

The Effect of Mesenchymal Stem Cells on Mitochondrial DNA Copy Number of Expanded Umbilical Cord Blood CD34+ Cells

BY : FATEMEH MANSOORI

➤ Introduction

➤ Design and
Methods

➤ Results

➤ Discussion



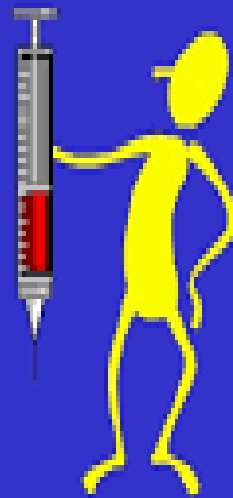
Introduction

Benefits of CB

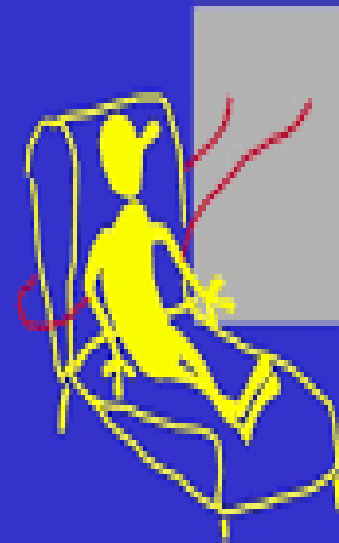
- Extends donor pool
- Lack of donor attrition
- Shortened time to transplant
- No safety risks for mother or child with CB collection
- HLA-mismatch well tolerated
- Thought to be lower incidence of (chronic) GVHD

