PARPs, New Players in the Pathogenesis of Exocrine Pancreatic Diseases

Short Running Head: Role of PARPs in Pancreatitis and Pancreatic Cancer

Neus Martínez-Bosch,[1] Martin E. Fernández-Zapico,[2] Pilar Navarro,[1]* and José Yélamos[1,3,4]

[1]*Cancer Research Program, IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain*

[2] *Schulze Center for Novel Therapeutics, Division of Oncology Research, Mayo Clinic, Rochester, Minnesota, USA*

[3]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain*

[4]*Department of Immunology, Hospital del Mar, Barcelona, Spain.*

*corresponding author

Address correspondence to Pilar Navarro, PhD, Cancer Research Program, IMIM (Hospital del Mar Medical Research Institute), Dr. Aiguader 88, 08003-Barcelona, Spain. E-mail: pnavarro@imim.es

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Abstract

The poly(ADP-ribose) polymerase (PARP) enzymes were initially characterized as sensors of DNA breaks but are now known to play key roles not only in the DNA damage response but also in regulating numerous molecular processes, such as gene transcription. Further, these polymerases have emerged as key players in the pathogenesis of multiple diseases, providing promising therapeutic targets for pathologies such as cardiovascular disorders, neurodegenerative diseases, and cancer. In recent years, PARPs have been implicated in the pathogenesis of pancreatitis and pancreatic cancer, and PARP inhibition has been proposed as a valuable strategy for treating these two important gastrointestinal disorders. For instance, in preclinical mouse models, pancreatitis was significantly attenuated after genetic or pharmacological PARP inactivation, and several clinical trials have demonstrated promising responses to PARP inhibitors in pancreatic cancer patients. In this review, we summarize the current understanding of PARP functions in these two dismal pathologies and discuss the next steps necessary to determine whether PARP inhibitors will finally make the difference in treating pancreatitis and pancreatic cancer successfully.

Introduction

Pancreatitis (acute or chronic) and pancreatic cancer are the most common disorders of the exocrine pancreas. In developed countries, pancreatitis is one of the most frequent gastrointestinal causes for hospital admission, and pancreatic cancer is the fourth cause of cancer-related deaths, highlighting the drastic impact of these pathologies. Although valuable insight into the molecular basis of these disorders has been gained, efficient treatments are still lacking, so that patients are resigned to suffer the symptoms of pancreatitis or to be treated with inefficient chemotherapy for pancreatic cancer. However, new therapies for these pancreatic pathologies are in the pipeline, and many research groups are joining efforts towards this direction.

Poly(ADP-ribose) polymerase (PARP) inhibitors have recently been shown to have a great potential as a tool for attenuating the inflammatory response and in cancer treatment. Importantly, recent clinical trials with PARP inhibitors have shown high effectiveness against hereditary cancers in people with mutations in DNA repair pathways, like *BRCA* (1). A number of clinical studies have been launched in light of these promising results. Here, we provide a detailed overview on the role of PARP proteins in pancreatitis and pancreatic cancer and discuss the use of PARP inhibitors for treating these two dismal gastrointestinal pathologies.

Biochemical and Cellular Biological Properties of the PARP Family of Proteins

PARylation and the PARP Superfamily

Poly(ADP-ribosyl)ation (PARylation) is a post-translational modification of proteins, mediated by members of the PARP family, in which ADP-ribose units from donor NAD^+ molecules are covalently transferred to acceptor amino acid residues of target proteins (2). This protein modification, first detected over 50 years ago, is a dynamic process, as indicated by the short half-life of the ADP-ribose polymer (PAR), which is rapidly degraded by the poly(ADP-ribose) glycohydrolase (PARG) and the poly(ADP-ribose)

hydrolase 3 (ARH3) enzymes (Figure 1). The PARP superfamily includes seventeen members that share a conserved catalytic domain with the “PARP signature motif”, a highly conserved sequence that forms the active site. However, only six PARP proteins (PARP-1, PARP-2, PARP-3, PARP-4, PARP-5A, and PARP 5B) can be considered as *bona fide* PARP proteins, as these contain a conserved glutamate residue that defines the PARP catalytic activity. Several PARP members lack this critical residue for PAR synthesis and therefore display only mono(ADP-ribosyl) transferase activity. Further, other family members are catalytically inactive or require a specific and as-of-yet undetermined cofactor to trigger ADP-ribosylation (2). PARylation plays a critical role in many cellular processes involved in physiological functions, such as DNA damage detection and repair, modulation of chromatin structure, transcription regulation, control of cell division, mitochondrial function, and cell differentiation (2;3). Therefore, PAR levels and PARylation are finely regulated through the opposite actions of PARPs and PARG to prevent dramatic consequences for cellular physiology.

