The metabolic landscape of RAS-driven cancers from biology to therapy

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Our understanding of how the RAS protein family, and in particular mutant KRAS, promotes metabolic dysregulation in cancer cells has advanced substantially over the last decade. In this Review, we discuss the metabolic reprogramming mediated by oncogenic RAS in cancer and elucidate the underlying mechanisms that could translate to novel therapeutic opportunities to target metabolic vulnerabilities in RAS-driven cancers.

The RAS family of proto-oncogenes, including KRAS, NRAS and HRAS, encodes a group of small GTPases that are activated in response to growth factors and other extracellular stimuli and induce downstream signaling cascades, such as the mitogen-activated protein kinase (MAPK) pathway. When mutated, oncogenic RAS remains preferentially in the active, GTP-bound state and GTP hydrolysis by its GTPase function and enzymes such as GTPase-activating proteins is compromised. The resulting state of the RAS protein family, and in particular, is among the most frequently mutated oncogenes in cancer, and its mutation is commonly associated with therapeutic resistance and poor prognosis. As a critical cancer driver, RAS has been the focus of an intensive search for therapies. However, no effective RAS inhibitor has been approved for clinical use thus far. Recent preclinical and early clinical results on the efficacy of inhibitors against the KRAS-G12C mutant sparked excitement in the scientific community. However, the initial enthusiasm has been somewhat tempered by work suggesting that acquired resistance may constrain the efficacy of the inhibitors, indicating that combination therapies may be needed.

The difficulty in targeting RAS has fueled a long-standing interest in identifying alternative approaches for treating RAS-driven cancers, efforts that have been supported by increased understanding of RAS biology. It is now clear that the roles of oncogenic RAS extend far beyond its classic function of activating MAPK pathways. The links of RAS signaling to altered cellular metabolism are of particular interest in cancer research, given the potential to leverage RAS-related metabolic vulnerabilities to treat RAS-driven cancers. Here we discuss the biology that connects RAS to metabolic dysregulation in cancer and evaluate the possibility of exploiting these connections for drug discovery and therapy.

Mutation of RAS genes in cancer

Cancers that harbor mutations in RAS genes make up a heterogeneous subset of all cancers, with the frequency of mutations in each isoform and the specific mutations varying greatly across different cancer types. Most mutations of RAS family members occur at codons 12, 13 and 61, although the mutation frequency for each residue and isoform varies for cancer types that originate from different tissues. For instance, nearly 24% of oncogenic mutations in lung adenocarcinoma occur in KRAS, whereas only 1.4% of such mutations occur in NRAS in skin cutaneous melanoma. The variation in substitution type is also striking. For instance, the G12C substitution is dominant in lung adenocarcinoma, whereas G12D is dominant in pancreatic adenocarcinoma. Overall, KRAS is mutated in many cancers (predominantly adenocarcinomas), whereas NRAS mutations are prominent in melanoma and myeloid cancers. RAS is mutated relatively infrequently; when mutations do occur, they are primarily in bladder and head and neck squamous cell carcinomas. These observations indicate a fundamental difference in the biological effects of specific mutations on different RAS isoforms and in different tissues. Consequently, treatment efficacy cannot be extrapolated from one RAS-driven cancer to another, but rather therapeutic approaches must be tailored to the isoform, mutation and tissue. Altogether, despite a greater understanding of the RAS signaling cascade’s complexity, fundamental questions remain concerning the role of different oncogenic RAS mutations affecting different isoforms in patients with cancer.

RAS and tumor metabolism

The reprogramming of cellular metabolism to support the energetic and biomass needs of uncontrolled proliferation is a hallmark of cancer. Use of fluorodeoxyglucose (FDG) positron emission tomography (PET) imaging as a way to stage cancer and of antimetabolites as chemotherapeutic agents in treating several cancers further underscores the clinical importance of tumor metabolism. Recognition that oncogenes, including RAS genes, can promote aerobic glycolysis, commonly known as the Warburg effect, and activate anabolic pathways has increased efforts to understand the molecular underpinnings of altered metabolism in cancer. In the subsequent sections, we discuss the
various manners in which oncogenic RAS reprograms metabolism, how these adaptations result in tumor-specific metabolic alterations that in turn modulate oncogenic signaling networks and how these metabolic changes may be targeted therapeutically. We focus primarily on KRAS, given the wealth of literature on this major oncogenic driver, and note the roles of other isoforms where these are known.

Interplay between oncogenic RAS and glucose metabolism. Altered glucose metabolism, for example, through the Warburg effect, is one of the most common metabolic changes differentiating normal and cancer cells. The breakdown of glucose through glycolysis, in addition to generating ATP, produces metabolic intermediates such as amino acids and precursors for fatty acids and nucleotides that are required for cell growth and proliferation (Fig. 2).

