miRNA Expression Associated with HbF in Saudi Sickle Cell Anemia

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Abstract: Background and Objectives: Sickle cell anemia (SCA) is a hereditary monogenic disease due to a single β-globin gene mutation that codes for the production of sickle hemoglobin. Its phenotype is modulated by fetal hemoglobin (HbF), a product of γ-globin genes. Exploring the molecules that regulate γ-globin genes at both transcriptional and translational levels, including microRNA (miRNA), might help identify alternative therapeutic targets. Materials and Methods: Using next-generation sequencing we identified pre-miRNAs and mature miRNA expression signatures associated with different HbF levels in patients homozygous for the sickle hemoglobin gene. The involvement of identified miRNAs in potential SCD-related pathways was investigated with the DIANA TOOL and miRWalk 2.0 database. Results: miR-184 were most highly upregulated in reticulocytes. miR-3609 and miR-483-5p were most highly downregulated in sickle cell anemia with high HbF. miR-370-3p that regulates LIN28A, and miR-451a which is effective in modulating α- and β- globin levels were also significantly upregulated. miRNA targeted gene pathway interaction identified BCL7A, BCL2L1, LIN28A, KLF6, GATA6, solute carrier family genes and ZNF genes associated with erythropoiesis, cell cycle regulation, glycosphingolipid biosynthesis, cAMP, cGMP-PKG, mTOR, MAPK and PI3K-AKT signaling pathways and cancer pathways. Conclusions: miRNA signatures and their target genes identified novel miRNAs that could regulate fetal hemoglobin production and might be exploited therapeutically.

Keywords: miRNA; SCA; hemoglobinopathies; HbF; globin expression

1. Introduction

Sickle cell anemia (SCA) is a result of homozygosity for a mutation in codon 6 of the β-globin gene (HBB; GAG→GTG) that leads to the production of sickle hemoglobin (HbS; glu7val; α2βS2). Fetal hemoglobin (HbF; α2γ2) encoded by HBG2 and HBG1 (HBG1/2) prevents the polymerization of deoxyHbS, the proximate cause of the phenotype of SCA, and therefore the major modulator of this disease [1,2].

Circulating hairpin and mature microRNAs (miRNAs) are small noncoding RNAs that modulate gene expression by inhibition of translation or stimulating degradation of gene transcripts. miRNAs are derived from distinct hairpin precursors (pre-miRNAs) and in contrast to mature miRNAs, profiling of pre-miRNAs is limited. These miRNA hairpin precursors, which fold into stem loop structures are ideal candidates for small
molecule binding. A recent study reported knockdown of BCL11A using a miRNA-adapted short hairpin RNA for post-transcriptional silencing of BCL11A to treat SCD [3]. More than 1000 miRNAs are encoded within the human genome, and some were associated with HbF induction [4–6]. For example, BCL11A, a major suppressor HBG2/1 expression, is targeted by miR-210, Lin28B, miR-486-3p, miR-30a and Let-7 family [7–10]. Another HBG2/1 repressor, MYB, was targeted by miR-126 and miR-15a/-16-1 which increased HBG2/1 expression [11,12].

HbF levels vary 2 to 4-fold among the five common HBB haplotypes associated with the HbS gene. The Arab-Indian (AI) haplotype is associated with HbF that averages ~17% in adults compared with 5 to 7% in the Bantu, Benin and Cameroon haplotypes and 10% in the Senegal haplotype [2,13–15]. Even within the AI haplotype there is variation of HbF levels. The causes of high HbF in AI haplotype SCA are not fully explained [16]. To determine whether miRNAs have an association with HbF levels in AI haplotype SCA we explored the complete hairpin and mature miRNA expression profile of HbS homozygotes with “high” and “low” HbF levels.