PARPs in DNA Damage and Repair

PARP-1 and PARP-2 are the best characterized members of the family, and both act as molecular sensors of DNA breaks (2) (Figure 1). These two enzymes are activated by DNA-strand interruptions, which increase their catalytic activity by more than 500-fold. PARylation mediated by PARP-1 and PARP-2 causes chromatin decondensation around damaged sites, recruitment of repair machineries, and acceleration of DNA damage repair. PARP-1 and PARP-2 form heterodimers and share nuclear partners, with some redundant functions. This is probably the reason that *Parp-1 and Parp-2* double-knockout mice have an embryonic lethal phenotype (4), whereas mice with a single *Parp-1* or *Parp-2* gene disruption are viable and fertile (5;6). Interestingly, *Parp-1* and *Parp-2* single knockout mice are both very sensitive to ionizing radiation and alkylating agents (4). Together, these data indicate a role of PARP-1 and PARP-2 in the DNA damage response (2;7).

PARPs in the Regulation of Gene Transcription and Cell Division

PARP-1 and PARP-2 also appear to play key roles in regulating other molecular and cellular processes besides the DNA damage response (Figure 1). One of the best examples is that of PARP-1 and PARP-2 in gene transcription regulation, which occurs by one of two main mechanisms: through regulation of chromatin structure by PARylating histones and destabilizing nucleosomes, or through direct interaction with transcription factors and cofactors, such as NF-*k*B or NFAT (7;8). Another important physiological role of PARPs is the regulation of cell division. PARP-1 and PARP-2, as well as other PARP family members, are associated with components of the mitotic apparatus, like centromeres and centrosomes, thereby controlling microtubule organization during mitosis and chromosome segregation (7).

PARPs in the Regulation of Angiogenesis, EMT, Cell Death, and Energy Homeostasis

PARP-1 has also been shown to modulate angiogenesis through its regulation of several factors, such as hypoxia inducible factor-1α (HIF-1α) (9) and vascular endothelial growth factor (VEGF) (10). Furthermore, PARP-1 has also been shown to regulate the epithelial-mesenchymal transition (EMT) by modulating key inducers of this cellular conversion, like vimentin and Snail1 (9). In addition, different types of cell death processes, such as apoptosis, parthanatos, necroptosis, and autophagy, have been shown to be modulated by PARylation (11). Finally, PARylation plays important roles in mitochondrial respiration regulation and energy homeostasis (3).

Taken together, these studies have provided the rationale for evaluating PARP proteins as pharmacological targets in several human diseases, particularly those involving genotoxic and inflammatory stress responses.

PARP Inhibition Attenuates Pancreatitis

Pancreatitis, which can occur either in an acute or a chronic form, is an inflammatory disorder of the pancreas that involves a complex cascade of local and systemic events,

resulting in severity that ranges from mild and self-limiting to a severe form (12). A yet-unknown triggering event converts digestive proenzymes within the pancreas into their active forms, leading to membrane disruption, edema, interstitial hemorrhage, and necrosis, accompanied by an inflammatory response with infiltrating neutrophils and leukocytes that contributes to the progression of both local pancreatic destruction and systemic manifestations (12). Many of the genes encoding these inflammatory mediators are regulated by NF-*k*B (13), whose activity is largely regulated by PARP-1 (7). PARP-1 can be involved in acute and chronic inflammatory disorders by increasing the synthesis of pro-inflammatory mediators, like iNOS, through activation of NF-*k*B and AP-1 transcription factors (7;14). This generates reactive hydroxyl radicals that cause extensive DNA damage that, in turn, activates PARP-1. The resulting PARP-1 activation induces necrotic cell death and release of intracellular content, causing tissue damage and exacerbating the inflammatory reaction. Accordingly, the severity of acute pancreatitis and pancreatitis-associated lung injury has been shown to be significantly attenuated in mice lacking *Parp-1*, but not in those lacking *Parp-2*, as compared to wild-type mice (15). Moreover, administration of PARP inhibitors in animal models of acute pancreatitis markedly decreased acute pancreatitis severity and pulmonary- and kidney-associated injuries (15-18). Future clinical trials in pancreatitis will be necessary to define the therapeutic efficacy of PARP inhibitors in a clinical setting.

