Seminars in Cell and Developmental Biology 98 (2020) 54–62

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Exploiting metabolic vulnerabilities of Non small cell lung carcinoma

Blanca Majem[a], Ernest Nadal[b,c,*], Cristina Muñoz-Pinedo[a,*]

[a] Cell Death Regulation Group, Oncobell Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet, Spain
[b] Clinical Research in Solid Tumors (CReST) Group, Oncobell Program, IDIBELL, L'Hospitalet, Spain
[c] Department of Medical Oncology, Catalan Institute of Oncology (ICO), L'Hospitalet, Spain

ARTICLE INFO

Keywords:
Non-Small cell lung carcinoma
Cancer metabolism
Mitochondria
Glucose

ABSTRACT

Lung cancer is the main cause of cancer death worldwide. Non-Small Cell Lung Carcinoma (NSCLC) is the most common subtype of lung cancer, and the prognosis of NSCLC patients in advanced stages is still very poor. Given the need for new therapies, the metabolism of NSCLC has been widely studied in the past two decades to identify vulnerabilities that could be translated into novel anti-metabolic therapeutic approaches. A number of studies have highlighted the role of glucose and mitochondrial metabolism in the development of NSCLC. The metabolic properties of lung tumors have been characterized in detail *in vivo*, and they include high glucose and lactate use and high heterogeneity regarding the use of nutrients and mitochondrial pathways. This heterogeneity has also been observed in patients infused with labeled nutrients. We will summarize here the knowledge about the use of amino acids, fatty acids and carbohydrates in NSCLC that could lead to new combination treatments.

1. Characteristic features of NSCLC and association with metabolic parameters

Lung cancer is the main cause of cancer death worldwide in both sexes [1]. Non-small cell lung cancer (NSCLC) accounts for about 85% of lung cancer and is classified into three major histological subtypes: lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and large cell carcinoma. During the last decade, a significant improvement in the clinical outcome was achieved, mainly due to the incorporation of targeted therapies in genetically selected subpopulations of patients and the impressive achievements of immunotherapy in advanced and locally advanced NSCLC [2]. However, only a minority of NSCLC patients will achieve long-term survival [3] and therefore, deeper knowledge about lung cancer biology is needed to unravel effective therapeutic strategies.

Deregulation of cellular energy metabolism to support continuous cell growth and proliferation is a hallmark of cancer [4]. In spite of significant advances on the understanding of vulnerabilities of cancer cells *in vitro*, the complexity of the tumor microenvironment has hindered the discovery of effective metabolic inhibitors.

Glucose is an essential nutrient required for multiple metabolic pathways in proliferating cells (Fig. 1). It is possible that a high supply of glucose benefits tumors. In fact, high blood glucose and a clinical diagnosis of diabetes have been associated with a higher incidence and mortality in many types of cancer, including NSCLC [5]. In particular, epidemiological studies suggested that pre-existing diabetes was associated with lung cancer mortality [6]. A role for diabetes in the prognosis of NSCLC patients is now becoming established. Some retrospective studies showed that diabetes as well as high levels of fasting plasma glucose were associated with worse outcome of NSCLC patients treated with surgical resection or concurrent chemoradiotherapy [7], while this association was not observed in small cell lung cancer (SCLC) patients [8]. The reason, however, why diabetes is associated with poor prognosis is unclear. Diabetes could be linked to NSCLC through a direct effect of hyperglycemia on the tumor that would promote metabolic advantages to glucose-avid cells. However, its effects could also be indirect, due to the effects of hyperinsulinemia on the tumor, inflammation associated with diabetes or shared risk factors like obesity.

According to Warburg's hypothesis, hyperglycemia could accelerate the proliferation of cancer cells, as they can obtain essential metabolites and energy mainly from glucose fermentation, even in aerobic conditions [9]. Data from patients and mouse models indicate that lung tumors are indeed dependent on glucose metabolism, and increased expression of the glucose transporter GLUT1 or glycolytic enzymes correlate with poor prognosis in advanced [10–12] and early stage lung cancer patients [13]. GLUT1 overexpression was independently associated with worse overall survival in patients with surgically resected squamous NSCLC. *In vitro*, it has been described that glucose levels determine sensitivity to radio- and chemotherapy in multiple cell types: cells growing in low glucose show more sensitivity. This suggests that

* Corresponding authors.
E-mail addresses: bmajem@idibell.cat (B. Majem), esnadal@iconcologia.net (E. Nadal), cmunoz@idibell.cat (C. Muñoz-Pinedo).

https://doi.org/10.1016/j.semcdb.2019.06.004
Received 19 March 2019; Received in revised form 16 June 2019; Accepted 17 June 2019
Available online 02 July 2019

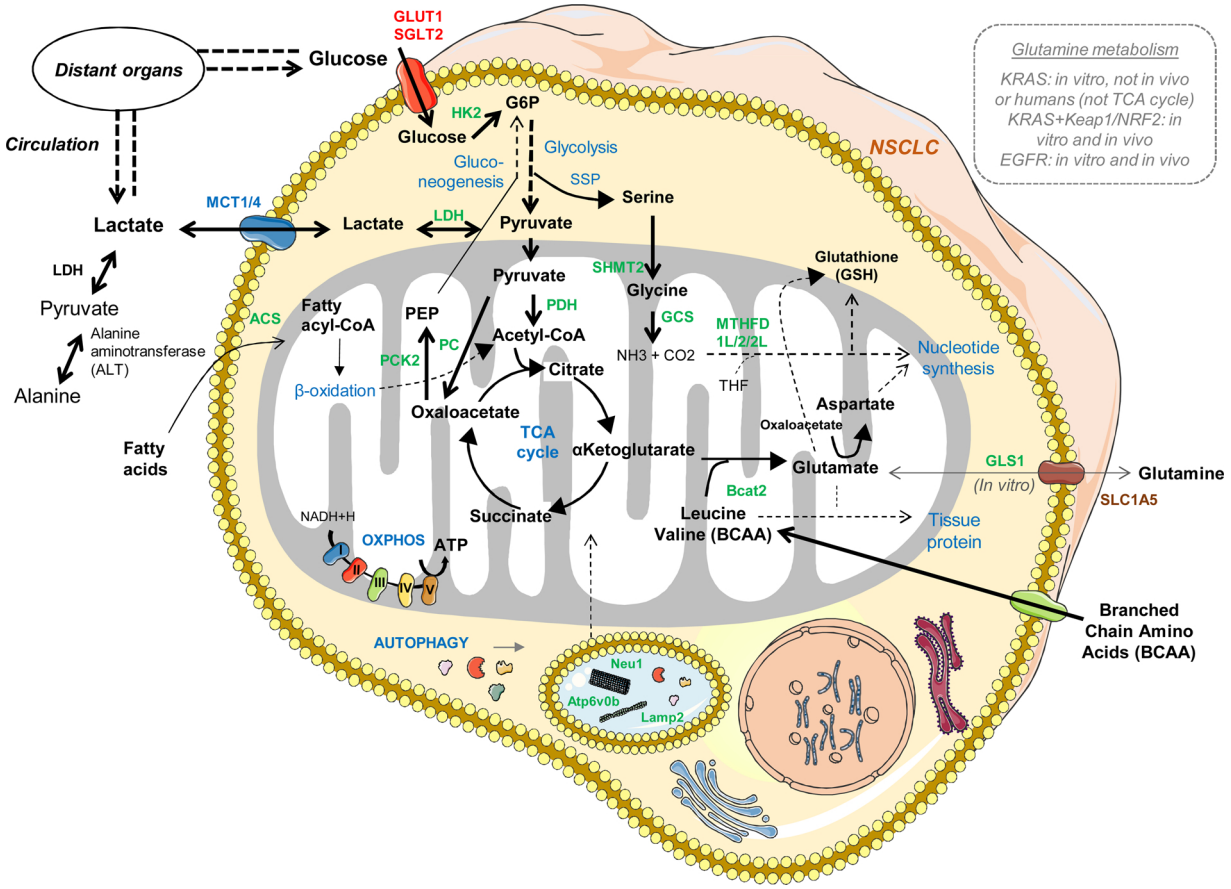

Fig. 1. Metabolism of NSCLC.
Abbreviations: ACS, Acyl-CoA synthetase. GCS, glycine cleavage system. GLS1, glutaminase 1. LDH, lactate dehydrogenase. PC, pyruvate carboxylase. PDH, pyruvate dehydrogenase. SHMT2, serine hydroxymethyltransferase 2. SSP, serine synthesis pathway.

hyperglycemia may be related to poor prognosis after treatment because it would reduce cell death induced by the therapy [14]. Hyperglycemia was also associated with a reduced antiproliferative effect of chemotherapy in preclinical models [15], although these results have not been confirmed yet in clinical trials.

While the association of high glucose with poor prognosis is clear, whether glucose is mostly used anaerobically through glycolysis in NSCLC is not. In this review, we will revise the metabolic properties of NSCLC that could explain clinical associations with metabolic parameters and that may help to unravel novel therapeutic opportunities.

2. NSCLC is driven by mutations that promote metabolic rewiring

Some advanced NSCLC tumors have been shown to be driven by the oncogenic activation of tyrosine kinases. Mutations in the epidermal growth factor receptor (EGFR) or v-Raf murine sarcoma viral oncogene homolog B (B-RAF) and chromosomal rearrangements of the anaplastic lymphoma kinase (ALK) gene and ROS1 are targetable, and this has improved the treatment of patients in advanced stages [16]. There is increasing knowledge indicating that these kinases also produce metabolic changes in the tumor cells. The specific mechanisms by which EGFR rewires NSCLC metabolism are widely studied, and it is known that oncogenic EGFR signaling is involved in the regulation of glucose metabolism [17], which will be described in more detail below.

