Impaired Function and Epigenetic Changes of Human Cord Blood-Derived CD133+/C-kit^{+}Lin^{-} Endothelial Progenitor Cells in Preeclampsia

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Impaired Function and Epigenetic Changes of Human Cord Blood-derived CD133+/C-kit^{+}Lin^{-} Endothelial Progenitor Cells in Preeclampsia

Directed by Professor Ja-Young Kwon

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This certifies that the Master's Thesis of
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ABSTRACT

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Objective: Recent evidence suggests that offspring delivered from preeclamptic women remains increased risk for long-term, adulthood issues such as cardiovascular and metabolic diseases which may be contributed to aberrant in utero environment causing fetal cell reprogramming. We aim to investigate functional impairment and epigenetic change of progenitor cells in offsprings born to preeclamptic mother.

Materials and methods: Human umbilical cord blood endothelial progenitor cells (EPCs) purified with anti-CD133/C-kit/Lin2 (CKL^{-}) microbeads using a magnetic cell sorter device in severe preeclampsia (n=10) and gestationally matched normal pregnant women (n=10) were retrospectively analyzed. Differentiation of EPCs to outgrowth endothelial cells (OECs) was assessed by morphology, differentiation day and the number of colonies using light microscopy in both groups. Angiogenic function of OECs differentiated from CKL^{-}EPCs of both groups was evaluated by migration, adhesion and tube formation assay. To identify the change of differentiation potency of EPCs and angiogenic function of OECs by environmental factors, EPCs from both groups were cultured in normal and preeclampsia derived serum conditioned media, respectively. Analysis of H3K4, H3K9, and H3K27 trimethylation of EPC in preeclampsia and normal pregnancy was assayed by using western blot.

Results: Differentiation day of CKL^{-}EPCs was significantly delayed (10 days

vs 16 days; $p<0.05$) and the number of OEC colonies were significantly reduced in preeclampsia compared with normal pregnancy. In addition, activity of migration, adhesion and tube formation of OECs was significantly diminished in preeclampsia compared with normal pregnancy. Reduced differentiation potency of EPCs from preeclampsia was not recovered in normal serum while normal EPC was poorly differentiated under fetal serum from preeclampsia.

Conclusions: In preeclampsia, differentiation potency of cord blood CKL^-EPCs was reduced, which was irreversible despite of environmental improvement such as serum condition. Moreover, angiogenic function of OECs was significantly diminished in preeclampsia. These results suggest that EPCs in preeclampsia may have irreversible epigenetic change which leads to defect on angiogenic function.

Key words: Preeclampsia, CKL^-Endothelial Progenitor Cells, Epigenetic changes, Outgrowth Endothelial Cell, Cord Blood

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I. INTRODUCTION

Recent studies have focused on the effect on offsprings after growing up whose mother had preeclampsia during pregnancy. The offsprings who exposed to maternal preeclampsia during in utero period have higher risks for cardiovascular disease or metabolic disease such as hypertension, type 2 diabetes mellitus (DM), metabolic syndrome after growing up than whose mother had no preeclampsia [1-4]. The Hypothesis suggests that increased inflammation, antiangiogenic factors and autoimmune response influences placental permeability in preeclampsia followed by altering the level of serum soluble factors including cytokines, sFlt-1, ROS, etc[5-7]. Therefore, the impact of these changes on epigenetic changes or cellular remodeling during in utero period could lead to increase the risks of the cardiovascular disease or metabolic disease in offsprings after growing up[3].

Endothelial progenitor cells (EPCs) are one of progenitor cell which are differentiated from hemangioblast and express CD133, C-kit as cell surface markers[8]. EPCs have potential to differentiate into outgrowth endothelial cells (OECs) and OECs compose vasculature in embryonic period. Meanwhile, circulating EPCs have potential to act on repairing vascular damage after differentiation into fibroblast-mimic cells[9].

Many studies have reported that level of circulating EPCs decreased in human

cardiovascular disease such as coronary artery disease, diabetic vasculopathy, atherosclerosis, systemic lupus erythematosis, and metabolic syndrome[10-14]. Moreover, many studies were also reported that the number of cord blood-derived EPCs were decreased in preeclampsia and were increased senescence[15-18]. There are controversies on the function of EPCs in preeclampsia and rare report on angiogenic or vasculogenic function of OECs differentiated from EPCs in preeclampsia[17-19]. According to these reports, we could get to association between maternal vasculopathy such as preeclampsia and fetal circulation. And it is reasonable to hypothesize that preeclampsia may induce in utero reprogramming of the function of fetal EPCs. And EPCs could reflect vascular health in terms of differentiation into endothelial cells which composes vasculature during in utero period.

