ABSTRACT

Beta-thalassemia is a prevalent monogenic blood disorders and, to date, more than 350 mutations have been identified in the β-globin gene that result in disrupted synthesis of adult haemoglobin and lead to severe anemia. Each year more than 40,000 infants manifest the disease and approximately 1.5% of the global population are carriers. HbE/β-thalassemia is a compound heterozygous state of the disease where one allele inherits HbE trait from one parent while the other inherits a β-thalassemia mutation, and is mostly prevalent in southeast Asia. This results in an extreme heterogenous group of patients with reduced β-chain synthesis leading to imbalance in globin chains and ineffective erythropoiesis. Owing to the dearth of established curative treatment methods (which are usually complex, challenging, expensive, and/ or fraught with risks) regular blood transfusion and iron chelation remains the mainstay for the management of the disease. Reactivation of developmentally suppressed fetal haemoglobin offers significant therapeutic potential. Recently, post-transcriptional regulation of fetal haemoglobin by non-coding RNAs, especially circular RNAs (circRNAs), has emerged as an attractive area of research. While circRNAs are currently opening up avenues in cancer research and neurodegenerative diseases, there is very limited understanding of the function of circRNAs in β-thalassemia. Therefore, this thesis seeks to investigate how circRNAs function as modifiers of fetal hemoglobin expression in the context of HbE/β-thalassemia. The project provides with a comprehensive pipeline to predict the landscape of circRNAs in maturing erythroid cells, which led to the identification of circNFATc3 as an upregulated circRNA in high fetal haemoglobin conditions and the identification of circNFATc3/let-7b/GATA2 axis. The mechanistics of this axis has been explored in details through various molecular and cellular approaches, including gain and loss-of-function studies. These evaluations have revealed that competitive splicing of circNFATc3, at the expense of its linear transcript, sponges let-7b miRNA, a member of the let-7 family known for its suppressive activity on HbF expression in adults. This sequestration, thereby, activates GATA2-mediated HbF expression, whose role in erythropoietic commitment and fetal haemoglobin induction deserves attention. Moreover, transcriptomic profiling of circNFATc3-overexpressed erythroid cells showed that its role is not only restricted to HbF induction but rather plays a broader role in erythropoiesis, which adds further complexity to the role of this circular transcript. A simultaneous increase in γ-globin levels, along with elevated expression of genes related to iron uptake, heme biosynthesis, and erythroid differentiation, collectively point to active erythropoiesis and high fetal haemoglobin expression. Therefore, this study demonstrates the pivotal role of circNFATc3 as a fetal haemoglobin inducer and places this circRNA as a promising modifier in erythroid transcription programs, opening avenues for novel therapeutic strategies in β-thalassemia.

Keywords: Beta-thalassemia, Fetal haemoglobin, Haemoglobin switching, Circular RNA, Erythropoiesis

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