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Fig. 1 | Frequency and distribution of mutations in RAS genes in human cancers. Human cancers differ by the RAS isoform mutated, the codon mutated and the resulting amino acid substitution. a, Distribution and frequency of mutations by RAS isoform (KRAS, NRAS and HRAS) across tumor types. Death rate per year (%) is based on the death rate per 100,000 men and women. Detailed information is provided in Supplementary Table 1. b, Types of cancer commonly associated with mutations in RAS genes. For each tumor type, the frequency of mutations in the most commonly mutated RAS genes is listed together with the most frequent amino acid substitutions. Detailed information is provided in Supplementary Tables 2 and 3. All human cancer types that had a sample size greater than or equal to 300 and a total RAS mutation rate greater than or equal to 15% are shown. Death rate data were collected from the National Cancer Institute’s SEER cancer statistics 2020 database.
Mutant KRAS is involved in glucose metabolism in multiple ways. Gene expression and metabolic flux analyses have shown that it upregulates expression of the GLUT1 glucose transporter to promote glucose uptake by cells, as well as inducing expression of hexokinase 1 and 2 (HK1 and HK2), rate-limiting enzymes of glycolysis, to increase glycolytic activity27–30 (Fig. 2). One recent study reported a role for the KRAS4A isoform of KRAS in carbon metabolism through direct regulation of the glycolytic enzyme HK1, which is of interest as it demonstrates direct GTP-dependent regulation through direct regulation of the glycolytic enzyme HK1, which

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Highly glycolytic RAS-mutant cells have been found to be vulnerable to inhibition of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with vitamin C, providing a mechanistic rationale for exploring the therapeutic use of this vitamin in KRAS- or BRAF-mutant colorectal cancer preclinical models37. Additionally, oncogenic HRAS mediates enhanced glycolysis rates, including increased glucose uptake, underscoring the fact that increased aerobic glycolysis is essential for RAS-mutant tumors to match energy production with the requirement for enhanced biosynthetic pathways38. Moreover, glycolytic KRAS-mutant cells produce increased amounts of potentially toxic byproducts of glycolysis such as methylglyoxal39. Methylglyoxal-mediated stress was shown to be involved in cancer progression40 and to be a potent activator of AKT signaling, suggesting that use of methylglyoxal scavengers in KRAS-mutant colorectal cancer cells might be more effective when combined with AKT inhibitors41. Mutant KRAS has also been implicated in induction of enzymes involved in the folate cycle42 and aberrant activation of mTOR, a key regulator of both serine synthesis and the folate cycle43,44. Furthermore, the tumor suppressor LKB1, which activates the energy sensor and metabolic regulator AMPK, has been linked to serine metabolism and induction of tumorigenesis45. Of note, LKB1 loss is prevalent in KRAS-mutant lung cancers46, indicating that oncogenic KRAS not only induces mTOR activity, but might also upregulate one-carbon metabolism by undermining the inhibitory role of AMPK in the folate cycle. However, systematic investigation is required to explore the role of oncogenic KRAS in one-carbon metabolism in detail.

The interplay between oncogenic RAS and glycolysis provides a rationale for targeting glycolysis in RAS-driven cancers. A number of natural or synthetic products, including inhibitors of GLUT1–GLUT4, have been discovered over the years and validated through various preclinical cancer models before clinical trials47. A promising candidate is silybin, a natural flavonoid and potent inhibitor of GLUT1 and GLUT4, which was shown to be effective in a phase 1 trial of prostate cancer, with asymptotic liver toxicity as an adverse effect48. BAY-876, a potent GLUT1 inhibitor49, was separately shown
to be an effective candidate in the preclinical setting\(^{46}\). Several other compounds have been found to have inhibitory properties against glycolytic enzymes, and some have been included in clinical trials\(^{94,95}\). Although preclinical studies support the effectiveness of these small molecule inhibitors, in-depth study is warranted to explore their true therapeutic and clinical potential. Additionally, toxicity and target specificity are a major concern for any drug, and it is nontrivial to specifically inhibit these glycolytic enzymes while avoiding unwanted effects on normal cells. Further study is essential to identify potent inhibitors targeting glycolysis that would specifically impair RAS-driven cancer growth.

**Oncogenic RAS in glutaminolysis and redox homeostasis.** The nonessential amino acid glutamine is the most abundant amino acid in human serum and is necessary for cellular function and survival. Breakdown of glutamine through glutaminolysis gives rise to glutamate, a critical precursor of most other nonessential amino acids, including aspartate, alanine, arginine and proline\(^{27}\). Thus, in addition to its central role in nucleotide and protein production, glutamine-derived carbon in the form of glutamate can be an important anaplerotic substrate for the tricarboxylic acid (TCA) cycle (Fig. 2). The process of anaplerosis replenishes metabolic intermediates removed from the TCA cycle, such as citrate, thereby increasing their availability for fatty acid and cholesterol biosynthesis\(^{9}\). Glutamine is also a major source of nitrogen for proliferating cells\(^{40,42}\).

Many tumors driven by oncogenic KRAS and its downstream effector, the transcription factor MYC, exhibit metabolic reprogramming to consume and rely more on glutamine for both catalytic and anabolic pathways\(^{31,52}\). Oncogenic KRAS elevates the gene expression of enzymes involved in glutaminolysis\(^{41}\). For instance, KRAS-dependent upregulation of glutamate oxaloacetate transaminases 1 and 2 (GOT1 and GOT2) in pancreatic cancer facilitates production of aspartate for nucleotide biosynthesis and allows NADPH generation via malic enzyme 1 (ME1)\(^{37,66}\) (Fig. 2). In addition to activating the GOT2–GOT1–ME1 pathway, oncogenic KRAS activates the NRF2 antioxidant system by inducing NRF2 expression\(^{47,49}\) and by constitutively activating the battery of genes controlled by NRF2 to maintain the redox balance and promote tumorigenesis\(^{9,46}\). Activation of NRF2 causes glutamine dependence in KRAS-mutant lung and pancreatic cancer cells and preclinical models\(^{31,57,60}\), and BRAF mutants can similarly activate NRF2 to promote reactive oxygen species (ROS) detoxification\(^{49}\) (Fig. 2). Oncogenic KRAS maintains reduced glutathione pools by mediating signaling through the GOT2–GOT1–ME1 and NRF2 antioxidant pathways. However, KRAS has also been shown to promote cancer cell growth by stimulating alanine aminotransferase activity, leading to high levels of α-ketoglutarate for the TCA cycle and mitochondrial ROS generation, which was required for mutant KRAS-driven tumorigenesis in a mouse model of lung cancer\(^{44}\).

