Research paper

The BET inhibitor JQ1 attenuates double-strand break repair and sensitizes models of pancreatic ductal adenocarcinoma to PARP inhibitors

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Abstract

Background: DNA repair deficiency accumulates DNA damage and sensitizes tumor cells to PARP inhibitors (PARPi). Based on our observation that the BET inhibitor JQ1 increases levels of DNA damage, we evaluated the efficacy of JQ1 + the PARPi olaparib in preclinical models of pancreatic ductal adenocarcinoma (PDAC). We also addressed the mechanism by which JQ1 increased DNA damage.

Methods: The effect of JQ1 + olaparib on in vivo tumor growth was assessed with patient-derived xenograft (PDX) models of PDAC. Changes in protein expression were detected by immunohistochemistry and immunoblot. In vitro growth inhibition and mechanistic studies were done using alamarBlue, qRT-PCR, immunoblot, immunofluorescence, ChIP, and shRNA knockdown assays.

Findings: Tumors exposed in vivo to JQ1 had higher levels of the DNA damage marker γH2AX than tumors exposed to vehicle only. Increases in γH2AX was concomitant with decreased expression of DNA repair proteins Ku80 and RAD51, and shRNA data showed that expression of Ku80 and RAD51 was BRD4- and BRD2-dependent in PDAC cell lines. Interpretation: The data are consistent with the hypothesis that JQ1 confers a repair deficient phenotype and the consequent accumulation of DNA damage sensitizes PDAC cells to PARPi. Combinations of BET inhibitors with PARPi may provide a novel strategy for treating PDAC.

Fund: NIH grants R01CA208272 and R21CA205501; UAB CMB T32 predoctoral training grant.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, accounting for ~45,000 deaths annually in the United States [1]. Despite the use of aggressive chemotherapeutic regimens such as FOLFIRINOX, which supports a median survival of 11 months, the 5-year survival for patients with PDAC has remained at ~7% for the last 40 years [1,2]. Recently the bromodomain and extraterminal domain (BET) family of proteins has been investigated as a potentially effective therapeutic target for treating PDAC tumors. The four members of this family of proteins (BRD2, BRD3, BRD4, BRDT) function as scaffolds for the recruitment of transcriptional activators to promoter or super enhancer loci of genes whose transcription is regulated by RNA polymerase II [3]. BET proteins BRD2 and BRD3 promote PDAC cell proliferation and growth, likely by modulating the activity of members of the Gli family of transcription factors [4]. BRD4 promotes PDAC cell proliferation by affecting expression of proteins of the sonic hedgehog pathway [5]. Current literature indicates that JQ1 inhibits BET protein function by binding to the domain of BET that interacts directly with acetylated lysine residues on specific histones, thereby decreasing expression of proteins that rely on BET-dependent...
The BET bromodomain proteins are transcriptional regulators that control expression of genes that contribute to tumor progression. We and others showed that pharmacological inhibition of this family of proteins suppresses tumor growth of models of several tumor types. However, even in preclinical models, BET inhibitors as single agents do not achieve durable responses. Three published studies assessed the efficacy of a BET inhibitor in combination with a PARP inhibitor. These studies reported synergy with this combination in homologous recombination (HR)-proficient ovarian, breast, and prostate cancer cells in vitro. The combination also decreased tumor progression in xenograft models of ovarian and breast cancer.

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Added value of this study

This study investigated the effectiveness of combining the BET inhibitor JQ1 with a PARP inhibitor in several in vitro and in vivo pancreatic ductal adenocarcinoma (PDAC) models. Data in this report are the first to: 1) show synergy in vitro and efficacy in vivo (p < .001) of a BET inhibitor + a PARP inhibitor using models of PDAC; 2) demonstrate that this combination inhibits (p < .001) progression of independently derived, KRAS mutated patient-derived xenograft (PDX) models at nontoxic doses equivalent to those tolerated clinically; 3) document that JQ1 inhibits expression of not only the HR DNA repair protein RAD51 but also the non-homologous end joining (NHEJ) repair protein Ku80 in PDAC cells in vitro and tumors in vivo; 4) provide mechanistic data to confirm that expression of RAD51 and Ku80 is BRD2- and BRD4-dependent using ChiP and shRNA studies in PDAC cells. The work provides mechanistic and efficacy data that support assessing this combination in clinical trials for patients with PDAC.

