Dysregulation of miR144 and miR451 Expression in the Circulating Human Erythrocytes from the African American Adults

Ghadi Alsharif1, Ibrahim Jafri1, Ronald Berasa2, Nesreen Hag Ahmed3, Gail Nunlee-Bland3, Maurice B. Fluit1t, Kanwal K Gambhir3*

1Department of Genetics and Human Genetics, Howard University College of Medicine, United States
2Department of OB/GYN, Howard University Hospital, United States
3Diabetes Treatment Center, Howard University Hospital, United States
4Division Endocrinology and Metabolism, Department of Medicine, Howard University College of Medicine, United States

*Corresponding Author: Kanwal K Gambhir, Department of Genetics and Human Genetics, Howard University College of Medicine, United States. Email: kgambhir@howard.edu

Abstract: There are several mechanisms that drive the aging process in the human body. Cellular senescence which leads to permanent cell growth arrest and oxidative stress are examples of aging promoting mechanisms. Moreover, genetics and epigenetic play important roles in accelerating and/or delaying the onset of the aging process. miRNAs are important players in controlling OS, aging, and cellular senescence. The purpose of this study was to evaluate circulating erythrocyte miR-451 and miR-144 expression as potential biomarker of cell aging. Blood samples were collected from consented volunteers and miRNAs were isolated from a control group and Type 2 Diabetes Mellitus. Taqman primers were used for detection and quantification of miR-144 and miR-451. Erythrocytes were further sub-fractionated into young, mid and old age by using discontinuous Percoll gradient. miRNA’s were isolated from erythrocytes using miRNA Isolation kit. The miR-451 expression was significantly down-regulated in old cells in T2DM group, while miR-144 expression was significantly up-regulated in old cells in T2DM. The findings of this study are consistent with our previous report of increase expression of miR-451 and miR-144 as the cell gets old. The current findings suggest that miR-451 and miR-144 may have a role in aging process.

Keywords: MicroRNA Expression, Cell Aging, Oxidative stress, microRNA, Erythrocytes, Type 2 Diabetes, Biomarkers of erythrocyte aging.

Abbreviations: Oxidative stress: OS, miRNA: miR, Erythrocytes: E, Young: y, mid: m, Old: o, Type 2 Diabetes Mellitus: T2DM, Red Blood Cells: RBCs, Insulin Receptor Substrate 1: IRS-1, Impaired Fasting, Glucose: IFG, Diabetes Mellitus: DM, Type 1 Diabetes mellitus: T1DM

1. INTRODUCTION

Diabetes mellitus (DM) is considered a global public health issue, affecting 463 million adults worldwide [1]. This count is estimated to rise above 640 million people by 2040 [2]. DM is caused by the failure of pancreas cells to produce insulin (Type1) or decrease insulin sensitivity (Type2). DM imposes a series of complex multi-organ and multi-system effects on the body over time and leads to cardiovascular disease, kidney failure, blindness, and nerve damage. Although many clinical procedures have been established to manage and treat DM, the underlying mechanisms of DM and its complications need to be fully understood in order to develop effective therapeutic strategies and identify novel biomarkers of disease [3].

Red blood cells (RBCs) (Erythrocytes) are the most abundant cells in blood stream, accounting for more than 90% of the cell population in peripheral blood. The function of RBCs is to carry oxygen throughout the body by binding to hemoglobin. Autoxidation of hemoglobin makes RBCs more susceptible to oxidative stress (OS) damage [4]. Recent reports highlight the emerging role of RBCs in several biological processes of importance to metabolic functions, including diabetes mellitus and its complications [5, 6]. These studies suggest that disturbances in RBC function and structure can promote oxidative stress and jeopardize antioxidant...
Dysregulation of miR144 and miR451 Expression in the Circulating Human Erythrocytes from the African American Adults

...resulting in red blood cell dysfunction and injury to peripheral tissues [5,7]. Thus, RBCs provide an efficient and attractive means to identify markers and mechanisms of cell aging in the setting of type 2 diabetes mellitus (T2DM). MicroRNAs (miRNA) are a class of small (18-25 nucleotides) non-coding RNA that are important in the regulation of gene expression by inhibiting translation or degrading target mRNAs [8]. MiRNAs are found and function throughout the body in both tissue and circulation. Previous work from our group and others has identified several miRNAs deregulated in T2DM [9-11]. We previously reported reduced expression of several miRNAs in erythrocytes of pre-diabetic adults [9-10], which suggests a potential regulatory role for miRNAs in these cells. MiR-451 and miR-144 are highly expressed and are important miRNAs in RBCs maturation and function [12]. MiR-451 reportedly protects erythrocytes against oxidant stress through a miR-451/14-3-3ζ/FoxO3 regulatory axis [12].

