제대혈 CD34 양성세포분획과 연관된 MicroRNA

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Identification of MicroRNA Related to the CD34+ Cell Fraction of Cord Blood Stem Cells

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Background: Cord blood (CB) is a reliable source of hematopoietic stem cells, and its utilization in stem cell transplantation is increasing continuously. The CD34+ cell count is arguably one of the most important parameters for evaluating the quality of a cord blood unit (CBU), but there is little evidence on the post-genetic modifications that can affect the CD34+ cell counts. In this study, the difference in the miRNA expression profiles between low and high CD34+ CBU was evaluated.

Methods: Paired CB and maternal samples with low (<0.06%) and high CD34+ cell counts (>0.9%) were selected for analysis. MicroRNA profiling was performed, and differentially expressed miRNA were identified. In addition, gene ontology analysis was conducted on the miRNA to elucidate the genes that could potentially affect the CD34+ cell count.

Results: Ten miRNA were identified to show significantly different expression between the low and high CD34+ groups. Four of the 10 miRNA were hematopoiesis-related (miR-199a-5p, miR-22-5p, miR-140-5p, and miR-181b-5p). From a total of 119 associated genes, nine (*CALCA*, *FARP2*, *FSHR*, *ITGAM*, *MELK*, *MLF1*, *PRG4*, *TREM2* and *VCAM1*) were associated with two or more of the aforementioned miRNA.

Conclusion: This is the first study that examined the difference in the miRNA expression profiles between high and low CD34+ CB cells and revealed the relevant genes associated with hematopoiesis. These results provide basic insight into the genetic processes involving hematopoietic stem cell proliferation. (**Korean J Blood Transfus** 2019;30:113-123)

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Introduction

Cord blood (CB) is a proven alternative source of viable hematopoietic stem cells to bone marrow and induced peripheral blood. The first successful CB transplantation was reported in 1989, whereby hematopoietic reconstitution in a Fanconi's anemia patient was achieved via the CB of a HLA-identical sibling [1]. In the US, CB is utilized in approximately 20% of hematopoietic stem cell transplantations [2]. In Japan, a total of 16,862 CB transplantations have been performed since the first transplantation in 1997 [3], whereas in Korea, 757 cases of CB transplantation have been reported from 2003 to 2017 [4].

After CB is obtained successfully after birth, it undergoes a rigorous process whereby the RBCs and plasma are removed by centrifugation, followed by the addition of cryoprotective agents. The processed CB is finally freeze-stored in liquid nitrogen tanks as a cord blood unit (CBU). If an appropriate HLA-matching recipient is found, the CBU is then thawed and transplanted into the recipient. The advantages of CB in hematopoietic stem cell transplantation are as follows. First, all pre-transplantation testing is conducted before storage, thereby reducing donor preparation times and the often underestimated struggles of donor refusal. Second, CB is more tolerant of higher HLA disparity. Of the three HLA loci (-A, -B, DR), it is recommended that there be at least 4/6 HLA matches (antigen level for HLA-A, -B, and allelic level for HLA-DRB1) [5]. Third, CB is associated with both a lower incidence and severity of Graft-versus-host disease (GVHD) [2].

The foremost parameters that influence the quality of a CBU are the total nucleated cell (TNC) count

and the CD34+ cell count. Logically, the TNC dose per unit should be adjusted according to the level of HLA matching. Although there are minor discrepancies depending on the transplant center and study groups, the general consensus is that $\geq 2.5\text{--}3.0 \times 10^7$ TNC/kg is the minimum cell dose for 5/6 or 6/6 HLA-matched units, and $\geq 5.0 \times 10^7$ TNC/kg is the minimum for 4/6 HLA-matched units [6]. Regarding the CD34+ cell counts, multiple studies have proven that CD34+ cell counts are positively correlated with enhanced neutrophil and platelet engraftment [7-10]. On the other hand, a range of maternal and fetal/neonatal parameters have been investigated owing to the large variability of the CD34+ cell counts among CB. A heavier fetal weight is associated with improved CB viability, TNC, CD34+ cell counts, and granulocyte/monocyte colony forming units (CFU-GM), whereas a longer gestational age showed higher TNC but lower CD34+ cell counts and CFU-GM [11]. First-born, large, term, female babies from younger mothers have been reported to have higher TNC and CD34+ cell counts [12]. Even blood group O, placental weight and method of delivery have been shown to affect the CB quality [13]. A consideration of the fetal parameters that may influence the CD34+ counts of CBU naturally lead to some interest in the genetic factors of the fetus.

