Bank, University and Hospital Centre of Coimbra, with approval by the Ethics Committee. Phosphorylated levels of JNK, p38, ERK1/2, PKA, Akt and PKC and the levels of LC3B-I and II were evaluated by Western Blot of total cell extracts from C28/I2 cells.

Results: Immunoreactivity for Y₁, Y₂ and Y₅ NPY receptors was observed in C28/I2 cells. In human cartilage, a positive signal was found for the Y₂ receptor in all samples while Y₅ receptor immunoreactivity was undetectable, regardless of disease state, gender and age of the donors. Y₁ receptor immunoreactivity was observed in male and female OA cartilage samples, as well as in those from non-OA females, but not in those from non-OA males. 50 nM NPY was sufficient to significantly increase the levels of phospho-JNK, -p38, -ERK1/2, -PKA, Akt and PKC with similar kinetics, but much slower than IL-1 β . A 6 hour stimulation with 50 or 100 nM NPY decreased LC3B-I and II levels in comparison with untreated cells. In the presence of chloroquine (ChQ), NPY increased LC3B-II levels relative to those found in cells treated with ChQ alone, indicating an increased delivery of LC3B-II to the lysosome consistent with autophagy activation by NPY.

Conclusions: This study shows that distinct NPY receptor subtypes are present and functional in C28/I2 cells and in human chondrocytes *in situ* in the articular cartilage, strongly suggesting that non-neuronal cells and tissues of the joints, namely chondrocytes, are relevant as NPY targets. The presence of each receptor subtype seems to be determined by gender and, in males, also by the disease state. The role of age is unclear as most cartilage donors were aged >55 years old. Future studies will be addressed at further elucidating the role of NPY and its receptors in modulating male and female chondrocyte functions, both in health, ageing and osteoarthritis.

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AB0097 INDIVIDUAL FUNCTIONS OF THE HISTONE-ACETYLTRANSFERASES CBP AND P300 IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: The close homologues cAMP-response element binding protein (CREB) binding protein (CBP) and p300 are writers of H3K27 histone acetylation marks found in active enhancers. In addition, their bromodomains are readers of acetylated lysine residues on histone tails and are subject of drug development for inflammatory and malignant diseases. CBP and p300 are widely accepted as redundant proteins and unique functions have not been investigated yet in depth.

Objectives: To analyse individual functions of CBP and p300 in rheumatoid arthritis synovial fibroblasts (SF).

Methods: SF were treated with the pan inhibitor I-CBP (1 μ M, 5 μ M), targeting the bromodomains of CBP and p300, in presence and absence of TNF- α (10 ng/ml) for 24 hour. The expression of CBP and p300 was silenced by transfection of antisense LNA gapmeRs (5 nM) in SF. Knockdown was verified by Western blotting. 24 hour after transfection cells were stimulated with TNF- α (10 ng/ml) for 24 hour. The mRNA expression of potential target genes was measured by quantitative Real-time PCR, using RPLP0 as an endogenous control. The protein expression of HOXD10 after CBP and p300 silencing was verified by Western blotting.

Results: I-CBP dose-dependently reduced the TNF- α -induced expression of MMP1 (p<0,05), MMP3, IL6 and IL8 in SF (n=3). Antisense LNA gapmeRs targeting CBP reduced the protein expression of CBP by 68,7% ($\pm$ 12,9%, p<0,01, n=5) in unstimulated cells and by 89,7% ($\pm$ 12,9%) in presence of TNF- α . The protein expression of p300 was reduced by 55,3% ($\pm$ 29,8%, p<0,05, n=6) in unstimulated cells and by 62,7% ($\pm$ 27,9%) in presence of TNF- α after transfection of LNA gapmeRs targeting p300. Silencing of CBP in SF (n=7) reduced the TNF- α -induced expression of IL6 (p<0,05), IL8 (p<0,05), MMP3 (p<0,05), as well as the basal (p<0,001) and the TNF- α -induced expression of MMP1 (p<0,05). In contrast, silencing of p300 induced the basal expression of IL6 (p<0,05), IL8 (p<0,01),

