Review Article

Fuel for thought: targeting metabolism in lung cancer

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Abstract: For over a century, we have appreciated that the biochemical processes through which micro- and macronutrients are anabolized and catabolized—collectively referred to as "cellular metabolism"—are reprogrammed in malignancies. Cancer cells in lung tumors rewire pathways of nutrient acquisition and metabolism to meet the bioenergetic demands for unchecked proliferation. Advances in precision medicine have ushered in routine genotyping of patient lung tumors, enabling a deeper understanding of the contribution of altered metabolism to tumor biology and patient outcomes. This paradigm shift in thoracic oncology has spawned a new enthusiasm for dissecting oncogenotype-specific metabolic phenotypes and creates opportunity for selective targeting of essential tumor metabolic pathways. In this review, we discuss metabolic states across histologic and molecular subtypes of lung cancers and the additional changes in tumor metabolic pathways that occur during acquired therapeutic resistance. We summarize the clinical investigation of metabolism-specific therapies, addressing successes and limitations to guide the evaluation of these novel strategies in the clinic. Beyond changes in tumor metabolism, we also highlight how non-cellular autonomous processes merit particular consideration when manipulating metabolic processes systemically, such as efforts to disentangle how lung tumor cells influence immunometabolism. As the future of metabolic therapeutics hinges on use of models that faithfully recapitulate metabolic rewiring in lung cancer, we also discuss best practices for harmonizing workflows to capture patient specimens for translational metabolic analyses.

Keywords: Lung cancer; metabolism; resistance; immunometabolism

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Introduction

One hundred years ago, the unexpected observation was made that cancers utilize fuel differently than normal tissues (1). Thus, for over a century, we have understood that cellular metabolic pathways are reprogrammed in malignancies (2,3). The building blocks of cellular infrastructure including amino acids, lipids, carbohydrates, and nucleotides are synthesized, processed, and broken down in distinctive ways in transformed cells compared to in normal cells (4). Networks of energy production are rewired to support the biosynthetic demands of higher proliferation rates (5), and redox balance is adapted to maintain cell survival despite high degrees of oxidative stress (6).

Normal cells that comprise lung parenchyma are highly metabolically active, as the pulmonary microenvironment

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is extensively vascularized and contains the highest oxygen tension in the human body. Thus, there is a substrate for altered metabolic states associated with malignant transformation. In the 1920s, Dr. Warburg and colleagues posited that tumor cells consume glucose and excrete lactate at much higher rates compared to healthy normal cells (2). Over time, the field has grown to appreciate that not all cancerous cells rely on fermentation, but that other pathways are indeed critical for adenosine triphosphate (ATP) generation and energy balance such as oxidative phosphorylation and glutaminolysis to fuel mitochondrial activity (7). These iterative developments in our understanding of how tumor cells are metabolically programmed have now collided with a deeper appreciation of genomic perturbations in cancer, thus setting the stage for identifying oncogenotype-specific metabolic liabilities for therapeutic purposes.

Beyond the recognition of histology-specific metabolic states in lung cancer (8), we are learning that distinct molecular events also shape tumor metabolism through oncogenic driver gains and tumor suppressor losses. As the era of precision medicine has ushered in the routine molecular genotyping of patient lung tumors, this has facilitated translational investigation of the impact of genomic alterations on unique aspects of tumor biology. This paradigm shift in broad molecular profiling has spawned a new enthusiasm for uncovering metabolic phenotypes associated with distinct biomarkers in lung cancer. Moreover, identification of metabolic dependencies offers the opportunity to manipulate tumor metabolic pathways for therapeutic benefit.

While metabolic dysregulation is a well-recognized hallmark of cancer (9) with an increasing breadth of studies describing discrete metabolic states in lung cancer, many unanswered questions remain. Do specific oncogenotypes confer dependence on essential metabolic targets? Can the molecular genotype of a lung tumor trump its histology through influencing metabolic states? Does metabolic rewiring account for non-genomic mechanisms of resistance in driving treatment-refractory disease? Is there a role for metabolic profiling of patient tumors? What is the optimal way to procure patient tumor specimens to functionally interrogate and manipulate metabolism? Are metabolic enzymes or modulators viable therapeutic targets in lung cancer?

Here, we discuss the current state of understanding of the landscape of discrete metabolic states in lung cancer and how metabolism is rewired in the context of therapeutic resistance. Furthermore, we review the mechanistic basis of how the genomic makeup of a lung tumor shapes metabolic networks through gain of oncogenic drivers, inactivation of tumor suppressors, or inhibition of synthetically lethal targets. We highlight how non-cellular autonomous processes in the form of immunometabolism merit particular consideration when manipulating metabolic processes systemically and discuss best practices for harmonizing workflows to capture patient specimens for translational metabolic studies.

Methods

A comprehensive literature search was conducted using PubMed database, with keywords including “lung cancer”, “metabolism”, “resistance,” and “immunometabolism” (Table S1). Studies published in English within the last 5 years were prioritized for inclusion. We reviewed each study for scientific quality and selected those that offered insights into metabolic reprogramming in lung cancer.

Metabolic rewiring in lung cancer

Hallmarks of tumor metabolism

Cellular metabolism refers to the complex, highly regulated array of biochemical reactions that allow cells to grow, divide, and carry out distinct functions. Tumor metabolism—in contradistinction to cellular metabolism—is defined by the differential usage of these pathways that facilitate cell proliferation and migration despite the nutrient constraints (10). For cancer cells to replicate and divide, they must acquire the nutrient building blocks that are used for synthesis of biomass (11,12). Examples of such nutrients include sugars such as glucose, amino acids such as glutamine, fatty acids, and vitamins such as folic acid. The requirements for these building blocks vary throughout the cell cycle, particularly during S-phase when deoxynucleotides are needed for DNA replication.

Given the interconnectedness of many biochemical pathways in the cell, sensing mechanisms are critical to ensure that adequate nutrient levels are maintained to support efficient growth (13). When amino acid levels are abundant, the mammalian target of rapamycin (mTOR) complex signals to promote active translation and inhibit autophagy-mediated protein breakdown. For bioenergetic reactions that utilize ATP as a co-factor, AMP kinase (AMPK) senses excessive ATP breakdown and provides inhibitory feedback signals to slow metabolism and allow

for ATP recovery. Even DNA damage signaling pathways, such as ATR, boost nucleotide production in response to replication fork arrest to promote necessary repairs and resumption of replication.

Beyond replication, there are additional pathways that help cells survive environmental stresses. Reactive oxygen species (ROS) pose a major threat to cells, damaging nucleic, proteins, and lipids to effect numerous forms of cell death (14). Under normal conditions, the oxidative stress sensor, Kelch-like ECH-associated protein (KEAP1), regulates the transcription factor NFE2L2 (nuclear factor erythroid 2-related factor 2, NRF2) through ubiquitylation and degradation (15). However, in the presence of oxidative stress, this negative KEAP1-mediated regulation is lost and NRF2 is free to translocate to the nucleus and promote transcription of several enzymes involved in detoxification of ROS. In lung cancers, tumors that harbor mutations in the KEAP1-NFE2L2 axis confer refractoriness to a both systemic therapies (e.g., chemotherapy, targeted therapy, immunotherapy) as well as local therapy (e.g., radiation) (16).

These aforementioned pathways are by no means exhaustive, but rather illustrate the importance of different types of metabolic activities that sustain the life of the tumor cell. The differential reliance—or dispensation—of these pathways can strongly influence the biology of growth or treatment resistance.

Stratification of metabolic states by lung tumor histology

Even prior to the current era of precision medicine, compelling examples illustrate how distinct metabolic states shape the biology of histologic subtypes of lung cancer. In fact, histologic characterization of lung cancer has a metabolic basis, as small cell lung cancer (SCLC) was originally distinguished from non-small cell lung cancer (NSCLC) for its smaller cell size, a reflection of the decreased biomass resulting from characteristic Rb loss that enables premature entry of S-phase for replication (17). We now appreciate that there are other discrete metabolic changes in SCLC beyond biomass regulation, as recent studies have pinpointed metabolic liabilities in SCLC centered around nucleotide handling, such as inosine monophosphate dehydrogenase (IMPDH) dependency in ASCL1-low subsets (18) and guanosine triphosphate (GTP) as a metabolic gate that links MYC-dependent ribosome biogenesis to nucleotide availability (19).

Metabolic features can also distinguish between histologic subsets within NSCLC. For example, differences in glucose metabolism can differentiate between lung adenocarcinomas and squamous cell lung carcinomas based on fluorodeoxyglucose (FDG)-uptake and expression of glycolytic markers. Squamous cell carcinomas are typically highly glycolytic at baseline with elevated expression of glucose and lactate transporters, glucose transporter 1 (GLUT1) and monocarboxylate transporter 1 (MCT1), respectively (20). While low-grade squamous tumors exhibit marked FDG-uptake irrespective of grade, FDG avidity may correlate with grade in adenocarcinoma (20,21). As FDG positron emission tomography (PET) provides a surrogate for glucose utilization through uptake of the analog FDG (^{18}F), there are ongoing investigational approaches that aim to use other metabolites beyond glucose to differentiate between lung cancer histologic subtypes. For instance, preclinical data suggests that spatial metabolite profiles can distinguish between (8) and within (22) NSCLC histologies.

