Molecular Cloning of hSC2 Encoding 5α-reductase-like Protein

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Background **: The human homologue of the SC2 gene from a human dermal papilla cell cDNA library has been isolated and designated hSC2. hSC2 protein also shares similarity with 5 -reductase, a protein important in testosterone metabolism.**

Objective **: Prior to knowing the functions of hSC2 in dermal papilla, we cloned it and analyzed its relative expression levels in adult tissues and cancer cell lines.**

Methods **: hSC2 was isolated from low abundant clones in dermal papilla cDNA library using cDNA array hibridization method. Full-length clone was sequenced and we studied its expression in different tissues by Northern blot hybridization.**

Results **: Sequence data reveals a single open reading frame, encoding a putative hydrophobic protein with a calculated molecular weight of 36 kDa. Its deduced amino acid sequences are almost 97.4% identical to those of rat protein. Northern blot hybridization shows that hSC2 cDNA recognizes a 1.35 kb transcript that was expressed in various epithelial and mesenchymal tissues including testis and liver.**

Conclusion **: We have cloned and analysed tissue distributions of hSC2. It was interesting that it had homology with 5α-reductase isozymes. Further studies will be needed to understand the involvement of hSC2 in androgen hormone signaling.**

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Key Words : 5α-reductase, Dermal papilla cDNA library, Human synaptic glycoprotein SC2

Androgens have paradoxically different actions on human hair follicles depending on their action sites of the body. They stimulate hair growth in many areas, such as the beard and pubis, and have almost no effect on protective hair follicles such as the eyelashes, but cause regression and balding on the scalp in genetically disposed individuals[1-3].

The dermal papilla is a mesenchyme-derived structure located at the base of the hair follicle, and is known to play an essential role to induce and maintain hair growth[4,5]. The current hypothesis on the androgen action in the hair follicle involves the circulating androgens acting on the cells of the dermal papilla to alter their production of the regulating factors with which they influence the other components of the hair follicle[2,3].

The mechanism of androgen action in human hair follicles appears to vary with the body site of the follicle. Androgen receptors are required for any androgen-dependent responses, as shown by the absence of body hair and scalp recession in the testicular feminization syndrome[6,7].

However, the hair distribution of 5α-reductase deficiency in men, who cannot convert testosterone to 5α-dihydrotestosterone intracellularly despite relatively normal levels of plasma testosterone, points to specific roles of testosterone and 5α-dihydrotestosterone in different follicles. These individuals produce little or no beard growth and do not proceed to baldness, but they form terminal

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hairs as a female pattern in the pubic and the axilla[6,8]. This suggests that 5α-dihydrotestosterone is the active androgen in beard and balding scalp follicles, but is not required for axillary and pubic growth.

Two steroid 5α-reductase isozymes (type 1 and type 2) have been identified in the human, rat, mouse, and the monkey[9,10]. The isozymes share about 50% of the sequence identity, the similar substrate preference, the similar gene structures, are present in the integral membrane proteins of the endoplasmic reticulum. However, they differ in their affinities to steroid substrates, pH optima, sensitivities to certain 4-azasteroid inhibitors, tissue distributions, and physiological functions[9].

In this study, the human homologue of the SC2 gene, that have similar gene structure with 5α-reductase isozymes in dermal papilla cells, has been cloned. Its relative expression levels in adult tissues and cancer cell lines have also been analyzed.

MATERIALS AND METHODS

Screening of low abundance cDNAs

The cDNA library was made with mRNA from human dermal papilla cells. Inserts over 400 bp were unidirectionally cloned into the lambda Uni-Zap phage vector to generate 10^7-10^8 recombinants. Bacterial transformants were obtained by cotransfection with a helper phage. Colonies were picked at random and minilysate DNA was prepared by alkaline lysis using REAL preps (Qiagen, USA). A total of 1,064 cDNAs were arrayed in nylon membranes in a 96-well format. Briefly, the membrane was cut to the size of the dot blot manifold and wet with 0.4 M Tris, pH 7.5, for 5 min. The membrane was placed into the manifold and clamped. The plasmid DNAs were denatured for 10 min at room temperature in 0.25 N NaOH/0.5 M NaCl. The DNAs were diluted in 0.1 x SSC/0.125 N NaOH to fix 200 ng DNA was fixed per dot. The DNA samples were loaded into the manifold and then suction was applied. The membranes were removed from the manifold, neutralized by rinsing in 0.5 M NaCl/0.5 M Tris, pH 7.5, dried in air, and then fixed by UV crosslinking at 1200 mJ using a Stratalinker (Stratagene, USA). The cDNAs used as probes were synthesized from total RNAs prepared from dermal papilla cells using an oligo(dT) primer and Superscript II reverse transcriptase. The cDNA mixture was radiolabeled with ^{32}P-dCTP by random priming and used as a probe to screen these 1,064 cDNAs. Over 200 clones were not detected with the complex cDNA probe and were subsequently considered as low abundance cDNAs. These cDNAs were sequenced from 5' end of the inserts using a Sequenase DNA sequencing kit. Sequences were compared with GenBank data base.

