Therapeutic targeting of amino acid dependency in cancer cells

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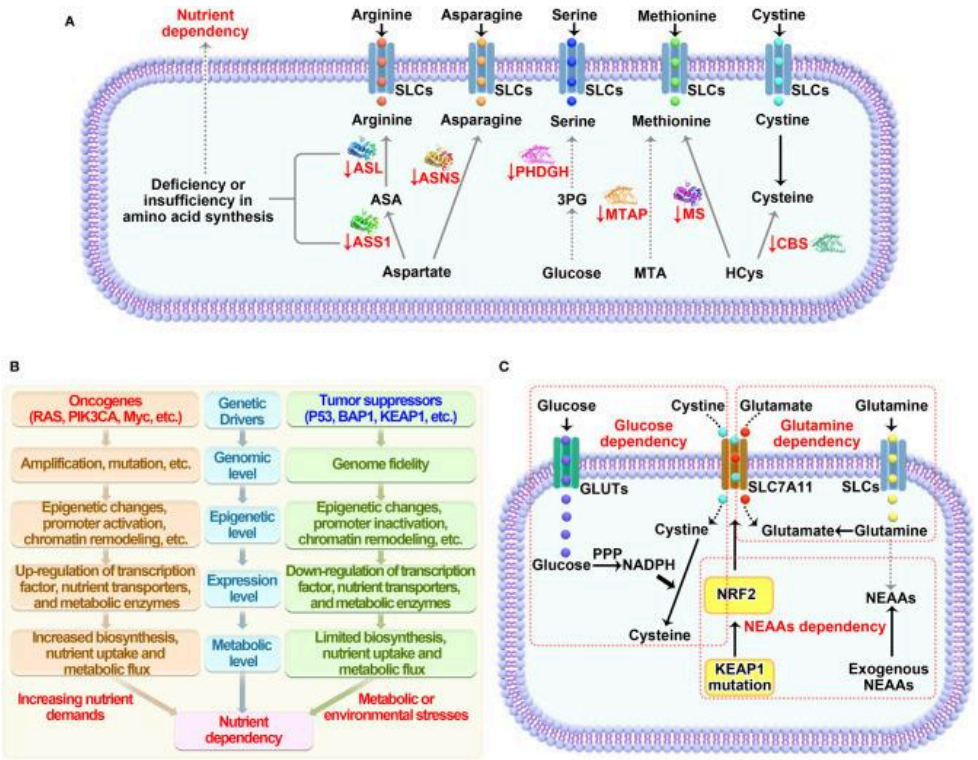
Abstract

Cancer cells often undergo changes to their metabolism when they mutate, to meet energetic and biosynthetic demands of their high proliferation rates and environment. Recent and ongoing research suggests that some of these nutrients and substances themselves can encourage oncogenic progression by altering cell signaling and blocking cellular differentiation. Such alterations in metabolism were once viewed as an indirect response to cell proliferation and survival signals, but studies show that the changes are as a direct result of modifications made for the cell to become cancerous.To satisfy mutant changes, cancer cells often require changes made in their metabolic pathways, requiring certain nutrients to progress and causing these cells to become dependent on these nutrients. Newly developed cancer therapy, still in the experimental stages, suggests eliminating or reducing these nutrients as an effective way to disrupt cancer cell activity and eventually, kill the cells. This paper focuses on the developed methods of targeting nutrients necessary to these new metabolic pathways, as a form of cancer treatment.

Keywords: amino acids, cancer metabolism, nutrient dependencies, metabolic therapy

Introduction

Nutrients are invaluable resources for the sustenance and survival of cells. Cancer cells alter their metabolism to suit their rapid proliferation which deviates from the normal cell cycle. Due to this rewiring which these cells undergo, especially the rewriting of specific pathways to suit their needs, oncogenic progression may lead to the dependence on certain non-essential nutrients which can be exploited (Fan et al., 2022; Garcia-Bermudez et al., 2019). The need for such nutrients can be influenced by diet, origin tissue from which the tumor developed, local microenvironment, tumor heterogeneity and functional demand. Nutrients such as glucose, lipids, vitamins and a number of amino acids including glutamine, arginine, serine, cysteine, methionine, are necessary for the growth of all cells as they play important roles in metabolic pathways.



[Figure 1] (A) Deficiency or insufficiency in amino acid synthesis within cells causes dependency on extracellular nutrients. **(B)** Genetic factors including oncogenes and tumor suppressors either directly regulate the expression of transporters and enzymes mediating nutrient metabolism or indirectly control the demands needed for cell growth, which impose specific dependencies on certain nutrients. **(C)** Nutrients involving crosstalk in their metabolic pathways are prone to be codependent on each other to maintain cellular homeostasis (Fan et al, 2022).