Considering all these contents mentioned above, the aim of this study is (1) to compare the function of EPCs between preeclampsia and normal pregnancy by evaluate the differentiation potency of EPCs and angiogenic function of OECs differentiated from EPCs in both groups, (2) to investigate if the environmental factors could affect EPCs from preeclampsia or normal pregnancy, and (3) to investigate the level of histone methylation to explore the factors which could affect function of EPCs.

II. MATERIALS AND METHODS

1. Study population and sample collection

Of the deliveries at our institute between September 2016 and July 2017, those performed by vaginal delivery and cesarean section at 36–41 weeks of gestation were included in this study. Umbilical cord blood for CKL^- cell isolation was obtained at the time of delivery after fetal expulsion from preeclampsia (n=10) and from normal pregnancy (n=10). All women in the PE group had severe PE, diagnosed in accordance with the American College of Obstetricians and Gynecologists guidelines[20], as the presence of hypertension in association with followings; thrombocytopenia (platelet count less than 100,000/microliter);.

impaired liver function indicated by abnormally elevated blood concentrations of liver enzymes (to twice normal concentration), severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnosis, or both; progressive renal insufficiency (serum creatinine concentration greater than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease); pulmonary edema; new-onset cerebral or visual disturbances. Hypertension was defined as blood pressure >160/110 mmHg on two occasions at least 4 hours apart while the patient is on bed rest. Pregnancies associated with premature rupture of membranes, fetal malformation, chromosome anomaly, multiple pregnancies, or renal or endocrine diseases except diabetes mellitus were excluded from the study. The sampling and use of medical records for research purposes were performed with the consent of patients. This study was approved by the Institutional Review Board (IRB) of Severance Hospital (4-2016-0607).

2. Isolation and cultivation of CKL$^-$ Cells

Blood samples (up to 50mL each) were collected from umbilical cords before placental exfoliation during delivery by gravity flow. EPCs were isolated by density gradient centrifugation over Biocoll (Biochrom, Berlin, Germany) for 30min at 400 ×g and washed three times in phosphate buffered saline(PBS) (Biochrom). CKL$^-$ EPCs were purified by positive and negative selection with anti-CD133/C-kit/Lin$^-$ microbeads (Miltenyi Biotec, Bergisch- Gladbach, Germany) using a magnetic cell sorter device (Miltenyi Biotec). Briefly, cord blood EPCs were incubated with anti-CD133 microbeads followed by cell washing to remove of unbounded antibodies. EPCs incubated with anti-CD133 microbeads were processed for positive selection, according to the manufacturer's instructions.

Then, CD133+ fraction was incubated with anti-C-kit microbeads and processed for running sensitive positive selection. For depletion of Lin+ cells from CD133+/C-kit+fractions, cells were incubated with anti-Lin microbeads and applied on column. Unbound cells were collected after being washed out.

This fraction was CD133+/C-kit+/Lin$^-$. Purity was over 98%, as assessed by fluorescence activated cell sorting analysis. CKL$^-$ EPCs were seeded onto 6-well plates coated with human fibronectin (Sigma, St. Louis, MO) in endothelial basal medium-2 (Clonetics, Cell Systems, St. Katharinen, Germany). The medium was supplemented with endothelial growth medium-2 (EGM-2; Clonetics, Cell Systems) containing fetal bovine serum, human VEGF-A, human fibroblast growth factor-B, human epidermal growth factor, IGF1, and ascorbic acid in appropriate amounts. CKL$^-$ cell identification was determined by staining cells with phycoerythrin-(PE-) conjugated human antibodies CD133-PE and C-kit-PE (BD Biosciences, Bedford, MA).

3. CKL$^-$ EPC differentiation assay

CKL$^-$ EPCs (5×10^5cells/well) from preeclampsia and normal pregnancy were seeded on 6-well plates and cultured in medium (EGM-2). The medium was changed every 2 days. The day of differentiation was defined as the first day on which a differentiated colony was observed from the time of seeding. Differentiation days and the number of colonies formed in each set were determined by light microscopy.

4. Migration assay

Cell migration was assayed using the Transwell system (Corning Costar, Acton, MA) with 6.5-mm diameter polycarbonate filters (8-mm pore size). Briefly, the lower surface of the filter was coated with 0.1% gelatin. OECs (10^5) were seeded onto chemotaxis filters in EBM plus 0.5% FBS. After the 4-hour migration period, non-migrating cells were removed from the top surface of the membrane.

Migrating cells adhering to the undersurface of the filters were measured by hematoxylin and eosin staining and quantified using Kodak 1D software (Eastman Kodak, Rochester,NY). Results were representative of four independent experiments.

5. Adhesion assay

Cell-matrix adhesion assays were conducted as following procedure [21]. The 96-well plates were coated overnight at 4 °C 10 μg/nl human fibronectin. OECs in adhesion buffer were seeded at 10^5 cells/well in a volume of 100 μl followed by incubation for 30 minutes at 37 °C. Non-adherent cells were removed by twice washing, and then adherent cells were measured by H&E staining and quantified in triplicate by counting adherent cells in five randomly selected fields per well (Axiovert 100; Carl Zeiss Micro-Imaging, Thornwood, NY). Results were representative of three different experiments performed in duplicate.

