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## The Metabolic Landscape of RAS-Driven Cancers from biology to therapy

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### Abstract

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

### Keywords

KRAS; metabolism; autophagy; glutaminolysis; glycolysis; macropinocytosis; chemoresistance; ferroptosis; cancer therapeutics

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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

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## Introduction

The RAS family proto-oncogenes *KRAS*, *NRAS*, and *HRAS* encode 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 MAPK pathway. When mutated, oncogenic RAS remains preferentially in the active GTP-bound state, whereas GTP hydrolysis by its GTPase function and enzymes such as GTPase-activating proteins, is compromised<sup>1,2</sup>. The resulting RAS-mediated signaling cascades drive tumor initiation, maintenance and progression by deregulating key cellular processes, for instance by increasing proliferation and suppressing apoptosis, but also by rewiring cellular metabolism and promoting alterations in the tumor microenvironment<sup>3</sup>.

Given that RAS dysregulation may cause aberrant cellular signaling and malignant transformation, the activation of the RAS signaling pathway is tightly controlled in normal cells<sup>1</sup>. However, *RAS* mutations and the resulting deregulated signaling events are responsible for one third of all human cancers<sup>4</sup> (Figure 1). *KRAS* in particular, is among the most frequently mutated oncogenes in cancer and is commonly associated with therapeutic resistance and poor prognosis<sup>1,5,6</sup>. Being a critical cancer driver, RAS has been the focus of an intensive search for therapies<sup>7</sup>. However, no effective RAS inhibitor has been approved for clinical use to date. Recent preclinical and early clinical results on the efficacy of inhibitors against the *KRAS* G12C mutant<sup>8–10</sup>, sparked excitement in the scientific community. However, the initial enthusiasm has been somewhat tempered by work suggesting that acquired resistance may constrain the inhibitors' efficacy, which indicates that combination therapies may be needed<sup>11–14</sup>.

The difficulty in targeting RAS has fueled a longstanding interest in identifying alternative approaches for treating RAS-driven cancers, efforts that have been supported by our 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.

## RAS mutations in cancer

Cancers that harbor RAS mutations comprise a heterogeneous subset of all cancers, with the frequency of each mutant isoform and the specific mutation varying greatly across different cancer types (Fig. 1a and 1b). Most mutations of *RAS* family members occur at codons 12, 13, and 61, although the mutation frequency at each residue and isoform varies between cancer types that originate from different tissues. For instance, 22% of oncogenic mutations in lung adenocarcinoma (LUAD) occur in *KRAS*, whereas nearly 24% of such mutations in skin cutaneous melanoma occur in *NRAS*<sup>15</sup> (Fig. 1b). The variation in substitution type is also striking. For instance, the G12C substitution is dominant in LUAD, whereas G12D is dominant in pancreatic adenocarcinoma (PDAC) (Fig. 1b). Overall, *KRAS* is mutated in many cancers (predominantly adenocarcinomas), whereas *NRAS* mutations are prominent in

melanoma and myeloid cancer<sup>4,15,16</sup> (Fig. 1). HRAS is mutated relatively infrequently, but when mutations do occur, they are primarily in bladder and head/neck squamous cell carcinoma. 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 and isoforms on 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<sup>17</sup>. The use of FDG-PET imaging as a way to stage cancer<sup>18,19</sup> and of antimetabolites as chemotherapeutic agents<sup>20</sup> in treating several cancers, further underscore the clinical importance of tumor metabolism<sup>21</sup>. The recognition that oncogenes, including RAS, can promote aerobic glycolysis, commonly known as the Warburg effect, and activate anabolic pathways<sup>22,23</sup>, has increased efforts to understand the molecular underpinnings of altered metabolism in cancer<sup>24</sup>. 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<sup>25,26</sup>, and how they 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. In addition to producing ATP, the breakdown of glucose through glycolysis produces metabolic intermediates, such as amino acids, and precursors for fatty acids and nucleotides that are required for cell growth and proliferation (Fig. 2a).

Mutant KRAS is involved in glucose metabolism in multiple ways. Gene expression and metabolic flux analyses have shown that it upregulates the 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 activity<sup>27–30</sup> (Fig. 2a). One recent study reported the role of KRAS4A KRAS isoform in carbon metabolism through the direct regulation of the glycolytic enzyme HK1, which is of interest as it identifies the direct GTP-dependent regulation of a metabolic enzyme<sup>29</sup> and further complicates the landscape of RAS mediated metabolism. Oncogenic KRAS also upregulates expression of other key glycolytic enzymes, such as PFK1, ENO1, and LDHA<sup>28,31–33</sup>, thereby promoting glycolytic flux and enabling the production of glycolytic intermediates that can be shunted into other anabolic pathways (Fig 2). KRAS also promotes the hexosamine biosynthesis pathway (HBP), which provides precursors for lipid and protein glycosylation<sup>34</sup>, and the non-oxidative arm of the pentose phosphate pathway

(PPP)<sup>28</sup>, which supplies ribose, the backbone for nucleic acid production<sup>35</sup> (Fig 2). KRAS regulates these pathways through MAPK-dependent signaling cascades, ultimately supporting cell survival and conferring a proliferative advantage on tumors<sup>28,36,37</sup>.

