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Proliferation and Differentiation Capacity of Mesenchymal Stem Cells, Age-Related Differences

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Mesenchymal stem cells (MSCs) isolated from human bone marrow have the capacity to proliferate and differentiate into chondrocytes, osteoblasts and adipocytes. Involvements of these cells in regeneration have been demonstrated for a number of tissues, including bone, cartilage and other mesenchymal tissues. For this reason, MSCs are of great therapeutic potential in stem cell strategies for repair of damaged organs. Questions arise to what extent MSCs are subject to aging and whether these MSC based strategies are suitable for patients of all ages. The aim of our study was to elucidate the effect of aging on proliferation and differentiation capacity of mesenchymal stem cells.

Methods and Materials Fifteen donors of different ages were involved in this study. They were divided into three groups: 7-12-year-old children, 20-55-year-old adults and 60- 85-year-age group. Each group had 5 donors. Proliferation and differentiation of expanded MSCs were assessed and compared among these donors, including number of colony forming unit fibroblasts (CFU-F), proliferation rate and the capacity to undergo chondrogenesis, osteogenesis and adipogenesis.

Proliferation of MSCs Mononuclear cells from bone marrow were seeded and allowed to adhere and proliferate for 5 days. CFU-F number was determined and subcultures were prepared to assess single cell cloning efficiency and proliferation rate.

Differentiation part Cells of passage 4 were subjected to differentiation into three distinct lineages. For chondrogenesis, micromass cultures were prepared and induced to differentiation in the presence of TGF-ß3 over 6 weeks. Chondrogenic differentiation was assessed by glycosaminoglycan (GAG) quantification and histologic/immunohistologic evaluation of Alcian blue and Collagen Type II staining. For osteogenesis, differentiated monolayer cells were evaluated for alkaline phosphatase (ALP) enzyme activities and mineralization by Alizarin Red S quantification. As a parameter for adipogenesis, lipid accumulation was quantified by Oil Red O staining.

Results We found no correlation between CFU-F frequency or proliferation rate of MSCs and donor age, but we observed a tendency of higher single cell cloning efficiency in isolates from young donors (P□0.01). Furthermore, our experimental data showed no correlation between chondrogenic and adipogenic differentiation capacity of MSC and age. There were no differences found in chondrogenic and adipogenic capacity of MSCs with age. But during osteogenic differentiation, both activities of bone related ALP activity and calcium deposition showed a positive correlation with age (P□0.05 and P□0.001 respectively), which indicates that osteogenic potential of MSCs increase with age.

Conclusion: Age dependent decrease of single cell cloning efficiency suggests an age dependent depletion of the stem cell pool capable to be activated for clonal expansion. However, osteogenic capacity correlated positively with increasing donor age, which sheds a promising light on the potential application of cell based bone remodelling strategies also in patients of older age. It seems promising to further study MSC populations from donors of older age to gain a better understanding about the molecular mechanisms regulating age-related changes in connective tissue biology and their possible consequences for regenerative medicine.