that CM is able to increase subchondral bone mineral density (BMD) in an experimental model of osteoporosis[1].
Objectives: To evaluate if CM could prevent the subchondral BMD alterations induced by OA, in association to an improvement in synovial membrane inflammation and cartilage damage in an OA model in rabbits.
Methods: Ten male New Zealand rabbits were submited knee surgery to induce OA by transection of anterior cruciate ligament. CM was performed using the chiropractic adjusting instrument ActivatorV 3 times a week during 8 weeks as follows: Force 2 setting was applied onto the tibial tubercle of the rabbit right hind limb (CM-OA group), at an angle of 90°, whereas the corresponding left hind limb received a false manipulation (FM-OA group) consisting of ActivatorV firing in the air and touching the tibial tubercle. Three healthy animals were used as controls. Following sacrifice, tibiae and femora were removed for mCT and histological evaluation. Synovial inflammation was evaluated by Krenn's score and the protein presence of VEGF, MMP3 and CollagenVI in the synovial membrane was evaluated by western blot.
Results: In the OA rabbits, subchondral BMD decreased in relation to control animals (*OA 4729±193 vs Control 5181±209 mg/cc*), been partially reversed in the tibiae of OA rabbits with CM (*TM-OA 5055±216 vs FM-OA 4404±170 mg/cc*). Subchondral trabecular bone structural parameters were analysed by microCT and a significant decrease of bone volume/trabecular volume (BV/TV), trabecular number (TbN) and trabecular thickness (TbTh) was observed in the OA rabbits, while trabecular separation (TbS) increased compared to control animals. TM-OA group showed a significant improvement of these parameters compare to FM-OA group. TM-OA had lower cartilage damage compare to FM-OA (*TM-OA 4 ±0,67 AU vs FM-OA 8±1,25* AU). TM-OA synovial membranes presented a total Krenn score lower than FM-OA joints (*TM-OA 3±0,35 vs FM-OA 4.5±0,38* AU). OA synovial membranes showed higher levels of CollagenVI respect to control ones; TM-OA synovial membranes presented less expression of CollagenVI than FM-OA group (*TM-OA 1.4±0,13 vs FM-OA 2.2±0,3* AU), been this associated with a decrease of both MMP3 (*TM-OA 1.2±0,1 vs FM-OA 1.7±0.2* AU) and VEGF (*TM-OA 1.2±0.14 vs FM-OA 1.9±0.26* AU).
Conclusions: These results support the hypothesis that CM may ameliorate subchondral BMD alterations induced by OA, in association to an improvement on synoviopathy and cartilage degradation.

REFERENCE:

[1] López-Herradón A, et al. Impact of Chiropractic Manipulation on Bone and Skeletal Muscle of Ovariectomized Rats.CalcifTissueInt 2017;101(5):519–529.

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AB0095 ATTEMPT TO DEVELOP A CHIMERIC CO-CULTURE SYSTEM TO DIFFERENTIATE MOUSE OSTEOCLASTS BY CULTURING MOUSE PRECURSOR CELLS WITH HUMAN SYNOVIAL FIBROBLASTS

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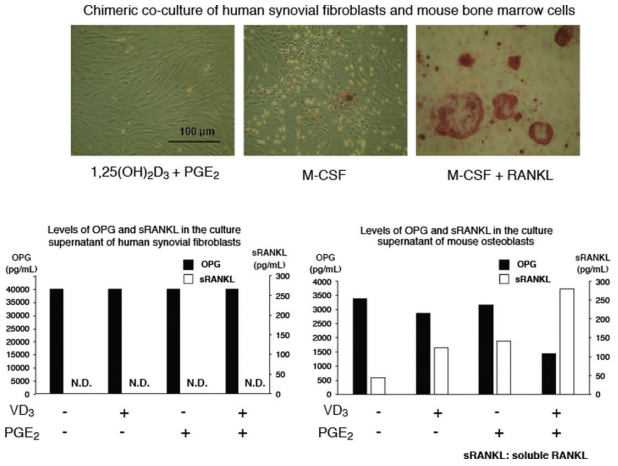
Background: In rheumatoid arthritis (RA), inflammatory synovial tissue called the pannus proliferates and erodes the articular cartilage and bone in the affected joints. Osteoclasts, multinucleated cells of monocyte/macrophage lineage, are implicated in the bone destruction in RA. Thus, osteoclasts are considered an important therapeutic target in the prevention of the joint destruction. Mouse bone marrow cells differentiate into osteoclasts when co-cultured with osteoblasts or stromal cells in the presence of reagents such as 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) and prostaglandin E_2 (PGE_2). There are no osteoblasts in the RA synovium, but there are fibroblasts and various inflammatory cells such as macrophages and lymphocytes. Thus, synovial fibroblasts may function as supporting cells for osteoclastogenesis in place of osteoblasts.
Objectives: The aim of this study was to establish a chimeric co-culture system of osteoclast differentiation using human synovial fibroblasts and mouse monocyte/macrophage lineage cells.
Methods: Synovial tissues were obtained from RA patients who underwent joint replacement surgery. Mouse osteoblasts were obtained from the calvariae of 2- or 3-day-old newborn C57BL/6 (B6) mice. Mouse bone marrow cells were prepared from femoral bones. Osteoclasts were visualised with tartrate-resistant acid phosphatase (TRAP) staining. The protein levels of RANKL and its decoy receptor, osteoprotegerin (OPG), in the culture supernatant were quantified using ELISA.
Results: We confirmed that mouse osteoclasts could be differentiated *in vitro* by culturing bone marrow cells in the presence of human M-CSF and RANKL. We then cultured mouse osteoclast precursors with human synovial fibroblasts in the presence of 1,25$(OH)_2D_3$ and PGE_2. The murine cells seemed to disappear in the course of the co-culture, whereas they survived when exogenous human M-CSF was added to the system. Interestingly, however, they did not become TRAP-positive multinuclear cells, suggesting that synovial fibroblasts do not provide a sufficient amount of the osteoclast differentiation factor, RANKL. Following these results, we used ELISA to quantify the level of human RANKL and OPG in the culture supernatant of synovial fibroblasts. Predictably, the level of RANKL was below the detection limit with or without the presence of 1,25$(OH)_2D_3$ and/or PGE_2. In contrast, that of OPG was very high, irrespective of the reagents added. We also quantified the levels of mouse RANKL and OPG in the culture supernatant of mouse osteoblasts. As expected, RANKL was detectable in this case. Interestingly, the level of OPG was very high and comparable to that of human OPG produced from synovial fibroblasts.



