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POSTER ABSTRACTS

605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Genome-Wide CRISPR/Cas9 Screens Identify *DDX19A/DDX19B* As a Critical Regulator of Intrinsic Apoptosis By Regulating MCL1 mRNA Cellular Localization

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Despite recent advancements, the prognosis of relapsed/refractory acute lymphoblastic leukemia (ALL) remains dismal, necessitating novel therapies. Selinexor, an XPO1 (Exportin-1) inhibitor, shows effectiveness in multiple myeloma (MM), diffuse large B-cell lymphoma (DLBCL), and ALL mouse models. However, its broad target spectrum could lead to off-target toxicities, limiting its clinical utility.

To elucidate Selinexor's molecular mechanisms in ALL we used CRISPR-Cas9 screens in the presence of Selinexor on ALL cell lines NALM-6 and KOPN-49. Among 18 genes displaying synthetic lethality in both cell lines with Selinexor, we focused on paralogous genes *DDX19A* and *DDX19B*, critical in nucleocytoplasmic transport of mRNA and translation initiation by dissociating protein complexes bound to mRNA.

Competition assays showed prominent growth inhibitions when *DDX19B* was knocked out prior to *DDX19A* depletion in both NALM-6 and KOPN-49, while single KO of either *DDX19A* or *DDX19B* did not remarkably inhibit cell growth. Additionally, in MOLT-3 with low *DDX19B* expression, significant growth inhibitions were observed upon single KO of *DDX19A* and these phenotypes were canceled by the overexpression of *DDX19B*, suggesting functional redundancy between *DDX19A* and *DDX19B*.

Further, we generated MOLT-3 that stably express HA-FKBP12 ^{F36V}-tagged *DDX19A* and knocked out endogenous *DDX19A*. Western blot analysis showed depletion of DDX19A by dTAG ^{V}-1 led to upregulate cleaved caspase-3 expression level within 4 hours, accompanied by a significant decrease in MCL1 protein levels in a BAX/BAK dependent manner. These phenotypes were rescued by the overexpression of *MCL1*, suggesting that the loss of *DDX19A/19B* induced intrinsic apoptosis through the downregulation of MCL1 expression.

Given that the depletion of *DDX19A/19B* led to the mislocalization of polyadenylated mRNA and that neither the stability of the MCL1 protein nor the translation of MCL1 mRNA was affected by this depletion, as confirmed by RNA-FISH, and Actinomycin-D and Cycloheximide chase assays respectively, we concluded that *DDX19A/19B* are crucial for the nucleocytoplasmic transport of MCL1 mRNA.

Furthermore, in exploring the relationship between the loss of *DDX19A/19B* and sensitivity to Selinexor, we found that combined treatment with Selinexor and the depletion of either *DDX19A* or *DDX19B* leads to a reduction in MCL1 protein, which can be rescued by overexpressing *MCL1*.

Through CRISPR-Cas9 screens, we identified *DDX19A/19B*, which are in a paralogous relationship, as potential therapeutic targets for ALL. Mechanistically, the loss of *DDX19A/19B* leads to impaired cytoplasmic mRNA transport, leading to the inhibition of novel synthesis and reduced expression of MCL1 protein, thereby inducing intrinsic apoptosis. Furthermore, we found that *DDX19A/19B* depletion exerts synergistic effects with Selinexor through the downregulation of MCL1 expression, suggesting that *DDX19A/19B* levels could serve as a biomarker for Selinexor treatment in ALL.

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