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Review Article

L-type amino acid transport and cancer: targeting the mTORC1 pathway to inhibit neoplasia

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Abstract: The L-type amino acid transporter (LAT) family are Na^+-independent transporters, which deliver neutral amino acids into cells. The four LATs, LAT1 (SLC7A5), LAT2 (SLC7A8), LAT3 (SLC43A1) and LAT4 (SLC43A2), are responsible for the majority of cellular leucine uptake. They show increased expression in many cancers, and are critical for control of protein translation and cell growth through the mTORC1 pathway. The increased transporter expression observed in cancers is regulated by transcriptional pathways such as hormone receptors, c-myc and nutrient starvation responses. We review the expression and function of the LAT family in cancer, as well as the recent development of specific inhibitors targeting LAT1 or LAT3. These LAT family inhibitors may be useful adjuvant therapeutics in multiple cancers.

Keywords: L-type amino acid, transport, cancer, mTORC1 pathway

Recent advances in therapeutics designed to target the PI3K/Akt/mTORC1 pathway have resulted in dozens of new anti-cancer compounds currently undergoing Phase I/II trials [1, 2]. This critical cell growth pathway is also regulated by nutrients, in particular the essential amino acid leucine, which is required for activation of the mTORC1/Ragulator complex. Leucine is the most common of the 20 proteinogenic amino acids present in proteins. It is thought that mTORC1 can only begin translation when sufficient levels of leucine, arginine or glutamine are available. The L-type amino acid transporters (LATs) are the major transporters that mediate uptake of leucine into cells, thereby regulating mTORC1 signaling and protein synthesis. This critical requirement for intracellular leucine is reflected in the increased expression of LATs in the majority of cancers, and in the diverse transcription factors that regulate their expression. The classification, structure and function of LAT family have been well reviewed recently [3, 4]. In our review, we provide an overview of recent studies focusing on the role and regulation of the four LAT family members (LAT1, LAT2, LAT3 and LAT4) in cancer. We have analyzed LAT family member expression levels, correlations with disease state and metastasis, and their role in cancer cell growth through the mTORC1 pathway. Furthermore, we discuss targeting of the LAT family as a novel anti-cancer approach and the current state of LAT inhibitors.

L-type amino acid transporter family

The L-type amino acid transporter (LAT) family consists of four Na^+-independent neutral amino acid transporters. The members of this family are grouped in two sub-families, namely, SLC7 (LAT1 and LAT2) and SLC43 (LAT3 and LAT4). Each member of the LAT family is believed to contain 12 transmembrane domains, however there are no current structures solved for any of the human LAT proteins. LAT1 (SLC7A5) and LAT2 (SLC7A8) associate with the 4F2hc (4F2 antigen heavy chain; CD98 heavy chain) glycoprotein, forming a heterodimeric obligatory exchanger with a high affinity [5-9]. LAT3 (SLC43A1) and LAT4 (SLC43A2) are facilitated diffusers of neutral amino acids with a low affinity, and do not appear to require a binding partner [10, 11]. LAT3 and LAT4 deliver a narrow range of neutral amino acids into cells, including leucine, isoleucine, valine, phenylalanine and

Table 1. LAT expression and function

Protein	Gene	Substrates	Expression pattern
LAT1	SLC7A5	Leu, Ile, Phe, Met, Tyr, His, Trp, Val	Brain, spleen, placenta, ovary, testis, colon, blood-brain barrier, fetal liver, activated lymphocytes [5, 6]
LAT2	SLC7A8	Gly, Ala, Ser, Thr, Asn, Gln, Met, Leu, Ile, Val, Phe, Tyr, Trp, His	Jejunum, ileum, kidney, placenta, brain, liver, skeletal muscle, prostate, ovaries, fetal liver, testis and heart [7-9]
LAT3	SLC43A1	Met, Leu, Ile, Val, Phe	Pancreas, skin, muscle, liver, kidney podocytes, prostate [10, 20, 23, 25]
LAT4	SLC43A2	Met, Leu, Ile, Val, Phe	Placenta, kidney, peripheral blood leukocytes [11]

methionine [10, 11]. Similarly, LAT1 and LAT2 can transport these same neutral amino acids, including additional amino acids such as tyrosine, histidine and tryptophan [5, 7-9] (**Table 1**).

In 1998, two independent groups cloned LAT1 from cancer cells [5, 6]. LAT1 has 507 amino acids with a molecular weight of 55 kDa. LAT1 mRNA is strongly expressed in brain, spleen, placenta, testis and colon (**Table 1**) [5]. LAT2 was subsequently cloned due to its homology with LAT1 [7-9]. Human LAT2 and LAT1 shows an amino acid sequence identify of 50%. LAT2 has 535 amino acids with a molecular weight of 58 kDa [7]. LAT2 transcripts are strongly expressed in jejunum, ileum, kidney, placenta, brain, and also detected in liver, skeletal muscle, prostate, ovaries and heart [7-9, 12]. Both LAT1 and LAT2 transport capacity is independent of sodium or chloride. However, binding of 4F2hc at cysteine 163 (LAT1) is required for the normal function and membrane localization of LAT1 and LAT2 [5, 7, 8]. Leucine transport by LAT1 is also dependent on L-glutamine, which is delivered by other amino acid transporters including ASCT2 (SLC1A5) [13-16].

LAT3 transcript was originally cloned from prostate cancer and named prostate cancer overexpressed gene 1 (POV1) [17, 18]. Later, POV1 was identified as a transporter and named LAT3 by expression cloning from the hepatocarcinoma-derived cell line FLC [10]. Mouse LAT3 contains 564 amino acids with a molecular weight of 62.6 kDa [19], while human LAT3 has 559 amino acids. A long intracellular loop predicted to exist between transmembrane domains 6 and 7, contains putative protein kinase C-dependent phosphorylation sites and a tyrosine phosphorylation site [19]. Human LAT4 exhibits 57% identity to human LAT3 [11]. LAT3 and LAT4 have a broad expression pattern in human tissues. Northern blot analysis showed that LAT3 mRNA is expressed in pancreas, liver, skeletal muscle and fetal liver at a high levels [10, 19] (**Table 1**). Human LAT4 mRNA is strongly expressed in placenta, kidney and peripheral blood leukocytes in human tissue. Mouse LAT4 is detected in intestine, kidney, brain, white adipose tissue, testis and heart, but not in liver [11] (**Table 1**). However, the physiologic functions of LAT3/LAT4 in these organs are still not fully understood. Although the expression of LAT3 in kidney is low, strong expression is detected at the apical plasma membrane of podocyte foot processes. LAT3 is important for the development and maintenance of podocyte function and structure [20, 21]. A recent study also showed that red blood cell development requires LAT3 expression for hemoglobin production. In LAT4 knockout mice, newborn mice are smaller than wild type mice [22], suggesting that LAT4 is important for growth and development.

LAT family expression and function in cancer

While the LAT family clearly play important roles in development and function of normal tissues, they are frequently increased in cancer samples. To effectively review this area, and highlight the important role of LAT family members in cancer, we have summarized publications across multiple cancers (**Table 2**). In addition, we have performed new analyses of Oncomine microarray/sequencing datasets to further highlight which cancers show a significant increase in LAT expression compared to normal tissue ($P < 0.05$, Fold change > 2; **Table 2**). These data clearly show that LAT1 is most commonly upregulated in multiple cancers, and accordingly LAT1 has been the most studied of the LAT family members. While the Oncomine data suggest that LAT2 is upregulated in 9 different cancer types, there are few studies that have validated its role in cancer cell growth. LAT3 and LAT4 show a more restricted expression pattern in 5 or 4 different cancer types