Highly glycolytic RAS-mutant cells have been found to be vulnerable to inhibition of the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with Vitamin C, providing a mechanistic rationale for exploring the therapeutic use of this vitamin against KRAS or BRAF mutant colorectal cancer preclinical models<sup>38</sup>. Additionally, oncogenic HRAS mediates enhanced glycolytic 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 pathways<sup>39</sup>. Moreover, glycolytic KRAS-mutant cells produce increased amounts of potentially toxic byproducts of glycolysis such as methylglyoxal<sup>40</sup>. Methylglyoxal-mediated stress was shown to be involved in cancer progression<sup>41</sup> and to be a potent activator of AKT signaling, suggesting that utilizing methylglyoxal scavengers in KRAS mutant colorectal cancer cells might be more effective when combined with AKT inhibitors<sup>40</sup>. Mutant KRAS has also been implicated in the induction of enzymes involved in the folate cycle<sup>42</sup> and the aberrant activation of mTOR, a key regulator for both serine synthesis and the folate cycle<sup>43,44</sup>. Furthermore, the tumor suppressor LKB1, which activates the energy sensor and metabolic regulator AMPK, has been linked to serine metabolism and induction of tumorigenesis<sup>45</sup>. Of note, LKB1 loss is prevalent in KRAS mutant lung cancers<sup>46</sup>, indicating that oncogenic KRAS not only induces mTOR activity, but might also upregulate one-carbon metabolism by undermining AMPK's inhibitory role 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 GLUT1–4, have been discovered over the years and validated through various pre-clinical cancer models before clinical trials<sup>47</sup>. A promising candidate is silybin, a natural flavonoid and potent inhibitor of GLUT1 and GLUT4, which was shown to be effective in phase I trial of prostate cancer with asymptotic liver toxicity as an adverse effect<sup>48</sup>. BAY-876, a potent GLUT1 inhibitor<sup>49</sup>, was separately shown as an effective candidate in pre-clinical setting<sup>50</sup>. Several other compounds have been found to have inhibitory properties against glycolytic enzymes and some have been included in clinical trials<sup>47,51</sup>. 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 non-trivial 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 sera and is necessary for cellular function and survival. The breakdown of glutamine through

glutaminolysis gives rise to glutamate, a critical precursor of most other nonessential amino acids, including aspartate, alanine, arginine, and proline<sup>52</sup>. 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. 2a). 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<sup>53</sup>. Glutamine is also a major source of nitrogen for proliferating cells<sup>52,54</sup>.

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 catabolic to anabolic pathways<sup>55,56</sup>. Oncogenic KRAS elevates the gene expression of enzymes involved in glutaminolysis<sup>27</sup>. For instance, KRAS-dependent upregulation of Glutamate Oxaloacetate Transaminase 1,2. (GOT) expression in pancreatic cancer facilitates production of aspartate for nucleotide biosynthesis, and allows NADPH generation via ME1<sup>57,58</sup> (Fig 2). In addition to activating the GOT2/GOT1/Malic Enzyme 1 (ME1) pathway, oncogenic KRAS activates the NRF2 antioxidant system by inducing NRF2 expression<sup>59,60</sup> and by constitutively activating the battery of genes controlled by NRF2 to maintain the redox balance and promote tumorigenesis<sup>61</sup>. The activation of NRF2 causes glutamine dependence in KRAS mutant lung and pancreatic cancers cells and preclinical mouse models<sup>60,62,63</sup>, and BRAF mutants can similarly activate NRF2 to promote reactive oxygen species (ROS) detoxification<sup>59</sup>. (Fig 2). Oncogenic KRAS maintains reduced glutathione pools by mediating GOT1,2/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  $\alpha$ -ketoglutarate for the TCA cycle and mitochondrial ROS generation, which was required for mutant KRAS-driven tumorigenesis in a mouse model of lung cancer<sup>64</sup>.

Glutamate metabolism is also being investigated as a therapeutic target<sup>54</sup>. Although no clinical-grade inhibitors for the GOT/ME1 axis currently exist, the dependency of certain KRAS-driven cancers, such as pancreatic cancer<sup>60</sup>, lung cancer<sup>62</sup>, on glutamine could 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 combined with chemotherapy is a viable means to halt pancreatic tumor growth in preclinical mouse models and is not toxic to normal cells<sup>58,60,65</sup>. Separately, the loss of LKB1 in mutant KRAS 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<sup>66</sup>. Additionally, mutations in the KEAP1 gene, which encodes a negative regulator of the NRF2, could point the way to treating lung adenocarcinoma, which is 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<sup>62</sup>. 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, higher frequency of metastasis, and therapy resistance<sup>67</sup>. This could be explained by the fact that loss of LKB1 sustains KRAS-mediated proliferation through autophagy and increased synthesis of essential

macromolecules, even under nutrient-deprived conditions<sup>68</sup>. Moreover, in oncogenic-KRAS-driven lung adenocarcinoma, loss of LKB1 often induces KEAP1 activation<sup>69</sup> and leads to metabolic alterations that could be counteracted by activation of NRF2<sup>70</sup>, 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 compared to normal counterparts<sup>66</sup>. Concurrent mutations in KRAS and LKB1 also confer vulnerabilities in pancreatic cancer, but the mechanisms are different from those in lung cancer<sup>45</sup>. In pancreatic cancer, such concurrent mutations support tumor growth by activating serine synthesis and increasing DNA methylation. Moreover, KRAS-driven lung adenocarcinomas with TP53 mutation induce immune cell production, while tumors with KEAP1 mutations rewire metabolism<sup>71</sup>. Exploiting these context-specific properties, either by depleting the immune cells in tumor tissues or by perturbing the altered metabolism, which could be effective in inhibiting tumor progression. This suggests that rather than considering 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<sup>71</sup>. 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<sup>72</sup>. However, environmental factors may also come into play, as for instance, the availability of extracellular as discussed below, can influence the dependence of cancer cells on glutamine metabolism<sup>73</sup>. Consistently, not all KRAS tumors are sensitive to inhibition of glutamine metabolism *in vivo*<sup>74,75</sup>, 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 cystine/glutamate antiporter that exports glutamate to the extracellular space and imports cysteine into the cytosol for the production of the amino acid cysteine, has been shown to be essential for oncogenic-KRAS-mediated transformation and involved in intracellular redox balancing<sup>76</sup>. Cystine import is key to KRAS-driven PDAC cell survival as deprivation of cysteine or xCT inhibition were shown to undergo ferroptosis<sup>77</sup>, an iron-dependent form of programmed cell death characterized by a lethal buildup of lipid peroxides<sup>78</sup>. Moreover, NRF2 enhances xCT activity to mediate glutathione synthesis<sup>60,63,73</sup> and also regulates glutathione peroxidase 4 (GPX4) activity, an enzyme that lies downstream of xCT and is involved in metabolic processing of ferroptosis<sup>78</sup>. In line with the known links of KRAS to NRF2, glutamine limitation was shown to induce pro-ferroptotic stimuli, including GPX4 inhibition, in KRAS mutant pancreatic cancer cells<sup>60</sup>, suggesting mutant RAS 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<sup>77</sup>, 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 mutants

