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Folate and Thiamine Transporters mediated by Facilitative Carriers (SLC19A1-3 and SLC46A1) and Folate Receptors

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Abstract

The reduced folate carrier (RFC,SLC19A1), thiamine transporter-1 (ThTr1,SLC19A2) and thiamine transporter-2 (ThTr2,SLC19A3) evolved from the same family of solute carriers. SLC19A1 transports folates but not thiamine. SLC19A2 and SLC19A3 transport thiamine but not folates. SLC19A1 and SLC19A2 deliver their substrates to systemic tissues; SLC19A3 mediates intestinal thiamine absorption. The proton-coupled folate transporter (PCFT,SLC46A1) is the mechanism by which folates are absorbed across the apical-brush-border membrane of the proximal small intestine. Two folate receptors (FOLR1 and FOLR2) mediate folate transport across epithelia by an endocytic process. Folate transporters are routes of delivery of drugs for the treatment of cancer and inflammatory diseases. There are autosomal recessive disorders associated with mutations in genes encoded for SLC46A1 (hereditary folate malabsorption), FOLR1 (cerebral folate deficiency), SLC19A2 (thiamine-responsive megaloblastic anemia), and SLC19A3 (biotin-responsive basal ganglia disease).

Keywords

SLC19A1; SLC19A2; SLC19A3; SLC46A1; folate; thiamine; hereditary folate malabsorption; thiamine-responsive megaloblastic anemia; folate receptor

1. Introduction

The folates and thiamine are members of the B family of vitamins. Both are metabolized to active forms that are poor export substrates and accumulate in cells where they sustain key metabolic reactions. Folates carry a negative charge, and thiamine a positive charge, at physiological pH (Figure 1A and 1B). Each is transported into cells by a specific member of the SLC19 family of solute carriers with a high degree of structural specificity (Table I). SLC19A1, the reduced folate carrier, transports folates but not thiamine; SLC19A2 (thiamine transporter 1- ThTr1) and SLC19A3 (thiamine transporter 2-ThTr2) transport thiamine but not folates. These transporters function optimally at physiological pH and are the major routes of delivery of folates and thiamine to systemic tissues. Intestinal absorption

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of folates is mediated by another member of the solute carrier family, SLC46A1 (Table II), the proton-coupled folate transporter (PCFT), also required for folate transport across the blood:choroid plexus:cerebrospinal fluid barrier. Folates are also transported by an endocytic mechanism mediated by two folate membrane receptors, FOLR1 and FOLR2. SLC19A1-3 are members of the 2A.48 subfamily of the Saier “transporter classification system” (<http://www.tcdp.org/>), while SLC46A1 is not classified in this system. The physiological roles of SLC19A2, SLC46A1, FOLR1, and to some extent SLC19A2 have been established by the autosomal recessive inherited disorders caused by loss-of-function mutations in these genes. Folate transporters also play an important role in the delivery of folate analogs to neoplastic and inflammatory cells for the treatment of cancer and inflammatory/autoimmune diseases, respectively. The recent development of diagnostic and therapeutic agents linked to folic acid, transported into malignant and immune cells via folate receptor mediated endocytosis, is a novel approach to the selective delivery of drugs to these tissues. Much of what has been learned about the mechanisms of folate transport has come from studies focused on folate analogues, in particular, methotrexate and, more recently, pemetrexed. Membrane transport of folates has been the subject of several recent reviews (Matherly et al., 2007; Matherly and Goldman, 2003; Zhao et al., 2009a; Zhao et al., 2011).

2. The Biological Roles of Folates and Thiamine

The major physiological folate is 5-methyltetrahydrofolate (Figure 1A). This is the predominant dietary form of folate, the folate absorbed in the intestine, the form in blood and delivered to mammalian tissues. The carbon at the N-5 position is donated to homocysteine in the synthesis of methionine, yielding tetrahydrofolate to which up to eight glutamate residues are added to the γ -carboxy of the glutamic acid moiety to form a series of polyglutamate derivatives. These are the preferred substrates for folate-requiring enzymes. These congeners are retained within cells, because they are poor substrates for folate transporters, where they acquire a carbon from formate or serine to form 10-formyltetrahydrofolate or 5,10-methylenetetrahydrofolate, required for the synthesis of purines and thymidylate, respectively, and ultimately the synthesis of DNA and RNA (Figure 1C) (Stokstad, 1990). Several of these reactions are inhibited by antifolates. For instance, of the agents currently in clinical use, methotrexate and pralatrexate inhibit the enzyme dihydrofolate reductase to suppress regeneration of tetrahydrofolate from dihydrofolate formed during the synthesis of thymidylate. This results in tetrahydrofolate depletion and cessation of tetrahydrofolate-cofactor-requiring reactions. Pemetrexed and raltitrexed, in their polyglutamate forms, inhibit formation of thymidylate by inhibiting the enzyme thymidylate synthase (Goldman et al., 2010). Higher pemetrexed polyglutamates also inhibit an enzyme required for *de novo* purine synthesis, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase, resulting in the build-up of ZMP with cells, activation of AMP kinase, and inhibition of mTOR (Rothbart et al., 2010).

Thiamine plays an entirely different role in cellular metabolism. Following its uptake into cells, thiamine is converted to thiamine pyrophosphate (TPP) by thiamine pyrophosphokinase (Figure 2). Thiamine pyrophosphate is a coenzyme required for several key enzymes involved in energy production from carbohydrate and amino acid metabolism. Enzymes that require thiamine pyrophosphate include: pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and transketolase (Frank et al., 2007). Like other phosphorylated derivatives of a variety of metabolites required for biosynthetic reactions and energy metabolism, these congeners are also retained and accumulate within cells.

3. SLC family transporters

3.1. The folate solute carriers

3.1.1. The reduced folate carrier (RFC;SLC19A1)—Soon after methotrexate was introduced for the treatment of acute leukemia, acquired resistance emerged as a major clinical challenge. Laboratory studies identified membrane transport as an important determinant of the activity of this agent and loss of transport as an important mechanism of acquired resistance in tumor cell lines (Zhao and Goldman, 2003). At about the same time as carrier-mediated mechanisms were being identified for a variety of major biological substrates, methotrexate transport in murine leukemia cells was shown to exhibit the properties of a facilitated process. This encompassed temperature and pH dependence, exchange phenomena with other folates, and uphill transport (Goldman et al., 1968). This transporter had a distinct structural specificity profile with a K_t for methotrexate, other antifolates, and the reduced folates in the 1-7 μ M range and a K_i for folic acid of ~200 μ M. More recently, antifolates have been developed that have SLC19A1 influx K_t 's of <0.5 μ M (Rosowsky et al., 1998). This transport activity was named the reduced folate carrier (RFC), which has persisted in the nomenclature. Another earlier designation, the reduced folate transporter (RFT), is no longer in use.

The *SLC46A1* gene encoding RFC was cloned in 1994 and shown to have the structure of a typical solute carrier with a topology, subsequently confirmed by epitope insertion and cysteine scanning mutagenesis, consisting of 12 transmembrane domains with N- and C-termini directed to the cytoplasm (Dixon et al., 1994;Ferguson and Flintoff, 1999;Cao and Matherly, 2004). There is one glycosylation site in the external loop between the first and second TMDs that is not required for function (Wong et al., 1998). SLC19A1 is expressed in all human and murine cells and, with a pH optimum of 7.4, is the major route of folate transport into systemic tissues. This transporter is also expressed in a variety of epithelia: the apical brush border membranes of the entire intestine, the choroid plexus and retinal pigment epithelium; the basolateral membrane of the proximal renal tubule and at the vascular blood brain barrier as illustrated in Figure 3 (Zhao and Goldman, 2003;Wang et al., 2001;Zhao et al., 2011;Chancy et al., 2000). Localization at these sites does not necessarily indicate function. For instance, while highly expressed at the apical brush-border membrane of the proximal small intestine (Wang et al., 2001), folate absorption at the acid pH within the microclimate at the surface of these cells is mediated by another process, SLC46A1 (Qiu et al., 2006). This was established by the observation that there is impaired intestinal folate absorption and severe folate deficiency when SLC46A1 function is lost in hereditary folate malabsorption (HFM), see below (Qiu et al., 2006;Zhao et al., 2007;Diop-Bove et al., 2011). Deletion of *SLC46A1* gene is embryonic lethal; while births of normal pups are possible with folate supplementation of the dams, without continued supplementation death of the pups ensues within two week due to failure of hematopoietic tissues (Zhao et al., 2001).

