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Review

Cancer Research

Amino Acid Transporters in Cancer and Their Relevance to "Glutamine Addiction": Novel Targets for the Design of a New Class of Anticancer Drugs

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Abstract

Tumor cells have an increased demand for amino acids because of their rapid proliferation rate. In addition to their need in protein synthesis, several amino acids have other roles in supporting cancer growth. There are approximately two-dozen amino acid transporters in humans, and tumor cells must upregulate one or more of these transporters to satisfy their demand for amino acids. If the transporters that specifically serve this purpose in tumor cells are identified, they can be targeted for the development of a brand new class of anticancer drugs; the logical basis of such a strategy would be to starve the tumor cells of an important class of nutrients. To date, four amino acid transporters have been found to be expressed at high levels in cancer: SLC1A5, SLC7A5, SLC7A11, and SLC6A14. Their induction occurs in a cancer type–specific manner with a direct or indirect involvement of the oncogene c-Myc. Further, these transporters are functionally coupled, thus maximizing their ability to promote cancer growth and chemoresistance. Progress has been made in preclinical studies, exploiting these transporters as drug targets in cancer therapy. These transporters also show promise in development of new tumor-imaging probes and in tumor-specific delivery of appropriately designed chemotherapeutic agents. *Cancer Res; 75(9); 1782–8. ©2015 AACR.*

Introduction

Amino acids represent an important class of major nutrients obligatory for the survival of any cell. Tumor cells have a notably increased demand for these nutrients to support their exceptionally fast proliferation rate. All mammalian cells, whether they are cancerous or not, have to obtain essential amino acids (Thr, Met, Phe, Trp, Val, Ile, Leu, and Lys) from external sources because they cannot synthesize these amino acids. Nonessential amino acids can be synthesized endogenously in all mammalian cells, but if the proliferation rate is fast as is the case with tumor cells, even these amino acids need to be obtained from external sources because the capacity of endogenous synthesis does not meet the increased demands of the highly proliferating cells. Although the primary function of the amino acids is to serve as the building blocks for protein synthesis, some amino acids have specific biologic functions. Glutamine, glycine, and aspartate are needed for nucleotide biosynthesis, a process critical for tumor cells for proliferation. Serine plays an important role as a one-carbon source that is critical in nucleotide synthesis and DNA methylation. Leucine, glutamine, and arginine serve as signaling molecules and activate mTOR. Recent studies have shown that tumor cells have altered metabolic pathways involving the amino acids glutamine, serine, threonine, and proline (1–4). In particular, the prominent role of glutamine in tumor-selective metabolic pathways has received considerable attention as evident from the widespread use of the terms such as "glutamine addiction" and "glutaminolysis" in the vocabulary of cancer biology.

Amino Acid Transporters in Cancer

All amino acids are hydrophilic and hence cannot traverse the plasma membrane without the aid of selective transport proteins. Mammalian cells express at least two-dozen amino acid transporters that are expressed differentially in a tissue-specific and development-specific manner (5, 6). Most amino acid transporters recognize more than one amino acid as substrates. Because tumor cells have a high demand for amino acids, it is logical to expect that these cells must upregulate certain selective amino acid transporters to meet this demand. Because the signaling pathways responsible for the initiation and propagation of tumor vary markedly from one tumor type to another, it is likely that not all tumor types rely on the same given set of amino acid transporters to support their growth. If there is a differential upregulation of selective amino acid transporters in specific tumors, we should be able to exploit such transporters for targeted cancer therapy. Tumor cells express these transporters at a higher level than normal cells because of the increased nutritional demands of the former. Therefore, blocking the function of such transporters should have detrimental effects specifically on tumor cells, leaving normal cells mostly unaffected. As such, the "starve the tumor cells to death" approach is logical for effective cancer treatment, but it has not received much attention in the field of cancer therapeutics. The widespread focus on the signaling pathways involving growth factor receptors and tyrosine kinases as targets for cancer treatment

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has largely overshadowed other potential therapeutic avenues. The purpose of this review is to highlight the recent advances in the area of amino acid transporters in cancer and pinpoint the potential of these transporters as molecular targets for the design and development of a brand new class of anticancer drugs.

