Poly-ADP-Ribose Polymerase as a Therapeutic Target in Pediatric Diffuse Intrinsic Pontine Glioma and Pediatric High-Grade Astrocytoma

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Abstract

Pediatric high-grade astrocytomas (pHGA) and diffuse intrinsic pontine gliomas (DIPG) are devastating malignancies for which no effective therapies exist. We investigated the therapeutic potential of PARP1 inhibition in preclinical models of pHGA and DIPG. PARP1 levels were characterized in pHGA and DIPG patient samples and tumor-derived cell lines. The effects of PARP inhibitors veliparib, olaparib, and niraparib as monotherapy or as radiosensitizers on cell viability, DNA damage, and PARP1 activity were evaluated in a panel of pHGA and DIPG cell lines. Survival benefit of niraparib was examined in an orthotopic xenograft model of pHGA. About 85% of pHGAs and 76% of DIPG tissue microarray samples expressed PARP1. Six of 8 primary cell lines highly expressed PARP1. Interestingly, across multiple cell lines, some PARP1 protein expression was required for response to PARP inhibition; however, there was no correlation between protein level or PARP1 activity and sensitivity to PARP inhibitors. Niraparib was the most effective at reducing cell viability and proliferation (MTT and Ki67). Niraparib induced DNA damage (γH2AX foci) and induced growth arrest. Pretreatment of pHGA cells with a sublethal dose of niraparib (1 μmol/L) before 2 Gy of ionizing radiation (IR) decreased the rate of DNA damage repair, colony growth, and relative cell number. Niraparib (50 mg/kg) inhibited PARP1 activity in vivo and extended survival of mice with orthotopic pHGA xenografts, when administered before IR (20 Gy, fractionated), relative to control mice (40 vs. 25 days). Our data provide in vitro and in vivo evidence that niraparib may be an effective radiosensitizer for pHGA and DIPG.

Introduction

Brain tumors account for 20% of all neoplasms in children and are the largest group of solid tumors in childhood (1). Among these tumors, pediatric supratentorial high-grade astrocytomas (pHGA) and diffuse intrinsic pontine gliomas (DIPG) are incurable pediatric malignancies for which no effective therapies exist. The prognosis is poor: the 5-year survival rate for pHGAs is 15% to 30% and the 2-year survival rate for DIPGs is <10% (2–7).

Clinical investigations into the effects of adjuvant radiotherapy or temozolomide on patient survival have shown that temozolomide is ineffective in DIPG. However, radiotherapy, the current standard of care, can increase overall survival of pHGA by 6.26 months (8, 9). Unfortunately, in both pHGA and DIPG, radiotherapy is predominantly palliative offering some symptom control for a limited period of time (10). Both temozolomide and radiotherapy also damage the developing cortex, deep brain structures, and posterior fossa, leading to adverse sequelae in patients, with higher risk when applied at a younger age (11–16).

Chemo- and radiosensitizers may offer the opportunity to improve the therapeutic index by increasing efficacy of radiotherapy or chemotherapeutic drugs while reducing toxicity and damage to developing brain (17, 18).

PARP enzymes catalyze the addition of PAR polymers onto acceptor proteins and are essential for single-strand break (SSB) DNA repair (19). Upon DNA damage, PARP1 binds to DNA via its N-terminal zinc finger motif and automodifies itself with long chains of PAR. This modification negatively charges PARP1 and allows it to dissociate from DNA while serving to recruit additional downstream DNA repair components (19, 20). PARP1 has been shown to be involved in delaying the replication fork progression in homologous recombination (HR)-proficient DNA damaged cells and in alternative pathways of nonhomologous end joining (NHEJ; refs. 21–27). PARP inhibitors are synthetically lethal in BRCA1/2-mutated tumors with DNA repair deficiencies and have been shown to be effective when combined with DNA-damaging agents in isogenic BRCA1/2-deleted cell lines and BRCA1-deficient mouse models (24–27).

