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Gene Section
Review

SLC1A5 (solute carrier family 1 (neutral amino acid transporter), member 5)

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Abstract

Review on human SLC1A5, with data on DNA/RNA, on the protein encoded and pathological and physiological implications.

Identity

Other names: AAAT, ASCT2, ATBO, M7V1, M7VS1, R16, RDRC

HGNC (Hugo): SLC1A5

Location: 19q13.32

Local order: Orientation: minus strand.

DNA/RNA

Description

The SLC1A5 gene, located at 19q13.3, counts 28692 nucleotides with 8 exons. It has been found in 56 different organisms (NCBI). The gene encodes a protein involved in sodium-dependent neutral amino acid transport (Kekuda et al., 1996; Pingitore et al., 2013).

Transcription

Three isoforms (transcripts) are reported either on NCBI and Ensembl databases for SLC1A5 human gene, deriving from different translation start. They differ in length, particularly at 5' extremity. The first variant NM_005628 represents the longest transcript, constituted by 2873 nucleotides and 8 exons. This transcript encodes a peptide of 541 amino acids. The second variant NM_001145144 is constituted by 1737 nucleotides and differs in the 5' UTR from the variant NM_005628.

In NM_001145144 the translation starts downstream the third exon generating a shorter peptide of 313 aa.

The third isoform NM_ 001145145 has 1927 nucleotides and lacks the first exon. It presents a different translation start at 5', coding a peptide of 339 amino acids. A longer transcript, XM_005259167, is reported only in NCBI database.

It has been identified by automated computational analysis. More than 400 SNP(s), both in coding and non-coding regions of the SLC1A5 gene, are reported in dbSNP database (dbSNP). More than 40 are responsible of amino acid substitutions with unknown significance. Only the variant SLC1A5-P17A (rs3027956) is associated with breast cancer (Savas et al., 2006). A region constituted by 907 bp upstream of the ASCT2 gene possesses promoter activity (Bungard and McGivan, 2004). In this region the following putative elements have been identified: an amino acid-regulatory element, a consensus site for binding of the transcription factor activator protein 1 (AP1) and a consensus binding sites for nuclear and hepatocyte nuclear factors.

Pseudogene

The gene is virtually present in all vertebrates. The better known orthologous of the human gene are those from rat, mouse and rabbit. Identity between the human and rat, mouse, rabbit are 79%, 82% and 85%, respectively.

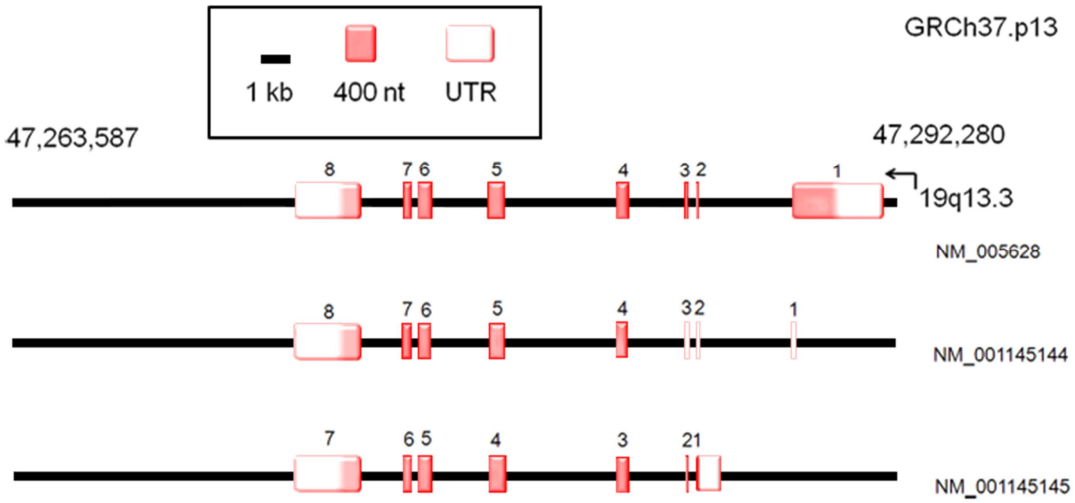

Figure 1. Isoforms of SLC1A5 gene. The three isoforms are present in the minus strand of the chromosome 19 in position 19q13.3. NM_005628: isoform one, encodes for the longest peptide and is constituted by 8 exons; NM_001145144: isoform two, due to alternative splicing is characterized by only four exons; NM_ 001145145: isoform three presents seven exons. The nucleotide sequence is depicted as black lines. Coding nucleotides and untranslated (UTR) regions are indicated by red and white boxes, respectively. Exons are indicated by roman numbers.