Implications of all the available evidence

Together the data strongly suggest that combinations of BET + PARP inhibitors merit further evaluation for treatment of solid tumors. Our in vivo work suggests further that this combination may be particularly effective for treating PDAC.

Research in context

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2. Materials and methods

2.1. Ethics statement

Animal protocols were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC-09186 and IACUC-20569).

2.2. Cell lines and compounds

Panc1, BxPC3, and MiaPaCa2 pancreatic cancer cells were purchased from the American Type Culture Collection (Manassa, VA, USA). Cells were cultured under recommended conditions. All cell lines were authenticated by short tandem repeat DNA profiling at the UAB Hepfin Center for Genomic Science (Birmingham, AL, USA). All pancreatic cancer cell lines used were tested for mycoplasma using MycoAlertTM PLUS Mycoplasma Detection Kit (Lonza, Walkersville, MD, USA) and results were negative. Olaparib (HY-10162, MedChem Express, Monmouth Junction, NJ, USA), JQ1 (a generous gift from Dr. Bradner; HY-13030, MedChem Express), and veliparib (a generous gift from Dr. Yang; ABT-888, Enzo Life Sciences, Farmingdale, NY, USA) were dissolved in DMSO.

2.3. Immunoblot analysis

Cell and tumor lysates were prepared in NP-40 lysis buffer with protease inhibitor cocktail or Cell Lysis Buffer (Cell signaling, Danvers, MA, USA) respectively. Analysis of lysates was carried out as previously described [7]. Primary antibodies used were: γH2AX (Cell Signaling), RAD51 (abcam, Cambridge, MA, USA), Ku80 (Bethyl Laboratories, Montgomery, TX, USA), BRD4 (Cell Signaling), BRD2 (Cell Signaling), vinculin (Santa Cruz Biotech, Dallas, TX, USA), GAPDH (Cell Signaling), β-actin (Cell Signaling), α-Tubulin (Cell Signaling), p21 (Cell Signaling), Cleaved PARP (Cell Signaling). Blots were quantitated using ImageStudio Lite (LI-COR Biosciences, Lincoln, NE, USA).

2.4. Immunohistochemistry

Immunohistochemistry staining was performed as previously described [7]. Ki67 and γH2AX indices: 10 random high magnification fields were taken, positive tumor cells counted and divided by the total number of tumor cells in two independent experiments. Expression indices were calculated by assessing each field of stained tumor tissue, and assigning a staining intensity (0, 1, 2, 3) to a given percent of tumor cells. The percentage was multiplied by the staining intensity and then these values were added to calculate a score between 0 and 300. Primary antibodies: Ki67 (abcam), γH2AX (Cell Signaling), cleaved caspase 3 (Cell Signaling), RAD51 (abcam), Ku80 (Bethyl Laboratories).

2.5. Histological analysis

Histological analysis was performed as previously described [7]. Photomicrographs were taken on an Olympus BH-2 microscope with DP71 camera and DPS-BSW v3.1 software (Center Valley, PA, USA).
2.6. In vitro cell viability assay

Cell viability assays were carried out as previously described [7]. Briefly, cells were seeded in 96 well plates. Serial dilutions of JQ1 and/or PARPi were added to the culture medium for 72 h. AlamarBlue reagent was applied according to the directions of the manufacturer, and fluorescence read on a Victor X5 microplate reader at 590 nm.

2.7. In vivo inhibition of tumor growth

Female 4-week old SCID CB 17/–/– mice were purchased from Taconic Farms (Newton, MA, USA) and housed in the AAALAC accredited vivarium at UAB Research Support Building. Development and characterization of the PDAC patient-derived xenografts (UAB-Pa4 and UAB-Pa16) have been previously described [18]. Mice bearing bilateral tumors were randomized into four groups of 5 mice/group (except vehicle control for UAB-Pa4 [4 mice/group]) when tumors reached ~200 mm³. Mice received intraperitoneal injections of vehicle (10% DMSO in 10% β-cyclodextrin x 2 injections), 50 mg/kg JQ1, 50 mg/kg olaparib, or the combination (50 mg/kg olaparib followed by 50 mg/kg JQ1) daily for 21 days. Drugs were prepared as instructed by the manufacturer. Tumor size was measured every other day using digital calipers, and tumor volume calculated using the formula v = (π/6) πd³. Twenty-four hours following final treatment mice were euthanized. Tumor tissue was harvested, formalin fixed and paraffin embedded or snap frozen in liquid nitrogen for further analysis. All values are presented as mean ± S.E.M. Tumor volumes were compared using two-way analysis of variance (ANOVA) using GraphPad Prism (version 7).