High levels of PARP1 expression are found in pHGA and correlate with reduced overall survival (17). Furthermore, in a small cohort of DIPG cases, we identified PARP1 expression combined with LOH in other DNA repair genes (5). The effects...
of PARP inhibitors have been investigated in cell and animal models as well as clinical trials as a monotherapy or with conventional DNA-damaging therapies in adult cancers, including breast, prostate, and lung cancer. This led us to hypothesize that PARP inhibition could potentially be used as a monotherapy or to enhance the therapeutic index of ionizing radiation (IR) in pHGA and DIPG. Veliparib, olaparib, and niraparib are three orally available PARP inhibitors which have all entered phase III clinical trials for adult cancer, demonstrating good tolerability in phase II studies (25–31). However, there is a lack of knowledge regarding the efficacy of these PARP inhibitors in pediatric brain tumors. Therefore, the present study aims to evaluate veliparib, olaparib, and niraparib, using in vitro and in vivo pHGA models.

Materials and Methods

Cell culture and drug treatments

Cells lines and their characteristics are summarized in Supplementary Table S1. Briefly, fetal nonimmortalized normal human astrocytes (NHA; Lonza) were derived from human fetal brain by Lonza and were grown in Clonetics AGM Astrocyte Growth Medium (Lonza), supplemented with 10% FBS (Invitrogen) and 1% penicillin/streptomycin (Invitrogen). SJG2, SF188, and KNS42 cells were derived from pHGA and were grown in DMEM (Wisent) containing 10% FBS and 1% penicillin/streptomycin (Invitrogen; refs. 32–35). Primary cell lines from primary DIPG and pHGA (DIPG58, 464, and 626) were derived by physically and chemically dissociating tumor tissue using sterile scissors, and DNase, trypsin, hyaluronidase, and kynurenic acid dissolved in human artificial cerebrospinal fluid, respectively. Chemical dissociation was inhibited using trypsin inhibitor, and cells were filtered through 40-μm nylon mesh and were grown as follows. Primary, low-passage DIPG cell lines DIPG (36), DIPG58, and SU-DIPG-IV (37) were propagated nonadherently, and primary pHGA cell lines, 462 and 626, were grown as adherent culture in poly-l-ornithine (Sigma-Aldrich; cat. 3184-13-2)/laminin (Sigma-Aldrich; cat. L2020). All primary lines were grown in stem cell media containing Neurocult NS-A Basal Medium (Stemcell Technologies), B27 supplement (2%; Invitrogen), N2 supplement (1%; Stemcell Technologies), and antibiotics/antimycotic (1×; Invitrogen). N2 supplement (1%; Stemcell Technologies), B27 supplement (2%; Gibco), 75 mg/mL BSA (Wisent), human EGF (hEGF; 20 ng/mL; Invitrogen), and human basic FGF (hFGF; 20 ng/mL; Biodose; ref. 36). All cells were grown in a humidified atmosphere at 37°C and 5% CO₂. Veliparib (Selleckchem; cat. S1060), and niraparib (ChemieTek; cat. CT-MK4827) were reconstituted in DMSO (Sigma-Aldrich) prior to treatment of cells at designated concentrations.

Immunohistochemistry and tissue microarrays

Tissue microarrays (TMA) were constructed from formalin-fixed, paraffin-embedded pHGA and DIPG samples obtained from the Hospital for Sick Children Pathology Department as described previously (5). Each TMA contained three 0.5-μm cores from each tumor as well as normal tissues as controls. Paraffin-embedded blocks were cut into 5-μm sections and were dewaxed in xylene followed by rehydration in a standard alcohol series. Antigen retrieval was achieved by pressure cooking for 20 minutes in citrate buffer (pH 6.0), followed by blocking of endogenous peroxidase in 0.3% H₂O₂. PARP1 antibody (1:500, Abcam; cat. ab6079) was added and incubated overnight at 4°C. Detection used biotinylated secondary anti-rabbit antibody (1:200, Vector Labs; cat. BA-1000) for 30 minutes at room temperature, the ABC reagent kit (Vector Labs; cat. PK-6102), and DAB chromagen (Vector Labs; cat. SK-4100). Sections were counterstained with hematoxylin (Fisher Scientific Inc.) for 30 seconds; dehydrated in 70%, 80%, and 100% ethanol; briefly washed in xylene; and mounted in Permperm (Fisher Scientific Inc.). Hematoxylin and eosin (H&E) sections were stained using standard protocols (Eosin Yellowish Solution, 1% w/v; Fisher Scientific Inc.). Two observers blinded to clinical data scored all slides for both intensity [negative (0), weak (1), moderate (2), or strong (3)] and distribution (0%–25%, 25%–50%, or >50%). Concordance between observers was 94%. If the distribution was greater than 25% and intensity greater than 1, a core was considered positive. If two of three cores were positive, a tumor was considered positive for PARP1.