Protein

Description

541 amino acids; molecular mass 56598,34 Da.

Human SLC1A5 is a permease (membrane transporter).

The 3D structure is not available. Homology modeling highlights a structure similar to that of the glutamate transporter of P. horikoshii (1XFH). N- and C-terminal ends are intracellular. Potential site of N-glycosylation and phosphorilation are predicted.

In the structural model, at least one glycosylation site is extracellular and the phosphorilation sites are intracellular (Fig. 2).

Expression

Human SLC1A5 has been originally named ASCT2 from AlaSerCysTransporter2 or ATB0.

The acronym ASCT2 is the most frequently used to designate this transport system.

It is expressed in many tissues, including brain, (Bröer and Brookes, 2001; Deitmer et al., 2003; Gliddon et al., 2009).

There is functional evidence of the expression of ASCT2 in kidney and intestine (Bode, 2001). Besides Caco-2 cells, apparently, also the HT-29 intestinal cell line functionally expresses ASCT2 (Kekuda et al., 1996; Kekuda et al., 1997). Poly(A)1 RNA isolated from several tissues of human origin revealed expression in placenta, lung, skeletal muscle, kidney, and pancreas (Kekuda et al., 1996).

Localisation

The protein is localized in the plasma membrane.

Function

Transport mediated by the human ASCT2 has been originally studied in intact cell systems over-expressing the transport protein (Kekuda et al., 1996; Kekuda et al., 1997).

Recently, hASCT2 was over-expressed in the yeast P. pastoris, purified and reconstituted in artificial phospholipid vesicles (proteoliposomes), in absence of other interfering transporters.

All experimental systems concur in demonstrating that hASCT2 is an obligate exchanger of neutral amino acid.

This antiport requires the presence of extracellular Na^+ which cannot be substituted by Li^+ or K^+. The Na^+_{ex}:amino $acid_{ex}$ stoichiometry of the human transporter is likely to be 1:1. Competition studies on ^{3}H-glutamine, ^{3}H-threonine or ^{3}H-alanine transport performed in cells indicated that other potential substrates of hASCT2 are valine, leucine, serine, cysteine, asparagine, methionine, isoleucine, tryptophan, histidine, phenylalanine. While glutamate, lysine, arginine along with MeAIB [α-(methylamino)isobutyric acid] and BCH [2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid] are neither transported nor inhibit hASCT2. Experiments with radioactive compounds confirmed the competition data (Torres-Zamorano et al., 1998). In proteoliposomes, inhibition has been confirmed for most but not for all of the amino acids. Moreover, proteoliposome studies highlighted an asymmetric specificity for amino acids allowing to distinguish the amino acids inwardly transported (alanine, cysteine, valine, methionine) from those bi-directionally transported (glutamine, serine, asparagine, and threonine).

