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REVIEW ARTICLE

Targeting metabolic reprogramming in KRAS-driven cancers

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Abstract Mutations of *KRAS* are found in a variety of human malignancies, including in pancreatic cancer, colorectal cancer, and non-small cell lung cancer at high frequency. To date, no effective treatments that target mutant variants of *KRAS* have been introduced into clinical practice. In recent years, a number of studies have shown that the oncogene *KRAS* plays a critical role in controlling cancer metabolism by orchestrating multiple metabolic changes. One of the metabolic hallmarks of malignant tumor cells is their dependency on aerobic glycolysis, known as the Warburg effect. The role of KRAS signaling in the regulation of aerobic glycolysis has been reported in several types of cancer. *KRAS*-driven cancers are characterized by altered metabolic pathways involving enhanced nutrients uptake, enhanced glycolysis, enhanced glutaminolysis, and elevated synthesis of fatty acids and nucleotides. However, Just how mutated *KRAS* can coordinate the metabolic shift to promote tumor growth and whether specific metabolic pathways are essential for the tumorigenesis of *KRAS*-driven cancers are questions which remain to be answered. In this context, the aim of this review is to summarize current data on *KRAS*-related metabolic alterations in cancer cells. Given that cancer cells rely on changes in metabolism to support their growth and survival, the targeting of metabolic processes may be a potential strategy for treating *KRAS*-driven cancers.

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Abbreviations

ACS	Acyl-CoA synthetase
ASNS	Asparagine synthetase
BCAA	Branched-chain amino acid
CMS	Consensus molecular subtype
CRC	Colorectal cancer
EGFR	Epidermal growth factor receptor
FA	Fatty acid
FASN	Fatty acid synthase
FDG	Fluorodeoxyglucose
FGFR	Fibroblast growth factor receptor
GLS	Glutaminase
GLUD1	Glutamate dehydrogenase 1
GLUT1	Glucose transporter-1
GOT	Glutamate–oxaloacetate transaminase
HBP	Hexosamine biosynthesis pathway
HK	Hexokinase
MDH1	Malate dehydrogenase 1
mTOR	Mammalian target of rapamycin
NSCLC	Non-small cell lung cancer
PDCA	Pancreatic ductal cell carcinoma
PET	Positron emission tomography
PKM2	Pyruvate kinase M2
PPP	Pentose phosphate pathway
ROS	Reactive oxygen species
TCA	Tricarboxylic acid
UPR	Unfolded protein response

Introduction

In recent years, there has been intense interest directed towards understanding the reprogramming of metabolic

processes in cancer cells [1–4]. The first recorded observations in cancer metabolism were made by Otto Warburg in the 1920s, who discovered that cancer cells showed increased glycolysis and lactate production regardless of oxygen availability [5]. Accumulating evidence also indicates that the reprogramming of cancer metabolism is under the control of various oncogenic signals [1].

The *KRAS* proto-oncogene encodes an approximately 21-kDa small GTPase, which cycles between the active guanosine triphosphate-bound state and the inactive guanosine diphosphate-bound state. Oncogenic activation of *KRAS* can influence several cellular processes that regulate morphology, proliferation, motility, and survival through the activation of its downstream pathways, such as the MAPK and PI3K/AKT/mTOR pathways [6, 7]. *KRAS* mutations occur in a variety of human malignancies, but they appear most frequently in pancreatic ductal cell carcinoma (PDCA), colorectal cancer (CRC), and non-small cell lung cancer (NSCLC). *KRAS*-driven cancers are largely resistant to therapeutic intervention, and KRAS itself has been considered to be "undruggable." To satisfy the increased needs for cellular building blocks as a result of enhanced tumor growth, metabolic pathways are rewired to divert nutrients, such as glucose and glutamine, into anabolic pathways [1]. While most cancers depend on a high rate of aerobic glycolysis for their growth, some cancer cells also display an addiction to glutamine despite glutamine being a non-essential amino acid that can be synthesized from glucose [8–10]. Recent studies have shown that oncogenic *KRAS* promotes metabolic reprogramming through the stimulation of glucose metabolism, differential channeling of glucose intermediates, reprogrammed glutamine metabolism, increased autophagy, and macropinocytosis [11–14]. The mechanism by which oncogenic *KRAS* coordinates the shift in metabolism to promote tumor growth remains an area of active investigation.

