Isolation and Functional Characterization of a Novel Organic Solute Carrier Protein, hOSCP1

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We succeeded in isolating a novel organic solute carrier from a human placenta cDNA library. The isolated cDNA consisted of 1137 base pairs that encoded a 379-amino acid protein, hOSCP1. Northern blot and reverse transcription PCR analyses revealed that the hOSCP1 mRNA is expressed in the placenta and testis and weakly expressed in the thymus and small intestine. When expressed in Xenopus laevis oocytes, hOSCP1 mediated the high affinity transport of p-aminohippurate (PAH) (Km = 35.0 ± 7.5 μM) and tetraethylammonium (Km = 62.3 ± 12.2 μM) in a sodium-independent manner. However, the hOSCP1-expressing oocyte did not mediate the transport of L-carnitine. The transport of PAH by hOSCP1 was sensitive to pH, but the tetraethylammonium was not transported at the high pH examined. hOSCP1 transported prostaglandin E2, prostaglandin F2α, estrone sulfate, glutarate, L-leucine, L-ascorbic acid, and tetracycline. Thus, hOSCP1 also showed broad substrate specificity. A wide range of structurally unrelated organic compounds inhibited the hOSCP1-mediated PAH uptake. Immunohistochemical analysis revealed that the hOSCP1 protein is localized in the basal membrane of the syncytiotrophoblast in the human placenta. Our results suggest that hOSCP1 is a novel polyspecific organic solute carrier protein responsible for drug clearance from the human placenta.

The plasma membrane of epithelial cells plays a central role in the elimination of a wide range of endogenous and exogenous ionic substrates including drugs and environmental toxicants (1–5). Recent molecular studies have demonstrated that transporters in the liver and kidney are primarily responsible for drug clearance. Such transporters are divided into five distinct families; the organic anion transporters (OATs; SLC22A), the organic cation transporters (OCTs; SLC22A), the ATP-dependent transporters (ABCs), the Na+-coupled bile acid transporters (NTCP; SLC10A1), and the Na+-independent organic anion transporting polypeptides (OATPs/oatps; SLC21/SLCO) (6–10). These transporters are mainly expressed in the brain, liver, and kidney and are involved in the elimination of various organic solutes (organic anions and cations) and conjugated substrates from the body. Thus, the transporters’ function serves as a drug clearance system for the protection of the body from toxic compounds (1–5, 11).

The blood-placenta barrier is well known to exist in the human placenta. The fetus is very sensitive to foreign substrates that enter the fetal circulation, and numerous drugs and chemicals have been known to cause malformations and growth defects. Whitsett (12) has revealed that the transport system of the syncytiotrophoblast mediating absorption and excretion is present in the syncytiotrophoblast and the basal membrane. In addition, Van der Aa et al. (13) have reported that PAH is extensively transported by the membrane vesicle isolated from the human placenta in a pH-dependent manner, suggesting that there exists a transport mechanism or mechanisms for the uptake of organic solutes in the placenta (13). Recent studies have revealed that organic ion transporters such as hOAT4 and OCT3 are abundantly expressed on the basal membrane (14, 15). However, there are still limited data concerning the molecular mechanism on the transport of organic solutes in the human placenta.

In the present report, therefore, we describe the molecular cloning and functional characterization of human organic solute carrier protein 1 (hOSCP1). Our results indicate that hOSCP1 is a novel gene that mediates various kinds of organic solutes in a pH-dependent and sodium-independent manner on the basal membrane of the placenta.

EXPERIMENTAL PROCEDURES

Materials—[14C]PAH (40.6 mCi/mmol) and [3H]valproate (55 Ci/mmol) were purchased from Moravek (Brea, CA). [14C]TEA (55 mCi/mmol), [3H]tetracycline (1 Ci/mmol), [14C]glutamate (55 mCi/mmol), L-[14C]leucine (55 mCi/mmol), and L-[3H]carnitine hydrochloride (80 Ci/mmol) were purchased from ARC Inc. (St. Louis, MO). [3H]Prostaglandin E2 (193.5 Ci/mol), [14C]testosterone (53.6 mCi/mmol), [3H]prostaglandin F2a (214.7 Ci/mol), [3H]estrone sulfate (57.3 Ci/mol), [14C]androstenedione (53.6 mCi/mmol), [14C]progesterone (55.4 mCi/mmol), [14C]ketoglutarate (54.5 mCi/mmol), L-[14C]ascorbic acid (4 mCi/mol), and [14C]salicylic acid (55.5 mCi/mmol) were purchased from PerkinElmer Life Sciences. [α-32P]dCTP (111 TBq/mmol) was obtained from Muromachi Yakuhin Kaisha, Ltd. (Tokyo, Japan). All other chemicals not listed here were of the highest grade commercially available.

