

Investigation of potential new targets for the diagnosis and/or the treatment of osteoarthritis

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P U R P O S E. Synovial membrane plays a key role in osteoarthritis (OA) pathophysiology. We have previously compared the gene expression pattern of synovial cells isolated from inflammatory (I) or normal/reactive (N/R) areas of a synovial membrane harvested from the same OA patient. We identified a large number of mediators belonging to key pathways involved in OA pathogenesis. The aim of this study was to validate different potential new targets for the diagnosis and/or the treatment OA. ■

M E T H O D S. Synovial cells (SC) were isolated from OA synovial specimens obtained from 12 patients undergoing knee replacement. The inflammatory status of the synovial membrane was characterized according to macroscopic criteria. The biopsies from N/R and I areas were cultured separately for a period of 7 days. Microarray gene expression profiling between N/R and I areas was performed. The biological relevance of up- and down-regulated genes was analyzed with Ingenuity Pathways Analysis. Western blot and immunohistochemistry confirmed the identified genes most differentially expressed in the key pathways. ■

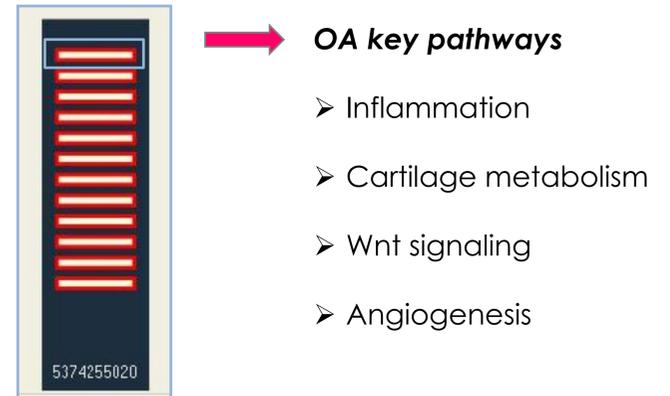


Fig.1 Differential gene expression between N/R and I synovial biopsies obtained by Human HT-12 BeadChip Array (illumina). 896 differentially expressed genes between N/R and I areas were identified. A significant number of the top ranking differentially expressed genes were identified as inflammatory, cartilage metabolism, Wnt or angiogenic pathways.

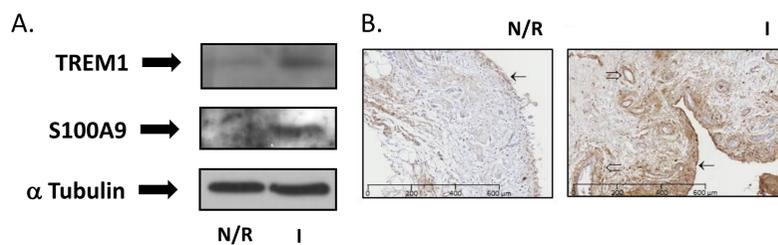


Fig 2
A. Increase of TREM1 and S100A9 protein production in I area compared to N/R area. Total protein extracts from N/R or I areas were analyzed by Western blotting with anti-TREM1, anti-S100A9 and anti- α Tubulin (control) antibodies.
B. Immunohistochemical detection of S100A9 in N/R and I synovial biopsies. In I synovial biopsies, a staining for S100A9 is observed in perivascular and sublining cells. N/R and I synovial biopsies were stained with anti-S100A9 antibody. (Magnification x10) (→) Intima lining, (⇒) Blood vessels.

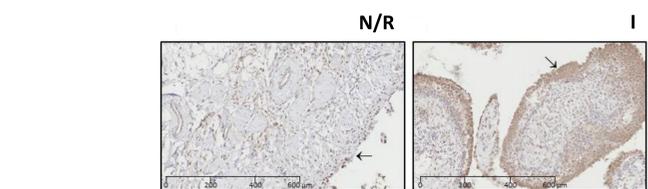


Fig 3
Immunohistochemical detection of HAS1 in N/R and I synovial biopsies. In I synovial biopsies, there are an increase in the HAS-1-positive cells with a predominance localization in the intima lining. N/R and I synovial biopsies were stained with anti-HAS1 antibody. (Magnification x10) (→) Intima lining.

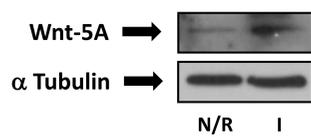


Fig. 4
Increase of Wnt-5A protein production in I area compared to N/R area. Total protein extracts from N/R or I areas were analyzed by Western blotting with anti-Wnt-5A and anti- α Tubulin (control) antibodies.

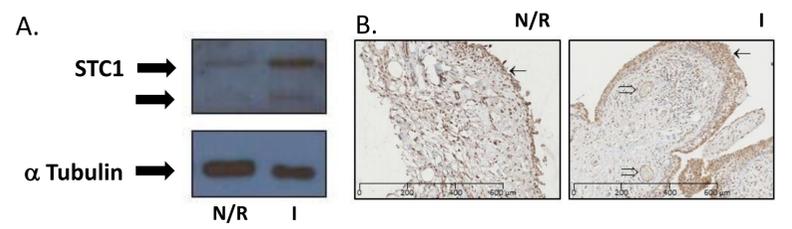


Fig. 5
A. Increase of STC1 protein production in I area compared to N/R area. Total protein extracts from N/R or I areas were analyzed by Western blotting with anti-STC1 and anti- α Tubulin (control) antibodies.
B. Immunohistochemical detection of STC1 in N/R and I synovial biopsies. In I synovial biopsies, a staining for STC1 is observed in perivascular and sublining cells. N/R and I synovial biopsies were stained with anti-STC1 antibody. (Magnification x10) (→) Intima lining, (⇒) Blood vessels.

C O N C L U S I O N. Synovial membrane inflammation is a key target for OA treatments. In this work, we have identified proteins involved in the synovitis pathways like *angiogenesis, cells infiltration* and *matrix remodeling*. These proteins could be targeted by drugs and used as companion biomarkers for evaluating their efficacy. Although qualitative, our results could also yield to the identification of markers of the disease. This investigation has to be further pursued. ■