Oncogene-Driven Metabolic Alterations in Cancer

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Abstract

Cancer is the leading cause of human deaths worldwide. Understanding the biology underlying the evolution of cancer is important for reducing the economic and social burden of cancer. In addition to genetic aberrations, recent studies demonstrate metabolic rewiring, such as aerobic glycolysis, glutamine dependency, accumulation of intermediates of glycolysis, and upregulation of lipid and amino acid synthesis, in several types of cancer to support their high demands on nutrients for building blocks and energy production. Moreover, oncogenic mutations are known to be associated with metabolic reprogramming in cancer, and these overall changes collectively influence tumor-microenvironment interactions and cancer progression. Accordingly, several agents targeting metabolic alterations in cancer have been extensively evaluated in preclinical and clinical settings. Additionally, metabolic reprogramming is considered a novel target to control cancers harboring un-targetable oncogenic alterations such as *KRAS*. Focusing on lung cancer, here, we highlight recent findings regarding metabolic rewiring in cancer, its association with oncogenic alterations, and therapeutic strategies to control deregulated metabolism in cancer.

Key Words: Cancer, Non-small cell lung cancer, Cancer metabolism, Metabolic reprogramming, Aerobic glycolysis, Oncogenic alteration

INTRODUCTION

Despite numerous efforts for cancer treatment, cancer is the leading cause of human deaths worldwide (Mathers and Loncar, 2006; Torre *et al*., 2015). Thus, understanding the biology underlying the evolution of cancer is important for reducing the economic and social burden of cancer. Recent investigations have demonstrated the impact of metabolic reprogramming on the development and progression of several types of human cancer, and deregulated metabolism is now regarded as one of the hallmarks of cancer (Hanahan and Weinberg, 2011; Pavlova and Thompson, 2016). Moreover, several findings demonstrate that mutations in oncogenes and/or tumor suppressor genes can mediate metabolic rewiring in cancer cells to support the high demands for building blocks and energy production in these cells (Iurlaro *et al*., 2014; Nagarajan *et al*., 2016; Kerr and Martins, 2017). Because cancer cells are prone to several oncogenic mutations such as *RAS*, *EGFR*, *MYC*, and *BRAF* mutations, these genes could also influence the metabolic changes in cancer. Based on several studies

on the association of oncogenic alterations with the metabolic reprogramming (Kroemer and Pouyssegur, 2008; Hanahan and Weinberg, 2011; Iurlaro *et al*., 2014; Nagarajan *et al*., 2016; Kerr and Martins, 2017), here, we summarize recent findings on the association of oncogenic alterations with metabolic reprogramming in cancer, focusing on lung cancer due to its great contribution to cancer incidence and mortality rates. Further, we discuss the impact of metabolic alterations on the tumor-microenvironment interaction and possible therapeutic options targeting metabolic reprogramming.

GENERAL FEATURES OF METABOLIC REPROGRAMMING IN CANCER

Cancer cells have been known to possess markedly different metabolic features compared with those of corresponding normal tissues (Tennant *et al*., 2010). Unlike normal cells, cancer cells rearrange their cellular metabolic networks to fulfill their high demands for building blocks and energy production
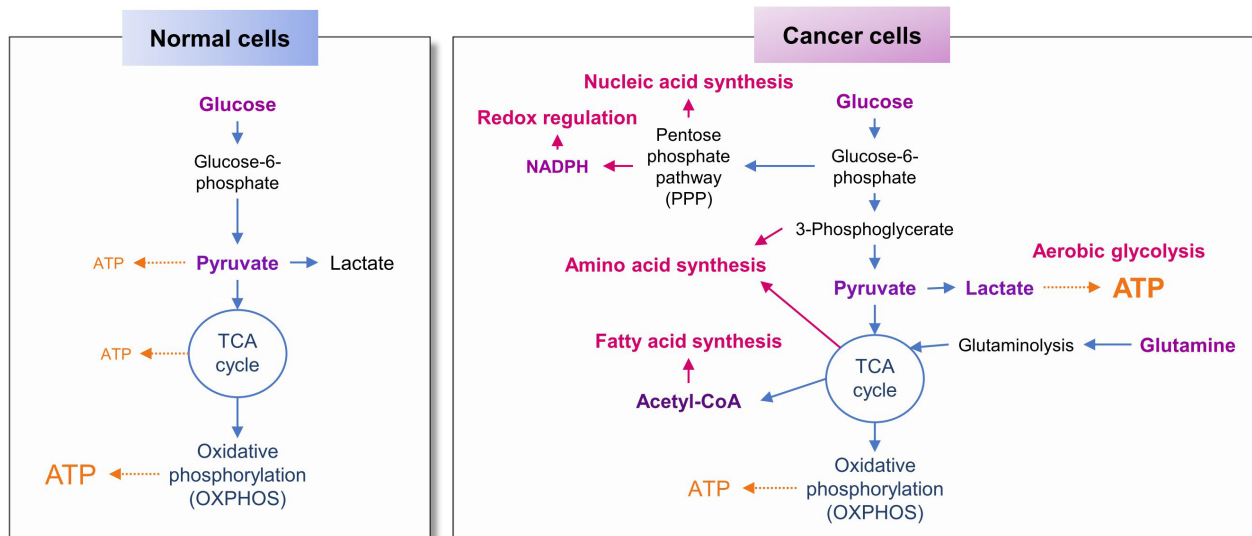
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Fig. 1. Metabolic reprogramming in cancer cells compared with normal cells.

