The greedy nature of mutant RAS: a boon for drug discovery targeting cancer metabolism?

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Abstract

RAS oncogene mutations are frequently detected in human cancers. Among RAS-mediated tumorigenesis, KRAS-driven cancers are the most frequently diagnosed and resistant to current therapies. Despite more than three decades of intensive efforts, there are still no specific therapies for mutant RAS proteins. While trying to block those well-established downstream pathways, such as the RAF-MAPK pathway and the PI3K-AKT pathway, attentions have been paid to potential effects of RAS on metabolic pathways and the feasibility for targeting these pathways. Recent studies have proved that RAS not only promotes aerobic glycolysis and glutamine metabolism reprogramming to provide energy, but it also facilitates branched metabolism pathways, autophagy, and macropinocytosis. These alterations generate building blocks for tumor growth and strengthen antioxidant defense in tumor cells. All of these metabolic changes meet different demands of RAS-driven cancers, making them distinct from normal cells. Indeed, some achievements have been made to inhibit tumor growth through targeting specific metabolism rewiring in preclinical models. Although there is still a long way to elucidate the landscape of altered metabolism, we believe that specific metabolic enzymes or pathways could be therapeutically targeted for selective inhibition of RAS-driven cancers.

Key words: RAS, cancer metabolism, bioenergetic metabolism, anabolic metabolism, scavenging pathway

Introduction

RAS was identified as an activated oncogene in human cancer cell lines in 1982. Subsequently, intensive sequencing of the cancer genome has revealed that the three RAS genes (HRAS, NRAS, and KRAS) are among the most commonly mutated oncogenes in human cancers, of which KRAS mutation is the most prevalent [1]. Three RAS genes encode four highly homologous RAS proteins: HRAS, NRAS, KRAS4A, and KRAS4B [2]. These RAS proteins bind GDP and GTP, respectively, to regulate signal transduction. Under physiological conditions, RAS proteins possess low intrinsic GTP hydrolysis regulated by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors [1]. Hydrolysis of GTP to GDP is the key reaction through which RAS signaling is turned off. However, the common oncogenic mutations at G12, G13, and Q61 impair both intrinsic and GAP-catalyzed hydrolysis, favoring persistent formation of RAS–GTP [3]. RAS–GTP binds and activates multiple downstream effectors [4]. Three primary effector pathways downstream of RAS, RAF-MEK-ERK, RalGEF-Ral, and PI3K-AKT play key driver roles in RAS-mediated oncogenesis. While great efforts have been made to directly target mutant RAS genes and their downstream pathways [5–9], no effective therapy is currently available in clinics [10,11]. Meanwhile, cancers with mutant RAS are often accompanied by...
poor prognosis and low survival rate, all of which highlight the need for new therapeutic strategies [1].

As metabolic disorder became one of the hallmarks of cancer, great attention has been paid to the crucial role of oncogene RAS in tumorigenesis where it orchestrates a metabolic reprogramming of tumors [12]. Indeed, oncogenic RAS plays an important role in regulating cancer cell metabolism by triggering several main metabolic changes. Activation of RAS up-regulates growth-promoting pathways controlled by PI3K-mTOR and MAP kinases [10]. Growing evidence suggests that there is a tight correlation between metabolism rewiring and these pathways. For example, RAS mutant cells are dependent on sufficient glucose uptake, PI3K can increase glycolysis through activation of AKT and stabilization of HIF-1, and the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) can drive a complex pathway to support glycolysis, the pentose phosphate pathway (PPP) and nucleotide biosynthesis [13–16]. RAS protein also increases autophagy and macropinocytosis to generate building blocks for growth as well as promote antioxidant defense. The metabolic changes driven by oncogenic RAS show great effect on oncogenesis and tumor progression, whereas normal tissues often lack the same reliance on such pathways. Enzymes involved in cancer metabolic pathways can be inhibited by small molecules [17]. This greedy nature of mutant RAS provides us an opportunity to explore the feasibility of targeting key enzymes in the altered metabolism to treat RAS-driven cancers.

In this review, we discuss the metabolic changes in RAS-driven cancers and the emerging role of RAS in these changes. We also show evidence that specific metabolic enzymes can be attractive therapeutic targets, although some unsolved issues and great challenges are still in this unpaved road.

