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Brief report

PARP-mediated repair, homologous recombination, and back-up non-homologous end joining-like repair of single-strand nicks

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ABSTRACT

Double-strand breaks (DSBs) in chromosomal DNA can induce both homologous recombination (HR) and non-homologous end-joining (NHEJ). Recently we showed that single-strand nicks induce HR with a significant reduction in toxicity and mutagenic effects associated with NHEJ. To further investigate the differences and similarities of DSB- and nick-induced repair, we used an integrated reporter system in human cells to measure HR and NHEJ produced by the homing endonuclease I-AniI and a designed 'nickase' variant that nicks the same target site, focusing on the PARP and HR repair pathways. PARP inhibitors, which block single-strand break repair, increased the rate of nick-induced HR up to 1.7-fold but did not affect DSB-induced HR or mutNHEJ. Additionally, expression of the PALB2 WD40 domain *in trans* acted as a dominant-negative inhibitor of both DSB- and nick-induced HR, sensitized cells to PARP inhibition, and revealed an alternative mutagenic repair pathway for nicks. Thus, while both DSB- and nick-induced HR use a common pathway, their substrates are differentially processed by cellular factors. These results also suggest that the synthetic lethality of PARP and BRCA may be due to repair of nicks through an error prone, NHEJ-like mechanism that is active when both PARP and HR pathways are blocked.

1. Introduction

Multiple, redundant pathways for the repair of cellular DNA are vital for cell survival in the face of constant DNA damage. Understanding of the mechanisms that govern these repair pathways has facilitated the development of targeted double strand break (DSB) technologies that are able to induce homologous recombination (HR)—a promising method for gene modification or repair with many applications. For example, the generation of a site-specific DSBs by highly site specific homing endonucleases, zinc finger nucleases, or TALE nucleases can substantially increase the rate of HR [1–4]. However, HR is not the only possible outcome of a chromosomal DSB: mutagenic non-homologous end-joining (mutNHEJ) and translocations at the site of the original DSB can also result, and mutNHEJ repair at additional off-target sites (due to non-specific cleavage by the endonuclease) can lead to significant toxicity in cells expressing such endonucleases.

We have previously shown that expression of an engineered variant of the homing endonuclease I-AniI which was designed to produce single-strand nicks instead of DSBs can induce HR with nearly undetectable levels of mutNHEJ and no detectible nick-induced toxicity [5,6]. This demonstration of nick-induced HR with decreased mutNHEJ has been confirmed in recent studies with both the I-AniI nickase [7,8], and with novel zinc-finger nickases [9], using a recently developed reporter system, termed the 'Traffic Light Reporter' (TLR) which quantifies HR and mutNHEJ outcomes from a single enzyme recognition site and repair reporter substrate [7].

The nick-induced HR pathway is currently not well characterized, but the observation that nicks induce HR with far lower toxicity and less mutNHEJ than DSBs argues that the nick-induced repair pathway may be distinct from the DSB-induced HR pathway, and has the potential for gene modification or repair with lower toxicity than DSB-induced HR repair. Comparative studies using I-AniI cleavase (termed 'I-AniIY2', as it contains two additional amino-acid substitutions, F13Y and S111Y, that enhance enzyme cleavage activity) and I-AniI nickase (termed 'I-AniIY2N', as it contains a single K227M substitution that inactivates one of the endonuclease active sites) comprise an ideal experimental system

Abbreviations: DSB, double-strand break; HR, homologous recombination; NHEJ, non-homologous end-joining; TLR, Traffic Light Reporter.

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for characterizing the repair of different types of breaks at the same target site. In this study, we employed the Traffic Light Reporter system to investigate the cellular repair factors, which are involved in nick- and DSB-initiated repair.

