Conceptual Framework for Cutting the Pancreatic Cancer Fuel Supply
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Abstract
Pancreatic ductal adenocarcinoma (a.k.a. pancreatic cancer) remains one of the most feared and clinically challenging diseases to treat despite continual improvements in therapies. The genetic landscape of pancreatic cancer shows near ubiquitous activating mutations of KRAS, and recurrent inactivating mutations of CDKN2A, SMAD4, and TP53. To date, attempts to develop agents to target KRAS to specifically kill cancer cells have been disappointing. In this regard, an understanding of cellular metabolic derangements in pancreatic cancer could lead to novel therapeutic approaches. Like other cancers, pancreatic cancer cells rely on fuel sources for homeostasis and proliferation; as such, interrupting the use of two major nutrients, glucose and glutamine, may provide new therapeutic avenues. In addition, KRAS-mutant pancreatic cancers have been documented to depend on autophagy, and the inhibition of autophagy in the preclinical setting has shown promise. Herein, the conceptual framework for blocking the pancreatic fuel supply is reviewed. Clin Cancer Res; 18(16); 4285–90. ©2012 AACR.

Introduction
Although inherited and acquired mutations are believed to cause pancreatic cancer (see review by Iacobuzio-Donahue and colleagues in this issue; ref. 1), common mutations in canonical oncogenes, such as AKT, MYC, PI3K, and RAS, and tumor suppressors, including TP53 and PTEN, also alter cancer metabolism to enable cancer cells to survive and proliferate in the hypoxic and nutrient-deprived tumor microenvironment (see review by Feig and colleagues in this issue; ref. 2). Oncogenes and tumor suppressors alter metabolism through transcriptional and posttranscriptional mechanisms. Mutations in genes encoding metabolic enzymes, such as succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH) have also been linked to tumorigenesis, underscoring the intimate connections between metabolism and cancer (11, 12). Loss-of-function SDH or FH mutations result in accumulation of precursors such as succinate or fumarate. Mutated IDH1 or IDH2, on the contrary, are neomorphic enzymes that reverse the chemical reaction to produce 2-hydroxyglutarate (2-HG) from α-ketoglutarate (α-KG; ref. 13). These accumulated metabolic intermediates all have the ability to inhibit α-KG–dependent dioxygenases, which are involved in histone or DNA demethylation and in prolyl hydroxylases that modulate the hypoxia-inducible factors (HIF; refs. 14–16). In this regard, mutations in enzymes cause epigenetic deregulation that contributes to tumorigenesis.

To survive and proliferate in an adverse tumor microenvironment with limited nutrients and oxygen, cancer cells must rely on their ability to reprogram canonical biochemical pathways to provide the necessary bioenergetics and precursors of proteins, nucleic acids, and membrane lipids (17–19; Fig. 1). In addition to rewiring metabolism, tumor cells can also activate autophagy, a process that permits recycling of cellular constituents as internal fuel sources when external nutrient supplies are limited (20–22). Although nutrient-rich conditions inhibit autophagy through activation of mTOR, nutrient-deprived conditions decrease ATP production, resulting in activated AMPK that stimulates autophagy (23, 24; Fig. 1). As such, cancer cells have multiple mechanisms for metabolic adaptation to the tumor microenvironment.

Proliferating cancer cells transport glucose and glutamine into the cell as major nutrient sources for the production of ATP and building blocks for macromolecular synthesis (3, 4, 6; Fig. 1). The mitochondrion serves not only as the cellular powerhouse, producing ATP efficiently from glucose and glutamine, but it is also a hub for the production of key intermediates involved in nucleic acid, fatty acid, and heme synthesis. The proliferating cancer cells, hence, also produce toxic by-products that must be eliminated or extruded for cell survival. Reactive oxygen species (ROS) from the mitochondrion are neutralized; superoxide is converted by SOD to hydrogen peroxide, which is neutralized by catalase via its conversion to water and oxygen (25–27). Lactate and carbon dioxide, produced from glucose and glutamine catabolism, are exported through monocarboxylate transporters or neutralized and extruded by...
carbonic anhydrase (28). Although this article focuses on blocking cancer fuel supply as a promising new approach to pancreatic cancer therapy, therapeutic opportunities may also exist in blocking the exhaust pipes that eliminate metabolic toxic by-products from cancer cells.