Cancer Metabolism

Proliferating cancer cells must alter their metabolism to meet the increased biosynthetic demands for growing tumor (Fig. 1) [18]. However, it is becoming clear that the activation of oncogenes and loss of tumor suppressors lead to tumor-specific metabolic reprogramming in order to support their growth and survival. In general, cancer cells alter the metabolic flux into multiple pathways to produce the cellular energy and building blocks for macromolecule synthesis that are required for cell growth and the generation of new cells. At the same time, the complex rewiring of cellular metabolism can provide enough reducing power to maintain redox balance and help cells to escape from increased oxidative stress [18,19].

When it comes to cancer metabolism, we may have to mention the Warburg effect. In 1920s, Otto Warburg found aerobic glycolysis and linked metabolism to cancer, and he showed that tumor cells take up excess glucose and produce lactate even in the presence of oxygen [20]. Meanwhile, glucose supplies many key biosynthetic intermediates that are required for the synthesis of proteins, lipids, nucleic acids, and complex sugars through the branched pathways such as PPP [21]. Just like the great demand for glucose, cancer cells also increase the glutamine uptake and utilize it through glutaminolysis [22]. Glutamine serves as a major anaplerotic substrate for the tricarboxylic acid (TCA) cycle, which is important to produce ATP for energy homeostasis, citrate for lipid synthesis, and amino acids as well as NADH for protein synthesis [23]. Under the stress of increased metabolic requirements, autophagy is consequently required for tumor maintenance [24]. These tumor-specific metabolic reprogramming makes them distinct from normal counterparts and provides the probability of a therapeutic window.

RAS and Bioenergetic Metabolism

It has been nearly a century since Otto Warburg showed that tumor tissues absorbed more glucose than normal tissues in the 1920s. Instead of oxidative phosphorylation (OXPHOS), cancer cells metabolize glucose to lactate and produce very little ATP (only two molecules of ATP versus the ~36 ATP units produced by TCA cycle from each glucose molecule) even in the presence of oxygen, which is an energetically less favorable process known as aerobic glycolysis or the Warburg effect [25,26]. This metabolic reprogramming is a prominent characteristic and now considered as a core hallmark of cancer [27,28]. Irrespective of these energy defects, aerobic glycolysis provides various intermediates for anabolic metabolism to sustain tumor growth. Besides increased glucose uptake, cancer cells also require more glutamine to supply energetic precursors for amino acid and lipid syntheses [21,22]. Actually, cancer cells are addicted primarily to glucose and glutamine; thus, they exhibit alternations in these bioenergetic processes, including aerobic glycolysis, glutaminolysis, and mitochondrial respiration [17,22,29].

Like other hallmark traits of human cancer, metabolic reprogramming also results in genetic alterations in key oncogenes or tumor suppressors, such as the activation of RAS, Myc, HIF-1α, IGF-1, and PI3K-AKT-mTORC1 pathways, or loss of the tumor suppressor p53 [28,30,31]. Oncogenic RAS, especially KRAS, has been widely reported to function in metabolic rewiring of tumor cells, although this also depends on tissue and genetic specificities. Indeed, several studies from cancer cell lines and genetically engineered mouse models have shown that mutant KRAS increases glucose and glutamine uptake to provide carbon source and to maintain redox balance [23,32]. Conversely, glucose deprivation can drive tumor cells to acquire KRAS pathway mutations [14]. These metabolic disturbances arise from transcriptional changes and the regulation of multiple rate-limiting steps mediated by the presence of oncogenic RAS. Since no effective targeted therapy currently exists for cancers with somatic KRAS mutations, targeting the enzymes involved in metabolic pathways may provide an alternative therapeutic approach for KRAS-driven cancer. Recently, several agents have been identified to inhibit these candidate targets and are now used in preclinical or clinical studies [17].