SLC7A5 and SLC1A5 in Cancer and the Relevance of Their Functional Coupling to Cancer Growth

SLC7A5 represents one of the System L (Leucine-preferring) amino acid transporters (7). As such, it is also known as LAT1 (L-Amino acid Transporter 1), the others being LAT2, LAT3, and LAT4. LAT1 shows high affinity for the transport of branched-chain amino acids (Val, Ile, and Leu) and also bulky amino acids (Phe, Tyr, Trp, Gln, Asn, and Met; Table 1). It does not interact with anionic or cationic amino acids. It is not an active transporter and is not coupled to any transmembrane ion gradients. More importantly, it is an obligatory exchanger, meaning that influx of one amino acid substrate into cells via this transporter is mandatorily coupled to efflux of another amino acid substrate. SLC7A5 is highly expressed in most cancers (8), raising the possibility that the transporter functions in the maintenance of amino acid nutrition in cancer cells. Recent studies have demonstrated that the hypoxia-inducible factor HIF2α upregulates SLC7A5 (9). Because hypoxia plays a critical role in cancer growth and progression, the control of SLC7A5 expression by HIF2α offers a molecular mechanism for the high expression of this transporter in cancer. MiRNAs may also play a role in the regulation of SLC7A5 expression. miR-126, which is downregulated in lung cancer, directly targets SLC7A5 (10). *SLC7A5* promoter also has canonical binding sites for the oncogene c-Myc, and overexpression of the oncogene results in increased expression of SLC7A5 (11).

In addition to the connection between SLC7A5 and the procarcinogenic transcription factors HIF2α and c-Myc, the ability of this transporter to mediate the cellular uptake of leucine with high affinity has further fueled the current interest in this transporter in cancer. Leucine is a well-known activator of mTOR signaling (12), and pharmacologic inhibition of SLC7A5 transport function suppresses mTOR signaling and tumor growth in a number of model systems (13–16). But how does SLC7A5, an obligatory exchanger, mediate the entry of leucine into cancer cells to impact on mTOR? The basis for this question is that without the presence of an outwardly directed concentration gradient for another substrate of SLC7A5, the transporter cannot facilitate the entry of leucine into cells. This conundrum was resolved with the finding that SLC7A5 does not act alone in mTOR activation; the transporter is functionally coupled to SLC1A5, another amino acid transporter (17). SLC1A5 is a Na^+-coupled transporter for alanine, serine, cysteine, and glutamine, but the transport process is an obligatory exchange in which Na^+-coupled entry of one amino acid substrate into cells is mandatorily coupled to Na^+-coupled efflux of another amino acid substrate out of the cells (Table 1). Thus, in the functional coupling between SLC7A5 and SLC1A5, glutamine enters the cancer cells via SLC1A5, which then effluxes out of the cells via SLC7A5 coupled to the entry of leucine (Fig. 1). Accordingly, loss of SLC1A5 or pharmacologic inhibition of SLC1A5 leads to inability of SLC7A5 to activate mTOR in cancer cells. Interestingly, SLC1A5 is also a target for c-Myc (11, 18), meaning that the two transporters are induced in cancer cells in a coordinated manner to optimize the functional coupling in the promotion of tumor growth. Further evidence in support of a role for SLC1A5 in cancer comes from recent studies, which show that the retinoblastoma protein, a well-documented tumor suppressor, regulates SLC1A5 by decreasing its expression (19).

