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Enhancement of proliferation of human umbilical cord blood–derived CD34$^+$ hematopoietic stem cells by a combination of hyper-interleukin-6 and small molecules

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ABSTRACT

Umbilical cord blood (UCB) is an alternative source of allogeneic hematopoietic stem cells (HSCs) for transplantation to treat various hematological disorders. The major limitation to the use of UCB-derived HSCs (UCB–HSCs) in transplantation, however, is the low numbers of HSCs in a unit of cord blood. To overcome this limitation, various cytokines or small molecules have been used to expand UCB-HSCs ex vivo. In this study, we investigated a synergistic effect of the combination of HIL-6, SR1, and UM171 on UCB-HSC culture and found that this combination resulted in the highest number of CD34$^+$ cells. These results suggest that the combination of SR1, UM171 and HIL-6 exerts a synergistic effect in the proliferation of HSCs from UCB and thus, SR1, UM171 and HIL-6 is the most suitable combination for obtaining HSCs from UCB for clinical transplantation.

1. Introduction

Umbilical cord blood (UCB) is an alternative to bone marrow or peripheral blood in transplantation and is a clinically useful source of hematopoietic stem cells (HSCs) for treatment of a wide variety of malignant and non-malignant disorders [1,2]. As a source for the engraftment of HSCs, UCB is readily available through cord blood banks and has relatively low levels of graft-versus-host disease after allogeneic transplantation [1,3]. However, the limited number of cells collected from a single donor unit is a major obstacle for the use of UCB-HSCs and may not be sufficient for engraftment in many adults [1]. Therefore, an optimal culture condition for expansion of human HSCs in ex vivo would greatly improve clinical transplantation [4].

To overcome this major limitation of UCB-HSCs, studies have been conducted on cytokines that enhance ex vivo expansion. A cocktail of stem cell factor (SCF), thrombopoietin (TPO), and FMS-like tyrosine kinase 3 (Flt-3) ligand (FL) has been accepted for the expansion of hematopoietic progenitor [5,6]. Together with these classical factors, interleukin-6 (IL-6) has been used to promote HSC proliferation in vitro [7,8]. Hyper-interleukin-6 (HIL-6), a fusion protein of IL-6 and its specific receptor, effectively enhances growth of precursor cells and maintenance of hematopoietic progenitor cells by activating the gp130 signaling at concentrations 100 to 1000 times lower than IL-6 [9–11].

Attempts to improve HSC expansion with small molecules, such as SR1 and UM171, have also been performed. SR1 is an antagonist of aryl hydrocarbon receptor; it expands cells expressing CD34, a surface marker for HSC, and maintains cord blood precursors [12]. UM171, which is a potent agonist of HSC renewal, retain the CD34$^+$ phenotype and efficiently expand human cord blood HSCs ex vivo [13,14].

In this study, we used a combination of HIL-6, SR1, and UM171 to investigate the synergistic effects on the expansion of human UCB-derived total nucleated cells (TNC) and CD34$^+$ cells. Here, we report a combination of small molecules and HIL-6 can improve the culture of HSCs from human UCB for increase in the proliferation of TNC and

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$CD34^+$ cells, which suggests our culture condition established in this study can be used to obtain a sufficient number of human UCB-derived HSCs for HSC based clinical applications.

2. Material and methods

2.1. Collection and processing of human $CD34^+$ UCB-HSCs

Human UCB samples were obtained from normal full-term deliveries with informed consent according to the institutional review board of Konkuk University Medical Center–approved ethical guidelines (IRB number: KUH1040066). A total of 10 human UCB samples were processed. UCB samples were processed within 4 h. $CD34^+$ UCB-HSCs were isolated using RosetteSep CD34 pre-enrichment cocktail followed by $CD34^+$ selection using an EasySep Human $CD34^+$ Selection kit (StemCell Technologies, Vancouver, BC, Canada). To confirm purity, isolated cells were stained in phosphate-buffered saline (PBS) supplemented with 2% fetal bovine serum (FBS) at 4 °C for 25 min with fluorescein isothiocyanate (FITC)-labelled anti-human CD34 (BD Biosciences, Franklin Lakes, NJ, USA) and APC-labelled anti-human CD45 (StemCell Technologies) [12,14]. Stained cells were washed once with PBS supplemented with 2% FBS and analyzed using a BD Accuri C6 flow cytometer (BD Biosciences).