MicroRNA (miRNA) is non-coding RNA consisting of 19~25 nucleotides, and plays an important role in post-transcriptional regulation by influencing cell renewal, differentiation, and proliferation. MiRNA was first identified in the nematode *Caenorhabditis elegans* in 2001 [14], and a microRNA registry (miRBase) was established in 2002 [15]. In recent years, more of the specific roles of various miRNAs

have been identified. Human miRNAs, miR-22, miR-29, miR-125, and miR-126, are expressed in hematopoietic stem cells and primitive hematopoietic progenitor cells, and have functions in the early phases of hematopoiesis [16]. The microRNA-223 dose levels were shown to regulate proliferation and differentiation in human CB progenitor cells and acute myeloid leukemia (AML), and higher levels were associated with a better AML prognosis [17].

In this study, the miRNA expression profiles of two cohorts (high vs. low CD34+ cell counts) consisting of paired CBU-maternal blood were examined. The resulting miRNAs with significantly different levels between the two groups were then analyzed for their roles in hematopoiesis. This study examined the difference in miRNA expression, as well as the genes associated with the CD34+ fraction where applicable, which can ultimately affect the clinical selection of CBU.

Materials and Methods

1. Study population

Reference CB and maternal samples cryopreserved at the Seoul Metropolitan Government Public Cord Blood Bank from 2012~2015 were used. Samples with low CD34 counts ($<0.06\%$) and high CD34 counts ($>0.9\%$) were selected for analysis. The set 0.06% and 0.9% CD34+ counts were calculated from the 1st and 99th percentiles of all the stored CBU (9328 units) at the cord blood bank from 2012~2015. A Wilcoxon rank-sum test was used to obtain the descriptive statistics comparing the two groups. The study was exempted from reviews by

the Institutional review board at SMG-SNU Boramae Medical Center (07-2017-3).

2. Cell viability

The cell viability was measured using the 0.4% trypan blue staining method upon receipt of the sample (primary) and before cryopreservation (secondary).

3. Complete blood cell count and CD34 count instruments

A XE-2100 (Sysmex, Kobe, Japan) was used for CBC analysis, and a FC-500 (Beckman-Coulter, USA) was used for CD34 analysis.

4. RNA quality check

The RNA purity and integrity were evaluated using an ND-1000 Spectrophotometer (NanoDrop, Wilmington, USA), Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA).

5. Affymetrix miRNA arrays methods

The Affymetrix Genechip miRNA 4.0 array (ThermoFisher Scientific, Waltham, MA, USA) process was performed according to the manufacturer's protocol. Briefly, it included a total of 30,434 mature miRNA probe sets, 2,578 human mature miRNA probe sets, 1,996 human snoRNA and scaRNA probe sets, and 2,025 human pre-miRNA probe sets. The RNA samples (1000 ng) extracted from the TNC of each CBU and maternal samples were labeled using a FlashTagTM Biotin RNA Labeling Kit (Genisphere, Hatfield, PA, USA). The labeled RNA was quantified, fractionated, and hybridized to the miRNA microarray according to the standard procedures provided by the manufacture. The labeled RNA was heated to 99°C for

5 minutes and then to 45°C for 5 minutes. RNA-array hybridization was performed with agitation at 60 rotations per minute for 16 hours at 48°C on an Affymetrix® 450 Fluidics Station. The chips were washed and stained using a Genechip Fluidics Station 450 (Affymetrix, Santa Clara, California, United States). The chips were then scanned with an Affymetrix GCS 3000 scanner (Affymetrix, Santa Clara, California, United States). The signal values were computed using the Affymetrix® GeneChip™ Command Console software.

6. Microarray data analysis

The array data were filtered by annotated Affymetrix® Expression Console™ Software. Comparative analyses between the test samples and control samples were carried out using the fold-change, independent T-test, and paired T-test, in which the null hypothesis was that no difference exists between the two groups. The false discovery rate (FDR) was controlled by adjusting the *P* value using the Benjamini-Hochberg algorithm. All Statistical tests and visualization of differentially expressed genes were conducted using R statistical language v. 3.1.2.

7. Gene enrichment and gene ontology analysis

The target genes of differentially expressed miRNAs were searched on three databases, such as microcosm (<http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/info.html>, version 5), MTI (<http://mirtarbase.mbc.nctu.edu.tw/index.php>, Version and download date: Release 4.5 - 11/01/2013), and miRecords (validated only): <http://mirecords.umn.edu/miRecords/doc.php>, download date April 27, 2013).