to support extensive proliferation and growth (Tennant *et al*., 2010; Kerr and Martins, 2017) (Fig. 1). The first defined cancer-specific metabolic alteration is the Warburg effect, an aerobic glycolytic process discovered by Otto Warburg in 1926 (Warburg, 1956). In this process, cancer cells are dependent on glycolysis for glucose metabolism even in the presence of oxygen, thereby producing high levels of lactate and reducing the use of the tricarboxylic acid (TCA) cycle (Levine and Puzio-Kuter, 2010). Because the TCA cycle and subsequent oxidative phosphorylation produce cellular energy more efficiently than glycolysis, this metabolic rewiring has been suggested as an alternative to compensate for mitochondrial dysfunction in cancer cells (Warburg, 1956; Kerr and Martins, 2017). Indeed, mutations in the TCA cycle-associated enzymes, such as succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH), have been found in several types of cancer including paraganglioma (mutations in *SDH*), phaeochromocytoma (mutations in *SDH*), renal carcinoma (mutations in *FH*), leiomyomatosis (mutations in *FH*), acute myeloid leukemia (mutations in *IDH*), and glioblastoma (mutations in *IDH*), and these alterations have been suggested to contribute to mitochondrial dysfunction in cancer and tumorigenesis (King *et al*., 2006; Dang *et al*., 2010; Galluzzi *et al*., 2013; Parker and Metallo, 2015). However, several recent findings have suggested the essential role of functional mitochondria in cancer cells (Magda *et al*., 2008; Whitaker-Menezes *et al*., 2011; Wallace, 2012). The upregulation of oxidative phosphorylation has been noted in cancer cells (Whitaker-Menezes *et al*., 2011), and the tumorigenic potential of cancer cells has also been shown to be significantly reduced by depletion of mitochondrial DNA (Magda *et al*., 2008). Therefore, in addition to ATP synthesis, metabolic switching to aerobic glycolysis appears to be a means of supplying cancer cells with the precursors of proteins, lipids, amino acids, and nucleic acids for building their cellular structure and maintaining their upregulated proliferation. Thus, mitochondria still play important roles in bioenergetics and biosynthesis in cancer cells (Wallace, 2012).

Recent findings demonstrate the additional metabolic rewir-

ing in cancer cells and consequent alterations in cellular signaling pathways and the tumor microenvironment, including changes in the metabolism of glucose, lipids, and amino acids; regulation of the cellular redox state to tolerate reactive oxygen species (ROS)-mediated damage in cellular compartments; and remodeling of the extracellular matrix surrounding cancer cells. For instance, cancer cells display elevated expression of the alternatively spliced form of pyruvate kinase (PK), PK muscle isozyme M2 (PKM2) (Kroemer and Pouyssegur, 2008; Dong *et al*., 2016). PK mediates the conversion of phosphoenolpyruvate (PEP) to pyruvate, the rate-limiting step of glycolysis (Dong *et al*., 2016). Owing to the reduced enzymatic activity of PKM2, the phosphorylated metabolites upstream of pyruvate in the glycolytic pathway accumulate and are finally diverted into several anabolic pathways to synthesize glycogen, triglycerides, phospholipids, nucleotides, and amino acids (Gatenby and Gillies, 2004; Kroemer and Pouyssegur, 2008; Dong *et al*., 2016). In addition, cancer cells introduce acetyl-CoA into a truncated TCA cycle, resulting in the export of acetyl-CoA into the cytosol, where it serves as a precursor of fatty acids, cholesterol, and isoprenoids, which are utilized for cell proliferation and growth (Kroemer and Pouyssegur, 2008). Fatty acid synthase and choline kinase, which mediate biosynthesis of long-chain fatty acids and phosphatidylcholines, respectively, are also known to be upregulated and activated in many types of cancer cells (Ramirez de Molina *et al*., 2002; Menendez and Lupu, 2007; Kroemer and Pouyssegur, 2008). In the case of amino acid metabolism, cancer cells express sensors of amino acid deficiency, such as GATOR1, folliculin, and the Ras-like small GTPase Rag complex, to ensure a sufficient supply of amino acids to activate rapamycin complex I (mTORC1) (Bar-Peled and Sabatini, 2014; Tsun and Possemato, 2015). The upregulated uptake of glutamine, a nonessential amino acid, through elevated expression of glutamine transporters such as SLC1A5 and SLC38A2 has been thought to play important roles in the supply of nitrogen, the uptake of essential amino acids, and the maintenance of mTORC1 activation in cancer cells (Wise and Thompson, 2010). Consistent with these hypotheses, elevated expression of these glu-

tamine transporters is correlated with poor clinical outcomes in breast and lung cancers (Hassanein *et al*., 2015; Jeon *et al*., 2015). Cancer cells also display extensive conversion of glutamine to glutamate and upregulation of several metabolic enzymes responsible for amino acid biosynthesis, including glutaminase (GLS), phosphoglycerate dehydrogenase (PHG-DH), and asparagine synthetase (ASNS) (Gao *et al*., 2009; Locasale *et al*., 2011; Possemato *et al*., 2011; Zhang *et al*., 2014a; Tsun and Possemato, 2015). Moreover, the generation of nicotinamide adenine dinucleotide phosphate (NADPH) by metabolizing glucose through the pentose phosphate pathway (PPP) supports the defense of cancer cells against oxidative or cellular stresses and the synthesis of fatty acids in cancer cells (Gatenby and Gillies, 2004; Kroemer and Pouyssegur, 2008; Levine and Puzio-Kuter, 2010). Further, the acidic tumor microenvironment is constructed through the overproduction of lactate through aerobic glycolysis, facilitating the invasion of tumor cells and blood vessels via matrix remodeling and suppressing anticancer immunity (Fischer *et al*., 2007; Hunt *et al*., 2007; Swietach *et al*., 2007; Kroemer and Pouyssegur, 2008; Levine and Puzio-Kuter, 2010). Collectively, these complex processes allow cancer cells to survive and proliferate, but the details are known to be context dependent and differentially regulated by various factors such as oncogenes/tumor suppressor genes, microenvironments, and tissue of origin (Levine and Puzio-Kuter, 2010; Yuneva *et al*., 2012; Hensley *et al*., 2016; Mayers *et al*., 2016; Kerr and Martins, 2017). Thus, understanding the influence of cellular or environmental factors, such as oncogene-induced metabolic switches, on cancer cell metabolism is important for the development of better anticancer therapeutics targeting altered metabolism in cancer cells.

