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## Isolation and characterization of novel human short-chain dehydrogenase/reductase SCDR10B which is highly expressed in the brain and acts as hydroxysteroid dehydrogenase\*

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Hydroxysteroid dehydrogenase belongs to the subfamily of short-chain dehydrogenases/reductases (SDR), and 11-β-hydroxysteroid dehydrogenase catalyzes the interconversion of inactive glucocorticoids (cortisone in human, dehydrocorticosterone in rodents) and active glucocorticoids (cortisol in human, corticosterone in rodents). We report here the cloning and characterization of a novel human SDR gene SCDR10B which encodes a protein with similarity to 11β-hydroxysteroid dehydrogenase 1. SCDR10B was isolated from a human brain cDNA library, and was mapped to chromosome 19p13.3 by browsing the UCSC genomic database. It contains an ORF with a length of 858 bp, encoding a protein with a transmembrane helix and SDR domain. Its molecular mass and isoelectric point are predicted to be 30.8 kDa and 10.3 kDa, respectively. SCDR10B protein is highly conserved in mammals and fish. Phylogenetic tree analysis indicated that SCDR10B stands for a new subgroup in the 11β-hydroxysteroid dehydrogenase family. Northern blot analysis showed that SCDR10B was highly expressed in brain, and a strong expression signal was detected in hippocampal neurons by immunohistochemical analysis. RT-PCR and immunohistochemical analysis showed that SCDR10B was up-regulated in lung-cancer cell lines and human lung cancer. SCDR10B can catalyze the dehydrogenation of cortisol in the presence of NADP+, and therefore it is a hydroxysteroid dehydrogenase.

Keywords: clone, hydroxysteroid dehydrogenase, brain, expression pattern

## INTRODUCTION

The short-chain dehydrogenase/reductase (SDR) family is a very large family of enzymes, most of which are NAD<sup>+</sup> or NADP<sup>+</sup> dependent oxidoreductases (Kallberg *et al.*, 2002). So far more than 3000 members from all forms of life are found in protein or nucleic acid databases. Members of this

family consist of 250–350 amino-acid residues (Jornvall *et al.*, 1995). All the members of the family only have a residue identity level of typically 15–20%, but two motifs are highly conserved in this superfamily (Oppermann *et al.*, 1997; Filling *et al.*, 2002): one is the GXXXGXG motif related to binding cofactor NAD(P)(H), the other is the YXXXK related to its catalytic activity.

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<sup>\*</sup>The sequence data of SCDR10B and its orthologs have been submitted to the GenBank database under accession numbers: AY634366, AY554048, AY554049, AY578179, AY578810, AY578812, AY578181, DQ862062, DQ862061, DQ832430.

Abbreviations: BLAT, BLAST-like alignment tool; EST, expressed sequence tag; 11 $\beta$ -HSD1 (HSD11B1), 11 $\beta$ -hydroxysteroid dehydrogenase 1; 11 $\beta$ -HSD2 (HSD11B2), 11 $\beta$ -hydroxysteroid dehydrogenase 2; GR, glucocorticoid receptor; IPTG, isopropyl  $\beta$ -p-thiogalactosidae; MR, mineralocorticoid receptor; LB, Luria-Bertani medium; SABC, streptavidin-biotin-peroxidase complex; SDR, short-chain dehydrogenase/reductase family; SCDR, short-chain dehydrogenase/reductase; ORF, open reading frame;  $\beta$ -MG,  $\beta$ -microglobulin.

SDR family members participate in the metabolism of steroids, prostaglandins, retinoids, aliphatic alcohols and xenobiotics. Hydroxysteroid dehydrogenase, a subfamily of SDR that includes 17β-hydroxysteroid dehydrogenase, 11 βhydroxysteroid dehydrogenase and 3<sup>β</sup>-hydroxysteroid dehydrogenase, contributes to the metabolism of steroids. 11β-hydroxysteroid dehydrogenase catalyzes the interconversion of inactive glucocorticoids (cortisone in human, dehydrocorticosterone in rodents) and active glucocorticoids (cortisol in human, corticosterone in rodents). Two isoenzymes (11β-HSD1 and 11β-HSD2) have been identified. 11β-HSD1, also called "liver" type 11β-hydroxysteroid dehydrogenase acts mainly as a NADPH-dependent cortisone reductase (Stewart et al., 1999). The 11β-HSD1 gene is located at chromosome 1q32.2, and is highly expressed in the liver, adipose tissue, bone and the brain (Ricketts et al., 1998; Brereton et al., 2001); it plays an important role in human obesity and insulin resistance (Stewart et al., 1999; Tomlinson et al., 2000; Wolf, 2002). 11β-HSD2, the "kidney" type 11β-hydroxysteroid dehydrogenase, acts as an NAD+-dependent cortisol dehydrogenase. The 11β-HSD2 gene is located at chromosome 16q22, it is highly expressed in classical mineralocorticoid-selective target tissues such as kidney, colon and sweat glands (Diaz et al., 1998; Brereton et al., 2001; Audige et al., 2002; Kataoka et al., 2002). 11β-HSD2 is linked to hypertension and apparent mineralocorticoid excess syndrome (Kotelevtsev et al., 1999; Krozowski et al., 1997; Lin-Su et al., 2004).

Active glucocorticoids (cortisol, corticosterone) and mineralocorticoids (aldosterone) play their roles through binding to their receptors (glucocorticoid receptor, GR; mineralocorticoid receptor, MR), and subsequently regulating the transcription of target genes (Diaz et al., 1998; De Kloet et al., 2000; Rabbitt et al., 2002). Cortisol is the main circulating glucocorticoid in the human and it is mainly synthesized and secreted in the zona fasciculata of adrenal gland. Aldosterone is the principal mineralocorticoid in the human and is mainly synthesized and secreted from the zona glomerulosa of the adrenal gland. The amount of glucocorticoids and mineralocorticoids available to activate GR and MR is not only dependent on the circulating levels but also on prereceptor metabolism by 11β-HSD1 and 11β-HSD2 occurring intracellularly (Rabbitt et al., 2002). 11β-HSD1 can activate glucocorticoids and make them access GR. The level of circulating glucocorticoids is 3 orders of magnitude higher than that of aldosterone (1 µM versus 1 nM, respectively), but glucocorticoids and aldosterone have an equivalent affinity for the mineralocorticoid receptor MR. Under normal physiological conditions glucocorticoids are expected to occupy the MR and regulate gene transcription,

instead of aldosterone. However, the enzyme 11 $\beta$ -HSD2 is coexpressed with the mineralocorticoid receptor, and unlike glucocorticoids, aldosterone does not react with 11 $\beta$ -HSD2. Thus, aldosterone can gain access to MR in epithelial and non-epithelial tissues and regulate transcription of genes that contribute to the mineralocorticoid response (Funder, 2005).