KRAS mutations occur in around 30% of NSCLC, mainly in the non-squamous subtype LUAD, and it is one of the most common driver mutations causing constitutive activation of the gene and its downstream signaling [18]. Many efforts have been done to target KRAS, from early discoveries on miRNA-based therapies [19] to more advanced therapeutic strategies such as the G12C inhibitor AMG510 under development (NCT03600883). However, to date, clinicians do not usually consider KRAS mutations in the clinical management since they are not actionable [16]. KRAS is one of the most widely studied oncogenes in terms of the metabolic changes that it produces, and the growing evidence of KRAS-dependent metabolic features will be likely translated into novel therapeutic avenues for NSCLC, as extensively reviewed by Kerr and Martins [20]. Another non-actionable gene mutation in NSCLC is LKB1, which is inactivated by mutation or deletion in almost 20% of NSCLC. LKB1 is widely studied as the kinase that activates AMPK to rewire metabolism in response to starvation, and its absence promotes sensitivity to drugs that target glucose or mitochondrial metabolism [21].

3. Oncogenic mutations drive glucose uptake and its use in the TCA

KRAS can drive glucose uptake and its mitochondrial oxidation. In particular, Kerr et al. [22] had described that glycolysis is acquired as a function dependent on the number of copies of mutant KRAS. They demonstrated (i) an increase in glucose utilization, expression of GLUT transporters and glycolytic enzymes coupled to increased channeling of glucose-derived metabolites into the TCA cycle, and (ii) a glucose-dependent management of reactive oxygen species (ROS) metabolism in mutant $KRAS^{G12D/G12D}$ NSCLC cells and murine tumors, but not in the early stage-$Kras^{G12D/WT}$ heterozygous ones [22].

It was recently shown in KRAS-mut/$p53^{-/-}$ NSCLC mice models that the genetic loss of AMPK, a kinase activated by low glucose or low ATP, reduced tumor growth [23]. This was accompanied with a

reduction in GLUT1 expression. In LKB1-deficient KRAS-mut/$p53^{-/-}$ lung tumor cells, the remaining levels of AMPK activity were sufficient to support survival [23]. These data helped to clarify the role of AMPK in lung cancer. Besides a role of this kinase in sensing low glucose and facilitating its uptake through GLUT1 transporter, AMPK activation induced the expression of core lysosomal genes, helping cells cope with nutrient stress [23]. The authors defined a core set of the 16 most AMPK-dependent lysosomal genes in lung cancer cells and applied this AMPK-dependent lysosomal gene signature to several public databases of lung cancer patients. Interestingly, elevated lysosomal gene expression correlated with accelerated disease recurrence [23].

Other common mutations in NSCLC, such as those in EGFR gene, have been shown to regulate not total GLUT1 expression but its localization to the plasma membrane in a PI3K/AKT-dependent manner [17]. Alternatively, glucose can enter into the cell through other than GLUT-dependent mechanisms, as shown for SGLT1 in EGFR mutant lung cancers [24] or SGLT2 in premalignant and early well differentiated KRAS LUADs. This increased glucose transport may be an early requirement to sustain the increased proliferation associated with progression from premalignant to invasive NSCLC [25]. Importantly, these distinct metabolic phenotypes during KRAS-dependent NSCLC progression have different prognosis and therefore, they offer different therapeutic susceptibilities.

Hexokinase 2 (HK2) catalyzes the phosphorylation of glucose to glucose-6-P creating a concentration gradient that facilitates glucose entry into the cancer cells (Fig. 1). Expression and activity of this enzyme was induced by KRAS-mutation [26] and EML4-ALK rearrangement [27] in NSCLC models. In particular, inhibition of HK2 impaired KRAS-mut lung cancer development in murine models and reduced lung cancer cell growth *in vitro* and *in vivo* by inhibiting nucleotide synthesis and the TCA cycle [26]. EML4-ALK rearrangement, another frequent oncogenic rearrangement in NSCLC, induced hypoxia-independent but glucose-dependent HIF1-α expression to drive HK2 expression and enhance glucose metabolism [27].

Several inhibitors of glucose transport and drugs targeting enzymes of the early phases of glycolysis like HK2 have been tested for decades against a variety of tumors (Fig. 2) [14]. In cells in culture and in mouse cancer models, 2-deoxyglucose and other inhibitors of glycolysis have shown effectiveness against LUAC and LUSC [28]. However, these inhibitors are still not used in the clinic, as results from clinical trials have been disappointing. It is possible that this is due to cells adapting their metabolism rather than undergoing cell death, or that the therapeutic window is small because other organs, including the immune system, require glucose. However, multiple studies indicate that glucose reduction confers sensitivity to chemotherapy, radiotherapy and targeted therapies. This, combined with the clinical data on GLUT1 as a prognosis marker suggests that it is worth testing combinations of therapies that include GLUT1 and hexokinase 2 inhibitors.

4. Mitochondrial metabolism in lung cancer patients and animal models: *glucose as fuel*

Glucose can be metabolized *via* glycolysis to pyruvate and, in the presence of oxygen, pyruvate is metabolized to CO_2 in the mitochondria through the Krebs/tricarboxylic acid (TCA) cycle and OXPHOS to generate large amounts of ATP [29]. In the absence of oxygen, pyruvate can be metabolized to lactate (anaerobic glycolysis), a biochemical reaction far less efficient than the TCA cycle and OXPHOS in generating ATP. However, rapidly proliferating cells (including cancer cells) can metabolize glucose to lactate in spite of the presence of oxygen, a process widely known as Warburg effect (or aerobic glycolysis), to quickly obtain energy and glucose-derived metabolites for macromolecular synthesis [29].

NSCLC, like many other cancer types, requires a high intake of glucose, and positron emission tomography (PET) imaging based on glucose-based tracer (F-FDG) became a standard diagnostic tool to assess tumor extension. However, whether glucose is utilized anaerobically to generate lactate in a classical "Warburg" manner, is still unclear in this type of cancer. The fate of glucose may depend on the mutational signature and even the intratumor location and perfusion of cells.

Metabolic flux analyses showed that $KRAS^{G12D}$ NSCLC cells cultured *in vitro* produce lactate from glucose [30]. While these cells still used the mitochondria, the majority of the TCA cycle intermediates came from glutamine metabolism. In contrast, *in vivo* metabolic flux analyses of lung cancer models demonstrated that concomitantly to the production of lactate (which was higher than in normal lungs due to higher glucose uptake in tumors), it's primarily glucose rather than glutamine that accounts for the fluxed carbon source into the TCA cycle [30]. By labeling glucose or pyruvate, Davidson et al. confirmed that glucose-derived metabolites enter the TCA cycle either through Pyruvate Dehydrogenase (PDH) or Pyruvate Carboxylase (PC) (Fig.1), which are required for glucose oxidation into the TCA cycle by converting pyruvate into acetyl-CoA and oxaloacetate, respectively [30]. In support of that, tumor development was impaired in genetically engineered lung cancer models by blocking the mitochondrial metabolism through the inhibition of PDH and PC. NSCLC cell lines, however, did not show reduced proliferation when these enzymes where knocked-out *in vitro*, underscoring the relevance of environmental impact on glucose utilization [30]. Altogether, these data suggest that tissue environment determines the fate of glucose in KRAS-driven lung cancer and point out that mitochondrial oxidative metabolism of glucose occurs *in vivo* and is necessary for tumor formation.

Recently, in a landmark study, Hensley et al. analyzed the fate of glucose in patients infused with radioactive glucose and found that NSCLC patients present inter- and intra-tumoral heterogeneity in terms of glucose oxidation, although they could not find associations between higher/lower glucose metabolism and specific mutations [31]. Overall, high glucose uptake by FDG-PET imaging was found in all tumors, and authors found higher production of lactate and TCA intermediates in tumors than in the normal lungs. They demonstrated that part of the produced acetyl-CoA could enter into the TCA cycle mainly *via* PDH and PC (at lesser extent), but acetyl-CoA could also be derived from lactate as demonstrated in mouse tumors [31]. In particular, by using DCE-MRI as a differential contrast technique, they found intra-tumor heterogeneity in terms of tumor perfusion. Then, they performed metabolite extraction and enrichment analysis to determine glucose metabolism in different areas. Low-perfused regions of the tumor presented higher rates of glycolysis coupled with increased mitochondrial metabolism of glucose through the TCA cycle and oxidative phosphorylation (OXPHOS), while well-perfused regions behave more like non-tumoral lungs and do not show such glucose oxidative metabolism. Well-perfused regions present lower PDH activity and lower citrate and glutamate production from glucose than the low-perfused regions [31]. In addition, RNA-seq data from these differentially perfused regions suggested that other nutrients could be fueling the TCA cycle of well-perfused areas, as genes related to lysosomes and amino acid metabolism appeared to be significantly deregulated [31]. Therefore, they postulated other possible fuels such as fatty acids, amino acids or lactate as alternative oxidized nutrients in well-perfused tumoral areas [31]. The authors suggested that since glucose is more efficient than other nutrients in terms of energy produced by oxygen consumed, and since glucose usually diffuses better in low-perfused regions, it might be the nutrient preferentially used in low-oxygen areas of these heterogenic tumors.

