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COMMENTARY

Targeting cancer metabolism at the plasma membrane by limiting amino acid access through SLC6A14

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Rapidly proliferating cancer cells increase flux through anabolic pathways to build the mass necessary to support cell division. Imported amino acids and glucose lie at the apex of the anabolic pyramid. Consistent with this, elevated expression of nutrient transporter proteins is characteristic of aggressive and highly malignant cancers. Because tumour cells are more dependent than their normal neighbours on accelerated nutrient import, these up-regulated transporters could be excellent targets for selective anti-cancer therapies. A study by Babu et al. in a recent issue of the *Biochemical Journal* definitively shows that SLC6A14 (where SLC is solute carrier) is one such cancer-specific amino acid transporter. Although mice completely lacking SLC6A14 are viable and exhibit normal mammary gland development,

these animals are highly resistant to mammary tumour initiation and progression driven by potent oncogenes. Because SLC6A14 is essential for tumour growth yet dispensable for normal development and tissue maintenance, small molecules that block amino acid import through this transporter could be effective and selective anti-cancer agents, particularly as components of rational drug combinations.

Key words: amino acid transporter, cancer metabolism, oestrogen receptor-positive (ER⁺) breast cancer, targeted therapy, SLC6A14 (ATB^{0,+}).

In order to support the increased biosynthesis that is required for proliferation, cancer cells express high levels of many different nutrient transporters on their surface [1,2]. For example, overexpression of c-Myc and oncogenic mutants of the Ras GTPase force cells into an anabolic programme and drive the expression of glucose and amino acid transporters to meet the increased demand that this metabolic re-wiring creates [3–7]. The accelerated glucose uptake conferred by elevated transporter expression is exploited clinically to highlight tumour cells in the midst of normal tissue in ¹⁸F fluorodeoxyglucose-positron emission tomography (¹⁸F-FDG-PET) scans; labelled glutamine may also have potential value as an *in vivo* imaging agent [8,9]. Although a non-essential amino acid, extracellular glutamine is required by many cancer cells because they cannot synthesize sufficient quantities to meet the demands of protein synthesis, hexosamine synthesis, redox balance and the anaplerotic reactions that supply the tricarboxylic acid cycle with 2-oxoglutarate (α -ketoglutarate) [9–11]. Cancers can also be addicted to other amino acids. Asparaginase has long been used against acute lymphoblastic leukaemias that require exogenous asparagine [12]. Enzymatic depletion of arginine can also trigger cancer-selective death, and requirements of some cancers for exogenous serine and possibly glycine have been recently uncovered [13–17]. Together, these studies have clearly established that imported amino acids that are essential for cancer cell growth and survival are often dispensable for normal cells. The hypersensitivity of cancer cells to amino acid deprivation may be explained by the fact that tumour cells carry mutations that lock them into an anabolic state, whereas normal cells can make compensatory metabolic adaptations to nutrient depletion. Despite these exciting proof-of-concept studies and the indisputable success of asparaginase, agents that limit

nutrient uptake are not in the clinical pipeline as a means to target ‘cancer metabolism’ [1,18]. One potential stumbling block for these kinds of therapies could be a narrow therapeutic index. For example, 2-deoxy-D-glucose proved too toxic in clinical trials at doses that limited glucose utilization [19]. Other approaches to limiting nutrient access have had unfortunate consequences. Although angiogenesis inhibitors such as bevacizumab, sorafenib and sunitinib successfully limit access to blood-borne nutrients, these agents have the undesirable property of creating a selective pressure for migration that drives metastasis [20]. Taken together, these published studies clearly establish the merits of targeting amino acid transporters as a novel approach to cancer therapy if selectively essential transporters can be identified and sufficiently potent small-molecule inhibitors can be developed.