Sources of Hematopoietic Stem Cells

Bone Marrow

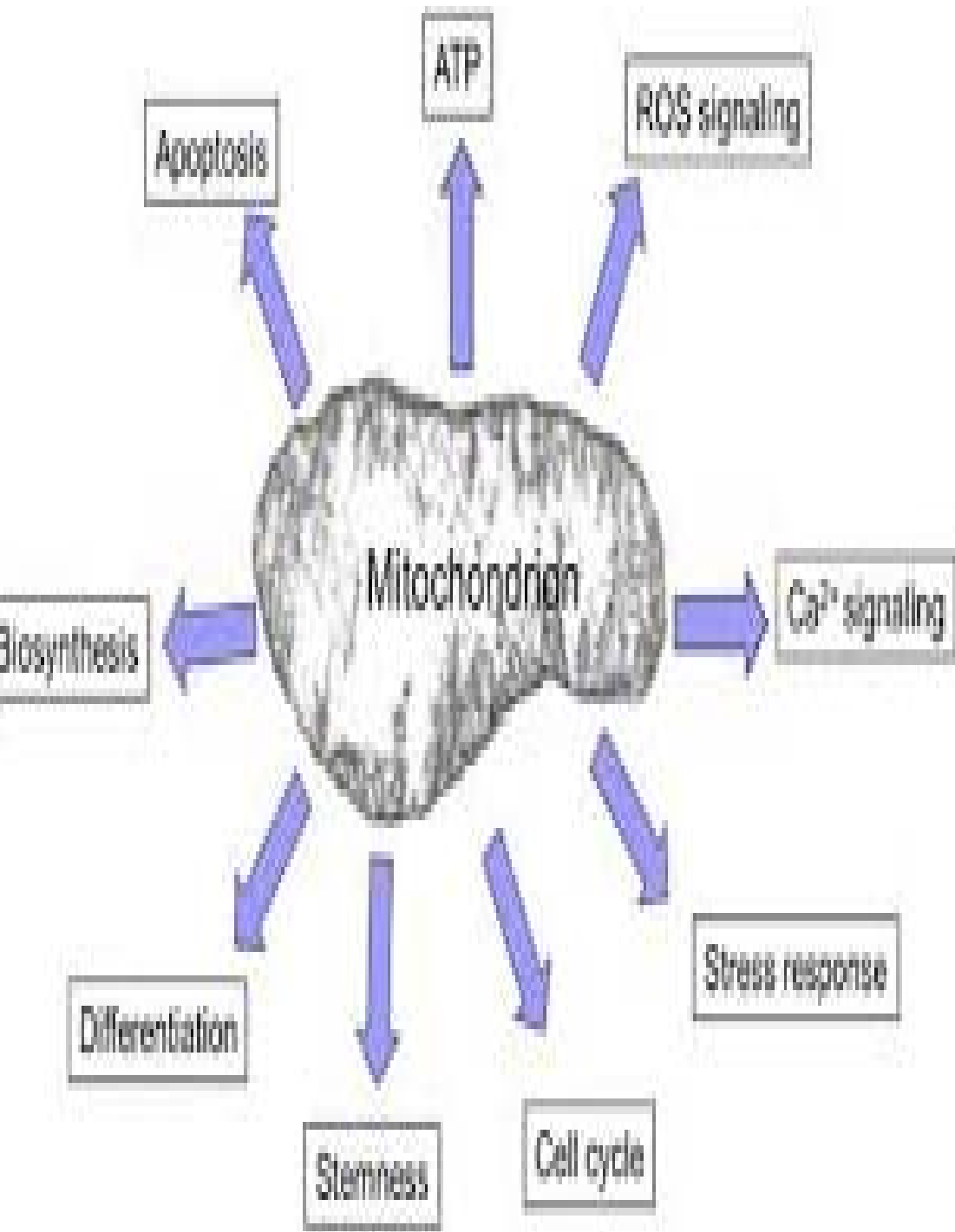


Peripheral Blood Stem Cells (PBSC)



