Differentiation of Cord Blood Stem Cells into Chondrogenic Derivatives

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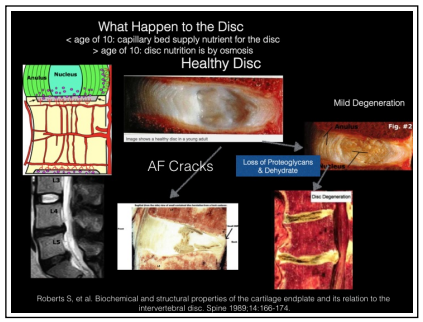
Introduction

Intervertebral disc stem cell based regenerative therapies using umbilical cord blood stem cells (CBSCs) has the potential to provide more primitive and promising populations of multipotent stem cells compared to bone marrow derived cells. However, the differentiation potential of these stem cells is not fully elucidated. This study focused on understanding the potential of CBSCs to differentiate into chondroprogenitors capable of yielding cells that produce extracellular matrix and glycosaminoglycans to restore the degenerated intervertebral disc.

Methods

Chondrogenic differentiation (image 1)of cord blood stem cells (CBSCs) was performed by spheroid culture technique. CBSCs (1X105 cells/ml) were centrifuged at 3K rpm for 10 mins. in chondrogenic medium consisting of high glucose DMEM, 10% fetal bovine serum, 10ng/ml recombinant human TGF-ß1, 10µg/ml insulin-like growth factor, 50µg/ml ascorbic acid and 100nM dexamethasone. Spheroids were incubated at 37ºC and 5% CO2, undisturbed, for five days, and then were transferred onto culture plates and cultured for an additional 30 days.

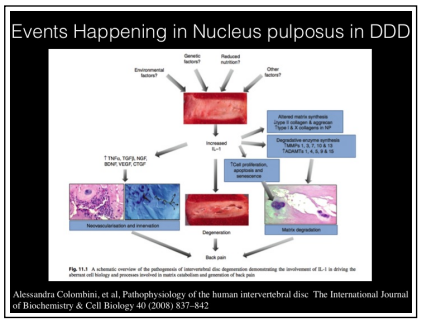


Methods (CONTINUED)

Chondrogenic differentiation was evaluated by Alcian Blue staining and RT-PCR analysis for expression of chondrogenic markers. Further in-vivo growth, differentiation, electron microscopy and PCR analysis was undertaken.

Results

The spheroids, cultured in the eppendorfs, allowed the majority of CBSCs to induce differentiation (fig. 2) towards the chondroprogenitor like cells. Transfer onto culture plates allowed the CBSCs to migrate from the spheroids and the uniformity of cell migration (fig. 1), cell morphology and the amount of extracellular matrix, were compared by light microscopy and Alcian Blue staining.



Results (CONTINUED)

It was also observed that CBSCs require a longer time to differentiate into chondroprogenitors than the 21 days usually necessary for other stem cell sources. Differentiated cells expressed chondrogenic markers (fig.3), such as Sox9, Aggrecan, and Col II determined by RT-PCR and immunocytochemical analysis.

Conclusions

This study demonstrated that CBSCs have reliable migration and differentiation capabilities into chondroprogenitors. Therefore, CBSCs can potentially be used as a standard cell source for autologous or allogenic intervertebral disc regenerative therapies.

References

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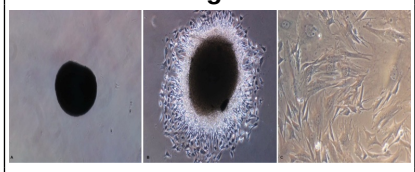
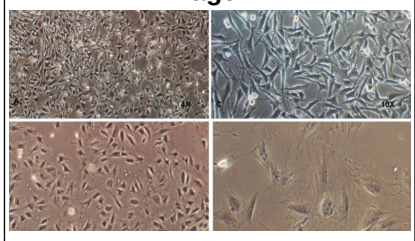
Image 1



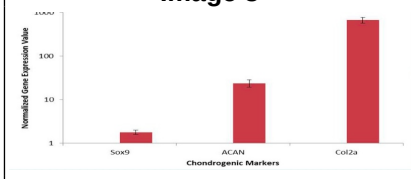
CBSCs spheroid (A), migration of cells outwardsfrom the spheroids at day 2 (B), differentiatedchondrogenic derivatives (C) in 3 weeks. LMs at 4X,10X.

Image 2



Outgrown cells from control (A,B) chondrogenic derivatives(C,D) of spheroidcultures for 3 weeks. Organels/extraellular matrix. LMs at 4X magnification (A,C) and 10X (B,D).

Image 3



Gene expression of Sox9, aggrecan (ACAN) andcollagen 2 (Col2a) were analyzed after 3 weeks of culture.