Autophagy is a highly conserved mechanism to degrade intracellular components and promote cell survival by providing energy in the form of ATP and building blocks (i.e., amino acids, lipids, sugars, and nucleotides) [15]. Autophagy is triggered by nutrient shortage, protein damage, and oxidative stress occurring through the inhibition of the AMP kinase and the mammalian target of rapamycin (mTOR) pathways and the activation of the unfolded protein response (UPR) system. To fuel metabolic processes, KRAS signaling leads to the scavenging of extracellular proteins and lipids, while also activating self-eating and protein recycling processes via autophagy. The role of autophagy in cancer is extremely complex.

Cancer cells are also able to absorb and degrade extracellular components through an endocytic process called macropinocytosis. In PDCA cells, *KRAS*-dependent upregulation of macropinocytosis acts as an important supply route for amino acids such as glutamine, with macropinocytosis inhibition shown to reduce *KRAS*-transformed cell growth [16]. Hydroxychloroquine is a compound approved for the treatment of malaria and several rheumatologic diseases that prevents lysosome acidification, thus inhibiting autophagy and macropinocytosis. Hydroxychloroquine is currently being tested in several ongoing trials involving patients with PDCA.

Here, we present a comprehensive review of the metabolic deregulations contributing to *KRAS*-driven cancer progression (Fig. 1; Table 1). These data reveal several key points that are of prime relevance to *KRAS*-driven cancers and tumor biology in general. Targeting distinct metabolic features of *KRAS*-driven cancers provides novel approaches for cancer treatment.

Pancreatic ductal cell adenocarcinoma (PDCA)

PDCA harbors a particular poor prognosis, with a 5-year survival rate of <5% [17]. The current systemic treatment of PDCA is dependent on chemotherapy, with targeted approaches having minimal success. Malignant progression from pancreatic intraepithelial neoplasia to invasive disease is accompanied by an early acquisition of *KRAS* mutations, which occurs in >90% of cases, and a subsequent loss of tumor suppressors such as *INK4A*, *TP53*, and *SMAD4*.

Glucose metabolism

Studies using the inducible *KRAS*^{G12D}-driven PDCA mouse model have been very informative for determining which aspects of tumor metabolism are most essential for *KRAS*-driven cancers. Using this mouse model, Ying et al. reported that mutated *KRAS* enhances the expression of glucose transporter-1 (GLUT1) and several rate-limiting glycolytic enzymes, including hexokinase and lactate dehydrogenase, and that mutated *KRAS* maintains tumor growth by stimulating glucose uptake and channeling glucose intermediates into the hexosamine biosynthesis pathway (HBP) and non-oxidative pentose phosphate pathway (PPP): *KRAS* mutations promote protein glycosylation through HBP and ribose production through non-oxidative PPP [18]. Notably, knockdown of either the HBP gene (*Gfpt1*) or non-oxidative PPP genes (*Rpia* or *Rpe*) lead to inhibition of *KRAS*-dependent tumor growth in vivo, indicating their potential as therapeutic targets.