2. Materials and Methods

2.1. Study Population and Samples

Participants aged from 3 to 42 years, 29 males (49%) and 30 females (51%), were clinically diagnosed by high performance liquid chromatography (HPLC) of hemoglobin. HbS homozygotes who were not having an acute vaso-occlusive episode or who were not currently on hydroxyurea, and normal hemoglobin controls were recruited from Qatif Central Hospital, Al-Qatif, Saudi Arabia. Blood samples (8–10 mL) were collected into EDTA tubes from 59 individuals that included 31 SCA patients and 28 healthy individuals without SCA. Blood counts were carried out by an automated clinical hematology analyzer (Sysmex, K-1000, Sysmex Corporation, Kobe, Japan), the percentage of HbS, HbA2 and HbF were measured by HPLC on the Bio-Rad Variant II Hb Testing System (Bio-Rad Laboratories, Hercules, CA, USA).

Ethical approval for the study was obtained from the Institutional Review Board Committee (IRB-2019-01-107) at Imam Abdulrahman bin Faisal University. The study was conducted according to the ethical principles of the Declaration of Helsinki and Good Clinical Practice Guidelines.

2.2. Magnetic Separation of CD71+ and CD235+ Cells

Peripheral blood mononuclear cells (PBMC) were isolated using density gradient Ficoll- Histopaque (Merck, St. Louis, MO, USA). PBMC were labeled with CD71 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany), which were used for the positive selection of human reticulocytes. The cell suspension was loaded onto a MACS® Column which was placed in the magnetic field of a superMACS separator. The magnetically labeled CD71+ cells were retained on the column. After removal of the column from the magnetic field, the magnetically retained CD71+ cells were eluted as the positively selected cell fraction. Similarly, Glycophorin A+ (CD235+) cells were magnetically separated after labeling with Glycophorin-A MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany). CD235a (Glycophorin A) MicroBeads were used for the positive selection of human erythroid cells as single-pass transmembrane glycoprotein is expressed on mature erythrocytes. These isolated cells were fluorescently stained with CD71-FITC and CD235-APC antibodies and confirmed by flow cytometry (Guava, Millipore, Burlington, MA, USA). Both these cell types were further processed for miRNA isolation.

2.3. RNA Extraction and Quantification

Total RNA was isolated using miRNeasy Mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer’s instructions to obtain 50 µL of extracted sample. The RNA isolation protocol utilizes QIAzol Lysis reagent, chloroform and ethanol. The final elution was with 50µL of RNase-free water and was immediately quantified using the
Qubit® microRNA Assay Kits and the Qubit® 4.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The quantified RNA was stored at −80 °C until library preparation.

2.4. miRNA Library Construction and Sequencing

Aliquots of 100ng of miRNAs, derived from the above-mentioned procedure, were used for the preparation of miRNA libraries. Sequencing-ready cDNA libraries using the Small RNASeq Library Prep kit for Illumina (Lexogen, Austria) were prepared according to the manufacturer’s instructions. Multiplexing indices were introduced during the PCR amplification step to distinguish each sample after the pooling phase, allowing multiplexing of up to 96 libraries [17]. The library product was cleaned and concentrated with a magnetic bead-based purification protocol [18]. Lastly, the sequencing step was performed with NGS technologies using Illumina platform NextSeq 500 and the NextSeq 500/550 High Output v2.5 kit (150 cycles).

2.5. Bioinformatic Analysis of the Raw Sequencing Data

FASTQ files were generated via Illumina bcl2fastq2 (Version 2.17.1.14-http://support.illumina.com/downloads/bcl-2fastq-conversion-software-v217.html accessed on 16 June 2021) starting from the raw sequencing reads produced by Illumina NextSeq sequencer. MiRNAs were mapped on miRbase hairpins using SHRiMP software. Differential expression analysis for miRNAs was performed with the R package DESeq2 (Ver 1.18.1) (R software, Vienna, Austria). MiRNAs with more than 5 counts were retained for further analysis. The miRNA expression is considered differentially significant if the log2(disease sample/healthy control) ≥ 1 and a False Discovery Rate (FDR) ≤ 0.1. Hence, a minimum |Log2FC| of 1 and an FDR lower than 0.1 as thresholds to differentially expressed genes to maximize the sensitivity of this analysis and to perform a massive screening and identification of candidate miRNAs. For comparative analysis, we dichotomized HbF levels into low (6.35 ± 3.57%) and high (21.31 ± 6.73%) groups [15,19].

