SF) stimulation was analysed by ELISA. FAO was inhibited by etomoxir or enhanced with exogenous carnitine supplements. Transcriptomics of RA blood monocytes and RA-SF macrophages was carried out by microarray.

Results We report that hypoxia exacerbates CCL20 and IL-1 β release in response to LPS and increases glycolytic intermediates at the expense of carnitines. Modulation of carnitine identified a novel role for FAO in the production of CCL20 in response to LPS. Transcriptomics of RA blood monocytes and RA-SF macrophages revealed that fatty acid metabolism was altered and CCL20 was increased when monocytes enter the RA milieu. *In vitro* analysis of monocytes showed that RA-SF increases carnitine abundance and CCL20 production in hypoxia, which was exacerbated by exogenous carnitine.

Conclusions This work has revealed a novel inflammatory mechanism in RA which links FAO to CCL20 production in human monocytes. This may contribute to RA disease pathogenesis by promoting the recruitment of Th17 cells and osteoclastogenesis.^{2, 3}

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P109

EXPRESSION LEVELS OF MIR-21 AND MIR-29 IN THE SERUM OF SYSTEMIC SCLEROSIS PATIENTS

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Career situation of first and presenting author Assistant.

Introduction Recent studies reveal the important role of microRNAs (miRNAs) in the pathogenesis of systemic sclerosis (SSc). miRNAs are involved in key biological pathways that regulate the fibrotic process in SSc.¹ Deregulated expression of miRNAs has been found in the serum, in skin tissues, in skin fibroblasts as well as in endothelial cells of patients with SSc.²

Objectives The aim of our study was to evaluate the expression levels of miR-21 and miR-29 in the serum of SSc patients and to determine their correlation with clinical and immunological parameters.

Methods 34 patients fulfilling the ACR/EULAR 2013 classification criteria for SSc were included in the study. miR-21 and miR-29 expression levels in the serum were determined by PCR (SYBR Green technology). 2^{- $\Delta\Delta C_t$} method was used for analysis. 14 healthy donors were used as controls.

Results Expression levels of miR-21 were upregulated in the serum of 17 (50.0%) of the patients. The expression of miR-29 was downregulated in 15 (44.12%) of the patients. Receiver operating characteristic (ROC) curve analysis was conducted in order to evaluate the diagnostic accuracy of the expression levels of the studied miRNA in the serum. Area

under the curve (AUC) for miR-21 was 0.634 (95% CI=0.479–0.790), p=0.147 with 64.7% sensitivity and 64.3% specificity. AUC for miR-29 was 0.605 (95% CI=0.420–0.790), with 64.3% sensitivity and 52.9% specificity but without statistical significance (p=0.257). The multimarker analysis of the ROC curves for both miRNAs showed AUC=0.714 (95% CI=0.569–0.860), p=0.021 with 79.4% sensitivity and 42.9% specificity. Levels of miR-29 correlated with the levels of miR-21 in the serum (with Spearman correlation coefficient 0.517, p=0.00017) and with the presence of anti-Scl70 antibodies in the serum (with Spearman correlation coefficient 0.438, p=0.010).

Conclusions Our data showed a deregulation of miR-21 and miR-29 in the serum of patients with SSc which could suggests their potential role in the disease pathogenesis. Further analysis with higher number of patients is needed to confirm if these miRNAs could be used in the clinical practice as diagnostic biomarkers as well as biomarkers for both disease activity and progression.

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P110/O18

EPIGENETIC CHANGES BY INHIBITION OF DOT1L AFFECT WNT SIGNALING, PROLIFERATION AND CELL CYCLE IN DERMAL FIBROBLASTS, WITH NO OVERALL EFFECT ON COLLAGEN DEPOSITION IN MODELS OF FIBROSIS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction The role of epigenetic factors in the pathophysiology of fibrosis, a hallmark of Systemic Sclerosis, is increasingly explored. DOT1L, the unique H3K79-methyltransferase, methylates histone 3 at the Lysine residue at position 79, thereby regulating gene expression programs. In cartilage and bone, DOT1L has cell-type specific effects on Wnt signaling, a pathway suggested to play an important role in fibrosis.

Objectives To study the role of DOT1L in fibrosis.

Methods Primary human dermal fibroblasts were treated with DOT1L-inhibitor EPZ-5676 or vehicle and stimulated with TGF- β . Expression of smooth muscle alpha 2 actin (ACTA2) and Wnt target genes was measured by RT-qPCR. Western Blot was done for dimethylated H3K79 and β -catenin. Picrosirius Red staining measured collagen deposition. 5-Bromo-2'-deoxy-uridine (BrdU) labeling for proliferation and flow cytometry with Propidium Iodide for cell cycle analysis was done. Col1a2;Cre-ERT²;DOT1L^{fl/fl} mice, injected

with tamoxifen to induce a fibroblast-specific DOT1L knockout, were injected subcutaneously with bleomycin or vehicle. Injected skin was analyzed by OH-prolin assay and by histology.