Table 2. LAT expression in tumors

	LAT1 (SLC7A5)		LAT2 (SLC7A8)		LAT3 (SLC43A1)		LAT4 (SLC43A2)		4F2hc (SLC3A2)	
Cancer	REF	ONC	REF	ONC	REF	ONC	REF	ONC	REF	ONC
Bladder	[92-94]	2/12								
Brain/CNS	[30, 42]									3/35
Breast	[34, 77, 95-97]	15/52		10/50				1/45	[34]	1/53
Cervical	[98]									
Cholangiocarcinoma	[66]									
Colorectal	[34]	23/34		4/34		2/34	[99]		[34]	6/37
Esophageal	[100]					3/9		1/6		1/9
Gastric	[36]	1/23								
Head and Neck	[34, 42, 99, 101]	7/32		2/32			[99]		[34]	3/32
Kidney		1/24				1/20		1/10		1/24
Leukemia	[87, 102]	4/28		4/27	[103]					2/31
Liver	[102, 104-107]				[108]				[33]	
Lung	[26, 35, 37, 67]	14/31							[37, 67]	
Lymphoma		8/30		1/29				2/21		
Melanoma	[15, 109]	3/7		1/7						1/7
Myeloma	[110-112]	1/8	[110]							
Ovarian	[113, 114]	3/14								
Pancreatic	[32, 115, 116]									
Pleural mesothelioma	[28]									
Prostate	[24, 25, 39]			1/20	[17, 18, 24, 25]	4/20				1/21
Sarcoma	[34, 42]	1/20							[34]	
Tongue cancer	[31]								[31]	
Thymic carcinomas	[29]									
Urinary tract	[92, 117]									
Uterine leiomyoma	[118]		[79]						[79]	
Other (seminoma)						3/3				
Other (skin)		4/4		4/4						1/4
Other (parathyroid)				3/3						

Expression of LAT family members in a variety of cancers was assessed using Pubmed to find published references (REF) and Oncomine (ONC) to detect significant upregulation ($P < 0.05$, fold change > 2) for each transporter. Oncomine numbers represent Datasets with Significant Upregulation/Total Number of Datasets.

respectively, with multiple publications on the critical role of LAT3 in prostate cancer [17, 18, 23-25]. **Table 2** shows the potential utility of targeting the LAT family in a variety of cancers, as well as highlighting a number of cancers that require further analysis of LAT family expression and function.

Immunohistochemical analysis in patient cohorts have shown that LAT1 is overexpressed in cancer and its expression correlates with cell proliferation and cancer progression. LAT1 is highly expressed in 52% of the large cell neuroendocrine carcinomas of the lung [26, 27], 50% of pleural mesothelioma [28], 75% of thymic carcinomas [29], 25% of high-grade gliomas [30], 61% of tongue cancer [31], 53% of pancreatic cancer [32] and 61% in hepatocellular carcinoma [33]. In these studies, a significant correlation was found between LAT1 expression and proliferation marker Ki-67, suggesting that LAT1 is important for proliferation in cancer cells [27, 29, 31-37].

LAT1 has also been used as biomarker for malignant cancer. Using Kaplan-Meier analysis of patients, low LAT1 expression patients showed a significant longer overall survival compared to high LAT1 expression patients, indicating that LAT1 could be a prognostic marker for predicting poor outcome after surgery [32, 33, 38]. In prostate cancer, LAT1 expression is correlated with prognosis in poor survival patients [39]. In breast cancer, LAT1 (SLC7A5) is also part of the 5 gene Mammostrat™ immunohistochemistry panel, where high expression is used to predict recurrence in ER$^+$ breast cancer during endocrine therapy [40, 41].

Studies determining the function of LAT1 in cancer have utilized a well characterized LAT family inhibitor, BCH (2-aminobicyclo-(2,2,1)-

heptane-2-carboxylic acid). BCH can inhibit all members of LAT family at a concentration above 10 mM. BCH treatment decreases leucine transport and suppresses mTORC1 signaling [13, 24]. Expression of cell cycle regulators is altered, such as up-regulation of p21 in glioma cells [30] and p27 in prostate cancer cells [24], down-regulation of CDK1, CDC20 and E2F1 [25]. Therefore, cell proliferation and DNA synthesis are suppressed.

When LAT1 is blocked by BCH, several studies also showed that apoptosis was enhanced in glioma cells [30], oral epidermoid carcinoma cells, osteogenic sarcoma cells [42]. Cleaved caspase 3 and cleaved PARP levels are increased after BCH treatment [30]. However, no apoptosis was observed using either BCH or knockdown LAT1 or LAT3 in prostate cancer cell lines [24]. This suggests that LAT-related cell apoptosis may be dependent on the cell type.

LATs and mTORC1 signaling

Perhaps the most important role of LATs is to transport neutral amino acids for protein synthesis. One of the major LAT substrates, leucine, is not only an essential amino acid, but also a regulator of mTORC1 (mammalian target of rapamycin complex 1) signaling. mTOR is a member of the phosphoinositide-3-kinase related kinase (PIKK) family that possess catalytic activity as a protein serine-threonine kinase. mTOR is present within the cell bound in two major complexes, mTORC1 and mTORC2. mTORC1 is formed by mTOR complexed with mLST8, RAPTOR, PRAS40 and DEPTOR, activating S6 kinase while repressing eIF-4E-binding protein (4E-BP1), thereby regulating protein translation [43, 44]. mTORC2 is formed by mTOR complexed with mLST8, RICTOR and mSin1, which can phosphorylate and activate Akt at Ser473 [45-47].

Both PI3K/Akt signaling and amino acids (especially leucine, arginine and glutamine) are required to activate mTORC1 signaling [13, 44, 48]. While the PI3K/Akt signaling pathway is well understood, the exact mechanism by which amino acids are sensed and activate mTORC1 remains unclear. Activated Akt phosphorylates TSC2 leading to the suppression of TSC2 activity. The inactivated TSC2 dissociates from the surface of lysosomes, releasing Rheb, a small GTPase, to activate mTORC1 on the lysosomal surface [49-53]. Recent studies have suggested that the intracellular level of leucine can be detected by a leucyl-tRNA synthetase (LRS), which can then catalyze the ATP-dependent ligation of L-leucine to leucyl-tRNA during protein synthesis [54, 55]. In the leucine rich environment, LRS with leucine may then interact and activate the Rag GTPase complex. Rag proteins are Ras-related small GTP-binding proteins that include four mammalian members, RagA, RagB, RagC and RagD. They form heterodimers consisting of RagA or RagB with RagC or RagD [56, 57]. Recent work showed that Rag GTPases are only essential for leucine- or arginine-activated mTORC1 signaling [58]. Glutamine-activated mTORC1 activation depends on adenosine diphosphate ribosylation factor-1 GTPase (Arf1) [58]. LRS may also bind to RAPTOR to activate mTORC1 signaling on the surface of lysosome [54, 55]. While the mechanism of leucine sensing remains unclear, low levels of intracellular amino acids lead to Rag heterodimer binding and recruitment of the TSC complex to the lysosome, thereby inhibiting Rheb and mTORC1 signaling [52, 53].

Another study has suggested that glutaminolysis and α-ketoglutarate are involved in glutamine and leucine sensing to activate mTORC1 signaling [48]. The enzyme glutaminase releases the amide group of glutamine to form glutamate. Leucine can directly bind and activate glutamate dehydrogenase, which subsequently converts glutamate to α-ketoglutarate (α-KG) [59]. α-KG is sufficient to stimulate recruitment of mTORC1 to the lysosome by activating RagB. The activated LRS or α-KG stimulates the transition of RagA/RagB GDP-RagC/RagD GTP to RagA/RagB GTP-RagC/RagD GDP [48].

Lysosomal membrane proteins, vacuolar adenosine triphosphatase (v-ATPase) and SLC-36A1, have been shown to interact with Rag GTPases, and may be necessary for mTORC1 activation by amino acids [60]. Lysosomal membrane amino acid transporter SLC38A9 was recently shown to interact with Ragulator/LA-MTOR complex, four RAG GTPases and VA0D1 of the v-ATPase [61, 62]. Purified SLC38A9 directly interacts with arginine making it a potential amino acid sensor for mTORC1 signaling [62]. When arginine binds to SLC38A9 substrate-binding site, SLC38A9 may undergo a

conformational change which affects its interactors, such as v-ATPase, RAG GTPases and Ragulator.

