Overcoming anticancer drug resistance is a major challenge in cancer therapy, requiring innovative strategies that consider the extensive tumor heterogeneity and adaptability. We provide recent evidence highlighting the key role of amino acid (AA) metabolic reprogramming in cancer cells and the supportive microenvironment in driving resistance to anticancer therapies. AAs sustain the acquisition of anticancer resistance by providing essential building blocks for biosynthetic pathways and for maintaining a balanced redox status, and modulating the epigenetic profile of both malignant and non-malignant cells. In addition, AAs support the reduced intrinsic susceptibility of cancer stem cells to antineoplastic therapies. These findings shed new light on the possibility of targeting non-responding tumors by modulating AA availability through pharmacological or dietary interventions.

The Multifaceted Factors behind Anticancer Drug Resistance

Despite constant progress towards developing effective cancer treatments, drug resistance currently remains a major challenge for cancer cure. The emergence of resistant clones results from the selection of both pre-existing and drug-induced resistance-mediating factors, which can be achieved by genetic and epigenetic alterations [1], together with the influence of the tumor microenvironment (TME) (see Glossary) [2] and the presence of cancer stem cells (CSCs) [3].

In this context, growing attention has been directed to the immense heterogeneity of tumor metabolism (Box 1). Drug-induced selective pressures favor the emergence of specific metabolic traits that confer the best resistance strategy. Several papers have described the importance of metabolic reprogramming in supporting drug resistance [4], mostly focusing on glucose metabolism and the energy requirements of resistant cells [5]. However, tumor metabolism is far more complex than a simple balance between glycolysis and oxidative phosphorylation (OXPHOS), and other aspects of the metabolic network, such as lipids [6] and amino acids (AAs), are recently emerging as key determinants of cancer progression and drug resistance. In this review we summarize recent findings demonstrating how cancer cells and the supportive microenvironment adapt their AA metabolism to overcome drug toxicity. This information offers new tools to meet the urgent need to develop innovative strategies to fight drug resistance.

Drug-Specific Adaptations in Amino Acid Metabolism Support Precise Resistance Strategies

The role of AAs is becoming an attractive topic in the field of cancer metabolism. AAs support almost all the biosynthetic pathways that are upregulated in cancer cells and are crucial mediators of the redox homeostasis balance (Box 2). Furthermore, AA metabolism provides resistant cells with specific adaptive traits to counteract the mechanism of action of the anticancer drugs they are exposed to [4] (Figure 1, Key Figure). Cancer cells that develop resistance to genotoxic therapies commonly display metabolic adaptations to prevent DNA damage-induced cell death [7], especially by promoting nucleotide biosynthesis [8]. In cisplatin (CPT)-resistant non-small cell lung cancer (NSCLC) cells, the adaptive mechanism relies on the increased CPT-induced expression of the gene encoding ornithine carbamoyltransferase (OCTT), a key enzyme of the citrulline pathway, and the subsequent accumulation of citrulline in the extracellular milieu. This leads to a reduced intracellular NAD+ level and facilitates the preservation of DNA repair capacity by increasing the availability of the citrulline metabolic pathway, which is one of the main AA metabolic pathways in cancer cells [9].

Highlights

Cancer cells reprogram their amino acid (AA) metabolism to sustain tumor progression and implement specific strategies of resistance to anticancer therapies.

Cancer cells establish metabolic crosstalk with cellular and non-cellular components of the tumor microenvironment that finally provides cancer cells with the nutrients necessary to support anticancer therapy resistance and cancer immune escape.

AA availability dictates the epigenetic status of all the components of the tumor, thereby contributing to the anticancer drug-resistance phenotype.

Altered AA metabolism contributes to the maintenance of cancer stem cell subpopulations, thus supporting tumor relapse and anticancer drug resistance.

Approaches targeting AA availability in the tumor microenvironment could be valid supportive tools for therapeutic interventions aimed at counteracting drug resistance.

1 Department of Experimental and Clinical Biomedical Sciences, University of Florence, Viale Morgagni 50, 50134 Florence, Italy
2 Laboratory of Cellular Metabolism and Metabolic Regulation, VIB-KU Leuven Center for Cancer Biology, VIB, Herestraat 49, 3000 Leuven, Belgium
3 Laboratory of Cellular Metabolism and Metabolic Regulation, Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Herestraat 49, 3000 Leuven, Belgium
4 Department of Experimental and Clinical Medicine, University of Florence, Viale Morgagni 50, 50134 Florence, Italy
5 These authors contributed equally

*Correspondence: marialetizia.taddei@unifi.it (M.L. Taddei).
cell lung cancer (NSCLC) cells, glutamine is mostly utilized for nucleotide biosynthesis rather than for bioenergetic, redox, or anaplerotic reactions, making these cells highly sensitive to glutamine deprivation [9]. Similarly, dietary methionine restriction resensitizes patient-derived xenograft (PDX) models of RAS-driven colorectal cancer resistant to 5-fluorouracil (5-FU), primarily affecting nucleotide metabolism and inducing increased methionine production from homocysteine, which consumes intracellular 5,10-methylene-tetrahydrofolate (CH2-THF) [10] (Box 3). In methotrexate (MTX)-resistant hematopoietic cells, depletion of two enzymes driving the histidine degradation pathway (formimidoyl transferase, FTCD; and histidine ammonia lyase, HAL) favors therapy resistance by decreasing the flux through the pathway, thus sparing the cellular THF pool for nucleotide biosynthesis under MTX treatment (Box 3). In vivo dietary supplementation of histidine sensitizes leukemia xenograft mouse models to MTX [11].

