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RAN contributes to bortezomib resistance in multiple myeloma via regulating the Wnt/PCP pathway

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Previous studies have shown that Ras-Related Nuclear Protein (RAN), a member of the RAS superfamily, is a small GTPase and an important oncogene in several cancers. However, its role in multiple myeloma (MM) and its potential contribution to drug resistance remain undetermined. Bioinformatics was employed to analyze differentially expressed genes in MM samples. RT-qPCR and western blotting were utilized for protein transcription and expression analysis. The CCK-8 assay was adopted to evaluate cell proliferation, and in vivo animal experiments were conducted to validate the results. The findings reveal that RAN represents one of the most significantly aberrant genes in MM, with its expression significantly elevated in both MM tissues and cells. Genetic manipulation experiments demonstrated that RAN promotes MM cell proliferation by activating the Wnt/PCP pathway. Concurrently, RAN governs the response of MM cells to the anti-cancer drug bortezomib (BTZ). Knockdown of RAN leads to increased sensitivity to BTZ. Mechanistic studies indicate that RAN influences drug response by regulating the activation of the JNK/c-Jun axis, thereby affecting the therapeutic response of MM cells. In summary, the upregulated expression of RAN in MM leads to BTZ resistance via activation of the Wnt/PCP pathway, potentially serving as a novel therapeutic target for MM.

Key words: multiple myeloma; RAN; Wnt/PCP pathway; bortezomib

Multiple myeloma (MM) is a malignant tumor derived from terminally differentiated plasma cells, which are a type of white blood cell responsible for producing antibodies [1, 2]. This disease primarily affects the bone marrow and can lead to various complications due to its impact on normal hematopoiesis and immune function [3]. The incidence rate of MM is only second to that of non-Hodgkin lymphoma, making it the second most common malignant tumor in the hematopoietic system. Despite advancements in treatment strategies that have improved patient outcomes over time, MM remains an incurable condition with a variable prognosis [4, 5]. Especially during chemotherapy treatment, drug resistance may develop, leading to treatment failure [6]. Therefore, exploring the mechanisms of resistance in MM treatment to chemotherapy will help improve survival rates while minimizing the adverse side effects of current treatments.

Ras-Related Nuclear Protein (RAN), a member of the RAS superfamily, is mainly distributed on the nuclear membrane of cells and has functions such as regulating material transport, regulating microtubule polymerization, and nuclear membrane assembly [7, 8]. Silencing the expression of the RAN gene in tumor cell lines can result in disorders in spindle formation during mitosis, absence of mitochondrial function, and cell apoptosis. Targeted gene therapy against this pathway may provide new cues for anti-tumor treatment [9]. High expression of RAN mRNA and protein in cell lines and tissues of various tumors is associated with poor prognosis in tumor patients, and inhibiting the expression of this gene can reduce tumor formation [10–13]. This suggests that RAN is an important oncogene in tumor development. To date, the study of RAN is largely unknown, and there are many controversies in MM. However, the mechanism of action of RAN in MM and the mechanism of RAN-mediated MM drug resistance remain unclear.

In the current study, we probed into the role of RAN in MM cells and its potential molecular mechanisms. Moreover, we explored the role of RAN in mediating bortezomib (BTZ) resistance in MM cells, which is likely to provide a promising therapeutic target for overcoming drug resistance in MM and furnish new insights into the pathogenesis of MM.

Patients and methods

Clinical samples. The bone marrow blood samples from MM patients collected from 20 patients with MM admitted to Zhuji People's Hospital of Zhejiang Province from June 2023 to June 2024 were selected as the research subjects. Meanwhile, we recruited 20 donors without malignancy (iron deficiency anemia patients, due to menorrhagia or hemorrhoid blood loss, and excluded malignant tumors) in the Zhuji People's Hospital of Zhejiang Province. This study was approved by the Ethics Committee of Zhuji People's Hospital of Zhejiang Province (Approval No. 2024-(1030)). Informed consent was obtained from participants or their legal representatives. All research processes are carried out in accordance with the Declaration of Helsinki.

Cell culture. MM cells RPMI-8226 and MM.1S were bought from the National Infrastructure of Cell Line Resource (Beijing, China), OPM2 was purchased from Tongpai (Shanghai) Biotechnology Co., Ltd. (Shanghai, China), and U266 was purchased from ATCC (Manassas, VA, USA). Cells were cultivated under the conditions of 37 °C and 5% CO_2 in RPMI-1640 medium (Solarbio Life Sciences, Beijing, China), which was supplemented with 10% fetal bovine serum (FBS, Excell Bio, Suzhou Excell Biotech Co., Ltd., Suzhou, China) and 1% penicillin/streptomycin (Biosharp Life Sciences, Beijing, China).

Establishment of BTZ-resistant MM cell lines. We established MM cell lines (OPM2 and MM.1S) resistant to BTZ through continuous induction of MM cells with low-dose BTZ (1.0 nM) and a gradual increase of the drug concentration to 10 nM over a period ranging from one to six months. We further confirmed the phenotype of acquired BTZ resistance in MM cells by measuring the IC_{50} of BTZ-resistant MM cells using the CCK8 assay.

Plasma cell separation. First, 5 ml of bone marrow fluid from healthy controls was taken and placed in a 15 ml centrifuge tube, followed by suspension with 15 ml of 1× PBS. In another sterile centrifuge tube, lymphocyte separation liquid was added at a ratio of 1:1 to the bone marrow fluid, and centrifugation was conducted at 24 °C and 240×g for 25 min. Subsequently, the centrifuge tube was removed, the intermediate white membrane layer was aspirated, and resuspended with 10 ml of 1× PBS. This process of gentle blowing and thorough mixing was repeated. Centrifugation was then performed again at 20 °C and 120×g for 10 min. The supernatant was removed to eliminate platelets mixed within individual nucleated cells, and this operation was repeated twice. Subsequently, magnetic bead sorting was carried out, followed by buffer suspension and the addition of 100 µl of CD138 antibody. The mixture was placed in an incubator at 4–8 °C for 15 min. A buffer was added for washing, and the cells were resuspended. The cell suspension was injected into the separation column to collect the $CD138^+$ cell suspension. The magnetic separation column was flushed with buffer as necessary. The above steps were repeated to obtain highly pure $CD138^+$ cells. Finally, the $CD138^+$ cells were resuspended in 1× PBS and filtered through a 70 µm filter. The isolated and purified primary $CD138^+$ bone marrow cells were cultured in RPMI-1640 (containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin).