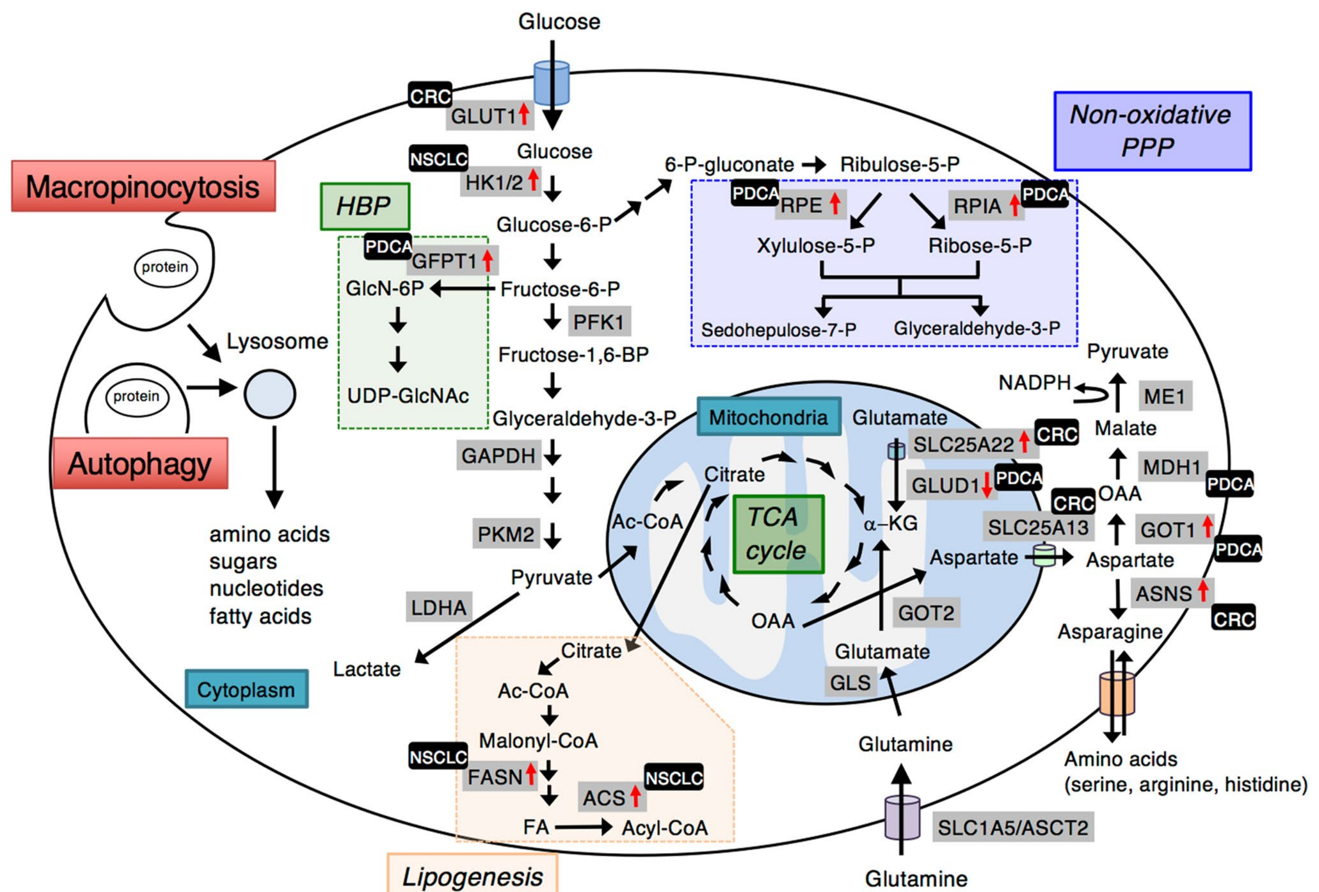

Fig. 1 Metabolic alterations in *KRAS*-driven cancers. Schematic representation of the metabolic routes involved in *KRAS*-driven cancers. *Black boxes* Cancer type in which the molecule is associated with mutated *KRAS*: *PDCA* pancreatic ductal cell carcinoma, *CRC* colorectal cancer, *NSCLC* non-small cell lung cancer. *Red arrows* Change in expression level that is modulated by mutated *KRAS*: *upwards arrow* upregulation, *downwards* arrow downregulation. *GLUT1* Glucose transporter 1, *HK1/2* hexokinase 1/2, *PFK1* phosphofructokinase 1, *GAPDH* glyceraldehyde 3-phosphate dehydrogenase, *PKM2* pyruvate kinase M2, *LDHA* lactate dehydrogenase A, *GFPT1* glucosamine-fructose-6-phosphate aminotransferase-1, *GlcN* glucosamine, *GlcNAc N*-acetylglucosamine, *RPE* ribulose-5-phosphate-3-epimerase, *RPIA* ribulose-5-phosphate isomerase, *GLS* glutaminase, *GLUD1* glutamate dehydrogenase 1, *GOT* glutamate–oxaloacetate transaminase, *MDH1* malate dehydrogenase 1, *ME1* malic enzyme 1, *ASNS* asparagine synthetase, *α-KG* α-ketoglutarate, *OAA* oxaloacetate, *Ac-CoA* Acetyl-CoA, *FA* fatty acid, *FASN* fatty acid synthase, *ACS* Acyl-CoA synthetase, *HBP* hexosamine biosynthesis pathway, *PPP* pentose phosphate pathway, *PDCA* pancreatic ductal cell carcinoma, *CRC* colorectal cancer, *NSCLC* non-small cell lung cancer

