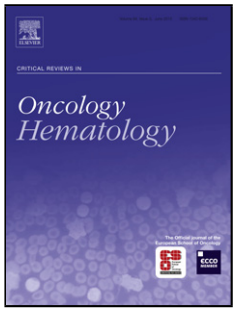


Title: Drug resistance in pancreatic <!–<query id="Q1">Please check the document header and correct if necessary. </query>–>cancer: Impact of altered energy metabolism

Authors: Cristoforo Grasso, Gerrit Jansen, Elisa Giovannetti

PII: S1040-8428(17)30095-1
DOI: http://dx.doi.org/doi:10.1016/j.critrevonc.2017.03.026
Reference: ONCH 2364

To appear in: *Critical Reviews in Oncology/Hematology*

Received date: 20-2-2017
Accepted date: 21-3-2017

Please cite this article as: {http://dx.doi.org/

Drug resistance in pancreatic cancer: impact of altered energy metabolism

Cristoforo Grasso[1] Gerrit Jansen[2], Elisa Giovannetti[1,3*]

1. Laboratory Medical Oncology, Department of Medical Oncology VU University Medical Center (VUmc), Amsterdam, The Netherlands. e.giovannetti@vumc.nl
2. Department of Rheumatology, Amsterdam Rheumatology and immunology Center, VUmc, Amsterdam, The Netherlands
3. Cancer Pharmacology Lab, AIRC Start-Up Unit, University of Pisa, Pisa, Italy

- *Corresponding author:*
Elisa Giovannetti, MD, PhD,
Department of Medical Oncology VU University Medical Center (VUmc)
Cancer Center Amsterdam, CCA room 1.52
De Boelelaan 1117,
1081 HV Amsterdam
The Netherlands
Tel: 0031204442267
E-mail: e.giovannetti@vumc.nl

Contents page

ABSTRACT

Pancreatic cancer is a highly deadly disease: almost all patients develop metastases and conventional treatments have little impact on survival. Therapeutically, this tumor is poorly responsive, largely due to drug resistance. Accumulating evidence suggest that this chemoresistance is intimately linked to specific metabolic aberrations of pancreatic cancer cells, notably an increased use of glucose and the amino acid glutamine fueling anabolic processes. Altered metabolism contributes also to modulation of apoptosis, angiogenesis and drug targets, conferring a resistant phenotype. As a modality to overcome chemoresistance, a variety of experimental compounds inhibiting key metabolic pathways emerged as a promising approach to potentiate the standard treatments for pancreatic cancer in preclinical studies. These results warrant confirmation in clinical trials. Thus, this review summarizes the impact of metabolic aberrations from the perspective of drug resistance and discusses possible novel applications of metabolic inhibition for the development of more effective drugs against pancreatic cancer.

Abbreviations: AMPK: AMP-activated protein kinase; HK2:hexokinase 2; LDHA, lactate dehydrogenase A; GLS1: glutaminase; GLUD, glutamate dehydrogenase GOT1; HIF1-a: Hypoxia-inducible factor 1-alpha; KRAS: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MCT: monocarboxylic acid transporter; mTOR: mammalian target of rapamycin; PK: pyruvate kinase PDH, pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase; PPP: pentose phosphate pathway; RRM1: Ribonucleoside-diphosphate reductase large subunit; SLC1A5: Neutral amino acid transporter B(0); TCA: tricarboxylic acid cycle.

Keywords: Cancer metabolism; drug resistance; new metabolically-targeted agents; pancreatic cancer.

1. Introduction

Pancreatic cancer is one of the most aggressive and deadliest malignancies. It is expected that by 2020 pancreatic ductal adenocarcinoma (PDAC) will surpass breast and colorectal cancer to become the second most common cause of cancer-related deaths.[1] Despite better understanding of its biology and pathogenesis, current treatment regimens are still insufficient.[2] To date, only 5% to 25% of PDACs are eligible for resection, and even after this intervention median survival covers only 12 - 20 months and the 5-year survival does not exceed 20%.[3,4] Given these poor statistics, there is a clear need to develop more effective pharmacological therapies.[5]

1.1 Pancreatic cancer chemoresistance

Chemoresistance is the major impediment for treating PDAC.[6] Currently, first and second-line therapy for PDAC chemotherapy relies on fluoropyrimidine- and gemcitabine-based regimen.[7,8,9] The drug combination of Folinic acid, 5-Fluorouracil (5-FU), Irinotecan, and Oxaliplatin (FOLFIRINOX) is now considered a standard treatment in first-line setting, since it provided PDAC patients with a 4.3 month increase in overall survival when compared to gemcitabine alone.[10] Despite this progress, not all patients benefit from this intense therapy and clinicians are lacking predictive markers to help choosing which individual patient will benefit or when chemoresistance will occur. Potential biomarker candidates include determinants of drug metabolism and activity, such as the enzyme of 5-FU catabolism dihydropyrimidine dehydrogenase (DPD), and the target enzyme thymidylate synthase (TS)[11]. For instance, Kurata et al[12] demonstrated that PDAC cells with high TS and/or DPD levels are more resistant to 5-FU. However, high TS immunoreactivity did not significantly influence

the OS of the patients with unresectable tumors, nor was an independent prognostic factor. Furthermore, in resectable patients, high TS expression levels were significantly correlated with a longer OS rate, vs lower OS for negative or low TS expression levels, suggesting a role for TS as a prognostic factor more than as a predictive biomarker.[13]

Data on potential biomarkers of resistance to platinum compounds in metastatic PDAC are also unclear. It has been demonstrated that cells able to repair platinum-DNA adducts present a profile of resistance to these drugs. The nucleotide excision repair system, which consists of at least 30 identified proteins, including ERCC1, play a key role in removal of damaged DNA.[14] However, the clinical role of ERCC1 staining as a biomarker for resistance to platinum drugs is limited by methodological issues since the currently used ERCC1 antibodies are not specific to detect the unique functional ERCC1 isoform.[15]

Cappelo et al focused on carboxyl esterase-2 (CES2), which activates irinotecan into SN-38, evaluating *in vitro* and *in vivo* models as well as extensive analyses of genetic databases, proteomics and tissue microarrays. High expression of CES2 was associated with longer OS and PFS in resectable and borderline-resectable patients treated with FOLFIRINOX in the neoadjuvant setting.[16] Remarkably, this is the first study reporting the associating of molecular features of pancreatic tumors and outcome of FOLFIRINOX treatment. However, the univariate and multivariate analyses were limited by the small number of patients included in the study (n = 22).

Gemcitabine (2,2-difluoro 2-deoxycytidine, dFdC) has been the standard of care for PDAC since 1997. This drug is a deoxycytidine analogue, whose cytotoxic activity is based on interference of DNA synthesis. Efficacy of gemcitabine-based therapy for PDAC is limited by emerging drug resistance, which can be intrinsic, or acquired after multiple treatment cycles, and is multifactorial.[17] Resistance can indeed result from several molecular and cellular changes, affecting nucleotide metabolism enzymes, apoptosis pathway, drug efflux pumps, cancer stem cells or epithelial-to-mesenchymal

transition (EMT) pathway, as well as up- or down-regulated expression of specific microRNA (miRNA).[18] For instance, Dhayat and collaborators suggested that consistent miR expression profiles (miR-21-5p, miR-31*, miR-125b-5p, miR-210-3p, miR-330-3p, miR-378a-3p, miR-422a and miR-486-5p) enhance proliferation by upregulating Bcl-2 expression in PDAC chemoresistant cells.[19]Alterations in the nucleoside transporter-1 (hENT1), an important element in gemcitabine uptake, as well as various gemcitabine metabolism gene products, including deoxycytidine kinase and ribonucleoside reductase subunits M1 and M2 (RRM1 and RRM2), were also contributing factors in gemcitabine resistance.[20],[21] Next, aberrant expression of genes associated with cellular survival and apoptosis have been implicated in gemcitabine resistance, such as for example the S100 family member S100A4, whose expression provokes resistance by regulation of the hypoxia-induced proapoptotic gene *BNIP3*.[22] Lastly, the phosphatidylinositol 3-kinase/Akt survival pathway has also been implicated in gemcitabine resistance[23] along with integrin-linked kinase (ILK).[24]

In particular, ILK increases gemcitabine chemoresistance in PDAC cells due to a chemoprotective effect occuring in association with suppression of caspase 3 activity.[24]

To overcome resistance modalities, several preclinical studies evaluated novel drugs alone and in combination with gemcitabine, and albumin-bound paclitaxel particles (nab-paclitaxel) revealed antitumor activity as a single agent and synergistic activity in combination with gemcitabine in murine models of PDAC.[25] Nab-paclitaxel is a nanoparticle albumin-bound paclitaxel, which achieves a higher tumor accumulation vs paclitaxel, both due to the lack of drug-sequestering solvent micelles and to albumin-mediated transcytosis.[26] The presence of albumin-binding proteins, such as secreted protein acidic and rich in cysteine (SPARC), which is overexpressed in the stromal fibroblasts surrounding PDAC, is another hypothesized mechanism to be responsible of the higher tumor accumulation of this drug.[27] However, SPARC failed as a predictive biomarker and as a potential selection criteria for treatment with nab-paclitaxel.[25]

Additional studies showed that nab-paclitaxel improved the intratumoral concentration of gemcitabine, though the inactivation of cytidine deaminase, the main gemcitabine catabolizing enzyme.[28] After promising phase II trials, a phase III trial enrolled 861 patients, with median OS of 8.5 months vs 6.7 months, respectively, favouring the combination of nab-paclitaxel and gemcitabine vs. gemcitabine monotherapy ($P < 0.001$).[25] However, no validated biomarkers to guide gemcitabine-nab-paclitaxel treatment are available and further studies on determinants of drug resistance are warranted. The role of tubulin in resistance to taxane therapy has been widely investigated in different tumor types. Lung cancer patients with low tumor levels of class III B-tubulin isotype had a better response rate, longer PFS and OS, and this variable was not found to be predictive in patients receiving regimens without taxanes.[29] The role of tubulin expression was also investigated in gastric cancer: who showed a significantly shorter median PFS if class III B-tubulin expression was high.[30] However, no data on the role of this potential biomarker are available for PDAC patients.

In the era of actionable mutations and targeted agents it would be desirable to identify molecular factors or biomarkers to predict response or resistance to therapy in order to maximize the efficacy of treatment and avoid useless toxic effects for non-responding patients. High throughput sequencing and copy number studies of PDAC genome have identified and validated the known driver mutations in *K-RAS*, *TP53*, *CDKN2A*, and *SMAD4*, as well as novel gene mutations that may be involved in cell growth, DNA repair, invasiveness, angiogenesis and metabolism.[31] These studies can potentially bridge a shift of focus to novel targets for therapeutic intervention in PDAC, including metabolic reprogramming. Understanding how best to integrate inhibitors to metabolic pathways with existing chemotherapeutic agents as well as determining the appropriate combination of inhibitors of metabolic and key signalling pathways should pave the way to combat and overcome the complex landscape of PDAC chemoresistance.[32]

1.2 Impact of altered metabolism in pancreatic cancer chemoresistance

A recent emerging strategy to treat (chemoresistant) PDAC relies on exploiting aberrant metabolic processes in cancer cells in general and PDAC cells in particular.[33,34,35] In fact, cancer cells reprogram their metabolic pathways, a process regulated by a complex and still poorly defined combination of intrinsic and extrinsic factors. A prevailing view posits that a key function of oncogenes is to reprogram the cellular metabolism back to the building blocks that sustain unrestricted tumor growth.[36] An early event during malignant transformation is the acquisition of activating mutations in the *K-RAS* oncogene at codons 12, 13, 61, which occurs in more than 90% of PDAC patients.[37] PDACs are highly "addicted" to this oncogene for multiple parameters influencing tumor initiation, progression and maintenance.[38] Recent studies demonstrated that *K-RAS* mutations play a pivotal role in the metabolic reprogramming of cancer cells, shifting them towards an anabolic metabolism necessary to produce biomass and support unconstrained proliferation.[39] Since mutant *K-RAS* expression has been associated to reduced anticancer activity of both gemcitabine and paclitaxel, new therapeutic strategies to target the K-Ras-dependent metabolic aberrations should therefore inhibit tumor cell growth and counteract drug resistance.

Desmoplasia and the tumor microenvironment have been frequently reported as major contributors to chemoresistance in PDAC. Notably, PDAC cells are embedded in a very complex microenvironment together with stromal components that enhance oxidative stress and promote tumor metabolic modulation.[40] Furthermore, signal transduction between stromal and parenchymal cells promotes both tumor cell growth and metabolic aberrations.[41] Together, these factors typically prompt PDAC cells to exhibit metabolic plasticity. As a consequence, in addition to the wide range of inter-tumor genetic heterogeneity, there is also significant intra-tumor heterogeneity due to the presence of multiple subclones with distinct metabolic features, that can reduce drug sensitivity.