Western blotting and densitometry

Western blotting was performed as previously described (38). Briefly, cells were lysed with standard PLC lysis buffer containing protease and phosphatase inhibitors (Sigma-Aldrich; cat. 11-836-153-001). Protein concentration was determined using the bicinchoninic acid (BCA) assay (Pierce Chemical Co.; cat. 23225). Lysates containing 30-μg total protein were loaded onto 10% SDS-PAGE gels and electrophoresed. Proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore; cat. IPVH00010) using a semidy transfer apparatus (Bio-Rad). Membranes were probed for various proteins overnight in 5% BSA. Primary antibodies were PARP1 (1:1,000; Cell Signaling, 9542S), PAR (1:1,000; Millipore, 4336-BPC-100), GAPDH (1:1,000; Cell Signaling, D16H11), and β-actin (1:10,000; Sigma-Aldrich, A2066-2ML). After incubation, membranes were washed 3 times for 20 minutes with TBS with 0.1% Tween-20 (TBST; Sigma-Aldrich) and incubated with horseradish peroxidase–conjugated secondary antibodies specific for the primary antibody (1:5,000; Bio-Rad; cat. 170-6515) for 1 hour at room temperature. After incubation, membranes were washed again 3 times for 20 minutes with TBST. Binding was detected using Chemiluminescence Reagent Plus (PerkinElmer; cat. NEL103001EA). ImageJ software (http://imagej.nih.gov/ij/) was used to quantify images.

PARP activity assay

PARP activity assay was performed as previously described (39). Briefly, 5 × 10⁴ SJG2 cells were seeded in a 6-well plate and allowed to adhere overnight. The following day, cells were treated with 2 Gy of radiation, the indicated concentrations of PARP inhibitors were added, and cells were incubated for 6 hours at 37°C and 5% CO₂. Cells were washed in ice-cold PBS, trypsinized, and analyzed using Western blotting.

Cell viability and cell counts

MTT Cell Viability Kit I (Roche; cat. 11465007001) was used according to manufacturer’s instructions to evaluate the effects of veliparib, olaparib, and niraparib on cell viability. Cells were seeded in triplicate at the following densities: NHA, 7000 cells per well; SJG2, SF188, KNS42, 462, 626, 3,000 cells per well; DIPGM, DIPG58, SU-DIPG-IV, 8000 cells per well. Cells were allowed to acclimatize overnight. Media containing either DMSO vehicle control or designated concentration of PARP inhibitor were added.
the next day. After 7 days, cells were fixed, lysed, and analyzed at 592 nm using a fluorescence microplate reader (Molecular Devices VenaMax 190). IC_{50} values were calculated using MTT assay data. To evaluate the effect of niraparib on cell number, trypan blue total cells counts were used (Vi Cell Xr cell counter, Beckman Coulter). Briefly, 5 × 10^5 SIG2, SF188, 462, or DIPG58 cells were seeded in 6-well plates in triplicate, treated with DMSO or an indicated concentration of Niraparib and counted. SIG2 and SF188 cells were counted on day 3, and 462 and DIPG58 cells were counted on day 14. Time points were chosen to accommodate relative cell doubling times.

Colony-forming assay

Colony-forming assay was conducted as previously described (38). Briefly, to assess clonogenic survival in response to PARP inhibitors alone, SIG2 or SF188 cells were trypsinized and plated in triplicate in 6-well plates (500 cells per well). The next day, media were replaced with vehicle DMSO control or PARP inhibitors at the indicated concentrations. To assess the radiosensitizing effect of PARP inhibition, SIG2, SF188, or KNS42 cells were treated with 1 μmol/L niraparib, radiated with 2 Gy, or pretreated with 1 μmol/L niraparib 6 hours before receiving 2 Gy (Gamma-cell 40 Exactor). All cells were grown for 10 days, fixed with 4% paraformaldehyde (Electron Microscopy Sciences; cat. 15710), stained with 0.1% crystal violet (Sigma-Aldrich), and the colonies were counted.