SLC19A1-mediated transport is uphill but is neither sodium nor proton-dependent. Rather, SLC19A1 functions as an anion exchanger with organic phosphates that are synthesized and retained within cells. This is an antiport system in which the downhill flow of organic phosphates via SLC19A1 is linked to the uphill transport of folate substrates into cells via the same mechanism. Ultimately, the energy requirement of the system is met by the consumption of ATP in the synthesis of organic phosphates (Henderson and Zevely, 1983;Goldman, 1971).This model was validated by a very interesting and fortuitous interaction between thiamine and folates as illustrated in Figure 3. While thiamine is not a substrate for SLC19A1, following its transport into cells via thiamine transporter-1 (SLC19A2) or thiamine transporter-2 (SLC19A3), it forms mono- and di- phospho derivatives that are substrates for SLC19A1 (Zhao et al., 2000;Zhao et al., 2002). The K_t 's for thiamine mono- and di- phosphates, based upon inhibition of SLC19A1-mediated

methotrexate influx, are 26 and 32 μM , respectively. Methotrexate is, in turn, a competitive inhibitor of SLC19A1-mediated efflux of these thiamine derivatives, and increases net accumulation of thiamine phosphates within cells. In *SLC19A1*-null cells, efflux of thiamine mono and diphosphate is decreased and their net accumulation is increased as compared to cells that over-express SLC19A1 (Zhao et al., 2000; Zhao et al., 2002).

The impact of metabolic poisons on net transport of methotrexate in cells is unusual. At low substrate concentrations, the electrochemical-potential for methotrexate in the intracellular water exceeds the level in the extracellular compartment under steady-state conditions (Goldman et al., 1968). However, when cells are exposed to metabolic inhibitors, the intracellular methotrexate level increases (Goldman, 1969; Fry et al., 1980; Dembo et al., 1984). The basis for this apparent paradox is attributed to the following. First, in virtually all mammalian cells there are multiple ATP binding cassette exporters, such as the multidrug resistance-associated proteins (MRPs) and the breast cancer resistance protein (BCRP-ABCG2), many of which export folates and antifolate monoglutamates (Kruh and Belinsky, 2003). Hence, the level of free folates within cells is determined by the net impact of the carriers, that are concentrative, and the exporters. When the exporters are inhibited, efflux of folates decreases and their intracellular electrochemical-potential rises because the organic phosphate gradient is sustained. In the case of the adenine nucleotides, their affinity for SLC19A1 is not related to their energy charge; indeed, AMP is a more potent inhibitor of SLC19A1-mediated transport than ATP (Henderson and Zevely, 1983; Goldman, 1971). Hence, in the presence of metabolic poisons, the exporters are inhibited, ATP is converted to ADP and AMP, the “effective” organic gradient is sustained, and the transmembrane methotrexate gradient is now determined solely by the organic phosphate gradient across the cell membrane.

Human *SLC46A1* is regulated at the transcriptional level; there are six promoters and noncoding regions with up to 15 specific transcripts (Matherly et al., 2007). Sp, USF and tissue-specific AP1 and C/EBP transcription factors regulate *SLC46A1* transcription in different tissues (Matherly et al., 2007). While *SLC19A1* is not required for intestinal folate absorption, folate deficiency results in increased intestinal expression of this carrier (Qiu et al., 2007; Said et al., 2000).

3.1.2. The proton-coupled folate transporter (PCFT;SLC46A1)—SLC46A1 is the youngest member of the folate-specific transporters. Like SLC19A1, the properties of this transporter were defined long before it was characterized at the molecular level (Zhao et al., 2009a). The features of this transporter that clearly distinguished it from SLC19A1 are its low pH optimum, its high affinity for both folic acid and the reduced folates at low pH, and its energetics. These were properties noted in the analysis of folate transport in a variety of model systems: from absorption in small intestinal loops, everted sacs, and membrane vesicles to choroid plexus, retinal pigment epithelial cells, hepatic sinusoidal membranes, and in a variety of human tumors (Zhao and Goldman, 2003; Zhao et al., 2011). The molecular basis for this activity was recently identified with the cloning of the gene encoding PCFT, designated *SLC46A1* (Qiu et al., 2006). Its critical role in intestinal folate absorption was confirmed by the demonstration that there are loss-of-function mutations in this protein in HFM (see below). SLC46A1 is expressed in most tissues but at high levels at the apical brush-border membrane of the duodenum and proximal jejunum, the sinusoidal membrane of the liver, the apical brush-border membrane of the kidney, the basolateral membrane of the choroid plexus (see below) and retinal pigment epithelium, and the placenta, (Figure 3) (Qiu et al., 2006; Qiu et al., 2007; Urquhart et al., 2010; Shayeghi et al., 2005; Zhao et al., 2009b; Gnana-Prakasam et al., 2011). Recently, a *Slc46a1*-null mouse was shown to have a folate-deficiency phenotype similar to what is found in humans with loss-of-function mutations in *SLC46A1* gene (Salojin et al., 2011).

The secondary structure of SLC46A1 has been established based upon the substituted cysteine accessibility method (Zhao et al., 2010). There are 12 transmembrane domains, a large internal loop separating six transmembrane alpha helices on either side with N- and C-termini located within the cytoplasm. Two glycosylation sites within the first extracellular loop are not required for function (Unal et al., 2008). The transporter has a pH optimum in the 5.0 – 5.5 range with an influx K_t at this pH for many, but not all, folates and antifolates in the 0.2 to 5 μM range. However, as the pH is increased the K_t increases and the V_{max} falls but in a substrate-specific manner. For instance, while the K_t increases to 130 μM , and the V_{max} decreases by a factor of 5, for folic acid as the pH is increased from 5.5 to 7.4, the K_t for pemetrexed increases to only 12-15 μM and the V_{max} decreases by only 20% over this pH range (Zhao et al., 2004a; Qiu et al., 2006; Zhao et al., 2008). Folate transport mediated by SLC46A1 in *Xenopus* oocytes is electrogenic; maximum current and intracellular acidification occurs at low pH (Qiu et al., 2006; Qiu et al., 2007; Umapathy et al., 2007). As the voltage gradient is decreased, the current decreases; at pH 7.4, in the absence of a proton gradient, transport mediated by SLC46A1 is voltage-dependent. Some reports have suggested that SLC46A1 is also a low-affinity, pH independent heme transporter, with a K_t in the 125 μM range, that plays a role in intestinal heme-iron absorption (Shayeghi et al., 2005). Such an activity has not been confirmed by other laboratories (Qiu et al., 2006; Umapathy et al., 2007; Nakai et al., 2007). Humans with HFM that lack SLC46A1 function and *Slc46a1*-null mice are not iron deficient (Qiu et al., 2006).

Information on the regulation of *SLC46A1* expression is emerging. The regulatory region of this gene contains vitamin D response elements and expression is increased in response to vitamin D (Eloranta et al., 2009). There are three nuclear respiratory factor-1 (NRF1) sites in the *SLC46A1* promoter that are required for *SLC46A1* expression (Gonen and Assaraf, 2010). NRF1 plays an important role in mitochondrial biogenesis but is not regulated by hypoxia. It is unclear as to its role in the regulation of *SLC46A1* expression *in vivo*. T-lymphoblasts have low levels of transport activity at acidic pH and express low levels of *SLC46A1* message. This has been attributed to methylation of the *SLC46A1* promoter as is the case in a HeLa cell line with acquired loss of *SLC46A1* expression associated with methotrexate selective pressure (Gonen et al., 2008; Diop-Bove et al., 2009).

3.1.3. The role of the folate solute carriers in the delivery of antifolates to tumor cells—Most antifolate drugs currently in the clinic have a high affinity for SLC19A1 which is ubiquitously expressed in human tumors (Goldman et al., 2010). SLC19A1 is also expressed in normal tissues where it is the major route of delivery of physiological folates. Transport of antifolates by this mechanism into replicating cells of the bone marrow and intestinal tract is the basis for the major toxicities associated with these agents (Zhao and Goldman, 2003). While SLC46A1-mediated transport at physiological pH is very low, there is sufficient residual pemetrexed transport at neutral pH to sustain activity of this agent in the absence of SLC19A1 (Zhao et al., 2004b; Chattopadhyay et al., 2006). Beyond this, there may be a greater role for SLC46A1 in the delivery of antifolates to malignant tissues since tumor cells operate largely through anaerobic glycolysis and acidify their microenvironment. In addition, the vascular supply to cells within solid tumors is erratic and compromised limiting oxygen delivery thereby further promoting anaerobic glycolysis. One current strategy for the development of new-generation antifolates is the design of agents with very low affinity for SLC19A1 and high affinity for SLC46A1. In this way toxicity associated with delivery of drugs to normal tissues via SLC19A1 is minimized, delivery to normal tissues via SLC46A1 is also minimal because of the unfavorable physiological pH within their microenvironments. However, delivery to tumor cells is optimized by their surrounding low pH. A purine synthesis inhibitor with these properties has been shown to have antitumor activity both *in vitro* and *in vivo* (Desmoulin et al., 2011; Desmoulin et al., 2010).