The mechanism of action of several anticancer therapies relies on increased oxidative stress-mediated cell death (Box 3). In these cases, resistant cells adapt their metabolism to generate the crucial mediators of cellular redox balance, NADPH and reduced glutathione (GSH), allowing them to strengthen their antioxidant capacity and overcome reactive oxygen species (ROS)-induced cell death [12]. In particular, intracellular GSH is synthesized de novo by glutamate-cysteine ligase (GCL) and glutathione synthetase (GSS) in a two-step reaction from glutamate, cysteine, and glycine, this latter being mostly derived from serine. The most relevant factors contributing to GSH synthesis are GCL activity and cysteine availability [13]. Under oxidative stress, GCL levels are enhanced by nuclear factor erythroid 2-related factor 2 (NRF2), Kelch-like ECH-associated protein 1 (Keap1), and nuclear factor κ light-chain enhancer of activated B cells (NF-κB), allowing cancer cells to overcome stressful conditions. In particular, it has been found that increased expression of GCLM, the modulatory subunit of GCL, correlates with therapy resistance in breast cancer, highlighting GCL as an effective pharmacological target to potentiate antineoplastic treatments. Indeed, inhibiting GCL is effective in reducing intracellular GSH levels and impairs tumor growth in different cancer models [14].

Cysteine and glutamate availability is regulated by the cystine/glutamate exchanger transporter (system xc−) that mediates cysteine uptake and glutamate export, finely tuning the intracellular GSH concentration. The xc− system acts as a Na+-independent and Cl−-dependent antiporter of the two anionic forms of these AAs. The transporter light chain xCT (encoded by the SLC7A11 gene) is specific for the import of cysteine, and it is overexpressed in many tumor cells and patient samples correlating with poor prognosis [15]. Furthermore, AA precursors of GSH are generated through the degradation of extracellular GSH by γ-glutamyl transferase (GGT), whose overexpression is associated with chemoresistance and worse outcome in breast cancer and sarcoma patients [16]. Ultimately, GSH content also depends on glutathione reductase (GR) activity, which reduces the oxidized form GSSG to GSH to maintain cellular redox homeostasis [17].

Alterations in the metabolism of GSH-related AAs are frequently associated with resistance to oxidative stress-inducing agents [15]. Increased serine metabolism correlates with acquired resistance to bortezomib (BTZ) in multiple myeloma. Strong upregulation of the serine synthesis pathway (SSP) allows BTZ-resistant cells to maintain intracellular levels of GSH in vitro, thus increasing their antioxidant capacity. Accordingly, higher phosphoglycerate dehydrogenase (PHGDH) levels have been detected in CD138+ plasma cells from patients with multiple myeloma refractory to BTZ therapy [18]. In both in vitro and in vivo models of hepatocellular carcinoma (HCC) resistant to the tyrosine kinase inhibitor (TKI) sorafenib, enhanced glutamine utilization enables resistant cells to maintain redox balance by increasing NADPH and GSH levels [19]. In addition, CPT-resistant NSCLC cells show increased extracellular glutamine uptake, glutaminase
(GLS) activity, and glutamate secretion through the xc− system. The upregulation of the glutamine/glutamate axis flux allows resistant cells to potentiate GSH generation, thus countering CPT-induced oxidative stress. Consequently, CPT-resistant cells are susceptible to both glutamine deprivation and treatment with system xc− inhibitors [20]. In breast cancer, enhanced resistance to oxidative stress is mediated by the incorporation of glutamate into GSH driven by the oncocogenic PI3K/Akt signaling pathway, and contributes to reduced sensitivity to CPT [21]. In epidermal growth factor receptor (EGFR) mutant lung cancer cells, continuous treatment with sublethal doses of EGFR TKIs establishes persistent drug-resistant cells through epigenetic-mediated upregulation of branched-chain amino acid aminotransferase 1 (BCAT1). This regulation leads to increased transamination of branched-chain amino acids (BCAAs) and the consequent accumulation of glutamate, which is converted to GSH, allowing resistant cells to counteract drug-induced oxidative damage, in both in vitro and in vivo PDX models. BCAT1 expression is higher in patients with EGFR mutant lung cancer who exhibit a reduced response to EGFR-TKi treatment [22]. Enhancing the drug-induced oxidative stress by inhibiting the xc− system is emerging as a valid strategy for the induction of ferroptosis [23]. For instance, treatment with the xc− system inhibitor erastin potentiates the toxic effect of CPT in an in vitro model of resistant ovarian cancer [24]. A possible mechanism of resistance to ferroptosis is provided by BCAT2-driven intracellular accumulation of glutamate. In this context, the combined administration of different ferroptosis inducers has a synergistic effect in inhibiting BCAT2 expression, thereby inducing ferroptotic death in various in vivo models of liver and pancreatic cancers [25].

Alternative adaptive strategies were described in endocrine therapy (ET) resistance, where activation of the autophagic process helps resistant cells to survive nutritional stresses. Enhanced miR-23b-3p expression was found in in vitro models of ET-resistant breast cancer, and conferred catabolic and anabolic advantages by increasing the expression of solute carrier 1A2 (SLC1A2) glutamate/aspartate transporter and activating autophagy. These findings were also validated in an in vivo PDX model and retrospective clinical data of ET-treated patients [26]. In tamoxifen (TMX)-resistant ER+ breast cancer cells, SLC7A5 cell-surface localization promotes leucine uptake and allows mammalian target of rapamycin (mTOR) activation, thereby sustaining cell proliferation under nutritional stress. High levels of SLC7A5 correlate with poor survival of ER+ breast cancer patients treated with TMX [27]. In accordance, silencing the neutral AA transporter complex SLC7A5/SLC3A2 increases breast cancer cell sensitivity to TMX. Moreover, SLC7A5/SLC3A2 expression identifies a cohort of ER+/HER2− breast cancer patients who fail to benefit from ET [28].

Cancer cells resistant to targeted therapies can also acquire peculiar metabolic traits because of the activation of specific signaling pathways. Resistance to BRAF kinase inhibitors (BRAFi) frequently occurs through the recovery of mitogen-activated protein kinase (MAPK)/Erk signaling or activation of the PI3K/Akt pathway, two mechanisms that finally converge on MYC activation. In BRAFi-resistant melanoma cells, enhanced MYC-mediated glutamine metabolism supplies building blocks for fatty acid and pyrimidine biosynthesis. In addition, MYC mediates increased dependency on the enzymes implicated in serine/glycine metabolism to fuel one-carbon metabolism and purine biosynthesis [29].

