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SPECIAL REPORT

New avenues in pancreatic cancer: exploiting microRNAs as predictive biomarkers and new approaches to target aberrant metabolism

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ABSTRACT

Introduction: Most pancreatic cancer patients are diagnosed at advanced-stages and first-line regimens (FOLFIRINOX and gemcitabine/nab-paclitaxel) provide limited survival advantage and are associated with considerable toxicities. In this grim scenario, novel treatments and biomarkers are warranted.

Areas covered: MicroRNAs (miRNAs) emerged as biomarkers for cancer prognosis and chemoresistance and blood-based miRNAs are being evaluated as indicators of therapeutic activity. Moreover, aberrant metabolism, such as aerobic glycolysis, has been correlated to tumor aggressiveness and poor prognosis. Against this background, innovative approaches to tackle metabolic aberrations are being implemented and glycolytic inhibitors targeting lactate dehydrogenase-A (LDH-A) showed promising effects in preclinical models. A PubMed search was used to compile relevant publications until February 2019.

Expert opinion: Analysis of tissue/circulating miRNA might improve selection for optimal treatment regimens. For instance, miR-181a modulation seems to predict response to FOLFIRINOX. However, we need further studies to validate predictive miRNA profiles, as well as to exploit miRNAs for treatment-tailoring. Several miRNAs have also a key role in regulating metabolic aberrations. Since preliminary evidence supports the development of new agents targeting these aberrations, such as LDH-A inhibitors, the identification of biomarkers for these treatments, including the above-mentioned miRNAs, should shorten the gap between preclinical studies and personalized therapies.

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FOLFIRINOX; gemcitabine; metabolic reprogramming; microRNAs; nab-paclitaxel pancreatic cancer; Warburg effect

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is currently the seventh leading cause of cancer-related deaths worldwide and despite the continuous improvements in its detection and management, the 5-year survival rate still stands below 9% [1]. This dismal prognosis is mostly due to late diagnosis as well as poor efficacy of current treatments [2]. In fact, because of the lack of biomarkers for early detection and of the retroperitoneal location, which often determines the absence of signs or symptoms in the early stages, this tumor is generally diagnosed at advanced stages. Though for resectable or borderline resectable patients surgical resection is the primary treatment for PDAC, the diagnosis at advanced stages and the invasive nature of this tumor impede the potential curative resection, rendering chemotherapy the sole treatment option for patients with metastatic or locally advanced PDAC [3].

In the last few years, several novel chemotherapeutic regimes have been developed and some progress in term of clinical response has been made. However, the impact on patient overall survival (OS) is rather limited. Such failure is caused, at least in part, by inherent or acquired chemoresistance [4]. In addition, several new regimens cause a significant increase in hematologic and extra-hematologic toxicities compared to gemcitabine-alone. Thus, we urgently need studies to identify molecular biomarkers, such as microRNAs, that could predict response to therapy in order to maximize the efficacy of treatments and avoid useless side effects for non-responding patients.

More effective therapeutic approaches are also warranted and the renewed interest in tumor metabolism has generated hope that a new class of anti-cancer treatment strategies could target aggressive tumors such as PDAC.

In fact, most PDAC cells exhibit profound metabolic alterations [5]. Among these abnormalities, one of the most common is the Warburg effect, which consists in an increased glycolysis even in the presence of oxygen [6]. Therefore, compounds that inhibit components of the glycolytic pathway could represent an innovative and effective anticancer strategy.

In the present review, we summarize the main therapeutic options for PDAC, and then critically discuss the use of microRNAs as novel potential biomarkers to predict drug activity. Moreover, we reported new experimental compounds that target glycolytic metabolism, analyzing their potential use to improve current therapies against PDAC. To cover these issues a PubMed search was used to compile relevant publications, until February 2019.

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Article Highlights

- Pancreatic cancer has a dismal prognosis mostly due to late diagnosis and poor efficacy of current treatments: new treatments and biomarkers are warranted
- MicroRNAs (miRNAs) are emerging as predictive biomarkers of response and should be investigated to optimize new therapeutic strategies
- Innovative approaches to tackle cancer metabolic aberrations, such as glycolytic inhibitors targeting lactate dehydrogenase-A (LDH-A), showed promising effects in preclinical models
- The parallel development of new drugs targeting LDH-A and of biomarkers for these treatments, including miRNAs, should shorten the gap between preclinical studies and personalized therapies.

2. Standard treatments

2.1. Gemcitabine and gemcitabine-based combinations

Gemcitabine is a pyrimidine analogue (2′,2′-difluorodeoxycytidine, dFdC) which exerts its antiproliferative action after conversion into active triphosphorylated nucleotides, interfering with DNA synthesis and targeting ribonucleotide reductase. It is extensively prescribed to treat a variety of other solid tumors such as pancreatic, breast, ovarian, bladder or non-small-cell lung (NSCLC) cancers [7]. Until a few years ago, gemcitabine monotherapy has been used as the first-line treatment for metastatic PDAC, since it provided an increased clinical benefit compared to 5-fluorouracil (5-FU). However, the median OS observed in metastatic PDAC patients administrated with gemcitabine monotherapy was only 5.65 months, with a very low response rate (i.e. 5.4%) [8]. For this reason, several studies were performed to improve patients' prognosis, testing the combination of gemcitabine with other treatment modalities.