Construction of cDNA Library and Isolation of hOSCP1—A non-directional cDNA library was prepared from human placenta poly(A)+ RNA using the Superscript choice system (Invitrogen) and was ligated into a phage vector AZiPlox EcoRI arms (Invitrogen). An expressed sequence tag data base searched for “query hOAT2,” and an expressed sequence tag clone (BX327815) was identified. The EST clone was labeled with [α-32P]dCTP by random priming (Quick Prime Kit, Amersham Biosciences) (16), and the library was screened with an expressed sequence tag clone as a probe under low stringency condi-
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sections. Replicate filters of a phage library were hybridized overnight in a hybridization solution (50% formamide, 5× standard saline citrate, 3× Denhardt’s solution, 0.2% SDS, 10% dextran sulfate, 0.3 μg/ml denatured salmon sperm DNA, 25 mM sodium pyrophosphate, 25 mM MES, and 0.03% antifoam A, pH 6.5) at 37 °C overnight. The filters were washed in 3× standard saline citrate and 0.5% SDS at 37 °C. cDNA inserts in positive ZipLox phage were recovered in a plasmid pZL1 vector by in vitro excision.

DNA Sequence and Hydropathy Analysis—Double-stranded cDNA of isolated clones were sequenced in both directions. Deleted clones, obtained by a KiloSequence deletion kit (Takara, Tokyo, Japan), and specifically synthesized oligonucleotide primers were used for sequencing hOSCP1, which was sequenced by the dye terminator method using a dye primer cycle sequencing kit (Applied Biosystems, Foster City, CA) and an automated Applied Biosystems 310 DNA sequencer. The sequence, membrane topology, and presence of possible signal peptides were assembled and analyzed using DNASIS-Pro (HITACHI Software Engineering, Yokohama, Japan).

Total RNA Isolation, Northern Blotting, and RT-PCR Analyses—Total RNA was isolated from various tissues using the acid guanidium thiocyanate (GTC)-phenol-chloroform extraction method as described by Chomczynski and Sacchi (17). The tissue was homogenized in GTC solution (4 M GTC containing 25 mM sodium citrate (pH 7.0), 0.5% Sarkosyl, and 0.1 M 2-mercaptoethanol; 10 ml of GTC solution was used per 100 mg of liver tissue). The RNA was extracted twice into phenol and chloroform (1 volume of phenol, 0.2 volume of chloroform, and 1 volume of GTC solution) and precipitated with isopropanol at room temperature. The pellet was dissolved in 0.3 ml GTC solution and precipitated with isopropanol at −20 °C. The resulting RNA pellet was washed with ice-cold 80% ethanol and dissolved in an appropriate volume of diethylpyrocarbonate-treated water. The RNA yield, purity, and integrity were determined by measuring the absorbance ratio at 260/280 nm (>1.6) and checked with 1% agarose/formamide gel. A commercially available hybridization blot containing poly(A)+ RNA from various human tissues (human multiple tissue Northern (MTN) blots I and II, Clontech) and human cancer cell line MTN blots (Clontech) were used for the Northern blot analysis. Total RNA from human thymus, liver, small intestine, testis, and placenta were purchased from Clontech. Two μg of poly(A)+ RNA prepared from various mouse and rat tissues were loaded onto 1% agarose/formaldehyde gel. After electrophoresis, the nucleic acids were transferred onto a nylon membrane (Hybond N+, Amersham Biosciences). These filters were hybridized at 42 °C overnight in a hybridization solution (50% formamide) with full-length cDNA of hOSCP1, which was randomly labeled with [32P]dCTP as described above. The membrane was washed in 0.1× standard saline citrate and 0.1% SDS at 42 °C. hOSCP1 cDNA was amplified by RT-PCR using the sense primer 5′-TGTCGTGCAGGTCGCACTTTACA-3′ and antisense primer 5′-GGATCTCGGATGTGCAAGT-3′ to yield a 192-bp fragment corresponding to the coding region between base pairs 331 and 522. RT-PCR was performed under the conditions of one cycle at 60 °C for 30 min, one cycle at 94 °C for 2 min, 25 cycles at 94 °C for 1 min, and annealing at 45 °C for 1 min and 72 °C for 3 min, followed by a final extension at 72 °C for 10 min according to the manufacturer’s instructions (Toyobo, Osaka, Japan).