2.2. $CD34^+$ UCB-HSCs culture

$CD34^+$ UCB-HSCs were cultured in expansion medium consisting of StemSpan SFEM (StemCell Technologies) supplemented with 1 × antibiotics and recombinant human SCF (100 ng/mL), TPO (100 ng/mL), and FL (50 ng/mL; all from Peprotech, Rocky Hill, NJ, USA) [12]. To optimize the HIL-6 concentration, $CD34^+$ UCB-HSPCs were cultured in HSC expansion medium supplemented with SCF, TPO, FL, UM171 (35 nM), SR1 (500 nM), and HIL-6 (0, 1, 5, 10, 20, 50, or 100 ng/mL) at 37 °C and 5% CO2 for 13 days (Supplementary Fig. S1). The cells were plated into a 24-well plate (1.2×10^4 cells in 500 μL per well). HIL-6 was added immediately after plating. Then cells were transferred to 6-well plates and fresh medium containing cytokines was added as needed to keep cell density between 3×10^5 and 1×10^6 cells/mL. Fresh medium was added every 2 days. The condition on the 13th day was 4 mL/well of 6-well plate.

2.3. Culture condition

STF, SCF (100 ng/mL), TPO (100 ng/mL), and FL (50 ng/mL); STF-DMSO, STF with DMSO (0.1%); STF-SR1-UM171, STF with SR1 (500 nM), UM171 (35 nM); STF-SR1-UM171-HIL-6, STF-SR1-UM171 with HIL-6 (1 ng/mL).

2.4. Flow cytometry analysis

For UCB-HSC phenotyping, cells were stained in PBS supplemented with 2% FBS at 4 °C for 25 min with FITC-labelled anti-human CD34 (BD Biosciences), PE-Cy7-labelled anti-human CD38 (BD Biosciences), PE-labelled anti-human CD45RA (BD Biosciences), APC-Cy7-labelled anti-human CD49f (BioLegend, San Diego, CA, USA), and APC-labelled anti-human CD90 (BioLegend). Stained cells were washed once with PBS supplemented with 2% FBS and analyzed. Multicolor analysis was performed on a BD FACSAria I flow cytometer (BD Biosciences) and the data were analyzed [12,14,15].

2.5. Colony-forming unit assay

Colony-forming units (CFU) were generated by seeding cells into MethoCult H4435 (StemCell Technologies) according to the manufacturer's recommendation [12]. After 14 days in culture, plates were visually scored for CFU-multilineage colonies.

