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MYELOID NEOPLASIA

PARP1 is required for chromosomal translocations

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Key Points

- Chromosomal translocations are mediated by PARP1 and can be suppressed by the clinical PARP1 inhibitors.

Chromosomal translocations are common contributors to malignancy, yet little is known about the precise molecular mechanisms by which they are generated. Sequencing translocation junctions in acute leukemias revealed that the translocations were likely mediated by a DNA double-strand break repair pathway termed nonhomologous end-joining (NHEJ). There are major 2 types of NHEJ: (1) the classical pathway initiated by the Ku complex, and (2) the alternative pathway initiated by poly ADP-ribose polymerase 1 (PARP1). Recent reports suggest that classical NHEJ repair components repress translocations, whereas alternative NHEJ components were required for translocations. The rate-limiting step for initiation of alternative NHEJ is the displacement of the Ku complex by PARP1. Therefore, we asked whether PARP1 inhibition could prevent chromosomal translocations in 3 translocation reporter systems. We found that 2 PARP1 inhibitors or repression of PARP1 protein expression strongly repressed chromosomal translocations, implying that PARP1 is essential for this process. Finally, PARP1 inhibition also reduced both ionizing radiation–generated and VP16-generated translocations in 2 cell lines. These data define PARP1 as a critical mediator of chromosomal translocations and raise the possibility that oncogenic translocations occurring after high-dose chemotherapy or radiation could be prevented by treatment with a clinically available PARP1 inhibitor. (*Blood*. 2013;121(21):4359-4365)

Introduction

Chromosomal translocations both classify types of malignancies and are required for the origin of those malignancies.[1,2] Because of this trend, translocations have been widely studied, but their precise molecular mechanism remains poorly understood. It is intuitively and experimentally clear that simultaneous DNA double-strand breaks (DSBs) must occur in distinct chromosomes for a translocation to occur.[3] DNA DSBs can be repaired by 3 pathways: (1) homologous recombination (HR), where sequence integrity is preserved; or (2) single-strand annealing (SSA) and (3) nonhomologous end-joining (NHEJ), both of which generate deletions.[4] Sequencing junctions of leukemic translocations in patient samples revealed that these junctions often had deleted sequences, indicating that these translocations predominantly arose by SSA or NHEJ.[5] Therefore, HR is not thought to play a significant role in chromosomal translocations.[1,2] SSA and NHEJ can be distinguished by the presence of repeated sequences adjacent to the junction site that could mediate the annealing in SSA. Thus, when long repeated sequences are adjacent to DSBs, translocations occur more frequently via SSA vs NHEJ.[3] When no repeated sequences are present, then translocations are mediated by NHEJ.

There are 2 major NHEJ pathways: the dominant, classical (cNHEJ) pathway; and the alternative (aNHEJ) pathway.[4] cNHEJ begins when the Ku70/80 complex recognizes free DNA ends and recruits DNA-PKcs, which initiates free DNA end processing and DNA ligase IV complex for end ligation.[4] aNHEJ initiates when poly ADP-ribose polymerase 1 (PARP1) recognizes a DSB and successfully competes for the free DNA ends with the Ku complex of cNHEJ.[6] MRE11 and CtIP 5′ to 3′ resect the free DNA ends to generate single DNA strands to allow for a microhomology search.[7,8] The opposite strands anneal at the microhomology. This step is followed by trimming of the overhangs, and DNA ligase III then seals the resulting nicks.[9]

Surprisingly, defects in the cNHEJ components Ku70 and DNA ligase IV increased translocation rates, indicating that these cNHEJ components normally repress translocations.[10,11] These data would seem counter to the previous paradigm that NHEJ mediates translocations. However, this paradox was resolved when the aNHEJ components DNA ligase III and CtIP were shown to be required for chromosomal translocations.[8,9,12] Also, immunoglobulin class switch recombination, a DNA process related to translocations, can be mediated by aNHEJ and relies on PARP1.[13] Because PARP1 is the rate-limiting initial step of aNHEJ, we hypothesized that PARP1 inhibition could prevent chromosomal translocations. This hypothesis is particularly important because several PARP inhibitors have been in clinical trials and have been well tolerated.[14] Chromosomal translocations result in many forms of cancers, and a method

