Published in final edited form as:
Oncogene. 2010 January 21; 29(3): 313–324. doi:10.1038/onc.2009.358.

Q's next: The diverse functions of glutamine in metabolism, cell biology and cancer

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Abstract

Several decades of research have sought to characterize tumor cell metabolism in the hopes that tumor-specific activities can be exploited to treat cancer. Having originated from Warburg's seminal observation of aerobic glycolysis in tumor cells, most of this attention has focused on glucose metabolism. However, since the 1950s cancer biologists have also recognized the importance of glutamine (Q) as a tumor nutrient. Glutamine contributes to essentially every core metabolic task of proliferating tumor cells: it participates in bioenergetics, supports cell defenses against oxidative stress, and complements glucose metabolism in the production of macromolecules. Interest in glutamine metabolism has been heightened further by the recent findings that c-*myc* controls glutamine uptake and degradation, and that glutamine itself exerts influence over a number of signaling pathways that contribute to tumor growth. These observations are stimulating a renewed effort to understand the regulation of glutamine metabolism in tumors and to develop strategies to target glutamine metabolism in cancer. Here we review the protean roles of glutamine in cancer, both in the direct support of tumor growth and in mediating some of the complex effects on whole-body metabolism that are characteristic of tumor progression.

Keywords

Glutamine; metabolism; cancer; Warburg effect; cachexia

Introduction

Tumors are metabolic entities. They draw nutrients from the bloodstream, consume them through biochemical pathways, and secrete waste products which then become substrates for metabolism elsewhere in the body. The metabolism of tumors has fascinated cancer biologists since Warburg's experiments in the 1920s demonstrated that ascites tumor cells from the mouse were capable of unexpectedly high rates of glucose consumption and lactate secretion in the presence of oxygen (the Warburg effect). Because this behavior was so different from the differentiated tissues he used in comparison, those early observations stimulated the hope that the metabolic idiosyncrasies of tumors could be exploited to benefit cancer patients (Warburg,

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Conflict of interest
The authors declare no conflicts of interest.

1925; Warburg, 1956). In terms of tumor imaging, this has turned out to be true: the utility of ^{18}FDG-PET and ^{1}H magnetic resonance spectroscopy in human cancer depend precisely on the ability of these modalities to detect glucose uptake and lactate production. Interest in the Warburg effect as an Achilles' heel to be exploited in cancer treatment has been further stimulated by the demonstration that enhanced glucose metabolism is a common consequence of many of the mutations responsible for human cancer, and therefore may be a central process essential for tumor growth (Elstrom *et al.*, 2004; Flier *et al.*, 1987; Kroemer and Pouyssegur, 2008; Matoba *et al.*, 2006; Osthus *et al.*, 2000; Shim *et al.*, 1997).

In terms of developing strategies to treat cancer, however, tumor metabolism has so far proven to be more of a Holy Grail than an Achilles' heel. Part of the difficulty lies in the flexibility of metabolic systems and the panoply of nutrients to which tumors have access. Thus a complete picture of the metabolism of any tumor must consider the contribution of multiple nutrients simultaneously. Chief among the other nutrients available to tumors is glutamine, the most abundant amino acid in the plasma and the major carrier of nitrogen between organs. Glutamine is also second only to glucose in terms of persistent interest in its role in tumor cell metabolism, which now dates back more than 50 years (Eagle, 1955; Kvamme and Svenneby, 1960). In culture, tumor cells are avid glutamine consumers, metabolizing it at rates far in excess of any other amino acid (Eagle, 1955). The same is true in tumors implanted onto vascular pedicles in rats to allow precise measurements of the rates of amino acid extraction from the blood (Sauer and Dauchy, 1983; Sauer *et al.*, 1982). These observations led to the notion that glutamine metabolism stood with the Warburg effect as a major component of the general metabolic phenotype of proliferating tumor cells (Kovacevic and McGivan, 1983).

Glutamine's importance in tumor cell metabolism derives from characteristics it shares with glucose. Both nutrients help to satisfy two important needs for proliferating tumor cells: bioenergetics (ATP production) and the provision of intermediates for macromolecular synthesis (DeBerardinis *et al.*, 2008b). A number of excellent discussions on the utilization of glutamine in cancer have previously been presented (Medina *et al.*, 1992; Souba, 1993). Surprisingly, however, only recently has it been reported that oncogenes influence glutamine metabolism as they do for glucose, and that tumor genetics can dictate cellular dependence on glutamine for survival. Furthermore, other studies have been uncovering diverse and unexpected roles for glutamine and its by-products in cell signaling, linking glutamine metabolism to cell survival and growth in ways beyond its roles in intermediary metabolism. Here we review glutamine's metabolic and non-metabolic functions in tumor cells, the integration of glucose and glutamine metabolism in tumor growth, and aspects of whole-body glutamine metabolism that may influence the morbidity and mortality of cancer patients.

Glutamine's roles in intermediary metabolism: functions and consequences

Glutamine has traditionally been viewed as a nonessential amino acid whose primary functions are to store nitrogen in the muscle and to traffic it between organs. Although it contributes only 4% of the amino acid in muscle protein, glutamine accounts for more than 20% of the free amino acid pool in plasma and more than 40% in muscle (Bergstrom *et al.*, 1974; Kuhn *et al.*, 1999). Mammals can synthesize glutamine in most tissues, but during periods of rapid growth or illness, the cellular demand for glutamine outstrips its supply and glutamine becomes essential (hence its designation as a "conditionally" essential amino acid). Proliferating cells display an intense appetite for glutamine, reflecting its incredible versatility as a nutrient and mediator of other processes (Figure 1).

The metabolic fates of glutamine can roughly be divided into reactions that use glutamine for its γ-nitrogen (nucleotide synthesis and hexosamine synthesis) and those that utilize either the α-nitrogen or the carbon skeleton. The reactions in the second category use glutamate, not

glutamine, as the substrate. Although tumor cells tend to have large intracellular pools of glutamate, maintaining these pools rests on the ability to convert glutamine into glutamate, because glutamine is an abundant extracellular nutrient and glutamate is not. This process is largely due to the activity of phosphate-dependent glutaminase (GLS), a mitochondrial enzyme that is highly expressed in tumors and tumor cell lines. Classical experiments demonstrated that glutaminase activity correlates with tumor growth rates *in vivo* (Knox *et al.*, 1969; Linder-Horowitz *et al.*, 1969), and experimental models to limit glutaminase activity resulted in decreased growth rates of tumor cells and xenografts (Gao *et al.*, 2009; Lobo *et al.*, 2000). Thus, while not all the metabolic fates of glutamine require glutaminase activity, this enzyme is essential to the metabolic phenotype of growing tumors.

