Mol. Cells *34*, 177-183, August 31, 2012
DOI/10.1007/s10059-012-0090-6
pISSN: 1016-8478 eISSN: 0219-1032

Molecules and Cells
http://molcells.org

Dynamic Expression of Specific miRNAs during Erythroid Differentiation of Human Embryonic Stem Cells

Hong Lian Jin[1,3], Jong Soo Kim[1,3], Young June Kim[2], Su Jin Kim[1], Hal E. Broxmeyer[2], and Kye-Seong Kim[1,*]

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at post-transcriptional levels through mRNA degradation or translation inhibition. Little is known regarding miRNA participation in regulating hematopoietic, or more specifically erythroid differentiation. This study was aimed at identifying erythroid lineage-specific miRNAs expressed during *in vitro* erythropoiesis using human embryonic stem cells (hESCs) and human umbilical cord blood (CB) CD34+ cells. CD34+ hematopoietic cells were produced from hESCs *in vitro* and subsequently induced to differentiate into erythroid cells by culture in sequence on OP9 feeder cells and then with mesenchymal stromal cells (MSC) in the presence of cytokines. Expression profiles of erythroid lineage-specific miRNAs were analyzed by quantitative PCR during *in vitro* differentiation. Expression levels of miR-142-3p, miR-142-5p, miR-146a and miR-451 were dynamically changed during differentiation of hESCs to CD34+ hematopoietic cells, and in subsequent differentiation of the CD34+ cells into the erythroid lineage. This suggests that these four miRNAs might be involved in regulating erythropoiesis.

INTRODUCTION

MicroRNAs (miRNAs) are short regulatory non-coding RNAs that control gene expression via degradation of target mRNAs and/or inhibition of translation, thereby playing critical roles in dictating the fates of a variety of cells by regulating unique subsets of genes during differentiation (Gammell, 2007). Hundreds of miRNAs have been identified from many eukaryotic organisms, but specific roles of many of these miRNAs for organ development, homeostasis and metabolism of many organisms have yet to be revealed. Recently, miRNAs have been proposed to play regulatory roles in the process of erythropoiesis. Because a single miRNA can potentially target several hundred genes and conversely, a single gene can bind more than one miRNA (Lewis et al., 2005), multiple miRNAs may regulate target genes cooperatively for erythropoiesis. The first miRNA identified as being expressed in human erythrocytes was miR-451 (Rathjen et al., 2006). Later, more signature miRNAs were reported to correlate with common myeloid/erythroid progenitor cell commitment and subsequent maturation. Correlation analysis with umbilical cord blood (CB)-derived CD34+ cells showed that expression of miR-15b, miR-16, miR-22, and miR-185 positively correlated with the appearance of erythroid surface antigens: CD36, CD71 and CD235a, and with hemoglobin synthesis, while induction of miR-28 inversely correlated with expression of these erythroid surface antigens (Choong et al., 2007). When variation of miRNA expression was assessed in K562 cells, miR-126 was up-regulated while miR-103, miR-130a, miR-210, and miR-18b were down-regulated after erythroid cell induction. The same miRNA expression tendency was observed in CD34+ cells isolated from human CB following erythroid induction (Yang et al., 2009). miRNA profile analysis using mouse erythroid precursors also showed that expression of miR-15b, miR-22, miR-26a, miR-29a, miR-30a-5p, miR-144, miR-292-5p and miR-451 was increased upon erythroid differentiation (Zhan et al., 2007). miR-451 affects cellular functions such as epithelial cell polarity formation (Tsuchiya et al., 2009) and cancer development (Bandres et al., 2009). Although microarray analyses for identifying changes in miRNA expression levels during erythropoiesis are informative, these reported results have not been consistent in the literature. miR-142 is involved in hematopoiesis, immune responses, and T cell differentiation (Chen and Lodish, 2005; Ramkissoon et al., 2006; Visone et al., 2009; Wu et al., 2007). miR-146a is known to suppress metastasis (Edmonds et al., 2009; Li et al., 2010) and more recently it has also been implicated in modulating immune responses (Tsitsiou and Lindsay, 2009; Williams et al., 2008). miR-451 plays a role in regulating erythropoiesis by repressing negative regulators for erythropoiesis, such as Gata2 (Dore et al., 2008; Kuang et al., 2009; Zhan et al., 2007). However, it is largely unknown whether these miRNAs are involved in early stages of hematopoiesis. In particular, regulatory roles of these miRNAs in development of human CD34+ hematopoietic cells

[1]Graduate School of Biomedical Science and Engineering, College of Medicine, Department of Anatomy and Cell Biology, Hanyang University, Seoul 133-791, Korea, [2]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis 46202, USA, [3]These authors contributed equally to this work.
*Correspondence: ks66kim@hanyang.ac.kr

Received March 23, 2012; revised April 16, 2012; accepted May 16, 2012; published online July 4, 2012

Keywords: dynamic expression, erythrogenesis, human embryonic stem cells, miRNAs

from human embryonic stem cells (hESC) and subsequent erythroid differentiation of human CD34+ hematopoietic cells (enriched for stem and progenitor cells) remains to be delineated. In this study we focused particularly on investigating expression changes of miR-142-3p, miR-142-5p, miR-146a, and miR-451 in the hematopoietic system. Here, we report dynamic variations of miR-142-3p, miR-142-5p, miR-146a and miR-451 expression during transition from hESC to CD34+ cells and from hESC derived CD34+ cells to erythroid cells *in vitro*. Our results suggest that miRNAs may be regulatory targets for possible engagement in erythroid cell commitment.