METABOLIC ALTERATIONS IN NON-SMALL CELL LUNG CANCER

Lung cancer is one of the main types of cancer due to its high prevalence and poor survival rates (Mathers and Loncar, 2006; Torre *et al*., 2015). Approximately 85% of all cases of lung cancer are non-small cell lung cancer (NSCLC) (Molina *et al*., 2008). The three major types of NSCLC (adenocarcinoma (ADC), squamous cell carcinoma (SQCC), and large cell carcinoma) are classified based on histological and molecular/genetic features (Clinical Lung Cancer Genome Project (CLCGP) and Network Genomic Medicine (NGM), 2013; Pikor *et al*., 2013). Mutations in *KRAS* and *EGFR* as well as *ALK* rearrangements, among others, are mainly found in lung ADC, which accounts for 30-40% of NSCLCs (Pikor *et al*., 2013). Lung ADCs carrying these genetic alterations are addicted to the associated signaling pathways for cell proliferation, growth, and survival and thus can be vulnerable to the disruption of these signaling pathways (Hrustanovic *et al*., 2015; Lin and Shaw, 2016). Indeed, several anticancer drugs specifically targeting EGFR or ALK have been clinically used as a first-line therapy for patients with lung ADC harboring these mutations (Saintigny and Burger, 2012). However, none of these drugs have shown remarkable clinical benefits, and drug resistance is still a large obstacle for efficient anticancer treatment using these regimens (Lin and Shaw, 2016). Moreover, there is no therapeutic option to control lung ADC carrying mutant *KRAS*. Although several alternative approaches

have been suggested, including targeting the functional outputs of mutant *KRAS* or cellular addiction caused by mutant *KRAS* (Kerr and Martins, 2017), it is important to develop novel therapeutic strategies to meet clinical needs for the treatment of lung cancer, especially lung ADC carrying mutations in oncogenes such as *KRAS*.

In line with the general metabolic reprogramming in cancer cells that has been described previously, recent studies have demonstrated metabolic alterations in NSCLC. Studies using NSCLC tumors surgically resected from patients after radioisotope-labeled glucose (^{13}C-glucose) infusion, NSCLC cells displayed enhancements in glycolysis and the TCA cycle and subsequent enrichment of TCA cycle intermediates compared with adjacent normal or benign lung tissues (Fan *et al*., 2009; Hensley *et al*., 2016). In addition, the activity of pyruvate carboxylase (PC), an enzyme mediating the irreversible carboxylation of pyruvate to generate oxaloacetate (Gray *et al*., 2014), was elevated in NSCLC tumors (Sellers *et al*., 2015; Hensley *et al*., 2016). Because upregulated PC activity plays a role in the replenishment of TCA intermediates that have been utilized in biosynthetic reactions (Kerr and Martins, 2017), this enhancement indicates the rewiring of glucose metabolism to meet the high metabolic demands of cancer cells. Moreover, silencing PC expression significantly reduced the proliferative, colony-forming, and tumorigenic abilities of NSCLC cells, suggesting that NSCLC cells are addicted to PC-mediated anaplerosis (the reduction of TCA intermediates due to biosynthetic reactions). Thus, PC has the potential to be a novel cellular target for anticancer drug development (Sellers *et al*., 2015). A recent study shows that a subset of NSCLC cells utilizes glycolysis for energy production and that these high glycolytic cells possess elevated hexokinase 2 expression (Wu *et al*., 2015). Another recent study also demonstrates the utilization of lactate as the main carbon source for the TCA cycle in tumors from NSCLC patients and NSCLC tumor xenografts (Faubert *et al*., 2017).

In addition to these changes in glucose metabolism, NSCLC cells exhibit alterations in the metabolism of lipids, amino acids, and nucleic acids. For example, the expression of acetyl-CoA carboxylase 1 (ACC1), one of the key regulators of fatty acid synthesis, was elevated in NSCLC cells. Further, pharmacological inhibition of ACC1 displayed significant antitumor effects in a preclinical model of NSCLC (Svensson *et al*., 2016; Svensson and Shaw, 2016). The expression and activity of ATP citrate lyase (ACLY), another key fatty acid synthesis enzyme involved in the generation of cytosolic acetyl-CoA and oxaloacetate, were also upregulated in NSCLC (Migita *et al*., 2008) and are associated with poor clinical outcomes in NSCLC patients (Migita *et al*., 2008). Consistent with the results of experiments targeting ACC1, siRNA-based ablation of *ACLY* expression exhibited significant inhibitory effects on proliferation and lipogenesis (Migita *et al*., 2008). Glycine decarboxylase (GLDC), a component of a multienzyme complex responsible for glycine decarboxylation and serine biosynthesis (Go *et al*., 2014) and involved in pyrimidine metabolism (Newman and Maddocks, 2017), was also upregulated in lung tumor-initiating cells and promoted cell transformation and tumorigenesis (Zhang *et al*., 2012). Elevated GLDC expression was associated with poor survival in patients with NSCLC (Zhang *et al*., 2012).

However, compared to altered glucose metabolism in NSCLC, the rewiring of other metabolic pathways in NSCLC
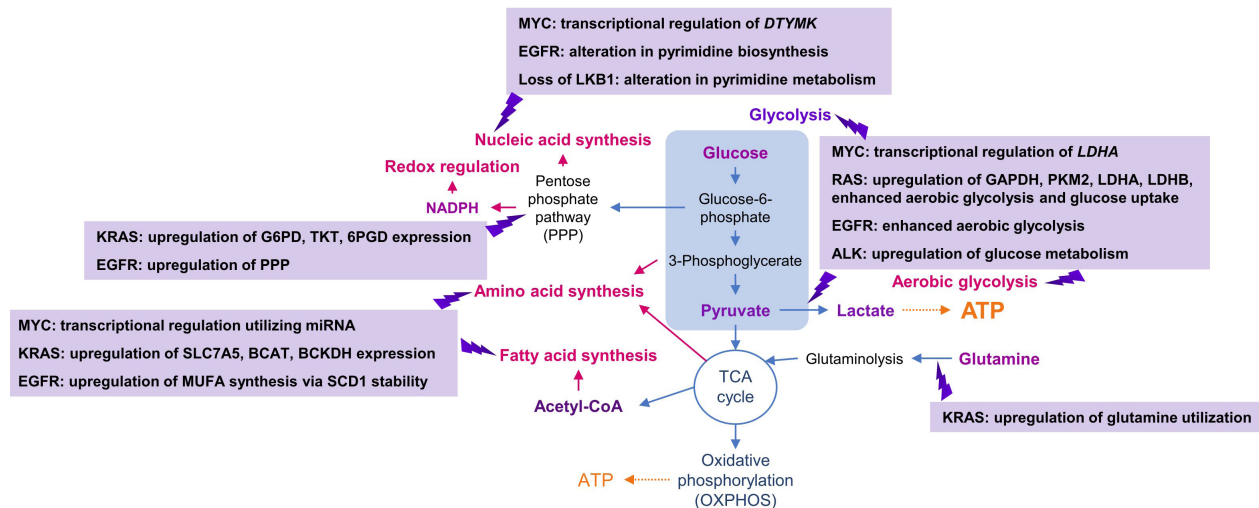
Fig. 2. Contribution of genetic alterations to metabolic reprogramming in cancer.

