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Metabolic control of cancer metastasis: role of amino acids at secondary organ sites

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Abstract

Most cancer-related deaths are caused by the metastases, which commonly develop at multiple organ sites including the brain, bone, and lungs. Despite longstanding observations that the spread of cancer is not random, our understanding of the mechanisms that underlie metastatic spread to specific organs remains limited. However, metabolism has recently emerged as an important contributor to metastasis. Amino acids are a significant nutrient source to cancer cells and their metabolism which can serve to fuel biosynthetic pathways capable of facilitating cell survival and tumor expansion while also defending against oxidative stress. Compared to the primary tumor, each of the common metastatic sites exhibit vastly different nutrient compositions and environmental stressors, necessitating the need of cancer cells to metabolically thrive in their new environment during colonization and outgrowth. This review seeks to summarize the current literature on amino acid metabolism pathways that support metastasis to common secondary sites, including impacts on immune responses. Understanding the role of amino acids in secondary organ sites may offer opportunities for therapeutic inhibition of cancer metastasis.

INTRODUCTION

The metastatic spread of tumor cells from a primary tumor to distant organs is the main contributor to cancer-related death and is still considered to be largely incurable. Despite the undeniable clinical prevalence and impact of metastases, the metastatic cascade itself is highly inefficient—only 0.02% of circulating tumor cells (CTCs) will go on to produce

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COMPETING INTERESTS

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clinically detectable metastases [1]. In 1889, English surgeon Stephen Paget observed that the secondary sites to which tumors metastasized did not seem to be completely random. He hypothesized a “seed and soil” model of metastasis, postulating that CTCs, the “seeds,” could not successfully take root and establish secondary tumors if the “soil” or the metastatic site, was not a suitable environment for outgrowth. To extend Paget’s metaphor, recent evidence indicates that the prevalence of nutrients in this “soil” could be a key aspect in the fitness of tumor cells.

Since the first observations of the Warburg effect, researchers have been investigating how nutrients influence the behaviors of tumor cells. Today, cancer-associated metabolic adaptation is widely considered to be a hallmark of cancer and an essential component of metastatic capability. Of particular significance, cancer cells utilize amino acids and their various metabolic byproducts in a myriad of critical processes that support the survival and progression of cancer cells (Fig. 1). First, and perhaps most obviously, rapidly dividing cancer cells must maintain a sufficient amino acid pool from which to pull building blocks for protein biosynthesis [2]. These amino acids are capable of entering the tricarboxylic acid (TCA) cycle to contribute to the cell’s supply of ATP [2] and of being converted to lipids and nucleosides [3–5]. Beyond this, amino acids can act as nutrient signals, including as neurotransmitters, to trigger the activation of important signaling pathways, and play key roles in the epigenetic modification and regulation of gene expression [6–8]. Finally, these important biomolecules function in the maintenance of intracellular redox status via the production of the antioxidant glutathione [3, 5, 9].

In this review, we will discuss the ways in which tumor cells leverage amino acid metabolism to effectively survive and form macro-metastases in a variety of secondary tissues. While other critical metabolic pathways like glycolysis and fatty acid oxidation contribute to amino acid biosynthesis in cancer cells, their importance in metastasis has been reviewed elsewhere [2–5, 9–11]. We also summarize the impact of amino acid on immune cells in tumor microenvironment and potentials for leveraging this knowledge for therapeutic intervention.

METABOLIC ADAPTATION IN METASTASIS

The metastatic cascade consists of multiple steps, including invasion of tumor cells into surrounding tissues, intravasation and survival in the circulation, and extravasation and outgrowth into distant niches (Fig. 2). In the earliest steps, metastatic cells at the primary tumor acquire migratory and invasive capabilities that are energy demanding, necessitating sufficient ATP supplies [12]. Indeed, enhancing amino acid metabolism can support the necessary elevation in ATP production. Invasive ovarian cancer cell lines are more glutamine dependent than those that are non-invasive, while genetically targeting glutaminase (GLS), an enzyme that converts glutamine to glutamate, reduces the invasive and metastatic capacity of colorectal cancer cells [13–15]. Similarly, overexpression of PSAT1, an important enzyme involved in the serine biosynthetic pathway, promotes invasion and metastatic colonization of non-small cell lung cancer cells [16]. Asparagine utilization supports a migratory and invasive phenotype in breast cancer cells through induction of the epithelial-to-mesenchymal transition (EMT) [17], indicating that metabolic rewiring can directly support metastatic

progression. Other evidence suggests that reprogramming of amino acid metabolism may be a consequence of oncogenic signaling or environmental stressors. Increased expression of asparagine synthetase, glutamic oxaloacetic transaminase 2, and *GLS* are observed upon activation of SOX12, a transcription factor that promotes EMT and is associated with metastatic progression in colorectal and breast cancer patients [18]. Likewise, environmental factors such as hypoxia and purine depletion support elevated glutamine metabolism and serine biosynthesis, respectively [15, 19].

As disseminating tumor cells enter the vasculature, they encounter a stressful environment where few escaping cells survive. Most circulating tumor cells (CTC) will succumb to anoikis. While evidence related to amino acid metabolism in CTCs is limited, glutamine metabolism may also support survival of CTCs. The conversion of glutamate to α-ketoglutarate by glutamate dehydrogenase promotes anoikis resistance in metastatic lung cancer cells [20]. Consistent with enhanced glutamine metabolism in CTCs, the plasma of metastatic esophageal squamous cell carcinoma patients was found to exhibit reduced glutamine but elevated glutamate levels [21]. Breast cancer CTCs also display a preference for oxidative phosphorylation (OXPHOS) [22–26], possibly reflecting an increased requirement for ATP synthesis. Indeed, glutamine-derived α-ketoglutarate feeds into OXPHOS and ATP production through the TCA cycle. While additional studies are necessary to fully elucidate the role of amino acid metabolism in CTCs, it is intriguing to speculate that many of the same pathways that contribute to survival and migration within the primary tumor may also support metastatic spread through the circulation.