In a recent issue of the *Biochemical Journal*, Babu et al. [21] tackle the first problem, placing SLC6A14 (where SLC is solute carrier) in the therapeutic cross-hairs by demonstrating that this transporter is required only in cancer cells [21]. Although SLC6A14 transports all neutral amino acids, it is of particular interest due to its role in supplying amino acids with important roles in cancer cell growth, specifically glutamine, arginine and leucine [22,23]. As highlighted above, many cancer cells depend on imported glutamine. Although glutamine can also be imported through SLC1A5 (system ASC amino acid transporter 2, ASCT2) and SLC38A1–SLC38A5 (system N amino acid transporter 1–5, SNAT1–SNAT5), the concentrating ability of SLC6A14 (sodium- and chloride-dependent neutral and basic amino acid transporter, ATB^{0,+}) makes it stand out as a potential key supplier [24]. It is also important to consider that SLC6A14 can import amino acids that allow exchangers such as SLC1A5 (ASCT2) and

Abbreviations: α -MT, α -methyltryptophan; ASCT2, system ASC amino acid transporter 2; ATB^{0,+}, sodium- and chloride-dependent neutral and basic amino acid transporter; ER⁺, oestrogen receptor-positive; LAT1, L-type amino acid transporter 1; MMTV, mouse mammary tumor virus; mTOR, mammalian target of rapamycin; PyMT, polyoma middle T antigen; SLC, solute carrier.

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SLC7A5 (L-type amino acid transporter 1, LAT1) to function [25]. Thus, glutamine limitation could actually starve cancer cells of multiple amino acids, increasing metabolic stress and decreasing the development of resistance. Limiting the substrates available to cancer cells is a good strategy only if the normal cells are not harmed. For this reason, targeting amino acid transporters that are selectively up-regulated in tumours would be the ideal. By this criterion, SLC6A14 is also a highly desirable target. *Slc6a14* expression is low to undetectable in healthy adult tissues but is significantly increased in colorectal, cervical, pancreatic and oestrogen receptor-positive (ER⁺) breast cancers [25]. The TCGA database also indicates that the *Slc6a14* gene is amplified in a subset of prostate, glioma and head and neck cancers, suggesting that SLC6A14 inhibitors might have even broader utility than previously appreciated [26,27]. Its cancer-weighted expression profile, ability to concentrate amino acids intracellularly and potential role in glutamine uptake and mammalian target of rapamycin (mTOR) activation all suggest that SLC6A14-mediated amino acid import could be a rate-limiting step in the anabolic growth of certain classes of cancer cells [21,23,25,28,29].

The *Slc6a14*-knockout mice created by Babu et al. [21] finally allow a definitive assessment of the relative contribution of SLC6A14 to the maintenance and growth of normal and transformed tissues [21]. Given that previously generated knockouts of two amino acid transporters critical for cell growth, SLC7A5 (LAT1) or SLC7A1 (high-affinity cationic amino acid transporter 1, CAT-1), have led to either embryonic or perinatal lethality [30,31], it is significant that *Slc6a14*-knockout animals were viable, showed no overt phenotype and exhibited normal fertility and mammary gland development [21]. Interestingly, compensatory up-regulation of other nutrient transporters was not observed in normal tissues, supporting the model that *Slc6a14* deletion does not produce amino acid limitation in non-transformed cells [21]. Whereas *Slc6a14*^{-/-} mice were normal and healthy, deletion of *Slc6a14* cut tumour incidence in half, significantly increased the tumour-free interval and dramatically reduced the tumour growth rate in mouse mammary tumor virus-polyoma middle T antigen (MMTV-PyMT) and MMTV-Neu transgenic models of breast cancer [21]. Molecular characterization of the PyMT-*Slc6a14*^{-/-} tumours showed the expected signs of amino acid starvation, including decreased mTOR signalling and up-regulation of asparagine synthetase and CCAAT-enhancer binding protein (C/EBP) homologous protein (CHOP), suggesting that the loss of amino acid import through SLC6A14 is in fact responsible for the poor tumour growth [21]. Microarray analysis comparing *Slc6a14*^{+/+} and *Slc6a14*^{-/-} tumours uncovered compensatory up-regulation of two amino acid transporters suggesting that, unlike normal tissues, tumour cells do experience amino acid limitation when SLC6A14 is absent. Interestingly, a number of immunoglobulin genes were also up-regulated in the tumours of the knockout mice, suggesting that the loss of *Slc6a14* might also stimulate the anti-tumour immune response [21]. If increased anti-tumour immunity is detected in future studies, it will be interesting to test whether this results from necrotic cell death or from increased amino acid availability to immune cells infiltrating tumours because the cancer cells are less able to compete for amino acids without SLC6A14 [32,33].

