TRIM33 Loss in Multiple Myeloma Impairs the DNA Damage Response Resulting in Sensitivity to PARP and ATR Inhibitors

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Introduction

Chromosomal instability is a hallmark of Multiple Myeloma (MM), with most patients displaying cytogenetic abnormalities which can arise due to DNA damage response (DDR) defects. TRIM33 is an E3 ligase and transcriptional co-repressor located on chromosome 1p13.2, a region frequently deleted in MM. Previous studies have shown that TRIM33 plays a role in the DDR and can regulate chromosomal stability, but its precise function remains unknown. In this study we investigated the impact of TRIM33 loss in MM on genomic stability and DDR pathways and whether this could be exploited therapeutically.

Methods

The CoMMpass dataset (IA15 release) was screened to identify patients with copy number (CN) loss of TRIM33 and this was correlated with overall survival (OS) and structural variants. TRIM33 shRNA knockdown models were established in JJN3 and U266 cells. The effect on DDR signalling was determined by western blotting and immunofluorescence. The Selleckchem DNA Damage/Repair Compound Library was screened on the JJN3 model in a high-throughput manner using the CellTox™ Green cytotoxicity assay. Validation of selected compounds was performed using CellTiter® Glo viability assay or clonogenic assays. Combination indices (CI) were calculated using CompuSyn software.

Results

Data on CN, OS and structural variants were available for 730 newly diagnosed MM patients and of these, 69 (9.5%) were identified to have a CN loss of TRIM33. These patients have poorer OS compared to those without TRIM33 loss (52.3 months vs 72.6 months; $p<0.0001$). Moreover, they exhibit a significantly higher median number of structural variants (deletions, duplications, inversions, and translocations; 38 vs 26; $p<0.0001$), indicative of increased chromosomal instability.

Our data in MM cell lines has showed that TRIM33 is rapidly recruited to chromatin within 5 minutes of induced DNA damage. TRIM33 knockdown led to an increase in 53BP1 foci formation and endogenous γH2AX ($P<0.001$) indicating unrepaired DNA double-strand breaks (DSBs) typical of a DDR defect. In response to these DSBs both ATM and ATR kinases were activated as demonstrated by increased pKAP1^{Ser824} and pCHK1^{Ser345} respectively ($p<0.001$). Additionally, we observed a reduction in RAD51 ($p<0.05$) indicative of a potential defect in the DSB repair pathway homologous recombination (HR).

To identify therapeutic vulnerabilities relating to TRIM33 loss, we performed a high-throughput screen to assess sensitivity to 160 unique DNA damaging compounds. TRIM33 knockdown cells exhibited increased sensitivity to 27 compounds across a range of drug classes. Additional studies confirmed that compared to control cells, TRIM33 knockdown sensitized cells to the PARP inhibitor Olaparib and ATR inhibitors BAY-1895344 and VE-821. Further investigation with VE-821 demonstrated that whilst treatment induced PARP cleavage and DSBs in both control and knockdown cells within 48 hours, knockdown cells exhibited significantly more pCHK1^{Ser345} inhibition ($p<0.01$). Furthermore, combining VE-821 with bortezomib yielded synergistic effects in TRIM33

knockdown cells across a range of doses (CI range 0.57-0.9) while no synergy was observed in control cells (CI>1 for all combinations).

Conclusion

We have identified a subset of MM patients with TRIM33 loss who display high-risk disease characterized by chromosomal abnormalities and defective DDR. Alongside this we have identified PARP and ATR inhibitors as therapeutic vulnerabilities in cell line models of TRIM33 loss. Moreover, we demonstrate that ATR inhibition increases the efficacy of bortezomib in TRIM33 knockdown cells. Further investigation into these compounds could lead to novel therapies for patients with TRIM33 loss.