Plasmid construction and cell transfection. The *RAN* (NM_006325.5) gene was entrusted to Tsingke Biotechnology Co., Ltd. for synthesis and construction into the pcDNA3 vector, thereby establishing the overexpression vector pcDNA3-RAN (oe-RAN). Moreover, the RAN knockdown shRNA sequence GTGGATATTAAGGACAGGAAATCTCGAGATTTCCTGTCCTTAATAT CCAC was synthesized by Tsingke Biotechnology Co., Ltd. and incorporated into the pSilencer 2.1-U6-Neo to obtain the RAN interference plasmid pSilencer 2.1-sh-RAN (sh-RAN).

Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) was employed for plasmid transfection and interference experiments following the instructions. The cells were seeded in cell culture dishes one day before the transfection experiment. When the cell density reached 70–80% confluence, the cells were subjected to the transfection experiment. When the cell density reached 40–50% confluence, the cells were subjected to the interference experiment.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR). Total RNA was extracted from the specimens with the TRIzol RNA Extraction Kit (Beyotime Biotechnology, Shanghai, China), and subsequently, the RNA was reverse transcribed into cDNA. The relative expression level of RAN mRNA was determined by the real-time fluorescence quantitative PCR (qPCR) approach using the real-time fluorescence quantitative PCR kit (Beyotime Biotechnology, Shanghai, China). GAPDH was employed as the internal reference gene, and the relative expression was represented by the $2^{-\Delta\Delta Ct}$ value. The qPCR primer sequences were as follows: RAN-qPCR-F: 5'-GGTGGTACTGGAAAAACGACC-3', RAN-qPCR-R: 5'-CCCAAGGT GGCTACATACTTCT-3'; GAPDH-qPCR-F: 5'-CTGGGCTACACTGAGCACC-3', GAPDH-qPCR-R: 5'-AAGTGGTCGTTGAGGGCAATG-3'.

Cell counting kit-8 (CCK-8) assay. Following transfection, MM cells were cultivated for 48 h and subsequently digested with trypsin. Thereafter, they were re-seeded at a density of 2×10^3 cells/well in 96-well plates. CCK-8 reagent was supplemented to the wells on a daily basis. On the fourth day after the addition of CCK-8 reagent, the wells were placed in a culture incubator in the dark for 1–2 h, and subsequently, the absorbance at 450 nm was measured using an enzyme-linked immunosorbent assay (ELISA) reader. The experiment was replicated three times, and the cell proliferation curve was plotted.

Western blotting assays. Total proteins were extracted from the cells employing RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) and subsequently quantified by the BCA method after sonication. Equal amounts of total proteins were separated through SDS-PAGE and transferred onto a PVDF membrane (Millipore Corpora-

tion, USA). The PVDF membrane was incubated with RAN (1:1000, #10469-1-AP, Proteintech, Wuhan, China), JNK (1:5000, #24164-1-AP, Proteintech, Wuhan, China), p-JNK (1:2000, #80024-1-RR, Proteintech, Wuhan, China), Wnt5A (1:500, #R26111, ZenBio, Chengdu, China), c-Jun (1:3000, #24909-1-AP, Proteintech, Wuhan, China), p-c-Jun (1:1000, #28891-1-AP, Proteintech, Wuhan, China), and GAPDH (1:10000, #10494-1-AP, Proteintech, Wuhan, China) at 4 °C overnight, respectively. Then, it was incubated with HRP-conjugated secondary antibody (1:1000, #SA00001-2, Proteintech, Wuhan, China) at room temperature for 2 h. The protein bands were detected via ECL chemiluminescence. Images were captured and quantified using the FluorChem 8900 image analysis system (Alpha Innotech Corporation, USA). The ratio of the target protein to the corresponding GAPDH protein was utilized as the relative expression level of the target protein.

***In vivo* animal experiments.** MM.1S-BR cells stably carried Lenti-sh-NC and Lenti-sh-RAN by lentivirus-mediated infection, and 2×10^6 MM.1S-BR cells were subcutaneously inoculated in the right flank of 6- to 8-week-old NOD/SCID female mice. When the tumor sizes were ~5 mm in diameter, the BTZ (0.5 mg/kg) was administered intraperitoneally for 2 weeks. The mice were euthanized by intraperitoneal injection of sodium pentobarbital, and the tumor weight and size were recorded. The animal study was approved by the Animal Ethical and Welfare Committee of HZNU (Ethic Approval Number: HSD-20241104-05) in compliance with the national guidelines for the care and use of animals.

Statistical analysis. The data were subjected to statistical analysis by means of SPSS version 17.0. Quantitative data were presented as mean ± standard deviation, and pairwise comparisons were carried out using independent samples t-tests. A p-value of <0.05 was regarded as statistically significant.

Results

RAN is highly expressed in MM. To delve into the pathogenesis of MM, we scrutinized the relevant data from the GEO database (GSE6691) and executed a differential expression analysis, thereby uncovering differentially expressed genes (Figure 1A). The heatmap of the significantly differentially expressed genes, encompassing RAN, is depicted in Figure 1B, where it is conspicuous that RAN is highly expressed in MM patients (Figure 1C). Subsequently, we verified the expression of RAN in the bone marrow blood samples of MM patients and found that the expression level of RAN in the bone marrow blood samples of MM patients was significantly higher than that of patients with polycythemia who were confirmed to have no malignant tumors. (Figure 1D). The protein levels were verified by western blotting in 4 MM patients and 4 controls (Figure 1E). Ultimately, we analyzed the expression level of RAN in 4 MM cell lines (OPM2, MM.1S, U266, and RPMI-8226) and normal plasma cells and ascertained that the expression level of RAN was significantly elevated in MM cells compared to normal plasma cells (Figure 1F). In sum, we found that RAN was upregulated in both MM patients and MM cell lines, suggesting that it might play a role in the development and progression of MM.

