Metabolic reprogramming by driver mutation-tumor microenvironment interplay in pancreatic cancer: new therapeutic targets

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Funding

Related work in the author's laboratory is funded by the Danish Cancer Society (#A12359, #A15823), the Novo Nordisk Foundation (#NNF19OC0058262, NNF19OC0057739), Independent Research Fund Denmark (#0135-00139B), the Carlsberg Foundation (#CF20-0491), and the European Union (H2020-MSCA-ITN-2018, grant 813834) (all SFP).

Acknowledgements

The authors are grateful to all members of the Pedersen lab for stimulating feedback and discussions that helped shape this review.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers globally with a mortality rate exceeding 95% and very limited therapeutic options. A hallmark of PDAC is its acidic tumor microenvironment, further characterised by excessive fibrosis and depletion of oxygen and nutrients due to poor vascularity. The combination of PDAC driver mutations and adaptation to this hostile environment drives extensive metabolic reprogramming of the cancer cells toward non-canonical metabolic pathways and increases reliance on scavenging mechanisms such as autophagy and macropinocytosis. In addition, the cancer cells benefit from metabolic cross-talk with nonmalignant cells within the tumor microenvironment, including stellate cells, fibroblasts, endothelial and immune cells. Increasing evidence shows that this metabolic rewiring is closely related to chemo- and radioresistance and immunosuppression, causing extensive treatment failure. Indeed, stratification of human PDAC tumors into subtypes based on their metabolic profiles was shown to predict disease outcome. Accordingly, an increasing number of clinical trials target pro-tumorigenic metabolic pathways, either as stand-alone treatment or in conjunction with chemotherapy. In this review, we highlight key findings and potential future directions of pancreatic cancer metabolism research, specifically focusing on novel therapeutic opportunities.

Keywords

PDAC, metabolic subtypes, lipid metabolism, glycolysis, acidosis, clinical trials

Statements and Declarations

The authors declare no conflict of interest.

1. INTRODUCTION

Pancreatic cancer has the lowest 5-year survival rate (less than 10%) of all cancers, and it is thought to become the second leading cause of cancer-related death worldwide by 2030 [1] . Pancreatic ductal adenocarcinoma (PDAC) is the primary type of pancreatic cancer accounting for about 95% of tumors found in pancreas. Because of the lack of diagnostic biomarkers and early-stage symptoms, diagnosis frequently occurs at advanced, invasive stages. The current treatment options are surgery if possible, or chemo- (gemcitabine, FOLFIRINOX and albumin-bound paclitaxel) and radiotherapy, but all have limited efficiency and only marginally prolong survival [2–4]. The unique tumor microenvironment (TME) and metabolism of pancreatic cancers are new areas of interest for treatment strategies. For instance, inhibitors targeting tricyclic acid (TCA) cycle enzymes synergized with chemotherapeutic treatment of pancreatic cancer patients [5]. Furthermore, accumulating evidence suggests that the glycolytic metabolism of the cancer cells allows them to resist chemotherapy through modulation of angiogenesis, apoptosis, and drug transport and -targets [6,7].

The unique PDAC TME evolves throughout cancer progression and comprises an excessive extracellular matrix (ECM) and abundant non-neoplastic cells such as cancer-associated fibroblasts (CAFs), pancreatic stellate cells (PSCs), immune cells and endothelial cells [8]. PDACs are nutrient-poor, with high levels of oxidative and inflammatory stress, extracellular acidosis, hypoxia, and high interstitial pressure, vascular collapse, and hypoperfusion [4,8–13]. This provides strong selection pressure, and only cells that have adapted their metabolism to these hostile conditions can survive and proliferate. Notably, accumulating evidence suggests that these adaptations also render the cancer cells more motile, invasive, and resistant to chemotherapeutic treatment [14]. While the metabolic rewiring of pancreatic cancer cells opens a new avenue of therapeutic opportunities [9], the pronounced genetic and metabolic heterogeneity and plasticity of pancreatic cancer cells [15–17], makes it a challenging target. Exploitation of metabolic synthetic lethalities has shown great potential in

other types of cancer [18,19], but to our knowledge, apart from a few examples [20,21], it is still not well developed in pancreatic cancer.

In this review, we summarize and critically discuss the current knowledge on the interplay between PDAC driver mutations and metabolism. We suggest that targeting this interplay is a promising future direction that may lead to improved PDAC treatment strategies and we outline open questions that urgently need to be addressed to reach this goal.

2. PANCREATIC CANCER CLASSIFICATIONS

Pancreatic cancers are not only highly diverse in their genetic profiles and responsiveness to treatments, between patients, but also exhibit extensive intratumoral heterogeneity, both genetically and phenotypically [21]. Traditional classification of pancreatic tumors developed by pathologists is based on phenotypic features and histological characteristics. However, increasing molecular understanding of pancreatic cancers has revealed that cancers with similar ~~morphology and~~ histological appearance may have completely different genomic alternations, resulting in different clinical outcomes [22,23].

2.1. Molecular subtypes

Development of PDAC occurs through driver mutations, four of which have been extensively characterized: Oncogenic KRAS mutations are found in nearly all PDAC cases, and inactivating mutations in tumor suppressor genes such as TP53, SMAD4 and CDKN2A (encoding p16) are also detected with high frequency [24].

Several key studies have further detailed PDAC molecular subtypes based on gene expression profiles and patient overall survival. Collisson et al. (2011) described three subtypes, based on microdissected tumor samples: classical, exocrine-like and mesenchymal [25]. In 2015, Moffitt et al. defined two “tumor-specific subtypes” (classical and basal-like) and two additional “stroma subtypes” (normal and activated) [26]. Shortly thereafter, Bailey et al. classified PDAC into four subtypes: squamous, pancreatic progenitor, immunogenic and aberrantly differentiated endocrine exocrine (ADEX) [27]. The existence of the ADEX subtype

has since become controversial [26,28]. Thus, Puleo et al. found evidence that the ADEX tumor subtype was a result of contamination by acinar cells in normal pancreatic tissue adjacent to the tumor [28]. Collisson et al. also did not detect exocrine-like subtypes in both human and mice [25], whereas Noll et al. described three [29], and Zhao et al. six subtypes, with one resembling Bailey´s ADEX subtype [30].

2.2. Metabolic signatures of PDAC

Recently, gene expression profiling allowed stratification of pancreatic cancer into subtypes with distinct prognoses and responses to therapy, based on differences in metabolism (Table 1). The subgroups are briefly described here, followed in section 3 by a general overview of pancreatic cancer metabolism, and its links to frequent driver mutations and the characteristic TME of this malignancy.

The glycolytic subtype. Metabolite profiling and transcriptional analysis of 38 pancreatic cancer cell lines by Daemen et al. [15] showed high levels of gene expression and metabolites from the glycolytic, serine and pentose phosphate pathways, classifying them as belonging to the glycolytic subtype. Glycolytic cell lines were found to be sensitive to inhibitors targeting aerobic glycolysis and glutaminolysis (Table 1,[15]). They also showed higher levels of fatty acid uptake than the lipogenic group and were more sensitive to media with reduced lipid concentration, suggesting that these cells rely on fatty acid uptake pathways for generating lipids. Based on a 42-gene set, the glycolytic subtype was associated with the quasi-mesenchymal molecular subtype, which is characterized by extremely poor prognosis, rapid growth, metastases, and resistance to chemotherapy [15]. Karasinska et al. profiled the expression of glycolysis and cholesterol biosynthesis genes in 325 clinical pancreatic cancer samples. In addition to upregulation of the glycolytic pathway, they found that the glycolytic subtype showed amplification of KRAS and Myc oncogenes and was correlated with the basal, squamous and quasi-mesenchymal subtypes, all of which are associated with a very poor survival outcome (Table 1) [17]. Finally, Zhao et al. [30] performed retrospective meta-analysis on whole transcriptome data from more than 1200 PDAC patients and stratified clinical

samples into six groups, three of which were associated with metabolism. Of these, the so-called L2 subtype was characterized by enriched glycolysis, down-regulation of lipid metabolism genes, and poor survival outcome, and was concluded to correspond to the basal, squamous and quasi-mesenchymal subtypes described by the molecular classification systems [30].

The lipogenic subtype. The lipogenic subtype described by Daemen et al. [15] was characterized by upregulation of lipogenic genes and metabolites involved in cholesterol and lipid synthesis and mitochondrial oxidative phosphorylation. As predicted, lipogenic cell lines were sensitive to inhibitors targeting *de novo* lipid synthesis. The lipogenic subtype was strongly correlated with epithelial features, and resembled the classical molecular subtype, which has a better prognosis than the other subtypes (Table 1,[15]). Karasinska's so-called cholesterogenic subtype was characterized by upregulation of cholesterol biosynthesis genes and the highest proportion of pancreatic progenitor subtype cases as defined by Bailey et al. [27]. Importantly, the progenitor subtype was previously found to be enriched in steroid hormone synthesis genes [17], a pathway downstream of cholesterol synthesis. In line with Daemen's study, Karasinska's cholesterogenic subtype was reported to have the longest median survival of all subtypes (Table 1, [17]).

