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Blood 142 (2023) 2670–2671

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Capturing Endothelial-to-Hematopoietic Transition Using a Highly Efficient in Vitro Platform

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Introduction

De novo generation of hematopoietic stem and progenitor cells (HSPCs) for therapeutic use has been a long-standing goal of regenerative medicine. During development, the first definitive hematopoietic stem cells (HSCs) derive from a small number of specialized vascular endothelial cells (EC), named hemogenic endothelial cells (HECs), located in the Aorta-Gonado-Mesonephrose (AGM). The process of HEC conversion to HSPCs is known as endothelial-to-hematopoietic transition (EHT). During EHT, endothelial features are lost while switching on hematopoietic transcriptional programs leading to drastic functional and morphological changes that generate free-floating, round hematopoietic cells from large, flat, anchorage-dependent ECs. Key steps regulating EHT are difficult to elucidate using *in vivo* models because the hemogenic capacity of HECs occurs within a 2-day developmental window. Only a tiny number of cells undergo EHT *in vivo*. New model systems that overcome current *in vivo* obstacles are required so the details of EHT and the mechanisms that lead to the generation of HSPCs can be better understood. We previously reported that fully-committed ECs can be converted to functional HSPCs by ectopic expression of transcription factors *Spi1, FOSB, GFI1, and Runx1* (SFGR) and an appropriate vascular niche (Sandler et al. Nature, 2014; Lis et al. Nature, 2017). But conversion using this method required several weeks and was associated with variable and low efficiency. Here, we show that our new *in vitro* EHT platform (ivEHT), very closely recapitulates the process of EHT *in vivo* and can be used to uncover heretofore unrecognized steps necessary for EC conversion to HSPCs.

Methods

All steps in the *in vitro* conversion of ECs to HSPCs were systematically analyzed and optimized. The refined model utilized new media constituents and polycistronic constructs to ensure ectopic expression of transcription factors *Spi1, FOSB, GFI1 and Runx1* (SFGR)in human umbilical vein-ECs (HUVECs) in appropriate ratios and sequence.

Results

The new ivEHT platform yields efficient EC conversion to hematopoietic like CD45+ cells (≥80%) and high temporal reproducibility of conversion that is largely complete within 96 hrs. The ivEHT sequence of conversion closely follows the timing and sequence of EHT *in vivo*. Initially, transduced HUVECs are large, flat cells with CDH5-marked intercellular junctions and firm adhesion to basement membrane. Within the first 24hrs of induction, SFGR-HUVECs downregulate ECs markers CD31, KDR, CDH5 and activate the endogenous RUNX1 locus detected by the expression of nuclear GFP (GFPn) under control of the human RUNX1+24 enhancer (detected using a lentiviral reporter vector). Day 2 (D2) is characterized by complete clearance of endothelial-specific Weibel-Palade Bodies (WPBs, marked by Von Willebrand Factor, VWF) and excess mitochondria. This window also initiates morphologic changes in RUNX1-GFPn marked cells with loss of intercellular junctions and attachment to basement membrane and initial expression of hematopoietic markers such as CD45. Days 3-4 are marked by loss of cell volume and cytoplasm, cellular rounding and detachment from matrix, and increased expression of CD45 and other hematopoietic markers like cKIT, MPL, CD43. These stages of conversion are only identified in RUNX1-GFPn marked cells and by the end of D5, ~80% of cells have fully converted to CD45+ cells. Conversion is not accompanied by cell proliferation, but after D5 cell numbers expand rapidly. The ivEHT platform is scalable and highly reproducible making it amenable to higher-throughput testing in 96-well format using flow cytometry readouts. Using the ivEHT platform, we have captured and validated in AGM explants previously unobserved aspects of early EHT that is complete between D2-D3.

Discussion

This work promises a reproducible, faithful, and scalable platform which generates hematopoietic like cells while successfully recapitulating early steps of the EHT in the AGM. Importantly, this platform is readily accessible and is easily customized for discovery of EHT molecular details currently hidden to science, allowing significant advances in the understanding of how HSPCs emergence *in vivo*.

Disclosures No relevant conflicts of interest to declare.

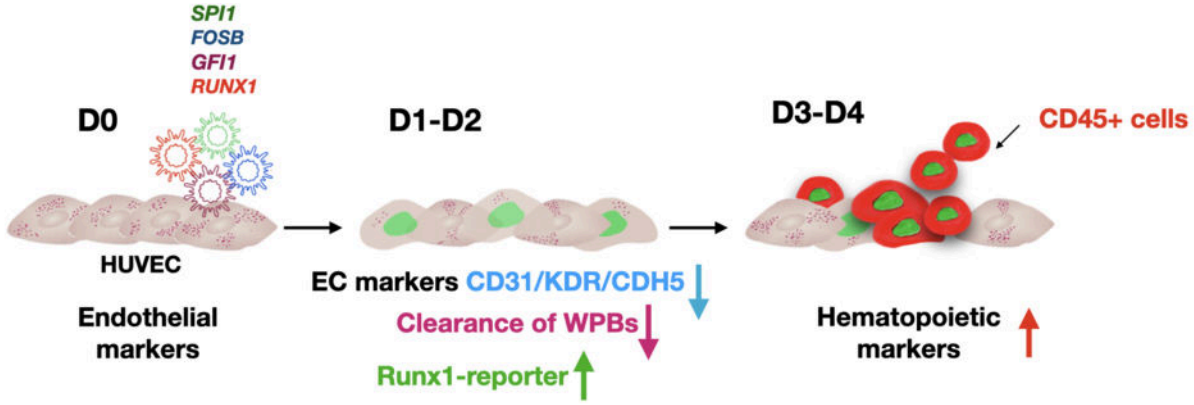

Figure1: Schematic representation of *in vitro* endothelial cell conversion to hematopoietic like cells stages and timing using ivEHT platform: freshly isolated HUVEC cells are transduced with 4 transcription factors Spi1, Runx1, GFI1 and FOSB. Upon induction of SFGR: D0-D2: SFGR-HUVEC down-regulate endothelial markers, CDH5, CD31, and KDR; D1-D3: transitioning cells upregulate Runx1-reporter (GFP), lose WPBs, become smaller and detach. D3-D4: GFP expressing cells become hematopoietic like CD45+ cells.

Figure 1

https://doi.org/10.1182/blood-2023-190072