Ultimately, there is a preference for targeting certain nutrients in cancer treatment due to the damage caused when these nutrients are removed. These nutrients are glucose and amino acids. One technique involves attacking the glycolysis pathway of the tumor cells, as this disrupts part of the generation of ATP for the cells. Cancerous cells show an increased uptake of glucose as necessary for their proliferative activities, thus requiring a higher rate of glycolysis. According to Ganapathy-Kanniappan and Geschwind, (2013), eliminating this pathway or disturbing it would incapacitate the cells from carrying out activities such as replication without the required ATP. This process can be applied by glucose deprivation, targeting specific enzymes such as HKII, or by blockage of GLUTs, preventing glucose entry into the cell. These procedures have been successful though they are yet to be translated to the clinic due to extensive toxicities as damage often spreads to non-cancerous cells. (Ganapathy-Kanniappan and Geschwind, 2013).

However, more success is seen in techniques which target amino acids instead. Amino acids primarily serve in cellular biosynthesis of proteins, nucleotides, and fatty acids especially with sufficient nutrient access; additional nutrients like cysteine, glycine and glutamate are also critical for the biosynthesis of glutathione, an anti-oxidative molecule. Therefore, deprivation of

amino acids in cells can induce a more rapid and potent necrosis of the cell, leading to theories of more effective treatments with this route. Limiting essential amino acid (EAA) metabolism is bound to procure challenges similar to those from inhibition of glucose metabolism as our cells do not synthesize the enzymes required to produce this group of amino acids. Instead, focus was turned to targeting non-essential amino acid (NEAA) - amino acids that can be synthesized by the human cells - metabolism to reduce toxicity, though this approach has its own limitations. It was noticed that while undergoing mutation, cancerous cells had lost the ability to synthesize these specific amino acids; their limitation was seen as a viable route to cancer therapy (Pathria and Ronai, 2021). According to Fan et al., (2022), there are three methods of removing these nutrients from the pathways: depletion of nutrients in the extracellular context, suppression of transport and uptake of nutrients by the cell, and inhibition of the nutrient-dependent metabolic pathway.

Nutrient restriction via enzymatic degradation or dietary restriction

Recent research has discovered many enzymes capable of degrading individual amino acids both *in vitro* and *in vivo*. As such, Asparaginase (ASNase), arginase (ARGase), arginine deiminase (ADI), methioninase and cyst(e)inase are now successfully developed enzymatic drugs targeting the amino acids asparagine, arginine, methionine and cyst(e)inase respectively (Garcia-Bermudez et al., 2019). This technique involves using serums, such as the one obtained from guinea pigs containing asparaginase, containing specific enzymes which deplete the targeted nutrient, reducing availability and therefore uptake of this nutrient for cancerous cells.

The main advantage of this method is its success with very limited or controllable toxicity. However, concerns of allergies and other immune responses have risen due to use of enzymes of non-human origin, showing symptoms such as anaphylactic shock (Butler et al., 2021). Additionally, though these enzymes are highly specific in action, they may exhibit dual-enzyme activities which may lead to the target of secondary substrate, more likely if the primary target is present in very low levels. This can be seen in the case of ASNase: it mostly catalyzes the hydrolysis of asparagine; however, asparaginase also exhibits activity similar to glutaminase in that it generates glutamate from glutamine. This extra function is not required for its anti-cancer capability and has the side effect of inducing cytotoxicity in leukemia cells as proven in Parmentier et al., (2015). Strategies such as chemical modification and use of engineered-biomaterials have been employed to boost the efficiency of the therapeutic enzymes while limiting any secondary functions. Further experimentation has led to the development of pegylated enzymes, in which the enzymes are modified by covalent linkage to polyethylene glycol (PEG), cresting substances such as PEG-asparaginase, PEG-arginase, PEG-arginine deiminase and PEG-methioninase. These, unlike their non-pegylated counterparts, have been found to exhibit decreased immunogenicity and prolonged half-life (Santo et al., 2018). This, among other findings, show how enzymatic amino acid degradation can be applied clinically in cancer treatment with minimal side effects.

On the other hand, dietary restriction of amino acids is more difficult than expected due to the nutritional complexity of ingested food items. Currently, only dietary restriction of methionine has moved to clinical trials. However, according to Epner et al., 2002, a methionine-free diet produced better patient responses when combined with chemotherapy using 5-fluorouracil and

mitomycin C in patients with gastric cancer compared with control treatment (methionine supplementation combined with the same chemotherapy). So far, the clinical evaluation of this method is undetermined, requiring additional approaches which explore targeting nutrient dependency as combined with other therapies.