Amino Acids Drive Epigenetic Modulation of Drug-Resistant Cancer Cells

Epigenetic regulations are emerging as key contributors to anticancer drug resistance, and modulating the availability of AAs implicated in the epigenetic program is a promising strategy to target the transcriptional plasticity of therapy-resistant cancer cells. Histone and DNA methylation mediated by S-adenosylmethionine (SAM) is particularly sensitive to nutrient fluctuations. Indeed, methionine deprivation is sufficient to deplete SAM levels, thereby inducing epigenetic...
...cells to both epigenetically modulate resistance-related gene expression and impair the anticancer immune response. Tumor cells can outcompete CD8+ T cells for extracellular methionine by upregulating the methionine transporter SLC43A2, thereby depriving immune cells of both methionine and SAM, with consequent impairment of T cell immunity [31]. In paclitaxel-resistant triple-negative breast cancer (TNBC) cells, downregulation of methionine metabolism and a decreased rate of SAM synthesis contribute to an overall global decrease in DNA methylation [32]. The emergence of neuroendocrine prostate cancer (NEPC), an aggressive variant of prostate cancer that is resistant to androgen receptor (AR) targeted therapies, is promoted by mTORC1/activating transcription factor 4 (ATF4)-mediated upregulation of SSP and the consequent rise in SAM-mediated DNA methylation that is responsible for NEPC differentiation [33]. Notably, the metabolic dependency of cellular epigenetic status may underlie the selection of resistant clones in specific regions of solid tumors according to the heterogeneity of nutrient distribution. In particular, the intracellular content of glutamine-derived α-ketoglutarate (αKG) is significant because it supports the activity of the Jumonji domain-containing histone lysine demethylases. This fact implies that, in the core region of solid tumors displaying lower levels of glutamine and αKG compared with the periphery, cancer cells present a global profile of histone H3 hypermethylation that, in V600EBRAF melanoma cells, favors the emergence of dedifferentiated BRAFi-resistant subpopulations [34]. In different in vivo xenograft models of melanoma, dietary glutamine supplementation can resensitize BRAFi-resistant cells by increasing αKG-dependent hypomethylation of H3K4me3 [35].

**Tumor Microenvironment-Derived Amino Acids Confer Anticancer Therapy Resistance**

The efficacy of a therapeutic approach is strongly influenced by the dynamic crosstalk established among all the components of the TME. Metabolic reprogramming of both tumor and non-tumor cells establishes a balanced network that supports tumor progression and facilitates the selection of therapy-resistant clones [36]. AAs are emerging as essential elements in the complex crosstalk...
inside the TME as well as in the regulation of tumor-induced immunotolerance, underlining their role in chemotherapy and cancer immunotherapy resistance [37].

**Tumor-Induced Metabolic Education of Non-tumor Cells**

Cancer cells educate stromal cells to adapt their metabolism to provide nutrients, such as AAs, that are essential for tumor progression [38]. This metabolic crosstalk supports tumor survival under nutrient deprivation [39] and facilitates the establishment of resistant clones by providing AAs that are necessary to overcome stressful conditions and drug-induced damage. In pancreatic ductal adenocarcinoma (PDAC), cancer cells can educate tumor-associated macrophages (TAMs) to potentiate pyrimidine biosynthesis. The specific increase of TAM-derived deoxycytidine...
release confers gemcitabine (GEM) resistance in cancer cells by competing with deoxycytidine kinase (DCK) for GEM, thus reducing the effective intracellular levels of the drug [40]. A study conducted on persisting acute myeloid leukemia (AML) cells, isolated during the maximal response to cytarabine/doxorubicin (DOXO)-based induction chemotherapy (iCT) regimen, demonstrated that chemotherapy selects for a small tumor subpopulation able to survive the treatment by increasing pyrimidine and GSH biosynthesis. Interestingly, this metabolic adaptation is strongly dependent on aspartate provided by a subpopulation of leptin receptor (LepR)+ CXCL12+ mesenchymal stromal cells which are educated by AML [41]. In addition, melanoma cells promote chemoresistance and tumor growth by providing oxidizable substrates for the pentose phosphate pathway (PPP) [140], and proline catabolism via proline dehydrogenase 1 (PRDH) is activated in metastasis [98]. Cysteine drives cancer cell metabolic rewiring by supporting carbon, sulfur, and energy metabolism, and by acting as a precursor of GSH through glutamate cysteine ligase (GCL) activity. Cancer cells mainly depend on exogenous cysteine by upregulating the import, mediated by system xc−, of cysteine in exchange for glutamate [144]. Serine is also concerned with the endogenous serine synthesis pathway (SSP), primarily by upexpressing serine-cleaving dehydrogenase (PHGDH), the first enzyme of the pathway. In proliferating cells, serine is essential for redox balance, contributes to NADPH and GSH production [142], and supports the synthesis of sphingolipids, resulting in mitochondrial stability [143]. The SSP can activate the mTORC1 signaling pathway through the production of NADPH [142]. Moreover, serine catabolism generates glycine that is incorporated into the purine ring and GSH and provides one-carbon units through its oxidation by the glycine cleavage system [140]. Methionine is another component of one-carbon metabolism, and contributes to nucleotide synthesis via the folate cycle. It acts as a methyl donor and regulates polyamine and protein biosynthesis. Furthermore, methionine conversion into homocysteine via the transulfuration pathway protects cancer cells from oxidative damage [145]. By contrast, tetrahydrofolate (THF) is consumed in the last step of the histidine degradation pathway, which transfers the formiminogroup to THF, finally forming glutamate from histidine. Tumor cells are generally auxotrophic for arginine because of loss of arginosuccinate synthetase 1 (ASS1) and diversion of urea cycle intermediates from arginine synthesis towards pyrimidine production [146]. ASS1 deficiency also leads to the accumulation of aspartate, that is essential for nucleotide biosynthesis [147], and can be utilized in cancer cells as an anaplerotic substrate under tricarboxylic acid (TCA) cycle impairment [148]. Asparagine acts as an AA exchange factor and regulates the uptake of other AAs that are necessary to activate mTOR signaling and the biosynthesis of proteins and nucleotides [149]. Although leukemia cells lack asparagine synthetase (ASNS) and strongly depend on exogenous asparagine, overexpression of ASNS in different solid cancers is associated with chemoresistance and metastasis [150].
Figure 1. Major AA metabolic pathways altered in cancer cells. AAs are essential energy sources for cancer growth because they support biosynthetic pathways, maintain intracellular redox balance, and mediate epigenetic and post-transcriptional modifications. AAs provide sources to overcome anticancer drug-induced damage by providing essential building blocks for nucleotide biosynthesis and DNA damage repair. Asp and Gln are utilized by cancer cells for pyrimidine and, together with Gly, tryptophan biosynthesis.

