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Inhibition of p300 increases cytotoxicity of cisplatin in pancreatic cancer cells

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Abstract

Pancreatic cancer is a notoriously deadly disease with a five-year survival rate around 10 percent. Since early detection of these tumors is difficult, pancreatic cancers are often diagnosed at advanced stages. At this point, genotoxic chemotherapeutics can be used to manage tumor growth. However, side effects of these drugs are severe, limiting the amount of treatment that can be given and resulting in sub-optimal dosing. Thus, there is an urgent need to identify chemo-sensitizing agents that can lower the effective dose of genotoxic agents and as a result reduce the side effects. Here, we use transformed and non-transformed pancreatic cell lines to evaluate DNA repair inhibitors as chemo-sensitizing agents. We used a novel next generation sequencing approach to demonstrate that pancreatic cancer cells have a reduced ability to faithfully repair DNA damage. We then determine the extent that two DNA repair inhibitors (CCS1477, a small molecule inhibitor of p300, and ART558, a small molecule inhibitor of polymerase theta) can exploit this repair deficiency to make pancreatic cancer cells more sensitive to cisplatin, a commonly used genotoxic chemotherapeutic. Immunofluorescence microscopy and cell viability assays show that CCS1477 delayed repair and significantly sensitized pancreatic cancer cells to cisplatin. The

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Taylor Bugbee: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Mary Gathoni:** Investigation. **Carlie Payne:** Investigation. **Morgan Blubaugh:** Investigation. **Kaydn Matlock:** Investigation. **Taylor Wixson:** Investigation. **Andrea Lu:** Software, Investigation, Data curation. **Steven Stancic:** Software, Investigation, Data curation. **Peter A. Chung:** Validation, Investigation, Resources. **Rachel Palinski:** Methodology, Software, Validation, Formal analysis, Data curation, Visualization. **Nicholas Wallace:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2023.147762.

increased toxicity was not seen in a non-transformed pancreatic cell line. We also found that while ART558 sensitizes pancreatic cancer cells to cisplatin, it also sensitized non-transformed pancreatic cancer cells.

Keywords

P300; DNA repair; Pancreatic cancer; Synthetic lethality; Alternative end joining

1. Introduction

Management of pancreatic cancer remains extremely challenging and aggressive treatment options are often used. Surgical resection is commonly followed by radiation or chemotherapy. Despite these approaches and significant resources being employed to improve outcomes, the average 5-year survival rate for people with pancreatic cancer remains under 10% (Torphy et al., 2020; Neoptolemos et al., 2018; Cao et al., 2021; Yang et al., 2016). One of the reasons why patient outcomes are so poor is that severe side effects from chemotherapy often prevent optimal dosing (Oun et al., 2018). As a result, there is great interest in developing sensitizing agents that could make these drugs work at lower and generally less toxic concentrations. For this strategy to be successful, the sensitizing agent needs to specifically target changes present in transformed pancreatic cells that are absent in non-transformed cells.

Manipulation of the DNA repair processes that protect cells against genomic instability are a way to sensitize tumors to genotoxic chemotherapies. Genomic instability is an established hallmark of cancer and is typically caused by defects in DNA repair processes (Yoshioka et al., 2021; Roos et al., 2016; Kiwerska and Szyfter, 2019; Arce et al., 2019; Roos and Kaina, 2013; Alberg et al., 2013). The DNA repair response consists of a myriad of pathways, each dedicated to repairing a specific type of DNA damage, often during certain parts of the cell cycle. When the flaws in DNA repair can be identified in tumors, they can be targeted by small molecule inhibitors (that exacerbate the flaw in the pathway or impede back up responses), genotoxic agents that cause the type of damage the impaired pathway is dedicated to fixing, or a combination of these approaches (O'Connor, 2015; Hopkins et al., 2022; Perkhofer et al., 2021). The ideal scenario is to identify two drugs that can be given at concentrations that are not toxic to non-transformed cells but are highly toxic to a transformed cell. This relationship is known as synthetic lethality. The therapeutic targeting of DNA repair pathways and the use of DNA repair inhibitors to create synthetic lethalities has been successful in the clinic and remains an active area of research (Setton et al., 2021; Guo et al., 2011; Evers et al., 2010; Wallace, 2020; Koniaras et al., 2001). BRCA1/2 mutations and PARP1 inhibition is a common synthetic lethality that is being targeted in breast, ovarian, and pancreatic cancers (Lord and Ashworth, 2017; Farmer et al., 2005; McCabe et al., 2006; Kaufman et al., 2015; Golan et al., 2019; Donawho et al., 2007; Balmaña et al., 2014). A problem that is being faced in pancreatic cancers is resistance to PARPi; which leaves a need for other synthetic lethalities (Pant et al., 2019; Mateo et al., 2019).

While there is an abundance of genotoxic chemotherapeutics, the work in this paper focuses on cisplatin, a platinum-based DNA cross-linking agent that kills cells by inducing highly lethal double strand breaks in DNA (DSBs) (Ghosh, 2019). Similarly, there are a multitude of DNA repair factors that can be inhibited. However, the mechanism of action of cisplatin focused our attention on repair factors that were required for DSB repair. DSBs can be repaired by a multiple DNA repair pathways and DSB repair inhibitors that target a single pathway or work more broadly exist.

