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## Data Article

# Data on the role of miR-144 in regulating fetal hemoglobin production in retinal pigmented epithelial cells

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## ABSTRACT

The data presented in this article are connected to our related article entitled "Inhibiting microRNA-144 potentiates Nrf2-dependent antioxidant signaling in retinal pigmented epithelial cells (RPE) and protects against oxidative stress-induced outer retinal degeneration" [1] where, we have shown that miR-144 induces oxidative stress in RPE cells by targeting Nrf2 expression. Previous studies from our laboratory have shown that like erythroid cells, RPE cells express  $\alpha$ ,  $\beta$  and  $\gamma$ -globin and produce hemoglobin locally in retina. Further, the ability to therapeutically reactivate fetal hemoglobin production in these cells, a strategy of high potential benefit in the treatment of complications of sickle cell disease, including retinopathy, is impacted by Nrf2-mediated signaling [2,3]. Studies by others [4,5] provide compelling evidence of a regulatory role for miR-144 and Nrf2 in fetal hemoglobin production in erythroid cells. Our current work confirms this finding in human RPE. We additionally show that miR-144-mediated regulation of fetal hemoglobin production in RPE cells is independent of kruppel like factor 1 (KLF-1). This supports the plausibility that in RPE, hemoglobin, particularly fetal hemoglobin, may be important for functions other than oxygen transport (e.g., antioxidant defense). Indeed, our new data on miR-144 in RPE

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supports strongly the potential mechanistic between fetal hemoglobin production and the regulation of oxidative stress in this cell type [1].

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#### Specifications Table

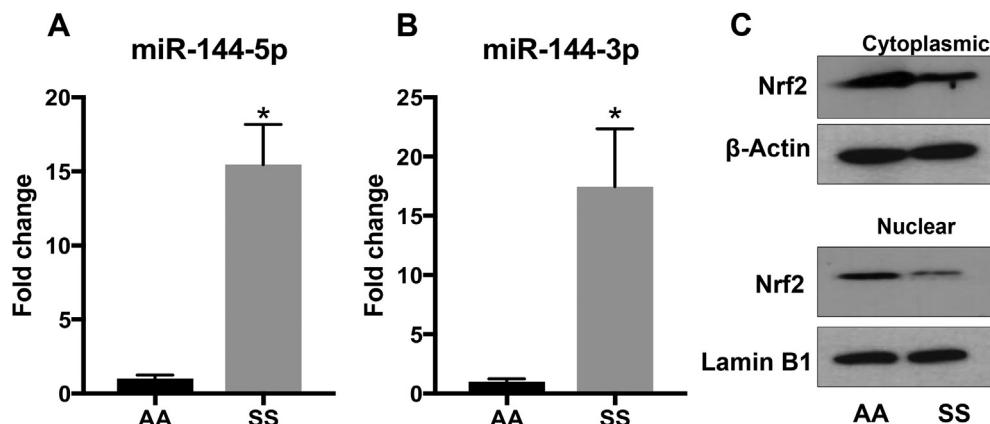
Subject area	<i>Molecular Biology</i>
More specific subject area	<i>miRNA and gene expression</i>
Type of data	<i>Graphs</i>
How data was acquired	<i>qPCR and western blotting</i>
Data format	<i>Raw and Analyzed</i>
Parameters for data collection	<i>Human retinal pigmented epithelial cells were transfected with miR-144-3p and miR-144-5p mimic and expression of fetal hemoglobin and related genes were evaluated.</i>
Description of data collection	<i>Total miRNA, RNA and Proteins were extracted from mouse and human retinal pigment epithelial cell line to perform qPCR and western blot analysis.</i>
Data source location	<i>Augusta University, Augusta, Georgia, USA.</i>
Data accessibility	<i>All data are included in this article</i>
Related research article	<i>Jadeja RN, Jones MA, Abdelrahman AA, Powell FL, Thounaojam MC, Gutsaeva D, Bartoli M, Martin PM. Inhibiting microRNA-144 potentiates Nrf2-dependent antioxidant signaling in RPE and protects against oxidative stress-induced outer retinal degeneration, Redox Biology 28 (2020) 101336. [1]</i>

