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# Specific transport of 3-fluoro-L-α-methyl-tyrosine by LAT1 explains its specificity to malignant tumors in imaging

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**Key words**

Amino acid transporter, biomarker, molecular probe, PET, tumor imaging

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**3-$^{18}$F-L-α-methyl-tyrosine ([$^{18}$F]FAMT), a PET probe for tumor imaging, has advantages of high cancer-specificity and lower physiologic background. FAMT-PET has been proved useful in clinical studies for the prediction of prognosis, the assessment of therapy response and the differentiation of malignant tumors from inflammation and benign lesions. The tumor uptake of [$^{18}$F]FAMT in PET is strongly correlated with the expression of L-type amino acid transporter 1 (LAT1), an isoform of system L upregulated in cancers. In this study, to assess the transporter-mediated mechanisms in FAMT uptake by tumors, we examined amino acid transporters for FAMT transport. We synthesized [$^{14}$C]FAMT and measured its transport by human amino acid transporters expressed in *Xenopus* oocytes. The transport of FAMT was compared with that of L-methionine, a well-studied amino acid PET probe. The significance of LAT1 in FAMT uptake by tumor cells was confirmed by siRNA knockdown. Among amino acid transporters, [$^{14}$C]FAMT was specifically transported by LAT1, whereas L-[$^{14}$C]methionine was taken up by most of the transporters. $K_m$ of LAT1-mediated [$^{14}$C]FAMT transport was 72.7 μM, similar to that for endogenous substrates. Knockdown of LAT1 resulted in the marked reduction of [$^{14}$C]FAMT transport in HeLa S3 cells, confirming the contribution of LAT1 in FAMT uptake by tumor cells. FAMT is highly specific to cancer-type amino acid transporter LAT1, which explains the cancer-specific accumulation of [$^{18}$F]FAMT in PET. This, *vice versa*, further supports the cancer-specific expression of LAT1. This study has established FAMT as a LAT1-specific molecular probe to monitor the expression of a potential tumor biomarker LAT1.**

The most commonly used positron emission tomography (PET) probe for tumor imaging is 2-$^{18}$F-fluoro-2-deoxy-D-glucose ([$^{18}$F]FDG), a glucose analog taken up by tumor cells via glucose transporters.[1] Although [$^{18}$F]FDG PET has been successfully used for the diagnosis and staging of malignant tumors, it has been recognized that [$^{18}$F]FDG is accumulated in inflammatory tissues, benign lesions and some normal tissues such as brain, causing false-positives and physiologic backgrounds.[2] To overcome such disadvantages of [$^{18}$F]FDG PET, various chemical compounds including amino acid derivatives that generally exhibit more tumor-selective properties have been developed as candidates for tumor-specific PET tracers. For example, L-[$^{11}$C-methyl]methionine ([$^{11}$C]MET) and *O*-(2-[$^{18}$F]fluoroethyl)-L-tyrosine ([$^{18}$F]FET) (Fig. S1) are, compared with [$^{18}$F]FDG, less accumulated in brain and inflammatory tissues.[3] However, [$^{11}$C]MET and [$^{18}$F]FET, as well as most of the other amino acid tracers, still suffer from some false-positives and physiologic backgrounds in PET.[4,5]

An amino acid tracer for single photon emission computed tomography (SPECT), 3-$^{123}$I-L-α-methyltyrosine ([$^{123}$I]IMT) (Fig. S1), is known to accumulate in malignant tumors with less false positives and low brain-background.[3] By substituting $^{123}$I of [$^{123}$I]IMT with $^{18}$F, 3-$^{18}$F-L-α-methyltyrosine ([$^{18}$F]FAMT) (Fig. S1) was developed as a PET tracer for tumor imaging.[6] In clinical studies, we and others have shown that [$^{18}$F]FAMT specifically accumulates in tumors and is useful for the prediction of prognosis, the assessment of therapy response and the differentiation of malignant tumors from inflammation and benign lesions.[7–14] It has, therefore, been speculated that [$^{18}$F]FAMT is taken up by cancer cells via amino acid transporters specifically expressed in cancer cells. The tumor uptake of [$^{18}$F]FAMT in PET is, in fact, well correlated with the level of expression of L-type amino acid transporter 1 (LAT1, SLC7A5) in various tumors, including non-small cell lung cancer, oral squamous cell carcinoma and esophageal cancer.[9,10,12,14]