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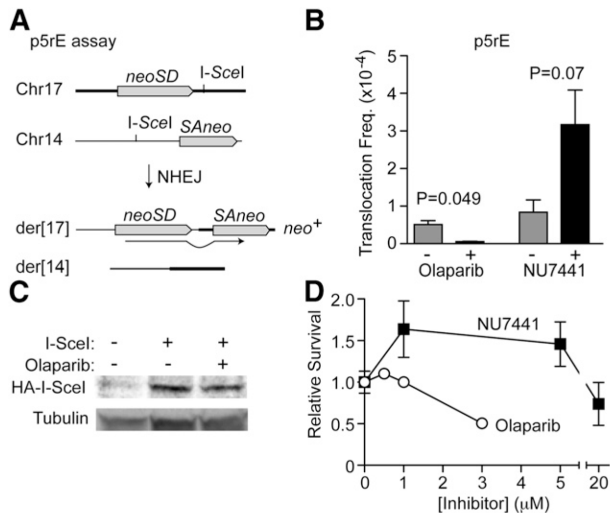

Figure 1. PARP1 inhibition suppresses and DNA-PKcs inhibition enhances chromosomal translocations. (A) The p5rE dual I-*Sce*I translocation reporter system has 5′ and 3′ neo segments with splice donor (SD) and splice acceptor (SA) signals on chromosomes 14 and 17. After translocation NHEJ reconstitutes a functional neo on der[17], which can be quantified by clonal survival in G418. (B) p5rE cells were treated with the PARP1 inhibitor olaparib (3 μM), the DNA-PKcs inhibitor NU7441 (5 μM), or the DMSO vehicle as a control during and after translocation induction with I-*Sce*I. Values are averages (± standard error of the mean [SEM]) for 3 or more determinations. (C) Olaparib does not alter expression of the transfected I-*Sce*I. (D) NU7441 does not reduce survival duration of the p5rE cells, but olaparib decreases survival duration moderately. However, all results were normalized for survival in inhibitors.

to prevent translocations might prevent some malignancies. This could be especially important in acute myeloid leukemia occurring after radiation or chemotherapy, where specific treatment regimens have defined risks for secondary leukemia. These risks might be reduced if concurrent preventive measures were taken, but at present, no such measures are available.

In this study, we demonstrate that PARP1 inhibition with olaparib, rucaparib, or small-interfering RNA (siRNA) results in markedly decreased translocations, defining PARP1 as a key factor in the molecular mechanism of translocations and providing a possible future strategy for the prevention of oncogenic translocations.

Methods

Chromosome translocation assays

Three translocation assays were used in this study. In 2 assays, simultaneous DSBs were generated at introduced I-*Sce*I sites in chromosomes 14 and 17 of p5rE or p5pF murine embryonic stem (ES) cells,[3] with the der[17] translocation reconstituting an intact neomycin phosphotransferase gene for both p5rE and p5pF, and also a puromycin resistance gene for p5pF cells (Figures 1A and 2A). The p5rE reporter system detects translocations by aNHEJ or cNHEJ. The p5pF cells have Alu direct repeats (290 bp) and shared puromycin gene regions (265 bp) adjacent to the I-*Sce*I sites in both chromosomes, which allow translocations via SSA that reconstitute a functional puro gene on der[14] and a functional neo gene on der[17] (Figure 2A). The p5pF reporter thus distinguishes translocations via SSA or either type of NHEJ. DSBs were induced following electroporation of an I-*Sce*I expression vector as described previously.[15] Translocation frequencies were calculated as the number of G418- or puromycin-resistant colonies per viable cell. Cells were pretreated for 16 hours before and 18 hours after I-*Sce*I transfection with 3 μM of olaparib (PARP1 inhibitor), 5 μM of NU7441 (DNA-PKcs inhibitor), or a vehicle control. This time and concentration of olaparib were chosen to allow maximal exposure with acceptable toxicity of the above ES cell lines (50%). This concentration of olaparib (3 μM) is equivalent to 1.2 μg/mL, which is below the serum concentrations achieved during early-phase clinical trials.[16]

In the third translocation assay, DSBs were induced in chromosomes 1 and 3 by lipofection of zinc finger nucleases (ZFNs; Sigma, St. Louis, MO) in human embryonic kidney (HEK) 293T or Jurkat cells. Single ZFNs of the pair, which cut just a single DNA strand, served as controls. We measured the rate of formation of der[3] translocation at 24 and 48 hours after ZFN lipofection using semiquantitative polymerase chain reaction (PCR) and nested primers with and without 16 hours of prior exposure with olaparib (3 μM) or another PARP1 inhibitor, rucaparib (400 ηM). After ZFN transduction, the PARP1 inhibitors were replenished and remained until harvest. In all translocation reporter systems in this study, translocation events were normalized to the effects of olaparib or rucaparib without DSB induction. To assess potential off-target effects of olaparib or rucaparib, PARP1 was repressed in the ZFN assay using commercial siRNA (Dharmacon, Lafayette, CO) lipofected before DSB induction. PARP1 enzymatic activity was measured using a PARP1 activity assay (Trevigen, Gaithersburg, MD) to confirm the effect of olaparib exposure.