Cloning and Sequencing of hSC2 cDNA

One of these low abundance cDNAs(clone P1115) was selected on the basis of significant homology with rat SC2. Therefore, the cDNA and the encoding protein are referred to 'human SC2' (hSC2). DNA sequencing was performed with Mn^{2+} reagent kit using Sequenase Version 2.0 by the manufacturer's protocol.

Multiple tissue Northern blots

To examine the tissue-specific expression of hSC2, the distribution of hSC2 mRNA in different human tissues was analyzed by Northern blot analysis using Multiple Tissue Northern blots (Clontech, USA). The multiple tissue Northern blots containing size-fractionated mRNA extracted from various human tissues were prehybridized for 30 minutes at 68℃ in ExpressHyb solution. After prehybridization, the fluid was removed and replaced with fresh ExpressHyb solution containing $2x10^6$ cpm radioactive DNA probe per 1 ml solution and the membranes were hybridized for 1 hour. The purified P1115 cDNA was radioactively labeled with Megaprime DNA labelling system according to the random priming method, and used as a probe. After hybridization, the membranes were removed and washed in 2xSSC/0.05% SDS at room temperature for 40 minutes with several changes, in 0.1xSSC/0.1% SDS at 50ŸY for 40 minutes with one change, and then exposed to X-ray film at -70℃.

RESULTS

A cDNA library was constructed from poly(A^+) mRNA extracted from cultured human dermal papilla cells. After dot blotting, 1,064 cDNAs were screened using a probe derived from the same cDNA mixture. The clones that showed low levels of expression signal were considered as low abundance cDNAs and were partially sequenced (Fig. 1).

One of these clones (P1115) was selected on the basis of significant homology with rat SC2 gene. The 1,111-bp P1115 insert has been sequenced in its entirety. The size of this cDNA insert appears very close to that of the corresponding mRNA suggesting that this cDNA is very near full-length. This clone was designated as 'human SC2' (hSC2).

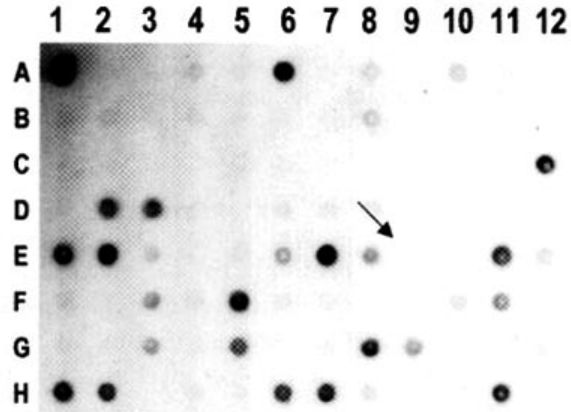

Fig. 1. Selection of low abundance cDNA using cDNA array method. Arrow indicates clone P1115.

The hSC2 sequence contains an open reading frame of 924 nucleotides, encoding a putative 308-amino acid protein with a calculated molecular weight of 36 kDa. The first in-frame ATG encoding the presumed initiating methionine is assumed to be the start methionine for two reasons; the sequences around the ATG sequences satisfies the Kosak consensus sequence, and the sequences from this ATG site is very similar to rat SC2 gene. The 3'-untranslated sequence contained a polyadenylation signal, AATAAA, followed by a poly(A) tail (Fig. 2). The sequence of our hSC2 has been registered in GenBank and its access number is AF222742.

