Metabolic adaptations to glutamine deprivation in pancreatic cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC), a poorly vascularized malignancy, is one of the most lethal human cancers. Despite using chemotherapy, radiation, and surgery in the treatment of pancreatic cancer, the survival rate remains largely dismal. Several *in vivo* and patient-based metabolomics analyses revealed that, compared to normal tissues, PDAC tumors are depleted of glutamine, a major metabolic substrate. Yet the mechanisms by which PDAC cells adapt to low glutamine levels are still unclear. Thus, it is imperative to understand the differential metabolic mechanisms in pancreatic cancer. Here, we review the current understanding of metabolic rewiring in pancreatic cancer in response to glutamine deprivation. The elucidation of these adaptive strategies may highlight new opportunities to improve PDAC diagnosis, as well as, shed insight towards novel therapeutic developments.

Keywords: Pancreatic cancer; metabolic stress; glutamine deprivation; metabolic adaptation.

Pancreatic cancer is considered one of the most lethal cancers with an increasing number of incidences and poor clinical outcomes. In 2019, the NIH estimates 56,770 new cases of pancreatic cancer and 45,750 death in the United States. Additionally, with a global increase in incidences, pancreatic cancer is expected to be the second leading cause of cancer-related death by 2020 (1). Anatomically, pancreatic tumors are classified into two main types: either forming in the exocrine gland or the endocrine gland. The most common pancreatic cancers, pancreatic ductal adenocarcinomas (PDACs), is an exocrine tumor compromising 95% of all pancreatic cancers (1). Despite advances in pancreatic cancer research, the five-year survival rate remains around 5-7%, largely because most cases are diagnosed at advanced stages lowering the benefits from surgical or chemotherapeutic interventions (2, 3). This grim prognosis is caused by the inaccessible location of the pancreas (4), lack of visible symptoms, and tumor biomarkers for early diagnosis (5). Therefore, early-stage detection methods and more effective preventive strategies are urgently needed for improving PDAC patients' survival rates (6).

Besides the fast progression of this disease, PDAC tumorigenesis is often accompanied by morphological and genetic alterations. The aberrant signaling pathways and metabolic alterations in PDAC tumors influence cell proliferation and growth even under harsh conditions in the microenvironment. More critically, the remodeling of PDAC genetics and metabolism can induce resistance to systemic therapies and hinder the success of other targeted therapies (7). Current understanding of how PDAC cells survive low levels of nutrients and the molecular pathways mediating these adaptions are still undergoing. This review focuses on recently discovered adaptive strategies used by PDAC cells under metabolic stress, with a specific focus on glutamine deprivation, to promote survival and resistance.

PDAC microenvironment

Compared to other cancer types, PDAC is characterized by a stroma-rich environment, which occupies the majority of the tumor mass (8). The PDAC stroma is heterogeneous and consists of a dynamic assortment of extracellular matrix components (ECM) and nonneoplastic cells. The accumulation of ECM components,

such as collagen, fibronectin, proteoglycans, hyaluronic acid (HA), catalytically active enzymes and proteinases, induces a change in the normal architecture of pancreatic tissue forming a dense barrier and leading to an abnormal configuration of blood and lymphatic vessels (9). Specifically, the excessive HA accumulation causes fluid pressure and compresses blood vessels potentially contributing to drug resistance in PDAC (10). Moreover, fibroblasts, pancreatic stellate cells (PaSCs), immune cells, and blood vessels form the cellular component of the desmoplasia and interact with cancer cells to influence tumor progression and invasion. For instance, cross-talk with PaSCs has been shown to enhance PDAC cell proliferation and migration (11).

This dense tumor mass also forms a highly hypoxic and nutrient-poor microenvironment (1, 8, 9, 12), due to the generation of solid stress and fluid pressure in tumors, and compression of the surrounding vessels. Importantly, a recent metabolomics study compared PDAC patient samples to benign adjacent tissue by using mass spectrometry and indicated that poor perfusion in tumors led to significant depletion of not only oxygen, but also nutrients such as glucose, glutamine, and fatty acids (13). This study indicates that PDAC cancer cells must adapt to the stress from microenvironment by exhibiting modified and unconventional pathways to survive.