Immunofluorescence

Cells were seeded on glass coverslips and incubated overnight to allow for adherence. For cells growing in suspension, poly-L-ornithine (Sigma-Aldrich; cat. 3184-13-2)/laminin (Sigma-Aldrich; cat. L2020)-coated slides were used. The following day cells were treated with vehicle, irradiated, or incubated with variable concentrations of niraparib for indicated times. Cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences; permeated with 0.5% Triton X-100 (Sigma-Aldrich), and blocked with 5% BSA diluted in TBST at 37°C for 1 hour. Slides were incubated with primary antibody overnight at 4°C. Primary antibodies were as follows: hH2AX (1:1,000; Millipore; cat. 05-636, clone JBW301) and Ki67 (1:50; Dako; cat. M7240). After incubation, slides were washed three times for 5 minutes each and xed with 4% paraformaldehyde, permeated with 0.5% Triton X-100 (Sigma-Aldrich), and blocked with 5% BSA diluted in TBST and incubated for 1 hour, at room temperature, in a dark environment with a fluorescent-conjugated antibody specific for the primary antibody (FITC 1:200; Life Technologies; cat. A16079). Slides were washed three times with PBS before being mounted in VECTASHIELD DAPI containing Mounting Medium (Vector Labs; cat. H-1200) and sealed with clear nail polish. Images were captured using a Zeiss AxioVert 200M quorum spinning disk confocal microscope and quantified with ImageJ.

DNA damage repair assay

The assay was conducted as previously described (17). Briefly, a minimum of 0.200 × 10^5 SIG2, SF188, KN42, 462, and DIPG58 cells were seeded on glass coverslips, incubated overnight to allow for adherence. 462 and DIPG58 coverslips were coated with poly-L-ornithine/laminin. Cells were treated with 1 μmol/L niraparib, 2 Gy, or pretreated with 1 μmol/L niraparib for 6 hours prior administration of 2 Gy. Cells were fixed at indicated time points, stained for hH2AX, counterstained with 4,6-diamidino-2-phenylindole (DAPI), and analyzed.

Cell-cycle analysis

Following a 72-hour treatment of niraparib or DMSO control, 1 × 10^6 cells were washed in ice-cold PBS and fixed with ice-cold 80% ethanol for 30 minutes. Cells were then resuspended in RNase A for 5 minutes, followed by incubation with propidium iodide (Sigma-Aldrich) and NP-40 (Sigma-Aldrich) for 30 minutes. Cells were then filtered through 85-μm Nitex mesh and analyzed via flow cytometry (LSRII) for the population of cells in each stage of the cell cycle. Cytometry data were analyzed using FlowJo (http://www.flowjo.com/).

Orthotopic xenografts

Stereotactic-guided intracranial implantations in NOD/SCID gamma (NSG) mice were performed as previously described (38). Briefly, 50,000 SIG2 cells were injected into the frontal cortex (Coordinates: x = −1.0 mm, y = 1.5 mm, z = 2.4 mm, with Bregma serving as reference point). Drugs were administered by intraperitoneal injections on the fifth day postsurgery. Experimental arms included saline (vehicle), niraparib 50 mg/kg daily for two 5-day cycles, 2 Gy IR once a day for two 5-day cycles (total of 20 Gy), or niraparib administered 6 hours before IR—combined arm (40, 41). Mice were sacrificed upon signs of sickness. Animal-use protocols (AIIP) were approved by the University Health Network (UHN) animal care committee (ACC).

Statistical analysis

Experiments were performed in triplicate. Linear regression, 2-tailed t tests, ANOVA, and Kaplan–Meier analyses were used to analyze data. ANOVA for multigroup comparisons was followed by a post-hoc Dunnett test or a post-hoc Tukey test, where appropriate. All statistic analyses were completed by GraphPad Prism 6.0 (http://www.graphpad.com)

Results

PARP1 protein is expressed in pHGA and DIPG patient samples and cell lines

PARP1 protein levels in pHGA and DIPG patient tumor samples were evaluated using TMA. Immunohistochemical analysis demonstrated positive PARP1 protein expression in 28 of 33 pHGA and 13 of 17 DIPG cases, respectively (Supplementary Fig. S1A). PARP1 protein levels in DIPG and normal brain tissues were also characterized by Western blotting. Two of three DIPGs exhibited higher PARP1 levels in tumor relative to normal brain (Fig. 1A and B). A panel cell of lines was characterized for PARP1 and PAR. Three of five pHGA (2 primary lines: 462 and 626) and 3 of 3 primary DIPG cell lines demonstrated elevated PARP1 expression (Fig. 1C and D). PARP activity was elevated in SIG2, DIPG58, and SU-DIPG-B (Fig. 1C and E). Both low PARP1 protein expression and low PARP1 activity were observed in NHA.

