   L   E   T   D   E   V   V   R   S   L   I   D   G   I   L   T   N   K   K   M   I   F   V   P   S   Y   I   N   I
811 TTT CTG AGA CTA CAG AAG TTT CTT CCT GAA CGC GCC TCA GCG ATT TTA AAT CGT ATG CAG AAT ATT CAA TTT GAA GCA GTG GTT GGC CAC
    F   L   R   L   Q   K   F   L   P   E   R   A   S   A   I   L   N   R   M   Q   N   I   Q   F   E   A   V   V   G   H
901 AAA ATC AAA ATG AAA TGA ATA AAT AAG CTC CAG CCA GAG ATG TAT GCA TGA TAA TGA TAT GAA TAG TTT CGA ATC AAT GCT GCA AAG CTT
    K   I   K   M   K   *
991 TAT TTC ACA TTT TTT CAG TCC TGA TAA TAT TAA AAA CAT TGG TTT GGC AC
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Figure 1. Nucleotide and amino-acid sequences of SCDR9.
NAD(P)H binding domain and active center are boxed. Asterisk represents the stop codon.

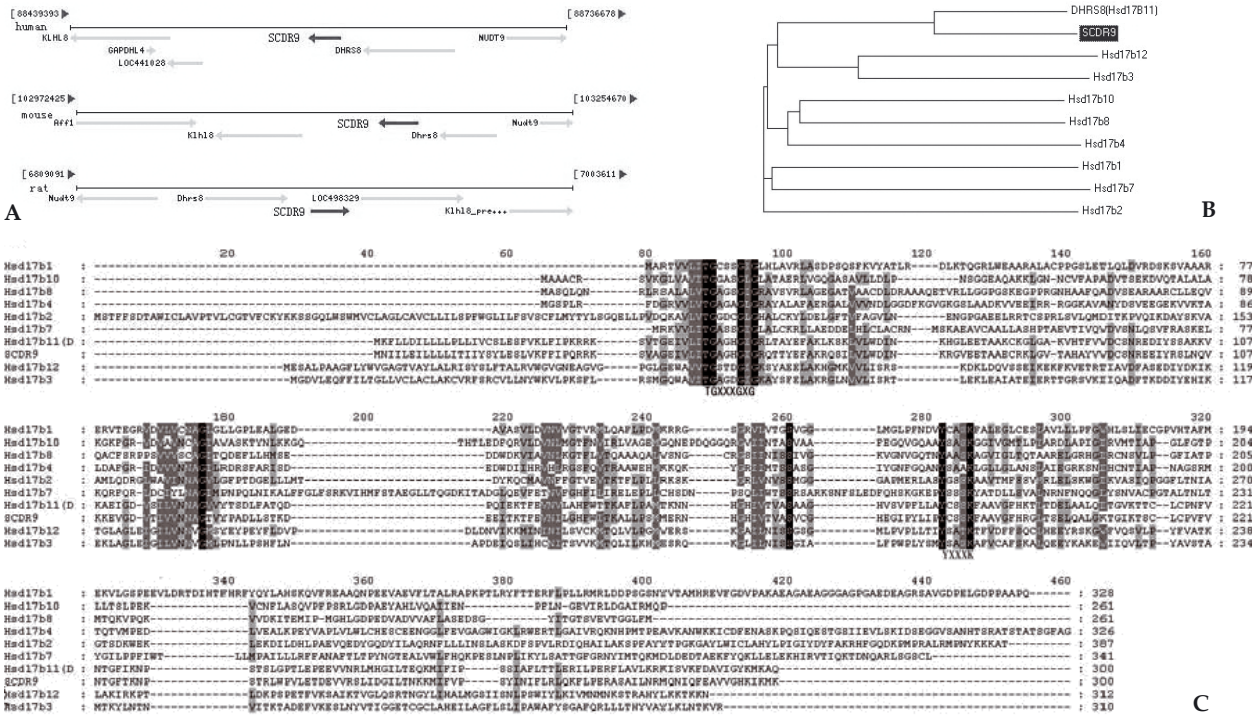


Figure 2. A. Genome location of *SCDR9* and *DHRS8* genes in human, mouse and rat using NCBI BLAST program. These data shows that these two genes are close together in the genome locus. **B and C**. Phylogenetic tree analysis and amino-acid sequence alignments of SCDR9 gene and other human 17β-hydroxysteroid dehydrogenases. Accession numbers of the full-length proteins are: Hsd17B1, NM_000413; Hsd17B2, NM_002153; Hsd17B3, NM_000197; Hsd17B4, NM_000414; Hsd17B7, NM_016371; Hsd17B8, NM_014234; Hsd17B10, NM_004493; DHRS8 (Hsd17B11) NM_016245; Hsd17B12, NM_016142. Note: the tree in Fig. 2B does not include HSD17B5, B6 and B9 because B5 belongs to AKR family while B6 and B9 do not exist in human. In Fig. 2C, in the alignment table only 1–326 amino acids of HSD17B4 are used because it has additional two domains beside the ada_short domain, while other enzymes have a single domain only.

