

TRIM28 functions as a key protein homeostasis regulator and mediates PI resistance in Multiple myeloma

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Introduction:

The intracellular protein homeostasis represents a key biological process essential for the survival of multiple myeloma (MM) cells. Tripartite motif (TRIM) proteins are defined as a subfamily of the RING-type E3 ubiquitin ligase family and play key roles in protein quality control. Here, we investigated the function of TRIM28, a member of TRIM family, as a protein homeostasis regulator in MM.

Method:

Bioinformatic analysis was used to clarify the correlation between TRIM28 with genes related to proteasome and autophagy mediated protein degradation pathway. shRNA knock-down (KD) and over-expression (OE) experiments were used to investigate the function of TRIM28. ChIP-seq and IP-MS were examined to clarify the potential downstream target of TRIM28.

Results:

Survival analysis revealed that the MM patients have poor outcome with high level of TRIM28 in MMRF-CoMMpass and in-house datasets, which were in the context of treatment with bortezomib. Bioinformatic analysis revealed a robust correlation between TRIM28 expression and the expression of proteasome subunits and autophagy-related genes in MM datasets. RNA-seq data showed that both the proteasome pathway and autophagy pathway were down-regulated after TRIM28 KD. TRIM28 KD led to significant decrease in chymotrypsin-like, caspase-like, and trypsin-like proteasome activities, along with ubiquitinated proteins accumulation. Meanwhile, we observed a decrease in autophagosome formation in TRIM28 KD cells, suggesting that TRIM28 activates autophagy. These results underscore TRIM28's critical function in maintaining protein homeostasis by regulating both proteasome and autophagy pathways.

ChIP-seq and IP/MS analyses were employed to delve into TRIM28's mechanism in maintaining protein homeostasis. ChIP-seq showed TRIM28 binding to proteasome gene promoters (e.g., PSMB1, PSMD2, PSMD4), with its knockdown reducing proteasome subunit expression. This implies the role of TRIM28 in transcriptionally activating multiple proteasome genes. IP-MS and Co-IP assay demonstrated that TRIM28 interacts with 14-3-3 ζ , a well-known negative regulator of autophagy. We

observed a significant decrease in the polyubiquitination level of 14-3-3 ζ in TRIM28 KD cells. TRIM28, acting as an E3 ligase, promoted ubiquitin-dependent degradation of 14-3-3 ζ , thus enhancing autophagy in MM.

The role of TRIM28 on MM cell sensitivity to proteasome inhibitors (PIs) was investigated due to its role in protein homeostasis. The results revealed that TRIM28 OE led to a notable reduction in the sensitivity of MM cells to bortezomib both in vitro and in vivo.

In addition to protein homeostasis regulation, TRIM28 could regulate cell proliferation, apoptosis and cell cycle in MM, indicating that TRIM28 plays a multifaceted role in MM.

Conclusion: Our study provided novel important insights into the key role of TRIM28 in maintain protein homeostasis in MM. TRIM28 triggers activation of proteasome and autophagy pathway, which represents a promising therapeutic target in MM.