Cytogenetic assay of radiation- and VP16-induced translocations

WI38 cells were grown in Dulbecco modified Eagle medium supplemented with 10% phosphate-buffered saline and 1% antibiotics. 32D cells were grown in RPMI1640 medium supplemented with 10% phosphate-buffered saline, 1% antibiotics, and 5 μg/mL of IL-3. Cells in log phase were pretreated with 3 μM of olaparib for 2 hours before exposure to 4 Gy of ionizing radiation (IR), or to 1.25 μM of VP16 for 1 hour. After irradiation or VP16 exposure, cells were incubated in the same olaparib-containing media for another 24 hours. Fresh media with colcemid (final concentration, 0.25 μg/mL) and olaparib were added, and cells were further allowed to incubate for 24 hours before harvest. Chromosome preparation was made according to the standard air-drying procedure.[17] The cells were harvested, washed with prewarmed phosphate-buffered saline twice, hypotonically treated (0.56% KCl, 20 minutes at 37°C), and subsequently fixed in freshly prepared acetic acid-methanol (1:3). At least 3 changes of fixative were performed before the cell suspension was dropped on to a precleaned chilled microscopic glass slide and dried at room temperature for at least 1 day before staining. Giemsa staining was used to score metaphase chromosomes. Structural chromosome–type translocations, such as dicentric and ring chromosomes and robertsonian translocations, were scored under 63× magnification.[17] Z-tests were used for testing the differences between the 2 proportions of induced structural translocation, as observed in the olaparib group vs the vehicle group, before and after exposure to IR or to VP16.[17] For each data point, more than 100 metaphase spreads were scored. Assuming a WI38 modal chromosome number of 46 (32 in 32D cells), the total number of chromosomes observed in this study was calculated by multiplying the modal chromosome number with the number of metaphase spread scored for each datum point. Total structural translocation induced (subtracting the value of background aberration observed in untreated control cells) in both groups (assuming 46 × number of metaphase spreads scored for the purpose of determining proportion values) were compared using the Z-test to determine the statistical significance, calculated as follows: Let p1 and p2 denote, respectively, the induced structural translocation proportion with DNA damage (irradiation or VP16 treatment) with or without PARP1 inhibition. Our hypothesis was one of the following two: H_O: p1 = p2, the observed differences in structural translocation frequencies (aberration in total number of chromosomes scored) with or without PARP1 inhibition are the result of chance (ie, there is no difference in radiosensitivity regarding structural translocation induction between these 2 groups after drug treatment). The alternative hypothesis is H_A: p1 > p2 (ie, PARP1 inhibition reduces radiation or VP16-induced translocations). Our test hypothesis was the following: Where n1 and n2 denote the total number of chromosome observed (modal chromosome number, 46 × metaphase spreads scored) without or with PARP1 inhibition, respectively, while p1 and p2 denote the proportion of structural translocations (number of observed aberrations divided by total chromosome observed for

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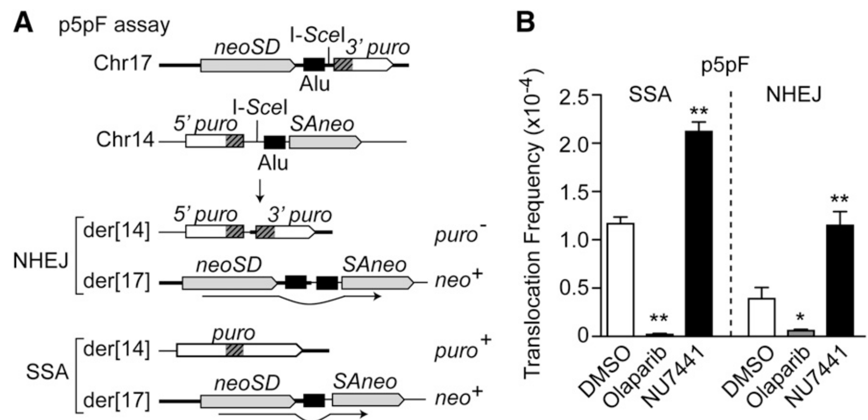

Figure 2. PARP1 inhibition suppresses SSA- as well as NHEJ-mediated translocations. (A) The p5pF translocation reporter system has puro and alu repeats (hatched and black boxes) flanking I-*Sce*I sites on chromosomes 14 and 17. A translocation mediated by NHEJ reconstitutes a functional neo gene on der[17], whereas SSA reconstitutes both neo on der[17] and a functional puro gene on der[14]. SSA-mediated translocation events confer neo and puro resistance, whereas NHEJ translocations confer only neo resistance. (B) Frequencies of SSA- and NHEJ-mediated translocations are averages (± SEM) for 3 determinations. $^*P < .05$; $^{**}P < .01$. All results were normalized for survival in inhibitors.

