Glutamine Metabolism: Nutritional and Clinical Significance

Glutamine and Cancer[1,2]

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ABSTRACT   Glutamine is the most abundant free amino acid in the human body; it is essential for the growth of normal and neoplastic cells and for the culture of many cell types. Cancer has been described as a nitrogen trap. The presence of a tumor produces great changes in host glutamine metabolism in such a way that host nitrogen metabolism is accommodated to the tumor-enhanced requirements of glutamine. To be used, glutamine must be transported into tumor mitochondria. Thus, an overview of the role of glutamine in cancer requires not only a discussion of host and tumor glutamine metabolism, but also its circulation and transport. Because glutamine depletion has adverse effects for the host, the effect of glutamine supplementation in the tumor-bearing state should also be studied. This communication reviews the state of knowledge of glutamine and cancer, including potential therapeutic implications.   J. Nutr. 131: 2539S–2542S, 2001.

KEY WORDS: • *glutamine* • *cancer* • *glutaminase* • *glutamine synthetase* • *transglutaminase*

Why are tumors highly glutaminolytic?

Neoplastic transformation is accompanied by adaptive increases in nucleotide and protein synthesis. The high rates of protein synthesis in rapidly growing tumors require a continuous supply of both essential and nonessential amino acids (Medina and Núñez de Castro 1988). Mider (1951) showed that tumors assimilate not only the nitrogen from the diet, but also the nitrogen from host proteins, raising the concept of tumors as "nitrogen traps," actively competing with the host for nitrogen compounds (Landel et al. 1985). Tumors use the incorporated amino acids for both oxidation and protein synthesis (Shapot 1979).

Because glutamine is the most abundant amino acid in the body and the main vehicle for circulation of ammonia in a nontoxic form (Medina et al. 1992), some authors consider that tumors behave indeed as "glutamine traps" (Klimberg and McClellan 1996, Souba 1993). The highly tumorigenic human breast cancer cell line, TSE cells, exhibit up-regulation of glutamine synthetase protein and mRNA levels and a decline in intracellular glutamine content upon chronic glutamine deprivation (Collins et al. 1997). The concept of tumor as a "glutamine trap" has sometimes been misunderstood and has been severely criticized. For example, it has been reported that glutamine oxidation is unlikely in hypoxic or anoxic tumor because oxygen is required for the reoxidation of essential coenzymes (Kallinowski et al. 1987). However, the main basis for this criticism is the incorrect assumption that glutamine is oxidized mainly by tumor cells. This does not seem to be true; in fact, tumors often waste energy and metabolic substrates. When Ehrlich ascites tumor cells are perfused with a continuous input of 0.5 mmol glutamine and steady state is reached, there is a perfect stoichiometry of one glutamate released per glutamine taken up (Segura et al. 1989). This behavior of tumors is also observed with other energy substrates, i.e., when 5 mmol glucose is used as a substrate, there is a perfect stoichiometry at steady state of two lactate molecules released per molecule of glucose taken up (Segura et al. 1989). Thus, it can be concluded that tumors are powerful energy dissipaters, consistent with the nonequilibrium thermodynamics developed by Prigogine (1980). There is experimental evidence to support tumor cell mitochondrial respiratory chain as an intracellular dissipative structure (IDS),[3] as espoused by Ji (1985). He defined IDS as disequilibrium distributions of chemicals inside cells that are maintained through a dissipation of free energy. The experimental evidence for this observation is termed a "noncommutativity test." A characteristic property of dissipative structures, not observed in equilibrium or near-equilibrium systems, is the possibility of successive metabolic bifurcations, which may drive the system to very different final states, depending on the order of substrate addition. In Ehrlich cells incubated with glucose and glutamine, the oxidative states of mitochondrial cytochromes at the end of incubation were very different, depending on the order of addition of glucose and glutamine. These results support Ji's IDS hypothesis (Medina and Núñez de Castro 1988).

Tumor cells are highly variable in their energy requirements. Hence, these cells are "efficacious" but "nonefficient" energy systems. They are "efficacious" in the sense that they

[3] Abbreviations used: GA, glutaminase; GS, glutamine synthetase; IDS, intracellular dissipative structure.

are able to utilize energy inputs from very different sources and under very different environmental circumstances. They are not "efficient" in the sense that they seem to waste most of the energy inputs without any apparent profit. This apparent wasteful spreading of energy has been theoretically justified on the basis of the quantitative principles of metabolic control theory (Crabtree and Newsholme 1985). In branded metabolic pathways, these authors demonstrated that if the flux through a metabolic branch is far higher than the flux through another branch, then the pathway with a small flux has a very high sensitivity to the modulators of the small flux. Both glycolysis and glutaminolysis represent wide metabolic branches, whereas the branches directed to macromolecule synthesis represent the small ones. In conclusion, these authors suggest that high rates of glycolysis and glutaminolysis are needed not for energy or precursor provision per se in cancer cells. Moreover, they are required for the pathways involved in macromolecule synthesis for specific regulators, thereby permitting high rates of proliferation when required.