The following are the major aspects of glutamine-based intermediary metabolism and their relevance to tumor cell growth. They are summarized in Figure 1.

1. Nucleotide biosynthesis

Glutamine is a required nitrogen donor for the *de novo* synthesis of both purines and pyrimidines and is therefore essential for the net production of nucleotides during cell proliferation. The γ (amido) nitrogens of two glutamine molecules are added to the growing purine ring, and a third is used in the conversion of xanthine monophosphate to guanosine monophosphate. Other nitrogens are supplied by glycine and aspartate, but many of these are also initially derived from glutamine (the α-nitrogen). Pyrimidine rings contain one nitrogen from glutamine's amido group and one from aspartate, and an additional amido nitrogen is added to uridine triphosphate to form cytidine triphosphate. The importance of glutamine nitrogen in nucleotide biosynthesis probably explains why some transformed cells exhibit delayed transit through S-phase in low-glutamine conditions (Gaglio *et al.*, 2009). However, the glutamine utilization rate exceeds nucleic acid synthesis by more than an order of magnitude in proliferating cells, and thus nitrogen donation to nucleotides accounts for only a small fraction of total glutamine consumption (Ardawi *et al.*, 1989).

2. Hexosamine biosynthesis and glycolsylation reactions

The rate-limiting step in the formation of hexosamine is catalyzed by glutamine:fructose-6-phosphate amidotransferase (GFAT), which transfers glutamine's amido group to fructose-6-phosphate to form glucosamine-6-phosphate, a precursor for N-linked and O-linked glycolsylation reactions. These reactions are necessary to modify proteins and lipids for their participation in signal transduction, trafficking/secretion and other processes. Impairment of glucosamine-6-phosphate production is thus predicted to reduce cell growth and to interfere with cell signaling. Surprisingly, GFAT ativity could be suppressed by expressing an antisense *GLS* cDNA to decrease glutaminase acitivty in breast cancer cells. This resulted in disturbances of O-linked glycolsylation pathways, altering the glycosylation status of the transcription factor Sp-1 and increasing its transcriptional activity (Donadio *et al.*, 2008). The mechanism by which loss of glutaminase activity influences glycolsylation is unclear, but the findings suggest that GFAT and perhaps other components of the glycosylation machinery are responsive to intracellular glutamine availability as determined by glutaminase.

3. Nonessential amino acids

Because of the high expression of glutaminase, tumor cells are poised to produce glutamate rapidly from glutamine, and a sizable fraction of their glutamate pool carries glutamine's α (amino) nitrogen. This nitrogen is then dispersed into various pools of nonessential amino acids (NEAA) through the activity of transaminases, particularly alanine aminotransferase and aspartate aminotransferase. These enzymes catalyze the reversible transfer of amino groups between glutamate and alanine or aspartate, respectively. Alanine is used in protein synthesis, but is also avidly secreted by tumor cells, carrying some of the excess carbon from glycolysis.

Aspartate, in contrast, remains inside the cell and contributes to the synthesis of proteins and nucleotides and to electron transfer reactions through the malate-aspartate shuttle. In this shuttle, aspartate exits the mitochondria and is converted to oxaloacetate (OAA) by aspartate aminotransferase. OAA is then reduced to malate, using electrons donated by NADH generated in glycolysis. The malate then enters the mitochondria and donates the electrons to Complex I of the electron transport chain. Thus, the shuttle facilitates ongoing ATP production in both the mitochondria (via oxidative phosphorylation) and the cytosol (by re-supplying NAD+ for glycolysis).

4. Glutathione (GSH)

At a concentration of approximately 5 mM, GSH is the major thiol-containing endogenous antioxidant and serves as a redox buffer against various sources of oxidative stress. In tumors, maintaining a supply of GSH is critical for cell survival because it allows cells to resist the oxidative stress associated with rapid metabolism, DNA damaging agents, inflammation and other sources (Estrela *et al.*, 2006). GSH is a tripeptide of glutamate, cysteine and glycine and its formation is highly dependent on glutamine. Not only does glutamine metabolism produce glutamate, but the glutamate pool is also necessary for cells to acquire cysteine, the limiting reagent for GSH production. This occurs by virtue of the X_c^- antiporter, which exports glutamate and imports cystine, as shown in Figure 1. Cystine can then be converted to cysteine inside the cell and used in GSH synthesis. Because of the key role of the X_c^- antiporter in maintaining glutathione levels, it has been suggested as a target for cancer therapy (Lo *et al.*, 2008).

5. Respiratory substrate

Oxidation of glutamine's carbon backbone in the mitochondria is a major metabolic fate of glutamine and a primary source of energy for proliferating cells, including lymphocytes, enterocytes, fibroblasts and some cancer cell lines (DeBerardinis *et al.*, 2008b; Miller, 1999; Reitzer *et al.*, 1979). This requires conversion of glutamine to α-ketoglutarate, typically via glutaminase activity followed by conversion of glutamate to α-ketoglutarate by either transaminases or glutamate dehydrogenase. However, other processes such as the donation of glutamine's amido nitrogen to nucleotides or hexosamines could contribute a fraction of the glutamate pool as well. Overall, this contribution is small, but it might become more prominent during stages of the cell cycle characterized by transient increases in nucleotide biosynthesis or other activities. Thus, proliferating cells, which exhibit high fluxes through a number of biosynthetic pathways, are well-positioned to make use of glutamine's carbon skeleton as a respiratory substrate. Complete oxidation of glutamine carbon involves exit from the TCA cycle as malate, conversion to pyruvate and then acetyl-CoA, and finally re-entry into the cycle (Figure 1). It should be noted, however, that careful studies of tumor cell glutamine metabolism reveal that neither its nitrogen nor its carbon are used to completion *in vitro*. Rather, a high fraction of both of glutamine's nitrogens are secreted from glioblastoma cells as they proliferate (as ammonia, alanine and glutamate), and at least half of its carbon is secreted as lactate (DeBerardinis *et al.*, 2007). This form of rapid glutamine utilization and secretion of glutamine by-products, similar to the Warburg effect in its apparent inefficiency, has been proposed to be an additional hallmark of tumor cell metabolism (Mazurek *et al.*, 2005).