6. Tube formation assay

Tube formation was assayed as previously described [22]. In brief, 250 μl Matrigel (BD Biosciences, Bedford, MA) was added to a 16-mm diameter tissue culture well and allowed to polymerize for 30 minutes at 37℃. After trypsinization, the harvested OECs were resuspended in EBM and were plated onto the layer of Matrigel (1.2 x 10^5 cells/well). Matrigel cultures were photographed at various time points (x200 magnification) during incubation at 37°C. The area covered by the tube network was determined with an optical imaging technique; pictures of the tubes were scanned into Adobe Photoshop and quantified using ImageJ software (National Institutes of Health).

7. Analysis of H3K4, H3K9, and H3K27, trimethylation

Trimethylation of H3K4, H3K9, and H3K27 were detected using specific antibodies for each protein. CKL^-EPCs were washed twice with phosphate-based saline and then lysed with radioimmunoprecipitation assay buffer (RIPA buffer). Cell lysates were electrophoresed on SDS-polyacrylamide gel electrophoresis (PAGE) and proteins were transferred onto polyvinylidene difluoride membranes. The blocked membranes were incubated with the appropriate antibody

[anti-H3K4me3 (Millipore, Millipore Corp., Bedford, MA, USA), anti-H3K9 and anti-H3K27me3 (Abcam, Cambridge, MA, USA) (1:1000 dilution)], and the immunoreactive bands were visualized with a chemiluminescent reagent, as recommended by Amersham Biosciences, Inc. (Piscataway, NJ, USA).

8. CKL-EPC differentiation assay under multiple serum-conditioned media

CKL^- EPCs (5×10^5cells/well) from preeclampsia and normal pregnancy were seeded on 6-well plates and cultured in EGM2 or conditioned media contained with maternal or fetal serum. After 3 days in culture, cell culture media were changed into each serum-conditioned media; EBM (negative control), EGM2 (20% FBS, positive control), normal maternal serum media (20% maternal serum), preeclamptic maternal serum media (20% maternal serum), normal fetal serum media (20% fetal serum), and preeclamptic fetal serum media (20% fetal serum). The medium was changed every 2 days. The day of differentiation was defined as the first day on which a differentiated colony was observed from the time of seeding.

Each serum for serum-conditioned media was separated by centrifugation from normal maternal blood, preeclamptic maternal blood, normal cord blood, and preeclamptic cord blood.

At least three assays were performed for each sample.

9. Statistical analyses

Data are shown as mean standard error (SE). Statistical comparisons between groups were performed using one-way analysis of variables followed by Tukey's post hoc tests.

III. RESULTS

1. Differential differentiation of human cord blood derived CKL^-EPCs into OECs between preeclampsia and normal pregnancy

Endothelial progenitor cell fraction was separated from cord blood using density gradient, and CD 133+/C-kit^{+}Lin^{-} (CKL^{-}) EPCs were sorted and purified. Cellular phenotype of CKL^{-} EPCs was confirmed by RT-PCR and immunostaining for CD133 and C-kit. After 10 days of culture, CKL^{-}EPCs from normal pregnancy spontaneously differentiated to OECs, which was confirmed by cell morphology and expression of lineage-specific markers. In contrast, CKL^{-}EPCs from preeclampsia showed delayed differentiation to OECs in terms of taking 16 days as differentiation day compared to normal pregnancy (Figure 1A) and delayed differentiation of CKL-EPCs in preeclampsia compared to normal pregnancy was confirmed by using quantification (Figure 1B). Moreover, the size of colony of OECs at same differentiation day was smaller in preeclampsia than in normal pregnancy as well as the number of colony of OECs was less in preeclampsia than in normal pregnancy (Figure 1C, 1D).

In preeclampsia, differentiation of CKL^{-}EPCs was delayed compared to normal pregnancy and characterization of colony of OECs differentiated from EPCs was less in size and in number than normal pregnancy. Taken together, these results suggest that differentiation potency was decreased in preeclampsia compared to normal pregnancy.

