Synergistic effect of poly (ADP-ribose) polymerase (PARP) inhibitor with chemotherapy on CXorf67-elevated posterior fossa group A ependymoma

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To the Editor: Posterior fossa group A (PFA) ependymoma is one of the most common and aggressive pediatric tumors, which currently lacks effective chemotherapies.[1,2] Previously, we revealed CXorf67, which is specially unregulated in PFA ependymoma, as a negative regulator of DNA homologous recombination (HR) repair, rendered PFA tumors highly sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors combined with radiotherapy through a mechanism of synthetic lethality.[3] However, PFA ependymoma mostly occurs in infants and young children, to whom ionizing radiation may pose great risks. Therefore, there is still an urgent need in the treatment of PFA ependymoma patients who could not or are reluctant to receive radiotherapy. Given that platinum-based drugs (cisplatin and carboplatin), as DNA damaging agents, are widely used as anticancer drugs. This study aimed to investigate whether the combination of PARP inhibitors and DNA-damaging chemotherapy could efficiently kill CXorf67-expressing tumor cells.

The Daoy medulloblastoma cell line was kindly provided by the Stem Cell Bank, Chinese Academy of Sciences, Beijing, China. The CXorf67 knockout (C67-KO) human medulloblastoma cell line Daoy was generated by our lab and grown in minimum essential media (MEM, Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Gibco). The PFA primary cells were isolated from corresponding patient derived xenograft (PDX) models as previously described.[4] All cells were grown at 37°C and 5% CO_2 and confirmed to be free of mycoplasma contamination.

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To test cell viability, the C67-KO Daoy cells, C67-KO with CXorf67 re-expressed cells, and patient derived PFA primary cells transfected with CXorf67-targeting short hairpin ribonucleic acid (shRNA, GGAGGGCTGAACAACGAAACC) viruses or control shRNA (ACAGTTAACCACTTTTTGAAT) were treated with cisplatin (0.1 μmol/L) or carboplatin (1 μmol/L) followed with different concentrations of niraparib, or treated with dimethyl sulfoxide (DMSO) or niraparib (1 μmol/L) combined with various concentrations of cisplatin. After treatment for 5 days, the cell viability was measured. The experiments for each treatment condition were repeated thrice.

All the animal experiments and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences (No. SIBCB1812030). Female BALB/c nude mice (6 weeks old) were purchased from Shanghai SLAC Laboratory animal Co, Ltd (China) and housed in a pathogen-free environment.

For Daoy subcutaneous xenograft models, 3×10^6 cells were mixed with matrigel and injected subcutaneously on the right flanks of nude mice. When tumors reached about 100 mm^3, the mice were randomly divided into four groups ($n = 5$ per group): Vehicle (0.5% methylcellulose in saline), niraparib (50 mg/kg in 0.5% methylcellulose,

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orally once daily for 5 days/week), carboplatin (10 mg/kg in saline, intraperitoneally once a week), and niraparib combined with carboplatin. The mouse tumor volume and weight were measured once weekly.

For the orthotopic xenograft models, C67-KO and C67-KO-re-expressed Daoy cells (2×10^5) with stable luciferase-expressing were implanted in the cerebellum of nude mice. The mice were confirmed tumor formation by bioluminescence and were randomized into four groups ($n = 5$ per group): Vehicle (0.5% methylcellulose in saline), niraparib (50 mg/kg in 0.5% methylcellulose, orally once daily for 5 days/week), cisplatin (2 mg/kg in saline, intraperitoneally once a week), and niraparib combined with cisplatin. The bioluminescence imaging levels were acquired every 7 days.

The PDX model of PFA (PFA-4) was established as previously described.[3] The tumor tissue was dissociated into single cells, and then mixed with matrigel and inoculated into right flanks of nude mice. When tumor volume reached about 100 mm^3, the mice were randomly divided into four groups ($n = 5$ per group) like the orthotopic xenograft models. The mouse tumor volume and weight were measured once weekly. For Kaplan–Meier survival plot, mice were euthanized when tumor volume reached 800 mm^3.

