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Establishment of human induced pluripotent stem cell-lines (IPSC) for in vitro modelling hand ostheoarthritis

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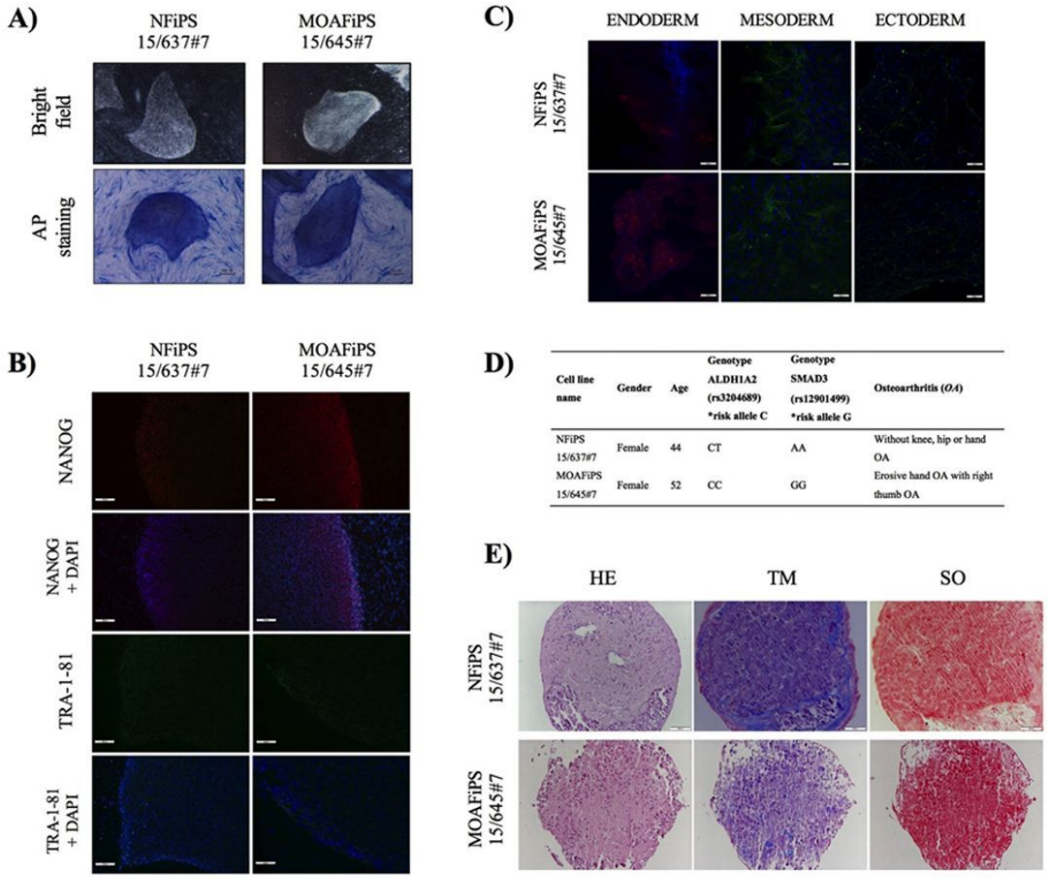
Background. Research results in the field of hand OA are currently limited due to the unavailability of tissue samples and lack of animal models replicating the features of the disease in humans. Cellular in vitro models are important tools to elucidate molecular mechanisms and pathways that are involved in hand OA. Specifically, induced pluripotent stem cells (iPSc) are considered ideal tools for this purpose since they allow the use of unlimited cells with chondrogenic differentiation potential. However, there are not studies published generating iPSc from patients with hand OA.

Objectives. To generate and characterize iPSc-lines from patients with radiographic hand OA and healthy donors, which can be used as cellular models of the disease, for studying the pathogenesis of the disease *in vitro* and for testing new drugs..

Methods. Patients with hand OA (non erosive hand OA with right thumb OA) and a healthy control were selected for the study. Using the explant culture technique, fibroblasts from skin biopsies of these patients were isolated. Transcriptional factors Oct4, Sox2, Klf4 and c-Myc were used for the reprogramming process; which was performed by using a non-integrating method, the Sendai virus. Cell lines obtained were morphologically, phenotypically and functionally characterized. To evaluate weather these iPSc lines could be used as cellular model of hand OA, presence of single nucleotide polymorphisms (SNPs) within the genes ALDH1A2 and SMAD3 were studied by Sanger sequencing, before and after reprogramming. Variants rs3204689 and rs12901499 respectively have been associated with severe OA of the hand (Styrkarsdottir *et al*., 2014; Shu-Thao Gao *et al*., 2018). Finally, chondrogenic differentiation capacity of the "healthy" and "ill" iPSc-lines was studied by means of histological techniques..

Results. Fibroblasts were isolated from one patient with radiographic hand OA and one healthy donor. Three weeks after reprogramming, embryonic stem cell-like colonies emerged in culture. These cells showed positivity for alkaline phosphatase activity (fig. 1A) and the pluripotency markers Tra1-81 and Nanog (fig. 1B). Molecular analyses showed high relative expression levels of the pluripotency-related genes OCT4, SOX2, NANOG and CRIPTO in the

iPSc. These cells were also able to give rise to cells from the three germ layers (fig. 1C). Indeed, during mesodermal differentiation, spontaneously beating cardiomyocytes were seen in culture. Regarding SNPs studies, cells from the patient with hand OA were homozygous for the at-risk allele in both genes studied, both before and after reprogramming (fig. 1D). The "ill" iPSc-line (MOAFiPS 15/645#7) showed worse chondrogenic differentiation than the "healthy" iPSc-line (NFiPS 15/637#7), as shown by the micromasses collagen and proteoglycan content (fig. 1E).



Cell line name	Gender	Age	Genotype ALDH1A2 (rs3204689) *risk allele C	Genotype SMAD3 (rs12901499) *risk allele G	Osteoarthritis (*OA*)
NFiPS 15/637#7	Female	44	CT	AA	Without knee, hip or hand OA
MOAFiPS 15/645#7	Female	52	CC	GG	Erosive hand OA with right thumb OA

Figure 1. (A) Bright field images and images after alkaline phosphatase staining (AP staining) of iPSc colonies from each cell line, x4 magnification. (B) Immunofluorescence images of pluripotency markers (NANOG and TRA-1-81) of representative clones from each cell line. Escale 100 µm (C) Immunofluorescence images of endoderm, mesoderm and ectoderm markers in each one of the cell lines. Escale 100 µm. (D) Summarize of the results obtained after single nucleotide polymorphism analysis. (E) Haematoxilin-Eosin (HE), Masson's Tricomic (TM) and Safranin-O (SO) staining after chondrogenic differentiation of the iPSC lines, x10 magnification.

Conclusion. The generation of one iPSc-line form patients with hand OA is reported for the first time. The presence of the at-risk alleles within the ALDH1A2 and SMAD3 genes were maintained after fibroblast reprogramming. The iPSC lines obtained shown differences in their chondrogenic differentiation capacity, showing their usefulness to model hand OA *in vitro*, and to deeper study the role of these genetic variants in the pathogenesis of hand OA..

References. [1] Styrkarsdottir U, et al. (2014) Nat Genet. 46 (5): 498-502.
[2] Shu-Tao Gao et al. (2018) Ther Clin Risk Manag. 14: 929–936.

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