Generation of functional platelets from human embryonic stem cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors

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Human embryonic stem cells (hESCs) could potentially represent an alternative source for blood transfusion therapies and a promising tool for studying the ontogeny of hematopoiesis. When we cultured hESCs on either C3H10T1/2 or OP-9 cells to facilitate hematopoiesis, we found that exogenous administration of vascular endothelial growth factor promoted the emergence of sac-like structures, which we named embryonic stem cell–derived sacs (ES-sacs). These ES-sacs consisted of multiple cysts demarcated by cellular monolayers that retained some of the properties of endothelial cells. The spherical cells inside ES-sacs expressed primarily CD34, along with VE-cadherin, CD31, CD41a, and CD45, and were able to form hematopoietic colonies in semisolid culture and to differentiate into mature megakaryocytes by day 24 in the presence of thrombopoietin. Apparently, ES-sacs provide a suitable environment for hematopoietic progenitors. Relatively large numbers of mature megakaryocytes could be induced from the hematopoietic progenitors within ES-sacs, which were then able to release platelets that displayed integrin αIIbβ3 activation and spreading in response to ADP or thrombin. This novel protocol thus provides a means of generating platelets from hESCs, which could serve as the basis for efficient production of platelets for clinical transfusion and studies of thrombopoiesis. (Blood. 2008;111:5298-5306) © 2008 by The American Society of Hematology

Methods

Reagents and cell lines

All reagents were from Sigma-Aldrich (St Louis, MO) unless indicated otherwise. Three hESC lines, Kyoto hESC-1 (KhES), KhES-2 and KhES-3, were obtained from the Institute for Frontier Medical Science, Kyoto University (Kyoto, Japan), with approval for hESC use granted by the Minister of Education, Culture, Sports, Science, and Technology.
of Japan. The Review Board of the Institute of Medical Science, University of Tokyo approved this research. The entire study was conducted in accordance with the Declaration of Helsinki. hESCs were maintained as described previously; they were cultured on irradiated mouse embryonic fibroblasts in a 1:1 mixture of Dulbecco modified Eagle medium and Ham F-12 medium supplemented with 0.1 mM nonessential amino acids (Invitrogen, Carlsbad, CA), 2 mM l-glutamine (Invitrogen), 20% knockout serum replacement (Invitrogen), 0.1 mM 2-mercaptoethanol, and 5 ng/mL basic fibroblast growth factor (bFGF; Upstate, Lake Placid, NY). The cells were passaged every 3 days to maintain them in an undifferentiated state. The mouse C3H10T1/2 cell line was purchased from the RIKEN BioResource Center (Tsukuba, Ibaraki, Japan) and was cultured in Eagle basal medium (Invitrogen) containing 10% fetal bovine serum (FBS) and 2 mM l-glutamine. The OP-9 cell line was a gift from Dr T. Nakano (Osaka University, Osaka, Japan) and was cultured in α-minimum essential medium (MEM; Invitrogen) containing 20% FBS and 2 mM l-glutamine. ESC differentiation medium was Iscove modified Dulbecco medium supplemented with a cocktail of 10 μg/mL human insulin, 5.5 μg/mL human transferrin, 5 mg/mL sodium selenite, 2 mM l-glutamine, 0.45 mM α-monomethylglucorlglycerol, 50 μg/mL ascorbic acid, and 15% highly filtered FBS (Collect Gold; ICN Biomedicals, Aurora, OH) in the absence or presence of the cytokines/mediators listed in Figures 3 and 5. Human vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and human bone morphogenetic protein-4 (BMP-4) were from R&D Systems (Minneapolis, MN). Human thymobopoietin (TPO), human interleukin-6 (IL-6), IL-11, insulin-like growth factor II (IGF-II), and human stem cell factor (SCF) were from Peprotech (Rocky Hill, NJ). The following antibodies were used: phycoerythrin (PE)-conjugated anti-CD31, PE- or fluorescein isothiocyanate (FITC)-conjugated anti-CD34, unconjugated CD41a (integrin αIIb subunit), PE-conjugated CD41a or allophycocyanin (APC)–conjugated anti-CD41a, FITC-conjugated anti-CD42a (GPIIX), PE-conjugated anti-CD42b (GP Ibα), Alexa 405–conjugated anti-CD45, unconjugated anti–vascular endothelial (VE)–cadherin, and APC-conjugated anti–VEGF-receptor 2 (VEGF-R2). Antihuman VEGF neutralizing antibody (bevacizumab) was from Roche (Basel, Switzerland). Anti-human c-Mpl antibody was a kind gift from Kirin (Tokyo, Japan). FITC-conjugated PAC-1 antibody (BD Biosciences, San Jose, CA) was used for integrin activation studies. Anti–tirofilin, a specific antagonist to human integrin αIIbβ3, was from Merck (Whitehouse Station, NJ).