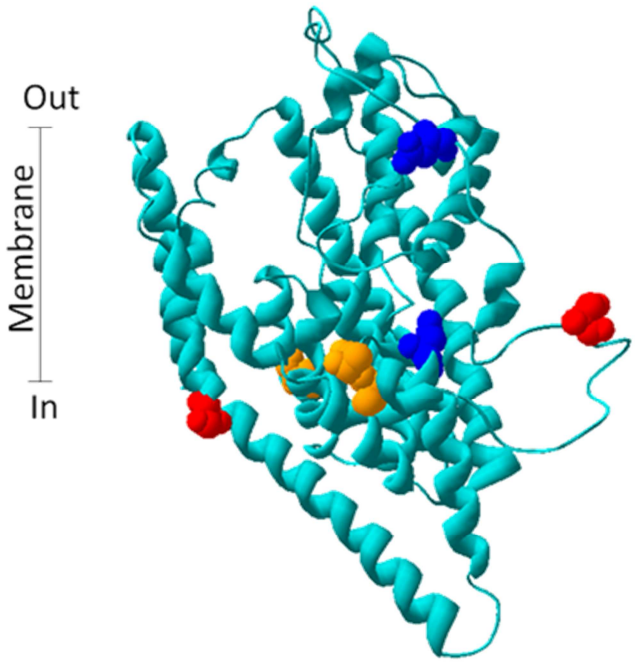

Figure 2. Homology structural model of hASCT2. Ribbon diagram viewing of the transporter from the lateral side. The model was built using the glutamate transporter Glpth from Pyrococcus horikoshii crystal structure (1XFH) as the template by Modeller V9.13. The homology model was represented using SpdbViewer 4.01. Asn 163 and 212, predicted as glycosilation sites, are highlighted in blue; Ser 183, 261 and Thr 206, 207, 329, predicted as phosphorilation sites are highlighted in red and orange, respectively. Prediction according to Scan Prosite.

The functional asymmetry was also confirmed by the kinetic analysis of [^{3}H]glutamine/glutamine antiport: different Km values were measured on the external and internal sides of proteoliposomes, 0,097 and 1,8 mM, respectively.

The SH reagents $HgCl_2$, mersalyl and pOHMB potently inhibited hASCT2 mediated transport (Pingitore et al., 2013).

The physiological role of hASCT2 consists in providing cells with some neutral amino acids exporting others on the basis of the metabolic need of cells consistently with the intra and extracellular amino acid concentrations. In brain, particularly, hASCT2 contributes to glutamine homeostasis of neurons and astrocytes. On the basis of experiments performed with animal models, it was hypothesized that hASCT2 mediates efflux of glutamine from astrocytes, a process that is critical for the functioning of the glutamate-glutamine cycle to recover synaptically released glutamate in exchange with glutamine efflux (Bröer et al., 1999). The glutamine-glutamate cycle has been shown also in placenta. Glutamine crosses the placenta and enters the fetal liver where it is deamidated to glutamate. About 90% of glutamate generated by the liver is taken up by the placenta and used in the metabolism. The glutamine-glutamate cycle between the placenta and the fetal liver is obligatory for the generation of NADPH in the placenta (Torres-Zamorano et al., 1998). Among other functions reported for hASCT2 there is the regulation of mTOR pathway, translation and autophagy. The transporter regulates an increase in the intracellular concentration of glutamine which is then used by another plasma membrane transporter, named LAT1 (SLC7A5) (Galluccio et al., 2013) as efflux substrate to regulate the uptake of extracellular leucine with subsequent activation of mTORC1 (Nicklin et al., 2009). Moreover, it has been proposed that a group of retroviruses specifically uses the hASCT2 as a common cell surface receptor following a co-evolution phenomenon. The orthologous murine transporter mASCT2 is inactive as a viral receptor (Marin et al., 2003).

Implicated in

Molecular basis of cancerogenesis

Note

Tumor cells acquire altered metabolism. Due to these changes, the expression of membrane transporters involved in providing nutrients is altered. The plasma membrane transporter for glutamine ASCT2 has been clearly associated to cancer development and progression, together with another amino acid membrane transporter, LAT1 specific for glutamine and other neutral amino acids (Fuchs and Bode, 2005). The energetic needs of cancer cells are different from normal ones due to the Warburg effect. According to this phenomenon ATP derives from anaerobic glycolisis bypassing

mitochondrial function (Ganapathy et al., 2009). In this scenario glutamine provided by means of ASCT2 and LAT1 transport function sustains tumor growth and signaling through mTOR pathway (Nicklin et al., 2009).
The importance of ASCT2 in this network is revealed by induction of apoptosis when silencing its gene in human hepatoma cells (Fuchs et al., 2004).
In the following paragraphs specific examples of human cancers are reported.

Prostate cancer

Note
Tissue microarray technology (TMA) has been used for studying ASCT2 in normal prostatic tissue, in benign prostatic hyperplasia and in prostate adenocarcinoma.
In particular, a negative prognosis and a shorter time of recurrence for adenocarcinoma were associated to hASCT2 expression. Moreover, a more aggressive behavior of adenocarcinoma is described (Li et al., 2003).