Metastatic tumors that develop at the secondary sites are traditionally thought to behave similarly to the primary tumors. Indeed, in some cases disruption of metabolic pathways leads to similar phenotypes in cancer cells both in primary tumors and secondary metastasis. However, recent studies in single-cell RNA-seq revealed distinct expression profiles in metabolic pathways between primary and secondary lesions [27–29]. Although the mechanism by which different metabolic states arise in primary tumors and metastases is not well understood, at least two possibilities, not mutually exclusive, could be envisioned. Because primary tumors contain heterogenous cancer cell populations, certain metabolic traits previously existing or acquired by subpopulations of cancer cells may have distinct advantages to survive during metastatic spread or during outgrowth in secondary organs. Alternatively, disseminated cancer cells may evolve and adapt to local environment to promote metastatic outgrowth. Regardless of parallel or unique metabolic attributes of primary versus metastatic tumors, organotropism of tumor metastasis could be metabolically affected by many factors, including nutrient availability in organ-specific niches and competition for nutrients between tumor cells and immune cells in the tumor microenvironment.

NUTRIENT AVAILABILITY IN ORGAN-SPECIFIC NICHES

Brain metastasis

Although the brain is an extremely energy-demanding organ, the interstitial environment does not contain a substantial energy reserve, allowing neuronal signaling fidelity [6]. Instead, this critical organ compensates for the lack of reserves through metabolic plasticity,

efficiently utilizing a variety of alternative metabolites when blood glucose is low [7, 8]. It is becoming clear that metastatic cancer cells must also demonstrate nutrient flexibility in order to survive in the brain.

While glucose reserves are more limiting, the interstitial space within the brain is highly abundant in both glutamine and branched-chain amino acids (BCAAs) [30–33]. In addition to its role as an energetic and biosynthetic substrate, the brain utilizes glutamine to support a high rate of glutamate synthesis, which can function as a neurotransmitter [34]. It is primarily BCAAs that serve to synthesize glutamate—at least two-thirds of the amino groups incorporated into brain glutamate are taken from BCAA-derived keto acids [32]. There is also evidence that brain metastatic tumor cells utilize these readily available BCAAs. Positron emission tomography detection of brain metastasis revealed improved sensitivity of ^{11}C-BCAA tracers compared to the traditional ^{18}FDG glucose analog, suggesting BCAAs are a more favored fuel source of brain metastatic cells [35]. Indeed, brain-tropic triple-negative breast cancer (TNBC) cells possess more activated branched-chain ketoacid dehydrogenase E1 and oxidize more BCAAs than their parental counterparts [36]. These findings suggest that brain metastatic cells efficiently utilize BCAAs to fulfill their energetic needs.

The importance of glutamine metabolism in brain metastatic cells was recently illustrated by Parida and colleagues in a HER2+ breast cancer brain metastasis model [37]. Cells derived from latent or metachronous metastases relied on glutamine metabolism, taking more time to influence the brain microenvironment. This is in contrast to cells derived from rapidly-forming synchronous tumors, which were highly proficient in glucose metabolism, allowing them to outcompete brain native cells for limited environmental glucose. These findings suggest that the interactions between the brain microenvironment and metabolic programming of brain metastatic tumor cells play a significant role in metastatic outgrowth. Importantly, glutamine-dependent disseminated tumor cells (DTCs) were found to be resistant to HER2-targeted therapies at least partially through improved protection against oxidative stress. Glutamine utilizing DTCs exhibited increased expression of the *SLC7A11*/xCT cystine/glutamate antiporter to support synthesis of glutathione (GSH), a prominent ROS sink [37]. Sensitivity to HER2 inhibitors was restored through xCT inhibition, suggesting that glutamine metabolism serves to support biosynthetic pathways as well as redox balance during metastatic outgrowth in the brain.

Metabolic adaptation to ROS is not the only way that brain metastatic cells modulate glutamine metabolism to fit into their new niche. In fact, brain metastatic breast cancer cells have been shown to parasitize a glutamate-dependent neuronal signaling pathway to steal nutrients [38]. Glutamate is released by excitatory glutamatergic presynaptic neurons and quickly taken up by postsynaptic neurons [39]. Brain metastatic breast cancer cells appear to co-opt this process, establishing pseudo-tripartite synapses to access glutamate secreted by presynaptic neurons (Fig. 3A). Targeting the glutamate N-methyl-D-aspartate receptor in breast cancer cells significantly decreased brain metastatic burden not affecting the growth of primary orthotopic tumors or lung metastases [38]. Unfortunately, targeting synaptic glutamate theft may be difficult to put into practice due to potential neurotoxicity. Of note, primary glioma cells also appear to “steal” glutamate from presynaptic neurons by

upregulating glutamine and glutamate transporters [34, 40, 41]. This similarity in adaptation strategies of brain metastatic and primary brain cancer cells supports the idea that the “soil” governs metabolic adaptations in cancer cells.

Despite the prevalence of glutamine and BCAAs in brain tissue, levels of other critical amino acids are greatly restricted, challenging metastatic tumor growth. To generate sufficient nucleotide pools, highly aggressive brain metastatic cells increase de novo biosynthesis of serine by enhancing expression of phosphoglycerate dehydrogenase (*PHGDH*), which catalyzes the rate-limiting step [42]. Pharmacological inhibition and genetic attenuation of PHGDH suppressed growth of brain metastases but not extracranial metastases or primary tumors [42]. Interestingly, aggressive brain metastatic clonal derivatives of the breast cancer cell line MDA-MB-231 exhibited a higher increase in glucose-derived serine than similar indolent lines. This observation dovetails nicely with Parida and colleagues’ model of aggressive glucose-dependent and more latent glutamine-dependent brain metastatic breast cancer cells [37]. Taken together, these data suggest that brain-tropic DTCs must adopt a similar metabolic cooperativity to cells native to the brain milieu to successfully compete for the limited energy reserves.

