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Isolation and characterization of human cord blood mesenchymal stem cells multipotent clones

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Background: Mesenchymal stem cells (MSCs) are self-renewing cells that have extensive capacity for *in vitro* expansion with high yield and immunomodulatory effects, that's why they are excellent choice in regenerative medicine. However, clonal analysis of these cells has revealed that they are a heterogeneous mixture of cells. Therefore, clones derived from a single cell would provide a homogeneous population of MSCs and therapeutic efficacy of MSCs population may be enhanced by the use of selected multilineage clones.

Aim of the Work: Cloning of umbilical cord blood MSCs, by limiting dilution method, could provide a better source for MSCs with high stemness calibre to meet clinical demands.

Methodology: Isolation, expansion and cloning by limiting dilution method of human umbilical cord blood MSCs were done. Two cord blood samples, Mother (A) and Mother (B) were compared with their corresponding daughter clones regarding their proliferation efficiency, surface antigen expression, pluripotent and proliferation genes expression, as well as, their differentiation potentials.

Results: MSCs were successfully isolated from two cord blood samples (Mother (A) and Mother (B)) and cloned by limiting dilution method to give 7 single clones for Mother (A) and 8 single clones for Mother (B). Mother (B) was fast growing, with higher PD and shorter PDT than Mother (A). It showed higher pluripotency and proliferation genes expression, as well as, a higher differentiation percent on the three mesodermal lineages level. The daughter clones (A2, A5) had a higher stemness criteria than their Mother (A) and other clones, whereas, Mother (B) showed a higher stemness characteristics than its daughter clones, except for clone B6 which showed comparable result to its mother.

Conclusion: Cloning of MSCs to obtain homogeneous populations of cells with efficient proliferation and differentiation potentiality could be of significance for clinical applications. Moreover, this study draw the attention to the importance of CD105 as a possible selection marker for MSCs with better stemness properties, represented as higher proliferation efficiency and/or stronger differentiation potentiality.

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