RAN acts as an oncogene by enhancing the proliferation of MM cells. To explore the role of RAN in MM, we initially fabricated plasmids for RAN overexpression and knockdown, and subsequently validated their efficacy. The outcomes demonstrated that overexpression of RAN in OPM2 and MM.1S cell lines significantly augmented the mRNA and protein levels of RAN, whereas knockdown of RAN led to a substantial decrement in its transcriptional and expression levels (Figures 2A, 2B). Subsequently, we probed the influences of alterations in RAN expression on MM cells. The CCK-8 assay results manifested that the overexpression of RAN significantly enhanced the proliferation of MM cells, while the knockdown of RAN brought about a marked reduction in the proliferation of MM cells (Figures 2C, 2D). These results suggest that RAN has a proliferative function in MM cells.

RAN activates the Wnt/PCP pathway in MM. To delve into the molecular mechanism by which RAN promotes the proliferation of MM cells in a profound manner, we employed the GSEA software (https://www.gsea-msigdb.org/gsea/index.jsp) to dissect the molecular mechanisms of RAN within GSE6691 [14]. The outcomes revealed a significant association between the expression level of RAN and the Wnt/JNK pathway, implying that RAN might exert regulatory control over the Wnt/JNK pathway (Figure 3A). Subsequently, we probed the alterations in the levels of JNK and its phosphorylated form in MM cells by manipulating the expression level of RAN. The results manifested that upon overexpression of RAN, the protein level of p-JNK/JNK underwent a significant escalation, while after knockdown of RAN, the protein level of p-JNK/JNK decreased (Figure 3B). In addition, to investigate whether RAN modulates JNK signaling via the Wnt/PCP pathway, we examined the expression levels of Wnt5A, a critical molecule in the Wnt/PCP signaling pathway, by overexpressing or knocking down RAN. Our findings revealed that RAN overexpression significantly upregulated Wnt5A expression, whereas RAN knockdown markedly reduced its expression (Figure 3B). These results demonstrated that RAN is capable of modulating JNK signaling via activating the Wnt/PCP pathway in MM cells.

RAN promotes MM cell proliferation via the JNK/c-Jun axis. We have evidenced that RAN is capable of activating the Wnt/PCP pathway; yet, the precise mechanism through which RAN promotes MM cell proliferation via the downstream signaling of the Wnt/PCP pathway remains ambiguous. Hence, we treated MM cells with the JNK inhibitor, SP600125, while concurrently overexpressing RAN and contrasted it with the untreated group. The results disclosed that the ratio p-JNK/JNK increased