Targeting Aerobic Glycolysis

Aerobic glycolysis and OXPHOS go on separate ways at the production of pyruvate after a series of enzymes catalyzing reactions starting from glucose. In normal cells, pyruvate enters mitochondria and is transformed into acetyl CoA to drive the TCA cycle. However, pyruvate is converted into lactate by lactate dehydrogenase (LDH) with little ATP production. In order to feed the elevated demand for energy and building blocks imposed by rapidly proliferating cells, cancer cells including these harboring KRAS mutation exhibit a remarkably higher uptake of glucose by increasing glucose transporter GLUT1 expression [11,33]. Thus, this first step of metabolic reprogramming launches a compelling strategy for cancer therapy. Pharmacological or genetic inhibition of GLUT1 exerts anticancer efficiency both in vitro and in vivo [34,35]. Silybin, a natural flavonoid from Silybum marianum, inhibits glucose uptake in a competitive manner through direct interaction with GLUT1 and GLUT4 and displays strong inhibitory effects on various cancer cells [36]. A Phase II clinical trial in patients with prostate cancer and a Phase I trial in patients with advanced hepatocellular carcinoma have been completed, and its effectiveness and safeness have been proved (https://www.clinicaltrials.gov/ct2/...
results?term=Silybin&Search=Search). Phloretin, a dihydrochalcone extracted from apple tree leaves, acts as a competitive inhibitor of GLUT1 to suppress tumor growth [37,38]. Other inhibitors of glucose transporter, such as cytochalasin B and its derivatives [39], fasentin [40], are also at the early stage of drug development for cancer therapy. However, it is not so easy to specifically inhibit this protein while avoiding unwanted effects on normal cells. Only a very few specific GLUT inhibitors have been developed so far. Recently, the exploitation of synthetic lethality in anticancer drug discovery emerges as a promising strategy by targeting at pathways that are specifically essential for cancer cells but not for normal cells [41]. Through this approach, a class of compounds, represented by STF-31, was identified, which selectively impaired renal carcinoma cells by directly binding to GLUT1 and blocking glucose uptake [42]. In addition, suppressing this target by means of small interfering RNA (siRNA) [43] or antisense nucleic acids [44,45] inhibits cell proliferation and glucose uptake in human cancer cells or sensitizes cancer cells to chemotherapeutic agents.

Hexokinase (HK) controls the first and rate-limiting step of glycolysis. HK1 and HK2 have also been reported to be up-regulated in KRAS-driven cancers [1,12] as well as in other highly malignant tumors. This enzyme together with GLUT1 plays a major role in the control system of glycolytic flux, thus revealing an attractive target for cancer therapy. Owing to its expression limited to skeletal muscles and adipose tissues, targeting HK seems to be highly safe and feasible for cancer patients [46]. Numerous compounds have been discovered showing HK-inhibitory properties, and some of them have entered clinical trials, such as lonidamine, 3-bromopyruvate (3-BrPA), and 2-deoxy-D-glucose (2-DG). Lonidamine has been identified as an efficient HK inhibitor that specifically inhibits mitochondria-bound HK [47]. Several studies have demonstrated its anti-proliferative activities in monotherapies or in combination with other agents to improve cancer therapy in various types of cancers [48,49]. However, a Phase III clinical trial in patients with benign prostatic hyperplasia was terminated due to its hepatic toxicity [38]. 3-BrPA was widely thought to be a HK2 inhibitor, but subsequent studies suggested that this drug might have a very wide range of possible targets [50]. Even so, 3-BrPA has shown remarkable efficacy in rapidly growing cancers used either as a single drug or in combination with others [51,52], leaving normal cells unaffected, and even eradicating advanced cancers in animal studies [53]. However, there is no ongoing human clinical trial involving 3-BrPA at present. Another HK inhibitor in clinical trials is 2-DG, a glucose analog that inhibits HK through competition with glucose [54]. As monotherapy, 2-DG did not seem to have significant effect on tumor growth, but it was proved to be effective in combination with chemotherapeutic drugs or other agents [38,55].
6-Phosphofructo-1-kinase (PFK1) is another enzyme up-regulated in KRAS-mutant cancer [1,12], which is under allosteric activation and catalyzes irreversible conversion of fructose-6-phosphate into fructose-1,6-bisphosphate. The potent allosteric activator of PFK1 is fructose-2,6-bisphosphate (F2,6BP), synthesized by PFKFB3 (PFK2) enzyme, leading to the increase of glycolytic flux [56]. The most important specific PFKFB3 inhibitor is represented by 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propanone-1-one (3PO), which decreases F2,6BP levels and therefore suppresses PFK1 activity and glycolytic flux, with the growth inhibition potency against cancer [36]. The expression of alpha-enolase (ENO1) was also found to be increased in KRAS-mutant cancer [32]. Knockdown of ENO1 decreased cancer cell proliferation and metastasis in vitro and in vivo [57,58]. However, the development of small-molecule inhibitors of enolase is at its initial phase and just starting to emerge [39]. Pyruvate kinase isozymes M2 (PKM2) is another key regulator of the Warburg effect [60]. The PKM2 tetramer catalyzes the conversion of phosphoenolpyruvate into pyruvate, while the PKM2 dimer can translocate into nucleus to activate transcription of various genes, promoting cancer cell survival and growth [61]. Because the expression and lower glycolytic enzyme activity of PKM2 are critical for the Warburg effect, and RAS-mutant cancers do show reliance on the Warburg effect, a direct or indirect correlation between PKM2 and RAS may exist. Besides the enzymes mentioned above, KRAS-mutant cells shows high level of lactate dehydrogenase A (LDHA) expression [1,12], which converts pyruvate to lactate, the last product of glycolysis. Knockdown of LDHA using short hairpin RNAs leads to a reduction in tumor growth [62]. At present, there is no specific inhibitor targeting at LDHA. Non-selective LDH inhibitor such as (R)-(-)-gossypol has entered clinical trials [38]. In general, LDHA could be a potential tumor target, but its side effects should be evaluated in patients with decreased LDH activity in muscles.

