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RAPID COMMUNICATION

Bortezomib depended on PRDM1 and TP53 to exert therapeutic effect in activated B-cell-like diffuse large B-cell lymphoma

PR/SET domain 1 (*PRDM1*) gene is located on chromosome 6q21, encoding the B lymphocyte-induced maturation protein 1 (BLIMP1).[1] It is reported that loss of PRDM1 function is exacerbated in activated B-cell-like (ABC)-diffuse large B cell lymphoma (DLBCL) and associated with inferior survival. However, it remains unclear what leads to PRDM1 inactivation and the drug resistance mechanism caused by abnormal inactivation of PRDM1. We investigated the contribution of *PRDM1* gene as a prognosis and potential therapeutic target for ABC-DLBCL patients and further clarified the possible mechanism of PRDM1 abnormal inactivation. We first proposed that *TP53* could regulate *PRDM1* by histone ubiquitination modification at the post-transcriptional level. Moreover, the therapeutic effect of bortezomib was dependent on *PRDM1* and *TP53*, and a synergistic effect of lenalidomide and bortezomib was observed in PRDM1-mutated ABC DLBCL cell lines, which provided a theoretical reference for overcoming drug resistance in *PRDM1*-mutated ABC-DLBCL patients.

We collected blood or tissue samples from 104 DLBCL patients to explore the prognostic effect of *PRDM1* mutation on DLBCL patients. Of the eight patients with PRDM1 mutations, six were categorized as a non-germinal center subtype (Table S1) and seven carried $MYD88^{L265P}$ and/or *CD79B* mutations. Compared with patients with wild-type *PRDM1*, patients with *PRDM1* mutation had significantly shorter progression-free survival and overall survival (Fig. S1). In ABC-DLBCL cell lines, *PRDM1* overexpression led to a significant increase in apoptosis (Fig. S2A, B) and inhibition of proliferation (Fig. S2C). At the same time, *PRDM1* knockout suppressed apoptosis (Fig. S2D) and promoted proliferation (Fig. S2E). In summary, the above results demonstrate that PRDM1 was a tumor suppressor gene in ABC-DLBCL cell lines.

In order to further investigate the potential mechanism of PRDM1 inactivation, we selected four ABC-DLBCL cell lines (SU-DHL2, HBL-1, U-2932, RI-1) and one multiple myeloma cell line (U-266) (Table S2). SU-DHL2, which had *PRDM1* exon2 mutation, showed high mRNA levels (both $PRDM1\alpha$ and $PRDM1\beta$) but low levels of PRDM1 protein expression (Fig. S3A, B). In the cycloheximide experiment, the half-life of PRDM1 protein was significantly higher in PRDM1 mutated cell lines than in PRDM1 wild-type cell lines (Fig. S3C). Bortezomib inhibited the degradation of PRDM1 protein caused by *PRDM1* mutation, restored the expression level of PRDM1 protein, inhibited the expression of downstream target genes including c-myc and BCL6 (Fig. 1A) and significantly increased apoptosis in a dose-dependent manner (Fig. S3D, E). The PRDM1 in the nucleus of SU-DHL2 cells showed a significant increase after the bortezomib treatment (Fig. S3F, G). The sensitivity of SU-DHL2 cells to bortezomib was sharply reduced after *PRDM1* knockout, but it was restored by the recovery of PRDM1 expression (Fig. S3H; Fig. 1B). In contrast, treating *PRDM1* wild-type cell line, U-2932, with bortezomib failed to increase PRDM1 protein expression or promote tumor cell apoptosis (Fig. S3I–K).