Lipids, including fatty acids, are an energy source in addition to glucose and glutamine, and proliferating cancer cells aberrantly activate lipid biosynthesis<sup>79</sup>. RAS-transformed cells depend on serum lipids for proliferation and survival<sup>80</sup>. Under metabolic stress, certain RAS-driven cancer cells stimulate lysophospholipid uptake and use it to support ATP production<sup>81</sup>. Oncogenic KRAS activates downstream signaling through AKT for the eventual activation of the ACLY enzyme, to enhance the conversion of citrate to acetyl-CoA, and increase *de novo* fatty acid and sterol biosynthesis<sup>82</sup> (Fig. 2a). Furthermore, KRAS reprograms lipid homeostasis to support tumorigenesis by upregulating ACSL3, an enzyme involved in lipid synthesis<sup>83</sup>. In line with these findings, mutant KRAS drives a lipogenic gene-expression program to promote *de novo* lipogenesis and activates lipogenesis by inducing FASN expression, which can be exploited therapeutically<sup>84,85</sup> (Fig 2). Inhibiting fatty acid oxidation in a mouse model of KRAS-driven pancreatic cancer was shown to reduce tumor recurrence<sup>86</sup>, suggesting potential therapeutic value in targeting lipid metabolism in RAS-driven cancers.

## Recycling pathways and nutrient scavenging in RAS mutants

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. 2a). One such process is autophagy<sup>87</sup>, 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<sup>88,89</sup>. The nexus between oncogenic KRAS and autophagy is also sustained by increasing the glycolytic rate<sup>90</sup> and supporting mitochondrial respiration<sup>91</sup>. 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<sup>88</sup>. Unlike normal cells, those harboring KRAS mutations upregulate basal autophagy by activating the MiT/TFE transcription program<sup>92</sup>. 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<sup>93</sup>. 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<sup>90,94,95</sup>.

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<sup>96</sup>. 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 non-specifically engulf material from the extracellular space<sup>93</sup>. 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<sup>97</sup> (Fig 2). Whereas oncogenic RAS enhances macropinocytosis, the process is initiated by growth factor-induced PI3K signaling<sup>98</sup>. In this context, the KRASG12R mutant was shown to be impaired in PI3K signaling and macropinocytosis, whereas the KRASG12D and G12V mutants relied on MYC to drive micropinocytosis in preclinical mouse models of pancreatic cancer<sup>99</sup>. 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<sup>+</sup>/H<sup>+</sup> exchange, reportedly inhibits macropinocytosis and sensitizes KRAS mutants to the mTOR inhibitor rapamycin<sup>80,98</sup>. Moreover, the vacuolar ATPase, a transmembrane protein complex that transduces protons across cellular and organellar membranes, is essential for RAS-mediated macropinocytosis. HRASG12V or KRASG12V 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<sup>100</sup>.

Given that RAS-driven tumors activate nutrient-scavenging pathways, such as autophagy and micropinocytosis, targeting these processes represents an interesting therapeutic approach<sup>93</sup>, 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 mutant-RAS tumor growth, although this concept remains to be determined 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<sup>101,102</sup>. For example, patients with mutant KRAS lung cancer had poor clinical outcomes following combined treatment with the EGFR inhibitor erlotinib and chemotherapy<sup>102</sup>. KRAS-dependent fibrosarcoma, colon and bladder cancer cell lines were also shown to become resistant to radiation therapy<sup>103,104</sup>. Both PI3K- and RAF-dependent, but MEK-independent signaling pathways have been suggested to underlie this KRAS-mediated radio-resistance in epithelial cells<sup>101</sup>. Consequently, targeting KRAS-mediated signaling can lead to the activation of compensatory pathways<sup>37,86,105</sup> 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<sup>106</sup> or mechanisms that rely on ROS to sustain proliferation. For example, ROS generated through mitochondrial metabolism was 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<sup>64</sup>. Separately, oncogenic KRAS was found to require ROS to promote the development of PDAC precursor lesions such as pancreatic intraepithelial neoplasia<sup>107</sup>. Moreover, oncogenic RAS-induced ROS was shown to be produced in a RAC1 and NADPH oxidase (Nox4)-dependent manner in zebrafish model system, leading to hyperproliferation and activation of DNA damage response pathways<sup>108</sup>. Thus, although mutant KRAS signaling reportedly leads to genotoxic stress stemming from ROS generation<sup>108</sup>, oncogenic KRAS can reprogram metabolism to favor glutathione biosynthesis and increase NADPH production. This may protect macromolecules from indiscriminate damage incurred by ROS (Fig. 2a). 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<sup>109</sup>. In summary, redox management may modulate tumor cell progression and therapeutic responses in RAS mutants.

Mutant KRAS tumor cells also shield themselves from the effects of stress stimuli and chemotherapy by promoting stress granule (SG) formation<sup>110</sup> through the production of the 15-d-PGJ2 prostaglandin. 15-d-PGJ2 is in turn responsible for NRF2 activation<sup>111</sup> and SG accumulation<sup>110</sup>, which could contribute to the ability of oncogenic KRAS cells to resist chemotherapy<sup>60,110</sup> (Fig 2). However, glutamine restriction was shown to reduce the capacity of 15-d-PGJ2 to form SGs in chemotherapy-treated KRAS-driven PDAC cells<sup>60</sup>, thereby limiting the cytoprotection this pathway provides against chemotherapy-induced stress stimuli. This connection of the KRAS-NRF2-SG axis and glutaminolysis suggests a potential approach to counteract mutant KRAS-mediated drug resistance. However, given that the process of SG accumulation is incompletely understood, elucidating the precise underlying mechanisms and metabolic links in RAS-driven cancer will determine the feasibility of SG-based therapeutic strategies. Taken together, targeting RAS-dependent metabolic alterations might be useful to counteract drug resistance and inhibit tumor growth.

