Trends in Cancer

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Review

Metabolic Reprogramming in Anticancer Drug Resistance: A Focus on Amino Acids

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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.

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.

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

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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 (CH_2-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 cystine 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 cystine, 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

Glossary

Anaplerotic reactions: chemical reactions which replenish a metabolic pathway of depleted intermediates. Most commonly refers to the tricarboxylic acid (TCA) cycle.
Autophagic process: a regulated, physiological mechanism of cellular component degradation and recycling. It acts as an adaptive response under nutrient starvation and participates in the intracellular clearance of unneeded macromolecules/organelles.
Auxotrophy: the inability of an organism to synthesize a particular organic compound required for its growth.
Cancer immunotherapy: a therapeutic approach that exploits the immune system to overcome cancer-induced immune escape. This class of therapeutics includes cellular immunotherapy [dendritic cell therapy and chimeric antigen receptor (CAR)-T cell therapy which adopt engineered T cells to recognize a specific tumor-associated antigen], antibody therapy (including the checkpoint blockade therapies, anti-CTLA-4, anti-PD-1 and anti-PDL-1, that target inhibitory pathways protecting the immune system from autoimmune reactions against self-proteins, namely 'immune checkpoints'), and cytokine therapy.
Cancer stem cells (CSCs)/tumor-initiating cells (TICs): a subpopulation of tumorigenic cells that have an unlimited ability to self-renew, to differentiate into several cell types, and to initiate and sustain tumor growth.
Endocrine therapy (ET): hormone modulation for medical treatment. In anticancer therapy ET is employed to decrease malignant cell proliferation by modulating hormone-dependent pathways. Anticancer ET includes antiestrogens (selective estrogen receptor modulators such as tamoxifen, selective estrogen receptor degraders such as fulvestrant, aromatase inhibitors, and antigonadotropins) and antiandrogens (androgen receptor agonists, androgen synthesis inhibitors, and antigonadotropins).
Ferroptosis: a form of iron-dependent programmed cell death. It is initiated by failure of the glutathione (GSH)-dependent antioxidant defenses, resulting in iron accumulation and lipid peroxidation.
Immunotolerance: a state of the immune system characterized by

(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 counteracting 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 oncogenic 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

unresponsiveness to a specific antigen or group of antigens to which an organism is normally reactive.

Jumonji domain-containing histone lysine demethylases: an enzyme superfamily of histone lysine demethylases which utilize α-ketoglutarate (αKG), molecular oxygen, and Fe^{2+} as cosubstrates/cofactors.

Reactive oxygen species (ROS): highly reactive chemical species generated by incomplete reduction of oxygen. ROS are both signaling molecules, that act as messengers in different cellular events (apoptosis, gene expression, signaling cascades), and oxidative stress inducers that produce oxidative damage to cellular components or DNA.

Tyrosine kinase inhibitors (TKis): a targeted therapy that inhibits specific tyrosine kinases involved in the growth and survival of tumor cells. Drugs in this class include sorafenib, that acts as an inhibitor of multiple kinases involved in cell proliferation or angiogenesis, BRAF/MEK inhibitors that reduce the proliferation of cancer cells harboring the oncogenic BRAF V600E activating mutation, and EGFR-TKis that bind to EGFR and inhibit cell growth.

Tumor microenvironment (TME): cellular (endothelial, immune, and stromal cells) and non-cellular (signaling molecules, extracellular matrix, hypoxia) components surrounding the tumor which are in close and constant interaction with cancer cells.

changes [30] and allowing cancer 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 ^{V600E}BRAF 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

Box 1. Tumor Metabolic Heterogeneity

Although tumor cells *in vitro* share numerous canonical hallmarks of proliferative metabolism, increasing evidence demonstrates that a single 'cancer metabolic profile' cannot be defined. Instead, a large heterogeneity of factors influences tumor metabolism *in vivo* [113], making the development of effective anticancer treatment particularly challenging.

Intertumor heterogeneity is mostly driven by different mutational backgrounds among patients [114]. Concurrently, the nutrient environment of the tissue strongly influences the metabolic profile of tumors. Indeed, the same oncogenic driver may result in divergent tumor metabolic signatures as a consequence of the influence of the parental tissue metabolic profile [115,116]. However, cancer cells from different primary tissues may converge to similar metabolic signatures in a given metastatic location [117].

