Introduction

In bone marrow (BM), stem and progenitor cells with haematopoietic differentiation potential exist in close proximity to another heterogeneous group of stem cells, with nonhaematopoietic (NH) differentiation potential termed marrow stromal cells (MSC). A subpopulation of these marrow stromal cells with unknown phenotype, can be isolated by plastic adherence (PA). Many attempts have been made to find the most specific antigen for this isolation of MSCs, such as CD105, CD117, CD271 (LNGFR), Stro-1 and CD7-Fib, a fibroblast marker. Isolated CD271+ bone marrow cells, as compared to plastic adherent bone marrow cells, were reported to show a one to three log greater expansion of MSCs in culture and a greater capacity to differentiate to adipocytes and osteoblasts. Therefore CD271+ has become one of the most promising markers for MSC isolation 1,2.

Methods

MSC isolation and cultivation were performed using the MSC Research Tool Box – CD271 (LNGFR)-APC and Anti-APC Microbeads for separation and NH Expansion Medium supplemented with CytoMix – CD271 (LNGFR) for cultivation. 2×10^6 bone marrow mononuclear cells (BM MNC) were separated using CD271 (LNGFR)-APC and Anti-APC Microbeads. The negative and positive fractions were cultivated with NH Expansion Medium and with and without CytoMix – CD271 in order to assess their proliferative capacity. These fractions were compared with MSCs isolated by PA and expanded using the same culture conditions as described for separated cells. The phenotype and population doubling (PD) as well as the cumulative population doubling (CDP) levels were determined over 41 days of cultivation using the equations shown below. To assess clonogenic potential, a colony-forming unit fibroblast (CFU-F) assay was used. The assay was performed with 1×10^4 BM MNC, 1×10^5 cells of the negative fraction or a sample of the positive fraction which were cultivated in NH Expansion Medium for 14 days. Furthermore, multipotent differentiation potential was investigated by culturing 5×10^5 MSCs in NH AdipoDiff and NH OsteoDiff Medium (Miltenyi Biotec GmbH). In order to observe chondrogenic differentiation, a micromass culture with 2×10^5 MSCs in NH ChondroDiff Medium (Miltenyi Biotec GmbH) was performed. A negative control was cultured in NH Expansion Medium only.

Results

1. Positive selection of CD271 (LNGFR)+ cells from BM MNC

2. Colony-forming unit fibroblast (CFU-F) assay

3. Expansion of MSCs isolated by CD271 separation vs. plastic adherence and assessment of the CPD

4. Phenotyping of in vitro expanded MSCs isolated by CD271 separation and plastic adherence

5. Differentiation potential of MSCs isolated by CD271 separation vs. plastic adherence

Conclusion

Highly purified CD271+ cells show greater expansion in number of fibroblastic cells compared to MSCs isolated by plastic adherence. They possess the ability to give rise to adipocytes and osteoblasts. A number of methods relying on specific physical properties have been used historically to isolate MSCs from sites at which they reside. The problem with this type of approach is that only two properties of MSCs have been unequivocally ascribed to MSCs. Therefore many different cell types are co-isolated, resulting in a mixed population of cells.