Targeting Glutaminolysis

Malignant cells, especially those harboring RAS mutation, rely heavily on glutamine for survival and proliferation [63,64]. Indeed, glutamine is the major anaerobic carbon source for TCA cycle in RAS-mutant cancer [32]. Glutamine is converted into glutamate by glutaminase-1 (GLS1) after being taken into the cell. Then, glutamate is converted to α-ketoglutarate by glutamate dehydrogenase (GLUD1) and enters the TCA cycle in the mitochondria to provide ATP and precursors for ana- bolic metabolism [30]. However, in pancreatic ductal adenocarci noma (PDAC) where KRAS mutation (>90%) is the signature event, the glutaminolysis process is reprogrammed into a non-canonical pathway. In this KRAS-regulated metabolic pathway, glutamine-derived aspartate is converted to oxaloacetate by the aspartate aminotransferase GOT1 in the cytoplasm, which is then converted to malate, and finally to pyruvate, providing abundant NADPH [64,65]. In this case, mutant KRAS increases GOT1 and represses GLUD1 gene expression, which is distinct from the normal pathway [54]. This specific pathway may provide novel therapeutic approaches to treat KRAS-mutant pancreatic tumors. However, whether this pathway is specific to the KRAS-mutant PDAC remains to be addressed. In addition, whether this characteristic of KRAS can be translated into other KRAS-mutant cancers is still an open question.

Knockdown of GOT1 and GOT2 can inhibit PDAC tumor growth [62], but no pharmaceutical-grade inhibitors of these enzymes are available at present. However, inhibitors targeting at GLS1 are available. Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-y1)ethyl sulfide 3 (BPTES) and its analogs are potent and selective inhibitors of GLS [66,67]. CB-839 is a potent, selective, and orally bioavailable inhibitor of both splice variants of GLS, and it displays significant antitumor activity in vitro and in vivo in triple-negative breast cancer [68]. CB-839 is currently under Phase I clinical study in patients with solid tumors. Study of GLS inhibitors in PDAC showed that it could disrupt the redox balance and synergize with treatments that increase reactive oxygen species (ROS) to inhibit pancreatic cancer growth. Therefore, a combination of glutamine inhibitor with therapies that increase ROS would be more reasonable and effective. This kind of combination has shown synergistic effect in pancreatic cancer models [64]. Our group has also developed a synthetic lethal chemical screening method in isogenic KRAS-mutant and wild-type cells to identify novel clinical drug pairs. We found that combinations of inhibitors of metabolism with other agents exhibit synergistic activity in KRAS-mutant cancer cells (data unpublished).

RAS and Anabolic Metabolism

Except for glycolysis, glucose can participate in another two important anabolic metabolism pathways: the PPP and the hexosamine biosynthesis pathway (HBP). In this section, we will discuss the regulation of PPP and HBP and the potential targets in RAS-mediated tumorigenesis.

The PPP pathway branches from glycolysis at the first committed step and exhibits great impacts on cell metabolism and survival, which can generate phosphopentoses and ribonucleotides for anabolism, and regulate the cellular redox state by generating NADPH [69]. Besides the PPP, another anabolic pathway up-regulated by RAS is the HBP. HBP can produce precursors used for glycosylation, which is important for post-translational protein modification and tumorigenesis [70]. UDP-N-acetylglucosamine is the end product of the HBP and can be used by O-GlcNAc transferase (O-GT) in the post-translational modification.

