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Uptake transporter organic anion transporting polypeptide 1B3 contributes to the growth of estrogen-dependent breast cancer

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ABSTRACT

Estrone-3-sulfate is one of the most abundant estrogen precursors in postmenopausal women. We previously showed that estrone-3-sulfate transporters are present in human breast cancer-derived MCF-7 cells (J. Pharmacol. Exp. Ther. 311 (2004) 1032–1037) and that inhibition of estrone-3-sulfate uptake resulted in the suppression of cell growth (Pharm. Res. 22 (2005) 1634–1641); therefore, estrone-3-sulfate transporter should be a novel target for therapy of hormone-dependent breast cancers. The purpose of the present study is to identify the transporter(s) responsible for the uptake of estrone-3-sulfate in breast cancer cells. We obtained two subclones of MCF-7 cells with different estrone-3-sulfate uptake activities and searched for differentially expressed transporter genes by means of DNA microarray analysis. Among several candidate transporters identified, OATP1B3 was further evaluated, since the uptake characteristics of estrone-3-sulfate by MCF-7 cells seemed consistent with the transport properties of OATP1B3. The contribution of OATP1B3 to estrone-3-sulfate uptake by MCF-7 cells was examined by the relative activity factor (RAF) method, and was calculated to amount to 6%. This result suggests that OATP1B3 is one of the transporters contributing to the supply of the estrogen precursor estrone-3-sulfate to estrogen-dependent breast cancer cells.

1. Introduction

Breast cancer is one of the major causes of cancer death in women. Since two-thirds of breast cancers are estrogen-dependent, estrogen is an important risk factor for progression of breast tumors [1]. However, breast cancers frequently occur during the postmenopausal period, when the circulating estrogen level is low because of loss of ovarian function [2,3]. Although the circulating level of estrogen is low, tissue concentrations of estrogen in breast cancer are significantly higher than those found in the plasma or in adjacent normal breast tissues, suggesting a specific mechanism of local biosynthesis from precursors [2]. The biologically active form of estrogen is estradiol, which is synthesized from the precursors through two main pathways; one is the aromatase pathway, in which aromatase converts androgens to estrogens, and the other is the sulfatase pathway, in which sulfatase converts estrone-3-sulfate to estrone. Both of them are currently molecular targets of endocrine therapy of breast cancer. Although estrone-3-sulfate is a biologically weak ligand of estrogen receptor, it is one of the most important forms of circulating estrogen. The plasma concentration of estrone-3-sulfate is about 5–10 times higher than that of other conjugated estrogens, and its half-life is longer than that of estradiol in postmenopausal women [4–6]. Moreover, sulfatase activity is 50–200 times higher than aromatase activity in breast cancer cells [6–8]. In addition, sulfatase activity in breast cancer cells is higher than that of normal breast cells [9]. Therefore, estrone-3-sulfate is thought to play an important role in the progression of breast cancer as a precursor of active estrogen.

Estrone-3-sulfate is highly hydrophilic, so that it cannot readily cross the plasma membrane, whereas unconjugated estrogens, such as estrone and estradiol, are lipophilic and can enter cells via simple diffusion. Thus, estrone-3-sulfate import into cells across the plasma membrane is expected to involve active transport. Indeed, we previously reported that the uptake of estrone-3-sulfate across the plasma membrane in T-47D cells and MCF-7 cells, which

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are estrogen-dependent breast cancer cell lines, is mediated by a specific transport mechanism [10,11].

Accordingly, we hypothesized that the transporter responsible for this transport process could be a novel target for endocrine therapy of estrogen-dependent breast cancers. To develop this concept further, it is necessary to identify the estrone-3-sulfate uptake transporter(s) in breast cancer cells. In our previous study, mRNA expression of some organic anion transporting peptide (OATP) and organic anion transporter (OAT) molecules was detected in MCF-7 cells by RT-PCR [11]. In addition, the uptake of estrone-3-sulfate by MCF-7 cells and T-47D cells was inhibited by anionic compounds, such as bromosulfophthalein, taurocholate, cholate, and probenecid, which are substrates of OATPs or OATs. Some of these transporters may be involved in the uptake of estrone-3-sulfate in MCF-7 cells, but their contribution remains to be examined.