SLC7A11 and Its Functional Coupling to SLC1A5 in Cancer: Relevance to Cancer Growth and Chemoresistance

SLC7A11 is a transporter for extracellular cystine (Cys-S-S-Cys) coupled to the efflux of intracellular glutamate; it represents the amino acid transport system x^-_c and is also known as xCT (catalytic subunit of the transport system x^-_c; ref. 7). This transporter is also a Na^+-independent obligatory exchanger, meaning that cystine entry into cells is mandatorily coupled to glutamate efflux from the cells (Table 1; Fig. 1). The primary function of this transporter was once thought to be the maintenance of glutathione status in cells via the provision of cysteine, the rate-limiting amino acid in the synthesis of this antioxidant peptide. Recent studies have shown that glutamate released into the extracellular environment via the transporter also plays a critical role in the biologic function of the transporter (20). SLC7A11 is upregulated

Table 1. Amino acid transporters that are upregulated in cancer

Gene	Alias	Transport mechanism	Substrates	Inhibitors/blockers	^{18}F-PET probes
SLC1A5	ASCT2	Obligatory exchange Na^+/AA exchanged for Na^+/AA Electroneutral	Ala, Ser, Cys, Thr, Gln	Benzylserine γ-Glu-*p*-nitroanilide	FACBC FPhPA
SLC7A5	LAT1	Obligatory exchange AA exchanged for AA Electroneutral	Large neutral amino acids	BCH JPH203	FAMT FET FPhPA FACBC
SLC7A11	xCT	Obligatory exchange AA exchanged for AA Electroneutral	Cystine, glutamate	Sulfasalazine Erastin Sorafenib (S)-4-Carboxyphenyl glycine	FSPG
SLC3A2	4F2hc CD98hc	Chaperone for SLC7A5 and SLC7A11			
SLC6A14	$ATB^{0,+}$	Unidirectional $Na^+/Cl^-/AA^{0,+}$ symport Electrogenic	All neutral amino acids All cationic amino acids	α-Methyl-L-Trp	FEMAET FET

Abbreviations: BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; FACBC, *trans*-1-amino-3-fluorocyclobutane carboxylic acid; FAMT, 3-fluoro-α-methyl-L-tyrosine; FET, *O*-(2-fluoro ethyl)-L-tyrosine; FEMAET, *O*-2-((2-fluoroethyl)methylamino)ethyl-L-tyrosine; FPhPA, 2-amino-5-(4-fluorophenyl)pent-4-ynoic acid; FSPG, (4*S*)-4-(3-fluoropropyl)-L-glutamate; JPH 203, (*S*)-2-amino-3-(4-((5-amino-2-phenylbenzo[d]oxazol-7-yl)methoxy)3,5-dichlorophenyl)propionic acid.