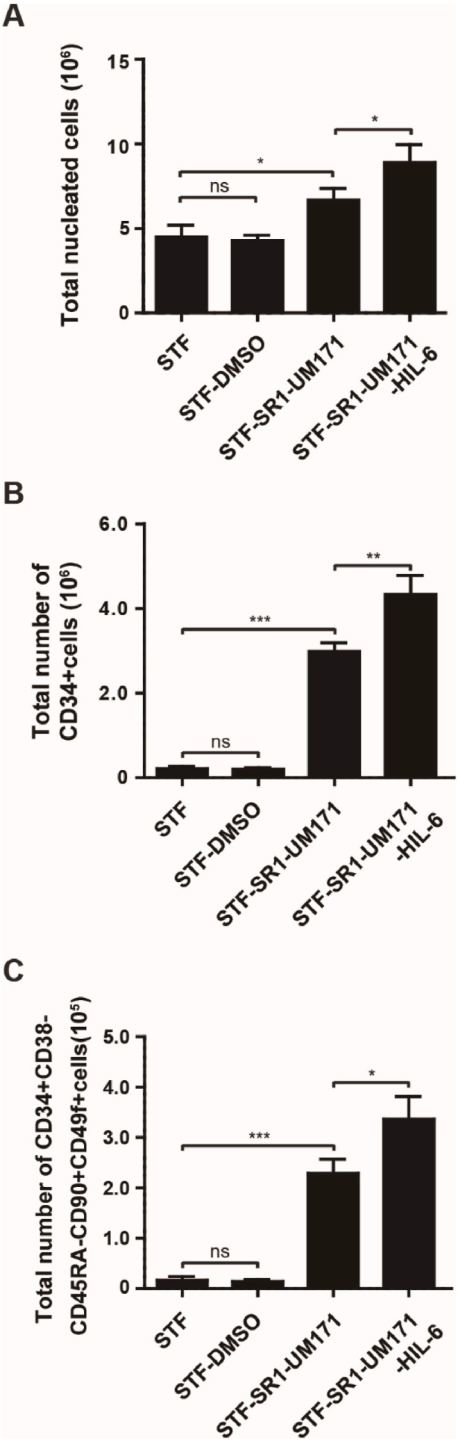

Fig. 1. Expansion of TNC and $CD34^+$ cells in the presence of HIL-6. (A) The total number of nucleated cells. (B) The total number of $CD34^+$ cells. (C) The number of CD34 + $CD38^-$CD45RA−CD90 + CD49f + cells. All data are means ± SD (n = 3). *p < 0.05, ***p < 0.001; ns, not significant.

2.6. Statistical analysis

All data are presented as means ± SD. All statistics were calculated using independent t-tests or analysis of variance (ANOVA) with least significant differences tests for post-hoc analysis. P values were generated in GraphPad Prism 5 for Windows (GraphPad Software, San Diego, CA, USA).

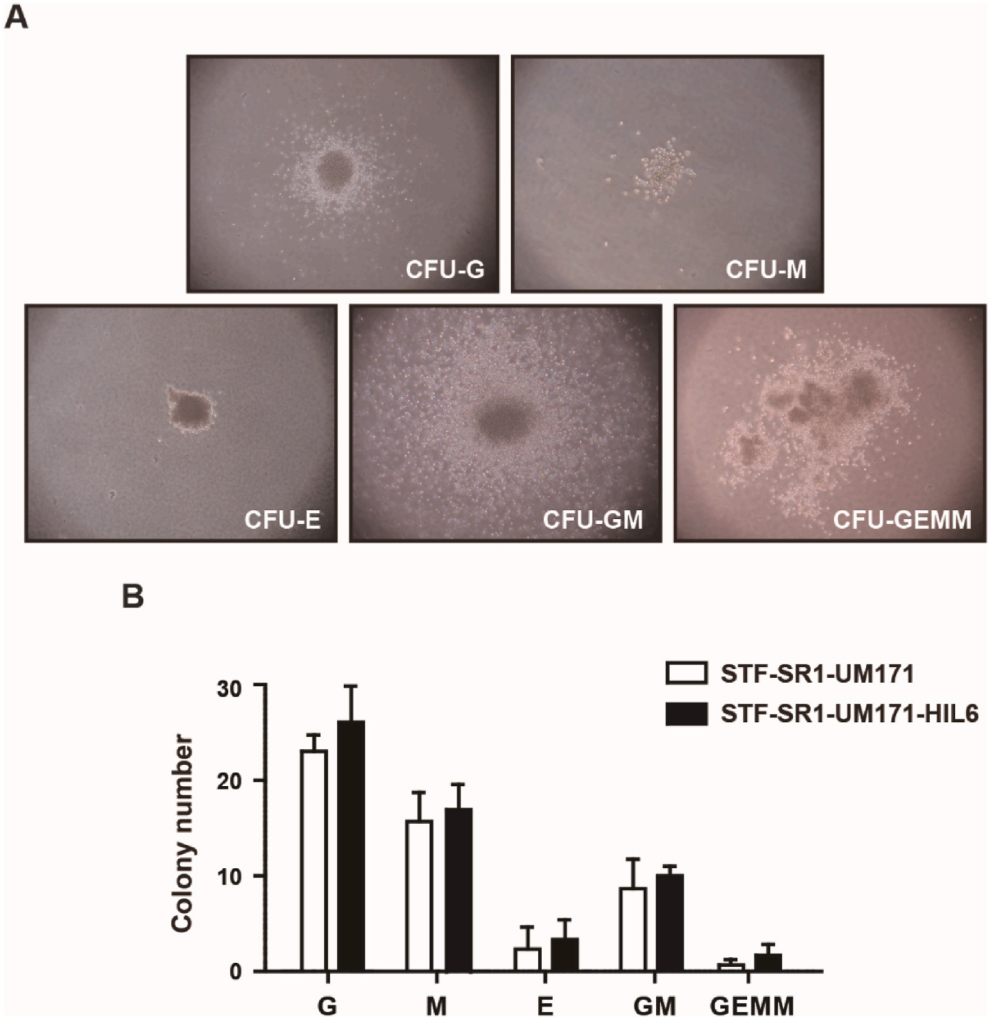

Fig. 2. Evaluation of in vitro functionality of CD34$^+$ UCB-HSCs using CFU assay. (A) Five different types of colony morphology observed with CD34$^+$ UCB-HSCs expanded for 13 days in the presence of HIL-6. (B) Numbers of colonies of expanded CD34$^+$ UCB-HSCs for 13 days. Data are means ± SD (n = 3). CFU-G, colony-forming unit of granulocyte; CFU-M, colony-forming unit of macrophage; CFU-E, colony-forming unit of erythroid; CFU-GM, colony-forming unit of granulocyte/macrophage; CFU-GEMM, colony-forming unit of granulocyte/erythroid/macrophage/megakaryocyte.

