transfections). These results were also validated using primary human OA chondrocytes.

Results: Sequencing of a 10kbp fragment upstream of human Pitx1 promoter allowed us to identify a SNP in a potential E2F respond element located in -3900/-3800 region of Pitx1 gene promoter. However, no association was found after validation of this SNP in a larger OA patient cohort (n=150). Nevertheless, using a 30bp probe, corresponding to the response element, and a DNA pull-down approach followed by mass spectrometry analysis (MS/MS), we discovered Prohibitin (PHB1) as the protein that interact with this response element. We confirmed this result with DNA pull-down followed by a Western blot against PHB1 and by chromatin immunoprecipitation (ChIP) assay. We have also found that in human OA chondrocytes, PHB1 is mostly accumulated in nucleus whereas in control subject it is mostly localized in cytoplasm. Overexpression of PHB1 in chondrocytes clearly demonstrated that PHB1 represses Pitx1 at mRNA and protein level. We also confirmed this repression using a gene reporter approach and point out that PHB1 use the -3900/-3800 response element discovered serendipitously. It has been shown that PHB1 represses the genes that are normally regulated by E2Fs transcription factor. Using a Luciferase reporter gene assay, we demonstrated that E2F1 is a transcriptional activator of Pitx1 gene and PHB1 over-expression block E2F1 effect on Pitx1 gene expression.

Conclusions: In healthy subjects, Pitx1 is normally regulated by E2F1, but in OA condition, accumulation of PHB1 in chondrocyte nucleus leads to Pitx1 gene repression. Further work will be undertaken to determine the mechanism that lead to nuclear PHB1 accumulation in OA chondrocytes.

THE PROTECTIVE ROLE OF THE PERICELLULAR MATRIX IN CHONDROYCYTE APOPTOSIS

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Purpose: In articular cartilage, pericellular matrix (PCM) plays a crucial role in homeostasis of chondrocytes. Apoptosis of chondrocytes contributes to common arthropathy such as osteoarthritis. This study attempted to quantify the protective role of the PCM in chondrocyte apoptosis using chondrons, which are a cartilage functional unit including a chondrocyte and its pericellular matrix.

Methods: Chondrocytes and chondrons were enzymatically isolated from human articular cartilage and exposed to monoiodoacetate (MIA) and staurosporine for apoptosis induction. To identify chondrons, a basic component of the PCM, type VI collagen, was fluorescently labeled. Apoptosis of chondrocytes and chondrons was examined using flow cytometry and microscopy. Apoptosis and cell death were separately calculated in chondrons, the type VI collagen positive cell population, and chondrocytes.

Results: Chondrocytes treated with MIA incurred a 26.97% increase in overall cell death compared to only 11.79% in chondrons. Chondrocytes treated with MIA underwent a 9.06% increase in apoptotic cells compared to only 1.64% in chondrons. TUNEL staining revealed that apoptotic chondrocytes often were the core of cell aggregates, while apoptotic chondrons did not attract cell aggregation. Caspase3 was expressed in both stressed chondrocytes and chondrons.

Conclusions: The PCM, a native microenvironment of chondrocytes, plays a protective role in chondrocyte survival. Retention of the PCM with chondrocytes is critically important in situations such as cartilage repair and tissue engineering, which depend on the survival of chondrocytes.

DIETARY HISTONE DEACETYLASE INHIBITORS AS CHONDROPROTECTIVE AGENTS

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Purpose: Organosulfur compounds such as sulforaphane and allicin occur naturally in plants and are part of the normal diet. Sulforaphane can inhibit tumourigenesis in animal models, has anti-inflammatory properties and of particular interest, is reported to inhibit histone deacetylase activity (Myszack et al; 2004). Allicin, the pungent compound found in garlic, and related allyl sulfur compounds have been shown to inhibit the proliferation of several human tumour cell lines but not normal cells. These compounds have been shown to modify DNA methylation and acetylation (Lea et al; 2002). Histone deacetylase inhibitors are potential chondroprotective agents (Young et al; 2005, Xu et al; 2006). The aims of this project are to test the relative efficacy of dietary organosulfur compounds as HDAC inhibitors in human chondrocytes and to test the ability of such compounds to inhibit cartilage resorption in a bovine nasal cartilage explant model.

Methods: Sulforaphane (SFN) and diallyl disulfide (DADS) were tested for their relative efficacy in modulating cytokine-induced metalloproteinase expression and histone acetylation in chondrocytes using quantitative RT-PCR and Western blotting. Furthermore, these compounds were tested for their ability to prevent cartilage destruction in the bovine nasal cartilage assay. Lactate dehydrogenase assays were used to test for toxicity.

