Metabolic Dependencies in RAS-Driven Cancers
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Abstract
The ability to inhibit the RAS oncogene has been the holy grail of oncology because of the critical role of this gene in a multitude of tumor types. In addition, RAS-mutant tumors are among the most aggressive and refractory to treatment. Although directly targeting the RAS oncogene has proven challenging, an alternative approach for treating RAS-driven cancers is to inhibit critical downstream events that are required for tumor maintenance. Indeed, much focus has been put on inhibiting signaling cascades downstream of RAS. Recent studies have shown that oncogenic RAS promotes a metabolic reprogramming of tumor cells, shifting them toward an anabolic metabolism necessary to produce biomass to support unconstrained proliferation. These cancers also use a diverse set of fuel sources to meet their metabolic needs and have even developed a variety of mechanisms to act as metabolic scavengers to obtain necessary metabolic substrates from both extracellular and intracellular sources. Collectively, these adaptations can create “metabolic bottle-necks” whereby tumor cells rely on particular pathways or rate-limiting metabolites. In this regard, inhibiting individual or combinations of these metabolic pathways can attenuate growth in preclinical models. Because these dependencies are tumor selective and downstream of oncogenic RAS, there is the opportunity for therapeutic intervention. Although targeting tumor metabolism is still in the early days of translation to patients, our continued advances in understanding critical metabolic adaptations in RAS-driven cancers, as well as the ability to study this altered metabolism in relevant tumor models, will accelerate the development of new therapeutic approaches. Clin Cancer Res; 21(8); 1828–34. ©2015 AACR.

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Introduction
The RAS oncogene has been the subject of intense study for the past few decades upon the identification of its potent transforming potential (reviewed in refs. 1–3). Indeed, the three RAS genes (HRAS, NRAS, and KRAS) are the most frequently mutated oncogenes in cancer, with KRAS being the most prevalent. RAS proteins serve as intracellular signaling molecules that transduce extracellular signals from receptor tyrosine kinases to downstream effector cascades. Although much of the early work had focused on the signal transduction related to proliferation, it is now understood that oncogenic RAS has wide-ranging effects on a cell that drives neoplastic transformation. Indeed, the oncogenic effects of the RAS oncogene are diverse and touch most, if not all, of the hallmarks of cancer (4). While there has been a resurgence in efforts to directly target mutant RAS genes (in particular, the highly prevalent KRAS oncogene), other approaches include targeting combinations of downstream effector pathways that are essential for tumor maintenance (5–10). Although progress is being made on both fronts, a potent RAS inhibitor has proven difficult to develop, and the approach of inhibiting downstream effectors, while showing promise in preclinical studies, is hampered by the fact that there are multiple downstream signaling pathways that are activated which may change dynamically under various conditions as well as differ in the context of particular tumor types.

Recently, it has become apparent that the RAS oncogene has yet another crucial role in tumorigenesis in that it orchestrates a metabolic reprogramming of tumors. Because many of these metabolic changes are critical for tumor growth and are dependent on the presence of oncogenic RAS, there are opportunities for a favorable therapeutic index. Moreover, the catalytic nature of the enzymes involved in metabolic pathways can be amenable to targeting using small molecules.

The purpose of this review is to discuss the emerging role of the RAS oncogene as a driver of metabolic changes in tumors. It is not meant to be a comprehensive review of tumor metabolism. Rather, it focuses on recent work illustrating that oncogenic RAS can promote a variety of metabolic dependencies in tumor cells and highlights how some of these adaptations may be attractive therapeutic targets. In particular, the focus will be on KRAS as this is the most commonly mutated RAS gene in human cancer.

Tumor Metabolism
The altered metabolism of tumors has been appreciated for many decades (11, 12). Indeed, it is clear that a cancer cell with the potential for unconstrained proliferation has distinct metabolic requirements from its normal counterpart. One key difference is the need to increase biomass, which is critical for a tumor cell that undergoes rapid cell division. Thus, there is a concerted shift to anabolic metabolism to produce the building blocks required to accomplish this (13, 14). In addition, tumor cells use diverse fuel
Figure 1.
Metabolic scavenging pathways have critical roles in RAS-driven cancers. Autophagy can degrade intracellular cargo by sequestering it in autophagosomes. These fuse with lysosomes where the cargo is degraded and subsequently recycled back into the cytosol. Macropinocytosis is associated with membrane ruffling that can lead to the internalization of extracellular material such as albumin with subsequent lysosomal degradation. Both pathways can supply metabolic substrates for various metabolic pathways. Chloroquine (CQ) and hydroxychloroquine (HCQ) inhibit lysosomal acidification and therefore block the degradation of cargo in both pathways. Ethylisopropylamiloride (EIPA) inhibits macropinocytosis.