(Figure legend continued at the bottom of the next page.)
Box 3. Anticancer Drugs That Selectively Affect Metabolic Processes

Several classes of antineoplastic drugs trigger cell death by directly or indirectly affecting cancer cell metabolism [151]. Antimetabolites are a class of drugs that are specifically designed to interfere with cell metabolism through their structural similarity to physiological metabolites. Antimetabolites interfere with the activity of enzymes involved in the synthesis of nucleotides or their precursors, thereby inducing apoptosis in highly proliferating cancer cells. Antimetabolites can be divided into antifolates (agonizing folic acid, i.e., methotrexate, MTX), purine analogs (i.e., 6-mercaptopurine), and pyrimidine analogs (i.e., 5-fluorouracil, 5-FU; gemcitabine, GEM; and azacitidine). The mechanisms of action of the most clinically relevant antimetabolites are reported here.

5-FU is an analog of uracil which, once converted into active metabolites, can either be incorporated into DNA and RNA, thus interfering with their structure and functionality, or competitively inhibits thymidylate synthase (TS). TS is the sole enzyme allowing the generation of dTMP through 5,10-methylene tetrahydrofolate (5,10-CH2-THF)-mediated reductive methylation of dUMP. In addition to general drug-resistance mechanisms (including drug efflux, DNA damage repair, and evasion of cell death), specific adaptations can be identified for antimetabolites. In particular, 5-FU resistance is induced by target-related mechanisms such as TS overexpression, dUMP accumulation, and reduced cytosolic levels of 5,10-CH2-THF.

MTX and its polyglutamate derivatives competitively inhibit dihydrofolate reductase (DHFR), which catalyzes the dihydrofolate conversion into THF that is necessary for nucleotide biosynthesis. Polyglutamate derivatives are also pyrimidine synthase and TS inhibitors, further interfering with DNA synthesis. MTX target-related resistance mechanisms include DHFR overexpression or mutations, decreased intracellular drug retention caused by diminished MTX polyglutamylation, and intracellular THF accumulation. GEM is a cytidine analog that is sequentially phosphorylated by deoxycytidine kinase (DCK), pyrimidine nucleoside monophosphate kinase (UMP/CMP kinase), and nucleoside diphosphate kinase (NDPK), resulting in active GEM derivatives. These are incorporated into DNA strands and generate ‘masked termination’, blocking DNA polymerase activity. GEM-targeted resistance mechanisms include dysregulation/inhibition of proteins participating in its metabolism, including DCK.

In addition to their primary mechanism of action, many anticancer drugs interfere with cancer metabolism by altering intracellular redox status and inducing reactive oxygen species (ROS) production, finally leading to apoptosis. Among them, platinum-based therapies (cisplatin, CPT; carboplatin; and oxaliplatin), anthracycline (doxorubicin, DOXO), proteasome inhibitors (PIs: bortezomib, BT2; and sorafenib) exploit cytotoxicity by inducing oxidative stress. Specifically, CPT forms conjugates with reducing equivalents such as GSH, thereby facilitating their export and elimination, and resulting in ROS accumulation; the redox cycling of the DOXO quinone moiety leads to ROS generation through a mechanism triggered by mitochondrial respiration-derived NADPH; PIs elicit endoplasmic reticulum (ER) stress which is related to ROS production; and sorafenib inhibits the xCT system, thereby impairing exogenous cystine uptake and its conversion to GSH.

immunotherapeutic approaches [44] (Figure 2). Increased IDO1 expression in cancer cells generates an immunosuppressive environment by depleting the TME of tryptophan and by enhancing kynurenine accumulation. Higher IDO activity in NSCLC patients correlates with intrinsic resistance to anti-PD1 treatment [45]. Accordingly, an increased kynurenine/tryptophan ratio in the serum of advanced melanoma and renal cell carcinoma patients treated with anti-PD1
nivolumab was associated with an adaptive resistance mechanism and a consequent worse overall survival [46].

Exogenous glutamine concurrently favors cancer cell growth and the generation of a protumoral immunosuppressive microenvironment. Indeed, high glutamine availability favors the acquisition of the protumorigenic M2 phenotype in macrophages [47]. Consistently, in immunotherapy-resistant mouse models of TNBC and Lewis lung carcinoma, impairing glutamine metabolism enhances the efficacy of checkpoint blockade therapy by favoring the differentiation of myeloid-derived suppressor cells (MDSCs) into proinflammatory TAMs. Indirectly, targeting glutamine metabolism also resensitizes resistant tumors to checkpoint blockade therapy by reducing the transcriptional activity of signal transducer and activator of transcription (STAT)1/3 and consequently decreasing IDO gene expression in tumor cells, thereby enhancing anticancer T cell functions [48]. This virtuous effect of glutamine antagonism is further potentiated by the
extraordinary metabolic plasticity of tumor-infiltrating CD8+ T cells that, unlike cancer cells, are able to overcome glutamine deprivation by reprogramming their energetic metabolism towards OXPHOS. This flexibility enables CD8+ T cells to increase their survival and to enhance effector and memory functions. T cells activated under glutamine blockade in vitro display a significant attenuation of αKG levels with a consequent increase in histone methylation status, reflecting the upregulated expression of memory T cell phenotype-related markers [49]. Similarly, systemic inhibition of the xc− system enhances the anticancer effect of anti–cytotoxic T lymphocyte antigen 4 (CTLA4) immunotherapy by inhibiting cancer cell proliferation, without affecting T cell antitumor efficacy in vivo [50].