Results: Both SFN (5-10μM) and DADS (4-32μM) significantly attenuated IL-1β/oncostatin M-induced MMP1, MMP3, MMP13, and ADAMTS4 expression in SW1353 chondrosarcoma cells. MMP2 and MMP28 were not affected. Basal ADAMTS5 expression was repressed by SFN at 5-15μM. Global and histone H3 acetylation was not affected by SFN or DADS treatment in the SW1353 cell line. Cytokine-induced cartilage destruction was abrogated in a dose dependent manner by SFN (5-30μM) and DADS (8-32μM), measured by collagen and glycosaminoglycan release.

Conclusions: Type II collagen and aggrecan are major structural components of cartilage. MMP-1 and MMP-13 are key collagenolytic metalloproteinases in arthritic disease since they can degrade collagen type II. ADAMTS-4 and ADAMTS-5 have aggrecanase activity. SFN and DADS can attenuate the induction of these genes in a dose dependent manner but do not appear to function through the inhibition of histone deacetylases. SFN and DADS are potential chondroprotective agents.

PHOSPHATE AND CALCIUM INFUX ARE REQUIRED FOR TGFβ-MEDIATED STIMULATION OF ANK (progressive ankylosis) EXPRESSION AND PPI TRANSPORT FUNCTION DURING CHONDROGENESIS

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Purpose: Genetic studies of familial chondrocalcinosis and cranial metaphyseal dysplasia have identified ANK, a multipass transmembrane protein, as a key player in biomineralization. The expression of ANK is stimulated by treatment of a variety of cell types with growth factors, including TGFβ. The purpose of this study was to determine whether TGFβ stimulation of ANK expression and function during chondrogenesis was dependent upon the influx of calcium and phosphate into the cells.

Methods: ATDC5 cells were differentiated from immaturity through hypertrophy with and without the addition of TGFβ1. Inhibitors of TGFβ1 signaling were used to identify the signaling pathway em-
employed in TGFβ regulation of ANK expression. Phosphate (Pi) uptake in the presence and absence of an inhibitor of the phos-
phate channels Pit-1 and Pit-2 was evaluated in the context of
TGFβ stimulation of ANK expression, as was Pi uptake in the presence and absence of the alkaline phosphatase inhibitor, lev-
amisole. Finally, inhibition of the L-type calcium channel alpha
1c was studied to determine its role in the regulation of ANK
expression and function in response to TGFβ.

Results: TGFβ produced an increase in ANK expression in all
phases of chondrogenic differentiation but most dramatically at
day 14 (proliferation) and day 32 (mineralizing hypertrophy)
of culture, suggesting a bimodal response to TGFβ during the
differentiative and hypertrophic phases of differentiation. To
determine the TGFβ-activated signaling pathway, cells with and without TGFβ were incubated in the presence of specific signaling pathway
inhibitors. The data show that by using the calcium-dependent PKC
inhibitor, G66976, there is an inhibition of ANK expression at cell
immaturity (day 4), proliferation (day 14), and mineralizing hyper-
trophy (day 32). To determine if Pi uptake was required for the
enhancement of ANK expression in response to TGFβ, we mea-
sured the effect of Pi uptake on TGFβ-stimulated ANK expression
during immaturity (day 4), proliferation (day 14) and hypertrophic
(day 32) phases of differentiation in the ATDC5 cells in the pres-
ence or absence of phosphonofomeric acid (PFA), a competitive
inhibitor of the type III Na+/Pi transporter. In the absence of PFA,
ANK expression is increased in response to TGFβ as expected.
However, in the presence of PFA, when Pi is unable to enter
the cells through the Pit-1 transporter, ANK expression is unres-
sponsive to treatment with TGFβ. We also explored the effect of
tissue non-specific alkaline phosphatase (TNAP) inhibition on
regulating ANK expression in response to TGFβ, particularly at
day 32 when the cells are hypertrophic and normally express high
levels of TNAP. In the presence of levamisole, ANK expression
in response to TGFβ was markedly reduced, as was its ability to
transport inorganic pyrophosphate (PPi) to the extracellular milieu.
These results suggest that Pi availability and uptake by the cells
is necessary for stimulation of ANK expression in response to TGFβ.
Furthermore, the data suggest that the higher the amount of Pi
available for uptake, the greater the stimulation of ANK expres-
sion in response to TGFβ. Since previous studies of endochondral
ossification in the growth plate have shown that L-type calcium
channels are essential for chondrogenesis, we investigated the
role of these channels in the TGFβ-stimulated ANK response in
ATDC5 cells. Our results indicated that treatment with nifedipine
to inhibit calcium influx via the L-type channel alpha 1c inhibited
the TGFβ-stimulated increase in ANK expression at all phases of
chondrogenesis.

