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Review

Opportunities for translation: Targeting DNA repair pathways in pancreatic cancer

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) remains one of the poorest prognosis neoplasms. It is typified by high levels of genomic aberrations and copy-number variation, intra-tumoural heterogeneity and resistance to conventional chemotherapy. Improved therapeutic options, ideally targeted against cancer-specific biological mechanisms, are urgently needed. Although induction of DNA damage and/or modulation of DNA damage response pathways are associated with the activity of a number of conventional PDAC chemotherapies, the effectiveness of this approach in the treatment of PDAC has not been comprehensively reviewed. Here, we review chemotherapeutic agents that have shown anti-cancer activity in PDAC and whose mechanisms of action involve modulation of DNA repair pathways. In addition, we highlight novel potential targets within these pathways based on the emerging understanding of PDAC biology and their exploitation as targets in other cancers.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the poorest prognosis cancers with a very high mortality, accounting for ~2% of cancer cases worldwide [1]. The common symptoms of PDAC include abdominal or back pain, obstructive jaundice, and weight loss. However, these usually appear towards the later stages of the disease and the vast majority of patients thus present with advanced inoperable, metastatic disease with a median survival of 4–11 months [2,3]. For patients who present with operable disease (<15% of cases) tumour resection (Whipple procedure) is the treatment of choice, and optimal surgery can result in a cure, although the median overall survival (OS) remains low at 15-19 months. Even in operable cases of PDAC the overall 5-year survival rate is only 11% without adjuvant chemotherapy, and 21% with [1,2]. The use of adjuvant chemotherapy has consistently demonstrated OS benefits above resection alone and may delay disease recurrence; thus it is recommended for all patients [4]. However, as PDAC is more prevalent amongst the elderly, with a median age of diagnosis of 72 years, additional medical conditions may contraindicate surgical procedures [5,6].

Abbreviations: ATR, ATM-Rad3-related; BER, base excision repair; Chk1, checkpoint kinase 1; DDR, DNA damage response and repair; DNA-PKcs, DNA-PK catalytic subunit; DOT1L, DOT1-like; DSB, double strand break; FOLFIRINOX, folinic acid, 5-FU, irinotecan, oxaliplatin; 5-FU, 5-fluorouracil; HR, homologous recombination; Mdm2, murine double minute 2; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NSCLC, non-small cell lung cancer; OS, overall survival; PARP, poly-ADP-ribose-polymerase; PARPBP, PARP1 binding protein; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; SSB, single-strand break; TDP, tyrosyl-DNA-phosphodiesterase; TIM, timeless; VCP, vasolin containing protein

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PDAC originates in the pancreatic ductal epithelium and evolves in a step-wise manner from pre-malignant lesions to fully invasive cancer [7]. PDAC development has been linked to a number of environmental factors such as tobacco smoking, which increases risk by ~2-fold [8]. In addition, advanced age and chronic medical conditions such as diabetes and pancreatitis are associated with increased risk [9,10]. Familial PDAC accounts for ~10% of cases and is associated with germline mutations in a number of genes including *BRCA2* and *PALB2* [11]. A characteristic set of molecular aberrations for PDAC development has been identified, with the genetic aberrations and associated cellular events underlying disease progression (e.g. cell growth and proliferation, apoptosis, migration) defined as early (*KRAS*, *ERBB2*), intermediate (*CDKN2A*) or late (*TP53*, *SMAD4*, *BRCA2*) occurrences during the process [12]. Extensive heterogeneity in the genetic aberrations detected both between PDAC patients [13,14] and within multiple clonal populations of individual tumours [15] have been documented. As such, a common specific molecular initiator of PDAC, if one exists, has yet to be defined. However, PDAC-associated genomic aberrations can be classified into core signalling pathways – including *KRAS* signalling, cell invasion and adhesion, DNA damage control, apoptosis [13,14,16] – thereby identifying key processes with the potential for therapeutic targeting.

For over 15 years, gemcitabine, either as single agent or in combination with other cytotoxic drugs, has been the standard chemotherapeutic agent for PDAC. However, while combining gemcitabine with other cytotoxic agents has improved progression-free survival (PFS) in some trials, a significant increase in OS, compared with gemcitabine monotherapy, has not been convincingly demonstrated in the majority [17–19]. Recently the gemicitabine-free FOLFIRINOX protocol (5-fluorouracil (5-FU), folinic acid, irinotecan, oxaliplatin) has demonstrated an increased overall survival to 11.1 months, from 6.8 months for gemcitabine alone [20]. However, toxicity and safety issues limit the routine administration of this regime to less than half of the advanced cases [20,21]. A more tolerable chemotherapy recently developed is the combination of gemcitabine with albumin-bound paclitaxel (*nab*-paclitaxel, Abraxane®) [22] which has demonstrated efficacy in patients with advanced PDAC [23–25], with a phase 3 clinical trial showing an improvement in median OS to 8.5 months, compared with 6.7 months for gemcitabine alone [23].