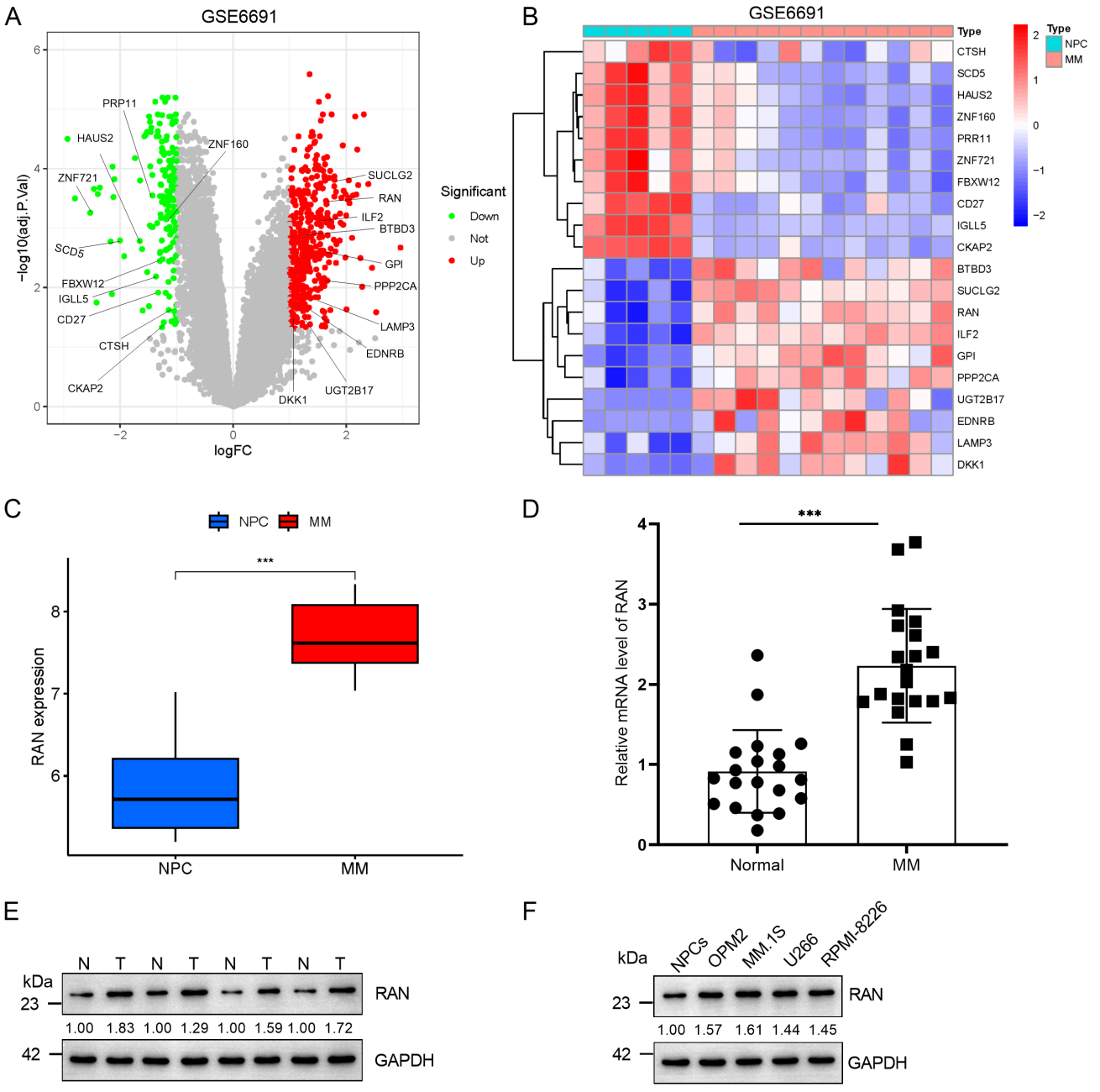

Figure 1. RAN level is upregulated in MM. A) A volcano map showing the 173 downregulated and 436 upregulated genes (cutoff, 1.5 folds) in the bone marrow (BM) samples from 12 patients with MM compared to the five normal plasma cells (NPC) from BM of healthy donors obtained from GSE6691 datasets. B) A heatmap listed the 20 differentially expressed genes from (A). C) RAN was upregulated in the BM samples of MM compared with the NPC of healthy donors. D) RT-qPCR was used to detect the mRNA levels of RNA in the BM blood samples from 20 MM patients and 20 patients with polycythemia who were confirmed to have no malignant tumors (Normal). E) Elevated RAN expression in the BM plasma cells from 4 MM patients (T) and four non-malignant samples (N) by western blotting. F) RAN protein expression was detected in MM cells and NPCs by western blotting. ***p<0.001

upon sole overexpression of RAN, while it was significantly suppressed in the RAN overexpression group after treatment with SP600125 (Figure 4A). Moreover, we examined the effect of JNK activation on the level of the downstream molecule c-Jun. The results showed that overexpression of RAN alone significantly increased the level of p-c-Jun/c-Jun, while treatment with SP600125 significantly reduced its expression level (Figure 4A). Next, we discovered via the CCK-8 assay that overexpression of RAN alone expedited MM cell proliferation, while the proliferative capacity of MM

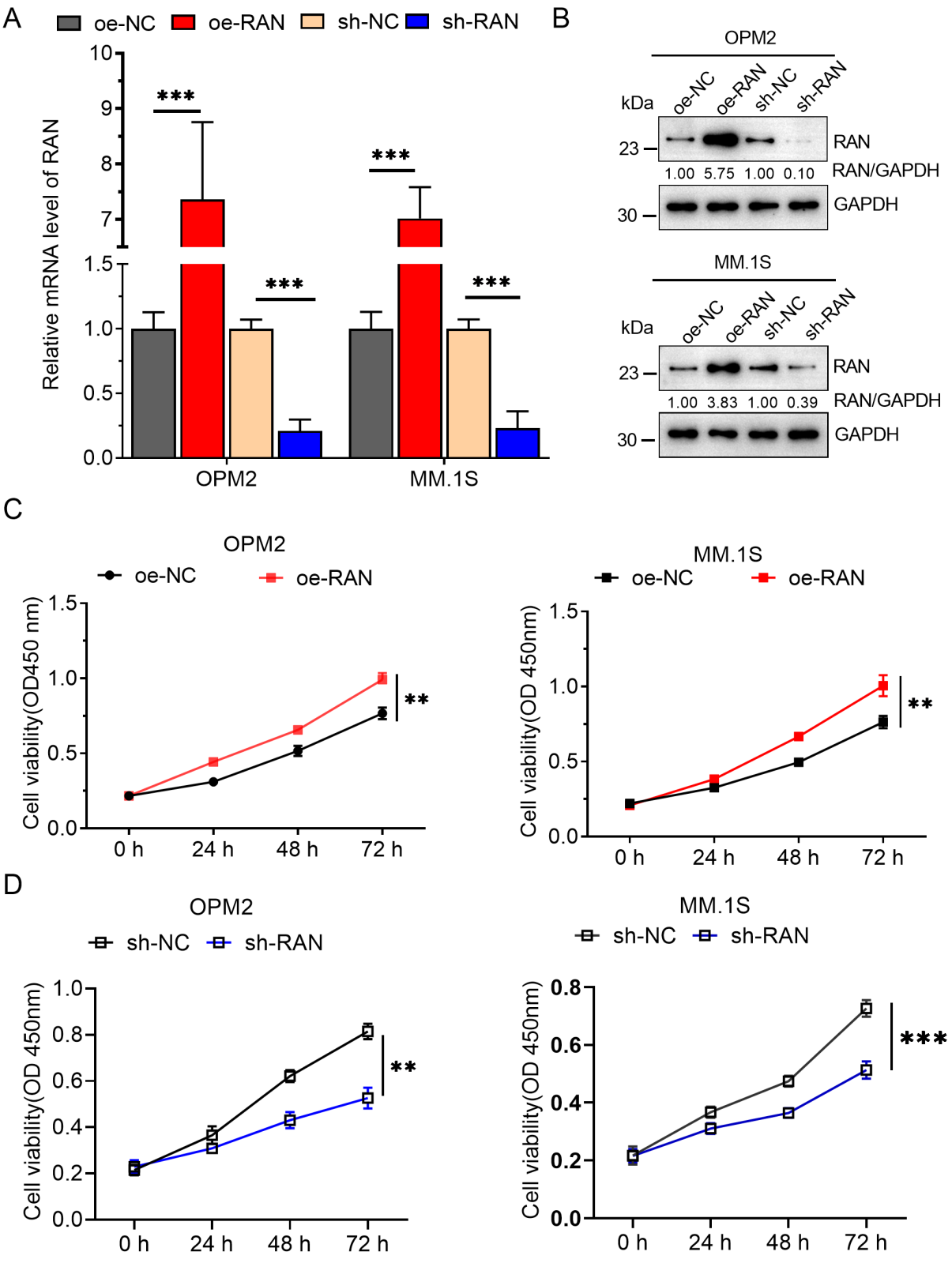

Figure 2. RAN increases the proliferation of MM cells. A) MM cells were transfected with the indicated vectors; the mRNA levels of RAN were detected by RT-qPCR. B) The expression of RAN was determined by western blotting. C, D) CCK-8 assay was used to detect the role of RAN overexpression (C) and RAN knockdown (D) on MM cell proliferation. **p<0.01, ***p<0.001