3.2. The thiamine solute carriers: thiamine transporter 1 (ThTr1;SLC19A2) and thiamine transporter 2 (ThTr2;SLC19A3)

Identification of the gene responsible for thiamine-responsive megaloblastic anemia (TRMA) led to cloning of the *SLC19A2* gene encoding thiamine transporter 1 (ThTr1) (Labay et al., 1999;Fleming et al., 1999;Diaz et al., 1999). The gene encoding SLC19A3 (ThTr2) was identified based upon homology screening (Eudy et al., 2000;Rajgopal et al., 2001). SLC19A2 and SLC19A3 share an identity of 48 % and both are ~ 40 % identical to SLC19A1. Despite the high homology, thiamine transporters do not transport folates or antifolates and SLC19A1 does not transport thiamine (Dutta et al., 1999;Rajgopal et al., 2001;Zhao et al., 2000). The predicted secondary structures of the thiamine transporters are similar to that of SLC19A1; however, the topology has not been confirmed experimentally (Zeng et al., 2005).

SLC19A2 is widely expressed in human tissues with the highest expression in skeletal muscle (Dutta et al., 1999). SLC19A3 is expressed at the highest level in the duodenum (Said et al., 2004). These transporters are also expressed in a variety of cells lines, such as: colon carcinoma CaCo-2, retinal pigment epithelial ARPE-19 cells, renal epithelial HEK-293 cells, and pancreatic beta-TC6 cells (Said et al., 2004;Subramanian et al., 2007;Ashokkumar et al., 2006;Mee et al., 2009).

Similar to SLC19A1-mediated folate influx, thiamine transport mediated by both SLC19A2 and SLC19A3 is optimum at neutral pH and decreases as extracellular pH becomes acidic (Dutta et al., 1999;Rajgopal et al., 2001). However, the affinities of SLC19A2 and SLC19A3 for thiamine are very different. SLC19A2 has an influx K_t in the 2.5 μ M range while SLC19A3 has a K_t for thiamine in the 25 nM range (Dutta et al., 1999;Said et al., 2004).SLC19A2 and SLC19A3 appear to target to different membranes in polarized cells. SLC19A2 targets predominantly to the basolateral membrane while SLC19A3 traffics to the apical membrane of MDCK cells indicating that they have different roles in thiamine transport across epithelia (Boulware et al., 2003).

The thiamine transporter that mediates intestinal absorption has been clarified. The observation that plasma thiamine levels were not reduced in subjects with thiamine responsive megaloblastic anemia (TRMA), due to loss-of-function mutations in *SLC19A2* gene, indicate that this transporter is not required for intestinal thiamine absorption (Neufeld et al., 2001). Recent studies in *Slc19a3*-deficient mice indicate that SLC19A3 is required for intestinal thiamine absorption while *Slc19a2*-null mice are not thiamine-deficient. *SLC19A2* expression was not altered in *Slc19a3*-deficient mice whereas *SLC19A3* expression was increased in *Slc19a2*-deficient mice (Reidling et al., 2010).

Expression of *SLC19A2* and *SLC19A3* is regulated. For example, the administration of alcohol orally to rats decreases intestinal SLC19A2 expression but reduces expression of both thiamine transporters in the kidney. Expression of both thiamine transporters in intestine was also reduced by enteropathogenic *E. coli* (Ashokkumar et al., 2009). Both thiamine transporters are adaptively regulated. In transgenic mice carrying *SLC19A2* and *SLC19A3* promoters, a thiamine-deficient diet increased activity of the *SLC19A3* promoter, but did not increase activity of the *SLC19A2* promoter in intestine. However, activity of both promoters was increased in kidneys of these mice with thiamine deficiency (Reidling and Said, 2005). Intestinal and renal *SLC19A2* and *SLC19A3* expression are also decreased in weaning, as compared to suckling, mice suggesting that thiamine transport may be developmentally regulated during early stages of life (Reidling et al., 2006). Recently, it was found that TSPAN1, a member of the tetraspamin family of proteins, interacts with SLC19A2 increasing the stability of this transporter suggesting its expression is also regulated at the protein level (Nabokina et al., 2011).

4. The folate receptors

4.1. Expression and function

Distinct from transport mediated by the solute carriers is folate-receptor mediated endocytosis involving two genetically distinct proteins, folate receptor-1 (FR encoded by the *FOLR1* gene) and folate-receptor-2 (FR encoded by the *FOLR2* gene), that are functionally similar but have a different pattern of tissue expression.

FOLR1 in adult mice is expressed almost exclusively in epithelia – the apical brush border membrane of the proximal renal tubule, the apical membrane of the choroid plexus in apposition to the cerebrospinal fluid (CSF), the basolateral membrane of retinal pigment epithelial cells, and the placenta as illustrated in Figure 3 (Kamen and Smith, 2004; Chancy et al., 2000; Zhao and Goldman, 2003; Zhao et al., 2011). All that is known about the functional role in these tissues is that in *Folr1*-null adult mice there is increased renal clearance of folate and decreased serum folate (Birn et al., 2005). The fact that these mice have a normal phenotype suggests that, if there are changes in other organs where FOLR1 is expressed, they are of little physiological consequences (Spiegelstein et al., 2004). A role for FOLR1 in folate transport across ependymal cells of the choroid plexus is indicated by the recent description of cerebral folate deficiency due to loss-of-function mutations in the *FOLR1* gene (see below).

The sequence of events in the endocytic process is initiated when a folate molecule binds to a folate receptor at the cell surface. This is followed by invagination of the cell membrane (clathrin-independent) containing the folate-receptor complex, formation of an endosome at the membrane-cytoplasmic interface followed by trafficking of the vesicle in the endosomal compartment where it acidifies releasing folate from the receptor which then exits the acidified endosome. The mechanism of export from the endosome is not clear. Presumably, this would be mediated by a process that is active at low pH such as SLC46A1 and, indeed, SLC46A1 is co-expressed with FOLR1 at the endosomal membrane (Zhao et al., 2009b; Bozard et al., 2010). This is similar to the role the divalent metal ion transporter, DMT1, plays in both intestinal iron absorption and the release of endosomal iron during receptor mediated endocytosis within reticulocytes (Gunshin et al., 1997; Andrews and Schmidt, 2007). However, while SLC46A1 expression augments receptor-mediated endocytosis of folates (Zhao et al., 2009b), this process can occur in at least some *SLC46A1*-deficient cells implying the presence of another mechanism of export from endosomes or nonspecific leakage (Deng et al., 2009). As indicated below, both SLC46A1 and FOLR1 are required for the transport of folates across choroid plexus ependymal cells. This linkage may be due to the requirement for SLC46A1 in FOLR1-mediated endocytosis in that organ.

4.2. Pharmacological applications of folate-receptor mediated endocytosis

Folate receptors are emerging as delivery vehicles for anticancer drugs and other reagents (Salazar and Ratnam, 2007). This is based upon the observation that FOLR1 is generally expressed in human tumors of epithelial origin (Parker et al., 2005; Toffoli et al., 1997), while FOLR2 is expressed in hematological malignancies and hepatic cancers (Wang et al., 2000; Ross et al., 1999; Elnakat and Ratnam, 2004). Beyond expression in epithelia noted above, folate receptor expression in normal tissues is negligible. Hence, agents that are transported solely by this route, and not by other mechanisms, would have inherent antitumor selectivity and minimal toxicity.

Two approaches are currently being pursued. The first is the development of antifolates with a very low affinity for SLC19A1, minimizing toxicity caused by transport into normal tissues by this route, but high affinity for folate receptors. One such agent, ONX0801, a

thymidylate synthase inhibitor is currently being evaluated in clinical trials (Gibbs et al., 2005; Leamon and Jackman, 2008). In a second approach, drugs structurally unrelated to the folates are coupled to folic acid through its glutamate moiety (Xia and Low, 2010). The coupling includes a spacer and cleavable sulfhydryl bond. This does not perturb the interaction between the folic acid portion of the molecule and the folate receptor so that the complex undergoes folate receptor-mediated endocytosis. In this case, as the endosome migrates in the endosomal compartment and the reducing potential of the endosomes increases, the sulfhydryl bond is broken, and the cytotoxic agent diffuses out of the endosome to reach its target within the cell. The first of this class of therapeutics in the clinic, EC145 - folic acid linked to desacetylvinblastine, is active in the treatment of platinum-resistant ovarian cancer (Dosio et al., 2010). This approach can also be utilized to deliver anti-inflammatory drugs to activated macrophages, which express high levels of FOLR2, in inflammatory disorders (Paulos et al., 2004).

There are also diagnostic applications of this technology. For instance, folic acid can be linked to ^{99}Tc to image tumors or sites of inflammation (Fisher et al., 2008). The former is utilized to establish that the tumor expresses functional receptor prior to administration of the folic acid-drug complex. Likewise, when administered after the tumor becomes resistant to the folic acid-drug complex, this reagent can establish whether the loss of activity is due to resistance to the chemotherapeutic moiety or to a loss of receptor and/or its function.