Beyond diagnostic considerations, there are also therapeutic differences that stratify histologic subsets of NSCLC, most notably with respect to sensitivity to the anti-folate pemetrexed between squamous and non-squamous lung tumors (23). Multiple lines of evidence support this observation, including increased glucose metabolism (24) and decreased folate receptor expression in squamous tumors (25,26).

As somatic mutational testing of lung tumors has improved our understanding of the molecular basis for different subtypes of lung cancer, the field has shifted from a histology-centric framework to one that incorporates histologic, immunophenotypic, and molecular features. This improved ability to molecularly stratify lung cancers has also fueled a deeper foundational knowledge of the biology that sustains tumor growth. In the following sections, we summarize the major molecular subsets of lung cancer in which metabolic changes influence tumor biology.

Oncogene-driven metabolic alterations in lung cancer

Oncogene-driven NSCLC is a class of lung cancers in which an early cancer-causing genomic perturbation is activation of an oncogene, whether by single nucleotide mutations, insertion/deletion, copy number gain, or translocation (27). Despite extensive characterization of oncogenic driver alterations and the development of matched targeted therapies, open questions remain regarding how distinct

disease biology results from oncogenic driver activation. For instance, what transduces the signals from the mutant oncogenic protein to exert a tumorigenic effect? While most investigation has traditionally focused on pro-proliferative signaling cascades downstream of the driver, there may be other mediators that link genomic alterations with carcinogenic effect, including rewiring of metabolism.

Receptor tyrosine kinase (RTK) activation and downstream metabolic reprogramming

Several RTKs have been described as oncogenic drivers in NSCLC, including epidermal growth factor receptor (*EGFR*), fibroblast growth factor receptor (*FGFR*), human epidermal growth factor receptor 2 (*HER2*), and mesenchymal-epithelial transition factor (*MET*) (28). Aberrant RTK activation can occur through gain-of-function mutations [e.g., *EGFR* exon 19 deletion or L858R (29), *ERBB2* exon 20 insertion (30)], genomic amplification [e.g., *MET* copy number gain (31)], chromosomal rearrangement [e.g., *FGFR* fusion (32)], constitutive activation due to loss of regulatory components [e.g., EGFR hyperphosphorylation (33)], or autocrine activation [e.g., HGF ligand activation of c-MET (34)].

EGFR mutations in lung adenocarcinomas typically occur in exons 18–21 (35) and have been shown to influence cancer metabolic reprogramming through a variety of mechanisms. EGFR signaling is linked to aerobic glycolysis, pentose phosphate pathways, and pyrimidine biosynthesis through activation of mTOR axis and upregulation of glucose transporter, GLUT1 (36), with reversal of this phenotype upon treatment with early generation EGFR inhibitors in the tyrosine kinase inhibitor (TKI)-sensitive context (37). Simultaneously crippling glucose and glutamine utilization via dual inhibition of EGFR with erlotinib and glutaminase (GLS) with CB-839 results in suppression of tumor growth *in vitro* and *in vivo* (38). Beyond energy metabolism, EGFR signaling has been implicated in regulation of lipid metabolism through direct regulation of stearoyl-CoA desaturase-1 (SCD1) (39).

Notably, diverse metabolic profiles have been observed in cell culture models of EGFR-mutant NSCLC, highlighting metabolic heterogeneity even within one molecular subset of NSCLC (40). Like the structure-based approach for defining functional groups of EGFR mutations based on structure-function relationships (35), it is possible that specific structural or mutational classes of EGFR may confer distinct metabolic dependencies. Beyond EGFR, there is a paucity of studies focusing on metabolic phenotypes conferred by other RTKs in NSCLC. Thus, further investigation is needed to crystalize whether there is a universal re-patterning of fuel utilization across all RTKs or whether there is oncogene-, mutation-, or structural class-specificity.

MAPK pathways alterations and metabolic dysregulation

Mutations in Kirsten rat sarcoma viral oncogene homologue (KRAS) are found in approximately a third of NSCLC cases and are typically associated with a heavy smoking history, high tumor mutational burden, and elevated programmed death-ligand 1 (PD-L1) (41). In 2021, the Food and Drug Administration (FDA) granted approvals for the first KRAS G12C inhibitors in NSCLC (42,43), with small molecule inhibitors of other mutant forms of KRAS currently in early phase clinical trials (NCT05737706). Despite being the first human oncogene identified and the most frequently mutated gene in human cancer (44), it was not until more recently when our understanding of how members of the RAS family promote metabolic dysregulation in cancer cells has evolved (45).

Early studies identified that activating mutations in KRAS or B-Raf proto-oncogene (BRAF) genes in colorectal cancer cell lines led to increased transcription of glucose transporter, GLUT1, enhanced glycolysis, and promoted survival in low-glucose conditions (46). Shortly after, it was described that the major function of glucose metabolism for Kras-induced anchorage-independent growth is to support the pentose phosphate pathway and that mitochondrial metabolism and mitochondrial ROS generation are essential for KRAS-induced cell proliferation and tumorigenesis (47). ROS are actively suppressed by oncogenic drivers such as KRAS, BRAF, and MYC, through transcriptional upregulation of the major coordinator of antioxidant response, Nrf2 (48). Activating mutations in KRAS are also observed in pancreatic and colorectal cancer. Interestingly, KRAS activation in lung cancer causes a greater utilization of glucose as compared to pancreatic cancer, where glutamine provides a relatively greater contribution as a fuel source (49). In lung cancer, codons 12, 13 and 61 are commonly altered, and there is preclinical evidence to suggest that the location of these alterations impacts glucose utilization (24). The study of KRAS-mutant lung cancer has revealed that prognosis is impacted by secondary modifications, notably in the tumor suppressor STK11, which encodes the kinase liver kinase B1 (LKB1) (50). This observation has highlighted the role of metabolic tumor suppressor inactivation as an additional modifier of lung cancer metabolism.

Metabolism and tumor suppressor inactivation

The characterization of metabolic tumor suppressors has largely occurred in the context of KRAS alterations, where co-occurring inactivation of STK11 (LKB1) or KEAP1 are associated with an aggressive clinical phenotype and reduced efficacy of immunotherapy (51,52). Characterization of the "KL" oncogenotype (co-occurring alterations in KRAS and LKB1) has revealed numerous metabolic differences compared to KRAS-mutant NSCLC with wild type LKB1 status. Loss of LKB1 signaling impairs the activation of several downstream signaling pathways, including the energy sensor AMPK (53). When this diminished energetic sensing occurs in the context of oncogene addiction, there is a paradoxically decreased ability to respond to metabolic stressors (54,55). Accordingly, KL NSCLC exhibits reliance on urea cycle enzymes, carbamoyl-phosphate synthase 1 (CPS1), through de-repression due to loss of LKB1 (56). CPS1 silencing in KL NSCLC cell lines results in perturbed nitrogen metabolism, pyrimidine depletion, and cell death in this molecular subset of NSCLC (56). Furthermore, mouse models and patient samples of KL NSCLC exhibit activation of hexosamine biosynthesis, another nitrogen-related metabolic pathway, with dependence on glutamine-fructose-6-phosphoate transaminase 2 (GSPT2) (57).

KRAS-mutant lung tumors can also feature co-occurring alterations in KEAP1, the negative regulator of the NRF2 antioxidant pathway. In genetically engineered mouse models (GEMMs) of KRAS-driven lung adenocarcinoma, loss of KEAP1 hyperactivates NRF2 which promotes dependency on glutaminolysis (58). Furthermore, GLS inhibition preferentially sensitizes KEAP1-mutant NSCLC cells to radiation therapy through depletion of glutathione and increased radiation-induced DNA damage (59). These studies set the stage for evaluation of therapeutically targeting GLS in distinct molecular subsets of NSCLC that are driven by KRAS and/or loss of KEAP1 (60,61) (further discussed below in the "Opportunities for therapeutic targeting of metabolism in lung cancer" section).

Interestingly, KL tumors differ metabolically from KL tumors that also contain loss of KEAP1 (KLK tumors). Activation of the KEAP1/NRF2 axis in LKB1-deficient cells cooperatively promote enhanced glutamine dependence and sensitized to the GLS inhibitor CB-839 both *in vitro* and *in vivo* (62). The metabolic reprogramming that occurs in these molecular subsets of KRAS-driven NSCLC highlight the importance of investigating tumor metabolism in the context of oncogenotype to elucidate potential therapeutic vulnerabilities.

There are still many unknowns in terms of how other co-occurring alterations with KRAS in NSCLC confer metabolic preferences and whether this contributes to the immunotherapy-refractory nature of certain subsets of KRAS-mutant-containing lung cancers (50). It is also unknown whether there are histology-specific effects on metabolism in KRAS-mutant lung tumors (i.e., KRAS G12C mutant adenocarcinoma vs squamous cell carcinoma) or whether different RAS family proto-oncogenes (i.e., *KRAS*, *NRAS*, *HRAS*) or RAS mutations (i.e., G12C vs G12D) alter downstream metabolic pathways differently. Beyond the impact for co-occurrence in KRAS mutant tumors, there may be histology-relevant associations for tumor suppressor alterations. For example, inactivating mutations in KEAP1, activating mutations in NFE2L2 (which encodes NRF2), or inactivating mutations in CUL3 (the ubiquitin ligase that inactivates NRF2) are all seen in squamous lung cancer (63,64), where constitutive activation of the NRF2 antioxidant pathway may predispose to more glycolytic phenotype.