6. Reducing equivalents

One of the benefits of converting glutamine to pyruvate is the reduction of $NADP^+$ to NADPH by malic enzyme (Figure 1). NADPH is a required electron donor for reductive steps in lipid synthesis, in nucleotide metabolism, and in maintaining GSH in its reduced state. Therefore proliferating cells must produce a large supply of it. Although cells contain numerous potential sources of NADPH, in glioblastoma cells the malic enzyme flux was estimated to be high

enough to supply all of the reductive power needed for lipid synthesis (DeBerardinis *et al.*, 2007).

7. Ammoniagenesis

Glutaminase activity generates free ammonia, a potentially toxic metabolite. In glioblastoma cells, the rate at which ammonia is secreted into the extracellular space is about 75% the rate of glutamine disappearance from the medium (our unpublished observations), consistent with a high fraction of glutamine being metabolized in the mitochondria by glutaminase. Without a mechanism to dispose of ammonia rapidly, intracellular ammonia concentrations would reach several hundred mmoles/L within a few hours. It is not known how tumor cells dispose of ammonia during rapid glutamine catabolism. The traditional view held that passive diffusion of the gaseous form (NH_3) across the lipid bilayer accounted for essentially all ammonia transport. This simple model no longer holds for some tissues with a high demand for ammonia transport. In the kidney, where ammonia metabolism is a key mediator of acid-base homeostasis, a number of protein transporters exist to traffic ammonia as NH_3 and/or NH_4^+. These systems include ion channels, aquaporins and Rh glycoproteins (Weiner and Hamm, 2007), some of which are over-expressed in tumors. Although the exact mechanism of tumor cell ammonia secretion has not been established, the process carries therapeutic potential. Blocking ammonia secretion would presumably either suppress net glutamine consumption or cause toxic intracellular accumulation of ammonia, both of which might impair cell survival and growth.

Glutamine's roles in cell signaling and gene expression

Beyond its roles in intermediary metabolism, glutamine exerts other effects that support cell survival and growth. Reflecting the importance of glutamine to anabolic metabolism, cells have developed glutamine-dependent mechanisms to control growth, including the modulation of signal transduction pathways. A recent study revealed the necessity of glutamine for the well-known stimulatory effect of essential amino acids on the mammalian target of rapamycin (mTOR) pathway (Nicklin *et al.*, 2009). In the study, stimulation of mTOR compelx-1 (mTORC1) in HeLa cells required bidirectional transport of glutamine: the cells imported glutamine through the Na^+-dependent transporter SLC1A5 and then exported it through the Na^+-independent transporter SLC7A5 (Figure 1). The latter step was accompanied by import of essential amino acids and subsequent activation of mTORC1, stimulating cell growth while suppressing autophagy. It was the sequential import and export of glutamine itself and not the by-products of glutamine metabolism that facilitated the uptake of essential amino acids, because addition of glutamate or α-ketoglutarate failed to rescue mTORC1 activation in the absence of glutamine. Thus, this mechanism of control relies on glutamine abundance exceeding the cells' metabolic needs, suggesting that glutamine excess is a signal to promote cell growth and suppress catabolism.

Other data has identified a role for glutamine in extracellular signal-regulated protein kinase (ERK) signaling pathways. This has been best characterized in intestinal epithelial cells, which consume glutamine as their major bioenergetic substrate and require glutamine both for proliferation and survival. Addition of glutamine was sufficient to stimulate ERK signaling within a few minutes in porcine intestinal epithelial cells, and it enhanced ^{3}H-thymidine incorporation (Rhoads *et al.*, 1997). In rat intestinal epithelial cells, glutamine was comparable to serum in preventing apoptosis, and it stimulated a sustained activation of ERK signaling (Larson *et al.*, 2007). In those cells, inhibitors of the ERK pathway eliminated the protective effect of glutamine supplementation. It was not clear from these studies whether glutamine import alone was required for the effects, or whether the cells needed to metabolize glutamine to activate ERK signaling.

Glutamate also influences signaling and tumor growth if it is secreted by tumor cells. In the central nervous system, the rate of glutamate secretion by glioma cells correlated with the ability of those cells to elicit regional neuronal cell death and to form tumors in the rat striatum. Both these effects were due to glutamate signaling through ionotropic receptors, because they were blocked by NMDA receptor antagonists (Takano *et al.*, 2001). Glutamate also has non-ionotropic signaling properties through its effects on metabotropic glutamate receptors (mGluRs) (Figure 1). These G-protein coupled receptors are widely expressed in both neuronal and non-neuronal tissues, implying that they have signaling duties beyond their traditional function in synaptogenesis (Nicoletti *et al.*, 2007; Skerry and Genever, 2001). Activation of mGluR isoforms leads to stimulation of ERK and phosphatidylinositol 3'-kinase (PI3K) signaling, supporting cell survival, growth and proliferation. This may translate into a role for glutamate signaling in cancer. The isoform mGluR1 is expressed in human melanoma cells but not in melanocytes or benign nevi, and overexpression of mGluR1 in mouse melanocytes caused hyperproliferation and occasional transformation into melanoma (Pollock *et al.*, 2003). In mice with doxycycline-repressible mGluR1 expression in melanocytes, the growth of existing melanomas could be impaired by administering doxycycline, and this was correlated with a reduction in activated ERK in the tumors (Ohtani *et al.*, 2008). Human glioblastomas also express mGluRs, and a small molecule inhibitor against mGluR2 and 3 inactivated ERK and PI3K signaling in glioma cell lines, inhibited cell proliferation, and suppressed the growth of tumors in both the brain and subcutaneous tissue (Arcella *et al.*, 2005). Thus these receptors might be useful targets in cancer therapy. Brain tumors may have access to sufficient extracellular glutamate to signal through these receptors without requiring glutamate secretion by the tumor itself. However, the glutamate concentration in the plasma is quite low (~ 50 μM). For melanomas and other tumors outside the central nervous system, mGluR signaling may require that the tumor secrete glutamate produced from glutamine metabolism. This could result in autocrine or paracrine effects on cell growth.

Consistent with glutamine's effects on cell signaling, a number of reports have demonstrated that it also influences gene expression (Brasse-Lagnel *et al.*, 2009). In cell lines, addition of glutamine increases expression of the pro-proliferation factors c-*jun* and c-*myc* within a few minutes (Kandil *et al.*, 1995; Rhoads *et al.*, 1997) and promotes cell survival through negative effects on growth-inhibitory and pro-apoptotis factors like CHOP, GADD45, Fas and ATF5 (Abcouwer *et al.*, 1999; Huang *et al.*, 1999; Watatani *et al.*, 2007; Yeo *et al.*, 2006). In Ehrich ascites tumor cells, glutaminase knockdown led to enhanced phosphorylation, DNA binding, and transcriptional activity of Sp1 (Segura *et al.*, 2005). In HepG2 hepatoma cells, glutamine was required for the induction of manganese superoxide dismutase (MnSOD) expression that accompanied the depletion of essential amino acids (Aiken *et al.*, 2008). Glutamine's involvement in MnSOD expression was blocked by inhibiting the TCA cycle, ERK1/2 or mTOR, suggesting that an integration between mitochondrial glutamine metabolism and signal transduction facilitates the effect.