## Targeting oncogenic RAS-related metabolism

As discussed in the previous sections, pleiotropic metabolic changes are among the primary events downstream of oncogenic KRAS expression (Fig. 3a), indicating that tumorigenesis progresses due to key oncogenic signaling that promotes metabolic adaptations to support proliferation<sup>112</sup> and present therapeutic opportunities. More specifically, RAS-driven cancer cells appear to function optimally when 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 (Fig 3b).

Potential metabolic targets of signaling pathways for precision therapy have been documented previously<sup>22</sup>, and the advancing research on the roles of mutant RAS on cancer metabolism, either through direct effects on metabolic enzymes and pathways as discussed above, or through the actions of downstream RAS effectors<sup>29,113–117</sup> provide many routes to explore 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<sup>114,116</sup>. Additionally, combining proximity-dependent proteomics with CRISPR screening identified a new set of functional RAS-associated proteins and several previously unrecognized direct RAS effectors, including metabolism-associated proteins, paving a way for the exploration of potential combinatorial therapies targeting the KRAS-effector pathway, RAS-mediated metabolic enzymes<sup>115</sup>, or other RAS-driven metabolic adaptations, including nutrient scavenging and stress response pathways (Fig 3a). As discussed further below, various signaling pathways often exist upstream metabolic processes to generate a common metabolic end product<sup>112</sup> and surging evidence suggests that genetic alterations are associated with specific rewired oncogenic metabolic pathways<sup>118</sup>, which supports the idea of using several drugs to target metabolism for a particular disease. Combining agents to target complimentary 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 KRASG12C, which have progressed to clinical trials<sup>8,9</sup>, now makes it possible to explore combinations of a RAS inhibitor with metabolism-focused treatment strategies. Indeed, one of the KRASG12C inhibitors, MRTX849, has revealed the 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 or SHP2 or CDK4/6 inhibitors will be necessary<sup>9</sup>. As discussed, targeting metabolic enzymes has proven effective in some mutant KRAS cancer cell lines and mouse models from certain KRAS mutants<sup>60,62,65,90,119,120</sup>, and several other pharmacological inhibitors targeting dysregulated cancer metabolism are under development or in different preclinical stages<sup>21</sup>. 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<sup>12</sup>. In addition, several clinical trials that target metabolic dysregulations in mutant-RAS 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 specific and/or tissue-dependent roles of different RAS mutations and isoforms. For example, different KRAS mutations likely have tissue-specific effects on metabolism; therefore, multiple strategies must be developed and matched to the RAS-mutant subsets. In line with this, there is considerable variation in glutamine dependencies between tissues based on their origin<sup>121–123</sup>, and various KRAS mutations can have different dependencies for as yet unclear reasons<sup>124</sup>. *In vivo* tumors also display variability in their glutamine dependencies compared to cell culture findings<sup>74</sup>, underscoring the importance of using appropriate model systems to draw firm conclusions. Further, various reports assessing

the transcriptomic, proteomic, phosphor-proteomic and metabolic profiles of oncogenic RAS variants, including HRAS, NRAS and KRAS isoforms, indicate possible differences between their phenotypic effects<sup>125–127</sup>. Nevertheless, the metabolic landscapes of HRAS- and NRAS-driven cancers remain less explored and it would 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<sup>128</sup>. The success with chemotherapies that target metabolism<sup>129</sup> intensifies hope that more metabolic therapies will ultimately reach the clinic. However, there are some limitations in targeting metabolism for therapeutic purposes<sup>130–132</sup>. Chief among them is the metabolic flexibility of cancer cells that can often switch their source of nutrients and energy and activate compensatory metabolic pathway for survival, when there is limitation in their favored metabolic pathways or they are deprived of preferred metabolic source<sup>75,133</sup>. This adaptive nature of cancer cells might limit the efficiency 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<sup>134</sup> similar to those utilized by tumor cells, which would make them vulnerable to the toxicity created by targeting metabolic pathways. Since affecting the metabolic processes of immune cells could potentially affect not only their anti-tumor 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 prospect 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 being 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<sup>135,136</sup>. 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 PanCancer Analysis of Whole Genomes Consortium samples<sup>135</sup> on the KEGG pathway database<sup>137</sup> depicts oncogenic driver genes involved in various metabolism processes in many cancer tissue types (Fig. 4a), with a gene-level analysis of each corresponding pathway unveiling driver genes that are co-altered in diverse tissue types

(Fig. 4b). Future work should focus on investigating the potential crosstalk between RAS and commonly mutated driver genes known to be involved in metabolic and RAS signaling pathways in diverse tumor types, taking into account also that tumors from different tissues may display divergent metabolic phenotypes irrespective of their genomic profile. Network analysis of the several driver mutation genes, including KMT2D<sup>138</sup>, PIK3CA<sup>139</sup>, PTEN<sup>140</sup>, and IDH1<sup>141</sup>, that regulate signaling and metabolic pathways in KRAS-driven cancers, connects 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<sup>142</sup>, or even c-Src<sup>143</sup>, to trigger PTEN, which may act through AKT to regulate KMT2D; through AP1<sup>143</sup> to regulate KMT2C, or through p300<sup>144</sup> to regulate the lipogenesis enzyme ACACA. KMT2D and KMT2C may in turn regulate c-MYC, SOX2, or CTNNB1 to control other metabolism driver genes, including GBA, IDH1, PTDSS1, POLE, and ACACA. Many of these circuits may feedback to NF1 to influence the RAS gene directly<sup>145</sup> or to impact the RAS protein through a feedback loop from c-MYC<sup>146</sup>, SOX2<sup>147</sup>, or CTNNB1<sup>148</sup>. Alternatively, KRAS may directly trigger PIK3CA<sup>139</sup>, which impacts CTNNB1 for subsequent regulation of many metabolism driver genes. Various levels of interplay are known between the PI3K-AKT and KRAS-MAPK signaling cascades<sup>139</sup>, including for potential therapy. For example, using a combination of PI3K and MEK inhibitors has been shown to treat KRAS-driven lung cancers in mouse models<sup>149</sup>. However, detailed study is needed to understand precisely how these two pathways and the other factors depicted in this broader signaling circuit (Fig. 4c) coordinate with each other for the metabolic rewiring needed to sustain uncontrolled proliferation in cancer<sup>43</sup>. 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 tumor origin, and metabolic alterations is not well understood. The crucial question of whether RAS-mediated altered 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. The elucidation of these events should also take into account the fact that metabolic phenotype is not uniform across different tumor types, and variability also exists 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.