5. Mitochondrial metabolism in lung cancer patients and animal models: lactate as a carbon source

Lactate has been recently shown to be a more common nutrient in tissues than previously thought [32]. Hui S. et al, measured the fluxes of circulating metabolites in mice using intravenous infusions of ^{13}C-

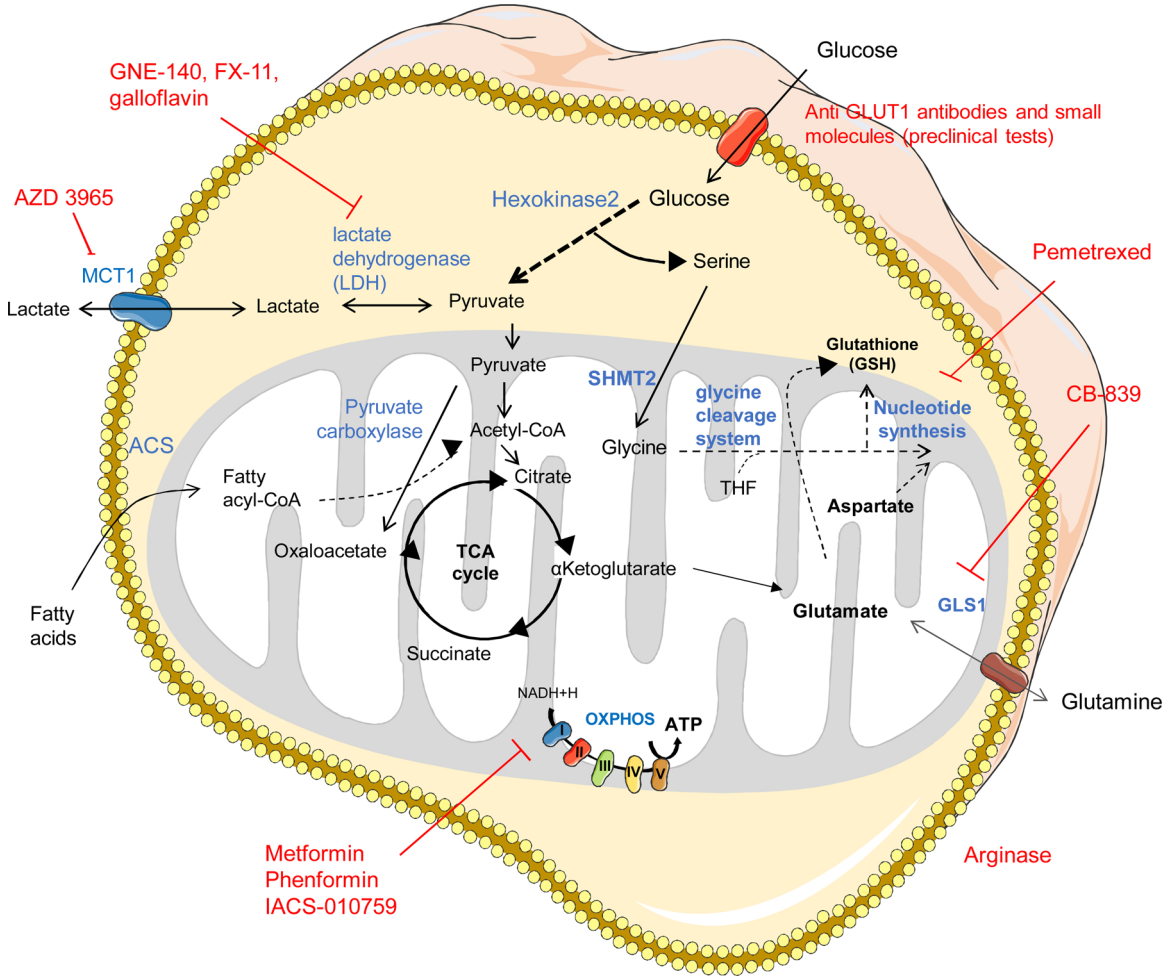

Fig. 2. Metabolic targets in NSCLC.
Potential target enzymes are shown in blue, and inhibitors in red. Inhibitors that have been tested in preclinical or clinical settings are included.

labelled nutrients and found that circulating lactate had higher turnover flux (consumption *vs*. excretion) than glucose, acetate, alanine, pyruvate, glycerol and glutamine [32]. They found that lactate is the primary carbon source for the TCA cycle in all tissues except in the brain. The authors also analyzed the contribution of glucose to the TCA cycle in genetically engineered KRAS-lung tumor mice and found that it occurs mostly indirectly, through circulating lactate, as infused ^{13}C-lactate extensively labels TCA cycle intermediates in these tumors [32]. They postulated that the high flux of lactate reflects a high fraction of pyruvate being excreted as lactate from some cells undergoing anaerobic glycolysis and a high fraction of tissue pyruvate being derived from circulating lactate. The activity of Lactate Dehydrogenase (LDH) A/B and lactate transporters monocarboxylate transporter-1/4 (MCT1/4) was required for the shuttling of lactate between tissues (Fig. 1). This shuttling may underlie the high flux of circulating lactate, which would be metabolized in the mitochondria of a different location (cell, tumor area, organ) [32]. In support of that, infusions of glucose and lactate in NSCLC human patients and orthotopic mice models revealed concomitant metabolism of both nutrients, with extensive labeling of TCA cycle metabolites from lactate, suggesting that glycolysis and the TCA are uncoupled [33]. In addition, it was found that deletion of MCT1 from tumor cells eliminated lactate-dependent metabolite labeling *in vivo* in NSCLC mice models [33]. In principle, the use of LDH to produce lactate from glucose/pyruvate and its use to produce pyruvate from circulating lactate seem contradictory. However, this still could be in line with the findings that the contribution of glucose to the TCA cycle is mostly through circulating lactate in lung cancer, with some cells producing lactate from glucose and other cells using the lactate for the TCA cycle [32] (Fig. 1). It should be noted that some controversy exists as to whether these labeling experiments would accurately reveal net contributions of each nutrient. Because lactate and pyruvate can be interconverted by LDH, a phenomenon named “isotope exchange” may occur, leading to overestimation of the net contribution of lactate to the TCA [34].

Regardless of whether lactate transporters contribute mainly to lung cancer by importing or exporting lactate, numerous data suggest that they could be a good therapeutic target against NSCLC and other tumors. AZD3965 was designed to selectively inhibit Monocarboxylate transporter-1 (MCT1) which extrudes lactate, and it has entered phase I clinical trials (Fig. 2). In a similar manner, LDH could contribute to cancer by promoting lactate catabolism or by supporting anaerobic glycolysis by generating lactate, a reaction favored by the LDH subunit isoform LDH-A (Fig. 2). Genetic deletion of LDH-A has been shown to induce tumor regression of established tumors and to impair tumor development of KRAS- and EGFR-driven lung cancer models in an allele-dependent manner [35]. In fact, this allele-dependent reduced tumor growth was accompanied by a decrease in lactate production and increased NADH and pyruvate accumulation [35]. This suggests that fermentative glycolysis is a key bioenergetic pathway in lung cancer development and progression. Importantly, the authors showed that LDH-A attenuation led to reduced glycolytic flux *in vitro*, *in vivo*, and *ex vivo*, and they only observed reactivation of the mitochondrial metabolism (labeled citrate and aspartate from glucose) *in vitro* [35]. This suggests that LDH-A inhibition could be useful for NSCLC patients and

highlights the role of the tumor microenvironment in metabolic reprogramming.

It is worth mentioning that, in human NSCLC patients, elevated mitochondrial oxidation of lactate at the time of diagnosis in stage I-II patients was associated with higher rates of recurrence and metastasis [33]. Furthermore, EGFR-mutant NSCLC tumors present lower lactate consumption and more homogeneous patterns of lactate oxidation within the tumor than the KRAS-mutant ones, which presented higher intra-tumor heterogeneity for lactate oxidation [33]. In other situations, lactate has been shown to serve as a gluconeogenic source for KRAS-mut lung cancer cells grown with low glucose *in vitro*. These cells generated glucose *via* gluconeogenesis through the mitochondrial enzyme PCK2 [36]. Also, in patient-derived xenografts (PDX) models of NSCLC fed with a labeled glucose-liquid diet, gluconeogenesis was increased in PDX tissues when compared with tumor slices infused *ex vivo,* as evidenced by a higher enrichment of labeled glucose-6 P and fructose-6P [37]. Together, glucose and lactate metabolism seem to be coupled and uncoupled depending on the anatomical site and driver mutation, following spatio-temporal dynamics regarding the intra-tumor oxygen availability and potentially involving the contribution of other organs (*i.e.* systemic metabolism) for cell growth and survival.

6. Glutamine metabolism

In some cancer cells, glutamine is used as a source of nitrogen for amino acid and nucleic acid synthesis, and it can be essential to replenish the TCA cycle. For this reason, several strategies to target glutamine metabolism have been proposed, including the use of inhibitors of glutamine uptake and catabolism and circulating glutamine-depleting enzymes. Many reports point towards the essentiality of glutamine for NSCLC lines *in vitro* [30,38,39]. Several oncogenes, including KRAS, could drive glutamine metabolism in these cells. Specifically, it was shown that glutamine metabolism is enhanced in KRAS-mut lung cancer cells *in vitro* either through PI3K/AKT [38] or ATF4 [39] dependent signaling.