LAT1 is one of the isoforms of system L that transports large neutral amino acids in a $Na^+$-independent manner.[15,16] LAT1 is predominantly expressed in a wide range of tumor cell lines, primary human tumors of various tissue origins, such as brain, lung, pancreas, breast, prostate, oral cavity, esophagus, stomach, liver, biliary tract, ovary, skin and bone, and their metastatic legions, where the expression of LAT1 correlates with tumor cell proliferation, angiogenesis and poor prognosis.[12,14,15,17–24] Because of such clinico-pathological

significance, LAT1 has been regarded as a candidate tumor biomarker.[24] Previously, using nonradiolabeled FAMT, we examined the interaction of FAMT with LAT1 in comparison with the other system L transporter LAT2 expressed in normal tissues[16] and suggested that FAMT is interacted with LAT1 but less so with LAT2.[25] We showed that α-methyl moiety of FAMT is responsible for its preference for LAT1 to LAT2.[25] In the present study, to reveal the transporter-mediated mechanisms of tumor-specific uptake of [$^{18}$F]FAMT and to establish FAMT as a molecular probe to monitor the expression of a potential tumor biomarker LAT1, we have synthesized $^{14}$C-labled FAMT, which is easier to handle in *in vitro* studies, and used it to obtain direct evidence of FAMT transport by LAT1 and further to examine whether FAMT is transported by the other amino acid transporters.

## Materials and Methods

**Chemicals.** For the synthesis of 3-fluoro-L-α-methyl [carboxyl-$^{14}$C]tyrosine ([$^{14}$C]FAMT), 3-fluoro-4-methoxyphenylacetone as a starting material was purchased from NARD Institute (Amagasaki, Japan). [$^{14}$C]FAMT was synthesized by Sekisui Medical (Tokyo, Japan) to obtain high specific radioactivity by Bücherer–Strecker reaction.[26] [$^{14}$C]FAMT was identified by the analysis of $^{1}$H-nuclear magnetic resonance (AV400M; Bruker Biospin, Rheinstetten, Germany), high performance liquid chromatograph (Agilent 1200) and mass spectrum (LTQXL; Thermo Fisher Scientific, Waltham, MA, USA).[25] The purity of the [$^{14}$C]FAMT determined on high-performance liquid chromatography was 99% and its specific radioactivity was 1.77 GBq/mmol.

L-[$^{14}$C]Leucine and L-[$^{14}$C]Alanine were purchased from Moravek Biochemicals (Brea, CA, USA). L-[$^{14}$C]Methionine and L-[$^{14}$C]Cystine were from American Radiolabeled Chemicals (St. Louis, MO, USA). L-[$^{14}$C]Glutamine (10.1 GBq/mmol) and L-[$^{14}$C]Tyrosine were from PerkinElmer (Boston, MA, USA) and Amersham Biosciences (Buckinghamshire, UK), respectively.

Non-radiolabeled FAMT was purchased from NARD Institute (Amagasaki, Japan).[25] Amino acids and 2-amino-2-norbornanecarboxylic acid (BCH) were from Sigma-Aldrich (St. Louis, MO, USA). Other general chemicals were from Wako (Osaka, Japan).

**Expression in *Xenopus laevis* oocytes and transport measurements.** cDNA for human transporters used in this study are presented in Table S1. cRNA were synthesized *in vitro* from the linearized plasmids using an mMessage mMachine Kit, polyadenylated with a Poly(A) Tailing Kit and purified with a MEGAclear Kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol.

For *Xenopus* oocyte expression, defolliculated oocytes were injected with polyadenylated cRNA (25 ng/oocyte).[15] For functional expression of LAT1, LAT2, $y^+$LAT1 and $y^+$LAT2, equimolar 4F2hc cRNA was co-injected.[15–17] For $B^0$AT1, equimolar collectrin cRNA was co-injected.[27]

Transport measurements were performed 2–4 days after injection as previously described.[15,17] In brief, the oocytes were incubated at room temperature with 500 μL uptake buffer containing $^{14}$C-labeled compounds. ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5) was used as the uptake buffer for $Na^+$-dependent transport. For $Na^+$-free uptake buffer, NaCl was replaced by choline-Cl. The radioactivity was determined by liquid scintillation counting. Functional expression of each transporter in *Xenopus* oocytes was confirmed by measuring the transport of its typical substrate as described in the legends to figures. To determine the concentration dependence of transport, the transport rate at each concentration was obtained by subtracting the uptake rate of control oocytes without cRNA injection from that of the oocytes expressing LAT1. The Michaelis constant ($K_m$) and maximal transport rate ($V_{max}$) were determined by plotting the transport rate against FAMT concentration and fitting to a Michaelis–Menten curve using enzyme kinetics module of Sigma Plot 12.5 (Systat Software, San Jose, CA, USA).

