



REVIEW

The challenge of drug resistance in pancreatic ductal adenocarcinoma: a current overview

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) has one of the highest mortality rates among all cancer types. Its delayed diagnosis precludes curative resection, thus most of the current therapies against PDAC are based on chemo- and radiotherapy. Unfortunately, these strategies are insufficient to improve its poor prognosis. Despite the advances made in chemotherapy (e.g. nab-paclitaxel and gemcitabine), many patients with PDAC are unable to benefit from them due to the rapid development of drug resistance. Currently, more than 165 genes have been found to be implicated in drug resistance of pancreatic tumors, including different integrins, mucins, NF- κ B, RAS and CXCR4. Moreover, drug resistance in PDAC is thought to be mediated by the modulation of miRNAs (e.g. miRNA-21, miRNA-145 and miRNA-155), which regulate genes that participate in cell proliferation, invasion and metastasis. Finally, cancer stem cells are intimately related to drug resistance in PDAC due to their ability to overexpress ABC genes -involved in drug transport-, and enzymes such as aldehyde dehydrogenases -implicated in cellular drug metabolism- and poly (ADP-ribose) polymerases -involved in drug-induced DNA damage repair. Understanding the mechanisms involved in drug resistance will contribute to the development of efficient therapeutic strategies and to improve the prognosis of patients with PDAC.

KEYWORDS

Pancreatic ductal adenocarcinoma; chemotherapy; drug resistance; cancer stem cells; therapeutic strategies

Introduction

Pancreatic ductal adenocarcinoma (PDAC) has one of the highest mortality rates among all cancer types and increases its incidence year by year. The 5-year survival rate is only 5%, even in patients undergoing complete tumor resection or treated with chemo- and radiotherapy¹. PDAC is the most frequent type of pancreatic cancer (PC), affecting 90% of patients with cancer in the pancreas, and it is the third cause of cancer-related death in the United States, following lung and colorectal cancer PDAC has no visible symptoms or biomarkers, which hinders its early diagnosis¹⁻². As a consequence, more than 50% of patients present with

metastatic disease at diagnosis, when no curative treatment can be offered. Many of the currently used drugs may increase the lifetime of patients and relieve their symptoms, but neither cancer eradication nor complete symptomatic relief is usually possible. Few drugs have been shown to be effective against PC over the years. gemcitabine, a nucleoside analogue used since the 90s as the clinical agent of reference² is the most common chemotherapy agent used in clinical practice. Unfortunately, low survival rates are still achieved with this drug. To increase treatment efficiency, formulations of gemcitabine encapsulated in albumin nanoparticles have been assayed *in vitro* and *in vivo*³. On the other hand, 5-fluorouracil (5-FU), a molecule widely used in colon cancer treatment due to its capacity to be inserted into the DNA and inhibit cell proliferation, lacks a therapeutic efficacy in PC, where no significant improvement in symptoms or life expectancy was demonstrated⁴. Clinical studies reporting the use of 5-FU along with gemcitabine did not show clinical benefits in comparison with gemcitabine alone, but a slight

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increase in side effects (neutropenia, diarrhea and anemia) was reported⁵. However, FOLFIRINOX, a formulation containing several drugs (including 5-FU), increased the survival of patients with advanced PC by two months⁶. Irinotecan, an inhibitor of Topoisomerase I present in FOLFIRINOX, showed effectiveness in the treatment of this type of cancer in some clinical trials⁷, and its liposomal encapsulation would improve the treatment of refractory PC⁸. Finally, Paclitaxel associated with human albumin (nab-paclitaxel) is also being used in PC⁹. Recently, a clinical trial demonstrated that nab-paclitaxel plus gemcitabine improved the survival of patients with advanced PC by two months, without significantly increasing drug toxicity¹⁰. Currently, the most effective therapies in clinical terms are nab-paclitaxel, gemcitabine and folfirinnox. However, this regime is not applicable to all patients given the increase in toxicity and the severe risk for patients in advanced stages of the disease.

In most cases, PC progresses to infiltration of other organs and distant metastasis, which have a high impact on survival. In these patients, conventional treatment does not improve the prognosis. A cornerstone of this therapeutic failure is the development of drug resistance. Accumulating evidence suggests that chemoresistance is intimately linked to the disruption of multiple genes involved in intracellular signaling, DNA repair, metabolism and regulation of cell replication¹¹. In addition, local recurrence of the tumor after surgical resection, chemo- and/or radiotherapy has been related to the presence of cancer stem cells (CSCs). These cells are characterized by a high treatment resistance and proliferation capacity, which explains the more aggressive nature of the recurrent tumor¹². In this review we analyze different genetic and protein resistance mechanisms by which PDAC cells reduce the efficacy of the available drugs, and the advances being made to avoid such drug resistance and decrease the current mortality rate of PC.