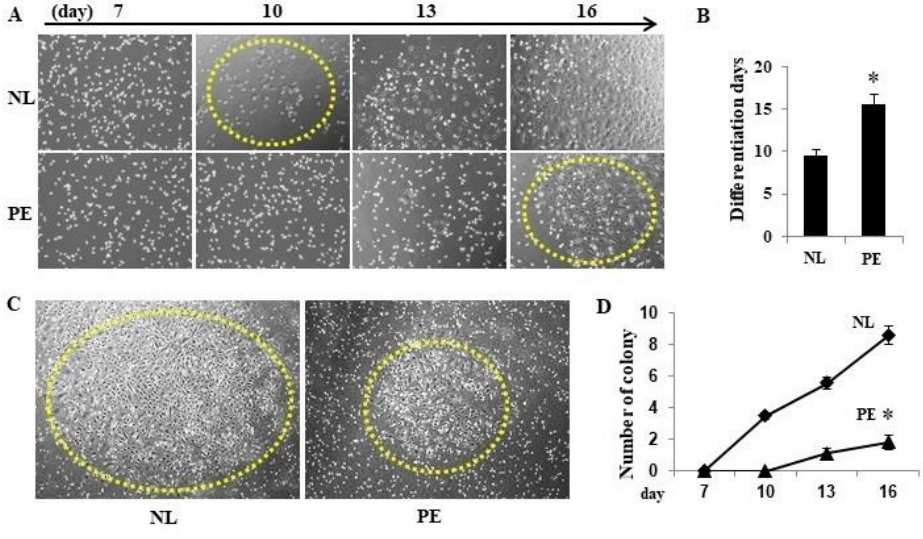

Figure 1. Decreased differentiation potency of EPCs in preeclampsia. **(A)**: Umbilical cord blood-derived CKL^{-}EPCs in preeclampsia differentiated in delayed manner compared to those in normal pregnancy. **(B)**: Differentiation day was significantly delayed in preeclampsia compared to normal pregnancy (*P<.05). **(C)**: At the same day after differentiation of EPCs into OECs, the size of colony was smaller in preeclampsia than in normal pregnancy as well as **(D)** the number of colony was fewer in preeclampsia than in normal pregnancy.

2. Diminished angiogenic potential of OECs differentiated from EPCs in preeclampsia compared to normal pregnancy

Migration assay, adhesion assay and tube formation assay were performed to investigate whether the angiogenic function of OECs differentiated from EPCs is different between in preeclampsia and in normal pregnancy. OECs in preeclampsia were poorly migrated through transwell chamber compared to those in normal pregnancy (Figure 2A) and the number of migrated OECs was significantly lower on quantitative analysis (Figure 2B). As shown in Figure 2C-2D, preeclampsia decreased adhesion of OECs. Tube formation potential was also decreased in OECs in preeclampsia compared to those in normal pregnancy. Morphologic differentiation of OECs was defective in preeclampsia (Figure 2E) as well as tube length of OECs was shorter in preeclampsia than that in normal pregnancy, which was confirmed by quantitative analysis (Figure 2F). These data demonstrate that preeclampsia causes malfunction of OECs in angiogenesis, even though successfully differentiated from EPCs to OECs.

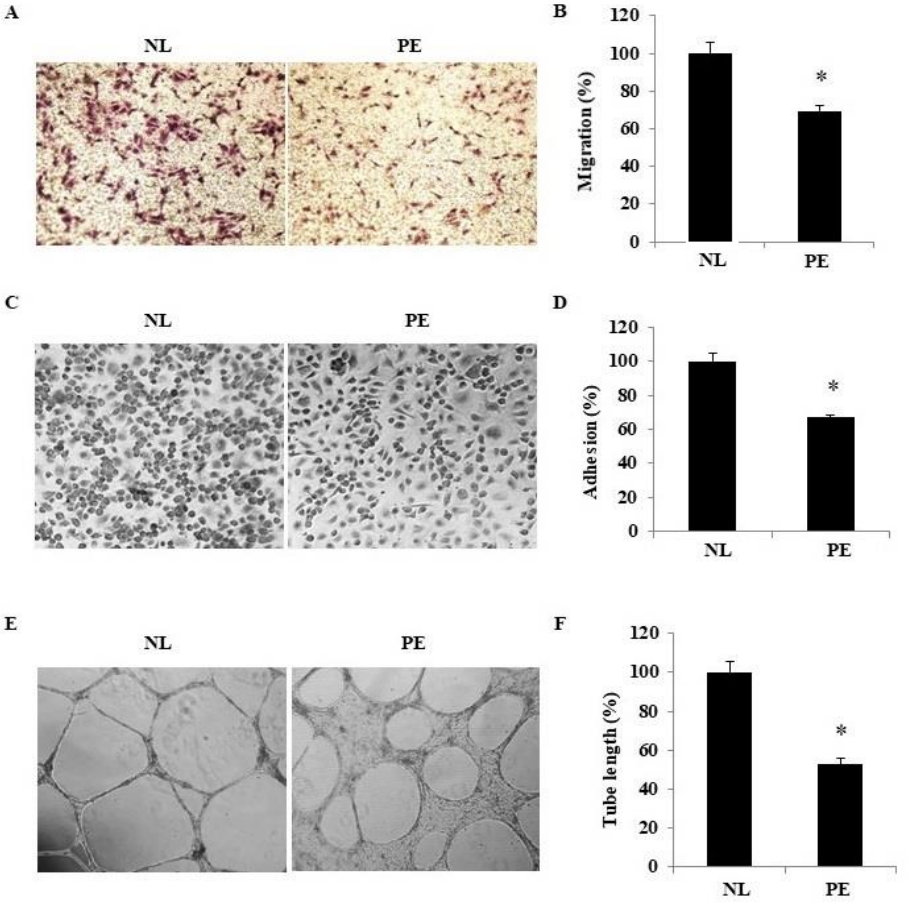

Figure 2. Comparison of angiogenic function of OECs between in preeclampsia and in normal pregnancy.