For transport measurements, 5–12 oocytes were used for each measurement. To confirm the reproducibility of the results, three separate experiments using different batches of oocytes were performed. Statistical differences were determined using Student's unpaired *t*-test. Differences were considered significant at $P < 0.05$.

**siRNA knockdown of LAT1.** Non-targeting control siRNA#1 (D-001810-01-05) and #2 (D-001810-02-05) were purchased from Thermo Fisher Scientific. LAT1 siRNA#1 (s15653), #2 (s15654) and #3 (s15655) were from Ambion. HeLa S3 cells were seeded in six-well plates at a density of $2 \times 10^5$ cells/well and transfected with 24 nM of siRNA using RNAiMax (Invitrogen, Carlsbad, CA, USA). Two days after transfection, cells were reseeded in a 24-well plate at $5 \times 10^4$ cells/well and in a 6-cm dish at $4 \times 10^5$ cells/dish for [$^{14}$C]FAMT transport measurement and western blot analysis, respectively.

**Transport measurement in HeLa S3 cells.** Transport measurement was carried out as described previously,[25] 2 days after reseeding of HeLa S3 cells treated or not treated with siRNA. Briefly, cells were incubated with 300 μL of Hanks balanced salt solution containing 1 μM [$^{14}$C]FAMT or L-[$^{14}$C]methionine for 1 min at 37°C. Radioactivity was measured by liquid scintillation counting.

**Western blot.** Two days after reseeding of HeLa S3 cells treated or not treated with siRNA, the cells were lysed in buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (pH 8.0), 1 mM phenylmethanesulfonyl fluoride, 1% NP40 and protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). The western blot was performed as described previously.[25] Antihuman LAT1 polyclonal antibody[17] and antihuman β-actin monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA) were used as primary antibodies at 1:5000 dilutions.

## Results

**[$^{14}$C]FAMT is transported only by LAT1 among amino acid transporters.** To determine the amino acid transporters responsible for FAMT uptake, we examined amino acid transport systems transporting aromatic amino acids such as systems L (LAT1~4), T (TAT1), $B^0$ ($B^0$AT1), $B^{0,+}$ ($ATB^{0,+}$), $b^{0,+}$ and $y^+$L ($y^+$LAT1, $y^+$LAT2) (Table S1).[27,28] In addition, we tested systems ASC (ASCT1, ASCT2) and N/A (SNAT1~5) mainly transporting small neutral amino acids to cover most neutral amino acid transporters (Table S1).[27,28] The transport of [$^{14}$C]FAMT by each transporter functionally expressed in *Xenopus* oocytes was measured and compared with that of L-[$^{14}$C]methionine.

Among the transporters from system L for large neutral amino acids, LAT1-expressing oocytes exhibited a high level of [$^{14}$C]FAMT uptake compared with control oocytes (Fig. 1a). Its transport rate was comparable to that of L-[$^{14}$C]leucine, a typical substrate of LAT1. LAT2, LAT3 and LAT4