Recent evidence suggests its role in the development of T2DM and its micro vascular complications [13-14]. In addition to miR-451, miR-144 has been shown to play an important role in T2DM. Investigators found that high expression of miR-144 significantly inhibit IRS-1 mRNA in T2DM and increase the glycemic status in those individuals [15]. Moreover, this study identified additional miRs that may participate in the regulation of insulin signaling and serve as biomarkers of disease [15]. The diagnostic, prognostic, and therapeutic potential of miRNAs in both tissue and circulation has been established and documented. However, additional studies are warranted to understand the complex nature and function of miRNAs in T2DM, especially those found in circulation (i.e. micro vesicles and RBCs). These circulating miRNAs can influence several biologically important processes, including cell aging. Moreover, circulating miRNAs provide an attractive early marker of disease progression. In this study, we evaluate miR-451 and miR-144 expression as a potential biomarker of cell aging in erythrocyte sub-fractions (young-, middle-, old-cells) isolated from type 2 diabetic adults.

2. MATERIALS AND METHODS

2.1. Participants and RBC Samples Processing

A total of 16 blood samples (T2DM=8, control=8) were collected and a written informed consent was obtained from all. Blood samples (~20 mL) were collected following an overnight fast of 8-10 hours in tubes containing heparin using a standard procedure. The study protocol was approved by Howard University Institutional Review Board (HUIRB) (IRB-13-MED-73). Whole blood was centrifuged for 20-minutes at 1200 rpm.

The plasma layer was removed and stored at -80 °C for future study. Isotonic choline chloride solution was added to RBCs and mixed gently by inversion. To obtain purified RBCs, RBCs were added to the wall of a glass tube contain a mixture of 1 mL Hypaque (H) (33.9%) and 2.4 mL Ficoll (F) (9%) and spun for 20 minutes at 1200 rpm. This procedure repeated twice. The purified RBCs separated into three subtractions (1.07-1.09 as y, 1.09-1.11 as m, and 1.11-1.12 as o) by layering them over a percoll gradient consisting of 35%, 40%, 45%, 50%, 55%, 65%, 80%, and 100% of percoll and the balance Gibco minimum essential medium [14] as shown in Figure 1. This was centrifuged at 1200 rpm for 30 minutes. Then the respective age subtractions were collected.

![Fig1: Schematic representation of percoll density for sub-fractionated RBCs: y = 1.07-1.09, m = 1.09-1.11, o = 1.11-1.12 g/mL.](image)

2.2. RNA Extraction

Total RNA was extracted from each age sub-fractionated RBCs of each sample using mirVana miR Isolation kit (Invitrogen) according to the manufacturer’s protocol. To
begin, the age sub-fractioned RBC sample (400 uL) disrupted in lysis solution. MiR homogenate additive was added to the sample, vortexed, and incubated for 10 minutes on ice. Acid-phenol chloroform was added in a volume equal to the total volume of the sample lysate, mixed, and centrifuged for 5 minutes at maximum speed at room temperature to extract. To extract, the upper phase removed carefully and transferred to a new tube. The RNA eluted in the elution solution. All samples stored at -80C.

2.3. cDNA Synthesis and Mirna Quantification

Real time PCR (RT-qPCR) was used for miR quantification in age sub-fractionated RBCs. Reverse transcription and PCR reaction were performed using TaqmanmiR transcription kit (Applied Biosystem). Applied biosystem uses total RNA that contains miR as the starting material for cDNA synthesis and allows for the detection of multiple miRs from a single cDNA preparation. The final reaction volume of reverse transcription was 20 uL. To quantify miR expression, a master mix was prepared following the manufacture’s procedure (for each 10 uL reaction: 7 uL Taqman master mix and 3 uL PCR primer). Amplification was carried out in 96 well plate using one step plus (Applied Biosystem) using the cycling conditions outlined by the manufacture. QRT-PCR was performed in duplication for each miRNA. RNU6 was used as a control.