Amino acid-activated signaling is also tightly regulated by proteins which interact with Rag GTPase or Ragulator. For example, folliculin and its interacting partner FNIP1/2 form a complex to activate RagC/D as a GTPase activating protein (GAP) [63]. Sestrins also bind to the heterodimeric Rag complexes and negatively regulate the activity of Rag complexes [64]. GATOR complexes interact with the Rag GTPase complex to negatively regulate leucine signaling. GATOR is composed of two subcomplexes named GATOR1 (DEPDC5, Nprl2 and Nprl3) and GATOR2 (Mios, WDR24, WDR59, Seh1L and Sec13) [65]. GATOR1 has GTPase-activating protein activity for RagA and RagB. GATOR1 components, such as NPRL2 and DEPDC5, contain deletion or mutation in multiple cancer cell lines, leading to hyperactivation of mTORC1 signaling and insensitivity to amino acid deprivation [65].

The role of LAT in metastasis

Several studies have suggested that LAT1 expression also correlates with metastasis. These data are across a range of cancers, including colon, breast, prostate, head and neck, lung, genital as well as soft-tissue sarcomas, all showing that LAT1 expression is significantly higher in the metastatic sites than in the primary sites [24, 27, 34]. LAT1 transport function may be critical in providing nutrients for metastatic cancer cells, as BCH treatment or knockdown of LAT1 expression by shRNA has been shown to decrease cell migration and invasion in cholangiocarcinoma cells *in vitro* [66]. This was also seen in prostate cancer, where LAT1 or LAT3 shRNA significantly inhibited metastasis *in vivo*, however this was confounded by a significant reduction in tumor size [25]. These effects were likely due to the transport function of the LATs, as microarray analysis showed significant downregulation of genes involved in cell cycle regulation, including CDK1, CDC20 and transcription factors E2F1 and E2F2 [25]. These same genes are highly expressed in metastatic prostate cancer, suggesting inhibition of LAT transporters may suppress metastatic prostate cancer proliferation [25].

LAT1 expression closely correlates with 4F2hc expression in human cancers, and has been shown to have a critical role in the metastatic process of diverse human neoplasms [31, 33, 34, 37, 67]. Apart from the transport activity of LAT1, regulation of metastasis may be mediated through integrin signaling, since 4F2hc has been shown to interact with β1-integrin and regulate β1-integrin affinity [68] and expression [69]. It was shown that the amino acid transporter function of LAT1 is not required for this effect of 4F2hc on integrin function. Further studies showed that 4F2hc interacts with the cytoplasmic domain of β1A integrin to reverse the suppression of integrin activation [70, 71]. The 4F2hc transmembrane domain also binds to integrin $\alpha_v\beta_3$ [72], suggesting that perhaps LAT1/4F2hc may be important in interactions with the metastatic niche. It is also possible that 4F2hc binding to integrins allows the cell to use LAT1 to “probe” the environment for nutrients.

Induction of LAT expression

Several factors, such as hormone stimulation, Myc/Rb oncogenic transcription, nutrient starvation and environmental stress have been shown to induce LAT expression, thereby providing the neutral amino acids required for cancer cell growth, survival and progression. The diverse nature of these stimuli highlight the critical requirement for nutrient supply to the cancer cell. For example, in prostate cancer LAT3 expression is driven by androgen receptor (AR) signaling, leading to high expression in primary prostate cancer [24, 25]. This is driven by direct AR transcription, confirmed by chromatin immunoprecipitation (ChIP) and promoter luciferase assays [24]. However, during anti-androgen therapies, LAT3 levels decrease, causing nutrient starvation. The reduction of amino acid levels activate the ATF4 nutrient stress signaling pathway through uncharged tRNAs in the cytoplasm. The general control non-derepressible 2 (GCN2) kinase binds to uncharged tRNAs, leading to phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α) on Serine 51 [73]. Activated eIF2α initiates the rapid translation of ATF4, which translocates to the nucleus, driving an adaptive response that includes transcription of amino acid transporters. The ATF4 knockout mice showed decreased expression of a number of amino acid transporters, with recent ChIP and promoter luciferase assays used to confirm ATF4 binding to amino acid response elements (AAREs) in LAT1,

4F2hc, ASCT2, ASCT1 and xCT [24, 25, 74-76]. Therefore, the ATF4 adaptive response to anti-androgen therapies restores intracellular amino acid levels through transporters including LAT1, allowing further protein translation and cell growth.

Other LAT family members also appear to be regulated by hormone receptors. In breast cancer cells, LAT1 expression is increased in response to estrodial which activates estrogen receptor (ER) [77]. LAT2 has been shown to increase expression in the presence of dihydrotestosterone (DHT), which activates AR [78]. Progesterone also activates ER to induce LAT2 mRNA level increase in primary human uterine leiomyoma smooth muscle (LSM) cells and tissues from premenopausal women [79]. These hormone driven responses are likely important drivers of proliferation during development, and their reactivation during oncogenic transformation is critical for subsequent cancer cell growth.

In addition to nutrient deprivation, oxygen-tension may also contribute to LAT1 expression. HIF2α binds to the SLC7A5 proximal promoter, increasing expression of LAT1 and activating mTORC1 signaling in renal carcinoma cells, as well as in normal liver and lung tissues [80]. These studies indicate that LAT1 is a key environmental sensor to regulate mTORC1 signaling.

The development of a LAT1 knockout mouse has provided further clues to regulation of LAT1 expression. Knockout T cells do not respond to antigen stimulation, thereby preventing T cell clonal expansion or effector cell differentiation [81]. Wild type T cells, however, respond to antigen or PKC activation (phorbol ester) by upregulating expression of LAT1 [81, 82]. Conditional knockdown of LAT1 in activated T cells suppressed c-Myc translation but not transcription. This is an mTORC1 independent process, as rapamycin did not prevent TCR-mediated elevation of c-Myc expression [81]. Since c-Myc has a short half-life (~15 min) [83], sustained expression is required for the maintenance of c-Myc protein. Therefore, LAT1 is critical in sustaining c-Myc levels. c-Myc is also important for metabolic processes including glycolytic switch and regulation of glutaminolysis, as well as for cell proliferation [84].

LAT family inhibitors

Amino acids such as leucine (**Figure 1A**) contain amine and carboxylic acid groups, as well as side chains, which are recognized by substrate binding sites of transporters. Generation of LAT inhibitors has therefore focused primarily on compounds that mimic LAT substrates, and can therefore compete for amino acid binding. However, this strategy has in general resulted in high effective concentrations of inhibitors, as is seen with the leucine analogue BCH (**Figure 1B**; ~10 mM). Furthermore, since the LAT family shares the majority of substrates, BCH targets all members of LAT family, which is undesirable for clinical development. Recently, several new inhibitors were developed to more potently target LAT1 and/or LAT3 (**Figure 1**).

JPH203 (also called KYT-0353; (S)-2-amino-3-(4-((5-amino-2-phenylbenzo[d]oxazol-7-yl)methoxy)-3,5-dichlorophenyl) propanoic acid; **Figure 1C**) is a novel tyrosine analog, which selectively inhibits LAT1 transport activity [85]. JPH203 showed a dramatic inhibition of leucine uptake (IC_{50}=0.06μM) and cell growth (IC_{50}=4.1 μM) in human colon cancer cells (HT-29) [85], human oral cancer cells (YD-38) [86] and leukemic cells [87]. In nude mice, significant inhibitory effects on tumor growth were observed after 7 days treatment with 12.5 mg/kg of this compound [85]. JPH203 suppressed activation of mTORC1 and Akt, decreased expression of c-myc in T-ALL (T-cell acute lymphoblastic leukemia) and T-LL (T-cell lymphoblastic lymphoma) [87]. JPH203 induced ATF4 translation initiation and an unfolded protein response mediated by CHOP (the C/EBP homologous protein), followed by cell death [87]. Importantly, JPH203 had no toxic effect on normal murine thymocytes, lymphocytes, erythrocytes, platelets, bone marrow mature cells, stem cells and early progenitors. Preclinical data from four patients showed no apoptotic effects of JPH203 on normal peripheral blood lymphocytes cells or cord blood mononuclear cells *ex vivo* [87]. Therefore, therapies targeting LAT1 in T-ALL is an attractive strategy that appears to have little side effects in normal cells. However, JPH203 biotransformation via phase II metabolism produces *N*-acetyl-JPH203 (NAc-JPH203), which may accumulate in the liver and kidney, and will need to be considered for future pre-clinical testing [88, 89]. Combined with chemotherapeutic drugs, such as rapamycin, dexametha-