Previous results have shown that there was no expression of PRDM1 protein in more than 40% of the ABC-DLBCL patients who were PRDM1 wild-type, and the expression level of wild-type *TP53* was positively correlated with the expression level of PRDM1 protein.[2] Yan et al suggested that a loop regulation relationship may exist between *TP53* and *PRDM1* in colorectal cancer cells, *TP53* up-regulated *PRDM1* transcription and *PRDM1* depletion increased p53 expression.[3] We discovered that *TP53* knockdown suppressed the expression of PRDM1 protein, while *TP53* overexpression enhanced the expression of PRDM1 protein (Fig. 1C). However, mRNA levels were inconsistent with protein levels. Determination of the half-life of PRDM1 protein confirmed that the degradation rate of PRDM1 protein increased after

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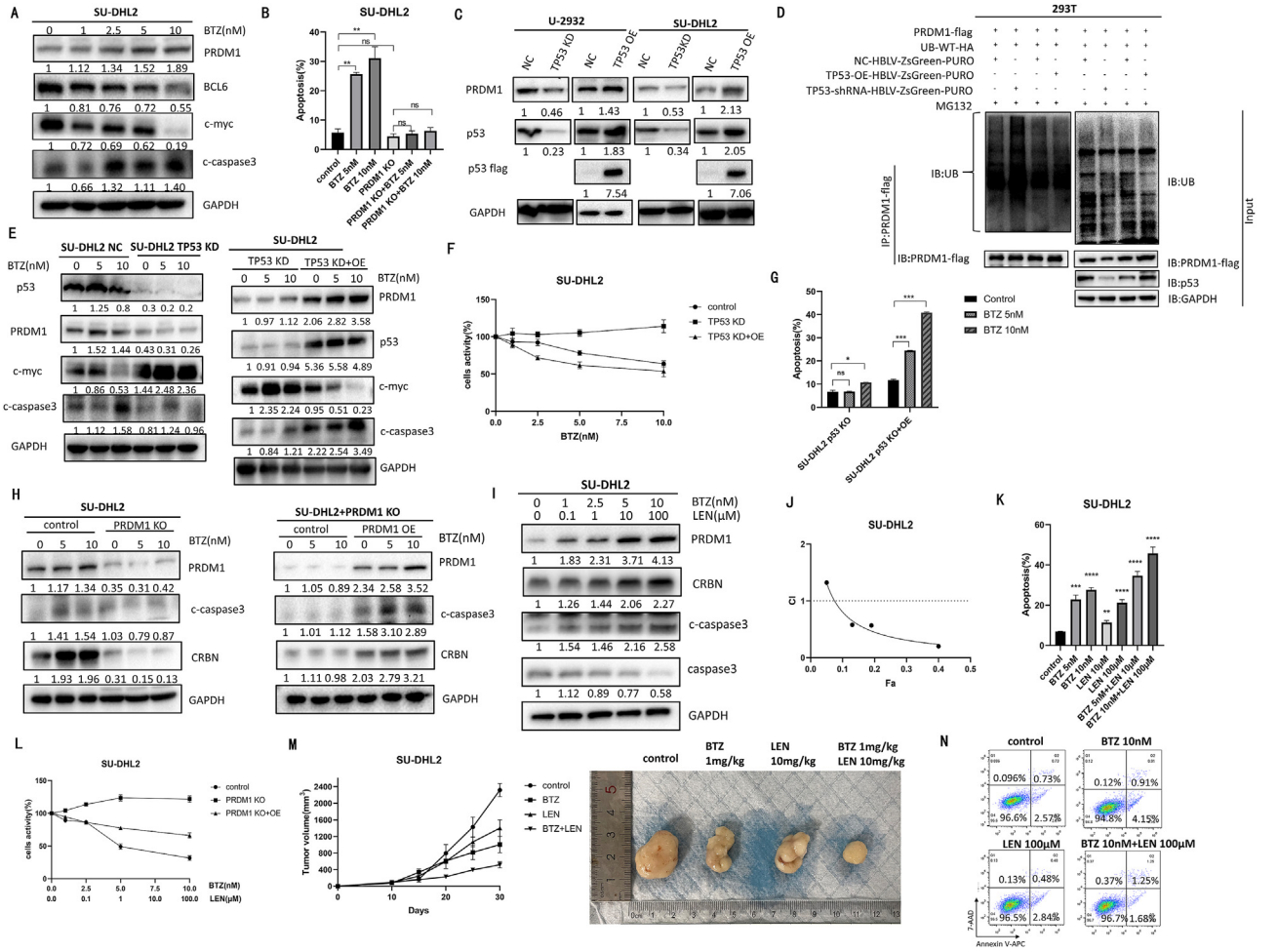

