Identification of Actionable Dependencies Among Direct Transcriptional Gene Targets of the NUP98-JARID1A Fusion Protein in Acute Myeloid Leukemia

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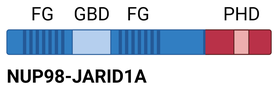
1. INTRODUCTION

NUP98-fusion proteins

- the N-terminal part of *NUP98* is fused to the C-terminal part of ~30 distinct partner genes forming oncogenic fusion proteins
- NUP98-fusion proteins occur in ~5% of pediatric AML patients [1]
- >20% of chemotherapy-resistant pediatric AML patients harbor *NUP98* gene rearrangements [2]
- NUP98-fusion-protein-driven AML is associated with poor prognosis as currently no targeted therapies are available

NUP98-JARID1A

- is the most common NUP98-fusion protein in infant AML
- JARID1A is an H3K4 histone demethylase
- NUP98-fusion proteins are associated with chromatin and regulate gene expression[3]



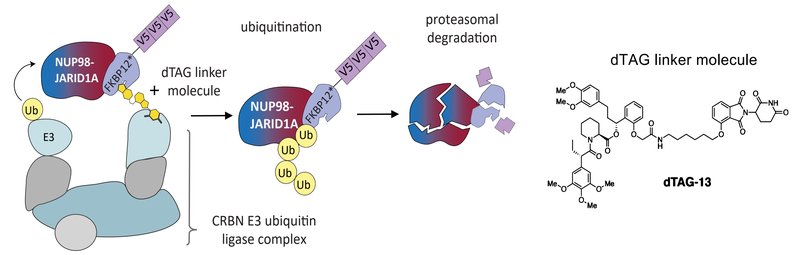
dTAG-system for ligand-induced protein degradation

- a heterobifunctional linker molecule binds mutated FKBP12 (=dTAG) fused to a protein of interest[4]
- recruitment of CRBN E3 ubiquitin ligase complex followed by ubiquitination
- fast and iducible proteasomal degradation of a protein of interest

2.HYPOTHESIS

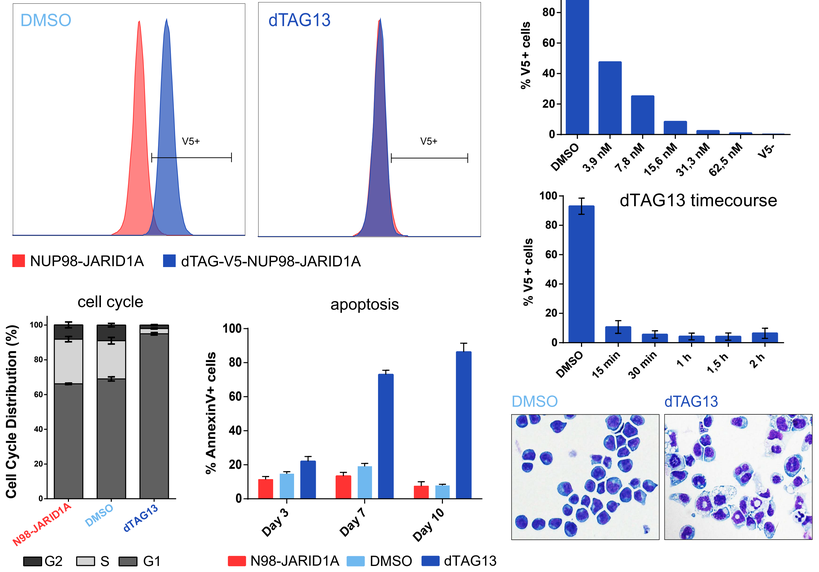
Oncogenic NUP98-fusion proteins are direct transcriptional regulators of target genes that are critical for leukemogenesis.

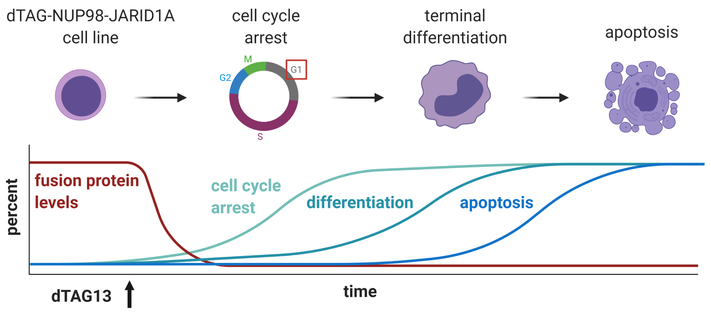
3. Establishment of a model for ligand-induced degradation of the NUP98-JARID1A fusion protein