Whereas Karunakaran et al. [23] had previously postulated that chemical inhibition of SLC6A14 should have minimal toxicity due to tumour-specific up-regulation of the protein, the lack of phenotype in the knockout mice generated by Babu et al. [21] provides clear evidence that a small-molecule inhibitor targeting this nutrient transporter is worth seeking. α -methyltryptophan

(α -MT) has been successfully employed against one SLC6A14-positive breast cancer xenograft model [23]. However, a more potent inhibitor would be desirable. Although the single agent activity of α -MT is encouraging and the effect of deleting SLC6A14 profound, it will be important to evaluate whether drug combinations will lead to even greater inhibition or regression and suppress the development of resistance. As oestrogen can drive SLC6A14 expression and potentially resistance to α -MT [23], combination with tamoxifen may be of value. If SLC6A14 restricts glutamine import as suggested by Babu et al. [21], compounds targeting enzymes important for glutaminolysis may also represent a logical combination with SLC6A14 inhibitors [34]. Given the group's earlier finding that α -MT stimulates autophagy [23], autophagy inhibitors may also potentiate the effects of SLC6A14 inhibitors. The *Slc6a14*-knockout mice described by Babu et al. [21] should prove to be an excellent tool for assessing the specificity of small-molecule inhibitors of this transporter.

While holding promise for novel approaches to breast cancer therapy, this intriguing study by Babu et al. [21] should also stimulate future research into the role of SLC6A14 in a wide range of cancer classes. In addition to ER⁺ breast tumours, SLC6A14 has already been established as a promising candidate for pharmacological inhibition in pancreatic, colon and cervical cancers [28,29,35]. Evaluating whether targeting SLC6A14 would have benefits in the subset of glioma, head and neck or prostate cancers in which *Slc6a14* is amplified would be worthwhile [26,27]. A conditional allele of *Slc6a14* would also be of great value in confirming that acute SLC6A14 inhibition in existing tumours can block tumour growth or lead to regression. Given the striking efficacy and cancer-selectivity of SLC6A14 deletion demonstrated by Babu et al. [21], it is exciting to speculate that additional 'kingpin' nutrient transporters might be identified as selectively up-regulated in other cancer classes and safely targeted by novel anti-cancer therapies. In summary, these studies with *Slc6a14*-knockout mice reported by Babu et al. [21] clearly establish that nutrient transporters deserve greater attention as a novel and effective means to target the constitutive anabolism of cancer cells.

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REFERENCES

- 1 McCracken, A.N. and Edinger, A.L. (2013) Nutrient transporters: the Achilles' heel of anabolism. *Trends Endocrinol. Metab.* **24**, 200–208 [CrossRef PubMed](#)
- 2 Edinger, A.L. (2007) Controlling cell growth and survival through regulated nutrient transporter expression. *Biochem. J.* **406**, 1–12 [CrossRef PubMed](#)
- 3 Wise, D.R., DeBerardinis, R.J., Mancuso, A., Sayed, N., Zhang, X.Y., Pfeiffer, H.K., Nissim, I., Daikhin, E., Yudkoff, M., McMahon, S.B. and Thompson, C.B. (2008) Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 18782–18787 [CrossRef PubMed](#)
- 4 Yun, J., Rago, C., Cheong, I., Pagliarini, R., Angenendt, P., Rajagopalan, H., Schmidt, K., Willson, J.K., Markowitz, S., Zhou, S. et al. (2009) Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. *Science* **325**, 1555–1559 [CrossRef PubMed](#)
- 5 Ying, H., Kimmelman, A.C., Lyssiotis, C.A., Hua, S., Chu, G.C., Fletcher-Sananikone, E., Locasale, J.W., Son, J., Zhang, H., Colloff, J.L. et al. (2012) Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* **149**, 656–670 [CrossRef PubMed](#)