is still unclear and needs to be further elucidated. Additionally, despite commonalities in metabolic reprogramming, the metabolic alterations in individual NSCLC cells or tumors are highly heterogeneous (Brunelli *et al*., 2014; Chen *et al*., 2014; Wu *et al*., 2015; Hensley *et al*., 2016). Considering a high mutation burden in lung cancer, especially lung ADC (Cancer Genome Atlas Research Network, 2014; Swanton and Govindan, 2016; Kerr and Martins, 2017), and the association of alterations in oncogenes or tumor suppressor genes with metabolic reprogramming (Levine and Puzio-Kuter, 2010; Iurlaro *et al*., 2014; Nagarajan *et al*., 2016; Kerr and Martins, 2017), the genetic heterogeneity of NSCLC appears to influence these metabolic diversities.

ROLE OF ONCOGENIC MUTATIONS IN METABOLIC REPROGRAMMING IN LUNG CANCER

Alterations in several oncogenes, such as *MYC*, *RAS*, and *BRAF*, have been known to play a role in metabolic reprogramming (Iurlaro *et al*., 2014; Nagarajan *et al*., 2016; Kerr and Martins, 2017). Briefly, MYC transcriptionally regulates some metabolic enzymes involved in DNA synthesis and glycolysis, including thymidylate kinase and lactate dehydrogenase A, respectively (Pusch *et al*., 1997; Shim *et al*., 1997). MYC is also involved in the metabolic reprogramming of fatty acids, glutamine, proline, and nucleic acids by direct transcriptional regulation or indirect regulation utilizing microRNAs (Mannava *et al*., 2008; Gao *et al*., 2009; Liu *et al*., 2012; Edmunds *et al*., 2014). In addition, increases in the uptake and interconversion of a polyamine spermine, the metabolism of inositol phospholipids, and aerobic glycolysis were observed in RAS-transformed cells (Huang *et al*., 1988; Pakala *et al*., 1988; Chiaradonna *et al*., 2006). Further, mutated *RAS* was found to mediate metabolic reprogramming in pancreatic cancer by stimulating glucose uptake, channeling glycolytic intermediates into the hexosamine biosynthesis pathway or pentose phosphate pathway, and directly regulating aspartate transaminases (Ying *et al*., 2012; Son *et al*., 2013; Nagarajan *et al*., 2016). BRAF is also known to regulate glucose and glutamine

metabolism in melanoma (Scott *et al*., 2011; Haq *et al*., 2013).

In the case of lung cancer, previous reports have suggested a link between genetic mutations and metabolic rewiring in NSCLC, especially lung ADC. The association of alterations in *KRAS*, *EGFR*, *ALK*, and *STK11* genetic abnormalities in lung ADC (Ji *et al*., 2007; Pikor *et al*., 2013) with metabolic changes is described as follows (Fig. 2).

Role of KRAS mutation in metabolic reprogramming in NSCLC

Mutations in the RAS oncogene are known to be a major driver of tumorigenesis (Cox and Der, 2010; Pylayeva-Gupta *et al*., 2011; Hobbs *et al*., 2016). Three isoforms of the RAS gene [Kirsten rat sarcoma viral oncogene homolog (*KRAS*), neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*) and Harvey rat sarcoma viral oncogene homolog (*HRAS*)] encode four RAS proteins (KRAS4A, KRAS4B, NRAS, and HRAS) (Pylayeva-Gupta *et al*., 2011; Hobbs *et al*., 2016). The two KRAS isoforms arise from alternative RNA splicing of the *KRAS* gene (Pylayeva-Gupta *et al*., 2011; Hobbs *et al*., 2016). Activating mutations have been identified at three hotspots within the RAS protein (G12, G13, and Q61), but the mutation frequency at each of the hotspots in the RAS isoform is known to be quite different in each isoform (Pylayeva-Gupta *et al*., 2011; Hobbs *et al*., 2016). The RAS protein is a small G protein whose activity is regulated by the GDP/GTP cycle (Cox and Der, 2010; Pylayeva-Gupta *et al*., 2011; Hobbs *et al*., 2016). The GTP-bound RAS, an activated form of the RAS protein, binds to downstream effectors and triggers activation of signal transduction pathways, such as the Raf-MEK-ERK pathway and the PI3K/Akt pathway, responsible for cell proliferation, survival, and growth (Cox and Der, 2010; Pylayeva-Gupta *et al*., 2011).