The purpose of this study is to identify the responsible transporter molecules among previously identified estrone-3-sulfate transporters, based on the correlation between mRNA expression levels and the uptake activity of estrone-3-sulfate in subclones of breast cancer-derived MCF-7 cells with differential transport activity.

2. Materials and methods

2.1. Materials

[^{3}H]Estrone-3-sulfate, ammonium salt (1702.0 Gbq/mmol) and [^{3}H]cholecystokinin octapeptide (CCK8) (3590 Gbq/mmol) were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA) and GE Healthcare UK Ltd. (Buckinghamshire, England), respectively. MCF-7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). Fetal bovine serum (FBS) was obtained from Invitrogen Life Technologies (Carlsbad, CA). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan).

2.2. DNA microarray analysis

MCF-7 cells were homogenized in Isogen (Wako Pure Chemical Industries) and the RNA phase was separated by using chloroform. Total RNA was precipitated with isopropyl alcohol, and the resultant pellet was washed with 70% ethanol for subsequent DNA microarray analysis. The expression profiles of transporter genes in the MCF-7 cells were analyzed by Kurabo Co. (Osaka, Japan) using a CodeLink™ Bioarray (GE Healthcare Bio-Sciences KK, Piscataway, NJ). Briefly, first-strand cDNA was transcribed from total RNA using T7 oligo primer and reverse transcriptase at 42 °C. The second-strand cDNA was synthesized from first-strand cDNA using DNA polymerase mix at 16 °C, then cleaned up with a QIAquick PCR purification Kit (QIAGEN, Valencia, CA). Biotin-labeled cRNA was synthesized from the double-stranded cDNA using T7 RNA polymerase-catalyzed *in vitro* transcription in the presence of biotin-labeled NTP, then fragmented at 94 °C. Biotin-labeled cRNA was heated at 90 °C for 5 min and was hybridized with the CodeLink™ Bioarray at 37 °C for 20 h. The CodeLink™ Bioarray was washed with buffer, stained with streptavidin-Cy5 solution, washed again, and scanned with a laser scanner (Applied Precision Inc., Pittsburgh, PA).

2.3. Cell culture

MCF-7 cells were grown in a humidified incubator at 37 °C, under 5% CO_2 in air, in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 100 units/mL penicillin G, and 100 μg/mL streptomycin.

2.4. Transport experiments with Xenopus laevis oocytes expressing OATP1B3

Uptake experiments were conducted with *X. laevis* oocytes that had been microinjected with complementary RNA (cRNA) of OATP1B3 (SLCO1B3) synthesized *in vitro* using T7 RNA polymerase (Ambion, Austin, TX). Briefly, defoliculated oocytes were injected with 50 nL of water containing 25 ng of cRNA, cultured for 3 days in modified Barth's solution (MBS, 88 mM NaCl, 1 mM KCl_2, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$ and 10 mM HEPES adjusted to pH 7.4 with NaOH), and used for uptake experiments. Uptakes of [^{3}H]estrone-3-sulfate and [^{3}H]CCK8 were measured at room temperature in MBS. Uptake was terminated by the addition of ice-cold MBS. Oocytes were washed three times by the addition of ice-cold MBS. For quantitation of test compounds, oocytes were solubilized in 5% sodium dodecyl sulfate solution, and the radioactivity was measured with a liquid scintillation counter after addition of liquid scintillation cocktail (Nacalai tesque, Kyoto, Japan). As the control, the same volume of water was injected into oocytes, which were then cultured for the same number of days, and the uptake was measured in the same manner as described above. To estimate kinetic parameters for two saturable components, the uptake rate (v) was fitted to the following equation by means of nonlinear least-squares regression analysis using KaleidaGraph (Synergy Software, Reading, PA).

$$v = \frac{V_{\max_1} * s}{(K_{m_1} + s)} + \frac{V_{\max_2} * s}{(K_{m_2} + s)}$$

where v and s are the uptake rate and concentration of substrate, respectively, and K_m and V_{max} represent the half-saturation concentration (Michaelis constant) and the maximum uptake rate, respectively. Here, suffixes 1 and 2 represent the high- and low-affinity components of saturable uptake, respectively.