Niraparib, when used as a monotherapy, is more effective at reducing tumor cell growth than veliparib or olaparib

We evaluated the monotherapeutic efficacy of three clinically relevant PARP inhibitors, veliparib, olaparib, and niraparib, using MTT viability and colony formation assays. All PARP inhibitors ablated auto-PARYlation of PARP1 in SIG2 cells in response to 2 Gy IR (Fig. 2A). Olaparib and niraparib significantly reduced cell viability across more cell lines and at lower
concentrations than veliparib (Supplementary Fig. S2A and S2B). Relative to other inhibitors, niraparib was the most effective monotherapeutic agent in 5 of 8 cell lines tested (SJG2, SF188, KNS42, DIPGM, and DIPG58; Fig. 2B, Supplementary Fig. S2A and S2B) and was equally effective as olaparib in the remaining three cell lines (462, 626, and SU-DIPG-IV). Niraparib, relative to olaparib, was more effective at reducing clonogenic growth in SJG2 (\( \sim 2 \) μmol/L) and SF188 cells (\( \sim 5 \) μmol/L; Fig. 2C and D). No significant correlations between PARP1 protein levels or activity and response to PARP inhibitors were found; however, some level of PARP1 protein appears to be needed for inhibitors to be effective (Supplementary Fig. S2C–S2E). There was no apparent dose-dependent toxicity to PARP inhibitors in NHAs (Fig. 2B and Supplementary Fig. S2A and S2B). Given its superior efficacy, we conducted all further experiments with niraparib.

**Niraparib causes formation of DNA damage and reduces cell proliferation**

We postulated that niraparib might inhibit endogenous DNA repair causing increased DNA damage and reduced cell proliferation. To determine the acute effects of niraparib on DNA damage and cell proliferation, SJG2, SF188, KNS42, and DIPG58 cells were incubated with 5 μmol/L niraparib for 24 hours. Levels of DNA damage and percentage of proliferating cells were evaluated using γH2AX and Ki67, as markers of DNA damage and proliferation, respectively (Supplementary Fig. S3A–S3D). Across all cell lines tested, niraparib significantly increased DNA damage (γH2AX levels) and reduced proliferation (Ki67 index).

**Niraparib induces growth arrest in SJG2 and SF188 cells**

DNA damage may result in apoptosis and/or growth arrest (42). SJG2 and SF188 cells were treated using an IC50 concentration of Niraparib at 72 hours (2 or 5 μmol/L, respectively). Niraparib significantly reduced cell viability as measured by MTT in both SJG2 and SF188 cells [Supplementary Fig. S4A and S4B]. Treatment with niraparib did not induce apoptosis as measured by cleaved PARP1 (Supplementary Fig. S4C). In SJG2 and SF188 cells, niraparib induced G2–M growth arrest (Supplementary Fig. S4D and S4E).

**Pretreatment with a low dose of niraparib results in persistence of DNA damage after irradiation and sensitizes pHGA and DIPG to radiation**

Because PARP1 plays key roles in repair of double-stranded breaks (DSBs; ref. 19), we hypothesized that niraparib may inhibit IR-induced DNA damage repair and sensitize cells to radiation. The presence of γH2AX foci was used to quantify DNA damage (17). In four pHGA cell lines, SJG2, SF188, KNS42, 462, and 626, and DIPG (DIPGM, DIPG58, SU-DIPG-IV) cell lines. **°,** \( P < 0.01; **°°,** \( P < 0.001; **°°°,** \( P < 0.0001. Error bars, SEM.**
DIPG58 cells, rapid formation of γH2AX foci was observed 30 minutes after IR (Fig. 3A–E and Supplementary Fig. S5A). At 1 μmol/L, niraparib did not increase the background levels of DNA damage. At 24 hours after IR, more γH2AX foci were observed in niraparib pretreated cells relative to cells treated with IR alone, except in SF188 cells. In SJG2, SF188 KNS42, 462, and DIPG58 cells, this difference persisted up to 72 hours after radiation. In SJG2, SF188, and KNS42 cells, pretreatment with 1 μmol/L niraparib was significantly more effective at reducing clonogenic survival when combined with 2 Gy of IR relative to niraparib or radiation alone (Fig. 3F). Similarly, in DIPG58 cells, pretreatment with 1 μmol/L niraparib significantly reduced total cell number relative to IR (Fig. 3G).