Ras and Metabolic Scavenging Pathways

Given the increased metabolic requirements of tumor cells, it is not surprising that certain tumor types have developed mechanisms to scavenge nutrients from both extracellular and intracellular sources. In particular, RAS-driven cancers have several different metabolic adaptations that allow them to recycle various metabolites. This serves two key purposes: (i) it provides metabolic flexibility and efficiency, and (ii) it ensures adequate availability of biosynthetic precursors. Importantly, these scavenging pathways have become critical to the metabolism of these cancers and may provide therapeutic opportunities.

Macrouautophagy (referred to hereafter as autophagy) is a catabolic process whereby a cell can degrade its intracellular components to support survival under stressful conditions (reviewed in refs. 19–23). This highly regulated process begins with cargo (damaged proteins, organelles, bulk cytoplasm) being sequestered in double-membraned vesicles called autophagosomes. These fuse to the lysosome, forming structures called autolysosomes, where the contents are degraded and the degraded products are recycled back into the cytosol where they can be used in a variety of biosynthetic and anabolic reactions (Fig. 1). Although the process was initially thought to be a nonselective bulk degradation pathway, it has become apparent recently, that there are also various forms of selective autophagy whereby specific cargo are brought to the autophagosome via autophagy receptors (24–26). Because of the diverse nature of what is degraded, it is evident that autophagy can provide substrates for multiple aspects of cell metabolism. These include nucleosides, fatty acids, amino acids, sugars, as well as critical elements such as iron (27, 28).

Although autophagy is present in all tissues at low levels for homeostatic functions, work from my laboratory as well as several other groups identified that autophagy is elevated in RAS-driven cancers and is critical for tumor growth (29–32). Indeed, inhibition of autophagy either pharmacologically with chloroquine (CQ) or its derivative hydroxychloroquine (HCQ), as well as genetically, demonstrated responses in cancers using in vitro and in vivo systems. These systems include Kras-driven autochthonous lung and pancreatic cancer models (29), (33–37). One of the common observations in these studies is that autophagy loss results in a metabolic dysfunction. In particular, mitochondrial
metabolism is significantly affected upon autophagy inhibition. Because CQ and HCQ have been used in patients for decades for a variety of indications, testing this therapeutic approach in patients is feasible and already under way. These agents act on the lysosome by inhibiting lysosomal acidification (ref. 19; Fig. 1). Therefore, they inhibit the late stages of autophagy at the level of autophagosome degradation. The inhibition of the lysosome likely has other cellular effects, some of which may also be antitumorigenic (see discussion of macropinocytosis below).

Currently more than 30 open trials are using HCQ in various cancers (clinicaltrials.gov). Cancers with a high frequency of KRAS mutations such as pancreatic cancers (duetal adenocarcinoma; >90%), lung (<30%), and colon adenocarcinomas (~40%) (1) are included in these trials. There have been some early reports on the efficacy of HCQ in various cancer types with mixed results and several key questions remain (38–43). One issue with these results is that it is not clear how effectively HCQ is inhibiting autophagy in patient tumors. This is a complicated issue for several reasons. First, measuring autophagy is complex (44), and it is nearly impossible to make the dynamic measurements (autophagic flux) one would need to in resected tumor specimens. Measuring flux is not possible in fixed biopsy tissue, and the surrogate markers used are static and may not accurately demonstrate autophagy inhibition. In addition, the majority of trials use combinations of various chemotherapeutics and targeted agents with HCQ. Although defining the most efficacious combinations will be critical, many of the other drugs activate autophagy as a reactive survival mechanism and therefore the measurements to an inhibition may be difficult to interpret (19). It should be noted that the pharmacologic properties of HCQ are not optimal and micromolar levels are required to inhibit autophagy and to see responses in model systems. Although this may be possible to achieve in vivo, it is not ideal (45, 46). Multiple other potentially targetable proteins are critical to autophagy (kinases such as ULK1 and ULK2; E1-like proteins such as ATG7; proteases such as ATG4b; ref. 47), and many pharmaceutical companies are pursuing such targets. Although having more potent and specific autophagy inhibitors will be exciting for the field, there are potential benefits to drugs targeting the lysosome. Indeed, in addition to the other pathways affected by blocking lysosomal function, inhibiting the later stages of autophagy results in an accumulation of cargo-filled autophagosomes. It is possible that this buildup may increase toxicity in cancer cells. Inhibition of proximal aspects of the autophagy pathway will abrogate autophagosome formation, which may have distinct cellular effects. The question still remains about how tolerable it will be to significantly inhibit autophagy in patients. As it is likely that HCQ is not completely abrogating autophagy, this question remains unanswered in patients. However, a recent study acutely deleted Agt7 in adult mice and showed that despite a complete blockade of autophagy, mice were viable for several months before they succumbed to sequelae of autophagy loss (34). Thus, intermittent dosing of effective autophagy inhibitors may be tolerated in patients.