Glutamate metabolism is also being investigated as a therapeutic target\(^{44}\). Although no clinical-grade inhibitors for the GOT2–GOT1–ME1 axis currently exist, the dependency of certain KRAS-driven cancers, such as pancreatic cancer\(^{46}\) and lung cancer\(^{56}\), on glutamine can be exploited by targeting glutaminase-1 (GLS1), the enzyme that restricts glutamine’s conversion to glutamate and its anaplerotic entry into the TCA cycle. Limiting glutamine use in combination with chemotherapy is a viable means to halt pancreatic cancer tumor growth in preclinical mouse models and is not toxic to normal cells\(^{97,98}\). Separately, loss of LKB1 in a KRAS-mutant non-small-cell lung cancer preclinical mouse model was found to promote NRF2-dependent metabolic alterations that increased the tumor cells’ dependence on glutamine and created a vulnerability to glutaminase inhibition\(^{46}\). Additionally, mutations in the KEAP1 gene, which encodes a negative regulator of NRF2, could point the way to treating lung adenocarcinoma driven by oncogenic KRAS. Cells from advanced lung tumors with oncogenic KRAS and loss-of-function Keap1 mutations were more dependent on increased amounts of glutamine than other cells, making them more susceptible to glutaminase inhibition\(^{42}\). KRAS activation is also commonly coupled with loss of LKB1 function. Co-occurrence of mutant KRAS and LKB1 deficiency in patients with lung cancer resulted in more aggressive tumors, a higher frequency of metastasis and therapy resistance\(^{45}\). This could be explained by the fact that loss of LKB1 sustains KRAS-mediated proliferation through autophagy and increases synthesis of essential macromolecules, even under nutrient-deprived conditions\(^{46}\). Moreover, in oncogenic KRAS-driven lung adenocarcinoma, loss of LKB1 often induces KEAP1 activation\(^{49}\) and leads to metabolic alterations that could be counteracted by activation of NRF2 (ref. \(^{49}\)), thereby maintaining redox homeostasis and fueling energy metabolism in a glutamine-dependent manner. Thus, cancer cells harboring KRAS, KEAP1 and LKB1 mutations may be more sensitive to glutaminase inhibition than their normal counterparts\(^{46}\). Concurrent mutations in KRAS and LKB1 also confer vulnerabilities in pancreatic cancer, but the mechanisms are different from those in lung cancer\(^{45}\). In pancreatic cancer, such concurrent mutations support tumor growth by activating serine synthesis and increasing DNA methylation. Moreover, KRAS-driven lung adenocarcinomas with TP53 mutations induce immune cell production, while tumors with KEAP1 mutations rewire metabolism\(^{47}\). Exploiting these context-specific properties, either by depleting the immune cells in tumor tissues or by perturbing the altered metabolism, could be effective in inhibiting tumor progression. This suggests that, rather than a one-size-fits-all approach to therapy, individualized precision therapies based on co-occurring mutations could be more effective for patients with KRAS-driven cancers\(^{46}\). Thinking more broadly about the interplay of metabolism with RAS signaling, targeting glutamine metabolism has also been found to suppress acquired resistance to MAPK inhibitors in melanoma cells\(^{48}\).

However, environmental factors may also come into play, as, for instance, the availability of extracellular cystine, as discussed below, can influence the dependence of cancer cells on glutamine metabolism\(^{53}\). In line with this, not all KRAS-driven tumors are sensitive to inhibition of glutamine metabolism in vivo\(^{49,50}\), indicating that a deeper understanding is required of the context in which KRAS-driven cancers would be most sensitive to agents that target this metabolic pathway.