man, mouse and rat (Fig. 2B). Since DHRS8 belong to the SDR type 17-beta HSD family, phylogenetics tree was analyzed (Fig. 2C). The result shows that SCDR9 might belong to the SDR type 17-β-HSD family. Multiple alignment of the SCDR9 gene and other SDR type 17-β-hydrogenases/reductases shows a high conservation of the coenzyme binding site and active center, although their residue identity is low (Fig. 2C) (http://www.ncbi.nlm.nih.gov/blast).

Tissue distribution of *SCDR9* cDNA

The result in Fig. 3 showed that SCDR9 was highly expressed in the liver. Expression in ovary, bone marrow, kidney, brain, lung, skeletal muscle, bladder, testis were also detectable, but at a much lower level. No expression was detected in uterus, spleen, large intestine, pancreas, thymus and colon.

Prokaryotic expression and purification

We used two expression vectors to express the protein in *E. coli* BL21 strain: pGEX-6p-1(GST-tagged) and pET-28A(+)(6×His tagged). The results of SDS/PAGE showed that the molecular mass of GST-tagged recombinant protein was about 58 kDa and of the 6×His-tagged protein is a little more than 31 kDa. Since the GST-tag is 25 kDa, the molecular mass of the SCDR9 protein should be around 33 kDa, similar to the prediction by OMIGA (Kramer, 2001). We manage to purify the protein, but since the pGEX-6P-1-encoded recombinant protein concentrated in inclusion body, only the pET28A(+)-encoded recombinant protein (6×His-tagged) could successfully be purified from the supernatant and was soluble in buffer (Fig. 4).

Subcellular localization

In order to investigate the cellular location of SCDR9 protein, the WoLF PSORT prediction tool was used, the predicted result indicated that SCDR9 is localized in the endoplasmic reticulum (ER), and there was also a high possibility of cytoplasmic localization. To verify whether SCDR is localized in the ER, pEGFP-SCDR9-N1 and pEGFP-SCDR9-C1 were transfected into HeLa cells and the ER-specific marker Concanavalin A/Texas Red was used. As shown in Fig. 5, the SCDR9 protein had little overlaps with ER. The protein concentrates in some site and does not scatter in the cytoplasm. However, the protein does not distribute in the cell nucleus. We

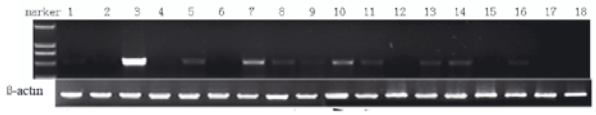


Figure 3. Expression pattern of *SCDR9* in 18 human tissues.
1, Stomach; 2, uterus; 3, liver; 4, spleen; 5, ovary; 6, large intestine; 7, bone marrow; 8, kidney; 9, brain; 10, lung; 11, skeletal muscle; 12, pancreas; 13, bladder; 14, testis; 15, prostate; 16, heart; 17, thymus; 18, colon. As control, the human β-actin gene was also amplified.

got the same result using pEGFP-N1 and pEGFP-C1 vector. Our result shows that prefered subcellular location according to of WoLF PSORT predictions was wrong.

DISCUSSION

In the present study, we isolated a human *SCDR9* cDNA. The short-chain dehydrogenases/reductases family is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. Most members of this family are proteins of about 250 to 300 amino-acid residues, most of them possess at least two conserved sites: the first binds the coenzyme, often NAD or NADP, and the second binds the substrate and catalyze. This gene also contains the two conserved sites. Commonly, there is little sequence identity, within 15–30%, between different SCDR enzymes, but a surprising finding was that DHRS8 protein (also named 17β-dehydrogenases/reductases 11) and SCDR9 protein were highly similar (78%). By searching the UCSC genome database, we found that *DHRS8* and *SCDR9* are both located in chromosome 4q22.1, and are close together in the human, mouse and rat genome (Fig. 2A). Since these two genes also have high nucleotide similarity, it is possible that