In a related manner, RAS-driven cancers also have developed the ability to utilize extracellular fuel sources. Pioneering work in the 1980s established that expression of the RAS oncogene can promote a process called macropinocytosis (48). During this endocytic process, membrane protrusions allow the engulfment of large portions of the extracellular space into large vesicles called macropinosomes. These vesicles go through a maturation process and eventually fuse with lysosomes (reviewed in refs. 49, 50; Fig. 1). Recent work has shown that RAS-transformed cells as well as tumor cells with oncogenic KRAS require macropinocytosis for growth (51). Tumor cells can take up extracellular albumin via macropinocytosis and degrade it into amino acids, which can then enter the TCA cycle. Although many of the proteins involved in the control of macropinocytosis have yet to be identified, this process can be inhibited by the drug EIPA (ethylisopropylamiloride) which blocks Na+/H+ exchangers (52). Treatment of KRAS-mutant pancreatic cancer xenografts with EIPA decreased their growth, whereas Kras WT tumors were not affected (51). In addition to albumin, RAS-transformed cells have been shown to take up exogenous lipids to provide cells with fatty acids, thereby decreasing the need for de novo synthesis (53). Whether this uptake is occurring by macropinocytosis is still unclear. Although specific and potent macropinocytosis inhibitors are not being utilized clinically, it is likely that lysosome inhibitors such as HCQ will inhibit both autophagy and macropinocytosis. Therefore, there is rationale that inhibiting scavenging at the level of the lysosome may have increased antitumor effects in RAS-driven cancers that rely both on autophagy and macropinocytosis to provide critical fuel sources. Another potential therapeutic approach is to actually harness macropinocytosis to deliver a therapeutic payload. Indeed, it is tempting to speculate that the recent clinical success seen with the drug nab-paclitaxel (paclitaxel conjugated to albumin) in pancreatic cancer patients (54) may be in part due to increased uptake of the albumin conjugate by macropinocytosis.

Ras Promotes Shifts in Anabolic Metabolism

Recently, advances in tumor models have allowed for a detailed assessment of what factors are required for tumor maintenance. Indeed, inducible oncogenic Kras alleles have been used to explore these issues in pancreatic cancer (55, 56). Although there are certainly many facets that mutant KRAS contributes to in order to sustain tumor growth, my group and other laboratories have shown that one of its prominent roles is to reprogram aspects of central carbon metabolism (55, 57–59). In pancreatic cancer cell lines and genetically engineered mouse models, oncogenic Kras expression increases glycolysis, but it also causes the shunting of glycolytic intermediates to specific anabolic pathways (Fig. 2A). One of the major mechanisms by which mutant Kras rewires metabolism is through regulating the expression of rate-limiting metabolic enzymes. In the case of glucose metabolism, it was demonstrated that Kras activates this transcriptional program through the activation of the Raf/Mek/Erk pathway, leading to upregulation of the Myc transcription factor (55). One of the pathways that is upregulated by oncogenic Kras is the hexosamine biosynthesis pathway (HBP). The HBP produces precursors needed for glycosylation, a critical posttranslational modification that has key roles in tumorigenesis (60). Inhibition of the Kras-regulated, rate-limiting HBP enzyme Gpt1 results in decreased overall cellular glycosylation levels in pancreatic cancer cells as well as attenuated growth in vitro and in vivo. Another key finding of the glucose rewiring was that oncogenic Kras shunted glucose-derived metabolites to the nonoxidative arm of the PPP to promote ribose biosynthesis, while not affecting flux to the oxidative arm (55). Thus, Kras decouples nucleotide biosynthesis using ribose produced via the nonoxidative arm from the NADPH production by the oxidative arm. Inhibition of Rpiα or Rpe, the
Kras-regulated PPP enzymes, decreased incorporation of glucose-derived ribose into DNA/RNA and decreased pancreatic cancer growth in vitro and in vivo.