Bone metastasis

Bone is among the most common sites of metastasis and bone lesions are a major cause of the morbidity [43]. A greater frequency of bone metastases is osteolytic, which stimulates bone destruction [8]. Breakdown of the hard bone tissue seems to be necessary to provide tumor cells with additional space to expand [44]. Notably, this bone destruction is not directly mediated by bone metastatic tumor cells themselves, but rather through activation of osteoclasts, bone-native cells that mediate bone resorption [45]. This phenomenon is known as the “vicious cycle” of bone metastasis, which is initiated by DTCs that secrete cytokines and other factors such as parathyroid hormone-related protein (PTHrP) to act on osteoblasts and trigger the production of RANKL and other osteolytic factors. These factors drive osteoclast differentiation and increased bone destruction that releases growth factors like TGF-β that further stimulate tumor cell proliferation [46]. While breast-to-bone metastases are often osteolytic, the majority of prostate cancer bone metastases are osteoblastic lesions, which stimulate abnormal bone formation [8]. While the advantages of osteoblastic lesions are less apparent, these cancer cells produce osteogenic factors to activate osteoblasts to deposit a new, but not yet mineralized matrix, that is rich in growth factors [47]. This especially fertile “soil” is quickly co-opted by tumor cells [47].

Like cancer cells, amino acids are integral to the identity and function of bone-remodeling cells. During differentiation, osteoblasts increase expression of the glutamine transporters *SLC1A5*/ASCT2 and *SLC7A7*/γ(+)-LAT1 to stimulate glutamine uptake [48]. Once inside the cell, glutamine catabolism by GLS has been shown to regulate lineage allocation of skeletal stem cells. Conditional ablation of GLS expression in mesenchymal progenitors decreased their proliferation and bone formation in mice [49]. Specifically, GLS was identified as a requirement for commitment to the osteoblast lineage since loss of GLS appears to bias toward adipogenesis [49]. While glutamine appears to be critical for osteoblast differentiation, evidence suggests that glutamine serves as an important precursor

to proline, which is necessary for translation of proline-rich bone matrix proteins (OCN, COL1A1) and other osteoblast-associated proteins (OSX, RUNX2). Expression was found to be dependent on sufficient exogenous glutamine and proline supplied by *SLC38A2*/SNAT2 uptake [50], suggesting that environmental supplies of proline or glutamine can significantly impact osteoblast differentiation and bone formation (Fig. 3B). Tumor cells have been demonstrated to be one of the primary consumers of glutamine in tumors, resulting in a limited supply of interstitial glutamine [51, 52]. Therefore, it is reasonable to postulate that amino acids may become limiting during metastatic outgrowth to alter normal osteoblast function [53].

Similar to osteoblasts, osteoclasts upregulate expression of *SLC1A5*/ASCT2 and *GLS* to enhance glutamine uptake and utilization required for activation [54]. However, α-ketoglutarate supplementation epigenetically promotes *SLC7A11*/xCT, reducing osteoclastogenesis through ROS-dependent induction of NFATc1 osteoclast transcriptional program [55]. While counterintuitive to osteolytic progression, the relative levels of glutamate and α-ketoglutarate may be limited through BCAA metabolism. RANKL-induced osteoclast differentiation steadily accumulates intracellular BCAAs, which are catalyzed by branched-chain aminotransferase 1 (BCAT1) to generate glutamate and alpha-keto acids [56]. Osteoclasts also appear to readily utilize arginine and methionine for energy production through anaplerosis or epigenetic repression of anti-osteoclastogenic genes upon RANKL stimulation [57, 58] (Fig. 3B). Lastly, evidence suggests a potential cooperation for serine may exists between osteoclasts and cancer cells. A bone tropic variant of the MDA-MB-231 human breast cancer cell line significantly upregulated expression of key genes involved in the serine synthesis pathway, including *PHGDH*, phosphoserine aminotransferase 1 (*PSAT1*), and phosphoserine phosphatase [59]. Excess serine exported into the tumor interstitium by ASCT2 [60] can serve to stimulate the differentiation of osteoclasts and bone resorption in vitro [59, 61]. Although bone resorption is a energetically demanding process, evidence supports the concept that osteoclasts are more metabolically adaptable to limiting nutrients within the bone metastatic microenvironment.

Lung metastasis

As with other organ sites, CTCs must successfully extravasate through the lung endothelium and adapt themselves to the nutrient milieu of their new home. Circulating neutrophils and platelets in the lung microvasculature interact with platelets and CTCs to support successful extravasation of CTCs into the lungs [62, 63]. Neutrophils can release web-like extracellular chromatin networks, called neutrophil extracellular traps (NETs), that can facilitate the clustering of CTCs by “catching” and holding them together, thus improving their chances of survival in circulation [63, 64] (Fig. 3C). Circulating NET levels were significantly increased in advanced esophageal, gastric, and lung cancer patients compared to patients with local disease or healthy controls [63]. Interestingly, the process of NETosis is highly dependent on amino acids, controlled by peptidyl arginine deaminase type IV, which converts arginine to citrulline on histones to support NET formation and nuclear membrane disruption [63, 65]. In platelets, the purine nucleotide adenine is converted to ATP and packaged in dense granules that are released upon interaction with CTCs [66]. Released adenine binds to purinergic receptors (P2Y2) on endothelial cells to facilitate extravasation

[66, 67]. Consistently, targeting this cellular interaction using chronic low-dose aspirin therapy or blockade of the adenosine-P2Y2 signaling axis reduced tumor cell metastasis in mouse models [66, 67]. Taken together, these findings indicate how amino acid metabolism may contribute to the high rate of lung metastases despite the relative competence of the lung endothelial barrier.