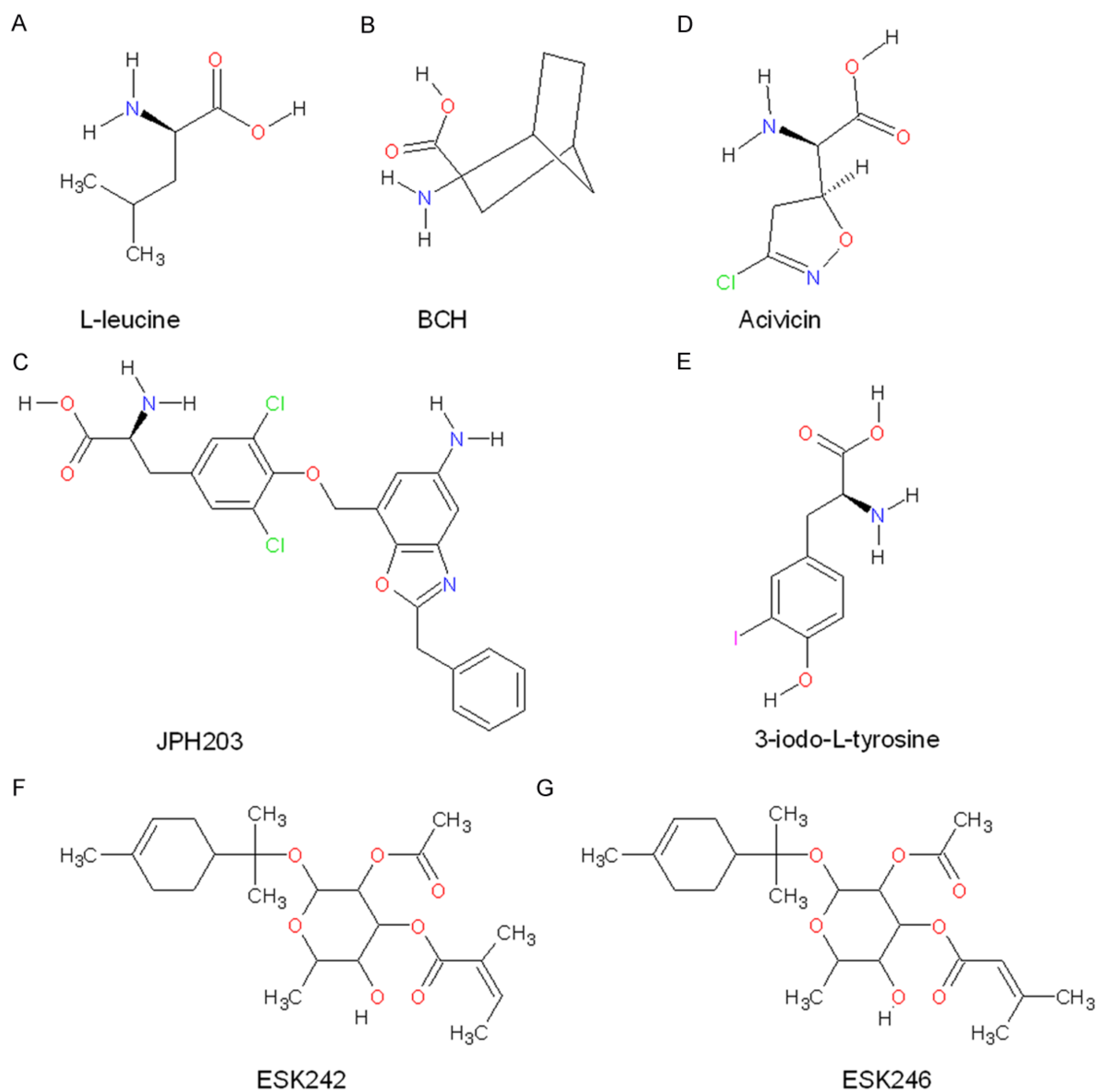

Figure 1. Structure of L-leucine and LAT family inhibitors. A. L-leucine; B. BCH; C. JPH203; D. Acivicin; E. 3-iodo-L-tyrosine; F. ESK242; G. ESK246.

sone, doxorubicin, Velcade and L-asparaginase, JPH203 showed synergistic effects, decreasing cell survival. The highest synergy was observed in combination with rapamycin [87]. Therefore, JPH203 could be an adjuvant therapeutic strategy to treat hematopoietic malignancies. However, the specificity of this compound was only examined for LAT1 and LAT2 in human colon cells HT-29 and mouse renal proximal tubule cells S2. It remains to be determined whether JPH203 can also inhibit LAT3 or LAT4 [85].

Recent structural analysis of membrane proteins have led to a number of publications modelling transporter structures [90]. The LAT1 structure was modelled based on the crystal structure of the arginine/agmatine transporter AdiC from *E. coli* in the outward–facing conformation. Virtual screening was then performed using DOCK3.5.54, to filter compounds from KEGG (Kyoto Encyclopedia of Genes and Genomes) DRUG and KEGG LIGAND COMPOUND database against the LAT1 model. The top-scoring compounds were validated *in vitro*, discovering two novel LAT1 ligands, acivicin (**Figure 1D**) and 3-iodo-L-tyrosine (**Figure 1E**) [90]. The IC_{50} of 3,5-diiodo-L-tyrosine (similar to 3-iodo-L-tyrosine) and acivicin is 7.9 μM and 340 μM, respectively. Both 3-iodo-L-tyrosine

and acivicin were shown to suppress GBM cancer proliferation [90].

Other than these *in silico* screening approaches, conventional high throughput screening strategies have also led to the discovery of novel LAT inhibitors. Using a natural compounds library (Nature Bank), two new monoterpene glycosides ESK242 (**Figure 1F**) and ESK246 (**Figure 1G**) were isolated, which inhibit LATs with a low IC_{50} [91]. These compounds were screened from more than 4500 fractions of biota samples, and specificity was determined using *Xenopus* oocytes expressing LAT1/4F2hc, LAT2/4F2hc, LAT3 or LAT4. ESK242 was found to inhibit LAT1 and LAT3 mediated leucine uptake, while ESK246 preferentially inhibits LAT3. So far, ESK246 is the first reported LAT3 specific inhibitor, which may be used to study the physiological function of LAT3 in the future. Comparison of these new inhibitors with BCH (IC_{50}=4060 μM in LNCaP prostate cancer cells), showed they are ~2 orders of magnitude more effective at inhibiting leucine uptake, with ESK246 and ESK242 having IC_{50} values of 8.1 μM and 29.6 μM respectively. ESK246 was also shown to significantly suppress LNCaP cell proliferation and cell cycle regulator expression at 50 μM [91]. While these compounds do not contain distinct amine and carboxylic acid groups, ESK242 has a side chain similar to isoleucine and ESK246 similar to leucine. Further studies are required to determine if these side chains mediate binding to LAT1/3. These data would assist in the development of more druglike inhibitors in the absence of LAT family structural information.

Conclusion

Over recent years, there has been substantial progress made on both the understanding of LAT family regulation and function in cancer, as well as the development of new inhibitors for this family of transporters. However, despite these advances, analysis of Oncomine data clearly shows that there are many more cancers where LAT family proteins may play an important role. Furthermore, a number of questions remain to be answered: 1) Since LAT1 and ASCT2 cooperate to regulate leucine transport, is it possible to target both transporters to more effectively suppress tumor growth? 2) Are there any proteins (other than 4F2hc) that directly interact with LATs to regulate amino acid transport? 3) Are there post-translational modifications, such as phosphorylation, that can regulate the LAT family? The answer to these questions may provide additional avenues for therapeutic strategies modulating LAT functions. In conclusion, while increased expression of the L-type amino acid transporter family is important for cancer growth and progression, further development of current inhibitors are required in order to reach their full therapeutic potential.