Abundant evidence suggests that glutamine also modulates immune responses, although it is unclear exactly how these changes are achieved. Conceivably glutamine could exert its effects via redox homeostasis, bioenergetics, nitrogen balance or other functions (Eliasen *et al.*, 2006; Roth, 2007). During radiation-induced oxidative stress in the rat abdomen, pre-treatment of the animals with glutamine significantly decreased tissue inflammation and expression of NF-κB, suggesting that in this case glutamine was used to buffer the redox capacity (Erbil *et al.*, 2005). NF-κB seems to be a key mediator linking glutamine availability to stress responses, since other reports also demonstrated an inverse correlation between glutamine abundance and NF-κB-mediated gene expression (Bobrovnikova-Marjon *et al.*, 2004; Hubert-Buron *et al.*, 2006). The role of glutamine as an immunomodulator in cancer has not been explored at the molecular level, but this is an area worth further study. If the avid consumption of glutamine

by tumors reduces its availability for neighboring cells, this could modulate local NF-κB signaling and expression of inflammatory mediators in the stroma.

Integrating glutamine and glucose metabolism in tumor cell growth

Because tumor cells are exposed to many nutrients simultaneously, achieving a comprehensive view of tumor metabolism requires understanding how cells integrate these pathways into an over-arching metabolic phenotype. Consequently, discussions about glutamine cannot ignore the rapid glucose utilization that also accompanies cell proliferation. In fact the rates of glucose and glutamine consumption far outpace the utilization of other nutrients available to the cell. Presumably this type of metabolism supports both bioenergetics and the production of precursor pools while sparing other energy-rich substrates like fatty acids and essential amino acids for direct incorporation into macromolecules (DeBerardinis *et al.*, 2006).

Warburg's work and many studies since then have focused on the production of lactate from glucose, on the low relative rate of glucose oxidation, and on the high apparent contribution of glycolysis to overall energetics in tumor cells. These have led to the generalization that tumors do not or cannot engage in oxidative metabolism, and that aerobic glycolysis (i.e. conversion of glucose to lactate in the presence of ample oxygen) is a *sine qua non* for the tumor metabolic phenotype. A few points should be made about these assumptions. First, metabolism of glucose to lactate is not limited to tumor cells, but is a common feature of rapid cell proliferation (Brand, 1985; Wang *et al.*, 1976). The potential advantages of this form of metabolism for proliferating cells have been reviewed (DeBerardinis *et al.*, 2008a). Second, while many tumor cell lines do exhibit high glycolytic rates, the contribution of glycolysis to total cellular ATP content varies widely, from over 50% as Warburg found to less than 5% in other cells (Zu and Guppy, 2004). Thus oxidative phosphorylation is not universally impaired in tumor cells. Third, mitochondrial metabolism directly contributes to cell growth because many macromolecular precursors are produced in the TCA cycle (DeBerardinis *et al.*, 2008b). Even if the glycolytic rate is high enough to support most of the cell's need for ATP synthesis, growth requires that the cells produce lipids, proteins and nucleic acids, and building blocks for these molecules come from the TCA cycle. Therefore, the aerobic glycolysis discovered by Warburg is only one piece of the puzzle of anabolic metabolism in tumor cells.

Our growing understanding of glutamine metabolism promises to help fill in this picture, as the concurrent consumption of glucose and glutamine has obvious theoretical advantages (Figure 2). The simultaneous metabolism of these two nutrients would support bioenergetics in cells exhibiting the Warburg effect by delivering glutamine-derived α-KG to the TCA cycle for oxidation. Moreover, the entry of α-KG offsets the export of intermediates used in biosynthetic pathways. The process of replenishing TCA cycle intermediates during cell growth (anaplerosis) is a keystone of biomass production and is much less active in quiescent cells. In rapidly proliferating cultured glioblastoma cells, for example, most of the acetyl-CoA pool comes from glucose, while essentially all of the anaplerotic carbon (i.e. the oxaloacetate) comes from glutamine (DeBerardinis *et al.*, 2007). This results in citrate molecules that contain carbon from both glucose and glutamine (purple arrows in Figure 2). After this citrate leaves the TCA cycle, the two glucose-derived carbons are released as acetyl-CoA and used in lipid synthesis. Protein and nucleic acid synthesis also require precursor molecules derived from glucose and glutamine metabolism. Thus during cell proliferation, glucose and glutamine are the two major nutrient inputs, and the primary outputs are biomass (nucleic acids, proteins and lipids) and the by-products secreted as a result of this type of metabolism: lactate, alanine and ammonia (Figure 2).

The importance of glucose and glutamine metabolism to tumor cell biology is underscored by the fact that mutations in tumor suppressors and oncogenes allow cells to by-pass the normal,

growth factor-dependent handling of these two nutrients. This has been extensively studied for glucose metabolism. As early as the 1980s it was demonstrated that over-expression of Ras or Myc was sufficient to drive glucose uptake in fibroblasts (Flier *et al.*, 1987). Subsequently, mutations in many other tumor suppressors and oncogenes have been implicated as drivers of the Warburg effect in tumor cells (reviewed in DeBerardinis, 2008). While much less is known about the regulation of glutamine metabolism, several reports have recently implicated c-Myc as a major player. Enhanced c-Myc activity was sufficient to drive glutamine metabolism and to impair cell survival in low-glutamine conditions (Wise *et al.*, 2008; Yuneva *et al.*, 2007). c-Myc regulates glutamine metabolism in part by stimulating the expression of surface transporters (Wise *et al.*, 2008). Interestingly, c-Myc also indirectly regulates the protein expression of glutaminase through effects on the microRNAs miR23a and miR23b. Normally, these microRNAs bind to the *GLS* 3'-UTR and prevent translation of the message. However, c-Myc suppresses miR-23a/b expression, and thus enhanced c-Myc activity de-repressed glutaminase translation and facilitated glutamine oxidation in the mitochondria (Gao *et al.*, 2009).