The main consequence of metabolic reprogramming is to provide energy and building blocks to tumor cells for proliferation and maintain their redox balance to defend against oxidative stress.

However, cancer cells acidify their microenvironment by promoting glycolysis to induce epithelial-mesenchymal transition (EMT) and the expression of matrix metalloproteinases (MMPs), thereby enhancing tumor metastatic potential.[42] A high glycolysis rate leads to more lactate production, which can stimulates angiogenesis and functions as a vasodilator to take over the limited energy availability, whereas angiotonics respond to abundant energy supplies.[43] Similarly, the hypoxic microenvironment of cancer cells triggers the upregulation of hypoxia-inducible factor-1α (HIF-1α), which mediates angiogenesis and the desmoplastic responses.[44] In keeping with these data, mathematical modelling of radiological/pathological data revealed that most PDAC patients harbor a few cells that are able to metastasize even when the tumor size is very and this tumor is a systemic chemoresistant disease, even at its inception.[45,46,47]

The fundamental idea of anticancer drugs is to target specific molecules and/or cellular processes that are essential for tumor cell survival and dispensable for normal cells. Aberrant cancer cell metabolism due to enrichment of genetic alterations that provide a survival advantage is recognized as a potential Achilles' heel in PDAC. In the next sections we will focus on PDAC metabolism in the context of chemoresistance and recent discoveries of small molecules that inhibit metabolism to improve standard treatment in PDAC.

2. Metabolism addiction in pancreatic cancer

2.1 Aerobic Glycolysis

Glucose metabolism is a complex process involving glycogenolysis and gluconeogenesis, both of which regulate blood glucose levels. The main function of glucose is to provide cellular energy for supporting various biochemical reactions. In contrast to normal differentiated cells, which rely primarily on mitochondrial oxidative phosphorylation to generate the energy needed for cellular processes, most cancer cells instead rely on aerobic glycolysis, a phenomenon termed "the Warburg effect".[48] Compared with oxidative phosphorylation (OXPHOS), glycolysis is a less efficient pathway

for producing adenosine triphosphate, but it clearly give a survival advantage to tumor cells by promoting rapid ATP production, producing key components for cellular biosynthesis and protecting towards reactive oxygen species (ROS) production [Figure 1].[49]

In PDAC, aberrant metabolism is driven by several cellular factors depending on the aberrant activity of specific oncogenes that guide the switching of nutrient utilization.[50] As such, cell-autonomous metabolic reprogramming is meaningfully driven by the activation of key genetic alterations and oncogenic signalling pathways. Not surprisingly, mutations in *K-RAS* and other canonical oncogenes (e.g., *MYC*) and tumor suppressors (e.g. *TP53*, *RB* and *PTEN*) were identified driving the accelerated growth of PDAC by directly reprogramming cellular metabolism.[51,52]

The K-ras protein plays an important role in PDAC glucose metabolism, which is featured by upregulation of glucose uptake and the increased expression of multiple key glycolytic enzymes, including glucose transporter type 1 (GLUT1), hexokinase 1/2 (HK1/2), phosphofructokinase, and lactate dehydrogenase A (LDHA).[53,54] K-ras also supports synthesis of building blocks (i.e. amino acids and nucleic acids) essential for cancer cell proliferation by shuttling glucose toward anabolic pathways, such as the pentose phosphate pathway (PPP), while preserving a low level of ROS and limiting ROS production and ROS-related apoptosis.[53] *TP53* contributes to the glycolytic switch via upregulation of GLUT1 and GLUT4 expression and loss of expression of TIGAR (TP53-inductible glycolytic and apoptotic regulator) which functions as a fructose-2,6-biphosphatase (FBP-ase).[55]

The hypoxic tumor microenvironment promotes activation of HIF-1α, leading to similar aberrant signalling due to oncogene activation of *K-RAS*, and *MYC*, inactivation of the tumor suppressor gene *TP53* and aberrations in the OXPHOS pathway.[56]Glycolytic change mediates also important interconnections between tumor stroma. Particularly, lactate may be an important vector for tumor-stroma interactions and symbiotic spatial energy fuel exchange between cell compartments within the tumor.[57] Hypoxic cancer cells produce lactate, which diffuses to the extracellular environment by

means of lactate transporter MCT-4 and is then taken up by normoxic cancer cells through MCT-1 to be used for oxidative metabolism, thereby saving glucose for hypoxic cancer cells. Lactate also "feeds" stromal cells providing a fuel source for OXPHOS.[58] Remarkably, microenvironment acidity also contributes to suppress immune cells by promoting chronic inflammation, while suppressing T-cell mediated adaptive immune response.[59] Collectively, high lactate concentrations and acidic pH, representative features of "glycolytic tumors", has been associated with poor prognosis and a more aggressive phenotype.[60]

2.2 Glutamine metabolism

Recent evidence demonstrated that some cancer cells use glutamine (Gln) to support anabolic processes to fuel proliferation.[61] Gln, as the most abundant free amino acid in humans, is utilized by tumor cells to maintain their pools of tricarboxylic acid (TCA) cycle amino acids, hexosamine, nucleotides and other molecules.[62] Recently, a study reported the identification of a non-canonical pathway of Gln utilization in PDAC cells that is required for tumor growth [Figure 2].

While most cells utilize glutamate dehydrogenase (GLUD1) to convert Gln-derived glutamate (Glu) into α-ketoglutarate in the mitochondria to fuel the TCA cycle,[63,64] PDAC relies on a distinct pathway to fuel the TCA cycle such that Gln-derived aspartate is transported into the cytoplasm where it can be converted into oxaloacetate (OAA) by aspartate transaminase (GOT1). Subsequently, OAA is converted into malate and then pyruvate to increase the NADPH/NADP+ ratio facilitating maintenance of the cellular redox state.[52] Relative to non-malignant cells, cancer cell growth relies on maintenance of proliferative signaling pathways with increased autonomy. [65] In Hela cells, excess Gln is exported in exchange for leucine and other essential amino acids. This exchange facilitates activation of the serine/threonine kinase mTOR, a major positive regulator of cell growth.[66] Importantly, PDAC cells are strongly dependent on this series of reactions, as Gln deprivation or genetic inhibition of any enzyme in this pathway leads to an increase in reactive oxygen species and

a reduction in reduced glutathione.[67] Moreover, knockdown of any component enzyme in this series of reactions also results in pronounced suppression of PDAC growth *in vitro* and *in vivo*.[68]

Abbreviations: GOT2: mitochondrial aspartate transaminase; GLUD1: glutamate dehydrogenase 1; Asp: aspartate; ME1: malic enzyme 1; ME2: malic enzyme 2; GLS1: glutaminase 1; MDH1: malate dehydrogenase 1; GOT1: cytosolic aspartate transaminase; OAA: oxaloacetate; αKG: α-ketoglutarate; TCA: tricarboxylic acid cycle.

2.3 Pancreatic cancer microenvironment

The dynamic relationship between tumors and their microenvironment holds promise for novel therapeutic interventions. There has been an increased interest in the potential targeting of the PDAC desmoplastic reaction, a cellular compartment containing cancer-associated fibroblasts (CAFs), extracellular matrix proteins, inflammatory, and endothelial cells.[69] The PDAC microenvironment is characterized by hypoxia and minimal vascularity compared to other tumor types [Figure 3].

Despite this, elevated pro-angiogenic vascular endothelial growth factor A (VEGF-A) levels have been observed in PDAC patients which correlate with increased vascular density of PDAC and greater disease progression.[70] However, the dense extracellular matrix of PDAC enables remarkable biophysical rigidity with increased intra-tumoral pressure. Increased pressure causes collapse of the vasculature and diminished diffusion into the tumor interstitium. This is hypothesized to be a major barrier in responding to therapies.[31] Furthermore, one of the major consequences of intra-tumoral hypoxia is the cells' metabolic reprogramming to meet the requirements of tumor proliferation under low oxygen and low nutrient supply because of lack of vasculature.[71]

Cancer associated fibroblasts (CAFs) are one of the most crucial components of the tumor microenvironment promoting growth and invasion of cancer cells by various mechanisms.[72] In PDAC, the CAFs are the main effector cells in the desmoplastic reaction, and are present in aberrantly high numbers.[73] These cells are distinct from normal fibroblasts and undergo metabolic reprogramming, resembling the phenotype associated with the Warburg effect. In particular, CAFs consume more glucose and secrete more lactate than normal fibroblasts in most solid tumors[74]. Moreover, a recent

study has shown that CAF-derived exosomes can strikingly reprogram the metabolic machinery following their uptake by cancer cells.[72] Notably, CAF-derived exosomes inhibit mitochondrial OXPHOS, thereby increasing glycolysis and Gln-dependent reductive carboxylation in cancer cells. Additionally, CAF-derived exosomes contain intact metabolites, including amino acids, lipids, and TCA-cycle intermediates to support tumor growth.[75]

In conclusion, tumor progression is driven by genetic mutations; meanwhile, environmental conditions such as hypoxia and metabolic energy supplies provide a selective advantage that allows cells with such mutations to clonally expand. Hypoxia initiates activation of HIF-1α along with oncogenic, inflammatory, oxidative and metabolic stress, the latter of which involves switching to anaerobic glycolysis.[76] Metabolic effects within the microenvironment, e.g. lactate secretion by cancer cells leading to acidification of the microenvironment, also trigger an inflammatory response through the release of cytokines and other factors that promote tumor progression.[77] Of further notice, lactate production by stromal cells can provide a bioenergetic substrate for cancer cells to further support their survival and growth.[78]

3. Role of metabolism alteration and anticancer drug resistance

Several metabolic alterations, driven by genetic and epigenetic factors, have been correlated to drug activity and clinical outcome, supporting the hypothesis that cancer metabolism is intimately linked to chemoreistance.[79] In addition, data from *in vitro* studies, proteomics platforms and ^{13}C metabolic flux analysis (MFA) provided insight into the complex metabolic mechanisms of cancer, enabling the selection of molecular targets for therapeutic interventions.[80] These issues are discussed in greater detail in the next paragraphs from the perspective of PDAC.

3.1 Metabolism-mediated modulation of survival and/or apoptosis pathways

Metabolic remodeling can contribute to key tumor features, thereby affecting cancer cell differentiation, proliferation and/or apoptosis, as well as therapeutic responses.[81] In particular,

several regulatory enzymes in glycolysis have been implicated in promoting a drug-resistant cancer phenotype [Figure 1]. The first metabolic step in the glycolytic pathway is catalyzed by the enzyme hexokinase (HK). Of the two isoforms of HK known; cytoplasmic HK1 and mitochondrial HK2, the latter is found up-regulated in many cancers and has the ability to inhibit mitochondrial apoptosis by direct insertion in the mitochondrial outer membrane.[82] HK2 was most highly expressed in PDAC metastases, suggesting a link between HK2 and pancreatic aggressive tumor biology.[83] Furthermore, survival pathways such as the PI3K/Akt/mTOR pathway can activate HK in cancer cells and induce drug resistance.[84] Due to its contribution in regulating apoptosis and cellular bioenergetics, HK2 is considered to be an important anticancer drug target. The HK2 inhibitor 3-bromopyruvate is able to reduce ATP reserves, and thereby reverse chemoresistance.[85] In contrast, elevated ATP levels as a result of increased glycolysis, activate HIF-1α and confer drug resistance.[86]

Fructose bisphosphate aldolase (FBA), which converts fructose 1,6-biphosphate into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate, is another metabolic enzyme overexpressed in PDAC.[64] Overexpression of FBA delays induction of apoptosis, as does G3P, by suppressing caspase-3 activity.[87] Additionally, overexpression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) prevents caspase-independent cell death, presumably by stimulating glycolysis, increasing cellular ATP levels, and promoting autophagy.[88]

PKM2 is the rate-limiting enzyme of the glycolytic pathway and converts phosphoenolpyruvate (PEP) and ADP into pyruvate and ATP. PK is a tetrameric enzyme composed of homotetramers or hybrid forms of one the four isotypes (M1, M2, L and R) being differentially expressed in different cell types. PKM2 is highly expressed in cancers, conceivably to drive higher glycolytic fluxes because of its high substrate affinity for PEP,[89] and maintains high lactate levels, which is potentially oncogenic. A recent study has demonstrated the role of lactate as a signalling intermediate in hypoxic conditions leading to activation of survival pathways. In addition, lactate-dependent stabilization was demonstrated of

the protein NDRG3, which binds to c-Raf and promotes neovascularization and survival.[90] Apart from promoting cell survival, lactate can attenuate immune signalling, and in particular, tumor-derived lactate can prevent the response of human T-cell,[91] which dominate the immune infiltrate in human PDAC. Of further notice, inhibiting the monocarboxylate family of transporters that include lactate transporters is being considered as a potential therapeutic option for cancer treatment, including PDAC.[92,93]

