Soluble factors from bone marrow endothelial cells regulate differentiation and proliferation of hematopoietic and endothelial lineages and embryonic stem cells

WANG Qi-Ru¹*, YAN Qi²

¹Department of Physiology, Xiangya Medical School of Central South University, Changsha 410078, China; ²Benaroya Research Institute, Departments of Biological Structure and Ophthalmology, University of Washington, Seattle, WA 98195, USA

Abstract: We have established a bone marrow endothelial cell line. This review focuses on the elucidation and analysis of the effects of this bone marrow endothelial cell-conditioned medium (BMEC-CM) on the differentiation and proliferation of hematopoietic and endothelial progenitors as well as embryonic stem cells (ESCs). We will review that (1) BMEC-CM promotes proliferation and differentiation of hematopoietic lineage; (2) BMEC-CM promotes proliferation and differentiation of endothelial lineage; (3) BMEC-CM induces differentiation of hematopoietic stem cells/progenitors into endothelial progenitors; and (4) BMEC-CM induces differentiation of ESCs into hematopoietic cells and endothelial cells. We conclude that the soluble factors secreted by BMECs are able to support the proliferation and differentiation of hematopoietic and endothelium lineages. Moreover, these soluble factors induce hematopoietic cells to differentiate to endothelial cells, and induce ESCs to differentiate towards both endothelial cells and hematopoietic cells. Therefore, this work provides evidence that a close relationship involved in the development of hematopoietic and endothelial lineage. This disclosure will be beneficial for therapy strategy in the treatment of ischemic and tumor diseases, and improve our understanding of the relationship between hematopoietic and endothelial lineages.

Key words: differentiation and proliferation; endothelial lineage; hematopoietic lineage; embryonic stem cells
Adult bone marrow contains cells of hematopoietic and endothelial lineages. These two lineages have a common precursor, hemangioblast. The hemangioblasts have the dual differentiation capacity that allow the more primitive precursors develop into hematopoietic or endothelial lineage cells\[^{[1]-[4]}\]. Recently, increasing evidence has indicated that not only the mature hematopoietic cells but also hematopoietic stem cells (HSCs) and the myeloid progenitors could differentiate into endothelial progenitor cells (EPCs)\[^{[5]-[12]}\]. These data indicate that an intimate relationship was involved in the development of hematopoietic and endothelial lineages.

Endothelial cell line is a useful tool for studying the relationship between hematopoietic and endothelial lineages. A variety of endothelial cell lines had been established and studied extensively, for example, bone marrow microvascular endothelial cell line\[^{[13]}\], human umbilical vein endothelial cell line\[^{[14]}\], bone marrow-derived endothelial cell line\[^{[15]}\], and CD34+ cord blood cell-derived endothelial cell line\[^{[16]}\]. We have established an endothelial cell line from bone marrow mono-nuclear cells\[^{[17]}\]. The cells from this line show all the characteristics of endothelial cells; the cells elaborate stimulatory and inhibitory factors that exert significant effects on the development of hematopoietic and endothelial cells\[^{[18, 19]}\]. The present article will review the effects of bone marrow endothelial cell-conditioned medium (BMEC-CM) on the development of hematopoietic and endothelial lineages as well as embryonic stem cells (ESCs), their earliest origin.

1 Bone marrow endothelial cells and BMEC-CM

The murine bone marrow-derived endothelial cell line was established in our laboratory\[^{[17]}\]. Serum-free BMEC-CM was collected. The cells showed typical endothelial-like cobblestone morphology (Fig. 1C), formed cord or tube-like structures (Fig. 1A) and EC colony (Fig. 1B). The cells were positive for Ac-LDL uptake (Fig. 1E) and UEA-1 staining (Fig. 1F). They were positive for endothelial cell markers flk-1 (Fig. 1G), CD144 (Fig. 1I), vWF (Fig. 1J), CD31 (Fig. 1K)

