Comparative analysis of cell proliferation, immunosuppressive action, and multi-lineage differentiation of immortalized MSC and MSC from bone marrow, adipose tissue, and umbilical cord blood

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Introduction

Mesenchymal stem cells (MSC) can be isolated from multiple tissue sources for use as research tools leading to potential cellular therapies. Numerous studies have reported benefits of MSC in tissue repair and regeneration. For example, MSC possess immunosuppressive properties, suppressing T-cells and modulating dendritic cell activities such that MSC can be used for allogeneic cell therapies. Additionally, adipose tissue-derived MSC have been shown to secrete a variety of bioactive molecules, which promote endothelial cell survival and proliferation (1).

A better understanding of differences in the characteristics and potential of MSC prepared from different tissue sources is critical for developing appropriate applications utilizing these primary cells. Further, the immunosuppressive and angiogenic capacity of hTERT immortalized MSC is largely unknown. To address these issues, we conducted a comparative study of bone marrow (BM)-, adipose tissue (AT)-, umbilical cord blood (UCB)-derived MSC along with an immortalized AT-MSC line (hTERT-MSC). In this study, we investigated differences in surface marker expression, immunosuppressive activity, cell proliferation, angiogenic capacity, and adipogenic, osteogenic and chondrogenic differentiation potential.

Methods

Proliferation and *in vitro* differentiation of MSC: AT-MSC (ATCC® PCS-500-011™), BM-MSC (ATCC® PCS-500-012™), UCB-MSC (ATCC® PCS-500-010™), and hTERT-MSC (ATCC® SCRC-4000™) were seeded at 5,000 cells/cm^2, cultured in ATCC's MSC growth media (ATCC® PCS-500-040™ or ATCC® PCS-500-041™) for several passages, and subsequently cultured in ATCC's adipogenic, osteogenic, or chondrogenic differentiation media (ATCC® PCS-500-050™, ATCC® PCS-500-051™, ATCC® PCS-500-052™, or ATCC® PCS-500-053™) for 3 weeks to compare their efficiency of multi-lineage differentiation.

Flow cytometry: Freshly harvested MSC were resuspended in D-PBS and incubated with PE-, FITC-, or PerCP-conjugated antibodies for 30 min on ice and washed twice with D-PBS prior to flow cytometric analysis of MSC.

T-cell immunosuppression assay: MSC were seeded at 40,000 cells/cm^2 and cultured overnight prior to Mitomycin C treatment. CD3/CD28 activated peripheral blood mononuclear cells (PBMC, ATCC® PCS-800-011™) were then added for co-culture with the growth arrested MSC for 3 days at a MSC:PBMC ratio of 1:5. T-cell proliferation was measured following an 18-hour pulse of BrdU followed by flow cytometry with APC-conjugated anti-CD45 and FITC-conjugated anti-BrdU antibodies.

Tubular structure formation of endothelial cells: AT-MSC or hTERT-MSC were seeded in a 24-well plate for 3 hours prior to plating immortalized human aortic endothelial cells (TeloHAEC, ATCC® CRL-4052™) on the MSC monolayer. MSC-induced tubular structure formation was monitored by immunocytochemistry of endothelial cells with primary anti-CD31 and Alexa Fluor®594-conjugated secondary antibodies after co-culture of MSC and TeloHAEC in ATCC angiogenesis media for 14 days.

Results

Characterization of MSC from BM, AT, and UCB and hTERT-MSC: To compare cell morphology, proliferation rate, and surface marker expression, four types of MSCs were cultured in their corresponding MSC growth media for at least ten passages. MSC exhibited a spindle-shaped morphology and similar growth rates although UCB-MSC appeared to have the highest growth rate (Figure 1). BM-, UCB-, and AT-MSC could be expanded for at least 15 population doubling levels (PDLs) post-thaw prior to senescence while hTERT-MSC demonstrated the highest proliferative capacity and could be cultured for more than 25 PDLs without any indication of senescence. Regarding surface marker expression, all cells were negative for CD14, CD19, CD34, and CD45 and positive for CD29, CD44, CD73, CD90, CD105, and CD166 (Table 1), which meets the International Society for Cellular Therapy (ISCT) guidelines.

Immunosuppressive and angiogenic potential of MSC: To assess differences in the immunosuppressive ability of MSC, four types of MSC were co-cultured with T cell-activated PBMC for 3 days. Compared to activated PBMC alone, all MSC tested significantly inhibited T-cell proliferation (P<0.0001, Figure 2). We are the first to establish an *in vitro* model of vascular network formation by co-culture of immortalized endothelial cells and MSC. Both hTERT-MSC and AT-MSC induced tubular structure formation of endothelial cells, although AT-MSC appeared to induce a more extensive vascular network (Figure 3).