## Data Availability Statement

For figure 1 and Supplementary Tables 1, 2, 3, genome-wide cancer mutation data were compiled from databases and public resources, including AACR Genie (Release 6.1-public)<sup>150</sup>, COSMIC (v90)<sup>151</sup>, and cBioPortal<sup>152,153</sup>, TCGA Research Network: <https://>

[www.cancer.gov/tcga](http://www.cancer.gov/tcga) and NCI's Genomic Data Commons (GDC)<sup>154</sup>, that are openly accessible to the public and are cited in the manuscript. The datasets derived from these resources that support the analyses and discussion presented in this article are available in the cited references. For figure 4a–b, previously published cancer driver mutation data were acquired from Ref<sup>135,136</sup> and the International Cancer Genome Consortium/The Cancer Genome Atlas via controlled access through rigorous application and are available from these resources.

## Code availability statement

For figure 4, Driver mutation genes were applied to an in-house pathway pattern extraction pipeline (PPEP) tool described in Ref<sup>155</sup> and implemented in customized R scripts ([www.r-project.org](http://www.r-project.org)). PPEP and corresponding databases (WPS version 2) can be downloaded from WPS homepage. This tool represents a pathway-based platform for discovery integration to maximize analysis power. The tool is freely available at [http://www.abcc.ncifcrf.gov/wps/wps\\_index.php](http://www.abcc.ncifcrf.gov/wps/wps_index.php)<sup>156</sup> or can be available on request from the correspondence author.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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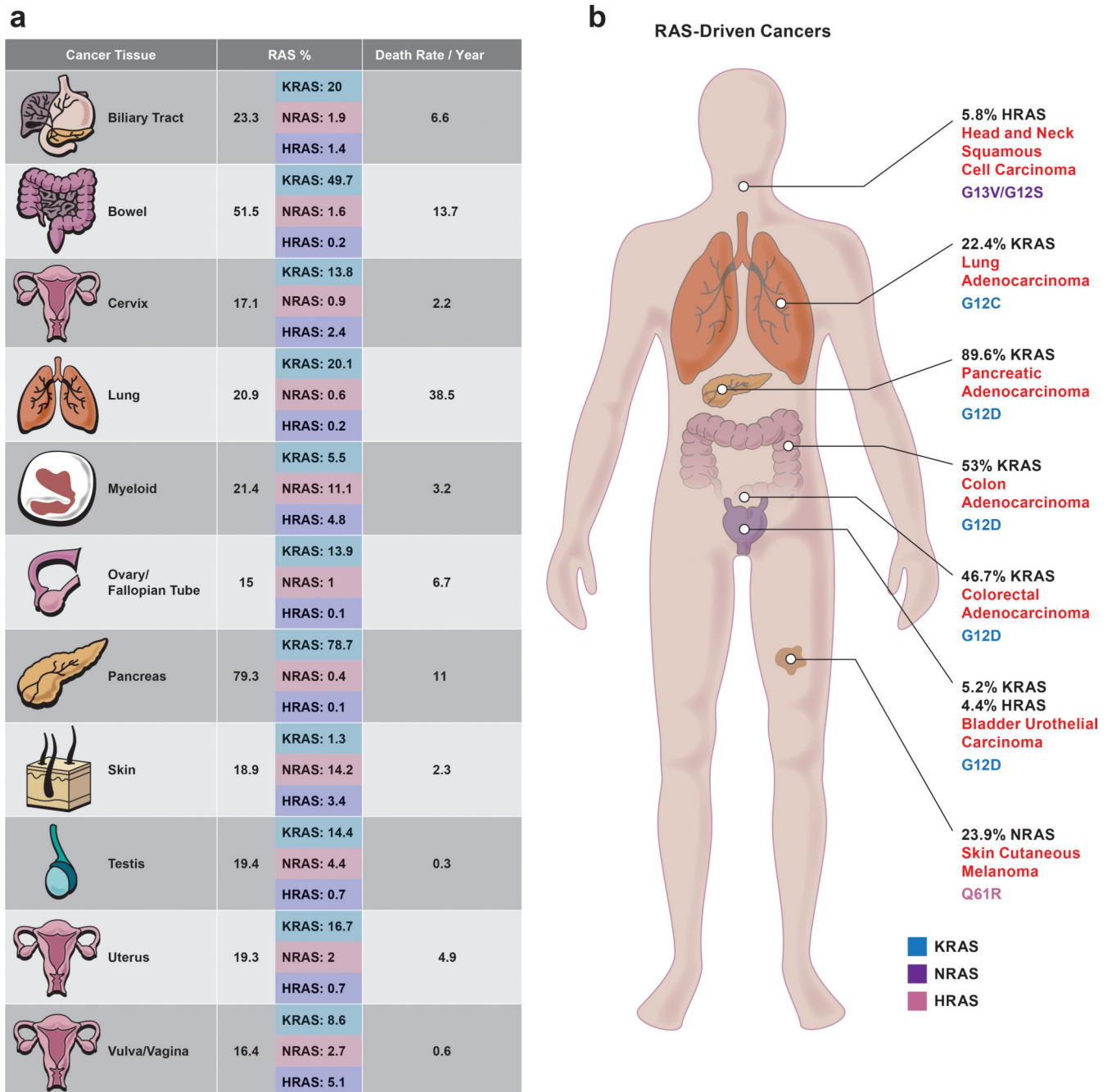


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**Figure 1. Frequency and distribution of RAS mutations in human cancers.**

