Background: Retrotransposon-derived DNA sequences occupy approximately 40% of the mammalian genome, compared with only 1.5% of protein coding genes, and are a source of variation in the genome. LTR-derived gene ZCCHC5 (Mart3) is a member of gag-related retrotransposon family that has lost the ability to retrotranspose. ZCCHC5 gene encode a protein of approximately 53 Kd and contains a nucleic acid binding domain (CX_2CX_4HX_4C), a gag-like region within the intact ORF and a homeobox associated leucine zipper motif indicating that this gene may have acquired new function(s) in the cell. Expression and function of ZCCHC5 in degenerative joint diseases such as OA or other diseases has not been explored.

Objectives: The aim of this study was to investigate whether ZCCHC5 is expressed in OA cartilage and chondrocytes and whether it is involved in the regulation of catabolic and/or anabolic factors in chondrocytes and its modulation under pathological conditions.

Methods: Chondrocytes were derived by the enzymatic digestion of human OA cartilage and chondrocytes and whether it is involved in the regulation of catabolic and/or anabolic factors in chondrocytes and its modulation under pathological conditions.

Results: The expression of ZCCHC5 was significantly decreased in normal cartilage and the unaffected cartilage of the same patient. mRNA expression was quantified using TaqMan assays. Protein expression was determined by immunohistochemistry (IHC) and Western blotting (WB) with validated antibodies, siRNA mediated depletion or plasmid mediated overexpression of ZCCHC5 gene was used to study its role in chondrocyte function under pathological conditions. Luciferase reporter vectors were used to study promoter activity in human chondrocytes.

Conclusions: In this study we have demonstrated the ability to use aqueous energy dissemination to successfully enrich MV with antibodies. These enriched MV are able to localise in the arthritic joint and deliver anti-inflammatory therapeutics. Overall, this study demonstrates the attainability of targeting a biological scaffold to the arthritic joint. The potential of co-delivering MV alongside anti-inflammatory therapeutics is paramount to simultaneously protect cartilage and reduce inflammation.

REFERENCES:
DISEASE MODIFYING EFFECTS OF THE CANINE IL4–10 FUSION PROTEIN IN THE CANINE GROOVE MODEL OF OSTEOARTHRITIS

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Background: An ideal disease modifying osteoarthritis (OA) drug should have analgesic, chondroprotective and anti-inflammatory effects. A fusion protein of Interleukin 4 and 10 (IL4–10FP) might have these effects.

Objectives: This study evaluates the effects of canine IL4–10FP (cIL4–10FP) in the canine Groove model of OA.

Methods: In 8 skeletally mature dogs, knee OA in the right leg was induced according to the Groove model. After 6 weeks of OA development, intra-articular injections in the affected knee with either PBS (500 μl; n=4) or cIL4–10FP (10 μg; 500 μl; n=4) were given weekly for 10 weeks. The contra-lateral joints served as a healthy control.

Force plate analysis (FPA) was used to determine joint loading as a surrogate marker of pain. The ratio of the affected over the contra-lateral control joint was calculated for each dog for each time point. FPA was performed before OA induction to determine baseline values, and before and 24 hours after the 1st, 6th and 9th intra-articular injection. A linear mixed model was used to evaluate effects of injections. After 10 weeks of treatment dogs were euthanized and tissue samples were harvested. Serum Immunoglobulin G (IgG) titers against cIL4–10FP were evaluated to check for potential antibody formation.

Cartilage proteoglycan content and release of proteoglycans were determined ex vivo by Alcian Blue assay. Synovial inflammation was evaluated by HE-staining using OARSI grading. Changes in outcomes in the affected/treated joints compared to contra-lateral control joints were calculated.

Results: After OA induction a clear reduction in joint loading (increase in pain) was found (standing force and braking force). After cIL4–10FP injections these forces increased toward normalisation (cIL4–10FP vs PBS group, p<0.002 and p=0.01, respectively). No IgG elevation was detected after 10 injections. Compared to contra-lateral controls, proteoglycan content of OA PBS injected knees suggested tissue degeneration (27 vs 34 mg/g). In the cIL4–10FP treated group proteoglycan content in right knees was not different from the contra-lateral controls (33 vs 31 mg/g). The mean change in proteoglycan content compared to contra-lateral controls was different with a p value of 0.057 (figure 1). A similar pattern was found for the change in release of proteoglycans from the cartilage, which was less increased in the cIL4–10FP group compared to the PBS group (3.4% vs 3.0%; p=0.029, figure 1).

Synovial inflammation was mild (characteristic of this model) and did not change after intra-articular injections (0.4 and 1.3 points out of 18 for cIL4–10FP and PBS respectively).

Conclusions: Repetitive intra-articular injection with canine IL4–10FP in dogs did not lead to antibody formation. Dogs treated with cIL4–10FP showed improved joint loading compared to PBS treated dogs, reflecting an analgesic effect. Recovery of proteoglycan content and normalisation of release in cIL4–10FP treated dogs indicate a chondroprotective effect. Synovial inflammation in the Groove model was too mild to be changed significantly. These results clearly warrant further research to develop IL4–10FP as a DMOAD.

Acknowledgements: This work is supported by the Dutch Arthritis Foundation project NR12–2–202.

Disclosure of Interest: None declared


CLAUDIN-11 REGULATES BONE HOMEOSTASIS VIA BIDIRECTIONAL EPHB4-EPHRINB2 SIGNALLING


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Background: Claudins (Cldns) are well-established components of tight junctions (TJs) that play a pivotal role in the modulation of paracellular permeability. Several studies have explored the physiologic aspects of Cldn family members in the nervous system myelin, on bone homeostasis. However, the effect of Cldn11, a major component of central nervous system myelin, on bone metabolism has not been reported.

Objectives: This study was performed to identify the effects of Cldn on bone metabolism via regulation of osteoclast and osteoblast differentiation and their function.

Methods: We performed various in vitro and in vivo studies using gain- and loss- of-function of Cldn11 that is belong to the Cldn group. Osteoclast formation from bone marrow cells (BMC) and Osteoblast formation was evaluated in specific condition with over-expression or down- regulation of Cldn11. The expression of osteoclast associated gene and osteoblast related gene mRNA were assessed.