**Cell culture**

C3H10T1/2 cells or OP-9 cells were irradiated (50 Gy) in 100-mm dishes before use. We compared 2 different protocols, as depicted in Figure 1Ai–ii. Protocol 1: Small clumps (>100 cells) of hESCs were transferred onto untreated OP9 cells and cultivated in the presence of 100 ng/mL TPO throughout the culture. On days 7 and 11, the cells were passed onto fresh OP-9 cells, leading to the generation of mature megakaryocytes on days 15 to 17, as previously demonstrated.16 Protocol 2: Small clumps of hESCs (suspended in PBS containing 0.25% trypsin, 1 mM CaCl2, and 20% KSR) were transferred onto C3H10T1/2 or OP-9 cells and cultured in hESC differentiation medium, which was refreshed every 3 days. On days 14 to 15 of culture, embryonic stem–derived sacs (ES-sacs) were collected into a 50-ml tube, gently crushed with a pipette, and passed through a 70-μm cell strainer to obtain hematopoietic progenitors. These cells were transferred onto fresh, irradiated feeder cells at a density of 2 to 3 × 10^6 cells per well in 6-well plates and maintained in differentiation medium supplemented with human TPO or other combinations of cytokines/mediators (human IL-6, IL-11, human SCF, and heparin; Pharmaica & Upjohn, Bridgewater, NJ). The medium was replaced every 3 days; nonadherent cells were collected and analyzed after 20 to 32 days.

**Immunohistochemical studies and flow cytometric analyses**

Immunohistochemical staining of ES-sacs was carried out on days 14 to 15. Intact ES-sacs were fixed with either 10% methanol or 4% formaldehyde in PBS and then stained with antibodies against CD31, CD34, VEGF-R2, and/or FITC-conjugated Ulex europaeus agglutinin-1 (UEA-1) lectin, an endothelial cell marker (Vector Laboratories, Burlingame, CA), after which they were labeled with secondary antibodies and observed under a fluorescence microscope (Leica DM IRBE; Leica Microsystems, Wetzlar, Germany).

To investigate the internal structures of ES-sacs, immunohistochemistry was carried out with serial 2-μm paraffin sections. Sections were fixed with 4% formaldehyde in PBS and stained with biotinylated UEA-I lectin, after which they were incubated with peroxidase-conjugated streptavidin (Nichirei, Tokyo, Japan). Peroxidase activity was visualized using 3′,3-diaminobenzidine in PBS with 0.01% H2O2. Parallel sections were also stained with hematoxylin and eosin. An ECLIPSESi light microscope (Nikon, Tokyo, Japan) was used for evaluation.

Expression of cell surface molecules was analyzed by flow cytometry (FACS Aria; Becton Dickinson Japan, Tokyo, Japan). To determine precise numbers of megakaryocytes, the cells were stained with anti–human CD41a, anti–human CD42a, and anti–human CD42b, and were accompanied with True Count Beads (BD Biosciences) when analyzed by flow cytometry.

**Semiquantitative RT-PCR**

On day 24 of culture, hematopoietic cells were sorted into CD34+/CD41a– and CD34+/CD41a+ fractions by flow cytometry and lysed with Trizol (Invitrogen). Total RNA was extracted as recommended by the manufacturer, after which cDNAs were obtained using a Thermo Script reverse-transcription–polymerase chain reaction (RT-PCR) system and oligo-dT primer (Invitrogen). Samples were normalized to intrinsic GAPDH. The following primer sequences (5′ to 3′) were used: for GAPDH, AAC AGC TTC AAG ATC ATC AGC (forward) and TTG GCA GTT TTT TCT AGA CGG (reverse); for GATA-1, TCA ATT CAG CAG CCT ATT CC (forward) and TTC GAG TCT GAA TCT ACC CC (reverse); and for FOG-1, GCC ACC GCA GTG ATC AAC AAA (forward) and AAG TGG CTG TAG AGG ATC TCC (reverse); for Fli-1, TAA GAA TAC AGA GCA ACG GCC (forward) and GGC ATG TAG GAG ATG TCA GAA (reverse); for NF-E2, ATG AGC TAT TGG CAA GGT ACC (forward) and TAC TCT TCA GGA GAG TAG CTG (reverse); for GP Ibα, AAT CCA CTA CTG ACG CAA CCC (forward) and GGT TAG AGA AAA GGG TCA TTT (reverse).