Amino acid metabolism

In addition to glucose, glutamine is also an important source of fuel for cancers. Glutamine is the most abundant circulating free amino acid in human plasma. It serves as a major anaplerotic substrate for the tricarboxylic acid (TCA) cycle, and also supplies nitrogen for nucleotides, non-essential amino acids, and hexosamine biosynthesis to fuel cell proliferation. Once transported into the cells via the glutamine transporter (SLC1A5/ASCT2), glutamine is first converted to glutamate by glutaminase (GLS) and then converted to the TCA cycle intermediate, α-ketoglutarate, by glutamate dehydrogenase 1 (GLUD1) or aminotransferases. The glutamine-derived α-ketoglutarate replenishes the TCA cycle by providing oxaloacetate that condenses with acetyl-CoA to maintain the TCA cycle and support fatty acid (FA) biosynthesis. In addition to providing carbon and nitrogen molecules for biosynthesis, glutamine plays a role in the uptake of essential amino acids and in maintaining mTOR signaling and NADPH production for redox control. mTOR complex 1 positively regulates GLS and glutamine flux through the S6K1 (p70 ribosomal S6 kinase 1)-dependent regulation of MYC [19]. The spectrum of glutamine-dependent tumors and the mechanisms by which glutamine supports cancer metabolism are being actively investigated [10, 20, 21]. Son et al. recently reported that *KRAS* mutations in PDCA regulate glutamine metabolism through its conversion to aspartate, thereby supporting growth by maintaining the cellular redox balance in the PDCA mouse model [22]. While most cells utilize GLUD1

Table 1 Promising metabolic targets for *KRAS*-driven cancers

Metabolic change	Targets[a]	Cancer type[b]	Pathway[c]	References
Glucose-related	GFPT1	PDCA	HBP	Ying et al. [18]
	RPIA	PDCA	Non-oxidative PPP	Ying et al. [18]
	RPE	PDCA	Non-oxidative PPP	Ying et al. [18]
	GLUT1	CRC	Glycolysis	Yun et al. [39]
	Vitamin C	CRC	Redox/glycolysis	Yun et al. [49]; Aguilera et al. [50]
	HK2	NSCLC	Glycolysis	Patra et al. [67]
Amino acid-related	GOT1	PDCA	Glutaminolysis	Son et al. [22]
	GLUD1	PDCA/CRC	Glutaminolysis	Son et al. [22]; Miyo et al. [62]
	MDH1	PDCA	Glutaminolysis	Wang et al. [23]
	ASNS	CRC	Glutaminolysis	Toda et al. [51]
	SLC25A22	CRC	Glutaminolysis	Wong et al. [61]
	SLC25A13	CRC	Glutaminolysis	Miyo et al. [62]
Lysosome-related	Hydroxychloroquine	PDCA	Macropinocytosis/autophagy	Yang et al. [26]; Guo et al. [27]; White et al. [29]; Wolpin et al. [32]
	EIPA	PDCA	Macropinocytosis	Commisso et al. [16]; Palm et al. [33]
	ATG7	NSCLC	Autophagy	Guo et al. [70]
Lipid-related	ACSL3	NSCLC	Lypogenesis	Padanad et al. [71]
	FASN	NSCLC	Lypogenesis	Gouw et al. [72]

[a] *GFPT1* Glucosamine-fructose-6-phosphate aminotransferase-1, *RPIA* ribulose-5-phosphate isomerase, *RPE* ribulose-5-phosphate-3-epimerase, *GLUT1* glucose transporter 1, *HK2* hexokinase 2, *GOT 1* glutamate-oxaloacetate transaminase 1, *GLUD1* glutamate dehydrogenase 1, *MDH1* malate dehydrogenase 1, *ASNS* asparagine synthetase, *EIPA* 5-(*N*-ethyl-*N*-isopropyl) amiloride, *ACS* Acyl-CoA synthetase, *FASN* fatty acid synthase

[b] *PDCA* Pancreatic ductal cell carcinoma, *CRC* colorectal cancer, *NSCLC* non-small cell lung cancer

[c] *HBP* hexosamine biosynthesis pathway, *PPP* pentose phosphate pathway

to convert glutamine-derived glutamate into α-ketoglutarate to fuel the TCA cycle, PDCA cells metabolize glutamine through an unconventional pathway in which glutamine is converted to non-essential amino acids, such as aspartate by glutamate–oxaloacetate transaminase 2 (GOT2). Glutamine-derived aspartate is converted into oxaloacetate by aspartate transaminase (GOT1) in the cytoplasm, then converted into malate, and finally converted into pyruvate, resulting in the production of NADPH to maintain the cellular redox balance. In the PDCA mouse model, mutated *KRAS* affects the reprogramming of glutamine metabolism by downregulating GLUD1 and upregulating GOT1. Importantly, interfering with glutamine metabolism by blocking aspartate transaminase or enzymes downstream could suppress tumor growth of *KRAS*-driven PDCA. Regarding the glutamine metabolism in PDCA, Wang et al. have reported that arginine methylation at R248 of malate dehydrogenase 1 (MDH1), which is catalyzed by CARM1, regulates glutamine metabolism and redox homeostasis of PDCA cells [23]. Importantly, R248 methylation of MDH1 is observed to be downregulated in clinical PDCA samples.

