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Generation of osteoarthritic mesenchymal stromal cell lines.

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

1. Universidade da Coruña (UDC), A Coruña, Spain..
2. Instituto de Investigación Biomédica de A Coruña (INIBIC), Spain.

Background. Bone-marrow mesenchymal stromal cells (MSCs) are multipotent self-renewal adult cells with high potential to regenerate the damaged tissues in degenerative diseases such as osteoarthritis (OA). Nevertheless, their usefulness for osteochondral Regenerative Medicine research is hampered by their proneness to senescence when in vitro cultured. Currently, MSC lines available are scarce and present limitations regarding their differentiation capacities. In addition, there is none OA MSC line available for research on this disease.

Objetives. The aim of this study was to generate and characterize immortalized human OA and non-OA MSC lines for their use in osteochondral Regenerative Medicine research.

Methods. For the generation of the immortalized MSC lines, SV40 large T antigen (SV40LT) and GFP-fused human telomerase reverse transcriptase (hTERT) were used. Primary MSCs derived from two hip OA patients and one hip fracture patient without OA were transduced by spinoculation at 800 xg for 45 minutes. Transgene expression was induced by valproic acid. Nuclear expression of SV40LT and GFP was tested by immunofluorescence. Proliferation and senescence were investigated through calculation of population doublings (PDs) at each passage after immortalization and β-galactosidase staining after 100 PDs for each MSC line. Maintenance of MSC characteristics in immortalized MSCs was tested by analysis of CD29, CD44, CD73, CD90, CD105, CD34 and CD45 expression by flow cytometry and cell differentiation experiments. Multi-lineage differentiation potential was analysed histochemical, immunohistochemical and molecularly.

Results: Three MSC lines have been generated: two OA and one non-OA. As shown by immunofluorescence, SV40LT is expressed in the nucleoplasm of these cells, while GFP-fused hTERT is expressed in the nucleoli. A constant proliferation rate thoroughout subculturing in addition to β-galactosidase negative staining confirms that immortalized MSC lines do not senesce, unlike primary MSCs. Expression of CD29, CD44, CD73 and CD90 and lack of CD34 and CD45 was conserved in immortalized MSC lines, while CD105 expression was altered for

transduction status and passage. Both OA and non-OA immortalized MSC lines maintain their multipotency (namely, osteogenic, chondrogenic and adipogenic differentiation capacity).

Conclusion. Both OA and non-OA MSCs are susceptible to immortalization by SV40LT and hTERT. For they increased lifespan combined with keeping of most of primary MSC characteristics, these MSC lines are expected to be valuable tools for the research on Regenerative Medicine for OA as part of Tissue Engineering in vitro models.

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