ASCT2 silencing regulates mammalian target-of-rapamycin growth and survival signaling in human hepatoma cells

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Fuchs BC, Finger RE, Onan MC, Bode BP. ASCT2 silencing regulates mammalian target-of-rapamycin growth and survival signaling in human hepatoma cells. Am J Physiol Cell Physiol 293: C55–C63, 2007. First published February 28, 2007; doi:10.1152/ajpcell.00330.2006. System ASC amino acid transporter-2 (ASCT2) was previously demonstrated to be essential for human hepatoma cell growth and survival, as its silencing via inducible antisense RNA expression results in complete apoptosis within 48 h by a mechanism that transcends its role in amino acid delivery. To gain mechanistic insights into the reliance of cancerous liver cells on ASCT2, the aim of this study was to determine the early consequences of its silencing on the growth and survival signaling that presage apoptosis. Induced antisense ASCT2 RNA in SK-Hep1 cells led to >90% suppression of ASCT2 mRNA by 6 h and inhibition of mammalian target-of-rapamycin (mTOR)/raptor (mTOR complex-1; mTORC1) signaling by 8 h, as manifested by diminished p70 ribosomal protein S6 kinase-1 and eukaryotic initiation factor-4E (eIF4E) binding protein-1 phosphorylation, while protein synthesis rates declined by nearly 50% despite no measurable decreases in the cap binding protein eIF4G or cellular ribosomal protein content. Depressed mTORC1 signaling occurred before detectable reduction in ASCT2 activity but coincided with a 30% decline in total cellular ASCT2 protein. By 12 h after ASCT2 silencing, further decrements were observed in protein synthesis rates and ASCT2 protein and activity, each by ~50%, while signaling from mTOR/ rictor (mTOR complex-2; mTORC2) was stimulated as indexed by enhanced phosphorylation of the Akt/PKB kinase on serine-473 and of its downstream target PKB (PKB/Akt) on Ser473, priming it for subsequent activating phosphorylation by phosphoinositide-dependent kinase-1 (PDK1) (39).

Amino acids stimulate mTOR activity via a poorly defined mechanism that involves the small GTPase Ras homology enriched in brain (Rheb) (24) and GβL (30). Likewise, a microarray study linked mTOR signaling to amino acid transporter expression, as rapamycin selectively inhibited System ASC amino acid transporter-2 (ASCT2), System L amino acid transporter-1 (LAT1), and 4F2 heavy chain (4F2hc) expression (33). ASCT2 is an Na+-dependent, broad-spectrum neutral amino acid exchanger that belongs to solute carrier family-1 (SLC1, the high-affinity glutamate and neutral amino acid transporter family) (27, 28). LAT1 is a catalytic “4F2 light chain” with 12 putative membrane-spanning domains that covalently attaches by a disulfide bridge to a type II membrane glycoprotein heavy chain (4F2hc) to form a functional heterodimeric transporter (42–44), and it belongs to SLC7 (the cationic amino acid transporter/glycoprotein-associated amino acid transporter family) (41). Evidence also exists showing that mTORC2 is required for nutrient transporter trafficking to the plasma membrane, including CD98 (4F2hc and associated light chains), as this process is mTOR dependent but rapamycin insensitive (12). While mTOR regulates the expression and trafficking of amino acid transporters, the work presented here assessed whether amino acid transporter expression reciprocally regulates mTOR signaling.

ASCT2 is the major glutamine transporter in human hepatoma cells (3) and specifically mediates >90% of glutamine uptake in SK-Hep cells (4). Even though glutamine is a major fuel for tumor cells (1, 35), expression of ASCT2 was shown to be essential for SK-Hep cell survival by a mechanism that transcended its role in glutamine/amino acid delivery, as cells undergo fulminant apoptosis within 48 h upon transporter silencing (17). Why suppression of ASCT2 ultimately leads to apoptosis remains unclear, so the aim of this study was to examine earlier consequences of ASCT2 silencing, especially to the growth (mTOR/protein synthesis) and survival (Akt) machinery within the cell, events that would be expected to
presage apoptosis. The effects of ASCT2 silencing on mTOR signaling through both complexes (mTORC1 and mTORC2) to downstream targets were measured in human hepatoma cells. The results show that silencing of ASCT2 expression leads to early growth repression, followed by enhanced survival signaling. This study identifies a novel regulatory relationship between ASCT2 and mTOR and further expands on the role of nutrient transporters in cell growth and survival.

