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**Novel mechanism of drug resistance to proteasome inhibitors in multiple myeloma**

Zhou J *et al*.Drug resistance in MM

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**Abstract**

Multiple myeloma (MM) is a cancer caused by uncontrolled proliferation of antibody-secreting plasma cells in bone marrow, which represents the second most common hematological malignancy. MM is a highly heterogeneous disease and can be classified into a spectrum of subgroups based on their molecular and cytogenetic abnormalities. In the past decade, novel therapies, especially, the first-in-class proteasome inhibitor bortezomib, have been revolutionary for the treatment of MM patients. Despite these remarkable achievements, myeloma remains incurable with a high frequency of patients suffering from a relapse, due to drug resistance. Mutation in the proteasome β5-subunit (PSMB5) was found in a bortezomib-resistant cell line generated *via* long-term coculture with increasing concentrations of bortezomib in 2008, but their actual implication in drug resistance in the clinic has not been reported until recently. A recent study discovered four resistance-inducing PSMB5 mutations from a relapsed MM patient receiving prolonged bortezomib treatment. Analysis of the dynamic clonal evolution revealed that two subclones existed at the onset of disease, while the other two subclones were induced. Protein structural modeling and functional assays demonstrated that all four mutations impaired the binding of bortezomib to the 20S proteasome, conferring different degrees of resistance. The authors further demonstrated two potential approaches to overcome drug resistance by using combination therapy for targeting proteolysis machinery independent of the 20S proteasome.

**Key words:** Multiple myeloma; Proteasome inhibitor; Bortezomib; Proteasome β5-subunit; Drug resistance; Clonal evolution; Combination therapy

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**Core tip:** Multiple myeloma (MM) is the second common hematological malignancy. An array of new treatments has been approved over the last decade. Hence, the survival of MM patients has improved steadily. Among these new drugs, the first-in-class proteasome inhibitor bortezomib has been revolutionary for targeted therapy. Now bortezomib is the backbone for treating MM. However, emerging drug resistance poses a major challenge for clinicians to use proteasome inhibitors. In this editorial, we discuss proteasome β5-subunit mutations as a novel resistant mechanism to bortezomib and its implication in tracking clonal evolution and suggest potential strategies to overcome drug resistance.

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**Introduction**

Multiple myeloma (MM) is the second most common hematological malignancy with approximate 138000 cases worldwide in 2016[1], so it is a significant social and economic burden globally. MM is characterized by uncontrolled proliferation of clonal antibody-secreting plasma cells in bone marrow. The aberrant accumulation of monoclonal proteins in blood and urine causes organ damage, summarized as calcium elevation, renal complications, anemia, and bone lesions[2]. MM is a highly heterogeneous disease and can be classified into a spectrum of subgroups based on their molecular and cytogenetic abnormalities[3]. According to primary genetic events, MM can be divided into hyperdiploid (HD) and non-hyperdiploid (NHD). There are several subgroups within NHD, such as t(11;14)(q13;q32) and CCND3, t(4;14)(p16;q32) and MMSET, FGFR3, t(14;16)(q32;q23), and other MAF translocations. These molecular cytogenetic subgroups and their associated prognosis are summarized in Table 1.

This heterogeneity is one of the factors contributing to the limited effect of chemotherapy (melphalan, cyclophosphamide, and doxorubicin) in general. In the last 12 years, significant progress has been made in the novel therapies for the treatment of MM patients[10]. These new agents, including proteasome inhibitors, immunomodulatory drugs (IMiDs), histone deacetylase (HDAC) inhibitors, and monoclonal antibodies, have significantly improved the survival of standard risk MM patients[11]. Especially, the first-in-class proteasome inhibitor bortezomib has been revolutionary for targeted therapy for MM[12]. Despite remarkable achievements in the past decade, myeloma remains incurable with a high frequency of patients suffering from a relapse, due to primary (inherent) and secondary (acquired) drug resistance[13]. Diversified molecular mechanisms underlying the resistance to proteasome inhibitor have been unveiled, including overexpression of a superfamily of ATP-binding cassette transporters (MDR, MRP1, *etc.*), enhanced aggresomal protein pathway, overexpression of heat shock proteins, bone marrow microenvironment, and appearance of mutations in proteasome subunits[14]. Although mutation in the proteasome β5-subunit (PSMB5) was found in a bortezomib-resistant cell line generated *via* long-term coculture with increasing concentrations of bortezomib in 2008[15], PSMB5 mutation has never been identified in relapsed or refractory MM patients until recently.