Cord Blood

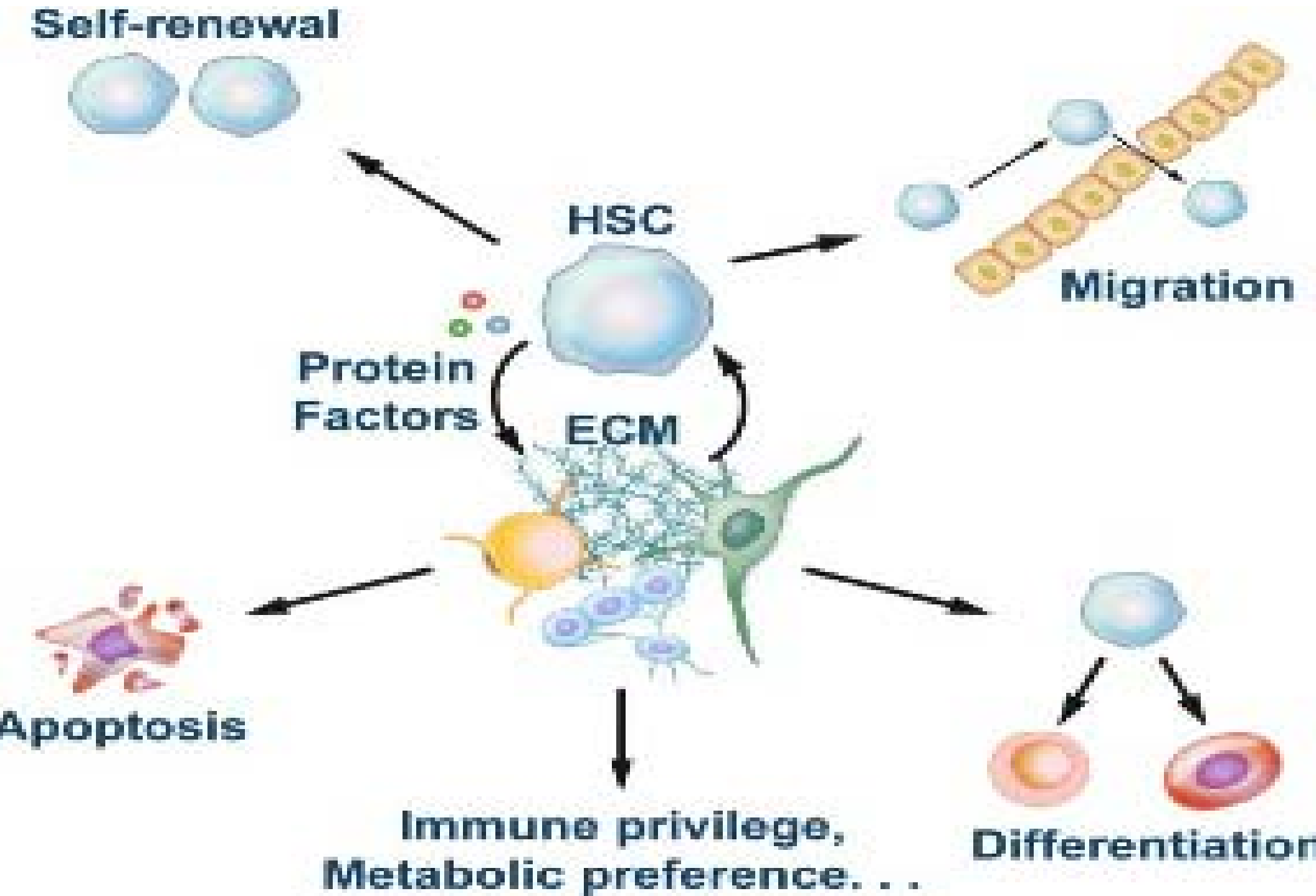




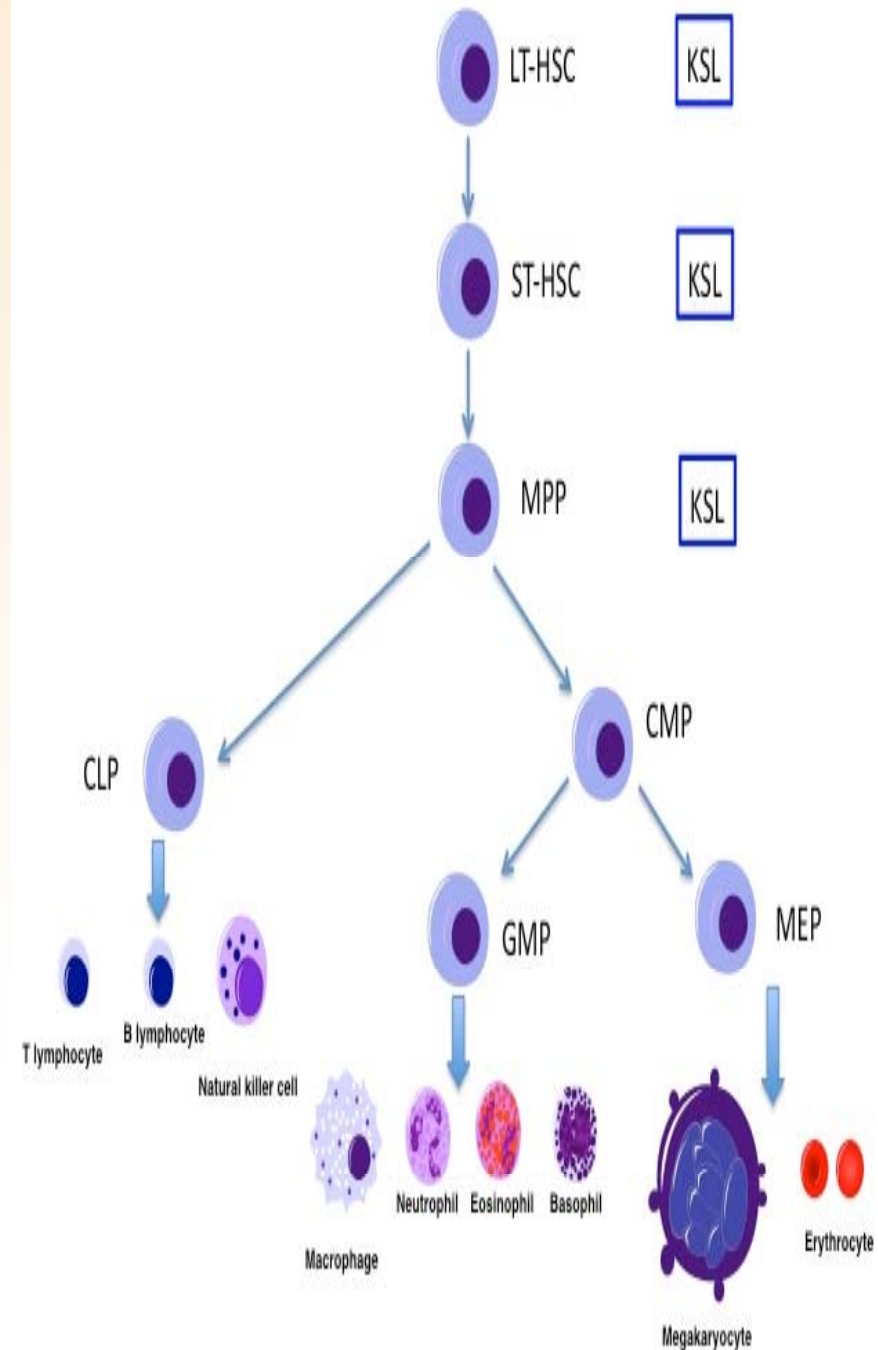
Mitochondria