5. Inherited diseases due to loss-of-function mutations in folate and thiamine transporters

5.1. Folate transporters

Targeted disruption of *Slc46a1* in mice is embryonic lethal unless the dams are supplemented with parenteral folate. When prenatal supplementation is sufficient, normal pups can be delivered but, without further supplementation, they die within two weeks due to atrophy of all hematopoietic tissues (bone marrow, liver, spleen, thymus) (Zhao et al., 2001). There is no known human counterpart. Thirty one families have now been described with loss-of-function *SLC46A1* gene mutations causing HFM (OMIM 229050) (Diop-Bove et al., 2011). In this autosomal recessive disorder, *SLC46A1*-null infants are normal at birth; signs and symptoms manifest within a few months after birth with several anemia, hypogammaglobulinemia with immune deficiency and infections, and virtually no folate in the cerebrospinal fluid (CSF) even when the blood folate level is normalized. If untreated, these subjects experience developmental delays, ataxia, other neurological signs, and ultimately seizures. Early treatment with high daily doses of oral 5-formyltetrahydrofolate or, preferably, parental administration of lower doses of the active isomer can obviate the manifestations of this disorder. The clinical aspects of HFM have been described in detail (Geller et al., 2002; Diop-Bove et al., 2011). A *Slc46a1*-null mouse has been developed which recapitulates many elements of the human disorder (Salojin et al., 2011).

Folr2-null mice do not have a phenotype; the physiological role of this protein is unclear (Piedrahita et al., 1999). The *Folr1*-null mouse is embryonic lethal (Piedrahita et al., 1999); however, with adequate folate supplementation during gestation, pups are normal and can be weaned off supplementation to produce adults with a normal phenotype (Spiegelstein et al., 2004). However, there are some physiological differences from the wild-type mice in that the *Folr1*-null mice have increased renal folate clearance and a modest decrease in serum folate (Birn et al., 2005). This occurs to a lesser extent in heterozygotes. Hence, while the *FOLR1* gene is required for embryonic development, it is not required for adult life.

Recently, several families have been reported with an autosomal recessive disorder in which there are progeny with loss-of-function mutations in *FOLR1* gene (OMIM-613068)

(Steinfeld et al., 2009;Caro et al., 2009;Grapp et al., 2012). Like the children with HFM, these children have very low CSF folate levels but unlike HFM, their serum folate levels are normal consistent with normal intestinal folate absorption. Also, in contrast to HFM, these subjects present with neurological consequences of folate deficiency several years after birth. The fact that both genetic defects produce a failure to transport folates across the choroid plexus suggests that these transporters act in series; disruption of either one results in the same defect. One possibility is that SLC46A1 is required for export of folates from acidified endosomes during receptor-mediated endocytosis in the ependymal cells of the choroid plexus (Zhao et al., 2009b). However, while SLC46A1 is expressed at the basolateral membrane in apposition to the blood (Zhao et al., 2009b), the pH at this site would be expected to be 7.4 unless there is active proton-sodium exchange at this site resulting in local acidification. Further, FOLR1 is expressed predominantly at the apical membrane in apposition to the CSF (Kennedy et al., 2003;Weitman et al., 1992a;Weitman et al., 1992b;Selhub and Franklin, 1984;Patrick et al., 1997), a location more likely to result in transport from CSF into the ependymal cells than transport into the CSF. Clearly, much more information is required to understand the physiology of folate transport across this organ and into the central nervous system.

5.2. Thiamine transporters

Loss-of-function mutations in the *SLC19A2* gene result in a rare autosomal recessive disorder, thiamine-responsive megaloblastic anemia (TRMA) (MIM 249270) (Labay et al., 1999;Fleming et al., 1999;Diaz et al., 1999). TRMA is characterized by megaloblastic anemia, sensorineural hearing loss and diabetes mellitus. The anemia is corrected by pharmacological doses of thiamine but erythrocytes remain macrocytic. Progressive hearing loss is irreversible and may not be prevented by thiamine supplementation. Affected individuals require lifelong administration of thiamine. All subjects with TRMA carry loss-of-function mutations in the *SLC19A2* gene (Oishi and Diaz, 1993). *Slc19a2*-null mice have a similar phenotype on a normal diet as also occurs with wild-type mice on a thiamine deficient diet (Oishi et al., 2002).

The first autosomal recessive disorder found to be associated with loss-of-function *SLC19A3* mutations was biotin-responsive basal ganglia disease (OMIM 60783,606152) (Ozand et al., 1998). This disorder begins in infancy as a subacute encephalopathy with confusion, dysarthria and dysphagia and, without treatment, progresses to rigidity, dystonia, quadriplegia, and death. Administration of high dose of biotin (5-10 mg/kg/day) early in the disorder eliminates these abnormalities that do not recur if treatment is continued. Despite the fact that this is a thiamine transporter, the disorder does not respond to thiamine (Ozand et al., 1998). SLC19A3 does not transport biotin (Subramanian et al., 2006), but biotin deficiency was shown to reduce expression of *SLC19A3* in human leukocytes (Vlasova et al., 2005).

Thiamine deficiency in the setting of alcoholism is associated with Wernicke's syndrome, a triad of acute mental confusion, ataxia, and ophthalmoplegia with late neuropsychiatric manifestations of memory loss and confabulation. A Wernicke's-like syndrome has also been associated with *SLC19A3* mutations (MIM 606152). In this case there was some residual thiamine transport function associated with compound heterozygous K44E and E320Q mutations (Kono et al., 2009).The disorder manifests clinically in the second decade of life with diplopia, nystagmus, ataxia, progressing to seizures. The patients are not thiamine deficient but respond to pharmacological doses of thiamine. Recently, a subject homozygous for a SLC19A3 E320Q mutation presented in early infancy with psychomotor retardation, seizures, and MRI findings of progressive brain atrophy and bilateral thalamus and basal ganglia lesions (Yamada et al., 2010).

6. Summation

Considerable progress has been made over the past two decades in the development of a comprehensive understanding of the mechanisms by which folates and thiamine are transported across epithelia and into human cells. Major advances were highlighted by identification of the genes encoding these vitamin transporters (Table I and II) employing diverse approaches and in diverse contexts. The impetus for cloning the *SLC19A1* gene was stimulated by the observation that acquired resistance to methotrexate in tumor cells was due to the loss of RFC activity. The *SLC19A2* gene was identified in the search for the genetic basis for the autosomal recessive disorder thiamine-responsive megaloblastic anemia, while *SLC46A1* was discovered by screening candidate genes for the long-sought folate transport activity that was optimal at acidic pH. Mouse knockout models for most of the folate and thiamine transporters are now available and will be very useful for further deciphering the roles and regulation of these transporters in key tissues, particularly under conditions of dietary folate and thiamine deficiency. The roles of *SCL19A2* in thiamine-responsive megaloblastic anemia and *SLC46A1* in hereditary folate malabsorption are well established and novel loss-of-function mutations in subjects with these disorders continue to be identified. The association of *SCL19A3* mutations with biotin-responsive basal ganglia disease or Wernicke's-like encephalopathy awaits further clarification as more mutations are identified and correlated with clinical findings. The observation that biotin-responsive basal ganglia disease responds to biotin, but not to thiamine, and that *SCL19A3* transports thiamine but not biotin, remains a puzzle. Loss-of-function mutations in *SLC46A1* and *FOLR1* both produce a marked deficiency of folate in the cerebral spinal fluid suggesting that these transporters act in series, but the mechanism by which this occurs is not fully understood. It remains to be seen if additional mutations or polymorphisms are identified that reduce, but do not abolish, activity of these vitamin transporters so that clinical manifestations only occur under conditions of dietary folate or thiamine deficiency and/or with heavy consumption of alcohol.

Abbreviations

RFC	reduced folate carrier
PCFT	proton-coupled folate transporter
ThTr1	thiamine transporter-1
ThTr2	thiamine transporter-2
FOLR1	folate receptor 1 or FR
FOLR2	folate receptor 2 or FR
CSF	cerebrospinal fluid
HFM	hereditary folate malabsorption
TRMA	thiamine-responsive megaloblastic anemia

References

- Andrews NC, Schmidt PJ. Iron Homeostasis. *Annu. Rev. Physiol.* 2007; 69:69–85. [PubMed: 17014365]
- Ashokkumar B, Kumar JS, Hecht GA, Said HM. Enteropathogenic *Escherichia Coli* Inhibits Intestinal Vitamin B1 (Thiamin) Uptake: Studies With Human-Derived Intestinal Epithelial Caco-2 Cells. *Am. J Physiol Gastrointest. Liver Physiol.* 2009; 297:G825–G833. [PubMed: 19628653]