2.6. Target Gene and Interaction Analysis

The involvement of identified miRNAs in potential SCD-related pathways (predicted target genes and predicted pathways) was investigated with the DIANA TOOL and miRWalk 2.0 database. Significantly differentially expressed miRNAs in SCA patients were considered for the analyses conducted on the mirWalk ver-2.0 to predict potential targets of these miRNAs [20]. Analysis was preferred for only validated results, using miRTarBase filter option and were compared using other popular algorithms, such as miRDB or Target Scan. DIANA-mirPath was used to hierarchical targeted genes clusters-based pathway identification utilizing the exact significance (p-value threshold set at 0.05) after FDR correction (Benjamini and Hochberg) and exclusion of pathways with few targeted genes nodes.

3. Results

Subject characteristics are shown in Table 1. Mean age in SCA was 22.45 ± 16.36 yrs. compared with 33.43 ± 13.2 yrs. in controls. Sixteen patients had low percentage of HbF (6.35 ± 3.57%) and 15 had high HbF% (21.31 ± 6.73%).

miR-184 was the most upregulated miRNA in the reticulocytes of the high HbF compared with the Low HbF cohort (fold = 2.83; p = 2.90 × 10⁻³; Supplementary Table S1). miR-4508 (fold = 4.36; p = 7 × 10⁻⁸), miR-451a (fold = 4.25; p = 3.6 × 10⁻⁸), miR-144 (fold = 2.78; p = 1.1 × 10⁻⁴) were upregulated in high HbF in reticulocytes compared with erythrocytes. In the high HbF cohort, the significantly downregulated miRNAs were mir-4655 (fold = −4.11; p = 1.70 × 10⁻⁵), mir-12118 (fold = −3.46; p = 4.40 × 10⁻⁴), mir-3125 (fold = −4.11; p = 5.90 × 10⁻⁴) and mir-7703 (fold = −3.49; p = 1.20 × 10⁻³) (Supplementary Table S2). miR-3609 (fold = −2.05; p = 2.50 × 10⁻²) in reticulocytes and miR-483-5p (fold = −3.6; p = 1.20 × 10⁻²) in erythrocytes were the most downregulated miRNA of high HbF compared with low HbF groups. The Principal Component Analysis
(PCA) plot illustrating genetic relatedness among hairpin and mature miRNA is shown in Figure 1.

Table 1. Baseline characteristics and hematological parameters.

|                               | Control (N = 28) | HB S Homozygote (N = 31) | Low HbF | High HbF | Control vs. SCA | Low HbF vs. High HbF |
|-------------------------------|------------------|---------------------------|---------|----------|-----------------|----------------------|
| Age (Y)                       | 33.43 ± 13.2     | 22.45 ± 16.36             | 17.34 ± 16.05 | 27.25 ± 15.64 | 0.01             | 0.092                |
| HbF (%)                       | 0.83 ± 0.32      | 14.98 ± 9.34              | 6.35 ± 3.57 | 21.31 ± 6.73 | <0.0001         | <0.0001              |
| HbA2 (%)                      | 3.54 ± 1.03      | 3.03 ± 0.5                | 2.91 ± 0.6 | 3.2 ± 0.29 | 0.12            | 0.193                |
| RBC (10⁹/mm³)                 | 5.79 ± 0.92      | 4.13 ± 0.74               | 3.9 ± 0.61 | 4.44 ± 0.83 | <0.0001         | 0.097                |
| Hb (g/dL)                     | 12.04 ± 1.58     | 10.65 ± 1.6               | 10.92 ± 1.42 | 10.3 ± 1.82 | 0.09            | 0.366                |
| Hematocrit (%)                | 40.18 ± 5.98     | 31.49 ± 5.24              | 31.97 ± 4.08 | 30.91 ± 6.56 | <0.0001         | 0.648                |
| MCV (fl)                      | 69.92 ± 8.72     | 78.17 ± 10.67             | 82.86 ± 10.14 | 72.55 ± 8.7  | 0.12            | 0.019                |
| MCH (pg)                      | 21.06 ± 2.97     | 26.42 ± 3.95              | 28.01 ± 3.43 | 24.51 ± 3.83 | 0.01            | 0.035                |
| MCHC (g/dL)                   | 30.04 ± 1.34     | 33.68 ± 1.56              | 33.9 ± 1.22 | 33.42 ± 1.93 | <0.0001         | 0.485                |
| Platelets (10³/mm³)           | 393.2 ± 72.9     | 271.7 ± 112.5             | 229.33 ± 75.03 | 322.6 ± 131.82 | 0.03            | 0.05                 |