MATERIALS AND METHODS

Microarray analysis

miRNAs profiles in hESCs, mesenchymal stem cells (MSCs) and CB CD34+ cells were analyzed using the Genopal miRNA microarray system (Mitsubishi Rayon Co., Ltd., Yokohama Japan) (Lee et al., 2010). Chip analysis was repeated at least twice, and hybridization signal intensities were analyzed as described previously (Lee et al., 2010).

Real time PCR

Gene expression levels were quantified using the ABI 7300 RT-PCR System (Applied Biosytems, USA). Expression levels of mature miRNAs were determined using TaqMan Gene Expression Assays (Applied Biosystems, USA). First strand cDNAs were synthesized from approximately 10 ng total RNAs using superscript II reverse transcriptase (Invitrogen, USA), and cDNAs were amplified for miRNAs by PCR using PCR premix (Gene Bio, Switzerland), Relative quantities of miRNA were calculated using different values between threshold cycles (Ct) of respective miRNAs and RNU6B used as endogenous control. Comparative real-time PCR assays with or without specific primers for miR-142-3p, miR-142-5p, miR-146a, miR-451 were performed in triplicate. Initial reactions were carried out at 95°C for 10 min, followed by additional 40 cycles of reactions at 95°C for 15s and 60°C for 1 min per cycle. Relative expression was calculated using the comparative C_t method.

hESC, OP9 cells and co-culture

hESC (H9 line) were maintained according to the research protocol of the WiCell Research Institute (USA). H9 colonies were passaged weekly on 10 mg/ml mitomycin C (Sigma, USA) treated mouse embryonic fibroblast feeder cells in DMEM/F-12 basal medium containing 20% serum replacement, 0.1 mM beta-mercaptoethanol, 0.1 mM non-essential amino acids solution, 100 unit/ml penicillin, 100 μg/ml streptomycin, and 4 ng/ml recombinant human FGF-2 (Invitrogen, USA). Differentiated hESC colonies were physically removed from undifferentiated hESCs at purity greater than 90% for each passage. The undifferentiated state of hESC was confirmed based on morphology and loss of OCT-4 expression by immnunocytochemical stain. The OP9 mouse bone marrow stromal cell line was purchased from ATCC, the Global Bioresource Center and maintained according to the manual provided. OP9 cells were grown on gelatin-coated culture dishes for at least five passages in α-modified minimum essential media (α-MEM) (Invitrogen, USA) supplemented with 20% defined fetal bovine serum not heat inactivated (FBS; HyClone Laboratories, USA) for adaptation before the cells were used as feeder cells for hESC differentiation. Hematopoietic differentiation of hESC on OP9 feeder cells was induced in α-MEM containing 10% FBS and 100 μM monothioglycerol (MTG, Sigma, USA) with complete medium change on day 1 followed by a half-medium change on days 4, 6, and 8. Finally on day 10, CD34+ hematopoietic cells were isolated for further differentiation into erythroid cells (Vodyanik and Slukvin, 2007; Vodyanik et al., 2005).

Isolation and differentiation of CD34+ cells

Aggregated cells were harvested from the co-culture and dissociated into single cells by treatment with 0.25% trypsin/EDTA solution (Gibco, USA) for 20-30 min at 37°C and passaged through 70-μm cell strainers (BD Bioscience, USA) after gentle pipetting. CD34+ cells were then isolated by MACS (Miltenyi Biotec, Germany) according to the manufacturer's instructions. Isolated CD34+ cells were induced to differentiate into erythroid lineage cells by culturing for the first 7 days in serum-free Iscove's modified Dulbecco's medium (IMDM, Gibco, USA) supplemented with three cytokines, SCF (Peprotech, USA) at 100 ng/ml, IL-3 (Peprotech, USA) at 5 ng/ml, EPO (STEMCELL Technologies, Canada) at 3 IU/ml, 1% human serum albumin (Sigma, USA), 200 μg/ml iron-saturated human transferrin (Sigma, USA), 50 μg/ml insulin (Sigma, USA), 4 mmol/L L-glutamine (Sigma, USA), 1.6×10^4 mol/l monothioglycerol (Sigma, USA) and 1% penicillin-streptomycin solution (Gibco, USA) with a half-medium change twice a week. The cells were continuously differentiated on human bone marrow (BM) derived MSC as feeder cells, by culturing for an additional 7 days in medium but with only erythropoietin (EPO) with a half-medium change every 2-3 days.