In activated T cells, high intracellular arginine promotes the generation of central memory-like cells with higher survival capacity, and potentiates the antitumor activity of CD8+ T cells both in vitro and in vivo [51]. Arginine supplementation improves α-programmed death ligand 1 (α-PD-L1) antibody efficacy in immunocompetent murine models bearing orthotopic or metastatic osteosarcoma by increasing CD8+ T cell activation, promoting cytotoxic T lymphocyte (CTL) infiltration, and protecting intratumor CTLS from exhaustion [52]. Taking advantage of this T cell auxotrophy for arginine, myeloid cells in the TME commonly increase their expression of arginase 1 (Arg1) and consequently deplete extracellular arginine as an immunosuppressive strategy. Therefore, treatment with Arg1 inhibitors is emerging as a compelling strategy to potentiate infiltrating T cell antitumor activity. Indeed, this approach enhanced the efficacy of GEM-induced immunosuppression, adoptive T cell and natural killer (NK) cell therapies, and checkpoint blockade therapy in several in vivo models [53], and improved CD33−chimeric antigen receptor (CAR)-T cell therapy against AML in vitro [54].

By a different mechanism, immunotherapy-activated CD8+ T cells may target tumor cells by promoting lipid peroxidation and ferroptosis through interferon γ (IFN-γ)-mediated downregulation of system xc− subunits [55]. Similarly, in high-grade serous ovarian carcinoma (HGSOC), CD8+ T cells repress CPT-resistance by interfering with the crosstalk between tumor cells and cancer-associated fibroblasts (CAFs). Indeed, CAFs confer resistance by decreasing CPT accumulation in tumor cells as a result of increased GSH and cysteine release. Through the uptake of these two thiol-based compounds, tumor cells increase their intracellular GSH content, which mediates CPT resistance via increased efflux of the GSH–platinum complex. In this scenario, CAFs support the nucleotide biosynthesis that is necessary for cancer cell proliferation, and cancer cell-derived glutamate preserves the oxidative balance in CAFs [58]. Moreover, PDAC cells are able to directly take up extracellular...
collagen and utilize collagen-derived proline to support the tricarboxylic acid (TCA) cycle via proline dehydrogenase 1 (PRODH1) upregulation under nutrient deficiency. Interestingly, PRODH1-mediated proline metabolism is essential for tumor growth, as demonstrated in KRAS mutant PDAC cells in vitro and in a PDAC mouse model in vivo [59], as well as for metastasis formation in breast cancer [60].

**Amino Acid Metabolic Regulation of Cancer Stem Cells Supports Cancer Aggressiveness**

Eliminating the CSC subpopulation represents a major challenge in cancer therapy because of their intrinsic lower sensitivity to antineoplastic agents. However, CSCs display specific aspects of AA metabolism that could be addressed to develop new therapeutic approaches. Interestingly, leukemia stem cells (LSCs) isolated from patient-derived primary AML specimens exhibit reduced metabolic flexibility and rely on AA metabolism to fuel OXPHOS [61], which is often upregulated in CSCs to prevent chemotherapy toxicity [62]. Therefore, LSCs are strongly dependent on AAs to survive, and treatment with azacytidine and the BCL-2 inhibitor venetoclax effectively eradicates the LSC population by decreasing AA uptake and their flux through OXPHOS. Nevertheless, LSCs derived from relapsed patients are more resistant to chemotherapy treatment than LSCs isolated from de novo AML patients, and also display a higher metabolic flexibility that allows them to adapt to AA depletion by upregulating fatty acid metabolism [61]. Among others, cysteine is fundamental for LSC survival, and treatment with the cysteine-degrading enzyme, cyst(e)inase, effectively eradicates LSCs derived from patients with both de novo and relapsed AML [63]. Accordingly, overexpression of the catalytic subunit of the xc− system in glioma cells leads to an increased percentage of cells with a CSC-like phenotype and consequent enhancement of temozolomide resistance [64]. Maintaining a strict redox regulation is a crucial feature for CSCs. In head and neck squamous cell carcinoma (HNSCC) cells, the xc− system and the glutamine transporter SLC1A5 are selectively upregulated in CD44 variant (CD44v)-expressing stem-like undifferentiated cells [65]. CD-44v-mediated xc− system subunit overexpression results in the acquisition of CPT resistance in both lung and urothelial cancer [66,67]. The xc− system thus represents a promising target for immunotherapy against undifferentiated cancer cells. In this perspective, DNA-based vaccination against a specific subunit of the xc− system has been demonstrated to exert antitumor activity in different breast cancer xenografts. This approach allows inhibition of xc− function in CSCs, thereby eradicating their self-renewal abilities and redox balance, leading to increased sensitivity to DOXO and impairment of pulmonary metastasis formation [68]. In addition to the xc− system, glutamine also participates in maintaining redox balance in CSCs. In HCC, glutamine deprivation or inhibition of GLS1 activity increases ROS accumulation, which suppresses the Wnt/β-catenin pathway, thereby reducing CSC marker expression and colony-forming potential both in vitro and in vivo. Accordingly, upregulation of GLS1 is associated with a stemness phenotype and advanced clinicopathological features in nondifferentiated HCC cell lines and HCC patient-derived samples [69]. A similar ROS/β-catenin-mediated mechanism was described in NSCLC, where glutamine deprivation or pharmacological targeting with L-asparaginases (ASNases) decreased the proportion of stem-like cancer cells in vitro and prevented tumorigenesis in in vivo xenograft models [70]. By contrast, tryptophan deprivation supports CSC maintenance by decreasing the endogenous levels of the tryptophan derivatve 2-(10H-indole-30-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE). ITE is essential to revert the stemness phenotype by inhibiting the transcription of the master pluripotency factor Oct4, and finally reduces the tumorigenicity of stem-like cancer cells in different in vivo tumor models [71].

