ELSEVIER

Blood 142 (2023) 2729

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Loss of EFL1 Which Causes a Shwachman-Diamond Syndrome Reduces Cell Proliferation and Alters Transcriptional Profiling during Neutrophil Differentiation

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Introduction. Shwachman-Diamond syndrome (SDS) is an inherited bone marrow failure syndrome characterized by neutropenia, exocrine pancreatic insufficiency, and skeletal abnormalities. Patients with SDS are predisposed to develop myeloid malignancy. Almost all individuals with SDS harbor biallelic mutations in *SBDS*. The partner of SBDS is EFL1, a ribosomal protein with GTPase activity. More than a dozen patients have been found with SDS due to biallelic mutations in *EFL1*. EFL1 serves to release EIF6, facilitating the assembly of the 80S ribosome from 60S and 40S ribosome subunits. Our lab has generated both *sbds* and *efl1* null zebrafish, which phenocopy much of the human syndrome. We recently reported that Sbds and Efl1 depletion leads to upregulation of *tp53* and *cdkn1a* (*p21*) and accumulation of Eif6 in SDS zebrafish models, which is hypothesized to cause bone marrow failure. However, the mechanism(s) of EFL1's loss-of-function leading to neutropenia is not known.

Methods. We used CRISPR/Cas9 editing to generate mutations in exon 17 of *Efl1* of 32Dcl3, an IL-3-dependent murine myeloid cell line that can be differentiated to mature neutrophils. We analyzed the effects of *Efl1* mutations on cell proliferation, survival, and differentiation with G-CSF. Immunoblotting and qPCR-based gene expression analysis were performed to identify affected mechanistic pathways.

Results. Two clones were isolated and were sequenced to verify biallelic deleterious mutations. A clone with compound heterozygous inframe deletion and frameshift mutations showed trace expression of EFL1 (EFL1-KD) and the other with compound heterozygous frameshift mutations showed no expression (EFL1-KO). They presented a myeloblast-like morphology indistinguishable from parental cells. They proliferated significantly less than parental cells, although there was no increased apoptosis. Both EFL1-KD and KO cells showed significantly increased protein expression of EIF6 and transcriptional expression of *Trp53* and *Cdkn1a*. EFL1 was downregulated during differentiation of parental 32Dcl3 cells to neutrophils, whereas EIF6 levels did not change. Withdrawal and replenishment of IL-3 from culturing media did not alter EFL1 protein expression. When EFL1-KD and KO were cultured in differentiating media, they did not proliferate and underwent greater apoptosis, particularly after 3 days of differentiation compared to parental 32Dcl3. The surviving EFL1-KD and KO matured into neutrophils after 7 days. Gene profiling for neutrophil differentiation identified equivalent expression of key transcriptional factors (*Cebpa*, *Cebpe*, and *Spi1*) of EFL1-KD and KO in the maintenance media, except for *Gfi1* which was two-fold and three-fold high in EFL1-KD and KO compared to parental cells, respectively. G-CSF induced significantly higher expression of *Cebpa* and lower expression of *Cebpe* in EFL1-KO from those in parental cells, respectively. Based on the observation that somatic genetic rescue by *EIF6* mutations occurs in human SDS patients and animal models, we transfected 32Dcl3 cells with siRNA against *Eif6*. In parental 32Dcl3 cells, reduction of EIF6 caused slow cell growth; however, transfection of siRNA to *Eif6* partially rescued proliferation of EFL1-KD and KO in both maintenance and differentiating media.

Conclusions. Our results suggested that imbalance of late maturing factors of ribosome associated with SDS may cause decreased proliferation in myeloid-committed cells, thereby leading to neutropenia in SDS. Newly established *Efl1*-deficient cells recapitulated key observation from SDS patients, with an increased proapoptotic profile in hematopoietic cells. Because *EFL1* has only been recognized recently as a rare cause of SDS, we do not know if EFL1 deficiency serves as a leukemia predisposition syndrome. We speculate that the aberrant normal neutrophil differentiation may form the foundation for myeloid neoplasia in SDS.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-187549