3.2 Metabolism-mediated regulation of drug targets, transport and catabolism

At present, altered metabolism is considered as one of the hallmarks of cancer cells, and accumulating studies are supportive of metabolic alterations being linked to cancer drug resistance and prompt development of new anticancer strategies to overcome chemoresistance.[65] However, the Warburg effect involves the complex control of the expression of multiple genes and pathways, and modulating one target or segment may not be sufficient to suppress tumors and might even result in drug resistance.[94] To challenge chemoresistance, research into the molecular mechanisms underlying chemoresistance is crucial. Altered metabolism comprises a multifactorial process of concerted action of genes, proteins and metabolites that generate a characteristic cancer phenotype. However, up to now, most studies focussed only on a few proteins involved in cancer metabolism and resistance towards anticancer drugs. One representative study showed the association of LDH-A and paclitaxel resistance in breast cancer cells and underscored the role of LDH-A in cancer therapeutics and drug sensitivity as the increased expression and activity of LDH-A in paclitaxel-resistant cells directly correlated with the sensitivity to the glycolysis inhibitor oxamate. Moreover, siRNA knockdown of LDH-A reversed taxol sensitivity in resistant cells.[95] More recently, a study on novel LDH-A inhibitors in PDAC cells lines showed a synergistic interaction with gemcitabine, which was attributed to modulation of gemcitabine metabolism, by enhanced expression of deoxycytidine kinase (dCK), overcoming the reduced synthesis of phosphorylated metabolites. Of note, acquired

resistance to gemcitabine in PDAC has been correlated on the differential expression of four genes involved in gemcitabine transport, activation and mechanism of action, i.e., hENT1, dCK, and RRM1, M2. Specifically, a decreased ratio of *hENT1* × *dCK*/*RRM1* × *RRM2* gene expression was a characteristic feature of gemcitabine-resistant subclones. The ratio of gene expression decreased progressively with development of acquired resistance in gemcitabine-resistant subclones. Furthermore, this expression ratio also significantly correlated with gemcitabine sensitivity in eight PDAC cell lines, whereas no single gene expression level correlated with the sensitivity.[20]

Another enzyme with a critical role in metabolism-mediated resistance is pyruvate dehydrogenase kinase 3 (PDK3), which catalyzes the first step of OXPHOS, and contributes to hypoxia-induced drug resistance in cervical and colon cancer.[96] Hypoxia induces PDK3 expression via upregulation of HIF-1α, which binds to the promoter of PDK3, resulting in a switch from mitochondrial respiration to glycolysis for energy production. Hypoxia-mediated PDK3 induction or forced PDK3 overexpression significantly inhibits cell apoptosis and increases resistance to cisplatin or paclitaxel in colorectal cancer and, considering the similar expression levels detected for this protein in PDAC tissues (http://www.proteinatlas.org/ENSG00000067992-PDK3/cancer), it might have the same effects in PDAC cells.[97]

Finally, fatty acid synthase (FASN) has been linked to acquired docetaxel/trastuzumab/adriamycin resistance in breast cancer and intrinsic gemcitabine and radiation resistance in PDAC.[98] In pancreatic tumors, a previous study demonstrated a positive correlation between FASN expression and resistance to chemo- or radio-therapy.[86] FASN expression is significantly upregulated in PDAC cells and inhibition of FASN by siRNA or the FASN inhibitor orlistat reduce gemcitabine resistance, whereas ectopic overexpression of FASN contributes to intrinsic resistance to gemcitabine and radiation .[99] FASN-induced radiation resistance may result from decrease in radiation mediated ceramide production, leading to reduced caspase-8 induced apoptosis. However, the precise mechanism of

FASN-induced gemcitabine resistance remains to be elucidated.[100] In gastric cancer glutaminolysis FASN was associated to cisplatin resistance via the activation of mammalian target of rapamycin complex 1 (mTORC1) signaling.[101] Notably, in vitro and in vivo experiments showed that the combination of AZD8055 and erlotinib synergistically inhibited the mTORC1/C2 signaling pathway, together with EGFR/AKT feedback activation, and cell growth, as well as suppressed the progression of PDAC in a xenograft model.[102] Therefore, new combinations of agents targeting of these pathways might also overcome chemoresistance caused by metabolic aberrations.

4. Critical pathways and targets in cancer metabolism

4.1 HIF-1α

Human cells require adequate supplies of O_2 on a continuous basis for use as the terminal electron acceptor in the process of mitochondrial respiration that generates ATP to power most biochemical reactions.[44] The intracellular O_2 concentration is tightly regulated, however, in cancer cells dysfunctions in the regulatory pathways, e.g. HIF-1α, are common.[103] HIF-1α is a master regulator of transcription of genes involved in cell proliferation and survival, as well as glucose and iron metabolism. HIF-1α stability, subcellular localization, as well as transcriptional activity are especially affected by oxygen levels.[104]

Hypoxia directly increases lactate production and excretion due to changes in mitochondrial redox status elicited by reduced oxygen availability.[105] Although hypoxia often leads to a reduction or cessation of proliferation through HIF-1α mediated upregulation of p21, in some cancers proliferation is maintained through the sustained activity of mTOR or Notch.[106] With hypoxia, in PDAC, the expression of HIF-1α is increased [Figure 1], just as the expression of glucose metabolic enzymes PDK1, LDH-A and PKM2.[107] Accordingly, knockdown of HIF-1α under hypoxic conditions inhibited the production of lactate and the expression of PDK1, LDH-A and PKM2. Knockdown of HIF-1α under hypoxia repressed the growth of the pancreatic cells BxPC-3 along with induction of

apoptosis.[107] Indeed, the stabilization and activity of HIF-1α in hypoxia strongly supports and even enhances the metabolic reprogramming of glycolysis through the upregulation of almost all glycolytic genes and the monocarboxylate transporters that export lactate.[108] HIF-1α has also been shown to upregulate the expression of genes encoding the glucose transporters Glut1 and Glut3, glycolytic enzymes such as the hexokinases HK1 and HK3, aldolase A and C and GAPDH.[109] HIF-1α mediated adaptation responses such as angiogenesis and anaerobic metabolism are induced to promote cell survival. Consistently, constitutive expression of HIF-1α confers apoptosis resistance in PDAC cells.[110] In the PDAC cells PCI-35, with constitutive HIF-1α expression, also Glut1 and aldolase A mRNAs were more abundantly expressed, thereby facilitating increased anaerobic metabolism and apoptosis resistance under conditions of hypoxia and glucose deprivation.[111] Another key enzyme upregulated by hypoxia-induced HIF-1α activity, and altering pyruvate metabolism, is PDK1. This HIF-1α -mediated effect leads to inactivation of the pyruvate dehydrogenase complex and subsequent loss of pyruvate oxidation.[112] The inhibition of the pyruvate dehydrogenase complex in hypoxia seems a protective mechanism, as it has recently been shown that activation of this enzyme complex by oncogenes is a key driver of oncogene-induced senescence through increased oxygen consumption and redox stress.[105] This is surprisingly similar to the phenotype shown in response to the inhibition of glycogen metabolism,[113] and points to the intriguing possibility of inducing oncogene-induced senescence in cancers through the inhibition of one or more obligate glucose-metabolising pathways.[105] This is further illustrated by the fact that inhibition of PDK1 expression impairs cell growth and increases oxygen consumption and cell death under hypoxia in human cancer cell lines.[97]

4.2 LDH

LDH catalyzes the reversible transformation of pyruvate to lactate under anaerobic conditions, coupled with the oxidation of NADH to NAD^+.[114] LDH is a tetrameric enzyme consisting of two types of subunits designated M (LDH-A gene product) and H (LDH-B gene product). Human cells contain

five different LDH isozymes as a result of the different hybrid forms of H and/or M subunits: LDH1 (H4); LDH2 (MH3); LDH3 (M2H2); LDH4 (M3H); LDH5 (M4)[115], with LDH-A identified as a target of both c-Myc and HIF-1.[116] LDH plays an essential role in regulating glycolysis by catalyzing the final passage of anaerobic glycolysis; therefore, its upregulation favours the efficiency of anaerobic glycolysis in tumor cells and allows ATP production in absence of oxygen.[117] Of all LDH isoenzyme forms, LDH-5 is the predominant isoform found in skeletal muscle and other highly glycolytic tissues, including tumor tissues, and has the highest efficiency to catalyze the conversion of pyruvate to lactate. LDH-5 is mainly localized to the cytoplasm, where it participates in glucose metabolism.[118] Several studies have illustrated the prognostic relevance of LDH in different tumour types including PDAC.[119] In tissue and xenograft studies it was demonstrated that inhibition of LDH-A activity due to lysine 5 acetylation, is reduced in human PDAC, thereby underlining its role in PDAC initiation and as a potential new target.[120] The potential oncogenic activity of LDH-A has also been reported in oesophageal carcinoma and gastric cancer.[121,122] Of further notice, the LDH gene promoter harbours two conserved hypoxia response elements (HREs) containing functionally essential binding sites for HIF-1α, which strongly suggest an oxygen dependent regulation of LDH-5 activity.[123] The relevance of LDH is further supported by elevated expression levels of LDH-A observed in PDAC samples compared with the matched normal tissues and by the fact that LDH-A promoted the growth of the PDAC, both *in vitro* and *in vivo*.[118] These results encourage further LDH-A-directed therapeutic interventions for PDAC.[124]

4.3 mTOR

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase, frequently activated in human cancers, including PDAC.[125] Whether or not the mTOR gene acquires oncogenic properties through somatic mutations, has remained unclear.[125] However, K-ras signaling, including PI3K/Akt, links ligation of growth factor receptors to the phosphorylation and activation of mTOR.[126]

mTOR engages a signalling program downstream from nutrient availability to stimulate metabolism and leading to cell cycle progression.[127] mTOR exists as two complexes: mTORC1, being sensitive to rapamycin inhibition, and mTORC2, being largely rapamycin inhibition insensitive.[128] mTORC1 interacts with the accessory protein Raptor-to-phosphorylate effectors S6 kinase 1, which ultimately enhances the translation of mRNAs, including ribosomal proteins, elongation factors, and insulin growth factor 2.[127]

The fact that mTOR signalling defects can cause both metabolic disorders and cancer suggests that mTOR links cancer development and metabolism[129]. This notion is supported by the observation that metformin inhibits mTORC1 signalling, via activation of AMPK and REDD1 and a Rag GTPase-sensitive mechanism, in addition to suppressing cancer.[130] Dowling et al. proposed that mTORC1 controls cell proliferation exclusively via 4E-BP while it regulates cell growth via S6K.[131] Evidence suggesting that mTOR links metabolism and cancer is further provided by a recent study demonstrating that LTsc1KO mice with hyperactive mTORC1 signaling display metabolic abnormalities, including defects in glucose and lipid homeostasis, and subsequently develop hepatocarcinoma.[132] Glutaminolysis constitutes another mTOR link between metabolism and cancer. Highly proliferating cancer cells are often glutamine-addicted, and tumor growth correlates with the activity of glutaminase (GLS), the enzyme that catalyzes the first step of glutaminolysis.[133] Lastly, it has been demonstrated that glutaminolysis also activates mTORC1, thereby promoting cell growth and inhibiting autophagy.[132] Of note, mTOR activity also impacts expression of HIF-1α, probably through the activation of S6K. As a consequence, inhibition of mTOR by rapamycin also suppresses HIF-1α expression.[134]

Remarkably, several preclinical data have demonstrated that inhibition of mTOR in specific *K-RAS*-dependent PDAC genetic subtypes leads to inhibition of tumorigenesis *in vitro* and *in vivo*. However, phase II trials of anti-mTOR regimens have not shown positive results.[135] Coordinated inhibition of

mTOR and other steps along the mTOR signaling pathway, including critical factors in tumor metabolism may lead to better responses by targeting pivotal mechanisms of tumor resistance.

