containing target protein, we established stable MH7A cell lines overexpressing GFP-tagged PLCH2. We also conducted *in vitro* and *in vivo* experiments using a recombinant adenovirus expressing the full length form of PLCH2 or C2 domain of PLCH2 (C2-PLCH2).

Results Several C2 domain-containing proteins were identified whose over-expression resulted in reduced proliferation and NF- κ B activity of RA-FLS. Among those, we focused on PLCH2 in this study. PLCH2 levels were significantly decreased in RA-FLS and synovium than OA. PLCH2 and C2-PLCH2 suppressed cell invasion and migration, cytokines production, and matrix metalloproteinase secretion in RA-FLS and stable MH7A cell lines compared to control. PLCH2 and C2-PLCH2 sensitised RA-FLS to apoptosis *in vitro* and *in vivo* model of matrigel implants engrafted into immunodeficient mice. PLCH2 and C2-PLCH2 further decreased arthritis severity in collagen-induced arthritis (CIA) mouse model.

Conclusions Our results showed that invasive characteristics of RA-FLS and inflammatory arthritis of CIA are reduced by PLCH2 and C2 domain of PLCH2. Therefore C2 domain of PLCH2 may have therapeutic potential for RA.

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P038

RECENT THYMIC EMIGRANT T CELLS INCREASE IN RHEUMATOID PATIENTS TREATED WITH ABATACEPT

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Introduction CD4⁺ T cells in rheumatoid arthritis (RA) display a peculiar restriction of the T-cell receptor (TCR) repertoire which compromises their ability to react to novel antigens.¹ We demonstrated that this process could be partially reverted by abatacept (ABA), which is a blocker of T lymphocyte co-stimulation, used in the treatment of RA.²

To better understand the mechanisms underlying this phenomenon, we speculated that ABA could influence the frequency of peripheral T cell subpopulations, even at early stages of maturation.

The homeostasis of the *naïve* T cell pool is maintained not only by thymus production, but also by mechanisms of peripheral replication involving TCR activation revealed by CD31 down-modulation. Indeed, CD4⁺CD45RA⁺CD31⁺ T cells have been described as *recent thymic emigrants* (RTE) newly produced by the thymus

and CD4⁺CD45RA⁺CD31⁺ T cells as *central naïve*, involved in a self-renewal peripheral process.

Objectives We aimed at profiling circulating *naïve* CD4⁺CD45RA⁺ T cells, by assessing their expression of CD31 (PECAM-1).³

Methods Thirty-one RA patients (median age [10th–90th percentile] 42 [25–64] years) were evaluated before and after 6 month of ABA therapy. Phenotypic analysis of peripheral blood T lymphocytes was made by flow-cytometry. Wilcoxon-signed rank test and Spearman rank correlation were used for the comparisons.

Results After ABA therapy, the absolute number of total T CD4⁺ increased from 780 [423–1351] to 1000 [658–1566] cells/mm³ (p=0.01). Total *naïve* CD4⁺ increased in percentage (33 [18–56] vs 40 [20–61]% of CD4⁺; p=0.02) and in absolute number (257 [82–568] vs 344 [62–689] cells/mm³; p=0.03). In parallel, the number of RTE increased in percentage (10.6 [2–26] vs 11.6 [3–25]% of CD4⁺; p=0.04) and in absolute number (51 [15–194] vs 110 [23–271] cells/mm³; p=0.01). The *central naïve* counterpart did not show significant variations in percentage (29 [23–40] vs 27 [20–38]% of CD4⁺; p=0.20) nor in absolute number (191 [41–404] vs 242 [76–526] cells/mm³; p=0.20). No correlation was found between age and RTE number at baseline.

Conclusions The number of total *naïve* T cells increases after therapy with ABA together with the number of RTE, suggesting a thymic output boost. Besides the peripheral effect in reducing the number of effector T cells which was showed by previous studies,⁴ ABA could have a role in promoting the immune reconstitution at the early stage of T cell development.

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P039

ROTATOR CUFF CALCIFIC TENDINOPATHY: CHONDROCYTE-LIKE CELLS SURROUNDING CALCIFIC DEPOSITS EXPRESS TNAP AND ENPP1, TWO KEY ENZYMES OF THE MINERALIZATION PROCESS

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Introduction Calcific tendinopathy is a frequent cause of chronic painful shoulders. It is caused by deposits of carbonated apatite in rotator cuff tendons. Although the disease is frequent, molecular and cellular mechanisms involved in this pathological mineralization process are not currently identified. **Objectives** The objective of the study was to analyse calcified tendon samples to understand the organisation of the deposits and to characterise the cells potentially involved in their formation.

Methods Samples were collected from cadaveric subjects. They were fixed in formalin 4%, decalcified in EDTA, dehydrated and embedded in paraffin. Some samples were not decalcified to allow a better characterisation of the calcific deposits. Hematoxylin and eosin (HE), Safranin O/Fast Green (SO/FG), Von Kossa (no decalcified samples) and Tartrate-Resistant Acid Phosphatase (TRAP)

staining were performed. Immunohistochemistry using anti-Runx2, anti-Sox9, anti-Collagen II and X, anti-CD31 and CD68 antibodies has been performed. We used also anti-TNAP (Tissue Nonspecific Alkaline Phosphatase) and ENPP1 (Ectonucleotide Pyrophosphatase/Phosphodiesterase 1) antibodies. Indeed, these two enzymes are essential in the physiological mineralization: extracellular inorganic pyrophosphates are provided by ENPP1 then hydrolyzed by TNAP to promote mineralization.