Fig. 1. Morphology and the marker expression of bone marrow endothelial cell (BMEC) line cells. The cells were cultured with 20% FBS. A: Cord structure. B: Endothelial cell colony. C: The confluence layer of BMEC cell line cells showed cobberstone morphology. D: Cells were co-cultured with zymosan for 45 min. The cells uptook Dil-acLD (E), and were positive for UEA-1 staining (F). They showed positive for flk-1 (G), c-kit (H), CD144 (I), vWF (J), CD31 (K), and hematopoietic marker CD45 (L). Scale bar: A–C, E–G: 50 µm; D, H–L: 20 µm.
and c-kit (Fig. 1H). They were also positive for intercellular adhesion molecule-2 (ICAM-2), ICAM-1, and for vascular cell adhesion molecule-1 (VCAM-1)[20]. Karyotypes of all the cells were aneuploidy with a greater percentage of hyperdiploid [21]. In addition, the cells elaborate various cytokines (see below).

In contrast to other endothelial cell lines described above, the origin of this BMEC line is derived from hematopoietic precursors based on the evidence of their strong phagocytosis ability (Fig. 1D) and CD45 expression (Fig. 1L).

Recently, several investigators reported that endothelial cells, including both vascular and bone marrow endothelial cells, support hematopoiesis. The underlying mechanism is attributed to the cytokines secreted by endothelial cells[22–25], extracellular matrix proteins[26, 27] and cell-to-cell interactions[27, 28]. Cytokines elaborated by endothelial cells have been analyzed in detail (Table 1). Rafii et al.[23] reported that bone marrow endothelial cells elaborate stem cell factor (SCF), IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF). Almeida-Porada and Ascensao[29] reported that they detected the presence of mRNA for GM-CSF, IL-1, SCF, IL-7, IL-6, IL-8 and transforming growth factor-β (TGF-β) in bone marrow endothelial cells. The supernatant of bone marrow endothelial cells contain SCF, IL-6, GM-CSF, IL-1, IL-11 and G-CSF[13]. We demonstrated that bone marrow endothelial cells contain SCF, IL-6, GM-CSF, IL-1, IL-11, TGF-β, bone morphogenetic protein 2 (BMP2), fibroblast growth factor receptor (FGFR), engrailed-2 (En-2), thymosin-b4 (Tb4), macrophage stimulating protein 1 (MSP-1), connective tissue growth factor, platelet-derived growth factor (PDGF), macrophage inflammatory protein 2 (MIP-2), placental growth factor (PLGF), epithelial neutrophil-activating protein 78 (ENA-78), IFN-γ, IL-13, Inhibin[18]. Among these cytokines and interleukins, SCF, GM-CSF, IL-6, IL-1, IL-11, IL-13, TGF-β, MSP-1, PDGF, IFN-γ and PLGF are related to the growth of hematopoietic progenitors, while IL-6, IL-11, SCF, GM-CSF and VEGF are critical factors promoting the growth of endothelial progenitors. This is one of the mechanisms explaining the inductive effects of BMEC-CM on the proliferation and differentiation of both hematopoietic and endothelial lineages. The thymosin-β4 (Tβ4) and MSP were identified as hematopoietic inhibitors in our recent publication [30, 31]. Tβ4 and MSP play roles in the combination with hematopoietic stimulators for the expansion of early hematopoietic progenitor cells (HPCs)[31,32].

Condition media derived from vascular endothelial cells such as HUVEC or microvascular endothelial cells showed similar but much weaker effects on the proliferation and differentiation of hematopoietic and endothelial lineages described above. The combination of cell growth stimulators such as VEGF and SCF, or EGF, IGF and bFGF or in EGM-2MV (endothelial cell basal medium-2, plus SingleQuots of growth supplements) also can induce the differentiation and proliferation of hematopoietic lineage towards endothelial lineage[33,34].