Colorectal carcinoma

Note
The expression of ASCT2 in colorectal carcinoma is normally associated to a decrease of percentage in patient survival (Witte et al., 2002).

Neuroblastoma and glioma

Note
Neuroblastoma are childhood tumors very often benign. In some cases, however, neuroblastoma became malignant. One of the biological marker of this second category is the increased uptake of glutamine and other neutral aminoacids via ASCT2 (Wasa et al., 2002). Human glioma C6 cells have been demonstrated to mediate uptake of glutamine via ASCT2 (Dolinska et al., 2003).

Hepatoma

Note
Hepatocell carcinoma (HCC) is the most common malignant tumor of liver and one of the main cause of death. A study reported that higher rate of glutamine uptake via ASCT2 is a common feature of six examined hepatoma cell line (Bode et al., 2002; Fuchs et al., 2004).

Lung cancer

Note
ASCT2 has been found over expressed in lung cancer by proteomic approach and then confirmed at molecular level. Pharmacologic and genetic targeting of ASCT2 decreased cell growth and viability in lung cancer cells, an effect mediated in part by mTOR signaling (Hassanein et al., 2013).

Breast cancer

Note
In breast cancer ASCT2 has been found over expressed together with other proteins related to glutamine metabolism like glutamminase and glutamate dehydrogenase (Kim et al., 2012).
The study revealed that this metabolism is essential for sustaining breast cancer development and that the protein levels are different according to different subtypes of cancer. The subtype HER2 showed the highest level of glutamine related proteins and that the basal-like breast cancers are more dependent on glutamine compared to luminal-likeones.

Other diseases

Note
Due to importance of glutamine in cell metabolism and the chromosomal localization of SLC1A5 gene, several association studies have been conducted to ascertain the involvement of hASCT2 in pathologies like cystinuria, cystic fibrosis, schizophrenia, Hartnup disorder and pre-eclampsia. However, no genetic associations have been revealed.

To be noted

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References

Kekuda R, Prasad PD, Fei YJ, Torres-Zamorano V, Sinha S, Yang-Feng TL, Leibach FH, Ganapathy V. Cloning of the sodium-dependent, broad-scope, neutral amino acid transporter Bo from a human placental choriocarcinoma cell line. J Biol Chem. 1996 Aug 2;271(31):18657-61

Kekuda R, Torres-Zamorano V, Fei YJ, Prasad PD, Li HW, Mader LD, Leibach FH, Ganapathy V. Molecular and functional characterization of intestinal Na(+)-dependent neutral amino acid transporter B0. Am J Physiol. 1997 Jun;272(6 Pt 1):G1463-72

Torres-Zamorano V, Leibach FH, Ganapathy V. Sodium-dependent homo- and hetero-exchange of neutral amino acids mediated by the amino acid transporter ATB degree. Biochem Biophys Res Commun. 1998 Apr 28;245(3):824-9

Bröer A, Brookes N, Ganapathy V, Dimmer KS, Wagner CA, Lang F, Bröer S. The astroglial ASCT2 amino acid transporter as a mediator of glutamine efflux. J Neurochem. 1999 Nov;73(5):2184-94

Bröer A, Wagner C, Lang F, Bröer S. Neutral amino acid transporter ASCT2 displays substrate-induced Na+ exchange and a substrate-gated anion conductance. Biochem J. 2000 Mar 15;346 Pt 3:705-10

Bode BP. Recent molecular advances in mammalian glutamine transport. J Nutr. 2001 Sep;131(9 Suppl):2475S-85S; discussion 2486S-7S

Bröer S, Brookes N. Transfer of glutamine between astrocytes and neurons. J Neurochem. 2001 May;77(3):705-19

Bode BP, Fuchs BC, Hurley BP, Conroy JL, Suetterlin JE, Tanabe KK, Rhoads DB, Abcouwer SF, Souba WW. Molecular and functional analysis of glutamine uptake in human hepatoma and liver-derived cells. Am J Physiol Gastrointest Liver Physiol. 2002 Nov;283(5):G1062-73