Abundant researches have proved that RAS mutation can increase the utilization of glucose in the nonoxidative PPP, whereas the oxidative branch is unaffected [71–73,74,32]. A recent study showed that the nonoxidative PPP branch glucanone-6-phosphate dehydrogenase (G6PD), glucose-6-phosphate dehydrogenase (6PGD), and transketolase (TKT) were up-regulated in A549 cells, whereas only G6PD was up-regulated in NCI-H460 cells, by using quantitative proteomic approach to analyze key enzymes involved in metabolic adaptations in two KRAS-mutated NSCLC cell lines (A549 and NCI-H460) and a non-tumoral bronchial cell line (BEAS-2B) [75]. In KRAS-mutant pancreatic tumor, G6PD was not altered; however, the expression levels of ribulose-5-phosphate isomerase (RPIA) and ribulose-5-phosphate-3-epimerase (RPE) were significantly up-regulated [32]. RPIA and RPE can help to generate the ribonucleotides required for nucleic acid biosynthesis. Meanwhile, much evidence has shown that RAS can elevate the expression and activation of Nrf2 (also known as NF-E2L2), a key transcription factor that is regulated by oxidative stress or xenobiotic stress [76–78]. Recently, studies indicated that Nrf2 plays an important role in increasing NADPH and nucleotide production, because it can bind to AU-rich element (ARE) and activate transcription of the PPP enzymes G6PDH, 6PDGH, TKT, and transaldolase (TALD) [79]. Although mutant RAS regulates different PPP enzymes in different cancers, it promotes PPP in general. Researches have shown that hyper-O-GlcNAcylatation in PDAC appears to inhibit apoptosis through NF-kB activation [80]. In KRAS-transfected cells, glucose starvation can induce cell death. Due to the lack of substrates, the
HBP stops providing metabolite for glycosylation, leading to the activation of the unfolded protein response (UPR) [81].

In addition to generate phosphotyrosines and ribonucleotides, the PPP is a primary source of NADPH and balances the cellular redox state [82]. ROS plays a very contradictory role in tumor cells. Low level of ROS can promote oncogenic mutations and facilitate cell proliferation and survival through the post-translational modification of kinases and phosphatases [82,83]. To some extent, ROS can induce the expression of HIF1-α and consequently trigger the expression of proteins, which provides pro-survival signals, such as the glucose transporter GLUT1 and vascular endothelial growth factor [84,85]. However, at higher levels, ROS may render cancer cells energetic and oxidative stress, causing DNA damage and triggering senescence and apoptosis [86–89]. Thus, it is well recognized that NADPH produced through the PPP can help to combat ROS. Besides, cancer cells including oncogenic KRAS-transfected fibroblasts can use glutamine to provide a significant amount of NADPH through malic enzyme (ME1), which oxidatively decarboxylates malic acid, producing carbon dioxide, NADPH, and pyruvate [33,90]. Indeed, in KRAS-driven PDAC, glutamine metabolism provides abundant NADPH and maintains redox balance [64,65]. As mentioned above, ROS can up-regulate Nfr2, and researchers have found that Nfr2 can promote ROS detoxication through activating the metabolic enzymes Hmox1, Nqo1, Gclc, Gclm, and Ggt1 [75,89]. Mutant KRAS drives this non-canonical pathway of glutamine use [64].