Migrated OECs was less in preeclampsia (PE) than normal pregnancy (NL) (A) and the number of migrated OECs was lower on quantitative analysis (B). The number of OECs adhered to well was fewer in PE than in NL (C), (D). Tube formation potential was decreased in OECs in preeclampsia compared to those in normal pregnancy. Morphologic differentiation of OECs from preeclampsia was incomplete compared to normal (E) and tube length of OECs in preeclampsia was shorter than that in normal pregnancy by quantitative analysis (F). (*P<0.05)

3. Differential differentiation pattern of cord blood derived EPCs in preeclampsia and normal pregnancy to OECs in conditioned-serum media

To investigate whether the environment regulates CKL^{-}EPC differentiation, CKL^{-}cells were cultured under human serum-conditioned media. Serum free media was set as negative control and EGM as positive control. Media was changed into human serum-conditioned media after attachment of CKL^{-}EPCs on the plate. CKL^{-}EPCs in cord blood from normal pregnancy were isolated and were differentiated in serum-conditioned media to investigate whether environmental factors could affect differentiation of cord blood-derived EPCs. EPCs were successfully differentiated under normal serum media and preeclamptic maternal serum media. However, differentiation of EPCs were failed in fetal serum of preeclampsia. Differentiation days of each media were same as positive control (EGM2) except under preeclamptic fetal serum media (Figure 3). CKL^{-}EPCs in cord blood from preeclampsia were isolated and were differentiated in serum-conditioned media to investigate whether environmental factors could affect differentiation of cord blood-derived EPCs or whether decreased differentiation potency could be reversible under non-affected serum media by preeclampsia. EPCs were differentiated only under positive control (EGM-2) with delayed manner. However, EPCs were failed to differentiate under both normal serum media and preeclamptic serum media (Figure 4).

Two to three weeks of change of serum environment could not influence on differentiation pattern of EPCs.

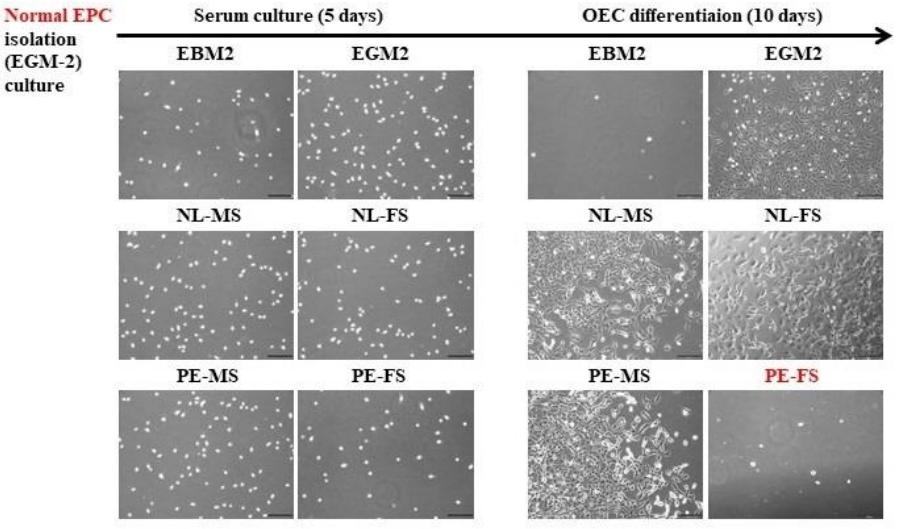

Figure3. Differentiation of Normal EPCs under serum-conditioned media. CKL^{-}EPCs in cord blood from normal pregnancy were isolated and were differentiated in serum-conditioned media to investigate whether environmental factors could affect differentiation of cord blood-derived EPCs. EPCs were successfully differentiated under multiple serum-conditioned media except in fetal serum of preeclampsia.