2.8. Immunofluorescence

Cells were seeded in chamber slides, and treated for 24 h. Cells were fixed in 10% NBF for 10 min, and permeabilized with 0.01% Triton X-100 for 10 min. Non-specific antibody binding was minimized by blocking in 2% BSA for 1 h. Cells were then incubated at 4 °C overnight with antibody anti-γH2AX (MilliporeSigma, Burlington, MA, USA) or anti-RAD51 (abcam). Cells were incubated with secondary antibody Alexa Fluor goat anti-rabbit (or -mouse) 488 (Fisher Scientific, Waltham, MA, USA) for 1 h at room temperature. Coverslips were mounted using ProLong Gold Antifade Reagent with DAPI (Cell Signaling). Immunofluorescent staining was visualized using the Zeiss Axio Observer Z1 microscope with the Zen 2011 Blue imaging software.

2.9. Clonogenic assays

Panc1 cells were plated at 200 cells/well in 24 well plates. Cells were then exposed to DMSO, JQ1 (1 μM), olaparib (1 μM), or the combination for 72 h. Drug-containing media was then removed, cells washed with PBS and overlaid with drug-free medium. On day 14, cells were fixed with 10% NBF and stained with 0.025% crystal violet. Cell groupings comprised of at least 50 cells were considered colonies. Two independent experiments were performed.

2.10. RNA isolation and qRT-PCR

Total RNA was isolated using TRIzol-chloroform extraction. qRT-PCR analysis was performed as previously described [7]. Primers used are listed in Table S1.

2.11. Chromatin immunoprecipitation (ChIP)

ChIP was performed using the SimpleChIP Plus Kit (Cell Signaling). Digested cellular chromatin was immunoprecipitated with a ChIP grade anti-BRD4 or anti-BRD2 antibody (Cell Signaling) or normal Rabbit IgG (Cell Signaling) used as the negative control. Levels of DNA sequences that co-precipitated with BRD4 or BRD2 were quantitated using qRT-PCR with primers that flanked regions of the Ku80 and RAD51 promoter loci marked by H3K27 acetylation. Data were analyzed relative to the percent input (2%) and then normalized to DMSO control. A minimum of two independent experiments were performed. Primers used are listed in Table S1.

2.12. Vectors and cell transfectants

BxPC3 and Panc1 cells were transfected with MISSION shRNA (MilliporeSigma) targeted for BRD4, BRD2 or the control shRNA for GFP (Addgene, Watertown, MA, USA) using PEI (Polysciences Inc., Warrington, VA, USA). After 8 h transfection, media was removed and regular medium was added and cells were allowed to recover for an additional 72 h. Stable transfectants were selected with media containing 10 μg/ml of puromycin (Enzo Life Sciences). The shRNA oligos used are listed in Table S1.

2.13. Statistical analysis

All statistical analyses were performed using GraphPad Prism 7 (San Diego, CA, USA). Unless otherwise specified statistical significance was calculated using the one-way ANOVA. p < .05 was considered significant.

3. Results

3.1. JQ1 increased the level of the DNA damage marker γH2AX in PDAC cells and tumors

BET proteins are essential for chromatin remodeling during the DNA damage response, and inhibition of BET protein function modifies chromatin structure and induces cell cycle arrest [3,10,19]. We hypothesized that BET inhibition would also minimize DNA repair. We addressed this hypothesis by exposing PDAC cells to the BET inhibitor JQ1 in vitro or in vivo and assessed levels of DNA damage as reflected by protein level and number of cells with detectable foci of the phosphorylated Ser139 variant of histone H2AX (γH2AX), an early cellular response to DNA strand breaks and a marker for DNA damage [20]. Immunofluorescence (IF) and immunoblot (IB) data using an antibody specific for γH2AX demonstrated that JQ1 increased the level of γH2AX in vitro and in vivo (Fig. 1). IF data demonstrated JQ1 increased γH2AX foci in BxPC3 PDAC cells, in a dose-dependent manner (p < .05, one-way ANOVA) (Fig. 1a). IB data showed that the increase in γH2AX levels was not unique to BxPC3 cells, as JQ1 also increased γH2AX in Panc1 and MiaPaCa2 PDAC cells in a dose-dependent manner (Fig. 1b, c).