Hematopoietic Stem cells

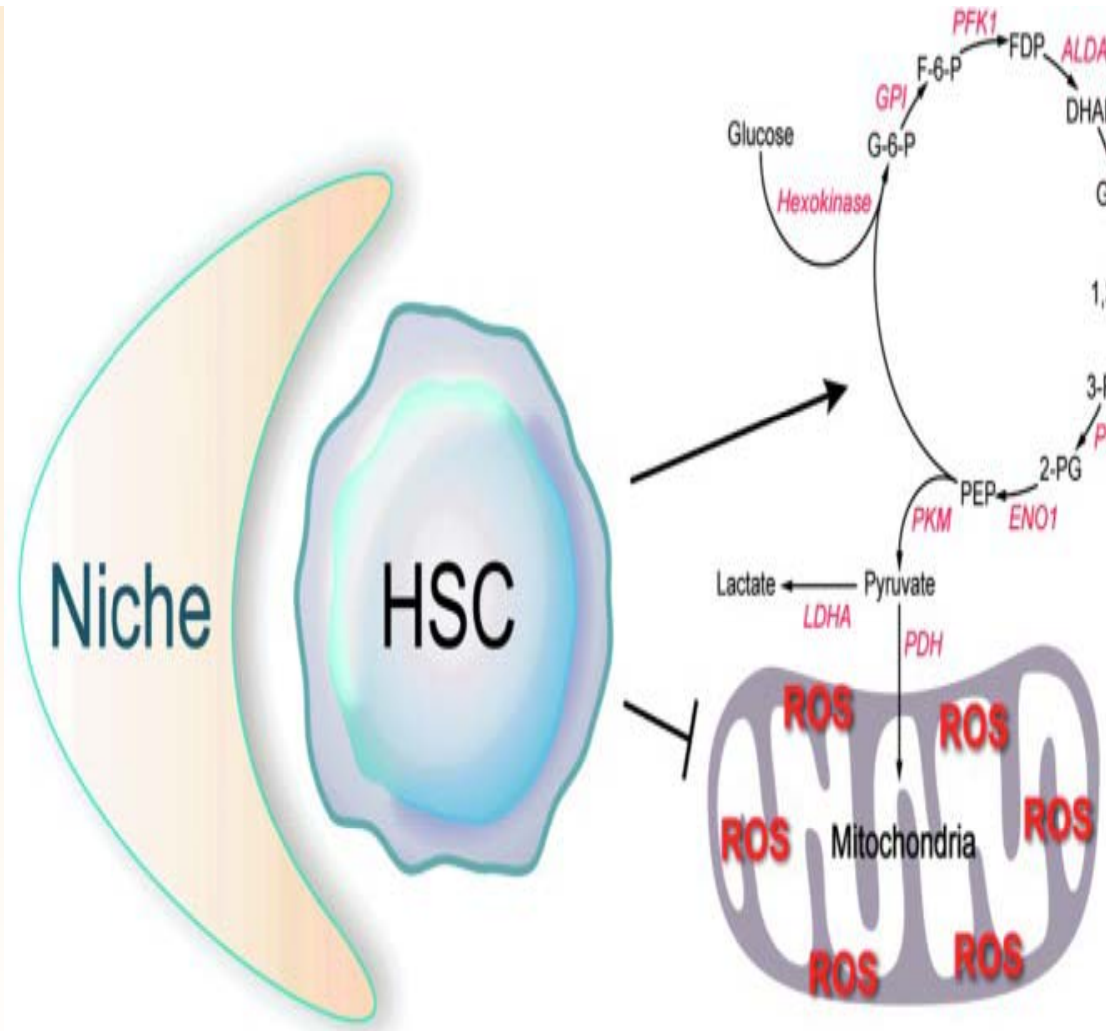




Long-term (LT-) HSC, those endowed with long-lasting self-renewal, have been shown to differ from more differentiated cells in energy metabolism.