Fusion-positive NSCLC and metabolic reprogramming

Fusion-positive lung tumors comprise approximately 8–10% of NSCLC and include lung cancers driven by translocations in anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene 1 (*ROS1*), Ret proto-oncogene (*RET*), neurotrophic tyrosine receptor kinase A/B/C (*NTRKA/B/C*), neurogenin 1 (*NRG1*), leukocyte receptor tyrosine kinase (*LTK*), *FGFR1/2/3*, *NUT*, *MET*, amongst others (32,65-67). These fusion-positive cancers are often associated with younger age at diagnosis and are enriched in patients without a history of cigarette use (68), thus likely yielding a distinct metabolite profile compared to lung tumors marked by DNA damage due to prolonged smoking exposure. While fusion-positive lung cancers exhibit oncogene dependency and are typically sensitive to inhibition using TKIs, resistance to targeted therapies always arises (28,69-74). As essentially all patients with fusion-positive NSCLC ultimately relapse, the development of new therapies for TKI-refractory disease is a major unmet need in oncogene-driven lung cancers.

Largely, the impact of fusion-positive NSCLC on tumor metabolism has not been well characterized. There are reports ALK$^+$ NSCLC having augmented glucose metabolism, as read out by FDG-PET CT, compared to *EGFR*-mutant NSCLC (75) and additional studies

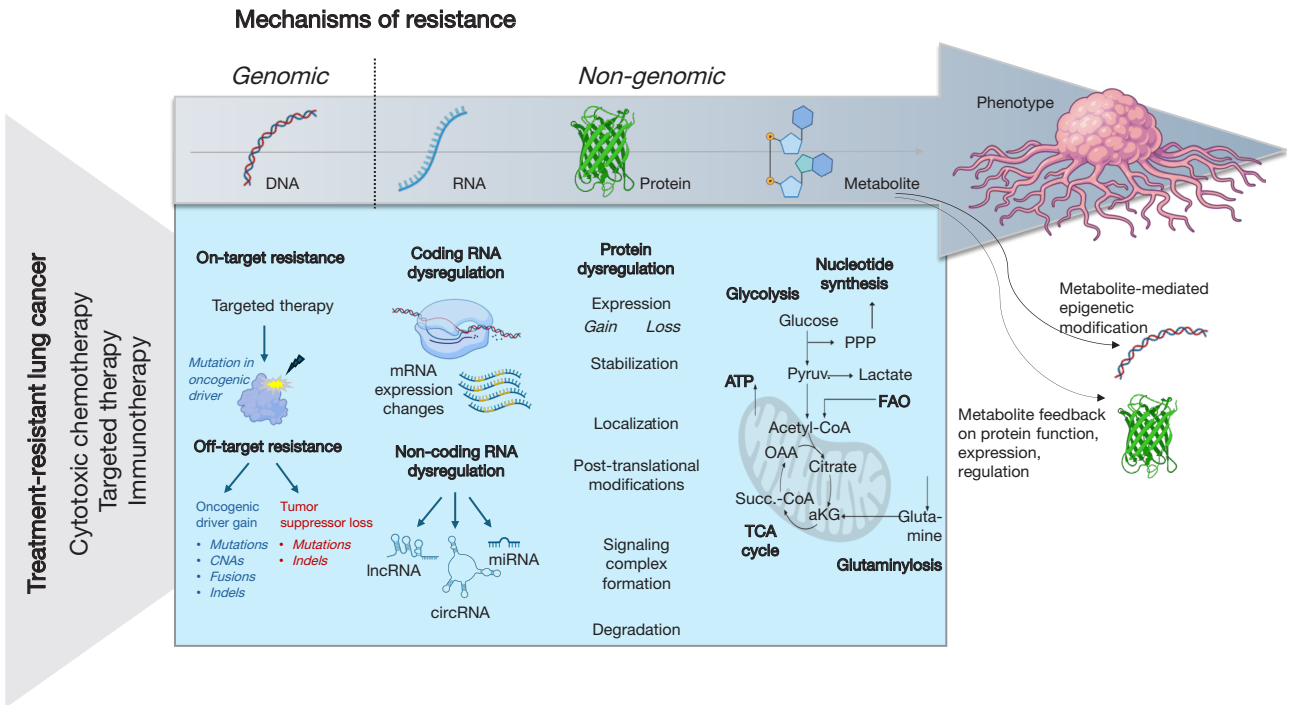

Figure 1 Mechanisms of resistance in lung cancer. Schematic showing mechanisms of resistance to systemic therapies in lung cancer, including alterations at the level of DNA, RNA, protein, and metabolite. Metabolite alterations can also regulate protein function and modify the epigenome. Some elements of some figures were created with BioRender.com. CNA, copy number alteration; Indel, insertions/deletion; lncRNA, long non-coding RNA; miRNA, microRNA; circRNA, circular RNA; Pyruv., pyruvate; OAA, oxaloacetate; Succ.-CoA, succinyl-CoA; FAO, fatty acid oxidation; PPP, pentose phosphate pathway; aKG, alpha-ketoglutarate; ATP, adenosine triphosphate; TCA, tricarboxylic acid cycle.

highlighting the role of glycolytic enzymes in ALK$^+$ disease (76,77). In ALK$^+$ anaplastic large cell lymphoma, cholesterol auxotrophy was shown to be a metabolic liability, with squalene protecting cancer cells from ferroptotic cell death and oxidative stress (78), but whether this is relevant in fusion-positive NSCLC remains to be determined.

Therapeutic resistance and metabolic rewiring

Despite the implementation of new classes of therapeutics in lung cancer care over the last two decades (e.g., targeted therapy, immunotherapy, antibody-drug conjugates), rarely is advanced lung cancer considered cured. Even in the early-stage setting, approximately 30–55% of patients who undergo curative surgery for early-stage NSCLC will have a recurrence and ultimately die of their disease (79,80). Many studies have shown that neoadjuvant or adjuvant chemotherapy confers modest benefit over surgery alone for resectable NSCLC (81,82). Furthermore, recent investigations using combined neoadjuvant chemotherapy plus immunotherapy (chemoIO) regimens for early-stage lung cancer have demonstrated that over 76–82% of patients still do not achieve a pathologic complete response (83,84). These data underscore the need to better understand the molecular basis for treatment resistance in distinct subpopulations of tumor cells and evaluate whether reprogramming of cellular metabolic networks contributes to primary refractory or acquired resistant disease (*Figure 1*).

Metabolic preferences in drug-tolerant persister cells (DTPs)

DTPs represent a small population of cancer cells that survive despite systemic treatment and have been shown to undergo metabolic rewiring to support the drug-tolerant persister state (85). DTPs in EGFR-mutant lung cancer can acquire new genomic mutations through activation of APOBEC3, which promotes acquired resistance by

facilitating evolution and trans-differentiation, allowing evasion of apoptosis, and driving disease relapse (86). However, prior to the acquisition of an increasingly complex array of mutations, DTPs employ non-genomic mechanisms of resistance to enter the reversible drug-tolerant state in response to treatment.

One study demonstrated the DTPs that re-enter the cell cycle (i.e., cycling persister cells) arise from a distinct cell lineage compared to non-cycling DTPs (87). Tracking clonal origin as well as proliferative and transcriptional states revealed that cycling persisters increase expression of antioxidant genes and exhibit a preferential reliance on fatty acid oxidation rather that glucose catabolism (87). Carnitine-linked fatty acids, substrates for beta-oxidation to sustain mitochondrial activity, were shown to be significantly enriched in cycling persister cells compared to non-cycling DTPs and were accompanied by an increase in flux through fatty acid oxidation over time with exposure to targeted therapy. This DTP phenotype was consistent across cell lines representing multiple cancer types, including in EGFR-mutant NSCLC and in patient lung tumor specimens (87).

Other studies have also uncovered a critical role for a coordinated antioxidant stress response in drug-tolerant tumor cell populations. Aldehyde dehydrogenase (ALDH) has been described as a critical mediator in controlling ROS in DTPs, and disruption of ALDH activity leads to DNA damage and apoptosis preferentially in the DTP subpopulation (88). Glutathione peroxidase 4 (GPX4) is another metabolic dependency described for therapy-resistant cell states across a wide range of cancers and drug treatments (89). Loss of GPX4 in DTPs induces ferroptotic cell death and prevents tumor recurrence in murine models. Taken together, interference with the preferred metabolic states of persister cells to cope with ROS exposure represents a potential strategy to delay or ultimately prevent disease recurrence.

Metabolic reprogramming in targeted therapy-refractory disease

The study of metabolic adaptations as a driver of acquired resistance in oncogene-driven lung cancers is a relatively nascent field. In melanoma and renal cell carcinoma models, compensatory upregulation and activation of proteins that regulate mitochondrial and bioenergetic metabolism have been described (90,91). For instance, selective pressure with chemotherapy plus a BRAF inhibitor in melanoma cells leads to upregulation of mitochondrial enzymes critical for oxidative phosphorylation that, when blocked, sensitizes to therapy (91).