Intratumor heterogeneity derives from intrinsic and extrinsic factors [118] (Figure I). Intrinsic factors include genetic and epigenetic alterations and lead to the coexistence of multiple clonal (epi-)genetically different populations [119], which are further diversified in the presence of distinct populations of CSCs that have their own specific metabolic profiles and nutrient dependencies [120]. Extrinsic factors include all the structural and cellular components of the TME [121,122]. The structurally aberrant vasculature generates uneven perfusion within the tumor mass, thereby providing different regions with variable amounts of oxygen and nutrients, and imposing metabolic changes in cells experiencing different local conditions [123]. Local nutrient distribution influences the epigenetic status of cells within the TME, further fostering subpopulation heterogeneity [34]. To face such nutritional variability, cancer cells localized in different regions adopt 'metabolic symbiosis' strategies [124]. For example, cells localized in hypoxic regions undergo a 'glycolytic switch' that supports normoxic cell metabolism by providing lactate [125]. In addition, heterogeneous cancer-associated fibroblast (CAF) subpopulations harbor distinct metabolic profiles [126]. CAFs and cancer cells mutually reprogram their metabolism [127], resulting in dynamic exchange of metabolites and entire organelles, such as mitochondria, which finally support malignant cell metabolism [128,129]. Conversely, cancer and immune cells share several metabolic features, resulting in nutritional competition that further increases the heterogeneity of the TME composition [130,131]. Moreover, cancer cells also generate a 'temporal heterogeneity' by changing their metabolism over time according to the ongoing process of cancer progression. An example is the diversity of metabolic adaptations acquired by cancer cells during the metastatic process [132,133]. Importantly, heterogeneity is not a synonym for random distribution. Indeed, the gradients of nutrients and metabolic waste products within the tumor mass act as signals to further orchestrate the phenotype and positioning of neighboring cells [134]. Importantly, the heterogeneity and flexibility of cancer metabolism fuel anticancer drug resistance [135]. Therefore, it is essential to consider tumor metabolic heterogeneity and the spatial organization of metabolic networks in the development of effective local-targeted therapies, and this opens new possibilities for precision medicine. Recently, the application of stable isotope tracers in *in vivo/ex vivo* models [136], and spatially resolved metabolomic analysis of patient-derived biopsies [137], are allowing better definition of the complexity of tumor metabolism for future therapeutic applications.