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Disclosure of conflict of interest

No potential conflicts of interest were disclosed.

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References

[1] Kruczek K, Ratterman M, Tolzien K, Sulo S, Lestingi TM and Nabhan C. A phase II study evaluating the toxicity and efficacy of single-agent temsirolimus in chemotherapy-naive castration-resistant prostate cancer. Br J Cancer 2013; 109: 1711-1716.

[2] Templeton AJ, Dutoit V, Cathomas R, Rothermundt C, Bartschi D, Droge C, Gautschi O, Borner M, Fechter E, Stenner F, Winterhalder R, Muller B, Schiess R, Wild PJ, Ruschoff JH, Thalmann G, Dietrich PY, Aebersold R, Klingbiel D, Gillessen S; Swiss Group for Clinical Cancer Research (SAKK). Phase 2 trial of single-agent everolimus in chemotherapy-naive patients with castration-resistant prostate cancer (SAKK 08/08). Eur Urol 2013; 64: 150-158.

[3] Fotiadis D, Kanai Y and Palacin M. The SLC3 and SLC7 families of amino acid transporters. Mol Aspects Med 2013; 34: 139-158.

[4] Bodoy S, Fotiadis D, Stoeger C, Kanai Y and Palacin M. The small SLC43 family: facilitator system l amino acid transporters and the orphan EEG1. Mol Aspects Med 2013; 34: 638-645.
[5] Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E and Endou H. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). J Biol Chem 1998; 273: 23629-23632.
[6] Mastroberardino L, Spindler B, Pfeiffer R, Skelly PJ, Loffing J, Shoemaker CB and Verrey F. Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. Nature 1998; 395: 288-291.
[7] Pineda M, Fernandez E, Torrents D, Estevez R, Lopez C, Camps M, Lloberas J, Zorzano A and Palacin M. Identification of a membrane protein, LAT-2, that co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. J Biol Chem 1999; 274: 19738-19744.
[8] Rossier G, Meier C, Bauch C, Summa V, Sordat B, Verrey F and Kuhn LC. LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. J Biol Chem 1999; 274: 34948-34954.
[9] Segawa H, Fukasawa Y, Miyamoto K, Takeda E, Endou H and Kanai Y. Identification and functional characterization of a Na+-independent neutral amino acid transporter with broad substrate selectivity. J Biol Chem 1999; 274: 19745-19751.
[10] Babu E, Kanai Y, Chairoungdua A, Kim DK, Iribe Y, Tangtrongsup S, Jutabha P, Li Y, Ahmed N, Sakamoto S, Anzai N, Nagamori S and Endou H. Identification of a novel system L amino acid transporter structurally distinct from heterodimeric amino acid transporters. J Biol Chem 2003; 278: 43838-43845.
[11] Bodoy S, Martin L, Zorzano A, Palacin M, Estevez R and Bertran J. Identification of LAT4, a novel amino acid transporter with system L activity. J Biol Chem 2005; 280: 12002-12011.
[12] Park SY, Kim JK, Kim IJ, Choi BK, Jung KY, Lee S, Park KJ, Chairoungdua A, Kanai Y, Endou H and Kim DK. Reabsorption of neutral amino acids mediated by amino acid transporter LAT2 and TAT1 in the basolateral membrane of proximal tubule. Arch Pharm Res 2005; 28: 421-432.
[13] 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 and Murphy LO. Bidirectional transport of amino acids regulates mTOR and autophagy. Cell 2009; 136: 521-534.
[14] Fuchs BC and Bode BP. Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? Semin Cancer Biol 2005; 15: 254-266.
[15] Wang Q, Beaumont KA, Otte NJ, Font J, Bailey CG, van Geldermalsen M, Sharp DM, Tiffen JC, Ryan RM, Jormakka M, Haass NK, Rasko JE and Holst J. Targeting glutamine transport to suppress melanoma cell growth. Int J Cancer 2014; 135: 1060-1071.
[16] Wang Q, Hardie RA, Hoy AJ, van Geldermalsen M, Gao D, Fazli L, Sadowski MC, Balaban S, Schreuder M, Nagarajah R, Wong JJ, Metierre C, Pinello N, Otte NJ, Lehman ML, Gleave M, Nelson CC, Bailey CG, Ritchie W, Rasko JE and Holst J. Targeting ASCT2-mediated glutamine uptake blocks prostate cancer growth and tumour development. J Pathol 2015; [Epub ahead of print].
[17] Chuaqui RF, Englert CR, Strup SE, Vocke CD, Zhuang Z, Duray PH, Bostwick DG, Linehan WM, Liotta LA and Emmert-Buck MR. Identification of a novel transcript up-regulated in a clinically aggressive prostate carcinoma. Urology 1997; 50: 302-307.
[18] Cole KA, Chuaqui RF, Katz K, Pack S, Zhuang Z, Cole CE, Lyne JC, Linehan WM, Liotta LA and Emmert-Buck MR. cDNA sequencing and analysis of POV1 (PB39): a novel gene up-regulated in prostate cancer. Genomics 1998; 51: 282-287.
[19] Fukuhara D, Kanai Y, Chairoungdua A, Babu E, Bessho F, Kawano T, Akimoto Y, Endou H and Yan K. Protein characterization of NA+-independent system L amino acid transporter 3 in mice: a potential role in supply of branched-chain amino acids under nutrient starvation. Am J Pathol 2007; 170: 888-898.
[20] Sekine Y, Nishibori Y, Akimoto Y, Kudo A, Ito N, Fukuhara D, Kurayama R, Higashihara E, Babu E, Kanai Y, Asanuma K, Nagata M, Majumdar A, Tryggvason K and Yan K. Amino acid transporter LAT3 is required for podocyte development and function. J Am Soc Nephrol 2009; 20: 1586-1596.
[21] Chung J, Bauer DE, Ghamari A, Nizzi CP, Deck KM, Kingsley PD, Yien YY, Huston NC, Chen C, Schultz IJ, Dalton AJ, Wittig JG, Palis J, Orkin SH, Lodish HF, Eisenstein RS, Cantor AB and Paw BH. The mTORC1/4E-BP pathway coordinates hemoglobin production with L-leucine availability. Sci Signal 2015; 8: ra34.
[22] Guetg A, Mariotta L, Bock L, Herzog B, Fingerhut R, Camargo SM and Verrey F. Essential amino acid transporter Lat4 (Slc43a2) is required for mouse development. J Physiol 2015; 593: 1273-89.
[23] Pritchard C, Mecham B, Dumpit R, Coleman I, Bhattacharjee M, Chen Q, Sikes RA and Nelson PS. Conserved gene expression programs inte-