Drug resistance at the molecular level

Although currently gemcitabine is the first-line treatment against PDAC, many patients are unable to benefit from it due to the rapid development of resistance to this drug by the tumor cells¹¹. Gene expression microarray analyses performed in PC cell lines showed more than 165 genes related to drug resistance. These genes were involved in a myriad of cell functions, including antioxidant activity, apoptosis, cell cycle regulation and transduction of signals, among others¹³ (**Figure 1**). gemcitabine inhibits cell proliferation and induces apoptosis of tumor cells through

the activation of the AMPK / mTOR pathway, increasing the expression of AMPK and decreasing that of mTOR, which results in cell autophagy¹⁴. In this route, ARK5, a kinase related to AMPK, induces the epithelial-mesenchymal transition (EMT) of PC cells, which is linked to drug resistance. Recently, the inhibition of ARK5 by modulating the oxygen conditions (normoxia/hypoxia) proved to sensitize pancreatic cells to gemcitabine¹⁵. The SRC tyrosine kinase, which has been used as a prognostic marker in PC, may also be involved in drug resistance of PC cells¹⁶. In fact, some natural compounds that act over this molecule were able to suppress tumor growth and decrease the chemoresistance of tumor cells against gemcitabine¹⁷. Similarly, the overexpression of integrin β 1, an adhesion molecule involved in the interaction between cells and the extracellular matrix, has been linked to chemotherapy resistance in solid cancers, including PDAC¹⁸. This integrin induced gemcitabine resistance by activating CDC42 and the PI3K pathway¹⁹, which regulate the cell cycle and apoptosis²⁰. Finally, mutations in the RAS proto-oncogenes -detected in a high proportion of human pancreatic tumors- have been associated with drug resistance²¹. The RT11-i (an antibody that inhibits the RAS / RAF / MEK and PI3K / AKT signaling pathways) reduced gemcitabine-resistance in PC cell lines²². In addition, the inhibition of RAB14, a member of this family of proteins, decreased the IC₅₀ of gemcitabine and increased apoptosis induction²³.

On the other hand, two of the most important glycoprotein families, i.e. ABC transporters and mucin proteins, have been related to PDAC resistance. ABC transporters extrude drugs out of the cell, decreasing their intracellular concentration. Cancer cells expressing these transporters are generally referred to as multidrug resistant (MDR) cells²⁴⁻²⁵. In this vein, overexpression of the ABCB1 gene was found to be deregulated in pancreatic tumors originated due to overexpression of the MYC oncogene²⁶. Similarly, MUC1 overexpression induces the expression of several drug-resistance genes in pancreatic adenocarcinoma²⁷. In addition, this glycoprotein induces the expression of HIF1 α and increases the metabolic rate and internalization of glucose. These processes have been implicated in an increased resistance to gemcitabine²⁸. Moreover, the deregulation of MUC4, a member of the MUC family, has been related to the first disturbances that result in carcinogenesis and drug resistance in PC²⁹. In fact, the overexpression of MUC4 was associated with a negative regulation of the expression of a nucleoside transporter (hCNT1) involved in cell internalization of gemcitabine³⁰⁻³¹.