Another key metabolic role of oncogenic KRAS is to maintain redox balance, and there are several mechanisms through which it can do this. One such aspect is its role in NADPH biosynthesis. NADPH has multiple critical roles and is particularly relevant for tumor growth. It is important for reductive biosynthesis for the production of fatty acids as well as in maintaining reduced glutathione pools for redox homeostasis (18). Given the above findings where Kras shunted glucose flux through the nonoxidative PPP, this suggested that alternative carbon sources were utilized to produce NADPH. Indeed, in PDAC cell lines, the amino acid glutamine was shown to be the critical carbon source that allows KRAS-driven PDAC to produce NADPH and maintain redox balance (57, 58). In this case, oncogenic KRAS regulates flux through a novel pathway whereby glutamine-derived aspartate produced by the mitochondrial aspartate aminotransferase (GOT2) is converted to oxaloacetate by the cytosolic aspartate aminotransferase (GOT1). This is then converted to malate by malic enzyme (ME1). Malic enzyme (ME1) then produces NADPH and pyruvate using this malate (refs. 57, 58; Fig. 2B). The NADPH produced by this pathway is essential for redox balance in these cells. In this case, KRAS increases the
expression of \textit{GOT1} and represses the expression of glutamate dehydrogenase (\textit{GLUD1}), shunting the flux of glutamate away from the canonical \textit{GLUD1} pathway. The downstream signaling pathways involved in these expression changes are still under investigation. Importantly, inhibition of this pathway at any level creates a redox imbalance as evidenced by an increase in reactive oxygen species (ROS) and causes a significant inhibition of growth in \textit{vitro} and \textit{in vivo} in xenografts. Growth can be restored simply by correcting the redox imbalance, suggesting that the critical role of the NADPH generated by this pathway is to maintain reduced glutathione pools \cite{57, 58}. Another mechanism whereby KRAS can promote redox balance in addition to its role in glutamine metabolism, is through its ability to upregulate \textit{NRF2}, a transcription factor that plays a critical role in the antioxidant response \cite{61}. \textit{NRF2} controls the expression of multiple genes that result in ROS detoxification, including \textit{ME1} \cite{62–64}. The precise mechanism by which the \textit{NRF2} response is integrated in the control of redox homeostasis with other aspects, such as glutamine metabolism, remains to be fully elucidated.

An important question also remains as to whether this novel glutamine pathway is active/required in all mutant KRAS tumors or is specific to the context of KRAS mutations in pancreatic cancer. Recent unpublished data from my group suggest the latter whereby this may not be a global property of all tumor types that possess KRAS mutations. Although more work needs to be done to assess the role of this pathway across a wide array of tumor types, this early work strongly supports the idea that metabolic dependencies are a product of both the genetic background (e.g., \textit{KRAS} mutations) as well as the tissue of origin \cite{A.C. Kimmelman; unpublished data}.

Together, this work suggests several potential therapeutic approaches. Although specific inhibitors are not available to the critical enzymes in the anabolic glucose metabolism discussed above, the fact that the MEK/ERK pathway controls expression of several of these enzymes provides an opportunity to utilize MEK inhibitors to suppress key aspects of anabolic glucose metabolism \cite{55}. Indeed, these inhibitors are currently being tested in multiple clinical trials \cite{65}. Integrating these therapies in the context of their roles in decreasing nucleotide biosynthesis and production of glycolysis precursors, will require a more detailed understanding of these pathways in various KRAS-driven cancers and which combinations would be the most efficacious. Indeed, since decreased nucleotide pools would be expected to impact DNA repair, therapies that cause DNA damage such as radiation or various chemotherapies (cisplatin for example) would be predicted to work well in combination with MEK inhibitors. Moreover, given the critical role of both autophagy and macrophagy in providing fuel sources to the cell, particularly for the TCA cycle, a combination of a MEK inhibitor with HCQ would have strong scientific rationale.

In the area of glutamine metabolism, there are also possible therapeutic approaches that are suggested by the available data. As shown in pancreatic cancer, interruption of glutamine metabolism at any level of the KRAS-regulated pathway results in a growth inhibition due to a redox imbalance. Therefore, inhibiting glutamine metabolism in combination with therapies that increase ROS should be synergistic. This is supported by preclinical data in pancreatic cancer models showing a synergy with therapies that increase ROS and inhibitors of glutamine metabolism \cite{57}. Although there are no selective inhibitors for \textit{GOT1}, \textit{MDH1}, or \textit{ME1}, there are currently inhibitors to glutaminase (\textit{GLS1}) that have just entered clinical trials \cite{66}. Moreover, therapies such as radiation treatment and various cytotoxic chemotherapies work in part via the creation of ROS and have the potential to be combined with inhibitors to aspects of glutamine metabolism.