A schematic representation of the protein appears in Fig. 3. The hSC2 protein appears to be generally hydrophobic in character and possesses two stretches of 23 amino acids (amino acids 87-109

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   1 CGC AGT TAG GCA GCA GCA GCC GCG GAC GAG TAG CCG CCG TGG GAG GGA GCC ATG AAG CAT
   1                                                                   M   K   H
  61 TAC GAG GTG GAG ATT CTG GAC GCA AAG ACA AGG GAG AAG CTG TGT TTC TTG GAC AAG GTG
   4   Y   E   V   E   I   L   D   A   K   T   R   E   K   L   C   F   L   D   K   V
 121 GAG CCC CAC GCC ACC ATT GCG GAG ATC AAG AAC CTC TTC ACT AAG ACC CAT CCG CAG TGG
  24   E   P   H   A   T   I   A   E   I   K   N   L   F   T   K   T   H   P   Q   W
 181 TAC CCC GCC CGC CAG TCC CTC CGC CTG GAC CCC AAG GGC AAG TCC CTG AAG GAT GAG GAT
  44   Y   P   A   R   Q   S   L   R   L   D   P   K   G   K   S   L   K   D   E   D
 241 GTT CTG CAG AAG CTG CCC GTG GGC ACC ACG GCC ACA CTG TAC TTC CGG GAC CTG GGG GCC
  64   V   L   Q   K   L   P   V   G   T   T   A   T   L   Y   F   R   D   L   G   A
 301 CAG ATC AGC TGG GTG ACG GTC TTC CTA ACA GAG TAC GCG GGG CCC CTT TTC ATC TAC CTG
  84   Q   I   S   W   V   T   V   F   L   T   E   Y   A   G   P   L   F   I   Y   L
 361 CTC TTC TAC TTC CGA GTG CCC TTC ATC TAT GGC CAC AAA TAT GAC TTT ACG TCC AGT CGG
 104   L   F   Y   F   R   V   P   F   I   Y   G   H   K   Y   D   F   T   S   S   R
 421 CAT ACA GTG GTG CAC CTC GCC TGC ATC TGT CAC TCA TTC CAC TAC ATC AAG CGC CTG CTG
 124   H   T   V   V   H   L   A   C   I   C   H   S   F   H   Y   I   K   R   L   L
 481 GAG ACG CTC TTC GTG CAC CGC TTC TCC CAT GGC ACT ATG CCT TTG CGC AAC ATC TTC AAG
 144   E   T   L   F   V   H   R   F   S   H   G   T   M   P   L   R   N   I   F   K
 541 AAC TGC ACC TAC TAC TGG GGC TTC GCC GCG TGG ATG GCC TAT TAC ATC AAT CAC CCT CTC
 164   N   C   T   Y   Y   W   G   F   A   A   W   M   A   Y   Y   I   N   H   P   L
 601 TAC ACT CCC CCT ACC TAC GGA GCT CAG CAG GTG AAA CTG GCG CTC GCC ATC TTT GTG ATC
 184   Y   T   P   P   T   Y   G   A   Q   Q   V   K   L   A   L   A   I   F   V   I
 661 TGC CAG CTC GGC AAC TTC TCC ATC CAC ATG GCC CTG CGG GAC CTG CGG CCC GCT GGG TCC
 204   C   Q   L   G   N   F   S   I   H   M   A   L   R   D   L   R   P   A   G   S
 721 AAG ACG CGG AAG ATC CCA TAC CCC ACC AAG AAC CCC TTC ACG TGG CTC TTC CTG CTG GTG
 224   K   T   R   K   I   P   Y   P   T   K   N   P   F   T   W   L   F   L   L   V
 781 TCC TGC CCC AAC TAC ACC TAC GAG GTG GGG TCC TGG ATC GGT TTC GCC ATC ATG ACG CAG
 244   S   C   P   N   Y   T   Y   E   V   G   S   W   I   G   F   A   I   M   T   Q
 841 TGT CTC CCA GTG GCC CTG TTC TCC CTG GTG GGC TTC ACC CAG ATG ACC ATC TGG GCC AAG
 264   C   L   P   V   A   L   F   S   L   V   G   F   T   Q   M   T   I   W   A   K
 901 GGC AAG CAC CGC AGC TAC CTG AAG GAG TTC CGG GAC TAC CCG CCC CTG CGC ATG CCC ATC
 284   G   K   H   R   S   Y   L   K   E   F   R   D   Y   P   P   L   R   M   P   I
 961 ATC CCC TTC CTG CTC TGA GCG CTC ACC CCT GCT GAG GCT CAG CCC CTC AAC CCG GTG GCA
 304   I   P   F   L   L   *
1021 TTC TGG GGG AGG AGT GGG GCC CAC AGC TCT CCA GCA CCC GGA ATA AAG CCC GCC TGC CCC
1081 AGT CAA AAA AAA AAA AAA AAA AAA AAA AAA A
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Fig. 2. cDNA and deduced amino acid sequence of hSC2. Corresponding predicted amino acid residues are shown in a single letter code. The polyadenylation signal is underlined.

and 256-278) that meet the requirement of Kyte and Doolittle[11] for a membrane-spanning domain. The hSC2 sequence also possesses three N-linked glycosylation consensus sequences consistent with the possibility that hSC2 is a glycoprotein. Examination of the hSC2 sequence, however, revealed that there was no hydrophobic signal sequence at the amino-terminal end of the putative protein.