### 2 BMEC-CM promotes the proliferation and differentiation of HSC/HPC

Hematopoietic stimulatory and inhibitory factors were both in the BMEC-CM. Stimulators were predominantly collected in the retentate (R-BMEC-CM) such as SCF, GM-CSF, PDGF, IL-6, IL-1, IL-11 and IL-13, and

<table>
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<tr>
<th>Table 1. Cytokines elaborated by bone marrow endothelial cell (BMEC)</th>
<th>GM-CSF</th>
<th>SCF</th>
<th>IL-6</th>
<th>IL-1</th>
<th>IL-11</th>
<th>G-CSF</th>
<th>TGF-β</th>
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<tr>
<td>BMEC +</td>
<td>+</td>
<td>+</td>
<td>+</td>
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GM-CSF, granulocyte-macrophage colony stimulating factor; SCF, stem cell factor; G-CSF, granulocyte colony stimulating factor; TGF-β, transforming growth factor-β; BMP2, bone morphogenetic protein 2; FGFR, fibroblast growth factor receptor; En-2, endothelin-2; Tβ4, Thymosin-b4; CoGF, connective tissue growth factor; PDGF, platelet-derived growth factor; MIP-2, macrophage inflammatory protein 2; PLGF, placental growth factor; ENA-78, epithelial neutrophil activating protein 78.
their molecular weights are more than 10 000 daltons. On the other hand, inhibitory factors, such as TGF-β, MSP-1, IFN-γ etc., their molecular weights are more than 10 000 daltons, were in retentate (R-BMEC-CM); but Tβ4 and AcSDKP, whose molecular weights are less than 10 000 daltons, were in filtrate (F-BMEC-CM). No hematopoietic stimulators were found in the filtrate (F-BMEC-CM). Both BMEC-CM and R-BMEC-CM stimulate proliferation and differentiation of hematopoietic stem cells and progenitors, but R-BMEC-CM was stronger than BMEC-CM for the differentiation of hematopoietic progenitors because the low molecular weight inhibitors were not involved in R-BMEC-CM. We found that R-BMEC-CM alone could stimulate CFU-GM and BFU-E colony formation in a dose-dependent manner. R-BMEC-CM supplemented with GM-CSF not only resulted in significant increase of CFU-GM in comparison with R-BMEC-CM or GM-CSF alone, but also stimulated high proliferative potential colony forming cell (HPP-CFC) formation in a dose-dependent manner. The data suggest that BMEC-CM could be used as the substitutes of hematopoietic growth factors to stimulate the formation of colony forming units-granulocytes/macrophages (CFU-GM), HPP-CFC, burst-forming unit-erythroid cells (BFU-E) and colony-forming unit-megakaryocyte (CFU-Meg) [35–37].

In addition, we demonstrated that the inhibitors in BMEC-CM together with hematopoietic stimulating factors have positive effects on the growth and maintenance of HSCs and HPCs. For example, when CD34+ cells were cultured with hematopoietic stimulators, they were associated with a significant loss of early HPCs [38]. We demonstrated that the early progenitors are expanded by several-fold when CD34+ cells are cultured with the same hematopoietic stimulators plus the early hematopoietic inhibitors such as TGF-β, MIP-1α, MSP or Tβ4 [31,32].

We reported that Tβ4 and MSP were elaborated by bone marrow endothelial cells [38], and they are hematopoietic inhibitors [31,32]. MSP inhibits the differentiation of early hematopoietic progenitor cells and expands the early hematopoietic progenitor cells in a liquid expansion culture system. For example, the percent of NBT positive cells for cytokines group (EPO+SCF+IL-3+IL-6+GM-CSF) was (22.75 ± 3.60)% and that of the MSP+cytokines group was (12.25 ± 3.43)% (P < 0.05). Analysis of hematopoietic colony showed bone marrow CFU-GEMM was significantly increased in cytokines+MSP group and significantly decreased in cytokines group (Table 2) [31].