Figure 1. Principal Component Analysis (PCA) plot illustrating genetic relatedness among hairpin and mature miRNA in study cohort.
In gene pathway interaction assay, BCL7A, BCL2L1, LIN28A, KLF6, GATA6, SLC4A8, SLC12A5, SLC30A4, SLC30A6, ZNF154, ZNF706, ZNF618, ZNF655, ZNF789, ZNF394 and others were identified. The overall biological and functional role of these miRNAs–target genes in KEGG pathways were interpreted using DIANA tool (mirPath v.3). Genes targeted by the miRNAs were significantly enriched in erythropoiesis, cell cycle regulation, cAMP, cGMP-PKG signaling pathway, glycosphingolipid biosynthesis, mTOR, MAPK and PI3K-AKT signaling pathways. The dysregulated miRNAs and their target genes and associated pathways are listed in Table 2, while node graphs generated for these dysregulated miRNA–target gene interactions are shown in Figure 2.

Table 2. Dysregulated miRNA, target gene interaction and associated pathways.

| Cohort          | Dysregulated miRNA | Target Genes                                                                 | Targeted Pathways                                                                 |
|-----------------|--------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Low HbF vs. High HbF upregulated | miR-184            | BCL7A, BTF3L4, CCND2, GNL1, HDGF3, HIF3A, HSP90AA1, IGF1R, LUC7L2, PDGFB,      | Arrhythmogenic right ventricular cardiomyopathy (ARVC)                           |
|                 |                    | RS66KA3, SFT2D2, SMG1, TRIM67, USB1                                           | Adherens junction                                                                |
|                 | miR-3125           | BCL7A, BIN3, LRRC8A                                                          | Adrenergic signaling in cardiomyocytes                                           |
|                 | miR-3169           | TMCC1, TTL                                                                  | Oocyte meiosis                                                                   |
|                 | miR-3668           | BTBD3                                                                        | Signaling pathways regulating pluripotency of stem cells                        |
|                 | miR-3976           | BCL7A, KLHL8                                                                 | Thyroid hormone synthesis                                                        |
|                 | miR-4261           | NFA5                                                                         | Glioma                                                                           |
|                 | miR-4327           | FNDC38, TMEM41B, TP53INP1                                                    | Choline metabolism in cancer                                                     |
|                 | miR-4654           | AB12, DCAF8, PMP22, PTP4A1, YWHAZ                                            | Pancreatic cancer                                                                |
|                 | miR-4754           | ARMCX6                                                                       | Hippo signaling pathway                                                          |
|                 | miR-5787           | C10orf67, CBX6, CELF1, GTF3C1, IBA57, ILK, MAPK8IP3, MEMO1, MLX, NCKAP5L,  | Oxytocin signaling pathway                                                       |
|                 |                    | PK4, PNMA8B, PTGIS, RAP1GAP2, RG56, SEC22C, SEPTIN14, TULP1                  | GnRH signaling pathway                                                           |
|                 | miR-6081           | ABL1, ASPH, BCL2L1, HEYL, HIF3A, HMGA1, NAB2, PLEKHA6, RPS28, UBE2D3         | Oxytocin signaling pathway                                                       |
|                 | miR-662            | MCFD2                                                                        | Mucin type O-Glycan biosynthesis                                                |
|                 | miR-7703           | CD226, CLOCK, FHL2, FNBP1L, GATA6, KCNN3, KIAA1958, LRPAP1, MRO, SLC12A5,   | Glycosphingolipid biosynthesis-ganglio series                                     |
|                 |                    | SLC30A4, TEF, TFDP2, TSPAN31                                                | Acute myeloid leukemia                                                           |
|                 | miR-922            | CALU, CDK6, CMTM4, GABPA, GALNT1, GOLGA7, LRRFIP2, PAK2, PHC3, RNFT2, TMEM248, TRMT9B, UBE2H, ZBTB10, ZBTB44, ZNF154, ZNF706 | Axon guidance                                                                    |
|                 |                    |                                                                              | Inflammatory mediator regulation of TRP channels                                 |
|                 |                    |                                                                              | Insulin secretion                                                                |
|                 |                    |                                                                              | Salivary secretion                                                               |
|                 |                    |                                                                              | Fatty acid elongation                                                             |
Table 2. Cont.