The simplest mechanism to explain the enhanced utilization of both glutamine and glucose by tumor cells is that metabolism of the two nutrients is co-regulated. However, recent findings suggest that they can be regulated by independent signaling pathways within the same cells. In a glioblastoma cell line with genomic c-*myc* amplification, inhibition of Akt signaling led to a decrease in glycolysis but had no effect on glutamine metabolism, which was only inhibited when c-Myc was suppressed to normal levels (Wise *et al.*, 2008). This raises the possibility that the complex metabolic phenotype observed in tumor cells is the result of multiple different signaling inputs, presumably through multiple mutations. This notion is consistent with the observation that serial transduction of human fibroblasts leads to step-wise changes in the cells' metabolic profile and dependence on particular pathways to sustain ATP supply (Ramanathan *et al.*, 2005).

Tumors often display regional heterogeneity in oxygen availability, and this can significantly influence intermediary metabolism independently of tumor genetics. Stabilization of the transcription factor hypoxia-inducible factor-1α (HIF-1α) in hypoxic cells enhances the expression of glucose transporters, glycolytic enzymes, and inhibitory kinases for the pyruvate dehydrogenase complex, all of which serve to increase the production of lactate from glucose (Kim *et al.*, 2006; Papandreou *et al.*, 2006; Semenza, 2003). Little is known about the effects of hypoxia on glutamine metabolism. Presumably in order to survive, cells would at least need to maintain some of the proximal steps of glutamine metabolism to satisfy homeostatic requirements for amino acid and nucleotide synthesis. One study showed that hypoxia could stimulate the import of glutamine in neuroblastoma cells, although downstream metabolism was not examined (Soh *et al.*, 2007). On the other hand, rat pheochromocytoma cells displayed increased rates of endogenous glutamine synthesis under hypoxia, perhaps reflecting a need for glutamine that exceeded transport capacity (Kobayashi and Millhorn, 2001). Further work is needed to determine how hypoxia influences glutamine metabolism and whether glutamine influences cell survival during hypoxic stress.

Glucose and glutamine metabolism also have overlapping functions in redox homeostasis, since both can produce NADPH and glutamine has the additional role of supporting GSH biosynthesis. This is an important consideration during cell proliferation, because NADPH is required for biosynthetic reactions and because some production of reactive oxygen species is inevitable during rapid nutrient metabolism. Glutamine withdrawal led to decreased GSH pools in fibroblasts with enhanced c-Myc activity, and to frank oxidative damage in hybridoma cells (Guerin *et al.*, 2006; Yuneva *et al.*, 2007). In neither case, however, was the redox stress sufficient to explain the loss of cell viability. On the other hand, in P-493 B lymphoma cells and PC3 prostate cancer cells the loss of cell proliferation and viability triggered by glutamine

deprivation or GLS knockdown could be partially reversed with antioxidants. Thus, while impaired glutamine metabolism limits the availability of GSH, this effect is not always responsible for the death of glutamine-dependent cells. It is likely that tumor cells differ in their use of glucose and glutamine to maintain redox balance, and exploiting these differences may be therapeutically useful, especially during therapies that induce oxidative stress.

Tumor growth and whole-body metabolism: is the tumor the tail that wags the dog?

The aggressive metabolism of glucose and glutamine by tumors begs the question of how cancer affects metabolism in the rest of the body. Two issues are particularly relevant to this discussion. First, how do tumors acquire the nutrients they need to grow? And second, what is the fate of the waste products secreted by tumor cells? Both these issues relate to the phenomenon of declining nutrition observed in patients with cancer or other chronic health problems. Cachexia, the progressive loss of muscle and adipose tissue mass, is a well-known and clinically important dimension of cancer, affecting more than half of all patients. It consists of a combination of complex changes in muscle and liver metabolism culminating in weight loss. Changes in appetite and taste sensation may accompany cachexia and can be exacerbated by chemotherapy, but these are not sufficient to explain the phenotype. Weight loss often predates the diagnosis and treatment of cancer, and normalizing the caloric intake usually does not reverse the loss of lean mass. These observations imply that fundamental changes in whole-body metabolism, including an increase in energy expenditure, are at the root of the problem. The clinical importance of cachexia is emphasized by the finding that it is the principal cause of death in one-third or more of cancer patients (NCI Website, 2009). Many aspects of the cachectic phenotype are stimulated (directly or indirectly) by the tumor, and evidence suggests that the tumor derives benefits from altered metabolism in the liver and muscle. Two aspects of cachexia are discussed here: the Cori cycle and muscle proteolysis, each of which has implications for the metabolism of growing tumors (Figure 3).

In the Cori cycle, lactate and alanine released from glucose-consuming tissues like the muscle and brain are returned to the liver and used as precursors for gluconeogenesis. As early as the 1950s, Hiatt demonstrated that a high fraction of glucose produced by the liver of tumor-bearing mice had been generated from 3-carbon units released by the tumor, suggesting a Cori-like cycle between the tumor and the liver (Hiatt, 1957). Similar experiments in humans also documented higher rates of Cori cycle metabolism in individuals with cancer of various types (Holroyde *et al.*, 1984; Reichard GA, 1963). The Cori cycle would thus be a way to minimize the apparent energetic inefficiency of the Warburg effect, since the “wasted” 3-carbon units could ultimately be returned to the tumor as glucose. However, while the cycle produces energy in the tumor (two ATPs per glucose used), this is more than offset by energy expenditure in the liver (6-12 ATP equivalents per glucose formed). Thus the Cori cycle imposes an energetic burden on whole-body metabolism. Increased flux through this pathway has been postulated to be one of the drivers of energy dissipation in cachectic cancer patients (Tisdale, 2009). Secretion of lactate by hypoxic cells within tumors can also have local effects on metabolism. If this lactate can reach better oxygenated cells within the tumor, it can be taken up and used as a respiratory fuel in the mitochondria. Sonveaux et al. identified the cell surface monocarboxylate transporter MCT1 as the enabler of such a metabolic symbiosis in xenografts. MCT1 allowed normoxic cells to take up lactate, presumably sparing glucose for the more hypoxic regions of the tumor. Inhibiting MCT1 significantly reduced tumor growth and increased the effect of radiation therapy (Sonveaux *et al.*, 2008).