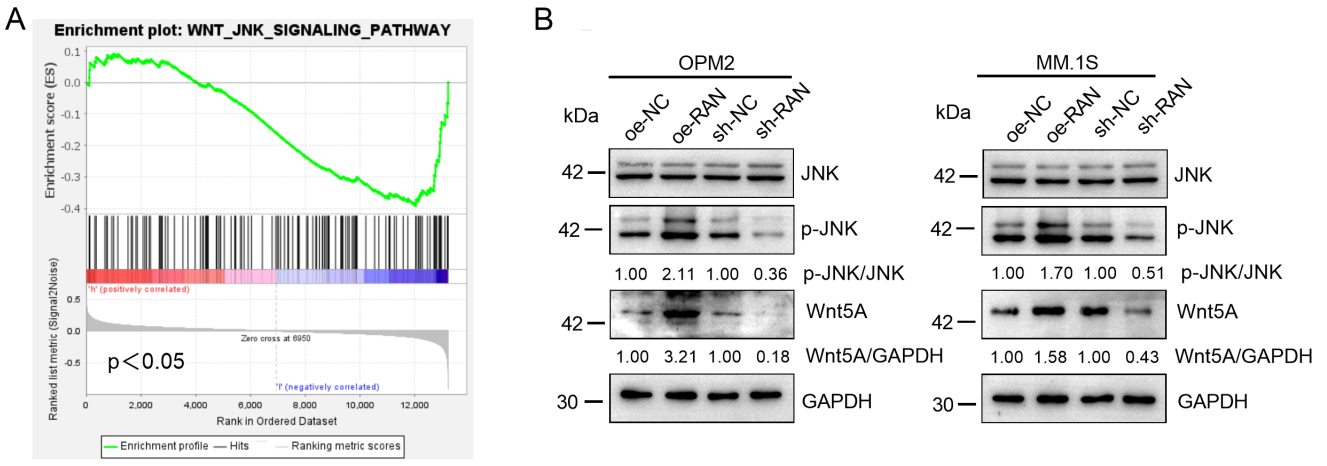

Figure 3. Wnt/PCP/JNK pathway is activated by RAN in MM. A) Gene Set Enrichment Analysis (GSEA) results revealed the enriched Wnt/JNK pathway that correlates with RAN expression in MM patients from the GSE6691 datasets. B) Western blotting experiments detected the protein levels of JNK, p-JNK, c-Jun, and p-c-Jun after transfected with RAN overexpressing or knockdown plasmids.

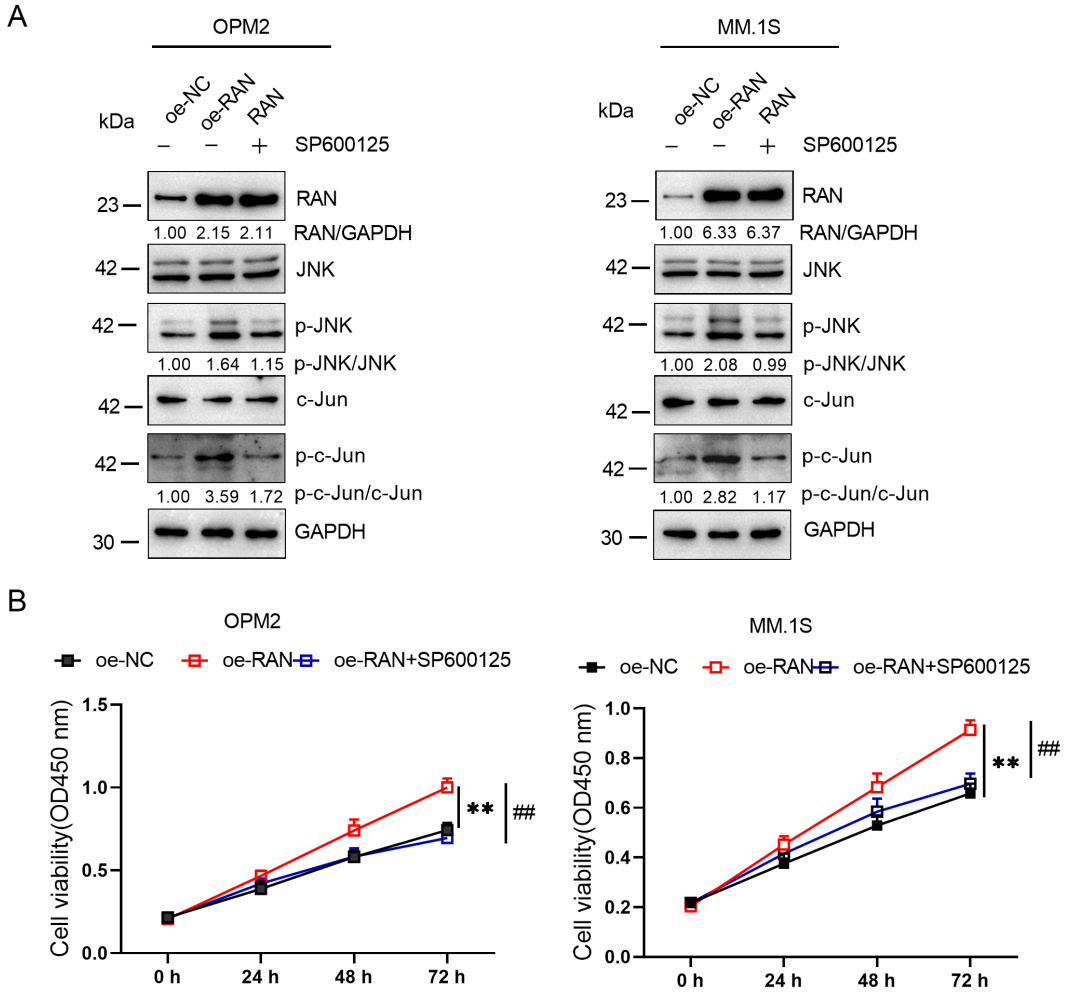

Figure 4. JNK inhibitor SP600125 abolishes MM cell proliferation induced by RAN overexpression. A) Levels of RAN, JNK, p-JNK, c-Jun, and p-c-Jun in MM cells transfected with the indicated plasmids, followed by treating with 25 μM of SP600125 for 24 h. B) The CCK-8 experiment was used to detect the role of SP600125 on MM cell proliferation in RAN overexpressing cells. **p<0.01, RAN vs. Vec-NC; ##p<0.01, RAN plus SP600125 vs. with RAN