2.5. Transport experiments with MCF-7 cells

Transport experiments were performed as described previously [12]. Briefly, after cultivation of MCF-7 cells in 15-cm dishes, the cells were harvested and suspended in the transport medium containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 25 mM Hepes, adjusted to pH 7.4. The cell suspension and a solution containing a radiolabeled test compound in the transport medium were separately incubated at 37 °C for 20 min and then transport was initiated by mixing them. At appropriate times, 100 μL aliquots of the mixture were withdrawn and the cells were separated from the transport medium by centrifugal filtration through a layer of a mixture of silicon oil (SH550; Dow Corning Toray Silicone Co. Ltd., Tokyo, Japan) and liquid paraffin (Wako Pure Chemicals) with a density of 1.03.

2.6. Estimation of estrone-3-sulfate uptake clearance by OATP1B3 in MCF-7 cells

To estimate the contribution of OATP1B3 to the overall reaction, we used the relative activity factor (RAF) method that has been described for cytochrome P450 [13]. This method has been applied to hepatic uptake transporters, OATP1B1 and OATP1B3 [14]. Based on this method, we estimated the contribution of OATP1B3 to the overall uptake of estrone-3-sulfate by MCF-7 cells. Because CCK8 is thought to be a selective substrate of OATP1B3, it was used as a reference compound for OATP1B3-mediated uptake [15]. The uptake clearance was obtained by dividing the uptake amount by the concentration of test compound in the uptake medium. The ratio of the uptake clearance of CCK8 in MCF-7 cells ($CL_{MCF,CCK8}$) to that in OATP1B3-expressing oocytes ($CL_{OATP1B3,CCK8}$) was calculated and defined as R_{act}. The OATP1B3-mediated uptake clearance

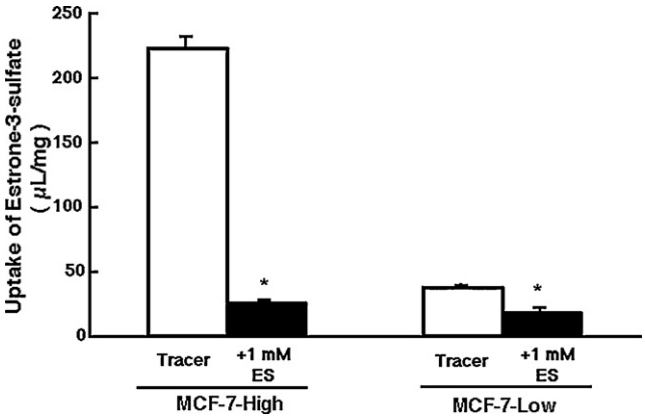

Fig. 1. Differential estrone-3-sulfate uptake activities of two subclones of MCF-7 cells. Uptake of [^{3}H]estrone-3-sulfate (10 nM) by MCF-7 cells was measured in the presence (closed columns, +ES) or absence (open columns) of 1 mM unlabeled estrone-3-sulfate for 10 min at 37 °C. MCF-7 cells with high uptake activity and low uptake activity were defined as MCF-7-High and MCF-7-Low, respectively. Each column represents the mean ± S.E.M. (n = 4). *Significantly different from the control by t-test (p < 0.01).

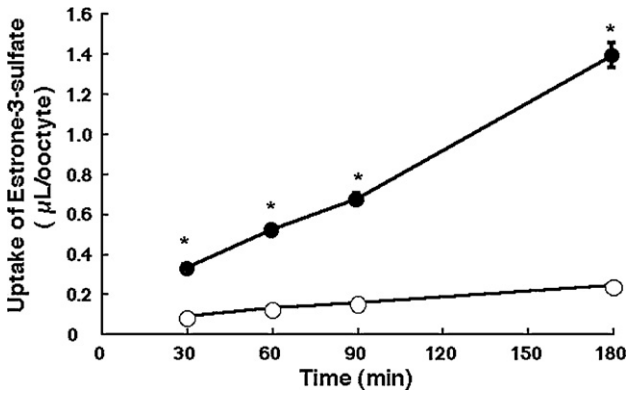

Fig. 2. Time course of [^{3}H]estrone-3-sulfate uptake by oocytes expressing OATP1B3. Uptake of [^{3}H]estrone-3-sulfate (10 nM) by oocytes injected with cRNA of OATP1B3 (closed circles) or water (open circles) were measured over 180 min at room temperature and pH 7.4. Each result represents the mean ± S.E.M. (n = 6–10). *Significantly different from the control by t-test (p < 0.01).