Wasa M, Wang HS, Okada A. Characterization of L-glutamine transport by a human neuroblastoma cell line. Am J Physiol Cell Physiol. 2002 Jun;282(6):C1246-53

Witte D, Ali N, Carlson N, Younes M. Overexpression of the neutral amino acid transporter ASCT2 in human colorectal adenocarcinoma. Anticancer Res. 2002 Sep-Oct;22(5):2555-7

Deitmer JW, Bröer A, Bröer S. Glutamine efflux from astrocytes is mediated by multiple pathways. J Neurochem. 2003 Oct;87(1):127-35

Dolińska M, Dybel A, Zabłocka B, Albrecht J. Glutamine transport in C6 glioma cells shows ASCT2 system characteristics. Neurochem Int. 2003 Sep-Oct;43(4-5):501-7

Li R, Younes M, Frolov A, Wheeler TM, Scardino P, Ohori M, Ayala G. Expression of neutral amino acid transporter ASCT2 in human prostate. Anticancer Res. 2003 Jul-Aug;23(4):3413-8

Marin M, Lavillette D, Kelly SM, Kabat D. N-linked glycosylation and sequence changes in a critical negative control region of the ASCT1 and ASCT2 neutral amino acid transporters determine their retroviral receptor functions. J Virol. 2003 Mar;77(5):2936-45

Bungard CI, McGivan JD. Glutamine availability up-regulates expression of the amino acid transporter protein ASCT2 in HepG2 cells and stimulates the ASCT2 promoter. Biochem J. 2004 Aug 15;382(Pt 1):27-32

Fuchs BC, Perez JC, Suetterlin JE, Chaudhry SB, Bode BP. Inducible antisense RNA targeting amino acid transporter ATB0/ASCT2 elicits apoptosis in human hepatoma cells. Am J Physiol Gastrointest Liver Physiol. 2004 Mar;286(3):G467-78

Fuchs BC, Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? Semin Cancer Biol. 2005 Aug;15(4):254-66

Savas S, Schmidt S, Jarjanazi H, Ozcelik H. Functional nsSNPs from carcinogenesis-related genes expressed in breast tissue: potential breast cancer risk alleles and their distribution across human populations. Hum Genomics. 2006 Mar;2(5):287-96

Ganapathy V, Thangaraju M, Prasad PD. Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. Pharmacol Ther. 2009 Jan;121(1):29-40

Gliddon CM, Shao Z, LeMaistre JL, Anderson CM. Cellular distribution of the neutral amino acid transporter subtype ASCT2 in mouse brain. J Neurochem. 2009 Jan;108(2):372-83

Nicklin P, Bergman P, Zhang B, Triantafellow E, Wang H, Nyfeler B, Yang H, Hild M, Kung C, Wilson C, Myer VE, MacKeigan JP, Porter JA, Wang YK, Cantley LC, Finan PM, Murphy LO. Bidirectional transport of amino acids regulates mTOR and autophagy. Cell. 2009 Feb 6;136(3):521-34

Galluccio M, Pingitore P, Scalise M, Indiveri C. Cloning, large scale over-expression in E. coli and purification of the components of the human LAT 1 (SLC7A5) amino acid transporter. Protein J. 2013 Aug;32(6):442-8

Hassanein M, Hoeksema MD, Shiota M, Qian J, Harris BK, Chen H, Clark JE, Alborn WE, Eisenberg R, Massion PP. SLC1A5 mediates glutamine transport required for lung cancer cell growth and survival. Clin Cancer Res. 2013 Feb 1;19(3):560-70

Kim S, Kim do H, Jung WH, Koo JS. Expression of glutamine metabolism-related proteins according to molecular subtype of breast cancer. Endocr Relat Cancer. 2013 Jun;20(3):339-48

Pingitore P, Pochini L, Scalise M, Galluccio M, Hedfalk K, Indiveri C. Large scale production of the active human ASCT2 (SLC1A5) transporter in Pichia pastoris--functional and kinetic asymmetry revealed in proteoliposomes. Biochim Biophys Acta. 2013 Sep;1828(9):2238-46

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