X. Laevis Oocyte Preparation, cRNA Synthesis, and Transport Assays—Isolation of stage V and stage VI defolliculated Xenopus oocytes was performed as described elsewhere (18). To remove the follicular layer from Xenopus oocytes, collagenase A (Roche Applied Science) was used at a final concentration of 2 mg/ml in an oocyte Ringer 2 solution (83 mM NaCl, 2 mM KCl, 1 mM MgCl2, and 5 mM HEPES, pH 7.5) for 1.5 h at room temperature. The isolated clone, hOSCP1, was linearized with BamHI, and capped cRNA was transcribed in vitro by T7 RNA polymerase (18). Defolliculated oocytes were microinjected with 50 ng of in vitro transcribed cRNA and incubated for 2 days in a modified Barth’s solution containing gentamicin (50 μg/ml) at 18 °C. Uptake experiments of radiolabeled substrates, as indicated in each experiment, were performed in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.4) at room temperature. For the Na+-dependent or -independent uptake experiments, we employed uptake buffers consisting of 96 mM NaCl, 96 mM LiCl, 96 mM choline chloride, or 96 mM mannitol. Oocytes were incubated in 450 μl of the same solution containing radiolabeled substrates for 1 h at room temperature. The uptake was terminated by the addition of 2 ml of ice-cold ND96 solution, and the oocytes were washed with the same solution at least five times. The oocytes were solubilized with 10% SDS, and accumulated radioactivity was determined with a liquid scintillation counter. The experiments were repeated with oocytes from at least five frogs.

Immunohistochemical Analysis—For immunohistochemical analysis, rabbits were immunized with a keyhole limpet hemocyanin-conjugated synthesized peptide, EFITEQRLSTSK, corresponding to cysteine and the 14 amino acids of the COOH terminus of hOSCP1. A 5-μm wax section of human placenta was obtained from BioChain Institute, Inc. (San Leandro, CA), and light microscopic immunohistochemical analysis was carried out using the streptavidin-biotin-horseradish peroxidase complex technique (Dako, Carpinteria, CA). Sections were dewaxed, rehydrated, and incubated with 3% H2O2 for 25 min to eliminate endogenous peroxidase activity. After rinsing in 0.05 M Trisbuffered saline containing 0.1% Tween 20, sections were treated with 10 μg/ml primary rabbit polyclonal antibody (4 °C overnight). Thereafter, the sections were incubated with the secondary biotinylated goat polyclonal antibody against rabbit immunoglobulin (Dako) for 30 min with horseradish peroxidase-labeled streptavidin. This step was followed by incubation with diaminobenzidine and hydrogen peroxide. The sections were counterstained with hematoxylin and examined under light microscopy. For the preabsorption experiment, the hOSCP1 peptide (200 μg/ml) was added to the hOSCP1-specific antibody solution and incubated overnight at 4 °C.

Kinetic Study—Concentration-dependent uptake experiments of [14C]PAH or [14C]TEA in oocytes expressing hOSCP1 were performed with each compound at final concentrations of 1, 5, 10, 20, 50, and 100 μM and 1, 10, 20, 50, 100, and 200 μM, respectively. The compounds were incubated with expressing hOSCP1 oocytes for 2 h at room temperature, stopped with ice-cold ND96 solution, and washed five times as described above. Individual oocytes were transferred to scintillation vials and dissolved in 0.25 ml of 10% SDS. A scintillation mixture was added, and radioactivity was counted. Counts in control uninjected oocytes were subtracted from the counts in cRNA-injected oocytes. Data are presented as mean ± S.E., except for kinetic constants for which the error represents the error of the fit. Km indicates the Michaelis-Menten constant (μM).