Characteristic Features of Metabolism in Cancers

A aberrant metabolism is now considered one of the hallmarks of cancer (29). As a result of genetic alterations and tumor hypoxia, cancer cells reprogram metabolism to meet increased energy demand for enhanced anabolism, cell proliferation, and protection from oxidative damage and cell death signals. The molecular underpinnings for the reprogrammed metabolism of cancer cells have been linked to the activation of oncogenes or loss of tumor suppressors, which can function independently of, or through constitutive stabilization of the HIF, HIF-1α. Activated Ras and AKT oncogenes can increase glycolysis, enhancing the conversion of glucose to lactate (3). The MYC oncogene, which encodes the transcription factor Myc, increases the expression of glycolytic genes, thereby enhancing glycolysis and production of lactate (35).

Glycolysis is a multi-enzymatic pathway that catalyzes glucose to pyruvate via more than a dozen different enzymes with several apparent rate-limiting steps. Phosphofructokinase provides the first rate-limiting step where fructose-1-phosphate, derived from glucose, is converted to fructose-1,6-bisphosphate. The other rate-limiting step is the conversion of phosphoenolpyruvate to pyruvate by pyruvate kinase (muscle form) PKM. Recent studies have raised an intense interest in the embryonic M2 isoform of pyruvate kinase (PKM2), which is highly expressed in human tumors. A study by Gao and colleagues (30) showed that dimeric, not tetrameric PKM2 that localizes the cellular nucleus, activates transcription of MEK5 by phosphorylating stat3 at Y705 using PEP as a phosphate donor. This protein kinase activity of PKM2 plays a role in promoting cell proliferation, revealing an important link between metabolism alteration and gene expression during tumor transformation and progression. The xenografted adenocarcinoma cells, which carry a mutant form of PKM2 that preferentially forms dimers, grow more rapidly and seem more aggressive than cells carrying wild-type (WT) PKM2. Although the dynamics of PKM2 oligomeric states and their roles in cancer remain confusing, these studies suggest that perturbation of glycolysis via manipulation of PKM2 could have a significant therapeutic effect.

Alteration of glucose metabolism in cancer is known as aerobic glycolysis or the Warburg Effect (3, 5, 31), which describes the ability of cancer cells to avidly metabolize glucose and glutamine via glycolysis and the TCA cycle. Both substrates contribute to the production of ATP, which when depleted would activate AMPK that in turn triggers autophagy. Autophagy increases lysosomal recycling of cellular constituents to recover ATP. GLS, glutaminase.
A manifestation of the Warburg Effect is the increased $\text{[^{18}F]fluoro-2-deoxy-D-glucose}$ import by pancreatic cancers as determined by positron emission tomographic (PET) scans. However, only about 50% of pancreatic cancers have positive clinical PET, suggesting additional complexities and the possibility that pancreatic cancers could use other fuels or resort to other metabolic survival modes (32, 33).

In addition to glucose, proliferating cancer cells also rely on glutamine as a major source of energy and building blocks. In fact, MYC, which is frequently amplified or overexpressed in pancreatic cancer (34), has been mechanistically linked to the regulation of glutamine metabolism (35). In this regard, it is reasonable to surmise that PET negative pancreatic cancers may use glutamine as a major nutrient source. The Myc transcription factor emerges as a master regulator of a plethora of genes involved in cell growth including those regulating ribosome and mitochondrial biogenesis and intermediary metabolism. The normal MYC:gene is under the scrutiny of many internal as well as extracellular cues such as nutrient and oxygen availability, such that deprivation of these supplies results in the downregulation of MYC expression. By contrast, many oncogenic pathways activate MYC or alterations of MYC itself resulting in a constitutive program of ribosome biogenesis and biomass accumulation that renders cancer cells addicted to nutrients. Indeed withdrawal of either glucose or glutamine triggers death of cells with MYC overexpression (35). In this regard, targeting enzymes involved in glucose or glutamine has also provided proof-of-concept that metabolic inhibition could provide a beneficial therapeutic effect (36).