- Ashokkumar B, Vaziri ND, Said HM. Thiamin Uptake by the Human-Derived Renal Epithelial (HEK-293) Cells: Cellular and Molecular Mechanisms. *Am. J Physiol Renal Physiol.* 2006; 291:F796–F805. [PubMed: 16705148]
- Assaraf YG, Sierra EE, Babani S, Goldman ID. Inhibitory Effects of Prostaglandin A1 on Membrane Transport of Folates Mediated by Both the Reduced Folate Carrier and ATP-Driven Exporters. *Biochem. Pharmacol.* 1999; 58:1321–1327. [PubMed: 10487535]
- Birn H, Spiegelstein O, Christensen EI, Finnell RH. Renal Tubular Reabsorption of Folate Mediated by Folate Binding Protein 1. *J. Am. Soc. Nephrol.* 2005; 16:608–615. [PubMed: 15703271]
- Boulware MJ, Subramanian VS, Said HM, Marchant JS. Polarized Expression of Members of the Solute Carrier SLC19A Gene Family of Water-Soluble Multivitamin Transporters: Implications for Physiological Function. *Biochem. J.* 2003; 376:43–48. [PubMed: 14602044]
- Bozard BR, Ganapathy P, Duplantier JN, Mysona B, Ha Y, Roon P, Smith R, Goldman ID, Prasad PD, Martin PM, Ganapathy V, Smith SB. Molecular and Biochemical Characterization of Folate Transport Proteins in Retinal Muller Cells. *Invest Ophthalmol. Vis. Sci.* 2010; 51:3226–3235. [PubMed: 20053979]
- Cao W, Matherly LH. Analysis of the Membrane Topology for Transmembrane Domains 7-12 of the Human Reduced Folate Carrier by Scanning Cysteine Accessibility Methods. *Biochem. J.* 2004; 378:201–206. [PubMed: 14602046]
- Cario H, Bode H, Debatin KM, Opladen T, Schwarz K. Congenital Null Mutations of the *FOLR1* Gene: a Progressive Neurologic Disease and Its Treatment. *Neurology.* 2009; 73:2127–2129. [PubMed: 20018644]
- Chancy CD, Kekuda R, Huang W, Prasad PD, Kuhnel JM, Sirotinak FM, Roon P, Ganapathy V, Smith SB. Expression and Differential Polarization of the Reduced-Folate Transporter-1 and the Folate Receptor Alpha in Mammalian Retinal Pigment Epithelium. *J. Biol. Chem.* 2000; 275:20676–20684. [PubMed: 10787414]
- Chattopadhyay S, Zhao R, Krupenko SA, Krupenko N, Goldman ID. The Inverse Relationship Between Reduced Folate Carrier Function and Pemetrexed Activity in a Human Colon Cancer Cell Line. *Mol. Cancer Ther.* 2006; 5:438–449. [PubMed: 16505119]
- Dembo M, Sirotinak FM, Moccio DM. Effects of Metabolic Deprivation on Methotrexate Transport in L1210 Leukemia Cells: Further Evidence for Separate Influx and Efflux Systems With Different Energetic Requirements. *J. Membr. Biol.* 1984; 78:9–17. [PubMed: 6708095]
- Deng Y, Zhou X, Kugel DS, Wu J, Cherian C, Hou Z, Matherly LH, Gangjee A. Synthesis and Biological Activity of a Novel Series of 6-Substituted Thieno[2,3-d]Pyrimidine Antifolate Inhibitors of Purine Biosynthesis With Selectivity for High Affinity Folate Receptors Over the Reduced Folate Carrier and Proton-Coupled Folate Transporter for Cellular Entry. *J. Med. Chem.* 2009; 52:2940–2951. [PubMed: 19371039]
- Desmoulin SK, Wang L, Hales E, Polin L, White K, Kushner J, Stout M, Hou Z, Cherian C, Gangjee A, Matherly LH. Therapeutic Targeting of a Novel 6-Substituted Pyrrolo[2,3-d]Pyrimidine Thienoyl Antifolate to Human Solid Tumors Based on Selective Uptake by the Proton-Coupled Folate Transporter. *Mol Pharmacol.* 2011; 80:1096–1107. [PubMed: 21940787]
- Desmoulin SK, Wang Y, Wu J, Stout M, Hou Z, Fulterer A, Chang MH, Romero MF, Cherian C, Gangjee A, Matherly LH. Targeting the Proton-Coupled Folate Transporter for Selective Delivery of 6-Substituted Pyrrolo[2,3-d]Pyrimidine Antifolate Inhibitors of De Novo Purine Biosynthesis in the Chemotherapy of Solid Tumors. *Mol Pharmacol.* 2010; 78:577–587. [PubMed: 20601456]
- Diaz GA, Banikazemi M, Oishi K, Desnick RJ, Gelb BD. Mutations in a New Gene Encoding a Thiamine Transporter Cause Thiamine- Responsive Megaloblastic Anaemia Syndrome. *Nat. Genet.* 1999; 22:309–312. [PubMed: 10391223]
- Diop-Bove, N.; Kronn, D.; Goldman, ID. Hereditary Folate Malabsorption. In: Pagon, RA.; Bird, TD.; Dolan, CR.; Stephens, K., editors. *GeneReviews* [Internet]. University of Washington, Seattle; Seattle (WA): 2011.
- Diop-Bove NK, Wu J, Zhao R, Locker J, Goldman ID. Hypermethylation of the Human Proton-Coupled Folate Transporter (SLC46A1) Minimal Transcriptional Regulatory Region in an Antifolate-Resistant HeLa Cell Line. *Molecular Cancer Therapeutics.* 2009; 8:2424–2431. [PubMed: 19671745]

- Dixon KH, Lanpher BC, Chiu J, Kelley K, Cowan KH. A Novel CDNA Restores Reduced Folate Carrier Activity and Methotrexate Sensitivity to Transport Deficient Cells. *J. Biol. Chem.* 1994; 269:17–20. [PubMed: 8276792]
- Dosio F, Milla P, Cattel L. EC-145, a Folate-Targeted Vinca Alkaloid Conjugate for the Potential Treatment of Folate Receptor-Expressing Cancers. *Curr. Opin. Investig. Drugs.* 2010; 11:1424–1433.
- Dutta B, Huang W, Molero M, Kekuda R, Leibach FH, Devoe LD, Ganapathy V, Prasad PD. Cloning of the Human Thiamine Transporter, a Member of the Folate Transporter Family. *J. Biol. Chem.* 1999; 274:31925–31929. [PubMed: 10542220]
- Elnakat H, Ratnam M. Distribution, Functionality and Gene Regulation of Folate Receptor Isoforms: Implications in Targeted Therapy. *Adv. Drug Deliv. Rev.* 2004; 56:1067–1084. [PubMed: 15094207]
- Eloranta JJ, Zair ZM, Hiller C, Hausler S, Stieger B, Kullak-Ublick GA. Vitamin D3 and Its Nuclear Receptor Increase the Expression and Activity of the Human Proton-Coupled Folate Transporter. *Mol Pharmacol.* 2009; 76:1062–1071. [PubMed: 19666701]
- Eudy JD, Spiegelstein O, Barber RC, Wlodarczyk BJ, Talbot J, Finnell RH. Identification and Characterization of the Human and Mouse SLC19A3 Gene: a Novel Member of the Reduced Folate Family of Micronutrient Transporter Genes. *Mol. Genet. Metab.* 2000; 71:581–590. [PubMed: 11136550]
- Ferguson PL, Flintoff WF. Topological and Functional Analysis of the Human Reduced Folate Carrier by Hemagglutinin Epitope Insertion. *J. Biol. Chem.* 1999; 274:16269–16278. [PubMed: 10347183]
- Fisher RE, Siegel BA, Edell SL, Oyesiku NM, Morgenstern DE, Messmann RA, Amato RJ. Exploratory Study of 99mTc-EC20 Imaging for Identifying Patients With Folate Receptor-Positive Solid Tumors. *J. Nucl. Med.* 2008; 49:899–906. [PubMed: 18483093]
- Fleming JC, Tartaglini E, Steinkamp MP, Schorderet DF, Cohen N, Neufeld EJ. The Gene Mutated in Thiamine-Responsive Anaemia With Diabetes and Deafness (TRMA) Encodes a Functional Thiamine Transporter. *Nat. Genet.* 1999; 22:305–308. [PubMed: 10391222]
- Frank RA, Leeper FJ, Luisi BF. Structure, Mechanism and Catalytic Duality of Thiamine-Dependent Enzymes. *Cell Mol Life Sci.* 2007; 64:892–905. [PubMed: 17429582]
- Fry DW, White JC, Goldman ID. Effects of 2,4-Dinitrophenol and Other Metabolic Inhibitors on the Bidirectional Carrier Fluxes, Net Transport, and Intracellular Binding of Methotrexate in Ehrlich Ascites Tumor Cells. *Cancer Res.* 1980; 40:3669–3673. [PubMed: 7438050]
- Geller J, Kronn D, Jayabose S, Sandoval C. Hereditary Folate Malabsorption: Family Report and Review of the Literature. *Medicine (Baltimore).* 2002; 81:51–68. [PubMed: 11807405]
- Gibbs DD, Theti DS, Wood N, Green M, Raynaud F, Valenti M, Forster MD, Mitchell F, Bavetsias V, Henderson E, Jackman AL. BGC 945, a Novel Tumor-Selective Thymidylate Synthase Inhibitor Targeted to Alpha-Folate Receptor-Overexpressing Tumors. *Cancer Res.* 2005; 65:11721–11728. [PubMed: 16357184]
- Gnana-Prakasam JP, Reddy SK, Veeranan-Karmegam R, Smith SB, Martin PM, Ganapathy V. Polarized Distribution of Heme Transporters in Retinal Pigment Epithelium and Their Regulation in the Iron-Overload Disease Hemochromatosis. *Invest Ophthalmol. Vis. Sci.* 2011; 52:9279–9286. [PubMed: 22058337]
- Goldman ID. Transport Energetics of the Folic Acid Analogue, Methotrexate, in L1210 Cells: Enhanced Accumulation by Metabolic Inhibitors. *J. Biol. Chem.* 1969; 244:3779–3785. [PubMed: 5805398]
- Goldman ID. The Characteristics of the Membrane Transport of Amethopterin and the Naturally Occurring Folates. *Ann. N. Y. Acad. Sci.* 1971; 186:400–422. [PubMed: 5289428]
- Goldman ID, Chattopadhyay S, Zhao R, Moran RG. The Antifolates: Evolution, New Agents in the Clinic, and How Targeting Delivery Via Specific Membrane Transporters Is Driving the Development of a Next Generation of Folate Analogs. *Curr. Opin. Investig. Drugs.* 2010; 11:1409–1423.