Unfortunately, the combination of gemcitabine with capecitabine or cisplatin showed only a marginal improvement in term of OS, and no statistically significant differences were observed when comparing different combinations of chemotherapeutic agents to gemcitabine monotherapy [9,10].

Multiple clinical trials were also conducted to test the combination of gemcitabine with biologically targeted agents, including epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, such as erlotinib. The combination of gemcitabine with erlotinib resulted in a slight, though significant, improvement in terms of survival benefit, but it was not considered clinically relevant [11]. This might be explained by the fact that EGFR mutations, that are used to guide the treatment of NSCLC with EGFR-tyrosine kinase inhibitors [12], are extremely rare in PDAC samples [13]. Skin rash was initially proposed as a surrogate marker of efficacy, but it failed to identify patients with clinical benefit, as reported in a randomized phase II dose escalation trial [14].

The first study of a gemcitabine-combined regimen which showed clinically relevant results was the phase III trial IMPACT, evaluating the combination of nab-paclitaxel (Abraxane®) with gemcitabine as the first-line option for patients with advanced or metastatic PDAC [15]. Nab-paclitaxel is a nanoparticle albumin-bound paclitaxel and represents the first nanotechnology-based drug in cancer treatment [16]. Given the reduced diameter of these particles, nab-paclitaxel has a greater distribution volume and a faster clearance than conventional paclitaxel [17]. Moreover, since albumin-paclitaxel complexes have sizes virtually identical to the endogenous albumin molecules in blood, they are fully capable of utilizing the physiological albumin pathways. Indeed, nab-paclitaxel uses transcytosis mediated by albumin, enhancing intracellular paclitaxel delivery, and should take advantage of the overexpression of albumin-binding proteins such as Secreted Protein Acid and Rich Cysteine (SPARC) in stroma fibroblasts surrounding the tumor tissue. This should increase the selective uptake of this drug in tumor cells [17,18]. SPARC has indeed been evaluated as a biomarker of the activity for gemcitabine/nab-paclitaxel regimens because 1) it should enhance the selective uptake of this drug in tumor cells and 2) SPARC protein expression has been correlated to cancer cell proliferation and metastatic features [19]. However, *in vivo* experimental studies in both patient-derived xenografts and genetically engineered mouse models showed that SPARC did not play a role in nab-paclitaxel internalization. In addition, despite initial promising data, immunohistochemical analyses in PDAC specimens from patients treated with gemcitabine/nab-paclitaxel showed that SPARC levels were not associated with clinical outcome [20].

Similarly, we do not have biomarkers which can predict the toxicity of the gemcitabine/nab-paclitaxel regimen, and a recent multicentre retrospective observational study in the South Eastern Region of Sweden showed that neutropenia, leukopenia, thrombocytopenia, and anemia were observed in 23%, 20%, 5%, and 4% of patients treated with this regimen, respectively [21].

2.2. Folfirinox

FOLFIRINOX is a therapeutic regimen based on a combination of a number of chemotherapeutic drugs including 5-FU, irinotecan, leucovorin, and oxaliplatin. Following the positive results of the phase III clinical trial PRODIGE-4/ACCORD-11, this regimen has been introduced as a standard treatment in metastatic PDAC [22]. In an initial phase II trial, FOLFIRINOX was tested on 46 patients with advanced PDAC, showing a response rate of 26% (including 4% complete response), a median time to progression of 8.2 months and a median OS of 10.2 months [23]. Therefore, considering also the good safety profile, and improved in quality of life (QOL), FOLFIRINOX was further assessed in the above-mentioned phase III trial. In particular, since oncologists were lacking data on the efficacy and safety of FOLFIRINOX as compared with gemcitabine, the randomized controlled trial enrolled 342 patients with histologically and/or cytologically confirmed metastatic pancreatic adenocarcinoma and who had not previously been treated with chemotherapy. The median overall survival was 11.1 months in the FOLFIRINOX group compared to 6.8 months in the gemcitabine group ($P < 0.001$), while the median progression-free survival (PFS) was 6.4 months in the FOLFIRINOX and 3.3 months in the gemcitabine group ($P < 0.001$). Moreover, FOLFIRINOX regimen was associated with an objective response rate of 31.6%. Unfortunately, this regimen has also shown a severe grade 3–4 toxicity profile with 45.7% of neutropenia, 5.4% of febrile neutropenia, 9.1% of

thrombocytopenia, 14.5% of vomiting and 12.7% of diarrhea. In conclusion, as compared with gemcitabine, FOLFIRINOX was associated with a survival advantage but it had also increased toxicity [22].

In this regard, many efforts have been made in order to reduce the toxic effects. A prospective phase II open-label study evaluated a modified version of FOLFIRINOX (mFOLFIRINOX) which consisted in 25% dose reductions of irinotecan and bolus 5-FU given every 2 weeks (until progression, unacceptable toxicity, or surgical resection) [24]. mFOLFIRINOX given along with prophylactic pegfilgrastim was associated with a similar response rate and improved tolerability compared to full dose FOLFIRINOX [24].