5. From the bench-to-the-bedside: translational studies on the impact of metabolism in pancreatic cancer

5.1 Determinants of cancer glycolytic metabolism as predictors of drug resistance and clinical outcome

PDAC is a highly aggressive and chemoresistant cancer. In search for biomarkers which may predict therapy response and/or drug resistance, several serum markers for PDAC have been investigated, including carbohydrate antigen 19-9 (CA 19-9), cell surface associated mucin (MUC1), carcinoembryonic antigen related cell adhesion protein molecule 1 (CEACAM1), and more recently a pyruvate kinase variant (M2-PK).[136] However, all these markers lack sensitivity and specificity, as they are infrequently elevated in the early stage of the cancerogenesis, and may also be over-expressed in various inflammatory conditions.[136] Still, high levels of HK2 and low levels of proliferating cell nuclear antigen (PCNA) expression may allow accurate identification of PDAC patients who benefit from intensive treatments and experience a longer survival.[137] Several reports have shown that increased acidosis is often linked to a tumor cell phenotype resistant to different anticancer therapies. For instance, overexpression of Glut1 in oral squamous cell carcinoma and HK2 in metastases of breast cancer predicts poor prognosis after radio- or chemotherapy.[138,139] Multivariate analysis showed that combined expression of PKM2 and LDH-A was an independent poor prognostic marker for survival in PDAC. Specifically, a high expression pattern of these two major glycolytic enzymes during pancreatic carcinogenesis featured aggressive tumours and had a significant adverse effect on survival.[140] High LDH-A is also associated with resistance to standard chemotherapy, poor progression-free survival and high performance status in patients with advanced colorectal cancer.[141] Conversely, low levels of LDH-A were significantly linked to improved responses to therapy in PDAC.[36]

Taken together, these findings suggest that LDH-A may serve as predictive marker for assessing the response of tumor cells to therapeutic agents, whereas inhibition of LDH-A may offer a novel approach in overcoming resistance to chemotherapy.[95] With respect to PDAC metabolism, high lactate levels are often associated with a worse prognosis, conceivably related to increased angiogenesis and metastasis.[142]

5.2 Metabolic inhibition to complement current treatment

Although Warburg effect (i.e., glycolysis preference of malignant cells to gain energy faster) was described as early as 1950s, targeting metabolic differences of cancer cells gained more popularity only in recent years. Altered metabolism is indeed now considered as one of the hallmarks of cancer.[65] Therefore, a better understanding of metabolic dysregulations in which characterize different tumor types, including PDAC, could lead to the discovery of novel therapeutic targets.[143] Targeting cancer metabolism may provide an additional strategy for PDAC treatment and drug resistance and will be discussed hereafter.

5.2.1 Focus on novel inhibitors of glycolysis

Several small molecule inhibitors of glycolysis, as single agents or in combination with other therapeutic modalities, exhibit promising anticancer activity both *in vitro* and *in vivo* [Figure 1].[144] Hexokinase (HK) catalyzes the first regulatory step in glucose metabolism by phosphorylating glucose to produce glucose-6-phosphate (G6P). Currently, HK inhibitors such as 2-deoxyglucose (2-DG), 3-bromopyruvate (3-BrPA) and lonidamine (LND) are evaluated in pre-clinical and early phase clinical trials, including a few trials in PDAC patients [Table 1].[99] In particular, 2-DG serves as a competitive inhibitor of HK blocking access of glucose to the enzyme. 2-DG is taken up by glucose transporters and phosphorylated by HK to 2-DG-P and subsequently accumulated intracellularly. 2-DG-P is not recognized as a substrate and metabolized by the next glycolytic enzyme, phosphoglucose isomerase. This results into the cellular retention of 2-DG-P and an impaired cellular ATP production.[145]

Combinations of 2-DG with radiotherapy or chemotherapy revealed synergistic effects in preclinical models of different tumor types, but only negligible effects were observed in clinical trials.[146]

Abbreviations: 2-DG, 2-Deoxyglucose; α-KGDH, Alpha-ketoglutarate dehydrogenase; GLS, glutaminase; HK, hexokinase; NCT, ClinicalTrials.gov identifier (https://clinicaltrials.gov/ct2/home), PDH, pyruvate dehydrogenase; PEXG, cisplatin, epirubicin, capecitabine, and gemcitabine regimen.

Lactate production occurs at the final stage of the glucose metabolism where LDH-A constitutes a major checkpoint for the switch to anaerobic glycolysis.[147] Remarkably, metastasis of tumors is promoted by lactate-induced secretion of hyaluronan by tumor-associated fibroblasts that create a milieu favorable for migration. Lactate itself has been found to induce the migration of cells and cell clusters.[148] Furthermore, under hypoxia, LDH-A provokes exacerbation of ROS production, which drives glycolysis.[149] Novel LDH-A inhibitors (NIH) emerged as very promising anticancer agents, by targeting both key mechanisms involved in the proliferation, cell-cycle control, apoptosis, stemness, and the migration properties of PDAC cells, especially under hypoxic conditions.[147] Promising results were also observed in several *in vivo* models. The inhibition of LDH by 3-bromopyruvate showed suppression of tumor engraftment and growth on chicken eggs and mice.[150] Of note, this study showed that 3-bromopyruvate enhanced gemcitabine efficacy by read-outs of expression markers of proliferation, apoptosis, self-renewal, and metastasis. Another LDH inhibitor, FLX11, impaired the growth of both lymphoma and PDAC xenografts.[151]

More recently, a novel and potent LDH inhibitor GNE-140, demonstrated its ability to modulate LDH-A activity both *in vitro* and *in vivo*.[152] Interestingly, GNE-140 action was potentiated in combination with phenformin, an inhibitor of complex I of the mitochondrial respiratory chain.[152] Phenformin is biguanide anti-diabetic drug, which harbors anticancer activity.[153] At the cellular level, biguanides act through activation of AMPK[154] via a mechanism that requires LKB1 and involves regulation of the downstream pathways relevant to the control of cellular proliferation resulting in a variety of effects distinct from their anti-diabetic activity. It has been demonstrated that PDAC cell lines sensitive to

GNE-140 were more dependent on glycolysis, whereas cell lines resistant to GNE-140 relied more on OXPHOS. Moreover, inhibition of glycolysis with GNE-140 and OXPHOS with phenformin resulted in synthetic lethality.[152] Consistent with the possibility that GNE-140 sensitive cell lines rely more on glycolysis than do resistant cell lines, sensitive lines had a lower baseline oxygen consumption rate (OCR). However, cells that are more reliant on OXPHOS may show greater sensitivity to GNE-140 upon co-treatment with phenformin.[152]

Another critical metabolic step in tumor cells, under hypoxia conditions and HIF-1α regulation, is the activation of HK and PDK to increase glycolysis and enhance lactate production.[155] PDK inhibition in tumor cells by dichloroacetate (DCA) elicits dual effects; on the one hand it decreases lactate production and the mitochondrial membrane potential, on the other hand it increases ROS and mitochondria-dependent apoptosis.[156] In Panc-1 cells, DCA-induced PDK inhibition stimulated metabolism via the Krebs cycle over glycolysis to impact Panc-1 proliferation and viability.[157] Preclinical results showed that DCA may synergize well with chemotherapeutic agents such as 5-FU and cisplatin via inhibition of glucose-dependent, hypoxia-induced chemoresistance.[156] Notably, PDH inhibition has also been associated with potent anticancer effects in human pancreatic and non-small cell lung cancer xenograft models.[158] In a phase I-II trial combining the PDH inhibitor CPI-613 with gemcitabine in patients with advanced solid tumors study, responses were observed in patients with stage IV pancreatic neuroendocrine tumor.[159] These encouraging results should prompt future trials.

The key role of glutamine in fueling tumor cell metabolism has spurred the development of inhibitors targeting enzymes along the glutamine metabolism pathway. In particular, CB-839, which is a highly selective, reversible, allosteric inhibitor of GLS is currently being evaluated clinically (NCT02071862).

Finally, a drug which has shown in numerous laboratory research and pharmacoepidemiology studies its capacity of attacking the bioenergetics reprogramming of cancer cells is the widely prescribed oral anti-diabetic drug metformin.[160] This is and inexpensive and safe-toxic-profile molecule and its ability

to decrease circulating insulin may be particularly relevant for the treatment of cancers well known for being associated with hyperinsulinemia. In particular, type 2 and type 1 diabetes mellitus augments the risk of PDAC, and almost 80 % of PDAC patients also have diabetes or impaired glucose tolerance.

Metformin activates AMP-activated protein kinase (AMPK), an energy sensor involved in regulating cellular metabolism in response to a rise in the cellular AMP:ATP ratio.[161] Currently, a number of clinical trials examining the use of metformin as a cancer therapeutic are underway including studies in prostate, breast, endometrial and PDAC patients.[162] In pancreatic cells, insulin enhances signalling triggered by insulin/IGF1R and multiple G protein-coupled receptors to promote cell growth.[152] Since the cross-talk between insulin/IGF1R and the G protein-coupled receptor signalling system depends on mTORC1, metformin was capable of disrupting this cross-talk via AMPK activation and had therapeutic effects in PDAC xenograft models.[163] However, in a recent preclinical study, several PDAC xenografts treated with metformin did not show any tumor growth inhibition.[164]

Similarly, a phase II clinical trial, testing the efficacy of supplementing metformin to systemic chemotherapy in patients with metastatic PDAC, showed that the addition of a conventional anti-diabetic dose of metformin to a polychemotherapeutic regimen with gemcitabine and cisplatin did not improve patient outcome.[165] In another trial, adding metformin to gemcitabine and erlotinib also did not improve the clinical outcome of unselected and heterogeneous patients with advanced PDAC.[166] Hence, the added value of metformin in current PDAC treatment remains controversial and additional research is warranted to explain the discordance between preclinical and clinical research. In particular, since the diabetic status of PDAC patients seems to have an impact on metformin outcome, the anticancer action could be metformin dose dependent and glucose level dependent. However, new studies should also address at which stage metformin therapy might be beneficial, and if molecular classification and grading of PDAC could improve outcome. Therefore, a more

rigorous planning of clinical trials, not only focusing on classical parameters but also on potential predictive biomarkers (AMPK, mTOR, HIF-1α, IGF-1R), is warranted.

6. Conclusions and future perspectives

Metabolic rewiring is central to the pathogenesis of PDAC and is a critical component of the tumorigenic program driven by *K-RAS*, the signature mutation in this malignancy. A key current challenge is to define how nutrient substrates are generated and utilized by these tumors and to understand how the multiple different cooperating genomic alterations found in PDAC influence these processes. Notably, numerous oncogenic activations of PDAC (such as *K-RAS*, *TP53*, and *MYC*) have glycolytic activity promoting effects with concomitant lactate production acidifying the tumor microenvironment. The metabolic changes in cancer cells, such as the Warburg effect, allow available resources to be converted into biomass in an efficient manner [Figure 4].

Aberrant (glycolytic) metabolism allows cancer cells to resist standard treatment through modulation of apoptosis and angiogenesis, as well as affecting drug transport and targets. Thus, compounds that influence deregulated cellular metabolism often have the ability to increase the efficacy or reduce resistance to current anticancer treatments.

In addition, there are many other important links between PDAC metabolism and drug resistance. Microenvironment conditions promote the Warburg effect in metabolism. Hypoxic conditions activate HIF-1α, which is the guardian sensor of oxygen concentration. HIF-1α activation leads to upregulation of glycolytic enzymes resulting in a higher glucose metabolism. In PDAC, CAFs have a role in creating an extracellular matrix structure and metabolic and immune reprogramming of the tumor microenvironment with an impact on adaptive resistance to chemotherapy. The pleiotropic actions of CAFs on tumour cells are probably reflective of them being a heterogeneous and plastic population with context-dependent influence on cancer.[167] Lactate produced by hypoxic tumor cells may indeed diffuse and be taken up by oxygenated tumor cells. Preferential utilization of lactate for

oxidative metabolism spares glucose, which may in turn reach hypoxic tumor cells.

Recently, encouraging results demonstrated that combining metabolism inhibitors to standard treatment yielded synergistic effects that potentiate cancer treatment. Although most inhibitors are still in the preclinical phase, the inhibition of glycolytic enzymes represents a promising novel approach for anticancer therapy in general and PDAC in particular. Unfortunately, many of these therapeutic strategies have still several drawbacks such as poor bioavailability, unfavourable pharmacokinetic profiles and associated nonspecific toxicities, hampering preclinical investigations. Nevertheless metabolic inhibitors appear a promising opportunity to exploit aberrant metabolism in PDAC as its Achilles heel.