- Goldman ID, Lichtenstein NS, Oliverio VT. Carrier-Mediated Transport of the Folic Acid Analogue Methotrexate, in the L1210 Leukemia Cell. *J. Biol. Chem.* 1968; 243:5007–5017. [PubMed: 5303004]
- Gonen N, Assaraf YG. The Obligatory Intestinal Folate Transporter PCFT (SLC46A1) Is Regulated by Nuclear Respiratory Factor 1. *J Biol. Chem.* 2010; 285:33602–33613. [PubMed: 20724482]
- Gonen N, Bram EE, Assaraf YG. PCFT Promoter Methylation and Restoration of Gene Expression in Human Leukemia Cells. *Biochem. Biophys. Res. Commun.* 2008; 376:787–792. [PubMed: 18817749]
- Grapp M, Just IA, Linnankivi T, Wolf P, Lucke T, Hausler M, Gartner J, Steinfeld R. Molecular Characterization of Folate Receptor 1 Mutations Delineates Cerebral Folate Transport Deficiency. *Brain.* 2012 Epub ahead of print.
- Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and Characterization of a Mammalian Proton-Coupled Metal-Ion Transporter. *Nature.* 1997; 388:482–488. [PubMed: 9242408]
- Henderson GB, Zevely EM. Structural Requirements for Anion Substrates of the Methotrexate Transport System of L1210 Cells. *Arch. Biochem. Biophys.* 1983; 221:438–446. [PubMed: 6838199]
- Kamen BA, Smith AK. A Review of Folate Receptor Alpha Cycling and 5-Methyltetrahydrofolate Accumulation With an Emphasis on Cell Models in Vitro. *Adv. Drug Deliv. Rev.* 2004; 56:1085–1097. [PubMed: 15094208]
- Kennedy MD, Jallad KN, Lu J, Low PS, Ben Amotz D. Evaluation of Folate Conjugate Uptake and Transport by the Choroid Plexus of Mice. *Pharm. Res.* 2003; 20:714–719. [PubMed: 12751625]
- Kitamura Y, Kusuhara H, Sugiyama Y. Basolateral Efflux Mediated by Multidrug Resistance-Associated Protein 3 (Mrp3/Abcc3) Facilitates Intestinal Absorption of Folates in Mouse. *Pharm. Res.* 2010; 27:665–672. [PubMed: 20148291]
- Kono S, Miyajima H, Yoshida K, Togawa A, Shirakawa K, Suzuki H. Mutations in a Thiamine-Transporter Gene and Wernicke's-Like Encephalopathy. *N. Engl. J Med.* 2009; 360:1792–1794. [PubMed: 19387023]
- Kruh GD, Belinsky MG. The MRP Family of Drug Efflux Pumps. *Oncogene.* 2003; 22:7537–7552. [PubMed: 14576857]
- Labay V, Raz T, Baron D, Mandel H, Williams H, Barrett T, Szargel R, McDonald L, Shalata A, Nosaka K, Gregory S, Cohen N. Mutations in SLC19A2 Cause Thiamine-Responsive Megaloblastic Anaemia Associated With Diabetes Mellitus and Deafness. *Nat. Genet.* 1999; 22:300–304. [PubMed: 10391221]
- Leamon CP, Jackman AL. Exploitation of the Folate Receptor in the Management of Cancer and Inflammatory Disease. *Vitam. Horm.* 2008; 79:203–233. [PubMed: 18804696]
- Matherly LH, Goldman DI. Membrane Transport of Folates. *Vitam. Horm.* 2003; 66:403–456. [PubMed: 12852262]
- Matherly LH, Hou Z, Deng Y. Human Reduced Folate Carrier: Translation of Basic Biology to Cancer Etiology and Therapy. *Cancer Metastasis Rev.* 2007; 26:111–128. [PubMed: 17334909]
- Mee L, Nabokina SM, Sekar VT, Subramanian VS, Maedler K, Said HM. Pancreatic Beta Cells and Islets Take Up Thiamin by a Regulated Carrier-Mediated Process: Studies Using Mice and Human Pancreatic Preparations. *Am. J Physiol Gastrointest. Liver Physiol.* 2009; 297:G197–G206. [PubMed: 19423748]
- Nabokina SM, Senthilkumar SR, Said HM. Tspan-1 Interacts With the Thiamine Transporter-1 in Human Intestinal Epithelial Cells and Modulates Its Stability. *Am. J Physiol Gastrointest. Liver Physiol.* 2011; 301:G808–G813. [PubMed: 21836059]
- Nakai Y, Inoue K, Abe N, Hatakeyama M, Ohta KY, Otagiri M, Hayashi Y, Yuasa H. Functional Characterization of Human PCFT/HCP1 Heterologously Expressed in Mammalian Cells As a Folate Transporter. *J. Pharmacol. Exp. Ther.* 2007; 322:469–476. [PubMed: 17475902]
- Neufeld EJ, Fleming JC, Tartaglini E, Steinkamp MP. Thiamine-Responsive Megaloblastic Anemia Syndrome: a Disorder of High-Affinity Thiamine Transport. *Blood Cells Mol. Dis.* 2001; 27:135–138. [PubMed: 11358373]

- Oishi, K.; Diaz, GA. Thiamine-Responsive Megaloblastic Anemia Syndrome. In: Pagon, RA.; Bird, TD.; Dolan, CR., editors. GeneReviews [Internet]. University of Washington; Seattle (WA): 1993.
- Oishi K, Hofmann S, Diaz GA, Brown T, Manwani D, Ng L, Young R, Vlassara H, Ioannou YA, Forrest D, Gelb BD. Targeted Disruption of Slc19a2, the Gene Encoding the High-Affinity Thiamin Transporter Thtr-1, Causes Diabetes Mellitus, Sensorineural Deafness and Megaloblastosis in Mice. *Hum. Mol. Genet.* 2002; 11:2951–2960. [PubMed: 12393806]
- Ozand PT, Gascon GG, Al Essa M, Joshi S, Al Jishi E, Bakheet S, Al Watban J, Al Kawi MZ, Dabbagh O. Biotin-Responsive Basal Ganglia Disease: a Novel Entity. *Brain.* 1998; 121:1267–1279. [PubMed: 9679779]
- Parker N, Turk MJ, Westrick E, Lewis JD, Low PS, Leamon CP. Folate Receptor Expression in Carcinomas and Normal Tissues Determined by a Quantitative Radioligand Binding Assay. *Anal. Biochem.* 2005; 338:284–293. [PubMed: 15745749]
- Patrick TA, Kranz DM, van Dyke TA, Roy EJ. Folate Receptors As Potential Therapeutic Targets in Choroid Plexus Tumors of SV40 Transgenic Mice. *J. Neurooncol.* 1997; 32:111–123. [PubMed: 9120540]
- Paulos CM, Turk MJ, Breur GJ, Low PS. Folate Receptor-Mediated Targeting of Therapeutic and Imaging Agents to Activated Macrophages in Rheumatoid Arthritis. *Adv. Drug Deliv. Rev.* 2004; 56:1205–1217. [PubMed: 15094216]
- Piedrahita JA, Oetama B, Bennett GD, van Waes J, Kamen BA, Richardson J, Lacey SW, Anderson RG, Finnell RH. Mice Lacking the Folic Acid-Binding Protein Folbp1 Are Defective in Early Embryonic Development. *Nat. Genet.* 1999; 23:228–232. [PubMed: 10508523]
- Qiu A, Jansen M, Sakaris A, Min SH, Chattopadhyay S, Tsai E, Sandoval C, Zhao R, Akabas MH, Goldman ID. Identification of an Intestinal Folate Transporter and the Molecular Basis for Hereditary Folate Malabsorption. *Cell.* 2006; 127:917–928. [PubMed: 17129779]
- Qiu A, Min SH, Jansen M, Malhotra U, Tsai E, Cabelof DC, Matherly LH, Zhao R, Akabas MH, Goldman ID. Rodent Intestinal Folate Transporters (SLC46A1): Secondary Structure, Functional Properties, and Response to Dietary Folate Restriction. *Am. J. Physiol Cell Physiol.* 2007; 293:C1669–C1678. [PubMed: 17898134]
- Rajgopal A, Edmondson A, Goldman ID, Zhao R. SLC19A3 Encodes a Second Thiamine Transporter, ThTr2. *Biochim. Biophys. Acta.* 2001; 1537:175–178. [PubMed: 11731220]
- Reidling JC, Lambrecht N, Kassir M, Said HM. Impaired Intestinal Vitamin B1 (Thiamin) Uptake in Thiamin Transporter-2-Deficient Mice. *Gastroenterology.* 2010; 138:1802–1809. [PubMed: 19879271]
- Reidling JC, Nabokina SM, Balamurugan K, Said HM. Developmental Maturation of Intestinal and Renal Thiamin Uptake: Studies in Wild-Type and Transgenic Mice Carrying Human THTR-1 and 2 Promoters. *J Cell Physiol.* 2006; 206:371–377. [PubMed: 16206251]
- Reidling JC, Said HM. Adaptive Regulation of Intestinal Thiamin Uptake: Molecular Mechanism Using Wild-Type and Transgenic Mice Carrying HTHTR-1 and -2 Promoters. *Am. J Physiol Gastrointest. Liver Physiol.* 2005; 288:G1127–G1134. [PubMed: 15705657]
- Rosowsky A, Wright JE, Vaidya CM, Bader H, Forsch RA, Mota CE, Pardo J, Chen CS, Chen YN. Synthesis and Potent Antifolate Activity and Cytotoxicity of B-Ring Deaza Analogues of the Nonpolyglutamatable Dihydrofolate Reductase Inhibitor Nalpha-(4-Amino-4-Deoxypteroyl)-Ndelta-Hemiphthaloyl-L-Ornithine (PT523). *J. Med. Chem.* 1998; 41:5310–5319. [PubMed: 9857098]
- Ross JF, Wang H, Behm FG, Mathew P, Wu M, Booth R, Ratnam M. Folate Receptor Type Beta Is a Neutrophilic Lineage Marker and Is Differentially Expressed in Myeloid Leukemia. *Cancer.* 1999; 85:348–357. [PubMed: 10023702]
- Rothbart SB, Racanelli AC, Moran RG. Pemetrexed Indirectly Activates the Metabolic Kinase AMPK in Human Carcinomas. *Cancer Res.* 2010; 70:10299–10309. [PubMed: 21159649]
- Said HM, Balamurugan K, Subramanian VS, Marchant JS. Expression and Functional Contribution of HTHTR-2 in Thiamin Absorption in Human Intestine. *Am. J Physiol Gastrointest. Liver Physiol.* 2004; 286:G491–G498. [PubMed: 14615284]