Since the bolus 5-FU contributes to the toxicity, Mahaseth and colleagues proposed a modified FOLFIRINOX regimen which included discontinuation of the bolus 5-FU and administration of growth factors to all patients. Therefore, on day-3 after chemotherapy, prophylactic pegfilgrastim (6 mg) was administered subcutaneously to each patient. This modified FOLFIRINOX regimen showed a decreased incidence of grade 3–4 neutropenia to 3%, with a satisfactory response rate (30%), showing an improved safety profile with maintained efficacy in metastatic PDAC [25]. Similar results were obtained in patients treated with FOLFIRINOX at 80% dose intensity with routine use of growth factor support [26].

A phase III randomized trial of The Gruppo Oncologico Nord Ovest (GONO) further demonstrated the efficacy of the simplified FOLFOXIRI regimen in metastatic colorectal cancer. This modified regimen included a higher dose of 5-FU continuous infusion and a slightly lower dose of irinotecan [27]. More recently, Vivaldi and colleagues used the GONO-FOLFOXIRI regimen (irinotecan 165 mg/m^2 over 1 h, followed by oxaliplatin 85 mg/m^2 and leucovorin 200 mg/m^2 concomitantly over 2 h through a Y-connector, on Day-1, followed by fluorouracil 3,200 mg/m^2 as a 48-h continuous infusion starting on Day-1) and a modified schedule (irinotecan 150 mg/m^2 over 1 h, followed by oxaliplatin 85 mg/m^2 and leucovorin 200 mg/m^2 concomitantly over 2 h through a Y-connector, on Day-1, and followed by 5-FU 2,800 mg/m^2 as a 48-h continuous infusion starting on Day-1) in 137 stage III/IV PDAC patients. One (0.6%) complete response and 52 (38%) partial responses were observed in the whole population, with a disease control rate of 72.2%. The median OS and median PFS were 12 and 8 months, respectively. Regarding the toxicity profile, the main hematologic grade 3–4 toxicity was neutropenia (35.7%), but only one patient (0.7%) experienced febrile neutropenia. The main G3-4 non-hematological adverse events included G3 diarrhea in 11 (8%), nausea in 10 (7.3%), stomatitis in 9 (6.5%) and liver toxicity in 6 (4.4%) patients [28].

In conclusion, FOLFIRINOX introduction is arguably one of the most important innovations in PDAC care since the introduction of gemcitabine in 1996. This regimen has indeed been shown to dramatically improve OS, but this comes at the price of significantly increased toxicity (neutropenia, febrile neutropenia, thrombocytopenia, diarrhea, neuropathy), despite careful patient selection and the generous use of growth factor support. Moreover, clinical outcome (in terms of both efficacy and toxicity) in individual patients is relatively unpredictable, even when current clinical selection criteria (young age, good PS, absence of a biliary stent) are employed. Here we propose to describe and analyze new tools to improve the current usage of FOLFIRINOX as well as of gemcitabine/nab-paclitaxel, exploring new potential pharmacogenetic markers and treatments.

3. New tools to overcome PDAC resistance

3.1. Personalizing treatments using microRNA as predictive biomarkers of response

The prognosis of patients with PDAC is very poor because of the inherent and/or acquired resistance to conventional treatment modalities, which are also causing severe toxicities, as described above for the FOLFIRINOX and gemcitabine/nab-paclitaxel regimens [29]. Despite the constant efforts to formulate new chemotherapy regimens, new strategies to target these treatments are urgently warranted in order to achieve significant clinical improvement [30,31].

In this regard, the discovery of appropriate markers could help to determine which tumors will respond to which treatments, predicting the likelihood of drug resistance and leading treatment decisions.

In the last few years, microRNAs (miRNAs) have emerged as predictive biomarkers of response to conventional anti-cancer treatments and miRNAs-based strategies could represent a promising method to select the most appropriate pharmacological agent for personalization of the treatment [32].

Chemoresistance in PDAC is mediated by both genetic or epigenetic alterations and miRNAs play a key role in the epigenetic control. Indeed, miRNAs quickly respond to the genotoxic stress environment caused by chemotherapy and quickly modulate mRNA translation in cancer cells [4]. Most importantly, each miRNA controls the expression of multiple gene transcripts offering the possibility to identify critical miRNAs that could be used as informative biomarkers for detection, diagnosis, and prognosis of tumors that result from the deregulation of multiple genes. For this reason, a number of miRNA profiling studies have been conducted to obtain diagnostic and prognostic signatures for a variety of tumor type [33]. Therefore, several miRNAs playing a crucial role in the regulation of gene expression offer new directions for the quest of cancer biomarkers [34]

In the last few years, miRNAs expression in PDAC tissues has been largely studied and the PDAC miRNome has been extensively profiled [35]. However, only a few studies evaluated the role of candidate miRNAs to predict the sensitivity/resistance to conventional chemotherapy.