Cancer also induces a negative nitrogen balance in the host characterized by loss of protein and amino acids from the muscle, eventually leading to loss of muscle mass. This observation predates even Warburg's work by several decades and has been credited to Müller (Parry-

Billings *et al.*, 1991), who compared the host response in cancer to that of a prolonged febrile illness. While the inciting agents that promote muscle catabolism have not been fully elucidated, many studies have implicated inflammatory mediators like transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and various cytokines, consistent with Müller's hypothesis (Tisdale, 2005). Clearly glutamine metabolism is tied to cachexia, since more than 90% of the body's glutamine stores are in the muscle, and glutamine is the major amino acid exported from the muscle during catabolic stress. Newsholme suggested that the demand for glutamine by rapidly proliferating cells like lymphocytes or tumor cells might be the trigger for cachexia (Newsholme *et al.*, 1985). Evidence in support of this hypothesis includes the observation that implantation of tumor tissue in rodents rapidly triggers an increase in muscle glutamine output and a drop in muscle glutamine stores (Chen *et al.*, 1993; Parry-Billings *et al.*, 1991). Similar changes have been suggested to occur with prolonged tumor burden in humans (Souba, 1993).

The process of muscle nitrogen loss during cachexia involves the transfer of amino acid nitrogen first to glutamate and then to glutamine, which is secreted into the bloodstream (Figure 3). Production of glutamine from glutamate is catalyzed by glutamine synthetase (also known as glutamate-ammonia ligase, *GLUL*). Tumor burden is associated with increased expression of the *GLUL* mRNA and activity in the muscle of mammals and birds, implicating this enzyme as an evolutionarily conserved mediator of cachexia (Chen *et al.*, 1993;Matsuno and Satoh, 1986;Quesada *et al.*, 1988). Glutamine synthesis consumes energy and thus would tend to increase energy expenditure in the muscle. It also requires ammonia. Interestingly, myocytes can take up and metabolize ammonia, and this is one of the mechanisms by which ammonia is cleared during exercise in humans (Bangsbo *et al.*, 1996). The source of muscle ammonia during glutamine secretion in cachexia is unknown, but presumably comes from an alteration in inter-organ ammonia handling. This could be similar to the changes that accompany chronic liver disease, when the muscle takes up excess ammonia and uses it to produce glutamine as a detoxification strategy (Olde Damink *et al.*, 2009). Thus, it is possible that some of the ammonia secreted by tumors as a result of their avid glutamine consumption is trafficked to the muscle to re-create glutamine from the intracellular glutamate pool (Figure 3). While speculative, such a system would be analogous to glucose handling in the tumor-liver Cori cycle.

The question of whether cancer patients with cachexia would benefit from glutamine supplementation has generated a large amount of interest and debate. A theoretical concern is that increasing glutamine availability might stimulate tumor growth, but it is not clear whether well-perfused tumors ever experience glutamine deprivation *in vivo*, and one study in sarcoma-bearing rats found no enhancement of tumor growth when the animals' diet was supplemented with glutamine (Klimberg *et al.*, 1990). A large number of clinical human trials have been performed to assess glutamine's utility in improving muscle mass or in limiting chemotherapeutic toxicity, alone or in combination with other dietary agents, but there is still no consensus as to whether glutamine is generally helpful.

In principle, one could also attempt to intervene at the level of glutamine release from the muscle. It is tempting to speculate that if muscle export is the major source of glutamine for the tumor, then suppressing either proteolysis or glutamine synthesis in the muscle might suppress tumor growth by reducing the supply of an essential nutrient. Transcription of the *GLUL* mRNA seems to be the pivotal level of regulation in this process. In various cell lines, *GLUL* expression is stimulated by glucocorticoids, which have also been implicated in the development of cancer cachexia (Gaunitz *et al.*, 2002; Wang and Watford, 2007). Perhaps targeting this system in the muscle could be of some benefit.

Conclusions and more questions

A number of landmark reports over the last decade cemented the intimate connections between oncogenes and glucose metabolism in tumors, and demonstrated that the reliance of transformed cells on the Warburg effect could be exploited experimentally to suppress tumor growth. The next wave of interest in tumor metabolism will likely focus increased attention on cellular glutamine handling, and should re-assess the question of whether the appetite for glutamine displayed by tumors can be used against them. Glutamine's diverse contributions to intermediary metabolism, cell signaling, gene expression and cachexia argue in favor of attempting to develop rational strategies to limit tumor glutamine uptake and to image glutamine metabolism in tumors. Presumably such maneuvers would be particularly useful in tumors containing enhanced c-Myc activity.

There are also a number of fundamental biological questions that deserve further study. For example, how do cells manage to allocate glutamine appropriately into the numerous pathways that use it? Are there mechanisms to channel glutamine towards the enzymes and cellular compartments that most need it, and are these systems responsive to stresses that change cellular patterns of glutamine use? The observation that some cells require glutamine export to activate mTOR signaling begs the question of how these cells can sense when glutamine is abundant enough to permit its secretion. It will also be interesting to determine how signals related to glutamine abundance/deprivation are transmitted to the nucleus to influence gene expression. A deeper understanding of these issues should help explain why glutamine pervades so many aspects of cell biology, and should support future efforts to manipulate glutamine metabolism in cancer and other diseases.

Acknowledgments

The authors thank Roland Knoblauch and Andrew Mullen for critically reading the manuscript. RJD is supported by grants from the NIH (DK072565) and the American Cancer Society (ACS-IRG-02-196).

Support: NIDDK (DK072565) and American Cancer Society (ACS-IRG-02-196)