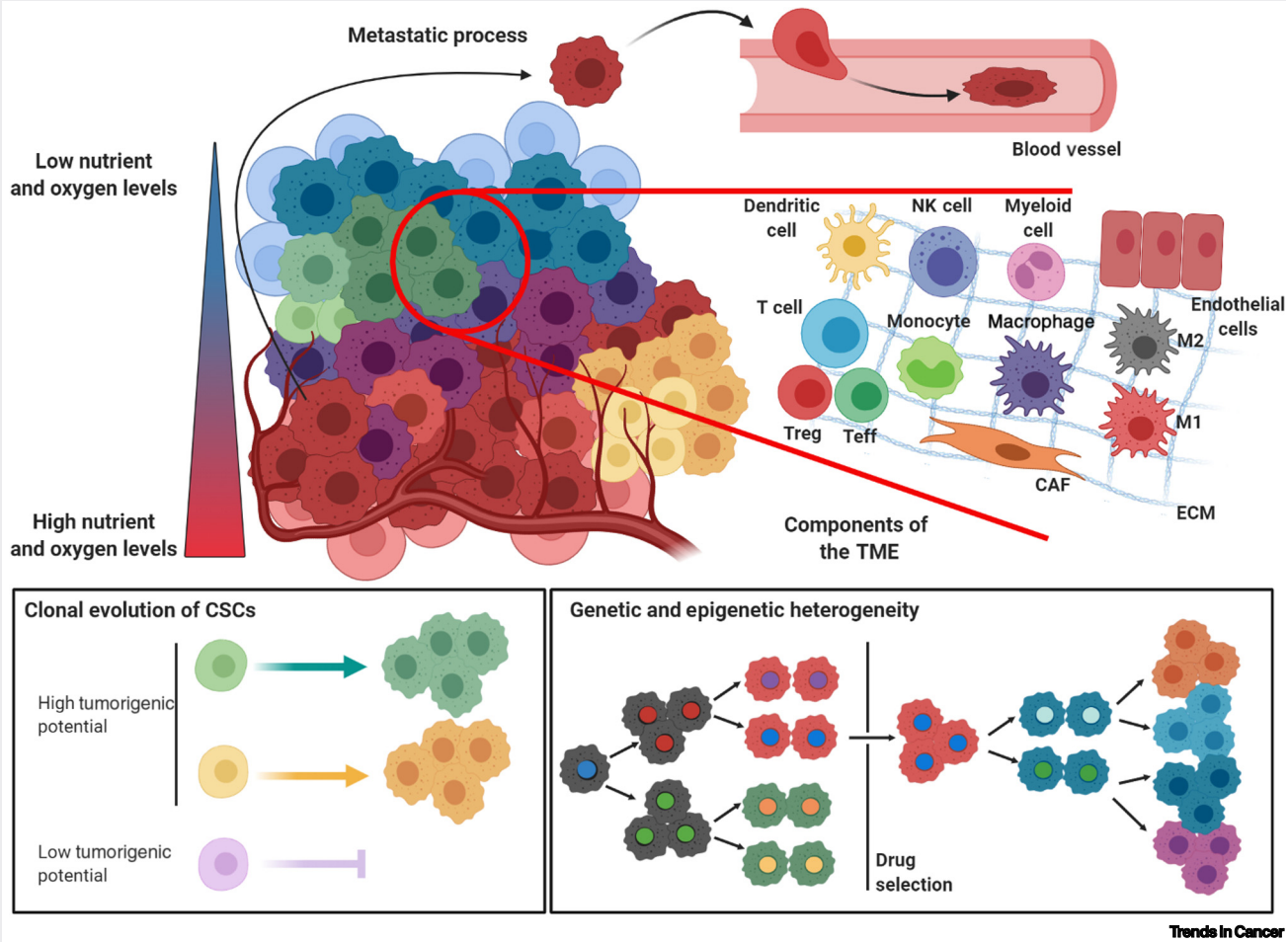

Figure I. Cellular and Non-cellular Factors Mediating Intratumor Heterogeneity. Cancer metabolism diverges in space depending on tumor cell position relative to the vasculature and the contribution of the tumor microenvironment (TME). The inefficient tumor vasculature generates a gradient of oxygen and nutrients, thereby influencing the metabolic phenotype of cells located in different positions within the tumor mass (represented by red or blue groups of cells in the tumor mass). By reciprocally exchanging nutrients, growth factors, and cytokines, cancer cells establish metabolic crosstalk with all the cellular components of the TME, including cancer-associated fibroblasts (CAFs), dendritic cells, endothelial cells, macrophages (influencing their differentiation in proinflammatory M1 or anti-inflammatory M2 phenotypes), myeloid cells, monocytes, and T cells [determining their differentiation into natural killer (NK) cells, regulatory, or effector T cells]. Moreover, cancer cells undergo specific metabolic alterations according to the step of tumorigenesis they are participating in. During metastasis formation, from epithelial-to-mesenchymal (EMT) transition to final successful metastatic colonization, cancer cells display different metabolic alterations. The presence of cancer stem cells (CSCs) further increases intratumor heterogeneity by generating different subpopulations within the tumor mass. The different (epi-)genetic status of cancer cells favors the selection of therapy-resistant subpopulations and promotes the coexistence of different cell phenotypes within the tumor. Figure created with BioRender.com. Abbreviation: Teff, effector T cell.

