Therapeutic Targeting of SETD8 Suppresses Multiple Myeloma Growth Through the IRF4/MYC Axis

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INTRODUCTION

- Multiple myeloma (MM) remains largely incurable due to relapse and therapeutic resistance.
- Epigenetic dysregulation, including alterations in DNA and histone methylation, contributes to MM pathogenesis, disease progression, and drug resistance.
- SETD8, the sole H4K20 monomethyl transferase, regulates cell cycle progression, chromatin organization, DNA repair, and genome stability; its overexpression in relapsed MM and association with poor prognosis highlight SETD8 as a potential therapeutic target.

AIM

- This study aims to determine whether pharmacological inhibition of SETD8 using our newly developed covalent inhibitor (Chen et al., 2026) suppresses MM growth in vitro and in vivo, and to identify the oncogenic pathways modulated by SETD8 blockade underlying this anti tumor effect.

METHOD

- SETD8 was depleted in OPM-2 and H929 MM cells using CRISPR/Cas9. SETD8 expression in BMPCs from primary MM patients was assessed by IHC. Bulk RNA-seq was performed following SETD8 depletion. Cell viability and apoptosis were evaluated by CellTiter Glo and Annexin V/7AAD assays, respectively. Cell cycle regulatory genes were analyzed by qPCR and Western blotting. The anti MM activity of the SETD8 inhibitor MS2928 was evaluated in MM mouse xenograft models by monitoring tumor regression.

CONCLUSIONS

- Our findings identify SETD8 as a therapeutic vulnerability in multiple myeloma, with elevated expression in relapsed disease. SETD8 inhibition disrupts oncogenic IRF4/MYC signaling and reduces H4K20me1 levels, leading to suppressed MM cell growth. In vivo, our covalent SETD8 inhibitor shows potent antitumor efficacy, supporting its potential as a therapeutic strategy for high-risk MM.

RESULTS

I. SETD8 is overexpressed in MM and associates with shorter PFS

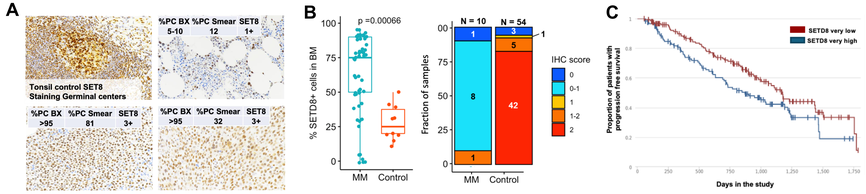


Figure I. Expression of SETD8 in MM patients. (A) Immunohistochemistry (IHC) of CD138⁺ Bone marrow plasma cells (BMPCs) from primary relapsed MM patients depicts heterogeneous SETD8 protein levels. **(B)** SETD8 is significantly overexpressed in BMPCs of primary relapsed MM patients (N=54) compared with healthy control (N=10). **(C)** Analysis of the MMRF CoMMpass study cohort demonstrates that high SETD8 mRNA expression is predictive of poor survival of MM patients. PFS: Progression-free survival

II. SETD8 silencing reduces H4K20me and inhibits MM growth

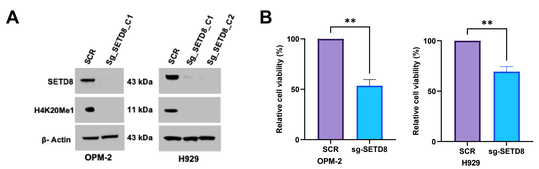


Figure II. Genetic depletion of SETD8 suppresses multiple myeloma cell growth. (A) CRISPR/Cas9-mediated SETD8 knockout reduced SETD8 and H4K20me1 levels in OPM-2 and H929 cells. (B) Cell proliferation was assessed at 72 h in SETD8 KO cells and SCR controls using the CellTiter-Glo assay. Statistical significance was determined using an unpaired Student's *t*-test. Data are presented as mean ± SD from two independent experiments. **p ≤ 0.01.