Other metabolic subtypes. Finally, Daemen et al. described a slow-proliferating PDAC subtype characterized by low levels of carbohydrates and amino acids and significantly higher doubling time than the lipogenic and glycolytic subtypes (Table 1,[15]). The study by Karasinska et al. also distinguished the so-called quiescent and mixed PDAC subtypes. Contrary to the quiescent subtype, which is associated with poor metabolic activity, the mixed subtype exhibited high metabolic activity and enrichment in both the glycolytic and cholesterol synthesis pathways. The quiescent subtype showed the highest frequency of ADEX and exocrine-like cases, implicating that these tumor cells may be involved in digestive enzyme secretion [17]. The L1 subtype described by Zhao et al. and characterized by up-regulation of both glycolytic and lipogenic genes corresponded to Karasinska's mixed subtype sharing the

characteristics of glycolytic and lipogenic groups. In addition, Zhao's L6 subtype correlated with up-regulation of genes related to digestive enzyme activity and protein metabolism, similar to Karasinska's quiescent subtype. L6 was classified as similar to the ADEX subtype. Both the L1 and L6 subtypes were related to intermediate patient survival (Table 1,[30]).

3. METABOLIC CHARACTERISTICS OF PDAC TUMORS

3.1. Glucose metabolism

The frequent shift of cancer cells to increased reliance on glycolytic metabolism even in the presence of oxygen has been widely studied since its discovery by Otto Warburg. Aerobic glycolysis is less efficient than oxidative phosphorylation in terms of adenosine triphosphate (ATP) synthesis (Fig. 1), but the shift is believed to benefit highly proliferating cancer cells by providing them with biosynthetic precursors for synthesis of nucleotides, proteins and lipids, supporting both growth and repair processes and thus favoring cancer development [31]. The phosphatidylinositol-3-kinase (PI3K)/Protein kinase B (Akt) and the mammalian target of rapamycin (mTOR) signaling pathways, as well as transcriptional regulation by hypoxia-inducible factor (HIF-1), Myc and TP53 all favor the switch to aerobic glycolysis [32]. Oncogenic KRAS promotes glycolysis in several ways: (i) by upregulation of glucose transporter 1 (GLUT1, SLC2A1) and rate-limiting glycolytic enzymes (e.g., hexokinase (HK)1 and -2 and lactate dehydrogenase A (LDHA)); (ii) by promoting mitochondrial translocation of pyruvate dehydrogenase kinase-1 (PDK1), limiting oxidative phosphorylation; and (iii) by driving the glucose intermediates into the hexosamine biosynthesis pathway (HBP) and the nonoxidative pentose phosphate pathway (PPP), decoupling ribose biogenesis from NADP/NADPH-mediated redox control [3,33].

3.2. Lipid metabolism

Cancer cells often exhibit increased reliance on lipid uptake and/or lipid synthesis compared to non-cancer cells [34,35]. Accordingly, genetic and metabolic analyses of large numbers of patient tumors have established that increased dependence on lipid metabolism is a

characteristic feature of a subset of pancreatic cancers [15,17,30]. Fatty acid availability is reduced in the nutrient-poor pancreatic tumor environment compared to the surrounding tissue [36]. Under normoxic conditions, *de novo* lipid synthesis can cover the demand of the cancer cells. Accordingly, fatty acid synthase (FASN) inhibition was shown to increase gemcitabine sensitivity in orthotopic PDAC tumor models [37], and inhibition of ATP citrate lyase (ACL), which converts citrate to acetyl-CoA enabling lipid production from glycolytic precursors, inhibited *in vivo* growth of pancreatic cancer xenografts bearing KRAS-G12D mutations [38]. Under hypoxic conditions, cancer cells must rely on import of fatty acids. A similar shift toward fatty acid import can, interestingly, also be driven by KRAS transformation in normoxic cells [39]. Pancreatic cancer cells are also dependent on exogenous cholesterol, and inhibition of receptor mediated cholesterol-LDL uptake inhibits their growth [40]. The dependence of pancreatic cancer progression on cholesterol is, however, more complex than might be anticipated from simple biosynthetic needs. The classical pancreatic cancer subtype [26] exhibits higher levels of cholesterol- and fatty acid metabolic genes than does the Basal subtype [17,41]. Paradoxically however, genetic or pharmacological inhibition of cholesterol biosynthesis caused a shift toward more aggressive forms of pancreatic cancer. This was shown to reflect reduced cholesterol-LDL-induced inhibition of SREBP1 binding to the TGFB1 promoter, leading to increased autocrine TGFβ1 signaling, which favors conversion of classical to the more aggressive basal subtype [41]. Further complicating prediction of net outcome, the effect of cholesterol biosynthesis was influenced by both KRAS and TP53 status [41]. Also the accumulation and utilization of lipids in the form of lipid droplets were recently shown to be driven by oncogenic KRAS-mediated regulation of hormone-sensitive lipase (HSL). Notably, the energy-demanding processes of migration and metastasis were found to be fueled by the use of stored lipids for oxidative metabolism [42], emphasizing the notion that despite their frequent glycolytic shift, cancer cells are also dependent on mitochondrial metabolism.

3.3. Amino acid metabolism

Pancreatic cancer cells rewire amino acid metabolism to meet the increased demand amid limited access to nutrients and oxygen [43]. Accordingly, amino acid transporters such as the L-type amino-acid transporter 1 (LAT1, SLC7A5) and SLC6A14 are highly upregulated in PDAC tumors and favor cancer cell growth and proliferation [44,45]. As in many other cancers, glutamine - the most abundant amino acid in blood plasma [46] - is a crucial source of nitrogen and carbon for proliferating PDAC cells [47,48]. In healthy pancreas, glutamate is converted into α-ketoglutarate by glutamate dehydrogenase (GLUD1) and utilized in the TCA cycle. Remodeling of glutamine consumption by KRAS-driven downregulation of GLUD1 and upregulation of several components of malate-aspartate shuttle: the aspartate aminotransferase 1 (Glutamic-Oxaloacetic Transaminase 1, GOT1) and the malate dehydrogenase 1 (MDH1) promotes PDAC growth [49]. PDAC cells rewire a noncanonical KRAS-mediated metabolic pathway in which glutamine is converted into aspartate by another isoform, GOT2, in mitochondria. After being transported to the cytoplasm, aspartate is processed by GOT1 into oxaloacetate, converted into malate and then pyruvate, resulting in elevated NADPH/NADP+ ratio and maintained cell redox homeostasis [50]. Interestingly, when exposed to an acidic microenvironment, PDAC cells upregulate GOT1 to control reactive oxygen species (ROS) levels and promote cancer cell survival [51]. This PDAC-specific metabolic pathway maintains relatively low levels of glutathione required for redox homeostasis [9]. Furthermore, pancreatic cancer cells utilize glutamine for glutathione synthesis and NADPH production for anabolic reactions and redox balance [52]. It is also notable that pancreatic cancer stem cells (CSCs) are characterised by increased expression of the tetraspanin CD9, which increases plasma membrane localization of the glutamine transporter ASCT2, favoring glutamine uptake [53].

Glutamine is not the only amino acid, metabolism of which is altered in PDAC. Several studies indicate that early in tumor progression, PDAC cells metabolize branched-chain amino acids (leucine, isoleucine and valine) secreted into blood plasma as a result of tissue breakdown [50,54,55]. Collagen-derived proline and cysteine promote PDAC cells growth, survival and

tumor progression under nutrient/oxygen-deprived conditions [56,57]. Finally, in PDAC tumors driven by obesity, the urea cycle pathway was found to be reprogrammed to fuel mitochondrial metabolism through upregulation of arginase 2 (ARG2) - a mitochondrial gene catalyzing hydrolysis of arginine to ornithine and urea [58].

3.4. Autophagy and macropinocytosis

Autophagy and macropinocytosis allow cancer cells - particularly in very nutrient-poor tumors such as PDAC - to salvage and recycle glucose, amino acids, nucleosides and lipids, and thereby adapt to intrinsic stresses. Moreover, both processes are strongly correlated with increased immune evasion [7]. While often anti-tumorigenic in normal cells and early cancer development, autophagy can support cancer progression in later stages by providing precursors for synthesis of ATP and other macromolecules [59,60]. *In vitro* inhibition of autophagy suppresses growth and proliferation of PDAC cells through increasing DNA damage, decreasing levels of metabolic substrates and disrupting redox state. In PDAC, constitutively upregulated autophagy is also often observed even under nutrient-rich conditions. This is in part dependent on the microphthalmia/transcription factor E (MiT/TFE) family which, upon dephosphorylation, translocates to the nucleus and upregulates transcription of e.g. autophagy genes [61,62]. Also oncogenic KRAS activates autophagy, favoring the non-oxidative PPP [7,63] and also favors macropinocytosis, supporting tumor growth. As such, macropinocytosis was found to provide alternative metabolic fuel sources like glutamate through degradation of internalized external proteins and feeding of central carbon metabolism, providing TCA cycle intermediates [54].