For gene ontology analysis, the terms, including hematopoiesis, regulation of hematopoiesis, positive regulation of hematopoiesis, and negative regulation of hematopoiesis, were searched.

Results

1. Study samples

A total of five low and four high CD34+ CB products were eligible for analysis. Table 1 lists the basic parameters and cord blood CBC results. Table 2 lists the specific CBU parameters. Table 3 presents the descriptive statistics comparing the two groups. No significant differences were observed in sex, weight, CBU volume, primary viability, white blood cell counts, hemoglobin levels, platelet counts, neutrophil counts, lymphocyte counts, secondary viability, weight of leukocyte-rich plasma transferred, weight of RBC transferred, total TNC count per unit, TNC recovery rates, total MNC count per unit, and MNC recovery rates. The high CD34+ CB group showed a lower gestational age, higher monocyte, eosinophil, and basophil counts, number of nucleated RBC per 100 WBCs, total CD34 counts per unit, and CD34 percentages.

2. Difference in miRNA expression profiles

A total of 10 miRNA (miR-199a-5p, miR-4284, miR-1260a, miR-7977, miR-1260b, miR-140-5p, miR-22-5p, miR-181b-5p, miR-3605-5p, miR-3205-3p) were found to have significant differences (fold change ≥ 1.5 , *P* value < 0.05) between the high and low C34+ CB groups (Table 4).

Table 1. Basic parameters and cord blood complete blood cell counts

Patient	Sex (M/F)	Weight (kg)	Age at birth (weeks+days)	Volume of CBU (mL)	Primary Viability (%)	WBC ($\times 10^3$ / μ L)	Hb (g/dL)	PLT ($\times 10^3$ / μ L)	Neutro (%)	Lympho (%)	Mono (%)	Eos (%)	Baso (%)	nRBC (/100WBC)
L1	M	3.45	41+2	96.67	98	9	11.7	195	50.5	38.9	8.1	2.4	0.1	0.3
L2	M	3.52	40+5	95.14	99	11.8	10.5	191	59.1	27.7	9.3	3.7	0.2	0
L3	F	3.18	41+2	103.24	97	9.5	12.4	217	49.6	41.8	7.3	0.9	0.4	0
L4	F	2.69	39+2	110.48	99	11.2	10.2	259	52.9	36.1	8.4	2.3	0.3	0.5
L5	F	3.58	41+1	115.71	99	9.2	11.3	222	55.4	34.5	8.6	1.3	0.2	0
H1	M	4.14	38+4	98.95	99	10	10.3	235	55.6	29.9	9.8	3.6	1.1	2.1
H2	M	3.20	39+0	102.57	99	12.9	11.5	157	56.8	26.5	9.9	6.0	0.8	5.0
H3	M	2.80	38+1	104.48	99	9.2	10.6	247	48.6	32.4	11.7	6.9	0.4	2.9
H4	F	3.68	37+4	120.1	99	8.5	11.1	149	46	39.1	9.3	4.5	1.1	9.8

Abbreviations: WBC, white blood cell count; Hb, hemoglobin; PLT, platelet; Neutro, neutrophil; Lympho, lymphocyte; Mono, monocyte; Eos, eosinophil; Baso, basophil; nRBC, nucleated red blood cell.

Table 2. Cord blood unit parameters of the study population

Patient	Secondary Viability (%)	Weight of LRP transferred (g)	Weight of RBC transferred (g)	Total TNC ($\times 10^8$ /unit)	TNC Recovery (%)	Total MNC ($\times 10^8$ /unit)	MNC Recovery (%)	Total CD34 count ($\times 10^6$ /unit)	CD34 (%)
L1	89	79	18.9	7.82	89.89	3.6	87.97	0.41	0.05
L2	87	80.1	18.9	10	89.07	3.4	81.85	0.53	0.05
L3	93	80.5	18.9	8.28	84.42	4	83.05	0.45	0.05
L4	93	87	18.9	8.64	87.87	3	80.45	0.42	0.05
L5	90	93.4	19.9	8.74	82.1	3.76	81.91	0.53	0.06
H1	91	82.2	18.9	8.2	82.87	3.24	82.45	7.71	0.94
H2	91	82	18.9	11.72	88.58	4.08	84.68	12.5	1.07
H3	91	85	18.9	7.82	81.36	3.34	78.78	9.09	1.16
H4	90	93	19.9	9	88.17	4.38	88.71	9.42	1.05

Abbreviations: LRP, leukocyte-rich plasma; TNC, total nucleated cell; MNC, mononuclear cell; CBU, cord blood unit.