Reverse transcriptase polymerase chain reaction

miRNA extracted from CD34+ cells was subjected to PCR using Tag polymerase and following primers forward primer (5′-CACATCTACCTCTGTGATAACCT-3′) and reverse primer (5′-CTTGATGTCACTTAGGATAGGAG-3′); GPA, forward primer (5′-GCATTAAGTACCACTGAGGTG-3′) and reverse primer (5′-GGAGGGTAAACAGTTCTAACAGA-3′); OCT4, forward primer (5′-AACTCGAGCAATTTGCCAAGCTCC-3′) and reverse primer (5′-TTCGGGCACTGCAGGAACAAATTC-3′); GATA4, forward primer (5′-CTCCTTCAGGCAGTGAGAGC-3′) and reverse primer (5′-GAGATGCAGTGTGCTCGTGC-3′); AFP, forward primer (5′-AGAACCTGTCACAAGCTGTG-3′) and reverse primer (5′-GACAGCAAGCTGAGGATGTC-3′); DCN, forward primer (5′-GAGCTCAGGAATTGAAAATG-3′) and reverse primer (5′-AAGCTTGTTGTTGTCCAAGT-3′); Brachury, forward primer (5′-GATCACAAAGAGATGATGGA-3′) and reverse primer (5′-TTGTCAGAATAGGTTGGAGA-3′); NeuroD1, forward primer (5′-ATTCTAAGACGCAGAAGCTG-3′) and reverse primer (5′-ACTGGTAGGAGTAGGGGTGT-3′); human actin, forward primer (5′-CAGATCATGTTGAGACCTTC-3′) and reverse primer (5′-ATGATGGAGTTGAAGGTAGTTT-3′). PCR was carried out by pre-incubation at 94°C for 5 min followed by 30 cycles of reaction at 94°C for 30 s, at 55°C for 30 s, at 72°C for 30 s and finally continuous reaction at 72°C for 7 min.

Imaging analysis

For immunological staining, isolated CD34+ cells were washed with PBS and attached to glass slides using Cytospin 3 (Shandon Scientific, USA). The cells were fixed with 4% formaldehyde, stained with anti-human CD34 monoclonal antibody at 1:100 ratio at 4°C overnight and FITC conjugated goat anti–mouse antibody at 1:500 ratio for 1 h at room temperature following PBS washing 3 times. After 3 washes with PBS, the cells were observed under a fluorescence microscope. In addition, cell morphology was examined with Wright-Giemsa staining under digital camera (Leica, Germany) at 1,000 fold magni-

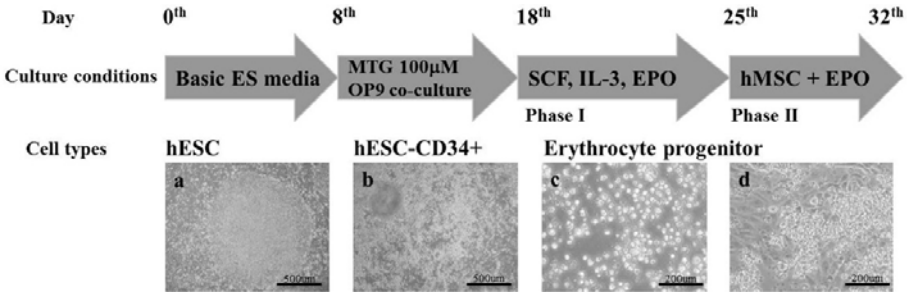

Fig. 1. Culture conditions under which H9 hESC differentiated into CD34+ hematopoietic cells and subsequently into erythroid cells. OP9 cells and hBM derived MSC cells were used as feeder cells for differentiation of H9 hESCs into CD34+ cells and subsequent erythroid cells, respectively. Contrast microscopic pictures were taken for the cells undergoing differentiation at a magnitude of 100× and 200× respectively. Characteristic morphological changes that occurred during the transition are shown; H9 hESC colony (a), CD34+ cells after a 10-day co-culture with OP9 (b), phase I erythroid progenitors after culture with SCF, IL-3 and EPO at day 25 (c), phase II erythrocyte after co-culture on hBM-MSC with EPO only at day 32 (d).

fication.

Flow cytometric analysis

Erythrocyte differentiation of CD34+ cells was assessed by staining with monoclonal antibodies to CD71 and glycophorin A conjugated with fluorescent isothiocyanate (FITC) and phycoerythrin (PE), respectively for 15 min on ice. Stained cells were analyzed with FACS Calibur and pro-CellQuest software (BD Medical Systems, USA).