Conflict of interest

All the authors declare no conflicts of interest

Vitae

Cristoforo Grasso received his BSc from the University of Catania, Italy in 2011. In September 2015 he was admitted as MSc student at the VU University of Amsterdam in the Netherlands, where he is attending a Master in Oncology. Currently, he is performing an internship at George Town University, Lombardi Cancer Center, Washington DC, US. He works, under the supervision of Anton Wellstein M.D., on pancreatic ductal adenocarcinoma heterogeneity and conditionally reprogrammed cell culture (CRC), in order to investigate whether CRC maintains in vitro heterogeneity of the human patient tumor biopsies.

Dr. Gerrit Jansen obtained his PhD degree in Biochemistry at the State University of Utrecht, The Netherlands in 1984. From 1985-1990 he held a post-doctoral position at the Utrecht University Medical Center (Dept. of Oncology). In 1991 he moved to the VU University Medical Center in Amsterdam (Dept. of Medical Oncology). From 1992-1994 he was a recipients of a fellowship of the Royal Netherlands Academy of Arts and Sciences, which was followed by a senior postdoctoral position at the VU University Medical Center (Dept. of Medical Oncology). In 2001 he moved to the Department of Rheumatology (currently Amsterdam Rheumatology and immunology Center) at the VU University Medical Center to become head of the laboratory for experimental rheumatology. Dr. Jansen's main research interest focuses on molecular mechanisms of drug resistance, with a special interest for resistance mechanisms to classical and experimental drugs from an anti-arthritic and anti-cancer perspective.

Dr. Elisa Giovannetti received her M.D. and Ph.D. with full marks and honours from the University of Pisa, Italy, in 2000 and 2007, respectively. Between 2001 and 2004, she worked as a clinical fellow in Pharmacology in the Department of Oncology of Pisa University, and contributed to clinical and preclinical studies on the relationship between gene expression/polymorphisms and anticancer drug

response in pancreatic and lung cancer. Since 2006 she collaborated with the Laboratory Medical Oncology at VUmc, Amsterdam, to set-up a new line of research characterizing novel predictive markers of drug activity and resistance in pancreatic cancer. She successfully requested funding from the Netherlands Organisation for Scientific Research (NWO, VENI grant), the European Initiative "Marie Curie for outgoing scientist", Italian Association for Research against Cancer (AIRC, Start-Up grant), Cancer Center Amsterdam (CCA) Foundation, and Dutch Cancer Society (KWF). She is actively involved, as elected member of the Steering Committee, in research projects within the "Pharmacology and Molecular Mechanism Group" group of the EORTC (EORTC-PAMM), as well as in studies of the European Pancreatic Club (EPC) and Italian Association for the Study of the Pancreas (AISP).

Acknowledgements
GJ would like to acknowledge the support by Cancer Center Amsterdam Foundation (CCA grant 2012-1-08), EG would like to acknowledge the support by CCA grants 2012-5-07, 2015-1-19 and 2016-5-32, Italian Association for Cancer Research (AIRC) Start- Up grant

REFERENCES

1. Rahib, L. *et al.* Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the united states. *Cancer Res.* **74,** 2913–2921 (2014).
2. Wysocka, O., Kulbacka, J. & Saczko, J. Adjuvant, neoadjuvant, and experimental regimens in overcoming pancreatic ductal adenocarcinoma. *Gastroenterol. Rev.* **11,** 155–162 (2016).
3. Helmut Oettle MD, P. *et al.* Adjuvant Chemotherapy With Gemcitabine vs Observation in Patients Undergoing Curative-Intent Resection of Pancreatic Cancer A Randomized Controlled Trial. *J. Am. Med. Assoc.* **297,** 267–277 (2007).
4. Ahn, D. H., Williams, T. M., Goldstein, D. A., El-Rayes, B. & Bekaii-Saab, T. Adjuvant therapy for pancreas cancer in an era of value based cancer care. *Cancer Treat. Rev.* **42,** 10–17 (2016).
5. Wagner, M. *et al.* Curative resection is the single most important factor determining outcome in patients with pancreatic adenocarcinoma. *Br. J. Surg.* **91,** 586–594 (2004).
6. Wang, C. *et al.* Toward targeted therapy in chemotherapy-resistant pancreatic cancer with a smart triptolide nanomedicine. *Oncotarget* **7,** 8360–8372 (2016).

7. Aroldi, F., Bertocchi, P., Savelli, G., Rosso, E. & Zaniboni, A. Pancreatic cancer: New hopes after first line treatment. *World J. Gastrointest. Oncol.* **8,** 682–687. (2016).
8. Walker, E. J. & Ko, A. H. Beyond first-line chemotherapy for advanced pancreatic cancer: An expanding array of therapeutic options? *World J. Gastroenterol.* **20,** 2224–2236 (2014).
9. Gall, T. M. H., Tsakok, M., Wasan, H. & Jiao, L. R. Pancreatic cancer: current management and treatment strategies. *Postgrad. Med. J.* **91,** 601–607 (2015).
10. Conroy, T. *et al.* FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* **364,** 1817–1825 (2011).
11. Wang, W. Bin *et al.* Recent studies of 5-fluorouracil resistance in pancreatic cancer. *World J. Gastroenterol.* **20,** 15682–15690 (2014).
12. Kurata, N. *et al.* Predicting the chemosensitivity of pancreatic cancer cells by quantifying the expression levels of genes associated with the metabolism of gemcitabine and 5-fluorouracil. *Int. J. Oncol.* **39,** 473–482 (2011).
13. Caparello, C. *et al.* FOLFIRINOX and translational studies: Towards personalized therapy in pancreatic cancer. *World J. Gastroenterol.* **22,** 6987–7005 (2016).
14. Chaney, S. G. & Sancar, A. REVIEW DNA repair: enzymatic mechanisms and relevance to drug response. *J. Natl. Cancer Inst.* **88,** 1346–1360 (1996).
15. Friboulet, L. *et al.* ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. *N. Engl. J. Med.* **368,** 1101–1110 (2013).
16. Capello, M. *et al.* Carboxylesterase 2 as a Determinant of Response to Irinotecan and Neoadjuvant FOLFIRINOX Therapy in Pancreatic Ductal Adenocarcinoma. *J. Natl. Cancer Inst.* **107,** 1–9 (2015).
17. De Sousa Cavalcante, L. & Monteiro, G. Gemcitabine: Metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. *Eur. J. Pharmacol.* **741,** 8–16 (2014).
18. Jia, Y. & Xie, J. Promising molecular mechanisms responsible for gemcitabine resistance in cancer. *Genes Dis.* **2,** 299–306 (2015).
19. Dhayat, S. A. *et al.* MicroRNA profiling implies new markers of gemcitabine chemoresistance in mutant p53 pancreatic ductal adenocarcinoma. *PLoS One* **10,** e0143755. (2015).
20. Nakano, Y. *et al.* Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. *Br. J. Cancer* **96,** 457–

463 (2007).

21. Duxbury, M. S. *et al.* Retrovirally mediated RNA interference targeting the M2 subunit of ribonucleotide reductase: A novel therapeutic strategy in pancreatic cancer. *Surgery* **136,** 261–269 (2004).
22. Erkan, M. *et al.* Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. *Oncogene* **24,** 4421–4432 (2005).
23. Bondar, V. M., Sweeney-Gotsch, B., Andreeff, M., Mills, G. B. & McConkey, D. J. Inhibition of the phosphatidylinositol 3 ’-kinase-AKT pathway induces apoptosis in pancreatic carcinoma cells in vitro and in vivo. *Mol Cancer Ther* **1,** 989–997 (2002).
24. Duxbury, M. S. *et al.* RNA interference demonstrates a novel role for integrin-linked kinase as a determinant of pancreatic adenocarcinoma cell gemcitabine chemoresistance. *Clin. Cancer Res.* **11,** 3433–3438 (2005).
25. Von Hoff, D. D. *et al.* Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N. Engl. J. Med.* **369,** 1691–1703 (2013).
26. Yardley, D. A. Nab-Paclitaxel mechanisms of action and delivery. *J. Control. Release* **170,** 365–372 (2013).
27. Desai, N., Trieu, V., Damascelli, B. & Soon-Shiong, P. SPARC Expression Correlates with Tumor Response to Albumin-Bound Paclitaxel in Head and Neck Cancer Patients. *Transl. Oncol.* **2,** 59–64 (2009).
28. Zhang, Y., Hochster, H., Stein, S. & Lacy, J. Gemcitabine plus nab-paclitaxel for advanced pancreatic cancer after first-line FOLFIRINOX: single institution retrospective review of efficacy and toxicity. *Exp. Hematol. Oncol.* **4,** 1–5 (2015).
29. Sève, P. *et al.* Class III beta-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel. *Mol. Cancer Ther.* **4,** 2001–2007 (2005).
30. Hwang, J.-E. *et al.* Class III β-tubulin is a predictive marker for taxane-based chemotherapy in recurrent and metastatic gastric cancer. *BMC Cancer* **13,** 431 (2013).
31. Chauhan, V. P. *et al.* Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature.* **26,** 495–501 (2015).
32. Chand, S., O’Hayer, K., Blanco, F. F., Winter, J. M. & Brody, J. R. The landscape of pancreatic cancer therapeutic resistance mechanisms. *Int. J. Biol. Sci.* **12,** 273–283 (2016).

33. Luo, J., Solimini, N. L. & Elledge, S. J. Principles of Cancer Therapy: Oncogene and Non-oncogene Addiction. *Cell* **136,** 823–837 (2009).
34. Vivanco, I. Targeting molecular addictions in cancer. *Br. J. Cancer* **111,** 2033–2038 (2014).
35. Belizário, J. E., Sangiuliano, B. A., Perez-Sosa, M., Neyra, J. M. & Moreira, D. F. Using Pharmacogenomic Databases for Discovering Patient-Target Genes and Small Molecule Candidates to Cancer Therapy. *Front. Pharmacol.* **7,** 1–16 (2016).
36. Yao, J. C. *et al.* Efficacy of RAD001 (Everolimus) and Octreotide LAR in Advanced Low- to Intermediate-Grade Neuroendocrine Tumors : Results of a Phase II Study. *J. Clin. Oncol. Clin. Oncol.* **26,** 4311–4318 (2008).
37. Takai, E. & Yachida, S. Genomic alterations in pancreatic cancer and their relevance to therapy. *World J. Gastrointest. Oncol.* **7,** 250–258 (2015).
38. Collins, M. a *et al.* Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer mice. *J. Clin. Invest.* **122,** 639–653 (2012).
39. Kimmelman, A. C. Metabolic Dependencies in RAS-Driven Cancers. *Clin Cancer Res.* **21,** 1828–1834 (2016).
40. Fiaschi, T. & Chiarugi, P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: A diabolic liaison. *Int. J. Cell Biol.* **10,** 762–770 (2012).
41. Kamiska, K. *et al.* The role of the cell-cell interactions in cancer progression. *J. Cell. Mol. Med.* **19,** 283–296 (2015).
42. Taddei, M. L., Giannoni, E., Comito, G. & Chiarugi, P. Microenvironment and tumor cell plasticity: An easy way out. *Cancer Lett.* **341,** 80–96 (2013).
43. Morais-Santos, F. *et al.* Targeting lactate transport suppresses in vivo breast tumour growth. *Oncotarget* **6,** 19177–19189 (2015).
44. Semenza, G. L. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci.* **33,** 207–214 (2013).
45. Haeno, H. *et al.* Metastasis Suggesting Optimum Treatment Strategies. *Cell.* **148,** 362–375 (2013).
46. Rhim, A. D. *et al.* EMT and dissemination precede pancreatic tumor formation. *Cell.* **148,** 349–361 (2013).
47. Zheng, X. *et al.* Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature.* **527,** 525–530 (2016).

48. Vander Heiden, M., Cantley, L. & Thompson, C. Understanding the Warburg effect: The metabolic Requiremetns of cell proliferation. *Science (80-.).* **324,** 1029–1033 (2009).

49. Hsu, P. P. & Sabatini, D. M. Cancer cell metabolism: Warburg and beyond. *Cell* **134,** 703–707 (2008).

50. Hezel, A. F., Kimmelman, A. C., Stanger, B. Z., Bardeesy, N. & Depinho. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev* **1,** 1218–1249 (2006).

51. DeBerardinis, R. J., Lum, J. J., Hatzivassiliou, G. & Thompson, C. B. The Biology of Cancer: Metabolic Reprogramming Fuels Cell Growth and Proliferation. *Cell Metab.* **7,** 11–20 (2008).

52. Cetinbas, N. M. *et al.* Glucose-dependent anaplerosis in cancer cells is required for cellular redox balance in the absence of glutamine. *Sci. Rep.* **6,** 32606–32618 (2016).