3. Hematopoiesis-associated miRNA and relevant genes

Four (miR-199a-5p, miR-22-5p, miR-140-5p, miR-181b-5p) of the 10 miRNA had the gene ontology terms related to hematopoiesis (Table 5). A total of 119 genes were related to the above four miRNA,

and nine genes (*CALCA*, *FARP2*, *FSHR*, *ITGAM*, *MELK*, *MLF1*, *PRG4*, *TREM2* and *VCAM1*) were associated with two or more miRNA.

4. Difference in miRNA expression profiles (matched maternal samples)

Two miRNA showed significantly different ex-

Table 3. Descriptive statistics of the study population

	Low CD34 group Median (IQR)	High CD34 group Median (IQR)	<i>P</i> value
Sex	M:F=2:3	M:F=3:1	0.41
Weight (kg)	3.5 (0.6)	3.4 (1.1)	0.56
Volume of CBU (mL)	100.7 (13.6)	103.5 (16.3)	0.41
Primary Viability (%)	99.0 (2.0)	99.0 (0.0)	0.41
WBC ($\times 10^3/\mu\text{L}$)	9.5 (2.4)	9.6 (3.5)	0.91
Hb (g/dL)	11.3 (1.7)	10.9 (1.03)	0.73
PLT ($\times 10^3/\mu\text{L}$)	217.0 (48.0)	196.0 (93.0)	0.73
Neutro (%)	52.9 (7.2)	52.1 (9.9)	0.73
Lym (%)	36.1 (9.2)	31.2 (10.1)	0.41
Mono (%)	8.4 (1.3)	9.9 (1.8)	0.02
Eos (%)	2.3 (2.0)	5.3 (2.9)	0.03
Baso (%)	0.2 (0.2)	1.0 (0.6)	0.02
nRBC (/100WBC)	0.0 (0.4)	4.0 (6.3)	0.02
Total TNC (/unit)	8.7 (1.7)	8.6 (3.1)	0.91
CD34 (%)	0.05 (0.01)	1.06 (0.17)	0.02

Abbreviations: IQR, interquartile range; WBC, White blood cell count; Hb, hemoglobin; PLT, platelet; Neutro, neutrophil; Lympho, lymphocyte; Mono, monocyte; Eos, eosinophil; Baso, basophil; nRBC, nucleated red blood cell; LRP, leukocyte-rich plasma; TNC, total nucleated cell; MNC, mononuclear cell; CBU, cord blood unit.

Table 4. miRNA with significantly different expression levels between the high/low CD34+ cord blood products (in descending order by the fold change)

miRNA	Mean fold change (high compared to low)	<i>P</i> value
miR-199a-5p	3.64	0.04
miR-4284	3.44	<0.01
miR-1260a	3.06	0.03
miR-7977	2.64	0.03
miR-1260b	1.96	<0.01
miR-140-5p	1.75	0.04
miR-22-5p	1.68	0.04
miR-181b-5p	1.53	0.018
miR-3605-5p	-1.56	0.02
miR-3200-3p	-2.13	0.04

pression between the two maternal groups: miR-22-53p and miR-140-5p (1.65 and 1.54 fold, respectively, data not shown). From the analysis, these two miRNA were not considered to be associated with hematopoiesis.

Discussion

Owing to the success of CB transplantation in treating hematologic patients, the demand is continuously on the rise. Over the years, attempts have been made to better predict the selection of high-quality CBU, and multiple studies have been published on various parameters associated with the CD34+ counts in CBU. On the other hand, the reported findings have largely been limited to ob-