**Functional analysis of platelet activation**

**Platelet preparation.** Platelets in culture medium were gently collected, and a one-ninth volume of acid citrate dextrose solution (85 mM sodium citrate, 104 mM glucose, and 65 mM citric acid) was added. The modified medium containing the cells was then centrifuged at 150g for 10 minutes to eliminate any large cells. The supernatant was transferred to a new tube, 1 μM prostaglandin E1 and 1 U/mL aprotinin were added to prevent platelet activation, and the mixture was centrifuged at 400g for 10 minutes to sediment a platelet pellet. The pellet was then resuspended in an appropriate volume of modified Tyrode-HEPES buffer at pH 7.4 (10 mM HEPES, 12 mM NaHCO3, 138 mM NaCl, 5.5 mM glucose, 2.9 mM KCl, and 1 mM MgCl2) and finally used after addition of 1 mM CaCl2.

**Studies of agonist-mediated integrin αIIbβ3 activation.** To investigate integrin αIIbβ3 activation, 50-μL aliquots of hESC-derived platelets in buffer were incubated for 20 minutes at room temperature with PE-conjugated anti-CD42b and FITC-conjugated PAC-1 in the absence or presence of human thrombin or ADP. The binding of PAC-1 to platelets was quantified using flow cytometry. Nonspecific binding was determined in the presence of 10 μM tirofilin, a specific antagonist to human integrin αIIbβ3.20 Specific binding was defined as total minus nonspecific binding.

**Confocal studies.** All observations of cytoskeletal changes in platelets were made using a confocal microscopic system (Leica TCS SP2; Leica Microsystems) equipped with a 63×/1.40 numeric aperture oil-immersion objective (Leica Microsystems). Images were assembled using Adobe Photoshop (Adobe Systems, San Jose, CA). Human washed platelets or
The protocol used to obtain ES-sacs required stromal cells, such as OP-9 or C3H10T1/2 cells. While OP-9 and C3H10T1/2 cells generated equal numbers of ES-sacs (data not shown), we primarily used C3H10T1/2 cells in the subsequent studies summarized in the results.

Each ES-sac consisted of a morphologically distinct external layer that enveloped several thousand spherical cells (Figure 1B). Immunohistochemical studies of ES-sacs revealed that the cells in the external layer preferentially expressed VEGF-R2, CD31, CD34, and UEA-1 lectin-binding activity (UEA-1), indicating differentiation along an endothelial cell lineage (Figure 2A arrows). Hematoxylin-eosin staining revealed that ES-sacs contain multiple cystic structures demarcated by UEA-1 (arrows in the higher-magnification view of Figure 2B). To characterize the spherical cells within ES-sacs, they were stained with anti-CD31, anti-CD34, anti-VEGF-R2, anti-VE-cadherin (a hematopoietic cell marker), anti-CD41a (an early hematopoietic progenitor and megakaryocyte marker), and anti-CD45 (a panhematopoietic cell marker) and then analyzed by flow cytometry. Most spherical cells within ES-sacs expressed CD31 (Figure 2C), whereas only approximately one-third of the CD31+ cells expressed CD34. VEGF-R2, VE-cadherin, CD41a, and CD45 were present on 8%, 70%, 50%, and 41% of CD34+ cells, respectively (Figure 2C). Moreover, these cells effectively formed multilineage colonies under semisolid liquid conditions (Figure S1).

Collectively, the results suggest the cells inside ES-sacs include multipotent hematopoietic progenitors. By contrast, hESC-derived clumps within cultures that did not form ES-sacs (they appeared in the absence of round cells) failed to differentiate into hematopoietic cells. Thus, our new protocol (protocol 2, summarized in Figure 1Aii) enabled hESCs to develop into hematopoietic progenitors, once they formed an ES-sac structure (Figure 2C). Indeed, when the cells inside ES-sacs were harvested, reseeded onto feeder cells in the presence of TPO, and cultured according to protocol 2, megakaryocytes were generated with much greater efficiency than has been seen with other in vitro methods, including protocol 1 and the floating cell method with protocol 2 (Figure 2D). This is noteworthy in that we recently succeeded in using the floating cells but not adherent cells to efficiently generate megakaryocytes and platelets from monkey ES cells (CMK6). There is thus a clear difference in the optimal methodologies between CMK6 ESCs and hESCs.