53. Ying, H. *et al.* Oncogenic kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* **149,** 656–670 (2012).

54. Wang, C.-W., Purkayastha, A., Jones, K. T., Thaker, S. K. & Banerjee, U. In vivo genetic dissection of tumor growth and the warburg effect. *Elife* **5,** e18126 (2016).

55. Bensaad, K. *et al.* TIGAR, a p53-Inducible Regulator of Glycolysis and Apoptosis. *Cell* **126,** 107–120 (2006).

56. Cairns, R., Harris, I. & Mak, T. Regulation of cancer cell metabolism. *Nat. Rev. Cancer* **11,** 85–95 (2011).

57. Romero-Garcia, S., Moreno-Altamirano, M. M. B., Prado-Garcia, H. & Sánchez-García, F. J. Lactate Contribution to the Tumor Microenvironment: Mechanisms, Effects on Immune Cells and Therapeutic Relevance. *Front. Immunol.* **7,** 52–61 (2016).

58. Feron, O. Pyruvate into lactate and back: From the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother. Oncol.* **92,** 329–333 (2009).

59. Choi, S. Y. C., Collins, C. C., Gout, P. W. & Wang, Y. Cancer-generated lactic acid: A regulatory, immunosuppressive metabolite? *J. Pathol.* **230,** 350–355 (2013).

60. Alfarouk, K. O. *et al.* Glycolysis, tumor metabolism, cancer growth and dissemination. A new pH-based etiopathogenic perspective and therapeutic approach to an old cancer question. *Oncoscience* **1,** 777–802 (2014).

61. Wise, D. R. & Thompson, C. B. Glutamine Addiction: A New Therapeutic Target in Cancer. *Trends Biochem. Sci.* **35,** 427–433 (2011).

62. Altman, B. J., Stine, Z. E. & Dang, C. V. From Krebs to clinic: glutamine metabolism to cancer

therapy. *Nat. Rev. Cancer* **16,** 619–634 (2016).

63. Guo, Y. *et al.* Glutaminolysis Was Induced by TGF-β1 through PP2Ac Regulated Raf-MEK-ERK Signaling in Endothelial Cells. *PLoS One* **11,** 240–248 (2016).
64. Chan, A. K., Bruce, J. I., Siriwardena Anthony KC Chan, A. K. & Siriwardena, A. K. Glucose metabolic phenotype of pancreatic cancer. *World J Gastroenterol* **22,** 3471–3485 (2016).
65. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: The next generation. *Cell* **144,** 646–674 (2011).
66. Nicklin, P. *et al.* Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* **136,** 521–534 (2013).
67. Son, J. *et al.* Glutamine supports pancreatic cancer growth through a Kras- regulated metabolic pathway. *Nature* **496,** 101–105 (2013).
68. Son, J. *et al.* Regulated Metabolic Pathway. *Nature* **496,** 101–105 (2013).
69. Carr, R. M. & Fernandez-Zapico, M. E. Pancreatic cancer microenvironment, to target or not to target? *EMBO Mol. Med.* **8,** 80–82 (2016).
70. Luo, J. *et al.* Pancreatic cancer cell-derived vascular endothelial growth factor is biologically active in vitro and enhances tumorigenicity in vivo. *Int. J. Cancer* **92,** 361–369 (2001).
71. Vasseur, S., Tomasini, R., Tournaire, R. & Iovanna, J. L. Hypoxia induced tumor metabolic switch contributes to pancreatic cancer aggressiveness. *Cancers (Basel).* **2,** 2138–2152 (2010).
72. Xing, F., Saidou, J. & Watabe, K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. *Front. Biosci. (Landmark Ed.* **15,** 166–79 (2010).
73. DF Quail and JA Joyce. Microenvironmental regulation of tumor progression and metastasis. *Nat Med.* **28,** 2903–2909 (2014).
74. Yoshida, G. J. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. *J. Exp. Clin. Cancer Res.* **34,** 111–120 (2015).
75. Zhao, H. *et al.* Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. *Elife.* **5,** 1–27 (2016).
76. Kroemer, G. & Pouyssegur, J. Review Tumor Cell Metabolism : Cancer ' s Achilles ' Heel. *Cancer Cell* **13,** 472–482 (2008).
77. Yabu, M., Shime, H., Hara, H., Saito, T. & Matsumoto, M. IL-23-dependent and -independent enhancement pathways of IL-17A production by lactic acid. *Int Immunol.* **23,** 29–41 (2011).
78. Balliet, R. M. *et al.* Understanding the 'lethal' drivers of tumor-stroma co-evolution. *Cacer Biol.*

Ther. **10,** 537–542 (2010).

79. Duckwall, C. S., Murphy, T. A. & Young, J. D. Mapping cancer cell metabolism with 13 C flux analysis : Recent progress and future challenges. *J Carcinog* **12,** 254–263 (2013).
80. Rahman, M. & Hasan, M. Cancer Metabolism and Drug Resistance. *Metabolites* **5,** 571–600 (2015).
81. Kinnaird, A., Zhao, S., Wellen, K. E. & Michelakis, E. D. Metabolic control of epigenetics in cancer. *Nat. Rev. Cancer* **16,** 694–707 (2016).
82. Jang, M., Kim, S. S. & Lee, J. Cancer cell metabolism : implications for therapeutic targets. *Exp. & Mol. Med.* **45,** 45–48 (2013).
83. Anderson, M., Marayati, R., Moffitt, R. & Jen Yeh, J. Hexokinase 2 promotes tumor growth and metastasis by regulating lactate production in pancreatic cancer. *Oncotarget* **2,** 3762–3763 (2016).
84. Kim, K. Il. INPP4B-mediated tumor resistance is associated with modulation of glucose metabolism via hexokinase 2 regulation in laryngeal cancer cells. *Biochem Biophys Res Commun* **440,** 137–142 (2013).
85. Gatenby, R. A. & Gillies, R. J. Glycolysis in cancer : A potential target for therapy. *Int J Biochem Cell Biol.* **39,** 1358–1366 (2007).
86. Morten, K. J., Badder, L. & Knowles, H. J. Differential regulation of HIF-mediated pathways increases mitochondrial metabolism and ATP production in hypoxic osteoclasts. *J Pathol.* **229,** 755–764 (2013).
87. Jang, M. *et al.* Glyceraldehyde-3-Phosphate , a Glycolytic Intermediate , Plays a Key Role in Controlling Cell Fate Via Inhibition of Caspase Activity. 559–563 (2009).
88. Colell, A. *et al.* GAPDH and Autophagy Preserve Survival after Apoptotic Cytochrome c Release in the Absence of Caspase Activation. 983–997 (2007).
89. van Veelen, W. M., Verbiest, H., Vlug, A. M. C., Rijksen, G. & Staal, E. J. Isoenzymes of pyruvate kinase from human brain, meningiomas and malignant gliomas. *Cancer Res.* **38,** 4681–4687 (1978).
90. Lee, D. C. *et al.* Article A Lactate-Induced Response to Hypoxia Article A Lactate-Induced Response to Hypoxia. *Cell* **161,** 595–609 (2015).
91. Fischer, K. *et al.* Inhibitory Effect of Tumor Cell-Derived Lactic Acid on Human T Cells. *Blood* **109,** 3812–3820 (2007).

92. Porporato, P. E., Dhup, S., Dadhich, R. K., Copetti, T. & Sonveaux, P. Anticancer targets in the glycolytic metabolism of tumors: A comprehensive review. *Front. Pharmacol.* **2,** 1–18 (2011).

93. Lai, I. L. *et al.* Targeting the Warburg effect with a novel glucose transporter inhibitor to overcome gemcitabine resistance in pancreatic cancer cells. *Carcinogenesis* **35,** 2203–2213 (2014).

94. Chen, X., Li, L., Guan, Y., Yang, J. & Cheng, Y. Anticancer strategies based on the metabolic profile of tumor cells : therapeutic targeting of the Warburg effect. *Nat. Publ. Gr.* **37,** 1013–1019 (2016).

95. Zhou, M. *et al.* Warburg effect in chemosensitivity : Targeting lactate dehydrogenase-A re-sensitizes Taxol-resistant cancer cells to Taxol. *Mol Cancer.* **9,** 1–12 (2010).

96. Zhao, Y. *et al.* Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis.* **71,** 4585–4597 (2012).

97. Lu, C. *et al.* Overexpression of Pyruvate Dehydrogenase Kinase 3 Increases Drug Resistance and Early Recurrence in Colon Cancer. *Am. J. Pathol.* **179,** 1405–1414 (2011).

98. Liu, H., Liu, Y. & Zhang, J. A new mechanism of drug resistance in breast cancer cells : fatty acid synthase overexpression-mediated palmitate overproduction. *Mol Cancer Ther* **7,** 263–270 (2008).

99. Zhao, Y., Butler, E. B. & Tan, M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis.* **4,** 532–536 (2013).

100. Yang, Y. *et al.* Role of fatty acid synthase in gemcitabine and radiation resistance of pancreatic cancers. *Int. J. Biochem. Mol. Biol.* **2,** 89–98 (2011).

101. Wang, J. *et al.* Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. *Cancer Cell.* **18,** 207–219 (2011).

102. Wei, F. *et al.* mTOR Inhibition Induces EGFR Feedback Activation in Association with Its Resistance to Human Pancreatic Cancer. *Int. J. Mol. Sci.* **16,** 3267–3282 (2015).

103. Kietzmann, T., Mennerich, D. & Dimova, E. Y. Hypoxia-Inducible Factors (HIFs) and Phosphorylation: Impact on Stability, Localization, and Transactivity. *Front Cell Dev Biol.* **4,** 1–14 (2016).

104. Greijer, A. E. & van der Wall, E. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. *J. Clin. Pathol.* **57,** 1009–1014 (2004).

105. Eales, K. L., Hollinshead, K. E. R. & Tennant, D. A. Hypoxia and metabolic adaptation of cancer

cells. *Oncogenesis* **5,** 190–198 (2016).

106. Deyoung, M. P., Horak, P., Sofer, A., Sgroi, D. & Ellisen, L. W. Hypoxia regulates TSC1/2–mTOR signaling and tumor suppression through REDD1-mediated 14–3–3 shuttling. *Genes Dev* **20,** 239–251 (2008).
107. He, G., Jiang, Y., Zhang, B. & Wu, G. The effect of HIF-1α on glucose metabolism, growth and apoptosis of pancreatic cancerous cells. *Asia Pac. J. Clin. Nutr.* **23,** 174–180 (2014).
108. Schofield, C. J. & Ratcliffe, P. J. Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell Biol.* **5,** 343–354 (2004).
109. Ziello, J. E., Jovin, I. S. & Huang, Y. Hypoxia-Inducible Factor (HIF) -1 Regulatory Pathway and its Potential for Therapeutic Intervention in Malignancy and Ischemia. *Yale J Biol Med.* **80,** 51–60 (2007).
110. Chen, J. *et al.* Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. *Am. J. Pathol.* **162,** 1283–1291 (2003).
111. Akakura, N. *et al.* Constitutive Expression of Hypoxia-inducible Factor-1␣ Renders Pancreatic Cancer Cells Resistant to Apoptosis Induced by Hypoxia and Nutrient Deprivation. *Cancer Res.* **61,** 6548–6554 (2001).
112. Golias, T. *et al.* Hypoxic repression of pyruvate dehydrogenase activity is necessary for metabolic reprogramming and growth of model tumours. *Sci. Rep.* **6,** 311–321 (2016).
113. Favaro, E. *et al.* Glucose utilization via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells. *Cell Metab.* **16,** 751–764 (2012).
114. Koukourakis, M. I. *et al.* Lactate dehydrogenase-5 (LDH-5) overexpression in non-small-cell lung cancer tissues is linked to tumour hypoxia , angiogenic factor production and poor prognosis. *Br J Cancer* **5,** 877–885 (2003).
115. Shim, H. *et al.* c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. *Proc. Natl. Acad. Sci. U. S. A.* **94,** 6658–63 (1997).
116. Gordan, J. D., Thompson, C. B. & Simon, M. C. HIF and c-Myc: Sibling Rivals for Control of Cancer Cell Metabolism and Proliferation. *Cancer Cell* **12,** 108–113 (2007).
117. Zheng, J. I. E. Energy metabolism of cancer : Glycolysis versus oxidative phosphorylation. *Oncol Lett.* **4,** 1151–1157 (2012).
118. Hryniewicz-jankowska, A. Lactate dehydrogenase 5 : An old friend and a new hope in the war

on cancer. *Cancer Lett.* **358,** 1–7 (2014).