Niraparib in combination with radiation prolongs survival in a pHGA orthotopic mouse model

To test the in vivo efficacy of niraparib, we used a SJG2 orthotopic xenograft NSG mouse model, previously established by our group (18). We conducted a small pilot experiment (n = 3) to ensure that SJG2 cells retain PARP1 expression in vivo and to test whether Niraparib was capable of penetrating into the tumor and inhibiting PARP activity (Fig. 4A and B). Previous studies demonstrated that 50 mg/kg doses of niraparib, alone or with radiation, were well tolerated in mice and when combined with radiation achieved the most significant tumor growth reduction (40, 41). The clearance rate of niraparib is 36 hours in humans and 24 hours in mice (30, 40). Taking this into account, we chose to administer either saline or niraparib at a dose of 50 mg/kg daily via intraperitoneal injections (total volume, 200 μL). For our pilot studies, niraparib was administered (1 dose/day × 3 days) 25 days after cell implantation. As in our previous work, mice developed tumors by this time (18). Mice were sacrificed 6 hours after the last dose of niraparib. Tumors and contra-lateral normal brain tissue were harvested and analyzed by Western blotting for PARP1 protein levels and PARP activity. Tumors retained PARP1 expression and a three-day course of niraparib was sufficient to completely inhibit PARP1 activity in vivo.

To test the in vivo effect of niraparib, either alone or in combination with IR, mice were randomized into four treatment arms: vehicle (n = 6), niraparib (50 mg/kg; n = 5), 20 Gy IR (2 Gy/fraction; d = 4), and niraparib + IR (50 mg/kg + 20 Gy; niraparib was administered intraperitoneally 6 hours before IR; n = 7; Fig. 4C and D). Compared with vehicle, treatment with IR or niraparib alone gave a small survival benefit (median survival, 25 days in controls vs. 27.5 and 32 days, respectively, for IR or niraparib alone). However, mice that were pretreated with 50
mg/kg niraparib before each fraction of IR had the longest survival benefit (median survival, 40 days, \( P < 0.001 \) vs. vehicle), 60% longer survival than vehicle mice.

**Discussion**

In the present study, we demonstrate that PARP1 is expressed in patients with pHGA and DIPG and serves as a potential therapeutic target in these cancers. Previous immunohistochemical analyses by our group and others demonstrated PARP1 expression in 54% of DIPG and from 24.1% to 100% of pHGA (5, 43, 44). In our patient cohort, PARP1 expression was found in the majority of pHGA (84%) and DIPG (76%) cases. We corroborated our clinical data by further characterization of DIPG normal tumor patient samples, as well as five pHGA and three DIPG cell lines. Vuurden and colleagues demonstrated PARP1 expression in some
PARP1 may be a potential therapeutic target (17). Cardnell and colleagues did not observe any correlation between overall PARP1 protein levels or enzymatic activity. Similarly, Barton and colleagues who reported that PARP1 does not have a dual mechanism of action by both inhibiting PARP activity and forming endogenous PARP1-DNA complexes at sites of DNA damage that are thought to be cytotoxic to dividing cells. PARP1 plays a role in repair of DNA breaks, and PARP inhibition is known to be particularly effective in cancers deficient in other DNA repair pathways like NHEJ, HR, or base excision repair sensitized DT40 cells to veliparib, olaparib, and niraparib (39). On the other hand, two primary pHGA cell lines, 462 and 626, which had very low levels of PARP1, did not have greater sensitivity. This suggests that even low levels of PARP1 may be sufficient to consider PARP inhibition a therapeutic option. Importantly, in nonirradiated brain, we were unable to detect PARP1 protein or enzymatic activity in keeping with Barton and colleagues who reported that PARP1 does not appear to be expressed in normal, nonneoplastic tissues (46) which should limit toxicity to PARP inhibitors.