Results The DOT1L-inhibitor EPZ-5676 reduced H3K79 dimethylation in all samples. In breast dermal fibroblasts, the induction of ACTA2 with TGF- β was reduced with DOT1L inhibition, while in abdominal dermal fibroblasts this induction was more pronounced. After 48 hours of TGF- β , collagen deposition was higher in DOT1L-inhibited fibroblasts. After 72 hours of TGF- β however, this deposition was comparable with controls. DOT1L inhibition induced canonical Wnt signaling in fibroblasts, with a small increase in active β -catenin and expression of Lymphoid enhancer-binding factor 1 (LEF1). With DOT1L inhibition, more proliferation of fibroblasts, but also proportionally more cells in the G1/G0 phase and less cells in S and M/G2 phase were seen. *In vivo*, subcutaneous bleomycin increased murine dermal thickness and skin collagen content. No difference was observed between wild type and mice with a fibroblast-specific deletion of DOT1L.

Conclusions In an *in vitro* model of fibrosis, ACTA2 induction in DOT1L-inhibited human dermal fibroblasts was dependent on the fibroblast origin. DOT1L inhibition resulted in an earlier deposition of collagen, without differences in deposition at the end points. Inhibition of DOT1L induced canonical Wnt signaling and proliferation but also led to a higher proportion of cells in the G0/G1 phase. In an *in vivo* murine model of skin fibrosis, no difference in bleomycin-induced skin thickness and collagen content was found when the DOT1L gene was deleted in fibroblasts.

Disclosure of Interest None declared.

P111

TENOCYTES EXTRACTED FROM ROTATOR CUFF TENDONS INDUCE TNAP-DEPENDENT MINERAL DEPOSITION AND EXPRESS GENES RELATED TO A HYPERTROPHIC CHONDROCYTE DIFFERENTIATION

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Career situation of first and presenting author Resident.

Introduction Calcific tendinopathy represents 10% to 42% of chronic painful shoulders. These calcium deposits are composed of carbonated apatite. Although the disease is frequent, its origin stays still largely unknown. Our previous results showed that calcific deposits are surrounded by chondrocyte-like cells expressing TNAP (Tissue Nonspecific Alkaline Phosphatase) and ENPP1 (Ectonucleotidepyrophosphatase/phosphodiesterase 1), two key enzymes involved in the mineralization process.

Objectives To study the ability of cells extracted from rotator cuff tendons to produce apatite crystals and to analyze the phenotype of these mineralizing cells.

Methods Tenocytes were extracted from rotator cuff tendons removed during shoulder total replacement. To evaluate their ability to mineralize, they were cultured in an osteogenic medium (OM) for 21 days. Mineral deposition then was assessed by staining with Alizarin red. Tenocytes total RNA

was extracted and analyzed by RT-qPCRs. TNAP enzymatic activity was also assessed in the cells. A TNAP inhibitor was used to delineate its implication in the mineralization process.

Results Tendon samples were obtained from 5 patients (age 69.6 ± 5.13 years). Cells extracted from these tendons expressed collagen I, collagen III, Scleraxis and Mxk (Mohawk homeobox), as expected for tenocytes. However, Tenomodulin was very weakly expressed and lost after passage 1. These cells were able to mineralize in the OM although no mineralization was observed in the control medium. qPCR analyses showed a significant increase of TNAP and ENPP1 expression by cells cultured in OM ($p < 0.05$). Osteoblast markers (Runx2, osteocalcin, osteopontin, BSP) were not increased by the OM. COMP (Cartilage Oligomeric Matrix Protein), a chondrocyte marker was significantly increased, as well as MMP13 (Matrix Metalloproteinase 13) and Collagen X suggesting a hypertrophic differentiation. In parallel, in the OM, TNAP enzymatic activity was significantly higher at 14 and 21 days compared to the control medium. An inhibitor of TNAP completely prevented mineral deposition in OM and reduced expression of the hypertrophic chondrocyte markers MMP13 and Collagen X.

Conclusions Tenocyte-like cells extracted from tendons of the rotator cuff are able to induce mineralization in an osteogenic medium. The cells express genes associated with a hypertrophic chondrocyte phenotype (TNAP, COL10 and MMP13) and TNAP seems to have a crucial role in the induced mineralization. These results suggest that tenocytes could differentiate into hypertrophic chondrocyte which induce TNAP-dependent apatite deposition in calcific tendonitis.

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Disclosure of Interest None declared.

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STUDYING CELLULAR SENESCENCE IN HUMAN LYMPH NODE STROMAL CELLS DURING THE EARLIEST PHASES OF RHEUMATOID ARTHRITIS

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Career situation of first and presenting author Student for a master or a PhD.

Introduction Cellular senescence is a state of proliferation arrest of cells. The persistence and accumulation of senescent cells has been implicated in the pathogenesis of age-related diseases. Ageing is an important risk factor of rheumatoid arthritis (RA), a prototypic autoimmune disease in which loss of immune tolerance and systemic autoimmunity precedes clinical onset of disease. Through their intimate contact with lymphocytes, lymph node stromal cells (LNSCs) are important regulators of peripheral tolerance. Therefore, malfunctioning senescent LNSCs may potentially lead to defective peripheral