Targeting Anabolic Process

The anabolic metabolism provides abundant metabolites for cancer cell proliferation and progression, which inspires us to target these pathways to block cancer. G6PD, as the first enzyme in PPP, should be considered. Dehydroepiandrosterone (DHEA), a naturally occurring adrenal steroid in Phase III clinical trials, is an uncompetitive inhibitor of G6PD. Several studies have proved that DHEA and its derivatives exhibit anti-proliferative actions on tumors by the inhibition of G6PD and increase of oxidative stress [91–95]. Adriamycin resistance in colon carcinoma cell lines could be reversed by treatment with DHEA [96]. Besides, 6-ammoniicotinamide (6AN), a competitive G6PD and 6PGD inhibitor, was even used in chemotherapy for various tumors [97–99]. When 6AN was combined with 2-DG, the radiosensitivity can be enhanced in human gliomas and squamous carcinoma cell lines, with the mechanism that the combination might down-regulate Nfr2 and induce oxidative stress [100–103]. At the same time, novel G6PD inhibitors are still under exploration [104]. Besides G6DP, another important enzyme TKT protein attracts great interest. In pancreatic cancer cells, TKT inhibitor oxothiamine can alter the dynamics of protein expression and trigger apoptosis [105]. Oxothiamine can also significantly inhibit the proliferation of metastatic ovarian cancer cells [106], and the combination of oxothiamine with DHEA shows better effects on impairing cancer cell growth [107]. An analog of thiamine, 5a [3'-5'-pyridyl thiamine]; 3-[6-methyl-2-amino-pyridin-3-ylmethyl] -5-(2-hydroxy-ethyl)-4-methyl-thiazol-3-ium chloride hydrochloride] can also inhibit TKT [108] and ring-opened analogs of 5a improve its pharmacokinetic profile and show better effect in vitro [109]. Consequently, a bienzymatic biosensor was designed to screen various human TKT inhibitors [110]. With the knockdown of TKT and G6PD in A549 cell xenograft mouse model, tumor volume was dramatically repressed, and the same phenomena was observed when Nfr2 was knocked down [79]. Recent reports have demonstrated that RPIA and RPE play important roles in oncogenic KRAS-induced pancreatic cancer, and knockdown of either of which can decrease the incorporation of glucose-derived ribose into DNA/RNA [32]. Phosphoglycerate mutase 1 (PGAM1) contributes to the regulation of biosynthesis in part by controlling intracellular levels of its substrate. Its inhibitor PGMI-004A attenuates cell proliferation and tumor growth through increasing 3-PG to inhibit G6PD [111]. In addition, PKM2 is an important enzyme involved in glycolysis, and its activators such as TEPP-46, SAICAR, and serine can limit the diversion of glucose toward the PPP, hence mediating antitumor activities [111,112].

Glycosyltransferases OGT inhibitors can reduce post-translational modification of nucleocytoplasmic proteins with O-linked N-acetyl-glucosamine residues [113]. It has the ability to inhibit cell proliferation of MIA PaCa-2 cells via suppression of hyper-OglcNAclyation [80]. When the KRAS-regulated, rate-limiting HBP enzyme Glp1 was knocked down in pancreatic cancer cells, the overall cellular glycosylation levels were decreased and the tumor cell growth was inhibited in vitro and in vivo [32].

As mentioned above, glutamate metabolism offers abundant ROS, and GLS inhibitor could destroy the redox balance in PDAC cancer models. Due to the specificity and essentiality of this metabolism pathway, it offers an accessible therapeutic window [64]. At present, many chemotherapies such as platinum and doxorubicin can induce DNA damage and increase ROS levels. This inspires us to block the PPP or glutamine metabolism to decrease the NADPH and nucleotide biosynthesis, eventually enhance the efficacy the chemotherapy agents. Knockdown of Nfr2 by miR-340 mimics, or Wogonin can overcome resistance to chemotherapy through suppressing Nfr2-dependent antioxidant pathway in cancer treatment [114–116].

Because few inhibitors of these enzymes are currently available, researchers have changed their focus to KRAS signaling pathways that regulate the transcriptional and metabolic changes in anabolic glucose metabolism. MEK inhibitor AZD8330 suppresses the expressions of several key enzymes in the nonoxidative PPPs [32]. There are some other MEK1/2 inhibitors that have entered multiple clinical trials in the field of cancer metabolism [117]. In pancreatic cancer, MEK inhibition suppresses KRAS-directed metabolism, while inhibition of mTOR signaling and PI3K signaling does not exhibit any significant impact [32]. Based on the facts that these two pathways play important roles in KRAS-driven cancers and different tumor types may differ in the metabolism patterns, specific inhibitors targeting at mTOR signaling and PI3K signaling should be considered to treat metabolic disorder in some other cancers.

