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 used 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 (Uhthoff, 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.

REFERENCE

1. Uhthoff HK. Calcifying tendinitis, an active cell-mediated calcification. *Virchows Arch a Pathol Anat Histol* 1975;**366**(1):51–8.

Disclosure of interest None declared

P040 | **INVOLVEMENT OF THE ANTI-AGEING PROTEIN KLOTHO IN CHONDROCYTE AUTOPHAGY AND APOPTOSIS DURING OSTEOARTHRITIS**

[1]E Le Tilly, [1]T Ong, [2]J Abadie, [1,3]J Guicheux, [1]L Beck, [1]C Vinatier*. [1]*INSERM UMR 1229 – RMeS, Regenerative Medicine and Skeleton, STEP Team, University of Nantes, UFR Odontology*; [2]*Animal Cancers as Models for Research in Comparative Oncology (AMaROC), ONIRIS*; [3]*PHU 4 OTONN, CHU Nantes, Nantes, France*

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**

[1]K Hatje, [1]T Kam-Thong, [2]D Hartl, [1]G Duchateau-Nguyen*. [1]*Roche Pharma Research and Early Development, Pharmaceutical Sciences*; [2]*Roche Pharma Research and Early Development, Immunology, Inflammation and Infectious Diseases, F. Hoffmann-La Roche Ltd, Basel, Switzerland*

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 Omnibus[2] 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 data[3] and signature enrichment was applied using the BioQC package.[4]Principal component