Statistical analysis

miRNA expression levels were evaluated as mean ± standard errors. Significance of data was determined using student's *t*-test and a P value less than 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Erythroid colony forming cells were generated *in vitro* from hESCs through CD34+ cells by co-culture with OP9 cells and MSC in the presence of appropriate cytokines

In vitro generation of mature hematopoietic cells from stem cells has been an intense research subject for many years. We attempted to establish a culture system to generate erythroid cells from hESC using a co-culture system in serum free medium supplemented with various cytokines. The first intermediate cell type we wanted to attain was CD34+ expressing cells. CD34+ is known to be expressed on hematopoietic stem/progenitor cells. This was achieved by co-culture of hESC on OP9 cells, a mouse stromal cell line as feeder cells in 100 mM monothioglycerol (MTG) for 10 days as described by Maxim et al. (Vodyanik et al., 2005) The CD34+ cells derived from hESC were continuously cultured in SCF, IL-3 and EPO for 7 days followed by additional culture on human BM derived MSC as feeder cells with EPO for another 7 days. The resulting cells formed typical erythroid colonies (Fig. 1).

hESCs were able to differentiate into CD34+ cells with loss of OCT4 and induction of AFP and DCN expression

We separated the CD34+ cells from undifferentiated hESC using magnetic-bead separation (MACS) kit and verified expression of CD34 mRNA. We then monitored expression of OCT4 (a marker for hESC), AFP (alpha fetoprotein; a marker for erythroid cells), and DCN (decorin; a marker for endotherm). RT-PCR results indicated that hESCs lost OCT4 on the cell surface after a 7-day differentiation culture (Fig. 2A). In contrast, we were able to detect CD34 expression on approximately 80% of the resulting cells, a manifestation of differentiation of hESCs into hematopoietic cells. Development of hematopoietic lineage cells was confirmed by expression of AFP and DCN. Similar to OCT4, we noticed diminished GATA-4 expression in hESC as a result of co-culture with OP9 cells. CD34 expression was detected in the generated cells as verified at a protein level by immunohistochemical analysis (Fig. 2B). Ninety five percent of the total cell population contained round-shaped nucleus stained with deep bluish-purple and little cytoplasm stained with pale gray-blue (Fig. 2C). These results indicate that hESCs were differentiated into hematopoietic CD34+ cells under the culture conditions adopted.

Differential miRNAs expression between hES and CB CD34+ cells

Next, we determined whether differentiation of hESCs into CD34+ cells was reflected by changes in specific miRNA expression. We assessed expression of four miRNAs (miR-142-3p, miR-142-5p, miR-146a and miR-451), and compared expression of these miRNAs by microarray analysis in hESCs, human embryoid bodies (hEB) generated from hESCs by a 15-day differentiation culture, hMSC, CB derived CD34+ cells and WI-38 cells, a human fibroblast-like lung cell line used as a negative control cells (Fig. 3A). Expression levels of all the miRNAs were highest in CD34+ CB cells among the cell types tested (Fig. 3A). Quantitative RT-PCR indicated higher levels of all the miRNAs in CD34+ CB cells compared to hESCs and WI-38 cells (Fig. 3B).

Differentiation of hESC derived CD34+ cells into erythrocyte progenitors was manifested by decrease of CD34 and increase of GPA-1 expression

Because induced CD34 expression in hESCs was apparent as a result of their co-culture with OP9 cells in the hematopoietic differentiation medium, we determined whether induced CD34 expression was lost as the CD34+ cells were further differentiated into erythroid cells. To this end, the hESC derived CD34+ cells were cultured in serum free medium supplemented with EPO and two other cytokines for first 7 days, and additional 7 days on human bone marrow derived MSC feeder cells with EPO only until 14 days of total culture, which lead to generation of more mature erythroid cells through two different developmental stages. The cells were then assessed for expression of CD34 and GPA-1, glycoprotein a marker for late stage erythroid and red blood cells. As shown in Fig. 4A, CD34 expression levels sharply declined at day 7 and were slightly evident at day 14. In contrast, expression levels of GPA-1 were increased

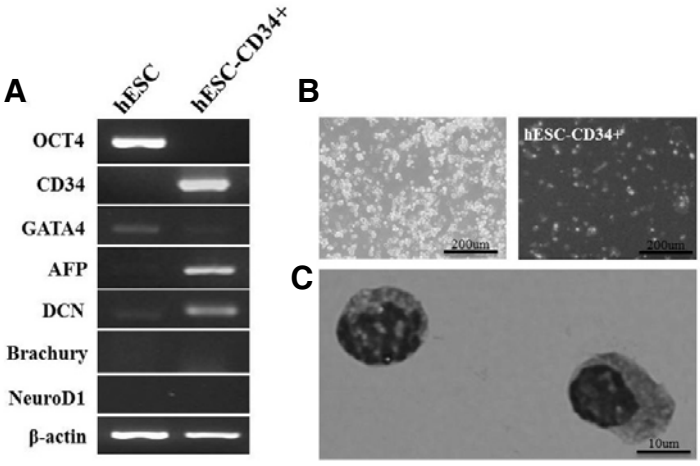

Fig. 2. Development of CD34+ cells expressing hematopoietic markers from hESCs. Initial hESCs and cells differentiated from hESC were compared for the mRNA expression levels of OCT4, CD34, GATA4, AFP, DCN, Brachury and NeuroD1 mRNA after a 10-day co-culture with OP9 cells in 100 μM MTG. The bands represent RT-PCR products from respective mRNA. Actin was used as an internal control protein (A). The cells developed from hESCs were stained with FITC conjugated anti-human CD34 antibody to detect CD34 at protein levels (B). The cells developed from hESCs were stained with Wright-Giemsa staining to evaluate morphological changes under light microscope at 1,000× magnification (C).