We have previously shown that the histone acetyltransferase p300 is essential for multiple DSB repair pathways (Howie et al., 2011; Dacus and Wallace, 2021). Inhibition of p300 via small molecule inhibitor, genetic knockout or knockdown, or viral mediated destabilization attenuates multiple repair pathways dedicated to preventing or repairing DSBs (Hu et al., 2020; Hu et al., 2022; Hu et al., 2023; Snow et al., 2019). The work described here uses a small molecule inhibitor of p300, CCS1477, to impair DNA repair. A growing number of reports have found that the repair of DSBs by a commonly yet rarely used repair mechanism (alternative end joining or Alt-EJ) is more common in tumor cells (Seol et al., 2018; Sallmyr and Tomkinson, 2018; Ceccaldi et al., 2015; Wood and Doublié, 2016; Lemée et al., 2010; Drzewiecka et al., 2022; Kawamura et al., 2004). We also evaluate ART558, a small molecular inhibitor of an essential Alt-EJ factor; polymerase theta (Wood and Doublié, 2016; Drzewiecka et al., 2022). The work presented in the following sections describes in more detail our rationale for suspecting that CCS1477 and ART558 would be able to augment the cytotoxicity of exposure to low concentrations of cisplatin. We provide strong *in vitro* evidence that this is the case for both inhibitors, but that only CCS1447 caused increased cytotoxicity, specifically in pancreatic cancer cells.

2. Materials and methods

2.1. Cell culture and reagents

HTERT HPNE (immortalized pancreatic duct epithelial cell line) cells (ATCC) were grown in 75% DMEM and 25% 3 M base supplemented with puromycin, epidermal growth factor (Corning), glucose, and 5% FBS. Capan-1(ATCC,) cells (pancreatic adenocarcinoma cell line) were grown in IMDM (ATCC) supplemented with 20% FBS (Lee et al., 2003; Fogh et al., 1977). Panc-1 cells (ATCC) (pancreatic epithelioid carcinoma cell line) were grown in DMEM (ATCC) supplemented with 10% FBS (Lieber et al., 1975). Cisplatin (Sigma-Aldrich) was used as our genotoxic chemotherapy to induce DNA damage. CCS1477 (Chemietek) was used to inhibit p300. ART558 (MedChem Express) was used to inhibit polθ activity. Alt-EJ plasmids (#113619, #113620, #113625, Addgene) were used to measure Alt-EJ efficiency (Bhargava et al., 2018; Tsai et al., 2020). DSB repair mutations assay plasmids (#JS825, Addgene) were used to induce DSB for sequencing (Hu et al., 2022; Hu et al., 2022).

2.2. Transfection

HTERT HPNE and Capan-1 cells were plated in 3 mL of complete growth medium in a 6 cm plate. Cells were used at 80% confluency. Two μg of plasmids were diluted in 300 μL Opti-MEM (Gibco). 10 μL Lipofectamine (Thermo Fisher) was added to 300 μL Opti-MEM

and incubated at room temperature for 5 min. The diluted Lipofectamine mixture was added to the plasmids and incubated at room temperature for 25 min. The transfection mixture was added to each well drop-wise and incubated for 6 hr at 37 °C. The transfection mixture was removed and replaced with 3 mL of complete growth media and incubated for 48 hr. Cells were harvested for DNA extraction and sequencing or flow cytometry (for Alt-EJ reporter).

2.3. Next-generation sequencing

The transfection protocol listed above was used to transfect HTERT HPNE and Capan-1 cells with the Cas9/SgRNA plasmids. A total of 42 primer sets were used to cover 0.1 Mb on each side of the Cas9 target site to produce an overlapping amplicon. Genomic DNA was extracted using MagAttract High Molecular Weight DNA kit (Qiagen). Target regions were amplified using primer pools coupled with KAPA HiFi Hotstart readymix (KAPA Biosystems) using 20 μM primers, 50/53 °C annealing temperature, and a 5-minute extension time. Highprep PCR cleanup system (Magbio) was used to remove primers from amplicons. Libraries were prepared from amplicons with Nextra XT DNA library preparation kit (Illumina) and sequenced on Nextseq 500 system. More detailed methods are available in previous publications (Hu et al., 2022; Hu et al., 2022).

2.4. Sequencing analyses

Mutation rates were identified as previously described (Hu et al., 2022; Hu et al., 2022). Briefly, raw reads were trimmed and mapped in CLC genomics workbench v21.0. Trimmed reads were normalized to transfection efficiency. Normalized reads were assessed for indels and structural variants and normalized for paired read variations in CLC Genomics Workbench v 20.0.4 (Qiagen). Next generation sequencing of mock transfected cells was used as a reference for determining mutations. Only mutations that did not exist in mock transfected cells were reported. More detailed methods are available in previous publications (Hu et al., 2022; Hu et al., 2022).

2.5. Immunofluorescence microscopy

Cells were seeded onto 96-well glass-bottom plates (Cellvis) and grown overnight. Cells treated with cisplatin and CCS1477 for the specified time and concentration were fixed with 4% formaldehyde. Then, cells were permeabilized with 0.1% Triton-X solution in PBS, followed by blocking with 3% bovine serum albumin in PBS for 30 min. Cells were then incubated with the pH2AX antibody (Cell Signaling). The cells were washed and stained with Alexa Fluor 594 goat anti-rabbit (Thermo Scientific A11012). After washing, the cells were stained with 30 μM DAPI in PBS and visualized with the Lionheart FX: Automated - Live Cell Microscope. Images were analyzed using ImageJ. For more details, please see our methods paper describing this approach (Murthy et al., 2018).