Irrespective of the regimen used, low response rates and a lack of sustained therapeutic efficacy remain a fundamental problem and feature of PDAC. Uncertainty continues to exist about the optimal use of combination chemotherapy regimens and sequencing in the treatment of this malignancy. Recent advances in the molecular understanding of PDAC suggests that targeting the DNA repair capacity of PDAC, in combination with DNA damaging agents, may represent an effective therapeutic strategy. Consistent with this, a number of studies have demonstrated the therapeutic potential of chemotherapy regimens incorporating DNA damaging agents, in particular platinum-based compounds, in PDAC [17–19]. The induction of DNA damage and/or modulation of DNA damage response (DDR) pathways has also been associated with the activity of a number of other chemotherapeutic agents proposed for use in PDAC. This review aims to discuss translational research progress in this area and highlights potential targets and strategies for improving outcome in PDAC.

2. DNA damage response/repair (DDR) pathways as chemotherapeutic targets in PDAC

Damage to the DNA of a cell can occur spontaneously, for example due to replication errors, or may be induced by exogenous factors such as radiation and environmental chemicals. As such, accurate repair mechanisms are crucial in order to maintain genome integrity, and consequently all cells are equipped with a number of DDR pathways. If cells are unable to repair the damage, they may undergo apoptosis in order to prevent replication of abnormal cells. DDR pathways are therefore vital to cell survival, and the presence of multiple repair processes ensures that if one is lost, an alternative mechanism may compensate. The importance of understanding DDR pathways in the context of cancer biology is evident as the mechanism of action of a number of chemotherapeutic drugs is via induction of DNA damage. DNA lesions induced by chemotherapeutic drugs include bulky adduct formation, base damage or misincorporation, DNA crosslinks, and DNA breaks, either single strand (SSB) or double strand (DSB). The key pathways which repair these lesions are the nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR), and non-homologous end joining (NHEJ) pathways. The principal processes and proteins involved in these pathways, as well as the common types of damage they recognise and repair, are listed in Table 1 (for detailed reviews of pathway mechanics see Jiricny [26]; Caldecott [27]; Leiber [28]; Cleaver et al. [29]; Moynahan and Jasin [30]).

Aberrant or dysregulated activation of DDR pathways are associated both with susceptibility to cancer, the tumourigenic process and resistance to chemo- and radio-therapy [31]. As such, disruption of these pathways has been identified as a strategic approach to increase therapeutic responses to DNA damaging agents in a number of cancer types (for excellent reviews see Al-Ejeh et al. [32]; Bouwman and Jonkers [33]; Lord and Ashworth [34]. The clinical relevance of targeting DDR pathways to enhance PDAC response to DNA damaging agents is highlighted by Jones et al., who identified DNA damage control as a core signalling pathway disrupted by PDAC-associated genetic aberrations. Additionally, polymorphisms within genes encoding DDR proteins have been associated with PDAC development and resistance to gemcitabine [35–38].

In addition to their application as enhancers of response to cytotoxic chemotherapy, the clinical development of DDR targeting drugs as anticancer agents has been designed around the concept of synthetic lethality, in which effectiveness is dependent on the genetic background of the cells with respect to competency in DDR pathways (Fig. 1). Poly-ADP-ribose-polymerase (PARP) inhibitors represent the most well known paradigm of synthetic lethality. PARPs are a family of nuclear enzymes, of which PARP-1, -2 and -3 are activated following binding to broken DNA ends. Subsequent to their activation, these proteins catalyse the formation of poly-ADP-ribose polymers which attract repair proteins, including XRCC1 and LIG3, to the sites of DNA damage. The best characterized member of this family is PARP-1, which is predominantly associated with BER-mediated repair of SSBs [39–42]. PARP-1 inhibition has been shown to be most effective in tumour cells with defective HR pathways, due to mutations in the *BRCA1* and/or *BRCA2* genes [43]. Conventionally, it has been understood that under these conditions PARP-1 inhibition prevents BER-mediated repair of SSBs, while DSB repair cannot occur due to inherent defects in HR pathways. The net result of this is accumulation of lethal levels of DNA damage [44,45]. *BRCA2* mutations have been associated with both sporadic and familial cases of PDAC [46–50]. Defects in other HR genes, namely *PALB2* (a BRCA protein binding protein) and *ATM* have been associated with PDAC [16,51]. The presence of such aberrations is likely to affect chemotherapy response, and as will be discussed later, should be a consideration when developing therapies incorporating DDR-targeting agents.

3. Gemcitabine and platinum compounds

Gemcitabine was established as the standard of care for PDAC following the pivotal phase III clinical trial in 1997 demonstrating its greater efficacy compared with 5-FU [52]. Conventionally, the mechanism of action of gemcitabine has been attributed to inhibition of DNA synthesis: as a nucleoside analogue, it is incorporated into replicating DNA in place of cytidine molecules. The position of the gemcitabine moiety as the penultimate nucleotide at the 3′ end of the nascent DNA strand is important in preventing its recognition and removal by DDR exonucleases, and its presence results in termination of the synthesis process. Additionally, gemcitabine actively inhibits DNA polymerase

Table 1
Key process and proteins involved in cell response to DNA lesions.