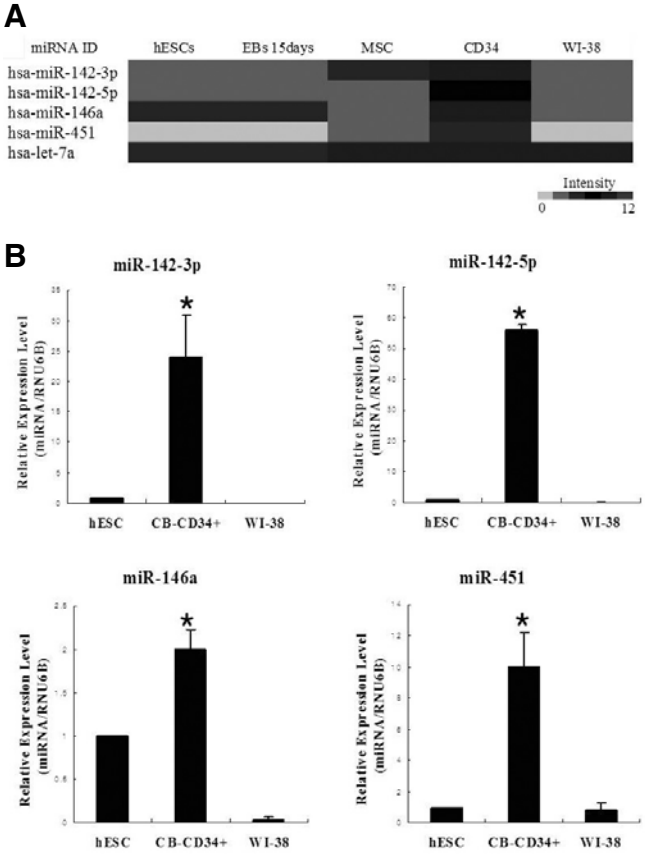

Fig. 3. Differential miRNAs expression in CB derived CD34+ cells. CD34+ cells, hESC, hEB, MSC and WI-38 cells were subject to Genopal miRNA microarray assays and predominant species of miRNAs were identified. Among various miRNAs miR-142-3p, miR-142-5p, miR-146a and miR-451 appeared to be significantly upregulated in CD34+ cells compared to hESCs, EBs, MSCs, and WI-38 cells (A). Quantitative real-time PCR for the four miRNAs was carried out with mRNA isolated from hESC, CD34+ cells and WI-38 cells. Relative miRNA levels of each cell type were depicted as quantity ratios of specific miRNA to RNU6B used as an endogenous control (B). *$P < 0.05$.

during the culture up to 14 days. Morphological changes were apparent: the nucleus was degraded during the differentiation culture (Fig. 4B), an indication of erythropoietic transition.

Flow cytometric analysis of the erythroid cells developed from hESC derived CD34+ cells for cell surface expression of CD71 and GPA-1, erythroid cell markers

We confirmed generation of erythroid cells from hESC derived CD34+ cells by using flow cytometry to determine induction of CD71, the transferrin receptor which is a marker for early but not mature stages of erythroid differentiation. CD71 expression was significantly increased at day 7 and remained at similar levels to day 14 during the erythroid differentiation culture. Consistent with the previous results (Fig. 4A), GPA-1 expression was increased continuously during the differentiation period (Fig. 5). This data demonstrated the potential of hESC derived CD34+ cells to differentiate into late erythroid stages.

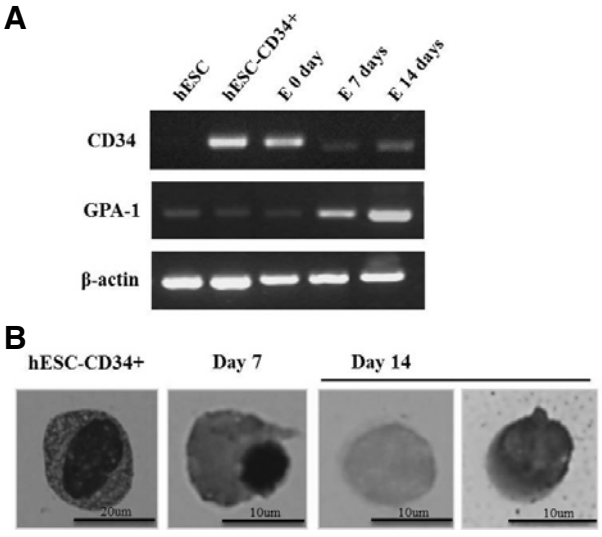

Fig. 4. Differentiation of hESC derived CD34+ cells into erythroid cells. hESC, hESC-derived CD34+ cells and CD34+ cells-derived erythrocyte progenitors at two stages were produced as described in Fig. 1 and CD34 and GPA-1 mRNA determined by RT-PCR. (A). The cells were harvested after 0, 7 and 14 days from erythroid inducing cultures and stained with Wright-Giemsa. Morphological changes indicative of erythroid cell development were observed under light microscope at 1,000× magnification (B).