Recent data has shown that lung metastatic breast cancer lesions upregulate the serine biosynthetic pathway compared to the primary tumor. The lung metastatic niche is enriched with the glycolytic product pyruvate, which lung metastatic cells utilize via MCT2 upregulation to fuel serine synthesis [68]. A key byproduct of PSAT1 in the serine biosynthetic pathway is α-ketoglutarate (α-KG), an important metabolic activator of mTORC1 signaling. Expression of the rate-limiting enzyme PHGDH was required for sensitivity to mTORC1 inhibitors in breast cancer lung metastases, but not primary tumors [68], indicating the importance of this pathway in lung metastasis. Consistent with this concept, the PHGDH inhibitor BI-4916 has no effect on the growth of primary breast tumors, but strongly inhibits pulmonary metastases in mice [69]. Further insight into the mechanism of how this metabolic switch facilitates breast cancer lung metastasis was provided in a study by Elia et al. where α-KG produced in lung-metastatic breast cancer cells activated collagen hydroxylation, allowing DTCs to favorably remodel the extracellular matrix in the lung metastatic niche [70]. In agreement, several studies have also indicated the importance of GLS and glutamine metabolism, an important producer of α-ketoglutarate, in establishment of lung metastasis in mouse models [52, 71]. Taken together, these data demonstrate how increased de novo serine synthesis is advantageous to lung-metastatic cells (Fig. 3C). It is interesting to note that while upregulation of glutamine metabolism and serine biosynthesis appears to be important in all three major metastatic sites discussed in this review, the mechanisms through which it exerts pro-metastatic effects do appear to be somewhat organ-specific.

The lung's function as the primary organ of respiration is one of the factors that makes this microenvironment unique. In this capacity, the lung is exposed to high levels of oxygen as well as inhaled toxins, leading to elevated levels of tissue-intrinsic oxidative stress [8, 72]. Logically, this implies that lung metastatic cancer cells would require a dependable system for dealing with ROS. Indeed, lung metastatic breast cancer cells upregulate expression of xCT, which supports GSH production in order to combat ROS [73]. Additionally, a study in colorectal cancer revealed that the $BRAF^{V600E}$ mutation induced expression of glutamate-cysteine ligase (GCL), the rate-limiting enzyme in GSH synthesis [74]. Quenching of ROS through GSH production was shown to be critical for lung metastasis formation but did not affect primary tumor growth or peritoneal metastasis [74]. Interestingly, the GSH pathway may also support development of lung metastasis by promoting cell adhesion. Glutathione peroxidase-1, which serves to reduce peroxides using GSH, acts as a redox safeguard of FAK kinase activation and cell attachment of TNBC cells, a pathway that is necessary for lung metastasis in vivo [75]. These data indicate that DTCs must develop the capacity to metabolically adapt to the increased oxidative stress intrinsic to the lung.

TUMOR–IMMUNE CELL INTERACTION IN THE MICROENVIRONMENT

Tumor cells at different metastatic sites encounter and interact with varied populations of tissue-specific cells. Aside from several specific examples of these interactions discussed above, there is an additional population of “neighbors” that DTCs must contend with no matter where in the body they end up – immune cells. This becomes significant as many of the metabolic pathways that are so integral to cancer cell growth and survival are important to all highly proliferative cells, including both pro- and antitumorigenic immune cells [76–78]. This shared demand for increasing amounts of cellular “fuel” can create a potentially competitive microenvironment as tumor cells and immune cells battle to take up greater amounts of dwindling nutrients. Notably, current work directly probing tumor-immune interactions in specific metastatic sites is limited. This is largely due to experimental challenges posed by specific metastatic models and sites. However, the possible crosstalk between tumor cells and immune cells cannot be discounted when conceptualizing the metastatic niche. For example, the availability of oxygen in the lung is likely not only to impact tumor cells as discussed above, but also affect immune cells. Consequently, investigating the interaction between organ-specific niche cells and immune cells will open up new avenues for targeting organ-specific metastasis.

Based on evidence from the first oncogenic drivers that were found to dysregulate cell metabolism, it was originally hypothesized that cancer cells were the predominant metabolic consumers within the tumor, serving to deplete nutrients in the tumor microenvironment. Early studies suggested that excessive glucose consumption by cancer cells could suppress antitumor functions of T cells [79, 80]. However, later experiments utilizing the labeled glucose analog ^{18}F-2DG showed that cancer cells were not, in fact, the primary consumers of glucose in the tumor microenvironment (TME);rather, experiments showed that they lagged behind both myeloid cells and, to a lesser degree, T cells in glucose uptake. Cancer cells were found to competitively out-consume immune cells for glutamine [51]. Several studies have indicated that targeting glutamine metabolism can increase glutamine availability and improve antitumor immune responses in solid tumors [52, 81, 82]. Inhibition of glutamine metabolism has been shown to further increase glucose uptake by immune cells, suggesting this metabolic partitioning may be a consequence of glutamine restriction [51].