grate mammalian prostate development and tumorigenesis. Cancer Res 2009; 69: 1739-1747.
[24] Wang Q, Bailey CG, Ng C, Tiffen J, Thoeng A, Minhas V, Lehman ML, Hendy SC, Buchanan G, Nelson CC, Rasko JE and Holst J. Androgen receptor and nutrient signaling pathways coordinate the demand for increased amino acid transport during prostate cancer progression. Cancer Res 2011; 71: 7525-7536.
[25] Wang Q, Tiffen J, Bailey CG, Lehman ML, Ritchie W, Fazli L, Metierre C, Feng YJ, Li E, Gleave M, Buchanan G, Nelson CC, Rasko JE and Holst J. Targeting amino acid transport in metastatic castration-resistant prostate cancer: effects on cell cycle, cell growth, and tumor development. J Natl Cancer Inst 2013; 105: 1463-1473.
[26] Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Tanaka S, Ishizuka T, Kanai Y, Endou H, Nakajima T and Mori M. Prognostic significance of L-type amino acid transporter 1 expression in resectable stage I-III nonsmall cell lung cancer. Br J Cancer 2008; 98: 742-748.
[27] Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Kawashima O, Iijima H, Ishizuka T, Kanai Y, Endou H, Nakajima T and Mori M. Expression of L-type amino acid transporter 1 (LAT1) in neuroendocrine tumors of the lung. Pathol Res Pract 2008; 204: 553-561.
[28] Kaira K, Oriuchi N, Takahashi T, Nakagawa K, Ohde Y, Okumura T, Murakami H, Shukuya T, Kenmotsu H, Naito T, Kanai Y, Endo M, Kondo H, Nakajima T and Yamamoto N. L-type amino acid transporter 1 (LAT1) expression in malignant pleural mesothelioma. Anticancer Res 2011; 31: 4075-4082.
[29] Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Ishizuka T, Kanai Y, Endou H, Nakajima T and Mori M. L-Type Amino Acid Transporter 1 (LAT1) Is Frequently Expressed in Thymic Carcinomas but Is Absent in Thymomas. J Surg Oncol 2009; 99: 433-438.
[30] Kobayashi K, Ohnishi A, Promsuk J, Shimizu S, Kanai Y, Shiokawa Y and Nagane M. Enhanced tumor growth elicited by L-type amino acid transporter 1 in human malignant glioma cells. Neurosurgery 2008; 62: 493-503; discussion 503-494.
[31] Toyoda M, Kaira K, Ohshima Y, Ishioka NS, Shino M, Sakakura K, Takayasu Y, Takahashi K, Tominaga H, Oriuchi N, Nagamori S, Kanai Y, Oyama T and Chikamatsu K. Prognostic significance of amino-acid transporter expression (LAT1, ASCT2, and xCT) in surgically resected tongue cancer. Br J Cancer 2014; 110: 2506-2513.
[32] Kaira K, Sunose Y, Arakawa K, Ogawa T, Sunaga N, Shimizu K, Tominaga H, Oriuchi N, Itoh H, Nagamori S, Kanai Y, Segawa A, Furuya M, Mori M, Oyama T and Takeyoshi I. Prognostic significance of L-type amino-acid transporter 1 expression in surgically resected pancreatic cancer. Br J Cancer 2012; 107: 632-638.
[33] Namikawa M, Kakizaki S, Kaira K, Tojima H, Yamazaki Y, Horiguchi N, Sato K, Oriuchi N, Tominaga H, Sunose Y, Nagamori S, Kanai Y, Oyama T, Takeyoshi I and Yamada M. Expression of amino acid transporters (LAT1, ASCT2 and xCT) as clinical significance in hepatocellular carcinoma. Hepatol Res 2014; [Epub ahead of print].
[34] Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Tanaka S, Ishizuka T, Kanai Y, Endou H, Nakajima T and Mori M. l-type amino acid transporter 1 and CD98 expression in primary and metastatic sites of human neoplasms. Cancer Sci 2008; 99: 2380-2386.
[35] Imai H, Kaira K, Oriuchi N, Yanagitani N, Sunaga N, Ishizuka T, Kanai Y, Endou H, Nakajima T and Mori M. L-type amino acid transporter 1 expression is a prognostic marker in patients with surgically resected stage I non-small cell lung cancer. Histopathology 2009; 54: 804-813.
[36] Ichinoe M, Mikami T, Yoshida T, Igawa I, Tsuruta T, Nakada N, Anzai N, Suzuki Y, Endou H and Okayasu I. High expression of L-type amino-acid transporter 1 (LAT1) in gastric carcinomas: comparison with non-cancerous lesions. Pathol Int 2011; 61: 281-289.
[37] Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Ishizuka T, Kanai Y, Endou H, Nakajima T and Mori M. Prognostic significance of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (CD98) expression in early stage squamous cell carcinoma of the lung. Cancer Sci 2009; 100: 248-254.
[38] Kaira K, Toyoda M, Shino M, Sakakura K, Takahashi K, Tominaga H, Oriuchi N, Kanai Y, Oyama T and Chikamatsu K. Clinicopathological significance of L-type amino acid transporter 1 (LAT1) expression in patients with adenoid cystic carcinoma. Pathol Oncol Res 2013; 19: 649-656.
[39] Sakata T, Ferdous G, Tsuruta T, Satoh T, Baba S, Muto T, Ueno A, Kanai Y, Endou H and Okayasu I. L-type amino-acid transporter 1 as a novel biomarker for high-grade malignancy in prostate cancer. Pathol Int 2009; 59: 7-18.
[40] Bartlett JMS, Thomas J, Ross DT, Seitz RS, Ring BZ, Beck RA, Pedersen HC, Munro A, Kunkler IH, Campbell FM, Jack W, Kerr GR, Johnstone L, Cameron DA and Chetty U. Mammostrat (R) as a tool to stratify breast cancer patients at

risk of recurrence during endocrine therapy. Breast Cancer Res 2010; 12: R47.
[41] Ring BZ, Seitz RS, Beck R, Shasteen WJ, Tarr SM, Cheang MC, Yoder BJ, Budd GT, Nielsen TO, Hicks DG, Estopinal NC and Ross DT. Novel prognostic immunohistochemical biomarker panel for estrogen receptor-positive breast cancer. J Clin Oncol 2006; 24: 3039-3047.
[42] Kim CS, Cho SH, Chun HS, Lee SY, Endou H, Kanai Y and Kim do K. BCH, an inhibitor of system L amino acid transporters, induces apoptosis in cancer cells. Biol Pharm Bull 2008; 31: 1096-1100.
[43] Chung J, Kuo CJ, Crabtree GR and Blenis J. Rapamycin-FKBP specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell 1992; 69: 1227-1236.
[44] Hara K, Yonezawa K, Kozlowski MT, Sugimoto T, Andrabi K, Weng QP, Kasuga M, Nishimoto I and Avruch J. Regulation of eIF-4E BP1 phosphorylation by mTOR. J Biol Chem 1997; 272: 26457-26463.
[45] Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P and Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol 2004; 14: 1296-1302.
[46] Sarbassov DD, Guertin DA, Ali SM and Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307: 1098-1101.
[47] Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, Huang Q, Qin J and Su B. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 2006; 127: 125-137.
[48] Duran RV, Oppliger W, Robitaille AM, Heiserich L, Skendaj R, Gottlieb E and Hall MN. Glutaminolysis activates Rag-mTORC1 signaling. Mol Cell 2012; 47: 349-358.
[49] Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y and Jiang Y. Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. Science 2007; 318: 977-980.
[50] Tee AR, Manning BD, Roux PP, Cantley LC and Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol 2003; 13: 1259-1268.
[51] Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA and Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat Cell Biol 2003; 5: 578-581.
[52] Demetriades C, Doumpas N and Teleman AA. Regulation of TORC1 in response to amino acid starvation via lysosomal recruitment of TSC2. Cell 2014; 156: 786-799.
[53] Menon S, Dibble CC, Talbott G, Hoxhaj G, Valvezan AJ, Takahashi H, Cantley LC and Manning BD. Spatial control of the TSC complex integrates insulin and nutrient regulation of mTORC1 at the lysosome. Cell 2014; 156: 771-785.
[54] Han JM, Jeong SJ, Park MC, Kim G, Kwon NH, Kim HK, Ha SH, Ryu SH and Kim S. Leucyl-tRNA synthetase is an intracellular leucine sensor for the mTORC1-signaling pathway. Cell 2012; 149: 410-424.
[55] Bonfils G, Jaquenoud M, Bontron S, Ostrowicz C, Ungermann C and De Virgilio C. Leucyl-tRNA synthetase controls TORC1 via the EGO complex. Mol Cell 2012; 46: 105-110.
[56] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L and Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science 2008; 320: 1496-1501.
[57] Kim E, Goraksha-Hicks P, Li L, Neufeld TP and Guan KL. Regulation of TORC1 by Rag GTPases in nutrient response. Nat Cell Biol 2008; 10: 935-945.
[58] Jewell JL, Kim YC, Russell RC, Yu FX, Park HW, Plouffe SW, Tagliabracci VS and Guan KL. Differential regulation of mTORC1 by leucine and glutamine. Science 2015; 347: 194-198.
[59] Sener A and Malaisse WJ. L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. Nature 1980; 288: 187-189.
[60] Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y and Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. Science 2011; 334: 678-683.
[61] Rebsamen M, Pochini L, Stasyk T, de Araujo ME, Galluccio M, Kandasamy RK, Snijder B, Fauster A, Rudashevskaya EL, Bruckner M, Scorzoni S, Filipek PA, Huber KV, Bigenzahn JW, Heinz LX, Kraft C, Bennett KL, Indiveri C, Huber LA and Superti-Furga G. SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. Nature 2015; 519: 477-81.
[62] Wang S, Tsun ZY, Wolfson RL, Shen K, Wyant GA, Plovanich ME, Yuan ED, Jones TD, Chantranupong L, Comb W, Wang T, Bar-Peled L, Zoncu R, Straub C, Kim C, Park J, Sabatini BL and Sabatini DM. Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. Science 2015; 347: 188-194.
[63] Tsun ZY, Bar-Peled L, Chantranupong L, Zoncu R, Wang T, Kim C, Spooner E and Sabatini DM. The Folliculin Tumor Suppressor Is a GAP for the RagC/D GTPases That Signal Amino Acid