Several studies have shown the role of miR-21 expression levels in predicting which patients achieve the optimal response. For instance, Hwang and colleagues evaluated miR-21 expression levels in two cohorts of PDAC patients treated with gem and 5-FU and their results suggested that miR-21 expression can affect the outcome of both gemcitabine and 5-FU-based treatment [36]. The association of miR-21 expression levels and chemoresistance to gemcitabine was also observed in a study conducted on laser-microdissected specimens and PDAC cells. In particular, tissues isolated by laser microdissection were collected from 81 patients with

metastatic (n = 31) or nonmetastatic (n = 50) PDAC and normal ductal samples. The results of this study demonstrated a correlation between high expression of miR-21 and worse outcome after gemcitabine treatment. In particular, the authors reported a significant association between miR-21 expression and both disease-free survival and OS. Patients with high miR-21 expression had a significantly shorter OS both in the metastatic and in the adjuvant setting. Moreover, miR-21 expression in primary cultures correlated with expression in their respective tissues and with gemcitabine resistance. Further analyses on PDAC cells delineated the mechanism of action by which miR-21 induces gemcitabine resistance. It has been indeed observed that miR-21-downregulated PTEN and was associated with the activation of PI3K/Akt/mTOR pathway, reducing apoptosis induction by gemcitabine [37].

More recently, Wei and colleagues have proposed a similar mechanism underlying the role of miR-21 in resistance to 5-FU in human PDAC cells through the downregulation of tumor suppressor genes, including PTEN and PDCD4 [38]. Their findings confirmed high expression levels of miR-21 in resistant primary cells in comparison to sensitive primary cells and demonstrated that the suppression of miR-21 expression sensitized cancer cells to 5-FU treatment. On the contrary, the overexpression of mir-21 conferred resistance to 5-FU and promoted proliferation, migration, and invasion of PATU8988 and PANC-1 cells.

However, a phase III randomized trial evaluated the expression of miR-21 in tumor cells or cancer-associated fibroblasts (CAFs) in a cohort of 229 PDAC patients treated with 5-FU o gemcitabine. The expression levels of miR-21 were assessed by in situ hybridization, showing that miR-21 expression in CAFs was associated with decreased OS in PDAC patients who received 5-FU, but not gemcitabine. Conversely, strong expression of miR-21 in tumor cells was not correlated with survival in gemcitabine or 5-FU treated patients [39].

MiR-21 is not the only miRNA that seems to play a crucial role in chemoresistance to cytotoxic agents. In fact, Donahue and colleagues have revealed that miR-142-5p would be a promising predictive marker for gemcitabine treatment in patients with resected PDAC. First, they identified 24 miRNAs candidates that were up- or down-regulated in gemcitabine resistant cells. The analysis of miRNA expression, in relation to the survival time of PDAC patients after curative resection, revealed that miR-142-5p expression correlated with survival in patients treated with gemcitabine after surgical resection of PDAC, but not in patients without gemcitabine treatment. These findings could aid to select the appropriate and efficient treatment of patients after resection of PDAC, improving their prognosis [40].

More recently, *in vitro* and *in vivo* analyses have revealed a correlation between miR-506 expression levels and chemosensitivity to gemcitabine in PDAC cells. This study has highlighted that, after the transfection with miR-506 mimics, the overexpression of this miRNA enhanced the chemosensitivity of the cells to gemcitabine, whereas miR-506 inhibitors significantly conferred chemoresistance to PDAC cells. Moreover, sequencing analysis have revealed that the hypermethylation of the promoter region of the miR-506 gene reduced the expression levels of this miRNA in PDAC and significantly associated with poor prognosis. Despite these promising findings, further examination are needed to confirm the possibility of using miR-506 as a predictive biomarker of response to gemcitabine [41].

MiR-509-5p and miR-1243 have also been proposed as potential biomarkers in PDAC. A recent study showed that the overexpression of miR-509-5p and miR-1243 increased the sensitivity of PDAC cells to gemcitabine, suggesting that the expression status of these two miRNAs might predict gemcitabine efficacy in patients with PDAC [42].

Moreover, Preis and colleagues proposed miR-10b as an important tool for clinicians to guide clinical decision-making about neoadjuvant treatment and surgery. The authors of this study suggested, for the first time, that miR-10b expression in samples from fine needle aspirates (FNA) can be used to delineate a subgroup of patients that will truly benefit from subsequent surgery. In fact, patients whose cancers express low levels of miR-10b are predicted to have greater than 50% survival rate after 2 years. In contrast, high levels of miR-10b predict poor outcome and early disease progression even after surgical resection. Moreover, miR-10b expression levels in FNA samples correlated with the response to multimodality neoadjuvant gemcitabine-based chemoradiotherapy, in patients with resectable or locally advanced disease [43].

Of note, it has been demonstrated that also miR-211 expression modulates chemoresistance to gemcitabine. This miRNA emerged from a microarray analyses in long versus short-survival patients. *In vitro* studies showed that the overexpression of miR-211, by transfection of PDAC cells with pre-miR-211, led to a significant reduction of the percentages of cell growth and an increased sensitivity to gemcitabine. Instead, transfection with anti-miR-211 resulted in the opposite effect. Most likely, miR-211 modulates sensitivity to gemcitabine through the direct control of RRM2 expression. Indeed, the overexpression of miR-211 leads to a reduction of RMM2 expression levels, which represents a target of gemcitabine activity [44]. These results were further confirmed in a more recent study, where Maftouh and colleagues explored the biological role of miRNA-211 in gemcitabine activity in two subclones of SUIT2 cell line (SUIT2-028 and SUIT2-007). Their results revealed that the less aggressive subclone SUIT2-028, which was more sensitive to gemcitabine than the more aggressive subclone SUIT2-007, reported higher miR-211 expression levels [45].

