Identification and isolation of committed osteogenic progenitors from a heterogeneous population of mesenchymal stem cells using a Runx2 reporter adenovirus

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BACKGROUND:
Mesenchymal stem cells (MSC) are a heterogeneous population of adult stem cells characterized by their self-renewal capability and their multidifferentiation potential into several cell types of the mesenchymal lineage. Current isolation methods of MSCs are still rudimentary due to the lack of unique markers that characterize this stem cell population. Given the heterogeneity of MSCs, inconsistent results are obvious, which in turn slows down the progress in stem cell biology and related applied disciplines such as tissue engineering. This circumstance suggests investigating novel or improved approaches for the isolation of more homogeneous subpopulations of MSCs. In this study, a novel method for the identification and isolation of a committed osteogenic subpopulation of MSCs was developed. This was approached by functionally identifying and selecting a subpopulation of MSCs due to the expression of Runx2, the key transcription factor in osteogenesis [1]. The expression of the key transcription factor is coupled to expression of green fluorescent protein (GFP), and on the basis of that, these fluorescent cell can be selected by means of fluorescent activated cell sorting (FACS). The resulting cell populations, namely Runx2 GFP+, Runx2 GFP-, and the original unsorted cells, were subjected to comparative in vitro investigation for their ability to differentiate into the osteogenic lineage. To substantiate the characterization of the cell populations, proliferative capacity of the cell populations was assessed because it has been proposed that MSC differentiation is accompanied by decreased proliferation [2]. We expect our results will lead to an improved and more reproducible method to isolate committed osteogenic MSC subpopulation(s).

MATERIALS AND METHODS:
Human bone marrow from three donors was obtained with full ethical permission from patients undergoing hip replacement. After Ficoll separation, the adherent cell fraction was passaged to P2. Cells were expanded in the presence of 5 ng/ml basic fibroblast growth factor (bFGF) prior to plating at 60,000 cells/cm² for transduction with the Runx2 reporter adenovirus the following day. High efficiency transduction of undifferentiated stem cells was achieved using lantehfection [3] at a MOI of 100. The cells were then cultured in osteogenic medium containing 10⁻⁷ M dexamethasone, 10 mM β-glycerophosphate and 50 µg/ml ascorbate-2-phosphate. After 3 days of osteogenic induction, cells were sorted by means of FACS due to the presence of green fluorescence. Runx2 GFP+, Runx2 GFP-, and unsorted cells were separately expanded in monolayer culture in presence of osteogenic induction, cells were sorted by means of fluorescent activated cell sorting (FACS). The resulting cell populations, namely Runx2 GFP+, Runx2 GFP-, and the original unsorted cells, were subjected to comparative in vitro investigation for their ability to differentiate into the osteogenic lineage. To substantiate the characterization of the cell populations, proliferative capacity of the cell populations was assessed because it has been proposed that MSC differentiation is accompanied by decreased proliferation [2]. We expect our results will lead to an improved and more reproducible method to isolate committed osteogenic MSC subpopulation(s).

RESULTS:
To investigate the osteogenic differentiation capability by examining the matrix mineralization, Ca45 incorporation of Runx2 GFP+, Runx2 GFP-, and unsorted cell populations was determined at day 21 of osteogenic differentiation. Ca45 incorporation, related to the DNA amount, at day 21. * notes significant difference (p<0.05). Results are presented as mean ± SD (n=12).

To assess the proliferative capacity of the cell populations, the three different cell populations were subjected to colony forming unit (CFU) assay. CFU analysis at day 14 revealed that the total number of CFUs for all three cell populations were similar. However, when distinguishing fast-growing from slow-growing colonies differing in the overall colony size, it arose that Runx2 GFP+ cells had the smallest fraction of fast-growing colonies at the expense of slow-growing ones. Runx2 GFP+ cell population showed a CFU behavior similar to the one of unsorted cell population (Table 1).

CONCLUSIONS:
Isolation of MSC subpopulations on the basis of GFP expression driven by the presence of the osteoprogenitor-specific transcription factor Runx2 resulted in a cell population that featured a more osteoblast like phenotype than the negative cells. Not only are they more osteoblast like, but also committed Runx2 GFP+ cells proliferate at a slower rate than the Runx2 GFP- cells and the unsorted population. This is also suggestive of a more differentiated phenotype. However, Runx2 GFP- cell population does not behave contrariwise to Runx2 GFP+ cell population, but rather like the unsorted cell population itself. This can be explained by the fact that withdrawal of Runx2 GFP+ cell population (ca. 5% of total cell population) from unsorted cell population, leading to Runx2 GFP+ cell population, only results in minor changes in the cellular behavior of the resulting cell population.

REFERENCES:

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