xCT, the cysteine/glutamate antiporter that exports glutamate to the extracellular space and imports cysteine into the cytosol for production of the amino acid cysteine, has been shown to be essential for oncogenic KRAS-mediated transformation and to be involved in intracellular redox homeostasis\(^{50}\). Cysteine import is key to the survival of KRAS-driven pancreatic ducal adenocarcinoma cells, as deprivation of cysteine or xCT inhibition was shown to cause cells to undergo ferroptosis\(^{50}\), an iron-dependent form of programmed cell death characterized by a lethal buildup of lipid peroxides\(^{51}\). Moreover, NRF2 enhances xCT activity to mediate glutathione synthesis\(^{51,52}\) and also regulates the activity of glutathione peroxidase 4 (GPX4), an enzyme that lies downstream of xCT and is involved in metabolic processing in ferroptosis\(^{53}\). In line with the known links of KRAS to NRF2, glutamine limitation was shown to induce pro-ferroptotic stimuli, including inhibition of GPX4, in KRAS-mutant pancreatic cancer cells\(^{54}\), suggesting that RAS-mutant cancer cells displaying high levels of glutaminolysis might be more susceptible to ferroptosis. Although KRAS-driven pancreatic tumors depend on cysteine metabolism to prevent ROS-induced ferroptosis, making cysteine depletion a potentially useful clinical strategy\(^{55}\), it is unclear whether ferroptosis can be selectively activated in all RAS-driven tumors. In-depth studies of the roles of oncogenic KRAS in cysteine metabolism are needed to determine possible therapeutic approaches.
Lipid metabolism and fatty acid biosynthesis in RAS-mutant cancers. Lipids, including fatty acids, are an energy source in addition to glucose and glutamine, and proliferating cancer cells aberrantly activate lipid biosynthesis. RAS-transformed cells depend on serum lipids for proliferation and survival. Under metabolic stress, certain RAS-driven cancer cells stimulate uptake of lysophospholipids, which they use to support ATP production. Oncogenic KRAS activates downstream signaling through AKT for eventual activation of the ACLY enzyme, to enhance the conversion of citrate to acetyl-CoA and increase de novo fatty acid and sterol biosynthesis (Fig. 2). Furthermore, KRAS reprograms lipid homeostasis to support tumorigenesis by upregulating ACSL3, an enzyme involved in lipid synthesis. In line with these findings, mutant KRAS drives a lipogenic gene expression program to promote de novo lipogenesis and activates lipogenesis by inducing expression of FASN, which can be exploited therapeutically (Fig. 2). Inhibiting fatty acid oxidation in a mouse model of KRAS-driven pancreatic cancer was shown to reduce tumor recurrence, suggesting potential therapeutic value in targeting lipid metabolism in RAS-driven cancers.

Recycling pathways and nutrient scavenging in RAS-mutant cancers. Oncogenic RAS-driven tumor cells develop distinct mechanisms to scavenge nutrients from extracellular sources and recycle intracellular fuel to provide metabolic flexibility and secure adequate nutrient availability (Fig. 2). One such process is autophagy, the regulated degradation and recycling of cellular components that is activated by starvation and stress and provides energy and building blocks, such as amino acids, nucleotides, lipids and sugars, necessary for cellular survival and organelle homeostasis. The role of autophagy in cancer is complex and context dependent, but this process is known to be elevated in cancer cells harboring KRAS mutations and is required for tumor maintenance and cellular viability. The nexus between oncogenic KRAS and autophagy is also sustained by increasing the glycolytic rate and supporting mitochondrial respiration. In particular, basal autophagy has been shown to be elevated in KRAS-driven pancreatic cancers, where it provides nutrients to fuel the TCA cycle necessary for cell growth and survival. Unlike normal cells, those harboring KRAS mutations upregulate basal autophagy by activating the MiT/TFE transcription program. Separately, autophagic deficiency in KRAS- and BRAF-mutant cancers is known to enhance glutamine dependence, suggesting that autophagic protein degradation supplies cancer cells with certain amino acids required for metabolic pathways, including glutamine. Blocking autophagy in a mutant RAS setting can deplete glutamine and block fatty acid consumption, which compromises tumor growth. This concept further suggests that inhibiting downstream effectors of KRAS in the MAPK pathway can upregulate autophagic flux, potentially as a metabolic adaptation of compromised mitochondrial activity. Thus, combinatorial inhibition of MAPK effectors and autophagy was shown to reduce KRAS-driven tumor growth in preclinical mouse models of pancreatic cancer.

Additionally, oncogenic KRAS upregulates mitophagy, a selective form of autophagy that clears damaged mitochondria and improves mitochondrial function under conditions of nutrient deficiency. Mutant KRAS stimulates a mitophagy receptor called NIX, leading to decreased mitochondrial metabolism and a shift toward glycolysis to stimulate cell proliferation and strengthen redox homeostasis. Given these findings, it may be worth exploring mitophagy as a target for RAS-driven metabolic malignancies.

Although autophagy can produce diverse nutrients, it cannot increase the cell's net biomass. To fuel elevated metabolic needs, KRAS-mutant tumors rely on lysosome-dependent macropinocytosis, the process in which cells nonspecifically engulf material from the extracellular space. For instance, RAS-stimulated macropinocytosis was shown to promote cellular uptake of extracellular albumin, followed by its degradation into amino acids (particularly glutamine) that could then enter the anaplerotic TCA cycle (Fig. 2). Whereas oncogenic RAS enhances macropinocytosis, the process is initiated by growth factor-induced phosphoinositide 3-kinase (PI3K) signaling. In this context, the KRAS-G12R mutant was shown to be impaired in PI3K signaling and macropinocytosis, whereas the KRAS-G12D and KRAS-G12V mutants relied on MYC to drive macropinocytosis in preclinical mouse models of pancreatic cancer. These mutant-specific effects indicate that further exploration is needed to elucidate how macropinocytosis and KRAS activity are interlinked and whether such allele-specific nutrient supply is active in other tumor types.

Although there is no clinically approved selective macropinocytosis inhibitor, EIPA, an inhibitor of Na+/H+ exchange, reportedly inhibits macropinocytosis and sensitizes KRAS-mutant cells to the mTOR inhibitor rapamycin. Moreover, the vacuolar ATPase, a transmembrane protein complex that transduces protons across cellular and organelle membranes, is essential for RAS-mediated macropinocytosis. HRAS-G12V or KRAS-G12V expression redistributed vacuolar ATPase from the cytoplasm to the plasma membrane of lung, pancreatic and colon cancer cells, raising the possibility that blocking macropinocytosis by targeting this complex may represent a new strategy to treat RAS-mutant cancers.