Some nuclear transcription factors have been implied in

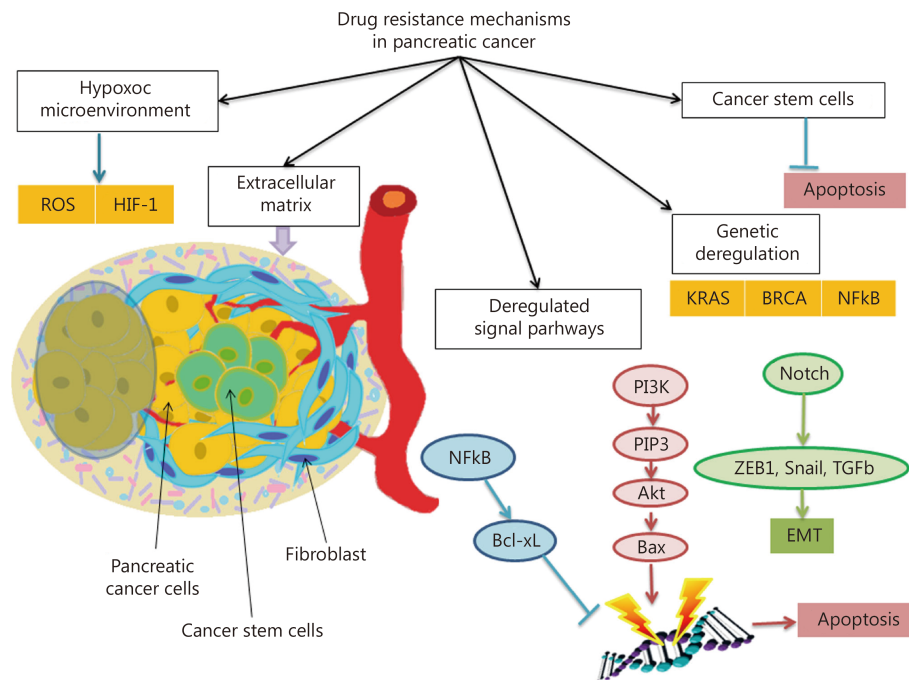


Figure 1 Different mechanisms of drug resistance in pancreatic cancer deregulated molecular signal pathways such as RAS, NFkB and PI3K pathways and, overcoat KRAS and BRCA genetic deregulation. These mechanisms comprise that tissular hypoxia increases HIF-1 levels and diminishes reactive oxygen species (ROS), a dense extracellular matrix impedes the diffusion of chemotherapeutic agents, and the existence of cancer stem cells escape to apoptosis.

drug resistance, as is the case with kappa β (NF- κ B), which modulates the immune and inflammatory responses³²⁻³³. Similarly, a factor related to NF- κ B, named TRIM31, was associated with a more aggressive phenotype of PC in which anti-apoptotic genes involved in gemcitabine-resistance were overexpressed. Therefore, a therapeutic strategy based on specific TRIM31 inhibitors could be useful to decrease gemcitabine-resistance in PC³⁴⁻³⁵. In addition, gemcitabine can induce the expression of NF- κ B and generate reactive oxygen species (ROS) through the activation of the P22 factor. The expression of NF- κ B regulates the signaling pathway of CXCR4, another factor that confers resistance against gemcitabine³⁶. The chemokine receptor CXCR4, involved in the first stages of organ development, is of great importance in the tumor genesis and metastatic spread of PC³⁷. Its overexpression is associated with a worse prognosis, probably because the overactivation of the CXCL12-CXCR4 signaling axis confers resistance against current therapies³⁸⁻³⁹. The basis of this resistance is that CXCR4 negatively regulates the expression of let-7a miRNA, which is responsible for the inhibition of cell proliferation, metastasis and drug resistance³⁹. Another nuclear factor that induces drug resistance is CHK1, a protein able to inhibit the progression of the cell cycle in response to DNA damage. Inhibitors of

CHK1 proved to decrease tumor cell resistance and favor the action of antiproliferative drugs (i.e. gemcitabine) in PC cells. Conversely, the inhibition of HSP90 -a CHK1-activating protein- did not increase the sensitivity of PC cells to gemcitabine⁴⁰. Recently, HSP27, another member of this family, has been demonstrated to be implicated in gemcitabine resistance⁴¹⁻⁴².

Role of non-coding RNA (ncRNA) in drug resistance

The ENCODE project estimates that ncRNA transcripts constitute approximately 70% of the human genome, having a number of cell regulatory functions. Within ncRNAs, miRNAs regulate 90% of gene expression and influence the processes of cell proliferation, invasion and metastasis. Subsequently, miRNAs have been implied in the diagnosis and prognosis of several cancer types, including PDAC⁴³⁻⁴⁵. In addition, some specific miRNAs play a role in the development of drug resistance in PC (**Table 1**).

For example, miRNA-21 proved to increase drug resistance through the inhibition of FasL expression, a factor that triggers apoptosis. This, in turn, is associated with a decrease in patient survival⁴⁶. Furthermore, the ability of