Relevant metabolic selective pressures may arise locally within the tumor microenvironment (TME), thus underscoring the need to profile metabolism across different metastatic niches. For instance, brain metastases have been shown to increase flux through glycolysis in MET-amplified NSCLC and exhibit heightened sensitivity to glucose deprivation (92). The brain is a highly metabolic organ, and studies of metabolic adaptation in breast cancer have shown that many nutrient synthesis pathways are upregulated to facilitate cancer cell growth in an otherwise depleted niche (93-95). Given growing evidence that metabolic reprogramming enables tumor cell survival despite effective blockage of oncogenic signaling achieved with use of a TKI, it will be critical to delineate metabolic shifts that occur in therapeutic resistance to TKIs in oncogene-driven lung cancer and whether this represents a viable treatment strategy.

To improve our understanding of these elements of acquired resistance, we must employ patient-derived preclinical models that faithfully recapitulate the full molecular and metabolic heterogeneity of patient tumors. Robust translational research programs are needed to fully integrate the collection of pre- and post-resistant patient specimens (discussed in the "Best practices for metabolic analyses using clinical specimens" section below) to execute functional, systematic analyses of metabolic mechanisms of resistance. Furthermore, the integration of genomic and non-genomic mechanisms of resistance (*Figure 1*) is critical to capture the full view of genomic and proteomic alterations influencing metabolites and metabolite-mediated feedback on gene and protein function.

Metabolic changes in chemotherapeutic resistance in SCLC

Despite the high response rates to chemotherapy, most SCLC patients will experience tumor relapse, leading to treatment-resistant or refractory disease. The highly proliferative nature in SCLC influences metabolic adaptations in treatment-resistant disease. With the appreciation that SCLC subtypes are defined by differential gene expression of key transcriptional regulators, ASCL1, NEUROD1, POU2F3, or YAP1 (96), discrete metabolic dependencies have emerged within this new framework. For instance, ASCL1-low SCLC cells and tumors contain elevated levels of guanosine nucleotides, an effect driven

by MYC-mediated expression of inosine monophosphate dehydrogenase-1 and -2 enzymes (IMPDH1 and IMPDH2) (18). Unsurprisingly, several metabolic phenotypes have been linked to MYC in SCLC, including a sensitivity to arginine deprivation (97), enhanced glycolysis (98), or GTP dependency (19). Elevated MYC in SCLC with acquired chemoresistance conferred dependency on IMPDH, raising the possibility of a targetable vulnerability in chemotherapy resistant MYC-high disease (19). While preclinical studies have identified the requirement for nucleotide synthesis to support growth in cell lines and murine tumor models of SCLC through dependencies on IMPDH (18) or DHODH (99), more studies will be needed to clarify their clinical significance.

Below, we discuss key examples of therapeutic targets of metabolism in lung cancer, with an emphasis on genotype-guided metabolic dependencies. While we highlight the successes in the field, we also acknowledge the challenges and limitations with these approaches.

Opportunities for therapeutic targeting of metabolism in lung cancer

Modulators of metabolism have been employed in oncology for decades, with the earliest forms of chemotherapy interfering with folate and nucleotide metabolism (100). Given the growing appreciation of metabolic pathways that are relevant in lung cancer biology and the increased recognition of metabolic states with routine molecular profiling, there is opportunity to determine whether metabolic vulnerabilities can be targeted for therapeutic purposes. Cytotoxic chemotherapy—the original class of antimetabolite-based therapies—is effective as it preferentially interferes with metabolite generation needed to sustain high proliferation rates in tumors (101). Beyond chemotherapy, targeted therapeutic inhibition of dysregulated nodes of signaling that control metabolism or interfering with metabolite generation may serve as more selective ways to disrupt tumor growth.

However, it is critical to recognize that genomic, transcriptomic, and proteomic alterations do not impact the metabolome with the same degree of genotype-phenotype correlation seen in oncogene addiction. Therefore, rigorous functional metabolic analyses are needed to fully uncover how metabolism is altered in tumors and required for growth and survival. Furthermore, lung tumors are not comprised solely of transformed cancer cells but rather, they include diverse array of stromal, endothelial, and immune cells. The complex cellular heterogeneity of the TME needs to be carefully considered when interrogating and modeling lung tumor metabolism. Moreover, cell type specificity represents a major challenge in developing therapeutic agents that target metabolic pathways, as systemic delivery of a compound that interferes with metabolite flux may influence cell types differentially.

Here, we summarize anti-metabolite therapeutics already in clinical use and novel targets actively being investigated to exploit metabolic vulnerabilities in lung cancer.

Next-generation approach to targeting metabolism in lung cancer

Metabolic reprogramming in malignancy is far from a "one size fits all". Metabolic dependencies in cancer are influenced by multiple factors including cancer lineage, histologic subtype, tissue microenvironment, and genetic events (102). Expanding the panels of molecular genotyping in lung cancer has enabled the identification of genomic alterations that may confer discrete metabolic adaptations, whether driven by a single genetic change or co-occurring alterations. Anchoring on molecular subtypes presents an ideal entry point for future work to metabolically profile lung cancer in ways that allow us to distinguish mere associations from true genotype-driven metabolic phenotype, the latter of which should be exploited for therapeutic targeting. Here, we review different strategies that are being employed for targeting metabolism in lung cancer (*Figure 2*), including manipulation of nutrient uptake, interfering with oncogenic signaling, targeting metabolic enzymes directly, and depleting metabolites through use of recombinant enzymes.

Metabolic targets in lung cancer

Blocking uptake of nutrients and metabolites utilized by cancer cells (i.e., glucose, lactate, amino acids) is one approach that has been explored to therapeutically manipulate metabolism in lung cancer. Preclinical models have shown efficacy of inhibitors that block transport of lactate (MCTs) (103), glucose (GLUTs) (104), glutamine (ASCT2) (105), or cystine (xCT, or SLC7A11) (106) transport. In lung cancer patients, some transport inhibitors have been tested in early-phase clinical trials (e.g., NCT01791595) (107), with more work needed to fully elucidate clinical efficacy and whether there are subsets of patients for which they would be more beneficial.

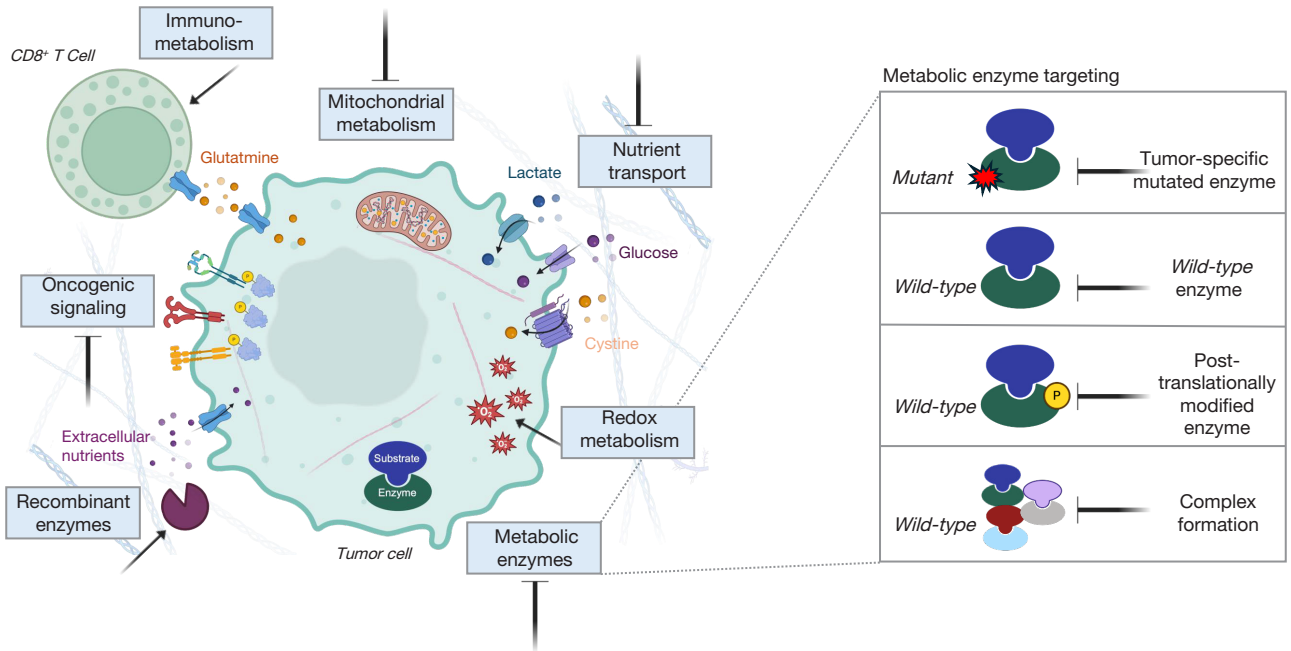

Figure 2 Targeting metabolic pathways in lung cancer. (Left) Schematic depicting opportunities for therapeutic targeting of tumor metabolism in lung cancer. (Right) Different conceptual approaches to modulation of metabolic enzymes. Some elements of some figures were created with BioRender.com.

