ELSEVIER

Blood 142 (2023) 4585–4586

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Downregulation of SLC7A11 in MDS-SF3B1 Patients Induces a High Sensitivity of Erythroid Precursors to Ferroptosis

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Introduction

Myelodysplastic syndromes with *SF3B1* mutation (MDS- *SF3B1*) represent a subtype of hematopoietic stem cell disorders characterized by bone marrow erythroid dysplasia and ring sideroblasts. They are associated with patient-impacting anemia and iron overload. Adequate iron levels are crucial for heme synthesis during erythropoiesis. An elevated iron level can enhance cell sensitivity to ferroptosis, a non-apoptotic iron-dependent form of cell death caused by lipid peroxidation. Recent studies have demonstrated that normal erythroblasts (EBs) are resistant to ferroptosis, expressing highly Glutathione Peroxidase 4 (GPX4), a ferroptosis suppressor, which requires Glutathione (GSH) as a cofactor. We hypothesize here that a loss of resistance to ferroptosis could occur in MDS- *SF3B1* EBs, contributing to ineffective erythropoiesis

Methods and results

We first used UT7-EPO and HUDEP-2 as cell models of *in vitro* erythropoiesis to study the effects of *SF3B1* knockdown (KD) using a shRNA lentiviral-based strategy. In both cell lines, we observed after *SF3B1* KD a higher level of ferroptosis, as assessed using BODIPY $^{581/591}$ C11 staining, both at basal state and after exposure to Ras-Selective Lethal 3 (RSL3), which degrades GPX4 and L-Buthionine- (S, R)-sulfoximine (BSO), which inhibits the cysteine import and subsequently GSH synthesis. In EBs derived from CD34 $^{+}$ cells, *SF3B1* KD induced a delayed differentiation together with an increased level of ferroptosis, and a decreased level of GSH. Since GSH production requires import of Cystine through the Solute Carrier family 7 member 11 (*SLC7A11*) transporters, we quantified this transporter by Western Blot and found it decreased in comparison to control. The use of γ-Glutamyl-Cysteine, which bypasses *SLC7A11* for GSH synthesis, decreased ferroptosis both at basal state and after RSL3 or BSO exposure.In normal EBs, GPX4 inhibition is not enough to induce a high ferroptosis. We hypothesized that, after *SF3B1* KD, the decreased GSH lowered the "trigger" for ferroptosis, but that an additional stimulus was required. Free iron appeared to be a good candidate since (i) it plays the central role in ferroptosis, (ii) an excess of free iron is observed in MDS- *SF3B1* EBs. We exposed EBs after *SF3B1* KD to free iron. We observed increased intracytoplasmic reactive oxygen species level (ROS), measured using the H2DCFDA probe and increased membrane lipid peroxidation as well as accumulation of intracytoplasmic iron assessed by FerroOrange probe while it decreased rapidly after a peak in the control conditions. Quantifying ferroportin revealed a decreased expression after *SF3B1* KD. α-Tocopherol, that reverts lipid peroxidation and N-Acetyl-Cysteine, that decreased intracellular ROS, restored ferroportin expression, iron excretion andoxidative stress. At last, we used primary bone marrow cells from 3 MDS- *SF3B1* patients and confirmed the phenotype observed in other cell models characterized by (i) an excess of basal ferroptosis, (ii) a decreased *SLC7A11* and ferroportin expression and (iii) an accumulation of intracellular free iron in comparison with normal EBs.

Conclusion

In summary, we show that *SF3B1* KD or mutation in primary cells from patients, impaired the natural resistance of EBs to ferroptosis together with a defect in erythroid differentiation and an increased mortality, potentially contributing to anemia. The heightened susceptibility to ferroptosis is associated with downregulation of SLC *7A11* expression, leading to a decreased

GSH synthesis. Moreover, the high level of ROS is associated with a decreased ferroportin expression, leading to accumulation of intracellular free iron and providing a permanent "fuel" for membrane lipid peroxidation that could be reverted by ferroptosis-blocking agents such as Vitamin E or Deferasirox. Both showed promise in restoring erythroid differentiation, preventing EB cell death, and may serve as an interesting therapeutic strategy for treating MDS- *SF3B1* patients.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-185703