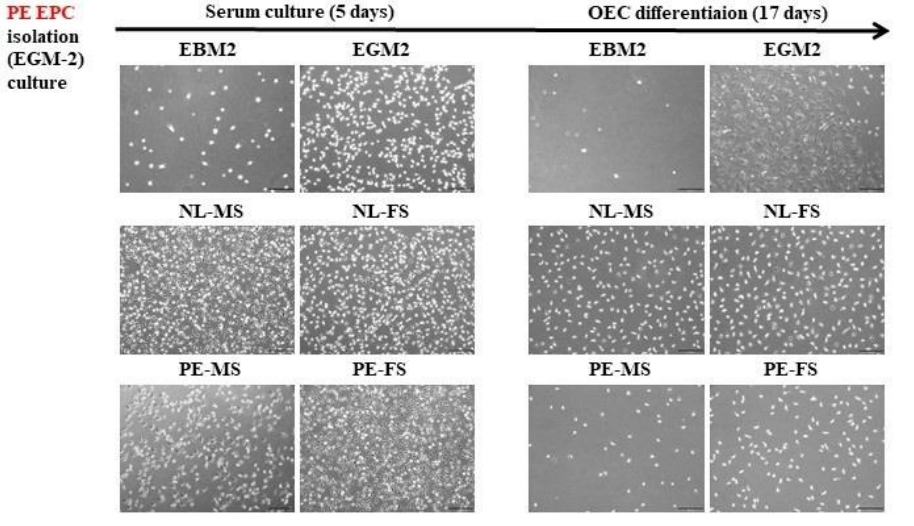

Figure4. Differentiation of Preeclamptic EPCs under serum-conditioned media. CKL^{-}EPCs in cord blood from preeclampsia were isolated and were differentiated in serum-conditioned media to investigate whether environmental factors could affect differentiation of cord blood-derived EPCs or whether decreased differentiation potency could be reversible under non-affected serum media by preeclampsia. EPCs were differentiated only under positive control (EGM-2) with delayed manner. However, preeclamptic EPCs were failed to differentiate under both normal and preeclamptic serum media.

4. Overall epigenetic changes of cord blood derived EPCs in preeclampsia compared to normal pregnancy

All the results of experiments above indicated that the mechanism was semi-permanent without temporary changes. To explore candidate mechanisms which affect function of EPCs in preeclampsia, especially of angiogenic function, we focused on the level of histone methylation as epigenetic markers. The level of trimethylation on H3 protein was analyzed by western blot to confirm the differential methylation of H3K4, H3K9, and H3K27 in preeclampsia and in normal pregnancy. Trimethylation level of H3K4 and H3K9 was significantly decreased in preeclamptic EPCs compared to in normal EPCs while there was no significant difference in the level of H3K27me3 between preeclampsia and normal pregnancy. Furthermore, the decreased level of trimethylation of H3K4 and H3K9 was aggravated in preeclamptic EPCs cultured with preeclamptic maternal serum(Figure5). This data suggests that some factors in maternal-fetal circulation, as soluble form in the serum causes inactivation of methylation level of histone protein in EPCs in the umbilical cord blood.

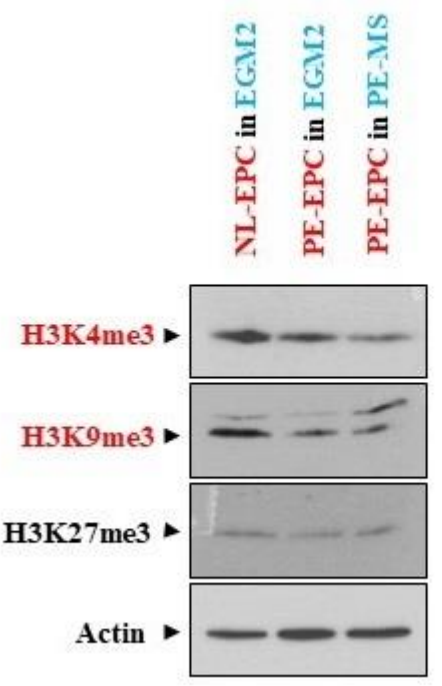

Figure 5. Decreased level of histone methylation in preeclamptic EPCs compared to normal EPCs. Expression of H3K4me3 was decreased in EPCs from cord blood in preeclampsia compared to those in normal pregnancy. And expression pattern of H3K4me3 of preeclamptic EPCs was decreased even more in preeclamptic maternal serum media than EGM2 media. Expression of H3K9me3 was also decreased EPCs from cord blood of preeclampsia compared to EPCs from cord blood of normal pregnancy.

IV. DISCUSSION

This research experimentally suggests that preeclampsia of mother can affect the vascular health of fetus during the pregnancy period.

Pathophysiological mechanism of preeclampsia on offspring has not been disclosed so far. However, it has been reported that incidence rate of cardiovascular and metabolic complications, such as hypertension, cardiovascular disorders, cerebrovascular disorders, imperfect brain development, type 2 diabetes, metabolic syndrome and hypercortisolism, increases for fetus born from mother with preeclampsia when they grow up [1-3,23-25]. There is also a report of increased pulmonary and peripheral vascular stiffness in fetus born from mother with preeclampsia in their school ages [26,27]. Baker proposed the hypothesis that the critical changes in intrauterine environment affect offspring in particular time, increasing incidence rate of cardiovascular and metabolic disorders when they grow up [28,29]. Current hypothesis is that preeclampsia triggers epigenetic change and cellular remodeling of offspring during pregnancy period, causing increase of morbidity to cardiovascular and metabolic diseases when offspring grow up [3].