It was previously reported that GR and MR are expressed in the brain (Diaz et al., 1998; De Kloet et al., 2000; Poletto et al., 2006). In fact, the rat mineralocorticoid receptor was actually first cloned from brain cDNA (Patel et al., 1989). In addition, some brain areas can locally produce aldosterone. MR sites, and their encoding mRNA, are highly expressed in the hippocampus (Watzka et al., 2000) and lateral septum, and in several brain stem nuclei, such as the motor XI nucleus (Arriza et al., 1988). 11β-HSD1 is expressed in the brain, with the highest levels found in the cerebellum, hippocampus, cortex, pituitary and hypothalamus, but its main function is activating glucocorticoids. 11β-HSD2 expressed in human fetal brain acts to protect the developing nervous system from the deleterious consequences of glucocorticoid overexposure, but 11β-HSD2 is barely expressed in the human adult brain. It is not understand why barely expressed 11β-HSD2 is sufficient to inactivate glucocorticoids and thereby adjusting the aldosterone to the MR and why it is sufficient to prevent the GR from becoming overactivated by cortisol in the human brain. The mechanism for aldosterone-specific actions in the brain becomes difficult to explain. It is still unknown whether another 11β-hydroxysteroid dehydrogenase is switched on in human adult brain in place of the switched off 11β-HSD2. The possibility of additional tissue-specific 11β-HSDs has been discussed for many years. However, such isoforms have not yet been characterized at either the biochemical or the molecular level. So we are interested in investigating whether additional brain-specific or more generalized 11β-HSD isoforms exist.

In the present study, the reported coding sequence of human 11β-HSD1 was submitted for a TBLASTN search of GenBank, which allowed us identify ESTs that were shown to have no homology to known 11β-hydroxysteroid dehydrogenase. A surprising finding is that many identified ESTs are transcribed from the brain. The new ESTs were assembled into a cDNA which encodes a protein with an integral ORF covered human HSD11B1 whole coding region. The cDNA was further cloned from the brain cDNA library and verified by sequencing. The cDNA was submitted to GenBank with the name SCDR10B and was given accession number AY268352. Northern blotting indicated that SCDR10B is highly expressed in the brain, and a strong expression signal was detected in the hippocampal neurons by immunohistochemical analysis. RT-PCR and immunohistochemical analysis showed that *SCDR10B* was upregulated in lung cancer cell lines and human lung cancer. *SCDR10B* can catalyze the dehydrogenation of cortisol in the presence of NADP<sup>+</sup>, therefore it is a hydroxysteroid dehydrogenase.

## MATERIALS AND METHODS

Cloning of SCDR10B. TBLASTN GenBank searches with the reported coding sequence of hu-11β-HSD1 sequence (GenBank accession man number NP 861420) allowed us to identify many new ESTs which were shown to have no homology to known HSD11B1. The new ESTs were assembled into a contig. To verify the sequence of the contig, primers of SCDR10B-up (5'-AGTGGGGGAAACT-GAGGCCTGAG-3' corresponding to nucleotides 183-205) and SCDR10B-down (5'-GTTCTGGCTC-CAGGGACAGTTGG-3' corresponding to nucleotides 1130-1152) were designed and synthesized (Shenggong BioTech, Com. China). PCR was performed with SCDR10B primers, and a human brain cDNA library (Clontech Co. Ltd) was used as a template. The PCR conditions were: 94°C for 5 min, 35 cycles of 45 s at 94°C, 45 s at 58°C, 100 s at 72°C, followed by a final extension of 5 min at 72°C. The PCR product was subjected to T-A cloning and sequencing.

In silico analysis. To determine the mapping information, the sequence of SCDR10B was submitted for genomic searching at http://genome.ucsc.edu. BLAST and TBLASTN tools (http://www.ncbi.nlm. nih.gov) were applied for identifing the orthologs of SCDR10B in different species. The SMART tool was used for domain searching (http://smart.emblheidelbeg.de), and the Vector NTI package (Informax, Co. Ltd) for protein molecular mass prediction. GeneDoc software was used for sequence alignment. Phylogenetic tree analysis of amino-acid sequences deduced from SCDR10B, HSD11B1 and HSD11B2 was performed using the bootstrap neighbor-joining method with 1000 replicates (Felsenstein, 1985). GenBank accession numbers of previously known HSD11B1, HSD11B2, the novel SCDR10B and its homologs' sequences used for analysis are HSD11B1: human (NP\_861420), monkey (AAB27374), dog (NP\_001005756), pig (NP\_999413), cat (AAN40687), mouse (NP\_032314), sheep (NP\_001009395), rat (NP\_ 058776), guinea pig (AAS47491), frog (AAH54291). HSD11B2: human (NP\_000187), cattle (NP\_777067), sheep (NP\_001009460), rabbit (P51976), horse (AAD31186), mouse (NP\_032315), rat (NP\_058777), pig (NP\_999078), frog (AAH74144), zebrafish (NP\_997885), Nile tilapia (AAO42610), rainbow trout (BAC76709). SCDR10B: human (NP\_941995),

macaque (AAS68529), cattle (NP\_996852), chicken (NP\_001001201), zebrafish (AAS89256), stickleback (AAS89257), frog (AAI06473), medaka (AAS89258), seasquirt (AK116129), pimephalas (DQ862062), wheat (DQ862061), pine (DQ832430), sesame (AAL13315), cress (NP\_680418).

Northern blot analysis of SCDR10B. Premade Multiple Tissue Northern (MTN) Blots of RNA isolated from eight human tissues (brain, heart, liver, lung, spleen, stomach, testis, skeletal muscle) were obtained from Shen Zhen King Grace Biotechnologies, Inc. (China). The blot was probed with  $[\alpha^{-32}P]dCTP$ labeled complete coding region of human SCDR10B cDNA. Hybridization was performed according to the manufacturer's recommendations. Briefly, hybridization was carried out with 5× SSC, 5× Denhardt's, 10% dextran sulfate, and denatured human DNA at 42°C overnight. After hybridization, the blots were washed three times at 42°C in 2× SSC containing 0.1% SDS for 15 min and twice in 0.1× SSC and 0.1% SDS at 42°C for 15 min, and then subjected to autoradiography at - 80°C. The blots were stripped by incubating for 10 min in 0.1× SSC and 0.5% SDS at 95°C. The membranes were reprobed with radio-labeled β-actin as an indicator of RNA loading.