Process	Key mediators
Nucleotide excision repair (NER) — recognises and repairs bulky- and helix-distorting adducts	
Adduct recognition	DDB1, DDB2, ERCC1, ERCC4, RAD23B, RPA1, XPA, XPC
DNA unwinding	ERCC2, ERCC3, ERCC6, ERCC8, RPA1
Adduct removal; damaged DNA excision	ERCC1, ERCC4, ERCC5, XPA
Gap repair - DNA synthesis and ligation	LIG1,PCNA, POLD, POLE, RFC1
Base excision repair (BER) — recognises and repairs non-helix-distorting base lesions and SSBs	
Lesion recognition	PARP1, XRCC1
Damaged 3′ and 5′ DNA end processing	APE1, APTX, FEN1, LIG3, PNKP, POLB, TDP1, XRCC1
Gap repair — DNA synthesis	FEN1, PARP1, PCNA, POLB, POLD, POLE, POLG, POLL, RFC, XRCC1
Gap repair — DNA ligation	LIG1, LIG3, PCNA, XRCC1
Mismatch repair (MMR) — recognises and repairs incorrect insertion, deletion and misincorporation of bases	
Base mismatch recognition	MSH2, MSH3, MSH6, MLH1, PMS2
Base mismatch removal	EXO1, RPA1, RFC, PCNA
Gap repair — DNA synthesis and ligation	LIG1, POLD
Non-homologous end joining (NHEJ) — recognises and repairs DNA DSBs	
DSB recognition	XRCC6 (Ku70), XRCC5 (Ku80)
Repair protein recruitment	XRCC6, XRCC5, H2AX
Repair protein activation	ATM, ATR, PRKDC
DSB end processing	DCLRE1C, FEN1, MRE11, NBN,PRKDC, RAD50
Gap repair — DNA synthesis and ligation	LIG4, XRCC4
Homologous recombination (HR) — recognises and repairs DNA DSBs	
DSB recognition; repair protein recruitment	RAD51, RAD52, H2AX
Repair protein recruitment/activation	ATM, ATR, MRE11, NBN, RAD50
DSB end processing and resection	BLM, BRCA1, BRCA2, BRIP1, EXO1, MRE11, NBN, PALB2, RAD51, RAD52, RAD54, RBBP8, RPA1
DSB resection — strand invasion/exchange	BLM, RPA1, RAD52, XRCC2, XRCC3, RAD51

and ribonucleoside reductase activity. This latter activity depletes the available cytidine pool, thereby favouring the incorporation of gemcitabine nucleotides [53]. Over the past few years, there has been an increase in the use of gemcitabine/cisplatin combination in the treatment of PDAC. This choice of combination was based on lack of overlapping dose-limiting toxicities and results of cell line model experiments [54]. The synergy between gemcitabine and cisplatin compounds has been related to increased induction of DNA damage and inhibition of DNA repair. The presence of gemcitabine nucleotides has also been suggested to facilitate structural changes in the DNA, thereby increasing the availability of platinum-binding sites [55,56]. Cisplatin preferentially forms adducts with DNA at the N-7 position of guanine and adenine, and the presence of these platinum-DNA adducts prevents or interferes with replication and transcription, leading to cell cycle arrest and cell death [57]. Recognition and removal of platinum-DNA adducts is mediated by the NER pathway, which may also directly activate the apoptotic programme [58]. However, where NER fails, or if apoptosis is not initiated, unrepaired adducts can progress to DNA strand breaks, either SSBs or DSBs (Fig. 2), whose repair and recognition are dependent on intact BER, HR and NHEJ pathways (see Table 1 for list of key steps and proteins in these pathways). Gemcitabine has been found to stabilise platinum-DNA adducts and DNA strand crosslinks (mediated by the adducts), indicating lack of removal and repair [56–63]. Additionally, direct inhibition of the NER and HR pathways by gemcitabine have

