npj | precision oncology

Article

Published in partnership with The Hormel Institute, University of Minnesota

https://doi.org/10.1038/s41698-025-00800-4

Defective homologous recombination and genomic instability predict increased responsiveness to carbon ion radiotherapy in pancreatic cancer

Brock J. Sishc[1,2], Janapriya Saha[1], Elizabeth M. Alves[1], Lianghao Ding[1], Huiming Lu[1], Shih-Ya Wang[1], Katy L. Swancutt[1], James H. Nicholson[1], Angelica Facoetti[3], Arnold Pompos[1], Mario Ciocca[3], Todd A. Aguilera[1], Michael D. Story[1,4] ✉ & Anthony J. Davis[1] ✉

Pancreatic ductal adenocarcinoma (PDAC) is notably resistant to conventional chemotherapy and radiation treatment. However, clinical trials indicate that carbon ion radiotherapy (CIRT) with concurrent gemcitabine is effective for unresectable locally advanced PDAC. This study aimed to identify patient characteristics predictive of CIRT response. We utilized a panel of human PDAC cell lines with diverse genetic profiles to determine their sensitivity to CIRT compared to γ-rays, assessing relative biological effectiveness (RBE) at 10% survival, which ranged from 1.96 to 3.04. Increased radiosensitivity was linked to impaired DNA double-strand break (DSB) repair, particularly in cell lines with deficiencies in the homologous recombination (HR) repair pathway and/or elevated genomic instability from replication stress. Furthermore, pretreatment with the HR inhibitor B02 significantly enhanced CIRT sensitivity in a radioresistant PDAC cell line when irradiated in the spread-out Bragg peak but not at the entry position of the beam. These findings suggest that PDAC tumors with HR pathway mutations or high replication stress are more likely to benefit from CIRT while minimizing normal tissue toxicity.

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in both men and women in the United States (U.S.)[1]. The primary curative treatment is surgery for resectable disease, however, only 15–20% of PDAC patients are eligible for this treatment option due to the location of the tumor and disease stage[2]. Gemcitabine (GEM) is the only active single-agent chemotherapeutic that improves survival, however, combination chemotherapy with either folinic acid, fluorouracil, irinotecan, oxaliplatin (FOLFIRINOX), or albumin-bound paclitaxel (nab-PTX)/GEM resulted in greater survival impact for localized and metastatic disease PDAC[3–5]. Thus, these combination chemotherapies are now considered standard of care. Clinical studies have shown that a combination of chemotherapy and radiation can transition an unresectable PDAC to a resectable state in roughly 20% of cases. However, the role of radiotherapy in improving survival in localized disease remains to be demonstrated, likely due to inherent tumor radioresistance, lack of biological triage, and/or an insufficient total dose of ionizing radiation (IR)[6]. Despite improvements in treatment options, the 5-year overall survival remains low at just over 10%[7]. Thus, there is a great need for the development and validation of additional innovative, potent, and targeted/selective treatment approaches.

Heavy ion therapy, such as carbon ion radiotherapy (CIRT), could be a game changer for PDAC. Particle radiotherapy in general, and CIRT in particular encompass numerous physical and biological therapeutic advantages when compared to conventional radiotherapy and some chemotherapeutics. The physical advantages include the generation of a spread-out Bragg peak (SOBP) to focus the most damaging portion of a particle track inside the tumor, an enhanced dose distribution that more effectively spares nearby, at-risk structures, lateral beam focusing to improve field homogeneity, dose verification allowing for real-time treatment

[1]Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX, USA. [2]Mayo Clinic Florida, Jacksonville, FL, USA. [3]Medical Physics Unit & Research Department, CNAO National Center for Oncological Hadrontherapy, Pavia, Italy. [4]Present address: Mayo Clinic Florida, Jacksonville, FL, USA.
✉e-mail: story.michael2@mayo.edu; anthony.davis@utsouthwestern.edu