119. Haas, M. *et al.* Prognostic value of CA 19-9, CEA, CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer: Results from a multicenter, pooled analysis of patients receiving palliative chemotherapy. *J. Cancer Res. Clin. Oncol.* **139,** 681–689 (2013).
120. Lin, G. G. & Scott, J. G. Physiological Glucose is Critical for Optimized Neuronal Viability and AMPK Responsiveness In Vitro. *J Neurosci Methods.* **100,** 130–134 (2012).
121. Yao, F., Zhao, T., Zhong, C., Zhu, J. & Zhao, H. LDHA is necessary for the tumorigenicity of esophageal squamous cell carcinoma. *Tumor Biol.* **34,** 25–31 (2013).
122. Cai, Z. *et al.* A combined proteomics and metabolomics profiling of gastric cardia cancer reveals characteristic dysregulations in glucose metabolism. *Mol. Cell. Proteomics* **9,** 2617–2628 (2010).
123. Leung, S. W. *et al.* Hypoxia Response Elements in the Aldolase A , Enolase 1 , and Lactate Dehydrogenase A Gene Promoters Contain Essential Binding Sites for. *J Biol Chem.* **271,** 2529–2537 (1997).
124. Fantin, V. R., St-pierre, J. & Leder, P. Attenuation of LDH-A expression uncovers a link between glycolysis , mitochondrial physiology , and tumor maintenance. *Cancer Cell* **9,** 425–434 (2006).
125. Yamaguchi, H. *et al.* Transforming somatic mutations of mammalian target of rapamycin kinase in human cancer. *Cancer Sci.* **106,** 1687–1692 (2015).
126. Faivre, S., Kroemer, G. & Raymond, E. Current development of mTOR inhibitors as anticancer agents. *Nature* **5,** 671–688 (2006).
127. Laplante, M. & Sabatini, D. M. Review mTOR Signaling in Growth Control and Disease. *Cell* **149,** 274–293 (2012).
128. Luo, Y. *et al.* Rapamycin inhibits mSin1 phosphorylation independently of mTORC1 and mTORC2. *Oncotarget* **6,** 4286–4298 (2015).
129. Yecies, J. L. & Manning, B. D. MTOR links oncogenic signaling to tumor cell metabolism. *J. Mol. Med.* **89,** 221–228 (2011).
130. Viollet, B. *et al.* Independent of AMPK , Inhibits mTORC1 in a Rag GTPase-Dependent Manner. *Cell Metab.* **11,** 390–401 (2009).
131. Dowling, R. J. O. *et al.* mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. *Science (80-.).* **328,** 1172–1176 (2010).
132. Oppliger, W. *et al.* Glutaminolysis Activates Rag-mTORC1 Signaling. *Mol Cell.* **136,** 349–358

(2012).

133. Boulahbel, H. & Tennant, D. A. Metabolic transformation in cancer. **30,** 1269–1280 (2009).
134. Thomas, G. V *et al.* Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat Med.* **12,** 122–127 (2006).
135. Iriana, S. *et al.* Targeting mTOR in Pancreatic Ductal Adenocarcinoma. *Front. Oncol.* **6,** 99 (2016).
136. Urayama, S., Zou, W., Brooks, K. & Tolstikov, V. Comprehensive mass spectrometry based metabolic profiling of blood plasma reveals potent discriminatory classifiers of pancreatic cancer Comprehensive mass spectrometry based metabolic profiling of blood plasma reveals potent discriminatory classifiers of. *Rapid Commun Mass Spectrom.* **24,** 613–620 (2010).
137. Lyshchik, A. *et al.* Expression of glucose transporter-1, hexokinase-II, proliferating cell nuclear antigen and survival of patients with pancreatic cancer. *Cancer Invest.* **25,** 154–162 (2007).
138. Reichert, T. E., Ph, D., Lehr, H. & Ph, D. Overexpression of Glut-1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma Overexpression of Glut-1 and Increased Glucose Metabolism in Tumors Are Associated with a Poor Prognosis. *Cancer.* **97,** 1015–1024 (2003).
139. Palmieri, D. Analyses of Resected Human Brain Metastases of Breast Cancer Reveal the Association between Up-Regulation of Hexokinase 2 and Poor Prognosis NIH Public Access. *Mol Cancer Res.* **7,** 1438–1445 (2016).
140. Mohammad, G. H., Damink, S. W. M. O. & Malago, M. Pyruvate Kinase M2 and Lactate Dehydrogenase A Are Overexpressed in Pancreatic Cancer and Correlate with Poor Outcome. *PLoS One* **11,** 1–17 (2016).
141. Ward PS, T. C. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell* **21,** 297–308 (2013).
142. Mainz, J. G., Dewhirst, M. W., Mueller-klieser, W. & Mainz, J. G. Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. *Int J Radiat Oncol Biol Phys.* **51,** 349–353. (2001).
143. Zhang, G. *et al.* Integration of metabolomics and transcriptomics revealed a fatty acid network exerting growth inhibitory effects in human pancreatic cancer. *Clin. Cancer Res.* **19,** 4983–4993 (2013).
144. Yu, L., Chen, X., Wang, L. & Chen, S. The sweet trap in tumors: aerobic glycolysis and potential

targets for therapy. *Oncotarget* **7,** 38908–38926 (2016).

145. Pelicano, H., Martin, D. S., Xu, R. & Huang, P. Glycolysis inhibition for anticancer treatment. *Oncogene* **25,** 4633–4646 (2006).
146. Raez, L.E. et al. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. Cancer Chemother Pharmacol. 71, 523-530 (2013)
147. Maftouh, M. *et al.* Synergistic interaction of novel lactate dehydrogenase inhibitors with gemcitabine against pancreatic cancer cells in hypoxia. *Br. J. Cancer* **110,** 172–82 (2014).
148. Hirschhaeuser, F., Sattler, U. G. A. & Mueller-Klieser, W. Lactate: A metabolic key player in cancer. *Cancer Res.* **71,** 6921–6925 (2011).
149. van der Mijn, J. C., Panka, D. J., Geissler, A. K., Verheul, H. M. & Mier, J. W. Novel drugs that target the metabolic reprogramming in renal cell cancer. *Cancer Metab.* **4,** 14–19 (2016).
150. Isayev, O. *et al.* Inhibition of glucose turnover by 3-bromopyruvate counteracts pancreatic cancer stem cell features and sensitizes cells to gemcitabine. *Oncotarget* **5,** 5177–5189 (2014).
151. Butler, E. B., Zhao, Y., Muñoz-Pinedo, C., Lu, J. & Tan, M. Stalling the engine of resistance: Targeting cancer metabolism to overcome therapeutic resistance. *Cancer Res.* **73,** 2709–2717 (2013).
152. Boudreau, A. *et al.* Metabolic plasticity underpins innate and acquired resistance to LDHA inhibition. *Nat. Chem. Biol.* **12,** 779–786 (2016).
153. Huang, X. *et al.* Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. *Biochem. J.* **412,** 211–221 (2008).
154. Kim, Y. D. *et al.* AMP-Activated Protein Kinase – Dependent Regulation of the Orphan Nuclear Receptor SHP. *Diabetes* **57,** 306–314 (2008).
155. Kim, J., Gao, P., Liu, Y.-C., Semenza, G. L. & Dang, C. V. Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. *Mol. Cell. Biol.* **27,** 7381–7393 (2007).
156. Sborov, D. W., Haverkos, B. M. & Harris, P. J. Investigational cancer drugs targeting cell metabolism in clinical development. *PMC* **24,** 79–94 (2015).
157. Anderson, K. M., Jajeh, J., Guinan, P. & Rubenstein, M. In vitro effects of dichloroacetate and CO2 on hypoxic HeLa cells. *Anticancer Res.* **29,** 4579–4588 (2009).

158. Stuart, S. D. *et al.* A strategically designed small molecule attacks alpha-ketoglutarate dehydrogenase in tumor cells through a redox process. *Cancer Metab.* **2,** 2–6 (2014).

159. Lee K, Khaira D, Rodriguez R, Maturo C, O'Donnell K1, S. R. Long term stable disease of stage IV pancreatic neuroendocrine tumors and without significant adverse effect by CPI-613, an investigational novel anticancer agent. *Case Study Case Rep.* **1,** 137–145 (2011).

160. Rattan, R., Ali Fehmi, R. & Munkarah, A. Metformin: An emerging new therapeutic option for targeting cancer stem cells and metastasis. *J. Oncol.* **2012,** 928–940 (2012).

161. Fontaine, E., Ave, N., Rigoulet, M. & Leverve, X. Dimethylbiguanide Inhibits Cell Respiration via an Indirect Effect Targeted on the Respiratory Chain Complex I *. **275,** 223–228 (2000).

162. Dowling, R. J. O., Goodwin, P. J. & Stambolic, V. Understanding the benefit of metformin use in cancer treatment. *BMC Med.* **9,** 33–43 (2011).

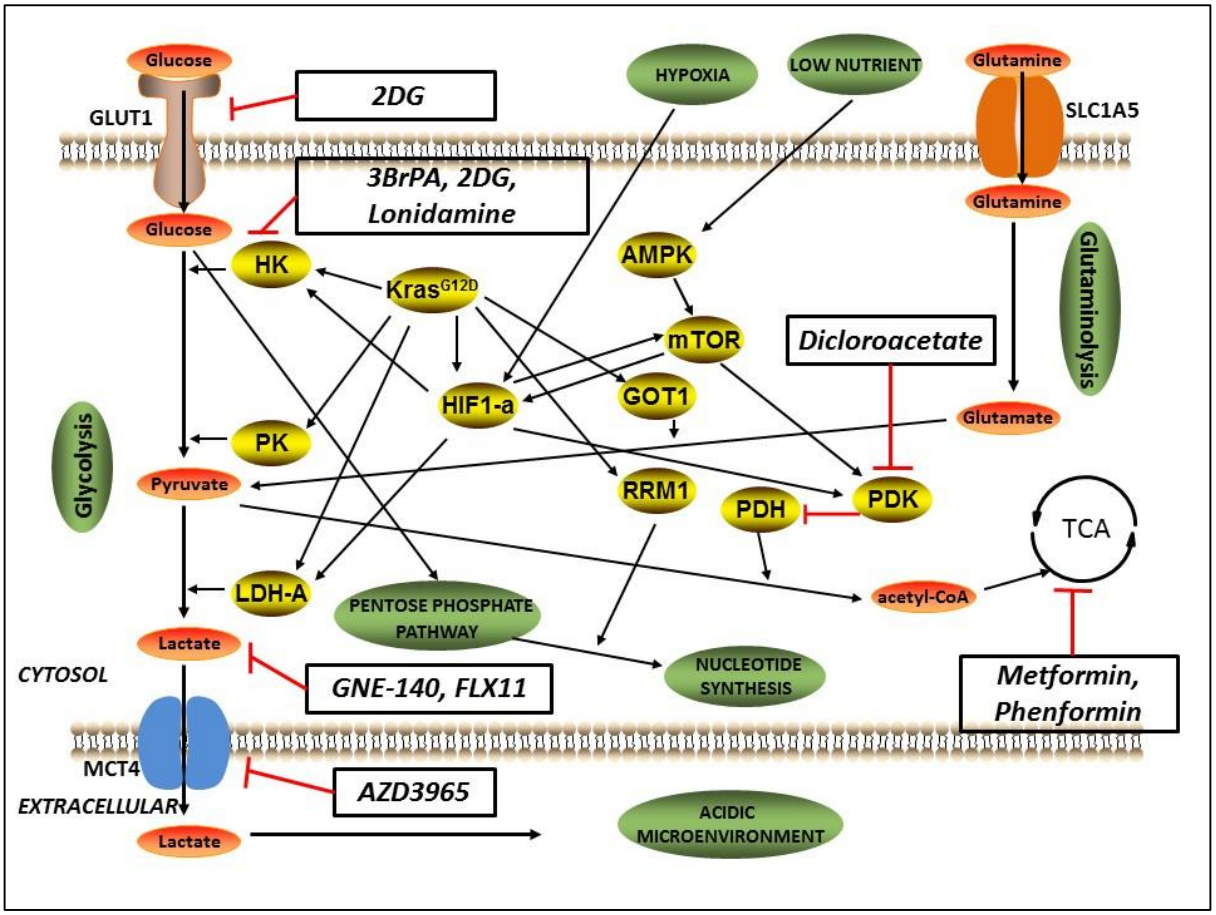
163. Rozengurt, E., Sinnett-smith, J. & Kisfalvi, K. Crosstalk between Insulin / Insulin-like Growth Factor-1 Receptors and G Protein-Coupled Receptor Signaling Systems : A Novel Target for the Antidiabetic Drug Metformin in Pancreatic Cancer. *Clin Cancer Res.* **16,** 2505–2511 (2010).