Mutations in the *KRAS* gene, including G12C, G12V, G12D, and G12A, are found in approximately 30% of NSCLC patients with ADC histology (Kempf *et al*., 2016). These mutations are found more frequently in smokers than in nonsmokers (25-35% in smokers and 5% in nonsmokers) (Mao *et al*., 2010; Dearden *et al*., 2013; Kempf *et al*., 2016). The *KRAS* mutation (G12D) is common in never-smokers, whereas the *KRAS*

mutation (G12C) is the most common mutation in NSCLC patients with a history of smoking (Kempf *et al.*, 2016). Mutations in *KRAS* and *EGFR* are mutually exclusive (Kempf *et al.*, 2016), but mutations in *STK11* or *TP53* are positively correlated with *KRAS* mutations (Kempf *et al.*, 2016). Although a recent report describes the weak prognostic impact of the *KRAS* mutations in NSCLC (Roberts and Stinchcombe, 2013), recent findings suggest a close association of the *KRAS* mutations with poor prognosis of patients with NSCLC (Meng *et al.*, 2013; Renaud *et al.*, 2015; Kempf *et al.*, 2016). Accordingly, several anticancer approaches targeting the RAS protein, including farnesyltransferase inhibitors, competitors disrupting the RAS-chaperone interaction, and inhibitors of the RAS effector or downstream signaling such as the MAPK pathway, mTOR, and Hsp90, have been evaluated in preclinical and clinical settings. None, however, has shown clinical benefits for anticancer treatment (Cox and Der, 2010; Kempf *et al.*, 2016), emphasizing the necessity of procuring alternative approaches to treat cancer carrying *RAS* mutations.

Numerous findings demonstrate the involvement of mutant *KRAS* in the metabolic rewiring of several types of human cancer (Pylayeva-Gupta *et al.*, 2011; Kimmelman, 2015; Lv *et al.*, 2016; Kawada *et al.*, 2017; Kerr and Martins, 2017), including upregulation of glucose uptake, glutamine utilization, and aerobic glycolysis (Onetti *et al.*, 1997; Ying *et al.*, 2012; Son *et al.*, 2013). Using patient-derived NSCLC tumors, cell lines, and animal models, several studies have consistently identified the influence of mutant *KRAS* on metabolic reprogramming in NSCLC. A recent study demonstrated the metabolism-related proteomic profiles of NSCLC cell lines carrying intrinsic mutant *KRAS* (A549 and H460) in comparison with those of normal bronchial epithelial cells (Martin-Bernabe *et al.*, 2014). These NSCLC cells expressed elevated levels of enzymes involved in glycolysis (GAPDH, PKM2, LDHA, and LDHB) and PPP (G6PD, TKT, and 6PGD) compared with normal cells, suggesting alterations in glucose metabolism in NSCLC cells carrying mutant *KRAS*. It is known that these two cell lines carry different *KRAS* mutations (G12S for A549; Q61H for H460) (Mahoney *et al.*, 2009; Acquaviva *et al.*, 2012) and that the different amino acid substitutions display distinct biological properties in terms of signaling activation and sensitivity to anticancer agents (Garassino *et al.*, 2011; Stolze *et al.*, 2015). Thus, cellular metabolism could be influenced by different *KRAS* mutations. In line with this notion, a recent study demonstrated the impact of different *KRAS* mutations on changes in metabolomic profiles (Brunelli *et al.*, 2014). In this study, different *KRAS* mutations at codon 12 (G12C, G12D, and G12V) were evaluated. NSCLC cells carrying each of these mutations displayed differential metabolic remodeling, including differences in redox buffering systems and glutamine dependency (Brunelli *et al.*, 2014). Among these mutations, mutant *KRAS* (G12C) showed the most prominent metabolic changes *in vitro*. Of note, these metabolic changes were maintained in a tumor xenograft model bearing the same NSCLC cell line (Brunelli *et al.*, 2014, 2016), suggesting that the *in vitro* cell line model can be utilized to investigate metabolic alterations in NSCLC patients. However, another independent study demonstrated discrepancies in glucose metabolism using *in vitro* versus *in vivo* models (Davidson *et al.*, 2016). In this study, several mouse models, including two autochthonous mouse models that develop spontaneous lung tumors (the *Kras*[LA2/+] mouse model and the *Kras*[LSL-G12D/+];*Trp53*^{fl/fl} (KP) mouse model

with intratracheal delivery of adenoviral Cre), a syngeneic xenograft model involving intratracheal inoculation with lung tumor cells derived from the KP mouse model, and a tumor xenograft model involving subcutaneous inoculation with human lung cancer cell lines, were used for determining metabolic changes *in vivo*. Tumor cells arising in the KP mouse model were used for *in vitro* determination of metabolic alterations (Davidson *et al.*, 2016). Both *in vitro* and *in vivo* models exhibited upregulated lactate production. However, in contrast to a dependence on glutamine for TCA cycle entry *in vitro*, lung tumors from these *in vivo* mouse models minimally utilized glutamine as a carbon source for TCA cycle entry. Additionally, some oxidative glucose metabolic enzymes, including pyruvate carboxylase and pyruvate dehydrogenase (which generate oxaloacetate and acetyl-CoA, respectively), were necessary for tumor formation and growth in these mouse models (Davidson *et al.*, 2016). Therefore, the environmental context needs to be taken into consideration in the investigation of physiologically relevant metabolic alterations, especially in the case of glucose metabolism.

Additional studies also suggest that mutant *KRAS* mediates the changes in the metabolism of amino acids, lipids, and folates. In a recent study using a mutant *Kras*-driven model of spontaneous lung tumorigenesis (the KP mouse model), the uptake and utilization of branched-chain amino acids (BCAAs), such as leucine and valine, were elevated in KP mice possessing lung tumors (Mayers *et al.*, 2016). The expression of enzymes responsible for the catabolism of BCAAs, including SLC7A5, BCAT, and BCKDH, was also upregulated in human NSCLC tumors, and ablation of *Bcat* expression resulted in decreases in *in vitro* NSCLC cell proliferation and *in vivo* NSCLC tumor growth (Mayers *et al.*, 2016), indicating the requirement of BCAA metabolism in NSCLC. In the same study, pancreatic ductal adenocarcinoma (PDAC) carrying the same genetic alterations did not utilize BCAA as a nitrogen source (Mayers *et al.*, 2016), suggesting the influence of tissue microenvironment-specific differences on metabolic reprogramming over genetic mutations. In addition to amino acid metabolism, mutant *KRAS* activated lipogenesis in lung ADC via induction of fatty acid synthase through the ERK2-mediated pathway (Gouw *et al.*, 2017). NSCLC cells carrying mutant *KRAS* also showed a tendency to be dependent on the folate metabolism pathway compared with those carrying wild-type *KRAS* (Moran *et al.*, 2014). Consistent with these findings, *KRAS* mutant NSCLC cells were sensitive to antifolates such as methotrexate and pemetrexed, and the expression level of enzymes related to folate metabolism, such as methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) was positively (Moran *et al.*, 2014).