Comparison of the derived amino acid sequence with all those present in the GenBank database revealed strong similarity between hSC2 and rat SC2. Rat SC2 is a novel brain cDNA isolated by a mixed polyclonal antibody directed against synaptic glycoproteins. A lineup of the human and rat SC2 protein sequences is shown in Fig. 4. The overall identity in amino acid sequences between these two proteins is 97.4%.

The hSC2 protein also shares similarity with 5α-reductase protein. 5α-reductase is a microsomal enzyme that plays a critical role in testosterone metabolism, converting testosterone to the more potent dihydrotestosterone. Homologies between hSC2 and either 5α-reductase type 1 or 5α-reductase type 2 were 22.9% and 24.7% overall, higher in the final 172 amino acids (28.7% and 28.2%, respectively) (Fig. 5). Glu^{251} in hSC2 correspond to Glu^{197} in 5α-reductase type 2 which is absolutely required for enzyme activity.

The size and tissue distribution of hSC2 mRNA was determined by Northern blot analysis. Figure 6 shows that a 1.35 kb mRNA was expressed preferentially in testis, liver, pancreas, skeletal muscle, kidney, heart, brain, placenta, spleen, thymus, prostate, and small intestine. The hSC2 mRNA expression was also clear in ovary, colon, lung and peripheral blood leukocytes. The hSC2 expression was also detected in various cell lines of epithelial or myeloid lineage.

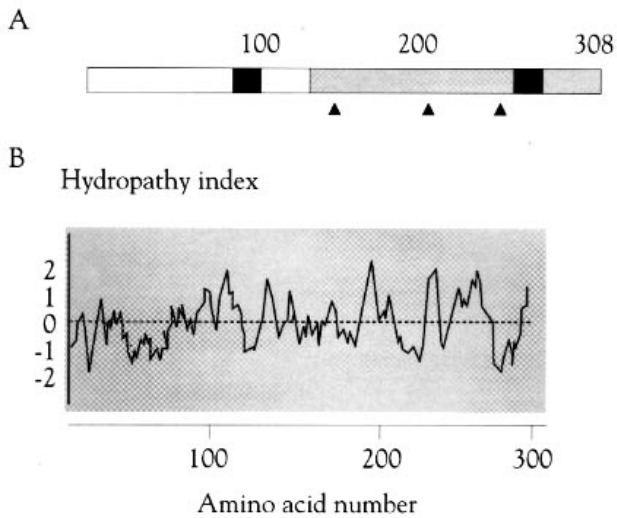

Fig. 3. Analysis of putative hSC2 primary structure. A: Schematic representation of hSC2 protein as derived from cDNA sequence. Shaded area represents areas of similarity with 5α-reductase; Black areas represent possible transmembrane domain; arrowheads represent N-linked glycosylation consensus sequences. B: Hydropathy profile of hSC2 protein. The putative amino acid sequence of the hSC2 protein was analyzed using the algorithm of Kyte and Doolittle averaged over six amino acids. Hydrophobic regions are assigned positive values.

