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Blood 142 (2023) 711–712

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

TRIP13 Is a Fetal-Enriched Therapeutic Target in NUP98-JARID1A $^{+}$ Pediatric Non-Down Syndrome AMKL

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Chromosomal rearrangements have been identified as the main drivers of pediatric Acute Megakaryoblastic Leukemia in absence of Down syndrome (non-DS-AMKL). The t(11;12) involving *NUP98* and *KDM5A/JARID1A* (NJ) accounts for approximately 15% of pediatric non-DS-AMKL cases and correlates with poor prognosis. An important aspect of AMKL is the strong enrichment in pediatric patients with frequent occurrence in infants (disease within 2 years post birth). In this study, we aimed to explore the role of cellular ontogeny in NJ-driven non-DS-AMKL and highlight TRIP13 as a fetal-enriched NJ-specific vulnerability acting through P53 regulation.

To investigate the mechanism of NJ transformation, we first transduced murine hematopoietic stem and progenitor cells (HSPCs) originating from fetal liver (mFL-HSPCs) and bone marrow of adult mice (mBM-HSPCs) with a lentiviral vector overexpressing the cDNA encoding human NJ. In line with other studies, NJacts as a potent oncogene *in vitro*, sustaining cell proliferation and arresting cell differentiation. Both mFL-HSPCs and mBM-HSPCs expressing NJ transformed and dominated the culture after 3-4 weeks with an immature immune-phenotype (Lin-, Sca1+ and c-Kit+). Notably, outgrowth of mFL-HSPCs was significantly accelerated compared to adult mBM-HSPCs, despite similar transduction rates. Furthermore, *in vivo* experiments revealed that NJ-expressing mFL-HSPCs displayed a more aggressive phenotype (latency of 36 days) compared to mBM-HSPCs cells expressing NJ (latency of 70 days; $P_{\text{Long-rank}} < 0.001$). Thus, both our *in vitro* and *in vivo* experiments strongly indicate an impact of fetal cell background.

Suspecting an impact of fetal gene programs on NJ-mediated transformation, we performed a CRISPR-Cas9 screening targeting fetal expression signatures with a library probing 880 genes found to be deregulated when comparing fetal versus adult murine and human primary HSPCs. By comparing our findings with two other fetal liver-derived leukemia models - representing DS AMKL and familial platelet disorder with predisposition to AML (FPD-AML) -, we identified *TRIP13* as a high-confidence candidate gene with exclusive dependency in NJ-driven non-DS-AMKL. Comparing human AML cell lines and NJ-overexpressing human fetal liver cells (hFL-HSPCs) confirmed selective targeting of NJ-driven cells by *TRIP13* loss.

To explore the molecular mechanism of TRIP13 sensitivity, we performed RNAseq. Surprisingly, gene set expression analysis (GSEA) revealed one highly significantly enriched pathway, i.e. TP53 signaling. Rescue experiments of *Trip13* knockout in NJ-driven non-DS-AMKL cells further supported this finding: the massive depletion upon loss of *Trip13* was completely reverted by either re-expression of a human *TRIP13* cDNA or by knockout of *Trp53*. Of note, neither activation of TP53 signaling nor cell depletion was seen in healthy hematopoietic cells from FL upon *Trip13* perturbation.

Finally, we aimed to leverage TRIP13 dependency therapeutically. To this end, we first explored *Trip13*-depletion in an *in vivo* setting. In a fluorescence-based competitive transplantation assay, *Trip13*-ablated leukemic blasts were significantly diminished in the bone marrow of recipient mice after 4 weeks, showing a mean depletion of 83%. Next, NJ-driven non-DS-AMKL mFL-HSPCs were treated with the TRIP13 inhibitor DCZ0415. Similarly to genetic *TRIP13* ablation, DCZ0415-treated NJ mFL-HSPCs showed a significantly higher sensitivity to the drug compared to models of DS AMKL, FPD-AML, and healthy HSPCs with fold changes of 1.6 (p-value = 0.024), 2.3 (p-value = 0.001) and 2.0 (p-value = 0.008), respectively. To exploit our mechanistic knowledge on TRIP13 ablation-mediated P53 activation, we further combined DCZ0415 with the MDM2 inhibitor Idasanutlin. In line with our mechanistic data, a high synergy (Bliss synergy score = 9.7) of TRIP13 and MDM2 inhibition was observed.

In conclusion, our study uncovers TRIP13 as a fetal-enriched vulnerability in NJ-driven non-DS-AMKL, mechanistically acting through P53, which we leveraged for a mechanism-driven treatment approach with dual TRIP13/MDM2 inhibition as a potential therapeutic strategy for the treatment of high-risk pediatric non-DS-AMKL.

Disclosures Klusmann: *Boehringer Ingelheim:* Consultancy; *Jazz Pharmaceuticals:* Consultancy.

https://doi.org/10.1182/blood-2023-182843