cells was restrained after overexpression of RAN followed by treatment with SP600125 (Figure 4B). These outcomes imply that RAN promotes MM cell proliferation by activating the JNK/c-Jun signaling.

RAN knockdown increases BTZ sensitivity in BTZ-resistant MM cells. BTZ has exhibited favorable clinical efficacy in the treatment of MM; nonetheless, the phenomena of primary resistance to BTZ or initial sensitivity followed by secondary resistance persist. Hence, exploring the molecular mechanism of BTZ resistance holds significant importance. Initially, we screened for MM cell lines resistant to BTZ (OPM2-BR and MM.1S-BR) (Figure 5A). Subsequently, we knocked down RAN in these BTZ-resistant MM cells and found a substantial reduction in RAN (Figure 5B). In MM-BR cells, upon knockdown of RAN and subsequent treatment with 5 nM BTZ, the results indicated a significant decrease in the proliferation capacity of both OPM2-BR and MM.1S-BR cells (Figure 5C). The *in vivo* animal experimentation results demonstrated that the tumorigenic ability of MM.1S-BR was inhibited after BTZ treatment (Figures 5D, 5E). These outcomes suggest that knockdown of RAN can increase the sensitivity of BTZ-resistant MM cells to BTZ.

RAN drives the BTZ resistance via activating the JNK/c-Jun axis in BTZ-resistant MM cells. The downregulation of RAN can enhance the sensitivity of MM-BR cells to BTZ, yet the associated molecular mechanisms remain elusive. The above studies have demonstrated that in MM-WT cells, RAN regulates the proliferation of MM cells through the JNK/c-Jun axis. Therefore, we subsequently explored whether RAN could activate the JNK/c-Jun axis in OPM2-BR and MM.1S-BR cells and whether SP600125 could inhibit this activation. Our results indicated that the expression of RAN alone could activate the JNK/c-Jun axis, but this activation was inhibited by the treatment with SP600125 (Figure 6A). The CCK-8 assay showed that the treatment of RAN-overexpressing MM resistant cells with SP600125 resulted in a marked decrease in cell proliferation compared to the cells with only RAN overexpression (Figures 6B, 6C). Similarly, we treated RAN-knockdown MM resistant cells with JNK activator (anisomycin), and the results revealed that the knockdown of RAN alone inhibited the JNK/c-Jun axis and suppressed the proliferation of MM resistant cells. However, the treatment with the JNK activator anisomycin was capable of activating the JNK/c-Jun axis and promoting the proliferation of MM resistant cells (Figures 6D, 6E). These results suggest that the BTZ resistance mediated by RAN in MM cells is modulated by the molecular mechanism involving the JNK/c-Jun axis.

Discussion

With the in-depth investigations into MM, coupled with improvements in treatment strategies and the development of novel drugs, the survival rate of patients with MM has witnessed a marked elevation. Nevertheless, owing to its heterogeneity, drug resistance, and proclivity for recurrence, the mortality and recurrence rates of MM persist at high levels, and the disease remains incurable, profoundly influencing the quality of life of patients [15–17]. Therefore, the study of the pathogenesis and drug resistance mechanisms of MM holds paramount significance for the clinical treatment of this malignancy.

RAN, or Ras-related nuclear protein, is a member of the RAS superfamily of small GTPases [18]. It plays a crucial role in various cellular processes, including nucleocytoplasmic transport, cell cycle regulation, and signal transduction [18, 19]. In the context of cancer biology, RAN has garnered attention due to its involvement in several mechanisms that contribute to tumorigenesis [20]. Abnormal expression levels of RAN have been observed in various types of tumors, suggesting that it may act as an oncogene [13, 21–23]. Wnt/PCP pathway is activated and plays a crucial role in the occurrence and development of tumors [24–27]. Herein, RAN has been identified as a pivotal molecule in the pathogenesis of MM, particularly through its interaction with the Wnt/PCP signaling pathway. The findings indicate that RAN not only enhances the expression of Wnt5A, a crucial component of the Wnt/PCP pathway, but also activates the JNK/c-Jun axis, which is integral to cellular proliferation. Specifically, RAN overexpression leads to an increase in p-JNK levels, suggesting that RAN modulates JNK signaling by activating the Wnt/PCP pathway. This relationship underscores the role of RAN as a regulator of downstream signaling pathways that influence MM cell proliferation. The diversity of Wnt signaling is exemplified by the functional divergence between the canonical pathway (Wnt/β-catenin-dependent) and non-canonical pathways, such as the PCP pathway. For instance, FZD3, a subtype of the Frizzled receptor, predominantly activates the canonical pathway via LRP5/6 co-receptors to promote β-catenin-dependent gene transcription. In contrast, FZD6 tends to form complexes with ROR2 or PTK7, initiating non-canonical signals that regulate cell polarity and migration [28]. This receptor-specific selectivity may underlie tissue-specific regulation mechanisms, although the precise molecular details remain to be fully elucidated. Within the PCP pathway, Frizzled receptors, including FZD6, activate Rho family GTPases (RhoA, Rac1, Cdc42) and downstream JNK kinases. Furthermore, recent studies have demonstrated that GEF-independent RAN protein activation regulates nuclear-cytoplasmic transport, thereby promoting cell proliferation [29]. These findings suggest potential crosstalk between non-canonical Wnt pathways and the RAN pathway in regulating cell cycle progression. For example, it would be valuable to investigate whether the ROR2-PTK7 complex enhances β-catenin-independent proliferation signals through RAN-dependent transport mechanisms.