Conclusions: TGFβ stimulation of ANK is dependent upon the
influx of phosphate and calcium into ATDC5 cells at all stages of
differentiation.

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LITHIUM INHIBITS CARTILAGE DEGRADATION AND MMP
PRODUCTION INDUCED BY PRO-INFLAMMATORY
CYTOKINES

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Purpose: Cartilage degradation leading to a loss of joint function
in the arthritis involves proteolytic cleavage of both aggrecan
and collagen. Pro-inflammatory cytokines, such as interleukin-1
(IL1) and tumour necrosis factor α (TNFα) induced matrix met-
alloproteinases (MMPs) are considered to play key roles in this
pathologic process. Lithium (LiCl) has been widely used as a drug
for the treatment of manic/depressive (bipolar) and depressive
disorders. Its function is considered to be by inhibition of glycogen
synthase kinase (GSK-3β), a serine/threonine kinase involved in
a multitude of cellular processes. GSK-3β activity is strictly linked
with the NF-κB, MAPK and JNK pathways, which in turn regu-
late IL1 and TNFα-mediated MMP regulation. GSK-3β inhibition
reduces joint inflammation and cartilage and bone damage in
murine arthritis model; these events play a key role in the patho-
physiology of arthritis. The aim of this study was to determine
the effects of the lithium (LiCl) on pro-inflammatory cytokine IL1,
IL1+oncostatin M (OSM) and TNFα mediated cartilage destruction
and to investigate the mechanisms involved.

Methods: Bovine nasal cartilage explants were stimulated with
cytokines ± LiCl or GSK-3 inhibitors (SB-415286 or TDZD-8) for
14 days. Hydroxyproline release was assayed as a measure of col-
lagen degradation, while collagenolytic activities (active and total)
and gelatinolytic activity in culture supernates were determined
by bioassay and zymography respectively. Immunoblotting was
used to determine the signalling pathways involved in the action
of LiCl.

Results: LiCl significantly decreased cytokine-induced collagen
release from bovine cartilage by the down-regulation of total and
active collagenolytic activities and gelatinolytic activity. The spe-
cific GSK-3 inhibitor, SB-415286 increased collagen release and
collagenolytic and gelatinolytic activity on cytokine treated bovine
cartilage, while TDZD-8 had no significant effect. LiCl decreased
IL1 induced HSP-27 phosphorylation, but had no significant effect
on IκB degradation or GSK3β phosphorylation.

Conclusions: This study demonstrates for the first time that
lithium, but not specific GSK-3 inhibitors, can protect cartilage
damage induced by pro-inflammatory cytokines via significantly
decreasing collagenase and gelatinase activities. These data indi-
cate that lithium mediated cartilage protection may act via inhibition
of an alternative (GSK-independent) signalling pathway and our
data indicates this to be the MAPK pathway p38. This study sug-
gests that lithium, or p38 inhibition, may represent a new therapy
to prevent joint damage.

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EFFECT OF FIBROBLAST GROWTH FACTOR-18 ON
ARTICULAR CARTILAGE FOLLOWING IMPACT LOADING

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Purpose: Joint impact injuries are known to increase the risk
of development of secondary osteoarthritis. Cartilage has poor
regenerative properties therefore treatments are being sought
that can assist in the repair of damaged cartilage within the
joint in an attempt to slow down disease progression. We have
shown previously that mechanical stimulation of human articular
cartilage increases the gene expression of fibroblast growth factor-
18 (FGF18). This novel growth factor is thought to be involved in
chondrocyte proliferation and cartilage matrix repair. This study
aimed to find out whether FGF18 has a reparative effect on car-
tilage that has been damaged by a traumatic impact load, and
to compare the effects of this growth factor with the more
extensively studied FGF2.

Methods: Full-depth circular explants (5 mm in diameter) of ar-
ticular cartilage were removed from the underlying subchondral
bone of bovine carpometacarpal joints (n=3) and cultured for 48
h. The explants were then subjected to a single impact load using
a 500 g mass dropped from a height of 25 mm in a drop tower.
The duration of each impact was approximately 3 ms with an im-
pact energy of 0.13 J. Following impact, the loaded explants
were cultured, along with unloaded controls, for 15 days in the pres-
ence of FGF2 or FGF18 (0, 10, 30 and 100 ng/ml). The medium