However, the essential nature of glutamine for the replenishment of the TCA cycle in NSCLC was not observed *in vivo*: glutamine flux analyses of KRAS-driven lung tumors in mice revealed only minimal labeling of glutamate and TCA intermediates [30]. Additionally, neither genetic deletion nor pharmacological inhibition of the mitochondrial enzyme glutaminase (GLS1) affected the growth of $KRAS^{G12D}$-induced lung tumors [30]. Moreover, in early stage NSCLC, no differences in GLS1 expression were found between human tumors and their normal counterparts, while pyruvate carboxylase was way higher in tumors than in nonmalignant lung samples [40]. This suggests that mitochondrial input into the TCA cycle (anaplerosis) occur mainly through glucose- instead of glutamine-derived metabolites. These results were supported by the low glutamine and high glucose utilization observed in lung tumor sections freshly "infused" *ex vivo* with labeled nutrients. In cancerous and non-cancerous tissues, GLS1 was found active (as tissue slices produced labeled glutamate) but no enrichment in labeled glutamine-derived metabolites was found in the TCA cycle [40]. In this model, suppression of pyruvate carboxylase reduced cell proliferation *in vitro* and *in vivo*, and this was accompanied by a decrease in anaplerotic input into the TCA cycle and reduced nucleotide and lipid biosynthesis. This could not be compensated by the abundant supply of glutamine [40]. One of the reasons that could explain the use of glutamine by lung cancer cells *in vitro* is the presence of cystine, which favors the use of glutamine by these cells for TCA anaplerosis [41]. On the other hand, these studies and a subsequent thorough examination of pyruvate carboxylase dependency on cell lines suggest that pyruvate carboxylase would be an interesting druggable target for a subset of NSCLC [42].

Certain mutations caused more dependence on glutamine metabolism than KRAS, such as those occurring in the KEAP1/NRF2 pathway, which have been identified in 23% of LUAC [18] and 31% of LUSC [43]. Loss of Keap1 caused the stabilization, nuclear localization and hyperactivation of NRF2 (a master transcriptional regulator of the oxidative stress response), and this was shown to accelerate KRAS-mut lung cancer progression *in vivo* [44]. Authors defined two genetic signatures related to "KEAP1 mutation" and to "NRF2 core target genes", and found that both were upregulated in stage IV lung tumors and associated with worse overall survival. Importantly, the authors showed that Keap1-mutant cancer cells presented a high dependency on the glutamine transporter *SLC1A5*, with increased glutamine consumption, and have subsequent higher sensitivity to glutaminase inhibition than KRAS-mut/$p53^{-/-}$ lung cancer cells *in vitro* and *in vivo*. Thus, the presence of Keap1 or Nrf2 mutation may serve to therapeutically stratify patients for pharmacological inhibition of glutaminase. This was further supported by the fact that human patients with KRAS-mut lung cancer harboring KEAP1 loss-of-function mutations or NRF2 gain-of-function mutations respond better to glutaminase inhibition [44]. Also, mutations in the EGFR gene (15%) [18] have been found to be involved in reprogramming glutamine amino acid metabolism. Conversely to what has been described for KRAS-mut lung cancer mice models *in vivo* [30], glutamine metabolism is increased in EGFR-mut lung cancers and inhibition of GLS in combination with the EGFR inhibitor erlotinib acted synergistically *in vitro* and *in vivo* inducing tumor regression [45]. In fact, the authors proved that the dual treatment reduced glucose and glutamine uptake and correlated with better response to treatment *in vivo*, by inducing energetic stress (decreased glutathione and ATP production) accompanied by an increased in the phosphorylated-AMPK and reduced MYC levels in treated lung tumor xenografts, finally resulting in cell death [45].

In summary, glutaminase inhibitors may prove effective for subtypes of NSCLC but their effect may likely be dependent on the driver mutations. The GLS1 inhibitor CB-839 is currently being tested in clinical trials for advanced NSCLC in combination with immunotherapy (NCT02771626) and will soon be tested in combination with the EGFR inhibitor osimertinib (NCT03831932).

7. Metabolism and essential role of other amino acids

Serine, a non-essential amino acid, is the major carbon source for one-carbon metabolism and it can be synthetized *de novo* from the glycolytic intermediate 3-phosphoglycerate (3-PG) through the serine synthesis pathway (SSP, Fig. 1) [46]. Serine is catabolized by the mitochondrial enzyme SHMT2 to glycine, which is then catabolized by the mitochondrial enzymatic complex GCS (glycine cleavage system; from which glycine decarboxylase is the core enzymatic component) to yield a one-carbon unit that is accepted by tetrahydrofolate (THF). Then, by the action of the mitochondrial enzymes MTHFD1L/2/2 L, the modified THF will donate its one-carbon unit for purine synthesis. Specifically in NSCLC, it was shown that cells with high NRF2 activity displayed elevated levels of serine consumption compared to cells with low NRF2 activity [47]. NRF2 regulated the serine/glycine metabolism by inducing the expression of SHMT2 (among other serine/glycine metabolic enzymes) *via* ATF4 to support glutathione and nucleotide production. In addition, the authors showed that elevated expression of a gene signature that includes enzymes of the serine synthesis pathway and SHMT2 was associated with poor prognosis in human NSCLC [47]. Similarly, it was demonstrated that glycine decarboxylase expression correlated with low survival in lung cancer patients and it drove cellular transformation and NSCLC tumorigenesis through its metabolic activity, specifically by modulating glucose metabolism and pyrimidine synthesis [48]. In particular, glycine decarboxylase was found elevated in lung tumor-initiating cells accompanied by high levels of the oncogenic stem cell factor LIN28B, among other serine/glycine metabolic enzymes [48]. Together, these results suggested that inhibition of the serine/glycine mitochondrial metabolism may open new avenues for lung cancer targeted therapies, specifically for the KRAS-Keap1/NRF2 oncotypes.

Other amino acids have been recently described to be relevant for the growth of KRAS-mut lung cancer. It was shown that KRAS-driven NSCLC can incorporate free branched-chain amino acids (BCAAs), such as leucine and valine, and use them as a nitrogen source for protein and nucleotide biosynthesis [49]. Levels of the mitochondrial enzyme Bcat2 were found increased in lung tumors compared to normal lung and, importantly, inhibition of BCAA catabolism blocked tumor growth *in vivo* [49]. Although the authors showed that their metabolism did not contribute to anaplerosis of the mitochondrial TCA cycle, they showed that BCAA can be used for aspartate synthesis in the mitochondria. This occurs *via* the transfer of nitrogen from the TCA cycle intermediate α-ketoglutarate to form glutamate by Bcat2, which then is transferred to oxaloacetate to form aspartate (see Fig.1), therefore contributing to the nitrogen pool for nucleotide biosynthesis. Accordingly, levels of BCAA in plasma where found to be lower in KRAS-mut lung cancer patients compared to KRAS-mut pancreatic cancer patients [49], altogether indicating that cancer metabolism is not only oncotype-dependent but also environmentally driven (*i.e.* local nutrient availability, tissue of origin and architecture, systemic metabolism, *etc.*).

Arginine auxotrophy, frequent in many tumors, has not been thoroughly described in NSCLC. However, a recent phase I clinical trial suggested that patients with argininosuccinate synthetase 1 (ASS1)–negative tumors could benefit from arginine depletion therapy through the use of ADI-PEG in combination with chemotherapy [50]. This finding should be confirmed in randomized trials and may not be applicable to the majority of NSCLC patients, and actually arginine depletion could potentially have the opposite effect. Arginine elimination from the microenvironment is a physiological immunosuppressive mechanism: myeloid-derived suppressor cells (MDSC) produce and secrete arginase. Along these lines, a role for arginine depletion in lung cancer was shown in a KRASG12D LUAD model, in which inhibition of arginase 1/2 promoted anti-tumor immunity and tumor clearance. Consistently, the concentration of arginine in the intracellular fluid of a lung cancer (KP) model has been shown to be much lower than plasma levels [51].

8. Mitochondrial lipid metabolism

Lipids are generated from glycolytic- and mitochondrial-derived metabolites, they can be used to produce ATP and they are essential for the synthesis of cellular membranes, vitamins, hormones, and other metabolites for intracellular signaling [52]. Some tumors require fatty acid synthesis, but NSCLC appears to be reliant on fatty acid catabolism. Fatty acid oxidation is a mitochondrial pathway that provides ATP and may offer therapeutic opportunities for KRAS-mut driven NSCLC. Acyl-CoA synthetase (ACS) is a mitochondrial outer membrane enzyme that catalyzes in the cytosol the first reaction before fatty acid oxidation starts in the mitochondrial matrix. This enzyme is upregulated by KRAS mutation and is highly expressed in human KRAS-mut NSCLC tissues [53]. In particular, the isoform ACSL3 mediates survival and tumorigenesis of KRAS-mut lung cancer cells by promoting uptake, retention, and β-oxidation of fatty acids [53]. Also, specific signaling lipids (*i.e.* phosphatidylinositol and arachidonic-containing phospholipids) were identified in KRAS-mut/high-MYC tumors but not in normal lungs; and were associated to MYC signaling through the increased activity of cPLA2 and COX-2 found in this model [54]. In addition, *in vivo* pharmacological inhibition of COX/5-LOX pathways resulted in reduced tumor burden of KRAS-mut/high-MYC tumors [54], suggesting that catabolism of certain types of lipids could be involved in KRAS-mut lung cancer progression and therefore serve as a potential target.

Strikingly, in a KRAS-mut lung cancer model, it has been recently suggested that the tumor can rewire systemic metabolism, which contributes to cancer progression [55]. Authors found that the tumor-secreted “waste” caused metabolic rewiring of the liver, including a pro-inflammatory response and altered glucose and lipid metabolism.