each datum point) and p = (n1p1 + n2p2)/(n1 + n2), q = (1 – p).[17] The *z* values were calculated to determine statistical significance at a level of 5% or 1%.[17]

Results

PARP1 inhibition with olaparib represses chromosomal translocations

Because aNHEJ components were found to be required for chromosomal translocations,[8,9,12] and PARP1 initiates aNHEJ,[6,13] we investigated whether clinical PARP1 small-molecule inhibitors could repress chromosomal translocations with 3 distinct reporter assays. In the p5rE translocation assay, simultaneous DSBs are induced at integrated I-*Sce*I sites in chromosomes 14 and 17 in murine ES cells, with the der[17] translocation reconstituting an intact neo gene via either type of NHEJ (Figure 1A).[3] Reconstitution of neo confers resistance to G418 yielding colonies harboring the t(14;17). We induced simultaneous DSBs in chromosomes 14 and 17 and measured der[17] G418–resistant colonies in the p5rE cells with and without olaparib (Figure 1A-B). We found that PARP1 inhibition with olaparib resulted in a 10-fold decrease in translocations in the p5rE reporter system (Figure 1B).

As a control, we also measured translocations in p5rE cells treated with the DNA-PKcs inhibitor NU7441 (Figure 1B). Consistent with prior studies of other cNHEJ components,[9-11] DNA-PKcs inhibition increased der[17] formation vs control levels by 54-fold. It was possible that olaparib decreased DSB induction by reducing I-*Sce*I expression; however, western analysis showed that olaparib did not alter the expression of I-*Sce*I in these cells (Figure 1C). Although olaparib showed some toxicity in the ES cell systems (Figure 1D), this finding cannot explain the reduced translocation frequency because such frequencies were normalized for cell survival. Also, 5 μM of NU7441 dramatically increased translocations yet had no effect on cell viability (Figure 1D). These results suggest that inhibition of cNHEJ with NU7441 results in more translocations because DSB repair is shunted to aNHEJ,[8-11] and support the view that cNHEJ represses translocations. Moreover, these results indicate that PARP1 plays a key role in promoting translocations via aNHEJ and also identify a clinically relevant PARP1 inhibitor that can prevent chromosomal translocations.

The human genome comprises nearly 50% repetitive elements that contribute significantly to genome instability, including translocations via SSA.[18,19] To investigate the role of PARP1 in aNHEJ- vs SSA-mediated chromosomal translocations, we used the p5pF translocation reporter system[3] (Figure 2A). This system is similar to the p5rE cells, in that I-*Sce*I induces DSBs in chromosomes 14 and 17, which can reconstitute neo. The distinction is that the p5pF cells have alu repeats flanking the I-*Sce*I sites on both chromosomes. Neo can be reconstituted on the der [17] using either SSA (via the alu repeats) or by NHEJ. In addition, if the translocation occurs via SSA but not via NHEJ, the der[14] reconstitutes puro, which can be measured by clonal survival in puromycin. Thus, dual resistance to G418 and puromycin defines a translocation event that occurred via SSA. As with the p5rE experiments, all results were normalized to p5pF survival in olaparib.

Olaparib markedly reduced total translocations (total neo-resistant clones, NHEJ, or SSA) by 330-fold in the p5pF system. Surprisingly, SSA-mediated translocations were further reduced by threefold by PARP1 inhibition. This finding demonstrates that PARP1 is important in translocation events arising from both aberrant aNHEJ and SSA DSB repair. It is possible that PARP1 is a previously unrecognized component of the SSA pathway and that inhibiting it blocked any SSA activity, regardless of translocation events. Consistent with the p5rE results, decreasing cNHEJ with the DNA-PKcs inhibitor NU7441 increased total p5pF translocations by 1.6-fold.