of estrone-3-sulfate in MCF-7 cells ($CL_{MCF,ES,OATP1B3}$) was calculated by multiplying the uptake clearance of estrone-3-sulfate in OATP1B3-expressing oocytes ($CL_{OATP1B3,ES}$) by R_{act}, as described in the following equations.

$$R_{act} = \frac{CL_{MCF,CCK8}}{CL_{OATP1B3,CCK8}} \quad (1)$$

$$CL_{MCF,ES,OATP1B3} = CL_{OATP1B3,ES} \times R_{act} \quad (2)$$

Finally, a contribution of OATP1B3 to the uptake of estrone-3-sulfate in MCF-7 cells was obtained by dividing $CL_{MCF,ES,OATP1B3}$ by a uptake clearance of estrone-3-sulfate in MCF-7 cells, $CL_{MCF,ES}$. To determine the uptake clearance by MCF-7 cells and oocytes expressing OATP1B3, we obtained the uptake clearance by calculating the slope of the uptake in the linear range. The saturable component of the uptake clearance was determined by subtracting the clearance in the presence of unlabeled substrate (excess) from that in the absence of unlabeled substrate (tracer alone).

2.7. Analytical methods

Cellular protein content was determined according to the method of Bradford using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard [16].

All data were expressed as mean ± S.E.M., and statistical analysis was performed by the use of Student's t-test with p < 0.01 or 0.05 as the criterion of significance.

3. Results

3.1. Microarray analysis

To identify transporters involved in the uptake of estrone-3-sulfate into MCF-7 cells, MCF-7 cells were subcloned and the uptake activity in each clone was measured. Those clones showed different uptake activities for estrone-3-sulfate (data not shown). Among them, two subclones were selected for further experiments, i.e., the one that exhibited the highest activity of estrone-3-sulfate uptake (MCF-7-High cells), and the one that exhibited the lowest activity (MCF-7-Low cells) (Fig. 1). The observed activities of [^{3}H]estrone-3-sulfate uptake in the two lines were decreased to the same extent in the presence of 1 mM unradiolabeled estrone-3-sulfate. Therefore, it was thought that the uptakes of estrone-3-sulfate by both lines were carrier-mediated, and that the responsible transporter was expressed at a higher level in MCF-7-High cells than in MCF-7-Low cells.

To identify the transporter, differential expression of transporter mRNAs was examined in the two clones by means of DNA microarray analysis. Messenger RNA were isolated from these cells and gene expression was evaluated by Kurabo Biomedical (Osaka, Japan) using the CodeLink™ Bioarray, which carries 20,469 records on slide glass, of which 209 encode fragments of solute carrier (SLC) transporter genes. Candidate SLC transporter genes, for which the expression level was apparently correlated with estrone-3-sulfate uptake activity, were chosen based on the criterion of a 1.5-fold or higher expression level in MCF-7-High cells than in Low cells. Twenty-six genes showed an expression ratio higher than 1.5 (Table 1). Among them, we focused on transporters which have previously been shown to function as influx transporters of estrone-3-sulfate and those for which no information about their substrates is available. Based on these results, two estrone-3-sulfate transporters, OATP1B3 and OAT3, and two uncharacterized transporters, BTR1 and MCT7, were selected. cDNAs of BTR1 and MCT7 were cloned and uptake studies were performed using oocytes injected with cRNA of BTR1 and MCT7 [17,18]. No transport activity for estrone-3-sulfate was observed in oocytes expressing either BTR1 or MCT7 (data not shown). We previously reported the inhibitory effect of various compounds on the uptake of estrone-3-sulfate by MCF-7 cells, and these results suggested a contribution of OATP transporters, not OAT transporters [11]. Therefore, we focused on OATP1B3 as a candidate transporter for uptake of estrone-3-sulfate in MCF-7 cells.

3.2. Characteristics of estrone-3-sulfate uptake by OATP1B3

The time course of estrone-3-sulfate (10 nM) uptake by oocytes injected with cRNA of OATP1B3 is shown in Fig. 2. The uptake by OATP1B3-expressing oocytes increased linearly up to 180 min, and was significantly higher than that by oocytes injected with water as the control. Thus, the uptake at 60 min was routinely measured as initial uptake in further studies. Fig. 3 shows the relationship between the initial uptake rate and estrone-3-sulfate concentration. The uptake, corrected by subtraction of the uptake by water-injected oocytes, reached saturation at over 500 μM (Fig. 3A). Eadie–Hofstee plot analysis showed biphasic kinetics (Fig. 3B). The values for the high- and low-affinity components of estrone-3-sulfate uptake were 1.24 ± 3.27 μM and 278 ± 11.3 μM (K_m) and 0.347 ± 0.276 pmol/oocyte/60 min and 71.1 ± 0.895 pmol/oocyte/60 min (V_{max}), respectively.

