TELOMERASE ACTIVITY IN CORD BLOOD

Thesis

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Clinical Pathology

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To a great Father

To my Mother, the source of hope in my life

To a precious Sisters

To a special Husband

To my sweet Kids

Abstract

Ex-vivo expansion of haematopoietic progenitor cells from umbilical cord blood is an interesting strategy to obtain a sufficient number of transplantable cells. Telomerase may contribute to the capacity for cell replication by compensating for telomere loss. Exploring the use of different cytokine combinations in increasing cellular replicative potential through telomerase activity may be useful for in vitro expansion of haematopoietic stem cells for transplantation. In this study, expansion of cord blood mononuclear cells was done in the presence of IL-3 and stem cell factor. Telomerase activity was assessed before and after one week of culture by a modified version of the telomeric repeat amplification protocol (TRAP). Cord blood samples expressed a basal level of telomerase activity which was increased in 80% of samples after expansion. However this increase did not reach a statistically significant level. The results of this work suggest that IL-3 and stem cell factor slightly increased telomerase activity of expanded cord blood cells. However, the addition of more cytokines to the culture might be useful to reach the optimal culture conditions allowing greater induction of telomerase activity and the generation of haematopoietic progenitor cells that retain their proliferative capacity.

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List of abbreviations

AA……Aplastic Anemia

ACD……Anticoagulant citrate dextrose.

ALL……Acute lymphoblastic leukemia.

AMD3100……An inhibitor of chemokine receptor 4 (CXCR4).

AML……Acute myeloid leukemia.

Anti-HCV ……Anti-Hepatitis C virus

APB……..Adult peripheral blood.

BCR……..B cell antigen receptor.

BFU-E……..……Burst forming unit-Erythroid.

BFU-MK ……Brust forming unit-Magkaryocyte.

BM ……...Bone marrow.

BMT……..Bone marrow transplant.

CB……..Cord blood.

CBSCT ……..Cord blood stem cell transplantation.

CBSCs…….Cord blood stem cells.

CCG……...A children's cancer group study.

CD …….Cluster of diffrentiation.

CFC ……Colony forming cells.

CFU-MK……Colony forming unit- megakaryocyte

CFU-GM……Colony forming unit-Granulocyte,Macrophage

CFU-GEMM……. Colony forming unit-Granylocyte, Eosinophil, Macrophage, Megakaryocyte

CIBMTR……....Center for International Blood and Marrow Transplant Research.

CLL ……Chronic lymphocytic leukemia.

CML…….Chronic myeloid leukemia

CMV……..…...Cytomegalovirus.

CNS ……Central nervous system.

CR……..… Complete response.

CSC……..Cord stem cell.

DCs …….Dendritic cells.

DIG……. Digoxigenin.

DMSO……..Dimethyl sulfoxide.

DNA ……Deoxyribose Nucleic Acid.

EBV-HLH…… Epstein-Barr virus associated hemophagocytic lymphohistiocytosis.

ELISA…… The Enzyme-Linked ImmunoSorbent Assay.

Epo……..…Erythropoietin.

EST……..…Ever short telomere.

FL…….……Fetal liver.

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

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

GVHD…….Graft versus host disease

GVL…….……Graft versus leukemia effect

HBsAg……. Hepatitis B surfes antigen

HCAb……....Hepatitis C antibody

HCL……..Hairy cell leukemia.

HEPA……..……..... High Efficiency Particulate Air (filter/mask).

HES……...Hydroxyethyl starch

HLA…….. Human leucocytic antigen

HPC……...…….Haematopoietic progenitor cell.

HRP…….. Horseradish peroxidase

HSC……..…Hematopoietic stem cell

HSCT……Hematopoietic Stem Cell Transplantation

HSPC……..Hematopoietic stem/progenitor cells

hTERT……..…. Human Telomerase Reverse Transcriptase

hTR……..…..Human telomerase RNA component

IGF-I…… Insulin-like growth factor-1.

IS…….. Internal standard.

IL-2…….Interleukin-2.

IL-3…….Interleukin-3.

IL-4……Interleukin-4.

IL-5…….Interleukin-5.

IL-6…….Interleukin-6.

Kbp……. Kilobasepairs.

LTC-ICs……..…Long term culture intiating cells.

MCL……....Mantle cell lymphoma.

MDS ……..…Myelodysplastic Syndrome.

MoAb……..…Monoclonal antibody.

MM……..... Multiple myloma.

MMF……..Mycophenolate mofetil

MNCs…….Mononuclear cells.

MTP…….. Microtiter plate.

NA……..... Not applicable.

NMDP……..…..The National Marrow Donor Program.

PB……..……Peripheral blood.

PBSCT……..… Peripheral blood stem cell transplantation.

PCR……....Polymerase chain reaction.

PNAs…….. ...Peptide nucleic acids.

RBCs……. Red blood Corpuscle.

rh G-CSF……Recombinant human granulocyte colony-stimulating factor.

rh GM-CSF……Recombinant human granulocyte-macrophage colony-stimulating factor.

rh SCF……Recombinant human stem cell factor.

RNA ……..…….Ribose Nucleic Acid.

SCF……..…… Stem cell factor.

STF…..…… flt3/flk2 ligand.

TBI ……Total-body irradiation.

TCR…… T cell receptor.

TMB……..Tetramethylbenzidine.

TNF…….. Tumor necrosis factor.

TP1……. Telomerase-associated protein 1.

Tpo…… Thrombopoietin.

TRAP……..Telomeric repeat amplification protocol.

TRF…….Telomere restriction fragments.

TrflP……..…Telomeric repeat binding factors-1.

Trf2P……Telomeric repeat binding factors-2.

TRLI……Transplantation-related lung injury.

UCB…….. Umbilical cord blood.

UCBT…… Umbilical cord blood transplantation.

VOD……. Veno-occlusive disease.

WBC…..……..… White blood cell.

Introduction And Aim of work

Introduction & Aim of the Work

Stem cells possess the unique ability of self-renewal and multilineage differentiation. These combined properties are reflected in the ability of a hematopoietic stem cell (HSC) to completely and durably reconstitute hematopoiesis of a myeloablated recipient and maintain it throughout the entire life span ***(Kondo et al., 2003).***

HSC self-renewal is not a perfect process and daughter cells have progressively reduced proliferative capacity, due in part to progressive telomere erosion with each cell division. The length of telomeres decreases with increase in age in vivo and with cell division in vitro in haemopoietic stem cell (***Vaziri et al., 1994).*** This, in turn, leads to proliferative senescence that can be observed both in vivo and in vitro.

Telomeres are structures at the end of eukaryotic chromosomes that protect chromosomes from degradation, fusion, and recombination. In mammalian cells, they consist of hexanucleotide (TTAGGG) repeats and several associated protein components. In the absence of compensatory mechanisms, dividing cells undergo gradual telomere erosion. When telomeres reach a critical degree of shortening, cells recognize this as DNA damage and initiate proapoptotic programs or enter senescence ***(Granger et al., 2002).***

Human telomerase, an RNA-dependent DNA polymerase, can compensate for the loss of telomere length by synthesizing new telomeric repeats (TTAGGG) n, complimentary to human telomerase RNA component (hTR) ***(Feng et al., 1995).***Telomerase is a ribonucleoprotein complex consisting of an RNA template complementary of telomeric