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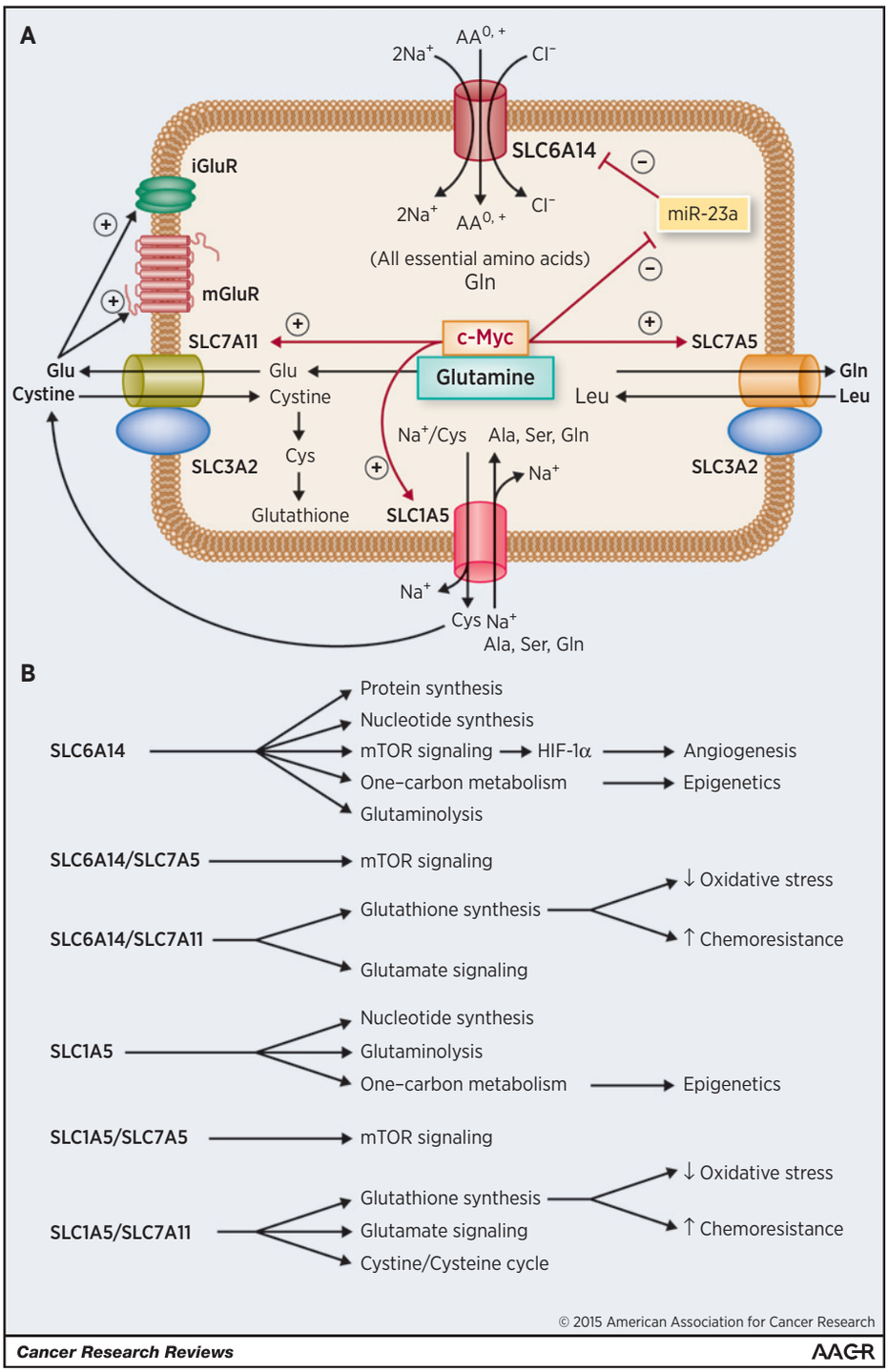

Figure 1.
A, the amino acid transporters that are upregulated in cancer and their functional inter-relationship. B, metabolic and signaling consequences of cancer-associated amino acid transporters and their functional coupling.

in a variety of cancers where the transporter-assisted promotion of glutathione synthesis reduces oxidative damage and protects the cancer cells from apoptosis (21). Equally important is the relevance of extracellular glutamate to cancer growth. This amino acid is well known for its excitotoxicity, but its role in the promotion of cancer is getting increasing attention in recent years. Glutamate acts on selective metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs) on cancer cells and potentiates oncogenic signaling (22, 23). The relevance of SLC7A11 as a promoter of cancer growth is evident from the findings that the transporter is stabilized in the plasma membrane by a specific cancer-associated variant of CD44 (24) and that the expression of the transporter is induced directly by c-Myc (25) and also by the proto-oncogene Ets-1 (26). This is also supported by

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several studies, which demonstrate the tumor-suppressing effects of SLC7A11 inhibitors (21). Two miRNAs, miR-26b and miR-27a, which are downregulated in several cancers, have been shown to target SLC7A11, thereby providing additional mechanisms for cancer-associated upregulation of this transporter (27, 28). However, because SLC7A11 promotes an antioxidant mechanism in cells, blocking the transporter function may have opposite effects in cancer prevention versus cancer treatment because while oxidative stress induces cell death in cancer cells, it increases the risk of carcinogenesis. This is evident from the findings that *Slc7a11*-null mice, though viable, fertile, and phenotypically normal, exhibit increased sensitivity to chemically induced carcinogenesis (29).

