



Title	Expression and Role of SNAT3 in the Placenta
Author(s)	Yoshioka, Chihiro; Yasuda, Satoru; Kimura, Fumitaka; Kobayashi, Masaki; Itagaki, Shirou; Hirano, Takeshi; Iseki, Ken
Citation	Placenta, 30(12), 1071-1077 https://doi.org/10.1016/j.placenta.2009.09.009
Issue Date	2009-12
Doc URL	http://hdl.handle.net/2115/42522
Type	article (author version)
File Information	PLA30-12_1071-1077.pdf



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Expression and role of SNAT3 in the placenta

Chihiro Yoshioka, Satoru Yasuda, Fumitaka Kimura, Masaki Kobayashi, Shirou Itagaki,
Takeshi Hirano¹, Ken Iseki *

Laboratory of Clinical Pharmaceutics & Therapeutics, Division of Pharmasciences, Faculty of
Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

¹ Present address: Department of Pharmacy, Kobe University Hospital, Kobe, Japan

*To whom correspondence should be addressed.

Ken Iseki, Ph. D., Laboratory of Clinical Pharmaceutics & Therapeutics, Division of
Pharmasciences, Faculty of Pharmaceutical Sciences, Hokkaido University

Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060-0812, Japan

Tel/Fax: +81-11-706-3770

e-mail: ken-i@pharm.hokudai.ac.jp

The number of characters in the abstract; 1079 characters without spaces

The number of characters in the entire manuscripts; 28922 characters without spaces

The number of illustrations; 8

The number of references; 40

Abstract

Glutamine is the most versatile amino acid and its plasma concentration is the highest of all amino acid. Many transporters are therefore involved in glutamine uptake or efflux. Glutamine is actively released from the placenta into fetal circulation. In this study, we examined the alteration of transporters that transport glutamine into fetal circulation as gestation progresses. High expression levels of system A and y^+L were found in the rat placenta in the late period of pregnancy and the expression levels of these transporters increased as gestation progressed ($p<0.05$). On the other hand, the expression of SNAT3, the system N transporter, was detected in the early period of pregnancy and its expression level decreased as gestation progressed ($p<0.05$). SNAT3 was also found to be expressed in isolated human primary cytotrophoblast cells and its expression level was decreased by their differentiation into syncytiotrophoblast cells ($p<0.05$). Since this regulation is closely related to glutamine synthetase expression, SNAT3 may play a key role in providing glutamine corresponding to glutamine synthetase function in the early period of gestation. This is the first report on the expression of SNAT3 in the placenta in the early stage of pregnancy.

Key Words

Placenta, SNAT3, glutamine, cytotrophoblast cell

Introduction

Supply of nutrients to the fetus is one of main functions of the placenta. Fetal development is dependent on placental nutrient supply, and insufficient nutrient supply increases the risk of fetal and perinatal diseases [1,2]. We previously showed that the expression levels of transporters that provide folate to the fetus change with progress of gestation [3]. It is therefore important to reconsider the expression and function of nutrient supply systems throughout the period of gestation, from the early stage of pregnancy to the end of pregnancy. More than twenty transporters are expressed in the placenta and they control the concentrations of amino acids as they work coordinately [4]. Although it is known that there is a relationship between deficiency of amino acid transporters and fetal growth deficiencies such as intrauterine growth restriction (IUGR) [1], the expression levels of amino acid transporters in each stage of gestation are unknown.

Glutamine is one of the components of protein, and it plays various roles in fetal growth and is therefore supplied into fetal circulation at the highest rate of all amino acids [5]. Glutamine is involved in the citric acid cycle, biosynthesis of purines and pyrimidines, and ammonia metabolism in addition to protein synthesis. Furthermore, glutaminase produces glutamate from glutamine in many organs, and glutamate acts as a precursor of GABA, a neurotransmitter, and glutathione, an antioxidant component [6]. This transformation between glutamine and glutamate also has an essential role in the regulation of intracellular pH [7]. Since glutamine and glutamate are involved in many biosynthesis and metabolism

processes, control of the concentrations of these amino acids between the placenta and fetus is essential. It has been reported that a large amount of glutamine is released from the placenta into fetal circulation and, reversely, glutamate is taken up by the placenta from fetal blood [8,9]. This glutamate-glutamine cycle in the placenta may play a major role in this exchange to contribute to the regulation of concentrations of these amino acids.

Systemic glutamine deficiency is related to multisystem disease and fatal multiple organ failure [10]. Thus, the placenta produces a large amount of glutamine to keep up with fetal demand [9]. Glutamine is produced by glutamine synthetase in mammalian cells, and this is an essential step in ammonium metabolism in the liver and brain [11]. The developing brain is more susceptible than the brain in adulthood, and ammonia toxicity could cause irreversible damage to the central nervous system such as cortical atrophy [12]. Production of glutamine by glutamine synthetase is directly associated with ammonia detoxification and it may decrease the risk of abnormal neurodevelopment. Cytotrophoblast cells, which are in contact with fetal circulation in the placenta, contain glutamine synthetase [13]. It is possible that cytotrophoblast cells play a key role in glutamine supply and ammonium metabolism in the placenta.

