Loss of TRIM33 Leads to Chromosomal Instability and Increased Endogenous DNA Damage in Multiple Myeloma

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INTRODUCTION

- 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.

The Structure of TRIM33. TRIM33 has an N-terminal RBBC domain typical of the RING E3 ligase family, and a C-terminal PHD-Bromodomain.

- 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 JJN3 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

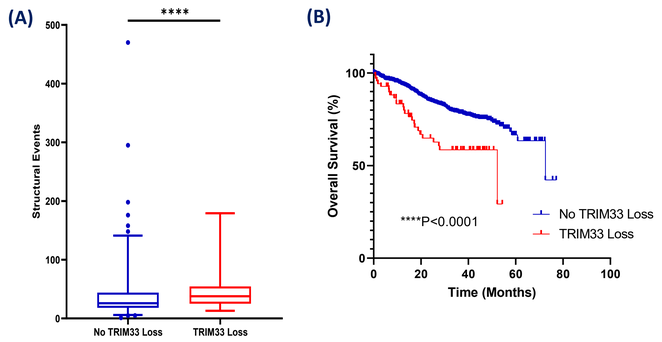


Figure 1. (A) Structural events (translocations, duplications, deletions & inversions) were counted for each patient. Patients with TRIM33 loss had a higher median of structural events compared to those without TRIM33 loss (38 events vs. 26 events) . **(B)** Kaplan-Meier analysis revealed patients with 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

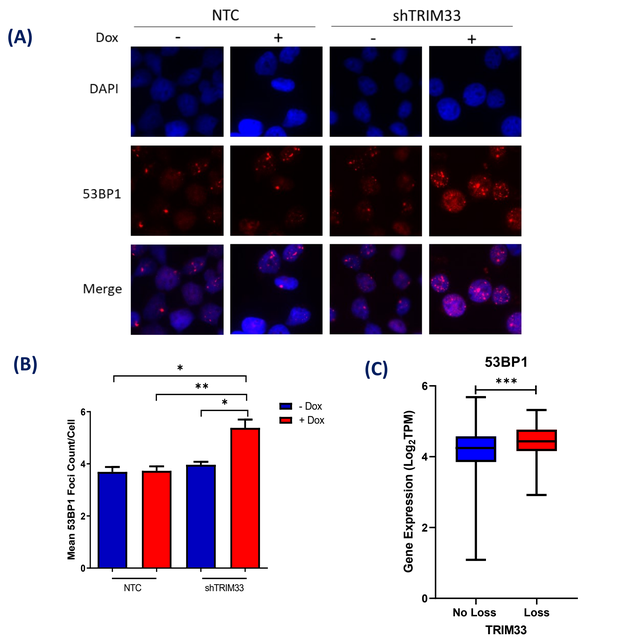


Figure 2. (A) Representative images of cells before and after induction of Tet-On system with doxycycline (dox) stained for 53BP1 by immunofluorescence. **(B)** Quantification of 53BP1 foci shown in (A). **(C)** 53BP1 gene expression is significantly higher in MM patients with TRIM33 loss. *p<0.05, **p<0.01, ***p<0.001.

RESULTS

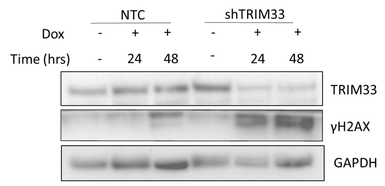
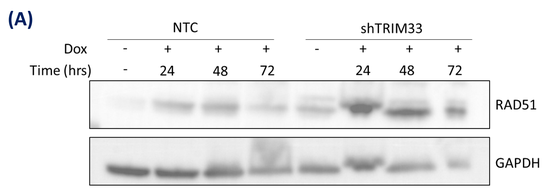


Figure 3. Expression of DSB marker γH2AX before and after induction of the Tet-On system with doxycycline (dox) as analysed by western blotting. Together with figure 2, results indicate an increase of DSBs upon loss of TRIM33.

TRIM33 loss is associated with increased RAD51 expression



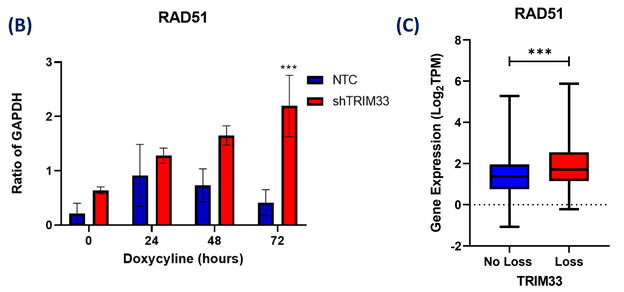


Figure 3. (A) Western blot analysis of basal RAD51 expression following activation of Tet-On system with doxycycline (dox). **(B)**.Densitometry quantification of part (A), ***p<0.001 **(C)** RAD51 gene expression in MM patients. RAD51 expression is higher in patients with TRIM33 loss compared to patients without loss.

TRIM33 interacts with ALC1 rapidly in response to DNA damage

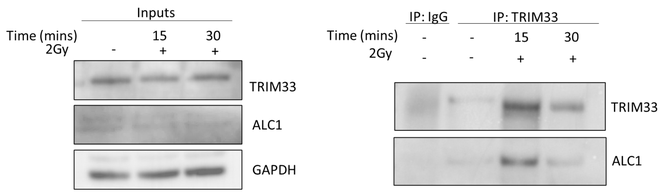


Figure 4. JJN3 cells were either left untreated or treated with 2Gy ionizing radiation (IR), and samples harvested 15 or 30 minutes following 2Gy IR. Co-immunoprecipitation analysis revealed a rapid, transient interaction between TRIM33 and ALC1 in response to 2Gy IR. ALC1 has previously been shown to participate in repair of DSBs by HR.

CONCLUSIONS

- A subset of MM patients have TRIM33 loss and this correlates with poor prognostic outcome and an increased number of chromosomal rearrangements.
- There is an increase in DSBs in the absence of TRIM33 suggesting a DDR defect.
- TRIM33 loss leads to increased expression of RAD51, indicating a potential imbalance of homologous recombination (HR) which may contribute to chromosomal instability.
- We have confirmed a rapid, transient interaction between TRIM33 and chromatin remodeller ALC1 in response to DNA damage in MM.
- Ultimately, a better understanding of the molecular function of TRIM33 in the DDR may uncover novel therapeutic opportunities to exploit MM patients with TRIM33 loss.

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