Table 1 miRNAs involved in drug resistance in pancreatic cancer

Name	Expression	Gene(s) regulated	Effect	Drug(s) involved	Reference
MiR-21	Overexpressed	FasL, PDCD4, PTEN (inh), Bcl2 (exp)	Inhibition of apoptotic and tumor suppressor genes	Gemcitabine, 5-FU	46-48,62
MiR-29c		USP22 (exp)	Autophagic process and inhibition of apoptotic process	Gemcitabine	49,50
MiR-155		DCK, ROS detoxification genes (exp)	Increased anti-apoptotic activity, ROS detoxification and gemcitabine metabolism		53,54
MiR-365		SHC1 (inh), BAX (inh)	Inhibition of pro-apoptotic genes		55
MiR-429		PDCD4 (inh)	Inhibition of tumor suppressor gene		63
MiR-181c		CTGF, BIRC5, BLC2L1, YAP, TAZ (exp)	Inactivation of antitumor pathway and increased expression of chemoresistance genes	Gemcitabine, 5-FU, Paclitaxel	56
MiR-221-3p		RB1 (inh)	Inhibition of tumor suppressor gene	5-FU	58
MiR-320a		PDCD4, β -cadherin, E-cadherin (inh), fibronectin, N-cadherin, Vimentin, ZEB1, Snail2 (exp)	Induction of epithelial-mesenchymal transition and inhibition of tumor suppressor genes		60
MiR-145	Underexpressed	RPS6KB1 (inh), miRNA-155 (exp)	Inhibition of cell migration, tumor growth and metastatic process	Gemcitabine	51,52
MiR-506		SPHK1, Akt, NF- κ B (exp)	Induction of tumorigenic pathway		64

inh: inhibited genes dependent on miRNA expression; exp: overexpressed genes dependent on miRNA expression.

miRNA-21 to induce drug resistance in tumor cells is mediated by the PI3K-AKT pathway, whose activation decreases cell susceptibility to apoptosis through an increased expression of the anti-apoptotic gene Bcl2⁴⁷⁻⁴⁸. Likewise, miRNA-29c seems to play a role in chemoresistance of PC cells. Its overexpression is associated with increased levels of USP22, which proved to induce autophagy and inhibition of apoptosis following treatment with gemcitabine⁴⁹⁻⁵⁰. Moreover, the inhibition of miRNA-145 and the increase in miRNA-155 expression have been also related to PDAC drug resistance. The former, a tumor suppressor that increases the sensitivity of tumor cells to gemcitabine, inhibits the signaling pathway of p70S6K1, a protein implicated in drug resistance, tumor growth and metastasis⁵¹⁻⁵².

The latter is involved in the increase in cellular anti-apoptotic activity⁵³ and in the deregulation of the DCK gene expression (implied in the metabolism of gemcitabine⁵⁴). In addition, miRNA-155 induces the secretion of exosomes, increases the expression of ROS detoxification genes and decreases DCK expression⁵³⁻⁵⁴. Finally, the overexpression of miR-365 through the repression of the pro-apoptotic genes SHC1 and BAX induced gemcitabine-resistance in PC cells⁵⁵.

Drug resistance in PDAC has been also associated with miRNAs. For example, miRNA-181c, which is highly expressed in advanced stages of PC, increases the chemoresistance against gemcitabine, 5-FU and Paclitaxel by

the inactivation of the Hippo antitumor signaling pathway⁵⁶. Interestingly, the lncRNA GAS5, an ncRNA of more than 200 bp, antagonizes the effect of miRNA-181c and should be explored as a therapeutic strategy⁵⁷. Moreover, miRNA-221-3p and MiR-320a have been implicated in 5-FU resistance. The former desensitizes PC cells to 5-FU through negative regulation of RB1, a tumor suppressor gene which has been implicated in the development of PC⁵⁸. The latter inhibits PDCD4, another tumor suppressor gene that increases the expression of molecular markers related to the EMT, promotes the proliferation and migration of tumor cells and makes them more invasive⁵⁹⁻⁶⁰. In fact, PDCD4 is regulated by several miRNAs such as miR183⁶¹ miRNA-21⁶² and miRNA-429⁶³, which may repress PDCD4 expression in PC cell lines, promoting tumor growth. Finally, the deregulation of miRNA-506 -which acts as a tumor suppressor- boosts the progression of pancreatic tumors, increasing chemoresistance through the modification of the signaling pathway in which NF- κ B participates⁶⁴. In sum, a large number of micro-RNAs are involved in the development of PDAC, acting in certain cases as proto-oncogenes or tumor suppressor genes depending on the signaling pathways regulated.