In mammalian organs, glutamine is transported via system A, system N, system y⁺L, ASCT2, B⁰AT, and system L [14-17]. In the placenta, system A is well studied for its essential role in amino acid uptake at microvillous membrane and its relationship between IUGR [1,18,19]. System A belongs to the SLC38 family. On the other hand, little is

known about the placental expression and function of system N, despite the fact that system N also belongs to the SLC38 family [20,21]. SNAT3 has been reported to be expressed in the liver, brain and kidney. Moreover, its expression in the placenta has been discussed for a long time [18,22]. SNAT3 mediates glutamine transport with sodium and proton coupled manner, and it contributes to ammonia metabolism in the liver and brain [23-25].

Since glutamine is critical for fetal growth, it is important to clarify the expression of transporters that mediate glutamine transport in the placenta and their alteration during pregnancy. However, little is known about the expression of nutrient transporters in the early period of gestation or their expression in cytotrophoblast cells [26]. Moreover, in the early stage of pregnancy before complete development of the placenta, cytotrophoblast cells may play a key role in the supply of glutamine. SNAT3 can contribute to the provision of glutamine to the fetus in the early stage of pregnancy. The aim of this study was to elucidate the expression pattern of glutamine transporters, including system A (SNAT1, SNAT2, SNAT4) , system B⁰ (ASCT2) , system y⁺L (y⁺LAT1, 4F2hc) , B⁰AT (slc6a19), system N (SNAT3, SNAT5) and new member of the slc38 family of transporters, SNAT6, in the placenta from the early stage to the end of gestation and to clarify the expression of SNAT3 in the placenta.

Materials and methods

Animals

Pregnant female Wistar rats were obtained from Slc (Hamamatsu, Japan). The rats were housed for at least 2 days before harvesting placentas. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals”.

Tissue collection

Full-term placentas were obtained from women who had caesarean sections with healthy babies and no complications of pregnancy at Sapporo Maternity Womens' Hospital in Japan. Written of informed consent was obtained from the women, and this study was approved by the Ethics Committee of Hokkaido University.

Isolation and culture of human trophoblast cells

Cytotrophoblast cells were isolated immediately after collection of the placenta as described in previous reports [27-29] with some modifications. Briefly, placental tissue was obtained within 1 h after the operation, and it was washed with cold saline. After removing blood, basal plate and placental blood vessels, placental villous tissue was cut into small fragments with scissors. Approximately 130 g of placental fragments was mixed with twice volume of enzyme solution: 0.125% dispase (Gibco/Invitrogen, Grand Island, NY) (2.16

U/ml) in the culture medium (DMEM/F12 [1:1] (Gibco/Invitrogen, Grand Island, NY), with 10% of FBS and 1% of penicillin/streptomycin (Sigma Aldrich, MO)). The mixture was incubated at 37°C for 60 min with continuous round shaking at 120 rounds/min. After enzyme treatment, the tissue suspension was filtered with gauze and this procedure was once repeated for the leftover. The filtrate was centrifuged at $700 \times g$ for 5 min, and the pellet was suspended with 10 ml of culture medium. The digested cells were put onto a 10-70% discontinuous percoll gradient (GE Healthcare Bio-sciences AB, Uppsala, Sweden) in a 50 ml centrifuge tube and centrifuged at $1500 \times g$ for 15 min. The purified cytotrophoblast cells were carefully collected in the region corresponding to a density of 1.035-1.064 g/l [30], and the percoll removing step was repeated. The cell pellet was suspended in 5 ml of medium and filtered with 30 μm MACS[®] Pre-Separation Filters (Miltenyi Biotec K.K, Tokyo, Japan). Finally, the cells were adjusted to 1.0×10^6 cells/ 1 ml in culture medium and incubated in 6-well culture plates (2 ml/well) at 37°C with 5% CO₂ and 95% humidity [31].

Hormone and enzyme analysis

Isolated cytotrophoblast cells were cultivated for up to 4 days. The culture medium was collected and centrifuged. Human chorionic gonadotropin (hCG) in the supernatant of the culture medium was detected by using an ELISA kit (Cosmo Bio, Tokyo, Japan).

Real-time RT-PCR analysis

Total RNA was prepared from rat placenta, human placenta, and isolated human cytotrophoblast cells using an Isogen (Nippon Gene, Tokyo) and an RNase-Free DNase Set (Qiagen, Hilden, Germany). Single-strand cDNA was made from 0.5-2.0 µg of total RNA by reverse transcription (RT) using an Omniscript RT Kit. PCR was performed using Tfi DNA polymerase (QIAGEN) with each nutrient transporter and GAPDH-specific primers through 23-40 cycles of 94°C for 30 sec, 53-61°C for 30 sec and 72°C for 20 sec. The primers were designed on the basis of sequence data in the GenBank™ database. The sequences of the specific primers are shown in Table 1. The PCR products were subjected to electrophoresis on a 2% agarose gel and then visualized by ethidium bromide staining. Quantitative real-time PCR was performed using an ABI PRISM 7700 Sequence Detector (Applied Biosystems, Foster City, CA) with Platinum[®] SYBR[®] Green qPCR SuperMix-UDG (Invitrogen) as per the manufacturer's protocol. The PCR products were normalized to amplified GAPDH, the internal reference gene.

