Uptake of an Estrogen Precursor in Estrogen Receptor-Positive Breast Cancer Cells: Focusing on Organic Anion Transporting Polypeptide 2B1

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Abstract

Uptake of estrogen precursors is important for cell proliferation in estrogen receptor (ER)-positive breast cancer. Estrone sulfate (E1S) is known as the main precursor of estradiol (E2). Since E1S is a hydrophilic compound, it likely requires a solute carrier to cross plasma membrane. The transporter for E1S therefore seems to be a good candidate as a new therapeutic target against ER-positive breast cancer. The aim of this study is to clarify the relationship between the expression level of the transporter for E1S and cell proliferation in ER-positive breast cancer. Organic anion transporting polypeptide (OATP, *SLCO*) is reported to be involved in uptake of various organic anions, including E1S. The expression of SLCO1A2, 2B1 and 3A1 mRNAs was detected in normal breast tissues, malignant breast tissues and MCF-7 cells (a breast cancer-derived cell line). The expression level of SLCO2B1 mRNA in malignant breast tumors was significantly higher than that in normal breast tissues. Significant positive correlations were observed between the expression level of SLCO2B1 mRNA and histological grade, expression of Ki-67 protein and STS mRNA in breast malignant tumors. Overexpression of OATP2B1 caused enhancements of E1S uptake, E2 secretion, ER-signal transduction and cell proliferation in MCF-7 cells. The uptake of E1S resulted in down-regulation of ER and induction of Ki-67 in MCF-7 cells. The present study has suggested that the transporter for E1S, such as OATP2B1, affects progression of ER-positive breast cancer, and inhibition of E1S uptake may be enumerated as a new therapeutic target against ER-positive breast cancer.

Objectives

To investigate the relationship between the expression of the transporter for E1S and cell proliferation in ER-positive breast cancer.

Experimental Methods

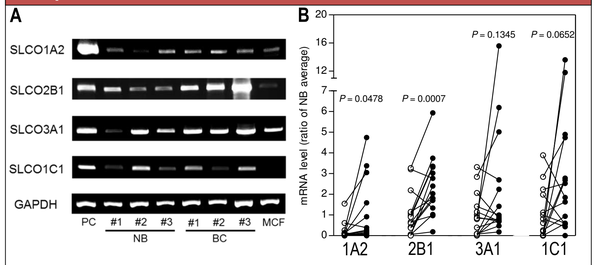
Cell-based assay

- Cell proliferation assay, reporter gene assay by using reporter plasmid containing estrogen response element and ELISA for E2 were conducted in MCF-7 cells with or without overexpression of OATP2B1.
- E1-S uptake experiment was carried out by using [^{3}H]E1-S.

Clinical Samples

- Normal breast and cancer tissues were collected from 16 and 49 patients, respectively. All patients provided written informed consent.
- This study was approved by the ethics committee of Graduate School of Medicine, Chiba University (approval No. 100).

1. Expression of SLCO mRNAs in breast cancer tissues

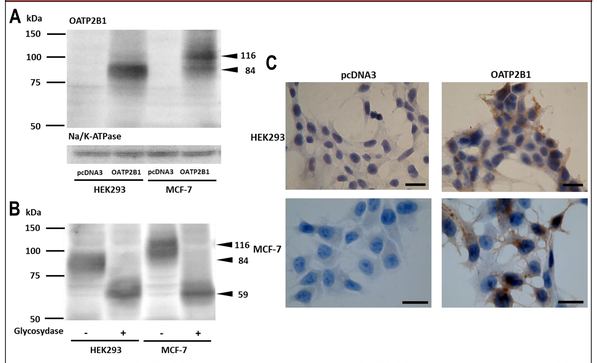


A, Expression of SLCO mRNAs in normal breast, cancer tissues and MCF-7 cells. NB, normal breast; BC, breast cancer. **B**, Differences in the expression levels of SLCO mRNAs between NB and BC.

2. The relationship between the expression of SLCO2B1 mRNA and clinicopathological parameters

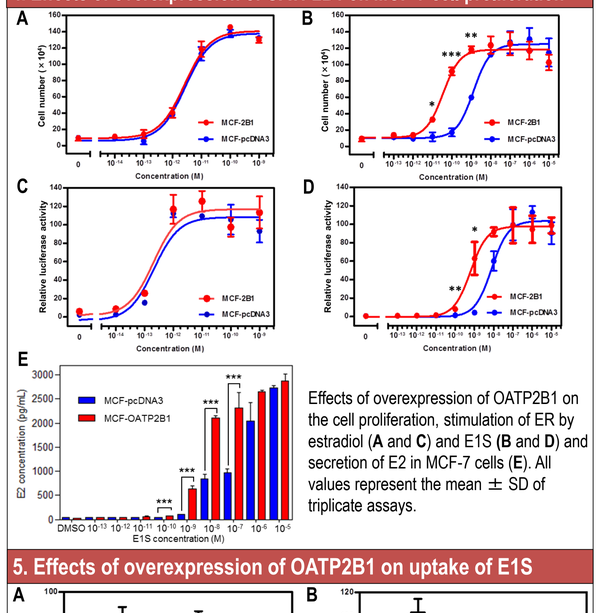
Clinical parameter		n	SLCO2B1 mRNA expression (mean ± SE)	*P* value
Age				
	<50 years	23	2.30 ± 0.47	0.220
	≥50 years	26	3.60 ± 0.93	
Tumour size				
	≤2 cm	18	3.16 ± 0.75	0.795
	>2 cm	31	2.89 ± 0.75	
Lymph node status				
	Positive	27	2.19 ± 0.41	0.132
	Negative	22	3.97 ± 1.08	
Histological grade				
	1	14	1.66 ± 0.26	0.022
	2 + 3	35	3.52 ± 0.74	
ER				
	Positive	40	3.71 ± 1.35	0.563
	Negative	9	2.83 ± 0.60	
PgR				
	Positive	37	5.10 ± 1.88	0.169
	Negative	12	2.30 ± 0.34	
HER2				
	Positive	7	2.67 ± 0.70	0.693
	Negative	42	3.04 ± 0.62	
Subtype				
	Luminal A-like	16	1.79 ± 0.28	0.072
	Luminal B-like	19	4.19 ± 1.29	
Ki-67 labelling index				
		49	r = 0.335	0.019
Estrogenic genes				
	Steroid sulfatase	49	r = 0.442	0.001
	ERα	49	r = -0.030	0.840
	Cyclin D_1	49	r = 0.177	0.223
	Aromatase	49	r = 0.009	0.954