| Cohort                        | Dysregulated miRNA | Target Genes                                                                 | Targeted Pathways                                                                 |
|-------------------------------|--------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Low HbF vs. High HbF downregulated | miR-3609           | AKR7A2, BCL2L11, BTF3L4, BZW1, CCDC71L, FOXJ3, KCNK10, KIAA0513, KIAA1191, MDM4, MYLIP, NFAT5, NIBAN1, NKIRAS1, POU2F1, PTPN4, RAB11FIP1, RAP1B, RUFY2, SREK11P1, TNKS2, WNK3, ZNF655, ZNF789, ZNF394 | Drug metabolism-cytochrome P450 Drug metabolism-cytochrome P450 Transcriptional misregulation in cancer Ubiquitin mediated proteolysis Lysine degradation PI3K-Akt signaling pathway Mucin type O-Glycan biosynthesis Neurotrophin signaling pathway |
|                               | miR-483-5p         | MAPK3                                                                         | Glycosaminoglycan biosynthesis - heparan sulfate/heparin Ubiquitin mediated proteolysis Gap junction Colorectal cancer Basal cell carcinoma Valine, leucine and isoleucine biosynthesis mTOR signaling pathway Tyrosine metabolism Long-term potentiation Endocytosis TGF-beta signaling pathway RNA degradation Osteoclast differentiation |
| Control vs. High HbF upregulated | miR-382-3p          | ATP13A3, CCNT1M, KLHL29, METAP1, SLC4A8                                       | Glycosaminoglycan biosynthesis - heparan sulfate/heparin Ubiquitin mediated proteolysis Gap junction Colorectal cancer Basal cell carcinoma Valine, leucine and isoleucine biosynthesis mTOR signaling pathway Tyrosine metabolism Long-term potentiation Endocytosis TGF-beta signaling pathway RNA degradation Osteoclast differentiation |
|                               | miR-451a           | VAPA                                                                          | Glycosaminoglycan biosynthesis - heparan sulfate/heparin Ubiquitin mediated proteolysis Gap junction Colorectal cancer Basal cell carcinoma Valine, leucine and isoleucine biosynthesis mTOR signaling pathway Tyrosine metabolism Long-term potentiation Endocytosis TGF-beta signaling pathway RNA degradation Osteoclast differentiation |
| Control vs. High HbF downregulated | miR-184            | CRISPLD2, EPB41L5, FBXO28, SF1                                                | Lysine degradation Pentose phosphate pathway Caffeine metabolism Biosynthesis of unsaturated fatty acids Taurine and hypotaurine metabolism |
|                               | miR-542-3p         | ASRGL1, EDEM3, GPATCH2L, GUCY1A2, HIPK3, MAP7, PLEKHM3, SAMD12, SLC30A6, VAPB, ZNF618 | Lysine degradation Pentose phosphate pathway Caffeine metabolism Biosynthesis of unsaturated fatty acids Taurine and hypotaurine metabolism |
| Control vs. Low HbF upregulated | miR-134-5p         | ANGPTL4                                                                       | Estrogen signaling pathway Purine metabolism Rap1 signaling pathway Long-term potentiation Proteoglycans in cancer Glioma Thyroid cancer Acute myeloid leukemia Prolactin signaling pathway Non-small cell lung cancer |
|                               | miR-181c-5p        | CPEB4, DDX3X, ETS1, KLF6, MECP2, MTX3, NAA50, OSBPL3, RCOR1, RPS6KA3, TRIM2   | Estrogen signaling pathway Purine metabolism Rap1 signaling pathway Long-term potentiation Proteoglycans in cancer Glioma Thyroid cancer Acute myeloid leukemia Prolactin signaling pathway Non-small cell lung cancer |
|                               | miR-370-3p         | CYB561D1, LIN28A, NF1, NSUN4, PARVB, TGFB2                                   | Estrogen signaling pathway Purine metabolism Rap1 signaling pathway Long-term potentiation Proteoglycans in cancer Glioma Thyroid cancer Acute myeloid leukemia Prolactin signaling pathway Non-small cell lung cancer |
4. Discussion