In vivo data were consistent with in vitro results. To first confirm that JQ1 increased DNA damage in vivo as well as in vitro at efficacious tolerated doses [7], we immunostained tumor tissue harvested 24 h following termination of treatment of vehicle control (VC) mice or mice treated with JQ1 50 mg/kg daily for 21 or 28 days, for levels of γH2AX (Fig. 1d). Results are presented as percent of tumor cells positive for γH2AX, and demonstrate that JQ1 in vivo increased γH2AX levels in UAB-Pa3, -Pa4, -Pa10, and -Pa30 tumors (Fig. 1e). The data show the novel observation that JQ1 accumulated DNA damage in vivo (p < .001, Student's t-test) as well as in vitro (p < .05, one-way ANOVA) in this tumor type.

3.2. JQ1 inhibited expression of DNA repair proteins Ku80 and RAD51 in PDAC cells

The BET protein BRD4, one of the principal targets of JQ1, facilitates gene expression and recruits DNA repair components such as 53BP1 to sites of DNA double-strand breaks to facilitate repair [11]. We hypothesized that the observed JQ1–induced increase in γH2AX, might not only regulate recruitment of repair components to sites of damaged DNA but might also decrease expression of specific DNA repair genes.
Double-strand break repair is accomplished by non-homologous end joining (NHEJ) and homologous recombination (HR) pathways [21]. Among DNA repair proteins involved in NHEJ and HR pathways, Ku80 and RAD51 are reported to be overexpressed in several tumor types including pancreatic cancer [22–25]. In addition, data from the web-portal UALCAN indicates that patients having tumors that express relatively high levels of Ku80 and RAD51 have a shorter duration of survival [26]. We first characterized expression of Ku80 and RAD51 in the primary tumors from which two models used in this study were derived: UAB-PA4 and UAB-PA16. Consistent with reports in the literature, each primary PDAC tumor expressed higher levels of the NHEJ protein Ku80 (2.6- to 3.4-fold) and HR protein RAD51 (1.7- to 1.8-fold) than normal pancreas (Fig. 2a) [22–25]. We then assessed the impact of JQ1 on expression of Ku80 and RAD51 in PDAC cell lines and in preclinical models derived from these two tumors.

qRT-PCR data demonstrate that a 48h exposure in vitro to JQ1 decreased mRNA levels of Ku80 and RAD51 by 40–78% in BxPC3 and Panc1 cells, compared to DMSO controls (p < .05, p < .01, two-way ANOVA) (Fig. 2b). Immunoblot (IB) analyses corroborate qRT-PCR data.
data and demonstrate that JQ1 reduced Ku80 and RAD51 protein levels in these cell lines (Fig. 2c), in a dose-dependent manner (Fig. 2d).

3.3. JQ1 + olaparib suppressed tumor growth in patient-derived xenograft (PDX) models of PDAC in vivo

Our data show that JQ1 increases the level of γH2AX and decreases expression of DNA repair proteins Ku80 and RAD51 (Fig. 1, 2). PARP inhibitors (PARPi) are selectively cytotoxic to cells deficient in double-strand DNA repair or to cells treated with DNA damaging agents [27,28]. We hypothesized that JQ1 would sensitize PDAC cells to PARPi and that JQ1 + the PARPi olaparib would have a greater than additive anti-proliferative effect. Olaparib has efficacy as a single agent and is approved for use in BRCA mutated ovarian cancer [29,30].