Targeting autophagy, even combined with chemotherapy, is, however, not a successful strategy in PDAC, due to compensatory upregulation of macropinocytosis [64,65]. Inhibition of autophagy leads to accumulation of Sequestosome 1 (p62/SQSTIM1), an autophagy adaptor protein which sequesters Kelch-like ECH-associated protein 1 (KEAP1) and activates Nuclear factor-erythroid factor 2-related factor 2 (NRF2), in turn driving transcription of macropinocytosis-controlling genes such as the Na^+/H^+ exchanger 1 (NHE1, SLC9A1), cell

division cycle protein 42 (CDC42) and syndecan 1 (SDC1), leading to upregulation of macropinocytosis [64].

In contrast, combined inhibition of autophagy and macropinocytosis was found to decrease tumor volume and increase survival in PDAC mouse models, in a manner associated with decreased cellular ATP and NADPH levels [64]. Interestingly, the antitumor activity of chloroquine (CQ), hydroxychloroquine (HCQ), and other lysosomotropic drugs could be uncoupled from their autophagy-inhibitory properties [66] and may involve inhibition of macropinocytosis and other lysosomal mechanisms such as lysosomal permeation [66–69]. Combined inhibition of autophagy using CQ or HCQ and inhibition of ERK signalling was found to be efficient in targeting PDAC in mouse models, as ERK inhibition led to autophagy dependence [63,70,71], see Table 3. Similarly, co-depletion of the KRAS effectors BRAF and CRAF together with depletion of autophagy E1 ligase ATG7 lead to G1 cell cycle arrest and increased apoptosis [72].

4. DRIVER MUTATIONS INFLUENCE PANCREATIC CANCER METABOLISM

As noted above, activating KRAS mutations are the single most common genetic abnormality in PDAC, present in over 90% of cases [24]. KRAS mutations together with other frequently mutated genes such as TP53, p16/CDKN2A and SMAD4/DPC4, play a crucial role in driving PDAC tumorigenesis and metastasis through different mechanisms (Table 2, [24]). Genetic alterations may also promote disease progression through reprogramming of glucose-, lipid- and AA metabolism and metabolic crosstalk within the TME (Fig.2).

4.1. Effects of KRAS mutations on PDAC metabolism

LC-MS/MS metabolomic studies indicate that KRAS-G12D tumors enhance glucose uptake via GLUT1 and increase lactate production by elevating levels of rate-limiting glycolytic enzymes like HKI/II, phosphofructokinase1 (PFK1) and LDHA (Fig. 2). Glycolytic flux in KRAS-G12D positive PDAC tumors is then redirected into ribose biogenesis through the nonoxidative arm of the PPP to promote DNA/RNA biosynthesis in tumor cells without affecting NADPH-

synthesizing oxidative arm (Fig.2, [33]). Studies also indicate a metabolic link between TP53 function, cancer metabolism and tumor cells survival [73]. Gene set enrichment analysis (GSEA) pointed to decreased steroid biosynthesis, pyrimidine metabolism and O-glycosylation in PDAC tumors [33]. Further, mutant KRAS upregulates ERK signaling, leading to Myc activation and upregulation of the PPP enzymes RPIA and RPE, favoring nucleotide synthesis and supporting PDAC growth [31,33,74].

Oncogenic KRAS also supports lipid metabolism in pancreatic cancer cells, favoring migration, invasion and matrix degradation through HSL downregulation, and increases PLIN-2 expression, increasing lipid storage and -utilization [42]. One of the unique features of PDAC metabolism is exploitation of NADPH levels, and this was also shown to be dependent on KRAS activity (Fig. 2). Thus, oncogenic KRAS regulates *de novo* synthesis of NADP+ through increased protein kinase C (PKC)-mediated phosphorylation of NAD+ kinase (NADK). This leads to NADK hyperactivation and sustained levels of NADP+/NADPH supporting cellular redox metabolism and thus survival [75]. Glucose metabolism of PDAC cells has been shown to rely on the expression of Nicotinamide Phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD salvage pathway, as the NAMPT inhibition resulted in significantly decreased glycolytic activity and a 30% drop in NAD+ levels in cancer cells leading to impaired tumor growth *in vitro* and reduced tumorigenesis *in vivo* [76].

As noted above, KRAS-G12D-positive PDAC cells are highly dependent on non-canonical glutamine utilization through the activity of GOT1, GOT2 and Malic Enzyme 1 (ME1) for proliferation and growth. It has been shown that knockdown of GOT1 and ME1 noticeably increases the NADP+/NADPH ratio, favoring redox balance and tumor proliferation, while inhibition of other sources of NADPH had no effect on NADP+/NADPH ratios or generation of ROS. This pathway has been reported only in PDAC cancer cells, and suggests a potential metabolic treatment target [50].

Finally, a novel oncogenic driver Lin28b, present in ~30-40% of PDAC tumors, was shown to driving progression of Sirtuin 6 (SIRT6)-deficient, KRAS-G12D-positive PDAC tumors via upregulation of glucose uptake and enhanced metastatic behavior *in vivo* [77].

4.2. Effects of TP53 mutations on PDAC metabolism

One of the key metabolic functions of TP53 is to control transcriptional activity of glycolytic enzymes, modulating signaling pathways to limit stages of glycolysis and enhance oxidative phosphorylation (Fig. 2) [78]. Accordingly, TP53 loss/mutation enhances glycolysis in pancreatic cancer cells [79]. The TP53-inducible glycolysis- and apoptosis regulator, TIGAR, has been shown to decrease the rate of glycolysis by downregulation of fructose-2,6-bisphosphate levels, reducing ROS levels and favoring survival of cancer cells [80]. In line with these findings, Rajeshkumar et al. showed that the loss of TP53 sensitizes pancreatic patient-derived xenografts (PDX) to inhibition of lactate dehydrogenase, indicating a link between glycolysis and TP53 expression [81]. Schofield et al., using gene expression profiles, discovered that one of the most common TP53 mutations, R273H (corresponding to murine R270H) alters pathways regulating the metabolism of amino acids, carbon sources, fatty acids, and autophagy. Furthermore, their functional analysis provided evidence that TP53-R273H expression reduces mitochondrial activity in pancreatic cancer cells, by interfering with branched chain amino acid (BCAA) metabolism [82]. Taken together, these results suggest a key role of TP53 in shaping pancreatic cancer metabolism.

4.3. Interplay between KRAS and TP53 mutations in metabolic changes in PDAC

TP53 activation was shown to trigger a metabolic shift in KRAS-G12D PDAC cells towards increased α-ketoglutarate (α-KG)/succinate ratio by integrating glucose-derived carbons, rather than glutamine-derived counterparts, into the TCA cycle. Multiple TCA cycle enzymes show altered expression upon inducible TP53 activation in PDAC cells, pointing to a metabolic link between TP53 function, cancer metabolism and tumor cell survival. Reinforcing this

notion, addition of cell-permeable α-KG leads to a less differentiated and more aggressive PDAC phenotype [73].

The lactonase paraoxonase 2 (PON2), which is usually transcriptionally repressed by p53, cooperates with KRAS-G12D to accelerate glycolysis by disrupting GLUT1-Stomatin (STOM) interaction and tuning GLUT1-mediated transport of glucose for PDAC needs [83]. Furthermore, PON2 has been shown to inhibit the AMPK-FOXO3A-PUMA pathway to promote PDAC growth and metastasis [83].

4.4. Effect of other PDAC driver mutations on PDAC metabolism

Loss of SMAD4 in PDAC cells induces the expression of the glycolytic enzyme PGK1, thus increasing the rate of glycolysis and driving tumor aggressiveness (Fig. 2). Intriguingly, in SMAD-null PDAC cells, nuclear PGK1 induces metastasis through induction of oxidative phosphorylation while cytoplasmic PGK1 functions as a glycolytic enzyme and enhances proliferation [84]. It was also shown that SMAD4 inactivation promotes resistance of PDAC cells to mitochondrial therapy [85]. Finally, upregulation of NADPH oxidase-4 (NOX4) upon p16INK4a/CDKN2A inactivation was found to favor NADH oxidation and glycolysis by generating NAD+, promoting PDAC cell growth (Fig.2) [86].

5. CANCER CELL - TME INTERPLAY IN METABOLIC REPROGRAMMING

5.1. Metabolites in the PDAC TME

Because of the poor vascularization and consequent reduced nutrient delivery and venting of metabolic waste products, PDAC tumor interstitial fluid (TIF) is high in lactate and low in glucose [87]. A recent quantification of TIF metabolites in mouse models of PDAC with oncogenic KRAS and TP53 knock-out showed increased levels of glycine, ornithine, and aspartate compared to plasma. Alanine was abundant in both TIF and plasma, and arginine, tryptophan, pyruvate and cysteine (metabolites important for immune cell function) were depleted in TIF relative to plasma [87]. In addition to these soluble metabolites, the abundant ECM in pancreatic cancer tumors serves as a source of amino acids for the cancer cells, which

can degrade collagens to peptides which are subsequently degraded to proline, that can enter the TCA cycle after conversion to glutamate [4].