The increased requirement for amino acids is a very early phenomenon during tumor development. Mayers et al. reported that metabolic reprogramming to provide cancer cells with branched-chain amino acids (BCAAs) preceded PDCA diagnosis by about 5 years and that elevated plasma levels of three BCAAs (isoleucine, leucine, and valine) were associated with future diagnosis of PDCA [24]. They also recently reported that *KRAS*-driven NSCLC depended on BCAA metabolism, whereas *KRAS*-driven PDCA did not, indicating the tissue of origin can affect the metabolic dependencies of tumors driven by the same genetic events [25]. NSCLC tumors have an increased expression of the BCAA transporter (*Slc7a5*) as well as BCAA catabolic enzymes (*Bcat2* and *Bckdh*), which enables BCAAs to be utilized as a nitrogen source, whereas PDCA tumors exhibit decreased expression of these genes.

Autophagy

Autophagy is a highly conserved mechanism which degrades intracellular components and promotes cell survival under metabolic stress by providing energy in the form of ATP and building blocks such as amino acids, lipids, sugars, and nucleotides [15]. While it appears that PDCA cells depend on autophagy for growth, the relationship between oncogenic *KRAS* and autophagy remains unclear. Genetic or pharmacological inhibition of

autophagy results in increased levels of reactive oxygen species (ROS), elevated DNA damage, and mitochondrial defects that have been shown to lead to decreased proliferation of PDCA cell lines in vitro as well as substantial tumor regression in vivo in PDCA mouse models [26, 27]. A recent study with a panel of 47 different cancer cell lines indicated that the *KRAS*-mutant cells used were no more dependent on autophagy than their wild-type counterparts [28]. Therefore, the specific role of *KRAS* mutations on autophagy remains controversial in PDCA. Clinical trials are under way in various cancers to test whether inhibiting both autophagy and macropinocytosis can compromise tumor growth by hydroxychloroquine, an inhibitor of lysosomal function. There have been some early reports on the efficacy of hydroxychloroquine in various types of cancer, but the results have been mixed [29–32].

Macropinocytosis

More recently, it has become apparent that *KRAS*-driven cancers acquire additional means to satisfy their nutritional needs, such as through fluid-phase endocytic uptake by macropinocytosis and self-cannibalization by autophagy. PDCA cells specifically harboring mutated *KRAS* utilize macropinocyotsis to transport extracellular protein into the cell [16] where it is used as a source of essential amino acids to sustain cell survival and proliferation [33]. The uptake of serum albumin by macropinocytosis provides amino acids, particularly glutamine, for multiple metabolic pathways, including TCA cycle anaplerosis and macromolecular synthesis. In addition to albumin, *RAS*-transformed mammalian cells take up exogenous lipids to provide cells with FAs, thereby decreasing the need for de novo synthesis [34]. Preclinical studies in which heterotopic xenografts-bearing mice were treated with a compound 5-(*N*-ethyl-*N*-isopropyl) amiloride, an inhibitor of macropinocytosis, showed an attenuation of tumor growth [16].

Colorectal cancer (CRC)

Carcinogenesis of CRC is caused by genetic mutations in various genes, such as *APC*, *KRAS*, *p53*, *SMAD4*, and *PTEN*, as well as by the epigenetic silencing of tumor suppressor genes [35]. *KRAS* mutations occur in approximately 40% of CRCs, and a number of studies have shown that *KRAS* mutations in CRC predict a lack of responses to therapies with antibodies targeting the epidermal growth factor receptor (EGFR) [36, 37]. Cetuximab and panitumumab, which are anti-EGFR monoclonal antibodies, are now recommended only for patients whose tumors have wild-type *KRAS*.

The international CRC Subtyping Consortium shares 18 large-scale data sets and has revealed that CRCs can be classified into four consensus molecular subtypes (CMSs), each with distinct features: CMS1 (hypermutated, microsatellite instability, and strong immune activation), CMS2 (epithelial, WNT and MYC activation), CMS3 (metabolic dysregulation) and CMS4 (transforming growth factor beta activation, stromal invasion, and angiogenesis) [38]. Notably, CMS3 is characterized by prominent metabolic dysregulation and is strongly correlated with *KRAS* mutations, which may suggest that the targeted intervention to the metabolic abnormalities is especially promising for *KRAS*-mutant CRCs.