Furthermore, altered one-carbon metabolism is crucial for the maintenance of the stem-like subpopulation and the consequent resistance to antineoplastic agents. Environmental serine...
availability profoundly affects squamous cell carcinoma initiation and the fate of epidermal stem cells (EpSCs). Indeed, abundant exogenous serine is essential for the expansion of tumor-initiating EpSCs. Accordingly, serine deprivation induces EpSCs to activate the de novo SSP, which in turn stimulates cK-G-dependent dioxygenases to remove the repressive histone modification H3K27me3, and finally sustains differentiation programs and suppresses tumor initiation in mice [72]. Similarly, in patient-derived AML stem cells, that are characterized by an upregulated BCAA degradation pathway, knockdown of BCAT1 results in cK-G accumulation and increased cK-G-dependent dioxygenase activity, leading to HIF1α protein degradation and suppression of tumor-initiating potential both in vitro and in vivo [73].

Through a different mechanism, methionine restriction reduces the CD44hi/CD24low CSC population in TNBC cell lines. In particular, in the absence of methionine, CSCs become strictly dependent on methionine adenosyltransferase 2A (MAT2A) activity for SAM biosynthesis, and the combination of methionine deprivation with the MAT2A inhibitor cycloleucine suppresses histone methylation, reduces stem-like properties in vitro, suppresses lung metastases, and induces apoptosis in primary tumors in vivo [74]. Similarly, tumor-initiating cells (TICs) derived from resected primary NSCLC display an elevated methionine cycle activity, thus becoming addicted to exogenous methionine. Therefore, methionine depletion impacts on the tumorigenic potential of TICs by imposing epigenetic alterations [75]. In patient-derived lung adenocarcinoma cells and xenograft models, mitochondrial methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) is essential to confer stem-like properties through the β-catenin pathway and ensure TKI-gefitinib resistance by modulating purine metabolism. Mechanistically, enhanced expression of MTHFD2 allows cancer cells to maintain low amounts of intracellular 5-aminoimidazole carboxamide ribonucleotide (AICAR), and consequently decreases AMPK activation, which partly contributes to drug resistance and stem-like properties [76]. Both ER+ and ER− breast CSCs (BCSCs) display upregulation of PHGDH under hypoxic conditions, and consequently PHGDH knockdown strongly abrogates the enrichment of ALDH+ BCSCs induced by DOXO or carboplatin treatment in vitro and in vivo orthotopic tumors, thereby increasing their sensitivity to chemotherapy [77]. Furthermore, in colorectal cancer, thrombopoietin (TPO)-dependent activation of lysine catabolism in CD110+ TICs drives the early stages of liver metastasis. In particular, lysine degradation generates acetyl-CoA, which supports LRP6 acetylation with consequent activation of Wnt signaling to promote self-renewal of CD110+ TICs, and produces glutamate that, through the modulation of redox homeostasis, promotes liver colonization and may confer resistance to anticancer drugs [78].

**Modulation of Amino Acid Availability for Cancer Therapy**

Given that AA metabolism adaptations contribute to acquired resistance to different antineoplastic agents, targeting these specific vulnerabilities has recently emerged as a strategy to develop successful anticancer therapy tools. The most common therapeutic approach is represented by the pharmacological inhibition of specific enzymes involved in cancer AA metabolism (reviewed in [79,80]). Furthermore, modulating the systemic availability of a specific nutrient can impair the progression of tumors that are unable to synthesize it. For example, melanoma cells that are arginine auxotrophic, due to lack of argininosuccinate synthetase (ASS1) expression, arrest cell proliferation under arginine deprivation in vitro and experience growth inhibition when injected into autophagy-defective mice – which display decreased serum arginine levels because the arginine-degrading enzyme Arg1 is released from the liver into the circulation [81].

Pharmacological depletion of specific AAs is an effective strategy to decrease their circulating levels and target auxotrophic tumors. In this context, one of the primary drugs used in ALL treatment, ASNase, acts by systematically depleting circulating asparagine. ASNase toxicity may be overcome by cancer cells through adaptive mechanisms, as demonstrated in a mouse model
of breast cancer metastasis, where SLC1A3 acts as a mediator of ASNase resistance by providing cancer cells with the aspartate and glutamate pools necessary to survive under ASNase treatment. Accordingly, inhibition of SLC1A3 overcomes resistance to ASNase therapy in prostate cancer cells in vitro [82]. In addition, cyst(e)inase treatment displays a greater efficacy in affecting chronic lymphocytic leukemia (CLL) compared with the standard-of-care drug, fludarabine, in both in vitro models and primary leukemia cells isolated from CLL drug-resistant patients [83]. Interestingly, cyst(e)inase treatment is effective in inducing ferroptosis in KRAS/p53 mutant pancreatic tumors in mice [23] and in EGFR mutant NSCLC xenograft models [84]. Similarly, reducing the circulating levels of methionine through administration of methioninase or its recombinant form rMETase demonstrated tolerability and efficacy in Phase I clinical trials [85]. Although this treatment option did not clinically advance over the past decade, recent reports have highlighted the efficacy of rMETase in BRAF V600E-negative melanoma and Ewing’s sarcoma patient-derived orthotopic xenograft (PDX) nude mouse models [86,87], and in an orthotopic mouse model of osteosarcoma [88,89]. Moreover, a pilot Phase I clinical study recently confirmed the absence of toxicity of rMETase therapy, indicating that pharmacological depletion of circulating methionine could be an interesting therapeutic strategy to be exploited for future applications [90]. Of note, different studies recently demonstrated that the efficacy of rMETase-based therapy is further potentiated in combination with other chemotherapeutic agents [91,92].

In addition, dietary AA deprivation and/or supplementation are emerging as novel promising methods to overcome chemotherapy resistance and delay cancer progression [93]. Given its pleiotropic role in tumor progression, together with pharmacological inhibition, dietary modulation of glutamine metabolism is one of the most-investigated therapeutic approaches, and demonstrates promising efficacy in suppressing tumor growth in various cancer types both in vitro and in vivo [94].