5.2. Acidosis in the PDAC TME

Extracellular acidosis is a key characteristic of many solid tumors ([88,89], Fig. 3). While the glycolytic shift of cancer cells is frequently "blamed" for this, the CO_2 resulting from oxidative phosphorylation also gives rise to acid. So, irrespective of which metabolic pathways dominate, solid tumors tend to exhibit strongly acidic regions, which only partly overlap with regions of hypoxia [89]. This is also the case in the few existing studies of the pH of the PDAC TME [10,12]. Studies of cells adapted to growth under acidic extracellular conditions have shown that such cells exhibit a profoundly altered metabolism, with increased dependence on lipid metabolism, accumulation of lipid droplets [90], and a shift away from glycolytic metabolism towards glutamine and oxidative phosphorylation [91,92]. The acidic TME is also likely to play a role in the frequent, context-dependent changes in mitochondrial morphology and dynamics in cancer cells, ranging from apparently fragmented, doughnut-shaped to hyperfused and elongated - a mitochondrial phenotype that is normally induced by acidic stress and associated with increased survival in hostile environments [93]. While these changes are yet incompletely understood, they may have therapeutic relevance in pancreatic cancer, where normalization of mitochondrial fragmentation limited tumor growth, chiefly through increased mitophagy, reducing oxidative phosphorylation capacity [94].

5.3. Other physicochemical properties of the PDAC TME: Mechanical properties and hypoxia

The dense ECM (desmoplasia) and numerous severely hypoxic regions in PDAC tumors add further selective pressure for malignant cells and push them to develop adaptive metabolic mechanisms [95–97]. In hypoxic conditions, PDAC cells undergo a glycolytic switch to support cell growth and increasingly rely on glutamine and glucose metabolism to promote their survival in a manner correlating with PDAC aggressiveness [48]. ECM stiffness *per se* has

also been shown to increase invasive potential, alter ATP turnover and rewire creatine phosphagen synthesis, favoring tumor progression [98–100]. Finally, ECM stiffness modulates glycolysis and lipid- and amino acid metabolism of cancer cells through ~~several signaling~~ pathways including integrin-associated focal adhesion kinase (FAK) - phosphatidylinositol 3-kinase (PI3K) - Akt, adenosine monophosphate-activated protein kinase (AMPK), Rho/Rho-associated protein kinase - actin cytoskeleton, Phosphatase and TENsin homolog deleted on chromosome 10 (PTEN), Yes-associated protein (YAP)/ Transcriptional coactivator with PDZ-binding motif (TAZ), and Thioredoxin Interacting Protein (TXNIP) [101].

6.STROMAL CELL METABOLISM IN PDAC

The stromal cell component of PDAC tumors has been extensively studied and is known to play a key role in driving progression and aggressiveness of the disease [4,102]. Interactions with stromal cells support PDAC cancer cell survival and aggressiveness in the hostile PDAC TME ([4,103]; Fig. 3). Furthermore, as discussed below, also the stromal cells themselves need to alter their metabolism to survive in the ~~hostile~~ PDAC TME.

6.1. Pancreatic stellate cells

In healthy pancreas, pancreatic stellate cells (PSCs) are located in close proximity to pancreatic acinar cells and represent 4-7% of pancreatic parenchyma [104]. In the quiescent state, PSCs express nestin, GFAP, vimentin, desmin and accumulate vitamin A-containing lipid droplets in their cytoplasm [35,105]. PDAC cells can initiate the switch of quiescent PSCs to an activated state, in turn favoring cancer cell growth, migration and disease progression [106] (Fig. 3). The activated PSCs secrete abundant ECM proteins (collagen type I and III, laminin, fibronectin [107]) and numerous cytokines, chemokines and growth factors (PDGF, TGFβ, CTGF, IL1, IL6, IL15) which support angiogenesis, fibrosis and epithelial-to-mesenchymal transition (EMT) and give rise to the dense PDAC ECM [104]. PSC~~s~~ activation is associated with expansion of their mitochondria and endoplasmic reticulum [105], and drastic remodeling of their lipidome to secrete excessive amount of lysophosphatidylcholines,

which are metabolized into lysophosphatidic acid, in turn stimulating PDAC aggressiveness by promoting migration and proliferation [106]. PDAC cells utilize alanine produced by the PSCs for fatty acid metabolism and further biomass increase [108]. PSCs also produce alanine, which is taken up by the PDAC cells via autophagy-dependent process and fuels the TCA cycle, sparing glucose for anabolic processes [7].

6.2. Cancer associated fibroblasts (CAFs)

The CAFs in the PDAC stroma may be tumor supportive or -suppressive, depending on their subtype [109]. PDAC CAFs stem from various types of cells, but their predominant origin seems to be activated PSCs and neighboring normal fibroblasts which have undergone differentiation induced by tumor cells [3] (Fig. 3). Fibroblasts can be driven to the CAF phenotype by multiple pathways including the sonic hedgehog (SHH) pathway, EMT, or stimulation by tumor necrosis factor α (TNF-α), and various cytokines [3,22]. Compared to normal fibroblasts, CAFs overexpress markers like smooth muscle alpha actin (α-SMA), fibroblast activation protein (FAP - a key enhancer of PDAC progression) and galectin. They also show enhanced glucose uptake capacity, lactic acid production, and elevated levels of LDHA, pyruvate kinase m2 (PKM2), and miR-21 (a miRNA shown to increase glycolysis in CAFs as well as several other contexts) [110,111]. Some activated CAFs switch from oxidative phosphorylation to preferential glycolysis, providing metabolites for the tumor cells (reverse Warburg effect) [111]. In this manner, CAFs secrete high-energy metabolites like lactate and pyruvate, enhance aerobic glycolysis, and glutamine-dependent reductive carboxylation and inhibit oxidative phosphorylation in the cancer cells. They also contribute to amino acid release through autophagy or exosome release and by producing collagen-rich ECM. The CAF-derived exosomes supply the cancer cells with TCA cycle intermediates and lipids [3,110]. Importantly, KRAS inhibition leads to rapid reduction of CAF activation, apparently reflecting the role of KRAS-driven cytokines in this process [109,112].

6.3. Endothelial cells

In healthy pancreas, endothelial cells (ECs) actively maintain the quiescent state and secrete autocrine, paracrine and endocrine factors to support cell survival [113]. ECs in stroma are more glycolytic than other healthy cell types and produce up to 85% of ATP from glycolysis. Accordingly, they are highly dependent on glucose and on the activity of the rate-limiting enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) [114]. When the tissue is deprived of oxygen and/or nutrients, ECs receive activating signals for vessel sprouting and become even more glucose dependent, increasing glycolysis rate by two-fold for cell proliferation [114]. They diminish PPP activity, in turn decreasing NADPH production and increasing ROS levels. This leads to GAPDH inhibition and activation of the polyol pathway, generating further ROS and toxic advanced glycation end (AGE) products. High levels of glucose also increase fatty acid oxidation and decrease glucose oxidation in PDAC ECs [115]. Furthermore, lactate secreted by the cancer cells induces EC activation through HIF-1α, promoting angiogenesis [116]. Finally, lactate has been shown to stimulate a pro-angiogenic and tumorigenic NF-kB/IL-8 pathway via IkBα degradation via the monocarboxylate transporter SLC16A1 (MCT1), further supporting tumor growth [117].

6.4. Immune cells

PDAC tumors are highly immunosuppressive [118,119], and most immune cells present in PDACs are pro-tumorigenic, including immunosuppressive tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells [120] (Fig.3). Both innate and adaptive immune responses impact PDAC cancer progression and metastasis [121]. Tumor associated neutrophils were shown to contribute to PDAC tumor angiogenesis by secreting MMP9 and VEGF *in vivo* [122], and B cells to favor tumor cell proliferation by secretion of IL-35 in KRAS-G12D-positive pancreatic neoplasms in mice [123]. Strikingly, exosomes secreted by PDAC cells initiated activation of tissue-resident macrophages in the liver, supporting the establishment of a pro-metastatic niche in livers of PDAC patients [124]. Several lines of evidence suggest that lactate secreted by tumor cells inhibits lactic acid export in CD8+ cytotoxic T cells, resulting in decreased immunogenicity, provokes M2-like

polarization of TAMs, and decreases the cytolytic function of natural killers (NK) cells in PDAC [125–127]. MDSCs were shown to favor tumor progression, survival and immunosuppressive activity by reprogramming their metabolism towards a higher rate of fatty acid oxidation and enhanced glycolysis [128,129]. According to several recent studies, MDSCs also inhibit T cell migration and NK cell cytotoxic activity, promote T cell inactivation through ROS secretion and support expansion of immunosuppressive regulatory T cells (Tregs; [130,131]). Mast cells were shown to crosstalk with PDAC cells and promote their migration, invasion, and proliferation through a MMP-dependent mechanism [132]. Finally, the TAMs seem to inhibit drug-induced apoptosis of PDAC cells by secreting IL-1β and enhancing cyclooxygenase 2 (COX-2) expression [133].