Targeting intracellular enzymes of major metabolic pathways has also been investigated, including manipulation of glutamine usage (108), lactate dehydrogenase (109), oxidative phosphorylation (110), mitochondrial complex components (111), purine nucleotide synthesis (112), or glutathione biosynthesis (113). These approaches were initially thought to hold promise but were ultimately constrained by metabolic adaptations and redundancies that can circumvent such inhibition. An additional layer of complexity is the narrow therapeutic window that occurs with targeting wild-type metabolic proteins that are ubiquitously expressed, both in normal and cancerous cells.

As discussed above in previous sections, the more recent strategies for manipulation of metabolism in cancer have emerged within the growing practice of precision medicine, with the idea that metabolism is more exploitable in discrete genetic contexts. There are now several examples of oncogenotype-specific metabolic dependencies in lung cancer for which therapeutics are being developed.

Deletion of methylthioadenosine phosphorylase (*MTAP*) is associated with multiple tumor types including NSCLC, mesothelioma, cholangiocarcinoma, glioblastoma, urothelial cancers, and pancreatic cancer. *MTAP* deletions occur in approximately 13% of NSCLC with an adenocarcinoma predominance yet also with occurrences squamous cell lung cancers and other NSCLC histologic subtypes (114). MTAP is a metabolic enzyme that regulates the methionine salvage and is responsible for generation of adenine nucleotides. Deletion of *MTAP* from tumors leads to buildup of its substrate methylthioadenosine (MTA) which binds and sequesters the protein arginine methyltransferase 5 (*PRMT5*). MTA-bound PRMT5 is kept in an inactive form, and thus *MTAP* deletion confers selective dependency on PRMT5 and synthetic lethality with PRMT5 inhibitors (115,116). Several PRMT5 inhibitors have been developed (117,118), with some compounds currently under evaluation clinical trials in patients with MTAP-deleted solid tumors (NCT05732831, NCT05275478, NCT05094336, NCT05975073).

There is also preclinical evidence to support combination strategies with PRMT inhibitors, for instance with PARP inhibitors in MTAP-null NSCLC (119). Targeting of metabolic enzymes upstream of PRMT5 is being explored in MTAP-deleted solid tumors, as in the case of the MAT2A inhibitor (120), which blocks the synthesis of S-adenosyl-methionine (SAM), a substrate for PRTM5-mediated

protein methylation (NCT05975073, NCT04794699). There may also be a place for targeting this selective metabolic liability in acquired resistance to targeted therapies, as 14% of patients with EGFR-mutant NSCLC with Osimertinib-refractory disease were found to harbor acquired *MTAP* deletion [American Society of Clinical Oncology (ASCO) abstract].

KEAP1-mutant NSCLC is another molecular subset of lung cancer that has ascribed metabolic dependencies. KEAP1-deficiency is associated with reliance on both glucose (104) and glutamine (58) catabolism. Introduction of *KEAP1* and *NRF2* mutations in murine models of Kras-driven NSCLC augments glutaminolysis and is sensitive to inhibition of GLS (58,62). A phase 2 randomized, multicenter, double-blind study of GLS inhibitor telaglenastat (CB-839) with standard of care chemoimmunotherapy in first-line KEAP1/NRF2-mutated non-squamous NSCLC was terminated early due to lack of clinical benefit (NCT04265534). There are other trials ongoing testing the glutaminolysis inhibitor telaglenastat in other contexts, including in EGFR-mutant NSCLC with osimertinib (NCT03831932) or in combination with another glutamine antagonistic DRP-104 [sirpiglenastat (121)] (NCT04250545). Other metabolic modulators are being tested in KRAS-mutant NSCLC including inhibition of fatty acid synthesis (TVB-2640, NCT03808558).

Beyond inhibition of wild-type metabolic enzymes

Apart from inhibition of metabolic enzymes in wild-type states, there are other ways to interfere with catalytic activities of metabolic enzymes (*Figure 2*). These include inhibition of mutant forms of a metabolic enzyme, interference with a regulatory post-translational modification, or disruption of a protein complex that is important for its function. The classic example of a mutant metabolic enzyme is the case of isocitrate dehydrogenase (*IDH1* or *IDH2*) mutations for which there are FDA-approved small molecule inhibitors in cholangiocarcinoma, myelodysplastic syndrome, acute myeloid leukemia.

To date, there are no approvals of small molecule agents directed against a mutated metabolic enzyme in lung cancer. Beyond targeting a somatic alteration, other approaches to manipulation of metabolism are conceivable, including interfering with a tumor-specific post-translational modification of a metabolic enzyme that is important for its catalytic activity or disrupting the spatial organization of a complex of metabolic enzymes. Drawing from the malignant hematology field, depletion of extracellular metabolites using recombinant enzymes may be a viable therapeutic strategy in lung cancer. For instance, asparaginase is approved in acute lymphoblastic leukemia (ALL) to exploit the fact that ALL cells are unable to synthesize asparagine and preferentially rely on circulating levels. Other amino acid-degrading enzymes are in preclinical development, with some in clinical trials, yet with a major caveat of the potential need for co-targeting with autophagy modulators to potentiate the deprivation (122,123).

Autophagy modulation in lung cancer

Beyond manipulation of select metabolic targets or pathways, targeting of autophagy in cancer is a way to modulate metabolism on a broader scale. Autophagy refers to the cellular process of breaking down and recycling macromolecules through lysosome-mediated degradation (124). Different types of autophagy including macroautophagy (125), microautophagy (126), and chaperone-mediated autophagy (127) are important for maintaining cellular metabolic homeostasis through regulating the availability and turnover of building blocks such as amino acids, carbohydrates, lipids, and nucleic acids (128).

The role of autophagy in cancers is complex, as it has been described as both tumor-promoting and tumor suppressing (129). While efforts to effectively target autophagy in a context-dependent manner are ongoing (130), studies using pre-clinical models implicate mitophagy, a type of autophagy that selectively eliminates dysfunctional mitochondria, in the initiation, progression, and metastatic potential of lung cancers (131). Effective clearance of damaged mitochondria through lysosomal degradation has been shown to be important for mediating metabolic adaptations in lung cancer stem-like cells, promoting innate immune sensing, driving chemotherapeutic resistance in SCLC, and inducing drug-tolerant persister cancer cells in lung adenocarcinoma (132-134).

While autophagy modulation in lung cancer has been tested in early-phase clinical trials with modest results (135,136), further investigation is needed to evaluate more selective inhibitors of autophagy (e.g., next-generation agents that interfere with specific autophagy machinery rather than by modifying lysosomal pH) and whether there are relevant biomarkers in lung cancer that may confer benefit to autophagy manipulation in select patient populations.

Immunometabolism in lung cancer

There are many unique features of immune microenvironment in the lung, as the entirety of the lung mucous membrane and respiratory epithelial surface interfaces with the outside environment. Additionally, the chronic exposure to environmental insults and carcinogens as well as the fluctuating oxygen and carbon dioxide tensions all serve to modulate the metabolic demands of lung-resident immune cells. Beyond the intrinsic metabolic requirements to sustain immune cell activation in the appropriate physiologic setting, lung-resident immune cells can also be regulated by tumor cells. Thus, one major challenge is disentangling how lung tumor cells influence immunometabolism and how the rapid changes in metabolic demands of proliferating immune cells influences the metabolic requirements of a tumor (137).

Interplay between tumor and immune cell metabolism

Comprehensive atlases of immune cells in lung cancer have revealed the presence of diverse immune cell types such as neutrophils, dendritic cells, macrophages, and T cells (137-139). These cells are highly susceptible to nutrient deprivation, immuno-modulatory metabolites, and proteins locally produced by tumor cells. Different lung cancer lineages may exert differential metabolic stresses on immune infiltrates in the tumor and surrounding tissues. For example, ^{18}F-FDG PET/computed tomography (CT) images of lung cancer patients revealed that squamous cell carcinomas exhibit a greater dependency on glucose and is associated with increased fatty acid and amino acid metabolism (140). This correlates with altered composition and metabolic signatures of the tumor-infiltrating immune cells (140).

Conversely, immune cells recruited and shaped by the metabolic rewiring of tumor cells, such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages, may directly promote tumor growth and abrogate anti-tumor immune responses (141-143). Tumor metabolism is also tightly linked to immune composition and function through numerous mechanisms involving hypoxia, glucose/amino acid deprivation, secretion of immunosuppressive metabolites (such as kynurenine, lactate, and adenosine), and elevated ROS (144,145). It still remains unclear how these mechanisms are specifically orchestrated in the context of human lung cancer. Furthermore, the relative contributions of the metabolic mechanisms involved in different molecular subsets of lung cancer and its impact on tumor-infiltrating immune cells remain unknown.