Results

Five calcified samples were collected On HE staining, voluminous calcium deposits were encapsulated by a fibrocartilaginous tissue. In one sample, we observed an intra-tendinous osseous metaplasia. This fibrocartilaginous area presented a red coloration (proteoglycan specific) on SO/FG staining but was collagen II negative whereas the fibrocartilage at the tendon attachment was strongly positive. Within this area, cells with round nuclei and pericellular lacunae were observed as previously described (Uthoff, 1975). These cells expressed Runx2 and Sox9 suggesting a chondrocyte differentiation but only a small number of them expressed type X collagen, hypertrophic chondrocytes-specific marker. These cells also expressed ENPP1 and TNAP. Interestingly, extracellular TNAP deposits were also present at the periphery of the deposits. We identified vessels surrounding the deposits on 4 of the 5 calcified samples. Finally, no CD68 positive cells or TRAP positive cells were detected around the deposits.

Conclusions Histological analyses of whole calcified tendon tissues showed a fibrocartilaginous area surrounding the calcium deposits with chondrocyte-like cells expressing ENPP1 and TNAP suggesting their crucial role in the deposition of apatite crystals. Further analyses are necessary to understand the origin of these cells and the regulatory factors involved in their differentiation.

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P040

INVOLVEMENT OF THE ANTI-AGEING PROTEIN KLOTHO IN CHONDROCYTE AUTOPHAGY AND APOPTOSIS DURING OSTEOARTHRITIS

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Introduction The pathogenesis of OA is not fully characterised, but is thought to be due to perturbation of chondrocytes homeostasis including an impairment in the autophagy process particularly during ageing. Among the anti-ageing factors, Klotho has been shown to regulate autophagy in a variety of cell types, and Klotho polymorphisms have been associated with higher risks of OA.

Objectives The aim of this project was to investigate the role of Klotho in OA.

Methods The expression of Klotho and autophagy markers (LC3b and Beclin-1) and OA onset were evaluated in ageing C57BL/6 mice as an age-related spontaneous model of OA. The cartilage integrity, autophagy and apoptosis status in

Klotho-deficient mice knee joints were also analysed. In parallel, to investigate the relationship between Klotho and autophagy, immature murine articular chondrocytes (iMACs) were stimulated with increasing doses of soluble recombinant Klotho. The effect of Klotho on autophagy was also evaluated in a pathological context, i.e. following IL1- β stimulation (10 ng/ml) for 24 hours. Bax/Bcl-2 ratio, a marker of the intrinsic apoptotic pathway, was also evaluated in IL1- β -treated chondrocytes.

Results In the knee joints of mice from escalating ages, the expression of Klotho correlated with LC3b and Beclin-1 expression and gradually decreased with age while OA features appear. Articular cartilage of KL-/- mice revealed an increase in the OARSI score, associated with increased chondrocyte death and LC3b expression, as well as caspase 3 and TUNEL expression. In IL1- β -treated chondrocytes, the autophagy markers and Bax/Bcl-2 ratio were overexpressed, while the addition of Klotho counteracted this effect.

Conclusions In summary, we described an early articular cartilage degradation in the absence of Klotho, suggesting a potential protective role of Klotho in OA development. The increase in autophagic process in Klotho mutant mice associated with the decrease in autophagy and Klotho expression with age clearly indicate intimate relationships between these two players. *In vitro* experiments suggested that Klotho may not have a direct effect on autophagy but rather through reducing apoptosis induced by pro-inflammatory cytokines such as IL1- β . Our study revealed the close relationship between the anti-ageing Klotho protein and chondrocyte death in articular cartilage, unveiling Klotho as a potential target to enhance chondrocyte survival.

Disclosure of interest None declared

P041

TISSUE GENE PROFILING UNCOVERS CADHERIN 11 RELATED SIGNATURES IN RHEUMATOID ARTHRITIS PATIENTS

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Introduction Cadherin 11 is selectively expressed by synovial fibroblasts and plays a role in the pathogenesis of rheumatoid arthritis (RA).¹ Consequently, blocking cadherin 11 function in inflamed tissues may represent a potentially effective and novel therapy for RA.

Objectives Here we interrogated publicly available transcriptomics datasets from RA patients synovial tissue and healthy controls using pre-defined primary cell gene signatures to better understand the heterogeneity of the underlying pathology. In addition, we analysed the association of these gene signatures with the expression of cadherin 11 gene to narrow down the underlying mechanistic network on which targeted treatments and biomarkers can be developed.

Methods We used two publicly available transcriptomics studies from NCBI Gene Expression Omnibus² performed on synovial tissue of RA patients and healthy controls: GSE7307 (RA=5, healthy=7) and GSE77298 (RA=16, healthy=7). The pre-defined gene signatures were derived from ENCODE primary cell expression data³ and signature enrichment was applied using the BioQC package.⁴ Principal component