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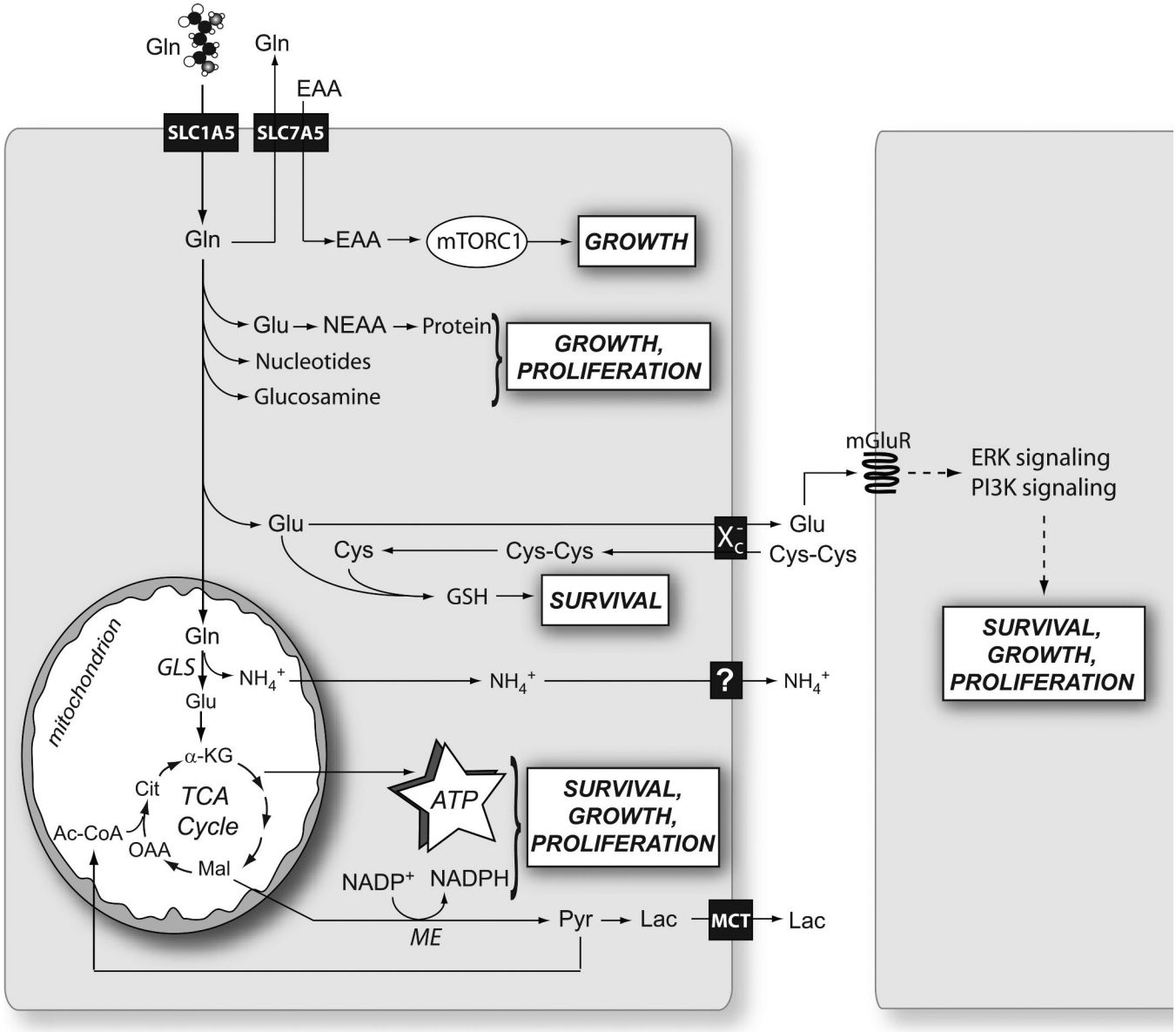

Figure 1. Glutamine supports cell survival, growth and proliferation through metabolic and non-metabolic mechanisms

After its import through surface transporters like SLC1A5, glutamine (Gln) is either exported in exchange with the import of essential amino acids (EAA) or consumed in various pathways that together support the basic metabolic functions needed for cell survival, growth and proliferation. In cancer cells, the mitochondrial enzyme glutaminase (GLS) appears to account for the largest fraction of net glutamine consumption. This enzyme produces NH_4^+, which is exported, perhaps through carrier-mediated mechanisms. Abbreviations: mTORC1, mammalian Target of Rapamycin Complex 1; Glu, glutamate; NEAA, nonessential amino acids; Cys, cysteine; Cys-Cys, cystine; GSH, glutathione; mGluR, metabotropic glutamate receptor; ERK, extracellular signal-regulated protein kinase; PI3K, phosphatidylinositol 3'-kinase; α-KG, α-ketoglutarate; Mal, malate; OAA, oxaloacetate; Ac-CoA, acetyl-CoA; Cit, citrate; Pyr, pyruvate; Lac, lactate; TCA, tricarboxylic acid; GLS, glutaminase; ME, malic enzyme.

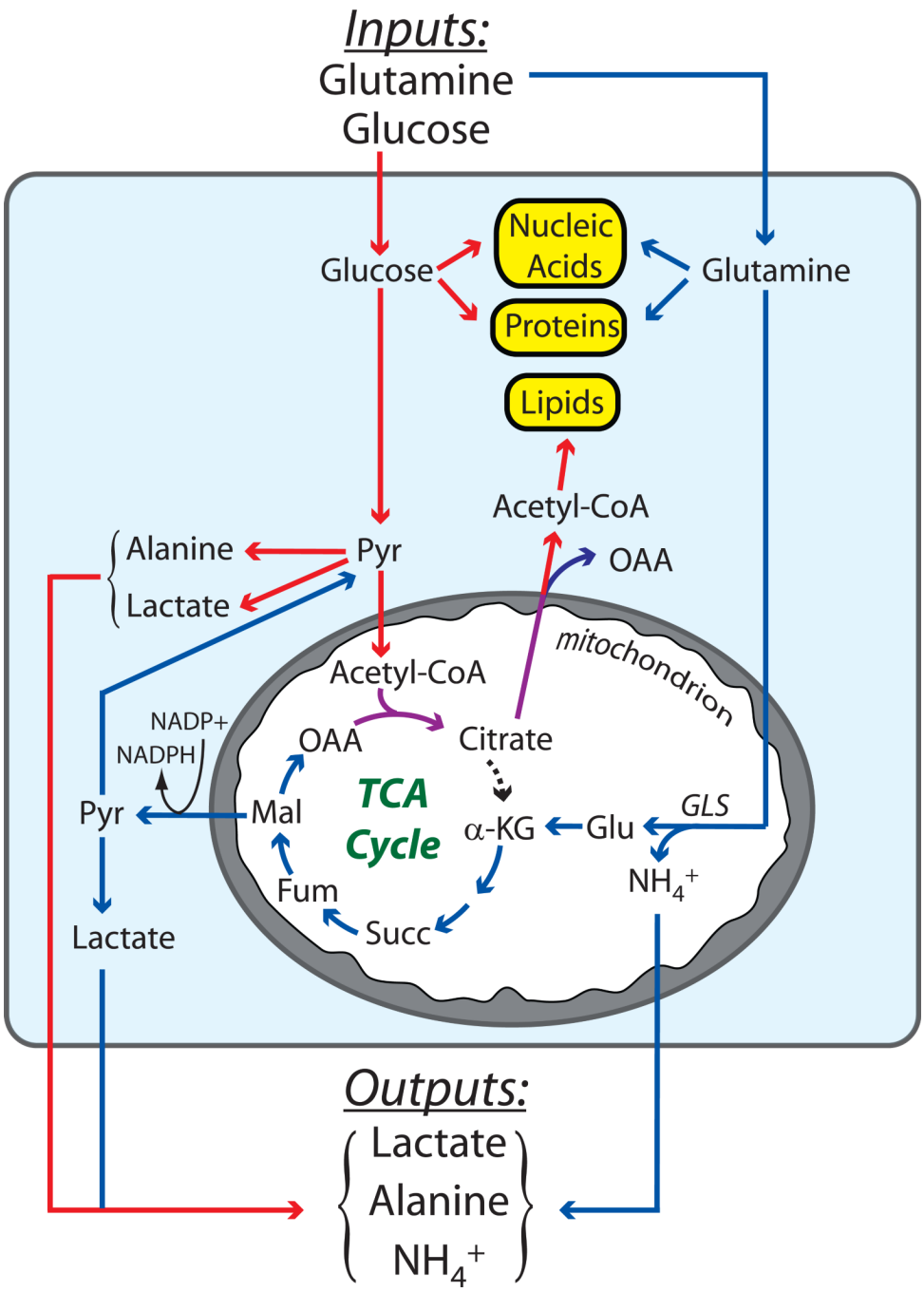

Figure 2. Cooperativity between glucose and glutamine metabolism in growing tumors