#### Value of the data

- Studies by others [4,5] have demonstrated convincingly a regulatory role for miR-144 and Nrf2 in fetal hemoglobin production in erythroid cells. However, the data in this article is the first documentation of miR-144 upregulation in the retinal pigment epithelium (RPE) of a humanized mouse model of sickle cell disease.
- The data set on the involvement of miR-144 in regulating fetal hemoglobin is highly important for further research on sickle cell retinopathy. Based on these data, miR-144 levels may be used as a potential diagnostic marker to predict occurrence of retinopathy in patients with sickle cell disease.
- Further, this data in combination with our recent report [1] shows that inhibiting miR-144 expression could improve fetal hemoglobin production and limit oxidative stress in RPE thereby, providing an effective avenue to prevent and treat retinopathy characteristic of sickle cell and other degenerative retinal diseases.

## 1. Data description

RPE/eye cup was dissected out from 10 months old HbAA (normal hemoglobin)- and HbSS (sickle hemoglobin)-expressing Townes humanized knockin mice and used for qPCR and western blotting assays. As shown in Fig. 1A–B, expression of miR-144-5p and miR-144-3p were significantly up regulated in HbSS mice compared to HbAA mice. Inversely, expression of Nrf2, a target of miR-144-5p and miR-144-3p was significantly down regulated in HbSS mice compared to HbAA mice (Fig. 1C). The raw data related to Fig. 1, is shown in Sheet 1 of [supplementary data](#). Next, we overexpressed both miR-144 variants by transfecting human retinal-pigmented epithelial cells (ARPE-19) with miR144-5p and miR-144-3p mimics (Figs. 2 and 3, respectively). Overexpression of miR-144-5p and miR-144-3p significantly reduced  $\gamma$ -globin gene expression and subsequently suppressed fetal hemoglobin production (Figs. 2A and D and 3A and D). Interestingly, the expression of BCL11A (BAF chromatin remodeling complex subunit BCL11A), a negative regulator of fetal hemoglobin synthesis (Figs. 2B and 3B) was significantly increased with overexpression of both miR-144-5p and miR-144-3p while that of KLF-1, a miR144 target and an important regulator of fetal hemoglobin synthesis in hematopoietic cells,



**Fig. 1.** Evaluation of miR-144-5p and miR-144-3p expression and Nrf2 levels in retinal pigmented epithelial cells from HbAA and HbSS mouse eyes. RPE/eyecup was obtained from 10-month-old HbAA (normal hemoglobin)- and HbSS (sickle hemoglobin)-expressing Townes humanized knockin sickle cell disease (SCD) mice to isolate miRNA and proteins. The expression of (A–B) miR-144 (5p and 3p sub units) and (C) Nrf2 protein levels were evaluated in these cells by qPCR and western blotting, respectively. Data are expressed as mean  $\pm$  S.E.M for  $n = 4$ . \* $p < 0.05$  vs. HbAA.

was not significantly altered (Figs. 2C and 3C). The raw data related to Figs. 2 and 3, are shown in sheets 2 and 3 of [supplementary data](#) respectively.

