Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Acta Astronautica 56 (2005) 918–922
ACTA ASTRONAUTICA
www.elsevier.com/locate/actaastro

Induction of vascular endothelial phenotype and cellular proliferation from human cord blood stem cells cultured in simulated microgravity

Brian Chiu[a,*], Jim Z-M Wan[a], Doris Abley[b], John Akabutu[b]

[a] *Department of Laboratory Medicine and Pathology, University of Alberta Hospitals, 5B4 14 Walter Mackenzie Centre, 8440 112 Street, Edmonton, Alberta, Canada T6G 2B7*
[b] *Alberta Cord Blood Bank, Edmonton, Alberta, Canada*

Available online 8 March 2005

Abstract

Recent studies have demonstrated that stem cells derived from adult hematopoietic tissues are capable of trans-differentiation into non-hematopoietic cells, and that the culture in microgravity (μg) may modulate the proliferation and differentiation. We investigated the application of μg to human umbilical cord blood stem cells (CBSC) in the induction of vascular endothelial phenotype expression and cellular proliferation.

CD34+ mononuclear cells were isolated from waste human umbilical cord blood samples and cultured in simulated μg for 14 days. The cells were seeded in rotary wall vessels (RWV) with or without microcarrier beads (MCB) and vascular endothelial growth factor was added during culture. Controls consisted of culture in 1 G. The cell cultures in RWV were examined by inverted microscopy. Cell counts, endothelial cell and leukocyte markers performed by flow-cytometry and FACS scan were assayed at days 1, 4, 7 and at the termination of the experiments. Culture in RWV revealed significantly increased cellular proliferation with three-dimensional (3D) tissue-like aggregates. At day 4, CD34+ cells cultured in RWV bioreactor without MCB developed vascular tubular assemblies and exhibited endothelial phenotypic markers.

These data suggest that CD34+ human umbilical cord blood progenitors are capable of trans-differentiation into vascular endothelial cell phenotype and assemble into 3D tissue structures. Culture of CBSC in simulated μg may be potentially beneficial in the fields of stem cell biology and somatic cell therapy.

*Corresponding author. Tel.: +780 407 6959; fax: +780 407 3009.
E-mail address: bchiu@cha.ab.ca (B. Chiu).

0094-5765/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.actaastro.2005.01.018

1. Introduction

1.1. Human umbilical cord blood stem cell (CBSC) and adult stem cell plasticity

In recent years, there has been an increased interest directed towards research in the biology of stem cells. It is generally assumed that once a cell commits to a given somatic cell lineage and acquires a defined phenotype, it can no longer change its fate. However, recent studies have suggested that some tissue-specific stem cells or progenitors may have differentiation potential outside of their tissue of origin. Under certain conditions, stem cells derived from adult hematopoietic cells are capable of trans-differentiating into non-hematopoietic cells such as brain, liver and cardiovascular cells [1]. Moreover, stem cells derived from non-hematopoietic tissue have been found to differentiate into hematopoietic cells [1].

Recent data have also shown that human umbilical cord blood contains a large number of pluripotent hematopoietic stem cells (HSC) [2]. The umbilical cord blood stem cells (CBSC) can be accurately identified by single platform flow cytometry using CD34 positive marker [3]. Transplanted cord blood-derived progenitor cells develop capillary-like structures and exhibit multiple endothelial phenotypic markers, such as CD31, KDR and von Willebrand factor [4].

1.2. Simulated microgravity culture environment

Several in vitro culture systems have been developed for investigating the cellular events in the formation of blood vessels. Cell cultures performed at unit gravity constrain cells to propagate, differentiate and interact in gravity-driven convection and sedimentation. The restricted diffusion of nutrients and oxygen limits their size with spheroids larger than 1 mm in diameter generally containing hypoxic and necrotic centre surrounded by a rim of viable cells [5].

Recently, rotating wall vessel (RWV) bioreactors, an earth-based culture system that simulates microgravity, have been developed at the National Aeronautics and Space Agency (NASA). The RWV consists of a family of vessels that are horizontally rotated, fluid-filled culture vessels equipped with membrane diffusion gas exchange to optimize gas/oxygen supply. The rotational speed is adjusted so that the culture medium and individual cells, pre-aggregated cell constructs or organ cultures rotate synchronously with the vessel, thus providing for low-shear stress, low-turbulence without gas/fluid interface [6]. The time-averaged gravitational vector acting on the cellular assemblies is reduced to about 10^{-2} G [7]. Low shear stress promotes close apposition of the cells forming three-dimensional (3D) structures. The randomized gravitational vectors either directly affect gene expression or indirectly facilitate paracrine/autocrine intercellular signaling through restricted diffusion of differentiative humoral factors [8].

Since its invention in 1992, the RWV bioreactor has been used in terrestrial laboratories and has demonstrated success with many different cell types [6], tumor organoids and microorganisms. Plett and colleagues recently studied human bone marrow CD34+ HSC cultured in simulated microgravity. Their data suggested that microgravity cultures stimulate proliferation of HSC and progenitors and cellular proliferation lasted a longer period of time than control cultured at 1 G [9].

2. Goals and objectives of this study

In this study, we hypothesized that trans-differentiation of HSC to vascular cells is influenced by microenvironment. Our aims were to enhance the microenvironment of cultured CBSC to encourage trans-differentiation into vascular cells, and to characterize and compare the morphologies and cellular marker expressions of trans-differentiated cells.

3. Experimental design

3.1. Human umbilical CBSC enumeration and isolation

Human umbilical cord blood samples (50 ml each) were collected in sterile blood packs containing citrate-dextrose solution according to established protocols. Written informed consent was obtained from all mothers before labor and delivery. Established protocols for processing human umbilical cord blood had been approved by the University of Alberta Ethics Review Board [4]. Enumeration and isolation