Table 1
Gene expression ratios between MCF-7-Low and MCF-7-High.

Gene name	Protein name	Ratio (high/low)	MCF-7-Low		MCF-7-High	
			Normalized intensity	Quality flag	Normalized intensity	Quality flag
SLC1A1	EAAC1,EAAT3	5.84	0.12	L	0.72	G
SLC1A4	ASCT1,SATT	2.96	0.72	G	2.12	G
SLC2A9	GLUT9	2.96	0.37	L	1.11	G
SLC2A10	GLUT10	13.66	3.11	G	42.86	G
SLC4A1	AE1	1.86	12.20	G	22.86	G
SLC4A11	BTR1	2.36	0.58	L	1.37	G
SLC6A4	SERT	2.26	0.33	L	0.75	G
SLC7A8	LAT2	2.46	1.27	G	3.12	G
SLC7A11	xCT	1.61	0.65	L	1.05	G
SLC8A1	NCX1	2.53	0.25	L	0.64	G
SLC12A2	NKCC1	6.32	1.82	G	11.52	G
SLC12A7	KCC4	4.03	4.31	G	17.36	G
SLC13A2	NaDC1	1.66	0.85	G	1.41	G
SLC16A6	MCT7	19.76	0.16	L	3.18	G
SLC19A2	ThTr1	2.66	5.82	G	15.48	G
SLC19A3	ThTr2	2.11	0.29	L	0.60	G
SLCO1B3	OATP1B3,SLC21A8	1.52	1.41	G	2.14	G
SLC22A3	OCT3	2.10	0.40	G	0.85	G
SLC22A8	OAT3	1.95	0.31	G	0.60	G
SLC25A16	GDC	5.07	2.28	G	11.55	G
SLC26A7		1.56	0.60	G	0.94	G
SLC29A2	ENT2	1.82	0.64	G	1.16	G
SLC30A5	ZNT5	1.56	8.94	G	13.94	G
SLC35A1	CMP-Sia transporter	2.66	8.65	G	23.02	G
SLC35A3	UDP-GlcNAc transporter	2.35	1.27	G	2.98	G
SLC39A2	HZIP2	1.51	0.91	G	1.37	G

Expression levels are shown as normalized intensity of the discovery probe. The ratio was calculated as normalized intensity of MCF-7-High divided by normalized intensity of MCF-7-Low. In the record of quality flag, the symbol "G" and "L" represent reliable expression or unreliable expression, respectively.