Changes in host glutamine metabolism upon cancer onset

Tumors elicit a specific response in the host nitrogen metabolism, i.e., to mobilize and augment circulating glutamine (Carrascosa et al. 1984, Márquez and Núñez de Castro 1991, Quesada et al. 1988a, 1996). There is a net flux of glutamine from host to tumor, which is possibly due to a net production of glutamine by host tissues as a result of an increase in the glutamine synthetase (GS)/glutaminase (GA) ratio. Our group studied this topic extensively in the tumor model of Ehrlich cells growing in the peritoneum of mice. The changes in the GS/GA ratio are evident both at mRNA and enzyme activity levels as early as 24 h after tumor implantation in kidney and liver (Aledo et al. 2000, Quesada et al. 1988b). On the contrary, in spleen, an important organ of the immune system, there is a transient increase of GA mRNA and activity levels (Aledo et al. 1998). These results agree extensively with those reported by other authors in other tumor models (Medina et al. 1992, Souba 1993).

Glutamine transport and metabolism by tumor cells

The actual rate of glutamine consumption by tumor cells depends on the presence or absence of alternative energy substrates. The interactions between glutaminolysis and glycolysis in proliferating cells have been reviewed previously (Medina and Núñez de Castro 1990), but fatty acids also interfere with glutamine metabolism (Medina et al. 1988a).

Because glutamine metabolism takes place in the mitochondria, it must be transferred from extracellular medium through specific plasma membrane and inner mitochondrial membrane transporters. Plasma membrane glutamine transport is reviewed by Bode (2001) in this publication. As a general rule, malignant cells transport glutamine across their plasma membranes at a faster rate than their nonmalignant counterparts (Espat et al. 1995, Medina et al. 1991 and 1992, Souba 1993). After glutamine gains access to the cytoplasm, it must be transported into mitochondria. Kovacevic et al. (1970) first postulated the existence of a neutral uniport mechanism for glutamine uptake into liver mitochondria. Because the mitochondrial transport of metabolites is usually 1–2 orders of magnitude faster than the plasma membrane–related transport, special problems arise in transport studies conducted with isolated mitochondria. To allow transport measurements without any interference by the mitochondrial glutamine metabolism, our group used native vesicles isolated from mito-

chondrial inner membrane to characterize the mitochondrial glutamine transport system of Ehrlich ascites tumor cells. Data confirm the existence of a specific mitochondrial transport system with high capacity for L-glutamine, showing cooperativeness and strong inhibition by the thiol reagent p-chloromercuriphenylsulfonic acid and the glutamine analog L-glutamate-γ-hydroxamate (Molina et al. 1995).

In the mitochondria, glutamine is acted upon by glutaminase, an enzyme requiring high phosphate concentrations to be fully active. The high concentrations of inorganic phosphate found in the mitochondria of tumor cells (Medina et al. 1988b) could explain the high activity of tumor glutaminase in vivo. In fact, experimental evidence supports the correlation of glutaminase activity with the extent of malignant proliferation (Medina et al. 1992, Souba 1993). Tumor glutaminase reaches a maximum of expression and activity immediately before the maximum proliferation rate (Aledo et al. 1994, Gómez-Fabre et al. 2000).

Although Huang and Knox (1976) partially purified the enzyme from a mammary carcinoma, our group was the first one reporting a tumor glutaminase purification to homogeneity (Quesada et al. 1988b). Afterward, our own group provided two alternative purification procedures that increased yield and decreased purification time (Segura et al. 1995). Purified Ehrlich ascites tumor cell glutaminase has been studied extensively and characterized both kinetically and topographically (Aledo et al. 1997, Campos et al. 1998, Quesada et al. 1988b).

On the other hand, glutamine synthetase has classically been considered as a "dispensable" enzyme for tumors (Medina et al. 1992). However, as previously mentioned, at least some tumors exhibit glutamine synthetase up-regulation as an adaptive response to glutamine depletion (Collins et al. 1997).

Therapeutical, nutritional and pharmacologic aspects

In the 1980s, two glutamine-related antineoplastic therapies raised great expectations, i.e., glutamine clearance and the use of glutamine analogs to kill tumor cells by exhausting their provision of glutamine. The promising results obtained in model systems have not been confirmed by clinical trials due to multiple toxic effects, lack of specificity and/or ineffectiveness of the treatments (Medina et al. 1992, Souba 1993).