Applying metabolomics technologies, with the use of nuclear magnetic resonance and mass spectrometer–based stable isotope resolved metabolomics with $^{13}$C-labeled glucose and glutamine, a study by Le and colleagues (36) documents that cancer cells use either glucose or glutamine, depending on the availability of the nutrients. This flexibility of cancer metabolism enables cancer cells to proliferate and survive even under the hypoxic and nutrient-deprived conditions, which are often encountered in the tumor microenvironment. Moreover, in hypoxia, they observed the enhanced conversion of glutamine to glutathione, an important reducing agent for control of the accumulation of mitochondrial ROS. Most importantly, this study uncovered a previously unsuspected glucose-independent glutamine-driven tricarboxylic acid (TCA) cycle. Cancer cells subjected to glucose deficiency and/or hypoxia would benefit from such glucose-independent TCA cycle activity in the tumor microenvironment. Cell growth and survival can be sustained by glutamine metabolism alone. In addition to the links between oncogenes and tumor suppressors to altered cancer cell metabolism, mutations in specific TCA cycle enzymes contribute to tumorigenesis of familial or spontaneously acquired cancers. The study by Mullen and colleagues (37) uncovered other metabolic reprogrammed pathways in cancer cells, which have mutations in complex I or complex III of the electron transport chain (ETC). These are often found in patient-derived renal carcinoma cells with mutations in FH, and also in pharmacologically ETC-inhibited cells with normal mitochondria. These cancer cells use glutamine-dependent reductive carboxylation generating acetyl-CoA for lipid synthesis. These processes use mitochondrial and cytosolic isoforms of NADPH/NADP-dependent IDH. The ETC-deficient cancer cells, like FH mutations, cannot grow without glutamine. Hence, depending on the genetic makeup, a cancer cell could be distinctively addicted to glucose or glutamine.

Given that pancreatic cancers uniquely display increased levels of fibrotic stroma, the pancreatic cancer cell environment could be highly nutrient deficient (38, 39). In this nutrient-deprived state, increased AMPK activity could trigger autophagy (21). The process of self-eating or autophagy provides starved cells a means to survive by recycling cellular components as bioenergetic substrates for energy production and building blocks. As such, certain metabolic hubs should be exploitable for therapy, particularly if a specific cancer type is “addicted” to that pathway. Metabolic enzymes are readily pharmacologically targetable; in fact, many historical clinically effective chemotherapeutic drugs were termed “antimetabolites.” Seeking drugs that directly inhibit these new metabolic targets involved in cancer cell energy metabolism while sparing normal cells is among the most desirable goal to improve cancer therapy, especially for the treatment of pancreatic cancer.

**Blocking pancreatic cancer fuel supply**

Addiction to glucose could be exploited through targeted inhibition of enzymes involved in glycolysis. One such target is the glycolytic pathway, in which the most consistent abnormality is its ultimate step of conversion of pyruvate to lactic acid by lactate dehydrogenase A (LDHA) to regenerate NAD$^+$ that is required for the further glycolytic conversion of glucose to pyruvate to generate ATP. Recent studies (40, 41) have targeted this phenotype of altered metabolism for therapy. The first drug-like small molecule (called FX11) that inhibits LDHA was used as proof of concept for targeting aerobic glycolysis in cancer. This compound, 7-benzyl-2,3-dihydroxy-6-methyl-4-n-propyl-1-naphtholic acid, has shown an antitumorigenic effect in mouse models of human lymphoma and pancreatic cancer through the increased production of ROS and cell death (40). By blocking LDHA, FX11 diminishes the ability of malignant cells to metabolize pyruvate to lactate, and halts the regeneration of NAD$^+$ for glycolysis processing. This study also showed a strong synergy effect in vitro and in vivo with the use of FX11 in combination with FK866, an inhibitor of NAD$^+$ biosynthesis, which accentuates NAD$^+$ depletion.

Besides the Warburg Effect, cancer cells also maintain mitochondrial oxidation of glutamine by glutaminase that converts glutamine to glutamate, which enters the TCA cycle as 2-oxoglutarate. Two recent studies targeted glutaminolysis in cancer by a specific glutaminase inhibitor, bis-2-[5-((phenylacetamido)-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES;
ref. 42). The first study by Seltzer and colleagues (43) reported a preferable inhibition of mutant IDH1 cell growth by BPTES as compared with the WT enzyme. Mutation at the R132 residue of IDH1 creates a novel enzyme function that produces 2-HG from α-KG, which is from glutamate, a product of glutamine via glutaminase. Cancer cells with mutant IDH1 become addicted to glutamine and heavily depend on glutaminase. The addition of exogenous α-KG rescued growth suppression of mutant IDH1 cells by BPTES, which lowered glutamate and α-KG levels, inhibited glutaminase activity, and increased glycolytic intermediates. However, 2-HG levels were unaffected by BPTES. This presents a potential therapeutic opportunity. The study by Wang and colleagues (44) provided another aspect of targeting mitochondrial glutaminase activity inhibiting oncogenic transformation. They showed that glutaminase activity, which is dependent on Rho GTPases and NF-κB activity, increased in transformed fibroblasts and breast cancer cells. Targeting glutaminase activity by BPTES to inhibit oncogenic transformation had thus been shown, through a connection between Rho GTPase activation and cellular metabolism, to be a promising way forward. The importance of hypoxic glutamine metabolism in the study by Le and colleagues was also underscored by the antiproliferative therapeutic effect of BPTES on neoplastic cells in vitro and in a tumor xenograft model in vivo (36).