MiR-200b, miR-200c and many members of the tumor suppressor let-7 family have been also suggested as a potential biomarker of gemcitabine resistance. Indeed, it has been found that their expression was significantly down-regulated in gemcitabine-resistant cells [46].

Other possible miRNAs that could represent good candidates as predictive biomarkers of response to chemotherapy are miR-192 and miR-215. Indeed, their overexpression results in reduced levels of thymidylate synthase which is the main drug target of the fluoropyrimidine/(5-FU)-based therapy [47].

Not many data are available on miRNAs affecting nab-paclitaxel, but several miRNAs have been associatedwith resistance to paclitaxel. For instance, it has been demonstrated that the ectopic expression of miR-200c downregulated

TUBB3 and enhanced sensitivity to microtubule-targeting agents, including paclitaxel [48]. Furthermore, miR-17-5p has been identified as one of most significantly downregulated miRNAs in paclitaxel-resistant lung cancer cells and it could affect the sensitivity to paclitaxel through the regulation of beclin1, which is one of the most important autophagy modulators [49]. On the contrary, it has been observed that miR-17-5p is upregulated in PDAC where it is associated with a poor prognosis and plays important roles in pancreatic carcinogenesis and progression [50]. However, further studies are needed to better evaluate the role of this miRNA as a predictive biomarker in PDAC.

A recent study evaluated the use of circulating miRNAs to predict and/or monitor patients with advanced PDAC treated with FOLFIRINOX. Results obtained showed that a reduction of miR-181a-5p expression levels in plasma is able to monitor response to FOLFIRINOX chemotherapy in patients with advanced PDAC. Conversely the same miRNA did not predict the outcome of patients treated with gemcitabine/nab-paclitaxel. *In vitro* analysis confirmed these findings and allowed to study the potential mechanism exploited by miR-181a-5b to affects FOLFIRINOX sensitivity. Interestingly, the inhibition of miR-181a-5p led to increased expression of tumor-suppressor protein ATM, enhancing sensitivity to oxaliplatin. However, the exact mechanisms of miR-181a-5p in chemosensitivity to FOLFIRINOX therapy remain to be investigated and the clinical utility of miR-181a-5p as a predictive biomarker should be further validated in prospective, large-scale clinical studies [51].

3.2. Targeting cancer cell glycolytic metabolism

3.2.1. Metabolic reprogramming in primary tumor and cancer metastasis

Pioneer Otto Warburg first revealed that metabolic differences exist between malignant tumor cells and adjacent normal cells. The neoplastic diseases are indeed characterized by a chronic and often uncontrolled cell proliferation which results in corresponding adjustments of energy metabolism in order to fuel cell growth and division. Despite the presence of oxygen, cancer cells switch from oxidative phosphorylation (OXPHOS) to the aerobic glycolysis, resulting in high rate glycolysis followed by lactic acid fermentation. Therefore, cancer cells tend to promote glycolysis over mitochondrial respiration, even under aerobic conditions. In tribute to Otto Warburg, this metabolic alteration is known as 'Warburg effect' [52,53].

Though aerobic glycolysis is an inefficient way to generate energy, cancer cells use this peculiar metabolic adaptation to increase the uptake and the incorporation of nutrients (such as nucleotides, amino acids, and lipids) into the biomass, conferring to cancer cell the advantage to obtain sooner all the elements they need to produce new cells [54].

Of note, the Warburg effect is a metabolic phenomenon involving the primary tumor but once cancer cell begin to spread from the original tumor to other organs or tissue of the body, their energy requirements change [55]. Emerging data suggest that metabolic flexibility is required for the success of the metastatic dissemination and is critical for efficient colonization of distant sites. Cancer cells differentially engage distinct metabolic strategies depending on their metastatic site [56]. For instance, breast cancer cells enhance their metastatic fitness to the liver by engaging a dominant metabolic phenotype. Instead, lung and bone metastasis are more dependent on OXPHOS [57].

3.2.2. Anti-cancer agents targeting the Warburg effect

Anti-cancer agents targeting the Warburg Effect include small inhibitory molecules that target glycolysis through the inhibition of glucose uptake (mainly mediated by Glucose transporter 1 (GLUT-1)), glucose retention (due to the reaction catalyzed by hexokinase (HK)) and lactate production (through the inhibition of LDH-A) (Figure 1). Glycolysis initiation requires glucose uptake that is mediated by the glucose transporters GLUTs [58], and overexpression of GLUT-1 has been found in various tumor types. This evidence can be explained by the high glucose consumption rate of cancer cell, which can be compensated by increased glucose influx through the overexpression of this transporter [59].

Of note, Shibuya and colleagues supposed that the increased glycolytic metabolism, characteristically seen in human cancers, has a pivotal role in the maintenance of cancer stem cells (CSCs) in several human cancer types. *In vitro* and *in vivo* analysis demonstrated that WZB117, a specific GLUT-1 inhibitor, could inhibit the self-renewal and tumor-initiating capacity of the human CSCs from different cancer types, including PDAC. Therefore, glucose metabolism-targeted therapy through GLUT-1 inhibition, in combination with other therapeutic modalities that effectively control the tumor bulk, is expected to achieve better management of cancers through a CSC-directed cancer therapy [60]. Moreover, a recent study conducted on primary PDAC resistant cells demonstrated that the novel GLUT-1 inhibitor PGL13 supports the synergistic interaction between the Akt inhibitor perifosine and gemcitabine, restoring the repression of aerobic glycolysis induced by Akt inhibitors [61].