We studied miRNAs in Saudi HbS homozygotes with the AI haplotype from the South-Eastern Province, where α thalassemia is found in more than half of all people and where consanguinity is high, to see if additional insight might be gained into the mechanisms underlying HBG2/1 regulation in this unique haplotype. miRNAs previously associated with HbF expression in diverse SCA patients, including Lin28B, miR-486-3p, Let-7, miR-146a, miR-15a/-16-1, miR-126, miR-150, miR-199-5p, miR-221/-222, miR-23a, miR7a, miR-96, miR-26b, miR-144, miR-451 and miR-210. Let-7, mir-3609, miR-370-3p, miR-451a, mir-19b, mir-26a, and mir-15b were also dysregulated in AI haplotype SCA [21–23]. miRNA-370-3p (p = 7.90 × 10⁻³) was significantly upregulated in erythrocytes of the low HbF cohort. Gene target analysis showed that this miRNA regulates LIN28A. Lee et al. [7] assessed the capability of LIN28B to repress the expression of let-7 miRNA and determined that LIN28 overexpression or let-7 miRNA downregulation results in a 19–40% elevation in HbF levels in SCA. In addition, LIN28A obstructs the pre-let-7 processing by Dicer via recruiting terminal uridylyl transferase 4 [24]. Hence, miR-370-3p could be a novel target to regulate LIN28 for elevated HbF production.

Similarly, miR-451a was upregulated in reticulocytes (p = 7.10 × 10⁻⁴). Expression of miRNA-451 is elevated during erythroid cell differentiation. Fang and Bartel [25] reported that miR-451, which derives from a hairpin with a suboptimal terminal loop and a suboptimal stem length, accumulates 40-fold higher levels when clustered with a helper hairpin. miR-451 was upregulated in reticulocytes by approximately 2.5-fold in the high HbF cohort. miRNA-451 forms a cluster with miRNA-144 and regulates the expression of many genes necessary for erythropoiesis. In 6 SCA patients with HbF 23.5 ± 2.1% and 6 patients with HbF 3.41 ± 1.0%, all with undetermined haplotypes, miRNA-144 expression in reticulocytes was upregulated 8-fold in low vs. high HbF patient groups [26]. miR-144 and its mature forms were significantly dysregulated in the reticulocytes of high HbF cohort compared with erythrocytes. Functional analysis of normal and sickle erythroid progenitors showed that the miRNA-144 antagonist can facilitate HbF production while increasing NRF2 expression [26]. Among eight adult patients with the Bantu HbS haplotype, 22 of 798 miRNAs were differentially expressed with 13 associated with genes that regulate HBG2/1 expression like BCL11A (miR-148b-3p, miR-32-5p, miR-340-5p, and miR-29c-3p), MYB (miR-105-5p), and KLF3 (miR-106b-5), and SP1 (miR-29b-3p, miR-625-5p, miR-324-5p, miR-125a-5p, miR-99b-5p, miR-374b-5p, and miR-145-5p) [23].

