STEM/PROGENITOR CELLS IN HUMAN UMBILICAL CORD BLOOD: PROLIFERATIVE POTENTIAL FOR IN VITRO CARDIOGENESIS AND ANGIOGENESIS

THESIS

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Abstract

Recently, stem cell based cell therapy has become a realistic option to replace damaged cardiomyocytes. Most studies on stem cell transplantation therapy have focused on the use of undifferentiated stem cells. There is a strong possibility that some cardiogenic differentiation of stem cell in vitro prior to transplantation would result in higher engraftment efficiency, as well as enhanced myocardial regeneration and recovery of heart function. In this thesis we aimed to define the conditions for ex-vivo differentiation of cord blood stem cells to cardiomyocytes and endothelial cells. These conditions include the combination of VEGF; FGF-2 and PDGF-AB growth factors. Forty cord blood samples were included in this work. In this work, the percentage of CD 34+ cells and CD 34/31+ cells in MNC suspension was counted prior to culture (day zero), and day 10 in the different growth factors cocktails used as well as the control tube, then from which the fold increase of CD 34+ cells and CD 34/31+ cells was calculated. Detection of cardiac troponin I in the cultured cells to confirm the cardiac differentiation was done at day 10 using Mouse anti-troponin I monoclonal antibody. From the present study, it can be concluded that cytokines cocktail in protocol 2 (FGF2+VEGF+PDGF-AB) gives better in vitro trans-differentiation of stem/progenitor cells in umbilical cord blood into cardiomyocytes and endothelial cells than cytokines cocktail in protocol 1 (FGF2+VEGF) alone.