Given that RAS-driven tumors activate nutrient-scavenging pathways, such as autophagy and macropinocytosis, targeting these processes represents an interesting therapeutic approach, especially as non-cancer cells are less likely to rely on these metabolic alterations. For example, both autophagy and macropinocytosis involve the lysosome, suggesting that lysosome inhibitors may inhibit both these pathways to restrict RAS-mutant tumor growth, although this concept remains to be tested experimentally. In addition, further work will determine whether autophagy and/or macropinocytosis inhibition could be combined with established therapeutic approaches, such as chemotherapies.

Oncogenic RAS, metabolism and therapy resistance. Oncogenic KRAS mutations have been associated with reduced sensitivity to therapeutic agents. For example, patients with KRAS-mutant lung cancer had poor clinical outcomes following combined treatment with the epidermal growth factor receptor (EGFR) inhibitor erlotinib and chemotherapy. KRAS-dependent fibrosarcoma, colon and bladder cancer cell lines were also shown to become resistant to radiation therapy. Both PI3K- and RAF-dependent, but MAPK kinase (MEK)-independent, signaling pathways have been suggested to underlie this KRAS-mediated radioresistance in epithelial cells. Consequently, targeting KRAS-mediated signaling could lead to the activation of compensatory pathways, resulting in adaptive resistance to therapies. In line with this, most therapies induce ROS in cancer cells, with treatment-resistant tumors often developing ROS-inhibitory mechanisms or mechanisms that rely on ROS to sustain proliferation. For example, ROS generated through mitochondrial metabolism were shown to be required for KRAS-induced anchorage-independent growth and to be essential for cell proliferation and tumorigenesis in KRAS-driven mouse lung adenocarcinoma. Separately, oncogenic KRAS was found to require ROS to promote the development of precursor lesions in pancreatic ductal adenocarcinoma, as well as pancreatic intraepithelial neoplasia. Moreover, oncogenic RAS-induced ROS were shown to be produced in a Rac1- and NADPH oxidase (Nox4)-dependent manner in a zebrafish model system, leading to hyperproliferation and activation of DNA damage response pathways. Thus, although mutant KRAS signaling reportedly leads to genotoxic stress stemming from ROS generation, oncogenic KRAS can reprogram metabolism to favor glutathione biosynthesis and increase NADPH production. This may protect macromolecules from indiscriminate damage incurred from ROS.
Various drugs with a direct or indirect effect on ROS metabolism are now in clinical trials testing whether targeting tumor cell antioxidant capacity is an effective therapy. In summary, redox management may modulate tumor cell progression and therapeutic responses in RAS-mutant cancers.

KRAS-mutant tumor cells also shield themselves from the effects of stress stimuli and chemotherapy by promoting stress granule (SG) formation through production of the 15d-PGJ2 prostaglandin. 15d-PGJ2 is in turn responsible for NRF2 activation and SG accumulation, which could contribute to the...
ties. More specifically, RAS-driven cancer cells appear to function to support proliferation and presenting therapeutic opportunities. For instance, detailed analysis of protein and genetic interactions in the RAS-driven pathway identified links between metabolic enzymes and oncogenic RAS, opening up a new avenue for potential RAS therapeutics. Additionally, combining proximity-dependent proteomics with CRISPR screening identified a new set of functional RAS-associated proteins and several previously unrecognized enzymatic dynamics.

Targeting oncogenic RAS-related metabolism

As discussed in the previous sections, pleiotropic metabolic changes are among the primary downstream events of oncogenic KRAS expression, indicating that tumorigenesis progresses owing to key oncogenic signaling that promotes metabolic adaptations to support proliferation and presenting therapeutic opportunities. More specifically, RAS-driven cancer cells appear to function optimally when the nutrient supply is favorable but undergo rapid bioenergetic collapse when starved of glucose or glutamine because their demands for energy cannot be met in the absence of sustained glycolysis or glutaminolysis, the major mechanisms that fuel energy production. The limited tolerance of malignant cells for metabolic imbalance creates a vulnerability that could be exploited with drugs targeting tumor metabolism. In this setting, conditions of limited nutrient availability would imbalance the ratio of energy produced per nutrient consumed, thereby leading to alterations in bioenergetic dynamics.

Table 1: Select clinical studies with novel agents targeting metabolism in RAS-driven cancer