It was possible that the decrease in aNHEJ-mediated translocations was the result of the artificial DSB induction system used in the p5rE and p5pF cells. In those cells, the DSBs are created in engineered I-*Sce*I recognition sequences, not endogenous genomic sequences. To analyze the effect of PARP1 inhibition on translocations arising from endogenous vs introduced sequences, we modified a translocation reporter system on the basis of transfected ZFNs (Figure 3A).[20] We generated 2 pairs of ZFNs that produce simultaneous DSBs in chromosomes 1 and 3, yielding a t(1;3) (Figure 3A). Olaparib inhibited PARP1 activity by ~threefold after 48 hours (Figure 3B). It should be noted that the inhibition of cellular PARP1 activity by olaparib in this assay is likely an underestimate, as it is not a permanent covalent inhibitor of PARP1, and the time it takes to process samples for this assay, in buffers without olaparib, may decrease olaparib bound to PARP1. Olaparib did not alter ZFN expression (Figure 3D), nor did it affect survival duration of these cells (data not shown). We measured der[3] translocations at 24 and 48 hours after ZFN transfection by using semiquantitative PCR and nested primers. Measuring the amount of translocated PCR product compared with genomic GAPDH product provides a ratio of translocated DNA to total genomic DNA. This ratio can be used to define the effect of PARP1 inhibition on translocation events. Consistent with the results above, olaparib markedly inhibited ZFN-induced translocations by an average of fourfold at 24 hours and 49-fold at 48 hours after ZFN transfection in HEK 293T cells (Figure 4A). As expected, control transfection with single ZFNs,

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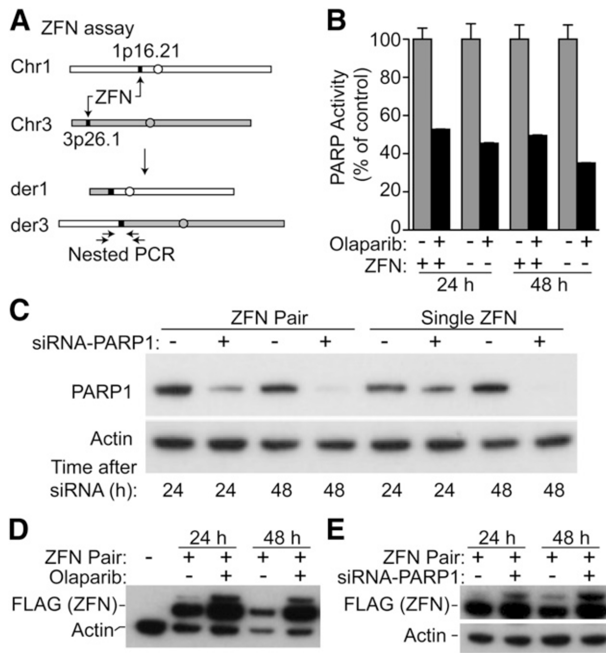

Figure 3. A ZFN chromosomal translocation assay. (A) Schematic of the ZFN translocation assay in HEK 293T cells, where simultaneous DSBs are induced in chromosomes 1 and 3, then nested semiquantitative PCR is used to identify the der [3] translocation product. A ZFN pair is required to nick both DNA strands for DSB generation. (B) In vitro assay showing that PARP1 poly(ADP-ribosyl)ation activity is inhibited up to 3.3-fold by olaparib in HEK 293T cells in the presence or absence of ZFNs. (C) siRNA against PARP1 repressed PARP1 protein expression. (D) PARP1 inhibition with olaparib does not decrease ZFN expression. (E) PARP1 siRNA does not decrease ZFN expression.

which cleave only 1 strand and do not create the requisite DSBs, did not generate any detectable translocations (Figure 4A).

We were interested in whether the reduction in chromosomal translocations with PARP1 inhibition could be generalized to other cell types, specifically to hematopoietic cells. Therefore, we investigated whether olaparib or rucaparib suppressed chromosomal translocations in the Jurkat leukemia cell line using transduced ZFNs to generate the t(1;3) translocation. These translocations were readily detected in Jurkat cells 2 days after ZFN transduction, and they continued to accumulate during a 6-day period, but olaparib reduced chromosomal translocations to undetectable levels in these cells (Figure 5). Olaparib had no effect on Jurkat survival duration or ZFN expression (not shown). Rucaparib, another PARP1 inhibitor that is currently being tested in clinical trials, also reduced translocations to undetectable levels (Figure 5). Thus, these data indicate that PARP1 inhibition decreases chromosomal translocations in a cell line of hematopoietic origin.

Repression of PARP1 with siRNA suppresses chromosomal translocations

Although olaparib and rucaparib are well-defined PARP1 inhibitors,[14,21] they also can inhibit PARP2 but to a lesser extent. In addition, other off-target effects of olaparib or rucaparib may influence translocations besides PARP2 inhibition. Therefore, we asked whether specifically depleting PARP1 protein could recapitulate the effects of olaparib. We repressed PARP1 protein expression using siRNA (Figure 3C), and measured der[3] translocation efficiency in the ZFN system in HEK 293T cells. Reducing PARP1 expression with siRNA markedly reduced chromosomal translocations (Figure 4A). Reducing PARP1 expression with siRNA had no effect on the protein levels of the ZFNs (Figure 3E). These data demonstrate that chromosomal translocations are indeed specifically mediated by PARP1, ruling out off-target effects.