- 6 Son, J., Lyssiotis, C.A., Ying, H., Wang, X., Hua, S., Ligorio, M., Perera, R.M., Ferrone, C.R., Mullarky, E., Shyh-Chang, N. et al. (2013) Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* **496**, 101–105 [CrossRef PubMed](#)
- 7 Osthus, R.C., Shim, H., Kim, S., Li, Q., Reddy, R., Mukherjee, M., Xu, Y., Woney, D., Lee, L.A. and Dang, C.V. (2000) Dereglulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *J. Biol. Chem.* **275**, 21797–21800 [CrossRef PubMed](#)
- 8 Yeluri, S., Madhok, B., Prasad, K.R., Quirke, P. and Jayne, D.G. (2009) Cancer's craving for sugar: an opportunity for clinical exploitation. *J. Cancer Res. Clin. Oncol.* **135**, 867–877 [CrossRef PubMed](#)
- 9 Rajagopalan, K.N. and DeBerardinis, R.J. (2011) Role of glutamine in cancer: therapeutic and imaging implications. *J. Nucl. Med.* **52**, 1005–1008 [CrossRef PubMed](#)
- 10 DeBerardinis, R.J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S. and Thompson, C.B. (2007) Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 19345–19350 [CrossRef PubMed](#)
- 11 DeBerardinis, R.J. and Cheng, T. (2010) Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene* **29**, 313–324 [CrossRef PubMed](#)
- 12 van den Berg, H. (2011) Asparaginase revisited. *Leuk. Lymphoma* **52**, 168–178 [CrossRef PubMed](#)
- 13 Phillips, M.M., Sheaff, M.T. and Szlosarek, P.W. (2013) Targeting arginine-dependent cancers with arginine-degrading enzymes: opportunities and challenges. *Cancer Res. Treat.* **45**, 251–262 [CrossRef PubMed](#)
- 14 Mussai, F., Egan, S., Higginbotham-Jones, J., Perry, T., Beggs, A., Odintsova, E., Loke, J., Pratt, G., U, K.P., Lo, A. et al. (2015) Arginine dependence of acute myeloid leukemia blast proliferation: a novel therapeutic target. *Blood* **125**, 2386–2396 [CrossRef PubMed](#)
- 15 Maddocks, O.D., Berkers, C.R., Mason, S.M., Zheng, L., Blyth, K., Gottlieb, E. and Vousden, K.H. (2013) Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells. *Nature* **493**, 542–546 [CrossRef PubMed](#)
- 16 Jain, M., Nilsson, R., Sharma, S., Madhusudhan, N., Kitami, T., Souza, A.L., Kafri, R., Kirschner, M.W., Clish, C.B. and Mootha, V.K. (2012) Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science* **336**, 1040–1044 [CrossRef PubMed](#)
- 17 Labuschagne, C.F., van den Broek, N.J., Mackay, G.M., Vousden, K.H. and Maddocks, O.D. (2014) Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. *Cell Rep.* **7**, 1248–1258 [CrossRef PubMed](#)
- 18 Galluzzi, L., Kepp, O., Vander Heiden, M.G. and Kroemer, G. (2013) Metabolic targets for cancer therapy. *Nat. Rev. Drug Discov.* **12**, 829–846 [CrossRef PubMed](#)
- 19 Vander Heiden, M.G. (2011) Targeting cancer metabolism: a therapeutic window opens. *Nat. Rev. Drug Discov.* **10**, 671–684 [CrossRef PubMed](#)
- 20 Bergers, G. and Hanahan, D. (2008) Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer* **8**, 592–603 [CrossRef PubMed](#)
- 21 Babu, E., Bhutia, Y.D., Ramachandran, S., Gnana-prakasam, J.P., Prasad, P.D., Thangaraju, M. and Ganapathy, V. (2015) Deletion of the amino acid transporter Slc6a14 suppresses tumor growth in spontaneous mouse models of breast cancer. *Biochem. J.* **469**, 17–23 [PubMed](#)
- 22 Sloan, J.L. and Mager, S. (1999) Cloning and functional expression of a human Na(+) and Cl(-)-dependent neutral and cationic amino acid transporter B(0+). *J. Biol. Chem.* **274**, 23740–23745 [CrossRef PubMed](#)