As noted above, PDACs are immunosuppressive ("cold") tumors, which have an extensive ability to evade the immune system [119]. Only a few types of inflammatory immune cells were found in PDAC stroma. Overall, tumor metabolism favors immunosuppression and T cell hyporesponsiveness facilitates tumor progression in several types of cancers [126]. While the mechanisms remain incompletely understood, this is at least in part due to the combined effects of reduced glucose, increased lactate, and acidosis in the PDAC TME [126,134]. With respect to the former, Chang et al. [134] showed that tumor glucose consumption metabolically restricts tumor-suppressive T cell functions by dampening their mTOR activity, glycolytic capacity and IFN-γ production. Inhibition of the mTOR pathway by rapamycin impaired glycolysis, revealing that IFN-γ production by T cells is glucose dependent, and that T cells and tumor cells compete for glucose. In progressively growing tumors the T cells were glucose-restricted, reducing their efficiency [134]. Of possible therapeutic interest, high levels of AMP in nutrient-deprived conditions activate AMPK signaling in T cells and serve as a boosting factor in immune expansion [115,135].

6.5. Metabolic symbiosis in PDAC tumors

Stromal cells sustain cancer cell survival and proliferation, not only through the reciprocal signaling events discussed above, but also through direct metabolic symbiosis. Classical

metabolic symbiosis occurs between PDAC cancer cells in differently oxidized regions of the tumor, with oxidative cells importing and utilizing the lactate produced and released by hypoxic cells undergoing anaerobic glycolysis [48]. In fact, circulating lactate, rather than glucose, is the main TCA cycle substrate in pancreatic tumors [136]. Accordingly, SLC16A3 (MCT4), which is a low affinity, high capacity transporter and thus best suited for lactate export, is preferentially expressed in hypoxic tumor regions [137,138], while MCT1, which has a higher lactate affinity and is more efficient in lactate uptake, is preferentially expressed in ~~the~~ normoxic regions. In recent years, a large number of studies have demonstrated the ~~key~~ role of lactate, not only as a nutrient for normoxic tumor cells, but also as a key signaling molecule, acting via the G protein coupled receptor GPR81 on both cancer- and immune cells [139]. As such, knockdown of GPR81 reduced growth of PDAC cells and -tumors [140]. Lactate also plays inhibitory non-cell autonomous roles in tumors, with several studies demonstrating lactate-mediated inhibition of anticancer immune cell activation and -function [141]. This notwithstanding, much remains to be understood about the precise roles of lactate in tumors, including the relative roles as nutrient and signaling protein, and the extent to which its reported functions can be separated from those of the acidosis that generally accompanies lactate accumulation in tumors [139].

In addition to the interactions between hypoxic and normoxic cancer cells, reciprocal interactions between pancreatic cancer cells and stromal cells contribute importantly to tumor development. An important such example is the substantial dependence of PDAC cells on alanine secreted by PSCs as a consequence of cancer cell-mediated induction of autophagy in the PSCs [108]. Furthermore, at least *in vitro*, macrophage polarization is dependent on MCT4-mediated lactate extrusion from the cancer cells [142]. In another striking example, KRAS-G12D mutations cell-autonomously reduce~~s~~ PDAC cell mitochondrial metabolism and favor~~s~~ the non-oxidative PPP - yet in the heterocellular TME, mitochondrial function of KRAS-G12D expressing cancer cells is restored in a manner involving SHH-driven reciprocal signaling between cancer cells and stromal cells [143].

7. TARGETING PDAC METABOLISM

PDAC metabolism is closely associated with chemo- and radiotherapy resistance and immunosuppression and therefore constitutes an excellent therapeutic target.

7.1. Chemotherapy

As mentioned above, the prevalent chemotherapy drugs in pancreatic cancer management, gemcitabine, FOLFIRINOX and albumin-bound paclitaxel, have minimal effect on overall patient survival [2]. As outlined in section 2, the glycolytic PDAC subtybe is characterized by chemotherapy resistance and extremely poor survival outcome, whereas the lipogenic subtype has a better response to chemotherapy and a better prognosis with longer median survival than the other subtypes.

Chemotherapy resistance is often multifactorial, involving both cancer cell-autonomous and TME-dependent events [6]. Pancreatic cancer, with its dense ECM and large stromal cell component, is particularly complex in this regard [144]. Accumulating evidence suggests that the glycolytic metabolism of the cancer cells contribute to chemotherapy resistance through modulating angiogenesis, apoptosis, and drug transport [6,7]. An example is oncogenic KRAS, which limits chemotherapy efficiency by driving the metabolic reprogramming of cancer cells towards the anabolic metabolism and aerobic glycolysis needed for their excessive growth and proliferation. Therefore, targeting KRAS-dependent metabolic aberrations~~,~~ might prevent drug resistance, increase the efficacy of chemotherapy treatment, and inhibit tumor cell growth [14]. Loss or mutation of TP53 and loss of SMAD4 shift cancer cell metabolism towards aerobic glycolysis, and the resulting metabolic changes might therefore also pose potential targets (Figure 1-2, Table 2). Thus, several ongoing strategies target cancer metabolism in general based on molecular/metabolic profiling, and more specifically by targeting the glycolytic pathway, cholesterol synthesis and autophagy (see table 3 and paragraphs 7.3.1-7.3.4). Given the importance of the ECM as a metabolic energy source for pancreatic cancer cells, strategies to target the ability of pancreatic cancer cells to degradade collagens to proline have also shown success in preclinical studies [4].

7.2. Radiotherapy

The exact signaling pathways and mechanisms conveying radiotherapy resistance are poorly defined, but the efficacy of radiation therapy is shown to inversely correlate with the glycolytic index of the tumors [145]. A potential metabolic target affecting radiotherapy resistance in PDAC is Mucin1 (MUC1), an oncogene overexpressed in PDAC and several other solid tumors. MUC1 was found to play a role in enhancing glycolysis, the PPP, and nucleotide biosynthesis. This led to radiation resistance, which could be reversed by pre-treatment with the glycolysis inhibitor 3-bromopyruvate (BrPA), both *in vitro* and *in vivo*. Accordingly, MUC1 KO sensitized three pancreatic cell lines (FG, HPAF II, and Capan2) to radiation therapy [146,147]. There are also sporadic indications that a ketogenic diet, forcing a shift toward mitochondrial metabolism, may support radiotherapy treatment efficacy in PDAC [148–150].

7.3. Treatments directly targeting metabolic pathways

A growing number of clinical studies target abnormal metabolic pathways developed by pancreatic cancer cells to improve the efficacy of the current first-line treatment (Table 3).

Metabolic combination treatments. Several drugs which have been used to inhibit PDAC metabolism, including metformin, doxycycline and mebendazole, are not generally associated with oncological treatment (Table 3). Metformin is an anti-diabetes medicine, which improves insulin sensitivity, lowers blood glucose, and downregulates glucose metabolism by blocking HKI/II [151]. However, metformin impacts multiple pathways, and has also been shown to regulate apoptosis [152] and decrease cell proliferation through ~~the~~ inhibition of Ras and mTOR [153–155]. Doxycycline is an antibiotic originally developed to treat acne. It may benefit cancer treatment by mediating DNA damage, degrading mitochondria and inhibiting MMPs, thus preventing metastatic spread [156]. Mebendazole, used as a treatment against parasitic worm infections, was shown to promote apoptosis in melanoma cells through inactivation of Bcl-2 and activation of caspases [157]. All three compounds are now in clinical trials in the context of pancreatic cancer (Table 3).

Targeting the glycolytic pathway. Several clinical trials study the efficacy of the combination of gemcitabine and imatinib - a tyrosine kinase inhibitor indicated for the treatment of leukemia and gastrointestinal stromal tumors (Table 3). Imatinib was previously shown to suppress glycolytic pathway in human chronic myeloid leukemia (CML) cells. Possibly a cause of concern for their use in PDAC is that downregulation of glycolysis in CML cells was associated with increased autophagy, resulting in maintained cell viability [158].

Targeting cholesterol synthesis. Cholesterol synthesis inhibitors, statins, are used to lower blood pressure and reduce the risk of cardiovascular diseases. This group of drugs regulates expression of pro- and anti-apoptotic genes and therefore may contribute to suppressing tumor growth [159]. Several ongoing clinical trials explore the efficacy of simvastatin in pancreatic cancer treatment (Table 3).

Targeting scavenging pathways. Chloroquine (CQ) and hydroxychloroquine (HCQ) were originally used to prevent and treat malaria. As inhibitors of autophagy, CQ and HCQ block nutrient recycling beneficial for cancer cells [160]. Moreover, CQ has been shown to activate the p53 pathway and induce apoptosis in human glioblastoma cells [161], and is now being tested for efficacy in PDAC (Table 3). It is also interesting to note that compounds commonly used as macropinocytosis inhibitors are in fact NHE1 inhibitors [54]. Thus, inhibition of these proteins may also inhibit PDAC growth by limiting extrusion of metabolically produced acid, resulting in intracellular acidification [89].