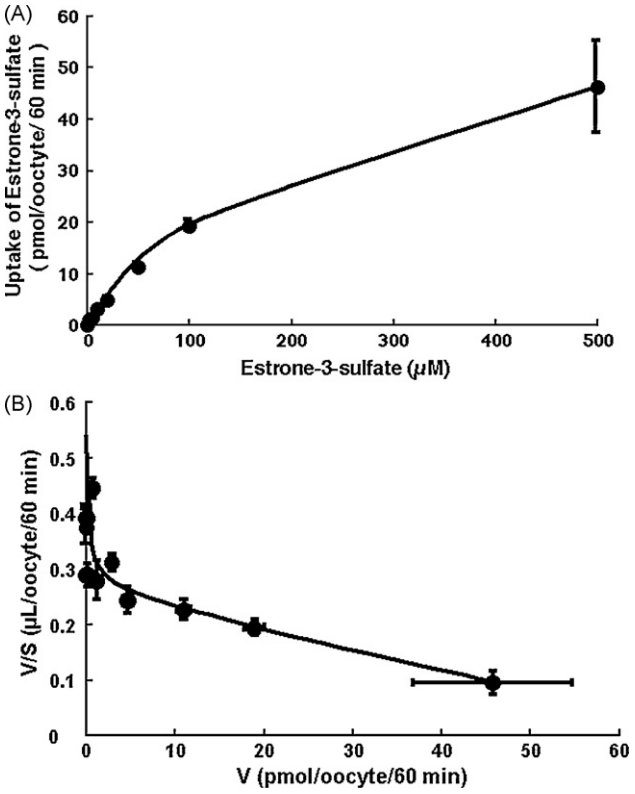

Fig. 3. Concentration dependence of estrone-3-sulfate uptake by oocytes expressing OATP1B3, shown by concentration-rate plot and Eadie–Hofstee plot. (A) OATP1B3-specific uptake of [^{3}H]estrone-3-sulfate, obtained by subtracting the uptake by water-injected oocytes from that by OATP1B3 cRNA-injected oocytes, was measured for 60 min at various concentrations from 0.1 μM to 500 μM. (B) OATP1B3-specific uptake of estrone-3-suflate at various concentrations is shown as an Eadie–Hofstee plot. The solid line represents the calculated values using the kinetic parameters obtained by nonlinear least-squares analysis. Each result represents the mean ± S.E.M. (n = 6–10).

3.3. Contribution of OATP1B3 to estrone-3-sulfate uptake by MCF-7 cells

To estimate the contribution of OATP1B3 to estrone-3-sulfate uptake by MCF-7 cells by means of the RAF method, the uptake clearance of CCK8 as a reference substrate of OATP1B3 [15] was estimated in MCF-7 cells and in OATP1B3-expressing oocytes. The uptakes of CCK8 increased linearly from 1 to 5 min and from 10 to 60 min, respectively (Figs. 4A and 5A). The uptake clearance by MCF-7 cells and OATP1B3-expressing oocytes were calculated as 0.520 μL/min/mg protein and 0.0126 μL/min/oocyte, respectively, from the slope values within these time periods. Consequently, R_{act} was calculated to be 40.9 oocyte/mg protein. Meanwhile, the uptake clearances of estrone-3-sulfate by MCF-7 cells and OATP1B3-expressing oocytes were calculated as 1.28 μL/min/mg protein and 0.002 μL/min/oocyte from the slope values from 1 to 5 min, and from 30 min to 90 min, respectively (Figs. 4A and 5A). From these data, the estimated uptake clearance of estrone-3-sulfate mediated by OATP1B3 in MCF-7 cells was 0.078 μL/min/mg protein. Thus, the contribution of OATP1B3 to the overall uptake of estrone-3-sulfate in MCF-7 cells was determined to be 6.0%.

4. Discussion

Estrone-3-sulfate is a major circulating estrogen and also participates in the progression of breast cancer cells [19]. Although estrone-3-sulfate itself is a weak ligand of the estrogen receptor, it is converted to the active estrogen, estradiol, by cytosolic sulfatase and 17β-hydroxysteroid dehydrogenase type 1. Since estrone-3-sulfate is hydrophilic, it was thought that a specific transporter would be involved in import of estrone-3-sulfate across the plasma membrane of breast cancer cells. We previously reported that estrone-3-sulfate is taken up by the estrogen-dependent breast cancer cell lines T-47D and MCF-7 via a specific transport mechanism [10,11]. Accordingly, we hypothesized that this transporter is a novel target for endocrine therapy of estrogen-dependent