## 2. Experimental design, materials and methods

### 2.1. Animals

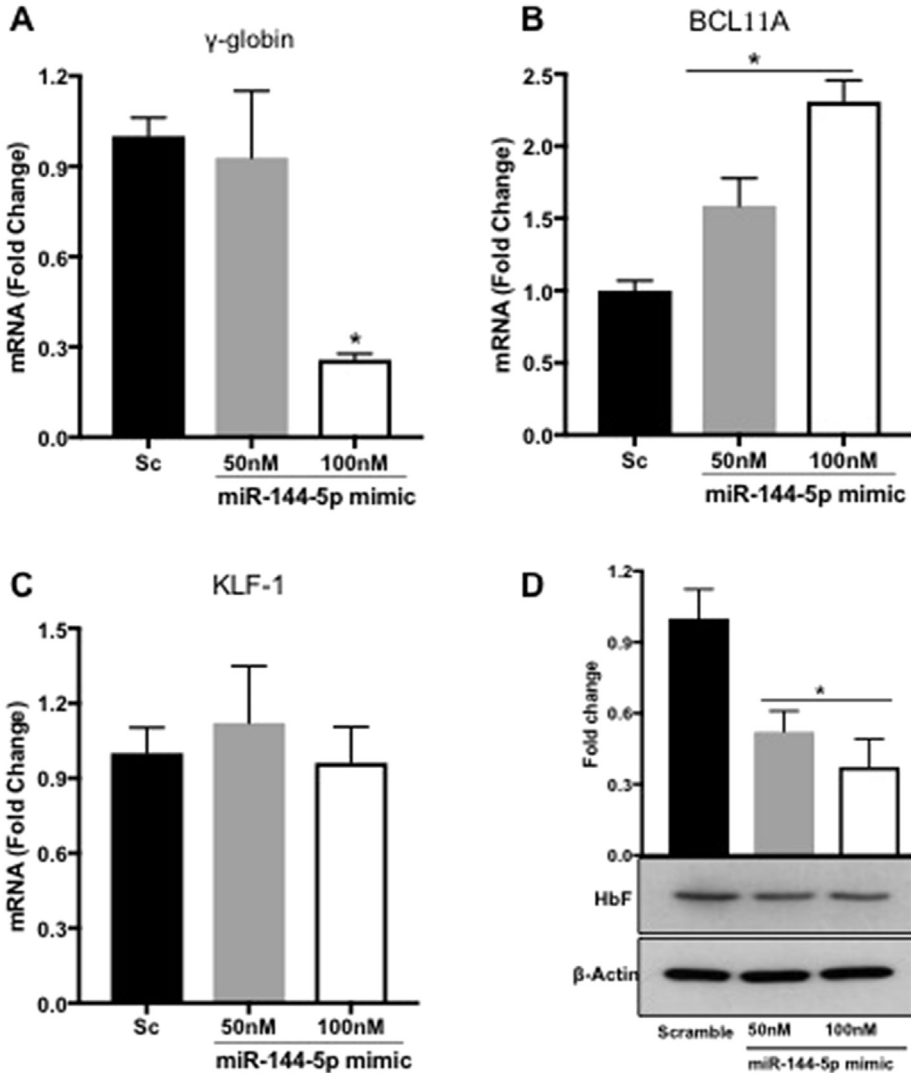
All experiments involving animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Augusta University (Augusta, GA) Institutional Animal Care and Use Committee. HbAA (normal hemoglobin)- and HbSS (sickle hemoglobin)-expressing Townes humanized knockin sickle cell disease (SCD) mice (Jackson Laboratories) have been described previously [2,3,6]. Animals were sacrificed at 10 months, the animals were euthanized by carbon dioxide asphyxiation followed immediately by thoracotomy and eyes were collected for further miRNA, mRNA and protein analyses.

### 2.2. Cell culture

Human retinal pigment epithelial (ARPE-19) cells were cultured in Dulbecco's modified Eagle medium DMEM/F12 medium (supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin) and maintained at 37 °C in a humidified chamber with 5% CO<sub>2</sub>. The culture medium was replaced with fresh medium every other day. Cultures were passaged by dissociation in 0.05% (w/v) trypsin in phosphate-buffered saline. Completely confluent, well-differentiated cultures were used for experimentation.