8. CONCLUSIONS, OPEN QUESTIONS AND FUTURE PERSPECTIVES

Pancreatic tumors are metabolically very different from normal pancreatic tissue, in a manner that reflects a combination of changes driven by PDAC mutations and the unique and very hostile TME of pancreatic tumors. A major obstacle for exploiting metabolism as a treatment target is that PDAC metabolism exhibits substantial heterogeneity and plasticity, both between patients and within single tumors, reflecting both TME differences and genetic intratumor heterogeneity. A key open question is which mechanisms drive these different environments,

including the interplay between driver mutations, TME and metabolism. Also the impact of these conditions on the stromal cells and how they in turn contribute to tumor progression remains vastly understudied. However, similar to what is emerging for other targeted treatments such as EGFR inhibitors (erlotinib), it seems highly probable that metabolic targeting in PDAC will require personalized protocols, possibly alongside conventional chemotherapy, as already seen in some ongoing clinical trials (Table 3). While this also requires much more detailed studies, the altered metabolite profiles found in PDAC tumors could also be used as biomarkers for diagnosis and therapy, using techniques like advanced imaging or liquid chromatography coupled to mass spectrometry [162]. This would allow stratification of tumors according to the combination of metabolic characteristics and driver mutations, potentially both improving diagnosis and enabling novel treatment strategies.

FIGURE LEGENDS

Fig. 1. Overview of PDAC metabolic pathways. Contrary to the glycolytic PDAC subtype, which is enriched in glycolytic pathway genes and metabolites, the lipogenic subgroup is characterized by upregulation of lipid metabolism genes. The mixed subtype presents high metabolic activity and enrichment in both the glycolytic and lipid synthesis pathways. The figure also presents examples of metabolism-targeting agents. See Table 3 for the full list of clinical trials directly targeting metabolic pathways in pancreatic cancer. Created with BioRender.com

Fig. 2. Driver mutations influence pancreatic cancer metabolism. See text for details. ↑ denotes upregulation, ↓ denotes downregulation. CAT3, Cationic Amino Acid Transporter 3; Eno, Enolase 1; Fru-2,6-P2, Fructose 2,6-bisphosphate; G6PD, Glucose-6-phosphate dehydrogenase; GLUT1, glucose transporter 1; GOT 1, Glutamic-oxaloacetic transaminase 1; HK 1, Hexokinase 1; HSL, Hormone-sensitive lipase; LDHA, Lactate dehydrogenase-A; Lin28b, Lin-28 Homolog B; ME 1, NADP-dependent malic enzyme 1; NADK, NAD Kinase; NRF2, Nuclear factor erythroid 2-related factor 2; NOX4, NADPH Oxidase 4; PDK2, Pyruvate Dehydrogenase Kinase 2; PFK 1, Phosphofructokinase 1; PGK1, Phosphoglycerate Kinase 1; PLIN-2, Perilipin 2; PON2, Paraoxonase 2; PPP, Pentose Phosphate Pathway; RPE, Ribulose-5-Phosphate-3-Epimerase; RPIA, Ribose 5-Phosphate Isomerase A; SCO2, Synthesis Of Cytochrome C Oxidase 2; TIGAR, TP53 Induced Glycolysis Regulatory Phosphatase. Created with BioRender.com

Fig. 3. Overview of the key components of the pancreatic tumor microenvironment (TME) and their metabolic cross-talk. In addition to the cancer cells, pancreatic tumors contain a large stromal component composed of extracellular matrix (ECM), pancreatic stellate cells (PSC) ,

cancer-associated fibroblasts (CAFs) and immune cells. See text for further details. Created with BioRender.com

Table 1. PDAC metabolic subgroups. ↑ - upregulation, ↓ - downregulation.

Metabolic subtype	Study	Characteristics	Prognosis	Correlation with molecular subtype
Glycolytic	Daemen et al. (Glycolytic) [15]	↑ glycolytic, pentose phosphate, serine pathways	Poor	Quasi-mesenchymal
	Karasinska et al. (Glycolytic) [17]	↑ glycolytic pathway, K-RAS and MYC amplification	Poor	Basal, squamous, quasi-mesenchymal
	Zhao et al. (L2) [30]	↑ glycolytic pathway ↓ lipid metabolism	Poor	Basal, squamous, quasi-mesenchymal
Lipogenic	Daemen et al. (Lipogenic) [15]	↑ lipid and cholesterol synthesis, mitochondrial OXPHOS	Good	Classical
	Karasinska et al. (Cholesterogenic) [17]	↑ cholesterol synthesis	Good	Progenitor
Mixed	Karasinska et al. (Mixed) [17]	↑ metabolic activity ↑ glycolytic and lipid synthesis pathways	-	-
	Zhao et al. (L1) [30]	↑ glycolytic and lipid synthesis pathways	Intermediate	-
ADEX	Karasinska et al. (Quiescent) [17]	↓ metabolic activity ↑ digestive enzyme activity	-	-
	Zhao et al. (L6) [30]	↑ digestive enzyme activity	Intermediate	-

		↑ protein metabolism		
Slow-proliferating	Daemen et al. [15]	↓ carbohydrates, amino acids ↑ cell doubling time	-	-

Table 2. Driver mutations influence pancreatic cancer metabolism. ↑ - upregulation, ↓ - downregulation.

Driver mutation	Main metabolic changes	Effect on downstream signaling	Mutational frequency in PDAC	Prognosis	Ref.
KRAS-G12D	↑ nonoxidative phase of PPP: ↑ Rpia, ↑ Rpe; PPP intermediates: ↑ sedohepulose-7-phosphate (S7P), ↑ sedoheptulose 1,7-bisphosphate (SBP); ↑ glycolytic flux; ↑ GLUT1; glycolytic enzymes: ↑ HK1, ↑ HK2, ↑ Pfk1, ↑ Eno1, ↑ LDHA; glycolytic intermediates: ↑ Glucose, ↑ glucose 6-phosphate (G6P), ↑ fructose-6-phosphate (F6P), ↑ fructose 1,6-bisphosphate (FBP), ↑ lactate ; ↑ HBP: ↑ Gfpt1; metabolites in HBP: ↑ Glucose, ↑ G6P, ↑ F6P, ↑ glucosamine-6-phosphate (GlcN-6P)	↑ proliferation, invasion via RAF/MAPK pathway; ↑ cell survival, proliferation via PI3K pathway; ↑ growth through mTOR pathway; ↑ cell survival, angiogenesis and invasion via NFκB pathway; triggers subsequent mutation in CDKN2A	70% - 95%; mutation on codon 12,exon 2; wild type GGT to: GAT (40%), GTT (33%), CGT (15%), GCT, AGT, TGT; or mutation on 13 codon, exon 2 (G13D, G13C, G13S, G13R) (7%), codon 61, exon 3 (Q61H, Q61L, Q61K, Q61R) (1–2%), codon 117 (K117) and codon 146 (A146), exon 4 (<1%)	Poor prognosis with complete tumor resection or locally advanced/metastatic PDAC, median survival is 12 months with 5-year survival rate <3%	[33,163–167]
TP53 mutants	adaptation to arginine starvation by ↑ Slc7a3; ↓ α-KG favoring tumor progression; ↑ GLUT 1, ↑ GLUT3 & ↑ GLUT4 favors glycolysis and cell energy supply; ↓ SCO2; ↓ mitochondrial respiration; ↑ PDK2; ↑ G6PD	↑ growth and survival by activation of mTORC1 pathway; ↓ p14ARF tumor suppressor pathway; ↑ glycolysis	63-76%	Poor overall survival with complete pancreatic resection in patients treated with FOLFRINOX; poor outcome in patients with p53Arg248, p53Arg282 mutations	[163,165,168–171]
CDKN2A ($p16^{INK4a}$)	↑ NOX4	↑ malignant transformation; dysregulation of p14ARF signaling pathway to facilitate advanced	58-85%	Tendency to a shorter overall survival of pancreatic cancer patients	[165,172,173]

		tumor progression and metastasis; ↑ tumor angiogenesis			
SMAD4	↑ PGK enzyme to facilitate glycolysis and tumor aggressiveness	↑ EMT	19%-61%	Poor prognosis in patients with resected pancreatic adenocarcinoma	[103,165,174]

Table 3. Clinical trials involving pancreatic cancer patients targeting metabolism. Source: https://clinicaltrials.gov/.

Target	Study title [Identifier]	Interventions	Summary	Phase	Partici-pants
Metabolism (general)	A Study of Therapy Selected by Molecular/Metabolic Profiling in Patients With Previously Treated Metastatic Pancreatic Cancer [NCT01196247]	Drug will be recommended based on IHC/Fish, CGH and Pan-XenoBank	The purpose of the study is selecting second line therapy for patients with pancreatic cancer using molecular profiling will improve 1 year survival.	2	35
	Study of the Safety, Tolerability and Efficacy of Metabolic Combination Treatments on Cancer [NCT02201381]	Metformin, Atorvastatin, Doxycycline, Mebendazole	The aim of this study is to determine the effectiveness of a regimen of selected metabolic treatments for cancer patients in a real world setting and conduct exploratory analysis on the relation between response and changes in biochemical markers (such as glucose and lipid levels).	3	207
Glycolytic pathway	Imatinib Mesylate and Gemcitabine in Treating Patients with locally Advanced, metastatic or Recurrent Pancreatic Cancer [NCT00281996]	Gemcitabine, Imatinib mesylate	This phase I/II trial is studying the side effects and best dose of giving imatinib mesylate together with gemcitabine and to see how well they work in treating patients with locally advanced, metastatic, or recurrent pancreatic cancer.	1	19
	Gemcitabine and Imatinib Mesylate as First-Line Therapy in Patients With Locally Adv. or Metastatic Pancreatic Cancer [NCT00161213]	Gemcitabine, Imatinib mesylate	This phase II trial is studying how well giving gemcitabine together with imatinib mesylate works as first-line therapy in treating patients with locally advanced or metastatic pancreatic cancer.	2	44
	Gemcitabine and Oxaliplatin (Gem-Ox) Plus Glivec in Gemcitabine-refractory Pancreatic Cancer (RPGOG1)	Gemcitabine, Oxiplatin,	The main research objective is to work out the optimal doses of the novel combination of gemcitabine, oxaliplatin and imatinib mesylate (glivec) in patients with advanced pancreatic cancer that	1	36