Tβ4, a hematopoietic inhibitor, also inhibits the differentiation of early hematopoietic progenitor cells and expands the early hematopoietic progenitor cells in a liquid expansion culture system. Hematopoietic stimulators include IL-3, IL-6, SCF, GM-CSF and erythropoietin (EPO). After 6 days of culture, the percentage of NBT positive cells for the stimulators group was (19.3 ± 3.2)% and that of the Tβ4 plus stimulators group was (9.7 ± 1.5)% (P < 0.01). Analysis of hematopoietic colony showed bone marrow HPP-CFC was significantly increased in stimulators plus Tβ4 group or significantly decreased in stimulators alone group in liquid culture system after 6 days of culture.

In conclusion, we have shown that bone marrow endothelial cells produce soluble factors including both stimulators and inhibitors. These stimulators and inhibitors have significant impacts on the proliferation and differentiation of hematopoietic progenitors. The work indicates that interaction between progenitor cells and their microenvironment is important for normal hematopoietic development.

<table>
<thead>
<tr>
<th></th>
<th>Cys</th>
<th>MIP-1α+Cys</th>
<th>MSP+Cys</th>
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<tr>
<td>CFU-GM</td>
<td>421 ± 161</td>
<td>570 ± 319</td>
<td>535 ± 92</td>
</tr>
<tr>
<td>CFU-GEMM</td>
<td>85.2 ± 10.0</td>
<td>166 ± 28*</td>
<td>172 ± 28**</td>
</tr>
<tr>
<td>NBT</td>
<td>22.75 ± 3.60</td>
<td>11.1 ± 2.73**</td>
<td>12.25 ± 3.43*</td>
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<tr>
<td>Apoptosis</td>
<td>5.42 ± 0.79</td>
<td>4.41 ± 0.58</td>
<td>4.11 ± 0.47</td>
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Bone marrow CD34+ cells were cultured in different liquid culture system for 6 days. *P < 0.05, **P < 0.01 vs Cys group. Cys: EPO+SCF+IL-3+IL-6+GM-CSF; MIP-1α+Cys: EPO+SCF+IL-3+IL-6+GM-CSF+MIP-1α; MSP+Cys: EPO+SCF+IL-3+IL-6+GM-CSF+MSP. EPO, erythropoietin; SCF, stem cell factor; GM-CSF, granulocyte-macrophage colony stimulating factor; CFU-GM, granulocyte-macrophage colony forming unit; CFU-GEMM, colony forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte; NBT, nitroblue tetrazolium. Data were reproduced from Ma et al. [31].
human bone marrow microvascular endothelial cells support long-term cell proliferation and differentiation of myeloid and megakaryocytic progenitors. Davis et al.\cite{28} reported that porcine brain microvascular endothelial cells support the expansion of primitive hematopoietic progenitor cells. Our results demonstrate that bone marrow endothelial cells elaborate a number of stimulators and inhibitors for proliferation and differentiation of hematopoietic progenitor cells. While the stimulators accelerate the hematopoietic development, the inhibitors maintain the hematopoietic stem cell in a quiescent state to protect them from exhaustion.

3 BMEC-CM enhances the proliferation and differentiation of endothelial colonies

To study the effects of murine BMEC-CM on the growth of bone marrow endothelial cells, serum-free BMEC-CM was collected, and ultrafiltration was performed with a centriprep 10 of cutting off molecular weight 10 kDa. The retentate of BMEC-CM (molecular weight >10 kDa) and the filtrate of BMEC-CM (molecular weight < 10 kDa) were collected. The BMEC-CM and the retentate of BMEC-CM (molecular weight >10 kDa) promote the proliferation of bone marrow endothelial cell colonies. Different concentrations of BMEC-CM exhibit a significant increase in the number of colonies and a positive correlation between the concentrations of BMEC-CM and the formation of endothelial cell (EC)-colonies (Fig. 2A), and it was similar between the concentrations of GM-CSF and the formation of EC-colonies (Fig. 2B). BMEC-CM increased [3H]-thymidine incorporation of bone marrow endothelial cells. The filtrate of BMEC-CM (molecular weight < 10 kDa) component did not affect the production of endothelial cell colonies and did not increase [3H]-thymidine incorporation of bone marrow endothelial cells \cite{19}.