MATERIALS AND METHODS

Cell culture. The human hepatoma cell line SK-Hep1 (American Type Culture Collection, Rockville, MD) was maintained at 37°C in a humidified atmosphere of 5% CO2-95% air in DMEM (4.5 mg/ml d-glucose) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin (all from Invitrogen Life Technologies, Carlsbad, CA). SK-Hep cells stably transfected with the GeneSwitch inducible mammalian expression system (Invivogen), previously described (17), express a transcript from a 1.333-kb ASCT2 cDNA fragment in the antisense (antisense I-1; AS-1-1) or sense orientation (sense 2-1; S 2-1) on induction with mitraphosphate (MFP). AS-1-1 and S 2-1 cells are maintained under antibiotic selective pressure in the above media supplemented with 300 μg/ml hygromycin B and 200 μg/ml Zeocin (both from Invitrogen). For amino acid deprivation studies, cells were grown in DMEM ± total amino acids containing 10% dialyzed FBS (dFBS), 100 U/ml penicillin G, and 100 μg/ml streptomycin and supplemented with hygromycin B and Zeocin in the case of stably transfected clones. Phorbol 12-myristate 13-acetate (PMA) was obtained from Sigma (St. Louis, MO).

Amino acid transport. Initial-rate Na+-dependent glutamine and valine uptake rates were measured by radiotracer analysis using the cluster tray method reported previously (3, 4). Briefly, cells were plated at a density of 5 × 10^4 cells/ml in 24-well plates (Corning) and allowed to grow for 2 days. At specific times after treatment, the initial-rate uptake of L-glutamine or L-valine was measured using radiotracers, L-[3H]glutamine (3 μCi/ml; Amersham Biosciences, Arlington Heights, IL), in the presence of 50 μM unlabeled glutamine or valine, respectively. Initial-rate transport velocities are expressed as the average ± SD of at least four separate determinations and normalized to total cellular protein as measured by the bicinchoninic acid (BCA) method. The transport data were analyzed for significant differences by t-test, with P < 0.050 considered significant.

Northern blot analysis. Total cellular RNA was analyzed by Northern blotting analysis as previously described (3, 17). Full-length 2.9-kb sense and antisense ASCT2 32P-labeled RNA riboprobes were generated by in vitro transcription from ATB0/ASCT2 cDNA in the pSport1 vector using a SP6/T7 MaxiScript kit (Ambion, Austin, TX) and α-32P]-UTP (Perkin Elmer, Boston, MA). The pSure1 vector was linearized with HindIII (Promega) and transcribed with T7 RNA polymerase to generate the sense probe that detects induced antisense ASCT2 RNA. Conversely, pSure1 was linearized with RsrII (New England Biolaboratories, Beverly, MA) and transcribed with the SP6 polymerase to make the antisense probe for detection of endogenous ASCT2 mRNA. A supplied pTRI-actin-mouse DNA template (250-bp KpnI-XhoI fragment) was transcribed in vitro with the SP6 polymerase to generate a 334-bp antisense β-actin riboprobe as a positive control (Ambion). Membrane hybridization and washing under high-stringency conditions were performed as previously described (17). Blots were analyzed with a Storm Phosphorimagmer with ImageQuant software (Amersham Biosciences, Piscataway, NJ). Band intensities were also quantified on X-ray film using the Kodak EDAS 290 system with 1D image analysis software (Eastman Kodak, New Haven, CT). Each Northern blot was repeated at least once to ensure reproducibility and to demonstrate qualitatively similar results.