Importantly, chronic pancreatitis is one of the major risk factors linked to pancreatic cancer development, thus suggesting that treatment of chronic pancreatitis patients with PARP inhibitors could protect the pancreas against cancer initiation by attenuating pancreatic inflammation and injury (15).

PARP in Pancreatic Cancer

Pancreatic cancer, and in particular pancreatic ductal adenocarcinoma (PDA), which accounts for more than 90% of pancreatic tumors, is the solid tumor with the worst

prognosis. PDA is currently the fourth leading cause of cancer-related deaths in developed countries and, as the outcome for PDA patients has not significantly improved during the past 40 years, is predicted to become the second leading cause of cancer-related death by 2020 (19). Thus, effective treatments are urgently needed. Given the success of PARP inhibition in the treatment of different tumors, several clinical studies have been conducted to explore this therapeutic strategy also in PDA (Table 1). The first results on the safety, tolerability, and maximum tolerated doses of olaparib in combination with gemcitabine were recently published (20). Promising—yet not statistically significant—results encourage ongoing clinical trials that consider selected patients with *BRCA* mutations in the absence of a chemotherapeutic backbone (Table 1) (21).

PARP, and in particular PARP-1, is frequently overexpressed in tumors (2), where its increased enzymatic activity might protect cancer cells against DNA damage and genetic instability. The involvement of these proteins in cancer is featured by the fact that high PARP levels correlate with poor prognosis in different tumor types (2;22). Accordingly, inhibition of PARP activity has emerged as a promising cancer therapy, either in combination with DNA-damaging agents (radio- or chemotherapy), or as a single-therapy in tumors harboring specific mutations in DNA-repair pathways, like *BRCA1/2*, to achieve synthetic lethality (23).

Nonetheless, PARP-1 expression and correlation with tumor prognosis and/or progression in PDA remains controversial. On the one hand, and in contrast to other tumors (2;22), high nuclear PARP-1 expression levels have been correlated with longer survival in PDA patients (24). On the other hand, our recent studies using normal, pre-neoplastic, and tumor samples from humans and mice support the hypothesis that PARP-1 expression is restricted to acinar cells (both normal and cancer), and that it is undetectable in normal and cancer ductal cells (25) (Figure 2). In fact, no significant survival differences were observed in transgenic mice that developed pancreatic

cancer under a *Parp-1* genetic deletion, as expected. However, more research is needed to explore whether expression of PARP-1 in ductal cancer cells can be upregulated in PDA patients after radiation or chemotherapy treatments. This information is critical to understand whether PARP inhibitors can be used as a combined-therapy with DNA-damaging agents. Recent studies have shown that PARP inhibitors can increase sensitivity to radio- and/or chemotherapy in several human pancreatic cell lines (26;27), and some *in vivo* studies using animal models have shown results along the same direction (28;29). However, further preclinical data and information from human samples is required prior to translating these results to the clinic.

Remarkably, beyond the putative use of PARP inhibitors to enhance the cytotoxic effects of radiation or DNA-methylating agents, recent clinical trials have demonstrated that PDA patients with mutations in *BRCA1* or *BRCA2* genes may benefit from single treatments of inhibiting PARP (30-32). Moreover, a recent phase Ib study using cisplatin chemotherapy and a PARP-1 inhibitor was successful for 56% of PDA patients with *BRCA* mutations, whereas no response was observed in patients with wild-type *BRCA* tumors (33). Another interesting study comes from a case report showing a notable clinical outcome of a PDA patient with an advanced, gemcitabine-resistant tumor that later responded to DNA-damaging agents (34). Further genetic studies of cancer cells from this patient revealed a biallelic inactivation of the *PALB2* gene (34), the protein of which links BRCA1 and BRCA2 and which is required for DNA damage repair. These data confirm that inactivation of DNA repair genes from the BRCA pathway sensitizes tumors to DNA-damaging agents and PARP inhibitors. Interestingly, mutations in *BRCA1* and *BRCA2* are the most common mutations in familial PDA, supporting the use of PARP inhibitors as a promising therapy for these patients (35). However, familial PDA is responsible for less than 10% of cases of pancreatic cancer (36), and the majority of PDA cases are sporadic without known