Reduction of mutagen-induced translocations

In the reporter systems above, the DSBs are generated enzymatically in defined genomic sequences. We asked whether DSB-induced chromosomal translocations generated by more clinically relevant causes, IR or VP16, were similarly suppressed by olaparib. We examined the effect of olaparib on IR-induced translocations in WI38 normal human fibroblasts. IR induces several types of translocation events that can be measured using conventional cytogenetics (Figure 6A). We found that olaparib exposure reduced the frequency of IR-induced rings and dicentric chromosomes by ~2.5-fold (Figure 6B). Topoisomerase IIα inhibitors such as VP16 are effective anticancer agents, but they can induce translocations that cause secondary malignancies.[22] Therefore, we tested whether olaparib could suppress translocations induced by VP16. As shown in Figure 6C, olaparib suppressed dicentric and ring chromosomes and robertsonian translocations (reciprocal acrocentric chromosomes) by ~twofold. These results indicate that PARP1 is also important for the formation of translocations in normal cells induced by IR or VP16.

Discussion

Chromosome translocations are common causes of oncogenesis, but little is known about the specific mechanisms that generate

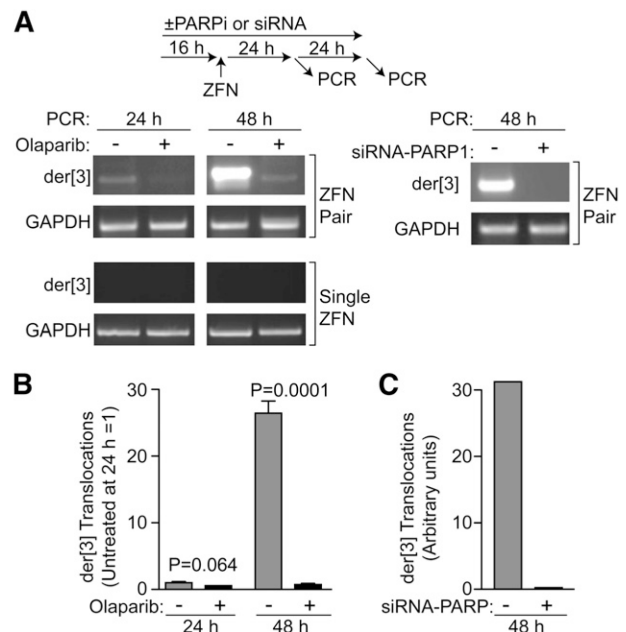

Figure 4. PARP1 inhibition with olaparib or siRNA decreases ZNF-induced translocations. (A) HEK 293T cells were treated with olaparib and transfected with siRNA and ZFNs at the times indicated in the schematic above. Olaparib and siRNA knockdown of PARP1 repressed ZFN-induced translocations, as assayed by semiquantitative PCR of the der[3] product. Data are representative of 3 independent determinations. Translocations are induced by two ZFN pairs that create 2 simultaneous DSBs in target chromosomes, but not by the negative control single ZFNs of each pair. (B) Quantification of ZFN-induced translocations with or without olaparib; values are averages (± SEM) for 3 determinations. (C) Quantification of ZFN-induced translocations with or without siRNA repression of PARP1; values are averages (± SEM) for 3 determinations, with SEM too small for bars to be visible.

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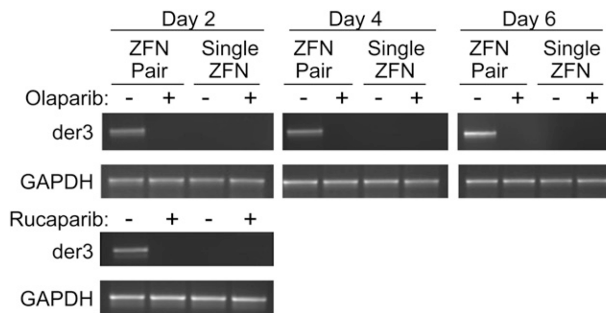

Figure 5. PARP1 inhibition decreases ZFN-mediated translocations in cells of hematopoietic origin. Jurkat cells were treated with olaparib or rucaparib, and translocations induced by ZFN pairs were measured by assaying der[3] formation by using semiquantitative PCR at the indicated times, as shown in Figure 4.

translocations. The first requirement for a translocation is the occurrence of 2 simultaneous DSBs in distinct chromosomes.[23] Recent studies have demonstrated that components of the cNHEJ pathway repress translocations, whereas aNHEJ factors CtIP and DNA ligase III are necessary for chromosomal translocations.[8,9] Because PARP1 initiates aNHEJ by outcompeting Ku70/80 for the free DNA DSB ends,[13] we hypothesized that PARP1 inhibition could prevent chromosomal translocations. Using 3 distinct translocation reporter systems in multiple cell types, and using IR and VP16 in normal cell lines, we found that 2 clinical PARP1 inhibitors (olaparib and rucaparib), as well as siRNA repression of PARP1, markedly reduces chromosomal translocations.