3. Results

In the presence of the optimal concentration of HIL-6 (1 ng/mL), the number of TNCs was about 1.33 times that in the STF-SR1-UM171 group (Fig. 1A and Supplementary Fig. S1) and the total number of CD34$^+$ and CD34 + CD38$^-$CD45RA−CD90 + CD49f + cells was about 1.5 times that in the STF-SR1-UM171 group (Fig. 1B and C). Interestingly, flow cytometry analysis showed that the percentage of CD34$^+$ cells, which include hematopoietic progenitor cells of HSCs (HSPCs), and CD34 + CD38$^-$CD45RA−CD90 + CD49f + cells, which are true HSCs, did not differ significantly between the STF-SR1-UM171 and STF-SR1-UM171-HIL-6 groups (Supplementary Fig. S2 and Supplementary Fig. S3). These results suggest that the combination of SR1, UM171, and HIL-6 promotes proliferation rather than stemness of HSCs.

To verify the differentiation ability of HSCs, we performed CFU assay and found that HSCs cultured in the presence of HIL-6 are functional (Fig. 2). Granulocyte colony-forming units (CFU-G), macrophage colony-forming units (CFU-M), erythroid colony-forming units (CFU-E), granulocyte/macrophage colony-forming units (CFU-GM), and granulocyte/erythroid/macrophage/megakaryocyte colony-forming units (CFU-GEMM) were observed, and each colony was classified and counted on the basis of its distinct characteristics (Fig. 2A). In the presence of STF-SR1-UM171-HIL-6, the numbers of CFU-G, CFU-M, CFU-E, CFU-GM, and CFU-GEMM colonies were similar to those in the presence of STF-SR1-UM171 only (Fig. 2B).

4. Discussion

Expansion of HSCs derived from UCB is critical to advancing technologies for transplantation for therapy of hematopoietic disorders [1, 16]. This can be achieved through self-renewal and amplification of HSCs induced by combinations of growth factors, small molecules, and cytokines [9–14]. In this study, we tried to find an optimal condition for expansion of HSCs obtained from UCB by developing the composition of the previously established HSC culture medium. In particular, we focused on a synergistic effect produced when HIL-6, a covalently linked IL-6/sIL-6R complex and a potent enhancer of HSC expansion [9–11] is added to medium containing SR1 and UM171.

First, we found that the culture containing SR1 and UM171 significantly increased the number of TNCs, CD34$^+$ cells (HSPCs), and CD34 + CD38$^-$CD45RA−CD90 + CD49f + cells (HSCs) in comparison with control cultures (STF and STF-DMSO), which is consistent with a previous study [14]. We found that both CD34$^+$ cells and CD34 + CD38$^-$CD45RA−CD90 + CD49f + cells were more abundant when HIL-6 was added to culture containing SR1 and UM171. Thus, combining HIL-6 and small molecules (SR1 and UM171) results in a synergistic effect on the increase in both HSPCs and HSCs. Interestingly, the ratio of CD34$^+$ and CD34 + CD38$^-$CD45RA−CD90 + CD49f + cells to the total cells in the presence of HIL-6 was similar to that in the presence of SR1 and UM171 only, while the addition of HIL-6 accelerated the proliferation of TNCs with maintenance of stemness in HPSCs and HSCs. These results imply that the increase in HSC abundance by adding HIL-6 results from an increase in the number of TNCs.

The importance of the combination of SR1, UM171, and HIL-6 was that it not only increased the number of TNC, but also the $CD34^+$ population than cells cultured in presence of SR1 and UM171 only. These results suggest that the combination of SR1, UM171 and HIL-6 helps to obtain sufficient HSCs from UCB for clinical transplantation.

Declaration of competing interest

The authors did not report any conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2022.101214.

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