Previous studies on epigenetic changes in preeclampsia mainly focuses on pathogenesis of the disease, explaining shallow placental implantation, endothelial dysfunction and inflammation due to the DNA methylation including genomic imprinting change such as STOX1 overexpression [30], promotor region change in SERPINA3 of placenta [31,32], hypomethylation of regulator in inflammatory modulator such as RXRα/PPARγ [33], and epigenetic change of HIF1α [34]. This studies only focuses on the placenta itself to explain pathophysiological mechanism of preeclampsia, not on the changes in offspring of mother affected by preeclampsia [35]. There are also many studies on analysis of miRNA of placenta and functional change of related genes, but these studies also focus only to find marker for early diagnosis or to explain pathophysiological mechanism of preeclampsia [36]. Recently reported studies on epigenetic changes related to the influence of preeclampsia on offspring mainly correlates neural development of fetus to the placental change of genetic imprinting, in relation to the hypoxia of

preeclampsia [37].

EPCs have been known as precursor of vascular endothelial cell, and it has been reported that vasculogenesis, angiogenesis, and neovascularization are possible after the fetal life [8,38]. Therefore, it could be considered that the abnormality of EPCs has association with vascular disease. In the previous studies, the number of circulating EPCs decreased in vascular and metabolic disorders such as coronary disease, diabetic vasculopathy, atherosclerosis, systemic lupus and metabolic syndrome [10-14]. Preeclampsia is also a hypertensive disorder occurred during pregnancy period, having decreased circulating EPCs in peripheral blood vessel of affected mother [39].

Cord blood is included in fetal circulation, and it is reasonable to state that CKL-EPCs of cord blood reflects the state of angiogenic potential of fetus, based on the previously reported study [40] that OECs differentiated from CKL-EPCs of cord blood has angiogenic potential. There is only a limited study about the EPCs function of fetus exposed to preeclampsia, while it can be found from research that the number of EPCs originated from cord blood of mother with preeclampsia decreased and those EPCs went through aging process [15-18]. In the result of this study, the function of CKL-EPCs originated from the cord blood of mother with preeclampsia declined, and angiogenic potential of differentiated OECs also decreased for mother with preeclampsia compared to the normal mother. It is also verified that differentiation potency of EPCs originated from cord blood of mother with preeclampsia was not recovered in the optimal condition that has been experimentally induced. It is possible to say that the change in EPCs function of cord blood exposed to the intrauterine environment of preeclampsia is irreversible.

Collectively, the intrauterine environment changed by preeclampsia can trigger modification of pathway involved in the maintenance of fetal vascular health.

The changes in soluble factor inside the serum of mother can explain the mechanism causing endothelial dysfunction of mother with preeclampsia. Mostly, soluble fms-like tyrosine kinase-2 (sFLT1) secreted from placenta increases, causes decrease of free VEGF and PIGF inside serum to destroy angiogenic homeostasis, while changing TGF-β level due to the soluble endoglin and

increasing systemic inflammation, eventually fails to maintain vascular health and vascular angiogenic homeostasis of mother with preeclampsia [41]. The explanation on any change in soluble factor of fetal serum in mother with preeclampsia will be the basis of sustainability of fetal vascular health. Staff el al. reported increased sFLT1 level in cord blood and amniotic fluid of mother with preeclampsia compared with those of normal mother, while the level of VEGF and PlGF decreased [42]. They mentioned fetal serum while reporting the concentration of sFLT1, VEGF and PlFG in umbilical vein, and it is possible to suppose that above soluble factor may trigger endothelial dysfunction or systemic inflammatory response in fetal vessel, as in the body of pregnant mother, considering that cord blood belongs to the fetal circulation. Also, recent study has reported that secretion of maternal placental VEGF is involved in angiogenic potency of HUVEC [43]. Therefore, based on the result of this study, a follow-up study about the process of changing vascular health of fetus in mother with preeclampsia, as well as about epigenetic change and angiogenic signaling pathway change on sFLT1, VEGF, or PlGF in cord blood.