In addition, we found that exogenous administration of VEGF significantly increased the number of ES-sacs (Figure 3A). While the synergistic action of IGF-II plus VEGF is required for more efficient production of hematopoietic progenitors from CMK6 ESCs, VEGF (up to 20 ng/mL), by itself, induced ES-sacs from hESCs as efficiently as VEGF plus IGF-II (Figure 3A). There is also a clear difference in the optimal methodologies between CMK6 ESCs and hESCs.

Results and discussion

Multipotent hematopoietic progenitors that efficiently generate mature megakaryocytes are enriched inside ES-sacs

Using the protocol depicted in Figure 1A, Gaur et al successfully generated megakaryocytes with high ploidy from H9 hESCs, but no release of platelets was observed. We also failed to obtain large numbers of platelet-like particles from hESCs when following this basic protocol, or even after application of additional hematopoietic cytokines, including SCF, IL-6, and IL-11 (data not shown). During the course of these studies, however, we noticed the appearance of inflated sac-like structures in cultures maintained for 2 weeks without additional reseeding procedures (Video S1, available on the Blood website; see the Supplemental Materials link at the top of the online article). What is more, some of these sac-like structures contained round, hematopoietic-like cells inside; those, we termed embryonic stem cell–derived sacs (ES-sacs).
We also found that VEGF increased the numbers of c-Mpl–expressing cells within ES-sacs on day 15 (Figure S2). Because it is well known that signaling mediated by the TPO/c-Mpl axis is indispensable for megakaryopoiesis,24,25 we suggest that augmented expression of c-Mpl mediated via a VEGF signaling pathway increases megakaryopoiesis and thrombopoiesis to levels not seen in the absence of VEGF (Figure 3A,B), which is consistent with a recent report.26

Because the cells making up ES-sacs manifest characteristics similar to those of endothelial cells (Figure 2A,B), and because bFGF and PlGF, like VEGF, are necessary for endothelial cell growth,27-29 we next assessed the respective capacities of bFGF or PlGF to promote the generation of ES-sacs and hematopoietic progenitors. Interestingly, bFGF at concentrations of 2.5 or 10 ng/mL exerted a dose-dependent inhibitory effect, while PlGF at 50 ng/mL tended to increase the generation of ES-sacs (Figure 3C) and platelets (data not shown). Moreover, whereas bFGF inhibited VEGF-augmented production of ES-sacs, PlGF acted additively with VEGF to further augment ES-sac production (Figure 3C).

We also compared generation of ES-sacs and platelets from the KhES-1, KhES-2, and KhES-3 lines, and found that the induction efficiencies of ES-sacs differed. The number of ES-sacs generated from KhES-3 was several-fold higher than those from KhES-1 or KhES-2 (data not shown). We therefore used KhES-3 cells for most of the results shown here.

Hematopoietic progenitors inside ES-sacs efficiently yield megakaryocytes

Coculture for an additional 7 to 9 days after day 14 on fresh, but irradiated, feeder cells in the presence of 100 ng/mL TPO (Figure 1Aii) promoted differentiation of many hematopoietic progenitors inside the ES-sacs into mature, proplatelet-forming megakaryocytes, as evidenced by cell surface markers revealed by flow cytometric analyses and cytospin-preparation staining (Figure 4A,B). Fifty percent to 60% of cells consistently expressed CD41a, CD42a, and CD42b, which is indicative of mature megakaryocytes.30,31 These hESC-derived megakaryocytes showed polyploidy upon May-Giemsa staining (Figure 4B) and proplatelet formation upon immunohistochemical staining (Figure 4C). In addition, some megakaryocytes appeared to be shedding their cytoplasmic membranes (Figure 4Civ). These morphologic features demonstrate that megakaryocytes cultured in vitro from hESCs possess the demarcation membrane system necessary for the generation of platelets.32