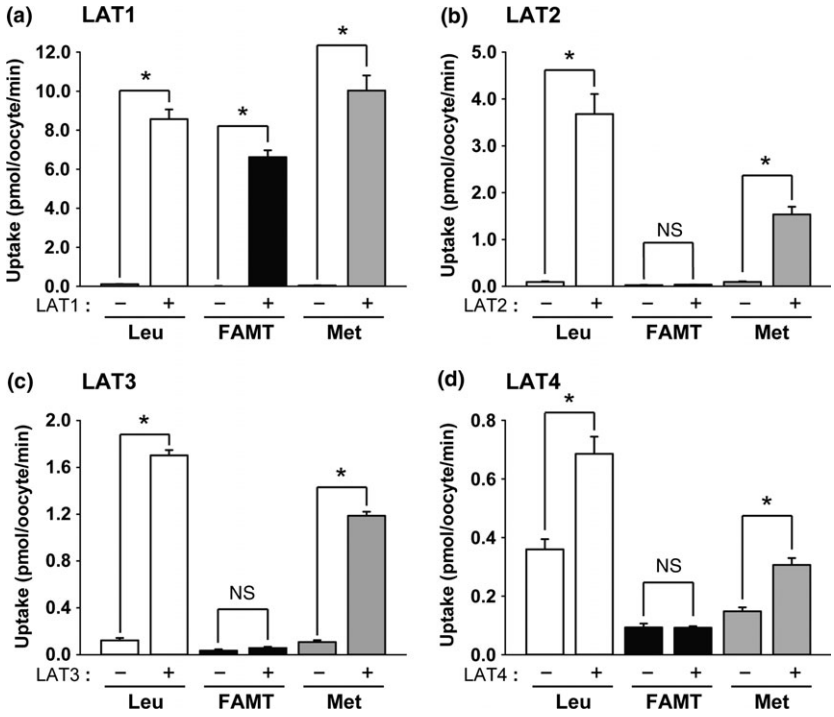


**Fig. 1.** [$^{14}$C]FAMT transport by system L transporters. The uptakes of 50 μM [$^{14}$C]FAMT (FAMT) and L-[$^{14}$C]methionine (Met) as well as a typical substrate L-[$^{14}$C]leucine (Leu) (50 μM) were measured for 15 min for LAT1 (a), LAT2 (b) and LAT3 (c), and for 30 min for LAT4 (d). The uptakes were measured in $Na^+$-free uptake buffer on the control oocytes ("−") and the oocytes expressing each transporter ("+"). The uptake rates were expressed as mean ± SEM ($n$ = 8–12). *$P$ < 0.05; n.s., not significant.

did not mediate [$^{14}$C]FAMT transport (Fig. 1b–d). In contrast, L-[$^{14}$C]methionine was transported by all the system L transporters (Fig. 1). Other amino acid transporters transporting aromatic amino acids, TAT1, $B^0$AT1, $ATB^{0,+}$, $b^{0,+}$, $y^+$LAT1 and $y^+$LAT2, did not transport [$^{14}$C]FAMT, whereas these transporters except TAT1 transported L-[$^{14}$C]methionine (Fig. 2). The transporters for small neutral amino acids, ASCT1, ASCT2, SNAT1, SNAT2, SNAT3, SNAT4 and SNAT5, did not transport [$^{14}$C]FAMT, whereas L-[$^{14}$C]methionine was transported by all except ASCT1 (Fig. S2). The results on the amino acid transporters are summarized in Table 1.

**Concentration-dependent [$^{14}$C]FAMT transport by LAT1.** Kinetic properties of [$^{14}$C]FAMT transport by LAT1 were determined. As shown in Figure 3, LAT1-mediated [$^{14}$C]FAMT transport was saturable and followed Michaelis–Menten kinetics. Its $K_m$ and $V_{max}$ values were 72.7 ± 11.0 μM and 12.1 ± 0.5 pmol/oocyte/min, respectively.

**LAT1 knockdown confirms the contribution of LAT1 in [$^{14}$C]FAMT uptake.** To confirm the role of LAT1 in FAMT uptake in cancer cells, we examined the effects of LAT1 knockdown with siRNA on the [$^{14}$C]FAMT uptake in HeLa S3 cells. As shown in Figure 4a, LAT1 protein expression was highly reduced by the treatment with LAT1 siRNA in HeLa S3 cells, whereas control siRNA had no effect on LAT1 protein level. Accordingly, significant reduction in [$^{14}$C]FAMT uptake was obtained by LAT1 knockdown (Fig. 4b). The uptake of L-[$^{14}$C]methionine was also reduced, although the reduction of [$^{14}$C]methoinine uptake by LAT1 knockdown was less than that of [$^{14}$C]FAMT uptake (Fig. 4c).

