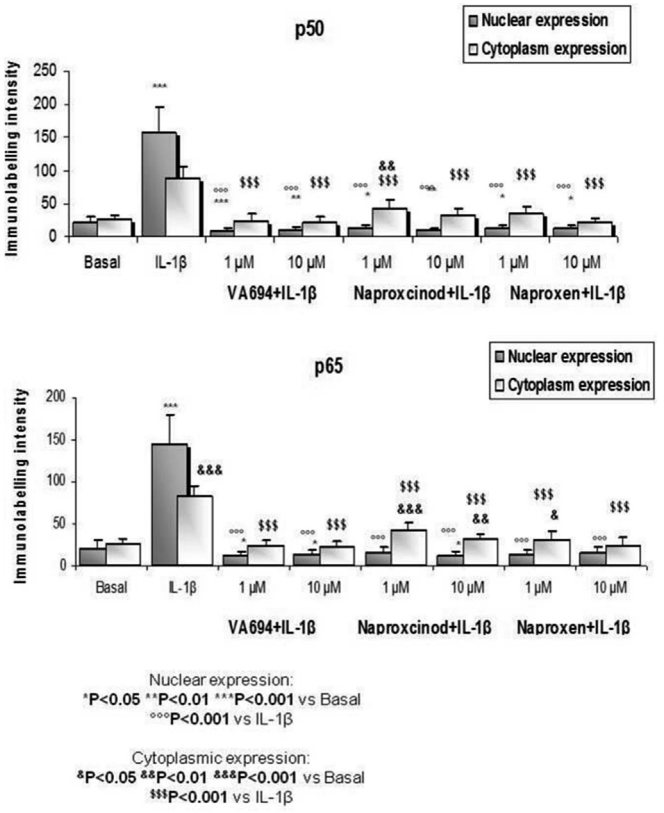
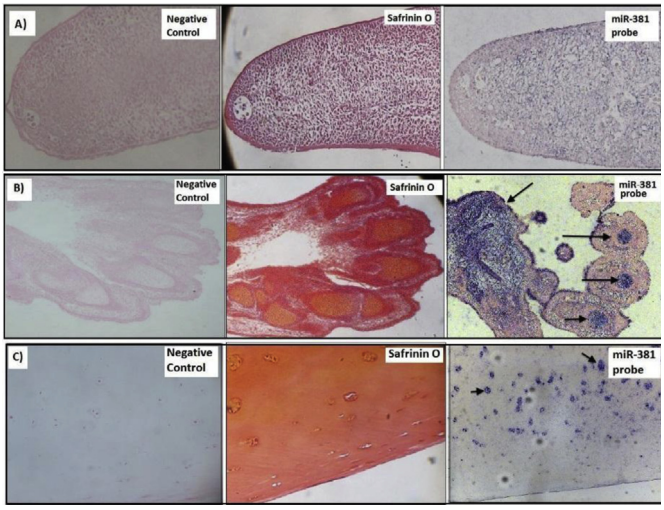
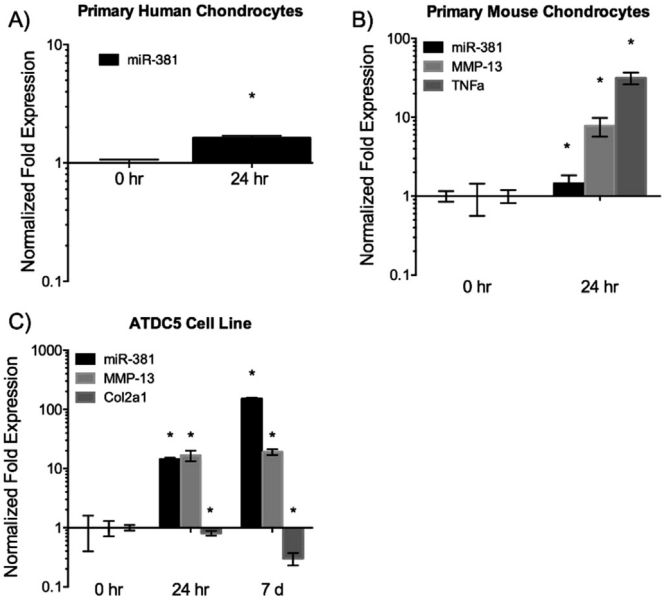
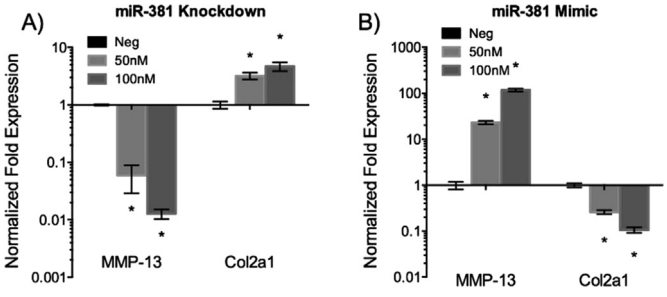


Fig 2. Immunolabelling intensity forp50andp65 subunits, calculated using the Image J software.







