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miRNA sequences. Inhibition of NF-κB and p38-MAPK activation with specific inhibitors down-regulated the expression of COX-2 but up-regulated the expression of miR-101_3, miR-199a* in human OA chondrocytes suggesting negative regulation of miR-101_3, miR-199a* by the activation of these pathways.
Conclusions: Our data implicate miR-199a* in the post transcriptional regulation of COX-2 expression in human OA chondrocytes. These results also identify miR-199a* as a novel therapeutic target for the treatment of OA.

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ROLE OF C-TYPE NATRIURETIC PEPTIDE (CNP) IN OSTEOGENESIS AND TREATMENT OF OSTEOPOROSIS

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Purpose: The recently described C-type natriuretic peptide (CNP) is critical for normal longitudinal growth in humans and plays a central role in osteoblast regulation in bone formation and regeneration. The purpose of this study was to determine whether CNP has therapeutic potential in osteoporosis or osteopenia secondary to inflammatory arthritis. Our goal was to test whether CNP overproduction can reverse the osteoporosis that develops in mouse disease models due to a lack of proper numbers and function of osteoblasts to balance the resorptive effect of osteoclasts.
Methods: *CNP*^{COL2A1TG} mice have been generated by cloning human CNP cDNA into a construct that contained the mouse collagen type II (*COL2A1*) promoter and enhancer (GenBank #m65161). We initially assessed the bone phenotype of mice that over-express CNP in chondrocytes *in vivo*. We then made primary chondrocyte and osteoblast cultures from mouse rib cage cartilage and calvarial osteoblasts. RNA and protein collected were used to measure osteoblastic activity. We induced osteoporosis/osteopenia by inducing chronic inflammatory arthritis in mice that over expressed CNP compared to wild type *in vivo*.
Results: Phenotypic characteristics of the CNP over-expressing mice became evident at 3-4 weeks of age. There was excessive elongation and noticeable widening of the distal ends of long bones. Increased bone trabeculation in both primary and secondary ossification centers was obvious in histology, at 8-9 weeks of age in CNP^{COL2A1TG} mice. Micro-CT analysis of age- and sex-matched littermates (4 female, 7 male), both wild type and CNP^{COL2A1TG}, at various ages were analyzed. *CNP*^{COL2A1TG} mice showed higher bone mineral density (BMD) and content (BMC) in the subchondral bone in proximal tibia (Table 1). Trabeculation in the subchondral bone and under the growth plate in *CNP*^{COL2A1TG} mice was also increased. CNP^{COL2A1TG} mouse serum showed significantly higher osteocalcin levels compared to wild type from 8-13 weeks ($p<0.005$).

Table 1. Spine m-CT

	Avg TbTh (μm)	Trabecular BMC (mg)	Cortical BMC (mg)
8 wk f WT (n=2)	50.57 (±1.02)	4.80 (±0.18)	7.65 (±0.2)
8 wk f CNP^{COL2A1TG} (n=2)	52.44 (±0.12)	6.95 (±0.03)	9.90 (±0.8)
14 wk f WT (n=4)	77.70 (±1.2)	3.51 (± 0.80)	8.16 (±0.9)
14 wk f CNP^{COL2A1TG} (n=2)	91.40 (±2.4)	7.43 (±1.20)	13.57 (±1.3)

Quantitative RT-PCR analysis showed that, in both primary chondrocytes and osteoblast cultures, collagen II, X, I, OPG, osteocalcin were expressed at significantly higher levels in the CNP^{COL2A1TG} mouse chondrocytes or when calvarial osteoblasts were treated with exogenous CNP (10ng/ml, > 3wk). However, RANKL mRNA was found to be decreased 2.5 fold in CNP^{COL2A1TG} mouse chondrocytes and osteoblasts. When we induced systemic arthritis using the K/BxN TCR serum model in mice (n=32) that were either CNP^{COL2A1TG} or wild type, the CNP^{COL2A1TG} mice did not develop endochondral delay or periarticular bone loss/osteoporosis even after 4 weeks of systemic arthritis compared to wild type. Serum osteocalcin levels, measured after the K/BxN TCR arthritis developed, showed decreased levels at 2.5, 3.5 and 5 months-of-age (Fig. 1). Interestingly, CNP^{COL2A1TG} mice continued to maintain high osteocalcin levels even after arthritis developed. Micro-CT analysis suggested that CNP^{COL2A1TG} mice that developed arthritis were resistant to both trabecular and cortical bone mineral density loss due to inflammatory changes in the joint. Trabecular thickness, inter-trabecular space and the connectivity of the trabecular structure were not different in wild type (WT) and K/BxN TCR mice.
Conclusions: Our observations from *in vivo* and *in vitro* experiments suggest a clear effect of CNP signaling in osteogenesis and suggest a role in osteoporosis treatment during systemic arthritis.