The first step in repairing DNA damage is recognition and recruitment of cellular repair factors to the site. PARP1 (poly [ADP-ribose] polymerase 1) is a cellular nick detector [10–12], which poly(ADP-ribosyl)ates itself and other proteins upon binding nicked DNA. PARP1 plays a key role in single-strand break repair (SSBR) by recruiting XRCC1 and DNA ligase III after autoribosylation [13], and may be involved in protection of hydroxyurea (HU)-induced stalled replication forks [14–16] and in the reversal of camptothecin-induced collapsed forks [17]. A deficiency or inhibition of PARP1 demonstrates synthetic lethality with BRCA1 and BRCA2, suggesting a degree of redundancy between the HR and SSBR pathways [18–20]. That observation has led to the use of PARP inhibitors in the therapy of BRCA-deficient tumors, although the mechanism of this synthetic lethality is not entirely understood. Several studies have demonstrated increased spontaneous HR-mediated sister chromatid exchange in PARP1-depleted or inhibited cells, as well as in cells from PARP1−/− mice [21,22]. In contrast, studies using the homing endonuclease I-SceI to trigger site-specific DSB-induced HR have found no effect of PARP inhibitors on HR [16,23]. A recent study showed that HR induced by HU-induced stalled replication forks was, in fact, reduced by PARP inhibitors [14]. Based on these results, we hypothesized that the increase in spontaneous HR due to PARP inhibition reflected a role for PARP in preventing nick-induced HR.

In order to further investigate the mechanistic basis for PARP1/BRCA synthetic lethality and the downstream cellular requirements for nick and DSB repair, we used the Traffic Light Reporter system to determine the effect of inhibiting the HR pathway. BRCA2 recruitment to BRCA1 repair foci has been shown to be mediated by PALB2 (Partner And Localizer of BRCA2) [24,25]. The structure of the PALB2 WD40 domain which binds BRCA2 has been well-characterized, as have mutations that interfere with binding [26,27]. These data allowed us to design a dominant negative truncation of PALB2 to determine the reliance of DSB- and nick-repair on the BRCA2 pathway. Our results help to clarify the differences and similarities between nick and DSB repair, and provide additional insight into the mechanism of the PARP/BRCA synthetic lethality.

2. Materials and methods

2.1. Traffic Light Reporter cell line generation

The 293/TLR-AIN cell line was based on the Traffic Light Reporter system [7], with the substitution of a G418/neomycin resistance cassette for the original puromycin selection marker. A lentivirus vector containing the TLR-AIN construct was produced by transfection of 293 cells as previously described [5]. 293 cells were transduced with a multiplicity of infection of less than 0.001 focus forming units per cell. Polyclonal populations of 100–500 G418-resistant colonies containing integrated copies of the TLR reporter were isolated and sorted to remove rare mCherry+ cells, which result from frameshift mutations during reverse transcription. All cells were grown in DMEM with 10% FBS, 1% penicillin/streptomycin in a humidified 5% CO_2 incubator at 37 °C.

2.2. Traffic Light Reporter assay

Polyclonal 293/TLR-AIN cells were seeded at 3.0×10^5 cells/well of a 12-well plate 1 day before transfection. Each well was transfected with 1.0 μg of plasmid DNA using X-tremeGENE 9 (Roche, Indianapolis, IN, USA). Cells were analyzed by flow cytometry 3 days after transfection using an LSRII system (Becton Dickinson Biosciences, San Jose, CA, USA) and FlowJo analysis software (Tree Star, Ashland, OR, USA). All percentages represent the number of GFP+ cells (HR) or 3 × mCherry+ cells (estimate of mutNHEJ) divided by the total number of live cells (gated by forward/side scatter).

The PARP inhibitors 3-aminobenzamide (Sigma–Aldrich, St. Louis, MO) and BSI-201, ABT-888, and AZD2281 (Selleck Chemicals, Houston, TX, USA) were dissolved in DMSO at 1000× the desired concentration. Medium was replaced 1 day after transfection with media containing drug or mock (DMSO alone), so that media for all conditions contained 0.1% DMSO.

2.3. I-AniIY2 and PALB2C expression plasmids

I-AniIY2 expression plasmids were derived from the pExodusY2 expression construct used previously [5]. A silent mutation (GGC to GGG) at G25 was introduced into pExodusY2 to remove a cryptic splice donor site. All constructs have an identical N-terminal HA-tag, a nuclear localization signal, and 'Y2' activating mutations [28]. I-AniI nickase (K227M) and a catalytically inactive control protein (D148E/K227M) were constructed as previously described [5,6]. The sequence coding for the human PALB2C terminal WD40 domain (residues 835–1186) was synthesized (GenScript, Piscataway, NJ, USA) and inserted into the same expression vector as I-AniIY2 in-frame with the HA-tag and nuclear localization signal. The mouse monoclonal antibody 6E2 was used for Western detection (Cell Signaling Technology, Danvers, MA, USA).