We then investigated which cells inside ES-sacs have the potential to differentiate into the megakaryocyte lineage. Data from earlier reports using human bone marrow– or umbilical cord blood–derived cells suggest that the development of hematopoietic progenitors into mature megakaryocytes is characterized and defined by the expression of CD34 and CD41a.33,34 We therefore isolated cells from inside ES-sacs on day 15, stained them with anti-CD34 and anti-CD41a antibodies, and
divided them into CD34+/CD41a−, CD34+/CD41a+, CD34−/CD41a+, and CD34−/CD41a− subpopulations using a cell sorter (Figure 4D top panel of dot plots on flow cytometry). Equal numbers of the sorted cells in each fraction were then transferred onto new feeder cells and maintained for an additional 9 days in the presence of 100 ng/mL TPO. Over that 9-day period, the CD34+/CD41a+ and CD34−/CD41a− populations continued to develop in culture, so that 80% to 90% of the differentiated nonadherent cells were CD41a+/CD42b+ on day 24. This means that CD41a+ cells within ES-sacs on day 15 were able to differentiate into mature megakaryocytes in our culture system. On the other hand, from the CD34+/CD41a− population, only 40% of nonadherent cells were CD41a+/CD42b+ megakaryocytes on day 24 (Figure 4D), which suggests that the CD34+/CD41a− population has the potential to differentiate into other lineages in addition to the megakaryocytic lineage. Furthermore RT-PCR analysis revealed that the development of CD34+/CD41a− progenitors into CD41a+ populations may reflect their expression of GATA-1, FOG-1, Fli-1, and/or NF-E2 (Figure 4E), which are required for megakaryopoiesis.35 Taken together, these results suggest that ES-sacs contain heterogeneous populations of cells at different stages during which CD34 and/or CD41a may be expressed, and that CD41a+ cells preferentially differentiate into the megakaryocyte lineage, which is consistent with the developmental behavior observed for hematopoietic cells in bone marrow and umbilical cord blood.33,34

ES-sac–derived megakaryocytes generate platelets

When the surface markers of hESC-derived platelet-like particles collected on day 24 were examined by flow cytometry using the same forward- and side-scatter gates used for plasma-derived adult human platelets, the CD41a+/CD42b+ particles were detected in
the culture supernatants (Figure 5A). Subsequent electron microscopic examination of the cytosolic structures of hESC-derived platelets revealed normal microtubules but fewer granules than were seen in plasma-derived human platelets (Figure 5B). Platelets generated in culture supernatant increased in number up to day 24, but thereafter their numbers declined in the presence of TPO alone (Figure 5C). To test whether other cytokines/mediators also affect platelet production, we examined the effects of the combinations shown in Figure 5D.25,36,37 Heparin (25 U/mL), SCF (50 ng/mL), and TPO (100 ng/mL) were the most effective stimulators of platelet production via protocol 2, whereas IL-6 and IL-11 had no effect on production, which is quite different from what is seen with mouse ESC-derived platelets (H. Nishikii, K.E., N. Tamura, K. Hattori, B. Heissig, T. Kanaji, A.S., S. Goto, J. Wave, H. Nakaushi, manuscript submitted). On average, 4.8 (± 0.2) × 10⁶ platelets were generated from an initial 10⁵ hESCs (KhES-3) on day 24 in a cocktail of TPO, SCF, and heparin. This can be considered the lower limit of the yield, as platelets are inevitably lost during the collection and purification procedures. C3H10T1/2 and OP-9 stromal cells proved equally efficacious in their ability to support platelet generation (data not shown).

Platelet production from mature megakaryocytes is likely influenced by multiple factors within the bone marrow microenvironment.32,38 Thus, while our new protocol introduces a number of intercellular or intratissue mediators that were lacking in earlier in vitro coculture systems, additional developments would be expected to increase the efficiency of platelet generation, thereby further advancing the process toward the ultimate aim of use in a clinical setting.

**hESC-derived platelets can activate integrin and undergo integrin-dependent actin cytoskeletal changes**

Stimulating platelets with an agonist changes the conformation of integrin αIIbβ3 and promotes clustering. This inside-out activation of αIIbβ3 promotes the binding of its ligands, principally fibronogen, but also von Willebrand factor. These ligands in turn promote outside-in signaling to induce the cytoskeletal changes and spreading required for production of stable platelet thrombi in vivo.39 To explore the functionality of hESC-derived platelets, we used flow cytometry to examine αIIbβ3 activation following stimulation with the major platelet agonists, thrombin and ADP.
Platelet-sized particles were collected from culture supernatants and incubated with anti-human CD42b (GPIb/IIb/H9251) antibody and with PAC-1, an antibody that mimics the specific fibrinogen binding to human GPIb/IIb/IIa in the activated state. Administration of ADP induced concentration-dependent increases in PAC-1 binding to hESC-derived platelets (Figure 6A,B). The binding was specific for αIIbβ3, as 10 μM tirofiban, a selective inhibitor to human αIIbβ3, reversed the agonist-induced PAC-1 binding. Similar results for PAC-1 binding were observed when platelets were stimulated with thrombin (data not shown).