Figure 1 *TP53* regulated *PRDM1* through ubiquitination modification and synergistic effects of bortezomib and lenalidomide. **(A)** Immunoblotting analysis of PRDM1, BCL6, c-myc, and cleaved-caspase3 in SU-DHL2 cells after bortezomib treatment for 24 h. **(B)** The apoptosis levels in SU-DHL2 cells and *PRDM1*-KO-SU-DHL2 cells after bortezomib treatment for 24 h were detected by flow cytometry. The percentage of apoptotic cells was assessed by flow cytometry following Annexin V-APC/7-AAD staining. **(C)** The PRDM1 protein expression in *TP53*-KD/OE-U-2932 and *TP53*-KD/OE-SU-DHL2 cells was determined by Western blot assays. The protein expression levels of the control groups were recorded below the gel images. **(D)** Co-immunoprecipitation analysis and quantitation of PRDM1 binding to ubiquitin in 293T cells in *PRDM1*-OE-control-lentivirus group, *PRDM1*-WT-*TP53*-KD lentivirus group, and *PRDM1*-OE-*TP53*-OE lentivirus group. **(E)** The comparison of PRDM1 after knocking down *TP53* and restoring *TP53* in SU-DHL2 cell line by Western blot assays. **(F)** The comparison of the bortezomib sensitivity of SU-DHL2 cells after knocking down *TP53* and restoring *TP53* by CCK8 method. The viability of the cells was compared with that of the corresponding controls. All viability values are means obtained from three independent experiments. **(G)** Annexin-V APC and 7AAD staining were used to detect the cell apoptosis of SU-DHL2 cells after knocking down *TP53* and restoring *TP53* by flow cytometry. **(H)** Western blots showed the changes of PRDM1, CRBN, and c-caspase3 between *PRDM1*-KO-SU-DHL2 cells and *PRDM1*-KO-SU-DHL2 cells with restored PRDM1 expression levels after bortezomib treatment. **(I)** Immunoblotting analysis of PRDM1, CRBN, and apoptosis-related proteins in SU-DHL2 cells after bortezomib and lenalidomide treatment for 24 h. **(J)** Combination indexes for different levels of growth inhibition (fraction affected) were calculated using the CompuSyn software. **(K)** The dot plots showing the difference in apoptosis of SU-DHL2 cells treated with bortezomib and lenalidomide for 24 h. **(L)** The correlation between *PRDM1* and lenalidomide sensitivity was detected by CCK8, and the synergistic effect of bortezomib and lenalidomide in the SU-DHL2 cell line was dependent on *PRDM1*. **(M)** Mice in each cohort were treated with PBS (control), bortezomib (1 mg/kg), or lenalidomide (10 mg/kg) twice weekly for 3 weeks. Tumor volumes were measured every 3–4 days. The tumors isolated from different groups were shown in the panel. **(N)** The dot plots showing the difference in apoptosis of U-2932 cells treated with bortezomib and lenalidomide for 24 h. BTZ, bortezomib; c-caspase3, cleaved caspase3; CHX, cycloheximide; LEN, lenalidomide; KD, knockdown; KO, knockout; OE, overexpression; UB, ubiquitination; WT, wild type. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. $^{****}P \leq 0.0001$.

knockdown of *TP53*, but decreased after overexpression of *TP53* (Fig. S3L). Co-immunoprecipitation experiments were performed to analyze the quantitation of PRDM1 binding to ubiquitin. It was found that the ubiquitination degradation rate of PRDM1 protein was enhanced after *TP53* knockdown but suppressed after the overexpression of *TP53* (Fig. 1D).