The need for intracellular glutamate for the optimal function of SLC7A11 in providing cysteine for glutathione synthesis inside the cells raises the issue of glutamate source. A recent study has addressed this issue by showing that extracellular glutamine supports SLC7A11-mediated cystine uptake in cancer cells (30). Therefore, it is likely that a glutamine transporter is coupled functionally to SLC7A11. An obvious candidate is SLC1A5, which is upregulated in cancer and is capable of mediating the influx of extracellular glutamine into cells in a Na^+-dependent manner (Fig. 1). The other potential candidate is SLC6A14, a Na^+/Cl^--coupled transporter for glutamine and multiple other amino acids (see below). Once glutamine enters the cancer cells, it can be converted into glutamate by glutaminase, thus providing the exchangeable amino acid substrate for SLC7A11-mediated cystine entry into cells. It is important to note that the c-Myc oncogene that induces SLC1A5 and SLC7A11 in cancer cells also induces glutaminase I (1, 2), thus making the functional coupling between the two transporters maximal. The proposed functional cooperation between SLC7A11 and SLC1A5 may also be relevant to the cystine/cysteine cycle that has been shown recently to play a role in inducing resistance to cell death in tumor cells (31). The overexpression of SLC7A11 in cancer cells mediates cystine entry into cells coupled to glutamate release; once inside the cell, cystine gets reduced to cysteine, a part of which is used in the synthesis of glutathione while the rest is secreted into the extracellular environment where it is oxidized again into cystine, thus completing the cystine/cysteine cycle (Fig. 1). The oxidation of cysteine into cystine outside tumor cells creates a reducing extracellular environment as an additional facilitator of resistance to cell death in tumor cells (31). The transporter responsible for the secretion of cysteine as a part of this cystine/cysteine cycle has not yet been identified, but we speculate that, among the amino acid transporters that are induced in cancer, SLC1A5 is best suited for this purpose because it is an obligatory exchanger. Because SLC7A11 and SLC1A5 are coexpressed at high levels in cancer cells, SLC1A5 provides glutamine, which is then converted into glutamate to fuel SLC7A11-mediated cystine entry; cystine is then converted into cysteine, which fuels SLC1A5-mediated glutamine entry (Fig. 1). The proposed coupling between SLC1A5/SLC6A14 and SLC7A11 in cancer cells would also mean that the ratio of glutamate to glutamine in the extracellular environment of tumors would be markedly higher than in normal tissue, resulting from increased clearance of glutamine from the extracellular medium via SLC1A5/SLC6A14-mediated entry into tumor cells following by increased glutaminolysis inside the cells coupled to increased release of SLC7A11-mediated release of glutamate into the extracellular medium where it can potentiate autocrine glutamate signaling in tumor cells via mGluRs/iGluRs. As such, the glutamate/glutamine ratio in the tumor extracellular environment might serve as a valuable indicator of the aggressiveness of tumor growth.

In addition to promoting tumor growth via mechanisms involving antioxidant machinery both inside and outside the cells and autocrine glutamate signaling, SLC7A11 also induces chemoresistance to selective drugs (28, 32–34). The underlying mechanism in this process also involves the ability of the transporter to modulate oxidative stress. Some anticancer drugs (e.g., geldanamycin) elicit their therapeutic effects through generation of reactive oxygen species, resulting in cell death in tumor cells; SLC7A11 interferes with the therapeutic efficacy of such drugs by reversing the oxidative stress (32). In case of certain other anticancer drugs, SLC7A11 suppresses the expression of the proteins that are targets for the drugs. For example, gemcitabine in the form of its diphosphate produces its anticancer effects by inhibiting ribonucleotide reductase; SLC7A11 reverses this effect by directly activating the enzyme with glutathione or inducing the expression of the enzyme with glutathione-dependent activation of p53 (35, 36). Therefore, pharmacologic blockade of the transport function of SLC7A11 not only interferes with tumor growth but also reverses resistance to certain chemotherapeutic agents.