RAS and Scavenging Pathways

Metabolic scavenging pathways basically include autophagy and macropinocytosis, and these biological mechanisms could provide nutrients for cells, including RAS-mutant tumors. Autophagy is a highly conserved mechanism, which is a degradation process that regulates synthetic metabolism in cytoplasmic organelles and macromolecules. It captures damaged or surplus intracellular proteins and damaged organelles in vesicles and then fuses with lysosomes where the cargo is degraded [118]. The products released from lysosomes can be used in metabolic and biosynthetic pathways [119]. The process of autophagy also affects the maintenance of mitochondrial operation and ATP levels, and the inhibitors of autophagy significantly affect mitochondrial metabolism [120]. In cancer cells, such as breast cancer and non-small cell lung carcinoma [121,122], the metabolic mechanism is rather complicated and the autophagy process is active under cellular
stavation, which then facilitates the cycle of cellular materials and nutrients [123]. Some studies have proved the association between autophagy induction and the activation of RAS in several human cancer cell lines. Elevated autophagy in RAS-driven cancer cells could help maintain oxidative metabolism and tumorigenesis, acting as a survival mechanism [24,124,125]. The critical substrates supplied by autophagy to metabolic pathways still need to be investigated. Autophagy deficiency in BRAFV600E-induced lung cancer enhances dependence on glutamine, suggesting that protein degradation by autophagy supplies amino acids and their derivatives to metabolic pathways, of which glutamine is particularly critical [126]. The role of other amino acids such as serine and glycine in the metabolism of RAS-driven cancers remains to be clarified [127].

In addition to the consumption of internal cargo by autophagy, RAS-mutant cancers also consume lipids and proteins from the extracellular space through a process termed as macropinocytosis [128,129]. Macropinocytosis belongs to endocytosis that is a caveolin- and clathrin-independent endocytotic process. The vesicles in this process are called macropinosomes, which ultimately fuse to the lysosome in a process similar to autophagy [129–131]. The relationship between oncogenic RAS and macropinocytosis has been confirmed in T24 cells, which are homozygous for the HRASG12V allele, and the level of macropinocytosis was increased when compared with the wild-type HRAS 5637 cells [132]. Besides, RAS-transfected pancreatic cancer cells use macropinocytosis to take up extracellular proteins into the cell. Then, these proteins undergo proteolytic degradation, yielding amino acid fuels for the TCA cycle [129]. Activation of EGFR and oncogenic RAS can induce macropinocytosis-mediated cellular uptake of exosomes [133]. Additionally, RAS-mutant cancer cells synthesize long-chain fatty acids from an extracellular fatty acid backbone rather than from de novo synthesis. This mechanism can reduce the demand for NADPH required for lipid synthesis, making it more available for antioxidant defense [128]. Taken together, these researches revealed that RAS-driven cancers develop multiple mechanisms to scavenge various metabolites to feed themselves, and this may be of therapeutic potential as normal cells are not likely to rely on this metabolic mode.

Targeting Autophagy and Macropinocytosis

Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ), the acknowledged inhibitor of autophagy, exhibit inhibitory effects on cancer cell proliferation in vitro and in vivo [120,134,135]. They have been used in clinical trials [136,137]. When essential autophagy regulators Atg5 and Atg7 were knocked down, a dramatic decrease in cell survival of the T24, H1299, and HCT116 cell lines could be observed [24]. The same phenomenon occurred when Atg7 was deleted in mouse models of spontaneous BRAFV600E-driven lung tumorigenesis [126]. The main mechanism of these effects is that the inhibition of autophagy caused a mitochondrial respiratory defect, and this process can be rescued by supplying substrates for the TCA cycle [11,120]. Bafilomycin A1, monensin and pepstatin A can suppress autophagy through affecting vacuolar ATPase, lysosomal pH and lysosomal protease cathepsin, respectively [138–140]. Some key autophagy proteins should be examined as potential targets, including the cysteine protease Atg4, the ULK1 and ULK2 serine–threonine protein kinases, as well as enzymes involved in ubiquitin-like conjugation systems (e.g. E1-like ubiquitination enzyme Atg7 and E2-like enzymes Atg3 or Atg10) [141]. Moreover, Vps34, a Class III PI3K, may be targeted, as it is a key autophagy regulator. Some PI3K inhibitors such as 3-methyladenine (3-MA), wortmannin, and LY294002 showed inhibitory activity on autophagy [142,143].

Compared with autophagy, our knowledge about the macropinocytosis process is still lacking. Important proteins involved in this process that can be potential targets should be investigated. Ethylisopropylamidolide can decrease the growth of KRAS-mutant pancreatic cancer xenografts [129] by inhibiting macropinocytosis through Na+/H+ exchangers [144]. Lysosomes are acidic organelles that play an essential degradation role in both autophagy and the macropinocytosis process. Hence, it is reasonable that lysosome inhibitors such as HCQ can inhibit macropinocytosis. Fatty acid desaturation is catalyzed by the oxygen-consuming enzyme stearoyl-CoA desaturase (SCD) 1 [128], and RAS-transfected cells show resistance to SCD1 inhibitors, suggesting that inhibition of lipid scavenging might be of therapeutic opportunities to target RAS-driven cancers.

