Novel Immortalized Human Bone Marrow–Derived Mesenchymal Stem Cells Overcome Primary Cell Limitations

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Abstract # 1382

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Abstract

Mesenchymal stem cells (MSCs) are adult stem cells that have the ability to differentiate into other types of cells. Additionally, MSCs can modulate the immune response and promote tissue repair. Due to their properties, primary MSCs have significant application potential in the treatment of various diseases. However, primary MSCs also have limitations such as limited life span, donor variability, source limitation, and heterogenicity, which hinders the utilization of primary MSCs in preclinical applications. These constraints could be overcome with cell immortalization. Cell immortalization technology enables the establishment of new cell models that retain normal physiology, demonstrate minimal cellular heterogeneity, and gain an indefinite lifespan. In this study, we generated a clonal cell line that was immortalized by stably expressing the human telomerase reverse transcriptase (hTERT) gene in normal human primary bone marrow–derived mesenchymal stem cells (BM-MSCs). This immortalized cell line has been cultured for over 200 days and has continued to proliferate over 120 population doublings. The immortalized BM-MSCs exhibited a normal karyotype and had a similar cell growth profile and cell doubling time as the parental primary cells. The immortalized BM-MSCs were positive for CD73, CD90, and CD105 and were negative for CD14, CD34, and CD45. In this study, we also investigated the adipogenic, osteogenic, and chondrogenic differentiation abilities of these immortalized BM-MSCs. In summary, the immortalized BM-MSCs present a novel cell model that avoids the limitations of primary cells and can be used as a valuable tool for cell and gene therapy.

Introduction

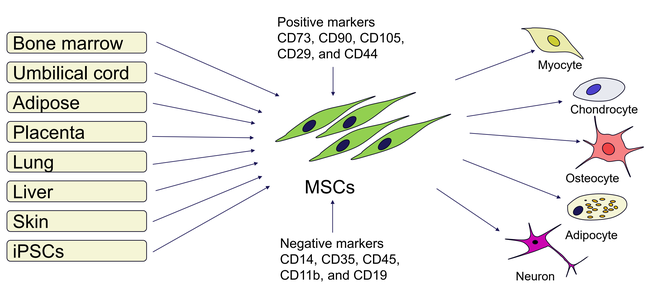

Figure 1: Isolation and differentiation of MSCs.

Table 1: Pros and cons of primary and immortalized cells

	Primary cells	hTERT-immortalized cells	Oncogene, Viral gene-immortalized cells
Lifespan	Short	Unlimited	Unlimited
Costs	+++	++	++
Supply	+	+++	+++
Relevance in vivo	+++	+++	++
Genomic stability	Diploid	Diploid/near diploid	Near diploid
Phenotype	+++	+++	++
Ease of use	+	+++	+++
Reproducibility of results	+	+++	+++

Results

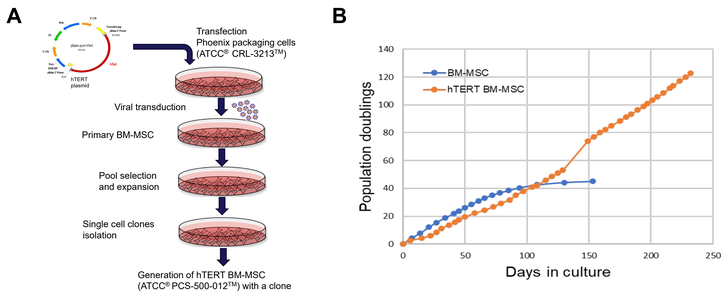

Figure 2: Immortalization of human primary bone marrow–derived mesenchymal stem cells (BM-MSC) with hTERT. (A) Primary BM-MSC (ATCC® PCS-500-012™) were transduced with hTERT retrovirus. The hTERT BM-MSC (ATCC® SCRC-4003™) cell line was generated with a clone. (B) hTERT BM-MSC (ATCC® SCRC-4003™) cells maintained consistent growth over > 122 population doublings while primary BM-MSC underwent senescence around 45 doublings.

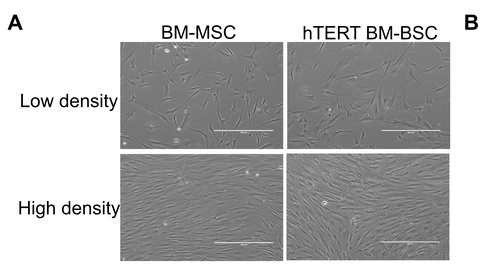

Figure 3: Evaluation of morphology and karyotype. (A) Morphology of primary BM-MSC and hTERT BM-MSC in low and high densities. hTERT BM-MSC showed similar morphology to that of primary cells. (B) hTERT BM-MSC retained a normal diploid karyotype.

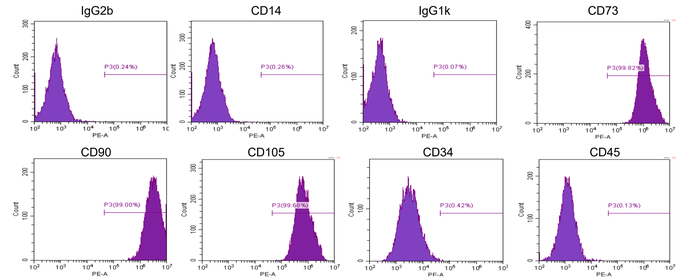

Figure 4: Evaluation of marker expression by flow cytometry. The hTERT BM-MSC cell line is positive for mesenchymal stem cell markers as shown via flow cytometry analysis. Cells were harvested and stained with PE Mouse Anti-Human CD73, CD90, CD105, CD14, CD34, and CD45 antibodies or PE Mouse IgG1κ and IgG2b isotype control antibodies. Positive markers: CD73, CD90, CD105; Negative markers: CD14, CD34, CD45; IgG 1K: Isotype control for CD73, CD90, CD105, CD34, CD45; IgG2b: Isotype control for CD14.

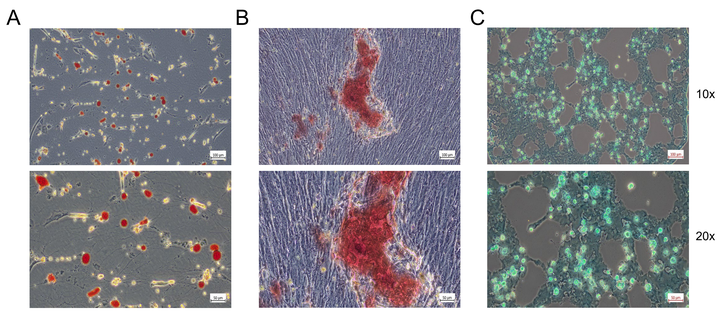

Figure 5: Immortalized cells can differentiate into adipocytes, osteocytes, and chondrocytes. (A) Adipogenic differentiation. Oil Red O (Lifeline® Cell Technology) staining of lipid droplets. (B) Osteogenic differentiation. Alizarin Red (Lifeline® Cell Technology) staining of calcium deposits. (C) Chondrogenic differentiation. Alcian Blue (Lifeline® Cell Technology) staining of sulfated proteoglycan deposits.

Conclusions

- Bone marrow–derived mesenchymal stem cells were successfully immortalized by hTERT.
- The immortalized hTERT BM-MSCs maintain mesenchymal stem cell morphology and marker expression.
- The immortalized hTERT BM-MSCs maintain differentiation abilities.
- hTERT BM-MSCs provide a valuable tool for cell and gene therapy.

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