Glucose metabolism

There are only a few reports on *KRAS*-related metabolic alterations in CRCs. Yun et al. reported particularly interesting data showing that the increase in GLUT1 expression and glucose uptake was critically dependent on *KRAS* and *BRAF* mutations in CRC cell lines and that this metabolic alteration provided a distinct survival advantage because CRC cells with mutated *KRAS* or *BRAF* were able to survive long-term in low-glucose culture environments [39]. In CRC cells with mutated *KRAS* or *BRAF*, increased glucose transport was associated with increased lactate production, although mitochondrial function and oxidative respiration were not affected [39]. Importantly, 3-bromopyruvate (3-BrPA), a glycolysis inhibitor, was highly effective on the xenografts that were derived from CRC cells with mutated *KRAS* or *BRAF* [39]. These results provide a proof of principle that glycolytic inhibitors can retard tumor growth at doses that are non-toxic to normal tissues.

Positron emission tomography with ^{18}F-fluorodeoxyglucose (FDG) is a diagnostic tool routinely used to detect cancers in the clinical setting. Using this technique clinicians are able to evaluate glucose metabolism in vivo by measuring the uptake of FDG, a glucose analog. Although FDG accumulation in tumor cells largely depends on GLUT1 and the rate-limiting glycolytic enzyme hexokinase type-2 (HK2), several recent studies on CRCs have reported that increased GLUT1 expression is the most essential factor for FDG accumulation [40]. In a retrospective study with primary and metastatic CRCs, we previously reported that FDG accumulation by *KRAS*-mutant CRCs was significantly higher than that by CRCs with wild-type *KRAS* [41–43]. There is also emerging evidence from other groups that FDG accumulation reflects the mutational status of *KRAS* in CRC and NSCLC [44–47]. In terms of the underlying mechanisms behind these clinical observations, we have recently shown that mutated *KRAS* causes higher FDG accumulation, possibly by upregulating GLUT1 and

at least partially by upregulating hypoxia-inducible factor 1-alpha (HIF-1α) induction under hypoxic condition [48].

Yun et al. recently reported that high levels of vitamin C were selectively toxic to CRCs with mutated KRAS or BRAF because the increased uptake of oxidized vitamin C via elevated GLUT1 expression disrupted redox homeostasis by depleting glutathione [49]. Accumulation of ROS mediated by an increased uptake of the oxidized vitamin C inhibited glycolysis at the level of glyceraldehyde 3-phosphate dehydrogenase, which led to an energetic crisis and ultimately cell death [49]. Regarding the antitumoral mechanism of vitamin C in *KRAS*-mutant CRC, Aguilera et al. have recently reported that vitamin C interferes with the downstream RAS/ERK pathway by facilitating the detachment of KRAS protein from the cell membrane, and then downregulates expression of GLUT1 and pyruvate kinase M2, which results in an energetic crisis [50]. Overall, these results provide a mechanistic rationale for the therapeutic use of vitamin C for *KRAS*-mutant CRCs.