Moreover, despite the metabolic switch to aerobic glycolysis in cancer cells, mitochondria are known to have a functional role in cell proliferation and tumorigenesis (Magda *et al.*, 2008; Whitaker-Menezes *et al.*, 2011; Wallace, 2012). Likewise, deregulation of mitochondrial function through the ablation of the expression of mitochondrial transcription factor A (TFAM) significantly suppressed mutant *Kras*-driven lung tumor formation (Weinberg *et al.*, 2010). In this study, mitochondrial ROS generated through Complex III was essential for mutant *KRAS*-induced anchorage-independent growth of cancer cells (Weinberg *et al.*, 2010). A previous report demonstrated the reduced expression of components of Complex I in *KRAS*-transformed cells (Baracca *et al.*, 2010). Consid-

ering that both Complex I and Complex II mediate electron transfer to Complex III (Mailloux, 2015), presumably, NSCLC cells expressing mutant *KRAS* might acquire an alternative method (e.g., upregulation of Complex II) of compensating for the *KRAS*-induced decrease in Complex I activity in order to maintain mitochondrial function.

Role of EGFR mutations in metabolic reprogramming in NSCLC

Approximately 15-30% of NSCLC patients carry abnormalities in *EGFR* (Gridelli *et al*., 2015). *EGFR* mutations are frequently observed in lung ADCs derived from Asian patients with no smoking history (Gridelli *et al*., 2015). The most common mutations in *EGFR* are a deletion at exon 19 (E746–A750) and substitutions at exon 18 (G719C, G719S, G719A) and exon 21 (L858R), all of which are sensitive to EGFR-targeted therapy (Pao and Miller, 2005; Gridelli *et al*., 2015). Aberrantly activated EGFR activates signaling pathways driving the mitogenic, prosurvival, and proinvasive phenotypes of the cancer cells (Zhang *et al*., 2010). In addition to the direct modulation of signal transduction, aberrant EGFR mediates metabolic reprogramming in NSCLC. For instance, global metabolic reprogramming, such as enhanced aerobic glycolysis and upregulation of PPP, alters pyrimidine biosynthesis and redox metabolism in *EGFR* mutant lung ADC cell lines (Makinoshima *et al*., 2014). Combination treatment with erlotinib and a glutaminase inhibitor (CB-839) drives *EGFR* mutant NSCLC cells to undergo metabolic crisis, thereby leading to enhanced cell death, decreased cell viability *in vitro*, and a rapid tumor regression *in vivo* (Momcilovic *et al*., 2017), indicating the necessity of glutamine as a source for bioenergetics and biosynthesis in NSCLCs carrying mutant *EGFR*. Moreover, EGFR increases monounsaturated fatty acid (MUFA) synthesis by phosphorylating stearoyl-CoA desaturase-1 (SCD1) via direct interaction and via maintaining the stability of the SCD1 protein (Zhang *et al*., 2017). The level of phosphorylated SCD1 expression was found to be an independent prognostic factor for poor survival in patients with NSCLC (Zhang *et al*., 2017). These results collectively indicate that targeting alterations in glucose or lipid metabolism would be an alternative combinatorial therapeutic approach for treatment of lung ADCs harboring mutant *EGFR*.

Role of ALK rearrangement in metabolic reprogramming in NSCLC

ALK rearrangement accounts for approximately 3-7% of NSCLC cases (Katayama *et al*., 2015). The most frequently observed ALK rearrangement is the *EML4-ALK* fusion (Katayama *et al*., 2015). Several ALK inhibitors, including crizotinib and ceritinib, have been clinically used for the treatment of patients with lung ADC harboring alterations in *ALK* (Katayama *et al*., 2015). The impact of *ALK* aberrations on metabolism in lung ADC has not been well characterized, but a recent report indicates presence of upregulated glucose metabolism and highly metastatic phenotypes in lung ADCs carrying *ALK* rearrangements (Choi *et al*., 2013).

Role of LKB1 loss in metabolic reprogramming in NSCLC

LKB1, encoded by the *STK11* gene, is a tumor suppressor gene which plays an important role in the regulation of cellular growth and metabolism by phosphorylation and activation of AMP-activated kinase (AMPK), an upstream kinase control-ling the mammalian target of rapamycin (mTOR) pathway, MARK/par-1, and other AMPK-related kinases (Shackelford and Shaw, 2009). Approximately 15-35% of NSCLC patients harbor mutations in *STK11* (Ji *et al*., 2007; Shackelford and Shaw, 2009), which is more frequently observed in lung ADC than in lung SQCC (Sanchez-Cespedes *et al*., 2002; Ji *et al*., 2007). According to its primary role in the regulation of cellular metabolism, loss of LKB1 leads to deregulation of cellular metabolism under conditions of energy stress (Carretero *et al*., 2007), causing enhanced sensitivity to therapies targeting metabolism such as phenformin (Shackelford *et al*., 2013) or therapies that induce energetic stress such as erlotinib (Whang *et al*., 2016). In addition, metabolic reprogramming in NSCLC harboring altered LKB1 has been demonstrated in a recently published study. Using NSCLC cell lines carrying either *KRAS* mutations alone or both *KRAS* mutations and loss of LKB1, this study identified that the additional loss of LKB1 resulted in the accumulation of metabolites associated with the urea cycle through upregulation of carbamoyl phosphate synthetase-1 (CPS1) (Kim *et al*., 2017). Silencing of CPS1 expression suppressed the growth of tumor xenografts derived from *KRAS*/*STK11*-mutant NSCLC cells through reduction of the pyrimidine to purine ratio, thereby disrupting DNA replication (Kim *et al*., 2017). These results indicate the existence of alterations in pyrimidine metabolism in LKB1-deficient NSCLC cells and provides a novel therapeutic target for the treatment of NSCLCs harboring loss of LKB1 expression.