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MICRORNA-381 IN CHONDROGENESIS AND INTERLEUKIN-1-β INDUCED CHONDROCYTE RESPONSES

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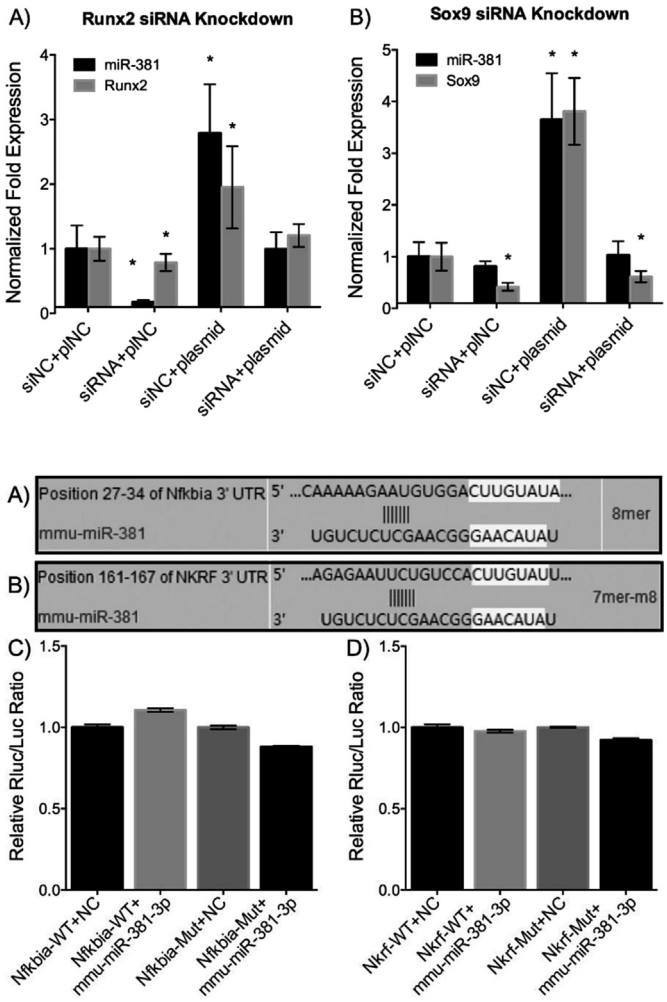
Purpose: Osteoarthritis is a debilitating joint condition characterized by cartilage degradation. The pathways that regulate cartilage degradation are still unclear. We previously identified miR-381 as a putative regulator of chondrogenesis related genes. Therefore, the aim of this study was to determine the role of miR-381 in chondrogenesis and cartilage degeneration.

Methods: miR-381 expression was assessed in vitro by qRT-PCR in response to IL-1β stimulation in primary human (PHC) and mouse (PMC) chondrocytes, and the chondrocyte-like ATDC5 cell induced to differentiate to chondrocytes with insulin, transferrin, and selenous acid (ITS) + premix. miR-381 expression was assessed in vivo in mouse embryos and osteoarthritic cartilage by in situ hybridization. The effect of miR-381 on chondrogenesis was assessed using a synthetic RNA mimic or inhibitor. Luciferase assays were used to assess the role of miR-381 in regulating NF-kB signaling. Upstream regulators were probed using siRNA or overexpression plasmids for Sox9 and Runx2.

Results: miR-381 expression was elevated in chondrogenic and hypertrophic ATDC5 cells. IL-1β-induced miR-381 expression in ATDC5 cells, PMCs, and PHCs. miR-381 was also expressed areas of cartilage degradation absorption in mouse embryos and human osteoarthritic cartilage. miR-381 expression was increased in ATDC5 cells over-expressing Runx2 or Sox9. miR-381 suppressed the expression of Col2a1 (collagen, type II, alpha 1) and enhanced the expression of metalloproteinase-13 (MMP-13), but did not regulate NFKBIA and NKRF activity.

Conclusions: miR-381 is highly expressed during chondrogenesis and in arthritic cartilage. It is likely regulated by Sox9 and Runx2. miR-381 may contribute to absorption of the cartilage matrix by repressing collagen II and inducing MMP-13.



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CRITICAL ROLE OF CD13/AMINOPEPTIDASE N IN BONE/CARTILAGE COMMUNICATION IN OSTEOARTHRITIS: ABILITY TO BIND 14-3-3E

