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Sickle cell disease: downregulation of GATA1 and oxidative stress response

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Objective

Ineffective erythropoiesis (IE) is generally considered to contribute little to sickle cell disease (SCD). However, a recent study provided compelling evidence for IE being a feature of SCD pathophysiology (1). Moreover, we recently developed a clinical index to measure IE and showed that SS, but not SC, patients presented with IE compared to healthy donors (HD) (2). However, the molecular mechanisms of IE in SCD, its relationship to disease heterogeneity and its potential as a therapeutic target remain unknown. Furthermore, GATA1, an essential erythroid transcription factor known to be downregulated in haematological conditions presenting with IE, has not been previously investigated in SCD. Thus, this project aims to investigate the molecular basis of IE in SCD by focusing on GATA1 functions in erythropoiesis.

Methods

HUDEP-2 erythroid cells, gene-edited sickle HUDEP-2 (SS HUDEP-2)(3) cells and CD34+ cells isolated from SS, SC and HD individuals were differentiated in vitro and (i) erythroid differentiation kinetics, (ii) oxidative stress, (iii) GATA1 expression and (iv) anti-oxidant response factors NRF2, FOXO3 and heme-oxygenase 1 (HO-1) were assayed. Moreover, the SS Berkeley transgenic mouse model was used as an in vivo model for SCD(4).

Results

No significant differences were observed in the differentiation kinetics and in levels of oxidative stress in in vitro differentiated HUDEP-2 and SS HUDEP-2 cells. Moreover, GATA1 levels were similar in both cases. Interestingly, in vitro differentiation of human SS CD34+ cells showed a striking delay accompanied by high oxidative stress and a decrease in GATA1 protein levels. We also find a significant decrease in FOXO3, NRF2 and HO-1 levels in SS cells, but not in SC cells. Of note, SC CD34+ differentiation kinetics were similar to HD cells, showing no differences in oxidative stress and in GATA1 levels. These findings agree with our previous publication showing IE not to be a significant feature of SC(2). We also investigated IE in the SS Berkeley transgenic mouse model. IE was evident in SS mice compared to AA transgenic controls. SS mice present with higher levels of reactive oxygen species (ROS) in both the bone marrow and the spleen. Moreover, we show a significant decrease in GATA1 protein in SS spleen cells. Histological studies show that the SS Berkley mouse has a very congested bone marrow with a disorganized architecture. We are currently investigating fibrosis in the bone marrow and the spleen tissue of the mice, as the latter is an indicator of inflammation.

Conclusion

Using in vitro differentiation of primary SS cells and an in vivo mouse model for SCD, we provide experimental evidence for IE being a feature of SCD. Furthermore, we show for the first time that IE in SCD is accompanied by a significant reduction in the protein levels of the GATA1 erythroid master transcription factor and of FOXO3 and NF-E2, important transcriptional regulators of the oxidative stress response. Another important finding of this study is that IE is not a feature of SC, emphasizing the need to discriminate between these two pathologies. References: 1. El Hoss S, Cochet S, Godard A, Yan H, Dussiot M, Frati G, et al. Fetal hemoglobin rescues ineffective erythropoiesis in sickle cell disease. Haematologica. 2020. 2. Brewin J, El Hoss S, Strouboulis J, Rees D. A novel index to evaluate ineffective erythropoiesis in hematological diseases offers insights into sickle cell disease. Haematologica. 2022;107(1): 338-41. 3. Demirci S, Gudmundsdottir B, Li Q, Haro-Mora JJ, Nassehi T, Drysdale C, et al. betaT87Q-Globin Gene Therapy Reduces Sickle Hemoglobin Production, Allowing for Ex Vivo Anti-sickling Activity in Human Erythroid Cells. Mol Ther Methods Clin Dev. 2020;17: 912-21. 4. Manci EA, Hillery CA, Bodian CA, Zhang ZG, Lutty GA, Coller BS. Pathology of Berkeley sickle cell mice: similarities and differences with human sickle cell disease. Blood. 2006;107(4): 1651-8.