Table 5. Hematopoiesis-associated miRNA and reported relevant genes

miRNA	Fold change (high vs. low CD34)	Associated genes (alphabetical order)
miR-199a-5p	3.64	<i>ACE, ACP6, ARIH2, BATF, BAX, CDKN1C, EBP, EPAS1, FARP2, HERC6, HES1, HIF1A, IL4R, JAK3, KRT75, LCK, LIF, LILRB3, LMO4, LRRC17, LTB, MLF1, MUC4, NDFIP1, NTRK1, PGM3, PIK3CD, PRDM16, PRG4, RELB, ROGDI, SCIN, SLC46A2, SLC8A3, SOS2, SPTB, TCF3, TREM2, VCAM1, WDR78</i>
miR-140-5p	1.75	<i>BCL2L11, BCL3, CALCA, CASP3, CASP9, DLL4, EFNA4, FES, FSHB, GATA1, GLRX5, GPC3, HDAC4, IKZF1, IL15, IL25, IL2RA, IRF4, ITGAM, MELK, NCOR1, PITX2, RDM1, RSAD2, SHB, SIX1, THRA, TRAF6, TREM2, VEGFA, ZNF683</i>
miR-22-5p	1.68	<i>AZI2, CALCA, CARTPT, CASP8, CSF2, DHRS2, FARP2, FCER1G, FSHR, FST, HHEX, HIPK1, HLA-G, IKZF3, IL6, ITGAM, NCKAP1L, PRG4, PRKCZ, SEMA4A, TSHR, TYR, VCAM1, ZBTB46</i>
miR-181b-5p	1.53	<i>ASXL2, CARD11, CSF3R, CYLD, DOCK7, DYRK3, ERBB2, FAS, FGFR2, FOXP1, FSHR, HLA-B, HMGB2, HOXB4, IFNA17, IFNA4, IFNG, IFNW1, IL2, INPP4, ITGA4, MELK, MERTK, MLF1, MYH9, NOTCH4, PLCL2, POLM, PRDX3, RASGRP4, RASSF2, SOS1, VCAM1</i>

Genes showing an association with more than two of the four hematopoiesis-associated miRNA are depicted in bold.

servational phenotypic findings. To the best of the authors' knowledge, this is the first study that examined the difference in miRNA expression profiles between high and low CD34+ CB cells.

Ten miRNA were found to have significantly different levels between the two groups (Table 4), from which four miRNA were subsequently discovered to have one or more than one hematopoiesis-associated gene: miR-199a-5p, miR-140-5p, miR-22-5p, and miR-181b-5p in Table 5. Owing to the large number of genes, this study focused mainly on those found to interact with more than two of the four hematopoiesis-associated miRNA. With the exception of miR-22 (in its miR-22-5p subtype form), miRNA that was previously reported to be associated with hematopoiesis, such as miR-29 and miR-223, showed no significant difference in expression between the high CD34+ CB and low CD34+ CB.

Owing to the complexity of hematopoiesis, no one specific gene is the determining factor for the CD34 levels other than directly encoding genes, such as the *CD34* gene itself. Instead, the genes discussed in this study interact with hematopoiesis in various, often indirect, ways. The *CALCA* gene, which is associated with miR-22-5p and miR-140-5p, partially encodes for calcitonin, which inhibits the osteoclast activity. Reduced osteoclastic activity has been reported to be associated with an increased hematopoietic stem cell (HSC) pool via an increase in bone mass [18]. The *CALCA* peptide, which is also known as CGRP, is involved in a signal pathway that is crucial for stress-induced hematopoiesis [19].

FSHR, which is associated with miR-22-5p and miR-199a-5p, encodes for the well-known follicle stimulating hormone receptor (FSHR) protein. Human hematopoietic stem-progenitor cells express the func-

tional receptors for sex hormones, including FSHR [20]. Moreover, in a murine study, the activation of pluripotent very small embryonic-like stem cells was more prominent after the FSH treatment [21]. *ITGAM*, which is associated with miR-22-5p and miR-140-5p, encodes for the integrin subunit alpha M peptide. As their name suggests, integrins are integral membrane proteins that are composed of an alpha and beta chain. The alpha M component plays roles in adhering the neutrophils/monocytes to stimulated endothelium and complement-aided phagocytosis. In addition to a glycosphosphatidylinositol-anchored surface protein called GPI-80, the ITGAM subunit co-localizes on the cell surface of the HSCs to promote self-renewal [22].

MELK, which is associated with miR-181b-5p and miR-140-5p, encodes the maternal embryonic leucine zipper kinase. Among its various kinase roles, MELK also plays a functional role in the transduction of cellular signals in certain hematopoietic cell lineages [23]. *PRG4* is associated with miR-22-5p and miR-199a-5p, and encodes the immunomodulatory factor, proteoglycan 4. In 2011, proteoglycan 4 was reported to be a novel parathyroid hormone (PTH)-responsive factor that regulates the immune cells and the action of PTH on hematopoietic progenitor cells in the bone marrow. *VCAM1*, associated with miR-22-5p, miR-199a-5p, and miR-181b-5p, encodes for vascular cell adhesion molecule 1. Macrophage expressing VCAM1 have been reported to be crucial for extramedullary myelopoiesis via the retention of HSCs in the spleen [24], whereas it has been noted as a regulator of the hematopoietic proliferation and differentiation of certain splenic stromal cell lines [25].