T cell metabolism in lung cancer

Among the most important cells involved in eliciting anti-tumor immunity are CD8$^+$ and CD4$^+$ T cells. With the emergence of immune checkpoint inhibitors (ICIs) and the emerging clinical role for adoptive T cell therapy, understanding how lung tumor metabolism impacts T cell responses is crucial. Lung cancer cells compete for glucose, glutamine, and other carbon/nitrogen sources or metabolites such as kynurenine (146,147), all of which could negatively impact T cell priming, proliferation, and differentiation. For example, some lung cancers highly express glutamine transporters such as SLC1A5, SLC38A2, and SLC38A3 which fuels glutamine dependency yet deprives immune infiltrates of the necessary amino acid, resulting in a so called "tug-of-war" for certain metabolites (148). *In vivo* tracing of ^{13}C-labeled metabolites revealed glutamine as a critical fuel required for eliciting effector CD8$^+$ T cell function *in vivo* (149). Carbon source availability has been shown to drive nutrient utilization and metabolic reprogramming of glucose catabolic pathways in CD8$^+$ T cells (150), highlighting the need to understand *in vivo* and *in situ* dynamics of how tumor cells and T cells share (or not) nutrient sources.

Conversely, deprivation of glutamine in the TME blunts GLS-dependent glutamate catabolism in T cells, which typically supports T cell activation through protein synthesis, redox balance, and flux through the tricarboxylic acid cycle (TCA) cycle (145). Furthermore, SLC38A2-dependent glutamine uptake by cDC1 was critical for T cell priming and therapeutic efficacy against tumors, highlighting the impact of glutamine deprivation on third-party immune cells critical for supporting T cell function (151).

Although glutamine depletion in the TME is generally associated with impaired tumor infiltrating lymphocyte functions, therapeutic targeting of GLS and its impact on anti-tumor T cell immunity remains complex. Although GLS deficiency attenuates early T cell activation, GLS inhibition increases T-bet expression which enhances differentiation and function of CD4$^+$ T helper 1 (Th1) and CD8$^+$ type 1 cytotoxic T (Tc1) cells (152). GLS inhibition also promotes oxidative metabolism of tumor infiltrating lymphocytes to increase cellular longevity and a memory-like phenotype (153). Also, glutamine deprivation impacts multiple immune cell types with opposing functions in the

TME (153). Thus, the effects of glutamine transporter inhibitors may be confounded by their collective effects across the heterogenous tumor landscape. Glutamine deprivation, along with many other immunosuppressive mechanisms, highlights one metabolic dependency of T cells in lung cancer. However, therapeutic manipulation to bolster anti-tumor T cell immunity remains a challenge due to a lack of specificity and off-target effects.

Myeloid cell metabolism in lung cancer

Myeloid cells also play a critical role in immune evasion and tumor progression in lung cancer (154,155). Immune profiling of human and mouse lung cancers demonstrated conserved myeloid populations across both individuals and species (156). Macrophages comprise a heterogeneous population of cells that reside in the lung and are particularly vulnerable to the metabolic demands and byproducts of lung cancer, which in turn regulate their recruitment and function (157,158). In murine models of NSCLC, LKB1-deficient tumors secrete high levels of metabolites such as lactate in an MCT4-dependent fashion to recruit MDSCs and immunosuppressive macrophages, which serve to impair anti-tumor immunity (159,160). Similarly, EGFR-mutated NSCLC has been shown to secrete high levels of surfactant and granulocyte macrophage-colony stimulating factor (GM-CSF) in order to induce tumor-associated alveolar macrophage (TA-AM) proliferation as a means to co-opt alveolar macrophage metabolism to support EGFR signaling (161). For example, GM-CSF-mediated rewiring of lipid metabolism in TA-AMs directly induces cholesterol synthesis and export, which subsequently drives EGFR phosphorylation of adjacent tumor cells and enhances tumor progression (161). Reprogramming macrophage metabolism may therefore serve as a promising avenue to repress the positive feedback loop utilized by lung cancer and improve anti-tumor immunity.

Immunometabolism and lung cancer immunotherapy

Cancer immunotherapy harnesses the host's immune system to recognize and eliminate cancer cells. Some ICIs in SCLC and NSCLC target programmed death-1 (PD-1) or PD-L1 to block inhibitory signals that prevent T cells from attacking cancer. However, not all patients benefit from these therapies, necessitating the identification of reliable predictive biomarkers and molecular targets to overcome resistance to these treatments (162).

To this end, one area of active investigation is the interrogation of shifting metabolic landscapes to predict response to ICIs. Peripheral blood systemic inflammation indices and serum metabolite biomarkers such as hypoxanthine and histidine have been reported as predictive in the response to PD-1 blockage in NSCLC (163,164). Prospective studies are needed to understand how these data can be used to influence clinical practice. It remains to be seen whether metabolic prognostic indices are sufficient to stand alone or whether they should be combined with other predictive biomarkers to guide intensification or de-intensification of treatment strategies.

Molecular programs used by lung cancers may dictate the functional outcome of tumor-infiltrating lymphocytes (TILs) and responsiveness of ICIs. Altered tumor-intrinsic metabolic programs are associated with changes in immune-regulatory properties of the tumor (165,166) and may directly drive resistance to PD-1-based immune checkpoint blockade (167). Interestingly, PD-L1 signaling itself play an important role in the regulation of tumor metabolism (168,169) and PD-1 blockade may bolster the ability of tumor cells to uptake glucose and other essential fuel sources (169,170). Changes in the metabolic microenvironment driven by the tumor-intrinsic properties and the effects of anti-PD-1 subsequently rewire the fate of intra-tumoral T cells and contributes to resistance to anti-PD-1 treatment. Numerous pathways can be targeted to re-engage T cell response against various tumor types, for example through amplification of one-carbon metabolism, formate supplementation, or modulation of pantothenate metabolism (171,172). While some studies have focused on a particular metabolic pathway, other investigations have pointed towards using a more global approach to improve mitochondrial function and overall metabolic fitness as a means to reinvigorate T cell function, prolong their longevity, and subvert T cell exhaustion (173).

Although metabolic interventions targeting tumor-immune crosstalk in murine models have helped shed light on the importance of metabolism in anti-tumor immunity, systemic treatments targeting metabolic pathway is constrained by the specificity of targeting. One path forward to enhance metabolic fitness of tumor-specific T cells may involve *ex vivo* manipulation of immune cells, for example using TIL, T cell receptor (TCR)-engineered or chimeric antigen receptor T (CAR-T) cell therapy. A recent study suggests that patient-derived CAR-T cell products over-expressing FOXO1 display superior mitochondrial

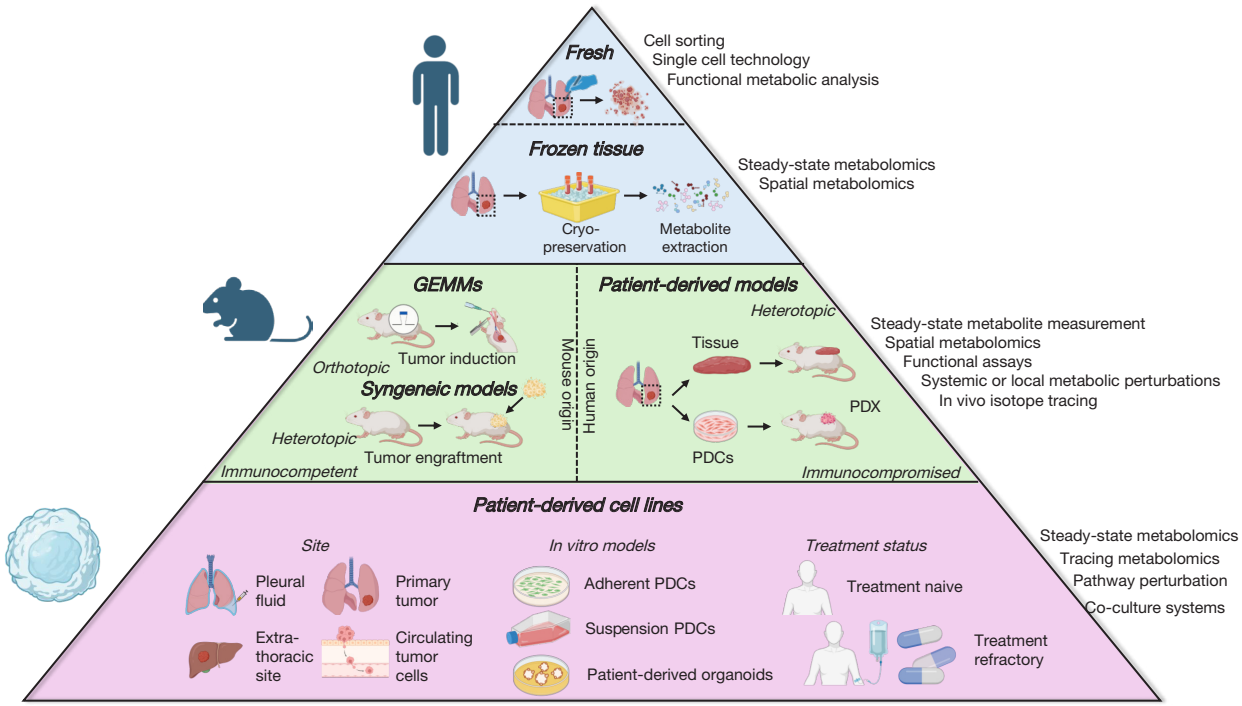

Figure 3 Model systems for metabolic analyses in clinical specimens. Diagram depicting models that can be used for interrogation of tumor metabolism, including fresh or snap-frozen patient specimens, GEMMs, patient-derived mouse models, and PDCs. Some elements of some figures were created with BioRender.com. GEMMS, genetically-engineered mouse models; PDCs, patient-derived cell lines; PDX, patient-derived xenograft.

fitness, persistence, and therapeutic efficacy in hematologic malignancies (174). Overcoming resistance to ICIs in lung cancer remains a key priority in lung cancer care, and as such, therapeutic targeting of immunometabolism may be one way to sensitize to immune manipulation.