9. Autophagy in NSCLC

Autophagy is a self-eating process that regulates normal cell homeostasis in response to stress, but at the same time is required for tumor maintenance since some metabolism by-products can potentially serve as metabolic fuels for cancer cell proliferation and energy production [56]. In this sense, extensive work has evidenced that autophagy is increased in KRAS-lung tumors and that it acts to maintain mitochondrial function and lipid catabolism [57], while its genetic abrogation caused tumor regression to oncocytomas, a benign lesion characterized by defective mitochondria and increased lipid storages [57,58]. In addition, other functions have been attributed to KRAS-dependent autophagy in lung cancer, such as the replenishment of the TCA cycle for nucleotide synthesis and the regulation of the cellular energy charge and redox homeostasis [59]. Autophagy inhibitors could be useful in combination with other therapies. For instance, KRAS/p53 deficient mouse models of lung cancer have been shown to be dependent on signaling by insulin receptor substrates 1/2 [60], and ablation of these proteins or inhibition of Insulin Receptor/IGF Receptor signaling led to a reduction of intracellular amino acids and sensitivity to autophagy inhibition. This, therefore, points to a potential two-pronged approach targeting these receptors and autophagy in KRAS-driven NSCLC. The link between autophagy, metabolism and cancer, however, goes beyond intracellular maintenance of nutrient pools. Autophagy is required to maintain glucose homeostasis, since its whole-body deletion impaired long-term survival of adult mice, which die of hypoglycemia when fasted, and drove many other systemic metabolic alterations [58]. Whole-body autophagy is also beneficial for lung cancer, as systemic deletion of autophagy also reduces tumorogenesis of an implanted KRAS/p53 NSCLC cell line [61]. This has recently been attributed to an unexpected effect of ATG7 deletion in the liver: the subsequent liver damage promotes the release of arginase I, an arginine-degrading enzyme. The reduction of circulating arginine prevented the growth of several tumors that are auxotroph for arginine, as is the case for the lung cancer cell line employed in this study [61].

10. OXPHOS as a target: metformin and phenformin

Mitochondrial drugs used to treat diabetes have long been associated with better prognosis of NSCLC. Metformin and phenformin effects have been attributed to inhibition of Complex I of the respiratory chain. Their effects on lung cancer are likely pleiotropic and could involve systemic effects as well as direct targeting of the tumor. *In vitro*, numerous studies indicate that metformin is toxic to NSCLC cell lines, although the doses used are much higher than those employed to treat diabetic patients. On the other hand, metformin acts on the liver and possibly on other organs to reduce systemic glucose levels by activating AMPK. Several epidemiologic studies assessed the impact of anti-diabetic therapy on cancer incidence and mortality and found an association among metformin and decreased cancer mortality [62]. Based on data from the Surveillance, Epidemiology and End Results registry linked to Medicare claims, diabetic patients with advanced NSCLC who were being treated with metformin at the time of lung cancer diagnosis achieved longer overall survival than those who were not treated with metformin [63]. In contrast, patients treated with insulin had higher mortality rates [62]. Accordingly, diabetic patients with locally advanced NSCLC treated with concurrent chemoradiotherapy who were receiving metformin had better clinical outcome than those being treated with insulin [64]. These data are not in accordance with the recent NRG-LU001 clinical trial, as the addition of metformin to concurrent chemoradiation did not improve clinical outcome in non-diabetic patients with unresectable stage III NSCLC [65]. Given the heterogenicity of NSCLC, we suggest that patients are selected based on their tumor dependency on OXPHOS *versus* glycolysis, which can be predicted, at least in Diffuse large B cell lymphoma, by the level of GAPDH expression [66].

Phenformin is another drug structurally similar to metformin that was widely used as an anti-diabetic agent but has been more recently discontinued due to unacceptable toxicity when used at effective doses in humans. In the clinic, links with patient prognosis are therefore less proven compared to metformin. Phenformin has been shown to be highly effective against LKB1 deficient tumors in animal models, which suggests that patient stratification according to driver mutations could be highly relevant when testing metabolic therapies in patients [67].

Other drugs targeting different complexes of the respiratory chain are being developed and tested in clinical trials against cancer. Among these, IACS-010759 has shown efficacy in mouse models with mutations in KRAS, p53 and the SWI/SNF chromatin remodeling complex [68].

11. Concluding remarks

Anti-metabolite drugs have been used to treat NSCLC for decades; amongst them, pemetrexed, an antifolate, is currently included in standard chemotherapy regimes in combination with cisplatin or carboplatin for non-squamous NSCLC. In the last two decades, the incorporation of novel techniques such as metabolic flux analyses, and the study of metabolic dependencies in murine models were crucial to unveil the potential of new drugs targeting other metabolic pathways beyond nucleotide synthesis, such as glycolysis, glutaminolysis or lactate transport and LDH. One of the limitations of this type of studies is that NSCLC is a highly heterogeneous disease, as has already been shown in metabolic flux data obtained from patients. Also, as the genomic context is highly relevant for tumor metabolism, larger studies assessing metabolic fluxes are needed to be able to stratify patients by driver mutations that confer distinct metabolic vulnerabilities. In this sense, extensive characterization of metabolic fluxes in multiple cell lines in culture have proven to be useful to predict sensitivity to pemetrexed and dependence on specific metabolic enzymes and nutrients [42]. Together, these studies will help to clinically define new realistic therapeutic avenues, specifically for patients harboring non-actionable mutation profiles or relapsed patients that become resistant to the current targeted therapies. Metabolic inhibitors could also be helpful in combination with tyrosine kinases inhibitors which may rewire metabolism. For instance, EGFR inhibitors promote a switch to oxidative phosphorylation (OXPHOS) that may be exploited to improve their effects [69]. Along these lines, targeting OXPHOS in combination with EGFR tyrosine kinase inhibitors (TKI) has been evaluated in a randomized phase 2 clinical trial (NCT03071705) that compared metformin plus EGFR TKI with EGFR TKI alone in patients harboring EGFR mutations [70]. Patients treated with this combination have significant benefit in terms of progression-free survival and overall survival compared with those treated with TKI alone [70]. However, another clinical trial (NCT01864681) did not show benefit when adding metformin to gefitinib compared to gefitinib alone in non-diabetic NSCLC patients harboring EGFR mutations [71].

Lastly, diet should be taken into consideration as an extra source of heterogeneity when studying metabolic parameters in patients, as diet composition influences strongly the circulating concentrations of glucose and amino acids [51]. We also consider that deeper understanding of the interplay between tumor and systemic metabolism will be translated into novel metabolic strategies for therapy, which still need to be prospectively studied with chemotherapy regimens and even in combination with immunotherapy or targeted therapy. We foresee a future in which modulation of systemic metabolism can be incorporated to the conventional treatment of lung cancer patients, where metabolic parameters might become as important as genetic mutations in the treatment of NSCLC.

Funding

CMP's lab is funded by the Ministry of Economy and Competitiveness-MINECO, Spain (cofunded by FEDER funds/European Regional Development Fund – a way to build Europe), [grant number BFU2016-78154-R] and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 766214 (META-CAN). EN has received support from the Department of Health of the Generalitat de Catalunya by the call "Acció instrumental d'intensificació de professionals de la salut" [grant number SLT006/17/00127], the PROYBAR17005NADA project funded by the AECC Barcelona (Spanish Association Against Cancer Barcelona), and the Instituto de Salud Carlos III [PI14/01109] co-funded by FEDER funds-a way to build Europe. We thank CERCA Program / Generalitat de Catalunya for their institutional support and grants 2017SGR332 and 2017SGR448.

Acknowledgements

The authors wish to thank D. Santamaría, A. Muir, M. Vander-Heiden, C. Frezza, F. Favaro, F. Püschel and M. Hernandez for input.