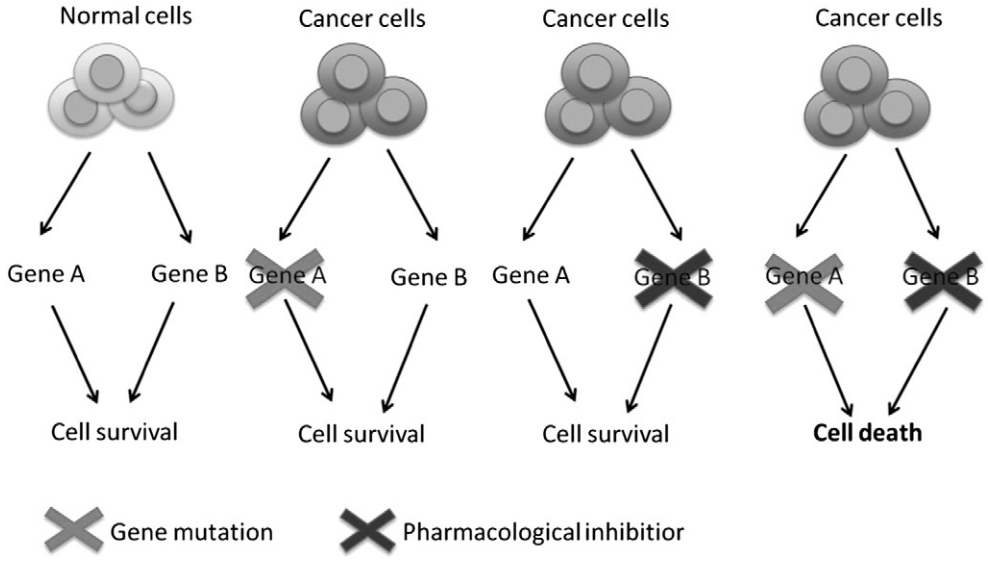

Fig. 1. Synthetic lethality: pharmacological inhibitor activity is dependent on genetic background. The concept of synthetic lethality is based on compensatory survival mechanisms of two genes. Under normal conditions both genes are functional, however in a cancer cell where the function of one is lost, either through mutation or pharmacological inhibition of its protein product, cell survival is possible through the action of the other gene alone. Targeting of both genes results in cell death. Pharmacological inhibition of both gene products may similarly result in a synthetically lethal outcome.

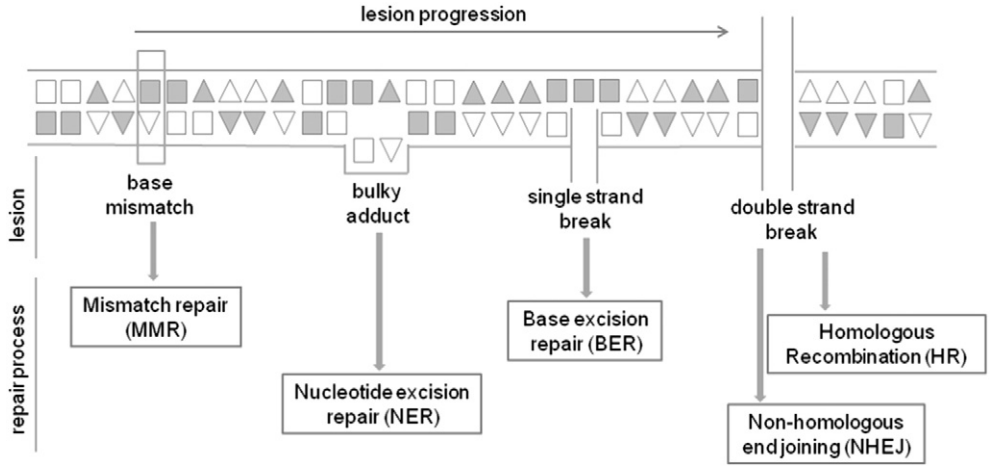

Fig. 2. Sequence of DNA lesion progression. The chemotherapeutic activity of DNA damaging compounds is partly based on the generation of DNA lesions. The initial lesions formed in response to platinum treatment are bulky platinum-DNA adducts. These may be removed by the NER pathway. However, if this fails and the adducts persist, more severe lesions are generated when DNA replication/transcription machinery encounter the adduct. Recognition and response to these lesions is regulated by activation of other DDR pathways shown. ▲ guanine, △ cytosine, ■ adenine, □ thymine.

been suggested as mechanisms underlying gemcitabine/platinum synergy [60,61,64].

NER is the primary process which functions to remove bulky platinum-DNA adducts [65]. This pathway also recognises and repairs helix-distorting DNA damage caused by other factors, including 6-4 photoproducts produced by UV light. Defects in NER are associated with a number of clinical conditions including the inherited disorder xeroderma pigmentosum, which is characterized by extreme UV sensitivity and predisposition to skin cancer [29]. Expression levels of ERCC1, an NER protein which functions in the excision of bulky adduct-DNA lesions, have been found to correlate inversely with response to gemcitabine/platinum chemotherapy, particularly in non-small cell lung cancer (NSCLC) [66–73] and testicular cancer [74–76]. In addition, polymorphisms within this gene, and that for another NER mediator ERCC2, have been associated with an improved response to gemcitabine/platinum treatment [71,77–81]. With regards to PDAC, the role of ERCC1 as a prognostic marker is ambiguous: increased ERCC1 expression has shown negative [82], positive [83] and neutral [84] association with survival following tumour resection. Although the patients in these studies received different adjuvant treatments (radiation + gemcitabine or 5-FU, none, or gemcitabine, respectively), the administration of chemotherapy was not found to correlate with ERCC1 expression levels. However, a small number of studies have agreed on a correlation, in ~40% of patients, between a polymorphism resulting in decreased translation of ERCC1 in PDAC tumours and a better response to platinum-based salvage chemotherapy [85,86]. A recent study however, has shown an association between *ERCC2* polymorphisms and PDAC response to gemcitabine/platinum treatment, but not to gemcitabine alone [81]. Together, these studies suggest that the expression/polymorphism status of NER proteins may be useful biomarkers for stratifying PDAC patients who will benefit the most from treatment with gemcitabine/platinum chemotherapy. Additionally, they suggest that PDAC resistance to cisplatin is partly due to NER-regulated DDR.