Abstract AB0095 – Figure 1

Conclusions: Synovial fibroblasts cannot be substituted for osteoblasts in a co-culture system of osteoclast differentiation. This is probably because synovial fibroblasts do not provide sufficient RANKL and M-CSF. Instead, they produce a large amount of OPG. This may be a mechanism by which ectopic osteoclastogenesis is inhibited. Thus, the osteoclasts observed in the pannus may be dependent on membrane-bound RANKL from other sources, like lymphocytes, or may be differentiated by stimulation with cytokines other than RANKL, such as TNF-α and IL-6.[1 2]

REFERENCES:

[1] Yokota K, et al. Arthritis Rheumatol 2014;66:121–9.
[2] O'Brien W, et al. Arthritis Rheumatol 2016;68:2889–2900.

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AB0096 EXPRESSION AND FUNCTION OF NEUROPEPTIDE Y RECEPTORS IN HUMAN ARTICULAR CARTILAGE: INFLUENCE OF GENDER AND OSTEOARTHRITIS

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Background: Elevated levels of neuropeptide Y (NPY) were reported in osteoarthritic (OA) joints[1]. No information exists regarding the role of NPY in OA joints, besides mediating or potentiating nociceptive transmission.
Objectives: To determine whether NPY receptors are present and functional in human chondrocytes, by evaluating the ability of NPY to activate important signalling pathways and to modulate autophagy, which was shown to be induced by this neuropeptide in hypothalamic neurons[2] and to be a crucial homeostatic mechanism in chondrocytes, whose reduction contributes to OA pathogenesis[3].
Methods: Immunofluorescence for the NPY receptor subtypes, Y_1, Y_2 and Y_5, was performed in the human chondrocytic cell line, C28/I2, and in human cartilage sections obtained from multi-organ donors (4 males, 55–75 years old, mean=67.25, 3 females, 33–68 years old, mean=55.33) at the Bone and Tissue

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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 Y_1, Y_2 and Y_5 NPY receptors was observed in C28/I2 cells. In human cartilage, a positive signal was found for the Y_2 receptor in all samples while Y_5 receptor immunoreactivity was undetectable, regardless of disease state, gender and age of the donors. Y_1 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-1β. 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.

REFERENCES:

[1] Wang, et al. BMC Musculoskelet Disord 2014;15:319.
[2] Aveleira, et al. PNAS 2015;112:E1642-E1651.
[3] Ribeiro, et al. Osteoarthritis Cartilage 2016;24:2116–2125.

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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 μM, 5 μM), 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% (±12,9%, p<0,01, n=5) in unstimulated cells and by 89,7% (±12,9%) in presence of TNF-α. The protein expression of p300 was reduced by 55,3% (±29,8%, p<0,05, n=6) in unstimulated cells and by 62,7% (±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±0,10 fold; p<0,01), HOXD11 (0,76±0,19 fold; p=0,098) and HOXA13 (0,75±0,17 fold; p=0,063), whereas HOXA9, HOXA10 and HOXA11 were not affected. Silencing of p300 reduced the expression of HOXD10 (0,65±0,24 fold; p=0,061), HOXD11 (0,45±0,10 fold; p<0,01), HOXA10 (0,70 ±0,14 fold; p<0,05) and HOXA13 (0,55±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±0,17	0,41±0,75	25,86±26,82	1,96±1,35
MMP3	0,97±0,31	0,64±0,35	2,60±1,62	3,05±2,89
IL6	1,21±0,43	0,29±0,17	4,51±3,32	1,90±1,20
IL8	8,27±9,70	0,52±0,20	207,8±96,17	4,75±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-1B 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 synoviocytes (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-1β expressed in RA-FLS promoted their pro-inflammatory effect. However, the roles of PGC-1β on regulating migration and invasion of RA-FLS remains to be identified.

Objectives: To investigate the role of PGC-1β 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-1β 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-1β 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-κB signalling pathway was detected by western blot.

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

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