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Purpose: Osteoarthritis (OA) is a whole-joint disease characterized by progressive destruction of articular cartilage which involves the three joint tissues (subchondral bone, articular cartilage and synovial membrane). Our team identified 14-3-3ε as a novel soluble mediator critical in the communication between subchondral bone and cartilage in OA. This protein acts as a potent MMP-3 and MMP-13-stimulatory factor in chondrocytes leading to catabolic phenotype. CD13/APN, potential receptor of this protein, was identified on the surface of chondrocytes suggesting its role in bone/cartilage communication.
Methods: Experiments were performed from murine cartilage of the C57BL/6J mice (5-6 days old), and from human cartilage samples, obtained from OA patients undergoing total joint replacement surgery for OA. CD13/APN was invalidated by siRNA in murine chondrocytes and blocked with anti-CD13 monoclonal antibodies in murine and human chondrocytes and then stimulated by 14-3-3ε. Chondrocyte gene expression and release of MMP-3 and MMP-13 was evaluated by RT-PCR and western-blotting or ELISA. Aminopeptidase N activity in murine and human chondrocytes was assessed in supernatant using the fluorescent substrate L-alanine B-naphtilamide. Interaction between 14-3-3ε and CD13/APN was measured using surface Plasmon Resonance (SPR) on a Biacore 3000 instrument.
Results: Invalidation of CD13/APN by siRNA or blocking antibody in articular chondrocytes reduced significantly mRNA expression and protein release of MMP-3 and MMP-13 induced by 14-3-3ε. Aminopeptidase N activity was identified in murine and human chondrocytes. Treatment with APNi (Specific inhibitor of Aminopeptidase N) inhibited strongly this activity. However, Aminopeptidase N was unchanged following stimulation with 14-3-3ε. Thus, these results suggest that CD13/APN is involved in 14-3-3ε signal transduction and establishement of procatabolic phenotype in murine and human articular chondrocytes, independently of its enzymatic activity. Finally, human recombinant CD13/APN was covalently immobilized on CM5 chip. An IgG1 isotype, a specific blocking antibody of human CD13 and human recombinant 14-3-3e were used as analytes. Results showed a direct interaction between recombinant CD13/APN and 14-3-3ε (78 RU), a higher binding with blocking antibody (170 RU) and no binding with IgG1 isotype (00 RU). Kinetic tests were performed then using increasing concentrations of recombinant 14-3-3ε and showed that 14-3-3ε has a low affinity to CD13/APN with a dissociation constant (KD) of 2.45 x 10-6 M and a $\chi 2$ value of 2.09 showing a good fit. These results suggest that CD13/APN interacts directly with extracellular 14-3-3ε to transmit its signal in chondrocytes. Thus, our results seem designate 14-3-3ε and CD13 as novel proteins to be explored in OA, either for therapeutic or prognostic purposes.
Conclusions: Our current data allowed to have a better understanding on mechanism of action of 14-3-3ε, and especially on its signalling pathways. Thus, this protein could be considered as a new target for treatment of OA by its interaction with CD13/APN and provide data to go further on preclinical step.

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ANALYSIS OF SINGLE ANION CHANNEL ACTIVITY IN CHONDROCYTES IN VITRO

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Purpose: Once osteoarthritis (OA) has progressed it is difficult to treat with drugs and frequently an operation (such as joint arthroplasty and autograft with arthroscopy) is required. Both of these options provide only symptomatic treatment and there are no effective cures. Therefore, elucidation of physiological function and construction of detailed cartilage models, including chondrocyte homeostatic maintenance mechanisms may facilitate future OA preventative treatments.
It is clear that mechanical activity of joints couples to chondrocyte metabolic activity, but the role of this coupling in OA is unknown. A simple way to mechanically disturb chondrocytes whilst simultaneously making electrophysiological recordings from chondrocytes is to use osmotic shock. In our previous chondrocyte electrophysiological work we have analyzed changes of both cation and anion channel activity in response to osmotic changes. In our recent anion work, we used whole-cell experiments to show that a volume-sensitive Cl- current (ICl,vol) is functionally expressed in rabbit articular chondrocytes and is activated by membrane stretch induced by hyposmotic solutions. Recently, it has also been suggested that aberrant activation of ICl,vol under isosmotic condition contributes to the cell shrinkage and apoptosis (apoptotic volume decrease, AVD) in various cell types including chondrocytes. We showed that various blockers of ICl,vol prevented AVD and subsequent cell death induced by ischemia-reperfusion stress or apoptotic inducers such as doxorubicin, Fas-ligand and TNF-α. The levels of several pro-inflammatory cytokines are elevated in osteoarthritis, and so the present study aimed to investigate the effects of inflammatory cytokines on functional expression of anion channels in chondrocytes.
Methods: Canine cartilage was collected from the stifle and elbow joints and isolated with type-II collagenase as reported previously (animals euthanized for unassociated veterinary reasons). Electrophysiological studies were carried out using up to 3rd passage canine chondrocytes Cytokine treatment was 72hrs TNF-α and IL-1β 1ng/ml each. Single-channel activity was recorded using cell-attached patch clamp of isolated chondrocytes (pipette solution containing 115/ 28mM (KG/KCl).
Results: Single-channel analyses of both normal (control) and cytokine treated canine chondrocytes show the presence of a large number of phenotypically distinct ion channels upon hypo-osmotic shock. In this study we selected for analysis, only ion channels with reversal potentials near to the predicted equilibrium potential for chloride (approximately +20mV under these recording conditions). We analyzed a total of 18 patches including 9 cytokine treated and 9 untreated. In untreated patches, 3/9 exhibited clear channel activity reversing near to ECl- ("chloride-like" channels) and the mean slope unitary conductance for