	[NCT01048320]	Imatinib mesylate	has progressed during or after treatment with first-line gemcitabine.		
Cholesterol synthesis	Trial of Simvastatin and Gemcitabine in Pancreatic Cancer Patients [NCT00944463]	Gemcitabine+ Simvastatin, Gemcitabine+Placebo	The purpose of this study is to determine whether simvastatin is effective in the treatment of advanced pancreatic cancer patients.	2	106
	Phase IB Metformin, Digoxin, Simvastatin in Solid Tumors [NCT03889795]	Metformin, Simvastatin, Digoxin	This is a single-center trial in subjects with pancreatic cancer and other advanced solid tumors.	1	15
	Cholesterol Disruption in Combination With FOLFIRINOX in Patients With Metastatic Pancreatic Adenocarcinoma [NCT04862260]	Cholesterol metabolism disruption	Addresses the effect of adding cholesterol shortage to FOLFIRINOX in newly diagnosed metastatic pancreatic adenocarcinomas. It is expected that drug-induced cholesterol shortage will slow or stop progression and increase response to chemotherapy.	1	12
Autophagy	A Phase I/II/Pharmacodynamic Study of Hydroxychloroquine + Gemcitabine/ Abraxane to inhibit autophagy in Pancreatic Cancer [NCT01506973]	Hydroxychloroquine, Gemcitabine, Abraxane	Phase I/II clinical trial investigating addition of Hydroxychloroquine to a gemcitabine/nab-paclitaxel in pancreatic cancer. This study will incorporate metabolic profiling by mass spectrometry, which will be related to mutations (including Kras) in pretreatment tumor specimens.	1-2	119
	Randomized Phase II Trial of Pre-Operative Gemcitabine and Nab-Paclitacel with or without Hydroxychloroquine [NCT01978184]	Gemcitabine, Abraxane, Hydroxychloroquine	This is a randomized phase II trial that will examine the ability of the hydroxychloroquine to improve the clinical activity of a pre-operative regimen of gemcitabine and nab-paclitaxel in subjects with potentially resectable adenocarcinoma of the pancreas.	2	104
	Paricalcitol and Hydroxychloroquine in Combination With Gemcitabine and Nab-Paclitaxel for the Treatment of Advanced or Metastatic Pancreatic Cancer [NCT04524702]	Gemcitabine, Hydroxychloroquine, Nab-paclitaxel, Paricalcitol	This phase II trial investigates how well paricalcitol and hydroxychloroquine work when combined with gemcitabine and nab-paclitaxel in treating patients with pancreatic cancer that has spread to other places in the body (advanced or metastatic).	2	21
	Pre-Operative Trial (PGHA vs. PGH) for Resectable Pancreatic Cancer [NCT03344172]	Gemcitabine, Nab-paclitaxel, Hydroxy-chloroquine, Avelumab	Randomized phase II trial examining the ability of Avelumab to improve clinical activity of pre-operative gemcitabine, nab-paclitaxel and hy-droxychloroquine in potentially resectable pacreatic adenocarcinoma	2	32
	Study of Pre-surgery Gemcitabine + Hydroxychloroquine (GcHc) in Stage IIb or III Adenocarcinoma of the Pancreas [NCT01128296]	Hydroxychloroquine, Gemcitabine	The primary goal is to determine whether treating pancreatic cancer patients with hydroxychloroquine in combination with gemcitabine before surgery is safe. The secondary goal is to determine if this new treatment regimen can effectively treat pancreatic cancer.	2	35
	Short Course Radiation Therapy With Proton or Photon Beam Capecitabine	Capecitabine, Hydroxychloroquine,	In this study the investigators are using standard photon radiation or a different type of radiation therapy called proton beam radiation	2	50

	and Hydroxychloroquine for Resectable Pancreatic Cancer [NCT01494155]	Radiation: Proton or Photon Radiation Therapy	and adding hydroxychloroquine to be used in combination with capecitabine.		
	Phase II Study of Paclitaxel Protein Bound+Gemcitabine+Cisplatin+ Hydro-chloroquine as Treatment in Untreated Pancreas Cancer [NCT04669197]	Paclitaxel, Gemcitabine, Cisplatin, Hydroxychloroquine	To evaluate the normalization rate of CA 19-9 of individuals with non-metastatic pancreas cancer following up to 6 months of neoadjuvant chemotherapy.	2	12
	Study of therapy selected by Molecular/Metabolic Profiling in patients with previously Treated Metastatic Pancreatic Cancer [NCT01196247]	Drug will be recommended based on IHC/Fish, CGH and Pan-XenoBank	The purpose of the study is selecting second line therapy for patients with pancreatic cancer using molecular profiling will improve 1 year survival.	2	35
	Trametinib and Hydroxychloroquine in Treating Patients With Pancreatic Cancer (THREAD) [NCT03825289]	Hydroxychloroquine, Trametinib	Phase I trial studying side effects and dosing of hydroxychloroquine plus trametinib in metastatic, nonresectable pancreatic cancer.	1	33