Western blot analysis

Total protein extracts were prepared from rat placenta and isolated human cytotrophoblast cells. Tissue and cells were suspended in lysis buffer containing 1.0% Triton X-100, 0.1% SDS, and 4.5 M urea. The suspension was left to stand for 5 min and sonicated for 15 min at 4°C. Then it was centrifuged at 12,000 × g for 15 min at 4°C, and the protein concentration in the clear supernatant was determined by the method of Lowry [32].

The samples were denatured at 100°C for 3 min in loading buffer containing 50 mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.002% BPB, and 3.6 M urea and then separated on 4.5% stacking and 10% SDS polyacrylamide gels. Proteins were transferred electrophoretically onto nitrocellulose membranes (Trans-Blot; Bio-Rad Laboratories, Richmond, CA) at 15 V for 90 min. The membranes were blocked with PBS containing 0.05% Tween 20 (PBS/T) and 10% non-fat dry milk for 1 h at room temperature. After being washed with PBS/T, the membranes were incubated with goat polyclonal anti-SNAT3 (Santa Cruz Biotechnology, Santa Cruz, CA, sc-33445) (dilution of 1:200) or mouse anti-actin monoclonal antibody (Chemicon, Temecula, CA) (dilution of 1:500) for 1 h at room temperature and then washed with PBS/T (3 times for 10 min each time). The membranes were subsequently incubated for 1 h at room temperature with horseradish peroxidase-conjugated donkey anti-goat or goat anti-mouse secondary antibody (Santa Cruz Biotechnology) at a dilution of 1:4,000 and washed with PBS/T (3 times for 10 min each time). The bands were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham Biosciences Corp., Piscataway, NJ).

Immunocytochemistry

Immunocytochemistry was performed according to the protocols recommended by Santa Cruz Biotechnology. Briefly, isolated cytotrophoblast cells were cultured for 24 h above the cover glass and then fixed in methanol at -18°C for 5 min. After fixation, cells

were washed with PBS and then incubated in a blocking buffer (10% FBS in PBS) for 60 min. Then the cells were incubated for 1 hour at 37°C with monoclonal anti-cytokeratin 7 (DaKo, Glostrup, Denmark) (dilution of 1:100). After washing, the cells were subsequently incubated for 60 min at 37°C with rhodamine-conjugated donkey anti-mouse secondary antibody (Santa Cruz Biotechnology) at a dilution of 1:400. Nuclei were stained with DAPI (0.1 µg/ml) for 5 min at room temperature and the cells were mounted in VECTASHIELD Mounting Medium (Vector Laboratories, Burlingame, CA). The expression of cytokeratin 7 was visualized by using a confocal microscope (Zeiss LSM-510; Carl Zeiss Inc., Thornwood, NY).

Statistical analyses

Comparisons between paired groups were performed using Student's t-test or Welch's t-test to determine the significance of differences. Bartlett's test and Friedman's test were performed against the relative alteration of SNAT3 expression in the isolated cytotrophoblast cells with their differentiation.

Results

Expression of glutamine transporters in the rat placenta

Expression of transporters that transport glutamine in the rat placenta was examined by RT-PCR. System A transporters were originally reported to be the main system for transporting glutamine in the placenta [18,33]. We used them as a positive control, and we found that they were expressed on both 14th and 20th day of gestation (Figure 1a, 1b, 1c). In addition, system B⁰ (ASCT2) and system y⁺L (y⁺LAT1 and its heavy-chain, 4F2hc) were also expressed on both 14th and 20th day of gestation (Figure 1d, 1e, 1f). Since the expression of some other transporters in the placenta is unknown, RT-PCR was performed against B⁰AT (slc6a19), SNAT3 (slc38a3), SNAT5 (slc38a5) and SNAT6 (slc38a6). Although B⁰AT was not detected in the placenta at any stage of pregnancy (Figure 1g), system N transporters (SNAT3, SNAT5) and new member of the slc38 family of transporters, SNAT6, were found in the placenta (Figure 1h, 1i, 1j).

Alteration of glutamine transporters during pregnancy

We then investigated alteration of the expression level of mRNA of each transporter in the placenta throughout the period of gestation using real-time RT-PCR. The expression levels of system A transporters (SNAT1, SNAT2 and SNAT4) (Figure 2a, 2b, 2c), system y⁺L transporter (y⁺LAT1 and 4F2hc) (Figure 2e, 2f) increased significantly with the progress of gestation ($p < 0.05$, $p < 0.01$). ASCT2 was expressed throughout the period of gestation, and

alteration in its expression was more moderate than that of other transporters (Figure 2d). These results are consistent with the results of previous studies showing the expressions of system A, system y⁺L and ASCT2 [18,33]. The mRNA expressions of SNAT5 and SNAT6 were also detected by real-time PCR (Figure 2g, 2h). Although SNAT5 and SNAT6 were expressed throughout the period of gestation, significant alteration in their expression was not found at any stage of pregnancy. On the other hand, mRNA of SNAT3 was expressed at a high level in the placenta in the early period of pregnancy and its expression level decreased as gestation progressed (Figure 3a) ($p < 0.05$). Moreover, we determined the protein levels of SNAT3 in the rat placenta by Western blotting analysis. We found that SNAT3 was expressed at a high level in the early period of pregnancy and that its expression level was reduced as gestation progressed (Figure 3b). This is the first study demonstrating that SNAT3 is expressed in the placenta.