| Disease | Biomarker | Therapy | Phase | Study identifier | Status |
|---------|-----------|---------|-------|-----------------|--------|
| Lung cancer: small cell or squamous | HRAS, KRAS and NRAS mutations | Auranofin, sirolimus | Phase 1/2 | NCT01737502 | Recruiting |
| Metastatic pancreatic carcinoma, stage II, III, IV pancreatic cancer, unresectable pancreatic carcinoma | KRAS mutations, exclusion criteria: glucose-6-phosphate dehydrogenase (G6PD) deficiency | Trametinib, hydroxychloroquine | Phase 1 | NCT03825289 | Recruiting |
| Metastatic pancreatic adenocarcinoma, stage IV pancreatic cancer | KRAS mutations | Hydroxychloroquine, binimetinib | Phase 1 | NCT04132505 | Recruiting |
| Lung cancer: non-small-cell lung carcinoma | KRAS mutations | CB-839, docetaxel | Phase 1 | NCT02071862 | Completed |
| Colon carcinoma | KRAS and BRAF mutations | Carbohydrate-restricted diet, vitamin C supplement | Phase 1/2 | NCT04035096 | Not yet recruiting |
| Metastatic melanoma | NRAS mutations | Trametinib, hydroxychloroquine | Phase 1b/2 | NCT03979651 | Recruiting |
| Non-small-cell lung carcinoma, colorectal carcinoma | KRAS mutations | CB-839, palbociclib | Phase 1/2 | NCT03965845 | Recruiting |
| Gastrointestinal adenocarcinoma | MAPK mutations: KRAS, NRAS, HRAS, BRAF (non-V600), MEK and ERK mutations | Ulixertinib, hydroxychloroquine | Phase 1/2 | NCT04145297 | Recruiting |
| Pancreatic cancer: metastatic adenocarcinoma | Mutant KRAS | Hydroxychloroquine, gemcitabine | Phase 1/2 | NCT01506973 | Active, not recruiting |
| Non-squamous-cell lung cancer | Wild-type and mutant KRAS | AZD2014, AZD6244 | Phase 1/2 | NCT02583542 | Active, not recruiting |
| Colon cancer | KRAS and BRAF mutation status | TVB-2640 | Phase 1 | NCT02980029 | Recruiting |
| Breast cancer, endometrial cancer, lung cancer, colorectal cancer, head and neck cancer | KRAS mutations | Serabelisib, canagliflozin | Phase 1/2 | NCT04073680 | Not yet recruiting |
| Metastatic colorectal cancer | Wild-type RAS | CB-839, panitumumab, irinotecan | Phase 1/2 | NCT03263429 | Recruiting |
| Colorectal cancer | Wild-type RAS | BAY94-9392, [1C]glutamine | Phase 1 | NCT03275974 | Recruiting |

Data are from My Cancer Genome and the ClinicalTrials.gov database.
direct RAS effectors, including metabolism-associated proteins, paving the way for exploration of potential combinatorial therapies targeting the KRAS effector pathway, RAS-driven metabolic enzymes or other RAS-mediated metabolic adaptations, including nutrient-scavenging and stress response pathways (Fig. 3a). As discussed further below, various signaling pathways often exist upstream of metabolic processes to generate a common metabolic end product, and surging evidence suggests that genetic alterations are associated with specific rewired oncogenic metabolic pathways, supporting the idea of using several drugs to target metabolism for a particular disease. Combining agents to target complementary metabolic pathways might be a suitable strategy for reducing the dose of individual drugs and eliminating unwanted toxicity levels in normal cells.

The successful development of potent inhibitors against KRAS-G12C, which have progressed to clinical trials, now makes it possible to explore combinations of a RAS inhibitor with metabolism-focused treatment strategies. Indeed, one of the KRAS-G12C inhibitors, MRTX849, has revealed potential resistance pathways that include the involvement of NRF2 in MRTX849 resistance, suggesting that a monotherapy approach might not work against RAS-driven cancers and that combinatorial therapies with mTOR, SHP2 or CDK4/CDK6 inhibitors will be necessary. As discussed, targeting metabolic enzymes has proven effective in some KRAS-mutant cancer cell lines and mouse models with certain KRAS mutants, and several other pharmacological inhibitors targeting dysregulated cancer metabolism are under development or in different preclinical stages. If successful, such approaches could be combined with RAS inhibitors. Some metabolic pathway inhibitors, including against mTOR, have already been tested in combination with MRTX849 in a preclinical setting, with encouraging results. In addition, several clinical trials targeting metabolic dysregulations in RAS-mutant cancers are underway, including strategies against glutaminolysis and autophagy (Table 1).

However, much work is still needed to fully explore the therapeutic potential of targeting altered metabolism in RAS-driven cancers. Among the complexities that require detailed study are the tissue-specific effects on metabolism; therefore, multiple strategies must be developed and matched to the mutant RAS subsets. In line with this, there is considerable variation in glutamine dependencies across tissues based on their origin, and various KRAS mutations can have different dependencies for reasons that are as yet unclear. In vivo, tumors also display variability in their glutamine dependencies when compared to cell culture findings, underscoring the importance of using appropriate model systems to draw firm conclusions. Furthermore, various reports assessing the transcriptomic, proteomic, phosphor-proteomic and metabolic profiles of oncogenic RAS variants, including HRAS, NRAS and KRAS isoforms, indicate possible differences among their phenotypic effects. Nevertheless, the metabolic landscapes of HRAS- and NRAS-driven cancers remain less explored, and it will be important to discover whether they resemble that of KRAS-driven tumors and whether different metabolic adaptations predominate in different tissue contexts for all RAS isoforms.