hereditary predisposition. Nonetheless, in the era of genomics, it would be worthwhile to develop screening programs to identify those PDA patients harboring mutations—such as *BRCA* or *PALB2*—who could benefit from PARP inhibition (37;38). Furthermore, recent data from other tumors have identified that besides BRCA deficiency, defects in other DNA damage repair machineries render cells highly sensitive to PARP inhibition (38-40), thereby expanding the subset of patients who could potentially be treated with this strategy. Remarkably, Waddell *et al.* (38) recently performed a whole-genome sequencing and copy number variation (CNV) analyses of 100 PDAs and identified a mutational signature of DNA damage repair deficiency, which included not only the *BRCA* genes but also other Fanconi anemia genes, like *PALB2* and *FANCM*, and *XRCC4/6*. These data could help to identify putative responders to platinum-based and PARP inhibition therapy, which would encourage high-throughput efforts to profile PDA patients, with important therapeutic implications. Finally, although rare, the possibility of *de novo* mutations, rather than germline mutations, in any of these DNA repair genes in sporadic PDA should also be considered. Indeed, spontaneous *BRCA* mutations have been demonstrated in sporadic breast cancer (41). Altogether, these data highlight the need to test the molecular signature of individual PDA patients, to identify targets that would allow a personalized cancer treatment and thereby broaden the population of patients who can benefit from PARP inhibitors.

In addition to the significant contribution that PARP inhibition could have in treating patients with advanced PDAs, our recent data demonstrate that PARP-1 may also play a role during tumor progression, suggesting a potential use for these inhibitors in early stages of tumorigenesis (25). Using a genetically engineered mouse model of PDA that recapitulates tumor progression (42) and *Parp-1* deficient mice, we have recently shown that PARP-1 expression induces acinar-to-ductal metaplasia. This event involves reprogramming of acinar cells to a ductal phenotype and is a key step in the

development of pancreatic cancer (43). Thus, using PARP-1 inhibitors at early stages of PDA, for instance in chronic pancreatitis, may hamper tumor initiation. Indeed, recent data have suggested the use of PARP inhibitors in chemopreventive regimens for high-risk individuals (*e.g.* for homologous repair–deficient tumors) (44). Still, we must take into account that preventive treatments with PARP inhibitors require further clinical evaluation, as long-term inhibition of PARP-1 may have adverse effects on health or even result in generating PARP-resistant tumors (45).

Last but not least, although the endocrine pancreas is out of the scope of this review, a role for PARP activation in the diabetes-associated inflammatory reaction, through a mechanism involving endothelial cells, has been recently reported (46). These data are highly relevant as diabetes is a potential cause, as well as a consequence, of pancreatic cancer.

Concluding remarks

PARP proteins play a critical role in the DNA damage response. Several pathological situations, such as inflammation and cancer, can induce the DNA damage response, leading to increased activity of PARPs. Accordingly, PARP inhibition has emerged as a promising novel therapeutic strategy for these pathologies, and several PARP inhibitors have been tested in clinical trials during the last decade, in breast, ovarian, prostate, lung, head and neck, and brain cancers, among others (1). Results have been promising for tumors with specific genetic alterations, such as BRCA1/2-deficient ovary and breast tumors. In pancreatic cancer, PARP inhibitors have demonstrated positive responses in patients with mutations in DNA repair pathways, who account for 5% to 8% of PDA patients. Figure 3 summarizes the state-of-the-art of PARP inhibition in pancreatitis and pancreatic cancer and speculates about the putative use of these compounds in precursor lesions, like acinar-to-ductal metaplasia or chronic pancreatitis, to hamper further tumor development.

Personalized medicine has recently emerged as a fundamental tool to stratify patients for response classification, which would help to avoid unnecessary treatments and save healthcare costs. In particular, identifying mutations in those DNA damage repair genes that have been demonstrated to increase the tumor sensitivity to PARP inhibition may be extremely helpful for determining which PDA patients would benefit from PARP inhibitors. These genes include *BRCA1*, *BRCA2*, and *PALB2*, for which positive results have been already demonstrated in clinical trials (30;32;33), but should also be expanded to new targets identified in other cancers, such as PTEN deficiency (39), ATM deficiency, and Aurora A overexpression (40). Moreover, although not tested, it is tempting to speculate that cancer cells with mutations in other DNA repair genes, such as ligase IV in T-cell lymphoma and *XRCC1* in non-melanoma skin cancer, may be selectively killed using PARP inhibitors (47). Future research and genetic screenings are needed to investigate whether these possibilities would also work in PDA patients.