RESULTS

The creation of stably transfected SK-Hep cells that express a 1.3-kb ASCT2 construct in either the sense (S 2-1) or antisense (AS 1-1) orientation after induction with MFP was described previously (17). In those studies, induction of AS 1-1 cells with MFP decreased ASCT2 mRNA expression by 85 and 73% after 14 and 24 h, respectively, compared with vehicle
Fig. 1. Time course of System ASC amino acid transporter-2 (ASCT2) mRNA, protein, and activity suppression. A: induced antisense RNA suppresses ASCT2 mRNA levels. Total RNA was isolated from cultures of SK-Hep cells stably transfected with a 1.3-kb ATB0/ASCT2 construct in the sense orientation (sense 2-1) or antisense orientation (antisense 1-1) after treatment with inducer (mifepristone, MFP; 10 nM) or vehicle (ethanol, 0.1%) for the indicated times. After size fractionation by denaturing agarose gel electrophoresis (20 μg RNA/lane; visualized by ethidium bromide, EtBr) and transfer to a nylon membrane, the blots were hybridized to full-length 2.9-kb sense and antisense ASCT2 radiolabeled RNA probes and a radiolabeled 334-bp β-actin RNA probe, all generated by in vitro transcription. Blots were analyzed with a Storm Phosphorimager during a 1-h exposure. ASCT2 band intensities were normalized to those of β-actin. Each Northern blot was repeated at least once to ensure reproducibility and to demonstrate qualitatively similar results.

B: Western blot analysis of ASCT2 protein. Total ASCT2 protein levels were assessed in cellular lysates by Western blot analysis, using antibodies directed against the NH2 (N) or COOH (C) terminus of the protein, at the indicated times after induction with MFP, and normalized to levels of tubulin in the same sample. Each Western blot was repeated at least once to ensure reproducibility and to demonstrate qualitatively similar results, which are graphically depicted at bottom of images (%vehicle control values) as individual experiments and their average as a function of time. S ASCT2, values for sense 2-1. C: time course of ASCT2 mRNA, protein, and activity after induced antisense RNA-mediated suppression. Cultures of antisense 1-1 cells were treated with MFP (10 nM) or vehicle (ethanol, 0.1%) at time 0. At the indicated times thereafter, the initial-rate (30 s) Na+-dependent transport velocity of 50 μM l-glutamine (+3 μCi/ml l-[3H]glutamine) was measured by radiotracer analysis. Results represent the average ± SD of 4 separate determinations. *P < 0.050 and **P < 0.010 vs. vehicle control (−MFP). Quantified values for cellular ASCT2 mRNA and protein levels from A and B are shown as well, expressed as %relevant controls for each time point.
controls, which corresponded to 49 and 65% losses in ASCT2 activity at the same time points. To determine the initial kinetics of the inducible antisense system, Northern and Western blot analyses and ASCT2 activity measurements were performed at earlier time points. As shown in Fig. 1, A and B, induced antisense RNA is detectable as early as 2 h after induction in the AS 1-1 cells, resulting in an approximate 85% loss in ASCT2 mRNA levels by 6 h. No antisense RNA was detected in S 2-1 cells after induction with MFP, and ASCT2 mRNA levels remain relatively constant in these cells (Fig. 1A). Cellular ASCT2 protein levels displayed no reproducible declines (85–110% of control) at 1 and 3 h after induction. However, substantial decrements in ASCT2 protein occurred after 8 h (25–30% reductions) and 14 h (50–60% reductions) compared with cognate controls (−MFP). Likewise, a slight but significant 16% decrease in ASCT2 activity (Na⁺-dependent glutamine uptake) was observed after 6 h (P < 0.050) but recovered to control values by 9 h (Fig. 1C). Sustained decrements in ASCT2 activity followed, with a 35% decrease beginning after 12 h that further diminished to 49 and 65% after 15 and 24 h.

Fig. 2. ASCT2 suppression inhibits mammalian target-of-rapamycin (mTOR)/regulatory-associated protein of mTOR (raptor) signaling. A: relative levels of total and phosphospecific (p-) eukaryotic initiation factor-4E (eIF4E) binding protein-1 (4EBP1) and p70 ribosomal protein S6 kinase-1 (S6K1) were determined by Western blot analysis using rabbit polyclonal antibodies directed against 4EBP1, phosphothreonine (Thr37/46) 4EBP1, S6K1, and phosphothreonine (Thr389) S6K1 in lysates from both experimental (antisense 1-1) and control (sense 2-1) SK-Hep cells at the indicated times after induction with 10 nM MFP (+) or 0.1% ethanol vehicle treatment (−). A monoclonal mouse anti-β-tubulin antibody was used to assess equal loading of protein samples. Two representative experiments are shown for antisense 1-1. B: quantification of the Western blots in A. Band intensities on X-ray film were quantified using the Kodak EDAS 290 system with 1D image analysis software, and the ratio of phosphorylated to total protein was calculated. For each protein, the ratio of the controls (antisense 1-1 −MFP at each time point) was set at a value of 100, and the values from the experimental treatments (antisense 1-1 + MFP) are expressed as a percentage of the corresponding controls (−MFP). Each Western blot was repeated at least once to ensure reproducibility and to demonstrate qualitatively similar results, and the results from 2 separate studies are shown for AS 1-1 along with their averaged (AVG) values. S, sense 2-1 results from A. A summary graph is shown for the averaged Western values and their temporal relationship to relative ASCT2 protein levels. mTORC1, mTOR complex 1.
respectively (*P* < 0.010), suggesting an approximate 6-h delay in the functional manifestation of ASCT2 mRNA loss (Fig. 1C).