Isolation of cytotrophoblast cells from term placenta

In order to determine whether SNAT3 is also expressed in human placenta, we investigated the expression pattern of SNAT3 by using cytotrophoblast cells from human term placenta. Isolated trophoblast cells were cultured for 4 days. On the first day of culture, cells were found to be mononuclear and positive for cytokeratin 7 by using immunostaining methods (Figure 4a). On the third day of culture, cells were fused and differentiated as they formed multinucleated syncytiotrophoblast cells. The differentiation

was confirmed by measurement of hCG secretion in the culture medium. The hCG level in the culture medium increased and peaked on the third day (Figure 4b), and this pattern of hCG secretion agrees well with the results of a previous study [29], reflecting the formation of syncytiotrophoblast cells [31]. These results showed that we successfully isolated cytotrophoblast cells and differentiated into syncytiotrophoblast cells.

Expression of SNAT3 in the human placenta

We then investigated the relationship between expression of SNAT3 and differentiation of cytotrophoblast cells. SNAT3 was expressed in the isolated cytotrophoblast cells, and the expression level of SNAT3 decreased significantly as differentiation of cytotrophoblast cells progressed (Figure 5a-5b, $p < 0.05$). SNAT3 expression had almost disappeared on day 3 of culture.

Discussion

Fetal development is dependent on nutrient supply from the placenta. Most of the nutrients are provided from maternal blood, while some of them are produced in the placenta. Since the fetus needs a large amount of glutamine from the early period of gestation, systemic glutamine deficiency leads to fatal neonatal multiple organ failure. Thus, we focused on the transporters that contribute to the provision of glutamine.

This study suggested that the expression levels of some glutamine transporters change during pregnancy, and its regulation is different. Most of the transporters are up-regulated as gestation progresses. System A and system y⁺L are expressed at high levels in the placenta in the late stage of gestation. These results tend to be consistent with the previous studies [26, 34, 35]. Moreover, it has been reported that system A plays a key role in glutamine transport from the maternal side into placental cells [18, 33]. Therefore, increased level of system A mRNA contributes to uptake of a large amount of glutamine from maternal circulation in the late period of gestation. On the other hand, the expression level of SNAT3, the system N transporter, decreases as gestation progresses. SNAT3 is expressed at a high level in the rat placenta in the early stage of pregnancy. We isolated cytotrophoblast cells from human term placentas and cultured them to clarify whether SNAT3 is expressed in human placental cells, and we found that expression levels of SNAT3 are higher in cytotrophoblast cells than in syncytiotrophoblast cells (Supplemental Figure 1). It has been reported that the expression of SNAT3 was not detected in term placenta and that the

transport of glutamine via SNAT3 was not detected either in term placenta or by BBMV uptake study [18,33]. It is possible that it was difficult to detect SNAT3 because it is expressed in the early stage of pregnancy or in cytotrophoblast cells. This finding that SNAT3 is expressed in the early period of gestation indicates the importance for understanding the nutrient supply system throughout gestation. The concentrations of glutamine in fetal circulation in mid gestation and late gestation are not different [5]. Therefore, there are some other systems to supply glutamine to the fetus in the early period of gestation instead of system A, the expression level of which is low at this time. Our results showed that there are the differences between system A and SNAT3 despite the fact that they belong to the same SLC family. System A is expressed at high levels in the late period of gestation and SNAT3 is mostly expressed in the early period of gestation. It is important to elucidate the route of nutrient supply in the early period of gestation because of its essential role in organogenesis. Taking these findings into consideration, we focused on SNAT3 and hypothesized that it is the main pathway of glutamine supply to the fetus in the early period of gestation.

There are three lines of evidence indicating an important role of SNAT3 in the placenta at the early period of pregnancy. First of all, the concentration of glutamine is higher in fetal blood than in maternal blood, and it must be controlled around 500 μM [24]. Therefore, it has been suggested that there is an active pathway that transports glutamine to the fetus. This property is well-fitted to SNAT3, which mediates glutamine efflux by low