2.4. Data Analysis

Differences in variance among the groups were analyzed using non-parametric one-way ANOVA. The differences in miRNAs expressions between control and T2DM were analyzed by using Student t-test. P<0.05 was considered statistically significant. Data analysis was performed using stats tester software.

3. RESULTS AND DISCUSSION

3.1. Differential Mir-144 and -451 Expression in Erythrocytes between Patients with T2DM and Control

We selected two miRs to be measured in human erythrocyte obtained from control and T2DM AA. These miRs are highly expressed in erythrocytes and have a known protective function in these cells [12, 16]. To identify if erythrocyte miRs expression differences in y, m, and o cells between control and T2DM individuals, we isolated total RNA from sub-fractionated purified erythrocytes and quantified miRs expression using qRT-PCR. MiR-451 was significantly reduced in T2DM group when compared to control group. Conversely, miR-144 was significantly higher in T2DM when compared to control group (fig 2).

We next sought to identify relationships between selected miRs expression in o cells and developing T2DM. We found there was a strong correlation between the miR-144 and development of T2DM. However, no such correlation with miR-451 was observed. The expression of miR-144 in o cells was significantly higher in T2DM group than control group (fig 3).
Dysregulation of miR144 and miR451 Expression in the Circulating Human Erythrocytes from the African American Adults

To determine the effect of T2DM on erythrocytes life span, we compared the expression of miR-451 in o cells between the two groups. We found a significant difference in miR-451 expression between control and T2DM. As mentioned previously, miR-451 is responsible for reducing the oxidative stress in erythrocytes. We found that T2DM group has a significant decrease in miR-451 in comparison with control group (fig 4).

Many studies have been reported that circulatory miRs can be served as non-invasive biomarkers of diseases such as cancer, diabetes, and heart diseases. Few studies have explored miR expression in erythrocytes. This study explores the regulatory role of these miRs as potential indicators of cell aging. Our previous report showed the significant difference between miR-451 and miR-144 expression in sub-fractionated erythrocytes [17]. To our knowledge, this is the first study to investigate the expression of miRs in sub-fractionated erythrocytes (y, m, and o) of T2DM in comparison with the controls. We reported that miR-451 are reduced in erythrocytes of the T2DM individuals. Also, miR-451 expressed more in o cells than m and y cells in both groups. MiR-451 regulates FOXO3 expression to protect erythrocytes from OS [18] by inhibiting the cytoplasmic adaptor protein 14-3-3ζ. This protein sequesters the production of FOXO3 that stimulates antioxidant genes such as catalase and glutathione peroxidases 1 [19].

Conversely, miR-144 expression was found to be up-regulated in T2DM in comparison with the control group. Moreover, miR-144 expressed more in o cells than m and y cells in both groups. MiR-144 expression correlate with several clinical characteristics associated with T2DM, including HbA1c making it an ideal candidate for genetic risk markers in AA. MiRNAs are reported to be involved in insulin production, secretion, and control insulin signaling pathway [20]. IRS proteins are known to be involved in the insulin signaling pathway. Dwi and colleagues found that miR-144 regulates IRS 1 gene [21]. Moreover, the study
reported that there is a linear relationship between miR-144 and increasing glycemic status in T2DM. Another study showed that miR-144 knockout mice were insulin resistant. This finding proved that miR-144 plays an important role in insulin signaling pathway [21].

Both miR-451 and miR-144 are the most abundant miRs present in erythrocytes. The findings of our study are consistent with the other reports of reduced expression of miR-451 in individuals with certain condition such as diabetes lead to shortened life span of erythrocytes [22]. Also, miR-144 expression increased in T2DM group in comparison with control group. Our findings also suggest that o cells expressed more miR than m and y cells.

4. Conclusion

This is the first study that we are reporting an increase in the expression of miR-451 and miR-144 in o cells than m and y cells in the erythrocytes from the individuals with T2DM. These findings could serve as potential biomarkers of red blood cell aging. These findings support similar cell aging studies. However, further studies are needed to clarify the potential role of RBC miRNAs as biomarkers for cell aging depending on the levels of which miR’s that are altered during cell aging.