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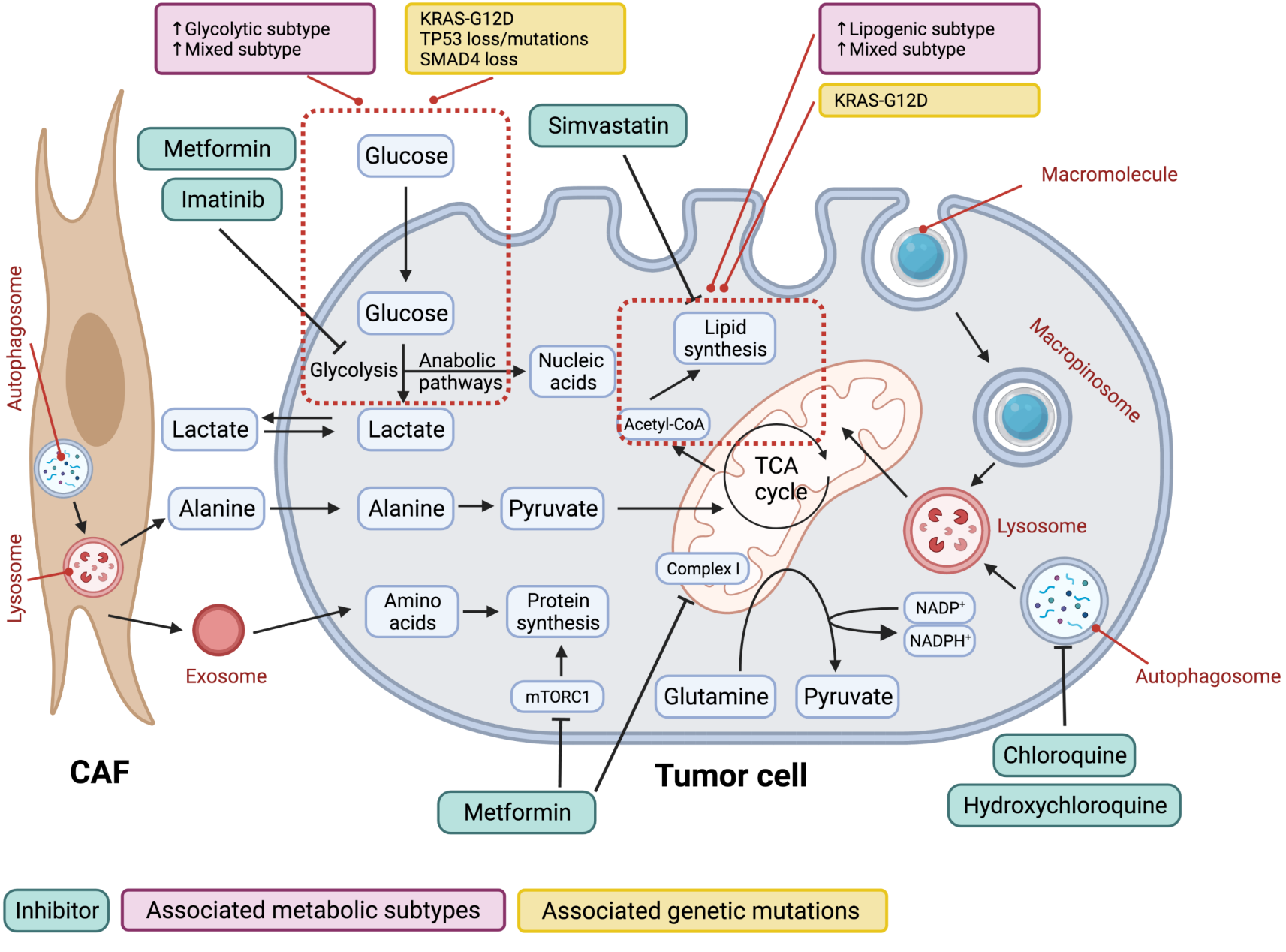
Fig. 1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Simvastatin
Glucose
Glucose
Glycolysis
Anabolic pathways
Nucleic acids
Lipid synthesis
Acetyl-CoA
TCA cycle
Lactate
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Pyruvate
Complex I
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Protein synthesis
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Glutamine
Pyruvate
Macromolecule
Macropinosome
Lysosome
Autophagosome
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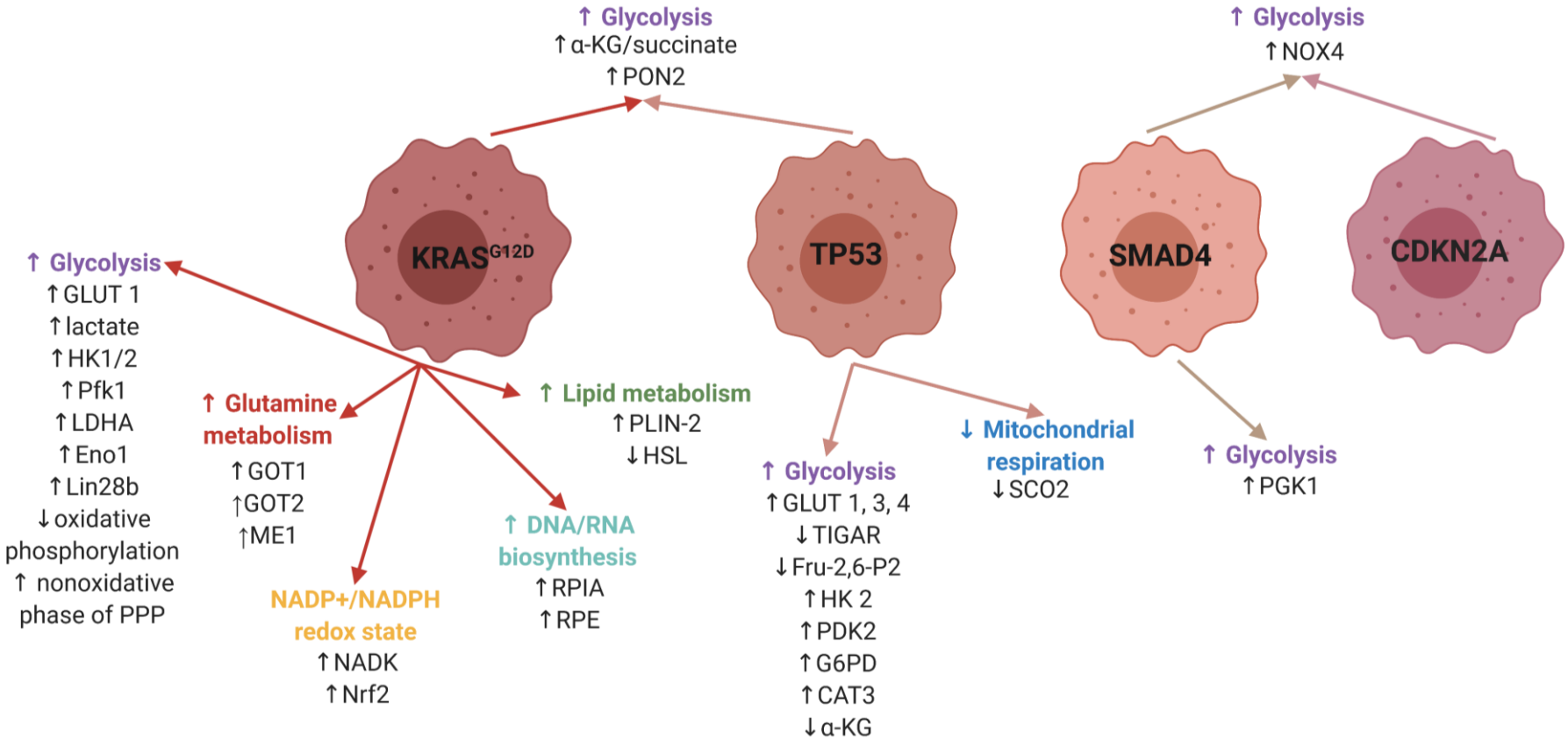
Inhibitor
Associated metabolic subtypes
Associated genetic mutations

Fig.2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NADP+/NADPH redox state
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↑ RPE
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↓ HSL
↑ Glycolysis
↑ GLUT 1, 3, 4
↓ TIGAR
↓ Fru-2,6-P2
↑ HK 2
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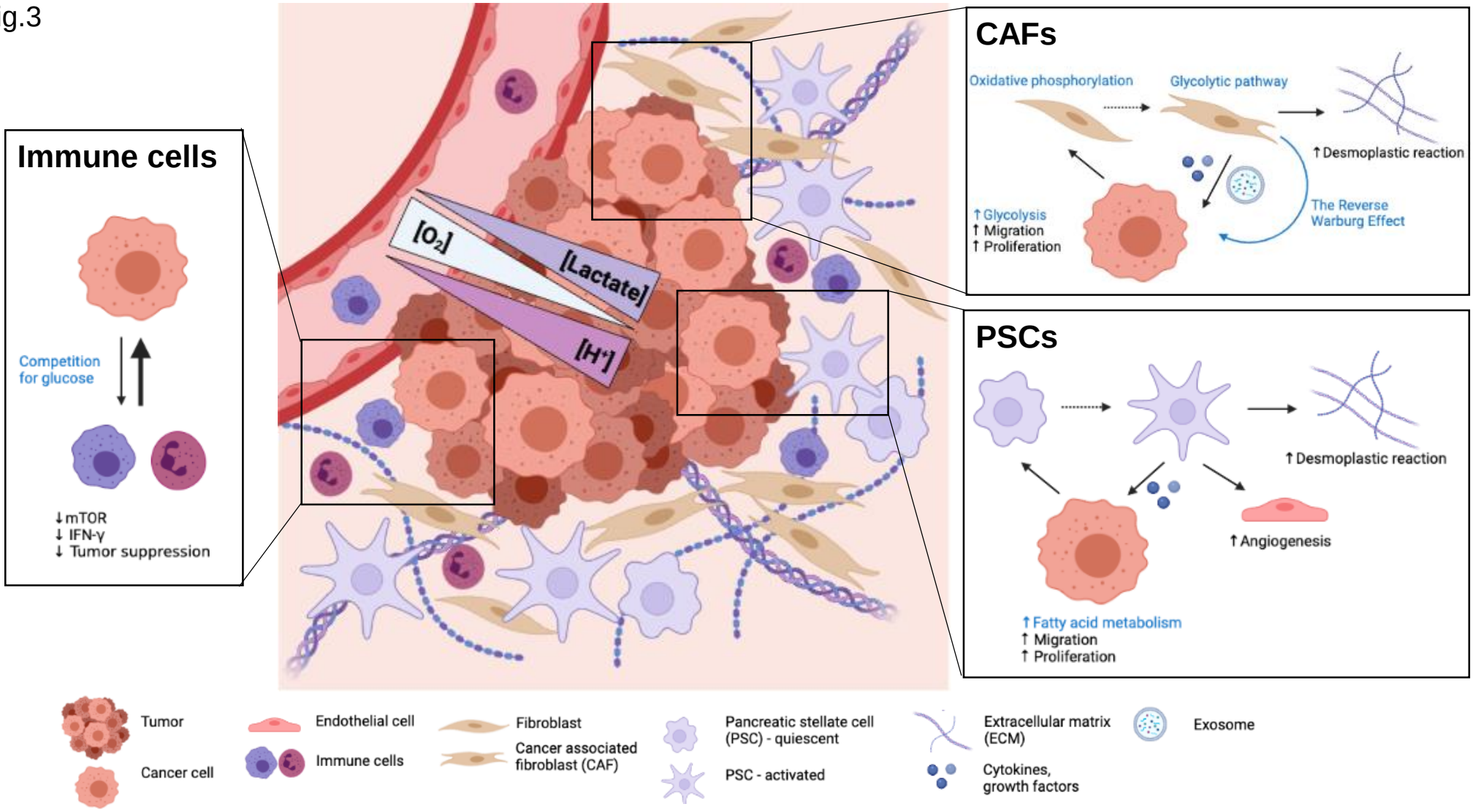
Fig.3


Immune cells
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↓ IFN-γ
↓ Tumor suppression
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CAFs
Oxidative phosphorylation
Glycolytic pathway
↑ Desmoplastic reaction
↑ Glycolysis
↑ Migration
↑ Proliferation
The Reverse Warburg Effect
PSCs
↑ Desmoplastic reaction
↑ Angiogenesis
↑ Fatty acid metabolism
↑ Migration
↑ Proliferation
Tumor
Cancer cell
Endothelial cell
Immune cells
Fibroblast
Cancer associated fibroblast (CAF)
Pancreatic stellate cell (PSC) - quiescent
PSC - activated
Extracellular matrix (ECM)
Cytokines, growth factors
Exosome