Stem Cell Research (2013) 11, 1074–1090

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

# Endothelial cells provide a niche for placental hematopoietic stem/progenitor cell expansion through broad transcriptomic modification☆

Christophe M. Raynaud[a], Jason M. Butler[b], Najeeb M. Halabi[b,c], Faizzan S. Ahmad[c], Badereldeen Ahmed[d], Shahin Rafii[b], Arash Rafii[b,c,*]

[a] Qatar Cardiovascular Research Center, Qatar Foundation, Qatar Science and Technology Park, Doha, Qatar
[b] Department of Genetic Medicine, Weill Cornell Medical College, NY, USA
[c] Stem Cell and Microenvironment Laboratory, Weill Cornell Medical College in Qatar Education City, Qatar Foundation, Doha, Qatar
[d] Maternal and Fetal Unit, Women's Hospital, Hamad Medical Corporation, Doha, Qatar

Received 9 January 2013; received in revised form 17 July 2013; accepted 31 July 2013
Available online 9 August 2013

**Abstract** Umbilical cord blood (UCB) is an attractive source of hematopoietic stem cells (HSCs). However, the number of HSCs in UCB is limited, and attempts to amplify them in vitro remain inefficient. Several publications have documented amplification of hematopoietic stem/progenitor cells (HSPCs) on endothelial or mesenchymal cells, but the lack of homogeneity in culture conditions and HSC definition impairs direct comparison of these results. We investigated the ability of different feeder layers, mesenchymal progenitors (MPs) and endothelial cells (ECs), to amplify hematopoietic stem/progenitor cells. Placental derived HSPCs (defined as $Lin^-CD45^{-/dim}CD34^+CD38^-CD90^+$) were maintained on confluent feeder layers and the number of cells and their marker expression were monitored over 21 days. Although both types of feeder layers supported hematopoietic expansion, only endothelial cells triggered amplification of $Lin^-CD45^{-/dim}CD34^+CD38^-CD90^+$ cells, which peaked at 14 days. The amplified cells differentiated into all cell lineages, as attested by in vitro colony-forming assays, and were capable of engraftment and multi-lineage differentiation in sub-lethally irradiated mice. Mesenchymal progenitors promoted amplification of $CD38^+$ cells, previously defined as precursors with more limited differentiation potential. A competitive assay demonstrated that hematopoietic stem/progenitor cells had a preference for interacting with endothelial cells in vitro. Cytokine and transcriptomic analysis of both feeder cell types identified differences in gene expression that correlated with propensity of ECs and MPs to support hematopoietic cell amplification and differentiation respectively. Finally, we used RNA sequencing of endothelial cells and HSPCs to uncover relevant networks illustrating the complex interaction between endothelial cells and HSPCs leading to stem/progenitor cell expansion.


☆ Author contributions: Christophe M. Raynaud: conception, design and conduct of experiments; data analysis; editing of the manuscript; Jason M. Butler: conception, design and analysis of in vivo analysis; Najeeb M. Halabi: genomic data analysis; Faizzan S. Ahmad: assistance in editing the manuscript; Ahmed Badreldeen: assistance with provision of tissue and cord blood samples; Shahin Rafii: assistance with design of the experiments and provision of the cell line; Arash Rafii: assistance with designing the experimental procedures; writing the manuscript.

* Corresponding author at: Department of Genetic Medicine and Obstetrics and Gynecology, Weill Cornell Medical College, Stem Cell and Microenvironment Laboratory, WCMC-Qatar, Education City, 24144 Doha, Qatar. Fax: +974 4492 8422.
*E-mail address:* jat2021@qatar-med.cornell.edu (A. Rafii).

1873-5061/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scr.2013.07.010

## Introduction

There is an increasing need of HSC transplantation for treatment of hematological disorders. Bone marrow is the main source of hematopoietic stem cells (HSCs) (Ballen, 2005; Broxmeyer et al., 1989, 1990, 1991; Copelan, 2006). However researchers have investigated other sources of HSCs to overcome the challenges of finding compatible donors and the invasiveness of the procedure. Umbilical cord blood derived HSCs have been extensively investigated and used in the clinical setting (Tse et al., 2008a, 2008b). However, single cord blood samples contain insufficient numbers of HSCs to effectively treat adults requiring bone marrow transplantation (Brunstein et al., 2007; Majhail et al., 2006).

To overcome these threshold limitations, researchers have investigated optimal methods for ex vivo HSC amplification. Schiedlmeier et al. used the ectopic expression of HOXB4 to obtain significant HSC expansion (Schiedlmeier et al., 2007). Several studies have investigated the use of multiple cytokines to increase HSC numbers in culture without being able to clearly define the optimal cocktail (Metcalf, 2008; Zhang and Lodish, 2008). Delaney et al. amplified HSCs on Jagged 1 coated plates, but the expanded HSCs failed to achieve long-term engraftment, suggesting amplification of hematopoietic progenitors (Delaney et al., 2010).