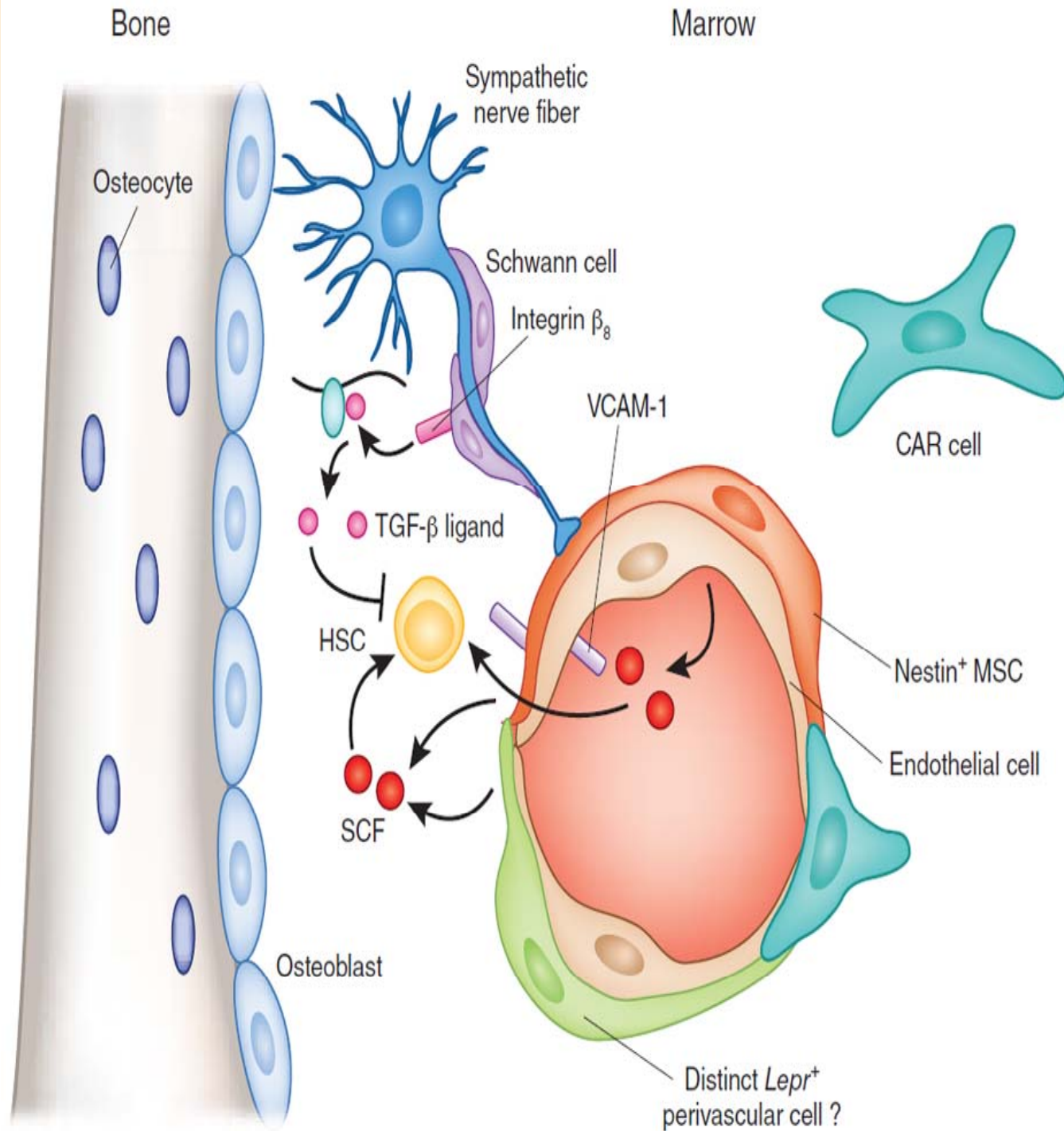


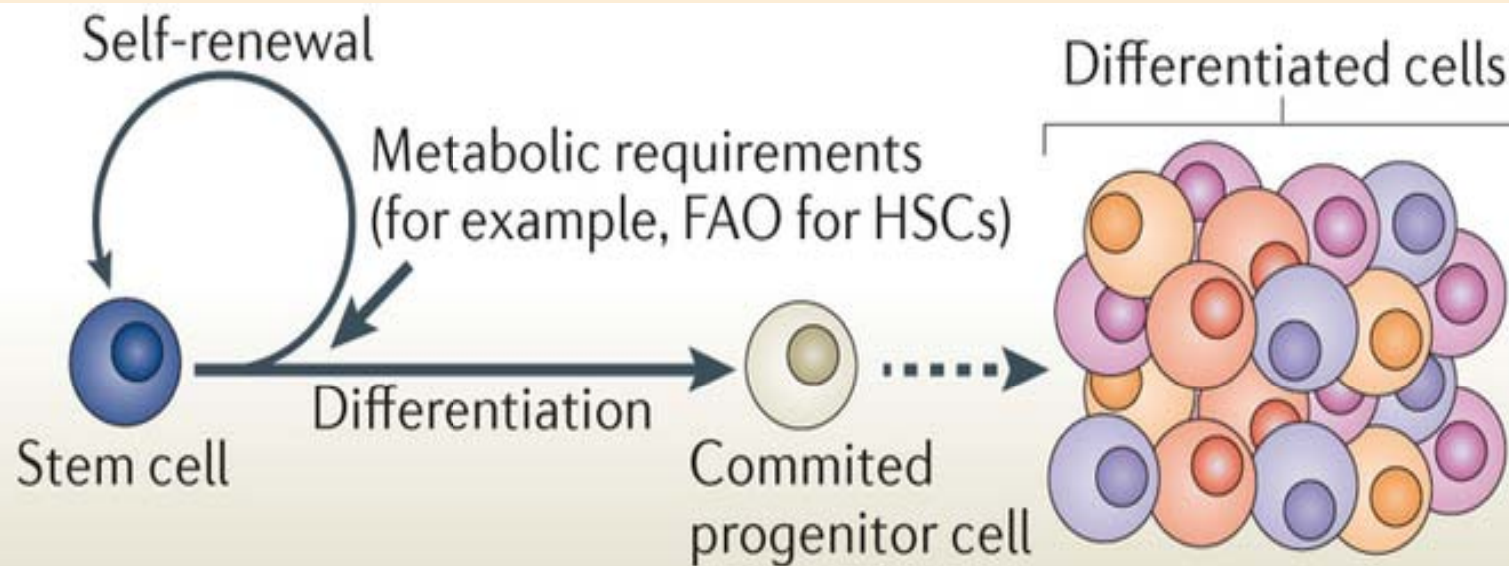
The lesser mitochondrial content in murine and human HSC might contribute to protect the cells from mitochondrial damage and subsequent apoptosis driven by ROS overproduction.



nestin+ mesenchymal stem cells

:
are directly
regulated by
sympathetic
nerve fibers,
which control
blood flow,
oxygen
availability