Loss of HR function has been found to prevent synergism between cisplatin and gemcitabine [61]. This suggests that HR competency should be considered when selecting for treatment with this combination. This may be particularly relevant for PDAC, as abnormalities in HR-related genes are associated with both sporadic and familial forms of the disease (see Section 2 for more details). HR is a process by which DSBs are repaired through the alignment of homologous sequences of DNA (see Table 1 for key steps and proteins). It requires an intact sister chromatid and occurs primarily during the S and G_2 phases of the cell cycle [30]. Polymorphisms in *XRCC3*, whose protein product functions in the final stages of HR, have been suggested to be predictive of response to gemcitabine/platiunum chemotherapy in lung and breast cancers [78,87]. With regards to PDAC, *XRCC3* polymorphisms have only been assessed in relation to response to radiotherapy [88]. However, the sensitivity of PDAC cells to gemcitabine alone has been shown to be increased, both *in vitro* and *in vivo*, following inhibition of HR activating proteins, namely ATR (ATM-Rad3-related) or Chk1 (checkpoint kinase 1) [89,90]. As such, HR competency status may also be useful in stratifying patients for treatment with gemcitabine alone, as well as selecting those who may benefit from additional HR-targeted therapies. A number of Chk1 inhibitors have been evaluated in clinical trials, and importantly they have shown activity in p53 deficient tumours, which account for the majority of PDAC cases [91]. PARP inhibitors have also been shown to sensitise HR-deficient PDAC cells to gemcitabine [92], and this class of agents will be discussed in the next section.

The NHEJ pathway may also be a target for increasing PDAC response to DNA damaging agents. Like HR, NHEJ functions in the repair of DSBs, but in comparison does not require homologous sister chromatids and therefore is error prone, but can be active at any stage of the cell cycle [28]. DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is a key component of NHEJ [93], and its inhibition has been shown to resensitise PDAC cell lines to cisplatin-induced apoptosis [94]. Inhibition of DNA-PKcs has also demonstrated potential as a radio-sensitising strategy for PDAC, and will be further discussed in the next section.

4. Targeting DNA repair directly: exploiting DDR deficiencies

As discussed previously, the therapeutic potential of DDR proteins is dependent on DDR pathway competency: the basis for this synthetic lethality is that the cells compensate for loss of one pathway by relying on the activity of another. As mentioned previously, defects in a number of HR genes have been associated with PDAC, in particular *BRCA2*, *PALB2* (a BRCA binding protein), and *ATM* [16,46,51,95,96]. Additionally, polymorphisms in the HR genes *RECQ1*, *RAD54L*, *XRCC2*, and *XRCC3* have been associated with decreased survival [88]. A deficiency in HR identifies a number of potential therapeutic and chemo-sensitisation strategies for PDAC, which will be discussed below.

4.1. PARP inhibition

Similar to ovarian and breast cancers, germline mutations in *BRCA2* are associated with familial PDAC. Such patients represent < 15% of this subpopulation of PDAC [46–49,95,96] suggesting limited, but

important therapeutic potential of PARP inhibitors in the treatment of PDAC. Although a small number of cases have been reported in which chemotherapy incorporating PARP inhibitors have been initially effective in PDAC patients harbouring *BRCA2* mutations, this response was not found to be sustained [97,98]. However, there are conflicting reports regarding the specificity of *BRCA2* mutational status as a predictor of PARP inhibitor response in PDAC: although reconstitution of BRCA2 protein expression, following restoration of the open reading frame via deletion of a frameshift mutation, in the Capan-1 PDAC cell line has been found to result in acquired resistance to PARP inhibition [99], a number of pre-clinical studies have shown PARP inhibitors to have a chemo- and radio-sensitizing effect in both *BRCA2* mutant and wild-type expressing PDAC cell lines, albeit to a greater extent in the former [92,100–103]. It has also been suggested that the effect of PARP inhibitors on *BRCA2* deficient PDAC cells is limited only to highly potent compounds [104]. However, it is possible that biomarkers other than, or in addition to *BRCA2* mutational status, may be required to predict response, and defects in other HR-related genes are likely candidates.

Although defects in *BRCA1* have also been associated with PDAC [47–49,96,104–107], there are few studies assessing its role in predicting response to PARP inhibitors. In comparison, cells deficient in *PALB2* – a BRCA protein binding partner, which is reported to be the second most commonly mutated gene associated with familial PDAC [51] – have been shown to exhibit greater sensitivity to PARP inhibitors [108]. In breast cancer and lymphoma cells, low levels of the HR protein *ATM* have also been associated with increased response to PARP inhibition [109–111]. This is particularly noteworthy as a recent study has reported *ATM* gene defects in 8% of sporadic PDAC cases [16], thereby suggesting that the therapeutic potential of PARP inhibitors may extend to a larger cohort of patients that initially indicated.