Once glucose is inside the cell, the first critical and irreversible step of glycolysis is performed by HK, which coverts glucose to glucose-6-phosphate to ensure that glucose will not diffuse back out of the cells [62,63]. For this reason, HK is considered another promising potential target for antiglycolytic therapies. As regard to the use of HK inhibitors in PDAC, Bhardway and colleagues demonstrated that 3-bromopyruvate (3BP), an inhibitor of HK-II, used in combination with IAA, an inhibitor of GAPDHase, effectively inhibited both energy production and cell signaling in the PDAC cell line PANC-1 [64].

Moreover, a recent study performed on PDAC cell lines, showed that human *in vitro* derived tumor-associated macrophages (TAMs) utilize Warburg metabolism to promote tumor growth and dissemination. Indeed, this alteration in macrophage metabolism supported angiogenesis, and augmented the extravasation of tumor cells in a VEGF-dependent manner. Furthermore, it induced epithelial-to-mesenchymal transition (EMT) in a TGFβ-dependent manner, facilitating tumor dissemination to secondary sites (such as the lung and liver). Instead, the inhibition of glycolysis in TAMs, with a competitive inhibitor to HK2, 2-deoxyglucose (2DG), was

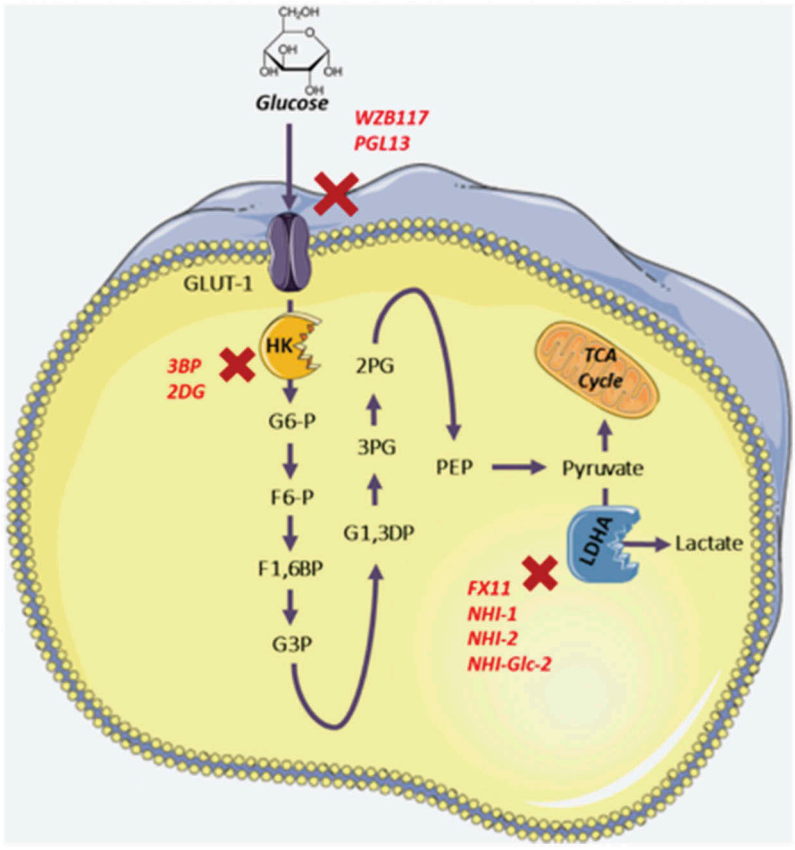

Figure 1. Scheme of selected components of the glycolysis pathway and inhibitors by small molecules targeting glucose transporter 1 (GLUT-1), hexokinase (HK) and lactate dehydrogenase (LDH-A) that have been studied or are undergoing evaluation as new anticancer treatments.

sufficient to disrupt this pro-metastatic phenotype, reversing the observed increases in TAM-supported angiogenesis, extravasation, and EMT [65]. In a phase I dose-escalation trial, the combination of 2-DG and docetaxel was tested in 34 patients with metastatic or advanced solid tumors. The results obtained prompt that the recommended dose of 2DG in combination with weekly docetaxel is 63 mg/kg/day with tolerable adverse effects [66], and further studies in PDAC patients seem warranted.

In the last step of glycolysis, pyruvate and ATP are formed. Then, pyruvate is taken up into mitochondria, converted into acetyl coenzyme A (Acetyl-CoA) which enters the tricarboxylic acid (TCA) cycle. But pyruvate can also be reduced to lactate by LDH-A, which has been proven to be overexpressed in many types of solid cancer, including PDAC [67]. FX11, a small-molecule inhibitor of LDH-A, negatively affects cellular energy supply, decreases cellular production of lactate, induces oxidative stress and finally provokes cell death. Of note, FX11 efficiently reduced cancer cell growth in PDAC cell and xenograft models [68,69]. This drug has been also tested in a more aggressive human pancreatic tumor xenograft LZ10.7, where it has been proven to be effective even as a single agent [68]. Unfortunately, to date, no clinical trial has been conducted to evaluate the clinical activity of FX-11.