THE HORMEL INSTITUTE
UNIVERSITY OF MINNESOTA

modification, an increased linear energy transfer (LET) that causes more damage to the tumor cells, and finally the capability to magnetically steer the ion beam leading to a more precise dose delivery[8,9]. The biological advantages of CIRT include a higher relative biological effectiveness (RBE) damaging cells more effectively per unit of physical dose as compared to photons or protons, a reduced dependency on molecular oxygen, which results in a lower oxygen enhancement ratio (OER) thus creating more damage in a hypoxic tumor, and the generation of complex DNA damage which leads to more persistent stress and cell death[8–10]. Furthermore, a carbon ion beam offers an ideal energy distribution that induces a maximum ionizing effect at the site of the tumor and less damage to the surrounding normal tissue, leading to the prediction that CIRT will result in better tumor control with fewer side effects than conventional radiotherapy.

Clinical data has been encouraging; as a Phase I dose escalation study in unresectable PDAC by the Japanese Working Group for Pancreatic Cancer showed a 2-year survival rate of 48% for patients treated with 45.6–55.2 GyE (Gray equivalent) and concurrent gemcitabine[11]. A follow-up single-institution study demonstrated a 53% 2-year survival for patients that received 55.2 GyE[12]. Local control in this latter group was 82%, suggesting that CIRT is improving local control of later-stage disease that is not observed with conventional or hypofractionated X-ray radiotherapy[6,12,13].

Due to the concentrated energy deposition within a confined volume, carbon ions cause DNA damage of greater complexity. A special feature of this densely ionizing radiation is the induction of clustered DNA lesions, which is defined as two or more DNA lesions, such as DNA double-strand breaks (DSBs), single-strand breaks, or base damage, within one or two helical turns of the DNA[14,15]. Multiple studies have revealed that as LET increases, DNA repair slows as complex DNA lesions are more difficult to repair[16–18]. In response to clustered DNA damage, cells activate multiple DNA damage response (DDR) pathways. The most toxic of the CIRT-induced DNA lesions are DSBs, which if left unrepaired or misrepaired can result in genomic instability or cell death by a number of mechanisms including apoptosis, mitotic catastrophe, or senescence. DSBs are repaired by four pathways: homologous recombination (HR), non-homologous end joining (NHEJ), alternative end joining (alt-EJ), and single-strand annealing (SSA)[19]. Multiple studies have addressed the different contributions of the NHEJ and HR pathways to DSB repair according to the complexity of the DSBs generated, with HR being more important for the repair of high-LET than low-LET generated DNA lesions[20–22]. However, the contributions of the NHEJ and HR pathways to the repair of clinical carbon ion beam-induced DSBs have not been clarified in human PDAC cell lines.

Sequencing and chromosomal copy number variation analyses of PDAC revealed a complex genomic landscape[23,24]. Activating mutations of *KRAS* are near ubiquitous and inactivation of *TP53*, *SMAD4*, and *CDKN2A* occurs at rates of >50%. Genomic classification via patterns of variation in chromosome structure identified four subtypes of PDAC that were termed (i) stable, (ii) locally arranged, (iii) scattered, and (iv) unstable[24]. The unstable subtype accounts for 15–20% of human PDACs and the majority of these tumors harbor a mutation(s) in a gene required for the DDR, including *BRCA1*, *BRCA2*, *PALB2*, and *ATM*. Similarly, mutations in DDR genes are commonly found in inherited forms of PDAC[25,26]. DDR deficiency renders some tumors preferentially sensitive to DNA-damaging agents such as platinum agents and PARP inhibitors[27–29]. Additionally, DDR deficiency has been observed to be predictive of FOLFIRINOX (platinum-containing regimen) efficacy in PDAC[30]. Unfortunately, FOLFIRINOX is suitable only for patients with good performance status given the drug combination's toxicity. Moreover, the burden of morbidity, and even mortality, associated with platinum chemotherapy is a major challenge complicating the use of this chemotherapeutic regimen[30]. Therefore, it is hypothesized that a less toxic, tailored, and targeted therapy for PDACs with either somatic or germline mutations in DDR genes could be leveraged to improve treatment outcomes and the overall toxicity profile.