EMT and drug resistance

Correct adhesion of tumor cells to the cellular matrix is a

hallmark of cancer progression. However, many of chemo- and radiotherapy-resistant tumors have been proved to originate blood circulating tumor cells from an EMT process⁶⁵. Cancer stem cells (CSCs) are essential in the genesis of tumors, and they produce a large number of signaling substances involved in cell proliferation and drug resistance. Interestingly, the cells activated during the process of EMT display a gene expression profile similar to that of CSCs, which would explain their ability to form new tumors with great resistance to chemo- and radiotherapy⁶⁶. In fact, Hangbin et al. were able to sensitize gemcitabine-resistant cell lines through the inhibition of EMT by means of hyperthermia⁶⁷. One of the metabolic pathways more involved in EMT and drug resistance is PI3K/AKT/mTOR. The deregulation of this pathway causes a decrease in chemotherapy-induced DNA damage, inhibition of apoptosis and a decrease in the expression of E-cadherin –a molecule associated with EMT-. The use of an inhibitor against this signaling pathway allows to inhibit the EMT and the migration of cancer cells, subsequently inhibiting tumor growth, metastasis and EMT in murine models⁶⁸. Another gene related to EMT is Slug, a transcriptional factor that suppresses E-cadherin expression, which confers resistance to gemcitabine in pancreatic CSCs through EMT. Thus, its suppression at the transcriptional level makes it possible to increase the sensitivity of PC cell lines, reducing their invasive and migratory capacity⁶⁹. Although different molecular pathways regulate EMT, miRNAs are essential factors in the control of this process. In this vein, miR-509-5p and miR-1243 inhibit the EMT process and their overexpression in PC cells increases the sensitivity to gemcitabine⁷⁰⁻⁷¹. In conclusion, the use of miRNA inhibitors of EMT, one of the processes that mostly influences drug resistance in PDAC, opens new possibilities in the treatment of this entity.

Role of cancer stem cells in drug resistance

For many years, cancer was thought to be composed of clonal, homogeneous cell populations. Nevertheless, over the years it became evident that tumors are highly heterogeneous systems constituted by cells with varying degrees of differentiation. In fact, it was observed that CSCs, a group of poorly-differentiated cells, are responsible for the self-renewal capacity of tumors¹². The presence of highly drug-resistant CSCs in the tumor is a cornerstone in understanding its recurrence (i.e. tumor relapse after chemo- or radiotherapy), a phenomenon associated with a worse prognosis⁷². Although several resistance mechanisms have

been described, three systems must be highlighted with regard to PDAC: overexpression of ABC transporters, detoxifying enzymes and proteins involved in cell death processes⁷³.

ABC transporters

The family of ABC transporters is present in most living beings, from the simplest forms of life (bacteria) to the most complex organisms (mammals). These molecules are involved in the transport of different metabolites between the cell membrane and the extracellular matrix against the concentration gradient, using the energy released from the hydrolysis of ATP. Their main functions are detoxification, prevention of intracellular oxidative stress and cell protection against xenobiotics⁷⁴. However, their detoxification activity serves as an escape mechanism for antitumor drugs and increases resistance to chemotherapy agents (**Figure 2**).

This resistance is mainly mediated by three receptors: MDR1, BCRP and MRP1⁷⁵. In addition, a high expression of the MRP4 protein has been detected in PDAC. This protein promotes cell proliferation and plays a role in the rapid formation of colonies from tumor cells⁷⁶. Other genes involved in the synthesis of ABC transporters, such as ABCB4/11, ABCC1/3/5/10 and ABCG2 are also overexpressed in PDAC tissues⁷⁷. Interestingly, CSCs from PC showed an increased expression of ABC transporters, which is associated with a worse response to chemotherapy. In particular, ABCB1 –which originates a protein known as p-glycoprotein- is of major relevance in PDAC as it is considered the ABC transporter more involved in drug resistance, not only in this tumor but in many other cancer types⁷⁸.

Aldehyde dehydrogenases

The aldehyde dehydrogenases (ALDH) are a family of enzymes whose function is to oxidize cellular aldehydes to carboxylic acids. These aldehydes are originated from the metabolism of several cellular components (proteins, nucleic acids) that often remain as cellular waste, and need to be eliminated. One of the primary functions performed by these enzymes concerns the metabolism of retinol (vitamin A), which is converted into retinoic acid. This molecule is essential for an adequate embryonic development, which makes a high expression of ALDH essential in stem cells⁷⁹. On the other hand, a great variety of aldehydes are generated from the metabolism of environmental agents and drugs, and they may induce cell damage and death. Therefore, the