However, it has been suggested that increased autophagy seen during anticancer drug treatment could also be a survival response of the dying cells rather than a cause of cell death [145]. Rapamycin, an inhibitor of mTOR, promotes autophagy and prevents tumor cell proliferation. However, clinical trials demonstrated that targeting mTOR is not successful because this intervention triggers autophagy as an alternate survival pathway in cancer cells [146]. Combination of phosphatidylinositol 3-kinase/protein kinase B inhibitors with rapamycin to target KRASG12D-induced mouse lung model may yield better clinical trial results. Similarly, combination of macropinocytosis inhibitor 5-(N-ethyl-N-isopropyl) amiloride with rapamycin is more effective to suppress mTORC1 in RAS-driven cancer cells [147]. Prereclinical studies using a Myc-induced mouse lymphoma model indicated that inhibition of autophagy by HCQ increased cell death and considerably decreased the recurrence of tumors after chemotherapy [148]. In the same way, many other anticancer drugs that induce autophagy have shown limited success in clinical trials. Thus, it is necessary to assess the function of autophagy in the mechanisms of action of these agents. Anyway, autophagy inhibition may provide new hope for anticancer chemotherapy, and a deeper understanding of the molecular basis of autophagy and macropinocytosis will help to identify more therapeutic targets for cancer that can be drugged individually or in combination.

Perspectives

Tumor cells harbor complicated genetic aberrations, such as mutations in oncogene that lead to its over-activation and produce uncontrolled cell proliferation. At the same time, they need extensive metabolic reprogramming to supply building blocks and energy, exhibiting a large number of potential drug targets. Many studies have demonstrated the relationship between oncogenic RAS and metabolic disorder. So, we may try new strategies to target cancer suppliers while blocking the well-established downstream pathways, such as RAF-MAPK and PI3K-AKT pathways.

The research evidence mentioned in this review reflects some of the vital metabolic pathways currently known to fuel cellular metabolism in RAS-mutant cancers, but there is still a lot to be done to elucidate each of the denoted metabolic pathways. In broad terms, we think that RAS mutation regulates some relative metabolism changes, but recently more evidence has shown that tumor type is another important factor [12]. So, when aiming at metabolism in different tumor types with RAS mutations, we should take the connection and differences between them into consideration. Of note, metabolic changes promoted by RAS are not absolutely specific. When the anabolic pathway for
neoplastic cells is inhibited, all other types of highly proliferating cells will also be affected. Whether there exists a therapeutic window for the clinical application of these metabolism inhibitors remains to be determined.

As listed above, achievements have been made in the development of novel inhibitors to various metabolic enzymes. For example, GLS inhibitors are already available for use. In addition, some old drugs including CQ and HCQ have the ability to inhibit autophagy and macropinocytosis. An understanding of how these available compounds perturb metabolic pathways will spark new ideas, even if other alkaloid may have equal or greater effect. Old drugs for diabetes may suppress key aspects of metabolism. A combination for existing inhibitors to the critical enzymes are still limited, especially for the anabolic pathways. The RAS downstream signaling pathways, such as the MEK-ERK pathway and the PI3K-AKT pathway, regulate the anabolic pathways. The RAS downstream signaling pathways, such as the MEK-ERK pathway and the PI3K-AKT pathway, regulate the expression of these enzymes; therefore, disrupting these pathways may suppress key aspects of metabolism. A combination for existing cancer treatments may also be used to target cancer metabolism. For example, compounds interfering ROS balance can be combined with radiotherapy and chemotherapies to strengthen the shots and suppress the emergence of resistance. Agents that inhibit autophagy can help to increase the efficacy of some antitumor chemotheraphy agents that can induce autophagy.

The accomplished work about targeting oncogenic RAS-driven metabolism changes is a tip of the iceberg. Future studies should be carried out to elucidate the extent of different tumors where the metabolic functions of RAS contribute to their biological activities. In addition, RNAi and CRISPR/Cas9 are powerful tools to identify potential targets. Further insights into the metabolism aspect of RAS-mutant cancer cell biology are expected to facilitate the development of more specific and efficient antineoplastic agents.

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References
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