2.4. Cytotoxicity assay

The PALB2C and PALB2CR constructs were inserted into the lentiviral vector LVX-IRES-Puro (Clontech, Mountain View, CA), and the vectors were used to generate polyclonal populations of 293T cells containing either LVX-IRES-Puro (empty vector) or the PALB2 expression vectors as above. After puromycin selection, 500 cells were plated in each well of a 6-well dish. After 1 day, media was replaced with media containing PARP inhibitor AZD2281 or DMSO alone (all media contained 0.1% DMSO). PARP inhibitor-containing media was changed after 5 days and surviving colonies (greater than 50 cells) were counted after 8–10 days.

2.5. Cloning of NHEJ mutation events

Genomic DNA was extracted from 293/TLR-AIN cells transfected with plasmids expressing I-AniIY2 nickase and the PALB2 WD40 domain using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). The TLR site was amplified with PfuUltra II Fusion HS Polymerase (Stratagene, La Jolla, CA) using primers TLRqF2c (5′-CGA CAGACTGAGTCGCCCGGA-3′) and TLRqR2c (5′-CCTCGGCGCGGGT CTTGTAGT-3′). PCR products were purified (QIAquick PCR Purification Kit, Qiagen), digested *in vitro* with I-AniIY2 cleavase, amplified again with nested primers TLRqF2b (5′-CGATCCT CGAGCGCCACCAT-3′) and TLRqR2b (5′-GCGCGGGTCTTGTAGTT GCC-3′), and purified and digested a second time. The band corresponding to the full-length PCR product was then gel extracted (QIAquick Gel Extraction Kit, Qiagen) and cloned using Zero Blunt PCR TOPO Cloning Kit (Life Technologies, Carlsbad, CA).

3. Results

3.1. PARP inhibitors increase nick-induced HR, but do not affect DSB repair

We measured the frequency of HR and mutNHEJ initiated from breaks in an integrated target site using the Traffic Light Reporter

system [7]. This reporter system employs an integrated GFP cassette, which has been inactivated by the insertion of an I-AniI endonuclease cleavage site (Fig. 1A). The reporter can be repaired by HR using an exogenous template to produce functional GFP. Mutagenic NHEJ, in contrast, produces an array of insertions and deletions of which approximately 1/3 will be frameshifted so that the mCherry open reading frame located downstream of GFP (originally in a +2 reading frame relative to GFP) can be translated. This system allows visualization and quantitation of DNA repair events, on a cell-by-cell basis, that are generated either by conservative HR or by non-conservative mutNHEJ repair (Fig. 1A).

A polyclonal population of 293 cells with an integrated Traffic Light Reporter was transfected with a donor template plasmid and a plasmid expressing one of three variants of the I-AniIY2 homing endonuclease (the I-AniIY2 'cleavase,' I-AniIY2N 'nickase,' or a catalytically inactive variant of the same enzyme, I-AniIY2 'inactive') that all bind the same I-AniI target site. Several variations on the design of the reporter were also generated, in which the I-AniI target site was introduced to generate either (1) a 21 bp insertion relative the intact GFP ORF; (2) a 21 bp mismatch; or (3) a 21 bp deletion, since previous work with a different reporter system suggested that mismatches between the repair target and template may affect nick-induced repair [5]. The frequency of nick-induced HR with all three target designs was 4.7–6.8% of DSB-induced HR (Fig. 1B and Supplementary Fig. S1).