## Discussion

Glucose analogues and amino acid derivatives have been used as PET tracers for tumor imaging on the basis that tumor cells take up more nutrients to compensate for increased cellular metabolism.[1–3] The upregulation of transporters responsible for the uptake of glucose and amino acids in tumor cells ensures the usefulness of such PET tracers in the diagnosis of malignant tumors.[1–3] Therefore, the assessment of the interaction of PET tracers with transporters could be beneficial to understand the mechanisms of their uptake in tumors and non-tumor tissues in PET. In this regard, many studies have been conducted on tumor cell lines to reveal amino acid transporters responsible for the uptake of amino acid tracers. Amino acid transport machineries on the plasma membrane were once distinguished and defined as amino acid transport systems such as L, A, $B^{0,+}$ and so on by means of inhibitors and based on $Na^+$-dependence.[28] Now it has been revealed that each transport system consists of more than one isoform, which cannot, in general, be distinguished by the classic maneuvers due to the limited specificity of inhibitors.[27] Therefore, in the previous study, to examine the transporter responsible for FAMT uptake, we used the culture cells stably expressing LAT1 or LAT2 and looked at the effects of cold FAMT on the uptake and efflux of a substrate of system L, L-[$^{14}$C]leucine.[25] However, except LAT1 and LAT2, culture cells are not appropriate for the exogenous expression of amino acid transporters due to high-background amino acid uptake. In the present study, to examine whether FAMT is transported by each amino acid transporter, we have expressed it individually into *Xenopus* oocytes, which have an advantage of low-background amino acid uptake.

Previously, based on the observations that non-labeled FAMT competitively inhibited LAT1-medicted L-[$^{14}$C]leucine uptake and that FAMT induced the efflux of L-[$^{14}$C]leucine mediated by LAT1 via an exchange mechanism, we suggested that FAMT is transported by LAT1.[25] In the present study, using radiolabeled FAMT, we directly showed that FAMT is transported by LAT1 (Fig. 1) and confirmed the significant contribution of LAT1 in FAMT uptake in tumor cells by means of knockdown of LAT1 (Fig. 4). The $K_m$ of FAMT transport determined by direct measurement of [$^{14}$C]FAMT uptake was 72.7 μM, which is a little higher than 27.5 μM obtained by measuring FAMT-induced $^{14}$C-leucine efflux in