TUMOR MICROENVIRONMENT-MEDIATED METABOLIC REPROGRAMMING IN CANCER

The interaction between tumors and the surrounding stromal cells that make up the tumor microenvironment has been known to be implicated in cancer development and progression (Quail and Joyce, 2013). Given the role of metabolic alterations in cancer, the tumor-microenvironment interaction could be affected by metabolic alterations in cancer cells and vice versa. For example, the differences in BCAA metabolism between lung cancer and PDAC (Mayers *et al*., 2016) and in glutamine dependent metabolism between *in vitro* and *in vivo* models (Davidson *et al*., 2016) appear to be influenced by the environmental context. Nutrient sharing, nutrient competition, and metabolite exchange between tumor and stromal cells are known to influence and shape the tumor-microenvironment interaction (Lyssiotis and Kimmelman, 2017). Indeed, lactate, amino acids, and fatty acids act as signaling molecules that can be exchanged between tumor and stromal cells, resulting in the regulation of signal transduction, gene expression, and characteristics of neighboring cells (Lyssiotis and Kimmelman, 2017). Macromolecules or organelles released from stromal cells can also support the biosynthetic and bioenergetic needs of cancer cells (Spees *et al*., 2006; Chaudhri *et al*., 2013; Lyssiotis and Kimmelman, 2017). Specifically, compared with normal fibroblasts, basal autophagy was elevated in lung cancer-associated fibroblasts (CAFs) through the influence of high glycolytic lung cancer cells, leading to the release of dipeptides that could support surrounding cancer cells (Chaudhri *et al*., 2013). Additionally, interactions with bone marrow-derived nonhematopoietic stem/progenitor cells or skin fibroblasts rescued lung cancer cells with mitochondrial defects and led to reactivation of their mitochondrial function including electron

Table 1. Compounds targeting cancer metabolism in clinical studies

Name	Target	Clinical development stage	Cancer types targeted
Agents targeting deregulated signaling pathways			
Rapamycin (Sirolimus)	mTOR	Phase I/II	Glioblastoma, Advanced cancer
Everolimus (RAD001)	mTOR	FDA approved	Advanced renal cell carcinoma, Pancreatic neuroendocrine tumors, Subependymal giant cell astrocytoma
Temsirolimus (CCI-779)	mTOR	FDA approved	Advanced renal cell carcinoma
Ridaforolimus	mTOR	Phase I/II/III	Advanced solid tumors
AZD8055 (MK-8669)	mTOR	Phase I	Advanced solid tumors
Metformin	AMPK	Phase I/II/III	Various advanced solid tumors
Agents targeting metabolic enzymes			
2-Deoxygluose (2-DG)	HK	Phase I/II	Various advanced solid tumors
TCD-717	CK	Phase I	Advanced solid tumors
Dichloroacetate	PDK1	Phase I/II	Advanced solid tumors, Head and neck carcinoma, Brain tumor
Indoximod	IDO	Phase I/II	Adult solid tumors, Advanced solid tumors, Acute myeloid leukemia
Ivosidenib (AG-120)	IDH1	Phase I/II	Acute myeloid leukemia, Glioma, Advanced cholangiocarcinoma, Advanced solid tumors
Enasidenib mesylate (AG-221)	IDH2	Phase I/II	Acute myeloid leukemia, Glioma, Advanced solid tumors
AG-881	IDH1 or IDH2	Phase I	Acute myeloid leukemia, Glioma
IDH1 peptide vaccine	IDH1	Phase I	Glioma
PEPIDH1M	IDH1	Phase I	Glioma
Agents depleting metabolites using recombinant enzymes (PEG-conjugated)			
Arginase 1	Arginine	Phase I/II	Acute myeloid leukemia, Hepatocellular carcinoma, Other solid tumors
Arginine deiminase	Arginine	Phase I/II/III	Advanced solid tumors, mesothelioma, small cell lung cancer, skin cancer
Asparaginase	Asparagine	Phase I/II/III	Various types of leukemia and lymphoma

mTOR: mammalian target of rapamycin, AMPK: AMP activated protein kinase, HK: hexokinase, CK: choline kinase, PDK1: pyruvate dehydrogenase kinase 1, IDO: indoleamine 2,3-dioxygenase.

transport chain activity (Spees *et al*., 2006). These phenomena occurred through the transfer of mitochondria or mitochondrial DNA from stem/progenitor cells or fibroblasts to lung cancer cells (Spees *et al*., 2006). Collectively, these findings suggest a crucial association between metabolic reprogramming and the tumor-microenvironment interaction. However, details regarding mechanisms of action, the lung microenvironment-specific consequences of these interactions, and their clinical impacts need to be explored in further studies.

TARGETING METABOLIC REPROGRAMMING FOR THE TREATMENT OF CANCER

According to the importance of metabolic alterations in the development and progression of cancer, several agents targeting cancer metabolism have been developed and evaluated under preclinical and clinical studies (Kroemer and Pouyssegur, 2008; Tennant *et al*., 2010; Nagarajan *et al*., 2016). Some metabolism-targeting agents, such as mTOR inhibitors [rapamycin (sirolimus), everolimus, and temsirolius] and metformin (AMPK activator and mitochondrial Complex I inhibitor) are now approved for clinical use (Carracedo *et al*., 2013; Nagarajan *et al*., 2016) (Table 1). Strategies targeting

metabolic alterations for anticancer therapy are detailed in the following sections (Nagarajan *et al*., 2016).