We first evaluated the efficacy of JQ1 + olaparib in PDAC PDX models UAB-PA4 and UAB-PA16. Both models harbor a G12D mutation in codon 12 of the KRAS gene, as is common in primary PDAC tumors. The TNM (tumor, nodes, metastasis) classification was pT3N0 and
Fig. 3. JQ1 + olaparib suppressed tumor growth, and decreased the expression of proliferation marker Ki67 in both UAB-PA4 and -PA16 patient-derived xenograft models of PDAC. Mice bearing bilateral tumors ~100–200 mm³ in size were randomized to four groups of 5 mice/group (except VC for UAB-PA4 [n = 4]). Mice were treated with vehicle control (VC), JQ1 (50 mg/kg), olaparib (50 mg/kg) or JQ1 (50 mg/kg) + olaparib (50 mg/kg) daily for 21 days. Data for UAB-PA4 tumors represent average tumor volume ± S.E.M. for 8 (VC, olaparib) or 10 (JQ1, JQ1 + olaparib) tumors/group. Data for UAB-PA16 tumors represent average ± S.E.M. for 9 (VC, olaparib) or 10 (JQ1, JQ1 + olaparib) tumors/group. We excluded tumors that did not grow. Average tumor volume (a, e; all values are presented as mean ± S.E.M.) for UAB-PA4 or -PA16 were calculated as described in Materials and Methods. Two-way ANOVA was performed (*p < .05, **p < .01, ****p < .0001). (b, f) Final tumor volume was analyzed by Student’s t-test (*p < .05, **p < .01, ***p < .001, ****p < .0001). (c, g) Tumor tissue was harvested 24 h after completion of treatment, H&E staining done to visualize morphology, and immunostaining done to detect the proliferation marker Ki67. Scale bar = 10 μm. (d, h) IHC data for Ki67 were quantitated as the number of Ki67 positive tumor cells divided by total number of tumor cells and are reported as bar graphs mean ± S.E.M and analyzed using one-way ANOVA (*p < .05, ****p < .0001).
Both proteins by up to ~95% in UAB-PA4 and -PA16 tumor models. Olaparib as a single agent inhibited growth of UAB-PA16 tumors (p < .01, two-way ANOVA) but did not inhibit growth of UAB-PA4 tumors (Fig. 3a, e). JQ1 as a single agent suppressed tumor growth in both models, compared to vehicle control (p < .0001, two-way ANOVA). The combination of JQ1 + olaparib was more effective than either drug alone in both UAB-PA4 (Fig. 3a, b) and -PA16 (Fig. 3e, f) PDX models. All mice in all treatment groups had consistent body weights (Fig. S1). Tumors were harvested at completion of therapy and archived for immunostaining and immunoblot analyses.

The observed inhibition of tumor growth was consistent with immunostaining data for the proliferation marker Ki67 (Fig. 3c, g). Ki67 levels in UAB-PA4 and -PA16 tumors exposed to JQ1 were lower than in tumors exposed to vehicle control (VC) (p < .0001, one-way ANOVA) (Fig. 3d, h). JQ1 + olaparib decreased Ki67 expression more than JQ1 or olaparib alone in both models. The data are consistent with tumor volume data and indicate that the combination was more effective than either drug as a single agent in inhibiting tumor cell proliferation in vivo.

3.4. JQ1 + olaparib decreased expression of DNA repair proteins Ku80 and RAD51 in vivo

Tumor tissue harvested at completion of efficacy studies were then immunoblotted (IB) or immunostained (IHC) to assess the effect of JQ1 ± olaparib on γH2AX, Ku80, and RAD51. IB data supported previous data showing that JQ1 increased levels of the DNA damage marker γH2AX 7- to 17-fold, and that JQ1 + olaparib increased γH2AX by 7.5- to 33-fold compared to vehicle controls (VC) in both PDX models (Fig. 4a, b). IHC data also show that exposure to JQ1 or JQ1 + olaparib in vivo increased the percent of γH2AX positive cells compared to VC (p < .05, one-way ANOVA) or to olaparib (p < .001, one-way ANOVA), and increased the apoptosis marker cleaved caspase 3 compared to VC or olaparib alone (p < .01, one-way ANOVA) (Fig. 4c-4f). Further, IB data demonstrated that JQ1 decreased expression of the NHEJ repair protein Ku80 and the HR DNA repair protein RAD51 by ~45-80%, and the combination of JQ1 + olaparib decreased in vivo expression of both proteins by up to ~95% in UAB-PA4 and -PA16 tumor models (Fig. 4a, 4b). IHC data corroborated these IB data (Fig. 4c-4f). The data suggest that decreased levels of DNA double-strand break repair proteins Ku80 and RAD51 lead to accumulation of DNA damage and increased apoptotic index, as reflected by the increase in cleaved caspase 3 (Fig. 4).