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hSC2      1 MKHYEVEILDAKTREKLCFLDKVEPHATIAEIKNLFTKTHPQWYPARQSLRLDPKGKSLK  60
            MKHYEVEI DAKTREKLCFLDKVEP ATI+EIK LFTKTHPQWYPARQSLRLDPKGKSLK
rat SC2   1 MKHYEVEIRDAKTREKLCFLDKVEPQATISEIKTLFTKTHPQWYPARQSLRLDPKGKSLK  60
hSC2     61 DEDVLQKLPVGTTATLYFRDLGAQISWVTVFLTEYAGPLFIYLLFYFRVPFIYGHKYDFT 120
            DEDVLQKLPVGTTATLYFRDLGAQISWVTVFLTEYAGPLFIYLLFYFRVPFIYG KYDFT
rat SC2   61 DEDVLQKLPVGTTATLYFRDLGAQISWVTVFLTEYAGPLFIYLLFYFRVPFIYGRKYDFT 120
hSC2    121 SSRHTVVHLACICHSFHYIKRLLETLFVHRFSHGTMPLRNIFKNCTYYWGFAAWMAYYIN 180
            SSRHTVVHLAC+CHSFHYIKRLLETLFVHRFSHGTMPLRNIFKNCTYYWGFAAWMAYYIN
rat SC2   121 SSRHTVVHLACMCHSFHYIKRLLETLFVHRFSHGTMPLRNIFKNCTYYWGFAAWMAYYIN 180
hSC2    181 HPLYTPPTYGAQQVKLALAIFVICQLGNFSIHMALRDLRPAGSKTRKIPYPTKNPFTWLF 240
            HPLYTPPTYG QQVKLALAIFVICQLGNFSIHMALRDLRPAGSKTRKIPYPTKNPFTWLF
rat SC2   181 HPLYTPPTYGVQQVKLALAIFVICQLGNFSIHMALRDLRPAGSKTRKIPYPTKNPFTWLF 240
hSC2    241 LLVSCPNYTYEVGSWIGFAIMTQCLPVALFSLVGFTQMTIWAKGKHRSYLKEFRDYPPLR 300
            LLVSCPNYTYEVGSWIGFAIMTQC+PVALFSLVGFTQMTIWAKGKHRSYLKEFRDYPPLR
rat SC2   241 LLVSCPNYTYEVGSWIGFAIMTQCVPVALFSLVGFTQMTIWAKGKHRSYLKEFRDYPPLR 300
hSC2    301 MPIIPFLL                                                     308
            MPIIPFLL
rat SC2   301 MPIIPFLL 308
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Fig. 4. Comparison of the hSC2 protein sequence with rat SC2.

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hSC2   137  HYIKRLLETLFVHRFSHGTMPLRNIFKNCTYYWGIAAWMAYYINHPLYTPPTYGAQQVKL
5AR2    90  HYFHRTFVYSLLNRGR—PYPAILILRGTAFCTGNGVLQGYYLIYCAEYPDGWYTD-IRF
hSC2   197  ALAVFVICQLGNFSIH—MALRDLRPAGSKTRKIPYPTKNPFTWLFLLVSCPNYTYEVGS
5AR2   147  SLGVFLFILGMGINIHSDYILRQLRKPGEISYRIPQGG———LFTYVSGANFLGEIIE
hSC2   255  WIGFAIMTQCLPVALFSLVGFTQMTIWAKGKHRSYLKEFRDYPPLRMPIIPFLL
5AR2   201  WIGYALATWSLPALAFAFFSLCFLGLRAFHHHRFYLKMFEDYPKSRKALIPFIF
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Fig. 5. Comparison of the hSC2 protein sequence with 5α-reductase type 2. Identical residues are marked with a line. One dot indicates amino acid substitutions that could occur with a single nucleotide change. Arrow head indicates the glutamate which is absolutely required for enzyme activity.

