Exploring the role of microRNAs in fetal hemoglobin regulation in β-thalassemia

Abstract

β-thalassemia is a blood disorder caused by the reduced or absent synthesis of the β-globin chains of the hemoglobin tetramer ($\alpha_2\beta_2$). The severity of β-thalassemia is related to the extent of imbalance between the α-globin and non-α-globin chains. Amongst the various non-α-globin chains of hemoglobin, is the γ-globin chain, which is an integral component of the fetal form of hemoglobin (fetal hemoglobin - HbF; $\alpha_2\gamma_2$). Expression of HbF is known to be favorable for patients with β-thalassemia and its re-activation is an area of prime focus for researchers globally. MicroRNAs (miRNAs), being small RNA molecules with their potential for gene-silencing are also considered as important molecular agents that could potentially be crucial during this HbF silencing process. Therefore, the overall aim of the project was to investigate the role of miRNAs in regulation of HbF in β-thalassemia. To achieve this aim, various aspects of γ-globin regulation were explored. This included identification of the effects of deleterious polymorphisms in BCL11A (key regulator of γ-globin) and various miRNA gene regions; exploration of suitable drug repurposing candidates for HbF induction based on integrative miRNA and gene expression analysis; and investigating the differences in miRNA expression profile in HbE/β-thalassemia in comparison with healthy individuals. Additionally, a web-based miRNA-pathway analysis tool "miRnalyze" and an experimentally validated miRNA-pathway-disease association database "miRwayDB" were developed. The *in silico* SNP analysis predicted several important candidates in genes (coding and non-coding regions) which are expected to alter the translation process and thereby the expression of HbF. Further, the drug repurposing strategy identified four candidates namely: Curcumin, Ginsenoside, Valproate, and Vorinostat, to be most suitable for HbF induction. Besides these findings, the results of miRNA expression profiling identified a total of 14 differentially expressed miRNAs. These miRNAs were found to be associated with MAPK and HIF-1 signaling pathways. Finally, knockdown of one differentially expressed miRNAs i.e., hsa-miR-146b-5p was performed to elucidate its role in γ-globin regulation and erythroid differentiation. In conclusion, the overall findings of these studies provide novel insights into the molecular mechanisms of HbF regulation and their therapeutic prospects.

Keywords: β-thalassemia, fetal hemoglobin, microRNA, single nucleotide polymorphism, drug repurposing