Glutamine metabolism in PDAC

Unlike the extensive understanding of the mutational mechanisms that initiate PDAC, which include common mutations in oncogenes such as AKT, MYC, PI3K, and RAS, and tumor suppressors, such as TP53 and PTEN, the metabolic rewiring in PDAC is still unclear. Reprogrammed cellular metabolism is crucial for tumor cells to sustain rapid cell growth and proliferation. As early as 1940, proliferating cancer cells were known to display a switch to aerobic glycolysis, also known as the Warburg effect. This allows for the utilization of glucose carbon as a major nutrient source to produce lactate and support ATP production and the necessary building blocks for anabolic processes. As this diverts glycolysis from the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, cancer cells increase their consumption of glutamine and other nutrients to maintain these pathways (14-16).

Glutamine, a non-essential amino acid, can serve as an important source of nitrogen in biosynthetic reactions, and a carbon source for glutathione production, and a precursor to nucleotides and lipid synthesis. The increased demand for glutamine by cancer cells was reported as early as the 1950s (17) and is now recognized as a hallmark of fast proliferative cancer cells (18, 19). In PDAC cells, the increased dependence on glutamine is reprogrammed by the activation of KRAS pathways that maintain its survival and growth (20). Oncogenic KRAS mutation is a significant genetic mutation occurring in over 90% of all patients and directly influences PDAC tumor initiation, progression, and growth. Specifically, the mutation in KRAS influences PDAC growth through metabolic rewiring of glutamine metabolism in two distinct ways. Firstly, cells express glutamate dehydrogenase (GLUD1) to convert glutamine-derived glutamate into α-ketoglutarate (α-KG) in the mitochondria to fuel the tricarboxylic acid cycle, as well as, serve as a cofactor for DNA and protein modifying dioxygenases. Secondly, KRAS expression directs the metabolism of glutamine through the noncanonical metabolic pathway (6). Mutant KRAS increases Glutamic-Oxaloacetic Transaminase 1 (GOT1) and decreases GLUD1 gene expression, resulting in increased flux through the GOT1-dependent pathway. Glutamine-derived aspartate is transported into cytoplasm where aspartate is converted to oxaloacetate, which is then converted to malate and further to pyruvate. The conversion of malate to pyruvate leads to an increased NADPH/NADP+ ratio which maintains the cellular redox state (21, 22).

As a consequence of pancreatic tumor interstitial localization and the surrounding dense stroma, PDAC cells are often situated in nutrient

deprivation microenvironment (23). Additionally, the paradox of increased glutamine dependency by PDAC cells leads to its depletion. Consistently, several studies in murine tumor models showed lower glutamine levels in solid tumors compared to adjacent normal tissues in a variety of tumors (24-26). More importantly, in vivo metabolomics studies demonstrated glutamine depletion in pancreatic tumors (13). Given the particular importance of glutamine as both a source of usable nitrogen and carbon that contributes to the TCA cycle, PDAC cells have to overcome these limitations and develop strategies to survive during glutamine deprivation.

Adaptation strategies to glutamine deprivation in PDAC cells

PDAC cells use different mechanisms to survive and grow under glutamine deprivation including autophagy, macropinocytosis, and reprogrammed cellular pathways.

Autophagy

PDAC cells activate the autophagic degradation of macromolecules when deprived of glutamine. Autophagy (or macroautophagy), a highly conserved cellular catabolic process, is used in PDAC cells to mediate degradation and utilization of biomolecules as well as whole organelles (1, 27). During autophagy, damaged organelles and their macromolecular components are degraded, providing recycled small molecule nutrients to feed into the intermediary metabolic pathways. Elevated autophagy is found in both primary PDAC tumors *in vivo* and cell lines *in vitro* (28). Genetic inhibition of autophagy in PDAC cells potently suppresses proliferation *in vitro* and elicits robust tumor repression and prolonged survival in pancreatic cancer xenografts and genetic mouse models (29). More importantly, under glutamine deprivation, PDAC cells rely on autophagy to maintain their survival (27, 30). Overall, these findings emphasize a role for autophagy in driving aggressive tumor formation and maintenance by providing intracellular nutrient supply to support cell survival.