After the knockdown of *TP53*, bortezomib failed to restore the expression level of PRDM1 protein and slowed down the degradation rate (Fig. S3M). With the recovery of p53 expression, bortezomib caused a significant increase in the expression level of PRDM1 protein again (Fig. 1E). Furthermore, the sensitivity of SU-DHL2 to bortezomib was significantly reduced after the knockdown of *TP53*, but restored after p53 expression recovered in *PRDM1* mutated cell lines (Fig. 1F, G). As suggested by the above results, bortezomib relies on *TP53* to eliminate tumor cells. Yet, there is no report on the regulatory relationship between *TP53* and *PRDM1* in DLBCL. Our study is the first to propose and confirm that *TP53* regulated the PRDM1 protein ubiquitination-proteasome degradation pathway in ABC-DLBCL. Post-translational modification initiates cellular processes by regulating the physicochemical properties, folding, conformation, stability, and activity of proteins. Phosphorylation, ubiquitination, glycosylation, acetylation, and SUMOylation have all been discovered to be capable of regulating signal transduction, epigenetics, and protein expression. Bortezomib failed to restore PRDM1 expression after *TP53* knockdown, which indicated that *TP53* may also regulate *PRDM1* through other post-translational modifications, which requires further research.

As revealed by a study on multiple myeloma, higher PRDM1 expression enhanced the drug susceptibility of lenalidomide.[4] CRBN has already been confirmed as the target of lenalidomide.[5] The expression level of PRDM1 was positively correlated with that of CRBN (Fig. S3N; Fig. 1H). By combining bortezomib and lenalidomide in the *PRDM1* mutated SU-DHL2 cell line, it was observed that there was a significant increase in the expression level of PRDM1, CRBN, and apoptosis-related proteins (Fig. 1I). The combination index calculated using the CompuSyn software confirmed the synergistic effect (combination index <1) in this combinatorial therapy quantitatively in SU-DHL2 cell line (Fig. 1J). After 24 h of combined treatment with bortezomib and lenalidomide, the apoptosis of SU-DHL2 cells was significantly increased (Fig. 1K). Moreover, the synergistic effect of bortezomib and lenalidomide was reversed in *PRDM1*-KO-SU-DHL2 cells, and it was restored when the expression level of PRDM1 was recovered (Fig. 1L). As confirmed by the *in vivo* experiments conducted on mice, the combined use of bortezomib and lenalidomide significantly reduced the tumor volume after SU-DHL2 tumor formation (Fig. 1M). However, there was no synergistic inhibitory effect observed in *PRDM1* wild-type cells (Fig. 1N), which proved that the synergy in ABC-DLBCL is specific to *PRDM1* mutated cell lines. Overall, our study confirmed the applicability of *PRDM1* gene as a prognosis and potential therapeutic target for ABC-DLBCL patients and clarified the possible mechanism of PRDM1 inactivation. At the genetic level, *PRDM1* mutation caused protein instability and degradation, while bortezomib reversed the inactivation of PRDM1 caused by *PRDM1* mutation. In addition, *TP53* regulated PRDM1 by histone ubiquitination modification after transcription and *TP53* knockdown mitigated the anti-tumor effect of bortezomib on *PRDM1* mutated cells. In *PRDM1* mutated cell lines, the application of bortezomib and lenalidomide in combination produced a synergistic effect by improving the expression level of PRDM1 and CRBN and enhancing the therapeutic effect, which is conducive to the treatment of drug-resistant ABC-DLBCL (Fig. S4).

Ethics declaration

All experiments involving human samples were approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (No. 2022-SR-319) and met all requirements of Helsinki. All animal experimental procedures were approved by Nanjing Medical University Institutional Animal Care and Use Committee (IACUC) (No. IACUC-2111048). The study was performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (eighth edition, NIH).

Author contributions

JT and LY conducted experiments, analyzed data, and wrote the paper. WX, YX, JT and LY performed conception and design. JZW carried out the data collection and analyses. JHL, HY and HRS participated in the data discussion and revised the manuscript. JYL, LW, YX and WX critically reviewed and approved the final content of the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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Data availability

The datasets generated during the current study are not publicly available since they will contain patient data and the informed consent agreement does not include sharing data publicly. An anonymized form of the data could be made available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.012.

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