To investigate whether PARP inhibitors combined with DNA damaging chemotherapy could efficiently kill CXorf67-expressing tumor cells, we exposed CXorf67-KO and C67-KO with Cxorf67 re-expressed Daoy cells to different doses of cisplatin in the presence or absence of niraparib. We found that C67-KO cells restored with CXorf67 re-expression were more sensitive to cisplatin than C67-KO cells. Moreover, the half-maximal inhibitory concentration (IC_{50}) of cisplatin was reduced from 451.00 nmol/L to 62.52 nmol/L in C67-KO cells with CXorf67 re-expression restored when niraparib was used [Figure 1A]. To further evaluate the combination effect, we used orthotopic xenograft models and implanted corresponding stable luciferase-expressing cells into the cerebellum of immune-compromised nude mice. We treated the mice with cisplatin, niraparib, and their combination. As shown in Figure 1B, the combination of niraparib and cisplatin showed the strongest effect on the suppression of tumor growth. We also tested the effect of niraparib and cisplatin combination on PFA-4 cell cultures, which had higher CXorf67 expression, as described previously.[3] We knocked down CXorf67 using shRNA in PFA-4 cells and found that CXorf67 knockdown reduced tumor cell sensitivity to cisplatin or its combination with niraparib compared with the control [Figure 1C]. In addition, we performed a combination treatment of niraparib with cisplatin in the PFA-4 model. Similar to the *in vitro* results observed in primary cell cultures, cisplatin in combination with niraparib showed a more substantial effect on tumor growth suppression compared with niraparib and cisplatin used alone [Figure 1D, E].

In summary, the treatment of PARP inhibitors combined with chemotherapy undoubtedly opens a new avenue for PFA ependymoma patients, especially those who cannot or are reluctant to receive radiotherapy. Moreover, it offers an option as a salvage regimen for recurrent PFA patients with CXorf67 high expression, for which no standard medical treatment exists, although further clinical researches are required. In addition, CXorf67 expression is also detected in a few other tumors, including diffuse midline gliomas, germinoma, and Merkel cell carcinoma.[4,5] Whether a combination of PARP inhibitors with DNA-damaging chemotherapy could also be effective in these CXorf67-expressing tumors remains unknown and warrants further studies.

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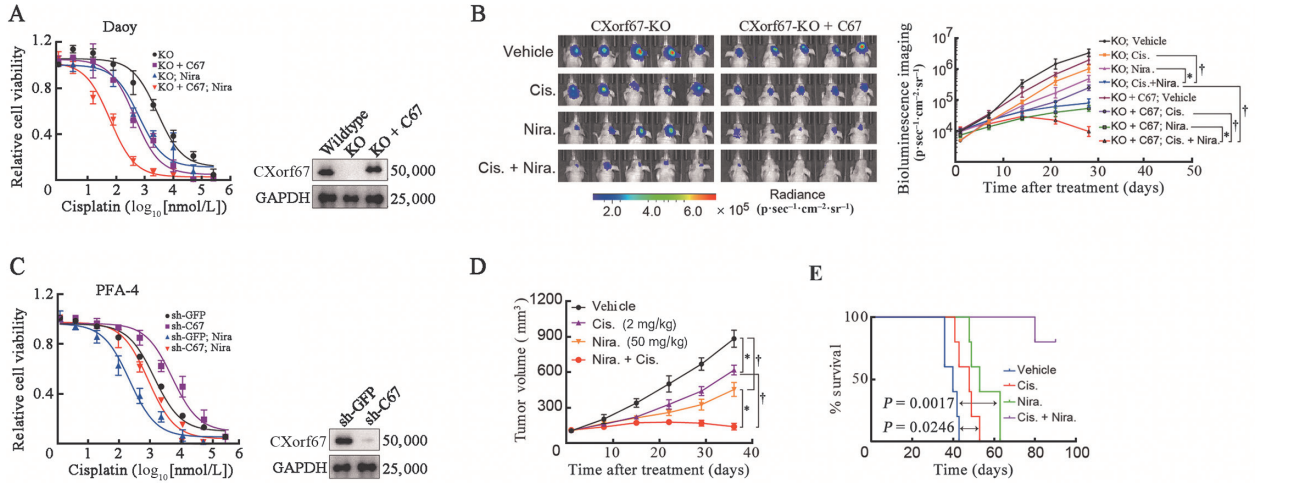

Figure 1: Effect of poly (ADP-ribose) polymerase inhibitor with combination of chemotherapy. (A) Dose-response curves for cisplatin with the indicated concentration in C67-KO and C67-KO with C67 re-expressed Daoy cells treated with 1 μmol/L of niraparib or DMSO. (B) Representative images for orthotopic xenograft experiment. (C) Combined treatment of niraparib with cisplatin enhanced killing of PFA ependymoma primary cell cultures. (D) Tumor volume measurements in the PFA-4 model. (E) Kaplan–Meier survival plots for PFA-4 PDX xenografts. Cis: Cisplatin; Con: Control; C67: CXorf67; DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3 phosphate dehydrogenase; GFP: Green fluorescent protein; KO: Knockout; Nira: Niraparib; PDX: Patient derived xenograft; PFA: Posterior fossa group A; sh-C67: CXorf67-targeting short hairpin ribonucleic acid; $^*P < 0.01$; $^\dagger P < 0.001$.

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Conflicts of interest

None.

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