There are several potential mechanisms by which Ku70/80 and PARP1 might regulate translocations. Ku70/80 is abundant and binds efficiently to free DNA ends, acting as an effective tether to prevent ends from drifting apart and rejoining with DSB ends on other chromosomes. PARP1 also binds to free DNA ends, but it may not tether ends as well as the Ku complex. In this model, when PARP1 outcompetes Ku70/80 for the free ends of a DSB, the free ends are no longer tethered; thus, translocations are more likely to occur. Another possible explanation is kinetic: Ku-mediated cNHEJ may simply be more efficient at end-joining than aNHEJ. In this model, when aNHEJ initiates repair of a DSB, it takes longer to complete than with cNHEJ, therefore leaving more time for free ends to drift apart, and be repaired by aberrant interchromosomal end-joining. Finally, it is also possible that PARP1 enhances CtIP and/or DNA ligase III translocation activity or represses cNHEJ accurate intrachromosomal end-joining. PARP1 could perform this by poly(ADP-ribosyl)ating these proteins or others involved in aNHEJ or cNHEJ. Note that these potential mechanisms are not mutually exclusive.

The considerations above raise the question of how cells choose between cNHEJ and aNHEJ to repair DSBs. One idea is that it is stochastic: the relative concentrations of the Ku complex and PARP1 at the DSB site determine which binds to the free ends.[6] However, it is also possible that different types of free ends may be more prone to repair by cNHEJ or aNHEJ. We found that PARP1 inhibition reduces translocations induced by nucleases (I-*Sce*I or ZFNs) to a greater extent than those induced by IR or etoposide. It is possible that PARP1 has greater affinity for "clean" ends produced by nucleases rather than ends that require processing, such as those created by IR, or when topoisomerase IIα is covalently bound to ends because of VP16. These differential effects of PARP1 inhibition can also be explained if the Ku-dependent cNHEJ pathway is required when ends require processing, a model consistent with the dominance of cNHEJ in V(D)J recombination, where Rag1/2 initiates V(D)J recombination by inducing DSBs that require processing before a second ligation.[24] Genomic sequences and structures may also influence the choice between cNHEJ and aNHEJ. Repeated sequences or noncanonic structures such as G-quadruplex DNA may favor one pathway vs another, which is consistent with findings that class switch recombination may favor aNHEJ vs cNHEJ in some circumstances.[13]

PARP1 inhibitors have gained interest because they are synthetically lethal in the inherited breast and ovarian cancers with defects in *BRCA1* or *BRCA2*.[25] In addition, another fraction of breast and ovarian cancers is not inherited but behaves like the *BRCA1/2* mutant cancers, giving rise to the term "BRCA-like" cancer. What all of these cancers have in common is a defect in HR. PARP1 inhibition blocks repair of single-strand breaks, which are converted to DSBs when encountered by a replication fork. *BRCA1* and *BRCA2* are required to repair replication-associated DSBs;

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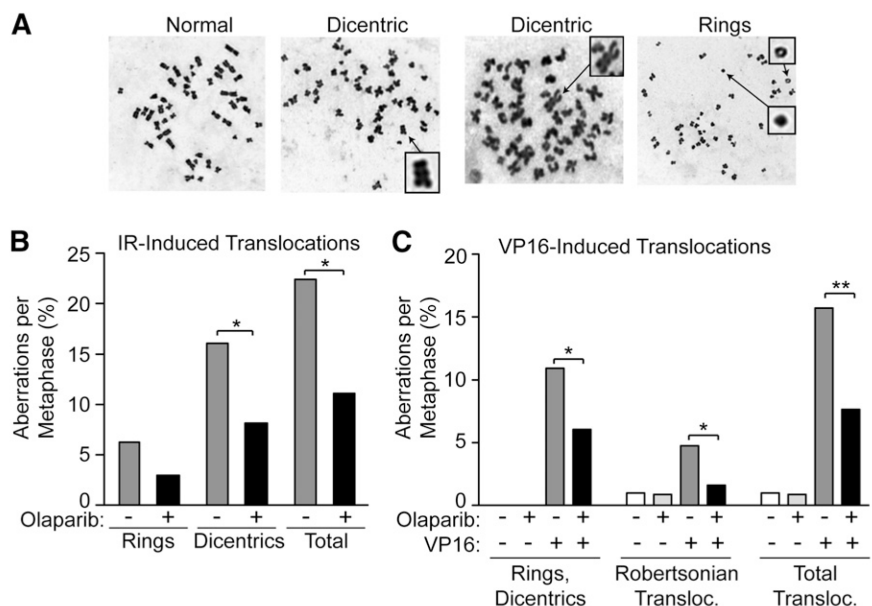