Inhibition Study—For inhibition experiments, oocytes expressing hOSCP1 were incubated for 1 h in ND96 solution containing 10 μM [14C]PAH in the presence or absence of various inhibitors at a final concentration of 1 mM, excluding PAH (2 mM). PAH, ibuprofen, captopril, enalapril, clarithromycin, rifampicin, quinidine, diltiazem, cimetidine, and famotidine were directly dissolved in ND96 solution from the stock solution. These stock solutions of the inhibitor were dissolved in dimethyl sulfoxide (Me2SO) and diluted to a final concentration as described above. The final concentration of Me2SO in the assay medium did not exceed 1%.

Statistical Analysis—Kinetic data from experiments measuring the uptake of radiolabeled substrates were fit to the Michaelis-Menten...
equation by nonlinear least squares regression analysis with the S.E. values derived from these curves. Comparisons of data measuring the initial rates of the uptake of radiolabeled substrates in the presence and absence of inhibitors were performed by the unpaired Student’s t test or one-way analysis of variance.

RESULTS

To search for a novel organic solute transport protein, we screened a human placenta cDNA library using BX327815 as a probe. After multiple rounds of screening, we isolated six positive clones. Two clones were identical to the phospholipid transfer protein (GenBank™ accession number AB076694); the remaining four clones had overlapping identical sequences and were identified as human organic solute carrier protein 1. hOSCP1 has 1137 bp with a single open reading frame encoding a 379-amino acid sequence with a calculated molecular mass of 43.3 kDa (GenBank™ accession number AB079075). There are two consensus sequences for O-glycosylation sites (Thr200 and Thr359) (Fig. 1, closed circles). Several membrane transporters have 12 predicted transmembrane domains, suggesting that these transporters may have a common membrane topology and perhaps a common evolutionary origin. However, Kyte-Doolittle hydropathy analysis revealed that hOSCP1 is likely to have three transmembrane domains. The phylogenetic tree analysis using the neighbor-joining and maximum-likelihood methods showed that hOSCP1 is positioned between the multidrug resistance protein 2 and rat liver-methotrexate carrier 1.

While this study was being conducted, a sequence from humans was submitted to the GenBank™ (oxidored-nitro domain-containing protein NOR1; accession number AF462348). The nucleotide sequence of hOSCP1 is 98% identical to that of NOR1 except for five bases (G at position 92 to C, T at position 246 to G, G at position 391 to A, G at position 919 to A, and G at position 1099 to A), resulting in an amino acid change (Arg31 to Pro, Thr131 to Ala, Gly306 to Arg, Ile352 to Ala, and Gly366 to Ser). We also identified several proteins of unknown function such as 1810007P19Rik protein (GenBank™ accession number AAH45150; Mus musculus) and an unnamed protein product (GenBank™ accession number BAC05326; Homo sapiens) that exhibited 93 and 96% predicted amino acid identity, respectively.

To elucidate the tissue distribution of the hOSCP1 gene in humans, Northern blot analysis was performed. As shown in Fig. 2A (left section), a strong 1.4-kb mRNA band and one weak band corresponding to a

FIGURE 1. The deduced amino acid sequence and phylogenetic analysis of hOSCP1. A, deduced amino acid sequence of the human organic anion carrier, hOSCP1. Putative O-glycosylation sites are indicated by closed circles (●). B, phylogenetic relationship between hOSCP1 and several transporters. The phylogenetic tree was constructed using DNAsis-Pro (Hitachi Software Engineering). Branch length is drawn to scale. The meanings of abbreviations not defined in the abbreviations footnote are as follows: hOCT, human organic cation transporter; hOCTN, human organic cation transporter novel; GLUT1, glucose transporter 1; hPGT, human prostaglandin transporter; hNaDC-1, human sodium dicarboxylate cotransporter 1; LAT, L-type amino acid transporter; Mrp2, multidrug resistance-related protein 2; RL-Mtx1, bile acid-sensitive rat liver methotrexate carrier 1; hENT1, human equilibrative nucleoside transporter 1; and rBAT, related to b₃ H₁₁01193 amino acid transporter.
shorter transcript (1 kb) were detected predominantly in the human testis. A significant 1.4-kb mRNA was also faintly detected in other tissues of the thymus, prostate, ovary, small intestine, heart, brain, placenta, lung, kidney, and pancreas. No positive signal was detected in the spleen, colon, peripheral blood leukocytes, liver, and skeletal muscle. To confirm this, we chose some tissues and subsequently performed RT-PCR analysis. As shown in Fig. 2A (right section), RT-PCR identified a signal for hOSCP1 cDNA in the thymus, small intestine, and placenta, but not in the liver.