We apologize to innumerable authors whose work could not be included here due to space constraints.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin. 68 (2018) 7–30, https://doi.org/10.3322/caac.21442.
[2] D. Planchard, S. Popat, K. Kerr, S. Novello, E.F. Smit, C. Faivre-Finn, T.S. Mok, M. Reck, P.E. Van Schil, M.D. Hellmann, S. Peters, Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 29 (2018) iv192–iv237, https://doi.org/10.1093/annonc/mdy275.
[3] E. Nadal, B. Massuti, M. Dómine, R. García-Campelo, M. Cobo, E. Felip, Immunotherapy with checkpoint inhibitors in non-small cell lung cancer: insights from long-term survivors, Cancer Immunol. Immunother. 68 (2019) 341–352, https://doi.org/10.1007/s00262-019-02310-2.
[4] D. Hanahan, R.A. Weinberg, Hallmarks of Cancer: the next generation, Cell. 144 (2011) 646–674, https://doi.org/10.1016/j.cell.2011.02.013.
[5] T.E.R.F. TERF Collaboration, Diabetes Mellitus, Fasting Glucose, and Risk of Cause-Specific Death, N. Engl. J. Med. 364 (2011) 829–841, https://doi.org/10.1056/NEJMoa1008862.
[6] J. Luo, M. Hendryx, L. Qi, G.Y. Ho, K.L. Margolis, Pre-existing diabetes and lung cancer prognosis, Br. J. Cancer 115 (2016) 76–79, https://doi.org/10.1038/bjc.2016.141.
[7] M. Bergamino, A.J. Rullan, M. Saigí, I. Peiró, E. Montanya, R. Palmero, J.C. Ruffinelli, A. Navarro, M.D. Arnaiz, I. Brao, S. Aso, S. Padrones, F. Cardenal, E. Nadal, Fasting plasma glucose is an independent predictor of survival in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy, BMC Cancer 19 (2019) 165, https://doi.org/10.1186/s12885-019-5370-5.
[8] L. Zhu, H. Cao, T. Zhang, H. Shen, W. Dong, L. Wang, J. Du, The effect of diabetes mellitus on lung Cancer prognosis: a PRISMA-compliant meta-analysis of cohort studies, Med. (United States). 95 (2016) e3528, https://doi.org/10.1097/MD.0000000000003528.
[9] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029–1033 https://doi.org/10.1126/science.1160809.
[10] O.C.J. Schuurbiers, T.W.H. Meijer, J.H.A. Kaanders, M.G. Looijen-Salamon, L.-F. de Geus-Oei, M.A. van der Drift, E.H.F. van der Heijden, W.J. Oyen, E.P. Visser, P.N. Span, J. Bussink, Glucose metabolism in NSCLC is histology-specific and diverges the prognostic potential of 18FDG-PET for adenocarcinoma and squamous cell carcinoma, J. Thorac. Oncol. 9 (2014) 1485–1493, https://doi.org/10.1097/JTO.0000000000000286.
[11] Y.W. Koh, S.J. Lee, S.Y. Park, Differential expression and prognostic significance of GLUT1 according to histologic type of non-small-cell lung cancer and its association with volume-dependent parameters, Lung Cancer. 104 (2017) 31–37, https://doi.org/10.1016/j.lungcan.2016.12.003.
[12] A. Cruz-Bermúdez, R.J. Vicente-Blanco, R. Laza-Briviesca, A. García-Grande, S. Laine-Menéndez, L. Gutiérrez, V. Calvo, A. Romero, P. Martín-Acosta, J.M. García, M. Provencio, PGC-1alpha levels correlate with survival in patients with stage III NSCLC and may define a new biomarker to metabolism-targeted therapy, Sci. Rep. 7 (2017) 16661, https://doi.org/10.1038/s41598-017-17009-6.
[13] E. Martínez-Terroba, C. Behrens, F.J. de Miguel, J. Agorreta, E. Monsó, L. Millares, C. Sainz, M. Mesa-Guzman, J.L. Pérez-Gracia, M.D. Lozano, J.J. Zulueta, R. Pio, I.I. Wistuba, L.M. Montuenga, M.J. Pajares, A novel protein-based prognostic signature improves risk stratification to guide clinical management in early-stage lung adenocarcinoma patients, J. Pathol. 245 (2018) 421–432, https://doi.org/10.1002/path.5096.
[14] N. El Mjiyad, A. Caro-Maldonado, S. Ramirez-Peinado, C. Munoz-Pinedo, Sugar-free approaches to cancer cell killing, Oncogene 30 (2011) 253–264 https://doi.org/10.1038/onc.2010.466.
[15] M.C. Gerards, D.L. van der Velden, J.W. Baars, D.P.M. Brandjes, J.B.L. Hoekstra,