3.5. Combinations of JQ1 + PARPi exerted synergistic cytotoxicity in PDAC cell lines

The observed efficacy of JQ1 + olaparib in PDAC PDX models were consistent with the original hypothesis that JQ1-induced accumulation of DNA damage would augment the anti-proliferative effects of PARPi. To address potential mechanistic aspects of the complementary activity of these two agents, we first confirmed that similar augmentation of efficacy was evident using in vitro models. We exposed BxPC3 or Panc1 PDAC cells to a range of concentrations of JQ1 (0.1-100 μM) plus the indicated fixed concentration of PARPi for 72 h and assessed cell viability by alamarBlue assay. In the presence of the PARPi veliparib, the IC50 of JQ1 decreased by ~18-fold in BxPC3 (Fig. 5a). Similarly, the PARPi olaparib decreased the IC50 of JQ1 by ~6-fold in Panc1 cells (Fig. 5b). If data were also consistent with in vivo data, exposure of BxPC3 cells to a fixed ratio of JQ1:3.5 μM + veliparib10 μM for 24 h increased levels of the DNA damage marker γH2AX, compared to JQ1 alone (p < .01, one-way ANOVA) (Fig. 5c). Further, we assessed the effect of JQ1 + olaparib (1:1 ratio) on colony formation, using Panc1 cells (Fig. 5d). Panc1 cells were exposed to drug(s) for 72 h, incubated for an additional 11 days in the absence of drug, and colonies counted on day 14. Notably, the anti-proliferative effect of the combination remained evident after a week in drug-free medium (p < .01, one-way ANOVA). These results suggest that it may be useful to compare the efficacy JQ1 + olaparib given on an intermittent schedule, rather than basing in vivo schedules of administration predominantly on the relatively short half-life (t1/2) of JQ1.

To facilitate calculation of combination indices (CI), we exposed BxPC3 and Panc1 cell lines to a fixed ratio (1:1) of JQ1 + veliparib or olaparib for 72 h and assessed cell viability with alamarBlue assays. CI values demonstrate that both combinations were synergistic in BxPC3 and Panc1 cell lines (Fig. 5e).

As above, both synergistic combinations were associated with an increase in γH2AX level in BxPC3 cells (Fig. 5f, c). Further, JQ1 decreased Ku80 and RAD51 expression and JQ1 + PARPi augmented this down-regulation. JQ1 + olaparib also increased p21 (CDKN1A) expression which is induced by DNA damage (Fig. 5f) [31]. Comparable data were obtained with Panc1 and MiaPaCa2 cells (Fig. S2).

3.6. JQ1 prevented formation of RAD51 foci in BxPC3 cells

Recruitment of RAD51 to sites of DNA damage is a critical step in HR repair, with increases in RAD51 foci formation reflecting levels of HR DNA double-strand break repair. To assess the effect of JQ1 on HR repair in cells, we exposed BxPC3 cells to 20 μM JQ1 for 24 h to increase DNA damage, and detect RAD51 foci using FITC-labeled anti-RAD51 (Fig. 5g). HR competent cells would be predicted to have more detectable foci, as RAD51 would be recruited to sites undergoing repair. As expected, JQ1 decreased RAD51 foci formation in BxPC3 cells (p < .01, Student’s t-test). Olaparib was used as a positive control since inhibition of PARP1 causes replication-associated DNA double-strand breaks which are predominantly repaired by the HR pathway [12,13,32].

3.7. Expression of Ku80 and RAD51 was BET-dependent

JQ1 associates with 96% of genetic loci to which BRD2 binds and with 99% of loci to which BRD4 binds, indicating that JQ1 targets BRD2 and BRD4 [33]. Current literature suggests that BRD4-associated mechanisms regulate expression of unique subsets of genes in different cell types. Specific gene products that depend on BRD4 or BRD2 for expression in pancreatic cancer cells have not been investigated. Data above suggest that in PDAC cells, BRD4 or BRD2 regulate expression of Ku80 and RAD51 and, conversely, that inhibition of either of these BET proteins decreases Ku80 and RAD51 expression. We addressed this hypothesis directly using chromatin immunoprecipitation (ChIP) assays to assess the occupancy of BRD4 and BRD2 on the promoter sequences of Ku80 and RAD51 in the presence and absence of JQ1 (Fig. 6a, b). The data demonstrate that JQ1 decreased the association of BRD4 with promoters of Ku80 and RAD51 by ~40% and ~63%, respectively, compared to DMSO controls, in BxPC3 cells (Fig. 6a), and decreased the association of Ku80 and RAD51 with the BRD4 promoters in Panc1 cells (Fig. 6b).
BRD2 with promoters of Ku80 and RAD51 by ~48% and ~55%, respectively in Panc1 cells (Fig. 6b).