Suppression of nutrient transporters

Cells depend on transporter proteins in the plasma member to intake necessary substances such as amino acids, glucose and other substrates. These transporters belong to the family of molecules known as solute carrier (SLC) transporters of over 300 differing members. With such a broad substrate specificity, it is important that whatever treatment is affected only involves the necessary nutrient transporters. Koppula et al., (2020) gives the example of SLC7A11/xCT, a transporter protein utilized in the uptake of amino acid cystine. Several molecules have been proven to be capable of suppressing this uptake, such as sulfasalazine, sorafenib, and erastin. Dixon et al., (2012) provides studies of the discovery of erastin's target of voltage-dependent anion channels, inducing non-apoptotic cell death, called ferroptosis. In a different and older study, sulfasalazine was noticed for its potential in inhibiting SLC7A11 and later became approved for treatment of a variant of diseases including Crohn's disease (Gout et al., 2001). Sorafenib, a multikinase inhibitor, was observed to primarily target RAF and VEGF and PDGF receptors tyrosine signaling. It "disrupts tumor microvasculature through antiproliferative, antiangiogenic and proapoptotic effects and its multiple molecular targets give it a wide range of action across various tumor types" (Wilhelm et al., 2008).

Despite these seeming successes, this method faced challenges in the effective target of only transporter proteins concerned with nutrient uptake. With the addition of other factors *in vivo,* interactions arise between the compounds which affect the specificity of the target. It is therefore necessary that context-dependent mechanisms which determine the efficacy of this treatment method *in vivo* are studied while more specific molecules for targeting nutrient transporters are generated.

Repression of metabolic pathways

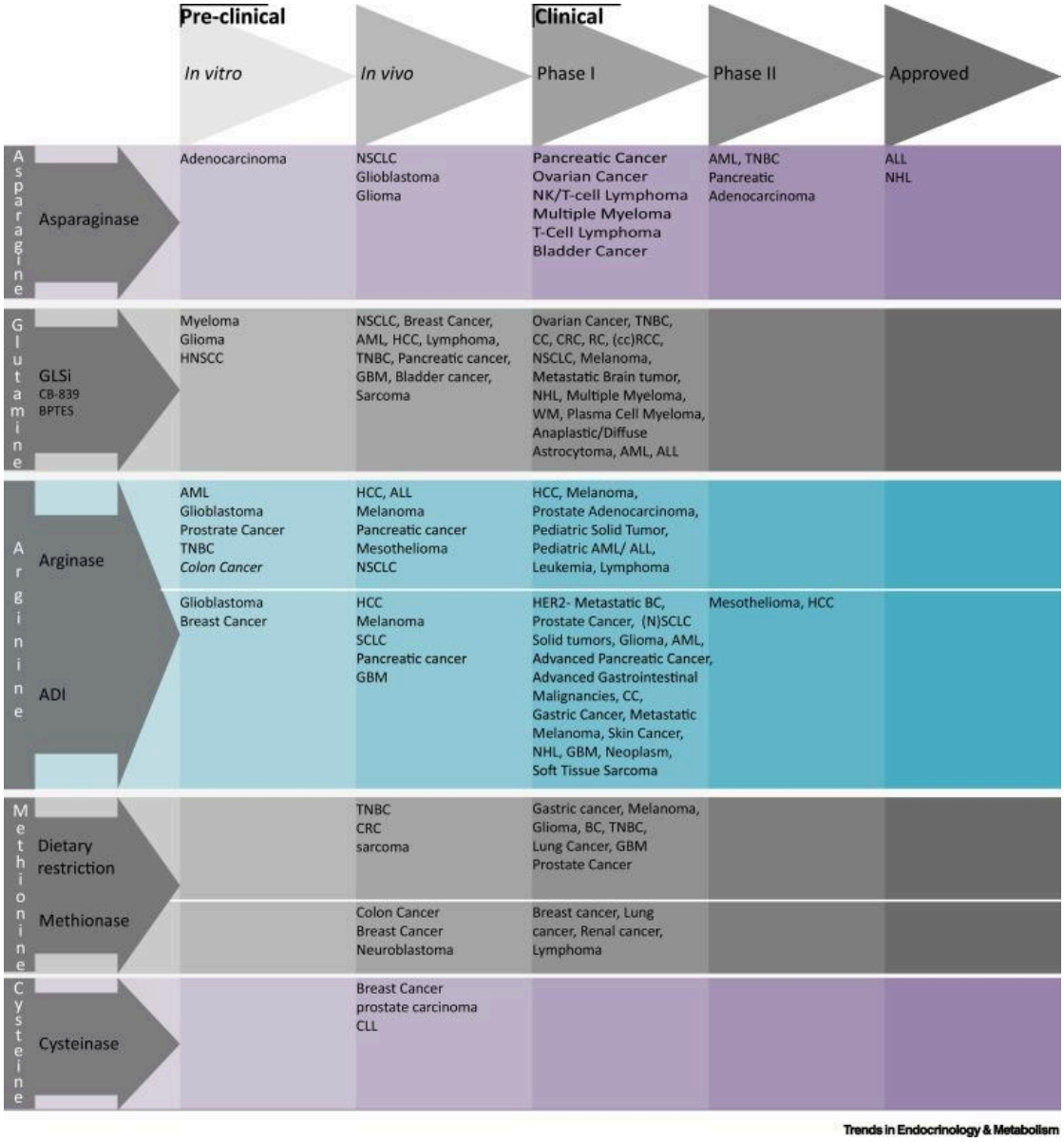
Recent strategies focus on the therapeutic target of the metabolic pathways developed by cancer cells to accommodate changes made to the cell biology, with focus especially on the enzymes involved. The clinical success of this strategy is attributed to the increased metabolic demand of these cells as required for their unorthodox nucleotide biosynthesis and DNA replication due to the mutation. However, nucleotide metabolism only covers one of the many metabolic needs of cancerous cells. Cell proliferation also affects metabolic demand, as proliferating cells differ in their nutritional requirements from non-proliferating cells. Proliferating cells, much unlike non-proliferating cells with their mainly catabolic demands, must balance diverging anabolic and catabolic requirements to sustain cellular homeostasis, all this during cellular replication. Their metabolic pathway is thus distinct from that of the origin tissue (Luengo et al., 2018).