Rapamycin decreases ASCT2 mRNA expression (33), so potential reciprocal effects of ASCT2 suppression on mTOR signaling were examined. As a center for cell growth control, mTOR stimulates protein synthesis by participating in the activation of S6K1 and inactivation of the eIF4E inhibitor, 4EBP1 (15, 34). Phosphorylation of these effectors is complex, but mTOR has been shown to phosphorylate S6K1 at Thr389 (8, 25) and 4EBP1 at Thr37/46 (7, 8, 18, 19). These events appear to be mediated by the mTOR/Raptor complex (mTORC1) (21, 29). Induction of AS 1-1 cells with MFP caused approximate decreases of 24–56% and 28–44% in S6K1 Thr389 phosphorylation after 8 and 14 h, respectively, compared with vehicle controls (Fig. 2). Similar effects were observed on 4EBP1 Thr37/46 phosphorylation, which decreased by ~3–22% and 38–44% after 8 and 14 h, respectively (Fig. 2). In general, diminished S6K1 phosphorylation was consistently observed before changes in 4EBP1 phosphorylation, sometimes as early as 3 h. Conversely, the phosphorylation levels of S6K1 and 4EBP1 were relatively constant in S 2-1 cells regardless of induction with MFP (Fig. 2). Thus mTORC1 signaling decreases shortly after the observed loss in ASCT2 mRNA expression and diminished cellular ASCT2 protein levels but before substantial decreases in ASCT2 activity, first detectable after 12 h (Fig. 1C).

Since both mTORC1 targets examined (S6K1 and 4EBP1) regulate growth-related translation, and in particular phosphorylation of 4EBP1 promotes cap-dependent translation (19), the functional effects of ASCT2 mRNA suppression on protein synthesis rates were measured. Induction of AS 1-1 cells with MFP significantly (*P* < 0.010) decreased protein synthesis rates by 42 and 54% after 8 and 14 h, respectively (Fig. 3). These effects cannot be attributed to decreased uptake of the radiotracer, since valine uptake rates did not change over the first 14 h (data not shown), nor did rates of protein synthesis in the cognate controls (−MFP). The onset of diminished protein synthesis rates coincided with the observed decreases in S6K1 and 4EBP1 phosphorylation, which occurred by 8 h as well (Fig. 2). Induction of the control S 2-1 cells with
MFP did not alter protein synthesis rates from vehicle controls (Fig. 3). Interestingly, overt amino acid deprivation of SK-Hep cells failed to cause a significant decrease in protein synthesis rates over the 14-h time course (Fig. 3). Moreover, levels of the 7-methylguanine cap binding protein and partner of eIF4E (20), eIF4G, were not diminished in response to ASCT2 silencing, except for a 28% decline after 14 h relative to vehicle controls (Fig. 4). Levels of ribosomal protein as indexed by S6 also did not change over the 14-h study, regardless of treatment (Fig. 4), suggesting that diminished protein synthetic rates in response to ASCT2 silencing cannot be attributed to loss of translational machinery. Taken together, the results thus far suggest that neither diminished amino acid delivery nor rote loss of translational capacity is alone sufficient to account for suppression of mTORC1 signaling on loss of ASCT2 expression.