III. SETD8 Inhibition Alters MYC and IRF4 Expression in HMCLs

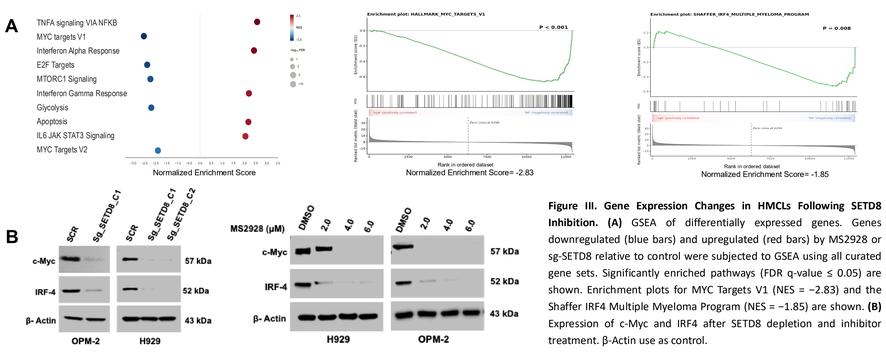


Figure III. Gene Expression Changes in HMCLs Following SETD8 Inhibition. (A) GSEA of differentially expressed genes. Genes downregulated (blue bars) and upregulated (red bars) by MS2928 or sg-SETD8 relative to control were subjected to GSEA using all curated gene sets. Significantly enriched pathways (FDR q-value ≤ 0.05) are shown. Enrichment plots for MYC Targets V1 (NES = −2.83) and the Shaffer IRF4 Multiple Myeloma Program (NES = −1.85) are shown. **(B)** Expression of c-Myc and IRF4 after SETD8 depletion and inhibitor treatment. β-Actin use as control.

IV. SETD8 inhibitor selectively kills MM cells while sparing normal PBMCs

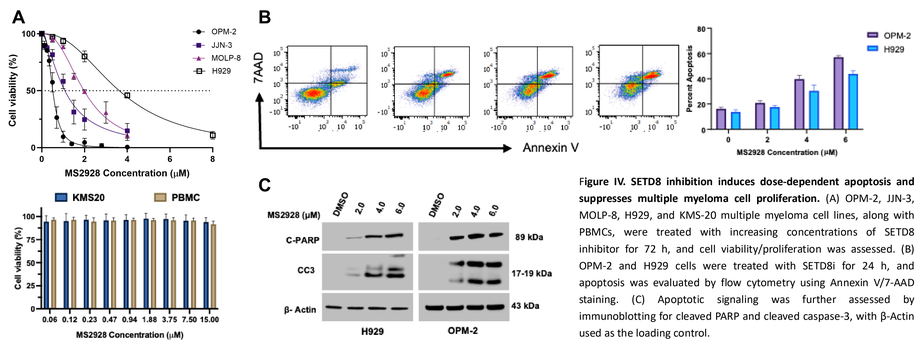


Figure IV. SETD8 inhibition induces dose-dependent apoptosis and suppresses multiple myeloma cell proliferation. (A) OPM-2, JJN-3, MOLP-8, H929, and KMS-20 multiple myeloma cell lines, along with PBMCs, were treated with increasing concentrations of SETD8 inhibitor for 72 h, and cell viability/proliferation was assessed. (B) OPM-2 and H929 cells were treated with SETD8i for 24 h, and apoptosis was evaluated by flow cytometry using Annexin V/7-AAD staining. (C) Apoptotic signaling was further assessed by immunoblotting for cleaved PARP and cleaved caspase-3, with β-Actin used as the loading control.

V. SETD8 Inhibition Activates the p53-p21 Axis

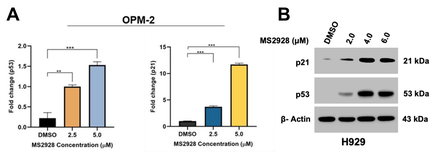


Figure V. SETD8 inhibition increases p53 and p21 expression. (A) OPM-2 cells were treated with SETD8 inhibitor for 12 h, and p53 and p21 mRNA levels were measured by qRT-PCR and normalized to β-actin. **p ≤ 0.01; ***p ≤ 0.001. **(B)** H929 cells were treated with SETD8i, and p53 and p21 protein levels were analyzed by immunoblotting.

VI. SETD8i suppresses the proliferation of MM cells in vivo

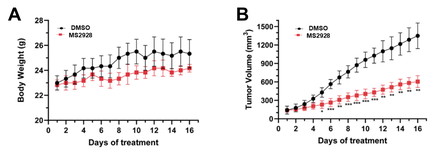
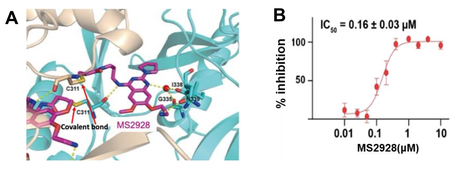


Figure VI. MS2928 is bioavailable and suppresses tumor growth in vivo. Mice bearing OPM-2 tumor xenografts were treated with MS2928 or saline twice daily at a dose of 25 mg/kg by intraperitoneal injection for 14 days. Tumor volume **(A)** and body weight **(B)** of OPM-2 mouse xenograft model were measured (n = 6) every day.

VII. Crystal structure of SETD8 in complex with MS2928 confirms covalent bond formation at C311

Figure VII. X-ray crystal structure of MS2928 in complex with SETD8. (A) The 2.05 Å resolution crystal structure of MS2928 bound to SETD8 (PDB ID: 9CR7) demonstrates covalent modification of the SETD8 catalytic C311 residue by MS2928, providing structural evidence for covalent inhibitor engagement. (B) MS2928 potently inhibited SETD8 methyltransferase activity in a biochemical assay, with an IC_{50} value of 0.16 ± 0.03 μM (n = 3). These structural and biochemical findings confirm the potent and covalent inhibition of SETD8 by MS2928.



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