Amino acid metabolism

Our group recently performed a comprehensive metabolomics analysis of isogenic CRC cell lines harboring mutated *KRAS* and wild-type *KRAS* and observed that *KRAS* mutations in CRC can cause alterations in amino acid metabolism [51]. These alterations are especially prominent in glutamine metabolism, where a marked decrease in aspartate level and an increase in asparagine level were observed [51]. We also found that asparagine synthetase (ASNS), the enzyme that synthesizes asparagine de novo from aspartate, was upregulated by the KRAS-activated signaling pathway, in particular by the PI3/K-AKT/mTOR pathway, and that *KRAS*-mutant CRC cells could become adaptive to glutamine depletion through asparagine biosynthesis synthesized via ASNS. Importantly, tumor growth in vivo of *KRAS*-mutant CRC cells was significantly suppressed upon ASNS knockdown, indicating that ASNS could be a novel therapeutic target. We also observed that mutated *KRAS* did not alter the expression of GLUD1 or GOT1 in CRC, although mutated *KRAS* has been reported to cause a decrease in GLUD1 and an increase in GOT1 in PDCA [22], which indicates the multifaceted roles of mutated *KRAS* in metabolism are cell type-dependent. In human glioma and neuroblastoma, asparagine plays a critical role in regulating cellular adaptation to glutamine depletion [52]. Asparagine is an essential component to suppress glutamine-withdrawal-induced apoptosis without restoring other non-essential amino acids or TCA cycle intermediates, and ASNS expression is statistically correlated with poor prognosis of glioma and neuroblastoma patients. Hettner et al. recently reported that ASNS silencing had the strongest inhibitory effect on sarcoma growth in a functional genomic short hairpin RNA (shRNA)-based screening of genetically engineered mouse sarcoma generated by oncogenic *KRAS* and disruption of *Cdkn2a* [53]. These authors also observed that ASNS inhibition significantly inhibited sarcoma growth in vivo only when combined with the depletion of plasma asparagine, which indicates that asparagine can promote cellular adaptation to metabolic stress such as glutamine depletion. ASNS is activated by mutated p53, protein limitation, and tumor microenvironmental stress [54, 55]. Although normal pancreatic tissues express high levels of ASNS, approximately half of PDCA express no ASNS or only low ASNS levels. These tumors may harbor an intrinsic fragility to asparagine depletion that could be exploited by L-asparaginase therapy [56]. Potent ASNS inhibitors has also been developed as new drugs for L-asparaginase-resistant acute lymphoblastic leukemia [57, 58]. The addition of asparagine to glutamine-deprived cells alters the transcriptional response, thereby suppressing the induction of apoptotic regulators of the UPR effectors CHOP and XBP1 [59]. Furthermore, a recent report indicates that intracellular asparagine promotes cancer cell proliferation as an exchange factor of extracellular amino acids (serine, arginine, and histidine), which is involved in the synthesis of proteins and nucleotides [59]. Taken together, these results suggest that asparagine may play a central role as an important regulator of cancer cell amino acid homeostasis, anabolic metabolism, and cell proliferation.

There is also emerging evidence from other research groups that *KRAS* mutations in CRC are associated with glutamine metabolism. Weinberg et al. reported that PPP, not glycolysis, was essential for *KRAS*-induced CRC cell growth under the aerobic condition and that glutamine conversion into the TCA cycle intermediate α-ketoglutarate via glutaminase and alanine aminotransferase was essential for *KRAS*-induced anchorage-independent growth in vitro [60]. Wong et al. have recently reported that CRC cells harboring mutations in *KRAS* and *APC/CTNNB1* are selectively sensitive to knockdown of the mitochondrial glutamine transporter SLC25A22 and that he knockdown of SLC25A22 suppresses glutaminolysis and aspartate biosynthesis via the TCA cycle, leading to apoptosis and cell cycle arrest [61]. In terms of its clinical significance, SLC25A22 overexpression is associated with poor prognosis in patients harboring *KRAS* mutations. Miyo et al. have very recently reported that the resistance to glucose-deprived conditions in CRC is associated with the levels of GLUD1 and SLC25A13 (a mitochondrial aspartate–glutamate carrier) and that combined expression of GLUD1 and SLC25A13 is significantly associated with tumor aggressiveness and poorer prognosis of patients with CRC [62]. Previous studies have demonstrated that mutated *KRAS* controls the reprogramming of glutamine metabolism by decreasing

GLUD1 and increasing GOT1 in the PDCA mouse model [22]. An important question remains as to whether these novel glutamine pathways are active in all *KRAS*-mutant tumors or if they are specific to PDCAs with *KRAS* mutations. Recent data on CRCs from our group [51] and other research groups [61, 62] suggest the latter whereby this specificity may not be a global property of all tumor types harboring *KRAS* mutations. The role of glutamine transporters, such as ASCT2 and LAT1, in cancer is also under investigation as part of the new era in the discovery of novel anticancer drugs [63, 64].

Non-small cell lung cancer (NSCLC)

NSCLC is a leading cause of cancer-related deaths worldwide, and *KRAS* mutations occur in approximately 30% of NSCLC [65]. In the vast majority of cases, *KRAS* mutations for *EGFR* or *ALK* are found in wild-type tumors; in other words, these mutations are non-overlapping with other oncogenic mutations found in NSCLC. Therefore, *KRAS* mutations define a distinct molecular subset of the disease. Unlike in CRC, *KRAS* mutations have not yet been shown in NSCLC to be negative predictors of a benefit of the anti-EGFR therapy. Recently, a systematic approach with a pool-based shRNA screening revealed that a combination of trametinib, a MEK inhibitor, plus fibroblast growth factor receptor (FGFR) inhibitor could be a promising strategy for treating *KRAS*-mutant NSCLC [66].

