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POSTER ABSTRACTS

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

The Spop/BRD4/c-Myc Axis Modulates IMiDs Sensitivity in Multiple Myeloma

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The development of novel agents including immunomodulatory drugs (IMiDs) lenalidomide (Len) and pomalidomide (Pom) has led to improved patient outcomes in multiple myeloma (MM); however, MM patients inevitably experience relapse and drug resistance. The expression level of CRBN, CRBN-binding proteins, and CRL4 ^{CRBN} ubiquitin ligase is considered to be associated with IMiDs resistance. Clinical studies have shown that MM patients can exhibit IMiDs resistance even without CRBN abnormalities, suggesting the molecular mechanisms of intrinsic resistance to IMiDs are not fully understood.
To delineate the molecular mechanisms underlying IMiDs resistance, we performed genome-wide knockout (KO) screening in IMiDs-sensitive MM cells. For *in vitro* work, we used the MM cell lines MM1S, and H929. We used sgRNA to KO *SPOP* and *BRD4* in MM cells.
After performing a CRISPR KO screen in MM cells, we discovered that in addition to CRBN and its associated genes, sgRNAs targeting SPOP (speckle-type POZ protein, a speckled zinc finger protein) were highly enriched after treatment with Poma. Importantly, *SPOP* KO MM cell lines acquired significant resistance to Pom and Len treatment. To examine whether *SPOP* KO-induced IMiDs resistance was CRBN-pathway dependent, we assessed CRBN and its downstream interacting protein levels. *SPOP* KO showed no effect on CRBN expression; moreover, IMiDs can still trigger IKZF1 and IKZF3 degradation, associated with the downregulation of IRF4, suggesting that SPOP mediates sensitivity to IMiDs in a mechanism independent of CRBN-IKZF1/3 axis.Compared to plasma cells from normal donors, we found that the expression levels of SPOP were significantly decreased in MGUS patients, smoldering myeloma patients, newly diagnosed MM patients, and refractory relapsed MM patients (p-values: 2.4e-2, 2.0e-4, 9.9e-3, 3.17e-6, respectively).
SPOP is the adaptor protein of the Cullin3-RING ubiquitin ligase complex. Therefore, the function of SPOP is determined by the characteristics of the substrates it recruits. We performed immunoprecipitation with overexpressed SPOP in MM cells, followed by mass spectrometry analysis, and identified BRD4 as a candidate protein binding to SPOP in MM, which was confirmed by Co-IP. KO of *SPOP* led to an upregulation of BRD4 protein levels in MM cells. As a downstream regulator of BRD4, the oncogene c-Myc showed a significant increase in protein expression in IMiDs-resistant MM patients. Furthermore, the KO of *SPOP* notably enhanced c-Myc expression in MM cells, while the KO of *BRD4* in *SPOP* KO MM cells decreaed c-Myc expression and restored MM cell sensitivity to IMiD treatment. These findings suggest that SPOP modulates c-Myc expression through BRD4 in a CRBN-IKZF1/3 independent manner, thereby mediating MM cell sensitivity to IMiDs.
BRD4 plays a significant role in the development and progression of various tumors. The BRD4 inhibitor JQ1 has been shown to exhibit anti-tumor effects in multiple types of cancer. We found that combination treatment with JQ-1 and IMiDs reversed IMiDs resistance of *SPOP* KO MM cell lines and primary MM cells from relapsed patients.
Our data show that SPOP is a CRBN-independent modulator of IMiDs sensitivity by regulating the BRD4/c-Myc axis and provides the preclinical rationale for combining IMiDs with BRD4 inhibitors to overcome IMiDs resistance and improve patient outcomes.

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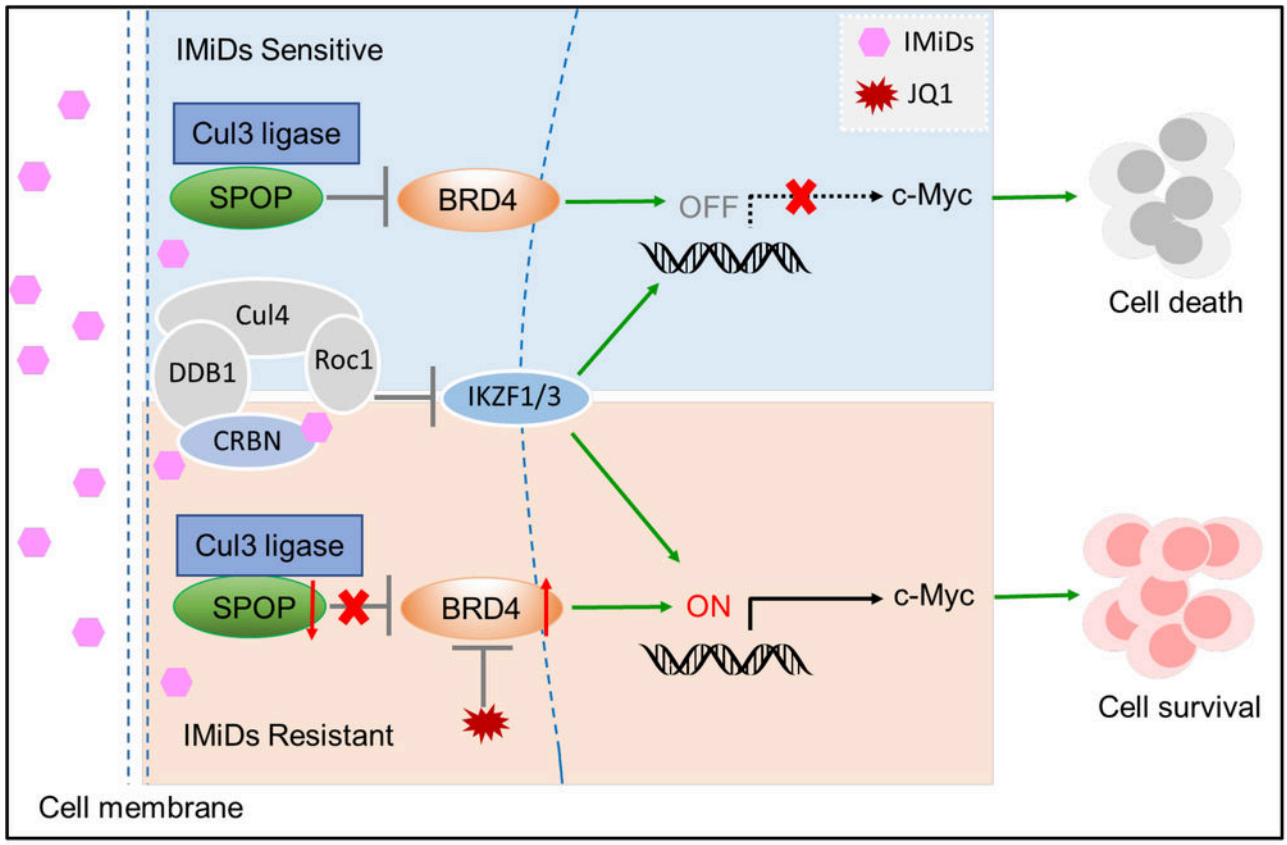

Figure 1: This schematic illustrates the role of SPOP in inducing IMiDs resistance through the BRD4/c-Myc axis in multiple myeloma (MM) and proposes potential strategies to overcome this resistance.

Figure 1

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