After treatment with the first-generation PARP inhibitor 3-aminobenzamide, we observed a significant increase in nick-induced HR but no effect on DSB-induced HR (Fig. 1C and D). This prompted us to assay more potent PARP inhibitors: BSI-201 (Iniparib), ABT-888 (Veliparib), and AZD2281 (Olaparib). Veliparib and Olaparib are both competitive inhibitors of the PARP1 active site, and were observed to increase the rate of nick-induced HR at nanomolar concentrations in a dose-dependent manner (Fig. 1D and Supplementary Fig. S2). In contrast, Iniparib (a noncompetitive inhibitor) did not display any effect on either DSB or nick repair at concentrations up to 50 μM. There was no effect of the PARP inhibitors on transfection efficiency in cells transfected with a GFP-expressing control plasmid, and there was no detectible HR or mutNHEJ induced by the inactive endonuclease with any of the PARP inhibitors. Thus, PARP inhibitors increase nick-induced HR up to 1.7-fold, to 1.2% of all live cells analyzed by flow cytometry, without generating detectible nick-induced mutagenic NHEJ or affecting DSB repair.

3.2. *PALB2 WD40 domain expression inhibits HR from DSBs and nicks and reveals back-up mutagenic nick repair*

The PALB2 C-terminal WD40 domain is known to bind to BRCA2, and it is responsible for recruiting BRCA2 to BRCA1 repair foci to initiate HR [24,25]. We reasoned that fusing a PALB2 WD40 domain to I-AniIY2 cleavase and nickase might bias repair pathway choice towards HR. However, in contrast to our original expectation, we found that the fusion protein instead reduced HR induced by both DSBs and nicks (Supplementary Fig. S3). Subsequent expression of the PALB2 WD40 domain (PALB2C) alone showed that this was due to a *trans* effect upon overexpression of this domain, suggesting that the mechanism of reduced HR might be competitive disruption of the normal PALB2–BRCA2 interaction (Fig. 2). The expression of this dominant negative PALB2 domain *in trans* strongly reduced HR due to both DSBs and nicks with the generation of a low but significant levels of nickase-induced mutagenic NHEJ (0.16% ± 0.02 estimated mutNHEJ, or 5-fold above background levels).

As a control, we also expressed a PALB2 WD40 domain with a single point mutation known to prevent binding of PALB2 to BRCA2 (A1025R [27]). This mutant (PALB2CR) was stably expressed, but had no effect on DSB- or nick-induced HR or mutNHEJ (Fig. 2). Thus,

