Main Topic:
Cancer Genomics

Subtopic
Functional Genomics

Presentation preference:
Oral presentation

Title:
THE SHEDDASE DOMAIN OF ADAM10 AUGMENTS THE INTERACTION OF LEUKEMIA CELLS WITH THE BONE MARROW NICHE IN VIVO AS SHOWN BY RECONSTITUTING PDX LEUKEMIA CELLS WITH CRISPR-CAS9-INDUCED KNOCKOUT

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Abstract Text:

Introduction
Tumor-microenvironment interactions are critical for leukemia formation and maintenance. Their interruption therefore represents an attractive therapeutic strategy. We developed a CRISPR-Cas9 screening approach for functional analysis of surface molecules in patient-derived xenograft (PDX) models of acute leukemias (AL) in vivo in order to decipher common and individual tumor specific vulnerabilities and gene dependencies.

Material and Methods
CRISPR library size was determined by genetic barcoding. Stable expression of Cas9 and sgRNA constructs in two PDX samples. Enrichment by MACS and injection into NSG mice. Gene depletion analysis using MAGeCK algorithm to screen and functional competitive in vivo assays to validate candidates. Characterization of the ADAM10 Knockout (KO) or inhibitor treated cells for engraftment capacity by homing assay, frequency of leukemic stem

cells by competitive limiting dilution transplantation assay (LDTA), sensitivity towards routine chemotherapy by in vivo competitive chemotherapy trials. Rescue assay by reconstitution of ADAM10 variants in competitive in vivo assays.

Results and Discussions

Running a customized CRISPR-Cas9 screen targeting about 100 cell surface molecules in two AL PDX samples, sample-specific as well as commonly depleted genes were identified. Candidates were confirmed using a competitive in vivo approach testing the PDX cells with and without KO in the same mouse. Among other candidates, ADAM10 was depleted in both PDX models. In vivo competitive experiments confirmed its essential role in PDX models from 6 additional patients with either acute lymphoblastic leukemia (ALL) or acute myeloblastic leukemia (AML). Treating PDX cells with ADAM10 inhibitor reduced the engraftment capacity into the bone marrow significantly. KO of ADAM10 reduced the frequency of leukemia stem cells and ADAM10 KO PDX samples in both lineages showed increased sensitivity towards routine chemotherapy treatments. Reconstitution of ADAM10 KO PDX cells with recombinant wildtype variant in vivo rescued the phenotype, while an enzymatic domain lacking variant did not, highlighting the importance of ADAM10's sheddase function in leukemia maintenance.

Conclusion

We established CRISPR-Cas9 drop-out screens in PDX models in vivo as technology to explore patient-specific tumor dependencies. Our data revealed a yet unknown role of ADAM10 to maintain patient leukemic cells in the bone marrow niche. ADAM10 thus represents an attractive future therapeutic target for the treatment of acute leukemia.