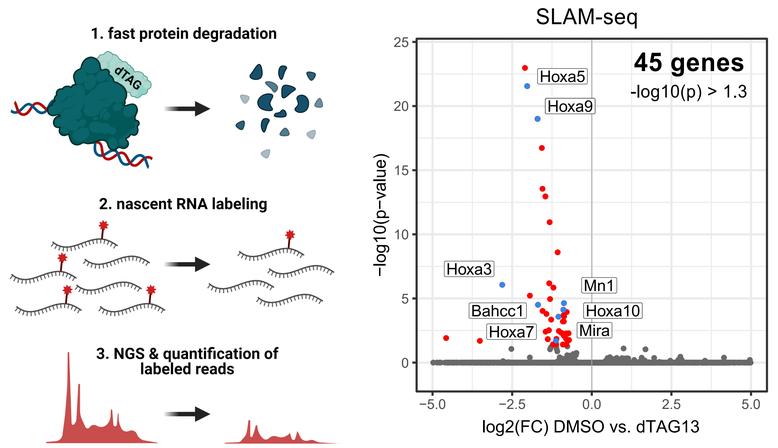
4.RESULTS

a) Characterization of the dTAG-NUP98-JARID1A cell line

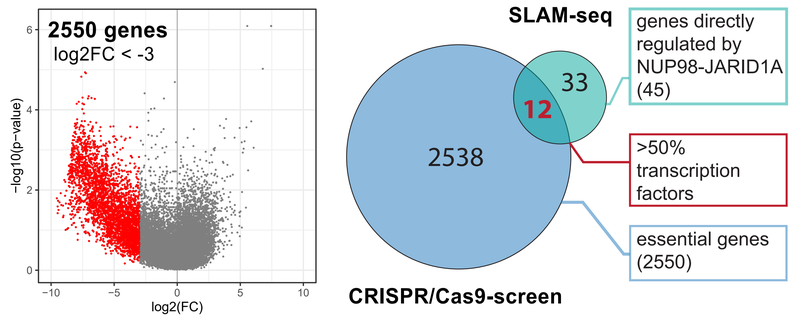




b) Global analysis of changes in nascent mRNA expression upon NUP98-JARID1A degradation by SLAM-seq

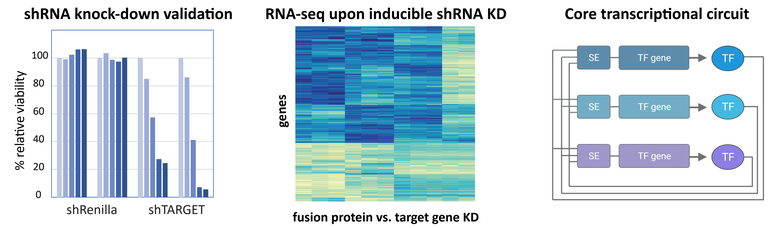


c) SLAM-seq data integration with genome-wide loss-of-function CRISPR/Cas9 screen in an NUP98-JARID1A dependent cell line



5. OUTLOOK

Validation of essential direct transcriptional target genes by inducible shRNA knock-down combined with RNA-seq analysis to recapitulate transcriptional changes induced by NUP98-JARID1A. Investigation of potential core transcriptional regulatory circuit maintained by the fusion protein by super enhancer mapping.



6. CONCLUSIONS

- **Development of a model for ligand-induced degradation of the NUP98-JARID1A fusion protein**
- **Degradation of NUP98-JARID1A leads to cell cycle arrest, differentiation and apoptosis**
- **Identification of essential direct transcriptional target genes of NUP98-JARID1A & potential therapeutic targets for treatment by SLAM-seq analysis and genome-wide CRISPR/Cas9 screening**

References:

1) Bisio, V. et al. (2016). NUP98-fusion transcripts characterize different biological entities within acute myeloid leukemia: a report from the AIEOP-AML group. Leukemia

2) McNeer, N. et al. (2019). Genetic mechanisms of primary chemotherapy resistance in pediatric acute myeloid leukemia. Leukemia

3) Schmoellerl, J. et al. (2020). CDK6 is an essential direct target of NUP98 fusion proteins in acute myeloid leukemia. Blood

4) Nabet, B. et al. (2018). The dTAG system for immediate and target-specific protein degradation. Nature Chemical Biology

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