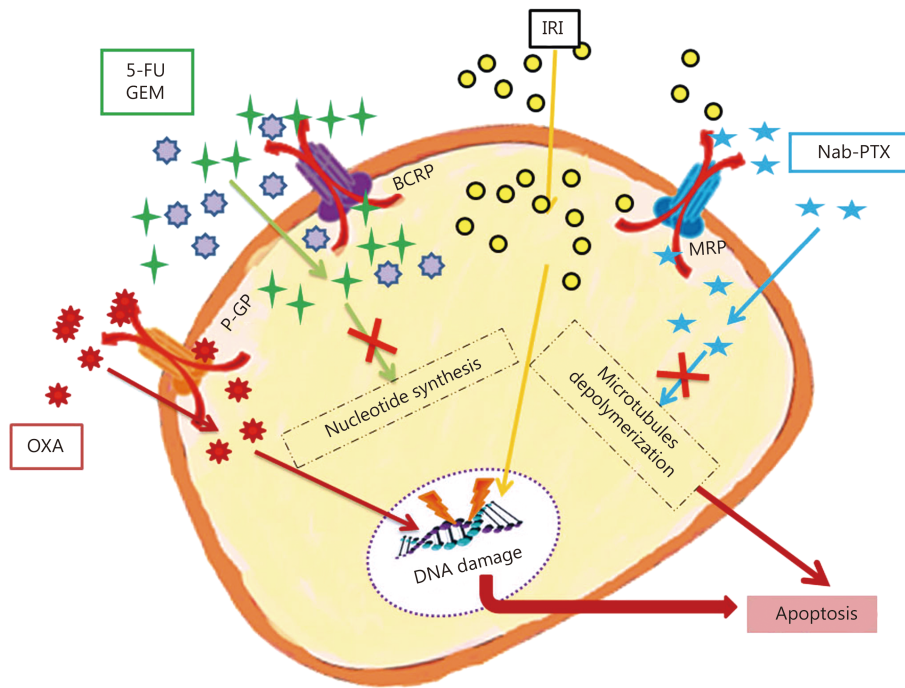


Figure 2 ABC transporters in detoxification of chemotherapeutic drugs in pancreatic cancer. The most common drugs are oxaliplatin (OXA), 5-fluorouracil (5-FU), gemcitabine (GEM), irinotecan (IRI) and Nab-paclitaxel (Nab-PTX). The main ABC transporters (ATP binding cassette) in pancreatic cancer was breast cancer resistant protein (BCRP), P- glycoprotein (P-gp) and multidrug resistance protein (MRP). MRP and BCRP transporters require the conjugation with glutathione.

overexpression of these enzymes protects against these toxic agents and promotes cell survival. In experiments using PDAC cell lines, overexpression of ALDH enzymes allowed to identify cell populations capable of originating tumors more efficiently⁸⁰.

Although there are several enzyme isotypes, the ALDH1A1 gene has commonly served as a marker to differentiate normal from CSCs in adult tissues⁸¹. Besides, the ALDH1B1 isotype is generally used as a marker of stem cells in the early stages of pancreas development and only a small population of cells that overexpress this marker remains in the adult⁸². This isotype also promotes tumor proliferation. Accordingly, two phenotypes of PC can be distinguished: those whose growth is favored by the overexpression of ALDH1A1, and those with a dominant ALDH1B1 phenotype⁸³. The inhibition of ALDH1A1 in PDAC cell lines proved to increase sensitivity to gemcitabine, indicating that ALDH1A1 overexpression may be paramount for drug resistance maintenance in tumor cells⁸⁴. In addition, gemcitabine-resistant PDAC cells showed a higher expression of membrane markers also present in CSCs -including ALDH1A1- and an overexpression of the SRC oncogene. The use of an SRC inhibitor along with gemcitabine proved to inhibit tumor proliferation, decreasing the expression of

ALDH1A1 and the number of CSCs in the tissue. This indicates that the expression of ALDH1A1 is of significance in both normal and cancer stem cells for the preservation of their phenotype⁸⁵⁻⁸⁶.

The PARP enzyme family

Poly (ADP-ribose) polymerases (PARPs) constitute a family of 18 proteins with a conserved catalytic domain capable of transferring several ADP-ribose units to their target proteins. They are involved in several cellular processes, including the regulation of proliferation and programmed cell death. Moreover, two of the most important members of this family, PARP1 and PARP2, play a role in DNA repair⁸⁷. Through their catalytic activity, these enzymes modify certain factors responsible for the recruitment of proteins involved in efficient DNA repair (**Figure 3**). PARP1 is overexpressed in pluripotent cells and its correct expression is essential for maintaining the unique characteristics of human stem cells, including CSCs. Its mechanism of operation is based on the addition of several units of ADP-ribose using NAD^+ as a substrate, resulting in a poly (ADP-ribose) chain that can contain up to 200 units⁸⁸. PARP1 modifies p53 and inhibits its binding to the genes that regulate the process of apoptosis.