2.6. Cell viability assay

10,000 Capan-1 cells/well, 3,000 HERT HPNE cells/well, and 5,000 Panc-1 cells/well were seeded on a 96-well plate and grown for 24 hr. Treatments of CCS1477/ART558 either alone or combined with 5 μM or 10 μM cisplatin were added to cells with indicated doses of inhibitor (CCS1477 or ART558) and incubated for 48 hr at 37 °C.

Forty-eight hours after treatment, 10 μL/well of MTT solution (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, 10 mg/mL MTT dissolved in PBS) was added for 24 hr. Subsequently, wells were incubated with 100 μL solubilization solution (990 mL DI water, 10 mL 1 M HCL, and 100 g SDS) for 24 hr and the optical density was measured at 640 nm. Doses for CCS1477: 0, 10, 20, 40 μM and ARTT558: 0, 1, 5, 25 μM.

2.7. Alt-EJ reporter assay

Alt-EJ activity was determined using a previously described reporter assay (Lieber et al., 1975; Bhargava et al., 2018) and as previously described (Hu et al., 2023). 48 h after reporter plasmid (Alt-EJ reporter using 4 nt microhomology) transfection, cells were harvested using trypsinization (Gibco). Cells were washed with PBS and fixed with 95% cold ethanol for 20 min at −20 °C. Samples were resuspended into 500 μL PBS. Samples were analyzed by a BD Accuri C6 Plus Flow Cytometer.

2.8. Statistical analysis

All values are represented as mean ± standard error (SE). Statistical differences between groups were measured by using Student's *t*-test. p-Values in all experiments were considered significant at less than 0.05.

3. Results

3.1. Pancreatic cancer Increases mutations during DNA repair

Genomic instability is an established hallmark of tumorigenesis, with tumors frequently acquiring mutations because of their reduced ability to repair DNA damage compared to non-transformed cells (Yoshioka et al., 2021; Roos et al., 2016; Kiwerska and Szyfter, 2019; Arce et al., 2019; Roos and Kaina, 2013; Alberg et al., 2013). To compare the DNA repair capablities between transformed and non-transformed pancreatic cells, we used HTERT HPNE as a model of non-transformed pancreatic cells and Capan-1 as a model of transformed pancreatic cells. To measure DNA repair capabilities, we used a Cas9 system to induce a DSB at a Cas9 site located on chromosome 12 and used next-generation sequencing to quantify the mutations associated with DNA damage repair (Hu et al., 2022; Hu et al., 2022). We transfected HTERT HPNE (non-transformed) and Capan-1 (transformed) pancreatic cell lines with Cas9 and sgRNA that induced a DSB at the at a CAS9 on chromosome 12 (Fig. 1A). A series of overlapping primers that target the 100 kb region upstream and downstream of the CAS9 target site were pooled and produced amplicons used in the sequencing. The raw reads were trimmed, mapped to the reference sequence, and assessed for mutations. This demonstrated that DSB repair was over 7-fold more mutagenic in Capan-1 cells compared to HTERT HPNE cells (Fig. 1B). We further analyzed the types of mutations that were increased and found that insertions, deletions, replacements, single nucleotide variations (SNV), and multi-nucleotide variation (MNV) were each more common in Capan-1 cells. We interpret these data as evidence that DSB repair fidelity is reduced in Capan-1 cells.

3.2. p300 inhibition delays DNA repair and Increases cytotoxicity

The therapeutic window for genotoxic agents (e.g., cisplatin) is often relatively small because of dose limiting side effects stemming from the non-specific nature of the DNA damage induced by these treatments. To address this issue, DNA repair inhibitors can be used to reduce the amount of genotoxin needed to kill transformed cells. This is especially true when the DNA repair inhibitor augments an existing defect(s) in the transformed cells. We hypothesized that p300 inhibition would specifically sensitize Capan-1 cells and Panc-1 cells to concentrations of cisplatin that are not typically toxic to cells for two reasons. First, the increase in mutations associated with DSB repair in Capan-1 cells indicates that these cells inherently have a reduced ability to faithfully repair DSBs. Second, our published work demonstrates that a small molecule inhibitor of p300 (CCS1477) broadly impairs DSB repair (Hu et al., 2022; Hu et al., 2023). To begin testing this hypothesis, we determined if CCS1477 could delay the repair of cisplatin-induced DSBs using immunofluorescence (IF) microscopy to detect a standard marker of DSBs (γH2AX foci). These lesions were detected 24, 48, and 72 h after HTERT HPNE, Capan-1, and Panc-1 cells were exposed to cisplatin (5 or 10 μM) for 24 h and then grown in media containing 10 μM CCS1477 or equal volume DMSO, the solvent for CCS1477 (Fig. 2A–F). Cisplatin and CCS1477 concentrations were determined experimentally by identifying concentrations that left ~ 80% of the cells viable. CCS1477 delayed resolution of γH2AX foci in Capan-1 cells and Panc-1 cells, but not in HTERT HPNE cells (Fig. 2A–D). This indicates that CCS1477 selectively delays the repair of cisplatin-induced DSBs in at least two transformed pancreatic cancer cell line.

