

Table 1. Baseline characteristics of patients:

Variable	PRP group (n=16)	HA group (n=22)	NALJ group (n=13)	P
Age (years) (x±SD)	59.56±8.70	63.32±5.49	62.23±7.73	NS*
BMI (kg/m ²) (x±SD)	30.31±3.75	30.3±3.36	31.99±5.94	NS*
Female (n,%)	8(50)	12(54.5)	7(53.8)	NS**
KOA stage (n,%)				NS**
II	5(33.3)	11(50)	6(46.2)	
III	10(66.7)	11(50)	7(53.8)	
Knee treated (n,%)				NS**
Left	4(25)	12(54.5)	7(53.8)	
Right	12(75)	10(45.5)	5(41.7)	
VAS pain (x±SD)	6.81±2.14	6.73±1.55	6.54±2.07	NS*
WOMAC (x±SD)	48.56 ±13.31	48.68 ±16.09	57.69±18.98	NS*
OARSI (x±SD)	63.92 ±17.48	57.03	70.80±13.51	NS*

NS: not significant. statistical significance based on: *the simple ANOVA test and ** on the Chi-square test.

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AB0101 SECRETOME ANALYSIS OF CHONDROCYTES AND SYNOVIAL FIBROBLASTS IN OSTEOARTHRITIS: MODULATION BY VIP

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Background: Osteoarthritis (OA) is a chronic, degenerative and multifactorial disease, and the main cause of pain and dysfunction among elder people. It is characterized by a progressive loss of function of synovial joints (1). The role of chondrocytes in this pathology has been widely studied (2), but other joint cells are also involved, including the synovial fibroblast (SF) (3-5). During joint destruction, inflammatory and degradative mediators are released by joint cells and from the extracellular matrix (ECM), including fibronectin degradation fragments (Fn-fs) (3, 4, 6). On the other hand, vasoactive intestinal peptide (VIP) exerts anti-inflammatory and immunomodulatory actions in several autoimmune and inflammatory disorders, including OA (3, 5). The study of the mediators released by joint cells and their modulation by pro- and anti-inflammatory mediators would be useful for the design of novel therapies for OA treatment.

Objectives: To analyse the mediators released from co-cultures of OA chondrocytes and SF, and to elucidate the effect of Fn-fs and VIP on these cells.

Methods: Human articular chondrocytes (HAC) and SF from 4 OA patients were provided by the Rheumatology Service at Complejo Hospitalario Universitario A Coruña. Isolated cells were recovered and plated in SILAC DMEM-Flex lacking Arginine and Lysine. In the case of medium and heavy media, isotope-labeled L-lysine and L-arginine were used. When 100% of labeling was reached, cells were put in co-culture and incubated in serum-free medium with or without Fn-fs (10⁻⁸M) or Fn-fs + VIP (10⁻⁸M) for 48h. Cell secretomes were separated on a 10% SDS PAGE gel. Gels were stained with Coomassie blue and the resulting lanes were cut into slices and subjected to in-gel digestion. Extracted peptide mixtures were desalted and concentrated via NuTip, subjected to liquid chromatography, using a Tempo nano LC equipped with a Sun Collect MALDI Spotter, and analyzed by MALDI-TOF/TOF. Identification of peptides and proteins and relative quantification were performed using Protein Pilot software (Sciex).

Results: Cell secretomes were analysed in 4 OA patients in duplicate. Database search (UniprotKB/Swissprot) and Venn's diagrams drawing tool (Venny 2.1.0) allowed us to identify 79 common proteins in the HACs-SF co-cultures. Among them, VIP was able to modulate 33 different proteins, significantly reducing 9 of them: CH3L1, PTX3, PGS2, MMP2, Complement C1R, Complement C3, TBA1, QSOX1, and CATB. These proteins include inflammatory and ECM proteins, proteases and complement system proteins among others, which play a main role in the OA pathogenesis.

Conclusion: VIP decreases inflammatory and degradative mediators in HAC-SF co-cultures, potentially slowing the progression of the disease and supporting its therapeutic role in osteoarthritis.

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AB0102 GENERATION OF OSTEOARTHRITIC MESENCHYMAL STROMAL CELL LINES

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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.

Objectives: 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 throughout 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