T.M. Vriesendorp, V.E.A. Gerdes, Impact of hyperglycemia on the efficacy of chemotherapy—a systematic review of preclinical studies, Crit. Rev. Oncol. Hematol. 113 (2017) 235–241, https://doi.org/10.1016/j.critrevonc.2017.03.007.
[16] D.B. Doroshow, R.S. Herbst, Treatment of advanced non–Small cell lung Cancer in 2018, JAMA Oncol. 4 (2018) 569, https://doi.org/10.1001/jamaoncol.2017.5190.
[17] H. Makinoshima, M. Takita, K. Saruwatari, S. Umemura, Y. Obata, G. Ishii, S. Matsumoto, E. Sugiyama, A. Ochiai, R. Abe, K. Goto, H. Esumi, K. Tsuchihara, Signaling through the phosphatidylinositol 3-Kinase (PI3K)/Mammalian target of rapamycin (mTOR) Axis Is responsible for aerobic glycolysis mediated by glucose transporter in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma, J. Biol. Chem. 290 (2015) 17495–17504, https://doi.org/10.1074/jbc.M115.660498.
[18] R.S. Herbst, D. Morgensztern, C. Boshoff, The biology and management of non-small cell lung cancer, Nature 553 (2018) 446, https://doi.org/10.1038/nature25183 2018 5537689.
[19] Q. Fan, X. Hu, H. Zhang, S. Wang, H. Zhang, C. You, C.-Y. Zhang, H. Liang, X. Chen, Y. Ba, MiR-193a-3p is an important tumour suppressor in lung Cancer and directly targets KRAS, Cell. Physiol. Biochem. 44 (2017) 1311–1324, https://doi.org/10.1159/000485491.
[20] E.M. Kerr, C.P. Martins, Metabolic rewiring in mutant Kras lung cancer, FEBS J. 285 (2018) 28–41, https://doi.org/10.1111/febs.14125.
[21] J. Carretero, P.P. Medina, R. Blanco, L. Smit, M. Tang, G. Roncador, L. Maestre, E. Conde, F. Lopez-Rios, H.C. Clevers, M. Sanchez-Cespedes, Dysfunctional AMPK activity, signalling through mTOR and survival in response to energetic stress in LKB1-deficient lung cancer, Oncogene 26 (2007) 1616–1625, https://doi.org/10.1038/sj.onc.1209951.
[22] E.M. Kerr, E. Gaude, F.K. Turrell, C. Frezza, C.P. Martins, Mutant Kras copy number defines metabolic reprogramming and therapeutic susceptibilities, Nature 531 (2016) 110–113, https://doi.org/10.1038/nature16967.
[23] L.J. Eichner, S.N. Brun, S. Herzig, N.P. Young, S.D. Curtis, D.B. Shackelford, M.N. Shokhirev, M. Leblanc, L.I. Vera, A. Hutchins, D.S. Ross, R.J. Shaw, R.U. Svensson, Genetic analysis reveals AMPK is required to support tumor growth in murine kras-dependent lung Cancer models, Cell Metab. 29 (2019) 285–302, https://doi.org/10.1016/j.cmet.2018.10.005 e7.
[24] Z. Weihua, R. Tsan, W.-C. Huang, Q. Wu, C.-H. Chiu, I.J. Fidler, M.-C. Hung, Survival of Cancer cells is maintained by EGFR independent of its kinase activity, Cancer Cell 13 (2008) 385–393, https://doi.org/10.1016/j.ccr.2008.03.015.
[25] C.R. Scafoglio, B. Villegas, G. Abdelhady, S.T. Bailey, J. Liu, A.S. Shirali, W.D. Wallace, C.E. Magyar, T.R. Grogan, D. Elashoff, T. Walser, J. Yanagawa, D.R. Aberle, J.R. Barrio, S.M. Dubinett, D.B. Shackelford, Sodium-glucose transporter 2 is a diagnostic and therapeutic target for early-stage lung adenocarcinoma, Sci. Transl. Med. 10 (2018), https://doi.org/10.1126/scitranslmed.aat5933 eaat5933.
[26] K.C. Patra, Q. Wang, P.T. Bhaskar, L. Miller, Z. Wang, W. Wheaton, N. Chandel, M. Laakso, W.J. Muller, E.L. Allen, A.K. Jha, G.A. Smolen, M.F. Clasquin, R.B. Robey, N. Hay, Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of Cancer, Cancer Cell 24 (2013) 213–228, https://doi.org/10.1016/j.ccr.2013.06.014.
[27] Y. Ma, C. Yu, E.M. Mohamed, H. Shao, L. Wang, G. Sundaresan, J. Zweit, M. Idowu, X. Fang, A causal link from ALK to hexokinase II overexpression and hyperactive glycolysis in EML4-ALK-positive lung cancer, Oncogene. 35 (2016) 6132–6142, https://doi.org/10.1038/onc.2016.150.
[28] J. Goodwin, M.L. Neugent, S.Y. Lee, J.H. Choe, H. Choi, D.M.R.J. Enkins, R.J. Ruthenborg, M.W. Robinson, J.Y. Jeong, M. Wake, H. Abe, N. Takeda, H. Endo, M. Inoue, Z. Xuan, H. Yoo, M. Chen, J.M. Ahn, J.D. Minna, K.L. Helke, P.K. Singh, D.B. Shackelford, J.W. Kim, The distinct metabolic phenotype of lung squamous cell carcinoma defines selective vulnerability to glycolytic inhibition, Nat. Commun. 8 (2017) 15503, https://doi.org/10.1038/ncomms15503.
[29] S.Y. Lunt, M.G. Vander Heiden, Aerobic glycolysis: meeting the metabolic requirements of cell proliferation, Annu. Rev. Cell Dev. Biol. 27 (2011) 441–464, https://doi.org/10.1146/annurev-cellbio-092910-154237.
[30] S.M. Davidson, T. Papagiannakopoulos, B.A. Olenchock, J.E. Heyman, M.A. Keibler, A. Luengo, M.R. Bauer, A.K. Jha, J.P. O'Brien, K.A. Pierce, D.Y. Gui, L.B. Sullivan, T.M. Wasylenko, L. Subbaraj, C.R. Chin, G. Stephanopolous, B.T. Mott, T. Jacks, C.B. Clish, M.G. Vander Heiden, environment impacts the metabolic dependencies of ras-driven non-small cell lung Cancer, Cell Metab. 23 (2016) 517–528, https://doi.org/10.1016/j.cmet.2016.01.007.
[31] C.T. Hensley, B. Faubert, Q. Yuan, N. Lev-Cohain, E. Jin, J. Kim, L. Jiang, B. Ko, R. Skelton, L. Loudat, M. Wodzak, C. Klimko, E. McMillan, Y. Butt, M. Ni, D. Oliver, J. Torrealba, C.R. Malloy, K. Kernstine, R.E. Lenkinski, R.J. DeBerardinis, Metabolic heterogeneity in human lung tumors, Cell. 164 (2016) 681–694, https://doi.org/10.1016/j.cell.2015.12.034.
[32] S. Hui, J.M. Ghergurovich, R.J. Morscher, C. Jang, X. Teng, W. Lu, L.A. Esparza, T. Reya, L. Le Zhan, J. Yanxiang Guo, E. White, J.D. Rabinowitz, Glucose feeds the TCA cycle via circulating lactate, Nature. 551 (2017) 115–118, https://doi.org/10.1038/nature24057.
[33] B. Faubert, K.Y. Li, L. Cai, C.T. Hensley, J. Kim, L.G. Zacharias, C. Yang, Q.N. Do, S. Doucette, D. Burguete, H. Li, G. Huet, Q. Yuan, T. Wigal, Y. Butt, M. Ni, J. Torrealba, D. Oliver, R.E. Lenkinski, C.R. Malloy, J.W. Wachsmann, J.D. Young, K. Kernstine, R.J. DeBerardinis, Lactate metabolism in human lung tumors, Cell. 171 (2017) 358–371, https://doi.org/10.1016/j.cell.2017.09.019 e9.
[34] M. Ying, C. Guo, X. Hu, The quantitative relationship between isotopic and net contributions of lactate and glucose to the TCA cycle, J. Biol. Chem. 294 (2019) 9615–9630, https://doi.org/10.1074/jbc.RA119.007841 jbc.RA119.007841.
[35] H. Xie, J. Hanai, J.-G. Ren, L. Kats, K. Burgess, P. Bhargava, S. Signoretti, J. Billiard, K.J. Duffy, A. Grant, X. Wang, P.K. Lorkiewicz, S. Schatzman, M. Bousamra, A.N. Lane, R.M. Higashi, T.W.M. Fan, P.P. Pandolfi, V.P. Sukhatme, P. Seth, Targeting lactate Dehydrogenase-A inhibits tumorigenesis and tumor progression in mouse models of lung Cancer and impacts tumor-initiating cells, Cell Metab. 19 (2014) 795–809, https://doi.org/10.1016/j.cmet.2014.03.003.
[36] K. Leithner, A. Hrzenjak, M. Trötzmüller, T. Moustafa, H.C. Köfeler, C. Wohlkoenig, E. Stacher, J. Lindenmann, A.L. Harris, A. Olschewski, H. Olschewski, PCK2 activation mediates an adaptive response to glucose depletion in lung cancer, Oncogene 34 (2015) 1044–1050, https://doi.org/10.1038/onc.2014.47.
[37] R.C. Sun, T.W.-M. Fan, P. Deng, R.M. Higashi, A.N. Lane, A.-T. Le, T.L. Scott, Q. Sun, M.O. Warmoes, Y. Yang, Noninvasive liquid diet delivery of stable isotopes into mouse models for deep metabolic network tracing, Nat. Commun. 8 (2017) 1646, https://doi.org/10.1038/s41467-017-01518-z.
[38] E. Caiola, L. Brunelli, M. Marabese, M. Broggini, M. Lupi, R. Pastorelli, Different metabolic responses to PI3K inhibition in NSCLC cells harboring wild-type and G12C mutant KRAS, Oncotarget. 7 (2016), https://doi.org/10.18632/oncotarget.9849.
[39] D.M. Gwinn, A.G. Lee, M. Briones-Martin-del-Campo, C.S. Conn, D.R. Simpson, A.I. Scott, A. Le, T.M. Cowan, D. Ruggero, E.A. Sweet-Cordero, Oncogenic KRAS regulates amino acid homeostasis and asparagine biosynthesis via ATF4 and alters sensitivity to L-Asparaginase, Cancer Cell 33 (2018) 91–107, https://doi.org/10.1016/j.ccell.2017.12.003 e6.
[40] K. Sellers, M.P. Fox, M. Bousamra, S.P. Slone, R.M. Higashi, D.M. Miller, Y. Wang, J. Yan, M.O. Yuneva, R. Deshpande, A.N. Lane, T.W.-M. Fan, Pyruvate carboxylase is critical for non–small-cell lung cancer proliferation, J. Clin. Invest. 125 (2015) 687–698, https://doi.org/10.1172/JCI72873.
[41] A. Muir, L.V. Danai, D.Y. Gui, C.Y. Waingarten, C.A. Lewis, M.G. Vander Heiden, Environmental cystine drives glutamine anaplerosis and sensitizes cancer cells to glutaminase inhibition, Elife. 6 (2017), https://doi.org/10.7554/eLife.27713.
[42] P.-H. Chen, L. Cai, K. Huffman, C. Yang, J. Kim, B. Faubert, L. Boroughs, B. Ko, J. Sudderth, E.A. McMillan, L. Girard, M. Peyton, M.D. Shields, D. Shames, H.S. Kim, B. Timmons, I. Sekine, R. Britt, S. Weber, L.A. Byers, J.V. Heymach, M.A. White, J.D. Minna, G. Xiao, R.J. DeBerardinis, Metabolic diversity in human non-small cell lung Cancer cells, BioRxiv. (2019) 561688, , https://doi.org/10.1101/561688.
[43] Comprehensive genomic characterization of squamous cell lung cancers, Nature. 489 (2012) 519–525, https://doi.org/10.1038/nature11404.
[44] R. Romero, V.I. Sayin, S.M. Davidson, M.R. Bauer, S.X. Singh, S.E. Leboeuf, T.R. Karakousi, D.C. Ellis, A. Bhutkar, F.J. Sánchez-Rivera, L. Subbaraj, B. Martinez, R.T. Bronson, J.R. Prigge, E.E. Schmidt, C.J. Thomas, C. Goparaju, A. Davies, I. Dolgalev, A. Heguy, V. Allaj, J.T. Poirier, A.L. Moreira, C.M. Rudin, H.I. Pass, M.G. Vander Heiden, T. Jacks, T. Papagiannakopoulos, Keap1 loss promotes Kras-driven lung cancer and results in dependence on glutaminolysis, Nat. Med. 23 (2017) 1362–1368, https://doi.org/10.1038/nm.4407.
[45] M. Momcilovic, S.T. Bailey, J.T. Lee, M.C. Fishbein, C. Magyar, D. Braas, T. Graeber, N.J. Jackson, J. Czernin, E. Emberley, M. Gross, J. Janes, A. Mackinnon, A. Pan, M. Rodriguez, M. Works, W. Zhang, F. Parlati, S. Demo, E. Garon, K. Krysan, T.C. Walser, S.M. Dubinett, S. Sadeghi, H.R. Christofk, D.B. Shackelford, Targeted inhibition of EGFR and glutaminase induces metabolic crisis in EGFR mutant lung Cancer, Cell Rep. 18 (2017) 601–610, https://doi.org/10.1016/j.celrep.2016.12.061.
[46] A.C. Newman, O.D.K. Maddocks, One-carbon metabolism in cancer, Br. J. Cancer 116 (2017) 1499–1504, https://doi.org/10.1038/bjc.2017.118.
[47] G.M. DeNicola, P.-H. Chen, E. Mullarky, J.A. Sudderth, Z. Hu, D. Wu, H. Tang, Y. Xie, J.M. Asara, K.E. Huffman, I.I. Wistuba, J.D. Minna, R.J. DeBerardinis, L.C. Cantley, NRF2 regulates serine biosynthesis in non–small cell lung cancer, Nat. Genet. 47 (2015) 1475–1481, https://doi.org/10.1038/ng.3421.
[48] W.C. Zhang, N. Shyh-Chang, H. Yang, A. Rai, S. Umashankar, S. Ma, B.S. Soh, L.L. Sun, B.C. Tai, M.E. Nga, K.K. Bhakoo, S.R. Jayapal, M. Nichane, Q. Yu, D.A. Ahmed, C. Tan, W.P. Sing, J. Tam, A. Thirugananam, M.S. Noghabi, Y. Huei Pang, H.S. Ang, W. Mitchell, P. Robson, P. Kaldis, R.A. Soo, S. Swarup, E.H. Lim, B. Lim, Glycine decarboxylase activity drives non-small cell lung Cancer Tumor-Initiating cells and tumorigenesis, Cell. 148 (2012) 259–272, https://doi.org/10.1016/j.cell.2011.11.050.
[49] J.R. Mayers, M.E. Torrence, L.V. Danai, T. Papagiannakopoulos, S.M. Davidson, M.R. Bauer, A.N. Lau, B.W. Ji, P.D. Dixit, A.M. Hosios, A. Muir, C.R. Chin, E. Freinkman, T. Jacks, B.M. Wolpin, D. Vitkup, M.G. Vander Heiden, Tissue of origin dictates branched-chain amino acid metabolism in mutant Kras-driven cancers, Science (80-.) 353 (2016) 1161–1165, https://doi.org/10.1126/science.aaf5171.
[50] E. Beddowes, J. Spicer, P.Y. Chan, R. Khadeir, J. Garcia Corbacho, D. Repana, J.P. Steele, P. Schmid, T. Szyszko, G. Cook, M. Diaz, X. Feng, A. Johnston, J. Thomson, M. Sheaff, B.W. Wu, J. Bomalaski, S. Pacey, P.W. Szlosarek, Phase 1 dose-escalation study of pegylated arginine deiminase, cisplatin, and pemetrexed in patients with argininosuccinate synthetase 1–deficient thoracic cancers, J. Clin. Oncol. 35 (2017) 1778–1785, https://doi.org/10.1200/JCO.2016.71.3230.
[51] M.R. Sullivan, L.V. Danai, C.A. Lewis, S.H. Chan, D.Y. Gui, T. Kunchok, E.A. Dennstedt, M.G. Vander Heiden, A. Muir, Quantification of microenvironmental metabolites in murine cancers reveals determinants of tumor nutrient availability, Elife. 8 (2019), https://doi.org/10.7554/elife.44235.
[52] N.S. Chandel, Navigating Metabolism, Cold Spring Harbor Laboratory Press, 2015.
[53] M.S. Padanad, G. Konstantinidou, N. Venkateswaran, M. Melegari, S. Rindhe, M. Mitsche, C. Yang, K. Batten, K.E. Huffman, J. Liu, X. Tang, J. Rodriguez-Canales, N. Kalhor, J.W. Shay, J.D. Minna, J. McDonald, I.I. Wistuba, R.J. DeBerardinis, P.P. Scaglioni, Fatty acid oxidation mediated by Acyl-CoA synthetase long chain 3 is required for mutant KRAS lung tumorigenesis, Cell Rep. 16 (2016) 1614–1628, https://doi.org/10.1016/j.celrep.2016.07.009.