Tumor cell culture and RT-PCR. Seven human tumor cell lines (SGC7901, stomach cancer; H1299, lung adenocarcinoma in which p53 is not expressed; K562, leukemia; SPCA-1, lung adenocarcinoma; H446, small cell lung cancer; H460, large cell lung carcinoma; SHG-44, neuroglioma) were purchased from ATCC (USA) and were cultured as previously reported (Huang et al., 2004). Total RNA was extracted from seven different tumor cell lines with a single-step isolation method using TRIzol reagent according to the manufacturer's instructions. cDNA was synthesized using 2 µg of total RNA, Superscript II reverse transcriptase (Gibco BRL) plus oligo(dT) 15 (Promega) according to the manufacturer's protocols. First-strand cDNA was subjected to RT-PCR amplification on an FS-918 DNA Amplifier (Shanghai Fusheng Institute of Biotechnology, China) using the primer pair of SCDR10B-RT-A (5'-TCTTCTACATCGCGGCGGACATG-3' corresponding to nucleotides 464-486) and SCDR10B-RT-B (5'-CCGCCGAGTAGGGAGTGGAGAAC-3' corresponding to nucleotides 741-763). PCR product size was 300 bp. The expression of the  $\beta$ -2-microglobulin ( $\beta$ -MG; GeneID: 567) was analyzed as a control: sense primer (5'-CTCGTGCTACTCTCTCTCTC-3'), antisense primer (5'-CATGTCTCGATCCCACTTAAC-3'). The PCR conditions were the same as in cloning except that the elongation time was changed to 30 s.

**Prokaryotic expression and purification.** The *SCDR10B* coding sequence was generated by PCR amplification using *Pfu* polymerase (Stratagene Clon-

ing Systems, La Jolla, CA, USA). The primers used to generate the sequence were: SCDR10B-pET32a-A: (5'-GCCGAATTCATGAAGGTGCTTCTCCTC-3') containing an EcoRI restriction site and SCDR10BpET32a-B: (5'-ATACTCGAGGGCTGCGCGGCCGT-GAC-3') containing an XholI restriction site. The PCR product was digested with restriction enzymes EcoRI and XholI, and the resulting product was ligated into the EcoRI/XholI restriction site of the pET32a vector. The obtained sequence of the construct was verified by automated DNA sequencing. Bacterial overexpression was performed with Escherichia coli strain BL21 (DE3). After transformation of the plasmid construct obtained in E. coli and plating on LB-agar (100 mg/ml ampicillin), a single clone was used to inoculate a 5 ml culture in LB-medium (200 mg/ml ampicillin), and the cells were grown at 37°C overnight. They were then inoculated into 500 ml of medium, and grown at 37°C until OD595 between 0.6 and 0.8. Overexpression of recombinant human SCDR10B was induced by adding IPTG to a final concentration of 0.3 mM and cells were incubated at 25°C for an additional 5 h. The cells were harvested by centrifugation at  $10000 \times g$  for 15 min, resuspended in lysis buffer, and then split by adding lysozyme to buffer. After a short period of ultrasonic action on ice, insoluble cell debris was removed by centrifugation at  $12000 \times g$  for 45 min twice at 4°C. The soluble extract was loaded onto Ni-NTA His-bind resin equilibrated with 1× Ni-NTA bind buffer (Novagen), washed with 2×20 ml 1× Ni-NTA wash buffer, then the protein was eluted with 4×0.5 ml 1× Ni-NTA elution buffer. The cell pellet and the eluted fractions were collected and analyzed by SDS/PAGE.

In vitro hydroxysteroid dehydrogenase activity assay. The activity of SCDR10B was measured with cortisol and cortisone as substrates (Ozols, 1995). The activities were assayed at 26°C by measuring the absorbance change at 340 nm in 5 min using spectrophotometer (U-3000, Hitachi). The molar absorption coefficient used for calculating rates is  $6220 \text{ M}^{-1}\text{cm}^{-1}$ . A standard assay mixture for dehydrogenation reactions contained 20 mM Tris/HCl (pH 8.5), 250  $\mu$ M NADP<sup>+</sup> (NAD<sup>+</sup>), 0–100  $\mu$ M cortisol substrate, and appropriate quantities of SCDR10B protein. When the reduction reaction was measured, the pH of Tris/HCl was adjusted to 7.0, and the cortisol substrate was replaced with cortisone, and 250  $\mu$ M NADPH (NADH) was used as a cofactor.

Rabbit anti human SCDR10B polyclonal antibody preparation and immunohistochemistry. Anti-SCDR10B antibodies were raised by immunizing rabbits with purified SCDR10B protein. Tissues were obtained from the Department of Pathology, Suzhou Medical College (Suzhou, China). In every case, tissues had been obtained from operative samples in accordance with local ethical committee approval. Five-micron thick formalin-fixed sections of normal cerebrum, hippocampus, cerebellar glioma and meningioma were cut and placed on coated glass slides. After dewaxing, slides were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. After washing in phosphate-buffered saline (PBS, 0.01 mol/l, pH 7.2), goat serum was used to block for 15 min at 37°C, and then slides were incubated with polyclonal antibody to human SCDR10B at a dilution of 1:300; omission of primary antibody was used as a control. A secondary antibody, goat anti-rabbit IgG biotin conjugated (1:100), was added to the sections for 20 min. SABC was added and incubated for 15 min, and slides were developed using 3,39'-diaminobenzidine and were counterstained with hematoxylin.

## RESULTS

#### Human SCDR10B gene and its genomic organization

In order to clone the SCDR10B gene by PCR, we first analyzed the tissue distribution of the ESTs from SCDR10B. We found that many ESTs were transcribed from the brain, so a human brain cDNA library was used as a template for PCR amplification. We found that the PCR products included three bands with different sizes in the agarose gel. These bands were excised and cloned into a pMD18-T vector (TaKaRa) and the clones were subjected to sequencing. The sequencing result indicated that these cDNAs were different alternative splicing product of the SCDR10 gene. These cDNA sequences were submitted to GenBank with the following GenBank accession numbers: SCDR10A, SCDR10B (short-chain dehydrogenases/reductases 10, isoform B AY268352), SCDR10C, SCDR10D, SCDR10E, SCDR10F and SCDR10G. The cDNA sequence of SCDR10B is consistent with the contig, and only the SCDR10B splice variant has a protein sequence similar to the whole coding region of 11β-HSD1. SCDR10B is 1677 bp in length, encoding a protein of 286 amino acids (Fig. 1). The molecular mass and isoelectric point are predicted to be 30.8 kDa and 10.3 kDa, respectively, using Vector NT I software. SCDR10B is composed of eight exons and seven introns (Table 1). The open reading frame is from nucleotide 223 to nucleotide 1080. The ATG start codon (nucleotides 455-457) is preceded by an in-frame stop codon TGA, and there is a typical polyadenylation signal AATAAA. The protein contains two conserved motifs which all SDR family members have: one is GANAGVG (GXXXGXG), a motif which is related to binding NAD(P)(H), the other is YSAAK (YXXXK) which is related to its catalytic activity. SCDR10B was