BMEC-CM also enhances the differentiation of endothelial cells. The bone marrow-derived endothelial cells formed capillary-like structures when cultured with 20% BMEC-CM plus 20% MSC or 50% BMEC-CM \cite{5}.

4 BMEC-CM induces hematopoietic cells into EPCs

Bone marrow cell line cells elaborated cytokines such as IL-6, IL-11, SCF, GM-CSF, VEGF, AcSDKP (N-Acetyl-Seryl-Aspartyl-Lysyl-Proline) and unidentified molecules. IL-6, IL-11, SCF, GM-CSF and VEGF promote the growth of EPCs\cite{19}. There are few studies of AcSDKP on endothelium. Liu et al.\cite{40} reported that AcSDKP is an effective agent for the induction of angiogenesis in vitro and in vivo. We are the first lab reporting that BMEC cell line cells elaborate Tβ4 and AcSDKP, and they are secreted into the BMEC-CM\cite{30}. AcSDKP or BMEC-CM which contained AcSDKP inhibits the growth of MSC or fibroblasts. By applying BMEC-CM in the culture condition, purified endothelial cells can be achieved without fibroblast or macrophage contaminations \cite{17, 41}. The method was used by Oikawa et al. for culture of endothelial cells from fresh bone marrow\cite{42}. The bone marrow cells were cultured on 0.1% gelatin in DMEM plus 20% fetal bovine serum supplemented with AcSDKP to avoid fibroblasts contamination. We described that HSC and HPC were significantly expanded by culturing with BMEC-CM for 24 h in liquid culture system. However, after longer time induction (5 or more days) with BMEC-CM, hematopoietic lineage cells differentiate into endothelial
progenitors or endothelial cells\(^5\). EPC cells were formed at day 5 after initial BMC culture in BMEC-CM. The cells were induced to form colonies after additional culture with BMEC-CM (Fig. 3). These colony cells expressed EC markers vWF and CD31 (Fig. 3\(D\), \(E\)), incorporated Ac-LDL, reacted with endothelial specific \textit{Ulex europaeus} lectin, showed endothelial morphology, exhibited a high proliferative capacity, and formed capillary-like structures; all of these indicate they are EPCs\(^5\). Colony cells were also positive for CD45 (Fig. 3\(B\)2), suggesting that the EPCs were derived from hematopoietic lineage.

The EPCs possess differential proliferative capacity depending on the hierarchy of hematopoietic progenitor that they are derived from. Quantitatively, there were \((671 \pm 93.5)\) EPCs per million of bone marrow mononuclear cells (BMMNCs) in culture with BMEC-CM. For the colony size, a small colony represents a limited proliferative capacity\(^8\), suggesting that it is derived from later hematopoietic progenitor. On the contrary, if the EPC is derived from HSC or early precursor in the lineage, this EPC has potent proliferative capacity, therefore, forming a large colony. The percentage of HPP-EPC is 1\%--2\% among all the EPC colonies. The cell number of per HPP-EPC colony in average was \((740.97 \pm 230.70) \times 10^4\) cells in eight HPP colonies in 14 weeks of culture\(^5\). Hematopoietic-derived HPP-EPC yields about \(7 \times 10^6\) cells, only the hematopoietic stem cells/early progenitor-originated or the transdifferentiated endothelial lineage cells can possess such high proliferative capacity. Some characteristics of these EPCs are: (1) they have higher proliferative capacity\(^5\) but much lower than the cord-blood-derived HPP-EPC which could be expanded 100 population doublings and yield \(10^{10}\) to \(10^{12}\) cells per EPC after 90-day incubation\(^{43}\); (2) they exhibit a significantly higher
phagocytic ability than vascular endothelial cells. They respond poorly to VEGF in the formation of capillary-like structures. The colony cells are positive for CD45 which emerge that the colonies are derived from hematopoietic lineage.