Erythroid cell generation from hESC derived CD34+ cells was associated with induction of four specific miRNAs

We assessed expression patterns of four different miRNAs, miR-142-3p, miR-142-5p, miR-146a and miR-451 by quantitative PCR analysis in the hESC derived CD34+ cells at two different stages of their erythroid differentiation during culture for 7 and 14 days. Expression levels of the four miRNAs were low in the hESC derived CD34+ cells, but increased dramatically as the hESC derived CD34+ cells differentiated into erythroid cells. However, induction patterns of these miRNAs differed during the differentiation culture. While expression levels of miR-142-5p and miR-451 were continuously increased throughout the 14 day culture, miRNA-142-3p and miRNA-146a reached a peak at day 7 but decreased by day 14 (Fig. 6). These results suggest that miR-142-3p, miR-142-5p, miR-146a, miR-451 may be induced in hematopoietic stem and/or progenitor cells upon erythroid cell differentiation, and each miRNA may play different roles for this differentiation.

For the last decade, miRNAs have been target molecules that many investigators have paid attention to due to their critical regulatory roles in cellular developmental processes. Comparative microarray assays has been used to identify miRNAs expressed in a variety of tissues including hematopoietic cells. For example, microarray analysis by Zhan et al. (2007). revealed that the overall miRNA expression levels are increased upon erythroid differentiation. This suggests that miRNAs may play an important role in promoting erythroid differentiation.

In this study we found that four miRNAs, miR-142-3p, miR-142-5p, miR-146a and miR-451 were most predominantly expressed in CD34+ cells by a microarray assay and extended this microarray study focusing on investigating expression of these four most prominent miRNAs by qRT-PCR during *in vitro* erythroid lineage differentiation of CD34+ cells at two different stages. We used two different sources of CD34+ cells, those generated from hESC and the others isolated from cryopreserved CB. Consistent with the results from microarray, qRT-PCR analysis indicated significantly higher expression of all the miRNAs in CB CD34+ cells compared to that in hESCs (Fig. 3B) and even higher as the CD34+ cells differentiated into erythroid progenitor cells (Fig. 6). However, expression profiles of the miRNA in erythroid progenitor cells were no longer synchronized as cells further matured; while miR-142-5p and miR-451 levels were continuously increased from day 7 to day 14 of the erythroid differentiation processes, miR-142-3p and miR-146a levels were decreased (Fig. 6). Erythroid differentiation consists of multiple maturation steps which may be coupled with expression of the specific miRNAs. Consequently, the individual miRNA expressed at an appropriate window of time may regulate specific target molecules necessary for dictating erythroid differentiation.

We characterized erythroid differentiation by determining induction of CD71 and GPA-1 on the cell surface. The significance of our study is that we were able to link expression profiles of the specific miRNAs and the degree of erythroid maturation defined by induction of CD71 using quantitative RT-PCR and flow cytometry, respectively. No regulatory target molecules of these miRNAs have been identified. Therefore, more studies have yet to be done using gene knockdown techniques for miRNAs in order to understand the exact target molecules and the roles of each miRNAs in erythropoiesis.

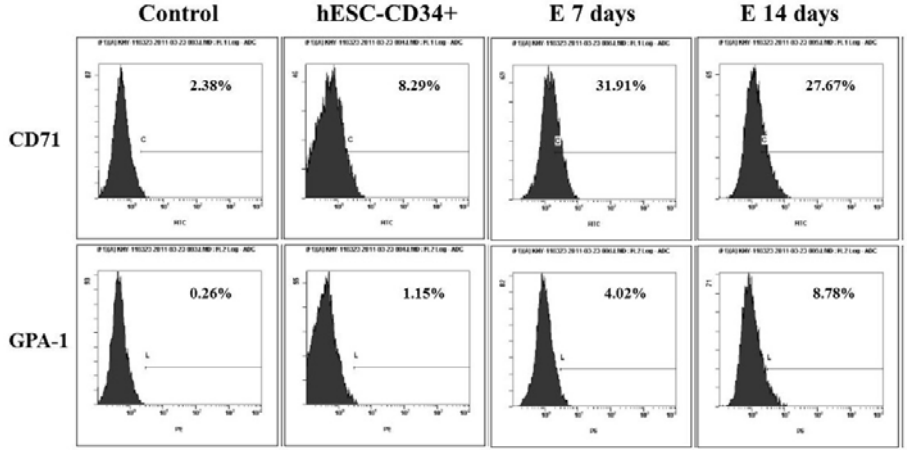

Fig. 5. Flow cytometric analysis of erythroid cells developed from hESC-derived CD34+ cells. Expression of CD71 and GPA-1, erythrocyte markers, on erythroid cells developed from hESC-derived CD34+ cells at two different phases (day 7 and day 14) was determined. Percent of the cells positive for CD71 and GPA-1 are denoted in the histograms. Control histograms represent the day 7 erythroid cells stained with isotype control antibody.