1	CGACGETEEGGEGETGGGTEEEEGAGGEEEGGEEEEEEEGGGAGGAGGTGGGETTEGAGTEAEGTGAEEE	72
73	GTGEEETAEGGGAGGGGGGGGGGGGGGGGGGGGGGGGGG	144
145	CGCGTGGCTCCCAGCCCGGGACCCCACCCCCGCTGGACAGTGGGGGGAAACTGAGGCCTGAGCGGGCCCACAC	216
217	AGGACCATGAAGGTGCTTCTCCTCACAGGGCTGGGGGCCCTGTTCTTCGCCTATTATTGGGATGACAACTTC	288
1	M K V L L L T G L G A L F F A Y Y W D D N F	22
289	GACCCAGCCAGCCTCCAGGGAGCGCGAGTGCTGCTGACAGGGGGCCAACGCTGGTGTGGTGAGGAGCTGGCC	360
23	D P A S L Q G A R V L L T <mark>G A N A G V G</mark> E E L A	46
	GXXXGXG	
361	TATCACTACGCGCGTCTGGGCTCCCACCTGGTGCTCACTGCCCACACTGAGGCTCTCCTGCAGAAGGTGGTA	432
47	YHYARLGSHLVLTAHTEALLQKVV	70
433	GGGAACTGEEGGAAGETGGGEGEEECECAAGGTETTETACATEGEGGEGGACATGGEETEEECTGAGGEGEEE	504
71	G N C R K L G A P K V F Y I A A D M A S P E A P	94
505	GAGAGEGTGGTGCAGTTTGEGETGGAEAAGETGGGEGGGETGGAETACETEGTGETGAAECAEATEGGEGGE	576
95	E S V V Q F A L D K L G G L D Y L V L N H I G G	118
577	GECEEGGEEGGEAGEEGGAGEEEGGAGEEECEAGGEAAETEGETGGETEATGEAGGTAAAETTTGTGAGETAE	648
119	A P A G T R A R S P Q A T R W L M Q V N F V S Y	142
649	GTGCAACTGACGTCGCGGGCGCTGCCCAGCCTGACGGACAGGACAGGGCTCCCTGGTGGTGGTGTCCTCGCTG	720
143	V Q L T S R A L P S L T D S K G S L V V V S S L	166
721	CTCGGCCGCGTGCCCACGTCGTTCTCCACTCCCTACTCGGCGGCCAAGTTTGCGCTGGACGGCTTCTTCGGC	792
167	L G R V P T S F S T P Y S A A K F A L D G F F G	190
	YXXXK	
793	TCCCTGCGGCGGGAGCTGGACGTGCAGGACGTGAACGTGGCCATCACCATGTGCGTCCTGGGCCTCCGAGAT	864
191	S L R R E L D V Q D V N V A I T M C V L G L R D	214
865	CGEGEETEEGEEGAGGEAGTEAGGGGAGTEAEGAGGGTEAAGGEGGEEEEGGGGEEEAAGGEAGEEETG	936
215	R A S A A E A V R G V T R V K A A P G P K A A L	238
937	GEEGTGATEEGEEGEEGEEGEEGEEGEEGEEGEEGEEGEEGEEGEEG	1008
239	A V I R G G A T R A A G V F Y P W R F R L L C L	262
1009	CTCCGGCGCTGGCTACCGCGCCCGCGGGCCTGGTTTATCCGCCAGGAGCTCAACGTCACGGCCGCGGCAGCC	1080
263	L R R W L P R P R A W F I R Q E L N V T A A A A	286
1081	TGAGEACEGGGGGGTGEECETEEAGTEECEAGAEGGEAATGTTEETEECTEEAAETGTEEETGGAGEEAGAAE	1152
287	*	288
1153	ACTCACAGAGACACCCCTGAGAGGGTGGCCACAGCCCAAGATGAAGTCATCAAGACAGAAAAGCAAAACCGA	1224
1225	GAAAAACGACGGGCACCTGGAACCAGTCACGGCTTGGGAGGTGCAGGTGCCCCGTGTTAGGCGCCTTTGTCG	1296
1297	GGGACTTGCAAGGCCTCACCTGTTTGGCCATGATTGATGACGTGACTGCTTCCATTTTGCAGATGAGGAAAC	1368
1369	TAAGGCTCAGAGAGGCCACGCCACCCTTGAGCCACCCATGGACCCCTCTCCATCTCCTGCCTG	1440
1441	GTCCCTGATTTATTCTTTCCATTCCATTCCGGGAGGAACCCCCCCAACTCCTGCCAGCTTCCCCTAGCT	1512
1513	GGGGTCTCTGGTACTCTTCACACCTGCAGGGGCGTCTACACTGTTCGTCTAC <u>CTGGTG</u> GCAGGGTCTGAGCG	1584
1585	GGAGGAGGAGGGAAAGAGTGTGTTCTGAGCTGGACCCAGCCTCTTGTTCGAG <mark>AATAAA</mark> AACTCTTCTTCTCT	1656
1657	тссалалалалалалал	1677

mapped to chromosome 19p13.3 by browsing the UCSC genomic database (http://genome.ucsc.edu). Result of searching in SMART database showed that the SCDR10B protein contains a transmembrane helix and SDR\_Short domain (http://smart.embl-heidel-beg.de).

## In silico clone of SCDR10B orthologs

TBLASTN GenBank searches with the coding sequence of human SCDR10B protein allowed us to identify many ESTs in different species including monkey, cattle, chicken, frog, zebrafish, medaka, stickleback, wheat and loblolly pine. These ESTs were extracted and assembled into contigs in different species, and we obtained the cDNA sequences of the orthologs of SCDR10B in monkey, cattle, chicken, frog, zebrafish, medaka and stickleback, as well as the homologs of the 11β-hydroxysteroid dehydrogenase family in the plants (wheat, loblolly pine). All the cDNA sequences encoding the protein with the whole ORF covered the whole human SCDR10B coding region. These sequences were submitted to GenBank with the accession numbers: monkey AY554048, cattle AY554049, chicken AY578179, zeFigure 1. Nucleotide sequence and deduced protein sequence of the human *SCDR10B* gene.