affinity ($K_m=1.1$ mM) and high capacity [17, 36]. Secondly, the environment of fetal blood is matched well with SNAT3 function. SNAT3 is sensitive to pH [37, 38], and it needs a proton anti-transport mechanism to release glutamine [36]. Since the acidity of fetal blood is slightly higher than that of maternal blood [39], this is the suitable environment for SNAT3. Finally, results of previous studies and our RT-PCR results show the corresponding alteration of glutamine synthetase with SNAT3 expression. It has been reported that there are two routes to provide glutamine to the fetus: uptake from maternal circulation and production in the placenta [7,9]. And we examined the characterization of glutamine transport in Bewo cells, cytotrophoblast cell model. The mRNAs of SNAT3 were found to be expressed in Bewo cells (Supplemental Figure 2(A)). The glutamine uptake by Bewo cells involved Na^+ -dependent manner and markedly increased with an increase in extracellular pH (Supplemental Figure 2(B)). These results corresponded with previous studies of SNAT3 function. Supplemental Figure 2(C-D) shows the concentration-dependent uptake of glutamine by Bewo cells. Eadie-Hofstee plot analysis showed that glutamine uptake was two-phase uptake (high-affinity phase-0.13mM, low-affinity phase-1.4mM). We suggest that K_m value of SNAT3 was similar to that of low affinity phase and SNAT3 transport glutamine in cytotrophoblast cells. In the rat placenta, the activity of glutamine synthetase is greatest on the 12th day of gestation [40]. This corresponds to our finding that the expression level of SNAT3 in the placenta is the highest in the 11th day of gestation. In addition to this finding, it was suggested that alterations of human placental SNAT3 and glutamine synthetase

correspond with each other. SNAT3 is contributed to provide glutamine, which is produced by glutamine synthetase in cytotrophoblast cells, to fetal circulation [13].

In the placenta, there is an active glutamate-glutamine cycle to provide glutamine to the fetus and to excrete glutamate and ammonia from fetal circulation [9]. The transporter that mediates glutamine efflux is not known [10,18]. It is possible that SNAT3 and glutamine synthetase in cytotrophoblast cells are responsible for ammonia metabolism in the early period of pregnancy, supposed at the organogenesis stage.

In conclusion, SNAT3 is expressed in the placenta and it is one of the routes of the supply of glutamine. Little is known about the nutrient transport system through the placenta in the early period of pregnancy. In this study, we found expression of SNAT3 in the early period of gestation or in cytotrophoblast cells.

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amino-acid metabolism in developing rat placenta. Eur J Biochem 1980; 110:289-293.

Figure Legends

Figure 1. Expression of glutamine transporters in the rat placenta. RT-PCR was performed against (a) SNAT1, (b) SNAT2, (c) SNAT4, (d) ASCT2, (e) γ^+ LAT1, (f) 4F2hc, (g) B⁰AT, (h) SNAT3, (i) SNAT5 and (j) SNAT6 using total RNA from the rat placenta of the 14th day of gestation (gd-14) and the 20th day of gestation (gd-20). As a positive control, total RNA from the kidney for B⁰AT, from the liver for SNAT3 and from the brain for SNAT5 was used.

Figure 2. Alterations of glutamine transporters in the rat placenta during pregnancy. Expression of mRNA of each transporter was detected by real-time RT-PCR. Each column represents the ratio of the expression level of mRNA to that of each mRNA on the 11th day of gestation (gd-11). The columns show the mean with S.D. of 3-5 measurements. *; significantly different from gd-11 at $p < 0.05$, **; at $p < 0.01$

Figure 3. SNAT3 expression in the rat placenta. Changes in expression level of SNAT3 was detected by real-time RT-PCR (a). Each column represents the ratio of the expression level of mRNA to that of each mRNA on the 11th day of gestation. The

columns show the mean with S.D. of 3-5 measurements. *; significantly different from gd-11 at $p<0.05$, **; at $p<0.01$. SNAT3 protein was detected by Western blotting (b). Representative data from 3-4 different placentas of each gestational day.

Figure 4. Detection of (a-c) cytokeratin 7 and (d) hCG secretion. Isolated cytotrophoblast cells was detected by immunocytochemistry against cytokeratin 7 (a, *red fluorescence*), DAPI staining of nuclei (b, *blue*) and merged image of them (c) in the cytotrophoblast cells. Scale bars: 20 μm . (d) Secretion of hCG, a marker of differentiation, was detected on each day. The columns show the mean with S.D. of 3 measurements. *; significantly different from the first day of culture at $p<0.05$, **; at $p<0.01$.

Figure 5. Expression of SNAT3 in human trophoblast cells. Detection of the expression of SNAT3 by real-time PCR (a) and SNAT3 by RT-PCR (b). (a); Each column represents the ratio of the expression level of mRNA to that of each mRNA on the first day of culture. The columns show the mean with S.D. of 3 measurements. (b); Representative data from 3 different cell isolations of different human term placentas. *; significantly different throughout the cells differentiation at $p<0.05$.

Supplemental figure 1. Expression of SNAT3 in human trophoblast cells.

Detection of the expression of SNAT3 by western blotting.

Supplemental figure 2. Expression of SNAT3 mRNA (A), Influence of extracellular pH and cations for glutamine uptake (B) and Kinetic analysis of glutamine uptake (C-D) in BeWo cells

(A) Expression of SNAT3 mRNA in BeWo cells was detected by RT-PCR and total RNA from HepG2 cells was used for positive control.

(B) Uptake of [³H] glutamine (total 100 μM; 4.5 nM radiolabeled plus unlabeled) was measured. Each point represents the mean ± S.D. of 3 measurements.

(C) Concentration-dependence of glutamine uptake. Uptake of [³H] glutamine (total 100 μM; 4.5 nM radiolabeled plus unlabeled in a Na⁺-containing buffer (●), and 22.5 nM radiolabeled plus unlabeled in a NMDG-containing buffer (○)) was measured. Each point represents the mean ± S.D. of 3 measurements.