Stable thrombus formation requires actin reorganization through αIIbβ3 integrin outside-in signaling. To observe integrin-mediated changes in the actin cytoskeleton, such as the assembly of filopodia, lamellipodia, and/or stress fibers, hESC-derived platelets were allowed to adhere to fibrinogen-coated cover glass and then stained for F-actin and labeled with anti-CD41a antibodies. As shown in Figure 6C, hESC-derived platelets formed filopodia, even in the absence of an agonist. In addition, they also formed lamellipodia and actin stress fibers when stimulated with 50 μM ADP or 1.0 U/mL thrombin. Thus our hESC-derived platelets exhibit a number of major functional responses established for plasma-derived platelets, at least in these in vitro assays.

In the present study, we report the establishment of a novel culture method for generating ES-sacs—unique, balloonlike structures that provide a microenvironment for the generation and differentiation of hematopoietic progenitors. Cells within ES-sacs are enriched in progenitors with multilineage differentiation potential, enabling megakaryocytes and platelets to be more efficiently produced in vitro than reported previously. Here we tested 2 different protocols for the production of platelets from hESCs (Figure 1Ai,ii). Protocol 2 differs from that of Gaur et al (protocol 1) in which megakaryocytes, but not platelets, were generated after multiple rounds of passaging hESC-derived progenitors grown on OP-9 cells. Our finding that generation of ES-sacs requires continuous culture for at least 2 weeks, without passaging, suggests protocol 1 does not allow sufficient time for cells to develop into ES-sacs. Moreover, the fact that ES-sacs appear to provide a suitable environment for highly prolific hematopoietic progenitors suggests the generation of ES-sacs may be indispensable for efficient production of fully differentiated hematopoietic cells, including platelets (Figures 4,5; Figure S1). It was previously reported that protocol 1 has the potential to generate 1 to 4 × 10⁴ megakaryocytes, but not platelets, from 10⁵ hESCs.
Figure 6. Integrin activation and actin cytoskeletal reorganization in hESC-derived platelets. (A) Representative dot plots for platelets binding FITC-conjugated PAC-1 in the absence (left panel) or presence (middle panel) of 50 μM ADP. The right panel shows inhibition of PAC-1 binding by 10 μM tirofiban. Numbers on plots are the percentages of total cells within each quadrant. (B) Binding of FITC-conjugated PAC-1 to hESC-derived platelets was quantified in the absence and presence of ADP by flow cytometry. Some specimens were also incubated with tirofiban. Data depict means (± SD) from more than 3 independent experiments. (C) hESC-derived platelets spreading on fibrinogen-coated cover glass in the absence and presence of 50 μM ADP or 1.0 U/mL thrombin. Cells were fixed, permeabilized, and stained with rhodamine-phalloidin to label F-actin (red) and anti-CD41a antibody followed by Alexa 488-conjugated secondary antibody (green). αIIbβ3-dependent formation of stress fibers, lamellipodia, and filopodia was observed. Bar represents 10 μm.

By contrast, protocol 2 consistently generated more than 2 to 5 × 10⁵ platelet-producing megakaryocytes, improving the efficiency by at least one order of magnitude (Figure 1Aii). Still, the megakaryocytes in this system yielded fewer platelets than megakaryocytes do in vivo, where approximately 2000 platelets can be generated per megakaryocyte.³⁸ This may indicate that some stimulus of thrombopoiesis (eg, shear flow⁴⁰) is lacking in the in vitro environment. Further improvement of our method will be required before we are able to obtain numbers of functional platelets that are sufficient for posttransfusion in vivo functional studies and, ultimately, clinical application. Genetic manipulation may prove to be a useful means of overcoming this challenge, as is exemplified by HoxB4 overexpression in ESC-derived hematopoietic stem cells.⁴¹ Nevertheless, our study provides a setting for future molecular studies of human thrombopoiesis and for further development of culture methods to generate platelets from hESCs.

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Authorship

Contribution: N.T., K.E., and H. Nakauchi designed the research and wrote the paper; K.E. edited the paper; N.T., H. Nishikii, J.U., H.T., A.S., and K.E. performed experiments and analyzed data; T.H. provided critical information for the novel methodology. Conflict-of-interest disclosure: N.T., H. Nishikii, K.E., and H. Nakauchi have applied for a patent related to the methodology described in the present work. The other authors declare no competing financial interests.

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References


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