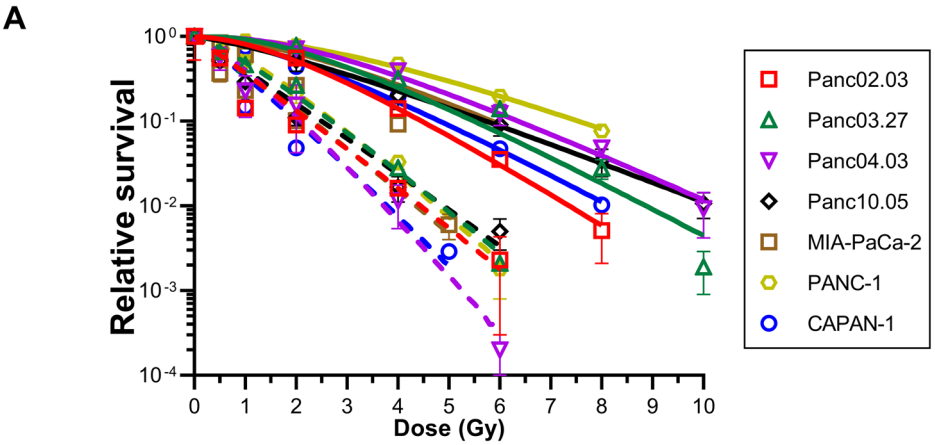
In this study, molecular mechanisms of CIRT were identified that impact the therapeutic response in PDAC cell lines. We investigated the contributions of the NHEJ and HR pathways to the repair of carbon ion-generated DNA lesions with the goal of identifying genomic features that can guide the treatment of pancreatic cancer and distinguish patients who would benefit the most from CIRT. We demonstrate that PDAC cell lines are more sensitive to CIRT than γ-rays and that this correlates with the burden of unrepaired DSBs. Furthermore, we found that the most sensitive cell lines are those with defects in the DDR. If we target a radioresistant PDAC cell line with the HR inhibitor, B02, there is an increased sensitivity to CIRT at the SOBP but not at the entry region of the depth-dose distribution in vitro, indicating an effect potentially driven by LET. Collectively, our data support the notion that PDAC patients or tumors with defects in DDR can benefit the greatest from CIRT. However, if a DDR defect is not identified then the HR pathway becomes a critical target to enhance response without complication of adverse normal tissue events.

Results

Differential response of PDAC cell lines to γ-ray and ^{12}C ion irradiation

PDAC has been shown to be effectively targeted by CIRT clinically[6,11,13]; however, it is unknown whether genetics affect the response to this treatment modality. To assess the impact of genetic variability, we examined the radiation response of a panel of seven human tumor-derived PDAC cell lines with varied genetic backgrounds to both γ-rays and CIRT. Each cell line contained a *KRAS* G12 mutation and additional mutation or deletion events in at least two of the other three commonly mutated genes (*TP53*, *CDKN2A*, and *SMAD4)* found in PDAC (Supplementary Fig. 1A). Furthermore, we noted various mutations of known or unknown significance in DNA damage response (DDR) genes and other putative oncogenic mutations (Supplementary Fig. 1B). Clonogenic survival curves were generated using ^{137}Cs for γ-ray (low LET) irradiation at UT Southwestern Medical Center (UTSW) in Dallas, TX and carbon ions (high LET) irradiation at the National Center for Oncological Radiotherapy (CNAO) in Pavia, Italy. As demonstrated in Fig. 1A, B, all cell lines were significantly more sensitive to carbon ions compared to γ-rays per unit physical dose (Gray/Gy), consistent with previous studies[31,32]. The surviving fraction at 2 Gy (SF2) to γ-rays ranged from 0.51 to 0.78 and at 3.5 Gy (SF3.5) from 0.20 to 0.51, indicating a broad range of inherent radioresponses. Alternatively, the SF2 values for carbon ions ranged from 0.09-0.23 and SF3.5 from 0.01 to 0.05, indicating much less cell-specific variability. Relative biological effectiveness (RBE) using 10% SF was estimated to be 1.96–3.04 and 2.38–3.53 when using the Mean Inactivation Dose (MID/$\bar{D}$)[33–35]. The most radioresistant cell lines to CIRT were PANC.03.27 and PANC-1 and the most radiosensitive cell lines were CAPAN-1 and PANC.04.03 (Fig. 1A, B).