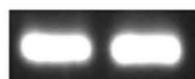
(D) Eadie-Hofstee plot of uptake of Na⁺ dependent glutamine

Table 1. Primer information

transporter name (gene name)	accession number	primer sequence	annealing temperature (°C)	product size (bp)
<i>Rat</i>				
SNAT1 (SLC38a1)	NM_138832	forward 5'- ccagagcacaggcgacattc -3'	61	113
		reverse 5'- ccagctcaaacaacgaggac -3'		
SNAT2 (SLC38a2)	NM_181090	forward 5'- cctccggatcgattacaatg -3'	61	147
		reverse 5'- ggaccagatagtcgccgttc -3'		
SNAT3 (SLC38a3)	NM_145776	forward 5'- acaccagagggctgaagac -3'	53	144
		reverse 5'- tgatggcgttgctgagattg -3'		
SNAT4 (SLC38a4)	NM_130748	forward 5'- tcccggagaaagatgcagac -3'	61	122
		reverse 5'- tggagcaactcgtcctcaac -3'		
SNAT5 (SLC38a5)	NM_138854	forward 5'- tggcgctatgtccagttacc -3'	61	157
		reverse 5'- catgagagccagaggcaaga -3'		
SNAT6 (SLC38a6)	NM_001013099	forward 5'- ccacattagcgtcaacatc -3'	61	218
		reverse 5'- actgaagcttccgaccacag -3'		
y ⁺ LAT1 (SLC7a7)	NM_031341	forward 5'- gccctggcactatactcagc -3'	61	116
		reverse 5'- atggaaatgccaatggagag -3'		

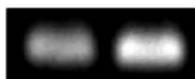
4F2hc (SLC3a2)	AB015433	forward	5'- ctgctcttctggctcggttg	-3'	61	170
		reverse	5'- ccagctatgcctctcgcttc	-3'		
ASCT2 (SLC1a5)	NM_175758	forward	5'- ttacagactccggcaacct	-3'	61	141
		reverse	5'- tgccatctccttgaataggg	-3'		
B ⁰ AT (SLC6a19)	NM_001039722	forward	5'- gccagtacatgctcacctg	-3'	61	130
		reverse	5'- cctccaggaccagaaggatg	-3'		
GAPDH (Gapdh)	AF106860	forward	5'- atgggaagctggatcaaac	-3'	53-61	221
		reverse	5'- gtggttcacacctcacaa	-3'		
<i>Human</i>						
SNAT3 (SLC38a3)	NM_006841	forward	5'- cagcatacaccatccccatc	-3'	61	193
		reverse	5'- gactccaccccgtttagaag	-3'		
GAPDH (GAPDH)	NM_002046	forward	5'- aaggtcatccctgagctgaa	-3'	61	95
		reverse	5'- ttctagacggcaggtcaggt	-3'		

(a) SNAT1



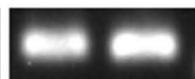
gd-14 gd-20

(b) SNAT2



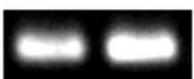
gd-14 gd-20

(c) SNAT4



gd-14 gd-20

(d) ASCT2



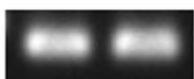
gd-14 gd-20

(e) γ^+ LAT1



gd-14 gd-20

(f) 4F2hc



gd-14 gd-20

(g) B⁰AT



gd-14 gd-20 Kidney

(h) SNAT3



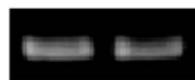
gd-14 gd-20 Liver

(i) SNAT5

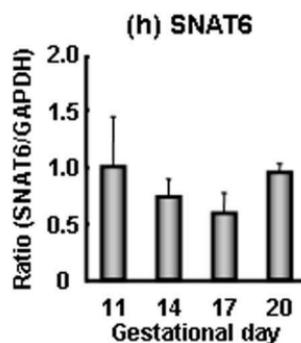
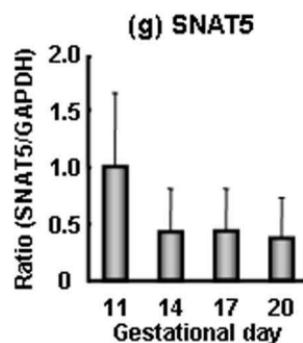
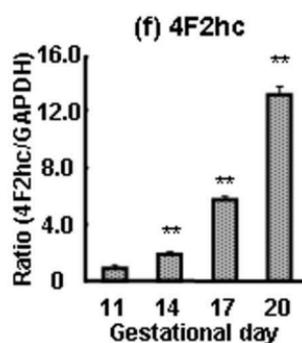
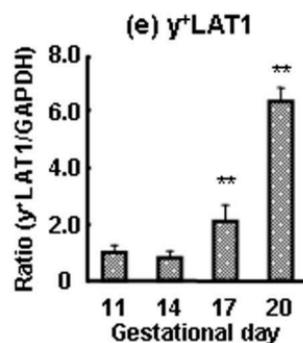
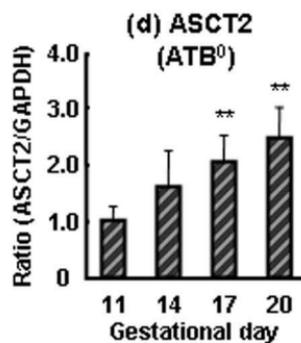
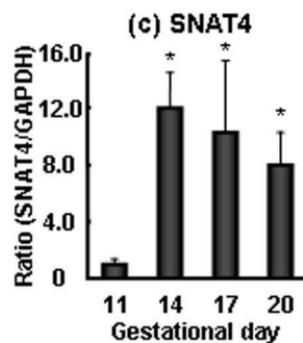
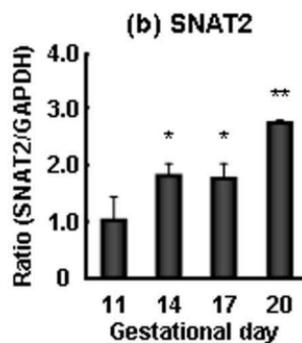
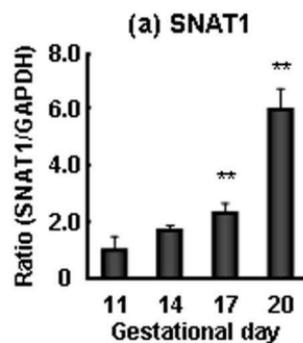