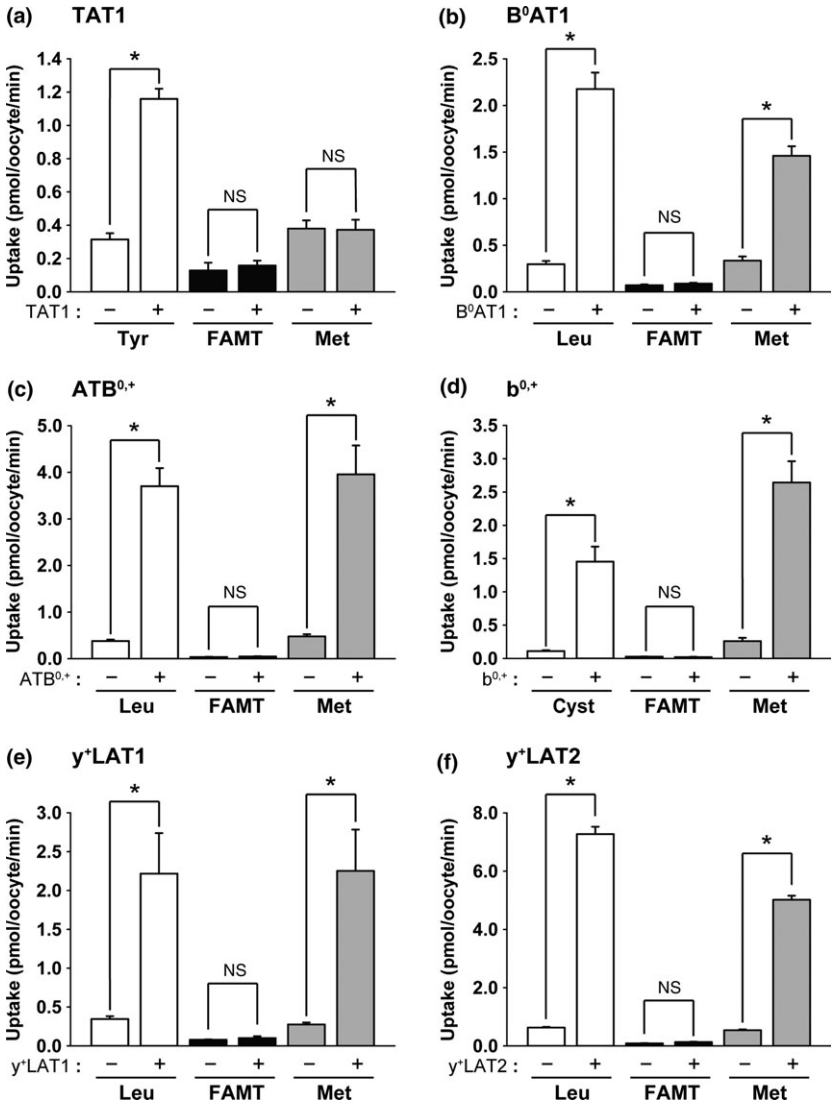


**Fig. 2.** [$^{14}$C]FAMT transport by neutral amino acid transporters other than system L that transport aromatic amino acids. The uptakes of 50 μM L-[$^{14}$C]tyrosine (Tyr), [$^{14}$C]FAMT (FAMT) and L-[$^{14}$C]methionine (Met) by TAT1 (a), the uptakes of 50 μM L-[$^{14}$C]leucine (Leu), [$^{14}$C]FAMT (FAMT) and L-[$^{14}$C]methionine (Met) by $B^0$AT1 (b), $ATB^{0,+}$ (c), $y^+$LAT1 (e) and $y^+$LAT2 (f), and the uptakes of 50 μM L-[$^{14}$C]cystine (Cyst), [$^{14}$C]FAMT (FAMT) and L-[$^{14}$C]methionine (Met) by $b^{0,+}$ (d) were measured on the control oocytes ("−") and the oocytes expressing each transporter ("+"). For the functional expression of $b^{0,+}$ in (d), rBAT, an auxiliary subunit of system $b^{0,+}$ transporter strongly inducing system $b^{0,+}$ activity in *Xenopus* oocytes, was expressed in *Xenopus* oocytes, therefore, the induced system $b^{0,+}$ activity was not that of human but of *Xenopus* although human rBAT was expressed.[16] L-[$^{14}$C]Tyrosine and L-[$^{14}$C]cystine were used as typical substrates of TAT1 and $b^{0,+}$, respectively. L-[$^{14}$C]Leucine was used as a typical substrate of $B^0$AT1, $ATB^{0,+}$, $y^+$LAT1 and $y^+$LAT2. $Na^+$-free uptake buffer was used for TAT1 and $b^{0,+}$, whereas ND96 solution was used for the others. Uptakes were measured for 30 min for $B^0$AT1 and for 15 min for the others. Uptake rates were expressed as mean ± SEM ($n$ = 5–10). *$P < 0.05$; n.s., not significant.

the previous study.[25] This is probably because of the underestimation of the transport based on the efflux measurement due to its limited linearity. The $K_m$ of LAT1-mediated [$^{14}$C]FAMT transport is similar to that of known LAT1 substrates,[17] which supports that FAMT is transported by LAT1 efficiently *in vivo*. Besides LAT1, FAMT was not transported by the other system L isoforms LAT2, LAT3 and LAT4 (Fig. 1). In the previous study, cold FAMT (40 μM) induced a small but significant amount of L-[$^{14}$C]leucine efflux in LAT2-expressing culture cells, which suggested that FAMT is transported by LAT2 at a low level,[25] whereas [$^{14}$C]FAMT (50 μM) was not transported by LAT2 expressed in *Xenopus* oocytes in the present study (Fig. 1). This discrepancy is due to the residual endogenous LAT1 remaining in LAT2-expressing culture cells, which we confirmed using a LAT1-specific inhibitor (data not shown).

In the present study, we furthermore examined transporters for aromatic amino acids and small neutral amino acids and provided direct evidence that FAMT is transported only by LAT1 among amino acid transporters (Table 1). Among them, it is intriguing that $B^0$AT1 and $ATB^{0,+}$, the broad scope transporters for system $B^0$ and system $B^{0,+}$, respectively, did not transport FAMT (Fig. 2). By using inhibitors and based on $Na^+$-dependence, systems $B^0$ and $B^{0,+}$ were suggested to transport a SPECT tracer [$^{123}$I]IMT structurally identical to FAMT except for the substitution in position 3 of an aromatic ring (Fig. S1).[29,30] At the moment, it is not clear whether this is because of the inaccuracy in assigning transport systems using less specific inhibitors in culture cells or the fundamental difference between IMT and FAMT in the interaction with $B^0$AT1 and $ATB^{0,+}$. Interestingly, it has recently been reported that a therapeutic drug for Parkinson's disease L-DOPA, in which a hydroxyl group is added to position 3 of the aromatic ring of L-tyrosine, is not transported by $B^0$AT1, although L-tyrosine is well transported.[31] Thus, it may be possible that the presence of fluorine or iodine in position 3 affects the interaction, particularly with $B^0$AT1.