One such pathway is glutaminolysis, the breakdown of glutamine into glutamate and other substances. Glutamine, a non-essential amino acid, is consumed far more excessively than

other amino acids by cancer cells *in vitro*, leading to their dependency on the amino acid, even from extracellular sources. Luengo et al., (2018) states that the cell covers this with high rates of glutaminolysis, supporting proliferation by replenishing depleted TCA cycle intermediates. Glutaminase, the key enzyme for this process, is considered a plausible target. In response to this study, small molecule CB-839 was developed as one of the few glutaminase inhibitors undergoing evaluation in clinical trials. Another is IPN6009, a glutaminase-1 selective inhibitor which has exhibited physicochemical properties in phase 1 of its clinical trials (Song et al., 2018). Investigations regarding other inhibitors such as PHDGH inhibitors, which aim to block serine biosynthesis, and mutant isocitrate dehydrogenase (IDH) inhibitors are yet to move into the clinical stage.

Discussion

For further movement in this form of treatment, the relationship between cancer cells and their microenvironment needs to be studied. This is especially important regarding the numerous developed regulators which restrict amino-acid influenced pathways. Though the regulators perform better than anti-glycolysis techniques, and with less toxicity, some may influence other characteristics of the cancer cells, making them more aggressive. Possible solutions involve a combination of different treatments as studies show better results when nutrient-targeted therapy is combined with other first-line cancer treatments. For instance, as observed in Fan et al., (2022), “glucose deprivation-induced inactivation of PRC1 (polycomb-repressive complex 1) promotes ER (endoplasmic reticulum) stress and cell death, leading to the strategic combination of PRC1 inhibitor and GLUTi treatment in cancer cells.”



[Figure 2] Progress of Amino Acid Depletion Therapies in the Treatment of Cancer in 2021 (Butler et al, 2021).

Despite the broad applicability of therapies targeting nutrient dependency in the treatment of cancer, consideration should be taken of possible antitumor effects that high levels of a specific amino acid may have. This could be either intrinsic to the tumor cells or involve extrinsic mechanisms such as the stimulation of an antitumor immune response. For example, Dietary Gln supplementation was shown to block melanoma tumor growth and prolong survival in a transgenic mouse model by affecting epigenetic reprogramming, whereas the supplementation of histidine (His) increased the sensitivity of leukemic xenografts to methotrexate (MTX) in a study by Butler et al., (2021). Furthermore, studies are being carried out concerning adaptation of the targeted cells to amino acid restriction. In response to low levels of intracellular amino acid, cells activate an elaborate transcriptional program, Amino Acid Response (AAR) signaling.

AAR signaling in turn rewrites cellular metabolic pathways to restore amino acid stability. This response is one of many challenges when pushing toward effective clinical translation of amino acid restriction techniques (Pathria and Ronai, 2021).

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