It has been reported that the hematopoietic stem cells and their progeny of myeloid lineage have been induced to differentiate into endothelial cells; likewise, TNFα also significantly facilitates the endothelial differentiation of myeloid cells in vitro and in vivo. Madlambayan et al. demonstrated that myeloid progenitor cells directly participate in new blood vessel formation in response to stromal cell derived factor 1α (SDF-1α).

They report that secondary transplantation of single hematopoietic stem cells showed HSCs are a long-term source for neovasculogenesis. Sekiguchi et al. separated freshly isolated mouse BMMNCs based on their fast or slow adherence in culture; they demonstrated that slow adherent cells from BMMNCs are EPC-enriched population. Cells under endothelial culture conditions not only express positive endothelial cell markers, but also have capability of proliferation and vascular formation. Slow adherent cells frequently expressed hematopoietic cell marker CD45 (92.1%–98.0%). In addition, Yang et al. isolated CD34+ cells, c-Kit+/Sca-1−/Lin− (KSL) cells, c-Kit+/Lin− (KL) cells and Sca-1+/Lin− (SL) cells from mouse BMMNCs. Their results showed that CD34+ cells isolated from BMMNCs exhibit a more adherent phenotype of endothelial progenitor cells under endothelial culture conditions. In EPC differentiation hierarchy, one type of EPC colonies is capable of differentiation into another type of EPC colonies, and the later can further differentiate into more mature EPCs in culture. The positive percentage of CD45 is 99% or 100% in EPCs. Our experiments provide evidence that hematopoietic cells differentiate into endothelium in a liquid culture in the presence of EC-CM. EC-CM contains various factors including VEGF, SDF-1, TNF-α and unidentified factors that play a synergistic effects in the induction of hematopoietic cells to endothelial cells. The various cytokines and unidentified molecules secreted by BMEC line and their synergistic effects played a significant role in promoting the adherence of hematopoietic stem cells in the initial stage of BMMNC culture; moreover, these factors not only facilitate the survival, proliferation, and differentiation of stem cell/progenitors, but also induce the stem cells to differentiate towards EPC lineage and stimulate their proliferation.

The secreted factors/molecules preserve the characteristics of hematopoietic stem cells during their differentiation towards EPC, such as high capacity of proliferation. Thus, one hematopoietic stem cell or progenitor cell can result in million of endothelial cells under the induction of BMEC-CM. This phenotype is extremely important given their potential in the therapeutic applications in ischemic diseases. For example, a recent study showed that intracoronary transplantation of bone marrow cells enriched in CD34+/CD45+ and CD133+/CD45+ cells in patients with ischemic heart disease significantly reduced the size of infarct area and increased the global ejection fraction as well as infarct wall movement velocity. Madlambayan et al. demonstrated in vivo that secondary transplantation of hematopoietic stem cells and showed HSCs are a long-term source for neovasculogenesis.

Under normal conditions, HSC/HPC proliferate and differentiate toward hematopoietic lineage. HSC/HPC could transdifferentiate to endothelium under pathological conditions such as in ischemic or tumor tissues where high levels of SDF-1, TNF-α are available. Our study showed that HSCs could differentiate to EPCs with the induction of BMEC-CM in vitro.

5 BMEC-CM promotes the differentiation of ESC into hematopoietic and endothelial lineages

BMEC-CM contained a variety of hematopoietic stimulating factors and inhibitors. These cytokines play important roles in regulating proliferation and differentiation of HSCs and HPCs, and play important roles in the growth of bone marrow ECs. These findings suggest that BMEC-CM may contribute to the differentiation of ES cells into hematopoietic and endothelial cells.