The high specificity of FAMT to LAT1 as described above allows FAMT to image LAT1. LAT1 is predominantly expressed in malignant tumors and metastases but not in benign lesions,[23,24] so that [$^{18}$F]FAMT is beneficial for imaging malignant tumors. In contrast, in the present study, we showed that L-[$^{14}$C]methionine is transported by most amino acid transporters widely expressed in the body (Table 1), which could result in the uptake by non-tumor tissues such as liver and inflammatory lesions in [$^{11}$C]MET PET.[4] In the

**Table 1. Transport of [$^{14}$C]FAMT and [$^{14}$C]Met by amino acid transporters**

| Amino acid transport system | Transporter | Gene | [$^{14}$C]FAMT | [$^{14}$C]Met |
|---|---|---|---|---|
| L | LAT1 | *SLC7A5* | +† | +‡ |
| L | LAT2 | *SLC7A8* | – | + |
| L | LAT3 | *SLC43A1* | – | + |
| L | LAT4 | *SLC43A2* | – | + |
| T | TAT1 | *SLC16A10* | – | – |
| $B^0$ | $B^0$AT1 | *SLC6A19* | – | + |
| $B^{0,+}$ | $ATB^{0,+}$ | *SLC6A14* | – | + |
| $b^{0,+}$† | | | – | + |
| $y^+L$ | $y^+$LAT1 | *SLC7A7* | – | + |
| $y^+L$ | $y^+$LAT2 | *SLC7A6* | – | + |
| ASC | ASCT1 | *SLC1A4* | – | – |
| ASC | ASCT2 | *SLC1A5* | – | + |
| N/A | SNAT1 | *SLC38A1* | – | + |
| N/A | SNAT2 | *SLC38A2* | – | + |
| N/A | SNAT3 | *SLC38A3* | – | + |
| N/A | SNAT4 | *SLC38A4* | – | + |
| N/A | SNAT5 | *SLC38A5* | – | + |

†"+" indicates that [$^{14}$C]FAMT or [$^{14}$C]Met is transported by the designated transporter, whereas "–" indicates that [$^{14}$C]FAMT or [$^{14}$C]Met is not transported. ‡$b^{0,+}$ activity was induced by expressing rBAT (SLC3A1), an auxiliary subunit of system $b^{0,+}$ transporter, in *Xenopus* oocytes (see the legend to Fig. 2).

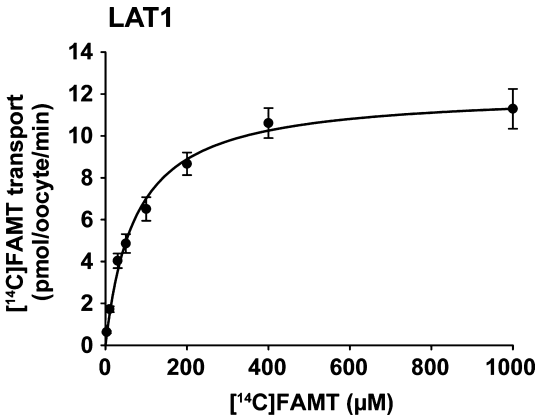


**Fig. 3.** Concentration dependence of [$^{14}$C]FAMT transport. Concentration dependence of [$^{14}$C]FAMT transport mediated by LAT1 was determined. LAT1-mediated transport of [$^{14}$C]FAMT at each concentration was measured for 15 min in $Na^+$-free uptake buffer. Transport rates were expressed as means ± SEM ($n$ = 7–9) and fit to Michaelis–Menten curve.