Best practices for metabolic analyses using clinical specimens

Considerations for patient specimen collection for metabolic studies

Interrogation of metabolism in patient samples can be stratified based on model system: direct use of patient tumor specimens, modeling of lung tumors (typically in rodents), or use of diverse patient-derived models (*Figure 3*). Arguably, the gold standard for studying tumor metabolism with the highest fidelity is direct using patient specimens. Cancer cells undergo diverse metabolic adaptations to meet energetic demands imposed by high proliferation rates and thus, analyzing patient lung tumors provides an opportunity to delineate cell-intrinsic and -extrinsic factors that may not be fully recapitulated in other models.

Stable isotope tracing by liquid chromatography-mass spectrometry (LC-MS) is a powerful technique that allows for probing of metabolic activity within intact tumors to delineate flux through discrete metabolic pathways (175-177). In this protocol, a stable isotope-labeled nutrient (i.e., [^{13}C]glucose) is introduced into the circulation prior to surgical resection followed by tumor removal, metabolite extraction, and analysis of the isotope labeling patterns with mass spectrometry to inform on which metabolic pathways utilize the infused nutrient within the tumor *in situ* (24). A major advantage of this methodology is the ability to capture metabolic properties of lung tumors *in vivo* that are reflective of the impact of cell non-autonomous factors, the heterogenous mixture of cells in tumors, and variable nutrient availability in the host (178). As this approach relies on clinical infusions and thus requires patient informed consent, all procedures must be approached by the institutional committee that oversees research on human subjects, with considerations for patient confidentiality and

adverse event monitoring.

Metabolite levels in patient lung tumor specimens can also be evaluated at steady state, without infusion of a labeled tracer. With institutional review board (IRB) approval, research collection of tumor specimens can be carried out, with subsequent processing of fresh tissue in real-time or snap-freezing for processing later. A benefit of fresh tissue is the ability to perform cell sorting or execute single cell technologies (179), although technologies are expanding to allow for single cell DNA-sequencing, assay for transposase-accessible chromatin (ATAC)-sequencing, and RNA-sequencing (RNA-seq) to be applied to archival specimens (180). Simultaneous collection of adjacent lung tissue allows for paired analysis of normal lung with patient-matched lung tumor, allowing for an additional internal benchmark against which lung tumor metabolites can be measured.

Cryopreserved frozen tumor specimens represents another valuable consideration for studying metabolism. The cellular metabolome is highly dynamic and thus, several factors may influence metabolite abundance, stability, interconversion, or detection in a tumor specimen. These factors include internal enzymatic activity, blood contamination, and the dynamic nature of metabolite concentrations, as well as external factors such as storage and handling (181). During collection, samples must be kept at the lowest possible temperatures and immediate snap-freezing is recommended to quench rapid degradation, prevent oxidation of labile metabolites, and halt enzymatic activity.

Therefore, it is critical for translational investigators to work closely with interventional radiology, interventional pulmonary, surgery, and pathology colleagues to develop standard operating protocols to ensure expedient cryostorage of tissues that are to be used to study metabolite levels. Despite best practices for patient sample collection, metabolites can be highly labile and susceptible to oxidation, aggregation, or degradation during sample handling, so internal quality assessment and quality control is critical (182,183). While there is no uniform defined metric to assess sample degradation for metabolomics in lung tumors, surrogates can be used including evaluating concentrations or ratios of metabolites that contribute to the energetic and redox balance (181,184), or RNA integrity number (RIN) when paired with transcriptomics (185).

Furthermore, interpreting metabolite enrichment and depletion data from bulk tumor metabolomics has additional caveats. Lung tumors are comprised of a complex heterogenous mixture of cell types. Single cell transcriptional analysis of patient lung tumors features additional cell types beyond cancer cells, including myeloid cells, fibroblasts, lymphoid cells, alveolar cells, epithelial cells, and endothelial cells (186). Cellular heterogeneity almost certainly translates to metabolic heterogeneity. To address this, spatial technologies are increasingly being used to demonstrate metabolite distributions *in situ* across a tumor. Furthermore, overlaying spatial distributions of metabolites allows for co-registration with other spatial technologies including spatial transcriptomics (187) or cyclic immunofluorescence (CycIF) (188). Integration of spatial metabolite analysis has the power to inform on pathways that are altered in a compartmentalized fashion, for instance in the case of high or low areas of immune infiltrate (22).

Since direct analysis of patient tumor specimens is not always feasible, proxies for altered metabolic states in malignancy can be considered. There is a growing body of literature on metabolite signatures in lung cancer patients that are detectable in diverse bodily fluids, including sputum, saliva, urine, plasma, and breath condensates (189,190). Studies have reported discrete metabolite profiles in sputum (191) and exhaled breath condensates (192) that can differentiate between healthy control and lung cancer patients. Metabolomic analysis of urinary extracellular vesicles also captured differential abundances in metabolites that distinguish between healthy control participants and patients with lung cancer (193). Blood-based metabolite measurements using both plasma and serum have been widely studied for lung cancer diagnosis and prognosis. For instance, metabolite signatures in plasma have been reported to discriminate between healthy controls and lung cancer patients as well as between early and advanced stages of disease and between lung cancer histologic subtypes (194). Interestingly, a panel of 27 serum metabolites has been reported to differentiate stage I lung adenocarcinoma from benign pulmonary nodules (195). More work is needed to develop clinical applications for non-invasive biomarker testing methods using biofluid metabolomics, with a major goal of serving as a screening tool for early-stage lung cancer when curative intent is still possible.

Metabolic analyses in patient-derived models

Complementary to direct use of patient tumors, patient-derived models have proven an invaluable part of the toolkit in the interrogation of dysregulated metabolic

states in lung cancer (*Figure 3*). Patient-derived mouse models, in the form of tumor graft models (or patient-derived xenografts, PDXs) or mouse xenografts of human tumor cells lines, have shown fidelity in predicting patient response to therapies (196,197). SCLC PDXs have also been generated from biopsies or circulating tumor cells, showing the promise of establishing a clinical-translational pipeline for tissue collection to interrogate disease biology and drug-sensitivities (198). However, PDXs require use immunocompromised mice to house the patient-derived implanted tissue or injected cells, and thus fail to reproduce tumor cell interactions with innate and adaptive immune components (199). Furthermore, while orthotopic implantation has been described for lung cancer (200), technical challenges with this approach have largely led to reliance on subcutaneous implantation for xenoengraftment for many NSCLC PDX model studies (199). This is an important consideration when studying metabolism, as there are stark differences in heterotopic, immunocompromised models including availability of circulating nutrients, oxygen tension, paracrine signaling, local stromal cells, lung resident cells, and tumor-associated immune infiltrate, all of which can impact metabolic states of tumor cells.

To circumvent the limitations of studying tumor metabolism in immunocompromised mice in heterotopic microenvironments, GEMMs have proven useful in dissection of tumor metabolism. Benefits of GEMMs include tissue specificity, inducibility, intact immune system, and autochthonous tumor initiation—albeit not of human origin. A study in GEMMs has underscored that tumor metabolic phenotyping can identify essential metabolic pathways. Infusion of mice with Kras-mutant NSCLC with isotope-labeled glucose or glutamine revealed that glucose carbon contribution to the TCA cycle is required for tumor formation *in vivo* (49). Furthermore, comparisons of the same oncogenically driven lung tumor subtype (Kras-mutant NSCLC) in different contexts—cultured cells versus murine tumor—have demonstrated that tumors arising in mice are less dependent on GLS than cultured cells, underscoring that tissue environments are a critical determinant of tumor metabolic phenotypes (49,201). Using GEMMs to pair genetic manipulation of tumors with *in vivo* metabolic flux (202) offers a powerful way to interrogate and manipulate cancer metabolism.

Patient-derived cells (PDCs) offer an additional tool to probe metabolism. While cell culture models may not fully capture metabolic features of a tumor *in situ*, particularly regarding cell-extrinsic factors, a major advantage is that these models allow for precise experimental controls and manipulations that are not necessarily feasible in patients. Functional interrogation of metabolic states can be easily executed in PDCs including through steady-state and tracing metabolomics, seahorse metabolic flux analysis, kit-based assays, redox state assessment, glucose utilization, and genetic or pharmacologic manipulation of a discrete metabolic pathways. Furthermore, PDCs allows for the investigation of cancer metabolic vulnerabilities in a large-scale, high-throughput fashion. Cancer metabolic diversity has been profiled in >900 cell lines across more than 20 cancer types, including in NSCLC and SCLC, and associated with functional genomic features and dependencies (203). Such resources offer unbiased associations linking the cancer metabolome to genetic alterations, epigenetic features, and gene dependencies. Use of PDCs also enables a deeper mechanistic dive into oncogenotype-specific metabolic dependencies, in the case of CPS1 dependency in Kras/STK11-mutant NSCLC (56).