### 2.3. miRNA transfection

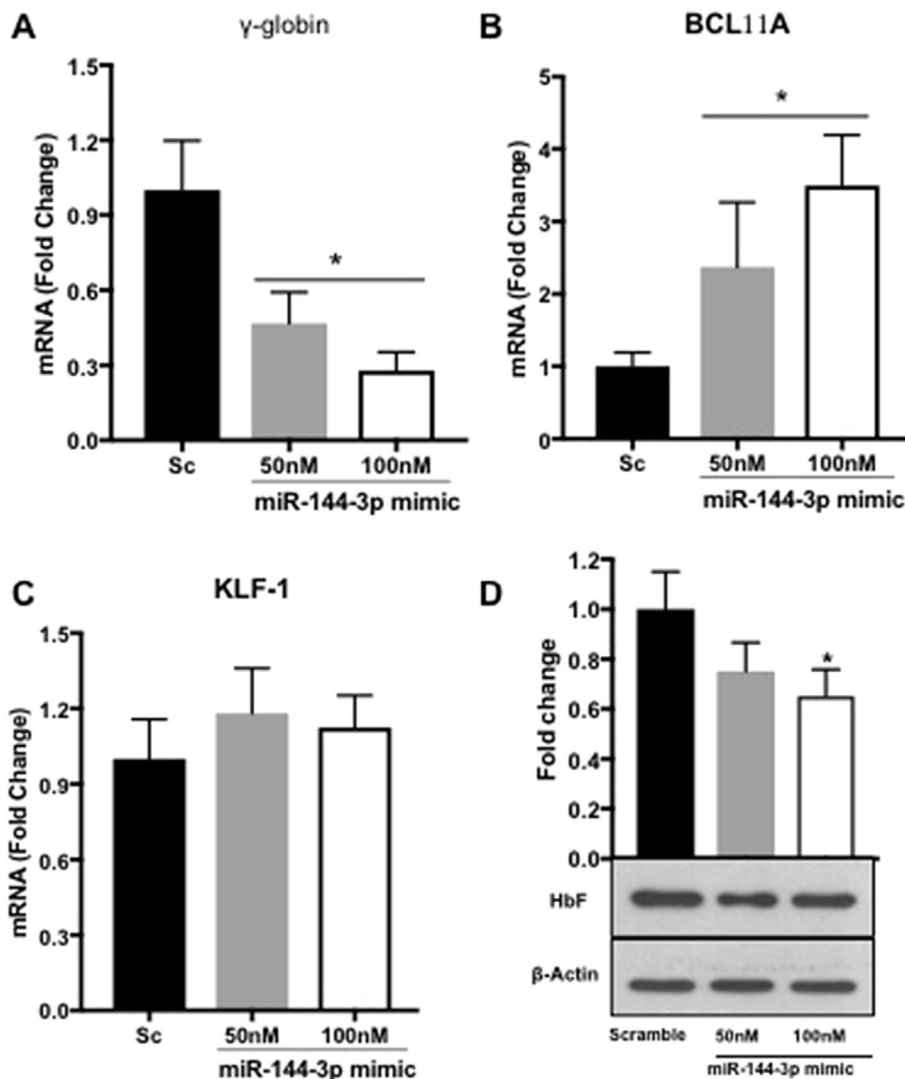
Human RPE cells (ARPE-19) were transfected with 50 or 100 nM of miR144-5p or miR-144-3p mimic using Hiperfect transfection reagent (Qiagen, USA). Cells were incubated at 37 °C, 5% CO<sub>2</sub> for 48 h and used for qPCR and Western blot assays. For each experiment, control cells were transfected with non-targeted (scrambled) miRNA.



**Fig. 2.** miR-144-5p regulates fetal hemoglobin production in human retinal pigmented epithelial (RPE) cells. Human RPE cells (ARPE-19) were transfected with non-targeted (scrambled, Sc) miRNA or 50 and 100 nM miR-144-5p mimic for 48 hr. Changes in the (A–C) mRNA expression of genes key to the regulation of fetal hemoglobin production and, (D) protein levels of fetal hemoglobin were evaluated by qPCR and Western blot respectively. Data are expressed as mean  $\pm$  S.E.M for  $n = 3$  independent experiments. \* $p < 0.05$  vs. scrambled miRNA transfected cells (Sc).

#### 2.4. Reverse transcription–quantitative polymerase chain reaction

Total miRNA and RNA was isolated from RPE/eyecup of mice or ARPE-19 cells using miRNAeasy mini and RNAeasy kits respectively (Qiagen, USA). cDNA was prepared from total miRNA and RNA using the miScript RT (Qiagen, USA) and iScript cDNA Synthesis Kit (Bio-Rad) respectively and subjected to qPCR assays. The reaction volume of 20  $\mu$ l for mRNA analysis contained 10.0  $\mu$ l SYBR green master mix (2X), 1  $\mu$ l cDNA, 1  $\mu$ l of each primer and 7  $\mu$ l nuclease-free water. Primer sequences are listed in Table 1. The following two-step thermal cycling profile was used (StepOnePlus Real-Time PCR, Life Technologies, Grand Island, NY):



**Fig. 3.** miR-144-3p regulates fetal hemoglobin production in human retinal pigmented epithelial (RPE) cells. Human RPE cells (ARPE-19) were transfected with non-targeted (scrambled, Sc) miRNA or 50 and 100 nM miR-144-3p mimic for 48 hr. Changes in the (A–C) mRNA expression of genes key to the regulation of fetal hemoglobin production and (D) protein levels of fetal hemoglobin were evaluated by qPCR and Western blotting respectively. Data are expressed as mean  $\pm$  S.E.M for  $n = 3$  independent experiments. \* $p < 0.05$  vs. scrambled miRNA transfected cells (Sc).