![Fig. 5. ASCT2 suppression enhances mTOR/rapamycin-insensitive companion of mTOR (rictor) signaling. A: relative levels of total and phosphospecific Akt were determined in cellular lysates by Western blot analysis, as described in MATERIALS AND METHODS, using rabbit polyclonal antibodies directed against Akt and phosphoserine (Ser473) Akt, respectively. Samples were harvested at the indicated times after induction with MFP (+) or vehicle control treatment (−) in control (sense 2-1) and experimental (antisense 1-1) SK-Hep cells. A monoclonal mouse anti-β-tubulin antibody was used to verify equal loading of protein samples. Bottom right: quantification of Western blots. Band intensities on X-ray film were quantified using the Kodak EDAS 290 system with 1D image analysis software, and the ratio of phosphorylated to total protein was calculated. For each protein, the ratio of the controls (−MFP) at each time point was set at a value of 100, and the values from the experimental treatments (+MFP) are expressed as a percentage of the corresponding controls. Each Western blot was repeated at least once to ensure reproducibility and to demonstrate qualitatively similar results, and the results from 2 separate studies are shown for AS 1-1 (AS) along with their averaged (AVG) values. S, sense 2-1 results. B: phosphorylation of the proapoptotic protein Bad in response to ASCT2 silencing. Procedures were exactly as in A, but rabbit polyclonal antibodies directed against Bad and phosphoserine (Ser136) Bad were utilized. Graph at bottom depicts results for antisense 1-1 (AS) and sense 2-1 (S).]
Recently, the mTOR/rictor complex (mTORC2) was identified as the elusive kinase that mediates phosphorylation of Akt at Ser473 (39). To assess the activity of mTORC2 after suppression of ASCT2, we measured the phosphorylation of Akt and one of its proapoptotic substrates, Bad. Akt promotes cell survival through its ability to phosphorylate the Bcl-2 family member Bad at Ser136 (9, 10). AS 1-1 cells induced with MFP exhibited a 1.5- to 2.4-fold increase in Akt Ser473 phosphorylation after 14 h compared with the vehicle controls (Fig. 5A). In one repeated study, Akt Ser473 phosphorylation increased twofold at 8 h after MFP induction (Fig. 5A). Furthermore, phosphorylation of its downstream target Bad at Ser136 was enhanced 4.8-fold at 14 h (Fig. 5B). In contrast, the phosphorylation levels of Akt and Bad were relatively constant in S 2-1 cells regardless of induction with MFP (Fig. 5A). Thus mTORC2 signaling increases only after diminished mTORC1 signaling to the translation effectors S6K1 and 4EBP1, consistent with what has been reported previously (39). The increase in mTORC2 signaling coincides with significant decreases not only in cellular ASCT2 protein but also in ASCT2-mediated transport, as observed in AS 1-1 cells induced with MFP after 12–14 h (Fig. 1C).

In conclusion, the data from this study suggest that loss of ASCT2 expression leads initially to diminished mTORC1 signaling, followed by an apparent compensatory mTORC2-mediated survival response but ultimately apoptosis of hepatoma cells (17). A model summarizing the results presented here is shown in Fig. 6.

**DISCUSSION**

The inducible antisense RNA system that was employed in this study was previously shown to effectively suppress ASCT2 expression and activity, which in turn elicits apoptosis of human hepatoma cells by the intrinsic (caspase 2- and 9-mediated) pathway (17). Cell loss is detectable by 24 h, and after 48 h, 98% of the cells perish because of loss of ASCT2 expression. The antiviral (double-stranded RNA-activated protein kinase, PKR) response was not involved in the mechanism, suggesting the apoptotic effects were ASCT2 specific (17).

ASCT2 was chosen for targeting because it is not expressed in normal human hepatocytes, and in human hepatoma cells it mediates the vast majority of heightened glutamine uptake, an amino acid long established to be required for tumor cell proliferation and survival (45). However, overt glutamine or total amino acid deprivation in SK-Hep cells was not alone sufficient to elicit the rapid apoptotic effects of ASCT2 silencing, suggesting that this transporter plays a key role in growth and cell survival that transcends its role in amino acid delivery (17).

Discrimination between ASCT2 expression and function was again confirmed in the present study, as ASCT2 suppression diminished mTORC1 signaling and protein synthesis (after 8 h) before detectable sustained changes in ASCT2 activity (12 h) (Figs. 1–3). The results suggest that suppression of ASCT2 protein levels coincides with decreased mTORC1 signaling and protein synthesis rates, which may contribute to the apoptotic phenotype. Inhibition of S6K1 activity, in turn, leads to increased Akt Ser473 phosphorylation (22, 40) by mTORC2 (39), a relationship confirmed in this study (Fig. 5A). It should be pointed out that S6K1 is not the only mTOR-regulated kinase associated with translational control; in addition, a related protein, S6K2, has also been shown to play an important role in growth regulation (32), and its potential role in this system and in human hepatocellular carcinoma (HCC) remains to be determined.