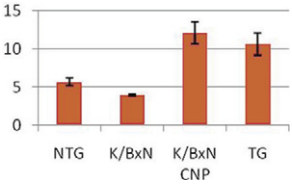

Figure 1. Osteocalcin (ng/ml).

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THE ROLE OF FIBROBLAST GROWTH FACTOR 2 (FGF2) IN THE CELLULAR RESPONSE TO INJURY FOLLOWING MURINE CARTILAGE EXPLANTATION

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Purpose: Studies from our lab have previously demonstrated that there is rapid activation of a number of intracellular signalling pathways upon explantation of porcine articular cartilage including the three mitogen activated protein kinases (MAPKs); c-jun N terminal kinase (JNK), p38, and the extracellularly regulated kinase (ERK) (Gruber, 2004), as well as activation of NFkB and PI3 kinase (unpublished). Re-cutting cartilage that has been rested in serum free medium for 48h results in activation of the ERK pathway only, and is due to release of FGF2 from the pericellular matrix. In order to determine the relative contribution of FGF2 to the explantation response, we set up signalling assays and studied gene induction in injured murine hip cartilage from wild type (WT) and FGF2 null mice.
Methods: Murine cartilage from 4-6 week old WT or FGF2 null mice was explanted from the intact hip by blunt dissection, and was either lysed immediately, or was cultured in serum free medium for up to 24h. Some explanted cartilage was rested in serum free medium (48h), then was re-cut. Protein was extracted from the hips (3-5 for signalling experiments) for phospho-western blotting for pERK, pJNK, pP38 and pIkappaB. Equal protein loading was confirmed by re-blotting for total ERK, IkB or tubulin. Gene analysis was performed on 5-8 hip explants (either explanted, re-cut or stimulated with FGF2). Messenger RNA was extracted using Trizol and purified. Quantitative RT-PCR for 48 pre-selected candidate genes using Taqman microfluidic cards was performed.
Results: Cartilage explantation led to early activation of MAPK pathways as well as causing phosphorylation of IkB, indicative of activation of NFkB. A very weak activation of inflammatory pathways was apparent when rested cartilage was re-cut. When FGF2 null cartilage was explanted there was an approximate 50% reduction in MAPK activation. A number of genes (selected from an unpublished murine OA microarray study) were regulated in the chondrocytes following explantation. These are listed in Table 1. Some of these genes were also regulated when the tissue was re-cut (rather than explanted), and those same genes were shown to be strongly induced by stimulation with recombinant FGF2. CCL2, arginase 1, IL-1α, CCL7 and

Table 1

Genes	Explantation	Recutting	FGF2 regulated
Arg1	✓	✗	✗
IL-1α	✓	✗	✗
CCL2	✓	✗	✗
CCL7	✓	✗	✗
HAS1	✓	✗	✗
CD14	✓	✓	✓
Activin	✓	✓	✓
COX2	✓	✓	✓
TNFRSF12a	✓	✓	✓
TSG6	✓	✓	✓
TIMP1	✓	✓	✓
PTGES	✓	✓	✓

Legend: Arg1 - arginase 1; CCL - CC chemokine ligand; HAS1 - hyaluronan synthase 1; CD14 - cluster differentiation 14; TNFRSF12a - TNF Receptor superfamily member 12a; TSG6 - TNF stimulated gene 6; TIMP1 - tissue inhibitor of metalloproteinase 1; Ptges - prostaglandin E synthase.