The activation of the JNK/c-Jun axis by RAN is further substantiated by the observation that inhibition of JNK signaling using SP600125 significantly reduces the proliferative capacity of MM cells overexpressing RAN. This

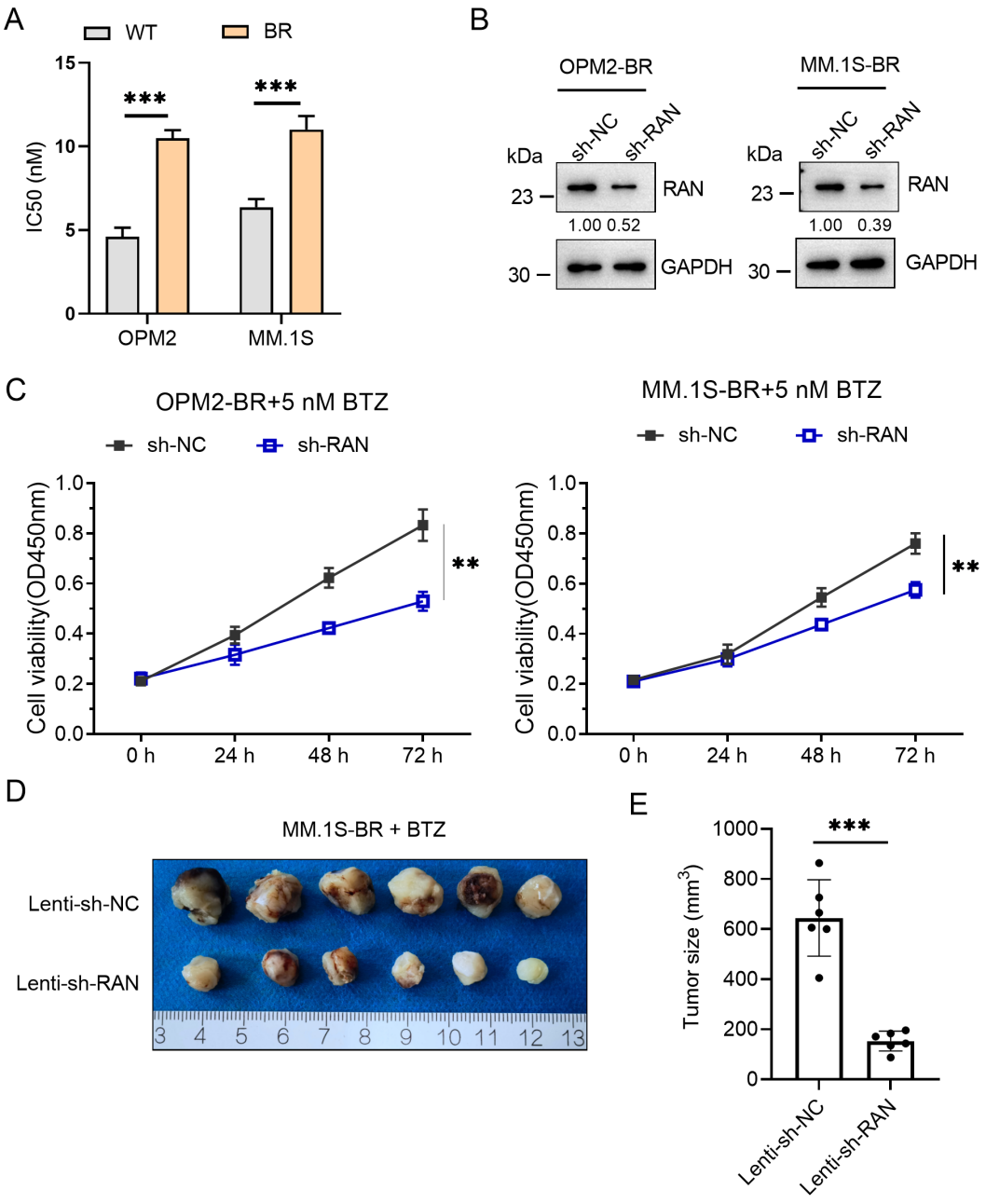

Figure 5. Downregulation of RAN enhances drug sensitivity in BTZ-resistant MM cells. A) Alteration of IC_{50} value in BTZ-resistant MM cells compared to the parental wild type (WT) cells. B) Western blotting experiments detected the protein levels of RAN after RAN knockdown. C) BTZ-resistant MM cells were transfected with RAN knockdown plasmids and the control plasmids for 24 h, then the cells were treated with 5 nM BTZ, and a CCK-8 assay was performed to detect the ability of cell proliferation. D) The images of tumors were taken from NOD/SCID female mice 4 weeks after subcutaneous inoculation and treatment with the BTZ (0.5 mg/kg) (n=6). E) The tumor volume was measured after the mice were euthanized. **p<0.01, ***p<0.001

indicates that RAN's oncogenic effects are mediated through the JNK/c-Jun signaling pathway, highlighting a critical molecular mechanism by which RAN promotes MM cell growth. In recent years, despite the remarkable progress achieved in the treatment of MM with BTZ, acquired resistance has constituted a major issue in the therapy of this disease [30]. After undergoing long-term BTZ treatment, patients inevitably develop resistance to the drug [31, 32]. Hence, a more profound comprehension of the mechanisms of MM resistance to BTZ will be conducive to the development of novel clinical treatment strategies. Herein, we downregulated the expression level of RAN in BTZ-resis-