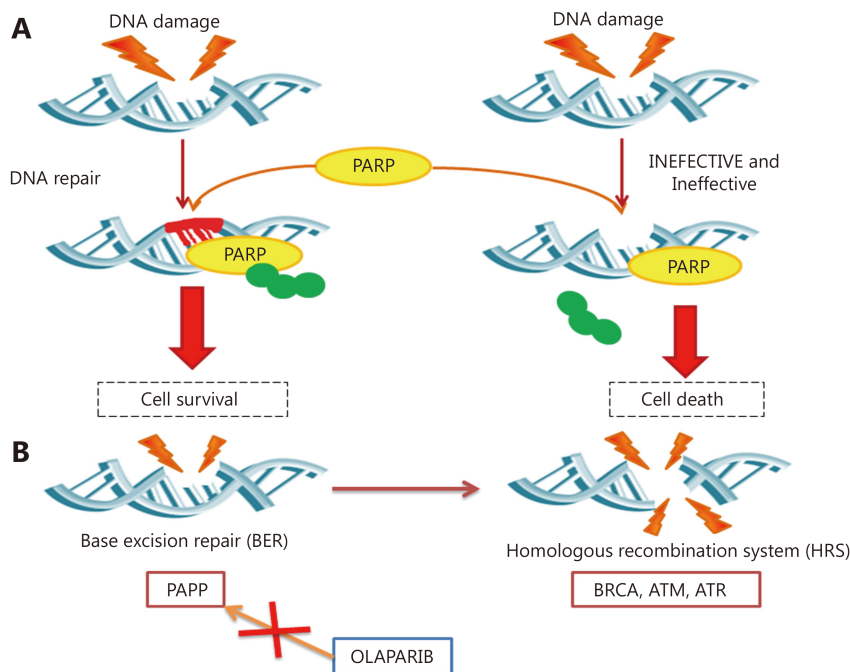


Figure 3 Mechanism of action of PARP (poly-ADP-ribose-polymerase). Single strand DNA damage induced by chemotherapeutic agents or radiotherapy is repaired by this enzyme leading to cell survival. In this process PARP accumulates ADP tails. However, big amounts of DNA damage cannot be repaired by PARP, so that, ADP tails are released and induce cell death (A). Mechanism of synthetic lethality. BER Base Excision Repair (RES) system repairs single chain DNA damage and Homologous recombination system (HRS) repair double strand DNA damage. These two systems are consecutive, so that, DNA damage and the ineffective of BER lead to the activation of HRS. HRS is made up by repair proteins like BRCA, ATM or ATR. Germinal of somatical mutations in these genes provoke an ineffective HRS dependent DNA repair. This fact could profit to trigger a syntetic lethality, inhibiting PARP with molecules such as olaparib. Therefore, the defective HRS due to genetic mutations added to BER system inhibition by PARP blockers lead to apoptosis of cancer cells (B).

The inhibition of p53 and the repair of DNA damage by PARP may act as mechanisms of drug resistance⁸⁹.

The overexpression of PARP1 has been associated with different cancer types in humans, including liver, lung, endometrium, ovary and skin⁹⁰. In 2010, it was discovered that the inhibition of PARP1 diminished cell proliferation in hepatocellular carcinoma by modulating the expression of genes implicated in tumor development and vasculogenesis⁹¹. Given the importance of PARP1/2 in DNA damage repair, several drugs aiming to inhibit their activity have been designed as a plausible strategy against cancer. This is the case with olaparib, an inhibitor of both PARP1 and PARP2 that induces S and G2/M arrest and apoptosis. Olaparib has been approved by the FDA since 2014 for the maintenance of patients with ovarian cancer who have mutations in BRCA1 and BRCA2⁹². Simultaneous inhibition of PARP1 and RAD51 proteins, which are capable of interacting with BRCA2 during homologous recombination, has the potential to sensitize cells to radiation therapy, leading to cell cycle arrest and apoptosis⁹³. The use of small molecules that mimic

the state of mutated BRCA2 can disrupt the BRCA2-RAD51 interaction, increasing Olaparib effectiveness and allowing the treatment of patients with wild BRCA2⁹⁴. In addition, specific inhibitors against the BET protein family are able to reduce the expression of RAD51, thus increasing sensitization to PARP1/2 inhibitors⁹⁵. Furthermore, PARP1/2 inhibitors have been used to delay DNA damage repair, allowing sensitization to proton beam irradiation⁹⁶. Finally, PARP inhibitors have been combined with agents that inhibit telomerase, an enzyme responsible for maintaining telomere length, in order to induce premature aging and apoptosis of PC cells⁹⁷.