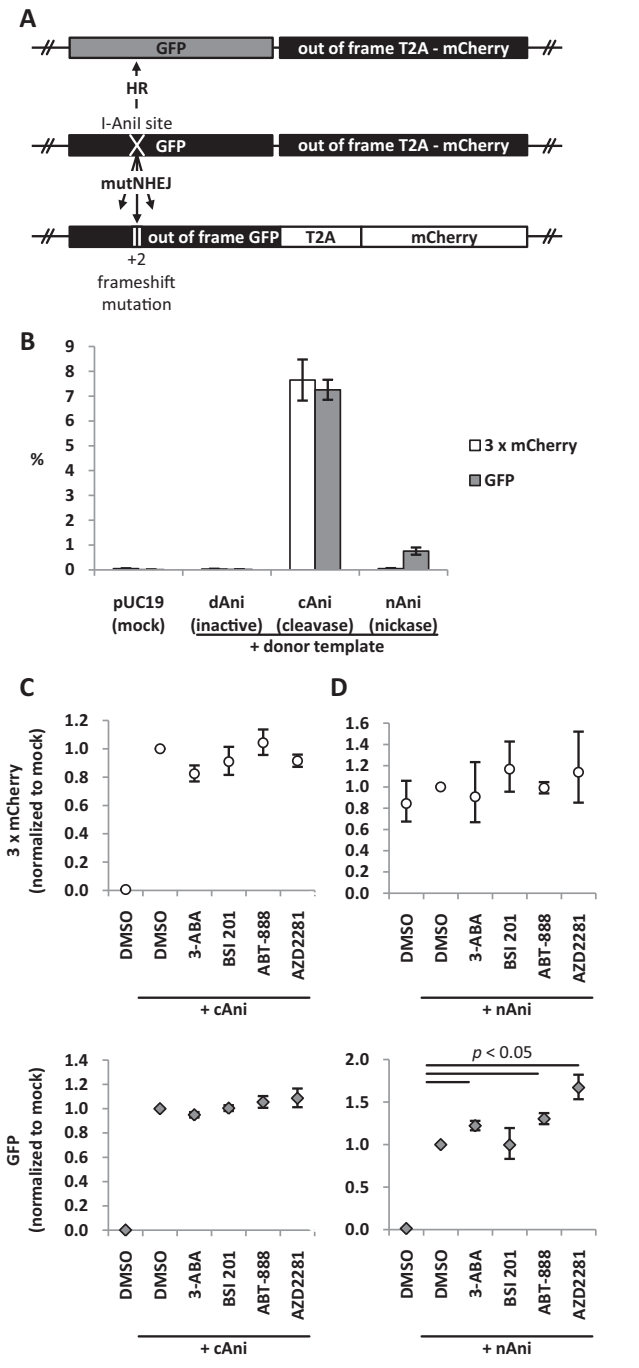

Fig. 1. PARP inhibitors increase the rate of nick-induced HR, but do not affect DSB repair. (A) Structure of the Traffic Light Reporter (TLR) is depicted in the center, with resulting GFP-expressing HR products (above) and mCherry-expressing mutNHEJ products (below). (B) 293/TLR-AIN cells were transfected with 0.5 μg donor plasmid and 0.5 μg of a plasmid expressing a variant of I-AniIY2. In PARP inhibition experiments, 1 day after transfection the culture medium was replaced with DMEM 10% FBS containing a PARP inhibitor in DMSO, or solvent alone (DMSO). The frequency of HR (% GFP+ cells) and a corresponding estimate of mutNHEJ events (derived from 3 times the frequency of mCherry+ cells; see text) were detected and quantified by flow cytometry 3 days after transfection with I-AniIY2 cleavase or nickase. The effect of PARP inhibitors on HR and mutNHEJ was determined as described above using TLR cells transfected with I-AniIY2 cleavase (C) or nickase (D) in the presence of 5 mM 3-aminobenzamide (3-ABA), 50 μM BSI-201 (Iniparib), 0.5 μM ABT-888 (Veliparib), or 0.5 μM AZD2281 (Olaparib). Each data point represents the mean (or geometric mean of normalized data) of three separate experiments ±s.e.m. Statistical significance was determined by two-tailed *t* test of paired values.