Similarly, cancer cells exhibit elevated consumption of other environmental amino acids, potentially depriving immune cells of these nutrients. It has recently been shown that cancer cells increase their uptake of extracellular methionine by upregulating the *SLC43A2*/LAT4 methionine transporter, leading to a decrease in available methionine and reduced intracellular SAM in $CD8^+$ T cells. Mechanistically, this results in loss of dimethylation at lysine 79 of histone H3 and a consequent decrease in expression of STAT5 and impaired antitumor $CD8^+$ T cell function [83]. Cancer cell consumption of extracellular arginine reduces arginine availability to T cells and consequently downregulates T cell mTORC1 activity, reducing T cell effector functions [84–87]. Finally, cancer cells have also been shown to take up the majority of extracellular serine and cysteine, the former of which is integral to T cell expansion and effector function and the latter of which is required for T cell activation [88–90].

Altered amino acid metabolism of cancer cells can not only impact immune cell responses through depleting nutrient supply but also by generating immunosuppressive metabolites. The most notable example comes from tryptophan, an essential amino acid that cannot be synthesized by the body and must instead be obtained entirely from the diet. The amount of extracellular tryptophan is an important factor in determining the magnitude and quality of a T cell response, and T cell proliferation and activation are strongly suppressed when cells are cultured in tryptophan-free media [91–93]. Cancer cells do not only take up the limited tryptophan in the microenvironment, but they also metabolize it to produce kynurenine. Kynurenine is the ligand for the aryl hydrocarbon receptor (AHR) which exerts a pro-tumor effect on T cells, including stimulating differentiation of $CD4^+$ T cells to immunosuppressive Tregs [94–96]. In $CD8^+$ T cells, kynurenine and AhR activation has been shown to induce expression of the immune checkpoint receptor PD-1 [94–98]. This example showcases a phenomenon wherein cancer cells “steal” essential nutrients from immune cells and release a processed form to further encourage immune suppression. Another example of this can be seen in the increased expression of Arginase-1 in pro-tumorigenic tumor-associated macrophages (TAMs). This leads to enhanced consumption of extracellular L-arginine, which, as previously stated, is essential for effective induction of T cell effector functions [84–87]. Additionally, the arginine that is consumed is used in the synthesis of highly immunosuppressive polyamines [99–102].

These effects and those more completely reviewed elsewhere [4, 103, 104] are important to consider in the context of metastatic tumors, but are likely significant to different degrees in distinct metastatic sites because of their other properties, including oxygen saturation and extracellular pH, which can also influence metabolic programming. Further research into tumor-immune metabolic communication in specific metastatic sites is necessary to more completely characterize these interactions and to identify metastasis-specific therapeutic targets.

TARGETING AMINO ACID METABOLISM IN METASTASIS

The critical nature of amino acid metabolism in the process of metastasis, including roles in cancer cell outgrowth and interactions with niche cells at various metastatic sites present attractive potential therapeutic targets. Such therapies have great potential to be used in concert with existing therapies, adding to the antitumor effect without substantially contributing to the side effect profile of the combination. Furthermore, influencing microenvironmental metabolism could theoretically lead to tumor cell starvation without indiscriminately inducing cytotoxicity. As this potential has become more apparent preclinically, an increasing number of new targeted drugs and drug combinations are being tested (Table 1, Fig. 1). Even though these novel therapies have not yet been widely tested in metastatic disease, the mechanisms through which they act are not constrained to primary tumors and thus may provide some benefits in the treatment of metastatic cancer.

Most therapies targeting amino acid metabolism are aimed at stymying glutamine metabolism in cancer cells, including reducing glutamine uptake or blocking its utilization. It has recently been shown in preclinical models that co-treatment with the S6K inhibitor PF-4708671 and the glutamine uptake inhibitor V-9302 reduces glutamine uptake in

paclitaxel-resistant ovarian cancer cells and re-sensitizes them to chemotherapy [105]. Furthermore, a separate study showed that treatment with V-9302 was able to selectively block glutamine uptake in triple-negative breast cancer cells but not in tumor-associated $CD8^+$ T cells [52]. This shows a particularly promising angle for targeting glutamine metabolism—not only can it work to starve tumors, but it appears to actively benefit nearby immune cells, thereby driving cancer cell death in multiple ways. Notably, another preclinical drug targeting glutamine metabolism has demonstrated similar effects on both cancer cells and immune cells by targeting glutamine utilization. DRP-104 is a novel pro-drug of the broad-acting glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON), which had shown high antitumor activity but was hampered by a particularly strong complement of side effects. DRP-104 is injected in an inactive form and preferentially converted to active DON at tumor sites, thereby mitigating side effects [106]. More targeted approaches include telaglenastat (CB-839), an orally bioavailable inhibitor of the GLS. Although it has not shown much promise as a monotherapy, it is currently part of several clinical trials in combination with various existing chemo- and immunotherapy drugs (Table 1, Fig. 1).

In addition to these approaches to target glutamine metabolism, some novel treatments targeting the uptake and metabolism of other amino acids are also being tested, including serine biosynthesis. NCT-502 and NCT-503, novel small molecule inhibitors of PHGDH, have each shown preclinical efficacy in suppressing the growth of PHGDH-dependent tumors in both cell culture and xenograft models [107]. Other approaches have sought to induce oxidative-induced ferroptotic cell death by blocking cysteine uptake, but with disappointing results. The erastin analog PRLX-93936 and the kinase inhibitor Sorafenib inhibit xCT to block cysteine uptake. Unfortunately, PRLX-93936 showed poor tolerability in Phase I clinical trials, while sorafenib, which is FDA approved for the treatment of unresectable HCC and advanced renal cell carcinoma, showed poor induction of ferroptosis in most cancer cell lines [108, 109]. Better clinical success has been observed through pharmacological arginine depletion using a PEGylated form of arginine deiminase (ADI-PEG-20). Preclinical studies demonstrated that ADI-PEG-20 enhanced the antitumor efficacy of doxorubicin in melanoma and breast cancer cell lines lacking arginosuccinate synthase 1 [110, 111]. A recent Phase I clinical trial (Table 1) indicated that this combination had some clinical benefit in metastatic HER2- breast cancer patients [112].