Human cancers differ by mutated RAS isoform, codon, and amino acid substitution. **a.** Distribution of RAS isoform (KRAS, NRAS, and HRAS) mutations across tumor types and frequency (%) of RAS mutations by isoform in each tumor type. Detailed information in Supplementary Table 1. **b.** Types of cancers commonly associated with RAS mutations. The frequency of the most commonly mutated RAS genes is listed by tumor type. For each tumor type, the amino acid substitutions that occur most frequently in the RAS isoform are shown. The color of the mutation refers to the mutated RAS gene (KRAS, blue; NRAS,

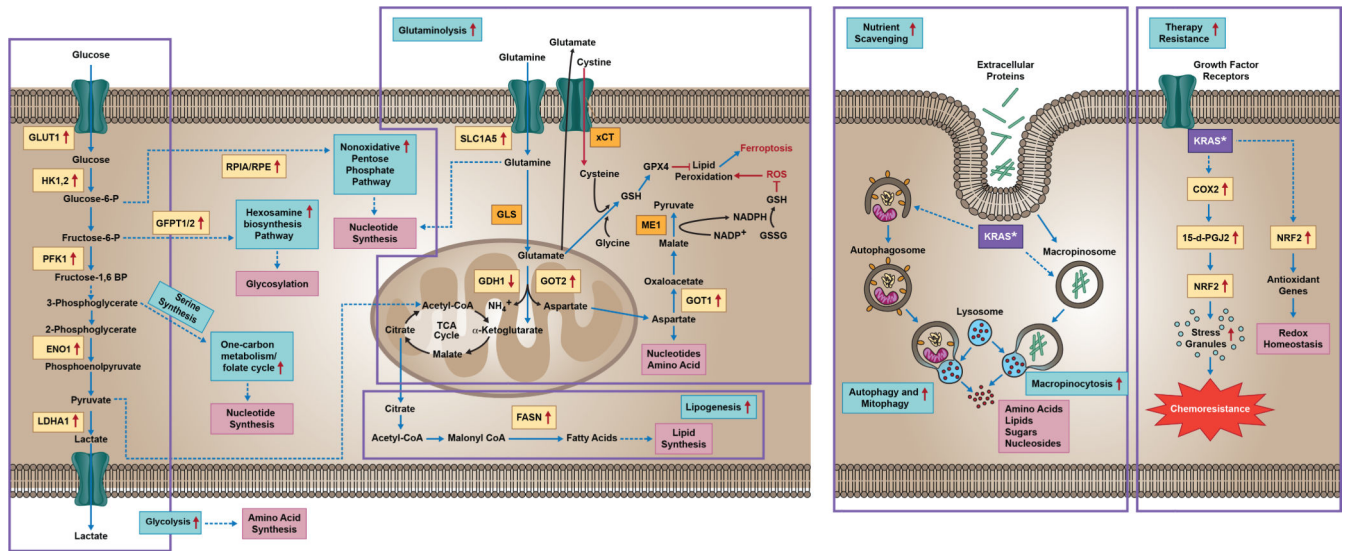
purple; and HRAS, pink). Detailed information in Supplementary Tables 2 and 3. All human cancers that had a sample size greater than or equal to 300 and a total RAS mutation rate greater than or equal to 15% are listed from data resources. Death rate/ year (%) is based on the death rate per 100,000 men and women. Data collected from the National Cancer Institute SEER cancer statistics 2020 database.

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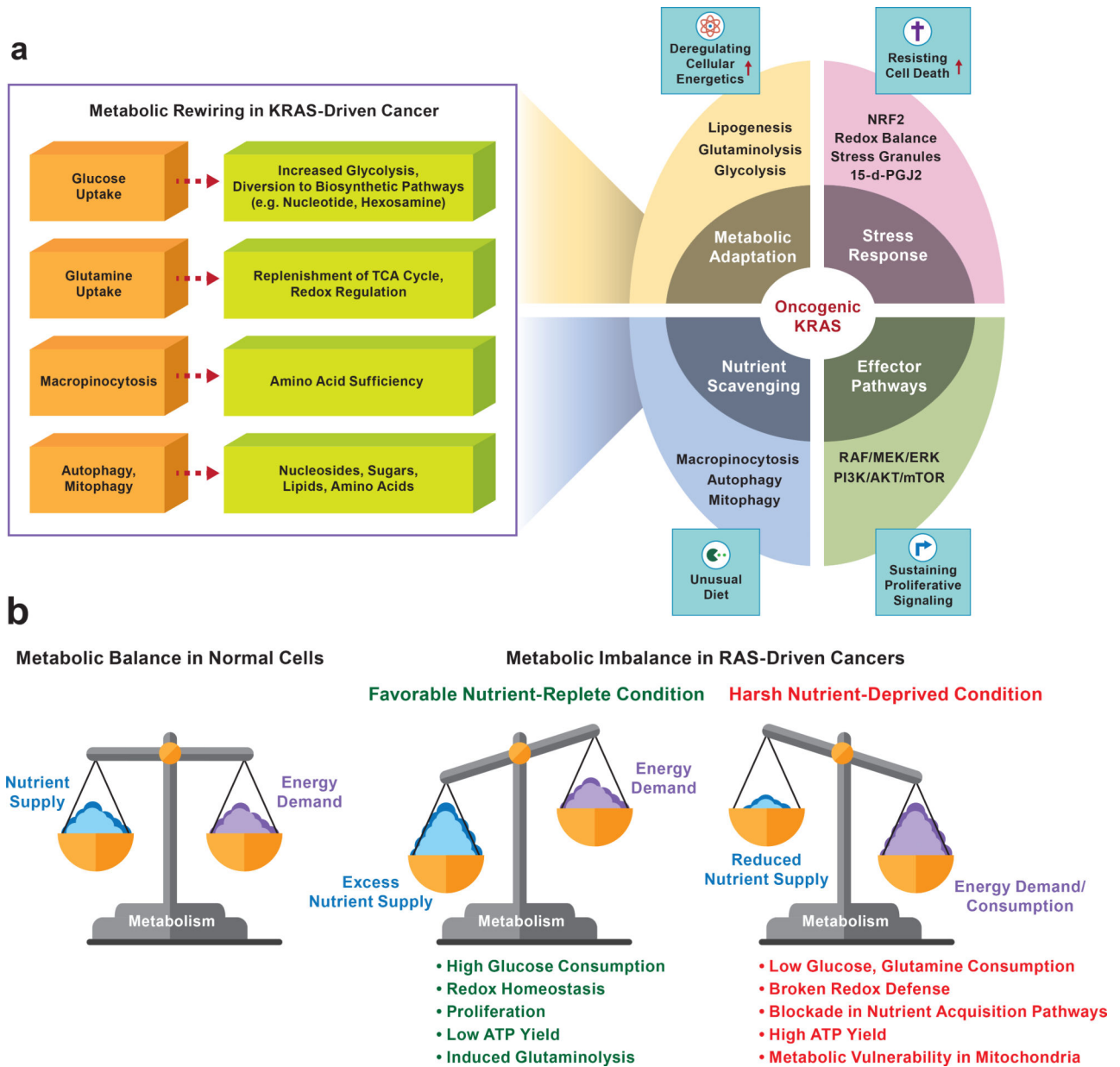
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**Figure 2. Oncogenic RAS-dependent control of cellular metabolism.**