- 23 Karunakaran, S., Ramachandran, S., Coothankandaswamy, V., Elangovan, S., Babu, E., Periyasamy-Thandavan, S., Gurav, A., Gnanaprakasam, J.P., Singh, N., Schoenlein, P.V. et al. (2011) SLC6A14 (ATB0, +) protein, a highly concentrative and broad specific amino acid transporter, is a novel and effective drug target for treatment of estrogen receptor-positive breast cancer. *J. Biol. Chem.* **286**, 31830–31838 [CrossRef PubMed](#)
- 24 Broer, S. and Palacin, M. (2011) The role of amino acid transporters in inherited and acquired diseases. *Biochem. J.* **436**, 193–211 [CrossRef PubMed](#)
- 25 Bhutia, Y.D., Babu, E., Prasad, P.D. and Ganapathy, V. (2014) The amino acid transporter SLC6A14 in cancer and its potential use in chemotherapy. *Asian J. Pharm. Sci.* **9**, 293–303 [CrossRef](#)
- 26 Cerami, E., Gao, J., Dogrusoz, U., Gross, B.E., Sumer, S.O., Aksoy, B.A., Jacobsen, A., Byrne, C.J., Heuer, M.L., Larsson, E., Antipin, Y. et al. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2**, 401–404 [CrossRef PubMed](#)
- 27 Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E. et al. (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **6**, pl1 [CrossRef PubMed](#)
- 28 Gupta, N., Miyauchi, S., Martindale, R.G., Herdman, A.V., Podolsky, R., Miyake, K., Mager, S., Prasad, P.D., Ganapathy, M.E. and Ganapathy, V. (2005) Upregulation of the amino acid transporter ATB0, + (SLC6A14) in colorectal cancer and metastasis in humans. *Biochim. Biophys. Acta* **1741**, 215–223 [CrossRef PubMed](#)
- 29 Gupta, N., Prasad, P.D., Ghamande, S., Moore-Martin, P., Herdman, A.V., Martindale, R.G., Podolsky, R., Mager, S., Ganapathy, M.E. and Ganapathy, V. (2006) Up-regulation of the amino acid transporter ATB(0, +) (SLC6A14) in carcinoma of the cervix. *Gynecol. Oncol.* **100**, 8–13 [CrossRef PubMed](#)
- 30 Poncet, N., Mitchell, F.E., Ibrahim, A.F., McGuire, V.A., English, G., Arthur, J.S., Shi, Y.B. and Taylor, P.M. (2014) The catalytic subunit of the system L1 amino acid transporter (slc7a5) facilitates nutrient signalling in mouse skeletal muscle. *PLoS One* **9**, e89547 [CrossRef PubMed](#)
- 31 Perkins, C.P., Mar, V., Shutter, J.R., del Castillo, J., Danilenko, D.M., Medlock, E.S., Ponting, I.L., Graham, M., Stark, K.L., Zuo, Y. et al. (1997) Anemia and perinatal death result from loss of the murine ecotropic retrovirus receptor mCAT-1. *Genes Dev.* **11**, 914–925 [CrossRef PubMed](#)
- 32 Pearce, E.L., Poffenberger, M.C., Chang, C.H. and Jones, R.G. (2013) Fueling immunity: insights into metabolism and lymphocyte function. *Science* **342**, 1242454 [CrossRef PubMed](#)
- 33 Edinger, A.L. and Thompson, C.B. (2002) Antigen-presenting cells control T cell proliferation by regulating amino acid availability. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 1107–1109 [CrossRef PubMed](#)
- 34 Hensley, C.T., Wasti, A.T. and DeBerardinis, R.J. (2013) Glutamine and cancer: cell biology, physiology, and clinical opportunities. *J. Clin. Invest.* **123**, 3678–3684 [CrossRef PubMed](#)
- 35 Bhutia, Y.D., Coothankandaswamy, V., Cao, S., Xu, Y. and Ganapathy, V. (2014) Abstract 4340: The amino acid transporter SLC6A14 is an effective drug target for treatment of pancreatic cancer. *Cancer Res.* **74**, 4340 [CrossRef](#)

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