Targeting amino acid metabolism is already showing tangible clinical potential in variety of tumor types. As more is learned about cancer metabolism and the different ways that the metabolism of the tumor microenvironment can be manipulated, these therapies will only improve. It remains to be determined, however, if these agents have efficacy in metastatic cancer. Due to the importance of amino acid metabolism in metastatic dissemination and outgrowth, it is intriguing to speculate that these same therapies may have a clinical benefit in the metastatic setting. Therapies that inhibit glutamine metabolism are particularly interesting, especially with availability of pre-clinical and clinical candidates. Bone metastatic tumors may be responsive to glutamine metabolism inhibition, particularly since these metastatic tumors are also characteristically reliant on glutamine metabolism [49, 50, 54]. One possible caveat to using these inhibitors in bone is the essential nature of glutamine for osteoblast lineage commitment [49]. However, it could be possible that impeding osteoblast activation could hinder further osteoclast activation to suppress the

"vicious cycle" of bone metastasis. While targeting glutamine metabolism may be beneficial in treating bone metastases, evidence suggests that these inhibitors may induce unfavorable neurotoxicity in brain metastatic cancers due to dysregulation of glutamate homeostasis [38]. However, it is also easy to see where inhibitors of PHGDH and de novo serine synthesis could be applied to metastatic disease at brain, bone, and lung. Elevated serine biosynthesis has been observed in cancer cells associated with all three organ sites [37, 42, 59–61, 68, 69], suggesting that PHGDH inhibition may be universally effective in metastatic disease. These inhibitors could potentially serve a dual purpose in the bone, not only preventing unchecked proliferation of tumor cells, but by reducing levels of osteoclast-activating serine in the extracellular space. However, it remains to be determined how these inhibitors will impact antitumor T cell activities. Collectively, despite the lack of specific testing of amino acid-targeted drugs in clinical setting, targeted therapies of amino acid metabolism have potential for specific metastatic targeting in combination with established regimens of care.

CONCLUSIONS AND FUTURE DIRECTIONS

A growing body of emerging evidence indicates the presence of organ-specific metabolic vulnerabilities in metastatic tumors. Targeting these vulnerabilities could lead to more successful treatments for existing metastatic lesions and, potentially, to amelioration or prevention of further metastatic spread. In this review, we have summarized evidence indicating that DTCs must adapt to the metabolic demands of the metastatic organ to survive and expand. Current treatment modalities targeting metastatic tumors do not take into consideration the different environments and vulnerabilities of distinct host tissues. Fortunately, as the potential of therapies targeting amino acid metabolism have been increasingly noted preclinically, an increasing number of promising new targeted drugs and drug combinations are being tested (Table 1). In addition, metabolic remodeling at metastatic sites affects the immune microenvironment and impacts antitumor immune response [4]. Understanding of how amino acids are utilized at secondary organs may also provide opportunities to improve immunotherapies. Although further work is inarguably necessary to understand the determinants of organotropism more completely in metastasis, our current knowledge has already resulted in the preliminary development of novel targeted therapies for largely incurable diseases. Metastasis is still the primary cause of cancer-associated death, but further insight into this process could lead to a world in which the successful treatment of metastatic tumors will become commonplace.

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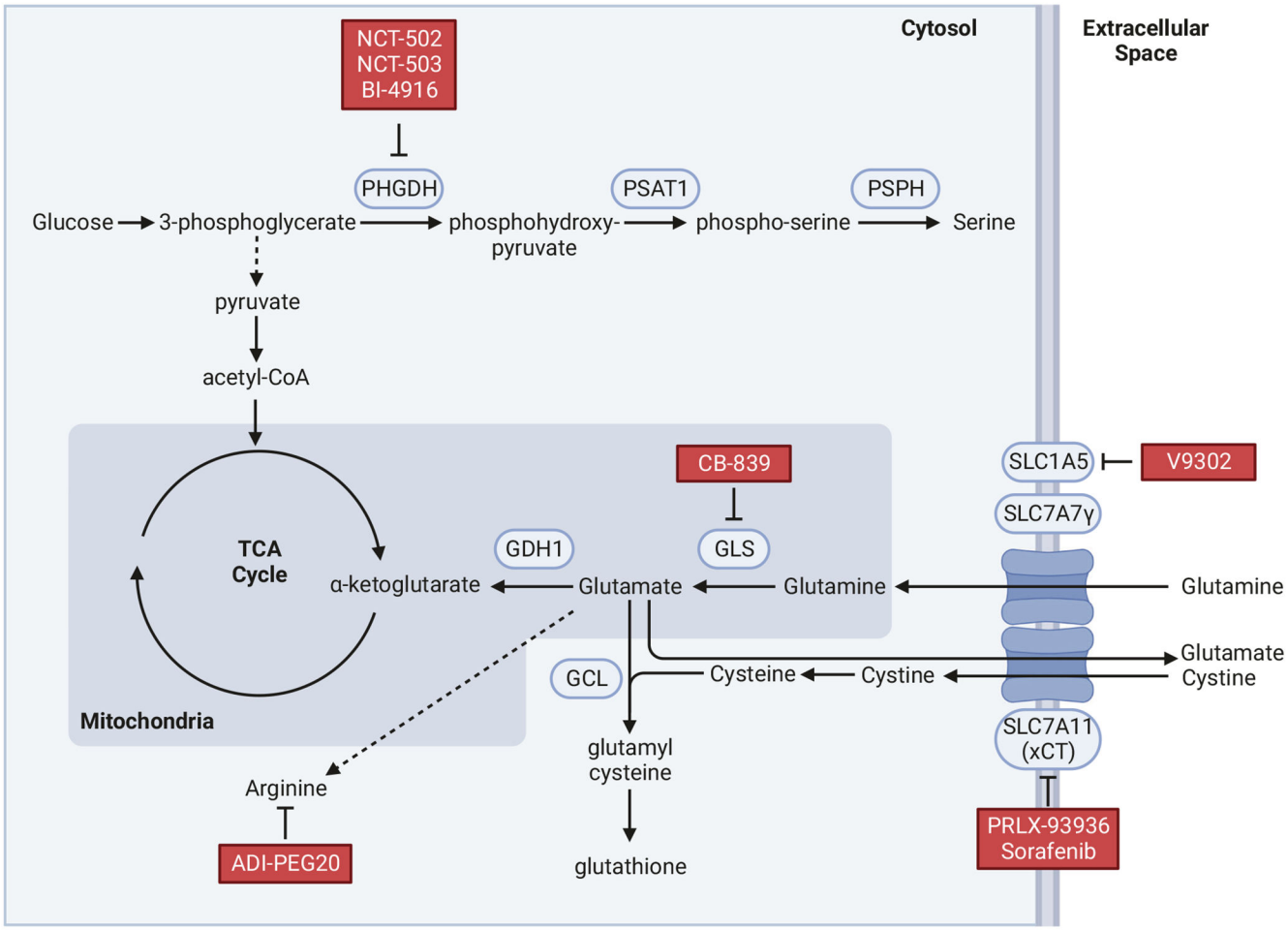