Acknowledgment

Authors thank fellows, clinicians and staff DTC for their kind support of this work.

Author’s Contribution: “GA “GA and IJ conducted studies and drafted manuscript; RB, NH, GNB recruited patients; MBF edited manuscript; KKG designed and supervised experimental protocol.”

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Dysregulation of miR144 and miR451 Expression in the Circulating Human Erythrocytes from the African American Adults

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Erythrocyte miRNA 144 and miRNA 451 as Cell Aging Biomarkers in African American Adults

Ibrahim Jafri¹, Ghadi Alsharif¹, Gail Nun-lee Bland¹ and Kanwal K. Gambhir²,*

¹Howard University College of Medicine, Genetics and Human Genetics, Washington DC 20059, United States
²Department of Medicine, Molecular Endocrinology Lab Division Endocrinology-Metabolism, Washington DC 20059, United States

Abstract:
Objective: MicroRNAs (miRNA) are novel critical regulators of cell proliferation and human disease, including diabetes mellitus and cancer. The aim of this study was to evaluate the expression of circulating erythrocytes (E) miRNA-144 and miRNA-451 expression in African Americans Adults (AAA) as a biomarker of cell aging.

Methods: The blood samples were collected from healthy controls [n=9] following an 8-12 hours fast. Erythrocytes were purified twice by Boyum gradient. Erythrocytes were further sub-fractionated into young (y) (1.07-1.09 g/ml), mid (m) (1.09-1.11 g/ml), and old (o) (1.11-1.12 g/ml) age cells by using discontinuous Percoll gradient (35%, 40%, 45%, 50%, 55%, 65%, 80%, and 100%) and total RNA extracted. MiRNA-144 and miRNA-451 were quantified in y, m, and o age E sub-fractions by qRT-PCR.

Results: MiRNA-451 expression was 82210.8271, 130922.476, and 149554.364 in y, m, and o cells, respectively. MiRNA-144 expression in y cells was 18.66410^9, m cells was 32.44136^2, and o cells was 57.81183^9. These results showed that o cells expressed both miRNA-144 and miRNA-451 more than that of m, and y cells.

Conclusion: The findings of this study showed that miRNAs expression differ in sub-fractionated erythrocytes. This study suggests that miRNA-144 and miRNA-451 have the potential to be used as biomarkers of RBC aging.

Keywords: Red blood cell, Cell aging, MicroRNA, African American adults, Erythrocytes, Proliferation, Biomarkers of cell aging.

1. INTRODUCTION

Red Blood Cells [RBCs] are considered to be the most abundant cells in circulation. About 2 million RBCs are produced per second and released into the blood stream in a steady state condition [1]. The first lineage of RBCs production is the erythroid. Proliferation is one of the important stages that help the precursor cells to amplify into huge number of cells that will differentiate to the final cell types [2]. Erythroblasts differentiate to erythrocytes (E) after going through numerous changes, such as cells hemoglobinization, nuclear condensation and enucleation. The process of RBCs synthesis is called erythropoiesis that must be effective and robust in order to regulate body homeostasis [3]. The whole process of erythrocyte formation is outlined in Fig. 1.

2. MIRNAS AND RED BLOOD CELL AGING

MicroRNA [miRNA] is a type of non-coding RNA that contains small 19-23 nucleotides that is important in regulation of gene expression by binding to specific mRNA to alter the expression of the targeted message. Changes in miRNA binding site nucleotide will lead to falsely binding to mRNA and
increase the gene expression that leads to increase the gene product [4]. MiRNA-144 and miRNA-451 are found to be highly expressed in RBCs specifically in erythroblasts. Both miRNA-144 and miRNA-451 are important for the proliferation and differentiation of erythrocytes [5].

MiRNA-144 and miRNA-451 are highly expressed in erythroblasts and their coding gene is located in chromosome 11 [7]. MiRNA-144 and miRNA-451 were found to be in several species other than humans, such as mice and zebrafish. These miRNAs [miRNA-144 and miRNA 451] are required for terminal erythropoiesis in all previous species [8 - 10]. One of the studies deleted miRNA-144 and miRNA-451 to determine the significance of these two miRNAs in erythropoiesis. The results revealed that loss of miRNA-144 and miRNA-451 caused mild anemia and reticulo-cytosis with moderate abnormalities in erythrocyte morphology [11]. MiRNA-451 functions, as studied in zebrafish embryos, reportedly functions to maintain late-stage erythrocytes maturation [12] (Fig. 2).