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

Box 2. Key Amino Acids in Cancer Metabolism

Glutamine provides cancer cells with carbons and nitrogens for protein, fatty acid, and nucleotide biosynthesis. The solute carrier 1A5 (SLC1A5) transporter imports glutamine into cells, whereas the SLC7A5/SLC3A2 antiporter exports glutamine in exchange for neutral amino acids (AAs), thereby regulating mammalian target of rapamycin (mTOR)-mediated cancer cell proliferation under nutritional stress. Glutamine affects ROS homeostasis by contributing to the synthesis of NADPH and reduced glutathione (GSH) from glutamate. Glutaminase (GLS), often upregulated in cancer, converts glutamine to glutamate, further catabolized to α-ketoglutarate (αKG) by glutamate dehydrogenase (GLUD) or aminotransferases, producing NADPH or essential AAs, respectively [138]. In this context, branched-chain amino acid (BCAA) aminotransferase 1 (BCAT1), the enzyme catalyzing the first step of exogenous BCAA degradation to the corresponding α-ketoacids and glutamate, is a prognostic cancer cell marker [139]. Proline is a principal component of collagen, and pyrroline-5-carboxylate reductase 1 (PYCR)-catalyzed conversion of Δ^1-pyrroline-5-carboxylate (P5C) to proline supports nucleotide biosynthesis and tumor growth by providing oxidizable substrates for the pentose phosphate pathway (PPP) [140], and proline catabolism via proline dehydrogenase 1 (PRODH) is activated in metastasis [60]. 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 cystine in exchange for glutamate [141]. Serine is a primary feeder of one-carbon metabolism in cancer, sustaining purine and thymidylate biosynthesis. Many tumors increase the endogenous serine synthesis pathway (SPP), primarily by overexpressing phosphoglycerate dehydrogenase (PHGDH), the first enzyme of the pathway. In proliferating cells, serine is also 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 αKG in breast cancer-derived lung metastasis [144]. 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 [142]. 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 formimino group to THF, finally forming glutamate from histidine. Tumor cells are generally auxotrophic for arginine because of loss of argininosuccinate 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].

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 to increase aspartate production from glutamine and to export it through SLC1A3. Blocking aspartate production in bone marrow stromal cells (BMSCs) partially sensitizes AML cells to iCT, confirming the chemoprotective effect of BMSC-derived aspartate [41]. In addition, melanoma cells promote immunotolerance through the paracrine release of Wnt5a to dendritic cells (DCs) that induces indoleamine 2,3-dioxygenase 1 (IDO1) activity in DCs, consequently decreasing the efficacy of anti-programmed cell death protein 1 (PD1) therapy [42]. Indeed, IDO1 overexpression generates an immunosuppressive microenvironment by depleting extracellular tryptophan necessary for T cell proliferation and facilitates the accumulation of kynurenine that supports Treg differentiation and activation, as well as effector T cell (Teff) function suppression [43].

Metabolic Reprogramming of Non-tumor Cells Favors an Immunosuppressive Microenvironment

Several AA metabolic traits of cancer and immune cells trigger tumor immune escape by generating a protumoral and immunosuppressive microenvironment that finally reduces the efficacy of