A more recently developed class of effective inhibitors of LDH-A include N-hydroxyindole-based compounds [70]. Maftouh and colleagues demonstrated the synergistic interaction between gemcitabine and two derivatives of these compounds (NHI-1 and NHI-2). Indeed, they revealed that in hypoxic models of PDAC, these two compounds affected apoptosis, spheroid-growth, and invasiveness, reducing the expression of metalloproteinases. The synergistic interaction with gemcitabine was attributed to modulation of gemcitabine metabolism, overcoming the reduced synthesis of phosphorylated metabolites in hypoxia [71].

The glucose-conjugated methyl ester NHI-Glc-2 is a weaker LDH-A inhibitor than NHI-2 on the isolate enzyme. But anyway, it exploits the GLUT-1 overexpression, leading to an increased uptake [72]. The combination of NHI-Glc-2 with deoxynyboquinone (DNQ) has been already tested in cancer cells and in a mouse model of lung cancer [73]. Moreover, preliminary *in vitro* and *in vivo* studies showed a potential synergistic interaction between NHI-Glc-2 and gemcitabine in PDAC models [74].

Oncogenic *KRAS* mutations play a pivotal role in the initiation and progression of pancreatic adenocarcinoma. These mutations prevent GAP stimulation and lead to an accumulation of persistently GTP-bound and active K-Ras. In its activated form, K-Ras can trigger multiple signaling pathways, such as MAPK and P13, impacting a wide range of cellular processes, including cell survival and proliferation [75]. To date, mutant K-Ras remains difficult to target and some progress has been made only regarding the selective inhibition of G12C mutant *KRAS* isoform with a small molecule, which is more common in lung cancer compared to PDAC [76,77].

However, several recent studies have reported that *KRAS* plays a critical role in controlling cancer metabolism through

the stimulation of glucose metabolism, differential channeling of glucose intermediates, reprogrammed glutamine metabolism, increased autophagy, and micropinocytosis [78–81]. These findings could provide a novel and appealing alternative opportunity to targeting such a key determinant in PDAC. In fact, specific compounds designed to target the effector pathways of KRAS, can both alter PDAC cells metabolism and impair the ability of the cancer cells to proliferate.

Notably, Ying and colleagues, using inducible *KRAS*-G12D-driven PDAC mouse model, have already established that mutated *KRAS* enhances the expression of GLUT1 and several rate-limiting glycolytic enzymes, including HK and LDH-A [82]. Moreover, preclinical studies conducted on HK2-knockout models have shown that the inhibition of this glycolytic enzyme may provide therapeutic benefit to mutant K-Ras lung tumors [83]. Similarly, Xie and colleagues have reported that inactivation of LDH-A, in mouse models of NSCLC driven by oncogenic *KRAS* or *EGFR*, leads to decreased tumourigenesis and disease regression in established tumors [84].

In conclusion, metabolic targeting strategies that have been preclinically validated in lung cancer models could also represent a valid method to effectively target K-Ras in other tumor types. In particular, further studies on the use of anti-cancer agents targeting the Warburg effect in pancreatic tumors driven by *KRAS* are warranted.

4. Conclusions

In the last few years, several progresses have been made in chemotherapeutic treatment of PDAC, but current available treatments provide a limited survival advantage and are associated with severe toxicities. During the last decade, the growing interest in glycolytic metabolism of cancer cells has generated hope that a new class of effective anti-cancer treatment strategies could finally be developed. In fact, over the years, numerous types of small molecules have been tested for their hypothesized ability to hampers glucose uptake and glycolysis, representing a promising tool to target the Warburg effect in cancer cells. Unfortunately, only a small portion of these small molecules has been tested in clinical trials and novel preclinical and clinical development strategies are warranted.

Personalized medicine could overcome, at least in part, the current limits in chemotherapeutic treatment of PDAC, having the potential to tailor therapy with the best response and highest safety margin to ensure better patient care. In this regard, miRNAs have been increasingly recognized as promising predictive biomarkers of response to chemotherapeutic agents. In fact, according to a number of studies, miR-21, miR-181a and other miRNAs seems to predict response to specific treatments in PDAC cells and/or patients. However, further studies are needed to confirm their possible use in clinical practice as well as to find new candidate miRNAs to predict response to new therapeutic strategies.

5. Expert opinion

Alterations of miRNAs expression have an impact on patient-specific pharmacokinetics and pharmacodynamics of cancer drugs, rendering them closely related with resistance or sensitization to specific cytotoxic drugs. For this reason, analysis of miRNAs expression could represent a valuable tool to predict the response to currently available treatments and to help tailor treatment appropriate for individual patients [85].

MiRNA expression can be evaluated in tissues specimens as well as in a tissue biopsy of the primary tumor. In fact, in clinical practice, the source of biological material typically comes from formalin-fixed paraffin-embedded tumor samples obtained during standard of care surgical procedures or biopsies. Unfortunately, the tissue extraction is risky and painful for the patient and in some cases the sample is limited because of 'inaccessible' tumor localization. Moreover, the procedure to obtain a tissue biopsy is expensive, and cannot be applied repeatedly [86]. In addition, because of intratumour genetic heterogeneity, the molecular profile of the primary tumor from the initial surgical specimen might significantly differ from the molecular profile in a tumor sample obtained from a biopsy and might not reflect molecular aberrations accumulated as a consequence of selection pressure caused by cancer therapies [87]. Importantly, liquid biopsies overcome most of the limitations of tissue biopsies as they represent a noninvasive, rapid, precise method, which can also bypass the heterogeneity of tumors. Therefore, analysis of miRNAs expression in liquid biopsies, such as in serum or plasma or other body fluids, could represent a more effective way to predict the response to cancer drugs and hopefully would replace tissue biopsy in the near future [88].

