Resistance to Olaparib is Dependent on Re-Emergence from G2/M Arrested Senescence

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Introduction

Inhibition of poly (ADP-ribose) polymerase (PARP) is an exciting treatment strategy recently approved for prostate cancer patients with DNA repair defects. Despite this advance in the field, there are important unanswered questions regarding PARP inhibitor (PARPi) use; 1) How do PARPi sensitive cells respond to treatment? 2) What mechanisms give rise to PARPi resistance? To address these questions, we sought to characterize response to PARP inhibition using PARPi sensitive LNCaP and C4-2B cells and two PARPi resistant cell line derivatives.

Methods

LN-OlapR and 2B-OlapR olaparib resistant cell lines were generated from LNCaP and C4-2B cells through chronic exposure to increasing doses of olaparib. Western blot was used to detect PARP activity, apoptosis, and DNA damage. Flow cytometry and beta-galactosidase activity assays tested response to PARPi's. CDK1 was inhibited using RNAi and small molecule drug, BMS-265246.

Results

OlapR cells exhibit marked resistance to olaparib versus parental cells. OlapR models are also cross-resistant to other clinically relevant PARPi's including rucaparib, niraparib, and talazoparib. Mechanistically, PARPi treatment inhibits PARP catalytic activity, induces DNA double strand breaks, and activates apoptosis in LNCaP and C4-2B cells. We also observed a cytostatic response in a significant proportion of cells. Flow cytometry showed a robust G2/M arrest in response to olaparib treatment, accompanied by marked increases in p21 expression and beta-galactosidase activity, suggestive of senescence. In contrast, OlapR cells do not exhibit G2/M arrest, increased p21, or senescence in response to PARP inhibition, suggesting that resistance is dependent upon re-emergence from p21 dependent senescence. CDK1 activity governs the G2/M cell cycle phases and is a primary p21 target. Thus, we tested if CDK1 inhibition re-sensitizes OlapR cells to PARPi treatment. Indeed, we found that CDK1 inhibition by either siRNA or BMS-265246 re-sensitized OlapR cells to treatment.

LNCaP and C4-2B derived LN-OlapR and 2B-OlapR cells display robust olaparib resistance

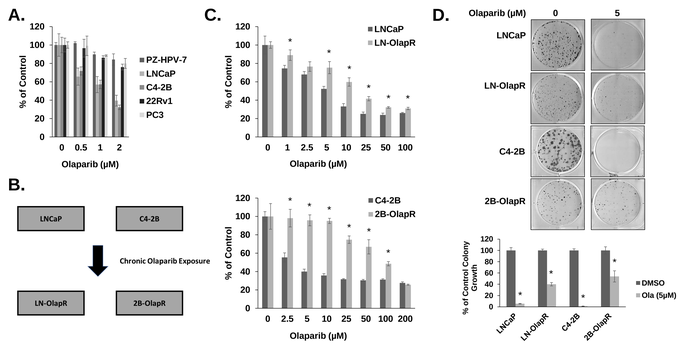

Figure 1: OlapR cell line models are resistant to olaparib. A. Cell growth assays show LNCaP and C4-2B cells are sensitive to olaparib. B. Schematic for OlapR creation. C. Cell growth assays and D. colony formation assays demonstrate resistance to olaparib in OlapR cells versus parental cells. * = p≤0.05.

OlapR cells are morphologically distinct and cross-resistant to clinically relevant PARP inhibitors

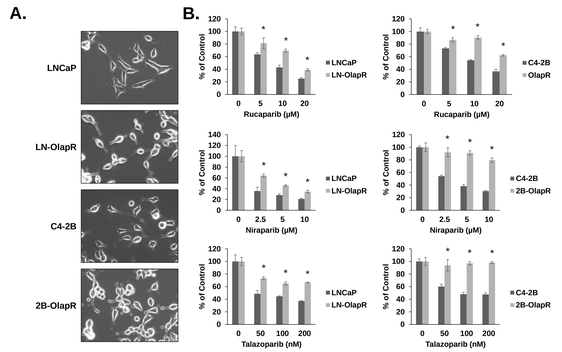

Figure 2: OlapR cell imaging and assessment of PARP inhibitor cross-resistance. A. Cell imaging demonstrates morphological distinction between OlapR cells and parental cells. B. Cell growth assays demonstrate cross-resistance of OlapR cells to other PARP inhibitors. * = p≤0.05.