Mutant RAS deregulates key metabolic processes, including glutaminolysis, glycolysis, autophagy, and macropinocytosis. Oncogenic KRAS directs glucose metabolism into hexosamine biosynthetic pathways by upregulating several key glycolytic enzymes, and induces the nonoxidative pentose phosphate pathway to support increased nucleic acid biosynthesis. RAS-driven cancer cells alter glutaminolysis to support rewired metabolism. Altered glutaminolysis is a key feature of KRAS-dependent cancer cells. KRAS regulated glutamine metabolic rewiring influenced the TCA cycle, which is critical for nucleotide biosynthesis to support cell growth and survival. KRAS-driven tumors require glutaminolysis for redox balance. KRAS-mediated activated NRF2 is depicted as a key transcription factor that modulates redox homeostasis for the survival of cells under oxidative stress. Cells harboring mutant RAS are characterized by increased macropinocytosis, autophagy, and mitophagy, processes which help generate the energy and macromolecules needed for accelerated tumor proliferation. Mutant RAS also regulates stress granule formation, which helps mediate chemoresistance. Yellow box indicates RAS-dependent gene and/or protein expression, with arrows indicating increased or decreased expression. Purple box indicates oncogenic KRAS. GDH1: Glutamate DeHydrogenase 1; TCA: Tricarboxylic Acid; COX2: Cyclooxygenase 2; HK1,2: Hexokinase 1 and 2; GLUT1: Glucose Transporter-1; PFK1: Phosphofructokinase-1; ENO1: alpha-enolase-1; LDHA: Lactate Dehydrogenase; ME1: Malic Enzyme-1; ROS: Reactive Oxygen Species; GOT1,2: Glutamate Oxaloacetate Transaminase 1,2.



**Figure 3. Metabolic alterations and vulnerabilities of RAS-driven cancers**

**a.** Schematic representation of the impact of oncogenic KRAS on cancer metabolism.

Various metabolic pathways involving KRAS-mutants critical in cancer cell proliferation and cell survival. Increased glucose uptake, induced glutaminolysis, autophagy and micropinocytosis are involved in deregulating energetics and nutrient scavenging which results in RAS-driven cancer cells' metabolic adaptation for the benefit of cell growth. **b.** The delicate balance of nutrient supply and demand dynamics in RAS-driven cancers. In a balanced state, nutrient supply is sufficient to maintain energy demand (left). Excessive supply of nutrient availability, in the absence of a parallel increase in energy demand, represents a situation in which the energy required to satisfy energy demand is lower than the available energy (middle). A nutrient-deprived condition provokes metabolic inequity



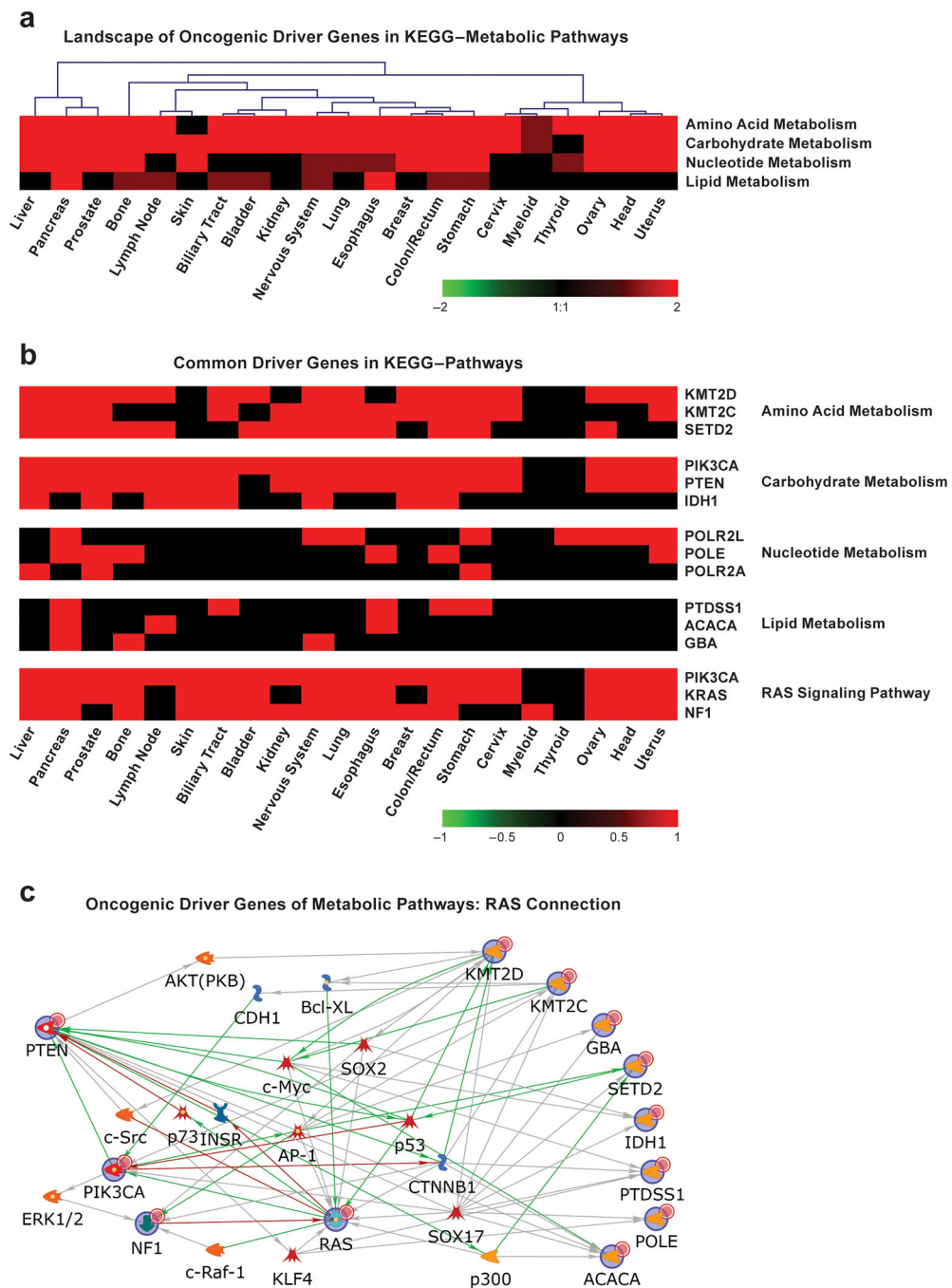
(energy supply < energy demand), leading to energetic stress and, ultimately, metabolic vulnerability (right). Nutrient-replete mutant RAS cells utilize rewired metabolism in their favor (middle panel). In the absence of glucose or glutamine, the metabolic vulnerabilities of mutant RAS cells intensify, leading to energetic stress and ultimately to cell death. Interventions that decrease nutrient consumption abolish redox defense and lead the cells to metabolic imbalance. This results in metabolic vulnerability, a potential therapeutic approach for RAS-driven cancer cells. (right panel).