Multi-lineage differentiation of MSC: Compared to other MSC, BM-MSC had the highest efficiency of osteogenic differentiation potential while there was no marked difference in adipogenic and chondrogenic differentiation potential among the four types (Figure 4).

Figure 1. Morphology and growth curves of cultured BM-, AT-, UCB-, and hTERT-MSC (10×)

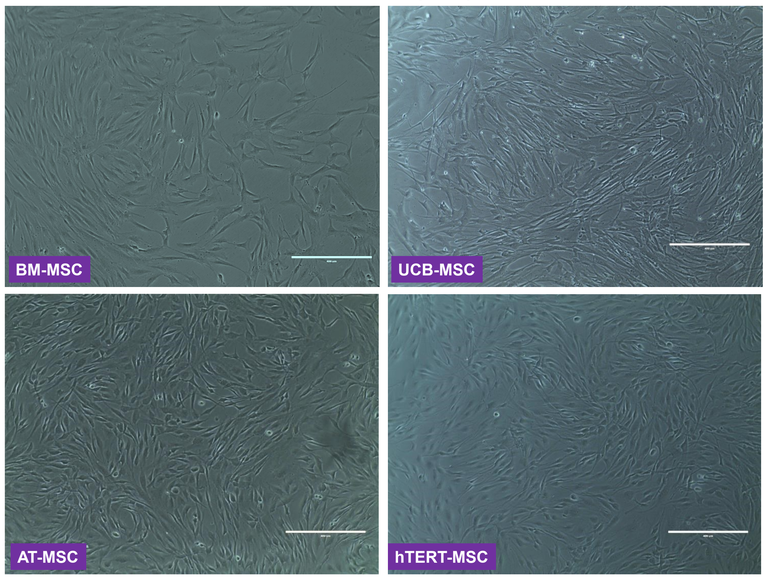
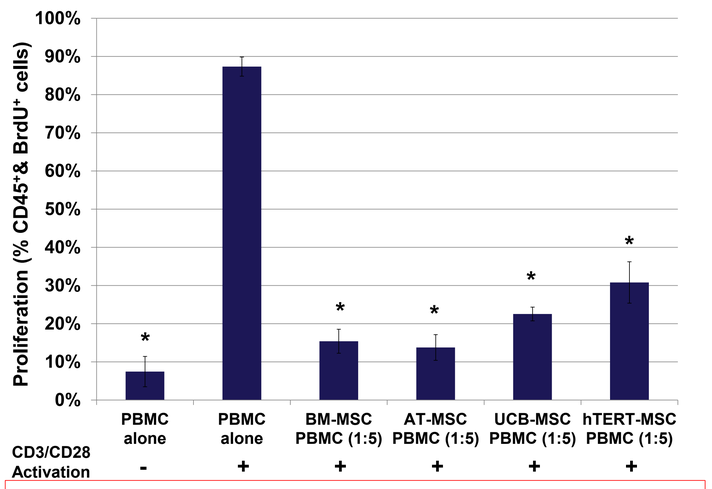


Table 1. Flow cytometric analysis of surface marker expression in BM-, AT-, UCB-, or hTERT-MSC

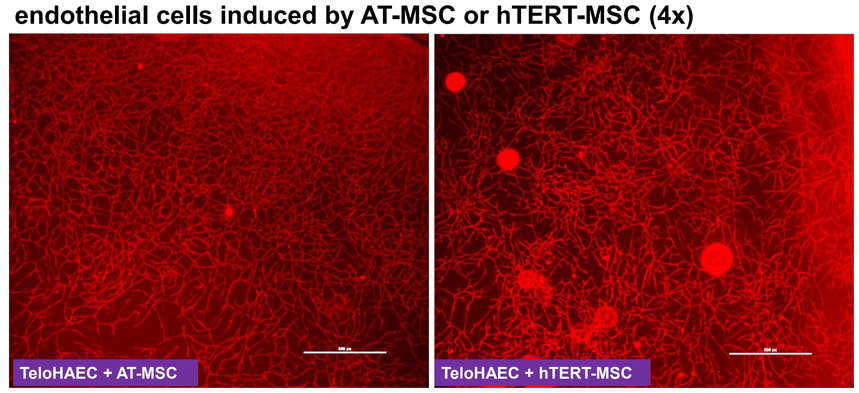
CD Marker	CD14 (%)	CD19 (%)	CD34 (%)	CD45 (%)	CD29 (%)	CD44 (%)	CD73 (%)	CD90 (%)	CD105 (%)	CD166 (%)
BM-MSC	0.26	0.07	2.91	0.15	100	99	100	100	100	94
AT-MSC	0.55	0.23	2.86	0.29	100	100	100	99	100	90
UCB-MSC	0.52	0.79	1.50	0.47	100	90	95	96	94	95
hTERT-MSC	0.25	0.23	0.99	0.55	100	100	100	100	99	96

