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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

CNOT4 Knockout Induces Proteasome Inhibitor Resistance in Multiple Myeloma Cells

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Background: The outcomes of multiple myeloma (MM) patients have been significantly improved with novel therapies including proteasome inhibitors (PI). However, many patients develop PI resistance and becoming refractory to PI. Although several studies have identified the mechanisms of PI resistance, such as mutation to the proteasome $\beta 5$ subunit, ER stress suppression, autophagy activation, and activation of efflux drug pumps, it remains unclear yet. To investigate new genes that may contribute to PI resistance, we performed a genome-wide CRISPR dropout screening in an MM cell line.
Materials and Methods: To identify the genes responsible for PI resistance, we used a CRISPR library (Kosuke Yusa, V3) targeting 18,740 human genes. The knockout cells, in which an CRISPR library lentivirus was introduced into a Cas9-expressing AMO1 MM cell line, were treated with ixazomib (IXA), carfilzomib (CFZ), and DMSO for two weeks. Next, we used next generation sequencing (NGS) to count sgRNA copy numbers of the pre-drug control group and the drug-treated group. NGS analysis of the treated cells showed that inactivated genes which induced higher levels of cell proliferation in the PI group than in the DMSO group were considered candidate genes for PI resistance.
Results: We identified 35 genes for PI resistance including the genes encoding subunits of the proteasome 19S complex, consistent with previous reports that shows reducing 19S subunits protects MM cells from bortezomib. Among 35 candidate genes, we chose the top hit gene, CNOT4, to further investigate. CNOT4 is a component of CCR4-NOT complex which removes the mRNA poly(A) tail for degradation of mRNA. In addition, CNOT4 has E3 ubiquitin ligase activity and is reported to control the proteasome complex formation.
To further validate the effect of PI resistance with inactivated CNOT4, we generated CNOT4 KO AMO1 cells and performed an MTS assay. We observed a decrease in PI sensitivity compared with AMO1 wildtype (WT) (IXA IC_{50}: 24.8 nM (KO) vs 13.0 nM (WT), CFZ IC_{50}: 7.45 nM (KO) vs 2.76 nM (WT)).
To investigate the influence of CNOT4 on proteasome complex formation and its activity, we first measured the expression of ubiquitinated proteins by Western blotting. In CNOT4 KO AMO1 cells, the expression of ubiquitinated proteins is increased with or without PI treatment, suggesting that inactivated CNOT4 can suppress the function of degrade ubiquitinated proteins in proteasome 19S complex.
To elucidate the mechanism underlying the resistance effect, we next checked the expression of ER stress marker proteins. In CNOT4 KO AMO1 cells, the expression of PERK and IRE1α proteins are decreased with PI treatment, suggesting that inactivated CNOT4 can suppress the ER stress levels in spite of the increases of ubiquitinated proteins.
Conclusion: Our CRISPR screen revealed that CNOT4 inactivation induced PI resistance in human MM cells. CNOT4 inactivation suppresses the function of 19S proteasome, which is reported the influence of PI resistance, and downregulates the ER stress signal leading to avoid PI induced cell death. Our findings demonstrate that CNOT4 plays an important role in PI sensitivity.

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