Studies showed that BMEC-CM promoted the generation of hematopoietic precursors from mouse embryonic stem cells in vitro. Studies proved that the cells induced from ESC express hematopoietic precursor cell antigens (c-kit, Sca-1, Thy-1 and CD34), transcription factors (c-myb, SCL and β-H1) and generated HPP-CFC and BFU-E. Sun et al. ’s study showed that BMEC-CM promotes differentiation of ESC into he-
matopoietic progenitors, and exerts the differentiation of ESC into endothelial cells. Sun et al.\cite{48} reported that after 4dEBs were cultured for 10–16 days with EC-CM, adherent cells were stained by Dil-Ac-LDL (Fig. 4B). These LDL-positive cells expressed CD31 (Fig. 4E), Flk-1 (Fig. 4F), ICAM2 (Fig. 4I) and also expressed ICAM1 (Fig. 4H) and VCAM1 (Fig. 4J) when they had been stimulated with TNF-α and IL-1. They could bind UEA1 lectin (Fig. 4G) and form vascular-like structures (Fig. 4L). The data suggest that the cell population consists of endothelial-like cells.

The combination of cytokines plays important roles in the development of endothelial cells and hematopoietic cells\cite{48, 51, 53–55}. Zhu et al.\cite{56} suggested that each of the factors appears to regulate specific steps of differentiation: BMP4 promotes the efficient formation of mesoderm; bFGF induces the differentiation of these mesodermal precursors to the hemangioblast fate; VEGF and thrombopoietin (TPO) are required for the production of committed hematopoietic progenitors. Stankovich et al.\cite{57} suggested that specific adhesion molecules influence ESC commitment toward hematopoietic and endothelial lineages. Angiopoietin-1 and BMP4 were revealed the essential role in determining the hematopoietic/endothelial fate\cite{58, 59}. EC-CM could induce differentiation of ESC into endothelial cells and hematopoietic cells (Fig. 4, Table 3), suggesting that EC-CM acts as a potent cytokine combination.

Table 3. Endothelial cell line-conditioned medium (EC-CM) promotes the differentiation of embryonic stem cells (ESC) into hematopoietic precursors

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cells (×10⁴)</th>
<th>CD34⁺ cells (×10⁴)</th>
<th>HPP-CFC (10⁵ EB cells)</th>
<th>BFU-E (10⁵ EB cells)</th>
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<tr>
<td>BECM</td>
<td>11.83 ± 0.43***</td>
<td>1.80 ± 0.065****</td>
<td>84.00 ± 8.39****</td>
<td>494.83 ± 19.59****</td>
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<tr>
<td>CYs</td>
<td>11.12 ± 0.38***</td>
<td>0.70 ± 0.024***</td>
<td>53.33 ± 8.26***</td>
<td>252.5 ± 22.23***</td>
</tr>
<tr>
<td>Control</td>
<td>1.96 ± 0.15</td>
<td>0.045 ± 0.035</td>
<td>0</td>
<td>146.00 ± 18.95</td>
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EB, embryoid body; HPP-CFC, high proliferative potential colony forming cell; BFU-E, the burst forming unit-erythroid; BECM, bone marrow endothelial cell-conditioned medium; CYs, cytokines, VEGF 5 ng/mL, SCF 50 ng/mL, Epo 2 U/mL. ***P < 0.001 compared with control group; ****P < 0.001 compared with CYs group. Data are reproduced from Zhao et al.\cite{51}.
In conclusion, the soluble factors secreted by bone marrow endothelial cell line cells are able to support the proliferation and differentiation of hematopoietic and endothelial lineages. Moreover, these soluble factors induce hematopoietic cells to differentiate into endothelial cells, and induce ESCs to differentiate towards both endothelial cells and hematopoietic cells (Fig. 5). This study will be beneficial for therapy strategy in the treatment of ischemic and tumor diseases, and improve our understanding of the relationship between hematopoietic and endothelial lineages.

* * *

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