Macropinocytosis

In addition to autophagy, PDAC cells activate salvage pathways for the uptake of extracellular protein to sustain the intracellular requirement for glutamine. Macropinocytosis is stimulated in PDAC cells by nutrient stress-induced activation of EGFR-Pak signaling and Src signal transduction (31, 32). In this process, extracellular proteins are demonstrated to be internalized for lysosomal degradation, which directly led to intracellular increased concentrations of glutamate and α-ketoglutarate (32), allowing starved cells to survive in low glutamine conditions (33). Mechanistically, the incoming protein-derived glutamine from macropinocytosis contributes substantially to the intracellular glutamine pool, supplying multiple metabolic pathways including the TCA cycle, redox homeostasis, nucleotide synthesis, and glycosylation (33). Thus, macropinocytosis provides a new model of metabolic flexibility to provide a source of glutamine enabling PDAC cells to adapt to the limited supply of glutamine in their unique macroenvironment (7).

Reprogrammed cellular pathways

Due to the selective usage of glutamine, PDAC cells eventually encounter a glutamine poor environment despite ongoing autophagy and macropinocytosis, which have been supported by a few studies using patient samples. First, as we mentioned previously, metabolomics analysis of over 49 patient PDAC samples vs adjacent benign tissues revealed that glutamine levels are significantly decreased in PDAC samples (13). Another recent publication analyzed over 200 patient samples to compare metabolomics data of plasma samples of pancreatic cancer patients versus chronic pancreatitis patients. The results from this study demonstrated that glutamine is significantly decreased (34). Moreover, a recent effort to use glutaminase inhibitor on PDAC tumors was faced with several difficulties since pancreatic cancer cells were shown to have adaptive metabolic networks that sustain proliferation in vitro and in

vivo upon targeted inhibition of glutamine metabolism (35).

In order to delineate some of these mechanisms that allow PDAC cells to combat glutamine deprivation, several studies have begun to explore potential adaptive pathways (36, 37). Izuishi et al. showed that high expression of PKB/AKT was associated with tolerance to nutrient starvation. When AKT antisense vectors were introduced into PANC-1 cells, cellular tolerance to glutamine deprivation was partially but significantly diminished by targeting both Akt1 and Akt2 (38). Additionally, dedifferentiation in PDAC cells, driven by mutations in p53 commonly associated with pancreatic cancer, was reversed by the accumulation of αKG, the downstream metabolite of glutamine. This indicates PDAC cells are able to survive and keep malignant progression under glutamine stress by the gain of function in mutant p53 (39-41). Consistently, our previous work discovered that activation of mutant p53 upon glutamine depletion regulates miR-135, which directly targets phosphofructokinase-1 (PFK1) and inhibits aerobic glycolysis, thereby promoting the utilization of glucose to support the TCA cycle. By upregulating miR-135, PDAC cells had high cell viability in glutamine deprived conditions (42). Moreover, Slc7a3, an arginine transporter, was increased upon glutamine deprivation. This influx of arginine was also dependent on p53 (43).

Conclusion

PDAC cells must contend with further metabolic constraints due to their hypovascular, fibrotic microenvironment, and ensuing hypoxia and limited nutrient availability. To support tumor growth, PDAC cells acquire multiple alterations in metabolic circuitry under glutamine deprivation, including activation of nutrient scavenging processes such as autophagy and macropinocytosis, as well as, metabolic signaling regulated by p53, miRNA, and transporters. Thus, it is important to study adaptive signaling pathways in PDAC cells when glutamine levels are low, or glutamine metabolism is inhibited. The elucidation of these adaptation strategies may highlight new opportunities to improve PDAC diagnosis as well as advanced the development of novel targeted therapeutics.

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