gd-14 gd-20 Brain

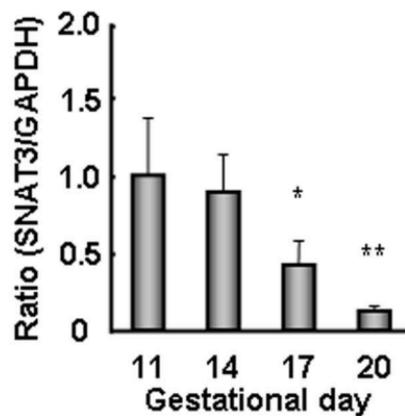
(j) SNAT6



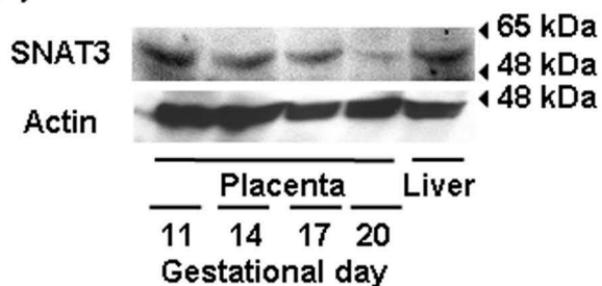
gd-14 gd-20



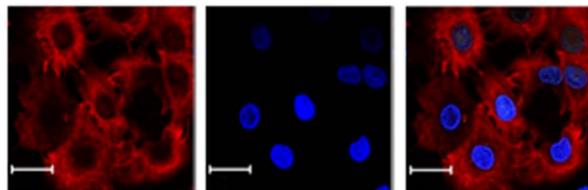
(a) SNAT3 mRNA



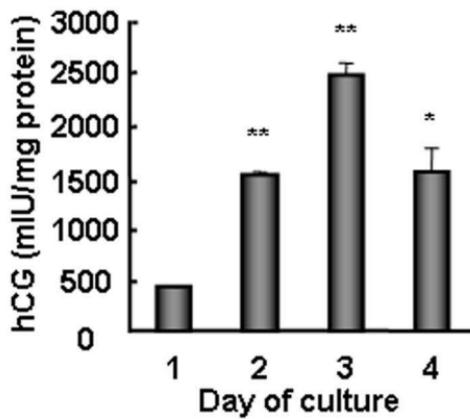
(b) SNAT3 Protein

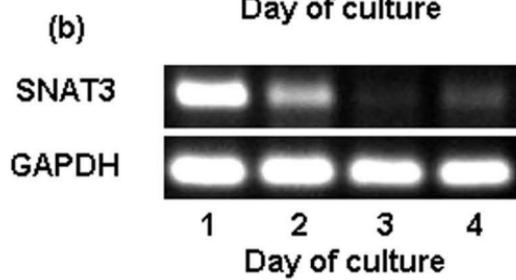
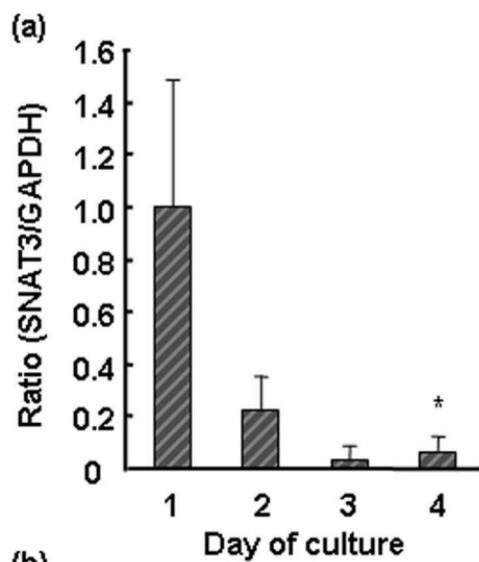