Based on these findings, we next examined the distribution of hOSCP1 mRNA in mouse and rat tissues by Northern blot analysis. As shown in Fig. 2B, hOSCP1 mRNA is abundantly expressed in the male mouse and rat testes. Hybridization signals could not be detected in mRNA isolated from other tissues such as the brain, eye, lung, heart, liver, kidney, pancreas, and skeletal muscle. However, no short transcript (1 kb) was detected in mouse and rat tissues as observed in human tissues. Thus, the expression of hOSCP1 mRNA in rodents was tissue-specific. We could not detect any bands corresponding to hOSCP1 mRNA in all tissues of the guinea pig (data not shown), indicating species-related differential distribution of the hOSCP1 gene. Interestingly, specific bands corresponding to hOSCP1 were detected in several human tumor-derived cell lines such as HL60, HeLa S3, MOLT-4, Raji, SW480, A549, and G361. In particular, hOSCP1 mRNA was highly expressed in A549 cells. No positive signal was detected in K562 cells.

Based on the structural similarities observed between rat liver-methotrexate carrier 1 and multidrug resistance-related protein 2 (Fig. 1), we assumed that hOSCP1 can transport organic solutes. Therefore, X. laevis oocytes injected with hOSCP1 cRNA were used for the transport characterization. Because the uptake of [14C]PAH by uninjected oocytes was equal to the oocytes injected with 50 nanoliters of water (data not shown), uninjected oocytes were used as the control instead of water-injected oocytes throughout this study. As shown in TABLE ONE, hOSCP1 cRNA-injected oocytes had higher transport of [3H]estrone sulfate, [3H]glutamate, L-[14C]leucine, L-[14C]ascorbic acid, [3H]tetraacycline, [3H]prostaglandin E2, [3H]prostaglandin F2α, [14C]PAH, and [14C]TEA. No significant transport activity was observed in the hOSCP1-mediated uptake of [14C]androstenedione, [14C]progesterone, [14C]testosterone, L-[1]H]carnitine, L-[14C]ketoglutarate, [14C]salicylate, and [3H]valproate. These findings indicate that hOSCP1 transports structurally dissimilar compounds.

It has been reported that the transport of organic compounds mediated by hOAT4 is sodium-independent, whereas the uptake of TEA mediated by OCT3 is sodium-dependent (14, 15). To elucidate further the transport characteristics of hOSCP1 based on their findings, we examined the effect of sodium-, pH-, time-, and concentration-dependent uptake. As shown in Fig. 3A, the uptake of [14C]PAH and [14C]TEA via hOSCP1 were not affected by the replacement of extracellular sodium with choline, lithium, and mannitol. These results indicate that hOSCP1 is sodium-independent. The effect of pH on the transport of PAH and TEA by hOSCP1-expressing oocytes was subsequently examined (Fig. 3B). There was a marked change in PAH transport with pH, namely a much higher transport at a low pH and lower transport at a high pH. However, the transport of TEA was not affected by changes in pH between 5.5 and 8.5. Thus, the uptake of anionic compounds mediated by hOSCP1 is sensitive to pH, but cationic substrates...
are not. Fig. 3C shows the time-dependent uptake of PAH via hOSCP1. The cell-associated count of [14C]PAH increased until 2.5 h of incubation on hOSCP1-expressing oocytes. Likewise, the transport of [14C]TEA via hOSCP1 showed time dependence up to 2.5 h (data not shown). This result indicates that hOSCP1 not only binds but also transports organic solutes into cytoplasm. The concentration dependence of the uptake of [14C]PAH and [14C]TEA via hOSCP1 is shown in Fig. 4. The hOSCP1-mediated uptake of [14C]PAH and [14C]TEA showed saturation kinetics and could be modeled by the Michaelis-Menten equation (Fig. 4, A and B). Nonlinear regression analyses yielded K_m values of 35.0 ± 7.5 μM and 62.3 ± 12.2 μM for PAH and TEA, respectively. These findings lead us to conclude that hOSCP1 mediated the transport of anionic organic solutes in a sodium-independent and pH-dependent manner.