Since CCS1477 only delayed DSB repair in Capan-1 and Panc-1 cells, we hypothesized that CCS1477 would increase the cytotoxicity of low concentrations of cisplatin specifically in those cell lines. To test this, we treated HTERT HPNE, Capan-1, and Panc-1 cells with CCS1477 with or without cisplatin for 48 h and used MTT to measure cell viability. We found that neither 5 μM cisplatin nor as much as 40 μM of CCS1477 caused significant decreases in HTERT HPNE cell survival (Fig. 3A). This was also true when HTERT HPNE cells were simultaneously exposed to cisplatin and CCS1477. Likewise, neither exposure to a range (10–40 μM) of CCS1477 concentrations nor 5 μM cisplatin for Capan-1 cells and 10 μM cisplatin for Panc-1 cells were significantly toxic when cells were exposed to the drugs individually (Fig. 3B–C). However, we observed a significant increase in cell death when concentrations of CCS1477 that minimally impacted cell growth (10 μM) were combined with equally modest concentrations of cisplatin. IC50 values based on these data were notably lower for CCS14477 and Cisplatin in combination in pancreatic cancer cells compared to immortalized pancreatic cells (Supplemental Table 1). Together these data suggest that the combination of CCS1477 and cisplatin is synthetically lethal in the context of at least two types of pancreatic cancer cells.

3.3. CCS1477 Increases usage of Alt-EJ but inhibiting Alt-EJ is not a viable therapeutic target

We have previously shown that CCS1477 caused primary foreskin keratinocytes to become more reliant on a rarely used and mutagenic DSB repair pathway, known as alternative end joining or Alt-EJ (Farmer et al., 2005). We hypothesized that inhibition of p300 would result in an increased use of Alt-EJ in Capan-1 cells. To test this hypothesis, we used an

established Alt-EJ reporter assay where a 46 nt insertion disrupts a GFP open reading frame (Pant et al., 2019). Cas9 endonuclease and sgRNA are used to induce a DSB upstream and a DSB downstream of the insertion. The use of the 4 nt microhomology as a template to repair the damage restores GFP expression. Capan-1 and HTERT HPNE cell lines were transiently transfected with the reporter and Cas9s. Both growth media containing either 10 μM CCS1477 or DMSO was added to cells 6 h after transfection. After another 48 h, the GFP signal was measured using flow cytometry (Fig. 4B–E). In HTERT HPNE, CCS1477 did not significantly alter the frequency of DSB repair by Alt-EJ. In contrast, CCS1477 increased repair by Alt-EJ in Capan-1 cells (Fig. 4B–E). These data indicate that the ability of CCS1477 to induce Alt-EJ is at least somewhat cell type specific. (Please note that direct comparisons of Alt-EJ frequency between cell lines is not possible because the impact of CCS1477 was not done in parallel.).

Since CCS1477 only increased the usage of Alt-EJ in Capan-1 cells, we hypothesized that an Alt-EJ inhibitor, ART558, could also be used to increase the cytotoxicity of cisplatin in Capan-1 cells. To test this hypothesis, we treated Capan-1 and HTERT HPNE cells with ART558 (0–25 μM) with and without cisplatin (5 μM) and used MTT assays to measure cell viability 48 h after exposure. We found that ART558 sensitized both pancreatic cell lines to sub-lethal doses of cisplatin (Fig. 5A–B). These data show a synthetic lethal relationship between ART558 and cisplatin. However, the synthetic lethality was not specific to transformed Capan-1 cells.

4. Discussion

DNA repair inhibition is an attractive chemo-sensitization strategy that is particularly relevant for hard-to-treat cancer types such as pancreatic cancer. The work described here makes several advances in this research area. Specifically, we used a novel combination of targeted next generation sequencing and a CAS9-directed DSB to demonstrate that DSB repair is over 7 times more mutagenic in transformed (Capan-1) compared to non-transformed (HTERT HPNE) pancreatic cells (Fig. 1). We demonstrated that DSB repair can be further and specifically impaired in Capan-1 cells and Panc-1 cells by CCS1477, a small molecule inhibitor of p300 (Fig. 2). We determined that 5 μM cisplatin and CCS1477 were individually minimally toxic to Capan-1 and HTERT HPNE cells, and 10 μM cisplatin and CCS1477 were minimally toxic to Panc-1 cells. The combination of these drugs remained minimally toxic in HTERT HPNE cells but killed more than half of the exposed Capan-1 and Panc-1 cells. Capan-1 and Panc-1 are cell lines derived from different pancreatic cancers and contain different driver mutations (Deer et al., 2010). Using cell lines with different genetic make-ups allowed us to illustrate that breadth of the potential of CCS1477 as a cisplatin sensitizing agent. We also demonstrated that while Capan-1 cells had a greater tendency to use Alt-EJ to repair DSBs than HTERT HPNE cells, inhibition of the pathway did not selectively sensitize Capan-1 cells cancer cells to 5 μM cisplatin.

CCS1477 is currently under evaluation for the treatment of metastatic castration-resistance prostate cancer in a phase 1/2 clinical trial (NCT03568656). The rationale for this trial is to target the role of p300 in androgen signaling (Welti et al., 2021; Eickhoff et al., 2022). CCS1477′s ability to bind and inhibit p300 is validated in the original manuscript that

characterizes CCS1477 (Welti et al., 2021). However, our data suggest that the potential utility of CCS1477 as a p300 inhibition is broader, including use as a chemo-sensitizing agent that could widen the therapeutic window of genotoxic drugs for care in pancreatic and perhaps other cancer types. However, limitations in our study have created significant knowledge gaps that need to be addressed. Our study only utilized MTT assays to show synthetic lethality. Because MTT assays measure metabolic activity as a surrogate for viability, our results could be improved by additional assays to measure other aspects of toxicity. A colony forming assay, for instance, would assess the ability of pancreatic cancer cells to go through multiple rounds of replication after treatment. Alternatively, there are luminescence-based viability assays that measure other aspects of metabolism that could be more or less responsive to CCS1477 and Cisplatin associated toxicity. Specifically, our data did not determine if CCS1477 was similarly able to sensitize other cancer cell lines to low concentrations of cisplatin. We also did not explore the cell death mechanism of the combined treatment of cisplatin and CCS1477. Likewise, to the best of our knowledge, *in vivo* animal models have not been used to test the chemo-sensitization capability of CCS1477.