The radioresponse to γ-ray and CIRT correlated with unrepaired DNA double-strand breaks (DSBs), as γ-H2AX (surrogate marker from DSBs) focus resolution was attenuated in a higher proportion in response to ^{12}C ions than γ-rays in the representative cell lines PANC.03.27, MIA-PaCa-2, and CAPAN-1 at 8- and 24-h post-IR (Fig. 1C). Moreover, the number of CIRT-induced γ-H2AX foci was significantly higher at 24 h in the known homologous recombination (HR)-defective cell line, CAPAN-1[36], compared to PANC.03.27 and MIA-PaCA-2 cells. The PANC.04.03 cell line had the highest RBE among the lines (Fig. 1A, B) likely due to its relative radioresistance to γ-rays, suggesting that this radioresistance was diminished when exposed to CIRT. Evaluation of the cBioPortal and COSMIC databases failed to identify a pathogenic mutation or deletion in a DDR gene in the PANC.04.03 cell line, indicating an unknown factor/phenotype is driving the increased sensitivity to CIRT. Clonogenic survival assays found that, unlike CAPAN-1, PANC.04.03 was not sensitive to treatment with the PARP inhibitor (PARPi) olaparib, indicating that this cell line is likely not HR-deficient (Fig. 2A)[36]. High γH2AX focus formation in G1 cells without exogenous stress indicates increased intrinsic replication stress[37]; therefore, we assessed if this was increased in PANC.04.03 cells. We found that PANC.04.03 has increased γH2AX foci in G1 cells without exogenous stress, suggesting that this cell line has elevated replication stress (Fig. 2B). Moreover, increased DNA damage in the PANC.04.03 cell line in the absence of treatment with an exogenous DNA damaging agent revealed that



B

PDAC Cell Line	γ-SF2	γ-SF3.5	^{12}C-SF2	^{12}C-SF3.5	$RBE_{SF10\%}$	$RBE_{\bar{D}\,AUC}$	p value
CAPAN-1	0.52	0.23	0.09	0.01	2.40	2.71	0.0120
MIA-PaCa-2	0.63	0.34	0.12	0.02	2.52	2.79	0.0161
PANC-1	0.78	0.51	0.23	0.04	2.76	2.88	0.0029
PANC.02.03	0.51	0.20	0.13	0.03	1.96	2.38	0.0115
PANC.03.27	0.68	0.33	0.23	0.05	2.04	2.43	0.0116
PANC.04.03	0.75	0.42	0.12	0.01	3.04	3.53	0.0088
PANC.10.05	0.53	0.29	0.18	0.04	2.29	2.50	0.0243

