GENERATION OF "OSTEOARTHRITIS" AND "HEALTHY" MESENCHYMAL CELL LINES FOR RESEARCH ON REGENERATIVE MEDICINE FOR OSTEOARTHRITIS

María Piñeiro-Ramil[1], Rocío Castro-Viñuelas[1], Clara Sanjurjo-Rodríguez[1], Tamara Hermida-Gómez[2,3], Isaac Fuentes-Boquete[1,2,3], Javier de Toro-Santos[1,3], Francisco Blanco-García[2,3], Silvia Díaz-Prado[1,2,3].

[1]Cell Therapy and Regenerative Medicine Group, Department of Biomedical Sciences, Medicine and Physiotherapy, Faculty of Health Sciences, University of A Coruña (UDC); Biomedical Research Institute of A Coruña (INIBIC); University Hospital Complex of A Coruña (CHUAC), Galician Health Service (SERGAS); A Coruña, Spain.

[2]Tissular Bioengineering and Cell Therapy Unit (GBTTC-CHUAC); Rheumatology Group, INIBIC; CHUAC, SERGAS; A Coruña, Spain.

[3]*Centro de Investigación Biomédica en Red* (CIBER*) de Bioingeniería, Biomateriales y Nanomedicina* (CIBER-BBN), Spain.

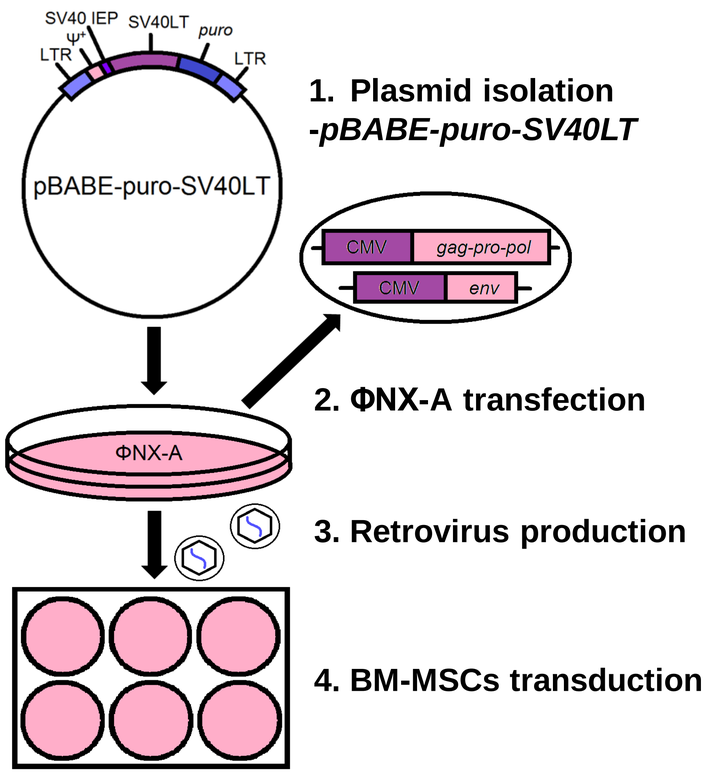
PURPOSE

Bone-marrow mesenchymal stem cells (BM-MSCs) are multipotent self-renewal adult cells with potential to regenerate the damaged tissues in degenerative diseases such as osteoarthritis (OA). Nevertheless, research require *in vitro* expansion of BM-MSCs, a process which eventually causes cell senescence. To overcome this problem cell lines can be used but, currently, BM-MSC lines available are scarce and present limitations regarding their differentiation capacities. For this reason, the aim of this study was to generate and characterize human BM-MSCs lines, derived from an OA patient and a healthy donor, with high chondrogenic and osteogenic capacities for their use in research on Regenerative Medicine for OA.

METHODS

For the generation of BM-MSC lines, SV40 large T antigen (SV40LT) was used. We developed a method for human BM-MSCs transduction employing ΦNX-A-produced retroviruses, spinoculation with addition of *Polybrene* and transgene expression induction by valproic acid (VA) (Figure 1). After antibiotic selection, nuclear expression of SV40LT was proven by immunocytochemistry. Population doubling level (PDL) values were calculated at each passage as a measure of proliferation of SV40LT-transduced BM-MSCs. Maintenance of BM-MSCs characteristics in SV40LT-transduced BM-MSCs was proven by analysis of clusters of differentiation expression by flow cytometry and cell differentiation experiments. Osteogenic and adipogenic differentiations were analyzed by Alizarin Red and Oil Red O hystochemical staining. Chondrogenic differentiation was analyzed by Safranin O and Masson's trichrome hystochemical stainings and also by immunohystochemical stainings for aggrecan and type II collagen.

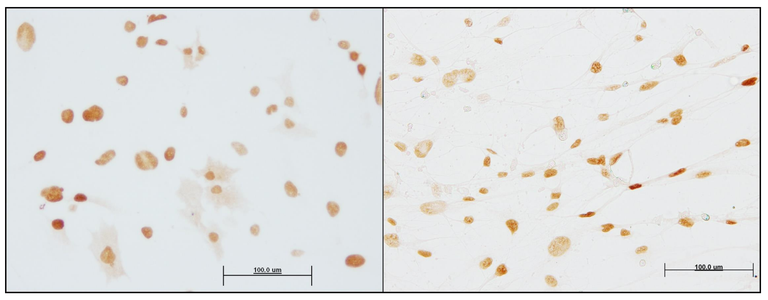
Figure 1. Human BM-MSCs transduction method, employing SV40LT, ΦNX-A-produced retroviruses, spinoculation with *Polybrene* and addition of VA.