In the study design, matched maternal samples were included to examine whether any differential expression of miRNA observed in CBU would also be shown in the corresponding maternal samples. Interestingly, only two miRNAs were shown to be different between the two maternal groups ($P < 0.05$): miR-22-53p and miR-140-5p (1.65 and 1.54 fold, respectively, data not shown), and these are not included in the miRNA showing different expression levels in CBU. The size of the miRNA and the size of the gaps in the fetoplacental-maternal barrier, where even relatively large nutrients can be exchanged, raises the question as to why there is a difference in miRNA expression levels between the CBU and their respective maternal samples. One possible answer may be that the miRNA clearance rates between adults and children differ, but further information is needed to properly explain this difference in a mother-fetus connection.

Despite the strengths of this study, including the well-controlled parameters between the two groups and also being the first study to examine the miRNA expression profiles of CB cells of low and high CD34+ groups, there were some limitations. First, the study sample size was extremely small, and a normal distribution cannot be assumed for the majority of statistical parameters. The small sample size also limits the ability to properly analyze the difference in miRNA expression between the basic parameters, such as sex, weight, birth age, etc. This limitation can be overcome relatively easily with larger sample sizes via longer study periods and multicenter participation. Secondly, although the study identified the different miRNA expressions and the relevant genes associated with hematopoi-

esis, it did not measure the gene expression levels directly. Therefore, it is uncertain whether there is a definite, causal association between the miRNA found in this study and the CD34+ levels of CBU. Further comprehensive studies should improve from this initial study by directly measuring the gene expression levels of those associated with the identified miRNA.

Although new roles of previously unknown miRNAs continue to be discovered, the function of miRNA in hematopoiesis requires continuous study. This paper presented the results of a novel study that identified the miRNA that showed significant differences between low and high CD34+ CB groups, and revealed the relevant hematopoiesis-related genes associated with the identified miRNA. These results provide basic information on the post-genetic modification involved with hematopoietic stem cell proliferation, and with further validation, can provide reliable genetic parameters that can improve the clinical selection of CBU.

Conclusion

This is the first study that examined the difference in the miRNA expression profiles between high and low CD34+ CB cells and revealed the relevant genes associated with hematopoiesis. These results provide basic insight into the genetic processes involving hematopoietic stem cell proliferation.

요약

배경: 조혈모세포이식에서 제대혈의 역할과 수요는 지속적으로 증가하고 있다. 제대혈제제의

질을 평가할 때 가장 중요하게 여겨지는 인자 중 하나는 바로 CD34+ 수치이나, 현재까지 제대혈에서의 CD34+ 수치를 예측할 수 있는 후성유전학적 지표는 없다. 본 연구에서는 CD34+ 수치가 낮은 군과 높은 군의 마이크로 RNA (miRNA) 발현 양상을 비교하였다.

방법: CD34가 낮은(<0.06%) 군과 높은(>0.9%) 군의 제대혈 검체, 그리고 각 제대혈의 모 검체를 포함하였다. 선정된 검체에 대해 miRNA 발현을 검사하였고, 두 군간 발현이 다른 miRNA를 식별하였다. 추가적으로, 검색된 miRNA에 대해 유전자 온톨로지 분석을 진행하여 CD34+ 분획에 영향을 줄 가능성이 있는 유전자를 식별하였다.

결과: 총 10개의 miRNA가 CD34+ 낮은 군과 높은 군에서 발현 정도에 차이를 보였다. 이중 4개는 조혈 기능과 관련이 있는 것으로 밝혀졌다 (miR-199a-5p, miR-22-5p, miR-140-5p, miR-181b-5p). 더불어, 조혈기능에 영향을 주는 miRNA와 관련된 총 119개의 유전자 중에서, 9개의 유전자 (*CALCA*, *FARP2*, *FSHR*, *ITGAM*, *MELK*, *MLF1*, *PRG4*, *TREM2* 그리고 *VCAM1*)가 2개 이상의 miRNA와 밀접하게 연관되어 있었다.

결론: 본 연구는 제대혈에서 CD34+ 분획에 따른 miRNA 발현 정도를 평가한 첫 보고이며, miRNA와 조혈 기능과 관련된 유전자를 식별하였다. 본 연구의 결과는 조혈모세포 증식과 관련된 유전적 과정에 대한 기초적인 정보를 제공할 수 있을 것으로 기대된다.

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