In addition to their roles in BER, PARP proteins have also been shown to be involved in coordination of the HR (PARP-1/2) and the NHEJ (PARP-1/3) pathways [42]. As such, the overall DNA damage repair competency of a tumour may affect the response to PARP-1 inhibitors, and may direct the most effective choice of chemotherapy/inhibitor combination. Of particular importance in this regard, the conflicting reports regarding the role of PARP in regulation of NHEJ must be considered: in HR-deficient cells, activation of the NHEJ mediator DNA-PKcs has been associated both with PARP-1 activity and treatment with PARP-1 inhibitors [42,112]. In the latter study, PARP inhibition in HR-deficient cells was linked to enhanced NHEJ activity, leading to an alternative model for PARP inhibitor toxicity whereby NHEJ-driven genomic instability leads to cell death. Under these circumstances, PARP inhibition would be of limited therapeutic use in NHEJ deficient tumours.

PARP inhibitors may also be effective in non-HR deficient PDAC when combined with direct inhibition of HR, for example using ATR or Chk1 inhibitors which have been found to sensitise PDAC cells to chemo- and radio-therapies [89,90,113–115]. PARP-1 binding protein (PARPBP) which has been established as an inhibitor of HR, has also been suggested as a therapeutic target due to its overexpression in PDAC [116–118]. PARBP overexpression is associated with increased genomic instability, likely due to compensatory action of other DDR pathways including NHEJ [117,118]. Somewhat paradoxically, knock-down of PARPBP in PDAC cells has been found to decrease viability and proliferation, while overexpressing cells have demonstrated increased sensitivity to DNA damaging agents, including PARP inhibitors [116–118]. Thus, PARPBP may represent both a therapeutic target and a biomarker for predicting response to DNA damaging agents.

Overall, it is clear that a greater understanding of the basis of PARP inhibitor response in PDAC is required for translational and clinical development of these agents, and in order to establish which patients may derive most therapeutic benefit from this class of inhibitors. This is particularly emphasized by conflicting data regarding the association between PARP-1 expression and survival: a recent study suggested that an association between high nuclear PARP-1 expression and longer survival indicates increased ability of the cells to respond to and repair genomic aberrations which might otherwise promote disease progression [119]. If this holds true, it would imply PARP-1 inhibition should be avoided where expression levels are high. However, a previous report has demonstrated no association between PARP-1/2 expression levels and survival [120].

4.2. DNA-PKcs inhibition

As mentioned previously, DNA-PKcs plays a crucial role in the repair of DSBs via the NHEJ pathway [93]. DNA-PKcs is a nuclear serine/threonine protein kinase which complexes with Ku70/80 heterodimers at DSB ends, forming the DNA-PK holoenzyme. This complex recruits the additional repair proteins required for the processing and ligation of the damaged DNA. Unlike DSB repair via HR, NHEJ does not rely on extensive homologies between the damaged DNA ends; therefore it can be active during any stage of the cell cycle. NHEJ is often prone to error, resulting in insertion or deletion of nucleotides at the ligated DSB junction (for a review of the NHEJ process see Lieber [28]).

Recently, inhibition of DNA-PKcs has been found to be synthetically lethal in ATM-deficient lymphoma [121]. However, we have found that inhibition of DNA-PKcs restores cisplatin sensitivity in HR competent ovarian cancer cell lines [94], and therefore it appears that HR status alone is insufficient to predict DNA-PKcs inhibitor response. The activity of DNA-PKcs inhibitors in both HR deficient and competent tumours may be due to crosstalk between DNA-PKcs and the AKT survival signalling pathway. Using a series of matched clinically platinum-sensitive/resistant paired ovarian cancer cell lines and lung and PDAC cell lines to assess the role of DNA-PKcs in the activation of AKT in response to cisplatin, we have shown that DNA-PKcs phosphorylates AKT at S473 in response to cisplatin-induced DNA damage in cells with clinically acquired-resistance to cisplatin but not in matched sensitive cells [94]. Furthermore we demonstrated co-localisation of DNA-PKcs and AKT in the nuclei of resistant but not sensitive tumour cells, and that inhibition of DNA-PKcs prevents AKT activation and enhances sensitivity to cisplatin in platinum-resistant cancer cells. We also showed that activation of AKT by DNA-PKcs occurs in response to cisplatin, but not insulin, across a range of tumour types, suggesting a nuclear, DNA-damage-mediated pathway distinct from canonical cell-surface phosphatidylinositol 3-kinase/AKT activation. Inhibition of DNA-PKcs has also been found to sensitise PDAC cells to radiation, and additionally, results in the prolonged accumulation of DSBs eventually leading to cell death [122]. We suggest therefore that inhibition of DNA-PKcs is a strong candidate for further development as a therapeutic strategy for PDAC.

5. Topoisomerase Inhibition

Topoisomerases are enzymes that catalyse the breaking and rejoining of the phosphodiester DNA backbone during unwinding of the DNA structure in preparation for transcription and replication. Their action is required to prevent the accumulation of supercoiled and tangled DNA structures during these processes. The anti-cancer activity of both topoisomerase I inhibitors (e.g. Topotecan, irinotecan) and topoisomerase II inhibitors (e.g. etoposide, doxorubicin) is well established, and conventionally these compounds function by "trapping" the enzyme in complex with the DNA — for this reason they are commonly referred to as topoisomerase poisons. The presence of these complexes prevents the re-ligation activity of the topoisomerase, resulting in the generation of DNA breaks which can affect genome integrity and lead to induction of apoptosis [123].