Overcoming chemotherapy resistance

Despite the large number of research lines dedicated to PC, the efficiency of current therapies remains too low. In order to avoid drug resistance, new formulations are being developed based on traditional drugs. One of the main

problems with gemcitabine is that its blood concentration is maintained for a short period of time, as the cytidine deaminase breaks it down in just one hour. Therefore, other formulations have been tested to increase drug efficiency. In this vein, the use of PEGylated liposomes allowed to reach a similar tumor concentration of gemcitabine with a 10-fold lower dose, reducing its rapid blood degradation⁹⁸. Likewise, the use of albumin nanoparticles along with gemcitabine decreased its toxicity and improved its biodistribution and efficiency in *in vitro* and *in vivo* assays using PDAC cells⁹⁹. Other nanoparticles containing gemcitabine and antisense oligonucleotides against the proto-oncogene miR-21 were found to have a high inhibitory effect on the proliferation of PC cells¹⁰⁰.

The addition of gemcitabine to cell cultures causes an increase in ROS, leading to apoptosis. However, this process is not totally efficient due to the existence of ROS detoxification enzymes capable of eliminating these molecules. To cope with this mechanism of resistance, Ju et al.¹⁰¹ proposed to target the molecular pathways that regulate the expression of detoxification enzymes and use inhibitors against some of these systems (e.g. GSH). Meanwhile, Aibani et al. prevented chemoresistance in PC cells by encapsulating three drugs (5-FU, Leucovorin and Doxorubicin) in PEG particles¹⁰². Finally, the use of a plant-derived compound (β -sitosterol) together with gemcitabine allowed to efficiently induce apoptosis in pancreatic cell lines through cell cycle arrest in G0/G1 and led to decrease the IC₅₀ of gemcitabine, revealing a synergistic effect of both drugs¹⁰³.

On the other hand, different strategies have been carried out to overcome drug resistance in PC at a clinical stage. For instance, EMT inhibition using antisense oligonucleotides such as Trabectedin has shown positive results in phase I/II clinical trials¹⁰⁴. Hyaluronic acid, one of the components of the extracellular matrix, plays an important role in drug resistance in pancreatic adenocarcinoma. Accordingly, hyaluronic acid-degrading enzymes (e.g. hyaluronidase) have been combined with chemotherapeutic agents to improve treatment efficacy, although contradictory outcomes have been reported. In fact, while phase II clinical trials using gemcitabine/Abraxane and hyaluronidase showed significant improvements in terms of progression-free survival, the combination of FOLFIRINOX and hyaluronidase led to poorer overall survival rates¹⁰⁵⁻¹⁰⁶.

Finally, although chemotherapy remains as the main treatment in PC, novel immunotherapy-based strategies are showing encouraging results¹⁰⁷. Immunotherapy aims to boost the immune response, subsequently increasing tumor

cell identification and elimination by the immune system. This can be achieved by means of both passive (e.g. antibodies, activated T-cell transfer) and active techniques (e.g. vaccines)¹⁰⁸. However, pancreatic adenocarcinoma has many properties that prevent its recognition by the immune system, including lack of tumor-infiltrating lymphocytes, highly dense extracellular matrix, and production of immunosuppressive cytokines by PC cells. These factors explain why novel immunotherapy treatments (e.g. Ipilimumab, Nivolumab, Pembrolizumab) are not totally effective at present¹⁰⁹.

Conclusions

Although remarkable progress has been made in cancer research within the last decade, PDAC still has very low survival rates. The current inability for early detection limits the application of effective treatments. In addition, the development of drug resistance is a key factor to understand the failure of current therapy in both the tumor and metastatic tissues. Drug resistance is mediated by different mechanisms, such as gene mutations involved in critical metabolic pathways and ncRNAs that modulate the expression of genes implied in cell behavior. On the other hand, CSCs from PDAC show a high drug resistance owing to several reasons, including overexpression of PARP enzymes, ABC transporters involved in drug elimination from the cell, and intracellular detoxification enzymes such as ALDHs. Therefore, the increase in survival of patients with PDAC should occur not just by means of discovering early serum markers, but rather due to the development of therapeutic strategies aimed to eliminate pancreatic CSCs and minimize drug resistance.

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Conflict of interest statement

No potential conflicts of interest are disclosed

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