The use of metabolic therapies may have some advantages over other approaches. Such therapies could offer heightened specificity given that tumor cells appear to be more sensitive to metabolic inhibitors than their normal counterparts. The success with chemotherapies targeting metabolism intensifies hope that more metabolic therapies will ultimately reach the clinic. However, there are some limitations in targeting metabolism for therapeutic purposes. Chief among them is the metabolic flexibility of cancer cells, which can often switch their source of nutrients and energy and activate compensatory metabolic pathways for survival when there is limitation in their favored metabolic pathways or they are deprived of a preferred metabolic source. This adaptive nature of cancer cells might limit the efficacy of targeting a single metabolic pathway for therapeutic purposes, a concern that could be addressed by combinatorial therapeutic approaches against multiple pathways, including known compensatory ones. Another major challenge in the development of drugs against cellular metabolism is the unwanted toxicity created by the effects of agents targeting metabolic enzymes in normal cells. Of particular concern is the dependency of immune cells on metabolic pathways similar to those utilized by tumor cells, which would make them vulnerable to the toxicity created by targeting metabolic pathways. Because affecting the metabolic processes of immune cells could potentially affect not only their antitumor activities but also the organism’s broader immune defenses, a detailed understanding of immunometabolism would be crucial in guiding the development and use of targeted therapies based on cancer metabolism. Although toxicity would limit the use of drugs in some cases, the fact that a therapeutic window may exist for many patients supports the notion that metabolic enzymes are attractive targets for cancer therapy. Better understanding of metabolic dependencies in specific tumor tissues, their links to different oncogenic alterations and signaling pathways, and potential toxicities of targeted approaches is key for defining metabolism’s prospects for improving the therapeutic index.

Connecting RAS to other oncogenic drivers and metabolic pathways. Oncogene-directed metabolic alterations can have extensive impact, with multiple metabolic pathways altered simultaneously in a single cancer type. Several oncogenes coordinate the transcriptional reprogramming that tumor cells need to thrive, with many cancer driver genes also perturbing metabolism. Thus, to target oncogenic RAS and its downstream signaling and metabolic programs effectively, it is important to also understand how these are linked to other oncogenic drivers and pathways.

Analysis of data from the Pan-Cancer Analysis of Whole Genomes Consortium using the KEGG pathway database mapped oncogenic driver genes to various metabolic processes in many cancer tissue types (Fig. 4a), with gene-level analysis of each corresponding
pathway unveiling driver genes that are simultaneously altered in diverse tissue types (Fig. 4b). Future work should focus on investigating the potential cross-talk between RAS and commonly mutated driver genes known to be involved in metabolic and RAS signaling pathways in diverse tumor types, also taking into account that tumors from different tissues may display divergent metabolic phenotypes irrespective of their genomic profile. Network analysis of several genes with driver mutations that regulate signaling and metabolic pathways in KRAS-driven cancers, including KMT2D\textsuperscript{137}, PIK3CA\textsuperscript{138}, PTEN\textsuperscript{139} and IDH1 (ref. \textsuperscript{140}), connected them to oncogenic RAS (Fig. 4c). This analysis suggests a hypothetical view of a broader transcriptional and signaling circuit coordinated by RAS together with PI3KCA, NF1 and...
PTEN for the tight regulation of metabolic pathways. For example, KRAS could signal through p73 (ref. 149) or even Src (150), to alter the function of PTEN, which may act through AKT to regulate KMT2D, through AP-1 (ref. 141) to regulate KMT2C or through p300 (ref. 141) to regulate the lipogenesis enzyme ACACA. KMT2D and KMT2C may in turn regulate MYC, SOX2 or CTNNB1 to control other metabolism driver genes, including GBA, IDH1, PTSS1, POLE and ACACA. Many of these circuits may feed back to NF1 to influence the RAS gene directly (151) or impact the RAS protein through a feedback loop from MYC (142), SOX2 (ref. 149) or CTNNB1 (ref. 143). Alternatively, KRAS may directly regulate PIK3CA (152), which impacts CTNNB1 for subsequent regulation of many metabolism driver genes. Various levels of interplay are known to exist between the PI3K–AKT and KRAS–MAPK signaling cascades (153), including for potential therapy. For example, using a combination of PI3K and MEK inhibitors has been shown to be effective in treating KRAS-driven lung cancers in mouse models (154). However, detailed study is needed to understand precisely how these two pathways and the other factors depicted in the broader signaling circuit (Fig. 4c) coordinate with each other for the metabolic rewiring needed to sustain uncontrolled proliferation in cancer (155). Such work will be instrumental for developing novel targeted therapies in RAS-driven cancers.

Conclusion and future perspectives

Although substantial progress has been made in unraveling the role of oncogenic RAS in metabolic pathways, many open questions remain about the links between RAS biology and metabolic dysregulation. For instance, the complex interplay between KRAS isoforms, oncogenic RAS alleles, tissues of origin for tumors and metabolic alterations is not well understood. The crucial question of whether RAS-mediated alterations to metabolic pathways are common to all RAS-driven cancers remains unanswered. Moreover, the connections between RAS and other oncogenic drivers and the signaling and metabolic processes they each control also require attention. Elucidation of these events should also take into account the fact that metabolic phenotype is not uniform across different tumor types, with variability also existing between different tumors of the same type. A deeper understanding of these complexities, combined with the renewed excitement around targeting oncogenic RAS, will pave the way for the development of well-tolerated and effective therapies for patients with RAS-driven cancer.

Reporting Summary.

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

For Fig. 1 and Supplementary Tables 1–3, genome-wide cancer mutation data were compiled from databases and public resources, including AACR Genie (release 6.1-public) (148), COSMIC (v90) (149), cbioPortal (151,152), the TCGA Research Network (https://www.cancer.gov/tcga) and NCI’s Genomic Data Commons (GDC) (153), that are openly accessible to the public and are cited in the paper. The data sets derived from these resources that support the analyses and discussion presented in this article are available in the cited references. For Fig. 4a,b, previously published cancer driver mutation data were acquired from refs. (134,135) and ICGC/TCGA via controlled access through rigorous application and are available from these resources.