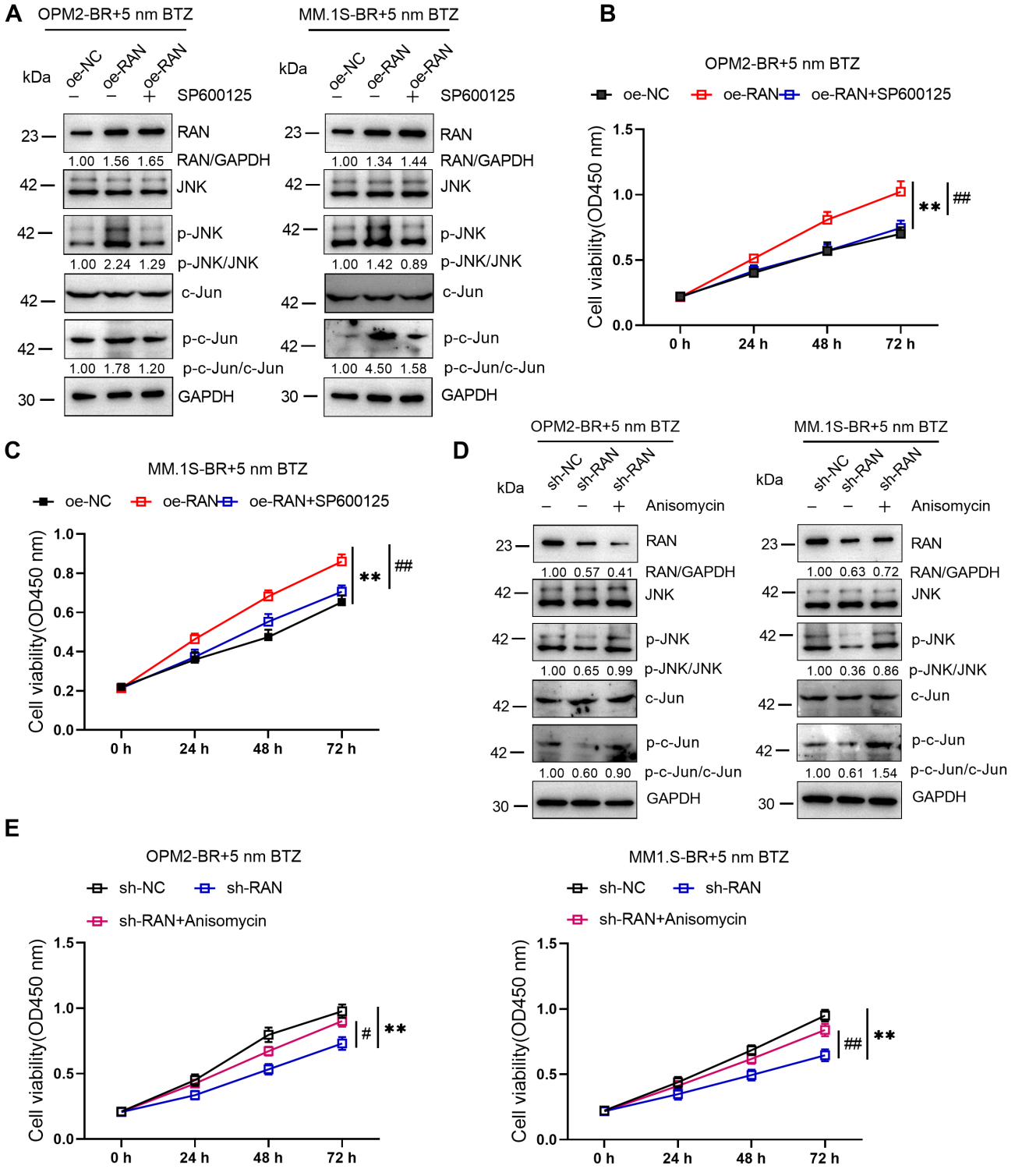

Figure 6. RAN regulates the drug resistance through the JNK/c-Jun axis in BTZ-resistant MM cells. A) Levels of RAN, JNK, p-JNK, c-Jun, and p-c-Jun in BTZ-resistant MM cells transfected with the control or RAN overexpressing plasmids, followed by treating with 25 μM of SP600125 plus 5 nM BTZ for 24 h. B) The CCK-8 experiment was used to detect the role of SP600125 on cell proliferation in RAN overexpressing BTZ-resistant MM cells. **p<0.01, RAN vs. Vec-NC; ##p<0.01, RAN plus SP600125 vs. RAN. C) Levels of RAN, JNK, p-JNK, c-Jun, and p-c-Jun in BTZ-resistant MM cells transfected with the control or RAN knockdown plasmids, followed by treating with 0.5 μM of anisomycin plus 5 nM BTZ for 24 h. D) The CCK-8 experiment was used to detect the role of anisomycin on cell proliferation in RAN knockdown BTZ-resistant MM cells. **p<0.01, sh-RAN vs. sh-NC; ##p<0.01, sh-RAN plus anisomycin vs. sh-RAN.

tant MM cell lines and discovered that the reduction of RAN augmented the sensitivity to BTZ both *in vitro* and *in vivo*, thereby suppressing the proliferation capacity of BTZ-resistant MM cells. Subsequently, we verified that the molecular mechanism through which RAN regulates BTZ resistance is by activating the JNK/c-Jun pathway. The interplay between RAN and these signaling pathways not only elucidates the molecular underpinnings of MM progression but also suggests potential therapeutic targets for overcoming drug resistance in MM, particularly in the context of BTZ resistance. Thus, targeting the RAN-mediated signaling pathways may offer a novel approach to enhance treatment efficacy in MM patients. While our study has shown that RAN can upregulate Wnt5A expression and thereby activate the Wnt/PCP pathway, it is important to acknowledge the notable limitations of our investigation. Exploring additional key molecules within the Wnt/PCP signaling pathway may provide deeper insights into the regulatory mechanisms governing this pathway.

In summary, our study revealed that RAN was upregulated in MM patients and acted as an oncogene in MM cells by activating the Wnt/PCP pathway. RAN could drive BTZ resistance of MM cells through Wnt/PCP pathway-mediated mechanism, which might be a novel therapeutic insight for MM.

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