To elucidate further the substrate specificity of hOSCP1, the inhibition of hOSCP1-mediated [14C]PAH uptake by various compounds was subsequently investigated. As shown in Fig. 5, cis-inhibitory effects were observed for structurally unrelated organic anions. The inhibition of the hOSCP1-mediated transport of [14C]PAH exhibited a rank order of enalapril > quinidine > captopril = famotidine = ibuprofen = cimetidine. The results indicate that hOSCP1 functions as a carrier with broad substrate specificity.

To determine the localization of hOSCP1 in the placenta, we subsequently carried out an immunohistochemical analysis. Light microscopy of 5-μm wax sections demonstrated that there was specific immunostaining of hOSCP1 in the placenta (Fig. 6B). Under high magnification, hOSCP1 was located in the basal membrane of the syncytiotrophoblast (Fig. 6C). By preincubation of the antibody with hOSCP1 peptide, the immunoreactivity was diminished (Fig. 6A). The specificity of the antibody for hOSCP1 was verified by these results.

DISCUSSION

The present study describes the isolation and functional characterization of a novel organic solute carrier protein, hOSCP1. The placenta consists of syncytiotrophoblasts or polarized cells as well as kidney proximal tubules and intestinal epithelial cells that mediate the absorption of essential nutrients and the excretion of waste products, including organic compounds for developing the fetus and protecting it from toxic compounds (12). In the placenta four distinct organic ion transporters, namely the amino acid transporter (19), the glucose transporter (20), the vitamin transporter (21), and the nucleotide transporter (22), have been isolated and well characterized to date. The role of these transporters is to facilitate the supply of essential compounds for fetal development. In contrast to these observations, only two organic solute transporters, hOAT4 and OCT3, have been isolated (14, 15). hOAT4 mediates the transport of anionic compounds such as estrone sulfate and dehydroepiandrosterone sulfate (15), whereas OCT3 mediates the transport of cationic compounds such as TEA and serotonin (14). Therefore, these transporters are considered to be major xenobiotic transporters in the placenta. Although hOAT4-expressing oocytes mediated the uptake of PAH (15), the transport rate was much lower than that of a previous report (13), suggesting that there is another transport system in the placenta. Because the elucidation of drug clearance from the placenta during pregnancy at a molecular level is important from a therapeutic point of view, many investigators have attempted to isolate the transporter responsible for the transport of organic solutes in the placenta; however, information on the transepithelial transport of organic solutes at the molecular level is still unknown.

The tissue distribution and the expression of the hOSCP1 gene were subsequently investigated. The expression of hOSCP1 mRNA in other tissues was low...
or nonexistent despite the fact that hOSCP1 mRNA is predominantly expressed in the testis; therefore, we chose some human tissues (testis, placenta, liver, small intestine, and thymus) and performed RT-PCR analysis to confirm whether the hOSCP1 gene is expressed. Although the liver is known to act as a secreting organ, no PCR product was found. At present, it is not clear whether hOSCP1 functions in the liver. Moreover, we found that hOSCP1 mRNA is abundantly expressed in tumor-derived cell lines, especially A549 cells (lung carcinoma), irrespective of the lower hOSCP1 gene expression in the normal lung. These findings suggest that hOSCP1 might be up-regulated to support the high protein synthesis for cell growth or cell activation.

Using a Xenopus oocyte expression system, hOSCP1-cRNA-injected oocytes were used for transport characterization. Of interest was the observation that hOSCP1 mediates the transport of both PAH and TEA with high affinity in a Na⁺-independent manner and that the transport of PAH via hOSCP1 exhibits pH dependence. Furthermore, oocytes expressing hOSCP1 do not transport L-carnitine, suggesting that a compound having both positive and negative charges (zwitterions) may not
be a substrate of hOSCP1. TEA is a prototypical substrate of OCT1, OCT2, OCT3, and OCTN1, whereas tetramethylammonium is not recognized by OCT3 (14). Therefore, tetramethylammonium might be a good substrate for investigating further detailed substrate selectivity of hOSCP1. To elucidate the substrate selectivity of hOSCP1 based on these findings, uptake studies were subsequently performed using several radiolabeled compounds. As expected, several structurally dissimilar compounds were transported via hOSCP1. These findings lead us to conclude that hOSCP1, as well as OAT and OATP/oatp, is an organic solute carrier protein with broad substrate specificity. However, we did not observe the trans-stimulatory effect of PAH on the hOSCP1-mediated efflux of PAH (data not shown). Thus, the driving force of the hOSCP1-mediated transport still remains unclear. In this respect, Li et al. (23) have revealed that Oatp1 (Sclt1a1) is an organic anion/glutathione one exchanger. Sekine et al. (8) have reported that OAT1 is an organic anion/dicarboxylic acid and substrates. Unspecified endogenous or exogenous substances might be involved in the hOSCP1-mediated transport. Although the physiological functions of hOSCP1 are still unknown, steroids (estrone sulfate) and eicosanoids (prostaglandin E2 and prostaglandin F2α) are transported by hOSCP1, suggesting that hOSCP1 may be involved in the regulation of cell functions; therefore, one possible function of hOSCP1 is to regulate cellular uptake and/or elimination of signaling molecules.