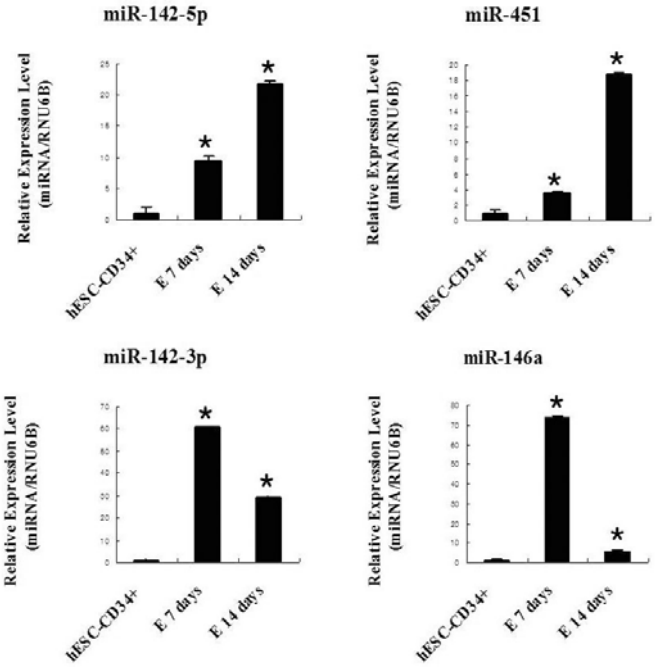

Fig. 6. Induction of four specific miRNAs expression in generated erythroid cells. hESC-derived CD34+ cells were induced to differentiate into erythroid cells in the presence of SCF, IL-3 and EPO (phase I), and EPO with MSC, feeder cells (phase II). Expression levels of four miRNAs, miR-142-3p, miR-142-5p, miR-146a and miR-451 by RT-PCR. RT-PCR products of the individual miRNAs isolated from hESC-derived CD34+ cells and erythrocyte progenitors at two different stages were compared by the ratios of specific miRNA to RNU6B, an endogenous control. *$p < 0.05$.

In recent years, human CB has served as a hematopoietic stem cell and progenitor cell source to repopulate the blood system (Broxmeyer and Smith, 2008) (Liao et al., 2011) for treatment of hematopoietic disorders such as leukemia (Lin et al., 2006; Miniero et al., 1995). However, the efficacy of stem cell transplantation with CB is somewhat limited due to the low number of hematopoietic stem cells contained in single CB units. Our results demonstrated that the CD34+ cells we generated from hESC *in vitro* were comparable to CD34+ cells isolated from cryopreserved CB in their ability to induce the four miRNAs we examined during erythroid cell differentiation *in vitro*. Thus, our study opens up a possibility that hESC may represent a potential future source of CD34+ cells and the miRNAs might be able to be utilized as needed as a clinical tool to promote erythropoiesis.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

ACKNOWLEDGMENTS

We are grateful to Mitsubishi Rayon Co., Ltd. for providing the Genopal microarray system and for excellent technical assistance. This work was supported part by Stem Cell Research Center (SC-2220) and Medical Research Center (2011-0028263) program funded by Ministry of Education, Science and Technology, Republic of Korea (to K.S.K.).

REFERENCES

Bandres, E., Bitarte, N., Arias, F., Agorreta, J., Fortes, P., Agirre, X., Zarate, R., Diaz-Gonzalez, J.A., Ramirez, N., Sola, J.J., et al. (2009). microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. Clin. Cancer Res. *15*, 2281-2290.

Broxmeyer, H.E., and Smith, F.O. (2008). Cord blood hematopoietic cell transplantation in Thomas' hematopoietic cell transplantation stem cell transplantation, 4th ed., F.R. Appelbaum and E.D. Thomas, eds. (Chichester, UK; Hoboken, NJ: Wiley-Blackwell), pp. 559-576.

Chen, C.Z., and Lodish, H.F. (2005). MicroRNAs as regulators of mammalian hematopoiesis. Semin. Immunol. *17*, 155-165.

Choong, M.L., Yang, H.H., and McNiece, I. (2007). MicroRNA expression profiling during human cord blood-derived CD34 cell erythropoiesis. Exp. Hematol. *35*, 551-564.

Dore, L.C., Amigo, J.D., Dos Santos, C.O., Zhang, Z., Gai, X., Tobias, J.W., Yu, D., Klein, A.M., Dorman, C., Wu, W., et al. (2008). A GATA-1-regulated microRNA locus essential for erythropoiesis. Proc. Natl. Acad. Sci. USA *105*, 3333-3338.