Similarly, methionine restriction rapidly leads to specific perturbations of methionine and sulfur metabolism, without altering the levels of other circulating AAs [10]. This approach has emerged as a potential strategy for cancer treatment that leads to cell-cycle inhibition and apoptosis in cancer cells [95,96]. Limiting dietary methionine also improves cancer therapy outcome in different drug refractory tumors [10,97]. Interestingly, methionine depletion synergizes with the humanized agonistic tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-R2 monoclonal antibody, lexatumumab, in both in vitro and in vivo TNBC models by increasing TRAIL-R2 mRNA levels and cell-surface expression [98]. A Phase II clinical trial is currently ongoing to confirm these promising preclinical findings (NCT03186937). Furthermore, methionine restriction acts as a stronger inhibitor of PDX colorectal cancer growth when provided 2 weeks before tumor inoculation, suggesting that this dietary intervention may exert its effects at the early stages of tumorigenesis [10].

In genetically engineered mouse models of serine-dependent tumors, such as Myc-driven lymphoma and inactive-Apc-driven intestinal tumors, administration of a serine and glycine (SG)-free diet reduces tumor growth. Of note, the efficacy of SG deprivation is strongly dependent on the genetic alterations of tumors, as demonstrated by the limited efficacy of SG starvation in affecting the survival of mice harboring PDAC cells induced to express activated KRAS, and which are able to respond to dietary SG restriction by upregulating SSP [99]. In addition, in mice harboring colon xenograft tumors, SG dietary restriction also promotes the accumulation of toxic deoxysphingolipids and mitigates tumor growth, an effect that can be further potentiated by inhibiting PHGDH [100]. Supporting the concept that the mutational background of tumors strongly affects their dependency on exogenous AAs, loss of Keap1 and the consequent chronic activation of the oxidative sensor NRF2 sensitizes cells to dietary or enzymatic depletion of nonessential AAs (NEAAs; namely asparagine, serine, and glycine) in vivo. Furthermore, pharmacologically decreasing intracellular glutamate levels further sensitizes cancer cells to NEAA depletion, and significantly reduces tumor growth.
In addition, feeding breast cancer-bearing mice with an asparagine-depleted diet leads to decreased cancer cell invasion and metastasis, partly through a reduction of epithelial-to-mesenchymal transition (EMT) proteins that are generally enriched in asparagine content [102]. However, resistance mechanisms to AA deprivation frequently undermine these therapeutic strategies. For instance, enhanced pyruvate carboxylase activity favors glutamine-independent growth by allowing cancer cells to utilize glucose-derived pyruvate instead of glutamine for anaplerosis [103]. Moreover, increased glutamine synthetase (GS) expression promotes resistance to glutamine restriction by enhancing glutamate-derived glutamine production [104]. In addition, p53-mediated induction of SLC7a3 leads to an increase in intracellular arginine levels and sustains cancer cell proliferation upon glutamine deprivation by activating mTORC1 [105]. Similarly, increased SLC1A3 expression mediates aspartate uptake to support nucleotide biosynthesis, and maintains electron transport chain (ETC) and TCA cycle activity through a p53-mediated adaptive mechanism to glutamine withdrawal [106]. Furthermore, sestrin 2 overexpression under glutamine deprivation facilitates mTORC2 activation, thereby favoring cancer cell growth by preserving intracellular ATP levels and maintaining redox balance [107]. In addition, arginine deprivation also induces adaptations in cancer cells, including reactivation of ASS1 expression through both genetic and epigenetic modulation. Activation of the Ras/ERK and PI3K/AKT signaling pathways results in phosphorylation and stabilization of c-Myc, finally leading to enhanced ASS1 expression [108]. ASS1 levels can also be epigenetically modulated by a chromatin-remodeling program that, under arginine starvation, induces HIF-1α degradation and the concomitant mobilization of c-Myc at the ASS1 promoter region [109].

Lastly, targeting metabolic crosstalk between tumor cells and the surrounding TME represents an attractive alternative strategy to inhibit tumor growth. Interestingly, cancer and immune cells often depend differently on a given AA for their survival and function. Because arginine is necessary for cancer cell growth, and its supplementation improves the survival and antitumor efficacy of central memory T cells [51], inhibiting arginine metabolism in tumor cells while avoiding arginine starvation in the T cell population could be a valid strategy to obtain clinical benefits. Similarly, methionine supplementation leads to increased T cell immunity [31], whereas methionine restriction impairs cancer cell growth, further underlining the importance of selectively targeting AA metabolism in different cell populations. A possible strategy to maximize the therapeutic potential of these approaches could be to modulate the dietary intervention according to the composition of the tumor immune infiltrate in a patient-specific manner. Indeed, although methionine restriction impairs antitumor T cell functions, it reverses the immunosuppressive activity of protumorigenic macrophages (M2) and facilitates TAM polarization towards antitumorigenic macrophages (M1), thus potentiating the efficacy of immunotherapies [110]. Following this evidence, methionine restriction could be more efficacious in patients displaying high M2 macrophage infiltration, as demonstrated by results obtained in in vivo models of advanced kidney and prostate cancers [110]. In addition, cotargeting specific metabolic features of different TME populations may potentiate dietary approaches. Promising results have been obtained in an orthotopic mouse model of ovarian carcinoma by simultaneously silencing GLS in cancer cells and GS in the corresponding CAFs. This approach depletes cancer cells of the CAF-derived glutamine they depend upon to support nucleotide biosynthesis, and consequently impairs cancer cell proliferation [111].