Key Figure

Amino Acid (AA) Metabolism in Cancer

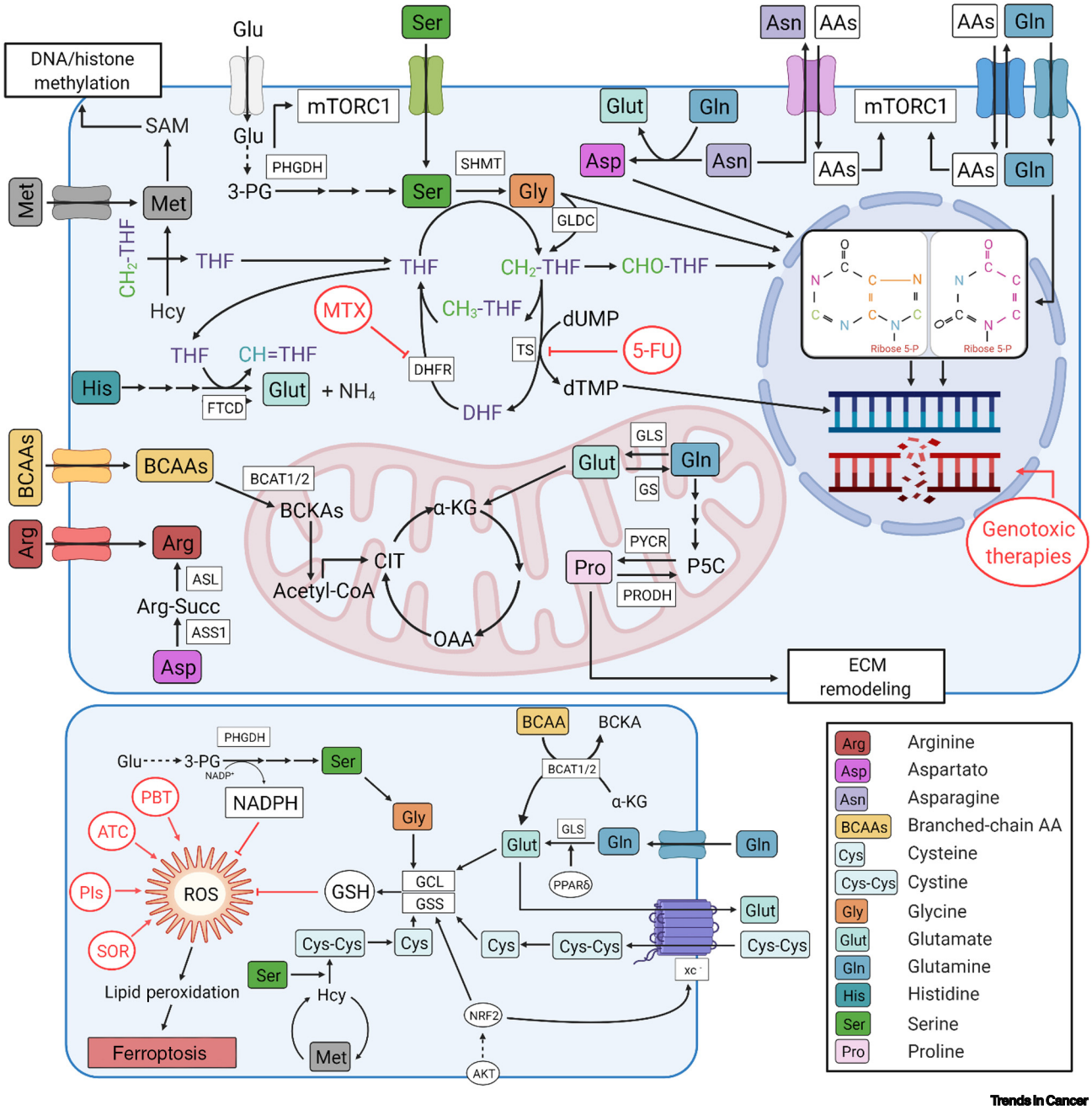

Figure 1. Major AA metabolic pathways altered in cancer cells. AAs are essential energy sources for cancer growth because they support biosynthetic pathways, maintain intracellular redox balance, and mediate epigenetic and post-transcriptional modifications. AAs provide sources to overcome anticancer 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,

(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 (antagonizing 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-methylenetetrahydrofolate (5,10-CH_2-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-CH_2-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, BTZ; 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 xc– 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

purine biosynthesis. Ser–Gly conversion contributes to purine and deoxythymidine monophosphate (dTMP) generation by fueling 5,10-methylene-tetrahydrofolate (CH_2-THF) into the folate cycle. The antimetabolites 5-fluorouracil (5-FU) and methotrexate (MTX) interfere with nucleotide metabolism by disrupting folate cycle flux through the inhibition of thymidylate synthase (TS) and dihydrofolate reductase (DHFR), respectively. Met metabolism participates in the folate cycle by providing THF, and is essential for the generation of *S*-adenosyl methionine (SAM). By contrast, His degradation consumes the cellular pool of THF through the activity of formimidoyltransferase cyclodeaminase (FTDC). AA transporters can regulate the activity of the mammalian target of rapamycin (mTOR) by modulating intracellular AA content. In breast cancer-derived lung metastasis, mTORC1 activation is modulated by phosphoglycerate dehydrogenase (PHGDH) activity. Branched-chain