PDCs can also be useful for interrogating metabolic rewiring during therapeutic resistance (204). However, obtaining treatment-naïve specimens derived from patients for comparison with treatment-refractory PDCs remains difficult. Procurement of pre-treatment specimens requires research protocols in place to allow for collection and processing of PDCs sometimes prior to the knowledge of a lung cancer diagnosis. Use of liquid biopsies concomitantly with tissue biopsies at has somewhat increased the pretest probability of a lung cancer diagnosis, but logistical challenges remain in identifying treatment-naïve patients for PDC generation.

Likewise, the gold standard of interrogating metabolic rewiring in therapeutic resistance is analysis of pre-treatment and post-treatment specimens from the same patient. However, obtaining patient-matched pre- and post-resistance PDCs is often challenging. Furthermore, some pre- and post-treatment specimens are collected from different tissue sites, i.e., pre-treatment specimen from the primary lung tumor and post-treatment specimen from a progressing liver metastasis (205), complicating interpretation of metabolic patterning attributable to resistance-driven or tissue-driven effects. Alternative approaches include interrogation of metabolism across multiple PDCs derived from different patients or from treatment-resistant PDCs derived in culture (206). The latter is useful as the treatment-sensitive parental line can be used as a comparator, but metabolic reprogramming may not necessarily be representative of that which occurs

in vivo, similar to limitations observed using *in vitro*-derived resistant models to study signaling pathways dysregulation in persister cell biology (85,207).

Whether investigating metabolism in PDCs or mouse models, neither fully recapitulate the nutrient availability of a patient tumor *in situ*, given the known differences in the makeup of mouse plasma or traditional cell culture media. Efforts to develop a culture medium with polar metabolite concentrations comparable to those of human plasma—also called human plasma-like medium, or HPLM—have been carried out to better mimic the physiologic metabolic milieu of a tumor (208). Several other studies have highlighted that the composition of culture media and sera, sometimes narrowed down to levels of a single nutrient, can head to differing sensitivities to metabolic pathway manipulation (209,210), underscoring that careful attention to PDC culture conditions is necessary to maximize fidelity of patient-derived models.

Immune profiling to capture tumor-immune cell crosstalk

Immune profiling involves the collection of patient-derived tumor samples and/or tumor-matched blood, which can be further processed and stored in the form of plasma, serum and buffy coat. Fresh tumor tissue can be either snap-frozen immediately following surgical resection or processed as a single cell suspension using appropriate digestive enzymes prior to cryopreservation. As discussed above, samples that are rapidly flash frozen are more likely to retain cellular and metabolite integrity.

The traditional approach to analyzing immune cell compartments in patient lung tumors has involved immunofluorescent-based assays, but only enables detection of a limited number of markers. Recent advances in spatial single cell-genomic/transcriptomic sequencing (211,212) and multiplexed protein-based spatial profiling techniques such as CycIF (213,214) and spatial mass cytometry by time of flight (CyTOF) (215,216) have enable comprehensive evaluation of a multitude of biomarkers to inform immune cell phenotype, function, and spatial distribution in patient samples. As the metabolic environment of a lung tumor is heterogenous, understanding the spatial distribution of immune cells corresponding to distinct metabolic environments may offer novel insights into mechanisms regulating anti-tumor immunity.

Frozen tissue samples may also be utilized for bulk genetic, epigenetic, transcriptomics, metabolomics or proteomic studies. Although a greater number of deconvolution techniques are now available to identify immune cell lineages (217,218), these approaches offer limited insights into immune cell diversity, fate, function, and the involved molecular mechanisms. Tumor interstitial fluid (TIF) extracted from tumor samples may serve as an alternative method to study the metabolites and proteins (i.e., cytokines, chemokines) which may provide an indirect readout of the local immune microenvironment (219,220).

Enzymatic digestion of freshly acquired patient sample for tissue dissociation has been utilized in the field of immune-oncology during the past decade, as it offers the benefit of high-fidelity single cell -omics, flow cytometry-based immune profiling, and functional studies of isolated immune cells. The ability to measure DNA, RNA or protein at single-cell resolution allows for precise characterization of cellular states and functions, which can be crucial for unraveling the complexity of immune cells in the TME. Fresh tissue, while sometimes difficult to obtain, is a preferred approach, as isolating immune cells from previously frozen tissue samples yields poor viability and may not be suitable for single cell -omics, with the exception of specialized techniques such as the single-nucleus RNA-seq (snRNA-seq) (221).

Furthermore, the use of single cell suspension from fresh patient samples offers the advantage of (I) encapsulating greater representation of a tissue instead of a single two-dimensional slice and (II) multiplexing and scaling up the total number of samples that can be run and analyzed. Flow cytometric or single-cell analysis of immune infiltrate in the tumor can be paired with analysis of peripheral blood mononuclear cells (PBMCs) since peripheral blood composition can be correlative and/or predictive of TME or tissue. Limitations of tissue dissociation procedures include altered transcriptomic and metabolic states of the cells, loss of cells sensitive to enzymatic digestion, and loss of spatial information. Thus, both fresh and frozen tissue procurement offer distinct advantages and collectively enable for profiling of immune cells and immune-modulatory factors in the TME.

Interrogation of immunometabolism in murine cancer models

Mouse models of cancers have proven to be a powerful tool to interrogate the effects of tumor metabolism on anti-tumor immunity. Despite the major differences in mice and humans, important biological mechanisms are often conserved across both species and have contributed to the

advancement of modern cancer immunotherapy.

As PDXs necessitate an immunocompromised murine background, these models generally prevent full assessment of the immune microenvironment and disregard the role of the endogenous immune system in the regulation of tumor progression. However, these models are frequently utilized to assess specific types of anti-tumor immunity through the adoptive transfer approach. In immuno-oncology, PDX models are most frequently used in the field of cellular therapy including CAR-T, TCR-transduced T cells and natural killer (NK) cells to examine tumor cell and transferred immune cell interactions. Recent advances in CAR-T cell engineering involve overcoming metabolic barriers in the tumor and/or increasing metabolic fitness of T cell products (174,222), and PDX model may serve as an appropriate model to study the contribution of tumor metabolism on cytotoxic lymphocytes.

Immunocompetent syngeneic tumor models are most frequently utilized in the field and involve tumor cell lines derived from the identical species as the host animal. These models offer a powerful tool for evaluating the complex interactions between tumor cells and the immune system and have facilitated the development of immunotherapies at pre-clinical stage. Importantly, syngeneic tumor models offer the advantage of scalability, and provide a diverse genetic toolkit to precisely control for tumor and immune cell function.

Lung cancer cell lines compatible with the commonly used C57BL/6 mouse strain, such as LLC ($Kras^{G12C}$ mutation) (223), CMT167 ($Kras^{G12V}$) (224), and EA1 cell lines (225), contain distinct molecular and genetic perturbations and can be administered orthotopically or subcutaneously to assess immune-tumor interactions. Although these models are frequently used in the field due the ability to precisely manipulate tumor and immune cell compartments, syngeneic tumor models often fail to recapitulate the complexity of the tumor progression and the full extent of the immune microenvironment.

To circumvent some of the limitations imposed by use of syngenetic models, GEMMs of lung cancer have been employed to study antitumor immunity in an immunocompetent, orthotopic, and autochthonous setting. The majority of NSCLC cancer GEMMs of involve $Kras^{G12D}$ and/or *TP53* in combination with other oncogenic drivers using the Cre-Lox recombination (226,227) or CRISPR-Cas9 system (228). GEMMs of SCLC are also routinely used and generally rely on conditional deletion of *Rb1* and *TP53* genes in the lungs of adult mice (229). Generally, lung adenocarcinoma GEMMs display immunosuppressive environments and the use of anti-PD-1 immunotherapy yield limited therapeutic response in comparison to syngeneic tumor models (230,231). Yet, GEMMs offer a unique avenue to genetically manipulate genes involved in immunometabolism and to explore more sophisticated mechanisms governing anti-tumor immunity that cannot be fully captured by syngeneic tumor models.

Conclusions

Precision medicine aims to deliver the right drug to the right patient, with the tailoring of treatments based on individual genomic profiles. The field is starting to appreciate that tumor genomic features may confer metabolic dependencies, both at initial presentation and through acquired resistance to therapy. In a full circle, altered metabolic states in tumors can, in return, influence epigenetic states (*Figure 1*) (232). Disentangling which insult drives tumor formation and propagation requires more investigation to inform on the appropriate therapeutic approach. Differentiating oncogene-associated metabolic phenotypes versus true metabolic dependencies is critical, the with latter holding promise for therapeutic intervention.

While progress has been made in identifying oncogenotype-specific metabolic dependencies in lung cancer, genomic features alone cannot fully predict metabolic profiles (24,102). This highlights that other yet-to-be-identified factors—beyond lineage, histology, and somatic alterations—also shape tumor metabolism. To address the ongoing unmet need in lung cancer treatment by harnessing the biology of altered metabolism, a thorough understanding of the differences between model systems can help to guide the identification of biomarkers essential for therapeutic responses. The failure to account for these variables is a potential common explanation for the lack of success in early phase clinical trials. Going forward, it will be necessary for investigators to accurately identify the full sequence of events that connect genotypes to growth and resistance phenotypes to expand the therapeutically targetable space in lung cancers and beyond.

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Footnote

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