3. Overexpression of OATP2B1 in MCF-7 cells



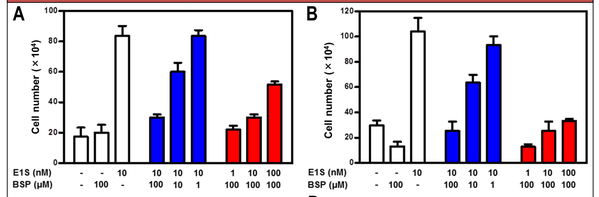
Detection of intact OATP2B1 (**A**) and deglycosylated OATP2B1 (**B**) expressed in MCF-7 cells. Na/K-ATPase was used as a loading control. Immunocytochemistry of OATP2B1 expressed in MCF-7 (**C**). Brown, OATP2B1; blue, nuclei. HEK293 cells stably expressing OATP2B1 were used as a positive control for OATP2B1 specific detection.

4. Effects of overexpression of OATP2B1 on MCF-7 cell proliferation



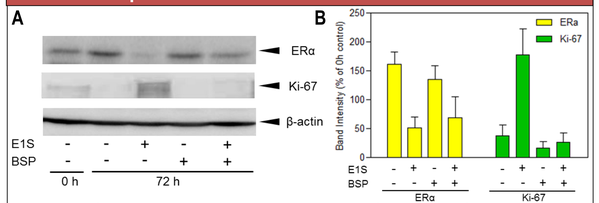
Effects of overexpression of OATP2B1 on the cell proliferation, stimulation of ER by estradiol (**A** and **C**) and E1S (**B** and **D**) and secretion of E2 in MCF-7 cells (**E**). All values represent the mean ± SD of triplicate assays.

5. Effects of overexpression of OATP2B1 on uptake of E1S



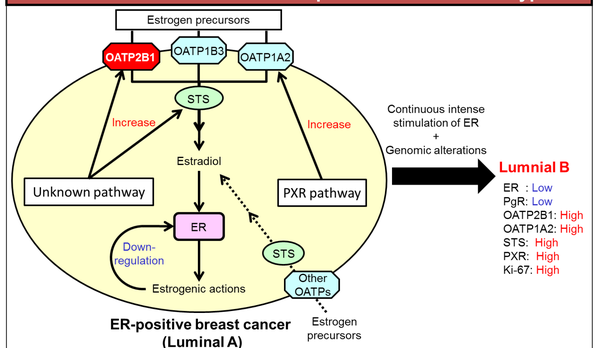
Effects of inhibition of E1S uptake by bromosulfophtalein (BSP, a specific inhibitor against OATPs) on the cell proliferation (**A**, MCF-pcDNA3; **B**, MCF-OATP2B1). Time course of [^{3}H]E1S (20 nM) uptake (**C**) and dose-concentration plot of E1S uptake by MCF-7 cells (**D**). Data represent the mean ± SD of triplicate experiments.

6. Alteration of the expression levels of ERα and Ki-67 by uptake of E1S in ER-positive breast cancer cells



A, Results of Western blot analysis. **B**, Band intensity of ERα and Ki-67 protein expression. All values represent the mean ± SD of triplicate experiments.

7. Putative relation between E1S uptake and luminal subtypes



STS, steroid sulfatase; PXR, pregnane X receptor, PgR, progesterone receptor

Results

- The expression level of SLCO2B1 mRNA in breast cancer tissues was significantly higher than that in normal breast tissues, and OATP2B1 was therefore investigated further.
- The expression level of SLCO2B1 mRNA was positively correlated with Ki-67 labeling index and STS mRNA expression.
- Cell-based assays showed that difference in the expression level of OATP2B1 affected the cell proliferation of ER-positive breast cancer by changing amount of estrogen in the cancer cells.
- The expression level of SLCO2B1 mRNA in Luminal B tended to be higher than that in Luminal A, and uptake of E1S caused down-regulation of ER and induction of Ki-67.

Discussion

- OATP2B1 may play a key role in supplying ER-positive breast cancer cells with E1S and supporting cell proliferation in ER-positive breast cancer.
- OATP2B1 might be useful as a novel therapeutic target against ER-positive breast cancer, especially luminal B-like cancer.

Conclusion

OATP2B1 may be involved in proliferation of ER-positive breast cancer cells by increasing the amount of estrogens in breast cancer cells.

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