In this review, we provided an overview of miRNAs that might predict response to standard treatments in PDAC. However, we also underlined the importance of new treatments, focussing on therapies targeting aberrant cancer glycolytic metabolism. These two topics should be further studied. To date, only a few studies evaluated miRNAs that could predict response to drugs targeting cancer cell glycolytic metabolism.

In particular, further studies should be conducted on miRNAs that, through their altered expression, might affect the expression of GLUT-1, HK2 or LDH-A (Figure 2). For instance, it has been demonstrated that miR-138, miR-150, miR-199a-3p and miR-532-5p downregulate GLUT-1 expression, whereas miR-19a, miR-19b, miR-130b, and miR-301a increase GLUT-1 expression in renal cell carcinoma. In addition, miR-144, which is downregulated in PDAC tissues and PANC-1 cells [89], was found to target GLUT-1 in lung cancer [90]. Therefore, the altered expression of these microRNAs, through the regulation of GLUT-1 expression, could modulate the chemoresistance toward GLUT-1 inhibitors such as WZB117 and PGL13.

In breast cancer cells miR-143 negatively regulate HK2 expression, whereas miR-155 indirectly promote HK2 transcription by repressing miR-143 [91]. Therefore, further studies on these two microRNAs could confirm their possible use as a marker of response to HK2 inhibitors such as *3BP* and *2DG*.

Finally, several microRNAs including miR-34a, miR-34c, miR-369-3p, miR-374a, miR-30a-5p, miR-142-3p, miR-30d-5p, miR-323a-3p, miR-199a-3p, miR-449a, and miR-4524a/b, have been found to target the mRNA of LDH-A [92–101]. In particular, it has been demonstrated that miR-34a re-sensitizes colorectal cancer cells to 5-FU by directly targeting the expression of LDH-A. These results suggest further studies on this miRNA as a predictive factor for response to a combined therapy of 5-FU with LDH-A inhibitors, such as FX11, NHI-1, NHI-2 and NHI-Glc-2 [102].

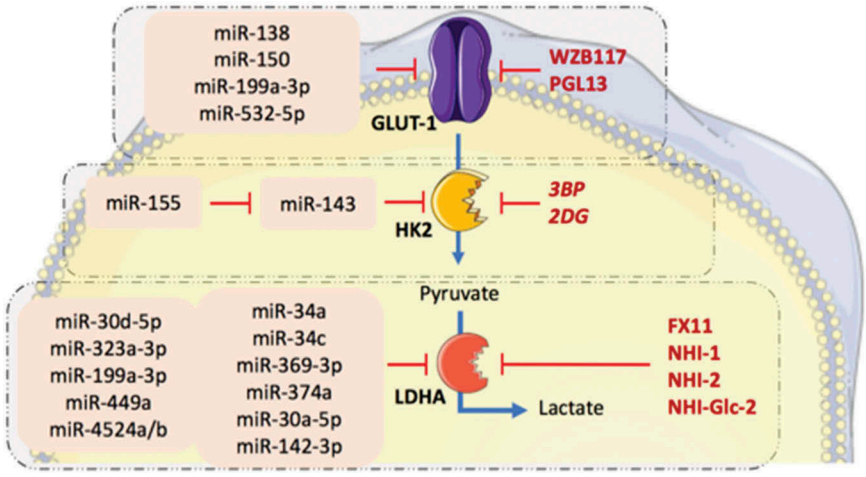

Figure 2. Scheme of potential mechanisms underlying the interaction or predictive potential of selected miRNAs with inhibitors of cancer glycolytic pathway. Four miRNAs (miR-138, miR-150, miR-199a-3p, miR-532-5p) can inhibit the glucose transporter GLUT-1, potentially modulating the sensitivity of cancer cells to the WZB117 and PGL13 compounds. Similarly, inhibition of HK2 can be modulated by a combinatorial effect of miR-143 and 3BP / 2DG inhibitors, while mir-155 can be used as predictive marker for 3BP or 2DG treatment response. A wide list of miRNAs have been found to target LDH-A and they may synergize the inhibitory effect on LDHA with FX11, NHI-1, NHI-2, NHI-Glc-2 small-molecule inhibitors.

In conclusion, future studies should evaluate the role of several emerging miRNAs as biomarkers in PDAC as well as to validate their use as potential predictive factors for the response to glycolysis inhibitors. Moreover, additional candidate miRNAs could be found by investigating the metabolism of glycolysis inhibitors. Indeed, miRNAs could modulate the expression of several drug-metabolizing enzymes, affecting the sensitivity to this class of new drugs. Hopefully, the identification of biomarkers of response, including the above-mentioned miRNAs, should shorten the gap between preclinical studies and personalized therapies using these novel treatments.

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Declaration of interest

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