The major nutrients consumed by tumors are glutamine and glucose, which provide precursors for nucleic acids, proteins and lipids, the three classes of macromolecules needed to produce daughter cells. The metabolism of glutamine (blue arrows) and glucose (red arrows) are complementary, converging on the production of citrate (purple arrows). Glutamine metabolism produces oxaloacetate (OAA) and NADPH, both of which are required to convert glucose carbon into macromolecules. Glutamine metabolism also supplements the pyruvate pool, which is predominantly formed from glucose. As a consequence of the rapid metabolism of these two nutrients, lactate, alanine and NH_4^+ are secreted by the tumor. Abbreviations:

GLS, glutaminase; Glu, glutamate; α-KG, α-ketoglutarate; Succ, succinate; Fum, fumarate; Mal, malate; Pyr, pyruvate; Lac, lactate; TCA, tricarboxylic acid.

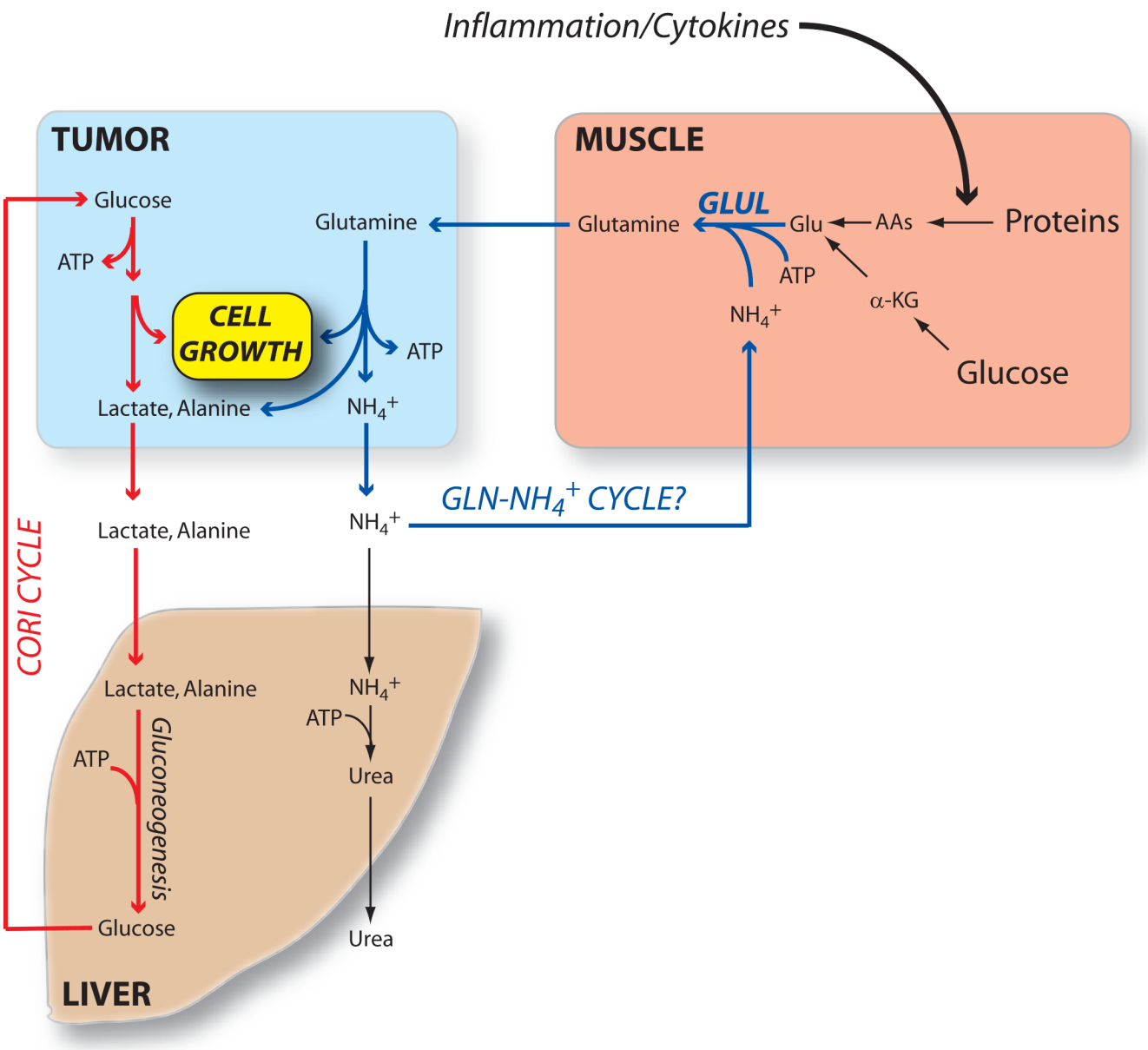

Figure 3. Proposed inter-organ metabolic cycles in cachectic cancer patients

As summarized in Figure 2, growth of the tumor involves consumption of glucose and glutamine with secretion of lactate, alanine and ammonia. Some of the lactate may be taken up by well-oxygenated regions of the tumor and used as a respiratory fuel. Other lactate and alanine are delivered to the liver and used to produce glucose, which can then return to the tumor (the Cori cycle). Meanwhile, the ammonia can be disposed through the urea cycle, or possibly delivered to the muscle for incorporation into new glutamine molecules produced during protein catabolism and glucose metabolism. Both the Cori cycle and the putative glutamine-ammonia cycle deliver energy to the tumor, but cost energy in the other organs involved, driving up whole-body energy expenditure as is typically observed in cancer cachexia. Glu, glutamate; α-KG, α-ketoglutarate; AAs, amino acids; GLUL, glutamate-ammonia ligase (i.e. glutamine synthetase).