Combining an AA-deprived diet with drugs that specifically target cellular components of the TME is another strategy to be evaluated. In PDAC, peripheral axons release serine to support exogenous serine-dependent tumor growth under SG deprivation. Pharmacologically blocking nerve infiltration further decreases tumor growth in mice on a SG-deprived diet [112].
Concluding Remarks and Future Perspectives

It has long been known that cancer cells can reprogram their metabolism to overcome stressful conditions and adapt their energy and biosynthetic needs. In particular, as highlighted in this review, AA metabolic rewiring may provide cancer cells with specific tools to bypass harsh conditions imposed by anticancer therapies. In this context, a significant contribution is made by the components of the TME – that adapt their AA metabolism to create a supportive environment for tumor progression and replenish cancer cells with the specific AAs necessary for their survival. Targeting the AA metabolic crosstalk within the TME thus represents a valid strategy to enhance the anticancer therapy response. In particular, the strong differences between cancer and immune cells in managing metabolic stress shed light to a still not completely investigated ‘metabolic checkpoint’ for anticancer immunotherapy. In addition, modulating plasma AA levels by pharmacological or dietary intervention is a promising option to improve anticancer therapies. Indeed, given the absence of toxicity and easy patient acceptability, dietary modulation of specific AAs is yielding good results. However, in adopting these strategies, attention must be addressed to some key aspects. First, it is necessary to consider the specific metabolic adaptations implemented by resistant cells to circumvent the mechanism of action of the drug they are exposed to, so as to selectively modulate the correct metabolic vulnerability. Moreover, choosing the appropriate dietary modulation according to the specific characteristics of the tumor is critical for successful therapy. Indeed, the individual genetic alterations and the metabolic profile of the parental tissue may provide cancer cells with a specific sensitivity to dietary modulation (see Outstanding Questions). Finally, besides the described benefits obtained by targeting cancer cell metabolic vulnerabilities, these approaches may also cause unwanted side effects on other cell types of both tumor and healthy tissues. Identifying the best balance between the downsides and advantages of any given metabolic intervention is essential to maximize their therapeutic efficacy. In conclusion, the crucial role of AA metabolism in driving the adaptive response of resistant cancers opens the possibility for novel therapeutic approaches to overcome therapy resistance, although both the genetic background of the tumor and the composition of the TME must be taken into consideration.

Acknowledgments

S.M.F. acknowledges funding from the European Research Council (ERC) under consolidator grant agreement 771486–MetaRegulation, Fonds Wetenschappelijk Onderzoek (FWO) research projects, KU Leuven Methusalem Co-Funding, and Fonds Ballet Latour. M.L.T. and P.P acknowledge funding from the University of Florence (Fondo ex-60%). P.P acknowledges funding from the Associazione Italiana Ricerca sul Cancro (AIRC) (project 19515 ‘Assaying tumor metabolic deregulation in live cells’). EP is supported by an Associazione Italiana per la Ricerca sul Cancro (AIRC) fellowship (project 24132, ‘Metabolic adaptations driving epigenetics of 5-fluorouracil-resistant colon cancer: the role of one carbon metabolism’).

Declaration of Interests

S.M.F. has received funding from Bayer AG, Merck, and Black Belt Therapeutics, and has consulted for Fund +. The other authors declare no conflicts of interest.

References


Outstanding Questions

Could targeting of stroma-derived AAs be exploited as a therapeutic strategy to impair tumor progression and drug resistance?

Cancer and immune cells display common metabolic vulnerabilities. Which therapeutic approach has the most translational potential in selectively targeting malignant cells without impairing the antitumor immune response?

Can we analyze plasma AA profiles to predict therapeutic response in cancer patients? Can these data be integrated with the other parameters considered in personalized medicine?

What strategies could be applied for selective targeting of AA metabolism in CSCs to prevent drug resistance and tumor relapse?

Can epigenetic therapy combined with specific dietary supplementation be used in clinic to inhibit the resurgence of therapy-resistant clones?

How far are we from developing strategies to target specific regions within the complex spatial heterogeneity of a tumor? Could these approaches be implemented by spatially resolved metabonomic investigations in tumor biopsies?

Will it be possible to temporally target AA metabolism in cancer cells during the ongoing process of tumorigenesis?
41. van Gastel, N. et al. (2020) Induction of a timed metabolic collapse to overcome cancer chemoresistance. Cell Metab. 32, 401–403 e6
52. He, X. et al. (2017) Combination therapy with L-arginine and α-L-FD-L antibody boosts immune response against osteosarcoma in immunocompetent mice. Cancer Biol. Ther. 18, 94–100
60. Bia, I. et al. (2017) Proline metabolism supports metastasis formation and could be inhibited to selectively target metastasizing cancer cells. Nat. Commun. 8, 15207
64. Polevski, M.D. et al. (2017) SLCL7A11 overexpression in glioblastoma is associated with increased cancer stem cell-like properties. Stem Cells Dev. 26, 1236–1246
71. Hegiwa, M. et al. (2018) Variant isoforms of CD44 involves acquisition of chemoresistance to cisplatin and has potential as a novel indicator for identifying a cisplatin-resistant population in uterine cancer. BMC Cancer 18, 113
75. Wang, Z. et al. (2019) Methionine nutrition restricts diet-induced growth of MCF10AT1-derived mammary tumors by increasing cell cycle inhibitors in athymic nude mice. BMC Cancer 19, 134


113. Kim, J. and DeBerardinis, R.J. (2019) Mechanisms and implica-
114. tions in the tumor microenvironment. Trends Cell Biol. 29, 2934–2939

115. Kim, J. and DelBarbera, R.J. (2019) Mechanisms and implica-

117. Loponte, S. et al. (2019) Metabolic landscape of the tumor microen-
118. vironment at single cell resolution. Trends Cancer 2, 736–746


123. Xiao, Z. et al. (2016) Metabolic landscape of the tumor microen-
124. vironment at single cell resolution. Nat. Commun. 10, 3763


126. Ippolito, L. et al. (2019) Cancer-associated fibroblasts promote prostate cancer malignancy via metabolic rewiring and mito-
127. chondrial transfer. Oncogene 38, 5309–5325


145. tions in the tumor microenvironment. Trends Cell Biol. 27, 863–875


149. Xiao, Z. et al. (2019) Metabolic landscape of the tumor microen-
150. vironment at single cell resolution. Nat. Commun. 10, 3763


153. Kraft, A.S. et al. (2016) Asparagine promotes cancer cell prolif-
154. eration through use as an amino acid exchange factor. Nat. Commun. 7, 11457