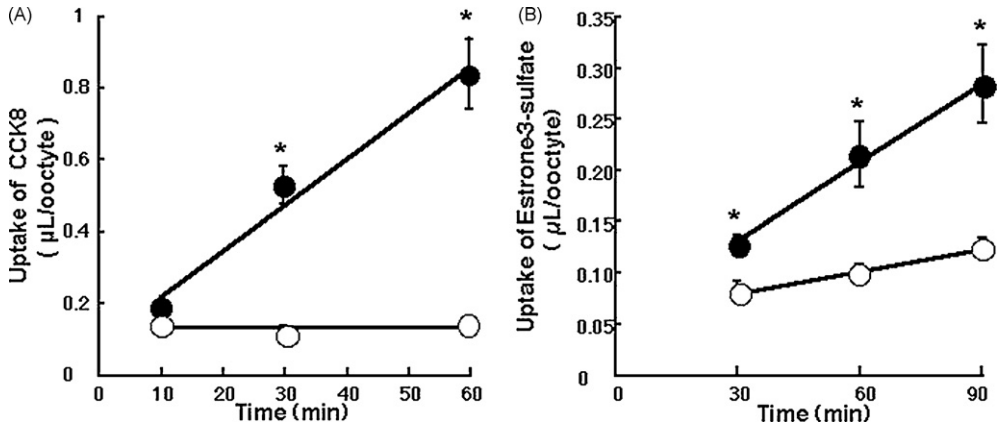

Fig. 4. Uptake clearance of [^{3}H]cholecystokinin octapeptide (CCK-8) and [^{3}H]estrone-3-sulfate by oocytes expressing OATP1B3. (A) Uptake of [^{3}H]CCK-8 (3 nM) by oocytes injected with cRNA of OATP1B3 (closed circles) or water (open circles) was measured over 60 min at room temperature and pH 7.4. (B) Uptake of [^{3}H]estrone-3-sulfate (10 nM) by oocytes injected with cRNA of OATP1B3 (closed circles) or water (open circles) was measured over 90 min at room temperature and pH 7.4. Each result represents the mean ± S.E.M. (n = 10). *Significantly different from the control by t-test (p < 0.05).

breast cancers. We have already demonstrated that inhibition of the transporter activity suppressed the cell proliferation induced by estrone-3-sulfate [11]. To confirm the idea that estrone-3-sulfate transporter could be a novel target for the treatment of breast cancers, it is necessary to identify the transporters involved in the uptake of estrone-3-sulfate by breast cancer cells.

To identify the responsible transporters, we examined the differential mRNA expression of transporter genes between two subclones of MCF-7 cells with different estrone-3-sulfate uptake activities by means of DNA microarray analysis. Among transporters that have been reported to transport estrone-3-sulfate, the mRNA expressions of OATP3A1, OATP4A1, OATP1B3, OAT2, OAT3, and Na^+/taurocolate cotransporting polypeptide (NTCP) were reliably detected by DNA microarray analysis. This result corresponds in part with the report that mRNA expression of OATP3A1 and OATP4A1, but not OATP2B1, was detected in human breast tissue by Northern blot analysis [20], and with our report that mRNA expression of OATP1A2, OATP3A1, OATP4A1, and OAT4 was detected in MCF-7 cells by RT-PCR [11]. Although there were some discrepancies, it may be due to the difference of detection sensitivity between DNA microarray and RT-PCR analysis and differences in the culture conditions of MCF-7 cells, such as cell density and culture period, between the two experiments, since it was reported that cell culture conditions affect the expression pattern of transporters [21]. Although OATP3A1, OATP1B3 and OAT3 showed positive correlations between mRNA expression level and estrone-3-sulfate uptake activity in this study, it was considered that OATP1B3 is the most important transporter, because the inhibitory effects of anionic compounds on the uptake of estrone-3-sulfate in OATP1B3 were consistent with those in MCF-7 cells [11,22,23].

Accordingly, we focused on OATP1B3 as a responsible transporter for uptake of a precursor of active estrogen in breast cancer. Uptake of estrone-3-sulfate by OATP1B3 was time- and concentration-dependent. Eadie–Hofstee plot analysis showed biphasic kinetics. The K_m value for the high-affinity component was 1.24 μM, which is similar to the value of 2.32 μM in MCF-7 cells [11], although the low-affinity component was not observed in MCF-7 cells. For these reasons, OATP1B3 was expected to contribute at least in part to the uptake of estrone-3-sulfate by MCF-7 cells. Thus, we estimated the quantitative contribution of OATP1B3 by applying the relative activity factor (RAF) method. The RAF method has been described for cytochrome P450 [13], and it has also been applied to hepatic uptake transporters [14,24]. Since CCK8 is a selective substrate for OATP1B3, it was used as a reference compound for OATP1B3-mediated uptake [15]. The ratio of the uptake clearance of CCK8 in MCF-7 cells to that in the OATP1B3-expressing system was calculated and defined as R_{act}. The uptake clearance by OATP1B3 in MCF-7 cells estimated by using R_{act} suggested that the contribution of OATP1B3 was about 6.0%. Although the evaluated contribution is unexpectedly small by this RAF analysis, the result

