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

Background: Inhibition of poly (ADP-ribose) polymerase (PARP) is an exciting treatment strategy recently approved for prostate cancer patients with homologous recombination 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.

Conclusions: We find that response to PARP inhibition is characterized largely by a G2/M arrested senescence, which may give rise to resistance through re-emergence from this state. PARPi induced senescence provides an escape route from PARPi cytotoxicity, creating a repository of persistent cells which can give rise to resistance. Targeting CDK1 may prove to be an efficacious strategy for the treatment of re-emerged, PARPi resistant prostate cancer.