amino acid (BCAA) aminotransferase 1 (BCAT1) catalyzes exogenous BCAA degradation. Pro metabolism is involved in extracellular matrix (ECM) remodeling. AA metabolism mediates the acquisition of resistance to oxidative stress-inducing agents by supporting glutathione (GSH) and NADPH production. GSH is synthesized *de novo* by glutamate-cysteine ligase (GCL) from Glu, Cys, and Gly, that is mostly derived from Ser. Nuclear factor erythroid 2-related factor 2 (NRF2) transcriptionally regulates GCL expression and mediates the cellular response to oxidative stress. The cysteine/glutamate exchange transporter xc– imports the AA Cys-Cys, the oxidized form of Cys, into cells in exchange for Glu to support cellular GSH synthesis. The Ser synthesis pathway is an essential source of cellular NADPH. Failure of these antioxidant defenses leads to reactive oxygen species (ROS) accumulation and may lead to the accumulation of lipid peroxides and consequently ferroptosis. Abbreviations: AKT, protein kinase B; Arg-Succ, argininosuccinate; ASL, argininosuccinate lyase; ASS1, argininosuccinate synthetase 1; ATC, anthracycline; BCKA, branched-chain ketoacid; BCAT1/2, BCAA transaminase cytosolic/mitochondrial; CIT, citric acid; DHF, dihydrofolic acid; GLDC, glycine dehydrogenase (decarboxylating); GLS, glutaminase; Glu, glucose; GS, glutamine synthetase; Hcy, homocysteine; α-KG, α-ketoglutarate; mTORC1, mammalian target of rapamicin 1; OAA, oxaloacetate; PARP, poly(ADP-ribose) polymerase; PBT, platinum-based therapies; P5C, pyrroline-5-carboxylate; 3-PG, 3-phosphoglyceric acid; PIs, proteasome inhibitors; PYCR, pyrroline-5-carboxylate reductase; PRODH, pyrroline-5-carboxylate dehydrogenase; SOR, sorafenib; SHMT, serine hydroxymethyltransferase. Figure created with BioRender.com.