Nutritional and pharmacologic aspects of glutamine are covered by other reviews in this publication. There is increasing evidence supporting a protective role for glutamine supplementation in enteral or total parenteral nutrition (Amores-Sánchez and Medina 1999). In relation to cancer, it seems that a supplementation of glutamine in the diet may be beneficial for several reasons. Tumor progression is associated with an avid consumption of host glutamine by tumor cells and a depression in the activity of natural killer cells due to a decrease in glutathione concentrations in these cells. Therefore, dietary supplementation of glutamine could have the beneficial effect of restoring the levels of glutathione inside natural killer cells; at the same time, however, it could have the deleterious effect of feeding the tumor. However, because glutamine consumption by tumors is almost absolutely dissipative, an increase in the growth rate of the tumor due to this process should not be expected (Austgen et al. 1992, Medina and Núñez de Castro 1990). In fact, there are experimental data that seem to indicate that a dietary supplement diminishes tumor growth by restoring the function of natural killer cells and improves protein metabolism of the host or patient (Fahr et al. 1994, Yoshida et al. 1995).

Additionally, an oral supplement of glutamine can increase the selectivity of antitumor drugs (Cao et al. 1999, Decker-

Baumann et al. 1999, Miller 1999) by protecting the patient from oxidative damage through an increase in glutathione contents (Rouse et al. 1995). Several groups have shown that glutamine can also protect against oxidative damage induced by radiotherapy (Jensen et al. 1994, Miller 1999, Yoshida et al. 1995).

However, there is no consensus on the usefulness of glutamine supplementation for cancer patients. For instance, a recent double-blind, randomized study on glutamine supplementation in cancer patients receiving chemotherapy concluded that glutamine did not have a significant effect on either tumor response or secondary effects of chemotherapy (Bozzetti et al. 1997).

Current and future trends

One of the most significant new advances in glutamine and cancer research has been the recent cloning of several glutaminase isoforms from two tumor cell cDNA libraries by two different independent groups. Elgadi et al. (1999) used a cDNA library from human colon adenocarcinoma cell line HT-29, and Gómez-Fabre et al. (2000) used a cDNA library from human breast cancer cell line ZR75–1. Interestingly, their tissue-specific expressions are different. Two of the isoforms identified by Elgadi et al. (1999) show high homology with rat kidney-type glutaminase, and a third isoform of glutaminase, previously identified by Imbert et al. (1996), is expressed only in cardiac and skeletal muscle. On the other hand, the glutaminase isoform cloned from ZR75–1 cells seems to be a liver-type glutaminase, with high expression levels in human liver and lower expression in pancreas and brain (Gómez-Fabre et al. 2000). This result is in sharp contrast with the view that kidney-type glutaminase is the isoform expressed in all tissues with glutaminase activity with the exception of postnatal liver. Furthermore, because Elgadi et al. (1999) found that one of the kidney-type isoforms is the predominant glutaminase expressed by the TSE breast carcinoma cell line, the implications of a possible expression of both kidney- and liver-type glutaminases in breast cancer should be analyzed in the immediate future.

Once the cloning of tumor glutaminase has been achieved, the future trends in this research area can be easily predicted. One of the main goals should be to provide further insight into tumor glutaminase gene expression and regulation by systematic studies of their promoter regions and by the identification of proteins interacting with tumor glutaminase.

A second important new development in this research area has been the successful inhibition of glutaminase expression by antisense mRNA (Lobo et al. 2000). Ehrlich ascites tumor cells transfected with a vector containing a 0.28-kb antisense segment of the C-terminal region of rat kidney-type glutaminase showed impairment in their growth rate and plating efficiency, as well as shortage in glutaminase protein and activity levels and remarkable changes in their morphology. Furthermore, these transfected cells lost their tumorigenic capacity in vivo, thus providing new ways for possible therapeutic applications. It can be anticipated that this will be a very active area of research in the near future.

Finally, a new interest is emerging for tissue transglutaminases. Four human transglutaminase genes have been identified (Dubbink et al. 1998). Tissue transglutaminase is a marker of apoptosis and has been postulated to play a role in cell adhesion, metastasis and extracellular matrix assembly (Hettasch et al. 1996, Rittmaster et al. 1999). Tissue transglutaminase-2 expression levels seem to correlate with drug resistance in cancer cells (Han and Park 1999, Mehta 1994).

On the other hand, tissue transglutaminase-4 is prostate specific and its expression is inhibited in most metastatic prostate cancers (An et al. 1999, Rittmaster et al. 1999). Very recently, it has been shown that tissue transglutaminase is directly involved in wound healing and angiogenesis (Haroon et al. 1999). A possible involvement of tissue transglutaminase in tumor angiogenesis should be evaluated. Furthermore, the tumor specificity and the positive or negative involvement of the different tissue transglutaminases in tumor progression will warrant future efforts in this important area of research.

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