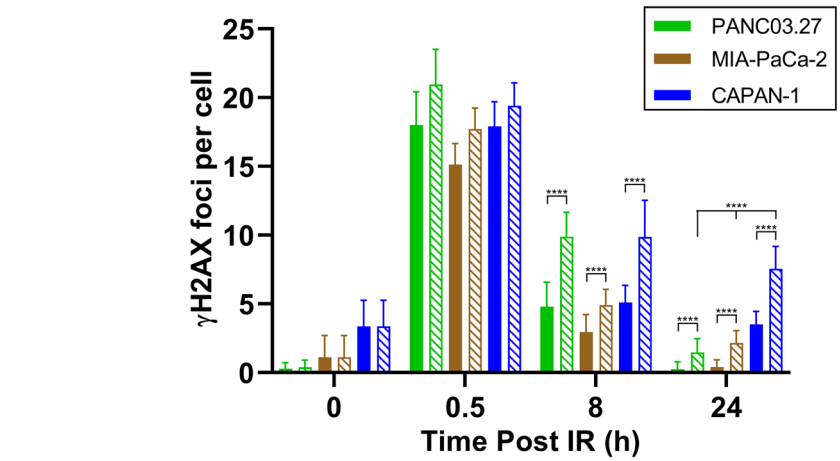


Fig. 1 | Response of PDAC cell lines to γ-ray and ^{12}C ion irradiation. A Clonogenic survival assays were performed to compare the radiation sensitivities of seven PDAC cell lines. Cells were irradiated at the indicated doses of γ-rays (solid) or carbon ions (dashed) and plated for analysis of survival and colony-forming ability. **B** Survival fraction (SF) at 2 Gy and 3.5 Gy in response to γ-rays and carbon ions. Relative biological effectiveness (RBE) is calculated using multiple methods. $RBE_{SF10\%}$, RBE calculated using 10% survival and RBE_{DAUC}, RBE calculated using mean inactivation dose derived from Reimann sum. Statistical comparisons (*p* values) of clonogenic survival curves of cells treated with γ-rays vs γ-rays were conducted using a student's *t*-test comparing the MID for each irradiation treatment. **C** Immunostaining of γH2AX foci in PANC03.27, MIA PaCA-2, and CAPAN-1 cells after exposure to 1 Gy of γ-rays (solid) or carbon ions (crosshatch). Cells were fixed at 0.5 h, 8 h, and 24 h after IR and immunostained for γH2AX foci. γH2AX foci were counted for each cell and averaged. Student's *t*-test (two-sided) was performed to assess statistical significance ($^{****}p < 0.0001$).

DNA-PKcs is autophosphorylated at serine 2056 and KAP1 and CHK2 are phosphorylated at serine 824 and threonine 68, respectively, in untreated cells (Fig. 2C, compare UT lanes). Finally, cells with increased replication stress are sensitive to ATR inhibition[27]. We found that PANC.04.03 cells are more sensitive to ATRi treatment than PANC.03.27 and MIA-PaCa-2, which further supports the notion that PANC.04.03 has elevated replication stress (Fig. 2D).

Non-homologous end joining and homologous recombination mediate the repair of ^{12}C ion-induced DSBs

The pathway or pathways integral to the repair of DSBs induced by therapy-relevant carbon ions in PDAC cell lines is still not clear. Thus, we aimed to determine if a specific DSB repair pathway is required for the repair of γ-ray and carbon ion-induced damage in PDAC cell lines. Specifically, we pretreated cells with either the DNA-PK_{cs} inhibitor NU7441, RAD51 inhibitor B02, or ATR inhibitor AZD6738 to inhibit the NHEJ, HR, and ATR-CHK1 pathways, respectively. Clonogenic survival assays show that inhibiting the NHEJ pathway resulted in a marked sensitivity to γ-rays in the PANC.03.27 (Fig. 3A, B) and MIA-PaCa2 (Fig. 3D, E) cells. No significant increase in radiosensitivity to γ-rays was observed when cells were pretreated with the RAD51 and ATR inhibitors (Fig. 3B, E). This observation correlated with unrepaired DNA double-strand breaks (DSBs), as treatment with the DNA-PK_{cs} inhibitor resulted in a significant increase in unrepaired DSBs as monitored by γH2AX foci resolution at 8- and 24-h post-IR (Fig. 3C, F). Next, we assessed clonogenic survival in response to CIRT with the three inhibitors. As shown in Fig. 3A, C, treatment with either the DNA-PK_{cs}, RAD51, or ATR inhibitor resulted in increased radiosensitivity to ^{12}C ions, with significant cell killing in response to pretreatment with the DNA-PK_{cs} and RAD51 inhibitors (Fig. 3B, E). Similar to the γ-ray data, the increased radiosensitivity correlated with unrepaired DSBs (Fig. 3C, F). Moreover, we assessed if ATM inhibition affected the response to CIRT. We found that pretreatment with the ATM inhibitor KU55933 resulted in a significant radiosensitization in PANC.03.27, MIA-PaCa-2, and PANC.04.03 (Supplementary Fig. 3A–C). We also examined if alt-EJ played a role in response to CIRT-induced DNA damage by examining if pretreatment with an inhibitor to PARP (olaparib) affects radiosensitization to CIRT in the PANC.03.27 cell line. We observed that treatment with olaparib did not result in increased radiosensitization to carbon ions (Supplementary