One of the causes triggering irreversible change by environment is epigenetic change. Stem and progenitor cells are characterized by a high prevalence of transcriptionally competent but poised genes that are marked by both active and repressive histone modifications that plays important roles in differentiation and development [44]. In our study, we confirmed that H3K4me3 and H3K9me3 in cord blood-derived CKL^{-}EPCs were reduced in preeclampsia. Histone modification may prime the activation of pro-angiogenic signaling pathways (histone H3 lysine 4 trimethylation [H3K4me3]) and repressive signaling pathways (histone H3 lysine 27 trimethylation [H3K27me3]). In addition, studies in endothelial cells have identified several signal transduction pathways such as VEGFR, CXCR4, VEGF, NOTCH, WNT, and SHH that coordinate survival, differentiation, arterial/venous specification, and blood-vessel morphogenesis [45-47]. And these signaling pathways are essential for angiogenesis and post-natal vasculogenesis. It would not be a coincidence that there has been a series of reports that those proteins in the angiogenic signaling pathways were altered in whole genome

sequencing or RNA sequencing of placenta of preeclampsia [48-50]. On the other hand, H3K9me3 has a role of reducing expression of HIF1α by decreasing description [51]. Generally, upregulation of HIF1α is found in preeclampsia, and HIF1α involved in the control of sFLT1 and soluble endoglin [52]. It is also reported that the decrease in H3K9me3 in stem cell is related to the increase of senescence [53]. Considering the report that senescence of EPCs originated from cord blood has been progressed [16], it is possible to state that there is a correlation between the decrease of H3K9me3 and dysfunction of EPCs in preeclampsia. Further study is required to determine which signaling pathway changes in the differentiation process of CKL^{-}EPCs and in the angiogenesis during in-utero development.

This study has following limitations. EPCs originated from cord blood has been verified to have angiogenic potency, therefore concluding that cord blood EPCs collected may reflect fetal vascular health by influencing cord blood EPCs in intrauterine environment changed by preeclampsia condition. However, additional research should be performed to suggest preeclampsia condition may affect long-term vascular health of offspring. Therefore, further study is necessary to see if dysfunction of cord blood EPCs would continue for a long time after separated from preeclampsia environment after the delivery.

V. CONCLUSION

In preeclampsia, differentiation potency of cord blood CKL^{-}EPCs was reduced, which was irreversible despite of environmental improvement such as serum condition. Moreover, angiogenic function of OECs was significantly diminished in preeclampsia. These results suggest that EPCs in preeclampsia may have irreversible epigenetic change which leads to defect on angiogenic function.

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ABSTRACT (IN KOREAN)

자간전증 산모에서 제대혈 유래 CD133+/C-kit^{+}Lin^{-} 혈관내피전구세포의 기능의 변화와 후생적 변화

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목적: 최근 자간전증 산모에서 출생한 후손이 성인으로 자란 이후에 심혈관 질환 및 대사성 질환에 높게 이환됨이 보고되었으며, 자간전증이 임신 기간동안 태아세포의 변형에 기여한다는 가능성이 제시된다. 이에 자간전증 산모에서 출생한 태아의 제대혈에서 혈관내피전구세포의 기능의 변화 및 후생적 변화를 연구하고자 하였다.

대상과 방법: 10명의 자간전증 산모와 10명의 정상 혈압 산모군에서 출생 주수를 일치시켜 획득한 제대혈에서 혈관내피전구세포를 분리하였다. 혈관내피전구세포에서 내피세포로의 분화능은 현미경을 사용하여 형태학적, 분화날짜, 군집의 수와 크기를 평가하였다. 내피세포의 혈관형성능은 세포의 이동능, 부착능, 관 형성능을 통해 평가되었다. 혈관내피전구세포의 분화능이 환경의 영향을 받는지 확인하기 위해 서로 다른 혈청을 첨가한 배지에서 각 군의 분화를 확인하였다. 각 군의 혈관내피전구세포에서 H3K4, H3K9, H3K27의 트리메틸레이션 정도를 분석하였다.

결과: 자간전증 산모에서 출생한 신생아의 제대혈에서 얻은 CKL-혈관내피전구세포의 분화는 유의하게 지연되며 (10일 대 16일; $p<0.05$), 내피세포의 군집의 수와 크기도 정상에 비해 감소되었다. 게다가 내피세포의 이동능, 부착능, 관 형성능이 전자간증에서 유의하게 감소되었다. 자간전증에서 감소된 혈관내피전구세포의 분화능은 혈청 환경을 변화시켜도 호전되지 않았다. 또한, 자간전증에서 유래된 혈관내피전구세포의 H3K4, H3K9의 트리메틸레이션 정도가 감소한 것이 관찰되었다.

결론: 자간전증에서 제대혈 유래 CKL- 혈관내피전구세포의 분화능은 감소되어 있으며, 혈청 환경 변화를 개선시키더라도 이러한 변화는 비가역적이다. 자간전증에서 유래한 내피세포의 혈관형성능 또한 저하된다. 이런 기능 저하는 자간전증에서 혈관내피전구세포의 혈관형성능의 저하 및 비가역적인 후생적 변화와 연관된 것으로 사료된다.

핵심되는 말: 자간전증, CKL-혈관내피전구세포, 후생적 변화, 혈관내피세포, 제대혈