Alternative end joining (Alt-EJ), also known as microhomology mediated end joining, is a recently discovered repair mechanism that is actively being characterized (Seol et al., 2018; Sallmyr and Tomkinson, 2018; Wood and Doublié, 2016). We have previously shown that, in foreskin-derived keratinocytes, p300 is a repressor of Alt-EJ. In this work, we were able to replicate this result in Capan-1 cells. However, the role of p300 in regulating Alt-EJ is more nuanced as we also demonstrated that p300 inhibition did not promote the use of Alt-EJ in HTERT HPNE cells. Thus, our data suggest that the ability of p300 to repress Alt-EJ is cell type specific. Further efforts are needed to identify the breadth of cell types where p300 negatively regulates Alt-EJ. However, the observation that p300 maintains this role in largely different cell types (pancreatic cancer cells and non-transformed keratinocytes) suggests that p300 maintains this ability in a wide range of cells and independent of the transformation status of the cell line. However, the observation that p300 did not negatively regulate Alt-EJ in both cell lines derived from pancreatic tissue suggests that more research is necessary to fully understand the role of p300 in regulating Alt-EJ. Given the expanding evidence that Alt-EJ is more commonly used for the repair of DSBs in transformed cells (Ceccaldi et al., 2015; Wood and Doublié, 2016; Lemée et al., 2010; Drzewiecka et al., 2022; Kawamura et al., 2004), understanding how the pathway is regulated and the extent that manipulating this regulation can sensitize cells to DNA damaging agents is important. An immediate next step from our work would be to determine if the combination of CCS1477, ART558, and cisplatin is synthetically lethal in Capan-1 (and ideally not in HTERT HPNE) cells.

5. Conclusions

The work described here demonstrates the leverage to control tumor cell growth that can be gained by understanding the molecular details of cancer cell biology as it relates to non-transformed cell biology. Here, we apply these principles to an *in vitro* model of pancreatic cancer to identify a specific synthetically lethal combination of a chemotherapeutic and a small molecule inhibitor that inhibits repair of DNA damage. Using this combination therapy led to persistent DNA damage in pancreatic cancer cells and not in non-transformed

cells. This delay in repair translated to increased cytotoxicity of chemotherapy specifically in pancreatic cancer cells. However, these principles can be applied to other targets as well as other tumor types.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

The data presented in this study are available in the NCBI GEO Dataset database with accession numbers SRR24111648, SRR24111649, SSR24111650, SRR24111651, SRR24111652, SRR24111653, SRR24111654.

Data availability

I have supplied the instructions to access the data at the end of the manuscript.

Abbreviations:

DSB	double stranded breaks
Alt-EJ	alternative end joining
IF	Immunofluorescence
μM	micromolar
GFP	green fluorescent protein
Nt	nucleotide

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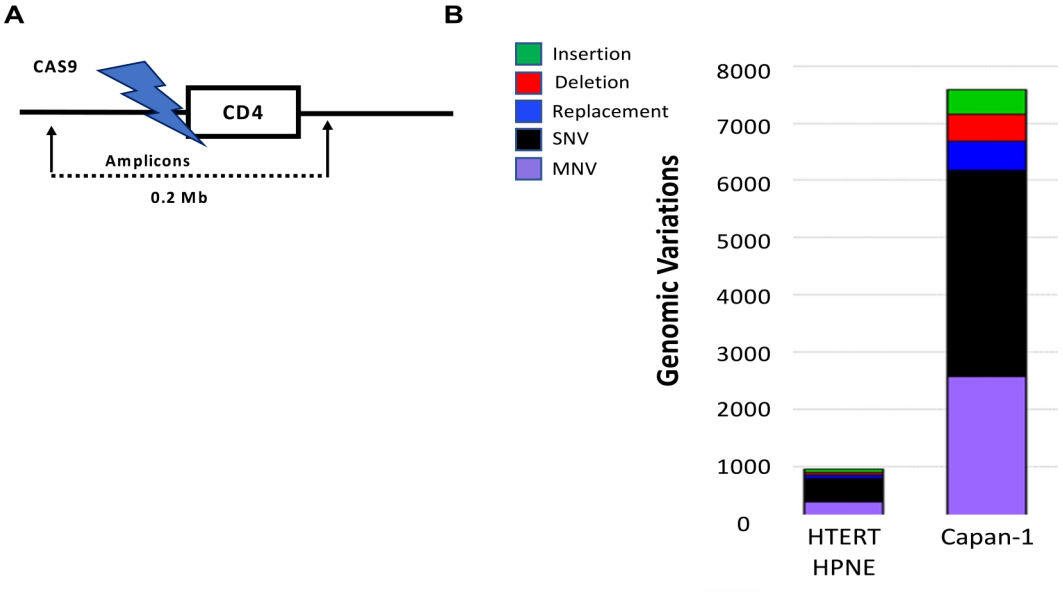

Fig. 1.

Capan-1 has Increased Mutagenic Events During DSB Repair. (**A**) Schematic of the placement of CAS9 induced DSB along the sequenced portion of the genome. (**B**) Genomic variations grouped by types of mutational events in HTERT HPNE and Capan-1. Insertion, deletion, replacement, SNV, and MNV events were compared between HTERT HPNE and Capan-1 cells.