Finally, despite the encouraging results obtained with PARP inhibitors in cancer clinical trials, there are still many hurdles to overcome: not only do we need to increase our knowledge about the mechanisms of resistance of tumor cells to PARP inhibition, but we also need to develop more specific and effective inhibitors. In this regard, we note that most of the current research has focused in the inhibition of the PARP-1 and PARP-2 enzymes, while the role(s) of the other PARP members have not been explored. In particular, considering the homology and sequence similarities among PARP proteins, it is likely that current PARP inhibitors may inhibit other members of the PARP family or even other proteins (48;49), highlighting the importance of extending our knowledge about the mechanisms of action of these inhibitors as well as the precise functions of the different PARP enzymes in physiology and pathology. Selective inhibitors are required not only to elucidate the specific functions of each PARP member but also to design cleaner therapeutic strategies. Interestingly, new tankyrase selective compounds have emerged as a plausible alternative to the classic

nicotinamide-based inhibitors (50). Moreover, studies on PARP structure offer a path towards increasing selectivity as well as the possibility to alter PARP-specific functions by developing agents that target acceptor sites or even non-catalytic domains (50).

Preclinical and clinical data point to an outstanding potential for PARP inhibitors in cancer treatment, both in combination with radio- and/or chemotherapy or used as single agents to achieve synthetic lethality in BRCA-deficient tumors. In pancreatic cancer in particular, whose dismal prognosis is highly worrying to the scientific community, much effort has been put into PARP targeting agents, with more than 10 clinical trials currently registered with encouraging preliminary results. Numerous issues must now be taken into account and deserve further research, such as optimization of dosage regimens, resistance to therapy, long-term therapeutic responses, putative use for pancreatitis treatment as PDA prevention, and careful patient selection, before we will know whether these compounds could finally mark a turning point in the treatment of this pathology.

Author contributions

P.N, J.Y, M.E.F.-Z., and N.M.-B. wrote the manuscript, prepared the figures and table, and had final approval of the submitted text.

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Table 1. Clinical Trials with PARP Inhibitors in Pancreatic Cancer

PARP inhibitor	Code name	Sponsor	Study phase	Status	In combination with	Indications	ClinicalTrials.gov ID
Olaparib	AZD2281	AstraZeneca	I	Completed	Gemcitabine	Pancreatic cancer	NCT00515866
Olaparib	AZD2281	AstraZeneca	II	Ongoing, but not recruiting	–	Pancreatic cancer with genetic BRCA1/2 mutation	NCT01078662
Veliparib	ABT-888	National Cancer Institute	II	Recruiting	Gemcitabine Cisplatin	Locally advanced or metastatic pancreatic cancer	NCT01585805
Veliparib	ABT-888	Georgetown University and Abbott	II	Recruiting	5-FU Oxaliplatin Leucovorin	Metastatic pancreatic cancer	NCT01489865
Veliparib	ABT-888	National Cancer Institute	I	Ongoing, but not recruiting	–	Recurrent pancreatic carcinoma	NCT00892736
Rucaparib	AG014699	Clovis Oncology, Inc.	II	Recruiting	–	Pancreatic cancer with deleterious BRCA mutation	NCT02042378
Talazoparib	BMN-673	BioMarin	I	Ongoing, but not recruiting	-	Pancreatic cancer	NCT01286987
Talazoparib	BMN-673	National Cancer Institute	Pilot trial	Recruiting	-	Advanced solid tumors and deleterious BRCA mutations	NCT01989546
Olaparib	AZD-2281	Astrazeneca	III	Recruiting		Mutated pancreatic cancer, not progressing on first-line platinum-based chemotherapy (POLO)	NCT02184195