SCDR10B encodes a polypeptide of 286 amino acid. Amino acids are identified by their one-letter code. Both nucleotides and amino acids are numbered at the left and right side of each line, respectively. \*, The termination codon  $\boxed{\GammaGA}$ ; **AATAAA** Polyadenylation signals; TGA, in-frame stop codon;  $\boxed{G \ A \ N \ A \ G \ V \ G}$  conservative motif binding to co-factor NAD(P)(H);  $\underbrace{Y \ S \ A \ A \ K}$ , conservative motif related to its catalytic activity.

brafish AY578810, medaka AY578812, stickleback AY578181, pimephalas DQ862062, wheat DQ862061, loblolly pine DQ832430. To our surprise, a BLAST search of the mouse and rat genomes and the Gen-Bank database with *SCDR10B*, we did not find any orthologs to human *SCDR10B* in rodents. In addition, in a blast search of the GenBank database with  $11\beta$ -HSD1, we did not find any orthologs in fish.

A search of published protein databases for sequences similar to that of SCDR10B indicated that it had considerable similarity to the 11 $\beta$ -HSD1 family. So SCDR10B is a new subgroup of the 11 $\beta$ -hydroxysteroid dehydrogenase family. This subgroup was named 11 $\beta$ -HSD3 (11 $\beta$ -hydroxysteroid dehydrogenase 3).

Multiple alignment analysis in Fig. 2a indicated that the orthologs of human SCDR10B are conserved in different species, the sequence identities of each ortholog to human SCDR10B is: 97% (macaque), 88% (cattle), 57% (chicken), 52% (frog), 50% (zebrafish), 52% (stickleback), 49% (medaka), 46% (pimephalas), 33% (seasquirt), and the similarity of human SCDR10B and its orthologs is higher in the NADP-binding region and active site region than in the C-terminus. The C-terminal region is

3' Splice acceptor	Exon	Size (bp)	5' Splice donor	Intron	Size (bp)
cDNA endCGACGCTCCG	1	208	GCCTGAGCGGgtgagtgcac	1	3547
ccctctgc <i>ag</i> TTCGACCCAG	2	87	TTCGACCCAG <i>gt</i> gagcacct	2	83
ctatggccagCCAGCCTCCA	3	131	CCTGCAGAAGgtgagccacc	3	1296
cccccccagGTGGTAGGGA	4	112	GACAAGCTGGgtgaggggct	4	372
tctgggccagGCGGGCTGGA	5	91	GCTCATGCAGgtgctccgct	5	290
ccgcctgcagGTAAACTTTG	6	94	TCGCTGCTCGgtgcgtgcac	6	127
gcgcccccagGCCGCGTGCC	7	166	AGGCAGTCAGgtgaggcccg	7	84
ctgtccccagGGGAGTCACG	8	770	CTTCTCTTGCaaaaaaaaaa	polyA	

## Table 1. Genomic structure of SCDR10B gene

Exon sizes are given in bp and the exonic and intronic sequences at the splice junction are shown in capital and lowercase letters, respectively. The exon-intron splicing signals gt and ag are in bold.

the steroid substrate binding region, which is obviously different between human SCDR10B and its fish homologs. All the members have conserved the YXXXK motif, but seasquirts do not have the conserved GXXXGXG motif.

Furthermore, we analyzed the evolutionary relationship among different  $11\beta$ -hydroxysteroid dehydrogenase family proteins with bootstrap N-J tree analysis (Fig. 2b), which shows that human SCDR10B and its orthologs in different species except seasquirt cluster together. SCDR10B is one of the proteins conserved during evolution.

## Expression pattern of the SCDR10B gene

Northern blotting and RT-PCR were used to determine the expression pattern of the gene. In order to investigate the tissue expression pattern of *SCDR10B*, Premade Multiple Tissue Northern (MTN) Blots including eight human tissues (brain, heart,