Edmonds, M.D., Hurst, D.R., Vaidya, K.S., Stafford, L.J., Chen, D., and Welch, D.R. (2009). Breast cancer metastasis suppressor 1 coordinately regulates metastasis-associated microRNA expression. Int. J. Cancer *125*, 1778-1785.

Gammell, P. (2007). MicroRNAs: recently discovered key regulators of proliferation and apoptosis in animal cells: Identification of miRNAs regulating growth and survival. Cytotechnology *53*, 55-63.

Kuang, W., Tan, J., Duan, Y., Duan, J., Wang, W., Jin, F., Jin, Z., Yuan, X., and Liu, Y. (2009). Cyclic stretch induced miR-146a upregulation delays C2C12 myogenic differentiation through inhibition of Numb. Biochem. Biophys. Res. Commun. *378*, 259-263.

Lee, M.R., Kim, J.S., and Kim, K.S. (2010). miR-124a is important for migratory cell fate transition during gastrulation of human embryonic stem cells. Stem Cells *28*, 1550-1559.

Lewis, B.P., Burge, C.B., and Bartel, D.P. (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell *120*, 15-20.

Li, Y., Vandenboom, T.G., 2nd, Wang, Z., Kong, D., Ali, S., Philip, P.A., and Sarkar, F.H. (2010). miR-146a suppresses invasion of pancreatic cancer cells. Cancer Res. *70*, 1486-1495.

Liao, Y., Geyer, M.B., Yang, A.J., and Cairo, M.S. (2011). Cord blood transplantation and stem cell regenerative potential. Exp. Hematol. *39*, 393-412.

Lin, Y.D., Shen, Y.K., and Li, Y.K. (2006). Unrelated umbilical cord blood stem cell transplantation in a patient with chronic myelocytic leukemia. Zhonghua Er Ke Za Zhi *44*, 586.

Miniero, R., Busca, A., Roncarolo, M.G., Saitta, M., Iavarone, A., Timeus, F., Biondi, A., Amoroso, A., Perugini, L., Ciuti, E., et al. (1995). HLA-haploidentical umbilical cord blood stem cell transplantation in a child with advanced leukemia: clinical outcome and analysis of hematopoietic recovery. Bone Marrow Transplant *16*, 229-240.

Ramkissoon, S.H., Mainwaring, L.A., Ogasawara, Y., Keyvanfar, K.,

McCoy, J.P., Jr., Sloand, E.M., Kajigaya, S., and Young, N.S. (2006). Hematopoietic-specific microRNA expression in human cells. Leuk. Res. *30*, 643-647.

Rathjen, T., Nicol, C., McConkey, G., and Dalmay, T. (2006). Analysis of short RNAs in the malaria parasite and its red blood cell host. FEBS Lett. *580*, 5185-5188.

Tsitsiou, E., and Lindsay, M.A. (2009). microRNAs and the immune response. Curr. Opin. Pharmacol. *9*, 514-520.

Tsuchiya, S., Oku, M., Imanaka, Y., Kunimoto, R., Okuno, Y., Terasawa, K., Sato, F., Tsujimoto, G., and Shimizu, K. (2009). MicroRNA-338-3p and microRNA-451 contribute to the formation of basolateral polarity in epithelial cells. Nucleic Acids Res. *37*, 3821-3827.

Visone, R., Rassenti, L.Z., Veronese, A., Taccioli, C., Costinean, S., Aguda, B.D., Volinia, S., Ferracin, M., Palatini, J., Balatti, V., et al. (2009). Karyotype-specific microRNA signature in chronic lymphocytic leukemia. Blood *114*, 3872-3879.

Vodyanik, M.A., and Slukvin, II. (2007). Hematoendothelial differentiation of human embryonic stem cells. Curr. Protoc. Cell Biol. *Chapter 23*, Unit 23.6.

Vodyanik, M.A., Bork, J.A., Thomson, J.A., and Slukvin, II. (2005). Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. Blood *105*, 617-626.

Williams, A.E., Perry, M.M., Moschos, S.A., Larner-Svensson, H.M., and Lindsay, M.A. (2008). Role of miRNA-146a in the regulation of the innate immune response and cancer. Biochem. Soc. Trans. *36*, 1211-1215.

Wu, H., Neilson, J.R., Kumar, P., Manocha, M., Shankar, P., Sharp, P.A., and Manjunath, N. (2007). miRNA profiling of naive, effector and memory CD8 T cells. PLoS One *2*, e1020.

Yang, G.H., Wang, F., Yu, J., Wang, X.S., Yuan, J.Y., and Zhang, J.W. (2009). MicroRNAs are involved in erythroid differentiation control. J. Cell Biochem. *107*, 548-556.

Zhan, M., Miller, C.P., Papayannopoulou, T., Stamatoyannopoulos, G., and Song, C.Z. (2007). MicroRNA expression dynamics during murine and human erythroid differentiation. Exp. Hematol. *35*, 1015-1025.