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**Figure 4. Oncogenic driver genes and their involvement in metabolic pathways**

a. 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 ListHits or numbers of cancer driver mutation genes from the tissue types involved in the composite metabolic pathways from the KEGG database. Red shows the number of driver genes from each tissue involved in each corresponding pathway. b. Gene-level heatmaps showing the three most common driver genes across tumor types for the KEGG metabolic pathways and the RAS signaling pathway. Red indicates an involved driver gene, while

black indicates that the gene is not involved. c. 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 (b) were used as seeds to retrieve their direct relations with each other or their indirect relations with other genes from the MetaCore™ database. 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. For further details on how analyses were performed see the Supplementary Note.

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

Diseases	Biomarkers	Therapies	Phase	Study Identifier	Status
Lung Cancer: Small Cell or Squamous	HRAS, KRAS and NRAS mutations in Codons 12, 13, 61, 117, and 146	Auranofin and Sirolimus	Phase 1/Phase 2	<a href="#">NCT01737502</a>	Recruiting
Metastatic Pancreatic Carcinoma, Stage II, III, IV Pancreatic Cancer, Unresectable Pancreatic Carcinoma	MEK	Trametinib, Hydroxychloroquine	Phase 1	<a href="#">NCT03825289</a>	Recruiting
Metastatic Pancreatic Adenocarcinoma, Stage IV Pancreatic Cancer	KRAS Mutation	Hydroxychloroquine, Binimetinib	Phase 1	<a href="#">NCT04132505</a>	Recruiting
Lung Cancer: Non-Small Cell Lung Carcinoma	KRAS Mutation	CB-839, Docetaxel	Phase 1	<a href="#">NCT02071862</a>	Completed
Colon Carcinoma	KRAS and BRAF mutation	Carbohydrate-Restricted Diet, Vitamin C Supplement	Phase 1/Phase 2	<a href="#">NCT04035096</a>	Not yet recruiting
Metastatic Melanoma	NRAS Mutation	Trametinib, Hydroxychloroquine	Phase 1b/Phase 2	<a href="#">NCT03979651</a>	Recruiting
Non-Small Cell Lung Carcinoma, Colorectal Carcinoma	KRAS Mutation	CB-839, Palbociclib	Phase 1/Phase 2	<a href="#">NCT03965845</a>	Recruiting
Gastrointestinal Adenocarcinomas	MAPK mutations: KRAS, NRAS, HRAS, BRAF non-V600, MEK, and ERK mutations	Ulixertinib, Hydroxychloroquine	Phase 1	<a href="#">NCT04145297</a>	Recruiting
Pancreatic Cancer: Metastatic Adenocarcinoma	Mutant KRAS	Hydroxychloroquine, Gemcitabine	Phase 1/Phase 2	<a href="#">NCT01506973</a>	Active, not recruiting
Non-squamous Cell Lung Cancer	Wild type and mutant KRAS	AZD2014, AZD6244	Phase 1/Phase 2	<a href="#">NCT02583542</a>	Active, not recruiting
Colon Cancer	KRAS, BRAF Mutation status	TVB-2640	Phase 1	<a href="#">NCT02980029</a>	Recruiting
Breast Cancer, Endometrial Cancer, Lung Cancer, Colorectal Cancer, Head and Neck Cancer	KRAS mutations	Serabelisib, Canagliflozin	Phase 1/Phase 2	<a href="#">NCT04073680</a>	Not yet recruiting
Metastatic Colorectal Cancer	RAS Wild Type	CB-839, Panitumumab, Irinotecan	Phase 1/Phase 2	<a href="#">NCT03263429</a>	Recruiting
Colorectal Cancer	RAS Wild Type	BAY94-9392, 11C-Glutamine	Phase 1	<a href="#">NCT03275974</a>	Recruiting

According to *My Cancer Genome* and [ClinicalTrials.gov](https://ClinicalTrials.gov) database