- Said HM, Chatterjee N, Haq RU, Subramanian VS, Ortiz A, Matherly LH, Sirotnak FM, Halsted C, Rubin SA. Adaptive Regulation of Intestinal Folate Uptake: Effect of Dietary Folate Deficiency. *Am. J. Physiol Cell Physiol.* 2000; 279:C1889–C1895. [PubMed: 11078704]
- Salazar MD, Ratnam M. The Folate Receptor: What Does It Promise in Tissue-Targeted Therapeutics? *Cancer Metastasis Rev.* 2007; 26:141–152. [PubMed: 17333345]
- Salojin KV, Cabrera RM, Sun W, Chang WC, Lin C, Duncan L, Platt KA, Read R, Vogel P, Liu Q, Finnell RH, Oravec T. A Mouse Model of Hereditary Folate Malabsorption: Deletion of the PCFT Gene Leads to Systemic Folate Deficiency. *Blood.* 2011; 117:4895–4904. [PubMed: 21346251]
- Selhub J, Franklin WA. The Folate-Binding Protein of Rat Kidney. Purification, Properties, and Cellular Distribution. *J. Biol. Chem.* 1984; 259:6601–6606. [PubMed: 6427219]
- Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, Khan Y, Warley A, McCann FE, Hider RC, Frazer DM, Anderson GJ, Vulpe CD, Simpson RJ, McKie AT. Identification of an Intestinal Heme Transporter. *Cell.* 2005; 122:789–801. [PubMed: 16143108]
- Spiegelstein O, Mitchell LE, Merriweather MY, Wicker NJ, Zhang Q, Lammer EJ, Finnell RH. Embryonic Development of Folate Binding Protein-1 (Folbp1) Knockout Mice: Effects of the Chemical Form, Dose, and Timing of Maternal Folate Supplementation. *Dev. Dyn.* 2004; 231:221–231. [PubMed: 15305303]
- Steinfeld R, Grapp M, Kraetzner R, Dreha-Kulaczewski S, Helms G, Dechent P, Wevers R, Grosso S, Gartner J. Folate Receptor Alpha Defect Causes Cerebral Folate Transport Deficiency: a Treatable Neurodegenerative Disorder Associated With Disturbed Myelin Metabolism. *Am. J. Hum. Genet.* 2009; 85:354–363. [PubMed: 19732866]
- Stokstad, ELR. Historical perspective on key advances in the biochemistry and physiology of folates. In: Picciano, MF.; Stokstad, ELR., editors. *Folic Acid Metabolism in Health and Disease*. Wiley-Liss; New York: 1990. p. 1-21.
- Subramanian VS, Marchant JS, Said HM. Biotin-Responsive Basal Ganglia Disease-Linked Mutations Inhibit Thiamine Transport Via HTHTR2: Biotin Is Not a Substrate for HTHTR2. *Am. J Physiol Cell Physiol.* 2006; 291:C851–C859. [PubMed: 16790503]
- Subramanian VS, Mohammed ZM, Molina A, Marchant JS, Vaziri ND, Said HM. Vitamin B1 (Thiamine) Uptake by Human Retinal Pigment Epithelial (ARPE-19) Cells: Mechanism and Regulation. *J Physiol.* 2007; 582:73–85. [PubMed: 17463047]
- Toffoli G, Cernigoi C, Russo A, Gallo A, Bagnoli M, Boiocchi M. Overexpression of Folate Binding Protein in Ovarian Cancers. *Int. J. Cancer.* 1997; 74:193–198. [PubMed: 9133455]
- Umapathy NS, Gnana-Prakasam JP, Martin PM, Mysona B, Dun Y, Smith SB, Ganapathy V, Prasad PD. Cloning and Functional Characterization of the Proton-Coupled Electrogenic Folate Transporter and Analysis of Its Expression in Retinal Cell Types. *Invest Ophthalmol. Vis. Sci.* 2007; 48:5299–5305. [PubMed: 17962486]
- Unal ES, Zhao R, Qiu A, Goldman ID. N-Linked Glycosylation and Its Impact on the Electrophoretic Mobility and Function of the Human Proton-Coupled Folate Transporter (HsPCFT). *Biochim. Biophys. Acta.* 2008; 1178:1407–1414. [PubMed: 18405659]
- Urquhart BL, Gregor JC, Chande N, Knauer MJ, Tirona RG, Kim RB. The Human Proton-Coupled Folate Transporter (HPCFT): Modulation of Intestinal Expression and Function by Drugs. *Am. J Physiol Gastrointest. Liver Physiol.* 2010; 298:G248–G254. [PubMed: 19762432]
- Vlasova TI, Stratton SL, Wells AM, Mock NI, Mock DM. Biotin Deficiency Reduces Expression of SLC19A3, a Potential Biotin Transporter, in Leukocytes From Human Blood. *J Nutr.* 2005; 135:42–47. [PubMed: 15623830]
- Wang H, Zheng X, Behm FG, Ratnam M. Differentiation-Independent Retinoid Induction of Folate Receptor Type Beta, a Potential Tumor Target in Myeloid Leukemia. *Blood.* 2000; 96:3529–3536. [PubMed: 11071651]
- Wang Y, Zhao R, Russell RG, Goldman ID. Localization of the Murine Reduced Folate Carrier As Assessed by Immunohistochemical Analysis. *Biochim. Biophys. Acta.* 2001; 1513:49–54. [PubMed: 11427193]

