ID: 01037
Type: Poster
Topic: Tumor treatment

PARP inhibitors, FOXM1 and CENPF in prostate cancer treatment

Sònia Farran[1] , Katia Ruggero[1] , Adrián Martínez[1] , Xieng Chen[1] , Alvaro Aytes[1]

1) Institut d'Investigació biomèdica de Bellvitge (IDIBELL), Barcelona, Spain

Introduction & objectives

Risk stratification and predictive biomarkers for individualized treatments is paramount in lieu of the increasing heterogeneity of prostate cancer (PCa) patients. This is exemplified in castration-resistant prostate cancer (CRPC), where between 20-25% of the patients have DNA damage repair (DDR) response gene alterations. PARP inhibitors (PARPi) have been shown to be effective in patients that are carriers of BRCA1/2 or ATM mutations, where the homologous repair (HR) is deficient and PARP acts as a backup plan for cancer cells, hence causing an effect known as synthetic lethality. While this has proven successful for a subset of PCa patients there is an urgent need for identifying (i) drug combinations that are synergistic with PARP inhibition, and (ii) biomarkers of HR deficient PCa that can identify patients that would most benefit from these therapies.

Here we will investigate the potential use of bromodomain and extra terminal BET family proteins inhibitors (BETi) to sensitize HR-proficient prostate cancer cells to PARP inhibition and we will investigate which is the mechanism of action. Exploiting a series of genetically engineered mouse models based on alterations in key tumor suppressors like *Pten*, *p53* and activation of oncogenes like *Kras*, we will assess the differential response to these drug combinations.

On the other hand, our group had identified FOXM1 and CENPF as prostate cancer malignancy synergic master regulators. Beyond their important roles in cell cycle progression, they have also been associated to the DDR and epigenetic regulation.. Therefore, we are investigating the cell-cycle independent mechanistic activities of FOXM1 and CENPF in prostate cancer (PCa) and determine whether their activity levels dictate the response to PARPi and combinations.

Materials & methods

NPp53 cells, derived from the $Nkx3.1^{CreERT2}$; $Pten^{floxed/floxed}$;$p53^{floxed/floxed}$ mice, were used to assess drug efficacy *in vitro* in survival assays for OTX015 (BETi); Rucaparib (PARPi), as well as colony formation assays. Cells were treated for 72h. and the synergistic effects were calculated using the Chou-Talalay method though the CompuSyn software or seeded at low densities and let grow for 10 days after which stained with crystal violet and quantified using ImageJ.

Stable silencing of FOXM1 and CENPF using lentiviral mediated delivery of specific shRNAs in 22rv1 and DU145 human PCa cell-lines has been carried out ot investigate whether. associated changes in histone modifications (Acetyl-histone H3 antibody sampler Kit, Methyl-Histone H3 antibody sampler Kit and Tri-Methyl-Histone H3 antibody sampler Kit, (Cell Signaling)) are associated and explain differences in drug sensitivity.

Results

Our preliminary data from the survival and clonogenic assays indicate that the PARPi and Rucaparib drug combination is highly synergistic. In particular, the sensitivity upon the

combined treatment of PARPi and BETi in NPp53 cells is statistically higher in comparison to either treatment alone, and the combination index shows a synergistic effect.

Regarding chromatin remodeling, we see a general dysregulation of the analyzed histone modifications upon FOXM1 and CENPF silencing for which we are currently assessing which of the histone residues modifications best predicts the differential response to drug combinations

Conclusion

We have observed that prostate cancer cells with a *Pten:p53* mutant background display synergistic response to the combined treatment with BETi and PARPi. We will now evaluate (i) whether this effect is background dependent or whether PCa cells with alternative genetic makeups have similar response rates, and (ii) the antitumor effects for these drug combinations *in vivo.* Finally, we will investigate the status of the HR-associated proteins to identify the mechanism of action and possible biomarkers.

On the other hand, FOXM1 targets chromatin-remodeling enzymes such as UHRF1 and HELLS, while CENPF silencing results in general chromatin decondensation. We are.performing CRISPRa-mediated gain-of-function assays to assess whteher FOXM1 and/or CENPF high tumors display a differential response to PARPi and combinations.

[files191=2;500;500][fileShow][/files]