MMP1 (p<0,05), and MMP3 (p<0,05), as well as the TNF- α -induced expression of IL6 (p=0,078), IL8 (p=0,078), MMP1 (p<0,05) and MMP3 (p=0,063). Silencing of CBP in hand SF (n=4) reduced the expression of hand-specific HOX genes including HOXD10 (0,53 $\pm$ 0,10 fold; p<0,01), HOXD11 (0,76 $\pm$ 0,19 fold; p=0,098) and HOXA13 (0,75 $\pm$ 0,17 fold; p=0,063), whereas HOXA9, HOXA10 and HOXA11 were not affected. Silencing of p300 reduced the expression of HOXD10 (0,65 $\pm$ 0,24 fold; p=0,061), HOXD11 (0,45 $\pm$ 0,10 fold; p<0,01), HOXA10 (0,70 $\pm$ 0,14 fold; p<0,05) and HOXA13 (0,55 $\pm$ 0,19 fold; p<0,05). The down regulation of HOXD10 after silencing of CBP and p300 in hand SF was confirmed on protein levels by Western blotting.

Abstract AB0097 – Table 1

	CBP gapmeR	CBP gapmeR +TNF	p300 gapmeR	p300 gapmeR +TNF
MMP1	0,46 $\pm$ 0,17	0,41 $\pm$ 0,75	25,86 $\pm$ 26,82	1,96 $\pm$ 1,35
MMP3	0,97 $\pm$ 0,31	0,64 $\pm$ 0,35	2,60 $\pm$ 1,62	3,05 $\pm$ 2,89
IL6	1,21 $\pm$ 0,43	0,29 $\pm$ 0,17	4,51 $\pm$ 3,32	1,90 $\pm$ 1,20
IL8	8,27 $\pm$ 9,70	0,52 $\pm$ 0,20	207,8 $\pm$ 96,17	4,75 $\pm$ 2,67

Conclusions: Our results unravel opposing functions of CBP and p300 in regulating the TNF- α -induced expression of inflammatory and matrix-degrading target genes in SF. In addition, CBP and p300 likely contribute to the maintenance of a joint-specific gene expression in SF by regulating the expression of hand-specific HOX genes.

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AB0098 PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA COACTIVATOR-1 β FACILITATES MIGRATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS VIA ACTIVATION OF CANONICAL AND NON-CANONICAL NF-KB SIGNALLING PATHWAY

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Background: Fibroblast-like synovocytes (FLS) in rheumatoid arthritis (RA) manifest tumor-like properties including increased proliferation, prolonged survival, apoptosis resistance, adherence and invasiveness of adjacent tissues. Peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) β is a transcriptional coactivator which plays important roles in regulating energy metabolism and cytokine signalling pathways. Our previous study showed that elevated PGC-1 β expressed in RA-FLS promoted their pro-inflammatory effect. However, the roles of PGC-1 β on regulating migration and invasion of RA-FLS remains to be identified.

Objectives: To investigate the role of PGC-1 β on regulating migration and invasion of RA-FLS and underlying mechanism.

Methods: Synovial tissues were obtained by closed needle biopsy from six patients with active RA and FLS were isolated and cultured. PGC-1 β in RA-FLS was down-regulated or over-expressed by lentivirus with same vectors marked Lv-sh-GFP or Lv-GFP as negative controls. Effects of PGC-1 β on migration and invasion capacity were detected by wound healing assays and transwell migration and invasion assays. The change of proteases in culture supernatants were detected by Proteome Profiler human protease array kit (R and D Systems, USA) and verified by qRT-PCR and western blot. The expression of key signalling molecules in canonical and non-canonical NF- κ B signalling pathway was detected by western blot.

Results: Down-regulation of PGC-1 β by Lv-sh-PGC-1 β transfection inhibited migration and invasion of RA-FLS compared with Lv-sh-GFP transfection group (wound healing: 1252 $\pm$ 214 vs. 764 $\pm$ 184 μ m, p<0.001; migration: 184 $\pm$ 74 vs. 642 $\pm$ 32 cells/field, p<0.001; invasion: 124 $\pm$ 47 vs. 445 $\pm$ 67 cells/field, p<0.001), while