Targeting deregulated signaling pathways

Recent studies demonstrate the effectiveness of targeting the signaling pathways downstream of oncogenes such as AMPK and mTOR, alone or in combination, in several types of cancer. For example, metformin, an AMPK activator, inhibited the biosynthesis of fatty acids and nucleic acids (Li *et al*., 2015), suppressed the proliferation of lung cancer and the self-renewal capacity of hepatocellular carcinoma stem cells by inducing apoptosis (Saito *et al*., 2013; Storozhuk *et al*., 2013), and increased the radiosensitivity of lung and breast cancer cells (Storozhuk *et al*., 2013; Zhang *et al*., 2014b). The mTOR inhibitor rapamycin also inhibited the cell proliferation in several types of cancer including colorectal cancer, glioma, pancreatic cancer, and recurrent glioblastoma (Houchens *et al*., 1983; Eng *et al*., 1984; Grewe *et al*., 1999; Cloughesy *et al*., 2008). In a phase I clinical trial, rapamycin showed anticancer activity in PTEN-deficient glioblastoma (Cloughesy *et al*., 2008). Rapamycin analogs with improved water solubility, such as everolimus and temsirolimus, also exhibited potent anticancer effects on several types of cancer alone or in combination with other anticancer agents (Vignot *et al*., 2005)

and have been clinically used for the treatment of advanced renal cell carcinoma, pancreatic neuroendocrine tumors, and subependymal giant cell astrocytoma (Benjamin *et al.*, 2011).

Targeting metabolic enzymes

2-Deoxyglucose (2-DG) has a similar structure to glucose and is unable to be metabolized in mammals (Nagarajan *et al.*, 2016). Thus, 2-DG can inhibit multiple glycolytic steps by competitively acting with glucose (Nagarajan *et al.*, 2016). 2-DG is phosphorylated by HK2 and phosphorylated 2-DG acts an inhibitor of HK2 (Wick *et al.*, 1957). In addition, various inhibitors targeting metabolic enzymes, including lonidamine and 3-bromopyruvate (hexokinase inhibitors), TLN-232 (a pyruvate kinase inhibitor), orlistat and cerulenin (fatty acid synthase inhibitors), dichloroacetate (a PDK1 inhibitor), MN58b and TCD-717 (choline kinase inhibitors), soraphen A (an acetyl-CoA carboxylase inhibitor), indoximod [an indoleamine 2,3-dioxygenase (IDO) inhibitor], ivosidenib (AG-120), enasidenib mesylate (AG-221 mesylate), AG-881, IDH305, PEPIDH1M (IDH1R132H-specific peptide vaccine) (inhibitors targeting mutated *IDH1* or *IDH2*), and SB-2049990 (an ATP citrate lyase inhibitor), have been evaluated in preclinical and clinical studies (Table 1) (Hatzivassiliou *et al.*, 2005; Wang *et al.*, 2005; Al-Saffar *et al.*, 2006; Beckers *et al.*, 2007; Kroemer and Pouyssegur, 2008; Tennant *et al.*, 2010; Mondesir *et al.*, 2016; Nagarajan *et al.*, 2016).

Depleting metabolites using recombinant enzymes

Strategies to inhibit a specific metabolic pathway using recombinant enzymes to reduce a specific oncogenic metabolite have been developed recently (Nagarajan *et al.*, 2016). For instance, recombinant arginine deiminase and arginase I (which degrade and deplete arginine) conjugated with polyethylene glycol (PEG) (pegylated arginine deiminase and pegylated arginase 1, respectively) have been evaluated in phase I/II clinical trials for the treatment of advanced melanoma and advanced hepatocellular carcinoma (Izzo *et al.*, 2004; Glazer *et al.*, 2010; Yang *et al.*, 2010; Ott *et al.*, 2013; Yau *et al.*, 2013; Nagarajan *et al.*, 2016). Recombinant L-asparaginase (which degrades and depletes asparagine) conjugated with PEG (PEG-asparaginase) is also in clinical trials for the treatment of pediatric and adult acute lymphoblastic leukemia, multiple myeloma, and advanced solid tumors (Taylor *et al.*, 2001; Agrawal *et al.*, 2003; Fu and Sakamoto, 2007; Kurtzberg *et al.*, 2011).

Specifically, in lung cancer, despite the various anticancer approaches targeting cancer metabolism described above, no metabolism-targeted drugs have been approved for lung cancer treatment. Currently, most metabolism-targeting agents for lung cancer are still under preclinical evaluation (Nagarajan *et al.*, 2016). Of note, agents targeting unique oncogene-driven metabolic rewiring have been relatively poorly developed and should be investigated in further studies. For lung cancer treatment, cellular markers specifically elevated in NSCLC cells harboring oncogenic alterations, including BCAT (Mayers *et al.*, 2016), SCD1 (Zhang *et al.*, 2017), and CPS1 (Kim *et al.*, 2017), could be potential candidates for developing novel anticancer agents specifically disrupting oncogene-driven metabolic reprogramming in NSCLC. In addition, metabolic synthetic lethality can be a valuable therapeutic approach considering the metabolic vulnerabilities of NSCLC carrying oncogenic mutations (Bensaad and Harris, 2013; Megchelen-

brink *et al.*, 2015; Kerr and Martins, 2017).

CONCLUSION

Cancer cells demand large nutrient supplies and thus reprogram their metabolic pathways to ensure metabolic flexibility, cellular homeostasis, energy production, cell proliferation, and survival. In addition to direct modulation of signal transduction pathways causing oncogenic addiction, alterations in oncogenes also contribute to metabolic rewiring in cancer cells, resulting in the promotion of cancer cell proliferation, survival, and metastatic dissemination. Accordingly, metabolic reprogramming is now considered an important characteristic of several types of cancer, including NSCLC. Despite several ongoing approaches to target cancer metabolism, metabolic reprogramming should be therapeutically explored in additional studies. In addition, the influence of metabolic rewiring on the interaction between cancer cells and the tumor microenvironment needs to be extensively investigated to comprehensively understand the course of cancer development and progression, providing mechanistic insights on several anticancer therapies targeting metabolism, microenvironmental interactions, and evasion of anticancer immunity. However, metabolic heterogeneity may reduce the responsiveness of metabolism-targeting anticancer drugs; thus, an in-depth exploration of metabolic status in cancer cells will be necessary to determine detailed metabolic changes at the cellular and molecular levels. Further, the clinical impact of metabolic alterations on cancer and the relevant biomarkers to predict or diagnose metabolic reprogramming should also be identified to develop tailored precision medicine targeting metabolic rewiring for the treatment of cancer.

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