Fig. 1. Amino acid metabolic pathways critical in metastasis and key Inhibitors.

A simplified diagram of major pathways of selected amino acid metabolism relevant to metastasis to the secondary sites. Inhibitors in Table 1 for specific enzymes and nutrient transporters are indicated.

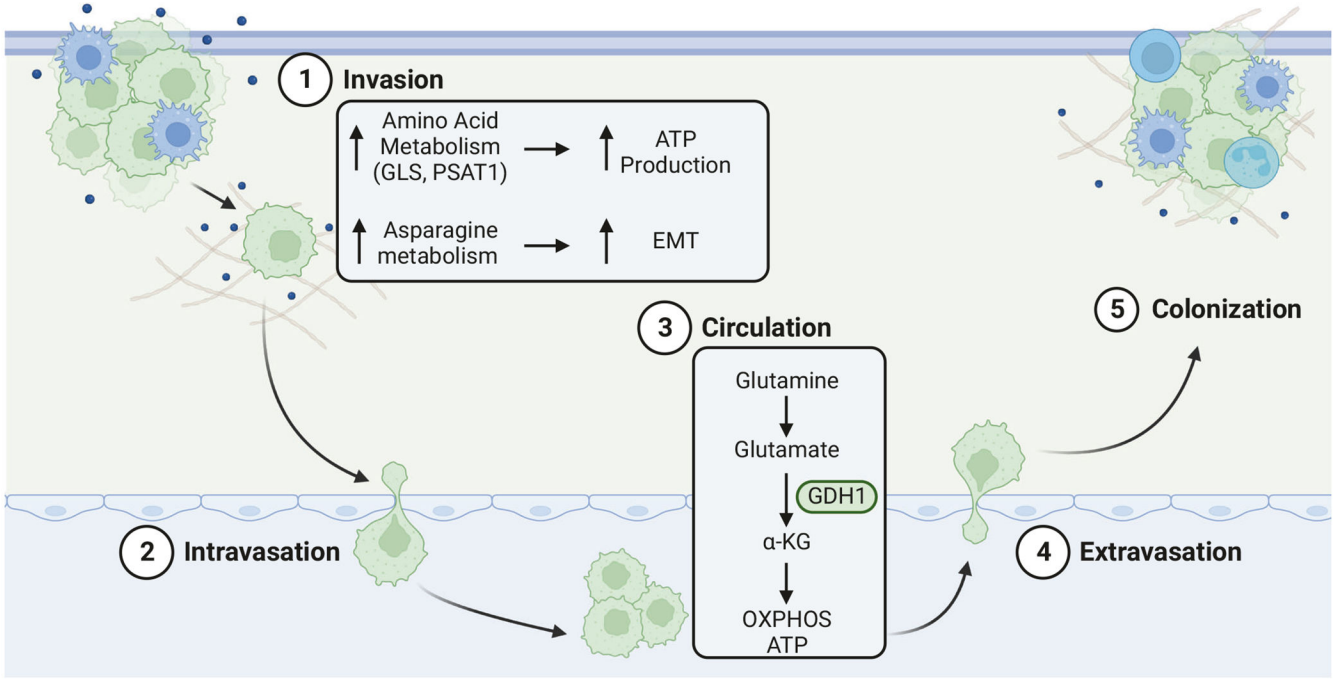

Fig. 2. Amino acid metabolism in the metastatic cascade.

The metastatic cascade consists of multiple steps, including (1) invasion of tumor cells into surrounding tissues, (2) intravasation and (3) survival in the circulation, and (4) extravasation and (5) outgrowth into distant niches.

