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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

The Resistance Mechanism to BET-Protac in Multiple Myeloma

Jianlin Qiao, PhD[1,2,3], *Yang Liu*[4], *Li Nian*[5], *Congyue Wang*[6], *Mingxiao Yu*[5], *Wenwen Zhang*[5], *Zhenyu Li*[4], *Chong Chen*[4]

[1]Blood Diseases Institute, Xuzhou Medical University, Xuzhou, China
[2]Department of Hematology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou City, China
[3]Department of Hematology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China
[4]The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China
[5]Xuzhou Medical University, Xuzhou, China
[6]Xuzhou Medical University, Xuzhou, China

Objective : Explore the resistance mechanism of BET protein degradation targeting chimera (BET-PROTAC) in multiple myeloma (MM) cells, and find strategies to enhance the efficacy of BET-PROTAC. So as to provide new theoretical basis for the treatment of multiple myeloma.
Methods : The changes of TCF7 and c-Myc mRNA level were detected by RT-qPCR after BET-PROTAC (ARV-771) treatment of RPMI-8226 cells, the transcriptional activity of β-catenin in ARV-771 tolerant cells was detected by double luciferase reporter assay. Constructing lentiCRISPR-v2 vector to knockout β-catenin in RPMI-8226 cells, and detecting the sensitivity changes to ARV-771 and the formation of drug-resistant clones; MM cell lines resistant to ARV-771 were induced by dose escalation method, and detect the sensitivity of drug-resistant cells to other BET inhibitors and anti-tumor drugs; RNA-seq was used to detect the significantly changed genes and signal modules in drug-resistant cells, and verify this in drug-resistant cells; The drug resistant cells were treated with inhibitors or knockout the resistance gene to observe whether the drug resistance was reversible; The CRISPR knockout gene library was used to screen the candidate genes related to the up-regulation of drug resistance genes in drug-resistant cells, the expression of candidate genes was interfered, and the expression of drug resistance proteins was detected. The correlation between these candidate genes and the prognosis of MM patients was analyzed through public databases.
Results : In ARV-771 tolerant RPMI-8226 cells, the mRNA expression of TCF7 was significantly elevated, but the expression of c-Myc was still at a low level. TOPFlash assay showed activation of β-catenin signal in tolerant cells; Knockout of β-catenin in RPMI-8226 cells by CRISPR/cas9 increased apoptosis induced by ARV-771. Colony formation assay showed that there was no significant difference in the formation rate and number of drug-resistant clones after β-catenin knockout. ARV-771 resistant RPMI-8226 cells were successfully induced by dose increasing method, and three drug-resistant clones (AR1, AR2 and AR3) were obtained, the IC50 of these cells were 80, 120 and 8 times higher than the parental cells, respectively. The drug-resistant cells were cultured in drug-free medium for another 6 months, but the sensitivity did not recover. AR1 and AR2 cells showed decreased sensitivity to ARV-825, MZ-1, OTX-015, I-BET151, Daunorubicin and Epirubicin. RNA-seq showed that ABCB1 was significantly up-regulated in drug-resistant cells, and we verified the up-regulation of ABCB1 at mRNA and protein levels in all drug-resistant cells. Combined use of ABCB1 inhibitors (verapamil, cyclosporin A, Elacridar) or knockout of ABCB1 could significantly reduce the IC50 of drug-resistant cells, increase the apoptosis rate after ARV-771 treatment, and increase the degradation of BRD4 and the down-regulation of c-Myc. Using CRISPR/Cas9 library screening technology, we preliminarily selected 39 genes that may be involved in the regulation of ABCB1 expression, among which the downregulation of 14 genes could reduce the protein expression of ABCB1 in drug-resistant cells. Further through public databases, we found that C1orf112, CCDC167 and CRIP2 are significantly correlated with the prognosis of myeloma patients and are highly expressed in a variety of tumor tissues. These three genes will be the focus of subsequent studies.
Conclusions : Our results showed that BET-PROTAC resistance in MM cells wasindependent of β-catenin activation. The up-regulation of ABCB1 expression was the key mechanism mediating the resistance of myeloma cells to BET-PROTAC. C1orf112, CCDC167 and CRIP2 might be associated with drug resistance in myeloma and could affect prognosis, and their mechanisms in myeloma need to be further investigated.
Keywords : Multiple myeloma; BET; Protein degradation targeting chimera; Drug resistance; CRISPR library; ABCB1

Disclosures No relevant conflicts of interest to declare.

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