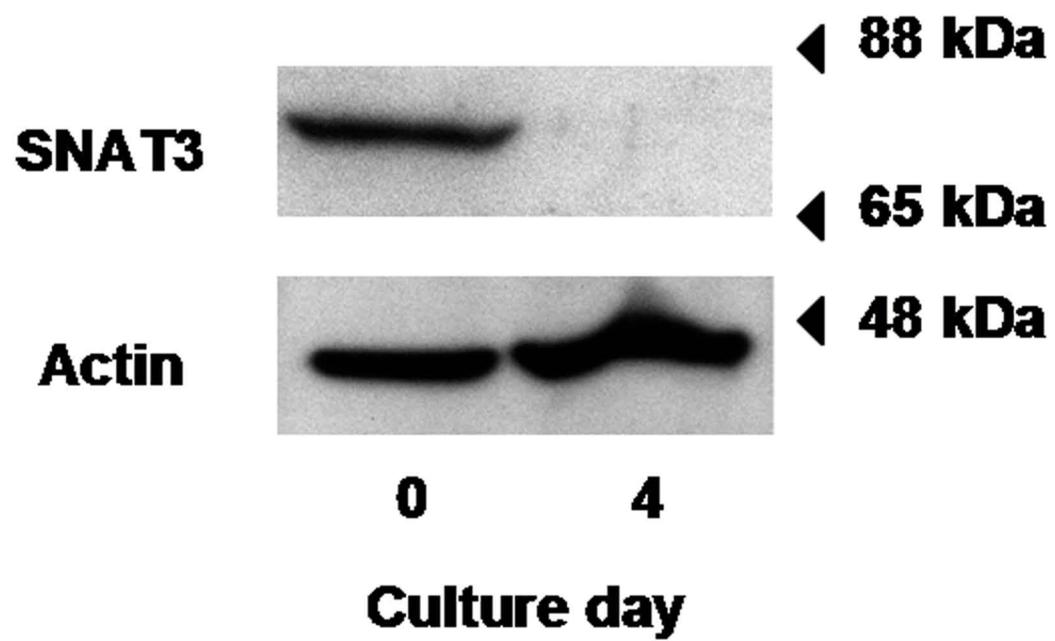
(a) Cytokeratin 7



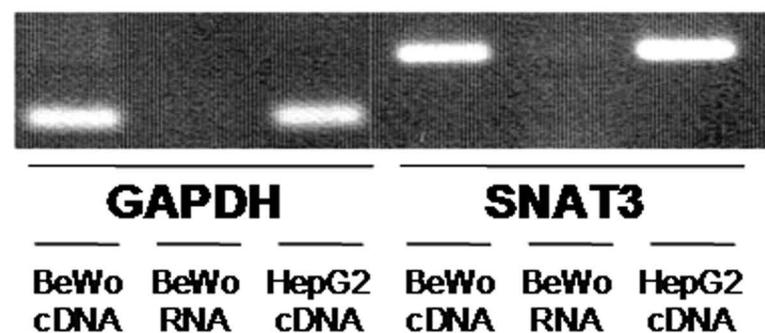
(b) hCG secretion



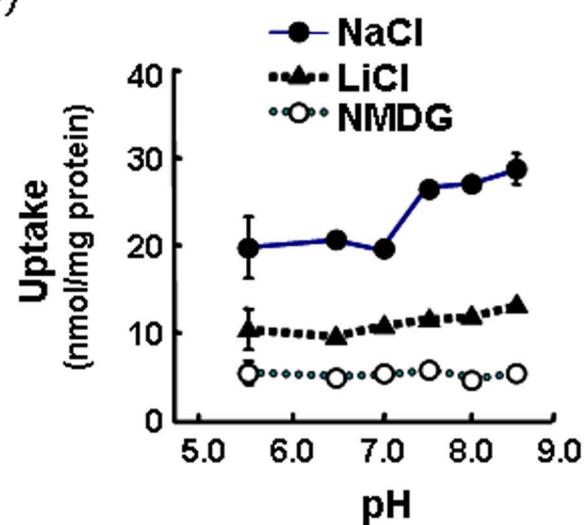




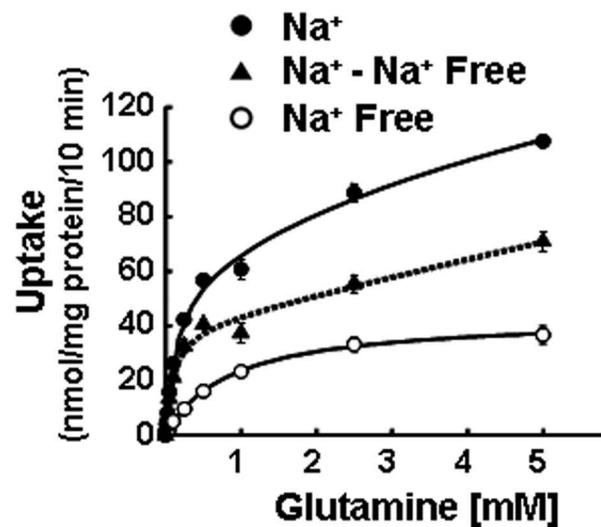
(A)



(B)



(C)



(D)