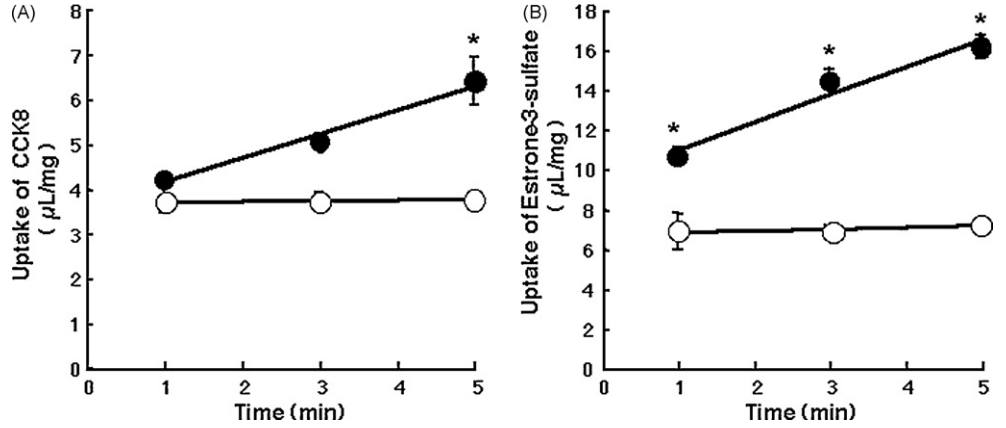

Fig. 5. Uptake clearance of [^{3}H]cholecystokinin octapeptide (CCK8) and [^{3}H]estrone-3-sulfate by MCF-7 cells. (A) Uptake of [^{3}H]CCK8 (3 nM) in MCF-7 cells was measured in the presence (open columns) or absence (closed columns) of 1 mM unlabeled estrone-3-sulfate at 37 °C. (B) Uptake of [^{3}H]estrone-3-sulfate (10 nM) in MCF-7 cells was measured in the presence (open columns) or absence (closed columns) of 1 mM unlabeled estrone-3-sulfate at 37 °C. Each result represents the mean ± S.E.M. (n = 4). *Significantly different from the control by t-test (p < 0.05).

demonstrated that OATP1B3 is involved in the uptake of estrone-3-sulfate in breast cancer cells. OATP1B3 is exclusively expressed in the liver under normal conditions, whereas it is broadly and abundantly expressed in cancer cells [25]. In addition, it was reported that expression of OATP1B3 is up-regulated by prolactin, which is a risk factor for postmenopausal breast cancers [26], and OATP1B3 expression was observed in a number of gastrointestinal cancers [25]. For these reasons, OATP1B3 might contribute more significantly in certain cases of cancers.

This study was based on the assumption that the uptake of estrone-3-sulfate would be correlated to the mRNA level of the responsible transporter. Since the mRNA expression level of OATP1B3 was low in MCF-7 cells (data not shown), it was thought that the contribution of OATP1B3 (6%) correlates with the expression level of OATP1B3 in MCF-7 cells. Therefore, it is possible that OATP1B3 expression level affects the estrogen-induced proliferation of the cells. However, there is a possibility that other molecules detected in the microarray assay may be involved in estrone-3-sulfate uptake by MCF-7 cells. Recently, it was reported that the expression level of OATP1A2, which is a member of the OATP transporter family as the same with OATP1B3 and exhibits transport activity of estrone-3-sulfate, was increased in breast cancer tissues expressing the ligand-activated nuclear receptor pregnane X receptor (PXR), and then the increase of OATP1A2 expression level induced the uptake of estrone-3-sulfate [27,28]. So, the authors suggested that OATP1A2 contributes to the supply of estrogen precursor to breast cancer cells. The approach they used was different from the current study, since they searched for the genes that are regulated by PXR. Furthermore, they used human breast-tumor tissues, which is different from current breast cancer-derived cell lines. So, it may be possible that different OATP molecules were found between our and those studies. However, it is interesting that OATP transporter family members were consistently found as the responsible transporters to supply a precursor of estrogen into breast cancer cells. Clarification of physiological roles and regulation mechanisms of those OATPs should be important for further understanding of hormone-dependent growth of cancers.

In conclusion, OATP1B3 was identified as one of the transporters involved in the uptake of estrone-3-sulfate in MCF-7 cells by differential expression profiles between breast-tumor derived cell lines that exhibited distinct transport activities for estrone-3-sulfate. Further studies to identify the responsible molecule for uptake of estrone-3-sulfate other than OATP1B3 should be needed to underpin the concept that inhibition of estrone-3-sulfate uptake transport is a viable novel strategy for treatment of hormone-dependent breast cancer.

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