To corroborate these data indicating that expression of these DNA repair enzymes Ku80 and RAD51 was BRD4- or BRD2-dependent in PDAC cells, we down-regulated BRD4 or BRD2 expression using two different short hairpin RNAs (shBRD4–1, shBRD4–2, shBRD2–1, shBRD2–2) and evaluated the effect of the direct decrease in BRD4 or BRD2 expression on Ku80 and RAD51 proteins. As shown in Fig. 6c, when the shBRD4–1 construct downregulated BRD4 expression by 53%, the level of Ku80 protein was decreased by 35% and RAD51 by 93% in BxPC3 cells. When BRD2 is down-regulated by 82% in Panc1 cells, the expression of Ku80 and RAD51 was decreased by 69% and
93% respectively (Fig. 6d). Similar results were obtained with the second set of shRNA's (Fig. S3). The data were consistent with Chip data, and we concluded that in PDAC cells, Ku80 and RAD51 expression was BRD4- and BRD2-dependent.

The data demonstrate that transcriptional repression of BRD4 or BRD2 by shRNA and pharmacological inhibition of BET protein activity by JQ1 decreased expression of DNA repair proteins Ku80 and RAD51 in PDAC cells. The data suggest that this inhibition may contribute to the synergy of JQ1 + PARPi in this tumor cell type.

In summary, JQ1 decreased expression of DNA repair proteins Ku80 and RAD51, induced apoptosis, facilitated an increase in levels of DNA damage, exerted synergistic cytotoxicity in vitro when combined with the PARPi veliparib or olaparib, and suppressed tumor growth in two PDX models of PDAC. Mechanistic data suggest that inhibition of BET-dependent expression of the HR DNA repair protein RAD51 and the NHEJ repair protein Ku80 contributes to the observed efficacy and synergy of JQ1 + olaparib.

4. Discussion

This study evaluated the anti-proliferative activity of the BET inhibitor JQ1 in combination with a PARPi inhibitor (veliparib or olaparib) in PDAC cell lines in vitro and of JQ1 + olaparib in independently derived PDX models in vivo. It also examined the effect of JQ1 as a single agent or in combination on levels of DNA damage and on expression of DNA repair proteins Ku80 and RAD51. The study addressed the hypothesis that inhibition of the BET proteins BRD4 or BRD2 attenuates DNA double-strand repair by reducing Ku80 and RAD51 expression, therefore augmenting the anti-proliferative activity of JQ1 in this tumor type. In vitro and in vivo data supported this hypothesis. The data show that JQ1 + olaparib is synergistic in vitro and has greater efficacy than either drug alone in vivo. The data also demonstrate that expression of DNA repair proteins Ku80 and RAD51 is regulated by BRD4 and BRD2 in PDAC cells.

Unlike potencies in the nanomolar range that have been reported for preclinical models of leukemic and other solid malignancies [34–36], JQ1 as a single agent exhibits IC50s in vitro in the micromolar range for BxPC3 and Panc1 pancreatic cancer cell lines. However, efficacious regimens of JQ1 in vivo are comparable to those reported for preclinical models of leukemic and other solid malignancies. A similar discrepancy between in vitro potency and in vivo utility has been documented for other agents such as vorinostat (SAHA) and erlotinib [37–42], and likely reflects differences in factors such as duration of exposure to drug in vitro (Fig. S4) and, most importantly, the therapeutic index of each agent. Notably, in vivo data in this report indicate a favorable therapeutic index for JQ1. The efficacy for BET inhibitors such as JQ1, however, may differ with tumor type. Recent literature suggests that BET proteins associate with specific subsets of promoters or super enhancers in tumor type-specific manner. Loven, et al. demonstrated that expression of super enhancer-associated genes c-Myc, IRF4, PRDM1/BLIMP-1 and XBP1 that actively contribute to multiple myeloma progression, decreased when BET function was inhibited [43]. In contrast, Lenhart et al reported that JQ1 did not inhibit c-Myc protein expression in small cell lung cancer cell lines, but did inhibit expression of the transcription factor achaete-scute homolog-1 (ASCL1) [44]. Identification of BET inhibitors that regulate expression of genes known to promote growth of specific types of tumors might maximize therapeutic indices and the utility of each BET inhibitor.