Hypersensitivity to PARP inhibitors has also been associated with DNA repair deficiency. In a recent study, we used siRNA to show that knockdown of PARP1 in SJG2 and SF188 led to alkylating agent sensitization (18). Furthermore, Murai and colleagues demonstrated knockdown of PARP1 is sufficient to abolish sensitivity to veliparib, olaparib, and niraparib (39). The idea that PARP1 may be a potential therapeutic target (17). Cardnell and colleagues did not find a significant correlation between PARP1 protein levels and sensitivity to BMN 673, a novel PARP1 inhibitor (45). However, our data and that of others imply that some PARP1 protein must be present for PARP inhibitors to be effective. The NHAs in our study had undetectable levels of PARP1 and enzymatic activity and were insensitive to PARP inhibition and Murai and colleagues demonstrated knockdown of PARP1 is sufficient to abolish sensitivity to veliparib, olaparib, and niraparib (39). On the other hand, two primary pHGA cell lines, 462 and 626, which had very low levels of PARP1, were sensitive to PARP inhibition, whereas cells with more PARP1 did not have greater sensitivity. This suggests that even low levels of PARP1 may be sufficient to consider PARP inhibition a therapeutic option. Importantly, in nonirradiated brain, we were unable to detect PARP1 protein or enzymatic activity in keeping with Barton and colleagues who reported that PARP1 does not appear to be expressed in normal, nonneoplastic tissues (46) which should limit toxicity to PARP inhibitors.

In support of this for PARP inhibitors, Chuang and colleagues found that olaparib was more effective than veliparib in reducing cell proliferation and increasing the formation of γH2AX and cleaved PARP1 in a panel of breast cancer cell lines (25). Interestingly, niraparib and olaparib, but not veliparib, are capable of inducing formation of PARP1-DNA damage complexes at sites of DNA damage that are thought to be cytotoxic to dividing cells (39). This suggests a possible explanation for the poor efficacy of veliparib as a monotherapy in our study; some inhibitors may have a dual mechanism of action by both inhibiting PARP activity and forming endogenous PARP1-DNA complexes (48, 49).

We found that niraparib induced DNA damage and reduced viability across multiple cell lines, suggesting that niraparib, when used as a monotherapy, may act as a DNA-damaging agent at higher doses. In both, SJG2 and SF188, the downstream effect of DNA damage was cell-cycle arrest. However, the effect of PARP inhibitor–related DNA damage is cell likely context dependent. More work is needed to identify markers predicting if a cell line is prone to apoptosis or growth arrest following DNA damage and whether the growth arrest can be manipulated into that of apoptosis.

The conventional mode of therapy for pHGA and DIPG is IR that produces SSB and DSB. PARP1 plays a role in repair of DNA breaks, and PARP inhibition is known to be particularly effective in cancers deficient in other DNA repair pathways (19, 26). This suggested to us that PARP inhibition may sensitize pHGA to IR. In all cell lines tested, pretreatment with a low dose of niraparib (1 μmol/L) did not induce DNA damage. Across a majority of cell lines tested, niraparib, when combined with IR, reduced the rate of
DNA damage repair, clonogenic growth, and cell number relative to niraparib or IR alone. Our data suggest that niraparib can act as a radiosensitizing agent in vitro. Similarly, niraparib was a reported to be a radiosensitizer in neuroblastoma as well as lung, breast, and prostate cancers (40, 41, 50).

One of the largest barriers to effective treatments in pediatric brain tumors is poor drug penetration across the blood–brain barrier (BBB). Here, we confirm effective niraparib penetration across the BBB as validated by our in vivo PARP assay. Importantly, we demonstrated in vivo that while niraparib or radiation alone significantly increases survival relative to vehicle-treated mice, combining both treatments together was most effective. Furthermore, as reported in non–brain tumor preclinical studies, we confirmed in our study that niraparib, whether alone or in combination with radiation, was well tolerated in mice (40). Niraparib phase I clinical trials have been recently published demonstrating that it is well tolerated in patients. In this study of 100 patients, only four patients suffered dose-limiting toxicities; grade 3 fatigue (one patient), grade 3 pneumonia (one patient), grade 4 thrombocytopenia (two patients; ref. 30). However, more work is needed to evaluate the safety and efficacy of niraparib in a pediatric population.

In summary, using patient tumor samples, primary and established disease relevant cell lines, and in vivo orthotopic xenograft model of pHGA, we demonstrate that not all PARP inhibitors are equal when used as a monotherapy, sublethal doses of niraparib are capable of sensitizing cells to IR, and niraparib may be an effective treatment option when combined with IR for pHGA and DIPG.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References


Molecular Cancer Therapeutics

Poly-ADP-Ribose Polymerase as a Therapeutic Target in Pediatric Diffuse Intrinsic Pontine Glioma and Pediatric High-Grade Astrocytoma

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