Legends

Table 1. Clinical Trials with PARP Inhibitors in Pancreatic Cancer

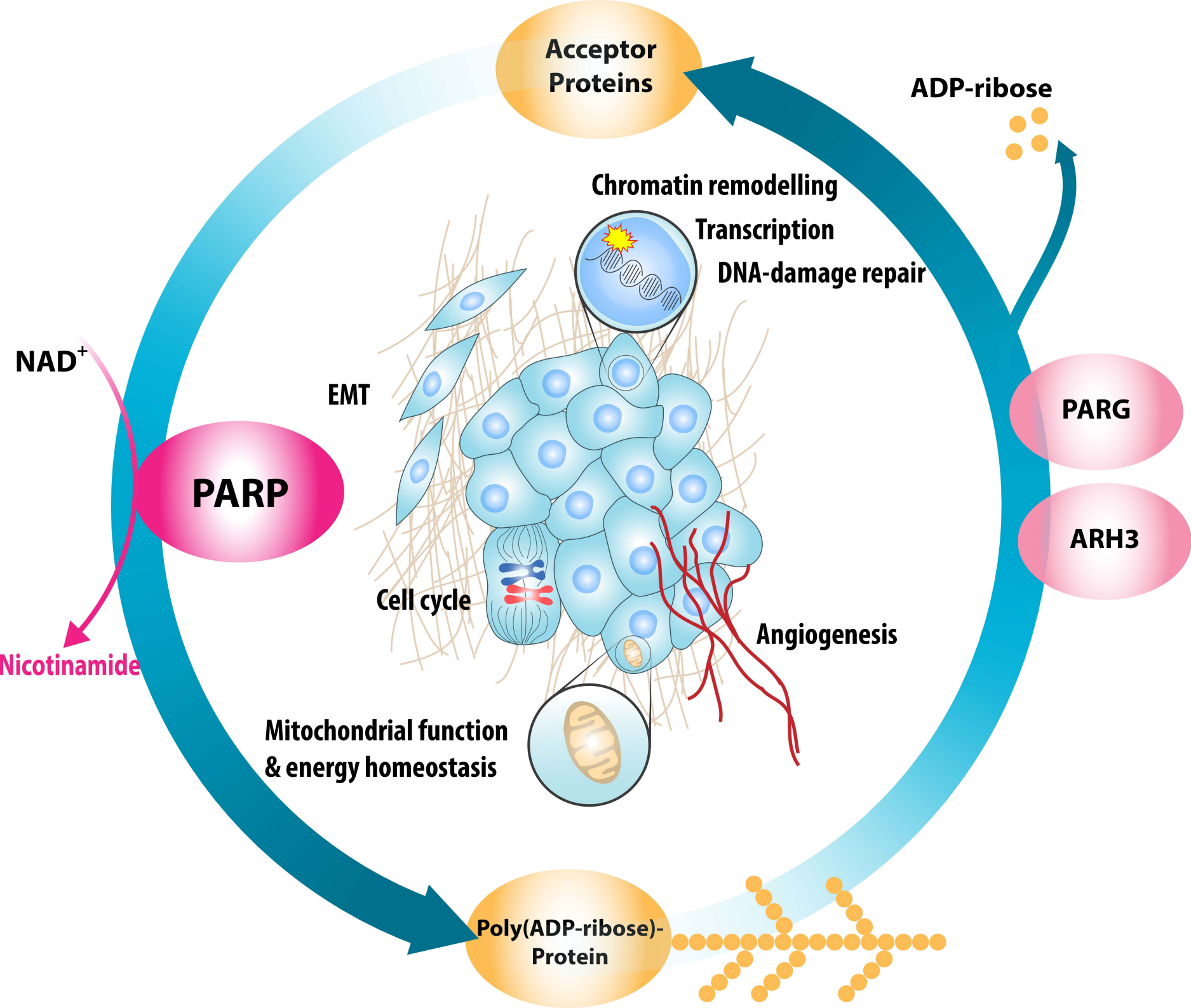
PARP inhibitors that have undergone or are currently in clinical trials are listed, with the code number, the clinical trial sponsor, type and state of the clinical trial, whether PARP inhibitors are being used in combination with chemotherapy, types of tumors included in the clinical trial, and the official ID given by clinicaltrials.org.