Glucose metabolism

Hexokinases catalyze the first committed step of glucose metabolism. HK2 was identified as an attractive target for *KRAS*-driven NSCLC and ErbB2-driven breast cancer because systemic whole-body deletion of *HK2* in these mouse models inhibited tumor initiation and maintenance [67]. HK2 was highly expressed in cancer cells and not in the normal cells, thus allowing for selective targeting of cancer cells. *HK2* deletion in *KRAS*-driven NSCLC cells suppressed glucose-derived ribonucleotide synthesis and impaired the incorporation of glutamine-derived carbon into TCA cycle intermediates [67].

Amino acid metabolism

Weinberg et al. reported that glutamine conversion into the TCA cycle intermediate α-ketoglutarate allowed the generation of ROS, which was required for *KRAS*-induced tumorigenicity through the ERK-MAPK pathway in the *KRAS*-driven NSCLC mouse model [60]. The role of other amino acids, such as serine and glycine, in the metabolism of *KRAS*-driven cancers remains to be investigated, although serine and glycine metabolism has recently been reported to play a key role in cancer cell proliferation and survival [68, 69].

Autophagy

In a mouse model of spontaneous *KRAS*^{G12D}-driven NSCLC, knockout of *Atg7*, an essential autophagy gene, caused the accumulation of dysfunctional mitochondria, suppressed tumor growth, and diverted the progression of carcinomas to more benign oncocytomas [70]. Autophagy deficiency in *KRAS*^{G12D}-driven NSCLC cells enhanced the dependence on glutamine, indicating that protein degradation by autophagy supplies amino acids and their derivatives to metabolic pathways, of which glutamine is partially critical [70].

Lipogenesis

Fatty acids are fundamental cellular components used for post-translational protein modification and energy generation through β-oxydation. De novo FA synthesis involves several enzymes: ATP citrate lyase generates acetyl-CoA from citrate; acetyl-CoA carboxylase catalyzes carboxylation of acetyl-CoA to form malonyl-CoA; fatty acid synthase (FASN) adds 2-carbon units to form long-chain FAs; acyl-CoA synthetase (ACS) converts long-chain FAs into acyl-CoA. The metabolism of FAs is emerging as a mechanism to cope with *KRAS* mutations. For example, mutated *KRAS* stimulates scavenging unsaturated FAs from lysophospholipids under hypoxic conditions in mammalian cells [34]. Padanad et al. have recently reported that mutated *KRAS* regulates lipid biosynthesis through ACS long-chain family member 3 (ACSL3) and that ACSL3 promotes cellular uptake, accumulation and β-oxydation of FAs, which is required for lung tumorigenesis in the *KRAS*-driven NSCLC mouse model [71]. Gouw et al. have recently reported that mutated *KRAS* activates lipogenesis through induction of FASN, which results in ERK2 activation and lipid signatures associated with human lung cancer cell lines [72].

Conclusions

Since cancer cells have distinct metabolic requirements that differ from those of their normal counterparts, they may be more reliant on specific fuel sources, thus providing unique therapeutic targets. However, there is still much work to be done to fully realize the potential of these approaches. It is likely that *KRAS* mutations have tissue-specific effects on

metabolism due to the intrinsic metabolic wiring in the tissue of origin of a particular tumor. It is also important to incorporate the tumor suppressor background when studying the effects of *KRAS* mutations on metabolism. In addition, more studies are required to investigate how these oncogenic *KRAS*-dependent metabolic changes are altered in vivo in the tumor microenvironment, such as hypoxia, limited nutrients, and crosstalk between tumor cells and stromal cells. Davidson et al. recently reported that *KRAS*-driven NSCLC cells in vitro use nutrients differently than lung tumors in vivo, especially with regard to glutamine metabolism; KRAS-driven lung tumors were less dependent on glutaminase than cultured cells [73]. This difference highlights the importance of studying cancer metabolism in a physiological context. About 20% of human tumors have mutations in *RAS*, most frequently in *KRAS* (85%), *NRAS* (15%), and *HRAS* (1%). Although about 20% of melanoma and about 2–4% of CRC have mutations in *NRAS*, little is known about the metabolic alterations of mutated *NRAS* signaling [74, 75].

In conclusion, we have summarized and highlighted the recently defined role of oncogenic *KRAS* in the regulation of altered metabolic signaling pathways in *KRAS*-driven cancers.

Compliance with ethical standards

Conflict of interest There are no potential conflicts of interest to be disclosed.

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