**STUDY ANALYSIS**

In a recent study by Barrio *et al*[16], four PSMB5 mutations from a MM patient receiving prolonged bortezomib treatment have been discovered and functionally validated. These investigators performed targeted deep-sequencing of 88 MM-related genes (M3P panel) on paired tumor-germline samples from 161 multi-refractory MM patients. They reported four subclonal mutations in PSMB5 gene: c.235G>A (p.A20T), c.256G>C (p.A27P), c.312G>C (p.M45I), and c.365G>A (p.C63Y) (protein positions refer to the cleaved mature protein). These mutations were further confirmed by whole exome sequencing. Interestingly, these subclonal lines were still sensitive to the combination of pomalidomide and elotuzumab as analysis of clonal evolution at different time points (TP) revealed that two subclonal lines (C63Y and A27P) become undetectable at TP4 and the remaining M45I and A20T also disappeared at TP5 (5 months later than TP4). Tracing back the samples available at TP1 (diagnosis) and TP2 (first relapse) confirmed the pre-existence of two of the variants, c.235G>A and c.365G>A, at these two earlier TPs. The illustration of the temporal order of clonal evolutionary trajectory in this patient adds to our growing understanding of MM evolution and therapeutic resistance. The co-existence of emergent new subclones after selection pressure (bortezomib treatment) on the original subclones confirms the “Big Bang” model of cancer evolution in a branching rather than in a “step-wise” linear progression[17,18]. Furthermore, the eradication of all these four subclones after a combination regime including the second-generation IMiD pomalidomide and the immunostimulatory monoclonal antibody elotuzumab underpins the importance of developing novel drugs for relapsed MM patients.

Notably, all four mutations occurred within a highly conserved region in exon 2, and protein structural analysis demonstrated that the mutations are located either within the S1 pocket (A20T, A27P, and M45I) or in proximity to the substrate-binding channel (C63Y). The authors performed *in vitro* functional assays and all the mutants impaired the binding of bortezomib to the proteasome, reduced catalytic proteasome activity, and conferred resistance to bortezomib and other proteasome inhibitors to varying degrees. Importantly, these PSMB5 mutant lines remain sensitive to p97/VCP AAA ATPase inhibitor, CB5083, which blocks proteolysis machinery independent of 20S proteasome. These results highlight another approach to overcome drug resistance to proteasome inhibitors by using p97/VCP inhibitors.

In conclusion, this study not only validated the importance of PSMB5 in mediating drug resistance to proteasome inhibitors, but also deciphered the dynamic and temporal effect of clonal evolution in the development of resistance and deepened our understanding of the relationship between clonal evolution and drug resistance in MM cells.

**CONCLUSION**

Drug resistance has been implicated in 90% of MM-related deaths, which poses a daunting challenge in the management of MM. The combination of the second-generation IMiD and antibody therapy or novel agents targeting proteasome-independent proteolysis machinery can override the resistance to proteasome inhibitors. These approaches hold promise to further improve the survival of relapsed and refractory MM patients. However, we have to wait for well-designed clinical trials to validate its efficacy and evaluate the toxicity. In addition, prospective biomarkers for prediction of drug resistance are absent. Owing to the heterogeneity of MM and various mechanisms involved in resistance, it is unlikely that one biomarker fits all MM. Nevertheless, screening PSMB5 mutations at diagnosis, during the treatment, and subsequent follow-ups should be useful in monitoring drug resistance to proteasome inhibitors. Furthermore, some important questions remain unanswered, for example, whether mutations in other 20S proteasome subunits, like PSMA5, exist. Finally, single-cell sequencing technology is particularly useful in tracking clonal evolution, providing opportunities to characterize MM subclones in unprecedented detail. We now have a better chance to conquer drug resistance and significantly further improve the outcome of MM patients.

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**Table 1 Molecular cytogenetic classification of multiple myeloma and prognosis**

|  |  |  |  |
| --- | --- | --- | --- |
| **Chromosomes affected (gene)** | **Ploidy** | **Prognosis** | **Ref.** |
| t(11;14)  *(CCND3)* | NH | Good | [4] |
| t(14;16)  *(c-MAF)* | NH | Poor | [5] |
| t(4;14)  (*FGFR3* and *NSD2*) | NH/H | Poor | [6,7] |
| Other IgH | NH/H | Poor | [8] |
| Hyperdiploidy | H | Good | [9] |

IgH: Immunoglobulin heavy chain; H: Hyperdiploidy; NH: Non-hyperdiploidy.