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Long term culture of CB-MSCs deteriorates their stemness nature through a series of bimolecular changes

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Background: Mesenchymal stem cells (MSCs) are an excellent candidate for cell therapy because their isolation is straight forward, they can be bio-preserved with minimal loss of potency, they are able to self-renew and no immunogenic reactions are observed with their transplants. The ease of culture expansion of MSCs represents one of their primary advantages and is a necessary component for most proposed clinical strategies using MSCs. However, MSCs have a finite life span, undergo senescence on long-term culture *in vitro* and may lose some of their differentiation potentials with increased time in culture. Therefore, the effects of *in vitro* serial propagation on MSCs should be investigated.

Aim: This study was designed to evaluate the effect of long term *in vitro* culture on morphology, proliferation, telomere length, pluripotency genes expression and differentiation potentials of cord blood (CB)-derived MSCs. This would allow us to choose the optimum passage number maintaining the MSCs stemness nature and providing the sufficient count intended for their clinical applications.

Methodology: Mononuclear cells were first isolated from CB and then they were expanded *in vitro* until reaching senescence. Cells from early and late passage cultures were subjected to pluripotency genes (Oct4, Sox2, Nanog, klf4 and c-Myc) and PDGFRa gene expression analysis, measurement of absolute telomere length by real-time PCR, measurement of telomerase activity and induction of differentiation into osteogenic, adipogenic and chondrogenic lineages.

Results: Upon repeated passaging, CB-MSCs telomere length decreased from 10.0168 kb±0.27 to 7.4186 kb±0.996 and this decrease was statistically highly significant (p<0.01) which coincided with negative telomerase activity. Pluripotency and proliferation genes expression decreased at the late passages. The adipogenic and chondrogenic differentiation potentials declined gradually whereas the osteogenic differentiation increased gradually and then dropped at the late passages.

Conclusion: Based on the results, *in vitro* expansion attenuated the parameters that were commonly used to define MSCs stemness nature. Therefore, we suggest considering CB-MSCs for cell and gene therapy at an early passage as soon as a clinically sufficient MSCs count is achieved.

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