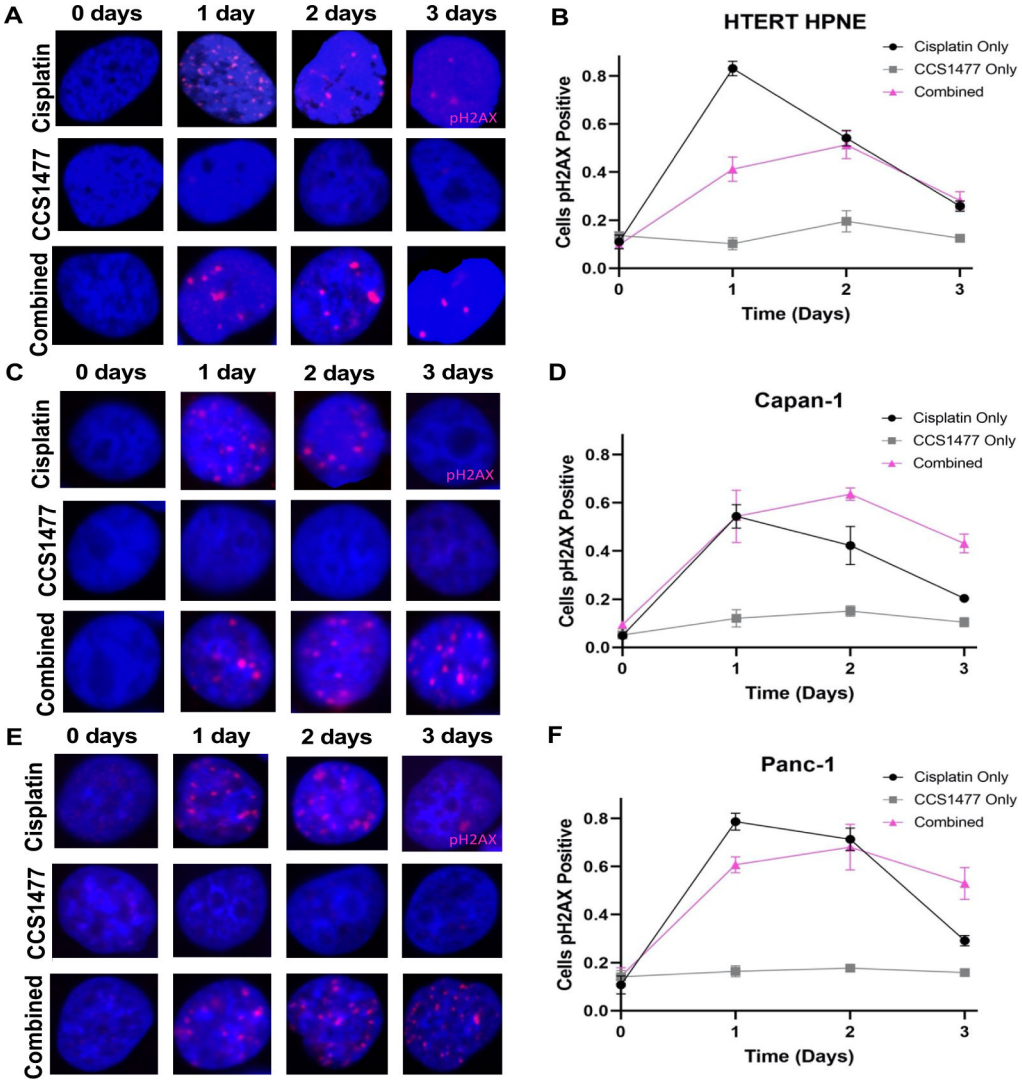

Fig. 2.

CCS1477 Increases Persistent DSB. (**A**) Representative IF images of pH2AX foci in HTERT HPNE cells treated with 5uM Cisplatin, 10uM CCS1477, or combined treatment of cisplatin and CCS1477 taken 0, 1, 2, or 3 days after treatment. (**B**) Fraction of HTERT HPNE cells positive for pH2AX foci following either cisplatin, CCS1477, or combined treatment. (**C**) Representative IF images of pH2AX foci in Capan-1 cells treated with 5uM Cisplatin, 10uM CCS1477, or combined treatment of cisplatin and CCS1477 0, 1, 2, or 3 days after treatment. (**D**) Fraction of Capan-1 cells positive for pH2AX foci following either cisplatin, CCS1477, or combined treatment. (**E**) Representative IF images of pH2AX foci in Panc-1 cells treated with 10uM Cisplatin, 10 uM CCS1477, or combined treatment 0, 1, 2, or 3 days after treatment. (**F**) Fraction of Panc-1 cells positive for pH2AX foci following treatment.

