BBA - Reviews on Cancer xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review

The plasticity of pancreatic cancer metabolism in tumor progression and therapeutic resistance

Douglas E. Biancur, Alec C. Kimmelman*

Perlmutter Cancer Center, Department of Radiation Oncology, NYU Medical School, New York 10016, NY, United States

ARTICLE INFO

Keywords:
Pancreatic cancer
Autophagy
Metabolism
Microenvironment
Macropinocytosis

ABSTRACT

Pancreatic ductal adenocarcinoma (PDA) is an aggressive cancer that is highly refractory to the current standards of care. The difficulty in treating this disease is due to a number of different factors, including altered metabolism. In PDA, the metabolic rewiring favors anabolic reactions which supply the cancer cell with necessary cellular building blocks for unconstrained growth. Furthermore, PDA cells display high levels of basal autophagy and macropinocytosis. *KRAS* is the driving oncogene in PDA and many of the metabolic changes are downstream of its activation. Together, these unique pathways for nutrient utilization and acquisition result in metabolic plasticity enabling cells to rapidly adapt to nutrient and oxygen fluctuations. This remarkable adaptability has been implicated as a cause of the intense therapeutic resistance. In this review, we discuss metabolic pathways in PDA tumors and highlight how they contribute to the pathogenesis and treatment of the disease.

1. Introduction

1.1. PDA Biology

Within the last 30 years considerable progress has been made in cancer detection and treatment, leading to a significant increase in survival rates of many cancer types. Despite these recent advances, the 5 year survival of pancreatic cancer remains dismal at ~8% [1]. In the United States there were 53,670 estimated new cases and 43,090 estimated deaths for 2017, making pancreatic cancer one of the deadliest cancers [1]. Despite only representing < 3% all cancer diagnoses, it is predicted to become the second leading cause of cancer deaths by 2020 [2]. The poor prognosis and aggressive nature of pancreatic cancer is linked to late onset of disease presentation, cancer cell intrinsic alterations, and microenvironmental factors.

The most common subtype of pancreatic cancer, accounting for about 90% of all cases, is pancreatic ductal adenocarcinoma (PDA) and is the focus of the review. The majority of PDA cases arise from pancreatic intraepithelial neoplasms (PanIN) which are microscopic lesions of dysplasia. The progression of PanIN to invasive carcinoma is a gradation brought on by accumulation of genetic alterations and development of the distinctive microenvironment [3]. The driving oncogene in PDA results from an activating mutation in the *KRAS* gene which is found in > 90% of tumors [4]. *KRAS* mutations are found in low grade PanIN lesions (PanIN-1A) and are likely an early event in malignant transformation. There is, however, discordance between the prevalence of benign precursor lesions and rates of cancer incidence, suggesting other genetic and environmental factors cooperate in the development of PDA [3]. Consistent with this observation, mouse models of pancreatic cancer driven by a pancreas-specific Cre-deleter and a latent knock-in allele of oncogenic *Kras* (LSL-$Kras^{G12D}$; Pdx^{Cre}) have long latency or require the loss of tumor suppressor genes (*TP53*, *CDKN2A*, *SMAD4*, and others) to develop a tumor [5,6].

Despite the complexity of PDA initiation, it has been established that the *KRAS* mutation is a key driver of tumor progression and multiple studies report that tumors are highly dependent on this oncogene for tumor maintenance [7,8]. The *KRAS* oncogene encodes for a small GTPase which acts as a molecular switch bridging signals from membrane bound receptors to central cellular signaling pathways. The activity of KRAS is regulated by GTPase activating proteins (GAPs) and guanine exchange factors (GEFs) which toggle the protein between inactive (GDP bound) and active (GTP bound) states [9]. In PDA, *KRAS* is most commonly mutated at the G12 residue preventing the interaction with GAPs which results in KRAS bound to GTP and is therefore constitutively active [10]. This results in aberrant downstream signaling through pathways such as mitogen-activated protein kinase (MAPK) and phosphoinsotide-3 kinase (PI3K). These signaling pathways are responsible for regulating a number of key cellular functions including growth and survival. Unchecked KRAS signaling results in increased proliferation, decreased apoptosis, and an invasive phenotype

* Corresponding author at: Perlmutter Cancer Center, Department of Radiation Oncology, NYU Medical School, New York 10016, NY, V: 646.501.8940, United States.
E-mail address: Alec.Kimmelman@nyumc.org (A.C. Kimmelman).

https://doi.org/10.1016/j.bbcan.2018.04.011
Received 31 January 2018; Accepted 20 April 2018
0304-419X/

[9]. While tumors require KRAS for growth, thus making it an attractive drug target, disrupting this oncogene pharmaceutically has proven problematic [11]. Recently, however, the development of inhibitors targeting specific KRAS mutations (G12C) have provided evidence that some mutations lead to vulnerabilities that can be exploited for therapeutic gain [12].

Appreciable effort is also underway to identify other frequently mutated genes in PDA as potential therapeutic targets. Whole exome sequencing of pancreatic tumors revealed reproducible alterations in *TP53*, *SMAD4*, and *CDK2NA*, but have yet to discover targetable recurrent mutations [4,10]. PDA tumors are characterized as heterogeneous and genetically complex with a number of chromosomal rearrangements which likely contributes to treatment resistance of the disease [13]. Sequencing of primary tumors has been challenging because of the lack of neoplastic cellularity, considering the bulk of the tumor is composed of stromal components [14]. Utilization of techniques such as laser-captured microdissection have aided in isolating malignant populations of cells for analysis [15].