Wang et al. (24) recently revealed that both organic solute transporter α (OST-α) and organic solute transporter β (OST-β) are required to achieve the transport of organic compounds. It is not clear at this time whether the hOSCP1 protein functions as monomers, multimers, or some native oocyte protein to form the activate transport molecule speculated as OST-α and OST-β. In this respect we have not yet tested this prediction on an experimental basis. Further detailed study will be required as to whether this protein is functioning as a transport activator or a regulator such as OST-α-OST-β and the 4F2 heavy chain for the L-type amino acid transporter 1 (LAT1) (25, 26) or whether the transport of organic solutes via hOSCP1 is a single transport system. It would be interesting to know whether hOSCP1 is coupled to other unidentified proteins, because hOSCP1 is expressed in the human body.

Additional experiments involving expression of hOSCP1 in transformed cell lines will better define the transport system associated with this clone.

It has been known that several membrane transporters such as OATs are predicted to have 12 membrane spanning domains (8). Seward et al. (25) have recently reported that OST-α and OST-β are predicted to have seven and one transmembrane domains, respectively (25). In contrast to these observations, Kyte-Doolittle hydropathy analysis has predicted that hOSCP1 is likely to have three membrane-spanning domains. Similar result has been obtained by Rose hydropathy analysis. These findings suggest that hOSCP1 may not have a similar membrane topology as that of OATs and OST-α/β and may have a different evolutionary origin. Although this prediction has not yet been determined experimentally, analysis of hOSCP1 with the SignalP version 1.1 program (www.cbs.dtu.dk/services/) predicted that the first 27 amino acids (amino acid 2–28) form a signal peptide. Because this region contains a putative transmembrane domain, the mature hOSCP1 protein would at least have a single transmembrane domain.

Based on the transport experiments, we subsequently studied the cis-inhibitory effect of hOSCP1-mediated uptake by various organic anions in order to clarify further the detailed substrate selectivity. The transport of PAH mediated by hOSCP1 interacted with some compounds such as ibuprofen, captopril, enalapril, quinidine, cimetidine, and famotidine. Because these drugs are known to cross the placental barrier, our results indicate that these drugs, at least partly, are candidates for the substrate of hOSCP1.

Kekuda et al. (14) and Cha et al. (15) have revealed that hOAT4 and OCT3 are expressed in the fetal facing basal membrane of the placenta (14, 15). For a comprehensive understanding of the role of hOSCP1 in the human placenta, immunohistochemical analysis was performed to determine the localization of hOSCP1. hOSCP1 was localized on the syncytiotrophoblast membrane facing the fetal blood, suggesting that hOSCP1 functions in the entry of organic solutes from fetal circulation into the placental trophoblast. Taken together, hOSCP1, as well as hOAT4 and OCT3, is likely to play a critical role in the placental handling of organic solutes to protect the fetus from toxic compounds.

The hOSCP1 gene was also predominantly expressed in human, rat, and mouse testes. The uptake of organic solutes across the blood-testis barrier has also been predicted. For example, Saigo (27) has reported that premedication administration of chlorpromazine to the parent rat influences the reproduction of the F0 and F1 generations (26). It would also be interesting to elucidate whether hOSCP1 is involved in the reproductive toxicity caused by various organic solutes.

In conclusion, we describe the molecular cloning and transport characteristics of a novel human organic solute carrier protein, hOSCP1. Our results, therefore, are expected to facilitate research on drug discovery and to provide clues in the search for still unidentified drug transporters in the human placenta. In addition, our results may provide new insights into drug clearance from the human body.

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