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Blood 142 (2023) 1405–1406

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Suppression of Super-Enhancer-Driven *TAL1* Expression By KLF4 in T-Cell Acute Lymphoblastic Leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy characterized by differentiation arrest and clonal proliferation of immature thymocytes. Many oncogenes in T-ALL are genes encoding transcription factors. TAL bHLH transcription factor 1 (*TAL1*) is one of the most frequently dysregulated transcription factors in T-ALL and is ectopically overexpressed in approximately 50% of T-ALL cases, owing to chromosome translocations, *SIL* (SCL-interrupting locus) -*TAL1* fusion, and 5′ super-enhancer (SE)-generating mutations. *TAL1* overexpression caused by 5′ *TAL1* SE is found in 5% of patients with T-ALL and is associated with unfavorable clinical outcomes. However, no clinically available drugs specifically inhibit *TAL1* or *TAL1* SE components.

Kruppel-like factor 4 (KLF4) is a member of the KLF family of transcription factors that exhibits both oncogenic and tumor-suppressive functions in a context-dependent manner. In T-cell malignancies, KLF4 is regarded as a tumor suppressor because *KLF4* expression is silenced by promoter methylation, and the induction of KLF4 promotes apoptosis through the BCL-2/BCL-XL pathway. However, whether KLF4 affects the expression of T-ALL-related transcription factors remains unclear.

We first analyzed three independent previously reported microarray datasets to explore the essential downstream targets of KLF4 in T-ALL cells. The following groups of genes were extracted: genes upregulated in patients with T-ALL compared to normal thymocytes (GSE46170), genes upregulated in Klf4-deficient T-ALL mice (GSE75663), and genes downregulated in KLF4-overexpressing human T-ALL cells (Li W *et al. Mol. Cancer.* 2015). Overlapping genes in these groups were identified as potential downstream targets of KLF4. Among these, we focused on *TAL1*, a well-known oncogenic transcription factor gene in T-ALL. We performed a functional analysis of KLF4 using TAL1-positive T-ALL cell lines (Jurkat, MOLT-3, CCRF-CEM, and RPMI-8402). Genetic induction of KLF4 showed stronger anti-leukemic effects in Jurkat and MOLT-3 cells, 5′ *TAL1* SE-positive T-ALL cell lines, compared with CCRF-CEM and RPMI-8402 cells, *SIL-TAL1*-positive T-ALL cell lines. KLF4 overexpression significantly reduced mRNA expression levels of *TAL1* in Jurkat and MOLT-3 cells but not in CCRF-CEM and RPMI-8402 cells. Thus, we hypothesized that KLF4 specifically suppressed SE-driven *TAL1* expression by impairing mutated *TAL1* enhancer activity. The luciferase reporter assay confirmed that the activity of the mutated *TAL1* enhancer was significantly reduced by the exogenous expression of KLF4, whereas that of the wild-type reporter was not affected. According to previous studies, mutations in *TAL1* enhancers in Jurkat and MOLT-3 cells create MYB primary motifs, and MYB binding at this location is essential for the formation of 5′ *TAL1* SE. Therefore, we examined whether KLF4 represses MYB expression. The chromatin immunoprecipitation assay and qPCR analysis revealed that KLF4 bound to the proximal gene promoter of *MYB* and directly downregulated its expression. The luciferase reporter assay using the *MYB* promoter showed a significant reduction of reporter activity upon KLF4 overexpression.

Finally, we investigated whether pharmacological induction of KLF4 using APTO-253, a small-molecule inducer ofKLF4, could control leukemia. APTO-253 inhibited the growth of all TAL1-positive T-ALL cell lines at nanomolar concentrations. The GI_{50} values of APTO-253 for Jurkat and MOLT-3 cells were lower than those for CCRF-CEM and RPMI-8402 cells, which was consistent with the KLF4-overexpression-induced growth inhibition of T-ALL cell lines. APTO-253 induced KLF4 expression and decreased TAL1 and MYB expression in Jurkat and MOLT-3 cells. Furthermore, exogenous MYB expression rescued

growth inhibition by APTO-253. Collectively, these results indicated that APTO-253 suppressed the growth of T-ALL cells by targeting SE-driven *TAL1* expression via the induction of KLF4.
In summary, we revealed that KLF4 suppressed SE-driven *TAL1* expression via the direct inhibition of *MYB*. Moreover, pharmacological induction of KLF4 demonstrated anti-leukemic activity in T-ALL cells. These findings propose a promising strategy for patients with T-ALL and 5′ *TAL1* SE.

Disclosures Kiyoi: *Perseus Proteomics:* Research Funding; *SymBio:* Honoraria; *Asahi Kasei:* Research Funding; *Ono Pharmaceuticals:* Honoraria; *Eisai:* Honoraria, Research Funding; *AstraZeneca:* Honoraria; *Daiichi Sankyo:* Honoraria, Research Funding; *Meiji Seika:* Honoraria; *Amgen:* Honoraria; *Sumitomo Dainippon Pharma:* Research Funding; *Nippon Kayaku:* Honoraria; *Zenyaku Kogyo:* Research Funding; *Bristol-Myers Squibb:* Honoraria; *Novartis:* Honoraria; *Chugai:* Honoraria, Research Funding; *Astellas Pharma:* Honoraria, Research Funding; *CURED:* Research Funding; *AbbVie:* Honoraria, Research Funding; *Otsuka Pharmaceutical:* Research Funding; *Kyowa Hakko Kirin:* Research Funding; *Pfizer:* Honoraria; *Towa:* Honoraria; *MSD:* Honoraria; *Pharma Essentia Japan:* Honoraria.

https://doi.org/10.1182/blood-2023-173640