3. MATERIAL AND METHOD

3.1. Ethical Statement

This study was carried out in accordance with the guidelines of the Howard University Institutional Review Board (HUIRB). The protocol was approved by HUIRB (IRB-13-MED-73). All participants of this study gave writing informed consent.

3.2. Subjects

Study participants were recruited from the Howard University Hospital Community. All samples were collected from the self-declared healthy individuals. Individuals who were pregnant, possessing malignant tumors, and smokers were excluded from this study. Whole venous blood was collected in heparinized vacutainer tubes following a period of fasting 8-12 hours. Each blood sample was centrifuged for 20 minutes at 1200 rpm to remove plasma within 2 hours after collection.

3.3. Isolation and Purification of erythrocytes

Boyum method as described by Gambhir et al. [13] was used to isolate erythrocytes. The erythrocyte pellet was purified by adding isotonic choline chloride solution (2x the volume of erythrocyte) and mixed by inversion. The erythrocyte suspension was layered on the tube (12 x 25 mm) containing a mixture of 1 ml Hypaque (H) (33.9%) and 2.4 mL Ficoll (F) (9%) (H-F mixture) and then spun at 1200 rpm for 20 minutes at room temperature. This procedure was repeated twice.

Separation of RBCs according to their age as y, m, and o was performed as described by Gambhir et al. [14]. The purified erythrocyte age sub-fractions were obtained by the differences in the specific densities of RBCs.

To create a distinctive layer of percoll gradient, 8 tubes were labeled 1 to 8, with tubes containing 1.3 mL medium and 0.7 mL percoll, 1.2 mL medium and 0.8 mL percoll, 1.1 mL medium and 0.9 mL percoll, 1.0 mL medium and 1.0 mL percoll, 0.9 mL medium and 1.1 mL percoll, 0.7 mL medium and 1.3 mL percoll, 0.4 mL medium and 1.6 mL percoll and 1 mL percoll, respectively. To the test tube 8 containing only percoll, dilutions from tube 7 to 1 were layered to stratify a discontinuous gradient containing 35%, 40%, 45%, 50%, 55%, 65%, 80% and 100% percoll. The purified erythrocyte pellet was layered to the wall of tube 8 (containing the discontinuous gradient) and then spun at 1200 rpm for 30 minutes. The three sub-fractions of percoll density were identified 1.08-1.09 as y, 1.09-1.11 as m and 1.11-1.12 as o as described in (Fig. 3).

3.4. RNA Isolation

To isolate total RNA from the age sub-fraction RBC, the commercially available kit, mirVana miRNA Isolation kit (Invitrogen; Thermo Fisher Scientific, Inc.), was used follow-
ing the protocol outlined by the manufacturer. Briefly, 400 µL age fractionated RBC sample was disrupted by adding 400 µL of lysis/binding buffer. Then 1/10 volume (80 µL) of miRNA Homogenate Additive was added to the age fractionated RBC sample and vortex. The samples were incubated 10 min on ice. Extracted with a volume of Acid-Phenol: Chloroform equal to the initial lysate volume (800 µL) and centrifuged at 10000 rpm for 5 min. The aqueous phase after centrifugation was recovered by transferring it to a fresh tube. Absolute ethanol was added to the lysed age fractionated RBC sample. Seven hundred µL of this sample was transferred to the fresh filter cartilage, then centrifuged for 15 seconds and discarded the supernatant. Seven hundred µL of wash 1 was added to the filter and centrifuged for 15 seconds. Five hundred µL of wash 2/3 was added and centrifuged for 15 seconds then discarded, and the supernatant saved and filtered. The wash 2/3 procedure was repeated twice. Eluted RNA with 100 µL 95°C Elution Solution or Nuclease-free Water in a new tube and spun for 20-30 seconds to recover the RNA. NanoDrop (ND-One) spectrophotometer (Thermo Scientific, Inc) was used to determine concentration and purification of RNAs.