Recently, investigators have tried to define the HSC niche in vivo (Levesque et al., 2010) in order to use its signaling pathways to expand HSCs while maintaining their stemness. The presence of HSCs in the cord blood suggests that the placenta and its cellular elements might also contain a niche for HSCs. Indeed hematopoietic progenitors and long-term culture-initiating cells have been found in the human placenta between 8 and 17 weeks of gestation (Barcena et al., 2009; Chen et al., 2004). Several follow-up studies also described the role of placenta in amplification of HSCs up to term (Robin et al., 2009; Serikov et al., 2009; Zhang et al., 2004).

The close proximity of HSCs to cellular elements in the placenta and the bone marrow suggests a requirement for complex signaling networks and has motivated the development of cellular based HSC expansion platforms. Many studies describe the expansion of HSCs on mesenchymal progenitor (MP) or endothelial cell (EC) feeder layers (Andrade et al., 2010; Butler et al., 2010; Fei et al., 2007; Hayashi et al., 2009; Huang et al., 2007; Jang et al., 2006; Levesque et al., 2010; Li et al., 2006; Mendez-Ferrer et al., 2010; Robin et al., 2009; Sasaki et al., 2010; Walenda et al., 2010; Yildirim et al., 2005; Zhang et al., 2004). HUVECs (human umbilical vein endothelial cells) have been used as a model for an endothelial niche (Butler et al., 2010; Kobayashi et al., 2010; Li et al., 2006; Yildirim et al., 2005) while bone marrow- and placental-derived mesenchymal progenitors (BM-MPs and PL-MPs) have been used as a surrogate for a mesenchymal niche (Andrade et al., 2010; Hayashi et al., 2009; Macmillan et al., 2009; Mendez-Ferrer et al., 2010; Resnick et al., 2010; Walenda et al., 2010). For instance, Robin et al. demonstrated that placenta derived perivascular stromal cells expressing CD13, CD29, CD44 and CD105 could support hematopoiesis in the placenta (Robin et al., 2009). Similarly, Yildirim et al. published that HUVECs could expand cord blood CD34+ cells (Yildirim et al., 2005). The hypothesis underlying these experiments is that secreted factors and membrane bound elements of the feeder cells will provide the signaling cues allowing cell expansion with maintenance of stemness. However, due to large variations in the cytokines used, a standardized protocol for the expansion of clinical grade HSCs is yet to be devised. Furthermore, the use of fetal bovine serum (FBS) may also modify the effects of components produced or secreted by the feeder cells themselves, confounding attempts to identify the key feeder derived factors required for HSC expansion.

Recently, our group elucidated the role of the endothelium and secretion of angiocrine factors in the expansion of mouse and human HSCs (Butler et al., 2010a, 2010b; Kobayashi et al., 2010). We developed a model of endothelial cells with autonomous Akt-activation (HUVECs-*E4ORF1*, referred to as $E4^+$ECs) that can survive in the absence of FBS and exhibit secretion of angiocrine factors. We demonstrated that the $E4^+$EC platform supported the expansion of serially engraftable HSCs. Similarly, we have isolated and comprehensively characterized mesenchymal progenitors from the placenta which are also able to survive in serum free conditions (Raynaud et al., 2012). However, few studies to date have systematically compared the capacity of endothelial and mesenchymal progenitors to facilitate the expansion of UCB-derived HSPCs. Therefore, in this study we compare the abilities of our previously engineered $E4^+$EC platform and mesenchymal progenitors to support hematopoietic stem/progenitor cell (HSPC) proliferation in a serum-free environment.

## Methods

### Cord/placental blood collection and fluorescence-activated cell sorting of HSPCs

Following approval from the Internal Review Board (HMC-IRB protocol 9109/09, Weill Cornell Medical College, Qatar), cord blood and placental tissue were collected from donors at the Women's Hospital at Hamad Medical Corporation immediately after elective Cesarean section. Exclusion criteria were absence of labor, preterm rupture of membrane (chorioamnionitis), and the presence of known chromosomal abnormalities.

In this study to retrieve the cord blood and placental hematopoietic stem and progenitor cells (HSPCs), we carefully aspirated and milked the placenta through the cord after detachment of the placenta. To obtain sufficient numbers of HSPCs, two to three cord/placenta samples were pooled for each experiment. Each experiment was repeated three to six times, and error bars in figures represent variability in the analysis of biological replicates.

Mono-nucleated cells were isolated after centrifugation on Ficoll gradients. $CD34^+$ cells were enriched using magnetic beads labeled with anti-CD34 monoclonal antibodies (Miltenyi Biotech, 130-046-702) and separated on an affinity column following the manufacturer's instructions. Hematopoietic stem/progenitor cells defined as $Lin^-CD45^{dim/-}CD34^+CD38^-CD90^+$ (Supplementary Fig. 1), were further purified by fluorescence-activated cell sorting (FACS). The following antibodies were used anti-CD34-APC-Cy7 (BD Biosciences, 343514), CD38-PerCP-Cy5.5 (BD Biosciences, 551400), CD90-AF700 (BD Biosciences, 550402), CD45-AmCyan (BD Biosciences,