Code availability

For Fig. 4, genes with driver mutations were applied to an in-house pathway pattern extraction pipeline (PPEP) tool described in ref. (154) and implemented in customized R scripts (https://www.r-project.org/). PPEP and corresponding databases (WPS version 2) can be downloaded from the WPS homepage. This tool represents a pathway-based platform for discovery integration to maximize analysis power. The tool (155) can be made available on request from the corresponding author.

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Author contributions

All authors conceived of the article, performed literature searches, integrated the information and wrote, discussed and edited the manuscript.

Competing interests

The authors are aware of no direct conflicts with the topic of the paper; however, M.G.V.H. is a scientific advisory board member for Agios Pharmaceuticals, Aeglea Biotherapeutics, iTeos Therapeutics, Faeth Therapeutics and Auron Therapeutics. F.M. is a consultant for the following companies: Amgen, Daiichi, Ideaya Biosciences, Kura Oncology, Leidos Biomedical Research, PellePharm, Pfizer, PMV Pharma and Quanta Therapeutics. F.M. is a consultant and co-founder for the following companies (with ownership interest including stock options): BridgeBio, DNAtrix, Olema Pharmaceuticals and Quartet. F.M. is the scientific director of the NCI RAS Initiative at the Frederick National Laboratory for Cancer Research/Leidos Biomedical Research. None of these affiliations represents a conflict of interest with respect to this paper.

Additional information

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Software and code

Policy information about availability of computer code

Data collection

Frequency and distribution of RAS mutations in human cancers: Genome-wide cancer mutation data was compiled from databases and acquired from different public resources—AACR Genie (Release 6.1-public)105, COSMIC (v90)106, and cBioPortal107,108 (including legacy TCGA studies, PanCancer Atlas, and data sets of published studies curated from literature up to November 2020)—for various tumor types. Expected mortality rate (%) is based on estimated new case incidence values from the National Cancer Institute SEER cancer statistics 2020 database.

Comprehensive distribution of oncogenic driver genes in metabolic pathways: Cancer driver mutation data were acquired from results in a) Sabarinathan, R. et al., doi:10.1101/190330 (2017) and b) Consortium, I. T. P.-C. A. o. W. G. Pan-cancer analysis of whole genomes. Nature 578, 82-93, doi:10.1038/s41586-020-1969-6 (2020) and downloaded from the International Cancer Genome Consortium/ The Cancer Genome Atlas via controlled access through rigorous application.

Proposed Ras' connection with oncogenic driver genes of metabolic pathways: KRAS, other top driver genes in the Ras pathway, and the top driver genes in KEGG metabolism pathways in (Fig 3b) were used as seeds to retrieve their direct relations with each other or their indirect relations with other genes from the MetaCore™ database.

Data analysis

Distribution of RAS isoform (KRAS, NRAS, and HRAS) mutations across tumor types and the frequency (%) of the RAS mutation by isoform in each tumor type. Detailed information is in Supplementary Table 1, 2, 3. All human cancers that had a sample size greater than or equal to 300 and a total RAS mutation rate greater than or equal to 15% are listed from data resources.

Pathway-level heatmap showing four KEGG composite metabolic pathways with the most hits of cancer driver mutation genes involved in various metabolic processes. Values are list hits or numbers of cancer driver mutation genes from the tissue types involved in the composite
metabolic pathways from the KEGG database. Gene-level heatmaps showing the three most common driver genes across tumor types for the KEGG metabolic pathways and the RAS signaling pathway. Driver mutation genes were applied to an in-house PPEP tool described in (Yi, M., Mudunuri, U., Che, A. & Stephens, R. M. Seeking unique and common biological themes in multiple gene lists or datasets: pathway pattern extraction pipeline for pathway-level comparative analysis. BMC Bioinformatics 10, 200, doi:10.1186/1471-2105-10-200 (2009)) and implemented in customized R scripts ([www.r-project.org](http://www.r-project.org)). In brief, pathway-level heatmaps were derived with different color ranges with a maximum color threshold at two to show the list size or number of genes from each tissue type involved in the composite metabolic pathways from the KEGG database ([described at www.genome.jp/kegg/pathway.html#metabolism](http://www.genome.jp/kegg/pathway.html#metabolism)). Red shows the number of driver genes from each tissue involved in each corresponding pathway. Gene-level heatmaps were derived for each corresponding pathway to show actual driver mutation genes from the tissue types. The driver genes involved in each metabolic pathway are plotted for each tissue.

With Clarivate Analytics' network-building tool ([https://clarivate.com/cortellis](https://clarivate.com/cortellis)), proposed RAS' connection with oncogenic driver genes of metabolic pathways were built into a relation network by using a shortest-path algorithm with a maximum number of two steps in the path, the goal being to maximize their connections as much as the algorithm setting allowed. The lines or edges between the gene nodes in the network indicate interaction either genetically or physically supported by published studies with assessed high confidence. Green lines indicate positive/activation relations, red lines indicate negative/inhibition relations, and gray lines indicate unspecified relations. The arrows indicate the relations' directions. The nodes with blue circles are the original seed genes, whereas other genes were added based on evidence to help connect these genes, if needed.

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