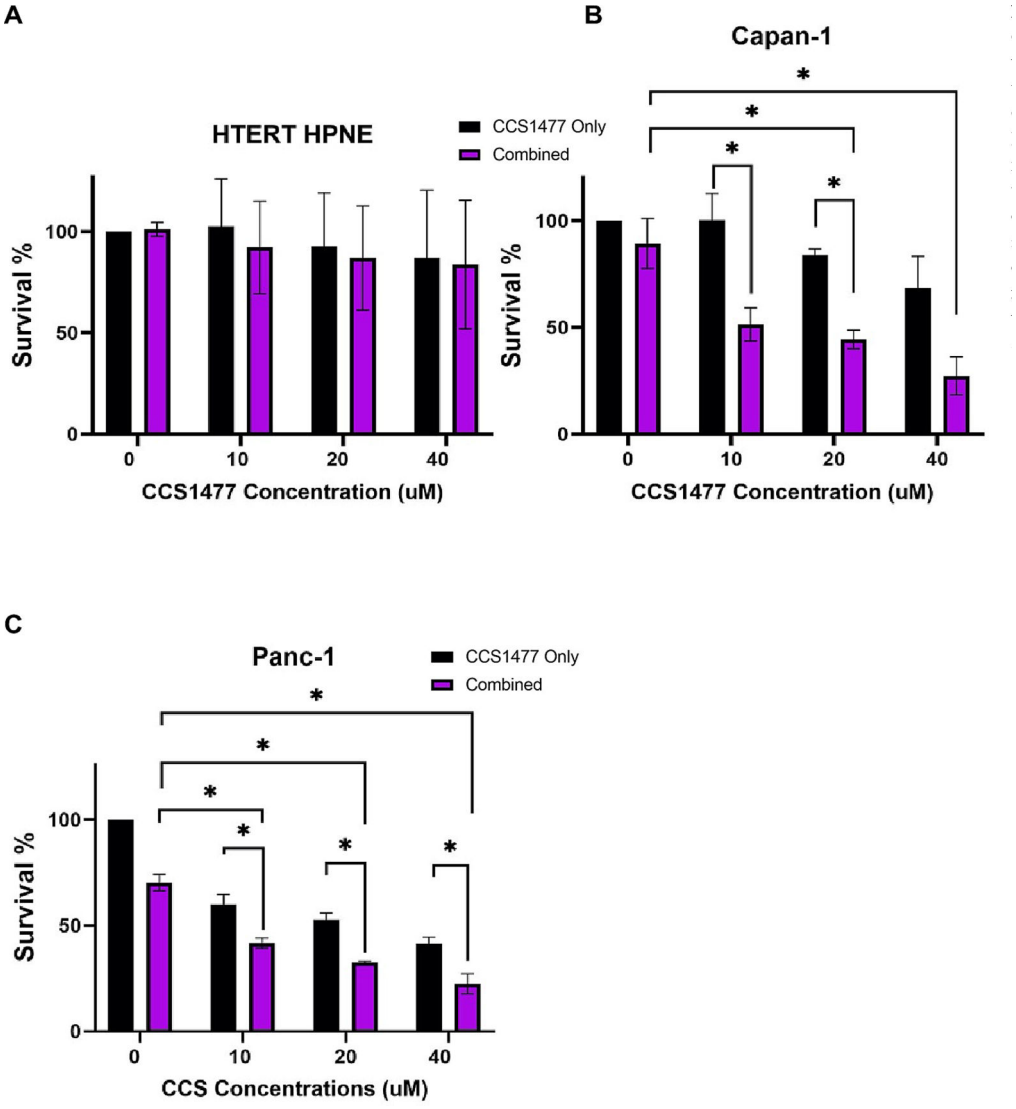

Fig. 3.

CCS1477 Increases Cytotoxicity of Cisplatin Treatment. (**A**) Relative cell viability of HTERT HPNE cells following treatments with different concentrations of CCS1477 or 5 uM cisplatin and CCS1477. (**B**) Relative cell viability of Capan-1 cells following treatments with different concentrations of CS1477 alone or with 5 uM cisplatin. All values are represented as mean ± standard error. The statistical significance of differences between cell lines were determined using Student's *t*-test. * represents p-value < 0.05. Each viability experiment had 3 independent replicates.