Conclusions/Critical Analysis

Because tumor cells have distinct metabolic requirements from their normal counterparts, they may be more reliant on specific pathways/fuel sources. This provides a unique opportunity for therapeutic targeting. Work in a variety of systems has demonstrated that the \textit{RAS} oncogene plays a critical role in these metabolic shifts. There is still much work to be done to fully realize the potential of these approaches. First, it is likely that \textit{RAS} mutations have tissue-specific effects on metabolism. This is due to the intrinsic metabolic wiring in the tissue of origin of a particular tumor and its interaction with oncogenic \textit{RAS}. Together this may create distinct metabolic dependencies for \textit{RAS} mutations in different tumor types and this needs to be explored in a systematic fashion. In a similar manner, \textit{RAS} mutations act in the context of other alterations in tumors, namely other oncogenic events as well as deletion/mutation of a constellation of tumor suppressor genes. Indeed, the tumor suppressor \textit{p53} has been shown to have an impact on multiple facets of cellular metabolism (reviewed in refs. 67–69). Therefore, it will be important to incorporate the tumor suppressor background when studying the impact of mutant \textit{RAS} on metabolism. Another aspect that requires additional study will be how these \textit{RAS}-dependent metabolic changes are altered \textit{in vivo} in the tumor microenvironment. This includes areas of hypoxia, limited nutrients, as well as potential metabolic crosstalk between tumor and stromal cells. To understand these complex relationships will require the use of sophisticated autochthonous tumor models as well as the ability to perform metabolic tracing studies \textit{in vivo}.

In terms of therapeutic targeting of altered metabolism, there are still several obstacles that need to be overcome. Despite the fact that the metabolic dependencies are created by \textit{RAS}, there may be physiologic situations where they are also required in normal tissues. Thus, it could be difficult to ensure a reasonable therapeutic index. This will require thorough preclinical studies using knockout mice for metabolic enzymes as well as tool compounds as they become available. In addition, we must identify adaptive responses of \textit{RAS}-driven cancers to inhibition of particular metabolic pathways that could promote therapeutic resistance. Many of the pathways of interest discussed above do not have inhibitors available at this point. Inhibitors that are available such as HCQ (autophagy and macrophagy) may have potency issues. We are still in the early days of targeting aberrant tumor metabolism and anticipate that more inhibitors will become available that will enable us to target relevant pathways. Understanding how best to integrate inhibitors to metabolic pathways with existing chemo- therapeutic agents as well as determining the appropriate combination of metabolic pathways to target will be critical and will
require important preclinical development. Even if one were to develop a potent and effective KRAS inhibitor, an important consideration is the development of resistance. Recent evidence has shown that when Kras is genetically ablated in Kras-dependent pancreatic cancer cells, there is a small population of resistant cells that remains dormant and is likely responsible for the ultimate relapse. Interestingly, these cells display distinct metabolic properties and are highly reliant on oxidative phosphorylation (70). Inhibition of oxidative phosphorylation decreased survival of these cells and decreased tumor recurrence. These results highlight the potential for integrating metabolic targeted therapies with other approaches to target KRAS.

This is an exciting time in the cancer biology field and the pace of discovery is rapid. The renewed excitement in targeting the RAS oncogenes, coupled with improvements in technology, brings hope that we will make significant progress. There have also been advancements in our understanding of how tumors driven by oncogenic Ras have altered metabolic needs as well as reliance on particular metabolic pathways. By identifying and inhibiting these metabolic dependencies that are created in Ras-mutant tumors, we have an opportunity to develop effective and well-tolerated therapies.

**Disclosure of Potential Conflicts of Interest**

A.C. Kimmelman reports receiving speakers bureau honoraria from Agios and the US Oncology Network, and is a consultant/advisory board member for Astellas Pharma, FORMA Therapeutics, and tCella. No other potential conflicts of interest were disclosed.

**Acknowledgments**

The author thanks Drs. Costas Lyssiotis and Haoqiang Ying for critical reading of the article and apologizes for the omission of any primary references.

**Grant Support**

A.C. Kimmelman was supported by the NCI of the NIH under award number R01CA157490, an American Cancer Society Research Scholar Grant (RSG-13-298-01-TBG), and the Lustgarten Foundation.

Received November 25, 2014; revised January 16, 2015; accepted February 20, 2015; published online April 15, 2015.

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**References**


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