Figure 2. Immunosuppression capacity of BM-, AT-, UCB-, and hTERT-MSC



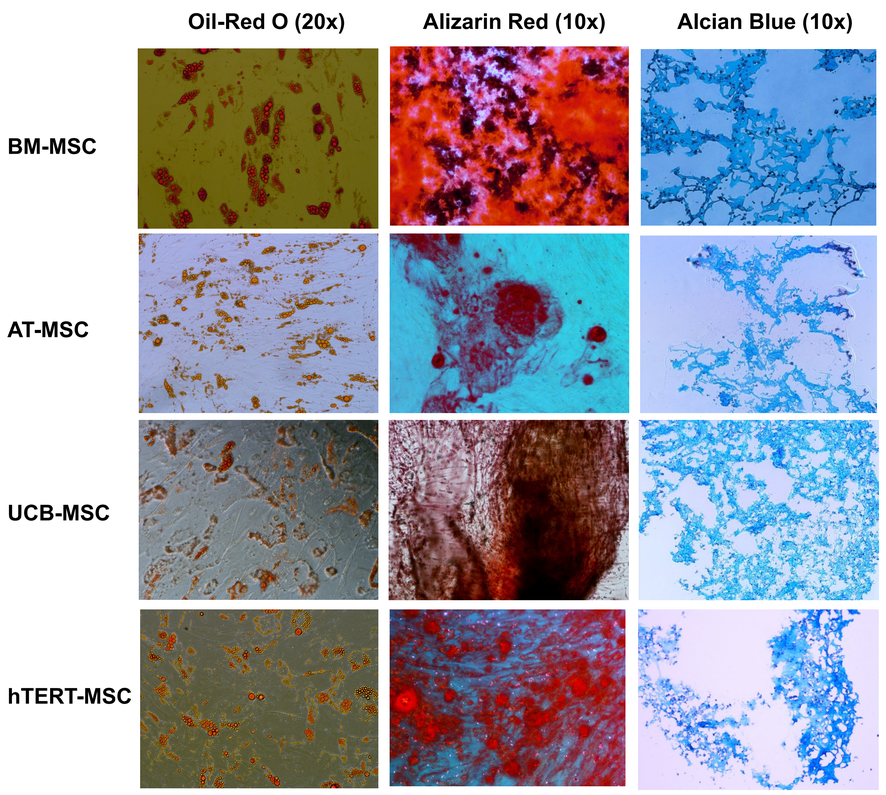
Data are mean ± STD; n =3
* Significantly different from activated PBMC alone (P < 0.0001)

Figure 3. Tubular structure formation of immortalized human aortic endothelial cells induced by AT-MSC or hTERT-MSC (4x)



Both hTERT-MSC and AT-MSC cells induced tubular structure formation of immortalized human aortic endothelial cells (TeloHAEC, ATCC® CRL-4052™). Endothelial cells were stained with an anti-CD31 antibody to visualize the network.

Figure 4. Adipogenic, osteogenic, and chondrocyte differentiation potential of BM-, AT-, UCB-, and hTERT-MSC



Summary

We performed comparative analysis to characterize and assess the potential of four types of MSC using ATCC's MSC growth and differentiation media. Overall, all MSC, including the hTERT immortalized MSC, exhibited similar morphology, surface marker expression, adipogenic and chondrogenic differentiation potential. However, UCB-MSC had the highest growth rate and BM-MSC displayed the highest efficiency of osteogenic differentiation. Regarding immunosuppressive capacity, all MSC significantly inhibited T-cell proliferation. Consistent with published findings (1), both AT-MSC and hTERT-MSC supported angiogenesis when co-cultured with endothelial cells. However, a slight increase in tubule formation was noted when AT- MSC cells were used. Nevertheless, hTERT-MSC exhibited significantly higher proliferative capacity (PDLs) and maintained all the characteristics and potential of primary MSC. Therefore, hTERT-MSC are a suitable alternative to primary cells for MSC-based assays. This comparative data and analysis allow for an informed decision regarding the choice of starting material for MSC-related research applications.

References

1. Merfeld-Clauss B.S. *et al*., Tissue Engineering: Part A. 2010, ***16***: 2953-2966