[54] Z. Hall, Z. Ament, C.H. Wilson, D.L. Burkhart, T. Ashmore, A. Koulman, T. Littlewood, G.I. Evan, J.L. Griffin, Myc expression drives aberrant lipid metabolism in lung Cancer, Cancer Res. 76 (2016) 4608–4618, https://doi.org/10.1158/0008-5472.CAN-15-3403.

[55] S. Masri, T. Papagiannakopoulos, K. Kinouchi, Y. Liu, M. Cervantes, P. Baldi, T. Jacks, P. Sassone-Corsi, Lung adenocarcinoma distally rewires hepatic circadian homeostasis, Cell 165 (2016) 896–909, https://doi.org/10.1016/j.cell.2016.04.039.

[56] A.C. Kimmelman, E. White, Autophagy and tumor metabolism, Cell Metab. 25 (2017) 1037–1043, https://doi.org/10.1016/j.cmet.2017.04.004.

[57] J.Y. Guo, G. Karsli-Uzunbas, R. Mathew, S.C. Aisner, J.J. Kamphorst, A.M. Strohecker, G. Chen, S. Price, W. Lu, X. Teng, E. Snyder, U. Santanam, R.S. DiPaola, T. Jacks, J.D. Rabinowitz, E. White, Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis, Genes Dev. 27 (2013) 1447–1461, https://doi.org/10.1101/gad.219642.113.

[58] G. Karsli-Uzunbas, J.Y. Guo, S. Price, X. Teng, S.V. Laddha, S. Khor, N.Y. Kalaany, T. Jacks, C.S. Chan, J.D. Rabinowitz, E. White, Autophagy is required for glucose homeostasis and lung tumor maintenance, Cancer Discov. 4 (2014) 914–927, https://doi.org/10.1158/2159-8290.CD-14-0363.

[59] J.Y. Guo, X. Teng, S.V. Laddha, S. Ma, S.C. Van Nostrand, Y. Yang, S. Khor, C.S. Chan, J.D. Rabinowitz, E. White, Autophagy provides metabolic substrates to maintain energy charge and nucleotide pools in Ras-driven lung cancer cells, Genes Dev. 30 (2016) 1704–1717, https://doi.org/10.1101/gad.283416.116.

[60] H. Xu, M.-S. Lee, P.-Y. Tsai, A.S. Adler, N.L. Curry, S. Challa, E. Freinkman, D.S. Hitchcock, K.D. Copps, M.F. White, R.T. Bronson, M. Marcotrigiano, Y. Wu, C.B. Clish, N.Y. Kalaany, Ablation of insulin receptor substrates 1 and 2 suppresses Kras -driven lung tumorigenesis, Proc. Natl. Acad. Sci. U. S. A 115 (2018) 4228–4233, https://doi.org/10.1073/pnas.1718414115.

[61] L. Poillet-Perez, X. Xie, L. Zhan, Y. Yang, D.W. Sharp, Z.S. Hu, X. Su, A. Maganti, C. Jiang, W. Lu, H. Zheng, M.W. Bosenberg, J.M. Mehnert, J.Y. Guo, E. Lattime, J.D. Rabinowitz, E. White, Autophagy maintains tumour growth through circulating arginine, Nature 563 (2018) 569–573, https://doi.org/10.1038/s41586-018-0697-7.

[62] G. Shlomai, B. Neel, D. LeRoith, E.J. Gallagher, Type 2 diabetes mellitus and Cancer: the role of pharmacotherapy, J. Clin. Oncol. 34 (2016) 4261–4269, https://doi.org/10.1200/JCO.2016.67.4044.

[63] J.J. Lin, E.J. Gallagher, K. Sigel, G. Mhango, M.D. Galsky, C.B. Smith, D. LeRoith, J.P. Wisnivesky, Survival of patients with stage IV lung Cancer with diabetes treated with metformin, Am. J. Respir. Crit. Care Med. 191 (2015) 448–454, https://doi.org/10.1164/rccm.201407-1395OC.

[64] M. Bergamino, A.J. Rullan, M. Saigí, I. Peiró, E. Montanya, R. Palmero, J.C. Ruffinelli, A. Navarro, M.D. Arnaiz, I. Brao, S. Aso, S. Padrones, F. Cardenal, E. Nadal, Fasting plasma glucose is an independent predictor of survival in patients with locally advanced non-small cell lung cancer treated with concurrent chemoradiotherapy, BMC Cancer 19 (2019) 165, https://doi.org/10.1186/s12885-019-5370-5.

[65] T. Tsakiridis, C. Hu, H.D. Skinner, R. Santana-Davila, B. Lu, J.J. Erasmus, A. Doemer, G.M.M. Videtic, J. Coster, X. Yang, R. Lee, M. Werner-Wasik, P.E. Schaner, S.E. McCormack, B. Esparaz, R. McGarry, J.G. Bazan, T. Struve, J.D. Bradley, Initial reporting of NRG-LU001 (NCT02186847), randomized phase II trial of concurrent chemoradiotherapy (CRT) +/- metformin in locally advanced Non-Small Cell Lung Cancer (NSCLC), J. Clin. Oncol. 37 (2019) 8502, https://doi.org/10.1200/JCO.2019.37.15_suppl.8502.

[66] J. Chiche, J. Reverso-Meinietti, A. Mouchotte, C. Rubio-Patiño, R. Mhaidly, E. Villa, J.P. Bossowski, E. Proics, M. Grima-Reyes, A. Paquet, K. Fragaki, S. Marchetti, J. Briere, D. Ambrosetti, J.-F. Michiels, T.J. Molina, C. Copie-Bergman, J. Lehmann-Che, I. Peyrottes, F. Peyrade, E. de Kerviler, B. Taillan, G. Garnier, E. Verhoeyen, V. Paquis-Flucklinger, L. Shintu, V. Delwail, C. Delpech-Debiais, R. Delarue, A. Bosly, T. Petrella, G. Brisou, B. Nadel, P. Barbry, N. Mounier, C. Thieblemont, J.-E. Ricci, GAPDH expression predicts the response to R-CHOP, the tumor metabolic status, and the response of DLBCL patients to metabolic inhibitors, Cell Metab. 29 (2019) 1243–1257, https://doi.org/10.1016/j.cmet.2019.02.002 e10.

[67] D.B. Shackelford, E. Abt, L. Gerken, D.S. Vasquez, A. Seki, M. Leblanc, L. Wei, M.C. Fishbein, J. Czernin, P.S. Mischel, R.J. Shaw, LKB1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin, Cancer Cell 23 (2013) 143–158, https://doi.org/10.1016/j.ccr.2012.12.008.

[68] Y. Lissanu Deribe, Y. Sun, C. Terranova, F. Khan, J. Martinez-Ledesma, J. Gay, G. Gao, R.A. Mullinax, T. Khor, N. Feng, Y.H. Lin, C.C. Wu, C. Reyes, Q. Peng, F. Robinson, A. Inoue, V. Kochat, C.G. Liu, J.M. Asara, C. Moran, F. Muller, J. Wang, B. Fang, V. Papadimitrakopoulou, I.I. Wistuba, K. Rai, J. Marszalek, P.A. Futreal, Mutations in the SWI/SNF complex induce a targetable dependence on oxidative phosphorylation in lung cancer article, Nat. Med. 24 (2018) 1047–1057, https://doi.org/10.1038/s41591-018-0019-5.

[69] V. De Rosa, F. Iommelli, M. Monti, R. Fonti, G. Votta, M.P. Stoppelli, S. Del Vecchio, Reversal of warburg effect and reactivation of oxidative phosphorylation by differential inhibition of EGFR signaling pathways in non-small cell lung Cancer, Clin. Cancer Res. 21 (2015) 5110–5120, https://doi.org/10.1158/1078-0432.CCR-15-0375.

[70] O.G. Arrieta Rodriguez, F.B. Barron, M.-Á. Salinas Padilla, L.A. Ramirez-Tirado, D. Flores-Estrada, G. Cruz-Rico, M.J. Arguelles Jiménez, A.F. Cardona Zorrilla, Combination of metformin plus TKI vs. TKI alone in EGFR(+) LUNG adenocarcinoma: a randomized phase II study, J Clin Oncol. 36 (2018) 9013, https://doi.org/10.1200/JCO.2018.36.15_suppl.9013.

[71] Y. He, L. Li, L. Jiang, Y. Wang, Y. Zhao, X. Zhang, G. Wu, X. Zhou, J. Sun, J. Bai, B. Ren, K. Tian, Z. Xu, H. Xiao, Q. Zhou, R. Han, H. Chen, H. Wang, Z. Yang, C. Gao, Combination of metformin and gefitinib as first-line therapy for nondiabetic advanced non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutations: a multicenter, randomized, double-blind, placebo-controlled phase II, J. Clin. Oncol. 37 (2019) 9035, https://doi.org/10.1200/JCO.2019.37.15_suppl.9035.