Step I (cycling): 95 °C for 5 min, 95 °C for 15 s, 60 °C for 30 s and 72 °C for 15s for 40 cycles. Step II (melting curve): 60 °C for 15 s, 60 °C 1 min and 95 °C for 30 s. The template amplification was confirmed by melting curve analysis. mRNA expression of genes were normalized to 18s expression and fold change in expression was calculated by  $2^{-\Delta\Delta Ct}$  method.

The reaction volume of 20  $\mu$ l for miRNA analysis contained 10.0  $\mu$ l Quantitact SYBR green master mix (2X), 2  $\mu$ l cDNA, 2  $\mu$ l of universal primer, 2  $\mu$ l of miRNA specific primer and 4  $\mu$ l nuclease-free water. The following two-step thermal cycling profile was used (StepOnePlus Real-Time PCR, Life Technologies, Grand Island, NY): Step I (cycling): 95 °C for 15 min, 94 °C for 15 s, 55 °C for 30 s and 70 °C for 30s for 40 cycles. Step II (melting curve): 60 °C for 15 s, 60 °C 1 min and 95 °C for 30 s. The template

**Table 1**

Primer sequences used for qPCR assays.

Gene	Forward (5'-3')	Reverse (5'-3')
$\gamma$ -Globin	ACTTCAAGCTCCTGGGAAATG	AAGCTCTGAATCATGGGCAGT
BCL11A	GCACGCCCATATTAGTGGT	TGTGTGAAGAAACAAGTGTCCG
KLF-1	GATGACTTCCTCAAGTGGTGGC	CAGCATATGCCCCAGAGTC
18S	CCAGAGCGAAAGCATTGCCAAGA	AGCATGCCAGAGTCTCGTTCGTTA

amplification was confirmed by melting curve analysis. miRNA expression of genes were normalized to 5S expression and fold change in expression was calculated by  $2^{-\Delta\Delta C_t}$  method.

### 2.5. Western blotting

Total protein was extracted from ARPE-19 cells using RIPA cell lysis buffer (Thermo Scientific, USA) containing protease and phosphatase inhibitors. Nuclear and cytoplasmic protein fractions from RPE/eyecup of mice were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Thermo Scientific, USA). The protein concentrations were determined using the Coomassie protein assay reagent (Sigma-Aldrich, USA). Equivalent amount of protein samples were subjected to SDS-PAGE, transferred to PVDF membranes, and then incubated with primary antibodies: Nrf2 (1:250, Cell signaling, USA), HbF (1:200, Abcam, USA) and Lamin B1 (1:500, Cell signaling, USA) overnight at 4 °C. Next day, blots were washed with TBST (Tris buffered saline-tween) and incubated with horseradish peroxidase conjugated secondary antibody (1:3000; Sigma-Aldrich, USA) for 60 min with gentle shaking at room temperature. Blots were then washed (with TBST) and developed with chemiluminescence reagent (Bio-Rad, Hercules, CA) using autoradiography films (Genesee Scientific, San Diego, CA).  $\beta$ -actin (1:3000; Sigma-Aldrich, USA) expression was evaluated to determine equivalent loading. Scanned images of blots were used to quantify protein expression using NIH ImageJ software (<http://rsb.info.nih.gov/ij/>).

### 2.6. Statistical analysis

All results are presented as mean  $\pm$  S.E.M for a minimum of three independent experiments. Statistical significance was defined as  $p < 0.05$  and determined using student's t-test (normally-distributed data) for single comparisons. Graphs were prepared using GraphPad Prism 7 software.

### Acknowledgements

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### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dib.2019.104874>.

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