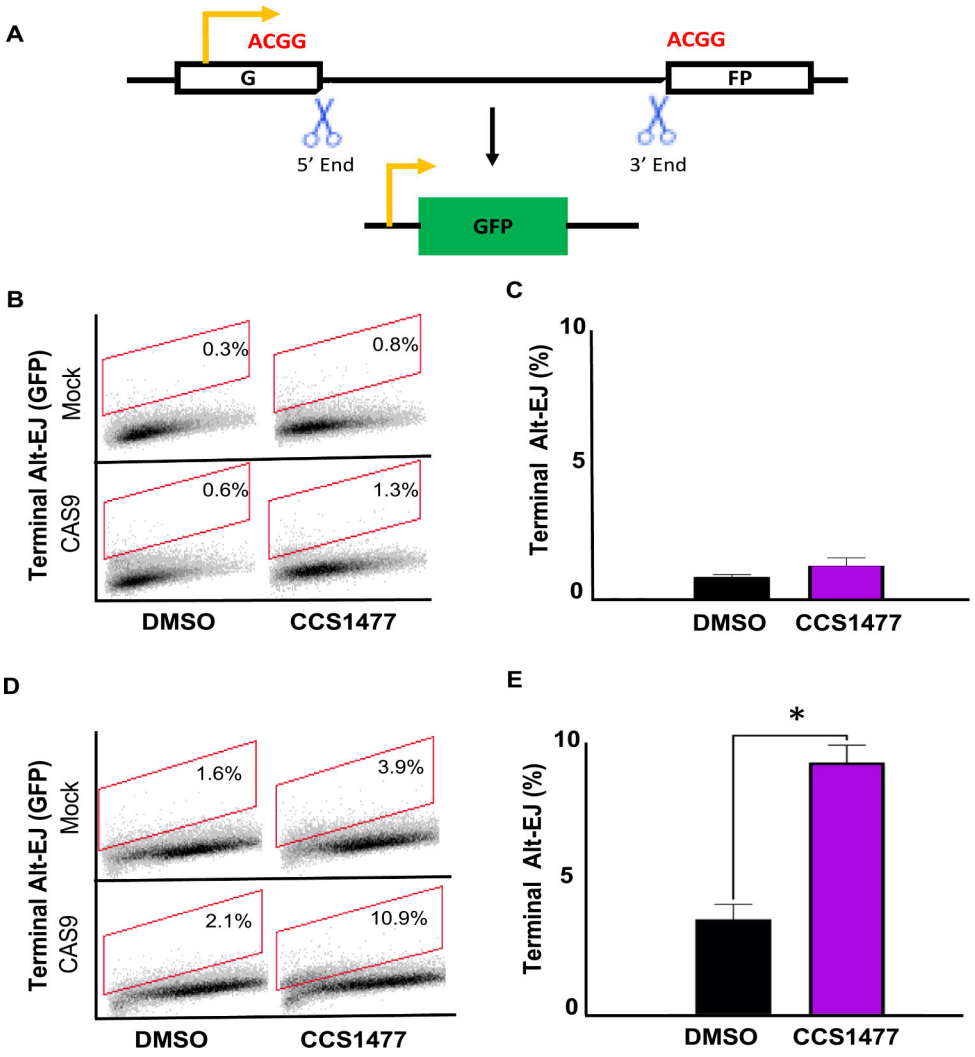

Fig. 4.

CCS1477 Increases Alt-EJ. (**A**) Schematic of Alt-EJ reporter assay. A microhomology-mediated Alt-EJ event will result in a restored GFP signal. (**B**) Representative images of flow cytometry results of HTERT HPNE cells that are GFP positive 48 hr after transfection with terminal Alt-EJ and treatment with DMSO or 10 uM CCS1477. The gating represents GFP positive based off mock transfected control. (**C**) Percent HTERT HPNE cells GFP positive following transfection with Alt-EJ reporter and treatment with DMSO or CCS1477 determined by flowcytometry. (**D**) Representative images of flow cytometry results of HTERT HPNE cells that are GFP positive 48 hr after transfection with terminal Alt-EJ and treatment with DMSO or 10 uM CCS1477. (**E**) Percent HTERT HPNE cells GFP positive following transfection with Alt-EJ reporter and treatment with DMSO or CCS1477 determined by flowcytometry. All values are represented as mean ± standard error. The statistical significance of differences between cell lines were determined using Student's *t*-test. * represents p-value < 0.05. 20,000 cells were measured for each independent flow cytometry experiment (n = 3).

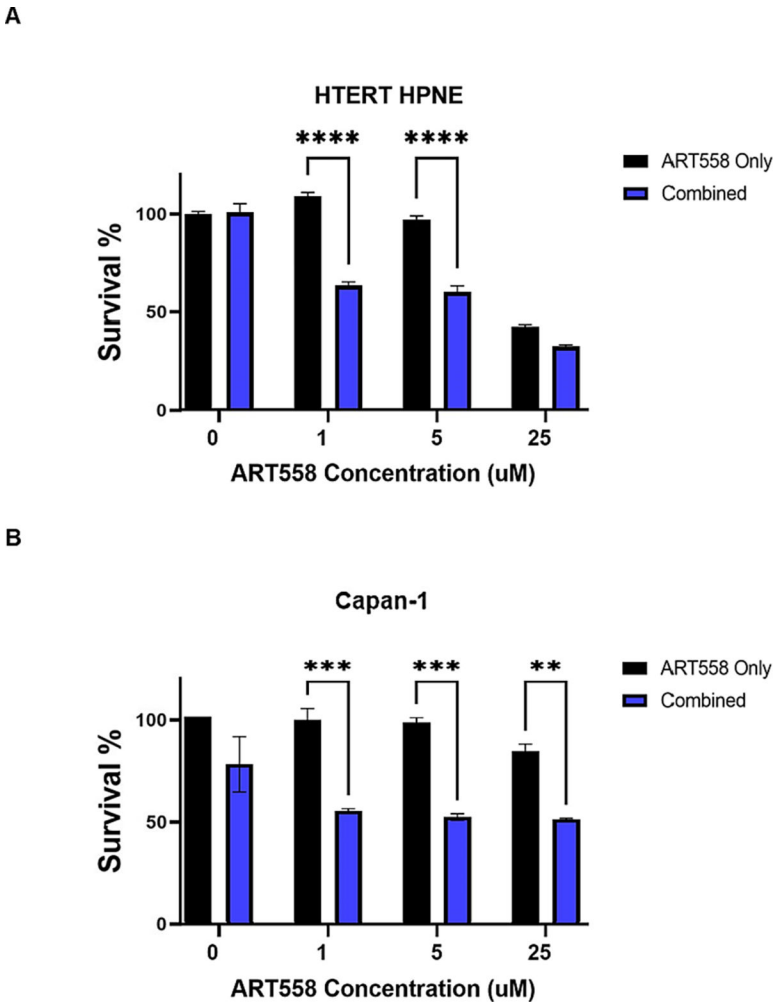

Fig. 5.

Inhibition of Alt-EJ does not selectively kill pancreatic cancer cells. (**A**) Relative cell viability of HTERT HPNE cells following treatments with different concentrations of ART558 or 5 uM cisplatin and ART558. (**B**) Relative cell viability of Capan-1 cells following treatments with different concentrations of ART558 alone or with 5 uM cisplatin. All values are represented as mean ± standard error. The statistical significance of differences between cell lines were determined using Student's *t*-test. * *represents p-value < 0.01, *** represents p-value < 0.001, **** represents p-value < 0.0001. Each viability experiment had 3 independent repeats.