Recent literature also documents encouraging results for the potential utility of BET inhibitors in combination with agents having complementary mechanisms of cytotoxicity. A noteworthy study demonstrated that in small cell lung cancer cells the combination of the BET inhibitor ABBV-075 and the BCL2 inhibitor venetoclax (ABT-199) induced apoptosis, as assessed by an increase in expression of the pro-apoptotic protein BIM and a decrease in anti-apoptotic proteins BCL-2 and BCL-xL. This combination showed strong synergy in vitro and tumor regressions in vivo [45]. A second study showed that combining JQ1 with the histone deacetylase (HDAC) inhibitor vorinostat (SAHA) improved overall survival in Kras/p53 mutant mice which develop pancreatic tumors with high penetrance [8]. A third study by Yang, et al. documented that JQ1 sensitized HR proficient ovarian and breast carcinoma tumor models to olaparib [46]. That study also demonstrated that JQ1 decreased expression of HR-associated repair proteins BRCA1 and RAD51 in cells exposed to JQ1 in vitro. Our results extend observations of Yang et al. in that in PDAC cells JQ1 suppressed expression of a protein associated with NHEJ repair (Ku80) as well as a protein associated with HR repair (RAD51). If, as Yang, et al. suggested, there is a compensatory upregulation of NHEJ when HR is inhibited, simultaneous inhibition of both processes might be therapeutically beneficial. Differences between the study of Yang, et al. and the data presented herein suggest that JQ1 may affect different genes in different cell types. Further, it is becoming evident that specific PARPi as single agents or in combination have unique characteristics with respect to efficacy [47]. For example, the DNA damaging agent temozolomide + olaparib was superior to temozolomide + veliparib, even though olaparib and veliparib had comparable potency as single agents in vitro in DT40 gallus bursa lymphoma cells [47]. Neither PARPi was effective in PARPi-deficient cells and each inhibitor synergized with temozolomide, although synergy was evident at lower concentrations of olaparib compared to veliparib. With the HR proficient PDAC cells in our study (BxPC3, Panc1), effects of veliparib and olaparib were comparable when combined with JQ1.

In this manuscript we document the novel observations that the combination of a BET inhibitor and a PARPi inhibitor exerts synergistic cytotoxicity in vitro and suppresses tumor growth greater than either agent alone in vivo, in PDAC models including two independently derived PDX models. Mechanistically, the data indicate that the observed synergy and efficacy likely depends on simultaneous inhibition of BRD4- and BRD2-dependent expression of HR DNA repair protein.
RAD51 and NHEJ repair protein Ku80 to confer DNA double-strand break repair deficiency in this tumor cell type. Work is ongoing to determine if in vitro mechanistic findings with BxPC3 and Panc1 cells are consistent among additional PDAC cell lines that express wild type compared to mutant KRAS and among PDX tumors of independent origin. We propose that a DNA repair deficient phenotype induced by JQ1 sensitized tumors to the PARP inhibitor olaparib. The work provides mechanistic and efficacy data to support assessing combinations of BET and PARP inhibitors in treating patients with PDAC.

**Funding Sources and Acknowledgements**

This study was supported by the National Institutes of Health (National Cancer Institute) grants R01CA208272 and R21CA205501 (K.J.Y.). A.L.M. is a recipient of the predoctoral training fellowship in Cell and Molecular Biology (CMB) T32 training grant (2017–2018). R.C.A.M.vW. is supported by DoD OCRP pilot grant W81XWH-15-1-0198. E.S·Y is supported by AstraZeneca, ASCO, Eli Lilly, Novartis and PUMA. The funding sources had no role in the study design, data analysis, or writing of the manuscript.
collection, analysis and interpretation, and writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflicts of interest (declaration of interests)

K.J.Y. receives grants from the National Institutes of Health (RO1CA208272 and R21CA205501). A.L.M. received the predoctoral training fellowship in CMB T32 training grant (2017–2018). R.C.A.M. received DoD OCRP pilot grant (W81XWH-15-1-0198). E.S.Y. receives DoD OCRP funds in support of work cited. All authors approved the final manuscript. All authors approved the final manuscript. All authors approved the final manuscript. All authors approved the final manuscript. All authors approved the final manuscript. All authors approved the final manuscript. All authors approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2019.05.035.

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