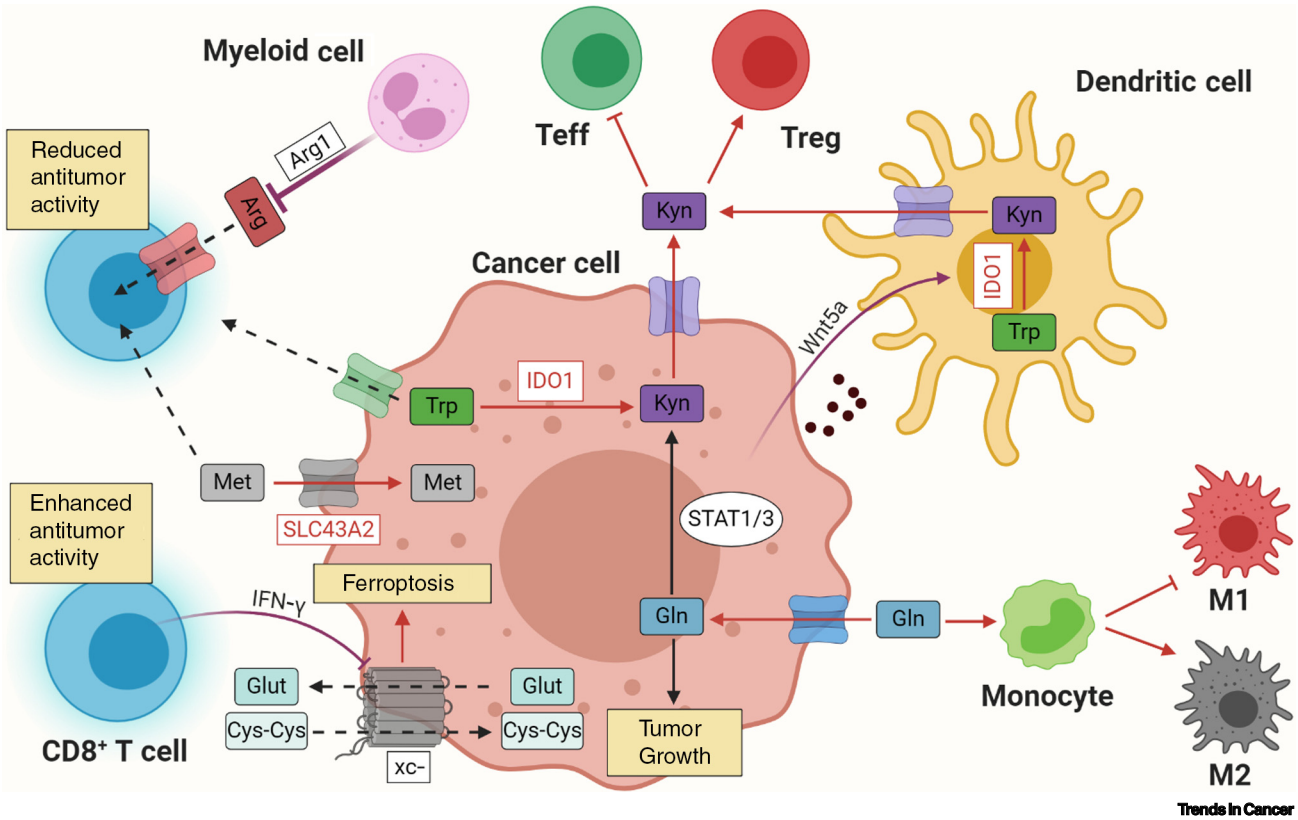

Figure 2. Amino Acids (AAs) Mediating Tumor Immune Evasion. Tumor cells establish complex metabolic crosstalk with different populations of immune cells (dendritic cells, myeloid cells, T cells, macrophages) within the tumor microenvironment (TME) to support cancer progression. AA metabolic reprogramming of both tumor and immune cells contributes to the generation of an immunosuppressive microenvironment and alters the anticancer immune response. Indoleamine 2,3-dioxygenase 1 (IDO1) upregulation in cancer or dendritic cells (DCs) increases the kynurenine (Kyn)/tryptophan (Trp) ratio, thereby inhibiting effector T cell (Teff) expansion, while promoting Treg activation. IDO1 expression in DCs is induced by paracrine release of Wnt5a from cancer cells. Inhibition of $CD8^+$ T cell function can be triggered by cancer cell-mediated Trp deprivation or myeloid cell-dependent arginase (Arg1) release into the TME, leading to exogenous arginine (Arg) deprivation. In addition, cancer cells outcompete $CD8^+$ T cells for exogenous methionine (Met), depriving T cells of Met and impairing immune cell functions. High glutamine (Gln) levels in the TME preferentially favor monocyte differentiation into protumorigenic M2 macrophages rather than into proinflammatory M1 macrophages. Gln is also utilized by cancer cells to promote signal transducer and activator of transcription (STAT)1/3-mediated upregulation of IDO1. By contrast, interferon γ (IFN-γ) release from $CD8^+$ T cells downregulates cystine (Cys-Cys)/glutamate (Glut) exchange transporter (xc–) expression and triggers ferroptotic death in cancer cells. The pathways upregulated or downregulated in cancer cells and the cellular components of the TME are highlighted with red or broken arrows, respectively; paracrine factors released by cancer or immune cells are highlighted with purple arrows. Figure created with BioRender.com.

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, $CD8^+$ T cell-derived IFN-γ abolishes the stromal protective activity by enhancing GSH degradation and repressing system xc– transcriptional expression in fibroblasts. Platinum-based chemotherapy-resistant HGSOC patients display a higher intratumoral content of stromal fibroblasts and lower levels of $CD8^+$ T cells compared with sensitive patients [56].

Non-cellular Components of the Tumor Microenvironment Rewire Cancer Amino Acid Metabolism

Non-cellular stromal components are additional sources which provide the AAs necessary to support tumor aggressiveness. In TMX-resistant breast cancer cells and *in vivo* xenograft models, hypoxia can induce ET resistance by promoting the expression and plasma-membrane localization of the glutamine transporter SNAT2. High levels of SNAT2 correlate with tumor hypoxia and low recurrence-free survival in breast cancer patients receiving antiestrogen therapy [57].

In addition, the extracellular matrix (ECM) directly or indirectly provides AAs to influence tumor progression. Indeed, ECM stiffening coordinates tumor–stroma metabolic crosstalk mediated by SLC1A3 overexpression, in which CAF-derived aspartate supports 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 derivative 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 (EpdSCs). Indeed, abundant exogenous serine is essential for the expansion of tumor-initiating EpdSCs. Accordingly, serine deprivation induces EpdSCs to activate the *de novo* SSP, which in turn stimulates αKG-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 αKG accumulation and increased αKG-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 $CD44^{hi}/CD24^{low}$ 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 (PDOX) 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 vivo [101]. 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 all 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.

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 insurgence 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 metabolomic investigations in tumor biopsies?

Will it be possible to temporally target AA metabolism in cancer cells during the ongoing process of tumorigenesis?

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Declaration of Interests

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