Stem cell marker

Positive

Negative

Differentiation marker

Negative

Positive

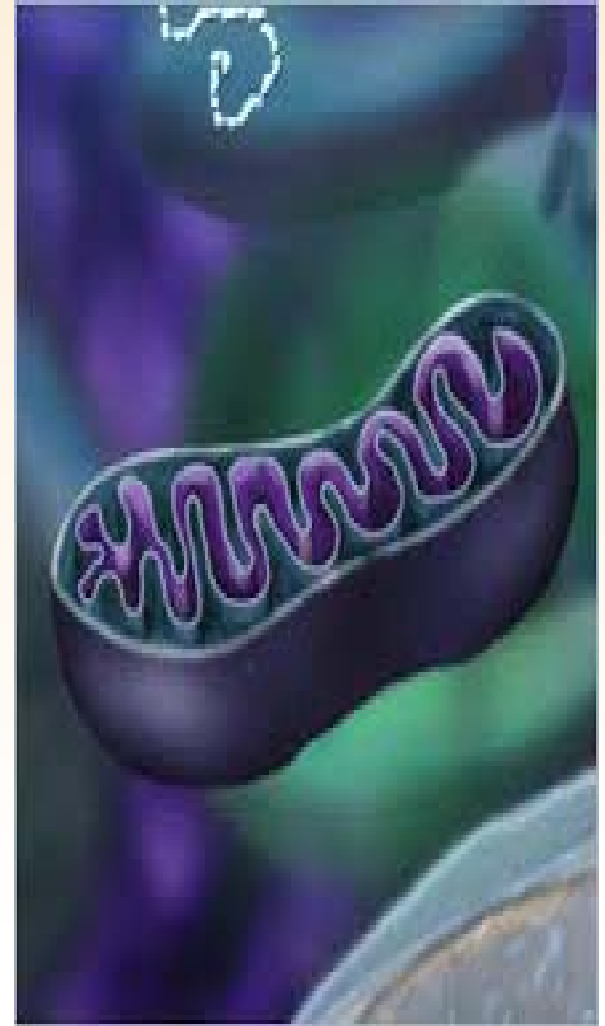
Glycolysis



OXPPOS



Mitochondria play key roles in HSC/HPC and have recently come under increased scrutiny because compelling evidence has revealed their role in numerous cellular processes, beyond ATP production and apoptosis regulation, and they have recently even been suggested to act as cell-fate or lineage determinants.

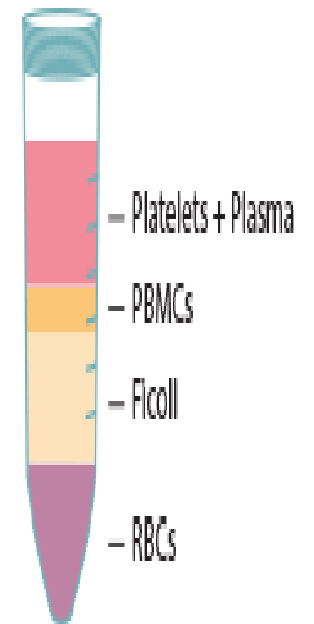
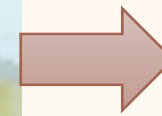
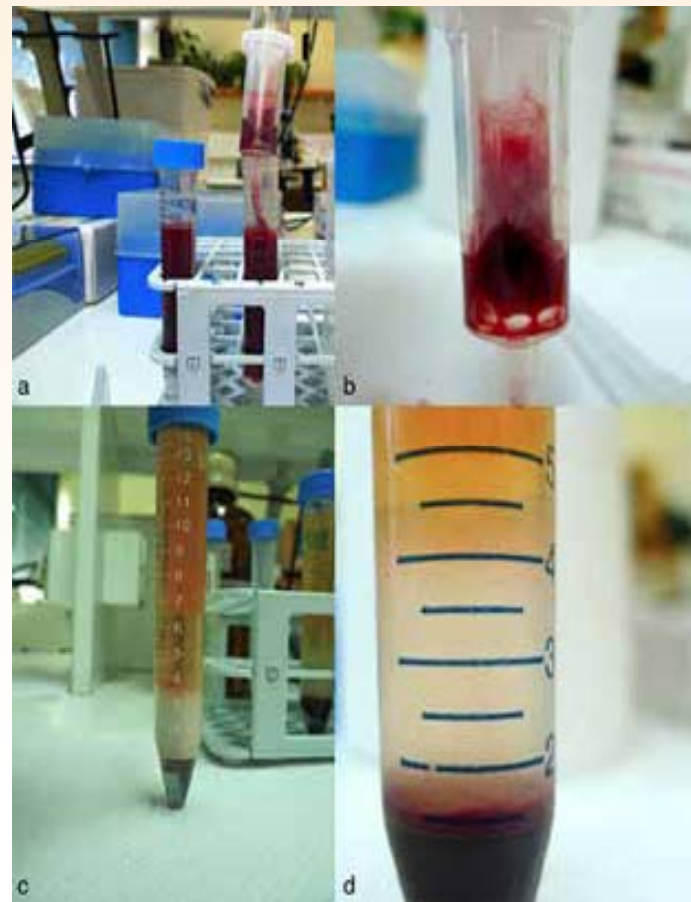