While mTOR activity is regulated by amino acids, it is currently unknown how mTOR senses intracellular amino acid levels, other than it requires the presence of the small GTPase Rheb (24) and GBL (30). One hypothesis is that mTOR senses charging of aminoacylated tRNAs (23); however, it has also been proposed that intracellular amino acids themselves, their metabolites, or amino acid-activated second messengers may regulate mTOR (2). A microarray study comparing the effects of amino acid starvation vs. rapamycin on BJAB leukemia cells suggested that mTOR senses a signal originating from other unidentified sources, not just amino acids (33). The data presented here suggest that phosphorylation of S6K1 and 4EBP1 by mTORC1 decreases largely after suppression of ASCT2 protein levels (Figs. 1 and 2). Consistent with this observation, protein synthesis rates decrease in concert with mTORC1 signaling and are refractory to overt amino acid starvation after 14 h (Fig. 3). Thus we propose that mTOR may directly or indirectly “sense” amino acid pools by monitoring intracellular amino acid transporter levels, possibly through its localization to the endoplasmic reticulum (ER) and Golgi apparatus (11).

Constitutively active Akt leads to cell autonomous nutrient transporter trafficking and growth factor-independent survival via an mTOR-dependent mechanism (13, 14). Among the transporters enhanced by this pathway is the heterodimeric amino acid transporter LAT1/4F2 heavy chain (CD98), whose cell surface expression is mTOR dependent but rapamycin insensitive (12), implicating mTORC2. Interestingly, ASCT2 and LAT1 are coordinately upregulated in a number of human cancers (16), and a recent study revealed that these two transporters exist in a plasma membrane complex with a monocarboxylate transporter (collectively termed the “metabolic activation-related complex”) in fibrosarcoma, breast, and colon carcinoma cells (46). The data in Fig. 5 indicate that mTORC2 signaling is substantially enhanced only after sub-

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**Fig. 6.** Model for relationship between ASCT2 silencing and mTOR signaling. On the basis of the results of this study, ASCT2 suppression results in early blunting of mTORC1 signaling and growth repression, followed by an mTORC2-mediated survival response. The mTORC1 signaling depicted is an average of S6K1 and 4EBP1 phosphorylation status (Fig. 2), while mTORC2 signaling is based on phosphorylation of Akt at Ser473 (Fig. 5). ASCT2 protein values are averaged from experiments shown in Fig. 1B.
stational decreases in cellular ASCT2 protein, a relationship summarized in Fig. 6. Given this temporal relationship, it is reasonable to speculate that enhanced mTORC2 signaling may in part be a compensatory response to traffic more transporters to the plasma membrane, a response that fails short based on the sustained suppression of ASCT2 activity after 12 h (Fig. 1C). While direct control of ASCT2 trafficking by mTORC2 has yet to be demonstrated, studies from our laboratory indicate that treatment of SK-Hep cells with the phosphor ester PMA downregulates ASCT2-mediated glutamine uptake (5) and induces a rapid loss of Akt Ser^473 phosphorylation that precedes the loss of ASCT2 activity (Onan MC and Bode BP, unpublished data). If the ASCT2/LAT1 transporter association is ubiquitous across cancer cell types (46), then similar to LAT1/CD98 (13, 14), the regulation of ASCT2 by mTORC2 follows logically.

In conclusion, the results presented here identify a link between amino acid transporter ASCT2 and mTOR signaling. Silencing of ASCT2 expression leads to early mTORC1 signaling inhibition and growth repression, followed by enhanced survival signaling via mTORC2, and ultimately to apoptosis of human hepatoma cells. These studies provide evidence for a novel mechanism whereby mTORC1 may indirectly monitor amino acid status through the perceived abundance of cognate transporters, the trafficking of which appear to be controlled by mTORC2 (12, 14, 26). This relationship may be particularly important, given that, among amino acid transporters, ASCT2 and LAT1 appear to be particularly coveted and augmented in cancerous human tissue, as recently reviewed (16). The specific mechanism by which mTOR putatively senses transporter levels remains to be determined in future studies.

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of which is rapamycin sensitive, have distinct roles in cell growth control. 