![Schematic biogenesis of MiRNA and its mechanism of action. Typical miRNA biogenesis pathway in a cell. miRNAs are transcribed by RNA polymerase II resulting in pri-miRNA transcripts. miRNAs are processed by microprocessor by a microprocessor complex including Drosha and DGCR8 having pre-miRNA which are exported from the cell nucleus via Exportin 5. Pre-miRNAs are processed by Dicer, the mature miRNA is loaded in the RISC complex. Mature miRNAs bind the 3’UTRs of target mRNAs and localized to bodies target miRNAs are deadenylated and degraded via CAF1and PABP or translationally repressed. RISC = ribonucleotide silencing complex Pol II = polymerase II [6].](image-url)
Fig. (3). Schematic representation of the protocol preparation of red blood cell purification and age sub-fraction. H:F Hypaque-ficoll gradient (Boyum gradient) CC: Choline Chloride.

Fig. (4). Total RNAs content of RBC age fractions.
Table 1. miRNA 144 and miRNA 451 expressions in Percoll density cell age gradient sub-fraction.

| Red Blood Cell subfraction | miR-451 expression | miR-451 relative expression | miR-144 expression | miR-144 relative expression |
|----------------------------|--------------------|----------------------------|-------------------|-----------------------------|
| Young Age cell (y)         | 82210.8271         | 1                          | 18.6641092        | 1                           |
| Middle Age cell (m)        | 130922.476         | 1.59                       | 32.4413621        | 1.74                        |
| Old Age cell (o)           | 149554.364         | 1.8                        | 57.8118394        | 3.1                         |

3.5. RT-qPCR

For each sample, 50 ng of total RNA containing miRNA was reverse transcribed into cDNA using the MicroRNA Reverse Transcription kit (AB Applied Biosystem) according to the manufacturer's protocol. The resulting cDNA served as a template for miRNA qPCR analysis (AB Applied Biosystem, Inc.). The amplification profile was on hold at 50°C for 2 minutes and at 95°C for 20 seconds followed by 40 cycles of 95°C for 1 second and 60°C for 20 seconds.

3.6. Statistical Analyses

The mean and standard deviation were calculated analysis of variance or paired t-test to determine differences in miRNA-144 and miRNA-451 expressions for all groups.

4. RESULTS

This study aimed to utilize miRNA-144 and miRNA-451 as biomarkers for cell aging in AAA. In screening for different miRNAs expressions in age fractionated erythrocyte, we performed total RNA isolation from y (0-40 days), m (41-80 days), and o (81-120 days) that revealed different amounts of RNA in each of the different cell age fractions (Fig. 4). We selected two miRNAs with the highest expression levels (miRNA-144 and miRNA-451) in RBCs. For miRNA-144 and miRNA-451 quantification, we used RT-qPCR Taqman probes which allow the detection of PCR products by generating a fluorescent signal. U6 was used as a control. The results of these PCR products are shown in (Table 1). miRNA-451 expression were 82210.8271, 130922.476, and 149554.364 in y, m, and o cells respectively. MiRNA-144 expression in y cells was 18.6641092, m cells was 32.4413621, and o cells was 57.8118394. To get relative quantification, we used y cells as the reference sample in order to analyze gene expression changes in sub-fractionated erythrocytes. To calculate the relative expression of each miRNA (with respect of y cell miRNAs expressions), we divided m and o cells miRNA expressions by miRNA expression in the y cells. For instance, we had 130922.476 and 82210.8271 as miRNA-451 expressions in m and y cells, respectively. We got 1.59 which is miRNA-451 relative expression by dividing 130922.476/82210.8271.

When these miR-451/144 expressions of fractionated erythrocytes were compared, the O RBCs demonstrate more miRNA-144 and miRNA-451 expression than m and y RBCs. There were significant differences observed in the expressions of these two miRNAs in y, m, and o cells. These values were expressed as 1:1.74:3.1 and 1:1.6:1.82 in y, m, and o, respectively (p<0.05). Moreover, miRNA 144 is relatively less expressed than miRNA-451 Fig. (5).