Figure 6. Olaparib represses chromosomal translocations induced by IR or VP16. (A) Representative metaphase spreads showing IR-induced dicentric and ring chromosomes. (B-C) Olaparib reduces IR-induced translocations in normal human WI38 fibroblasts and VP16-induced translocations in 32D murine hematopoietic progenitor cells. $*P < .05$; $**P < .01$, based on z values calculated from 135-192 metaphase spreads (B) or 103-248 metaphase spreads (C).

hence, PARP1 inhibitors are synthetically lethal in these cancers. The advantage of this synthetic lethal approach is that normal cells, harboring at least 1 normal allele of *BRCA1/2*, are not affected. Thus, PARP1 inhibition in a non-*BRCA1/2* mutant setting should be safe and effective for preventing leukemogenic translocations. Bolstering this concept is that PARP1 inhibitors have been quite safe in phase 1 and 2 trials,[26] and PARP1$^{-/-}$ mice have been viable and without major defects.[27]

It should be noted that olaparib and rucaparib can also inhibit PARP2.[14,21] We believe that the reduction in translocations with these drugs reflect specific inhibition of PARP1 because siRNA against PARP1 completely recapitulated the reduction in chromosomal translocations. Olaparib has some cytotoxicity to ES cells, but this cannot account for the reduced translocation frequencies because results were normalized to cell survival. Also, siRNA knockdown of PARP1 reduced translocations but had no effect on cell survival duration. It was possible that olaparib or siRNA knockdown of PARP1 could have reduced expression of the DSB-generating agents, I-*Sce*I or the ZFNs. However, western analysis showed that this was not the case; in fact, PARP1 inhibition occasionally increased ZFN expression, which should enhance translocations. In addition, PARP1, but not PARP2, plays a role in immunoglobulin class switch recombination,[13] a process related to chromosomal translocation.

That PARP1 inhibition decreased chromosomal translocations mediated by SSA was unexpected. There are several possible explanations for this result. First, PARP1 binding to free DNA ends could shunt DSB repair away from cNHEJ, allowing for more SSA as well as more aNHEJ on a simple kinetic basis. In this model, PARP1 is a negative regulator of cNHEJ but is not a positive initiating event for any other DSB repair pathway. However, this model does not account for the observation that PARP1 has an important role in initiating aNHEJ.[6] Second, PARP1 binding to free DNA ends could be an initial event for both SSA and aNHEJ. Certainly, whether PARP1 initiates SSA represents a new avenue worthy of further study.

We envision 2 potential clinical uses for the findings presented here. First, because PARP1 inhibition reduces chromosomal translocations, it is possible that increased PARP1 expression might increase the risk for a leukemogenic translocation, especially with high-risk therapies. If so, then PARP1 levels and activity could be measured in the bone marrow of patients before high-risk therapy is undertaken (eg, before a stem-cell transplant ablative preparative regimen) to predict the risk for secondary leukemia. Defining the risk for a secondary malignancy could assist in defining future long-term screening of these patients for cancer after exposure to the preparative regimen. The second potential application would be as an intervention to prevent secondary oncogenic translocations. Patients undergoing therapy that has a high risk of inducing oncogenic translocations may benefit from concurrent treatment with a PARP1 inhibitor to reduce the risk for translocations. Indeed, one could define a clinical stratification for such patients on the basis of PARP1 expression levels and known risks for treatment-induced translocations. In conclusion, this study raises for the first time the possibility that oncogenic translocations that occur in cancer survivors after therapy might be preventable.

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Authorship

Contribution: J.W. and E.A.W. designed, performed, and analyzed experiments; S.B.S., Y.W., Y.Z., and L.S. performed experiments; D.M.W. provided essential reagents and designed experiments; S.-H.L. analyzed experiments and wrote the manuscript; D.Z. performed and analyzed experiments; C.R.C., R.P., M.H.-J., and V.K. analyzed experiments; J.A.N. analyzed experiments and wrote the manuscript; and R.H. designed and analyzed experiments and wrote the manuscript.

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