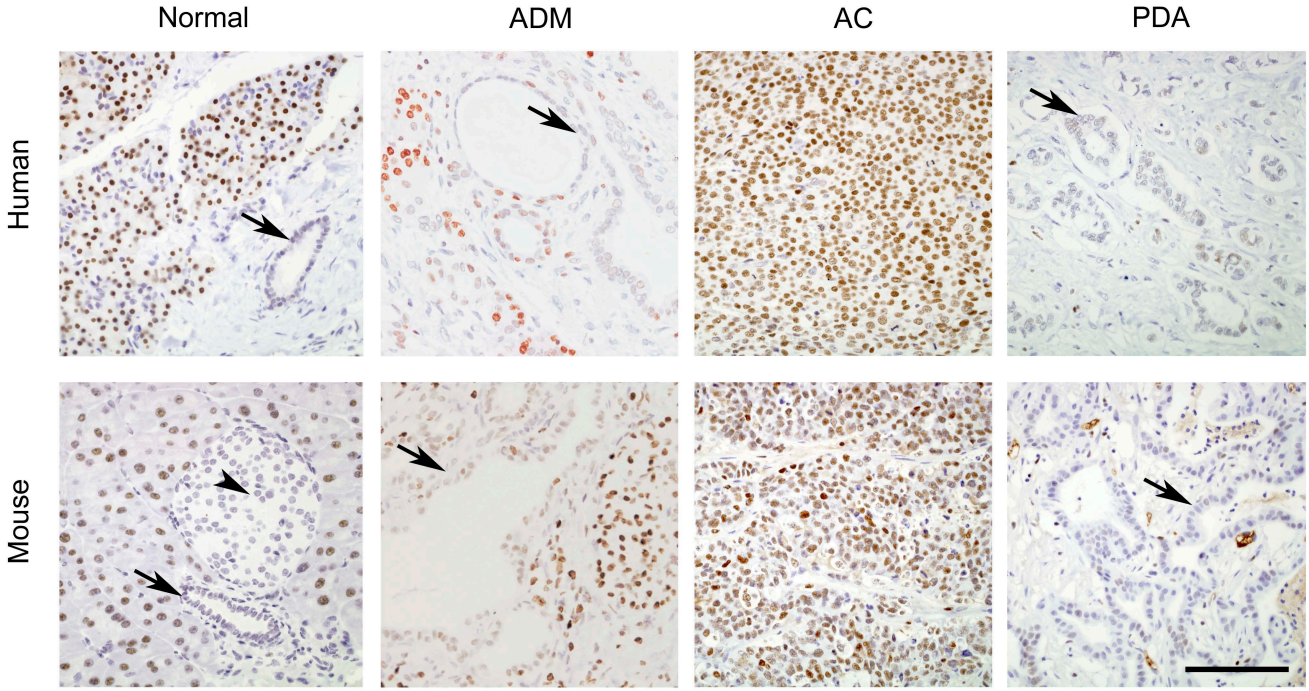
Figure 1. Biochemistry and biological functions of PARylation reaction mediated by PARP enzymes. Activated PARPs hydrolyse NAD^+, releasing nicotinamide, and catalyze the polymerization of ADP-ribose units onto acceptor proteins. The reaction is reversed by the activities of poly(ADP-ribose) glycohydrolase (PARG) and poly(ADP-ribose) hydrolase-3 (ARH3), which hydrolyze poly(ADP-ribose) into ADP-ribose units. Modification of aceptor proteins by PARylation has a critical role in many cellular processes, such as DNA damage repair, regulation of chromatin structure and transcription, control of cell division, mitochondrial metabolism, epithelial-mesenchymal transition (EMT), and angiogenesis.