human	:	MKVILLTGLGALFFAYYDD-NFDPASLQCARVLLTGANACVGEELAMHYARLGSHLVLTAHTEALLQKUVGNGR	:	74
macaque	:	MAVILLTGLGALFFAYYDD-NFDPASLQCARVLLTGASACVGEELAYHYARLGSHLVLAAHTEALIOKUVGNGR	:	74
cattle	:	MAVILLTGLGALFFLYYDD-NFDPASLH ARVLLTGVSA IGEELAYHYARLGSHLVLNAHTEALIOQUVGNGR	:	74
chicken	:	MMKPFGKVICAAGSLAVLLAFFWRD-TFQPEQLSGARVLLTGASAGIGEQMAVHYATFGAEIVLINARREAVIQEVMKKGL	:	79
frog	:	-MGIHVKRWCFL-ALVASSAYLLRD-SFDPATLANARVLVTGASTGIGEEIAYHYARAGAELVLDARREHALQEVKRRGL	:	77
zebrfish	5	-MKLYAKLELCS-ICVAFIAVR SAPSFNEESLK GARVLVIGASTGIGEQLAYHYARLGAQIVIDARRGNVLEQVVSK R	:	78
sticklebac	:	-MGTFTKILLSA-ACVAFLARTAPRFDAESLRGARVLVTGASTGIGEOMAYHYARFGAQVVIDARRDKVUOKVAEEGL	:	78
medaka	:	-MA-FTKVLIVS-LCVVFLAVKWTSPTFDPESIRGARVLVTGASTGIGEOMAYHYARFGAQVVITARREKVLQQVVEKGL	:	77
seasquirt	:	-MSYCFKALFVIIVAIVTYSFIQPD-DYDTGILKNKRVLITGASQNIGEELATQYAQSGAKIVIDARREDKLKEVAAKGR	:	78
pimephalas	:	-MKVYVSLFVLIAALMSYMTRD-TFDPDSVR JARVLITGASS IGEOMAYHYAKFSAQIIITARRLEALKKVAQKSV	:	75
		GXXXGXG		
human	:	KLGAPKVFMIAADMASPEAPESVVQFALDKLGGLDYLVLNHI-GGAPAGTRARSPQATRWMQVNFVSYVQLTSRAL	:	150
macaque	;	KLGAPKVFYIAADMASPEAPESVVQFALDKLGGLDYLVLNHI-GGVPAGTRARTPQATEWIMQVNFLSYVQLTSRAL	:	150
cattle	:	KLGAPKVFYIAADMASPEVPERVVOFALDKLGGLDYLVLNHL-GAAPAGTRVRSSOSTRWIMOMNFLSYVOLTSSAL	:	150
chicken	:	TLGAKKVFYIPADMSSPSEPDRVVOFAVONLGGLDYLVLNHI-GVSPFOMWGGDVEHTRWLMOVNFFSYVALATAAL	:	155
frog	:	ELGAKNVSLVVADMASHNAREPVVAEALSALGGLDYLVLNHI-SWTPFKMWDGDVNHTRW/MEVNFLSYTHLATSAL	:	153
zebrfish	:	EMGAOKAFYIPADMANPSDADLVVKYAIEOLGGLDYLVLNHI-GPSPYOMWDGDVOHTRWILEVNFLSYLOMAOKAL	:	154
sticklebac	:	RLGAOKALYVAADMASESDPDTVVDFALEHLGGLDYLVLNHI-CPSPFSMWEGDVEHTEWUMKVNFLSYVOMAWRSL	:	154
medaka	:	SLGAOKAVY TANDASTDADPDKVVDFALEELGGHDYLVINHI - CSIDLRMWDGDHEOVOOLMKDNFHSVVOMAGRAL	:	153
seasquirt	:	KAGAT SVD/IV/DMADIEOVKN/WKDAVALLGGLDOLVINHVTCLNHPGMWNNSOFNLDYAONSAIWNYVSYTHLSTHAT	:	158
pimephalas	÷	KLGAO MMYMTGDMSDPADPERVYKYAVEKLGGLDFLVLNHV-GDTNVEFWNRDADHVSSIMOVNEVSYTOMTAAAL		151
<b>rr</b>				
human	:	SUTDSK SUVVVSSLI RVPTSFSTPVSAAK GUDGBEGSTRRBDV-ODVIVATHEVD GTRDRA-SUAFAVRGVTRV		228
human macague	:	PSUTDSK SSLVVVSSLLCRVPTSF STPYSAAKCALDG OF GSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSK SLVVVSSLLCRVPTSF STPYSAAKCALDS OF GSLERELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV	:	228 228
human macaque cattle	:	PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAKFALDGGFGSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAKFALDSGFGSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSSPYSAAKFALDSGFSSLRRELDV-OEVNVAITMCVLGIRDRA-SAAEGVRGTRV	:	228 228 228
human macaque cattle chicken	: : : :	PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAXFALDG3FGSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAXFALDS3FGSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSSPYSAAXFALDS3FSSLRRELDV-OEVNVAITMCVLGIRDRA-SAAEGVRGITRV PTUEKNHGSVVVVSSLTCKTPTPFTTSVSATXFALDG3FSSLRRELTM-OKRNVSITLCILGIDTD-AALEKTRGKVFI	:	228 228 228 233
human macaque cattle chicken frog		PSUTDSKCSLVVVSSLLCRVPTSFSTPYSAAKALDGEFGSLRRELDV-QDVNVAITMCVLCIRDRA-SAAEAVRGVTRV PSUTDSKCSLVVVSSLLCRVPTSFSTPYSAAKFALDSEFGSLRRELDV-QDVNVAITMCVLCIRDRA-SAAEAVRGVTRV PSUTDSKCSLVVVSSLLCRVPTSFSSPYSAAKFALDSEFSSLRRELDV-QEVNVAITMCVLCIRDRA-SAAEGVRGTRV PTUEKNHCSVVVVSSLTCKIPTPFTTSMSATKFALDGEFSSLRRELTM-QKRNVSITLCILCIDTD-AALEKTRGKVFI PYHAOSKSLIVVSSLTCKIPTPFTTSMSATKFALGEFSSLRRELTM-QKRNVSITLCILCIDTD-AALEKTRGKVFI PYHAOSKSLIVVSSLTCKIPTPFTTSMAASKFALEGEFSSLRRELTM-QKRNVSITLCILCIDTD-SAKEKTKDKITL	: : : :	228 228 228 233 231
human macaque cattle chicken frog zebrfish		PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAKFALDGEFGSLRRELDV-QDVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAKFALDSEFGSLRRELDV-QDVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSSPYSAAKFALDSEFSSLRRELDV-QEVNVAITMCVLGIRDRA-SAAEGVRGITRV PTEKNHGSVVVVSSLLCRUPTFTSYSARKFALDGEFSSLRHELIM-QKRIVSITLCILGIDTD-AALEKTRGKVFI PTEKSKGSIVVVSSLLCRUPTFYSYAAKFALDGEFSSLRHELIM-QKRIVSITLCILGIDTQ-SAMEKIKGKITL PTEKSKGSIVVSSLLCRUFGFALVAASKFALGGEFSSLRHELIM-QNNEVSITLCILGIDTQ-SAMEKIKGKITL		228 228 228 233 231 232
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human macaque cattle chicken frog zebrfish sticklebac medaka seasquirt nimenhalas		PSUTDSK SSLVVVSSLLCRVPTSFSTPYSAAKFALDGEFGSLRRELDV-QDVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSK GSLVVVSSLLCRVPTSFSTPYSAAKFALDSEFGSLRRELDV-QDVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSK GSLVVVSSLLCRVPTSFSSPYSAAKFALDSEFSSLRRELDV-QEVNVAITMCVLGIRDRA-SAAEAVRGVTRV PTDEKNE SVVVVSSLLCKIPTPFTTSMAAKFALDGEFSSLRRELDV-QEVNVAITMCVLGIRDRA-SAAEGVRGTRV PTDEKNE SSLVVVSSLLCKIPTPFTTSMAAKFALGGEFSSLRRELDM-QKRIVSITLCILGIDTD-AALEKTRGKVFI PTDEKSK GSLVVVSSLLCKICGPFALPYASTKFALGGEFSSLRRELIM-QKRIVSITLCILGIDTD-SAMEKIKGKITL PTDEKSK GSLVVVSSLLCKICGPFALPYASTKFALGGEFGSLRHELIM-QKRIVSITLCILGIDTD-SAMEKIKGKITL PTDEKSK GSLVVVSSLLCKICGPFALPYASTKFALNGEFGGSLHHELAM-KKSNVSVSITLGIDTESAMEKIKGVINM ASVEQSK GSLVIVSSLLCRVSTPYVAPYTSTKFALNGEFGSLKHELVM-KKSNVSVSITLGIDTESAMEKIKGVINM PHERTGFALVVSSLCRVSTPYVAPYTSTKFALNGEFGSLCHBLAM-KKSNVSVSITLGIDTESAMEKIREFTDM PHUTESNGNLIVVSSLAGHVPTINTAINGATKASLNHEVASLRLDMAASERDPYSITIGMCPIETDRSRWSKFPDKVNM		228 228 233 231 232 232 232 232 231 238 229
human macaque cattle chicken frog zebrfish sticklebac medaka seasquirt pimephalas		PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAKFALDGEGSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSTPYSAAKFALDSEGSLRRELDV-ODVNVAITMCVLGIRDRA-SAAEAVRGVTRV PSUTDSKGSLVVVSSLLCRVPTSFSSPYSAAKFALDSEFSSLRRELDV-OEVNVAITMCVLGIRDRA-SAAEAVRGVTRV PTIEKNHGSVVVVSSLLCKIDTPFTTSMSARKFALDGEFSSLRHELTM-OKRNVSITLCILGIDTD-AALEKTRGKVFI PTUEKSKGSIVVVSSLLCKICGPFALPYASTKFALDGEFSSLRHELTM-OKRNVSITLCILGIDTQ-SAMEKIKGKITL PTUEKSKGSIVVVSSLLCKICGPFALPYASTKFALDGEFSSLRHELTM-OKRNVSITLCILGIDTQ-SAMEKIKGKITL ASTEQSKGSLVVVSSLLCKICGPFALPYASTKFALGGEFGSLNHELTM-OKSNVSITLCILGIDTQ-SAMEKIKGVINT PSUAQSKGSLVVVSSLLCKICGPFALPYASTKFALNGEFGSLNHELAM-KKSNVSVSITLGILDTD-SAMEKIKGVINT PSUERTGGALVVVSSLCRVSTPVAPYTSTKFALNGEFGSLRHELAM-KKSNVSISITLGILDTA-SAMEKIRGFTDM PHUTESNGLLIVVSSLACHVPTINTAIYGATKASLHHEYASIKLEMMASERDPYSITICMCGPIETDRSRWSKFPDKVIM PAUETSAGSLVVSSLACHVPTINTAIYGATKFAMIGEFGAMOSELAM-OKSNVSVSIMILGIDTE-SAMKKIRGFSTM SS VYXXK		228 228 233 231 232 232 232 231 238 229
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Figure 2a. Sequence alignments of human SCDR10B protein and its orthologs.

