

IN VITRO DIFFERENTIATION CAPABILITY OF CORD BLOOD DERIVED UNRESTRICTED SOMATIC STEM CELLS ALONG THE OSTEOGENIC AND CHONDROGENIC LINEAGE

Ö.Degistirici, I.Napierski, S.Schroot, A.Knipper

Orthopedics Group, Kourion Therapeutics Inc., Langenfeld, GER

INTRODUCTION: Human umbilical cord blood contains hematopoietic precursor and stem cells. In recent years it has become an important alternative source for transplantation of hematopoietic stem cells. Here we describe a novel CD45-negative stem cell termed USSC (unrestricted somatic stem cell). It is derived from human umbilical cord blood, placental blood and/or the blood of a newborn child. It is capable of differentiating into mesenchymal stem or progenitor cells, hematopoietic lineage stem or progenitor cells, neural stem or progenitor cells or endothelial stem or liver progenitor cells[1]. These cells represent the progenitor of the hematopoietic lineage as well as that of the mesenchymal lineage as well as that of the neural lineage. Our studies describe the differentiation of USSC towards the osteogenic and chondrogenic lineage.

METHODS: USSC were generated from human umbilical cord blood and expanded as adherent cell layers. Monolayers were harvested by trypsinization, washed and resuspended at a concentration of 1×10^6 /ml in PBS.

Osteogenic differentiation

Cells are plated at 8000 cells/cm² in 24 well plates. At 70% confluency the expansion medium is supplemented with 10^{-7} M Dexamethasone, 50µM Ascorbic acid-2 phosphate and 10 mM β-Glycerol phosphate (DAG) to induce osteogenic differentiation. Medium changes are performed every 3 to 4 days.

Suspension culture of cell aggregates

Cells were cultured in defined medium containing DMEM high-glucose supplemented with antibiotic (1:100 dilution), 100 nM dexamethasone, 35 µg/mL ascorbic acid-2- phosphate, 1 mM sodium pyruvate, and ITS+ (1:100 dilution), 10 ng/ml TGFβ1. Aliquots of 2×10^5 cells in 0.5 ml of medium were then centrifuged at 150g in 15 ml polypropylene conical tubes. The pelleted cells were incubated at 37°C and 5% CO₂. Cell aggregates were cultured for 21 days with medium changes every two to three days

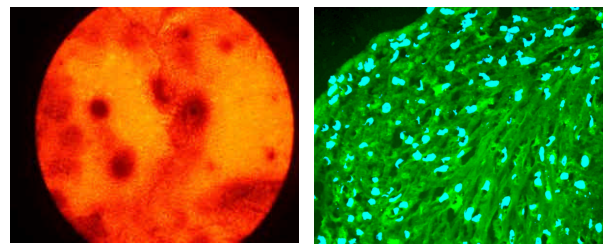
RESULTS: Two weeks of osteogenic stimulation result in the deposit of calcium phosphate which can be visualized by Alizarin Red staining and quantitated by a Calcium assay (Sigma). Osteogenic differentiation is further confirmed by visualization of alkaline phosphatase activity and RT-PCR analysis of distinct

osteogenic marker genes like Osteopontin, Osteocalcin alkaline phosphatase and bone sialoprotein.

In vitro micromass cultures demonstrate that USSC cultured in the presence of chondrogenic induction factors are capable of differentiating towards the chondrogenic lineage. The detection of both type II collagen and proteoglycan confirm chondrogenic differentiation. RT-PCR analysis reveal a chondrogenic differentiation as early as day 7 following stimulation.

Fig. 1: *Alizarin Red staining and collagen type II staining as characteristics of USSC differentiating along the osteogenic and chondrogenic lineages*

DISCUSSION & CONCLUSIONS: In summary, the adherent USSC population described here provides a novel and clinically advantageous pluripotent cell



population for the treatment for numbers of degenerative disorders. The establishment of a panel of HLA-typed, preferentially allogeneic USSC, fully tested for virological and microbiological parameters, will be a biological and logistical advantageous source of cells for tissue regeneration.

REFERENCES: ¹Wernet et al.(2001) Detection of Unrestricted Multipotential Stem Cells in Human Cord Blood, ASH01 Symposium

ACKNOWLEDGEMENTS: This study was supported by the Wirtschaftsministerium NRW, GER.