SLC3A2 in Cancer and Its Relevance to SLC7A5 and SLC7A11

Although SLC7A5 and SLC7A11 are the actual transporters, they need SLC3A2 (also known as 4F2hc or CD98hc) as a chaperone to recruit them to the plasma membrane. Both transporters exist in the plasma membrane in the form of heterodimers in complex with SLC3A2. The chaperone by itself does have any transport function, but its expression is obligatory for the recruitment and hence the transport function of SLC7A5 and SLC7A11 (7). Interestingly, SLC3A2 is also a target for c-Myc as are SLC7A5 and SLC7A11 (25), and is overexpressed in cancer. SLC3A2, however, has additional biologic functions that are relevant to tumor growth. Recent studies have shown that SLC3A2 promotes Ras-driven tumorigenesis by modulating integrin-mediated mechanotransduction, which involves an increase in extracellular matrix stiffness as well as potentiation of the tumor cells' response to matrix rigidity (37). On the other hand, tumor stiffness is known to influence the metastatic behavior negatively (38); what this means in terms of the impact of overexpression of SLC3A2 on cancer progression at the primary site versus spreading of the tumor to distant sites needs further investigation.

SLC6A14 in Cancer and the Relevance of Its Transport Function to SLC7A5 and SLC7A11

SLC6A14 is unique among the amino acid transporters and is very different from SLC1A5, SLC7A5, and SLC7A11 in that its transport function is highly concentrative being coupled to transmembrane gradients of Na^+ as well as Cl^- and also to membrane potential and that its substrate specificity is broad including all neutral and cationic amino acids (39). SLC6A14 is also known as $ATB^{0,+}$ (amino acid transporter with neutral, denoted by "0," and cationic, denoted by "+," amino acids as substrates). The substrates of SLC6A14 include all essential amino acids as well as glutamine. Unlike SLC1A5, SLC7A5, and SLC7A11, it is not an exchanger and it catalyzes almost a unidirectional influx of its substrates into cells. It is upregulated in several cancers, including

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estrogen receptor–positive breast cancer, colon cancer, cervical cancer, and pancreatic cancer (40). The extraordinary ability of SLC6A14 to concentrate glutamine in tumor cells leads to coupling of this transporter functionally to SLC7A5 and SLC7A11 (Fig. 1). Pharmacologic blockade of SLC6A14 in tumor cells causes amino acid starvation, autophagy followed by apoptosis, suppression of mTOR and HIF1α signaling, and inhibition of tumor growth *in vitro* and *in vivo* (41, 42). However, the upregulation of this transporter is not a common phenomenon in all cancers; for example, the transporter is not upregulated in estrogen receptor–negative breast cancer. *SLC6A14* is a target for estrogen receptor (42). c-Myc also influences SLC6A14 expression, not directly but indirectly via miR-23a; c-Myc suppresses the expression of miR-23a (11), and SLC6A14 is a documented target for miR-23a (43).

Utility of Amino Acid Transporters in Cancer Treatment, Tumor Imaging, and Tumor Cell–Selective Delivery of Anticancer Drugs

Nutrient transporters that are overexpressed in tumor cells have potential as drug targets for cancer therapy because pharmacologic blockade of such transporters would lead to nutrient deprivation in tumor cells and hence growth arrest and cell death (44, 45). Because these transporters are upregulated in cancer to meet the demands for nutrients, their expression is higher in tumor cells than in normal cells. Therefore, blocking such transporters may not affect the biology of normal cells. Several inhibitors and blockers have been identified that selectively target the four amino acid transporters that are upregulated in cancer (Table 1); these pharmacologic agents show promise in killing tumor cells in a manner specific for the transporter. However, none of these agents has yet progressed beyond the preclinical stage. The clinical and therapeutic potential of these agents will ultimately depend on whether or not their transporter targets have essential function in normal tissues. With regard to this important issue, some insight can be gained from the knockout mouse studies with selective deletion of these transporters. Mice with the deletion of Slc7a11, Slc1a5, and Slc6a14 are viable and fertile with no obviously noticeable phenotype (46–48). This suggests that pharmacologic blockade of these three transporters may not have any significant impact on normal cells. Therefore, these transporters have potential as drug targets in cancer therapy. In contrast, deletion of Slc7a5 in mice is embryonically lethal (49). This transporter is essential for the transfer of amino acids from blood into brain across the blood–brain barrier, suggesting a critical role for the transporter in the function and development of the brain. It is also expressed highly in placenta, implicating the transporter in fetal nutrition and development (50). The potential obligatory function of this transporter in early development might underlie the nonviability of *Slc7a5*-null mice. Therefore, whether pharmacologic blockade of SLC7A5 would be a feasible approach to treat cancer without any major impact on normal tissues remains to be seen. The same is true with Slc3a2, the chaperone for Slc7a5; deletion of *Slc3a2* in mice results in embryonic lethality (51). Nonetheless, conditional deletion of *Slc3a2* protects against Ras-driven tumorigenesis (37), implicating its role in the promotion of cancer. However, because Slc3a2 is multifunctional with its role as a chaperone of selective amino acid transporters such as Slc7a5 being just one of them, it is difficult to assess the relevance of amino acid nutrition to Slc3a2-dependent promotion of cancer.