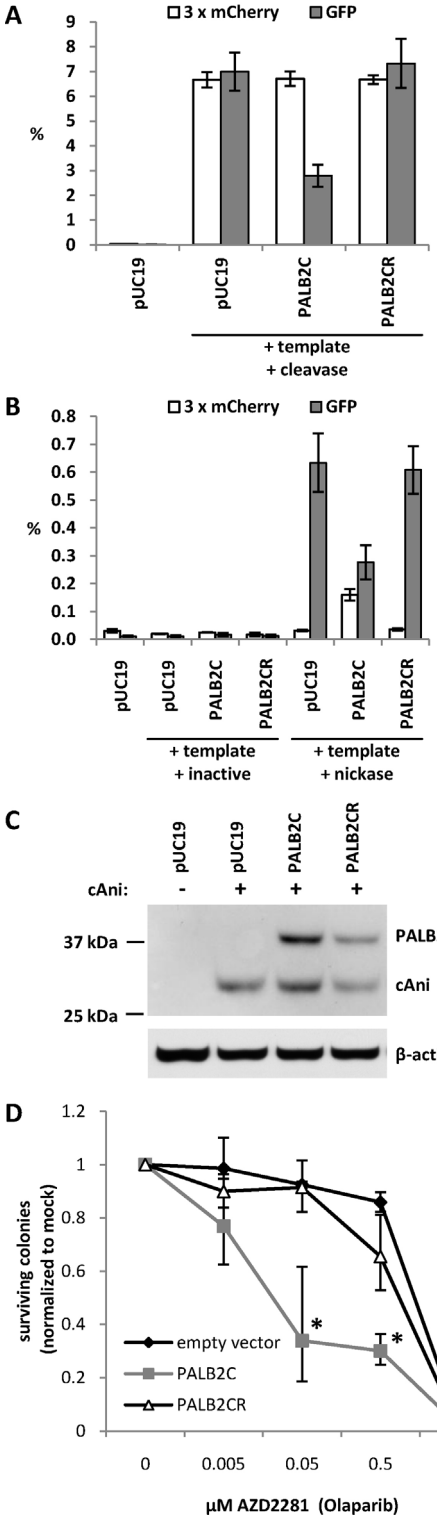

Fig. 2. Expression of the PALB2 WD40 domain *in trans* blocks both nick- and DSB-induced HR. 293/TLR-AIN cells were transfected with 0.4 μg donor plasmid, 0.2 μg of pUC19 or constructs expressing PALB2 C-terminal WD40 domain (PALB2C), or PALB2 C-terminal WD40 domain A1025R mutant (PALB2CR) and 0.2 μg of either I-AniIY2 cleavase (A) or nickase (B). HR and mutNHEJ were detected and quantified by flow cytometry 3 days after transfection. All data points represent the mean of three separate experiments (±s.e.m.). (C) Western blot analysis demonstrates expression of HA-tagged PALB2 C-terminal domains (top) and β-actin loading control (bottom) using cell lysates (5 μg total protein/lane) prepared at the time of flow cytometry. (D) Sensitivity of 293T cells stably expressing PALB2C and PALB2CR to PARP inhibitor was determined by detecting colony forming ability in the presence of AZD2281 for 8–10 days. Data points were normalized to cells only treated with DMSO and represent the geometric mean from three separate experiments (±s.e.m). Asterisks (*) indicate p values of <0.05 compared independently to both cell lines with PALB2CR and empty vector (two-tailed unpaired t test of log transformed normalized data).

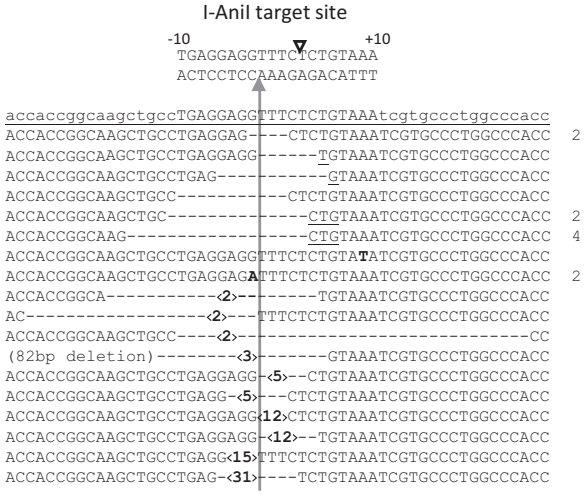

Fig. 3. Sequences of nick-induced NHEJ-like mutations after block of HR. Sequences of 24 mutated clones from cells expressing I-AniIY2 nickase and the PALB2 C-terminal WD40 domain. The I-AniI target sequence in the pTLR-AIN plasmid is shown underlined in the top row, with the I-AniI binding site in capital letters. I-AniI nickase cleaves the site marked by the filled arrowhead, I-AniI cleavase cleaves the sites marked by both arrowheads. Microhomologies are underlined, dashes mark deleted bases in individual clones and new base insertions in angled brackets.

the observed decrease in HR and appearance of mutagenic nick-repair appear to require a functional interaction between the PALB2 WD40 domain and BRCA2.

As we hypothesized that that the dominant negative PALB2C peptide interfered with the normal PALB2–BRCA2 interaction, we tested whether expression of PALB2C demonstrates synthetic lethality with a PARP inhibitor, as shown for cells deficient for BRCA2 [19]. 293T cells stably expressing PALB2C, PALB2CR, and an empty vector were grown in the presence of AZD2281 for 8–10 days. As predicted, we found that expression of PALB2C, but not PALB2CR increased the sensitivity of cells to AZD2281 toxicity at the concentrations, which showed the greatest increase in nick-induced HR (Fig. 2D).

Finally, we extracted DNA from cells expressing I-AniIY2 nickase and the PALB2 WD40 domain *in trans* and sequenced 24 clones with mutations in the targeted region (Fig. 3). Eleven of 24 sequences contained deletions, and 8 of these clones contained microhomologies of 1–3 bp at the deletion junctions. Three clones contained single base pair mismatches and may be due to polymerase error, rather than a true nickase-induced event. The remaining 10 clones contained a combination of deletions and untemplated additions. These indels extend both 5′ and 3′ of the nicked site. Regardless of the size or orientation of the mutation, every sequence except one (with a single point mutation) included a change of one of the two bases flanking the nicked phosphate bond (between position −3 and −2). Using the same methods, we were only able to identify a single point mutation (at position −3) in cells expressing I-AniIY2 nickase without PALB2C. These results confirm that mutagenic NHEJ events induced by I-AniIY2 nickase alone are not readily detectable, but that expression of the PALB2 WD40 domain can reduce HR-repair while promoting mutagenic NHEJ-like repair of the nicked site.

