International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.10, No.02, pp 109-113, 2017

Effect of Cryopreservation and cumulative population doublings on Senescence of Umbilical Cord Mesenchymal Stem Cells

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Abstract : Background: Multiple harvest explant method (MHEM) derived umbilical cord mesenchymal stem cells (UC-MSCs) was shown to undergo senescent beginning at passage-10 (P-10). However, for the same cells, there are no senescent data after cryopreservation and passage. Therefore, this study aimed to analyze the senescent profile of cryopreserved MHEM derived UC-MSCs after serial passages.
Methods: MHEM derived UC-MSCs were isolated and cultured in platelet lysate (PL) containing medium as described previously. The cells were cryopreserved at passage-1 (P-1) in 10% dimethyl sulfoxide (DMSO) and 10% PL containing alpha minimal essential medium (αMEM). Cell density at cryopreservation was 500 000 cells/mL. After one month, the cells were thawed and recultured until P-8 in six 12-well plate. At 80-90% confluent, two wells were harvested and the cells were recultured into six wells, and the four remaining wells were subjected to senescent (β-galactosidase) staining, which was done for all passages. Random photographs were taken from all stained wells, and senescent percentage was recorded.
Results: No senescent cells were observed at P-2. Senescent cells began to appear at P-3. Percentage (mean ± SD) of senescent cells from P-3 through P-8 were 0.04 ± 0.02, 1.18 ± 1.82, 0.14 ± 0.18, 0.49 ± 0.00, 0.58 ±0.91, and 0.07 ± 0.07, respectively.
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Conclusion: No senescent cells were observed at P-2 (cumulative population doubling [CPD] > 9.38). Senescent cells began to appear at P-3 (CPD > 14.06), but in all passages until P-8 (CPD >34.34) the senescent percentage was below 5%.
Keywords: MSC, umbilical cord, cryopreservation, passage, senescence.

International Journal of PharmTech Research, **Vol.10, No.2, pp 109-113 (2017)**

http://dx.doi.org/10.20902/IJPTR.2017.10116

Jeanne Adiwinata Pawitan *et al* /International Journal of PharmTech Research**,** 2017,10(2): 109-113.

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