One of the defining characteristics of PDA tumors is excessive desmoplasia, a heterogeneous mixture of extracellular matrix (ECM) proteins and a multitude of cell types [16]. Stellate cells, a specialized type of fibroblast found in the pancreas, constitute a major cell type in the tumor stroma and have multifaceted roles within the tumor microenvironment (TME) such as the production of ECM [17]. Other cells included within the stromal reaction are endothelial cells, immune cells and neurons. The contribution of these cells to pancreatic cancer progression is an active area of investigation. The PDA microenvironment is understood to be immunosuppressive and accordingly, immune checkpoint inhibitors have been largely unsuccessful as a monotherapy [18]. These results highlight the complexity of immunological reactions within the TME and suggest that successfully treating PDA will require a multipronged approach [19,20]. The switch of pancreatic stellate cells from a quiescent to an activated state results in deposition of the collagenous components of the ECM. This leads to extensive fibrosis which impinges on vasculature causing increased hydrostatic pressure, lower nutrient influx, and hypoxia [21]. The deficient vascular network in pancreatic tumors has been shown to impede drug delivery, further contributing to the highly refractory nature of these tumors [22]. Consistent with the lack of functional vasculature, PDA are likely nutrient depleted when compared to benign adjacent tissue [23]. To survive in such austere conditions, PDA tumors rely on a unique combination of adaptive metabolic networks for nutrient acquisition and utilization which are described in this review (Fig. 1).

1.2. Tumor metabolism

In order to support the ability to proliferate in an unconstrained manner, tumor cells have adapted their metabolism in many different ways. Perhaps the most well-known example comes from the observation that cancer cells have increased rates of aerobic glycolysis, known as the Warburg effect [24]. This leads to decreased glucose oxidation and more flux through the anabolic side branches of glycolysis, such as the pentose phosphate pathway (PPP). Indeed, a major consequence of many of the metabolic changes seen in tumors is to shift fuel sources into anabolic pathways in order to provide the cells with the necessary substrates to increase biomass. Interestingly, it has been recently appreciated that many of the oncogenes and tumor suppressor genes important in tumor pathogenesis can actually rewire metabolism to favor a more anabolic state and therefore support tumor growth. This includes oncogenes such as *KRAS* and *MYC* as well as the *TP53* tumor suppressor. Given that many of the metabolic adaptations occur downstream of these genes selectively in cancers, they may provide opportunities for therapeutic intervention. This review will focus on the metabolic alterations seen in PDA, with a particular emphasis on the metabolic plasticity that is characteristic of these tumors.

2. Nutrient scavenging

2.1. Autophagy

Macroautophagy is a highly conserved catabolic process that results in the lysosomal degradation of intracellular components and functions to maintain metabolic and cellular homeostasis through recycling of cytoplasmic materials to basic cellular building blocks (amino acids, fatty acids, nucleotides). This process involves sequestration of cytoplasmic material such as proteins or organelles into double membrane structures known as autophagosomes. The fusion of the autophagosome and the lysosome results in degradation of the cargo and release of recycled components back into the cytosol [25,26]. There are three different types of autophagy; macroautophagy, microautophagy and chaperone mediated autophagy which each utilize different mechanisms in delivering cargo to the lysosome [27]. This review will focus on the role of macroautophagy (hereafter autophagy) which plays a prominent role in the metabolism and progression of PDA.

Nearly all tissues have low levels of basal autophagy which generally serves as a protective mechanism, ridding the cells of damaged organelles and protein aggregates which have the potential to become cytotoxic. Indeed, dysregulation of autophagy has been implicated in a number of different pathological conditions, including neurodegenerative diseases, inflammation and cancer [28]. The major function of autophagy is understood to be pro-survival, as has been shown in the initial mouse studies where autophagy genes were deleted embryonically and resulted in death soon after weaning [29]. Consistently, autophagy flux is increased under cellular stress (nutrient deprivation, hypoxia, and chemotherapeutics).

Autophagy can be broken down into discreet steps beginning with initiation of the autophagosome. One of the most potent activators of autophagy is nutrient deprivation, which is tightly regulated by the MTOR pathway [30]. Through degradation of cytosolic components, autophagy can supply nutrients to essential metabolic pathways and promote survival during starvation. The canonical process is tightly controlled through a complex series of steps that involve > 30 autophagy related genes (ATG) and the process has been extensively reviewed elsewhere [27]. In brief, under nutrient replete conditions MTOR is active, and MTORC1 mediated signaling suppresses autophagy activation. In contrast, starvation stimulates autophagy through modulation of MTORC1 activity as well as AMPK signaling. Upon induction of autophagy, machinery involved in the nucleation of the phagophore is recruited. Following initiation is autophagosome elongation/maturation and lysosomal fusion. The maturation phase involves a concert of ATG proteins to form the autophagosome membrane. During this process, cytosolic components become engulfed by the forming autophagosome which are subsequently degraded by the lysosome following fusion. In addition to bulk autophagy, where cytosolic contents are degraded non-selectively, there are multiple forms of selective autophagy whereby cargo is selectively targeted to the autophagosome by autophagy receptors [31]. Importantly, perturbation at multiple steps of this process results in defective autophagosomes and diminished autophagic flux. Autophagy can also be blocked at the level of the lysosome with drugs such as hydroxychloroquine which inhibits lysosomal acidification.

In PDA, autophagy levels are basally high even when grown in complete media suggestive of alternative or supplemental means of autophagy activation. Indeed, it has been shown that the MiT/TFE family of transcription factors can stimulate high levels of basal autophagy independent of MTOR activity. These transcription factors are constitutively activated in PDA in a nutrient-independent manner and conversely, knockdown of MiT/TFE factors impairs PDA lysosomal function and autophagic flux [32]. Additionally, the identification of an ULK1 (ATG1) phosphatase (PP2A-B55alpha) that stimulates autophagy has also been shown to be required for high basal levels of autophagy in PDA [33].