Design and Methods

- Isolation cord blood CD34+ cells
- Expansion of cord blood CD34+ cells for 7 days
- Isolation DNA of cord blood CD34+ cells by QIAamp DNA Mini kit
- Study of DNA copy number by Real-Time PCR Taqman

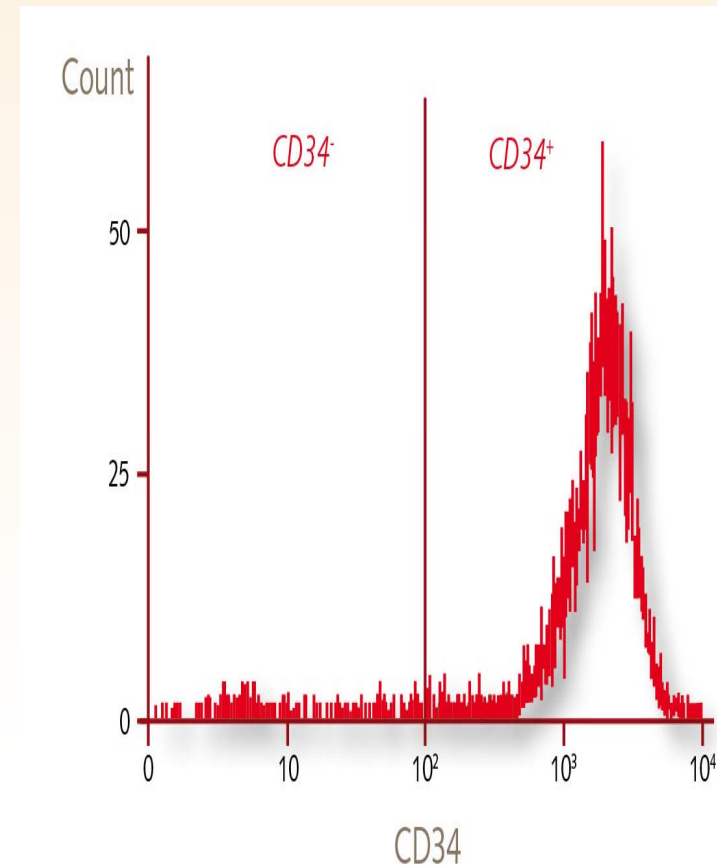
Cord blood collection and CD34+ cell isolation and culture

Mononuclear cells were isolated using Ficoll-Hypaque and after lysing the red cells



Cord blood collection and CD34+ cell isolation and culture

The purity of the CD34+ fraction was assessed by flow cytometry using an antiCD34-PE antibody (Miltenyi Biotec), and only CD34+ fractions showing purity higher than 90% were used.



Expansion of cord blood CD34+ cells for 7 days

expansion of 2×10^5
CD34+ cells in
presence of growth
factor:

- a) SCF 50 ngr/mL
- b) TPO 50 ngr/mL
- c) Flt-3L 25 ngr/mL
in StemLine II