Characterizing response to olaparib in parental and OlapR cells

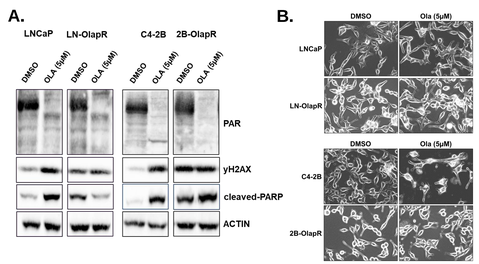

Figure 3: Characterization of response to olaparib. A. Western blots for various markers of olaparib function including PAR, γH2AX, and cleaved-PARP. Actin served as loading control. B. Imaging response to olaparib shows morphology indicative of senescence in parental cells, not OlapR cells.

Olaparib induces G2/M arrested senescence in LNCaP and C4-2B cells, but not OlapR cell line derivatives

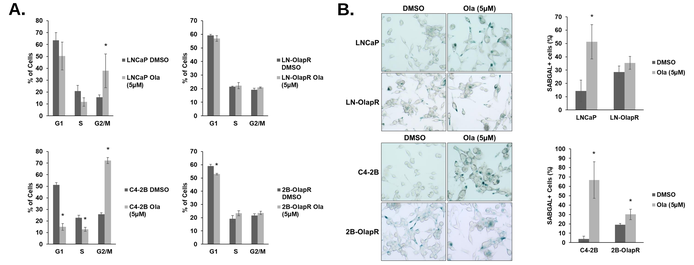

Figure 4: Olaparib induces G2/M arrested senescence in parental cells, not OlapR cells. A. Flow cytometry demonstrates olaparib induces G2/M arrest in parental cells, not OlapR cells. B. Beta-galactosidase activity assays suggest olaparib induces senescence in parental cells, not OlapR cells. * = p≤0.05.

Olaparib induced G2/M arrested senescence is p21-dependent

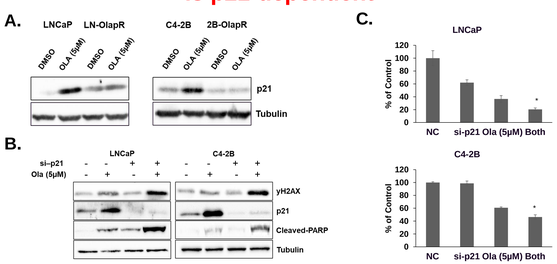

Figure 5: p21 mediates olaparib induced G2/M arrested senescence. A. Western blots show olaparib induces p21 expression in parental cells, not OlapR cells. Tubulin served as loading control. B. Western blots show that inhibition of p21 enhances DNA damage and cell death response in combination with olaparib. Tubulin served as loading control. C. Cell growth assays show inhibition of p21 enhances olaparib efficacy. * = p≤0.05.

Inhibition of CDK1 sensitizes OlapR cells to olaparib treatment

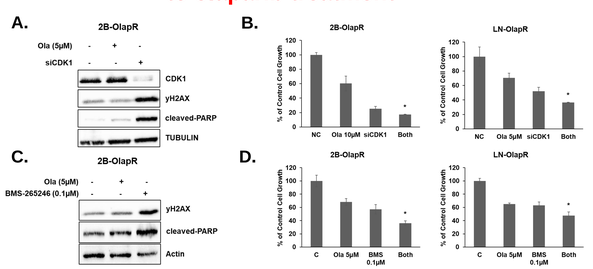

Figure 6: CDK1 inhibition enhances olaparib efficacy. A. Western blot for CDK1, γH2AX, and cleaved-PARP in response to siRNA mediated inhibition of CDK1 or olaparib treatment. Tubulin served as loading control. B. Cell growth assays show CDK1 inhibition sensitizes to olaparib. C. Western blot for γH2AX and cleaved-PARP in response to CDK1 inhibitor, BMS-265246 or olaparib. Actin served as loading control. D. Cell growth assays show BMS-265246 enhances olaparib efficacy. * = p≤0.05.

Conclusions

- Response to PARP inhibition is largely characterized by p21-dependent, G2/M arrested senescence
- Olaparib resistance appears to rely upon re-emergence from G2/M arrested senescence and restoration of cell cycle function
  - PARP inhibitor induced senescence may provide a repository of cells that escape cytotoxicity which may go on to give rise to resistant cell populations
- Inhibition of CDK1 overcomes olaparib resistance and enhances olaparib efficacy.

References

1. Antonarakis ES, Gomella LG and Petrylak DP. When and How to Use PARP Inhibitors in Prostate Cancer: A Systematic Review of the Literature with an Update on On-Going Trials. Eur Urol Oncol 2020;
2. Johnson N, Li YC, Walton ZE, Cheng KA, Li D, Rodig SJ, Moreau LA, Unitt C, Bronson RT, Thomas HD, Newell DR, D'Andrea AD, Curtin NJ, Wong KK and Shapiro GI. Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. Nat Med 2011; 17: 875-882.

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