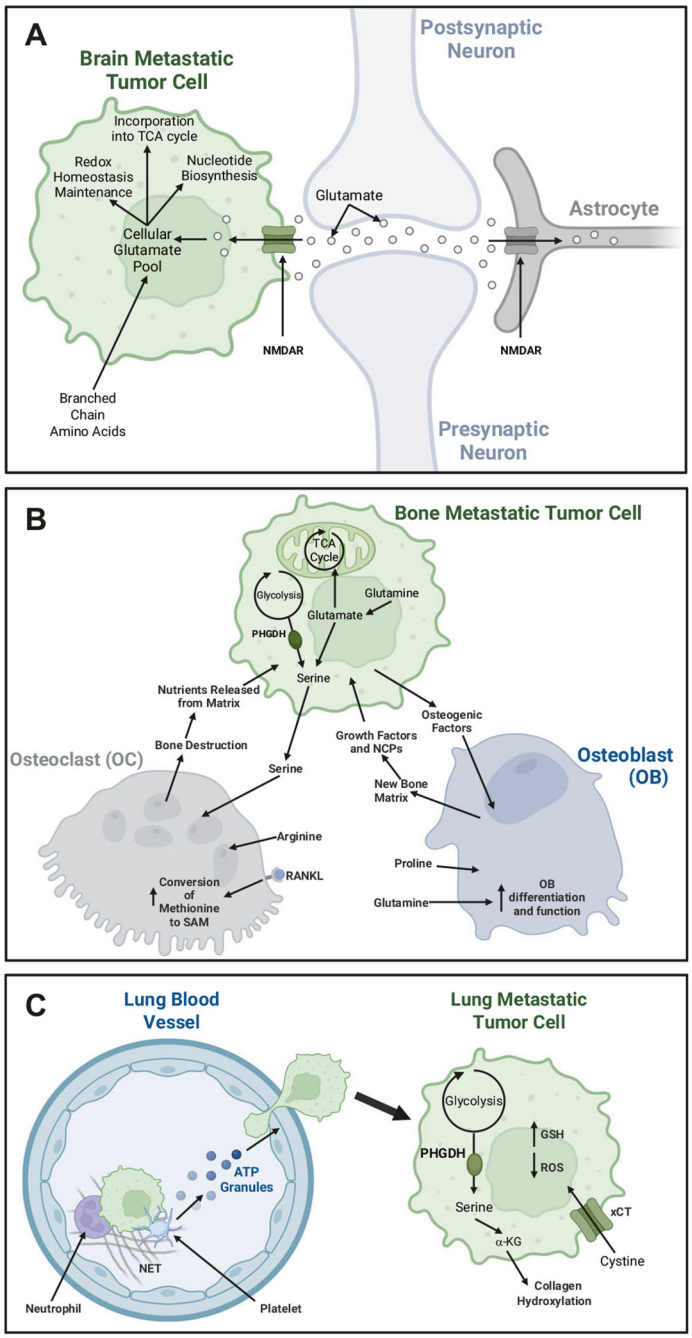

Fig. 3. Amino acid metabolism in secondary niches.

A Amino Acid Metabolism in the Brain Metastatic niche: Brain-metastatic cells take up large amounts of branched-chain amino acids (BCAAs) to meet their energetic needs and upregulate enzymes to oxidize glutamine and BCAAs. The metastatic cells also parasitize a glutamine-dependent neuronal signaling pathway to steal nutrients. **B** Amino Acid Metabolism in the Bone Metastatic Niche: In the bone niche, glutamine metabolism and proline are essential for osteoblast differentiation and linage commitment. While arginine and methionine appear to be critical in osteoclast differentiation and function. Bone

metastatic breast cancer cells have also been shown to promote osteoclast activation through modulation of serine metabolism. **C** Amino Acid Metabolism in the Lung Metastatic Niche: During metastasis to the lung, modification of arginine residues is essential to the formation of neutrophil extracellular traps (NETs), which allow CTCs to survive in the bloodstream. Lung metastatic breast cancer lesions, but not primary breast tumors, use phosphoglycerate dehydrogenase (PHGDH) and the serine biosynthesis pathway to support mTORC1-mediated growth signaling. Production of α-ketoglutarate (α-KG) activates collagen hydroxylation, allowing DTCs to favorably remodel the extracellular matrix in the lung metastatic niche. Increased xCT expression is also increased in metastatic cells, supporting glutathione (GSH) production to combat oxidative damages.

Table 1.

Selected agents targeting amino acid metabolism for cancer treatment.

	Target	Treatment	Stage	Cancer Type	Reference(s)
Glutamine	GLS1	CB-839+cabozantinib	Phase II	Renal cell carcinoma	NCT03428217
		CB-839 + talazoparib	Phase Ib/II	Solid tumors	NCT03875313
		CB-839 + paclitaxel	Phase II	TNBC	NCT03057600
		CB-839 hydrochloride + sapanisertib	Phase I/Ib	NSCLC	NCT04250545
		CB-839 + Panitumumab and Irinotecan	Phase I/II	Metastatic and refractory RAS wildtype colorectal	NCT03263429
		CB-839 + Azacitidine	Phase I/II	Advanced myelodysplastic syndrome	NCT03047993
		CB-839 + capecitabine	Phase I/II	Advanced solid tumors, colorectal cancer	NCT02861300
		ACB-839 + palbociclib	Phase Ib/II	Solid tumors	NCT03965845
	ASCT2	V-9302	Preclinical	In vivo mouse models and in vitro	[52, 105, 113, 114]
Cysteine	xCT	Sorafenib	In clinic	Kidney, liver, and thyroid cancer	207012
		PRLX 93936 (erastin analog)	Phase I	Solid tumors	NCT00528047
Serine	PHGDH	NCT-502	Preclinical	In vitro	[107, 115]
		NCT-503	Preclinical	In vitro and mouse	[107]
Arginine	Arginine auxotrophic cancer cells	ADI-PEG-20	Phase II	Metastatic Melanoma	NCT00450372
		ADI-PEG 20	Phase II	Non-Hodgkin’s Lymphoma	NCT01910025
		ADI-PEG 20	Phase I	Cutaneous Melanoma, Uveal Melanoma, Ovarian Carcinoma or Other Advanced Solid Tumors	NCT01665183
		ADI-PEG 20	Phase II	Acute Myeloid Leukemia	NCT01910012
		ADI-PEG 20	Phase I	HER2 Negative Metastatic Breast Cancer	NCT01948843