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# Temporal Changes of Heterogeneous Subpopulations of Human Adipose-Derived Stromal/Stem Cells in vitro

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**Abstract :** The application of adipose-derived stromal/stem cells (ASCs) in regenerative medicine is gaining more awareness due to their advanced translational potential and abundant source preparations. However, ASC-based translation has been confounded by high subpopulation heterogeneity, causing ambiguity about its precise therapeutic value. Some phenotypes defined by a unique combination of positive and negative surface markers have been found beneficial to the required roles. Therefore, the immunophenotypic repertoires of cultured ASCs and temporal changes of distinct subsets were investigated in this study. ASCs from three donors undergoing cosmetic liposuction were cultured in standard culturing methods, and the co-expression patterns based on the combination of selected markers at passages 1, 4, and 8 were analyzed by multi-chromatic flow cytometry. The results showed that the level of heterogeneity of subpopulations of ASCs became lower by in vitro expansion. After a few passages, most of the $CD166^+/CD274^+/CD271^+$ based subpopulations converged to CD166 single positive cells. Meanwhile, these $CD29^+CD201^+$ double-positive cells, in combination with CD36/Stro-1 expression or without, feathered only the major epitopes and maintained prevailing throughout the whole process. This study suggested that, upon in vitro expansion, the phenotype repertoire of ASCs redistributed and stabilized in a way that cells co-expressing exclusively the strong markers remained dominant. These preliminary findings provide a general overview of the distribution of heterogeneous subsets residents within human ASCs during expansion in vitro. It is a critical step to fully characterize ASCs before clinical application, although the biological effects of heterogeneous subpopulations still need to be clarified.

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