INRODUCTION

- Multiple Myeloma (MM) is an incurable haematological malignancy of plasma cells.
- Most patients have cytogenetic abnormalities which can drive disease progression.
- Deletion of chromosome 1p (del1p) is a common genetic event with prognostic significance.
- Located at 1p13.2 is TRIM33, a chromatin-associated E3 ligase which can function as a transcriptional co-repressor and often a tumour suppressor.
- Recent studies demonstrate a role for TRIM33 in the PARP-dependent DNA Damage Response (DDR).
- TRIM33 loss can result in the accumulation of chromosomal abnormalities across many cancers
- However, little is known about its precise role in response to DNA damage and in MM specifically.

AIMS & OBJECTIVES

- To explore the impact of TRIM33 loss in MM, focusing on genome stability.
- Uncover the molecular function of TRIM33 in the DDR.

METHODS

Analysis of patient data:
- The publicly available CoMMpass (Relating Clinical Outcomes in MM to Personal Assessment of Genetic Profile) data set (IA15 release) was used.
- 730 patients were screened to identify patients with a CN loss of TRIM33 of which there were 69 (9.5%).
- CN data was correlated with overall survival, structural variants and gene expression files.

In vitro studies:
- Tetracycline-inducible shRNA knockdown was performed on the JN35 cell line.
- Co-immunoprecipitation analysis of TRIM33 and ALC1 was performed using the Pierce™ Co-Immunoprecipitation Kit.
- Western blotting and immunofluorescence were used to determine protein expression.

RESULTS

**Loss of TRIM33 in MM patients correlates with chromosomal instability and poor outcome**

![Figure 1](image1)

- Structural events (translocations, duplications, deletions & inversions) were counted for each patient. Patients with TRIM33 loss had a higher median of structural events compared to those without TRIM33 loss (38 events vs. 26 events).
- Kaplan-Meier analysis revealed patients with TRIM33 loss have a significantly poorer overall survival compared to patients without loss of TRIM33 (median 52.3 months vs 72.6 months).

**There is an accumulation of endogenous DNA damage in the absence of TRIM33**

![Figure 2](image2)

- Representative images of cells before and after induction of Tet-On system with doxycycline (dox) stained for 53BP1 by immunofluorescence. (B) Quantification of 53BP1 foci shown in (A). (C) 53BP1 gene expression is significantly higher in MM patients with TRIM33 loss. *p<0.05, **p<0.01, ***p<0.001.

**TRIM33 interacts with ALC1 rapidly in response to DNA damage**

![Figure 3](image3)

- Western blot analysis of basal RAD51 expression following activation of Tet-On system with doxycycline (dox). (B) Densitometry quantification of (A). **p<0.001 (C) RAD51 gene expression in MM patients. RAD51 expression is higher in patients with TRIM33 loss compared to patients without loss.

**TRIM33 loss is associated with increased RAD51 expression**

![Figure 4](image4)

- Expression of DDR marker γH2AX before and after induction of the Tet-On system with doxycycline (dox) as analysed by western blotting. Together with figure 2, results indicate an increase of DSBRs upon loss of TRIM33.

REFERENCES


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