Levels to mTORC1. Molecular Cell 2013; 52: 495-505.
[64] Peng M, Yin N and Li MO. Sestrins Function as Guanine Nucleotide Dissociation Inhibitors for Rag GTPases to Control mTORC1 Signaling. Cell 2014; 159: 122-133.
[65] Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M and Sabatini DM. A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 2013; 340: 1100-1106.
[66] Janpipatkul K, Suksen K, Borwornpinyo S, Jearawiriyapaisarn N, Hongeng S, Piyachaturawat P and Chairoungdua A. Downregulation of LAT1 expression suppresses cholangiocarcinoma cell invasion and migration. Cell Signal 2014; 26: 1668-1679.
[67] Kaira K, Oriuchi N, Imai H, Shimizu K, Yanagitani N, Sunaga N, Hisada T, Kawashima O, Kamide Y, Ishizuka T, Kanai Y, Nakajima T and Mori M. Prognostic significance of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (CD98) expression in surgically resectable stage III non-small cell lung cancer. Exp Ther Med 2010; 1: 799-808.
[68] Fenczik CA, Sethi T, Ramos JW, Hughes PE and Ginsberg MH. Complementation of dominant suppression implicates CD98 in integrin activation. Nature 1997; 390: 81-85.
[69] Kim SM and Hahn JH. CD98 activation increases surface expression and clusteringof beta1 integrins in MCF-7 cells through FAK/Src- and cytoskeleton-independent mechanisms. Exp Mol Med 2008; 40: 261-270.
[70] Zent R, Fenczik CA, Calderwood DA, Liu S, Dellos M and Ginsberg MH. Class- and splice variant-specific association of CD98 with integrin beta cytoplasmic domains. J Biol Chem 2000; 275: 5059-5064.
[71] Kolesnikova TV, Mannion BA, Berditchevski F and Hemler ME. Beta1 integrins show specific association with CD98 protein in low density membranes. BMC Biochem 2001; 2: 10.
[72] Prager GW, Feral CC, Kim C, Han J and Ginsberg MH. CD98hc (SLC3A2) interaction with the integrin beta subunit cytoplasmic domain mediates adhesive signaling. J Biol Chem 2007; 282: 24477-24484.
[73] Dever TE, Feng L, Wek RC, Cigan AM, Donahue TF and Hinnebusch AG. Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast. Cell 1992; 68: 585-596.
[74] Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM and Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 2003; 11: 619-633.
[75] Ren P, Yue M, Xiao D, Xiu R, Gan L, Liu H and Qing G. ATF4 and N-Myc coordinate glutamine metabolism in MYCN-amplified neuroblastoma cells through ASCT2 activation. J Pathol 2015 ; 235: 90-100.
[76] Sato H, Nomura S, Maebara K, Sato K, Tamba M and Bannai S. Transcriptional control of cystine/glutamate transporter gene by amino acid deprivation. Biochem Biophys Res Commun 2004; 325: 109-116.
[77] Shennan DB, Thomson J, Gow IF, Travers MT and Barber MC. L-leucine transport in human breast cancer cells (MCF-7 and MDA-MB-231): kinetics, regulation by estrogen and molecular identity of the transporter. Biochim Biophys Acta 2004; 1664: 206-16.
[78] Hamdi MM and Mutungi G. Dihydrotestosterone stimulates amino acid uptake and the expression of LAT2 in mouse skeletal muscle fibres through an ERK1/2-dependent mechanism. J Physiol 2011; 589: 3623-3640.
[79] Luo X, Yin P, Reierstad S, Ishikawa H, Lin Z, Pavone ME, Zhao H, Marsh EE and Bulun SE. Progesterone and mifepristone regulate L-type amino acid transporter 2 and 4F2 heavy chain expression in uterine leiomyoma cells. J Clin Endocrinol Metab 2009; 94: 4533-4539.
[80] Elorza A, Soro-Arnáiz I, Meléndez-Rodríguez F, Rodríguez-Vaello V, Marsboom G, de Cárcer G, Acosta-Iborra B, Albacete-Albacete L, Ordóñez A, Serrano-Oviedo L, Giménez-Bachs J, Vara-Vega A, Salinas A, Sánchez-Prieto R, Martín Del Río R, Sánchez-Madrid F, Malumbres M, Landázuri M and Aragonés J. HIF2α Acts as an mTORC1 Activator through the Amino Acid Carrier SLC7A5. Mol Cell 2012; 48: 681-91.
[81] Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM and Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. Nat Immunol 2013; 14: 500-508.
[82] Nii T, Segawa H, Taketani Y, Tani Y, Ohkido M, Kishida S, Ito M, Endou H, Kanai Y, Takeda E and Miyamoto K. Molecular events involved in up-regulating human Na+-independent neutral amino acid transporter LAT1 during T-cell activation. Biochem J 2001; 358: 693-704.
[83] Hann SR and Eisenman RN. Proteins encoded by the human c-myc oncogene: differential expression in neoplastic cells. Mol Cell Biol 1984; 4: 2486-2497.
[84] Dang CV. MYC on the path to cancer. Cell 2012; 149: 22-35.
[85] Oda K, Hosoda N, Endo H, Saito K, Tsujihara K, Yamamura M, Sakata T, Anzai N, Wempe MF, Kanai Y and Endou H. L-type amino acid transporter 1 inhibitors inhibit tumor cell growth. Cancer Sci 2010; 101: 173-179.

[86] Yun DW, Lee SA, Park MG, Kim JS, Yu SK, Park MR, Kim SG, Oh JS, Kim CS, Kim HJ, Kim JS, Chun HS, Kanai Y, Endou H, Wempe MF and Kim do K. JPH203, an L-type amino acid transporter 1-selective compound, induces apoptosis of YD-38 human oral cancer cells. J Pharmacol Sci 2014; 124: 208-217.

[87] Rosilio C, Nebout M, Imbert V, Griessinger E, Neffati Z, Benadiba J, Hagenbeek T, Spits H, Reverso J, Ambrosetti D, Michiels JF, Bailly-Maitre B, Endou H, Wempe MF and Peyron JF. L-type amino acid transporter 1 (LAT1): a therapeutic target supporting growth and survival of t-cell lymphoblastic lymphoma/t-cell acute lymphoblastic leukemia. Leukemia 2014; [Epub ahead of print].

[88] Wempe MF, Rice PJ, Lightner JW, Jutabha P, Hayashi M, Anzai N, Wakui S, Kusuhara H, Sugiyama Y and Endou H. Metabolism and pharmacokinetic studies of JPH203, an L-amino acid transporter 1 (LAT1) selective compound. Drug Metab Pharmacokinet 2012; 27: 155-161.

[89] Toyoshima J, Kusuhara H, Wempe MF, Endou H and Sugiyama Y. Investigation of the role of transporters on the hepatic elimination of an LAT1 selective inhibitor JPH203. J Pharm Sci. 2013; 102: 3228-38.

[90] Geier EG, Schlessinger A, Fan H, Gable JE, Irwin JJ, Sali A and Giacomini KM. Structure-based ligand discovery for the Large-neutral Amino Acid Transporter 1, LAT-1. Proc Natl Acad Sci U S A 2013; 110: 5480-5485.