4. Discussion

This study explores parallels and differences in the repair pathways initiated by DSBs and nicks, and it adds experimental evidence to support the hypothesis that targeted nicks are not simply converted to frank DSBs prior to repair *via* homologous recombination (HR). Specifically, the initiation and processing of DSBs and nicks appear to follow different pathways despite making common use of a downstream PALB2/BRCA2-dependent pathway. These observations shed light on the complexity of HR repair, and on the mechanistic basis for the synthetic lethality of PARP1 inhibitors with BRCA mutants.

We first confirmed prior observations [5,7–9] that, in contrast to DSBs, single-strand nicks can induce HR with an exogenous template with significantly less mutNHEJ. In our previous study [5], we used two versions of an integrated reporter containing an inactive *lacZ* with an I-AniI target site that can be repaired by HR. One *lacZ* target required the deletion of 16 bp, whereas the other required the repair of a mismatch. While rates of DSB-induced HR were similar in the different target types, nick-induced HR was 10-fold higher with the mismatch target. We therefore had hypothesized that mismatch repair might be involved in nick-induced repair; however, we did not observe this discordant activity when using similar Traffic Light Reporter-based vectors. These results confirm that nick-induced HR follows a distinct pathway from DSB-induced HR, and indicate that the specific mismatch model of nick-induced HR we proposed previously (Fig. 8 in [5]) is likely to be a reporter-specific, as opposed to general, finding.

PARP1 has been shown to be involved in the initiation of SSBR [13], and the protection and reversal of stalled and collapsed replication forks [14–16]. PARP1 deletion or inhibition is also known to demonstrate synthetically lethality with deficiencies of BRCA1 or BRCA2 [18–20], a seminal observation that led to clinical trials of PARP inhibitors in BRCA-deficient human tumors. The mechanistic role of PARP1 in HR, however, has not been well-defined. PARP inhibition increases spontaneous HR [21,22], but PARP inhibition had no effect on DSB-induced HR [16,23] and a suppressive effect on HU-induced HR [14]. We demonstrate here that PARP inhibition stimulates nick-induced HR, with no effect on DSB-induced HR. This strongly suggests that the effect of PARP on spontaneous HR is due to its effect on single-strand nicks.

Our observation that Iniparib (BSI201), a prodrug of 4-iodo-3-nitrosobenzamide reported to kill cancer cell lines [29], did not suppress nick-induced HR in a dose-dependent fashion was surprising. This PARP inhibitor was purportedly unique among PARP inhibitors by virtue of its ability to covalently modify PARP1 as opposed to competitively inhibiting the PARP active site as do other inhibitors including 3-aminobenzamide, ABT-888 (Veliparib), and AZD2281 (Olaparib). The failure of Iniparib (BSI201) to increase the rate of nick-induced HR in this study thus is consistent with several recent studies that show Iniparib is not a specific PARP inhibitor [30–32]. This belated recognition is important in light of the reported failure of Iniparib in a phase III trial to treat triple-negative breast cancer. This failure led to premature questioning of the general strategy of using the PARP/BRCA synthetic lethality in cancer therapy [32,33].

PALB2 plays a critical role in the recruitment of BRCA2 to BRCA1 repair foci [24,25], and the interruption of the normal PALB2–BRCA2 interaction by the expression of the PALB2 WD40 domain alone significantly blocks HR induced by both DSBs and nicks, and also sensitizes cells to the PARP inhibitor AZD2281 (Fig. 2). This novel dominant negative inhibitor provided a useful reagent in the analysis of cellular HR pathways, as it reveals the use of a conserved PALB2–BRCA2 interaction in both DSB and nick-induced HR. Importantly, the dominant negative disruption by PALB2 WD40 expression revealed a nick-induced mutagenic NHEJ pathway that is only detected when the HR machinery is blocked. Since I-AniIY2 nickase-induced mutagenic NHEJ events are very rare in normal cells and have yet to be unambiguously detected, it is unclear whether the block of HR simply increases the rate of NHEJ-like mutation or alters the types of mutations that occur.