In addition to the proof-of-concept studies suggesting the feasibility of targeting glucose or glutamine metabolism in pancreatic cancer, it has been noted that pancreatic cancers display significant autophagic activities for survival (45–49). In fact, Ras-transformed cells depend on autophagy for survival (50). Hence, inhibition of autophagy with the antimalarial agent chloroquine has resulted in significant preclinical responses of pancreatic cancer xenografts and allografts in treated mice as compared with control (49, 51). Chloroquine also diminishes pancreatic tumorigenesis in a transgenic model (49). These studies suggest that 2 related and widely used agents with extremely favorable safety profiles, chloroquine or hydroxychloroquine, could have profound clinical effects. Indeed, there are now clinical trials testing this concept in pancreatic cancer. As for targeting glycolysis or glutaminolysis, the field is awaiting pharmaceutical companies to develop clinically safe, highly potent drugs to be tested in the clinic. Notwithstanding the inherent challenges for drug development, the concept of blocking the pancreatic cancer fuel supply provides a reasonable framework for the development of what is hoped to be a new class of anticancer agents.

**Future directions**

Although cancer cells can exhibit unique metabolic pathways, they also use the classic metabolic pathways of normal cells. This presents a great challenge to directly target metabolic pathways, especially metabolic enzymes as drug targets. The success of small molecular agents depends on how much cancer cells are “addicted” to the fuel nutrition versus normal cells, as shown in the studies mentioned above. The combination of multiomics technologies will give a functional perspective of cancer progression, beyond genes and protein expression profiles. The extensive data obtained from metabolic flux will be mined to identify specific pathways active in the tumor compared with untransformed cells. These data are essential for understanding the metabolic activity in tumor tissue, especially how cancer cells can respond to different environmental conditions and how they respond to therapy to detect likely responders and nonresponders to a particular chemotherapeutic agent. This is highly desirable because it is important to avoid the use of cytotoxic drugs that have no benefit. The ultimate goal is to characterize and enable targeted selection of patients based on predicted metabolic responses. The metabolic signatures that correlate with sensitivity of pancreatic cancers to metabolic inhibitors will also be used in the future to help us combine existing drugs to target multiple metabolic pathways and attack specific attributes of each patient’s cancer. In fact, metformin, which inhibits NADH dehydrogenase and mitochondrial respiration, has preclinical activity against pancreatic cancer xenografts (Kumar and colleagues, unpublished data) and is used in clinical trials. However, parameters that predict resistance or response remain poorly understood. Hence, defining the metabolic pathways of pancreatic cancer through metabolomics will pave the way for prediction of response and the identification of new enzyme targets for pancreatic cancer therapies. A major technical challenge in this arena is the heterogeneity of the pancreatic tumor tissue, which is laced with an extensive fibrotic stroma comprising of host immune cells. The potential for cancer cells to reprogram their metabolism to bypass the targeted enzymatic step may occur and hence poses a challenge to metabolic therapy. This issue is especially significant given the interconnectivity of the cellular metabolic network. In combination with appropriate computational tools (e.g., flux balance analysis), metabolomics offers a powerful way to identify possible “metabolic escape routes.” With the insight of this guide, we will eliminate these escape routes through rational combination therapies, for example, in combination with current anti-metabolites. This will allow for more cost-effective and personalized cancer treatment.

Further complicating attempts at developing pancreatic cancer therapies, there is emerging evidence of pancreatic cancer stem cells in pancreatic cancers and these cells are responsible for drug resistance to standard chemotherapy, resulting in relapse and metastasis of pancreatic adenocarcinoma (see review by Penchev and colleagues in this issue; ref. 52). Therefore, recognizing the heterogeneity of the subpopulation of pancreatic tumors and understanding the distinguished metabolic modes of each are critical important for targeting of these subpopulations.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: A. Maitra

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.V. Dang
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