Irinotecan is a topoisomerase I inhibitor which is included in the FOLFIRINOX chemotherapy regimen that has shown efficacy in patients with advanced PDAC [20]. Although irinotecan as a single agent has demonstrated marginal efficacy in PDAC patients [124–127], improved efficacy, in terms of longer PFS, is found when it is combined with

gemcitabine, compared to the single agents alone [128–130]. Irinotecan is synergistic with 5-FU and is the standard of care in colorectal cancer but has never been tested formally in PDAC (except in FOLFIRINOX with the addition of oxaliplatin). Interestingly, a direct role for gemcitabine in topoisomerase I inhibition has also been suggested [131]. Thus, the therapeutic application of irinotecan for PDAC lies predominantly in its combination with other agents.

Topoisomerase II poisoning has been associated with the cytotoxic activity of anthracyclines such as doxorubicin and danorubicin, both of which are primarily used for the treatment of haematological malignancies. Combination of doxorubicin with carboplatin has also been show to be effective in epithelial ovarian cancer, both as a first line treatment and for recurrent disease in platinum-sensitive patients [132]. However, the activity of these agents may also be due to DNA intercalation and the generation of reactive oxygen species. In comparison, etoposide is considered a specific topoisomerase II inhibitor, and is used in the treatment of a number of solid malignancies [123]. Two studies reported that the gene encoding topoisomerase II, *TOP2A*, is amplified in 60–90% of PDAC cases where it is frequently co-amplified with *ERBB2* which is found on the same chromosome [133,134]. However, the theranostic value of this is uncertain, and clinical trials involving topoisomerase II poisons have focused on their combination with other cytotoxic agents [124,135–139] but have not been found to be more effective or less toxic compared with other chemotherapeutic treatment approaches for PDAC. A number of novel compounds which inhibit the catalytic activity of topoisomerase II proteins have demonstrated single agent anticancer activity against PDAC cells *in vitro* [140–142], and the clinical efficacy of this approach remains to be investigated.

Given the generation of DNA breaks in response to topoisomerase inhibitiors, a rational therapeutic approach would be to combine them with DDR inhibitors. The choice of inhibitor is likely to depend greatly on DDR competency: topoisomerase II inhibitor-induced damage can be repaired by both HR and NHEJ, with NHEJ suggested as the more important mechanism [143,144]. Thus, where both pathways are functional, inhibition of one may not be sufficient to prevent repair of topoisomerase inhibitor-induced damage. Delay of HR has been suggested as the mechanism underlying the synergy observed in PDAC cells treated with both topoisomerase II and murine double minute 2 (Mdm2) inhibitors — however, it should be noted that the NHEJ capacity of these cells was not reported [145]. Although Mdm2 is best known for its role as a negative regulator of p53 function, a p53-independent interaction with the nibrin protein, which functions in the early stages of the HR pathway, links it with DSB responses [146]. This may also account for the synergy reported in PDAC cells co-treated with Mdm2 inhibitors and platinum compounds [147,148]. Further development of a therapeutic strategy combining topoisomerase inhibitors with DDR inhibitors will require clarification of which DDR pathway is the optimal target, and whether response is affect by repair competency status.

Another important consideration regarding the use of topoisimerase inhibitors in PDAC is clearly the genetic background of the tumour. The response of PDAC cells to combination treatment with topoisomerase II and Mdm2 inhibitors was found to be independent of p53 mutation status — this is of note as *TP53* mutations are detected in ~50–75% of PDAC cases [2] and thus, this strategy may represent a broad treatment option. However, the efficacy of topoisomerase inhibitor-based chemotherapy in PDAC is likely to vary with other genetic aberrations. For instance, PDAC cell lines harbouring inactivating defects of Smad4, a component of TGF-β signalling, have been found to be more sensitive to irinotecan compared with wild-type counterparts [127]. In contrast, overexpression of *HDAC2* is associated with PDAC resistance to etoposide [149]. Characterisation of such biomarkers of response will allow the design of rational combination chemotherapy regimens incorporating targeted agents: for example, a synergistic increase in apoptosis has been shown in PDAC cell lines treated with etoposide and valproic acid, which depletes HDAC2 [149], suggesting that a combination of these agents may be more beneficial in patients harbouring amplifications/mutations in *HDAC2*.

In addition to direct targeting of topoisomerases, inhibition of topoisomerase-induced DNA damage repair pathways also represents a potential therapeutic strategy for PDAC. As previously mentioned, the formation of topoisomerase-DNA complexes is an essential preparatory stage of DNA transcription and replication. In order for these processes to proceed, however, these complexes must be removed by tyrosyl-DNA-phosphodiesterases (TDP1 or TDP2), which are also involved in the repair of topoisomerase-mediated DNA damage [123]. Inhibitors of TDP1 have been shown to be most effective in cells deficient in the ERCC1/4 endonuclease pathways, due to redundancy between these repair mechanisms [150]. As discussed previously, the role of ERCC1 expression as a predictive marker in PDAC is unclear and is based on a small number of studies. Within these, low ERCC1 expression was reported in 15–50% of samples [82–86], suggesting a possible rationale for assessing TDP1 inhibitors as a novel therapeutic approach in PDAC.