Identical amino acids are shaded in black, similar amino acids are shaded in grey. The sequences used for alignment include: human (NP\_941995), macaque (AAS68529), cattle (NP\_996852), chicken (NP\_001001201), zebrfish (AAS89256), stickleback (AAS89257), frog (AAI06473), medaka (AAS89258), seasquirt (AK116129) and pimephalas (DQ862062).

#### PHYLIP\_1



liver, lung, spleen, stomach, testis, and skeletal muscle) were used for Northern blot analysis. The tissue distribution pattern of *SCDR10B* mRNA is shown in Fig. 3. The result revealed that *SCDR10B* was highly expressed in brain, and low expression was detected in lung, spleen, stomach, testis, and skeletal muscle. The size of the transcripts was about 1.7 kb. In heart and liver, almost no positive signal was detected. There are two isoforms of actin expressed in heart and muscle, so two bands observed in the control transcript ( $\beta$ -actin).

RT-PCR analysis was used to determine the expression pattern of *SCDR10B* mRNA in six tumor cell lines; the result in Fig. 4 showed that the *SCDR10B* gene was differentially expressed in these tumor cell lines. The expression level was higher in lung cancer cell lines SPCA-1, H460 and H446 than in stomach cancer cell line SGC7901, leukemia cell line K562 and neuroglioma cell line SHG-44.

# Expression and purification of recombinant SCDR10B protein in a prokaryote

In order to obtain the SCDR10B protein for characterizing its enzyme activity and for raising



A phylogenetic tree was constructed with the bootstrap N-J method using program PHYLIP with 1000 bootstrap trials. The number is the value found in 1000 trials.



## Figure 3. Northern blots analysis of *SCDR10B* in human tissues.

A commercially available Northern blot membrane (Shen Zhen King Grace Biotechonologies, Inc., China) containing multiple human tissues (brain, heart, liver, lung, spleen, stomach, testis, skeletal muscle) was probed with a <sup>32</sup>P-labeled *SCDR10B* cDNA. The blot was stripped and reprobed with  $\beta$ -actin cDNA probe supplied with the blot to confirm equal loading of mRNA on the blot. A transcript of 1.7 kb was detected in the adult human brain tissue.



Figure 4. Expression pattern of *SCDR10B* in seven human tumor cell lines.

Total RNA was extracted from seven different tumor cell lines with a single-step isolation method using TRIzol reagent. cDNA was synthesized using 2 mg of total RNA, SuperscriptII reverse transcriptase (Gibco BRL) plus oligo(dT)15 (Promega). First-strand cDNA was subjected to RT-PCR amplification using the primer pair of SCDR10B-(5'-TCTTCTACATCGCGGCGGACATG-3') RT-A and SCDR10B-RT-B (5'-CCGCCGAGTAGGGAGTGGAGAAC-3'). PCR product size was 300 bp. The expression of the  $\beta$ -microglobulin ( $\beta$ -MG) was analyzed as a control: sense (5'-CTCGTGCTACTCTCTCTTC-3'), primer antisense primer (5'-CATGTCTCGATCCCACTTAAC-3'). M, DNA marker; lane 1, stomach cancer SGC7901; lane 2, leukemia K562; lane 3, neuroglioma SHG-44; lane 4, lung adenocarcinoma H1299 in which P53 is not expressed; lane 5, lung adenocarcinoma SPCA-1; lane 6, large cell lung carcinoma H460; lane 7, small cell lung cancer H446.

rabbit anti-human SCDR10B polyclonal antibody, a SCDR10B-pET32a construct was transformed into *E. coli* strain BL21 (DE3). Recombinant SCDR10B protein was highly expressed after induction by 0.3 mM IPTG. We obtained high-purity SCDR10B protein by Ni-NTA His-Bind Resin affinity chromotography (Fig. 5).



Figure 5. Expression of recombinant SCDR10B in *E. coli* strain BL21(DE3).

Coomassie Blue-stained SDS/PAGE gel of the SCDR10B expression construct. M, molecular mass markers; lane 1, not induced by IPTG; lane 2 and 3, induced by adding IPTG to a final concentration of 0.3 mM; lane 4, purified SCDR10B protein.

## Hydroxysteroid dehydrogenase activity assay

SCDR10B was a new member of the short chain dehydrogenase superfamily, and its enzyme activity was still unknown. Considering that it has a high sequence similarity to HSD11B1, we were interested in investigating whether SCDR10B is capable of catalyzing the dehydrogenation of cortisol and the reduction of cortisone in the presence of NAD(P) (H). Our result in Fig. 6 revealed that SCDR10B can catalyze the dehydrogenation of cortisol in the presence of NADP<sup>+</sup>. We also detected very weak activity in the presence of NAD<sup>+</sup>. Nevertheless, the rate of the reverse reaction, the reduction of cortisone, was not detected in our experiment (not shown).

## Immunohistochemical analysis to investigate the tissue distribution of SCDR10B protein

SCDR10B was highly expressed in lung cancer cell lines (shown by RT-PCR), but only a weak expression was detected in normal lung (shown by Northern blotting), so we were interested in further investigating whether the expression of SCDR10B is up-regulated in lung cancer. Immunohistochemical analysis was applied to determine the expression level of the SCDR10B protein in normal lung and cancerous lung; the result in Fig. 7a indicated that SCDR10B was highly expressed in cancerous lung. A strong staining signal was observed in lung cancer but only a weak signal was observed in normal lung.