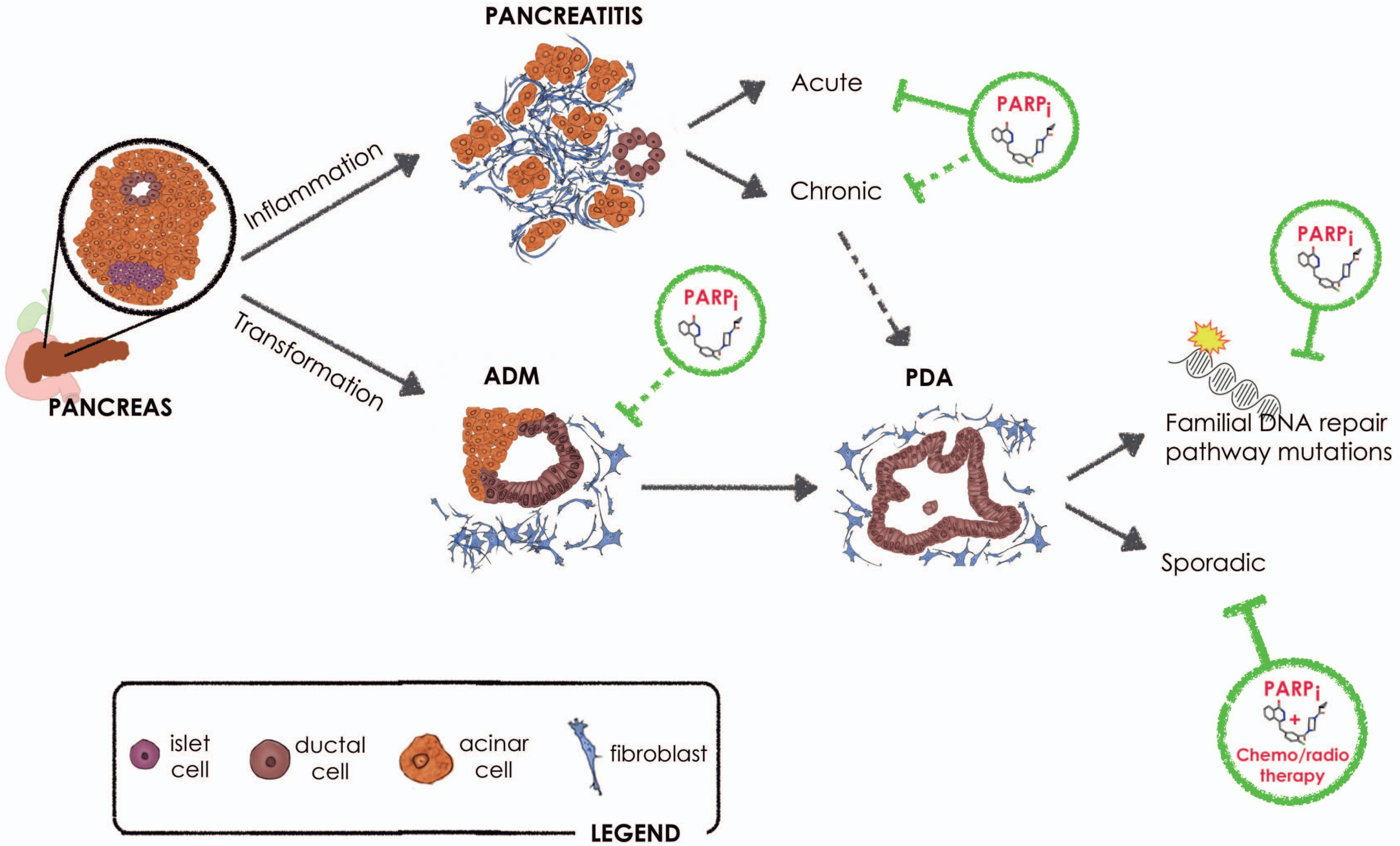
Figure 2. **PARP-1 expression in normal pancreas, acinar-to-ductal metaplasia (ADM), acinar and ductal tumors in humans, and the *Ela-myc* transgenic mouse model of pancreatic cancer.** PARP-1 nuclear expression is detected by immunohistochemistry and shows similar patterns of distribution in both human and mouse. PARP-1 is only expressed in acinar cells and is undetectable in ductal cells (arrows) or islets of Langerhans (arrowheads) in normal, metaplastic, or tumor samples. Scale, 100 μm.

Figure 3. Use of PARP inhibitors in exocrine pancreatic pathologies. Diagram showing the main exocrine pancreatic pathologies and how PARP inhibitors (PARPi) can help in their treatment. Effectiveness of these inhibitors has already been proven in preclinical studies of murine acute pancreatitis (15-18) and in PDA cell lines and clinical

trials, either as a single therapy—in patients with mutations in BRCA1/2 or PALB2—or in combination with DNA-damaging agents (PARPi, continuous green line) (26;27;30-34). Moreover, based on our results with *Parp-1*$^{-/-}$ mice (15;25), we speculate that PARPi can be useful for treating precursor lesions before the onset of a malignant PDA, such as acinar-to-ductal metaplasia (ADM) and chronic pancreatitis (PARPi, discontinuous green line).


Acceptor Proteins
ADP-ribose
Chromatin remodelling
Transcription
DNA-damage repair
NAD+
EMT
PARG
PARP
ARH3
Cell cycle
Angiogenesis
Nicotinamide
Mitochondrial function & energy homeostasis
Poly(ADP-ribose)-Protein


Normal
ADM
AC
PDA
Human
Mouse


PANCREATITIS
Acute
Chronic
PARPi
Inflammation
Transformation
PANCREAS
ADM
PDA
Familial DNA repair pathway mutations
Sporadic
PARPi + Chemo/radio therapy
islet cell
ductal cell
acinar cell
fibroblast
LEGEND