SLC38 gene family contains several amino acid transporters, many of them capable of mediating concentrative entry of glutamine into cells (52). Among them is SLC38A5 (also called SNAT5 or SN2) that catalyzes Na^+-coupled entry of glutamine into cells in exchange for H^+ (53, 54), indicating that this transporter has potential not only for supplying glutamine to cells but also regulating intracellular pH; both of these features are beneficial for tumor cells. Even though very little is known on the contribution of SLC38A5 to amino acid nutrition in cancer cells, it is important to note that SLC38A5 is a c-Myc target (18). Two recent studies (55, 56) have identified another member of this gene family, SLC38A9, as relevant to mTOR activation. But this transporter is located intracellularly on the lysosomal membrane, thus most likely involved in the efflux of amino acids from the lysosome into the cytoplasm; such a function might be relevant to autophagy in the maintenance of amino acid nutrition in cells, thus invoking a potential connection to cancer. However, the relationship of this transporter to cancer has not yet been investigated.

These amino acid transporters that are upregulated in cancer can also be exploited for tumor imaging by PET. This approach is analogous to the current use of ^{18}F-deoxyglucose (FDG) in cancer diagnosis based on the selective upregulation of the glucose transporter SLC2A1 (also known as GLUT1) in cancer. If a given transporter is expressed at higher density in tumors than in the surrounding normal tissue, ^{18}F-labeled substrates of the transporter can be used as a tracer for PET scanning to image the tumor. Several PET probes have been designed and validated for each of the four amino acid transporters discussed in this review (Table 1). Again, all of them are still at the preclinical stage. On the same rational basis, the amino acid transporters that are upregulated in cancers can also be exploited for tumor cell–selective delivery of chemotherapeutic agents. Some of these transporters are amenable for drug delivery (39). Anticancer drugs can be designed or chemically modified so that they are recognized as transportable substrates for these transporters, thus enabling the delivery of such drugs selectively into tumor cells. For example, the histone deacetylase inhibitors butyrate and propionate become substrates for the amino acid transporter SLC6A14 if they are presented in the form of amino acid esters (40). Similarly, selective amino acid derivatives of naproxen show enhanced potency in colorectal cancer prevention than the parent drug (57), most likely via preferential entry of the derivatives into cancer cells facilitated by some amino acid transporters.

Conclusion

Cancer has proven to be a very difficult disease to treat. Most of the currently used anticancer drugs are either nonselective for tumor cells or extend the lifespan of the patients only in terms of a few months to years. Obviously, new molecular targets are sorely needed to make progress in cancer treatment. Nutrient transporters that are upregulated in cancer provide logical and attractive, but yet unexplored, targets for the design and development of a brand new class of anticancer drugs. Because the transporters are cell-surface proteins, functional blockade of such targets can be achieved not only with selective small molecules but also with blocking antibodies. Metabolic reprogramming is a hallmark of cancer; in recent years, tumor

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cell–specific metabolic pathways have been gaining increasing attention, but the fact that nutrient transporters lie upstream of these metabolic pathways has gained relatively less attention. Interfering with the functions of these transporters with small molecules or monoclonals would automatically suppress the tumor-specific metabolic pathways because of the lack of the substrates to fuel the pathways. As such, this therapeutic approach is simple and logical, and is prime for exploitation as a novel new strategy for cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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