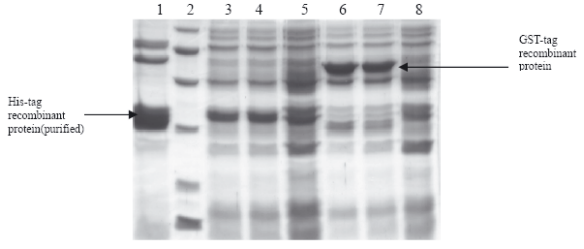
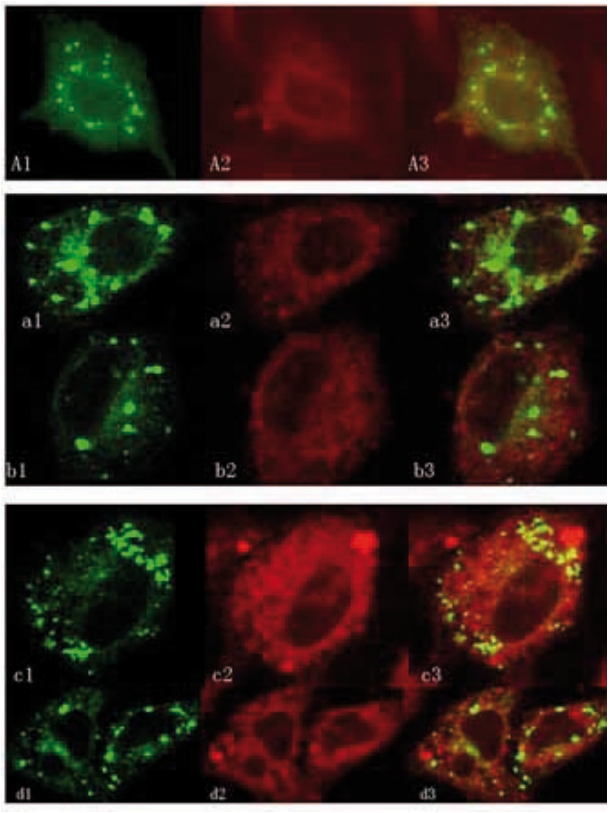


Figure 4. Expression of recombinant SCDR9 protein in *E. coli* strain BL21(DE3).
Coomassie Blue-stained SDS/PAGE gel of the SCDR9 expression construct. Lane 1, purified pET28A(+)recombinant SCDR9 protein (His-tag). Lane 2, marker, from top to botton is 97, 66, 43, 31, 20, 14 kDa. Lane 3 and 4, PET28A-SCDR9 induced by adding IPTG to a final concentration of 0.4 mM. Lane 5, control (no IPTG). Lane 6 and 7, pGEX-6P-1-SCDR9 induced by adding IPTG to a final concentration of 0.4 mM. Lane 8 control (no IPTG).



Figue 5. Subcellular localization of EGFP-SCDR9 in HeLa cells.
A1, a1, b1 are fluorescence images of HeLa cells transfected with pEGFP-N1-SCDR9; c1 and d1 are HeLa cells transfected with pEGFP-C1-SCDR9. Panels A1, A2, A3 are obtained using microscope equipped with fluorescence optics, other images were visualized with a Leica TCS-NT laser scanning microscope system. Panels A2, a2, b2, c2, d2 are the same cell shown in as A1,a1,b1,c1,d1 processed for ER specific fluorescence marker Concanavalin A/Texas Red® conjugate. These pictures show there is little overlapping with ER.

they originated from one single gene. Both express highly in the liver, although their tissue distribution is not exactly the same. In addition, the SCDR9 and DHRS8 proteins are located in the cytoplasm, but the SCDR9 protein is concentrated in some site of the cytoplasm, whereas DHRS8 is distributed more uniformly. SCDR9 has little overlap with the ER marker Con-A/Texas Red, so SCDR9 is not localized in the ER. Previous studies on DHRS8 protein indicated it metabolizes 3α-Adiol to androsterone, so it was also named 17β-dehydrogenase/reductase 11 (Brereton *et al*., 2001; Chai *et al*., 2003). Whether SCDR9 catalyzes the reverse reaction, conversion of androsterone into 3α-Adiol or SCDR9 metabolizes some other substrate needs a further study to make it clear.

The physiological functions of SDR enzymes make some SDR members attractive in pharmacological drug targets. Differential inhibition of 17β-HSDs is similarly interesting in all pharmacological fields aiming at controlling sex steroid metabolism,

e.g. in breast and prostate cancer treatment (Duax *et al.*, 2000; Hoffren *et al.*, 2001). Recent studies have shown that the DHRS8 gene is a cutaneous T-cell lymphoma-associated antigen and suggest it could be a potential target for diagnostic and prognostic purposes (Hartmann *et al.*, 2004). Thus, the SCDR9 gene, which is highly similar and locates close to DHRS8, might have a similar or contrary role in metabolism and tumor formation. Further study should be made to clarify the precise role of SCDR9.

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