The miRNA 144 and miRNA-451 are involved in the process of erythropoiesis and may also be participated as a marker for cell aging.
5. DISCUSSION

MiRNAs play an important role in cellular differentiation, apoptosis, and aging [15]. Circulating blood microRNAs can be considered as health and disorders biomarkers [16]. Our observation showed that among these RBCs miRNAs, we identified miRNA-144 and miRNA-451 as potential novel biomarkers of human RBC aging in AAA. We report, for the first time, the expression of miRNA-144 and miRNA-451 in sub-fraction RBCs are different. The ratio of miRNA-144 and miRNA-451 were expressed as 1:1.74:3.1 and 1:1.6:1.82 in y, m, and o respectively. As described in the Results section, these relative ratios are with respect to y cells. The deletion of miRNA 144 and miRNA 451 the most expressed erythroid miRNA gene, causes anemia and increased RBC to oxidant stress. The miRNA 144 and miRNA 451 expressions were highly up-regulated in o RBC than in y and m. The main objective of this study was to develop a simple and efficient method suitable for studying miRNA-144 and miRNA-451 as red blood cell aging biomarkers. Cell aging, known as cellular senescence, is a process of irreversible growth arrest that stops any damaged cells from growing. The role of cellular senescence is to protect cells against cancer; however, recent studies added more complex roles, such as cellular development, tissue repair, and age related disorders [17]. Chronic conditions, such as heart disease, diabetes, and cancers that are known to affect the whole organism function have many risk factors, such as aging. There is increasing evidence that chronic age-related diseases incidence will increase as the world’s population ages >60 years will double in the next four decades. In the United States, health disparities are linked to race. One study suggested that black individuals are aging faster than whites individuals [18]. Therefore, there is a need to investigate molecular biomarkers, such as miRNAs for early detection of cell aging in AAA. The purpose of this investigation was to determine whether miRNA-144 and miRNA-451 can be used as a possible marker of cell aging in AAA. Real time PCR of miRNA-144 and miRNA-451 was conducted, and the results showed that the expression profiles of RBC miRNA-144 and miRNA-451 of AAA individuals were different in different RBC sub-fractions. The results of real-time PCR indicated that RBC miRNA-144 and miRNA-451 expression levels were significantly increased in o cells compared with the m and y cells. Interestingly, the levels of miRNA 451 were also significantly higher in all cell sub-fractions than miRNA 144. The expression trend of both miRNAs provides direct evidence for the relationship between RBC miRNAs and cell aging. Many studies indicate that miRNA-144 plays a crucial role in the process of erythropoiesis as an erythroid-specific regulator [19]. Moreover, biological experiments showed that miRN-144 can increase the RBC number by inhibiting RAB14 transcription [20]. Therefore, it is possible that RBC-derived miRNA-144 influences the process of erythropoiesis by targeting RAB14. Papapetrou et al. observed that the expression level of miRNA-144 increases during erythropoiesis in HSCs and miRNA-144 can positively regulate erythrocyte differentiation [21]. Therefore, we speculate that miRNA-144 derived from erythrocyte vesicles may be taken up by the HSCs and inhibit the expression levels of target genes (e.g., RAB14), resulting in the promotion of erythropoiesis.

On the other hand, the target gene of miRNA-451 is not well determined. Experiments using Zebra fishes showed knockout of miRNA-451 induced severe reduction of RBC production, suggesting that miRNA 451 is one of the key molecules for erythropoiesis. One study suggested that miRNA-451-Ago2 complexes in mature RBCs may be playing roles for homeostasis of normal erythropoiesis, and the analysis of miRNAs-Ago2 complex in RBCs is relevant for understanding normal and pathological erythropoiesis [22].

CONCLUSION

These results are consistent with our hypothesis that miRNA-144 and miRNA-451 may be used as useful biomarkers of RBC aging. From these results, one also could speculate that circulating non-nucleated cells have evolved to require alternative means of regulating protein translation to maintain viability during 120 days (RBC) life span.

MiRNA expression differences in human HSC lineages enable regulated transgene expression [23]. However, further studies are needed to fully understand the role of these miRNAs as RBC aging biomarkers.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by Howard University Institutional Review Board (HUIRB) (IRB-13-MED-73) USA.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written consent was obtained from each participant.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

This work was partially supported by the JHU-UMD DRC Grant P30DK079637-08, Grant # 4525 and the NIH Research Grant 5G12MD007597 (Dr. Gambhir, PI).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Authors thank fellows, clinicians and staff DTC for their kind cooperation in getting the blood drawn of the subjects. The authors would also like to thank Dr. Maurice Fluitt for his diligent proofreading of this paper.
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