Key words: cord blood- stem cells- in vitro cardiogenesis- cardiomyocytes

List of Contents

List of Figures

List of tables

List of Abbreviations

Ab…… Amyloid beta peptide

AD…… Alzheimer's disease

ALS……Amyotrophic lateral sclerosis

AMI……Acute myocardial infarction

APC……Antigen presenting cells

ASC…….. ..Adult stem cells

BBB…… Blood-brain barrier

bFGF…… ..Basic Fibroblast growth factor

BM…….. …Bone marrow

BMSCs……Bone marrow stem cells

CABG…….Coronary artery bypass surgery

CB……. .Cord blood

CB-SCs…….....Cord blood stem cells

CI…….. ..Cerebral ischemia

CKD…… Chronic kidney disease

CM……...Conditioned medium

CMP……Common myeloid progenitors

CPCs……. …..Cardiac progenitor cells

CSCs……Cardiac stem cells

EB……. …..Epidermolysis bullosa

ECs……..Endothelial cells

EGC……. .Embryonic germ cells

EGF…….. .Epidermal growth factor

EMT……...Epithelialto-Mesenchymal transition

EO-EPCs…….. .Early-outgrowth endothelial progenitor cells

EPCs……..Endothelial progenitors

ESCs……..Embryonic stem cells

ESRD……..… …Endstage renal disease

FGF……Fibroblast growth factor

G-CSF……...Granulocyte colony stimulating factor

GFP……..Green fluorescent protein

GM-CSF……..Granulocyte/macrophage colony-stimulating factor

GvHD……...Gravt versus host disease

GvL……..Gravt versus leukaemia

hASCs……. Human adipose tissue-derived stem cells

HF……... Heart failure

HGF……Hepatocyte growth factor

HMGB…… High-mobility group box protein

HPCs……... Haematopoietic progenitors

HSCs……Haematopoietic stem cells

HUVEC……Human umbilical vein endothelial cells

i.v……..Intravenously

ICM……..Inner cell mass

IGF-1…… Insulin-like growth factor-1

IL……...Interleukin

iPS…….Induced pluripotent stem cells

KDR……. Kinase insert domain receptor

LIF…… Leukemia Inhibitory Factor

LO-EPCs…….. Late-outgrowth endothelial progenitor cells

LSCD…… Limbal SC deficiency

LV……..…Left ventricular

maGSCs…… Multipotent Adult Germline Stem Cells

MCAO……. Middle carotid artery occlusion

MHC…… Major histocompatibility complex

MI…….Myocardial infarction

MLP……..Multipotential lymphoid progenitors

MSCs…… Mesenchymal stem cells

NK…….Natural killer cells

NSCs…….Neural stem/progenitor cells

OA…….Osteoarthritis

PCI…….Percutaneous coronary intervention

PCNA…… Proliferating cell nuclear antigen

PD…….. Parkinson's disease

PDGF……..Platelet derived growth factor

PDMC…….. ..Placental-derived multipotent cells

PECAM-1……Platelet/endothelial cell adhesion molecule-1

PSCs……Pluripotent stem cells

SC……Stem Cells

SCF……. Stem cell factor

SDF…… Stromal cell– derived factor

SJS……..Stevenson-Johnson syndrome

SMαA+…….. Smooth muscle αA+

T1D……Type1Diabetes

TNF……Tumor necrosis factor

Tβ4…….Thymosin β4

UCB……Umbilical cord blood

USSC…….. Unrestricted somatic stem cells

VE……Vascular-endothelium

VEGF……Vascular endothelial growth factor

VEGFR…….Vascular endothelial growth factor receptor

VSEL…… Very small embryonic/epiblast-like

VSMCs……..Vascular smooth muscle cells.

WJ-MSCs……Wharton's jelly Mesenchymal stem cells

Introduction and aim of the work

The ability of stem cells to renew their own population and to differentiate into specialized cell types has always attracted researchers looking to exploit this potential for cellular replacement therapies, pharmaceutical testing and studying developmental pathways **(Walia et al., 2012)**.

Despite of vast improvements in treatment, myocardial infarction often leads to heart failure (HF) which remains the leading cause of death in developed countries. Other than heart transplantation, therapeutic options have a limited role in improving outcomes in patients with severe HF. It is therefore no surprise that cardiac cell therapy has raised many hopes as a novel therapeutic approach aimed at cardiac myocytes replacement - regeneration termed "cellular cardiomyoplasty"**(Pendyala et al.,2008).**

Regenerative medicine with vascular growth factor and stem cell therapy have within the last decennium had great interest and have been tested in clinical trials in patients with ischemic heart disease. The aim is to induce growth of new blood vessels or replacement of damaged myocardial cells either directly by trans-differentiation of stem cells or by a paracrine effect of cytokines secreted from the stem cells **(Kastrup, 2011).**

SCs show high plasticity, i.e. the complex ability to cross lineage barriers and adopt the expression profile and functional

phenotypes of the cells that are typical of other tissues. The plasticity can be explained by trans-differentiation (direct or indirect) and fusion **(Lodi et al., 2011).**

Stem cells have been categorized as (i) embryonic stem cells (ESCs), (ii) cord blood stem cells (CB-SCs), and (iii) adult stem cells (ASC). Each one of these populations has been characterized and further divided into sub-populations using genotyping assays and phenotypic expression of markers **(Francese and Fiorina, 2010).**

It has been shown that CB stem cells have the ability to regenerate numerous tissue types, and when transplanted into animals and humans, have produced measurable functional improvements. Generally, tissue-derived stem cells have been described for neural, muscle, retinal, pancreas, skin and liver tissues but these tissue-specific stem cells have limited self-renewing capabilities and are unable to reconstitute a whole organ system **(Harris, 2009)**.

Recently, cord blood (CB) is considered an important source of many types of stem cells, including haematopoietic stem cells (HSCs), endothelial progenitors (EPCs), mesenchymal stem cells (MSCs), very small embryonic/epiblast-like (VSEL) stem cells, and unrestricted somatic stem cells (USSC), potentially suitable for use in regenerative medicine **(Pelosi et al., 2012)**.