RESULTS

We have obtained two SV40LT-transduced human BM-MSC lines: one derived from an OA patient and the other derived from a healthy donor. SV40LT nuclear expression was proven in both lines (Figure 2). A constant increase in PDL values confirms that BM-MSCs lines do not senesce, unlike primary BM-MSCs (Figure 3). Expression of CD29, CD44, CD73, CD90 and CD105 and lack of CD34 and CD45 was conserved in OA BM-MSCs line (Figure 4). Although SV40LT-transduced BM-MSCs maintain their multipotency (osteogenic, chondrogenic and adipogenic differentiation capacity), differentiation potential was altered after SV40LT transduction. Osteogenic potential was increased while adipogenic potential was reduced in BM-MSCs lines (Figure 5). Both BM-MSCs lines showed chondrogenic potential and were capable of spheroid formation (Figure 6).

Figure 2. OA BM-MSC line (a) and healthy BM-MSC line (b) SV40LT immunocytochemistry.



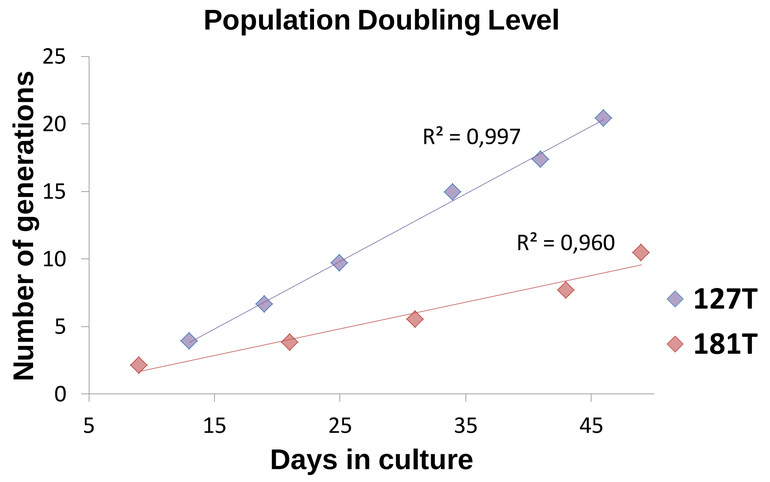


Figure 3. Number of generations calculated as (*log Nf – log Ni*) / *log* 2, being *Nf* the final cell population, *Ni* the number of cells in the inoculum and *log* the natural logarithm facing days in culture.

Figure 4. OA BM-MSCs and OA BM-MSC positive (CD29, CD44, CD73, CD90 and CD105) and negative (CD34 and CD45) mesenchymal superficial markers expression.

	CD29	CD44	CD73	CD90	CD105	CD34	CD45
OA BM-MSCs	99,2	99,6	98,4	94,9	85,2	9,9	0,1
OA MSC line	99,0	98,9	96,2	99,1	82,3	1,5	0,4

Figure 5. Alizarin Red and Oil Red O hystochemical stainings of osteogenic and adipogenic cell differentation experiments.

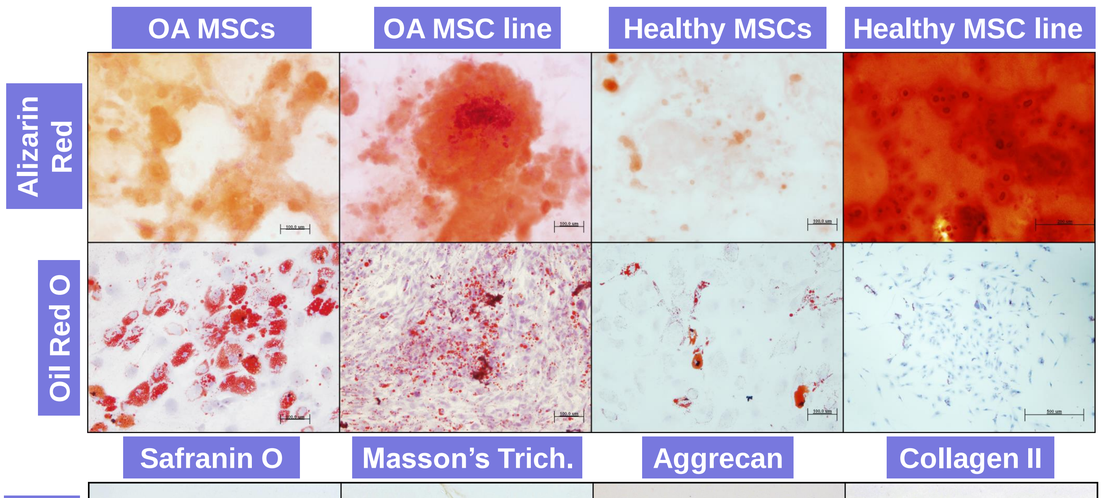


Figure 6. Safranin O and Masson's Trichrome hystochemical satianings and immunohystochemistry against aggrecan and type II collagen of shperoid-shaped chondrogenesis differentiation experiments for OA and healty MSC lines.

CONCLUSIONS

Two BM-MSCs lines were generated: one "OA" and one "healthy". BM-MSC lines shows an increased lifespan while maintaining primary BM-MSCs characteristics, although differentiation potential could be altered. Osteogenic potential may be increased by SV40LT transduction. The cell lines generated are expected to be very useful in research on Regenerative Medicine for OA.

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