- Weitman SD, Lark RH, Coney LR, Fort DW, Frasca V, Zurawski VR Jr, Kamen BA. Distribution of the Folate Receptor GP38 in Normal and Malignant Cell Lines and Tissues. *Cancer Res.* 1992a; 52:3396–3401. [PubMed: 1596899]
- Weitman SD, Weinberg AG, Coney LR, Zurawski VR, Jennings DS, Kamen BA. Cellular Localization of the Folate Receptor: Potential Role in Drug Toxicity and Folate Homeostasis. *Cancer Res.* 1992b; 52:6708–6711. [PubMed: 1330299]
- Wong SC, Zhang L, Proefke SA, Matherly LH. Effects of the Loss of Capacity for N-Glycosylation on the Transport Activity and Cellular Localization of the Human Reduced Folate Carrier. *Biochim. Biophys. Acta.* 1998; 1375:6–12. [PubMed: 9767079]
- Xia W, Low PS. Folate-Targeted Therapies for Cancer. *J Med Chem.* 2010; 53:6811–6824. [PubMed: 20666486]
- Yamada K, Miura K, Hara K, Suzuki M, Nakanishi K, Kumagai T, Ishihara N, Yamada Y, Kuwano R, Tsuji S, Wakamatsu N. A Wide Spectrum of Clinical and Brain MRI Findings in Patients With SLC19A3 Mutations. *BMC. Med Genet.* 2010; 11:171. 171. [PubMed: 21176162]
- Zeng WQ, Al Yamani E, Acierno JS Jr, Slaugenhaupt S, Gillis T, MacDonald ME, Ozand PT, Gusella JF. Biotin-Responsive Basal Ganglia Disease Maps to 2q36.3 and Is Due to Mutations in SLC19A3. *Am. J Hum. Genet.* 2005; 77:16–26. [PubMed: 15871139]
- Zhao R, Diop-Bove N, Visentin M, Goldman ID. Mechanisms of Membrane Transport of Folates into Cells and Across Epithelia. *Annu. Rev. Nutr.* 2011; 31:177–201. [PubMed: 21568705]
- Zhao R, Gao F, Goldman ID. Reduced Folate Carrier Transports Thiamine Monophosphate: an Alternative Route for Thiamine Delivery into Mammalian Cells. *Am. J. Physiol Cell Physiol.* 2002; 282:C1512–C1517. [PubMed: 11997266]
- Zhao R, Gao F, Hanscom M, Goldman ID. A Prominent Low-PH Methotrexate Transport Activity in Human Solid Tumor Cells: Contribution to the Preservation of Methotrexate Pharmacological Activity in HeLa Cells Lacking the Reduced Folate Carrier. *Clin. Cancer Res.* 2004a; 10:718–727. [PubMed: 14760095]
- Zhao R, Gao F, Wang Y, Diaz GA, Gelb BD, Goldman ID. Impact of the Reduced Folate Carrier on the Accumulation of Active Thiamin Metabolites in Murine Leukemia Cells. *J. Biol. Chem.* 2000; 276:1114–1118. [PubMed: 11038362]
- Zhao R, Goldman ID. Resistance to Antifolates. *Oncogene.* 2003; 22:7431–7457. [PubMed: 14576850]
- Zhao R, Hanscom M, Chattopadhyay S, Goldman ID. Selective Preservation of Pemetrexed Pharmacological Activity in HeLa Cells Lacking the Reduced Folate Carrier; Association With the Presence of a Secondary Transport Pathway. *Cancer Res.* 2004b; 64:3313–3319. [PubMed: 15126375]
- Zhao R, Matherly LH, Goldman ID. Membrane Transporters and Folate Homeostasis: Intestinal Absorption and Transport into Systemic Compartments and Tissues. *Expert. Rev. Mol. Med.* 2009a; 11:e4. [PubMed: 19173758]
- Zhao R, Min SH, Qiu A, Sakaris A, Goldberg GL, Sandoval C, Malatack JJ, Rosenblatt DS, Goldman ID. The Spectrum of Mutations in the PCFT Gene, Coding for an Intestinal Folate Transporter, That Are the Basis for Hereditary Folate Malabsorption. *Blood.* 2007; 110:1147–1152. [PubMed: 17446347]
- Zhao R, Min SH, Wang Y, Campanella E, Low PS, Goldman ID. A Role for the Proton-Coupled Folate Transporter (PCFT - SLC46A1) in Folate Receptor-Mediated Endocytosis. *J. Biol. Chem.* 2009b; 284:4267–4274. [PubMed: 19074442]
- Zhao R, Qiu A, Tsai E, Jansen M, Akabas MH, Goldman ID. The Proton-Coupled Folate Transporter (PCFT): Impact on Pemetrexed Transport and on Antifolate Activities As Compared to the Reduced Folate Carrier. *Mol. Pharmacol.* 2008; 74:854–862. [PubMed: 18524888]
- Zhao R, Russell RG, Wang Y, Liu L, Gao F, Kneitz B, Edelman W, Goldman ID. Rescue of Embryonic Lethality in Reduced Folate Carrier-Deficient Mice by Maternal Folic Acid Supplementation Reveals Early Neonatal Failure of Hematopoietic Organs. *J. Biol. Chem.* 2001; 276:10224–10228. [PubMed: 11266438]

Zhao R, Unal ES, Shin DS, Goldman ID. Membrane Topological Analysis of the Proton-Coupled Folate Transporter (PCFT-SLC46A1) by the Substituted Cysteine Accessibility Method. *Biochemistry*. 2010; 49:2925–2931. [PubMed: 20225891]

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Figure 1.

A: Structures of 5-methyltetrahydrofolate and thiamine. **B:** The sequence of metabolic events that occur after 5-methyltetrahydrofolate (5-methylTHF) enters cells and donates its carbon moiety to homocysteine in the synthesis of methionine yielding tetrahydrofolate (THF). THF then acquires another carbon moiety to form one-carbon derivatives required for thymidylate synthesis (5,10-methyleneTHF) or purine synthesis (10-formylTHF). Methotrexate and pralatrexate are inhibitors of dihydrofolate reductase which blocks the regeneration of THF from dihydrofolate (DHF) formed in the synthesis of thymidylate. The tri- to penta- glutamate derivatives of pemetrexed are direct inhibitors of thymidylate synthase

and one of the transformylases that provides a carbon moiety in the formation of the purine ring.

Figure 2.

A model of the transcellular fluxes of thiamine, its phosphorylated metabolites, and folates mediated by the reduced folate carrier (SLC19A1) and the thiamine transporters – SLC19A2 and SLC19A3. At physiological pH, folates/antifolates (FOL^-) have a negative charge while thiamine (T^+) has a positive charge. Thiamine (T^+) influx is mediated by SLC19A2 and SLC19A3 but not by SLC19A1; folates are transported only by SLC19A1. Thiamine is converted to TPP^- (thiamine pyrophosphate) and TMP^- (thiamine monophosphate) within cells; both have a negative charge. TPP^- and TMP^- are substrates for SLC19A1 and their efflux can be inhibited by folates (FOL^-).

Figure 3. The localization of folate transporters in cells and in epithelia

The location of folate-specific and other transporters in epithelial membranes is the basis for vectorial transport of folates and thiamine. SLC19A1 is expressed in all human cells and is the major route of delivery of folates at the neutral pH that encompasses these tissues. SLC46A1 is also widely expressed but is only active at low pH, such as in the microenvironment of the brush border membrane of the proximal small intestine (pH 5.8-6.0). A low pH may be present at other interfaces due to the presence and activity of sodium-hydrogen exchangers (i.e. possibly at the basolateral membrane of ependymal cells of the choroid plexus). ATP-binding cassette transporters are ubiquitously expressed in mammalian cells. They export folate monoglutamates opposing the concentrative activities of the folate-specific transporters (Assaraf et al., 1999). When these exporters are inhibited, net uptake of folates and antifolates is enhanced. ATP-binding cassette transporters located in epithelial membranes are important determinants of vectorial flows of folates. MRP-3, located in the basolateral membrane of the proximal small intestinal, plays an important role in the intestinal absorption of folates (Kitamura et al., 2010). SLC19A2 and SLC19A3 are located at different locations in intestine and renal proximal tubules and are also expressed to varying extents in mammalian tissues. Much less is known about their distribution and tissue function or the impact of ATP-binding cassette transporters on their cellular levels.

Table I

SLC19 – Folate/thiamine transporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transport type / coupling ions *)	Tissue distribution and cellular / subcellular expression	Link to disease	Human gene locus	Sequence accession ID
<i>SLC19A1</i>	RFC	RFC1,RFT	reduced folates and antifolates	Exchanger: organic phosphates	ubiquitous, plasma membrane	methotrexate resistance	21q22.3	NM_194255
<i>SLC19A2</i>	THTR1	ThTr1	thiamine	Facilitated transporter	ubiquitous, plasma membrane	TRMA-thiamine-responsive megaloblastic anemia syndrome	1q23.3	NM_006996
<i>SLC19A3</i>	THTR2	ThTr2	thiamine	Facilitated transporter	ubiquitous, plasma membrane	biotin-responsive basal ganglia disease, Wernicke's-like encephalopathy	2q37	NM_025243

Table 2

SLC46 –Folate transporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transport type / coupling ions *)	Tissue distribution and cellular / subcellular expression	Link to disease	Human gene locus	Sequence accession ID
<u><i>SLC46A1</i></u>	PCFT	HCP1, PCFT/HCP1	folates and antifolates	C:proton-symporter	intestine (apical), choroid plexus (basolateral), kidney - proximal tubule (apical), liver (sinusoidal), placenta	hereditary folate malabsorption	17q11.2	<u>NM_080669.3</u>
<u><i>SLC46A2</i></u>	TSCOT	Ly110	unknown		thymus, kidney		9q32	<u>NM_033051.3</u>
<u><i>SLC46A3</i></u>			unknown				13q12.3	<u>NM_181785.3</u> <u>NM_001135919</u>