The zinc finger proteins, ZNF802 and ZNF410 are HbF gene repressors. Induced deficiency of ZNF802 resulted in an increase in HbF to 35.0 ± 3.5% [27]. Knockout of ZNF410 reduced Chromodomain Helicase DNA Binding Protein 4 (CHD4) levels by 60%, enough to substantially derepress HbF [28]. ZNF154, ZNF706, ZNF655, ZNF789, ZNF394
and ZNF618 were among the miRNA targets of the present study. Similarly, other than the well-studied BCL11A, BCL7A and BCL2L1 were also among the target genes in the present study. BCL2L1 has been associated with HbF gene expression in patients with SCA, where overexpression of BCL2L1 resulted in increased HBG2/1 expression fourfold and F-cells by 13% [29]. Likewise, other than the known KLF1, an interaction between KLF6, a new potential regulator of erythropoiesis and miR-2355-5p was identified, and its downregulation significantly raised γ-globin mRNA [30]. miR-181c-5p targeted KLF6, and the significantly downregulated miR-3609 targeted the binding site of KLF6 [31].

Hematopoietic stem cell differentiation is associated with activation of multiple signaling pathways including MAPK and PI3K [32–34]. We found novel miRNAs, miR-5787, miR-3609 and miR-483-5p associated with these signaling pathways might be potentially exploited as therapeutic targets. Histone deacetylase inhibitors like Apicidin and Tricostatin, increase H3 and H4 acetylation in the region adjacent to the HBG2/1 promoters [35] and induce γ-globin gene expression [36] by activating MAPK signaling pathway.

5. Conclusions

miRNA signatures and their target gene-pathway analysis identified novel miRNAs that could regulate fetal hemoglobin production and might be exploited therapeutically. Additional studies in erythroid cell lines and primary erythroid progenitor cells are needed to define how any of the identified miRNAs might modulate HBG2/1 expression and the accumulation of HbF and if some of the HbF-associated miRNAs can account for the high levels of HbF in A1 haplotype SCA.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/medicina58101470/s1, Supplementary Table S1: List of upregulated miRNAs with more than 1.5-fold change with HbF level in SCA cohort; Supplementary Table S2: List of downregulated miRNAs with more than 1.5-fold change with HbF level in SCA cohort.

Author Contributions: C.C., C.V., A.A.-N., S.C., M.S.A., M.D., D.A. and A.A.-A. are involved in the design of the work, critically revising of protocol, patient recruitment, analysis, interpretation of data and drafting of the manuscript. C.C., S.C. and A.A.-A. are involved in the design of the work, laboratory work, analysis, interpretation of data and drafting of the manuscript. C.C., S.H.A. and M.H.S. are involved in the design of the work, critically revising of protocol, analysis, interpretation of data and drafting of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia, Grant Number-11-MED1422-46. This is a government agency.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board/Ethics Committee of Imam Abdulrahman bin Faisal University Ethical Committee (Reference IRB-2019-01-107).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to extend our gratitude to King Abdulaziz City for Science and Technology for sponsoring this project and for their continued support [Grant Number-11-MED1422-46]. We are also grateful to the patients, nurses, and technical staff at the hospital for their dedication and support.

Conflicts of Interest: The authors declare no conflict of interest.

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