



The 65th ASH Annual Meeting Abstracts

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.

Disclosures Akashi: Pfizer Japan Inc.: Honoraria; ONO PHARMACEUTICAL CO., LTD: Honoraria; Eisai Co., Ltd.: Honoraria; Takeda Pharmaceuticals Co., Ltd.: Research Funding; CHUGAI PHARMACEUTICAL CO., LTD.: Honoraria, Research Funding; AbbVie Inc.: Honoraria, Research Funding; ASAHIKASEI PHARMA CORPORATION CO., LTD: Research Funding; Kyowa

Kirin Co., Ltd.: Honoraria, Research Funding; Otsuka Pharmaceutical Co.,Ltd.: Research Funding; Bristol-Myers Squibb K.K.: Honoraria; Janssen Pharmaceutical K.K.: Honoraria.

<https://doi.org/10.1182/blood-2023-182620>