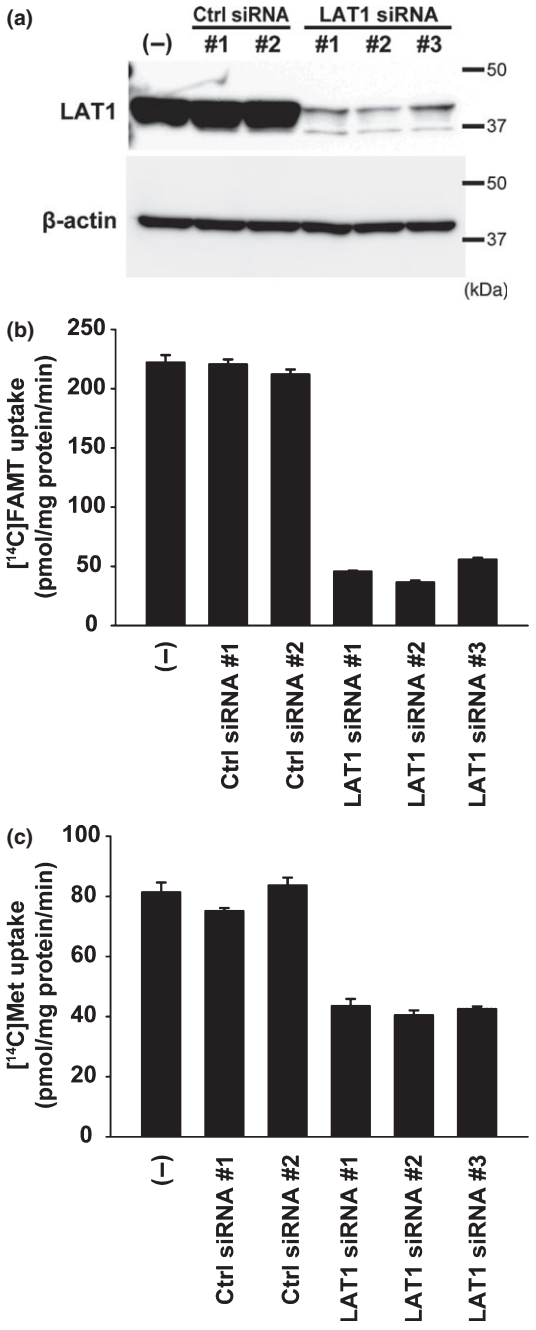


**Fig. 4.** Effect of LAT1 knockdown on the uptake of [$^{14}$C]FAMT and L-[$^{14}$C]methionine in HeLa S3 cells. HeLa S3 cells were transfected with LAT1 siRNA (LAT1 siRNA#1, #2, #3) or non-targeting control siRNA (Ctrl siRNA#1, #2) and compared with non-treated control ("(–)"). Knockdown of LAT1 with siRNA highly reduced LAT1 protein level in HeLa S3 cells in western blot (a). Accordingly, [$^{14}$C]FAMT uptake was decreased by the treatment with LAT1 siRNA (b). The uptake of L-[$^{14}$C]methionine (Met) was also reduced, although the reduction of [$^{14}$C]methoinine uptake by LAT1 knockdown was less than that of [$^{14}$C]FAMT uptake (c). Control siRNA did not affect the levels of LAT1 protein and the uptake of [$^{14}$C]FAMT and L-[$^{14}$C]methionine (a–c).

LAT1-knockdown experiments, the reduction of [$^{14}$C]methoinine uptake was less than that of [$^{14}$C]FAMT uptake (Fig. 4), which is consistent with the results summarized in Table 1 showing that [$^{14}$C]methionine is transported by multiple transporters, whereas [$^{14}$C]FAMT is transported by only LAT1. The high specificity of FAMT to LAT1 could explain the cancer-specific accumulation and low physiologic background in [$^{18}$F]FAMT PET. This, *vice versa*, further supports the cancer-specificity in the expression of LAT1. Because LAT1 is a potential therapeutic target as well as a diagnosis biomarker of cancer, the inhibitors of LAT1 have been developed to suppress tumor growth.[32] LAT1 can also be used for tumor-targeted delivery of anti-tumor agents such as melphalan[17,33] and para-boronophenylalanine in boron neutron capture therapy.[34] Therefore, the LAT1-specific probe established in the present study could also be useful for the selection of subjects and the monitoring of therapeutic effects in the LAT1-targeting therapies.

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## Disclosure Statement

The authors declare no conflict of interest.

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## Supporting Information

Additional supporting information may be found in the online version of this article:

**Fig. S1** Chemical structures of FAMT, IMT, FET and Met.

**Fig. S2** [$^{14}$C]FAMT transport by amino acid transporters for small neutral amino acids.

**Table S1** Human cDNA and their constructs for *Xenopus* oocytes expression.