6. Potential novel therapeutic targets for PDAC

The influence of genetic alterations in DDR genes on chemotherapy response has been described in a number of cancers, including PDAC. However, upregulated DDR proteins, whose increased expression in PDAC results from amplifications or mutations, may represent novel targets with therapeutic potential. As mentioned, Jones et al. [13], identified the DDR as a core signalling pathway dysregulated in PDAC. Of the 9 DDR-related genes identified as altered in PDAC in this study, 5 have previously been described as valid therapeutic targets in other cancer types: *VCP*, *CUL4B*, *DOT1L*, *ERCC4* and *TIM*. However, it must be noted that these genetic aberrations were detected in single patient cases within the study by Jones et al. [13]. In addition, their functional effects are unclear, and will determine whether or not direct targeting of the encoded proteins represent viable therapeutic approaches.

- The *VCP* gene encodes the valosin containing protein (VCP), an ATPase with functions in transcriptional regulation, proliferation, apoptosis, and protein degradation [151] was reported to be amplified by Jones et al. [13]. Elevated VCP serum levels were reported in 8/12 PDAC cases in another study [152], and increased VCP protein levels have been associated with progression of hepatocellular carcinoma and NSCLC [152–155]. siRNA-mediated or pharmacological inhibition of VCP has been found to reduce tumour proliferation and progression, and induce apoptosis.
- A missense mutation in *CUL4B* was detected in one sample by Jones et al. [13]. The product of this gene, cullin 4B, is an E3-ubiquitin ligase subunit, which plays a role in NER-mediated DNA repair [156]. Cell lines derived from human lymphoblastoid cells with *CUL4B* mutations have shown increased sensitivity to camptothecin, and this phenotype was recapitulated following siRNA-mediated inhibition of *CUL4B* [157].
- The *DOT1L* protein product, DOT1-like (DOT1L) is a histone H3 methyltransferase whose function has been associated with facilitating recruitment of the DSB sensor 53BP1 to sites of damage [158]. Recruitment of DOT1L to chromosomal translocations involving the *MLL* gene has been identified as a driver of mixed lineage leukaemia [159]. A number of small molecule inhibitors of DOT1L have been developed, and have demonstrated pre-clinical efficacy against this form of leukaemia [160]. Jones et al. [13], identified a missense mutation in *DOT1L*.
- A missense mutation in *ERCC4* (excision repair cross-complementing rodent repair deficiency, complementation group 4), which functions in the NER pathway, was also reported by Jones et al. [13]. Ovarian cancer, leukaemia, and testicular cancer cell lines defective in ERCC4 have demonstrated increased sensitivity to DNA damaging agents, including cisplatin [74,161,162]. Conversely, cisplatin-induced

upregulation of ERCC4 reduces the sensitivity of melanoma cells to the drug [163]. Strategies proposed to directly target ERCC4 include direct inhibition of its catalytic site, as well as disruption of its interaction with other NER proteins [164].

- Timeless (*TIM*) has reported roles in regulation of DNA replication and maintenance of genome stability [165,166], and its function has been associated with activation of ATR or ATM during the S and G_2/M phase cell cycle checkpoints, respectively [167,168]. Under normal conditions, loss of TIM confers reliance on the HR pathway for maintaining DNA synthesis and stability [166,167]. In cancer cells (colon and NSCLC), TIM downregulation has been found to increase sensitivity to DNA damaging agents [168,169]. However, low expression levels of *TIM* (mRNA) have recently been associated with PDAC [170], while Jones et al. [13], reported a missense *TIM* mutation in PDAC. This suggests that TIM status, in combination with HR competency status, may be useful in determining whether a PDAC patient will benefit from treatment with DNA damaging agents.

7. Conclusion

PDAC remains a disease associated with very poor response to chemotherapy and consequently reduced survival. Recent and historical data have highlighted the importance of DNA damaging agents in the treatment of PDAC and in evolving the understanding of DDR and its role in response and resistance to therapy. Novel targets such as PARP are have shown early promising data in clinical trials in biomarker pre-selected (*BRCA* mutated) small subgroups. Targets such as DNA-PKcs which links DNA damage to pro-survival signalling represent strong targets for future developments. DDR pathway competency is an important consideration when considering DDR-targeting chemotherapy for the treatment of PDAC. As such, successful development of this therapeutic strategy will require implementation in the context of putative response biomarkers, particularly mutations in HR-related genes such as *ATM*. Identifying the functional drivers of PDAC within DDR pathways and matching these to treatment strategies offers hope for real progress in the treatment of PDAC. As with other cancers, these therapeutic developments are likely to lead to improvements in biomarker selected subsets rather than one encompassing overall strategy for all PDAC patients. The challenge thus remains in both biomarker selection and overcoming pathway-mediated resistance.

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