Perturbing each arm of the PARP1/BRCA synthetic lethal interaction suggests a model in which the choice of nick repair pathway proceeds in manner distinct from that suggested for DSB repair (Fig. 4). Nick processing in the presence of PARP inhibition leads to more nicks being repaired by HR; if the HR pathway is blocked, nicks can be repaired through a back-up, mutagenic NHEJ process. If synthesis proceeds across nicked DNA, it will be converted to a single-ended break. Single-ended breaks are normally conservatively repaired (they may be prevented or reversed by PARP-mediated activity or repaired by HR), but if HR is blocked then the remaining conservative mechanisms are unable to repair all single-ended breaks, leading to mutagenic NHEJ-like repair. Single-ended breaks are not substrates for normal NHEJ repair. However, persistent single-ended breaks may increase the likelihood of double-ended DSB generation by a converging second replication fork, or by cleavage or breakage of the remaining single-stranded DNA template molecule. Evidence for elevated levels of nicks in PARP-inhibited cells has been contradictory [13,34], but it would not necessarily be predicted by our model (Fig. 4). This nick-induced model of PARP1/BRCA synthetic lethality is also consistent with the recent demonstration that synthetic lethality results from excessive NHEJ activity, and that inhibition of NHEJ can decrease toxicity [35]. Our model predicts that blocking both PARP1 and BRCA at the same time would produce an excess of nicks repaired through a mutagenic NHEJ-like mechanism, suggesting

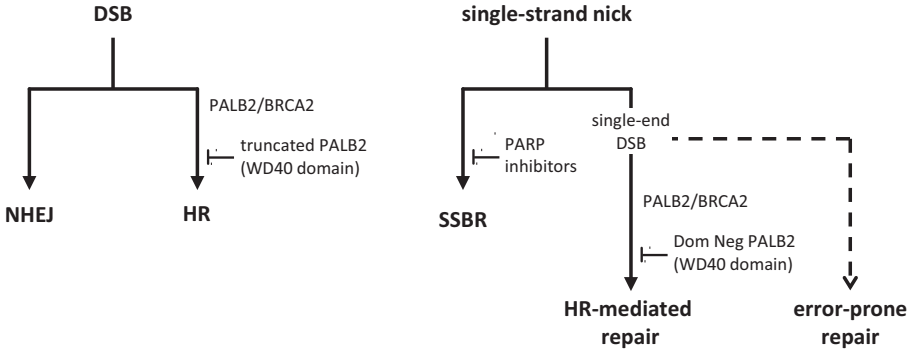

Fig. 4. Model of DSB and single-strand nick repair pathways. DSBs can be repaired through either the NHEJ or HR pathways in normal cells (left panel), and single-strand nicks by SSBR (single strand break repair) and HR pathways (right panel) in normal cells with no detectable nick-induced mutagenic NHEJ. HR may be triggered by a nick that leads to fork collapse to form a single-ended break. If HR is blocked, nick-induced fork collapse can be repaired through a latent error-prone repair pathway (dotted line), which may be triggered by a converging second replication fork converting the single-ended DSB to a double-ended DSB.

that PARP1/BRCA synthetic lethality may reflect the error-prone repair of single-strand nicks.

The need to suppress mutagenic repair of spontaneous nicks is likely to be an active process as dividing cells may generate large numbers of nicks—an estimated 10^4 per cell per day [36]. Our results also suggest a more nuanced view of nick- and DSB-induced repair, with different initiation and processing steps though the use of common, conserved BRCA2-dependent HR steps for completion. Mechanistic dissection of these pathways should shed additional light on the mechanism of synthetic lethality of PARP1 and BRCA in cancer treatment. This knowledge might also lead to more efficient nick-induced gene repair if additional selective inhibitors of PARP1/DNA ligase III-mediated nick repair can be identified.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.dnarep.2013.04.004.

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