Immunohistochemical analyses of human brain sections using SCDR10B polyclonal antibody indicated that SCDR10B was expressed in the cerebrum, hippocampal neurons, cerebellar glioma and meningioma (Fig. 7b). A strong staining signal was detected in the superficial layer of the cerebral cor-



Figure 6. Time scan at  $OD_{340}$  analyzing the enzyme activity of SCDR10B.

Assay mixture for dehydrogenation reactions contained 20 mM Tris/HCl (pH 8.5), 250  $\mu$ M NADP<sup>+</sup> (NAD<sup>+</sup>), 0–100  $\mu$ M cortisol substrate, and appropriate quantities of SCDR10B protein. The concentration of cortisol from bottom to top is 0, 13.3, 20, 26.7  $\mu$ M.

Α



Figure 7a. Representative immunohistochemistry images depicting SCDR10B protein expression in normal lung and lung cancer.

A, B, C and D: expression of SCDR10B-immunoreactive protein in a section of human lung cancer; E, F, G and H: expression of SCDR10B-immunoreactive protein in a section of human normal lung. The image of A, B, D, E, F and H was developed by horse-radish peroxidase coupled goat anti-rabbit IgG staining. The image of C and G was developed by hematoxylin-eosin staining. A, E, D and H: ×10 magnification. B and F: ×40 magnification. A, B, C, E, F and G are a group of sections stained with the antihuman SCDR10B antibody at a dilution of 1:300. Omission of primary antibody was used as a control in panels D and H. The thickness of the sections is 4  $\mu$ M. Scales bar 10  $\mu$ M.

Figure 7b. Representative immunohistochemistry images depicting SCDR10B protein expression in normal cerebrum, hippocampus, cerebellar glioma and meningioma.

A. B: expression of SCDR10B-immunoreactive protein in a section of human cerebellar glioma: A ×40 magnified region was showed in panel A; C, D: expression of SCDR10B-immunoreactive protein in a section of human meningioma; E, F: expression of SCDR10B-immunoreactive protein in a section of human normal cerebral corex; G, H: Expression of SCDR10B-immunoreactive protein in a section of human hippocampus. The image of B and D was developed by HE staining. F: ×40 magnified region was showed in panel E. H: ×40 magnification of G. Scales bar 10 µM.

tex. Hippocampal neurons also displayed strong staining. Strong and diffuse staining is observed in cerebellar glioma and meningioma.

## DISCUSSION

In the present study, we report the isolation and characterization of a novel human short-chain dehydrogenase/reductase gene *SCDR10B*, which maps to chromosome 19p13.3. It contains an ORF with length of 858 bp, encoding a protein with a short-chain dehydrogenase/reductase (SDR) domain. The SDR superfamily is a very large family consisting of more than 3000 members (Kallberg *et al.*, 2002). Analysis of sequence similarities revealed that SCDR10B shares homology with 11 $\beta$ -hydroxysteroid dehydrogenase family members. The result of an enzyme activity assay revealed that SCDR10B can catalyze the dehydrogenation of cortisol in the presence of NADP<sup>+</sup>. Therefore it is a hydroxysteroid dehydrogenase.

11β-HSD1 is highly expressed in the liver and adipose tissue, and is called "liver type" 11βhydroxysteroid dehydrogenase. 11β-HSD2 is highly expressed in the classical mineralocorticoid selective target tissues such as kidney, colon and sweat glands, and is called "kidney type" 11β-hydroxysteroid dehydrogenase. In contrast, the result of Northern blotting revealed that *SCDR10B* is highly expressed in the brain. Immunohistochemical analysis of human brain sections using SCDR10B polyclonal antibody revealed that SCDR10B was transcribed in the cerebrum, hippocampal neurons, cerebellar glioma and meningioma. These results suggest that SCDR10B is a "brain type" hydroxysteroid dehydrogenase.

SCDR10B was a highly conserved protein during evolution. Its homologs were widely found in different species including animals and plants (Baker, 2004). Its orthologs were cloned in mammals (monkey, cattle), aves (chicken), fish (zebrafish, medaka, stickleback, pimephales) and ascidiacea (sea squirt). Blast analysis also indicated that wheat, loblolly pine, sesame, arabidopsis and Oryza sativa have homologs to SCDR10B and HSD11B1. The ortholog in sesame is called steroleosin; it was previously determined that steroleosin exerts dehydrogenase activity on both 17β-hydroxysteroid (estradiol) and 11β-hydroxysteroid (corticosterone) substrates in the presence of NADP<sup>+</sup> (Lin et al., 2002). Our results also indicated that SCDR10B exerts dehydrogenase activity on the cortisol substrate in the presence of NADP<sup>+</sup>. SCDR10B has a higher dehydrogenase activity in the presence of NADP<sup>+</sup> than in the presence of NAD<sup>+</sup>. The sequence similarity of human SCDR10B and its orthologs is higher in the NADP-binding region and active site region than at the C-terminus. The C-terminal region is the steroid binding region, which is obviously different beween human SCDR10B and its homologs, which suggests that SCDR10B is likely to metabolize different substrates in mammals, fish, chicken, seasquirt and plants. Elucidating their catalytic mechanisms is needed in a future study.

Abnormal hormone signaling and action is a main feature in the pathogenesis of major cancers afflicting western populations (Henderson & Feigelson, 2000). For instance, breast cancer is linked to estrogen and progesterone, prostate cancer is associated with estrogen and testosterone, endometrial cancer is linked to estrogen, and thyroid cancer is linked to thyroid hormones and thyroid stimulating hormone. In contrast, the role of glucocorticoids in the pathophysiology and treatment of cancers is less known. Glucocorticoids inhibit the proliferation of many cell types. Reductase activity of 11β-HSD1 increases local cortisol levels and leads to increased differentiation (Bujalska et al., 2002a; 2002b). The dehydrogenase activity of 11β-HSD2 decreases the availability of active cortisol thereby facilitating proliferation (Rabbitt et al., 2003). It was reported that 11B-HSD2 is upregulated in different tumors, including breast cancer (Cohen, 2005; Koyama et al., 2001), colorectal cancer (Zbankova et al., 2004), and pituitary adenoma (Nigawara et al., 2006), which suggests that the inhibition of 11β-HSD2 activity can prevent the tumor from growing. The results of RT-PCR indicated that the expression of SCDR10B in lung cancer cell lines is upregulated; in agreement with this, immunohistochemical analysis also showed that SCDR10B is transcribed in the cancerous lung at a higher level than in normal lung tissue, which suggests that the abnormal expression of SCDR10B in the lung maybe related to the development of lung cancer.

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