164. Lipner, M. B. *et al.* Metformin treatment does not inhibit growth of pancreatic cancer patient-derived xenografts. *PLoS One* **11,** 1–15 (2016).

165. Belli, C. *et al.* (Ir) relevance of Metformin Treatment in Patients with Metastatic Pancreatic Cancer : An Open-Label , Randomized Phase II Trial (Ir) relevance of Metformin Treatment in Patients with Metastatic Pancreatic Cancer : An Open-. *Clin Cancer Res.* **22,** 1076–1085 (2016).

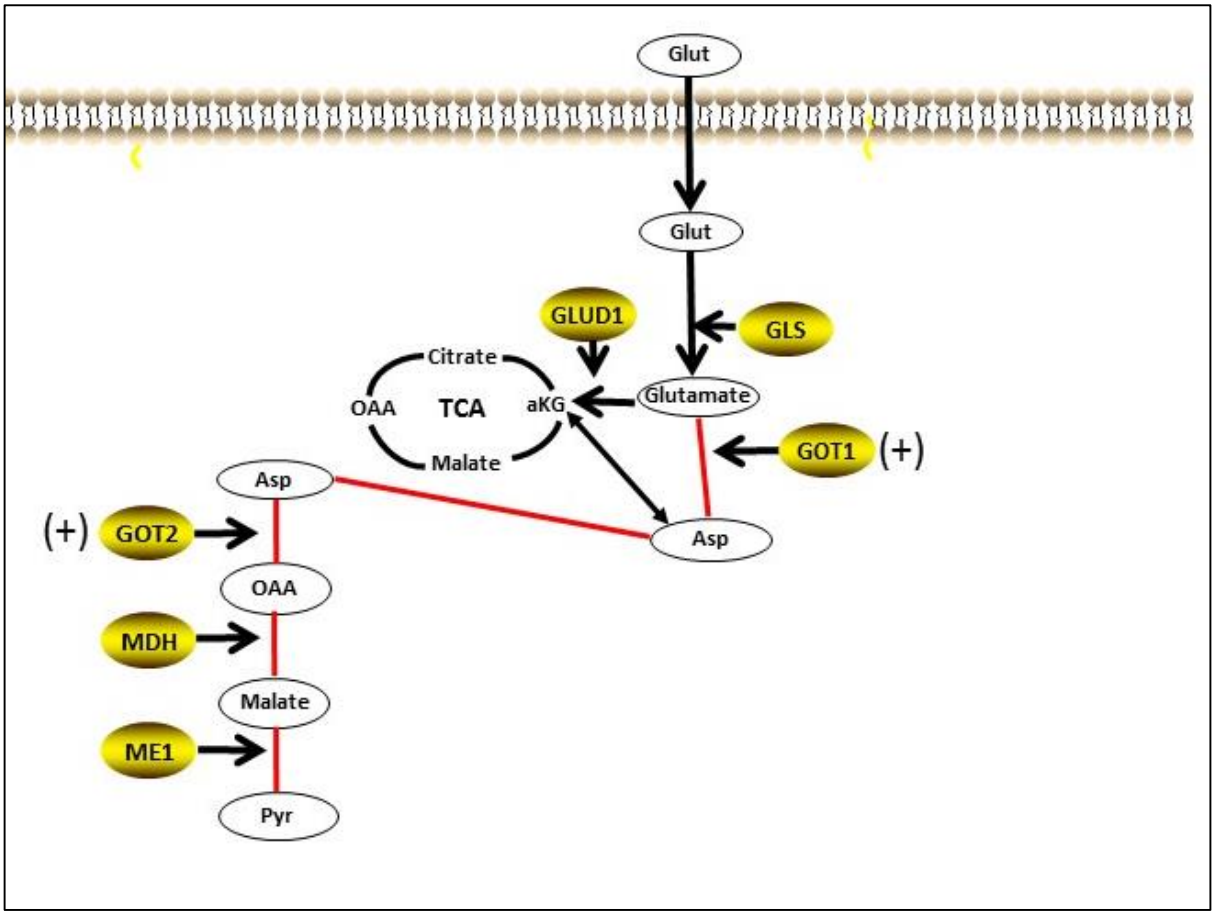
166. Sil Kordes, M. N. P. *et al.* Metformin in patients with advanced pancreatic cancer : A double-blind , randomised , placebo-controlled phase 2 trial. *Lancet Oncol.cet Oncol.* **16,** 839–847 (2015).

167. Kalluri, R. The biology and function of fibroblasts in cancer. *Nat. Rev. Cancer* **16,** 582–598 (2016).



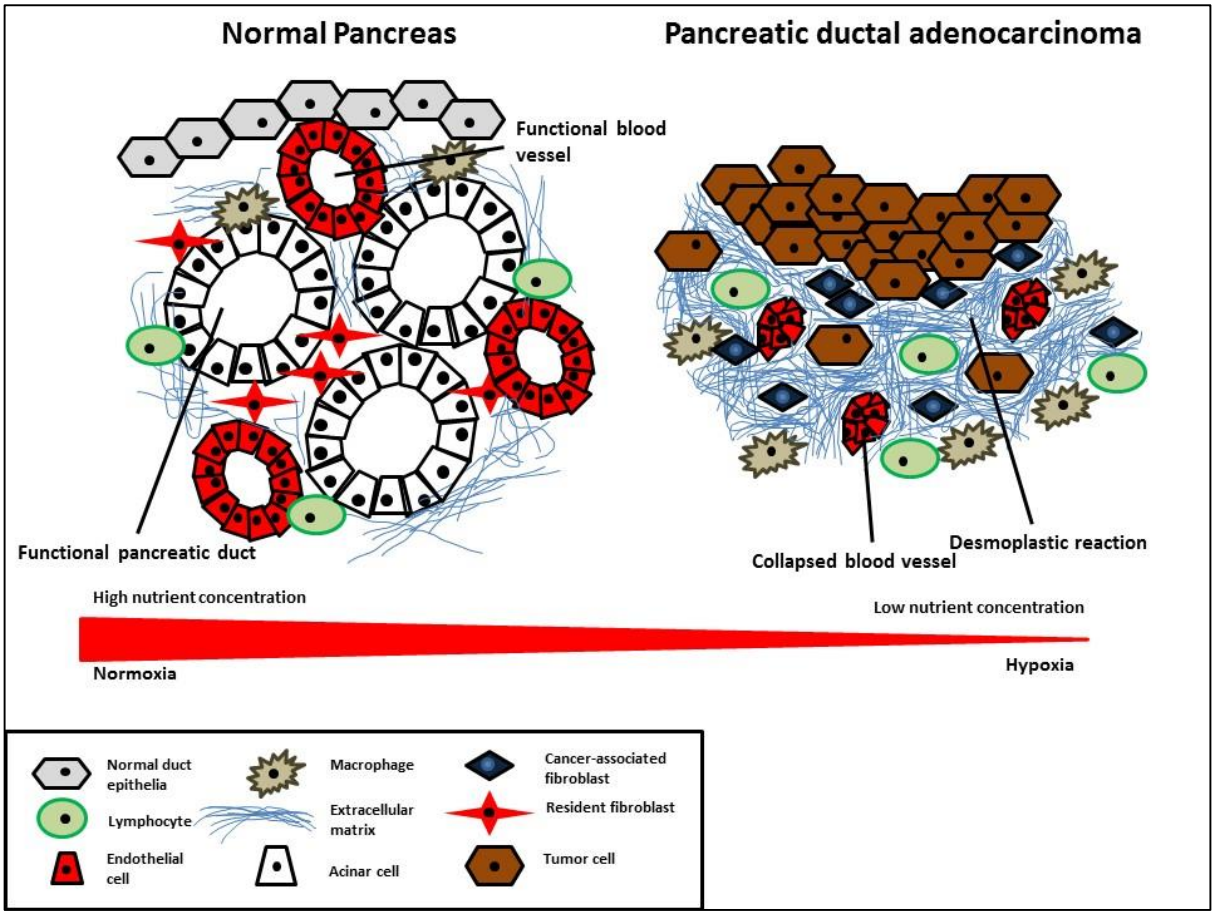
<InlineImage1>

Figure 1. Summary of pancreatic cancer metabolic reprogramming. Metabolic reprogramming is characterized by enhanced glycolysis, PPP, glutaminolysis, among others. These pathways provide cancer cells with not only essential energy but also important precursors to supply large-scale biosynthesis, rapid proliferation, growth, invasion, metastasis, and resistance to anti-cancer therapies.



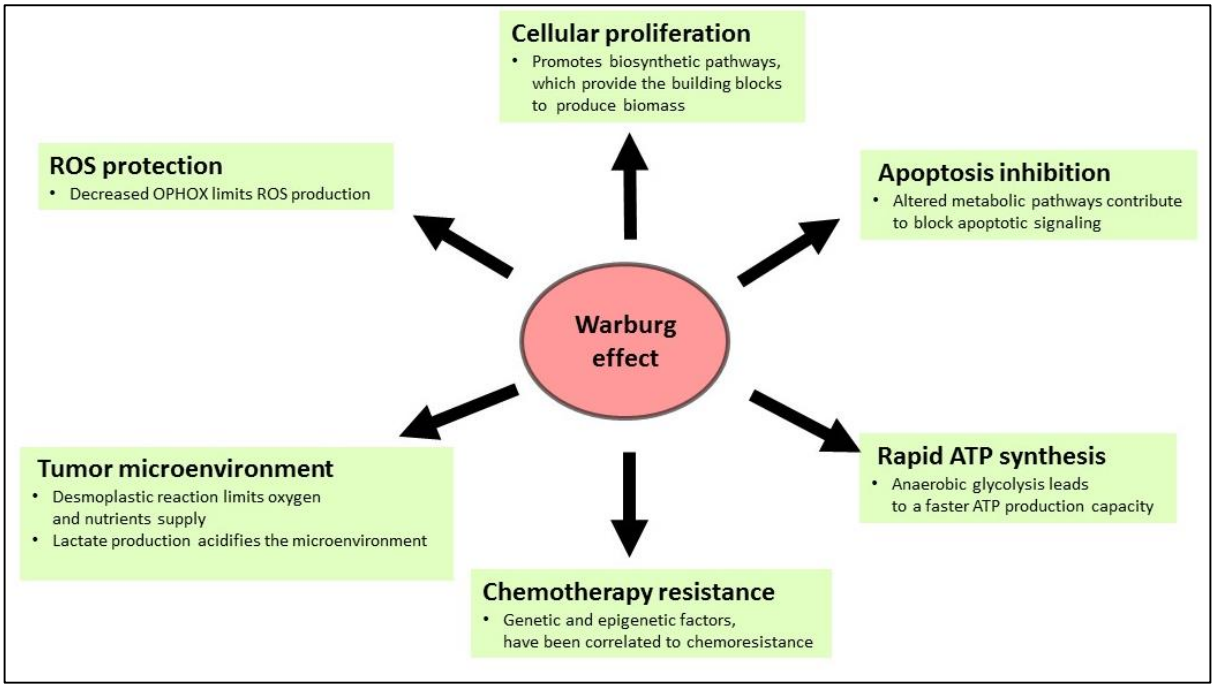
<InlineImage2>

Figure 2. KRAS enhance non-canonical pathway of glutamine metabolism in pancreatic cancer. In KRAS-mutant pancreatic cancer, mitochondrial glutamine flux is rewired to principally flux through GOT2, rather of the canonical GLUD1 pathway.



<InlineImage3>

Figure 3. Hypoxic and nutrient starvation in pancreatic tumor development. On the left, normal pancreas with functional pancreatic ducts and blood vessels. On the right, pancreatic tumor cells are surrounded by a dense stroma constituted by nerve fibers, immune cells, occluded blood vessels, cancer associated fibroblasts. Such desmoplasic reaction gradually reduces oxygen and nutrient supply to pancreatic cancer cells.



<InlineImage4>

Figure 4. Schematic representation of the processes related to Warburg effect.

Table 1: Clinical trials of inhibitors targeting metabolism in patients with pancreatic cancer.

Target	**Phase**	**Compound**	**Combined with**	**Status/Results**	**NCT identifier** [Reference]
HK	I/II	2-DG	alone or with docetaxel	Negligible effects	NCT00096707 [140]
PDH and α-KGDH	I/II	CPI-613	gemcitabine	Encouraging results	NCT00907166 [153]
GLS	I	CB-839	alone	Ongoing	NCT02071862
Mitochondrial Complex I	II	metformin	PEXG	Negative results	NCT01167738 [158]
Mitochondrial Complex I	II	metformin	gemcitabine and erlotinib	Negative results	NCT01210911 [159]