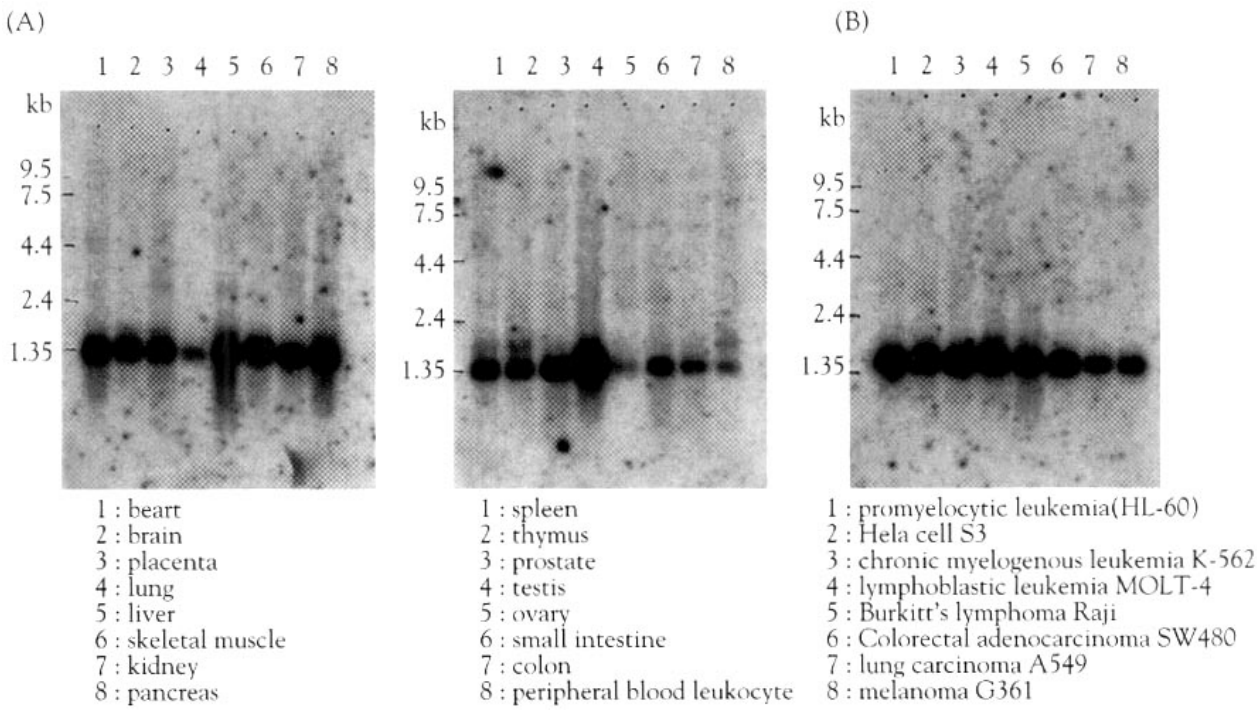

Fig. 6. Northern blot analysis of hSC2 mRNA expression.
A: human adult tissues, B: cancer cell lines.

DISCUSSION

cDNA sequence data suggests that the putative hSC2 protein is a 308-amino acid long molecule, with a calculated molecular weight of 36 kDa. Although the derived sequence possesses three potential N-linked glycosylation sites, as well as a potential membrane-spanning domain, an amino-terminal hydrophobic signal sequence is lacking.

The hSC2 shares 97.4% amino acid sequence identity with the rat SC2. It is a novel brain cDNA isolated using a mixed polyclonal antibody directed against concanavalin A-binding synaptic junctional glycoproteins[12]. Rat SC2 is detected throughout postnatal development and strongly expressed in many types of neurons in rat.

Northern blots from 16 different human tissues showed that hSC2 transcripts were ubiquitously expressed as 1.35 kb band. The level of transcript was relatively high in those tissues such as testis, liver, pancreas, skeletal muscle, kidney, heart, brain, placenta, spleen, thymus, prostate, small intestine and relatively low in ovary, colon, lung and peripheral blood leukocyte. hSC2 expression was

markedly detectable in various cell lines of epithelial or myeloid lineage tested.

Similarity searches revealed some similarity between hSC2 and the microsmal enzyme 5α-reductase type 1 and type 2[13-15]. The use of the simple algorithm of Doolittle[16] suggests that the level of similarity observed (both about 28% over 173 amino acids) is unlikely to occur by chance alone.

Steroid 5α-reductases catalyze the NADPH-dependent conversion of testosterone to dihydrotestosterone, which is a key step in steroid metabolism and is essential for the embryonic development of male external genitalia and prostate[9,17]. The importance of this reaction is evident from certain forms of hereditary male pseudohermaphroditism in humans that are caused by steroid 5α-reductase deficiency. Sequence analysis of the steroid 5α -reductase type 2 gene from affected families has identified a missense mutation that causes a conservative substitution of aspartate for Glu^{197}, corresponding to Glu^{251} in hSC2.

Sequence analysis of the 5α-reductases mRNA also revealed several points relevant to hSC2. Although 5α-reductases have been shown to be an integral membrane protein of the endoplasmic reticulum, sequence data indicates that the enzymes lack a cleavage signal sequence. Instead, the enzymes are generally hydrophobic in nature, with several prominent hydrophobic regions that may act as membrane-spanning domains. Although an internal signal has not been specifically identified, in vitro translation experiments show that nascent 5α-reductase peptide is incorporated into microsomes, as is normally expected with proteins possessing a conventional signal.

This extensive similarity to 5α-reductase isozymes suggests that hSC2 may perform a similar in *vivo* biochemical function as the 5α-reductase isozymes. To understand in vivo functions of hSC2, it will be important to assay enzyme activity in transfected cell lysate.

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