[91] Wang Q, Grkovic T, Font J, Bonham S, Pouwer RH, Bailey CG, Moran AM, Ryan RM, Rasko JE, Jormakka M, Quinn RJ and Holst J. Monoterpene glycoside ESK246 from Pittosporum targets LAT3 amino acid transport and prostate cancer cell growth. ACS Chem Biol 2014; 9: 1369-1376.

[92] Eltz S, Comperat E, Cussenot O and Roupret M. Molecular and histological markers in urothelial carcinomas of the upper urinary tract. BJU Int 2008; 102: 532-535.

[93] Kim DK, Kanai Y, Choi HW, Tangtrongsup S, Chairoungdua A, Babu E, Tachampa K, Anzai N, Iribe Y and Endou H. Characterization of the system L amino acid transporter in T24 human bladder carcinoma cells. Biochim Biophys Acta 2002; 1565: 112-121.

[94] Baniasadi S, Chairoungdua A, Iribe Y, Kanai Y, Endou H, Aisaki K, Igarashi K and Kanno J. Gene expression profiles in T24 human bladder carcinoma cells by inhibiting an L-type amino acid transporter, LAT1. Arch Pharm Res 2007; 30: 444-452.

[95] Furuya M, Horiguchi J, Nakajima H, Kanai Y and Oyama T. Correlation of L-type amino acid transporter 1 and CD98 expression with triple negative breast cancer prognosis. Cancer Sci 2012; 103: 382-389.

[96] Shennan DB and Thomson J. Inhibition of system L (LAT1/CD98hc) reduces the growth of cultured human breast cancer cells. Oncol Rep 2008; 20: 885-889.

[97] Shennan DB, Thomson J, Barber MC and Travers MT. Functional and molecular characteristics of system L in human breast cancer cells. Biochim Biophys Acta 2003; 1611: 81-90.

[98] Uno K, Kuwabara H, Terado Y, Kojima K, Kawakami T, Kamma H, Sakurai H, Sakamoto A and Kurata A. Divergent expression of L-type amino acid transporter 1 during uterine cervical carcinogenesis. Hum Pathol 2011; 42: 1660-1666.

[99] Haase C, Bergmann R, Fuechtner F, Hoepping A and Pietzsch J. L-type amino acid transporters LAT1 and LAT4 in cancer: uptake of 3-O-methyl-6-18F-fluoro-L-dopa in human adenocarcinoma and squamous cell carcinoma in vitro and in vivo. J Nucl Med 2007; 48: 2063-2071.

[100] Kobayashi H, Ishii Y and Takayama T. Expression of L-type amino acid transporter 1 (LAT1) in esophageal carcinoma. J Surg Oncol 2005; 90: 233-238.

[101] Yamauchi K, Sakurai H, Kimura T, Wiriyasermkul P, Nagamori S, Kanai Y and Kohno N. System L amino acid transporter inhibitor enhances anti-tumor activity of cisplatin in a head and neck squamous cell carcinoma cell line. Cancer Lett 2009; 276: 95-101.

[102] Yanagida O, Kanai Y, Chairoungdua A, Kim DK, Segawa H, Nii T, Cha SH, Matsuo H, Fukushima J, Fukasawa Y, Tani Y, Taketani Y, Uchino H, Kim JY, Inatomi J, Okayasu I, Miyamoto K, Takeda E, Goya T and Endou H. Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim Biophys Acta 2001; 1514: 291-302.

[103] Xu SM, Tang K, Meng L and Tang Y. Suppression of amino acid transporter LAT3 expression on proliferation of K562 cells. J Huazhong Univ Sci Technolog Med Sci 2013; 33: 632-635.

[104] Ohkame H, Masuda H, Ishii Y and Kanai Y. Expression of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (4F2hc) in liver tumor lesions of rat models. J Surg Oncol 2001; 78: 265-271; discussion 271-262.

[105] Tamai S, Masuda H, Ishii Y, Suzuki S, Kanai Y and Endou H. Expression of L-type amino acid transporter 1 in a rat model of liver metastasis: positive correlation with tumor size. Cancer Detect Prev 2001; 25: 439-445.

[106] Storey BT, Fugere C, Lesieur-Brooks A, Vaslet C and Thompson NL. Adenoviral modulation of the tumor-associated system L amino acid

transporter, LAT1, alters amino acid transport, cell growth and 4F2/CD98 expressionwith cell-type specific effects in cultured hepatic cells. Int J Cancer 2005; 117: 387-397.
[107] Kondoh N, Imazeki N, Arai M, Hada A, Hatsuse K, Matsuo H, Matsubara O, Ohkura S and Yamamoto M. Activation of a system A amino acid transporter, ATA1/SLC38A1, in human hepatocellular carcinoma and preneoplastic liver tissues. Int J Oncol 2007; 31: 81-87.
[108] Ritchie JW and Taylor PM. Tryptophan and iodothyronine transport interactions in HepG2 human hepatoma cells. Amino Acids 2010; 38: 1361-1367.
[109] Ochiai H, Morishita T, Onda K, Sugiyama H and Maruo T. Canine Lat1: molecular structure, distribution and its expression in cancer samples. J Vet Med Sci 2012; 74: 917-922.
[110] Kuhne A, Kaiser R, Schirmer M, Heider U, Muhlke S, Niere W, Overbeck T, Hohloch K, Trumper L, Sezer O and Brockmoller J. Genetic polymorphisms in the amino acid transporters LAT1 and LAT2 in relation to the pharmacokinetics and side effects of melphalan. Pharmacogenet Genomics 2007; 17: 505-517.
[111] Kuhne A, Tzvetkov MV, Hagos Y, Lage H, Burckhardt G and Brockmoller J. Influx and efflux transport as determinants of melphalan cytotoxicity: Resistance to melphalan in MDR1 overexpressing tumor cell lines. Biochemical Pharmacology 2009; 78: 45-53.
[112] Isoda A, Kaira K, Iwashina M, Oriuchi N, Tominaga H, Nagamori S, Kanai Y, Oyama T, Asao T, Matsumoto M and Sawamura M. Expression of L-type amino acid transporter 1 (LAT1) as a prognostic and therapeutic indicator in multiple myeloma. Cancer Sci 2014; 105: 1496-1502.
[113] Fan X, Ross DD, Arakawa H, Ganapathy V, Tamai I and Nakanishi T. Impact of system L amino acid transporter 1 (LAT1) on proliferation of human ovarian cancer cells: a possible target for combination therapy with anti-proliferative aminopeptidase inhibitors. Biochem Pharmacol 2010; 80: 811-818.
[114] Kaji M, Kabir-Salmani M, Anzai N, Jin CJ, Akimoto Y, Horita A, Sakamoto A, Kanai Y, Sakurai H and Iwashita M. Properties of L-type amino acid transporter 1 in epidermal ovarian cancer. Int J Gynecol Cancer 2010; 20: 329-336.
[115] Yanagisawa N, Ichinoe M, Mikami T, Nakada N, Hana K, Koizumi W, Endou H and Okayasu I. High expression of L-type amino acid transporter 1 (LAT1) predicts poor prognosis in pancreatic ductal adenocarcinomas. J Clin Pathol 2012; 65: 1019-1023.
[116] Hayashi K, Jutabha P, Endou H and Anzai N. c-Myc is crucial for the expression of LAT1 in MIA Paca-2 human pancreatic cancer cells. Oncology Reports 2012; 28: 862-866.
[117] Nakanishi K, Ogata S, Matsuo H, Kanai Y, Endou H, Hiroi S, Tominaga S, Aida S, Kasamatsu H and Kawai T. Expression of LAT1 predicts risk of progression of transitional cell carcinoma of the upper urinary tract. Virchows Arch 2007; 451: 681-690.
[118] Luo X, Coon VJS, Su E, Pearson EK, Yin P, Ishikawa H and Bulun SE. LAT1 Regulates Growth of Uterine Leiomyoma Smooth Muscle Cells. Reprod Sci 2010; 17: 791-797.