Activation of Diverse Eicosanoid Pathways in Osteoarthritic Cartilage
A Lipidomic and Genomic Analysis

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Abstract

Objective: Non-steroidal anti-inflammatory drugs (NSAIDs) that are prescribed for treatment of osteoarthritis (OA) symptoms including pain and inflammation target the production of eicosanoids which exhibit numerous functions in various cell types. In these studies, we have (a) identified the diverse eicosanoid pathways that are activated in human chondrocytes of normal and OA cartilage, (b) delineated the modulation of eicosanoids in the presence of NSAIDS and selective COX-2 inhibitors, and (c) characterized eicosanoid products and various transcripts modulated by various inhibitors of eicosanoids in human OA cartilage by gene expression arrays.

Methods: Immunoassay analysis of culture supernatants were utilized to determine the spectrum of eicosanoids derived from both the cyclooxygenase (COX) and lipoxygenase (LOX) pathways of normal and human OA cartilage in ex-vivo conditions. Human OA cartilage was incubated in ex-vivo conditions to examine spontaneous or IL-1 induced production of eicosanoids in the presence of various COX inhibitors. Gene expression analysis was performed to analyze the expression of mRNA in the presence and absence of COX-2 inhibitors in OA cartilage in ex-vivo conditions.

Results: Normal and OA human cartilage explants produced multiple eicosanoids of the COX and LOX pathways. PGF1α, PGF2α, PGE2 > TXB2, PGD2, and LTB4 were spontaneously generated by normal and OA cartilage. Among these, elevated levels of PGE2 and LTB4 were generated in OA as compared to normal cartilage. IL-1 treatment further enhanced these eicosanoids production. Treatment of OA cartilage explants with cyclooxygenase inhibitors (celecoxib & indomethacin) augmented LTB4 accumulation by 2- to 4-fold. A follow-up pharmacogenomic analysis identified approximately 90 cytokine and growth factor related transcripts that were modulated following selective COX-2 inhibition.

Conclusion: These studies for the first time demonstrate that normal and OA cartilage generates multiple and differential eicosanoid products. Inhibition of the COX- pathway in human OA cartilage caused accumulation of end products (LTB4) of the 5LO pathway. Furthermore, celecoxib, a selective COX-2 inhibitor, regulated numerous genes in cartilage, which are linked to the NFkB and AP-1 pathways at the mRNA level. In conclusion, these experiments demonstrate the complex and pleotropic role of eicosanoids in human cartilage homeostasis and pathophysiology of OA.

Human OA cartilage is a site of active production of cytokines and mediators classically associated with inflammation, similar to activated macrophages.1 These mediators include prostaglandin E2 (PGE2), nitric oxide, and cytokines, such as interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α).1,3 We have previously reported that human OA-affected cartilage expresses COX-2 and spontaneously produces PGE2, the levels of which can
be reduced by intracellular nitric oxide, tetracyclines, and non-steroidal anti-inflammatory drugs. However, the spectrum of eicosanoids and expression of arachidonate pathway enzymes has not been characterized in OA cartilage.

Prostaglandins are synthesized by enzyme cascades initiated by the release of arachidonic acid from membrane phospholipids due to the action of cytosolic phospholipase A2 (cPLA2) or secretory PLA2 (sPLA2). Unesterified arachidonate is converted via cyclooxygenase (COX)-1 and -2 to PGH2, which is converted to specific prostanoid end products, such as PGE2, PGF2α, PG12, PGD2, and thromboxane A2. The regulation of eicosanoid production occurs, in part, via the coordinated spatial-temporal expression of associated enzymatic pathways that vary according to cell type and upon the state of cellular activation. In resting cells, for example, constitutively expressed cPLA2, COX-1, and cytosolic PGE synthase (cPGES) produce PGE2 that mediates physiological functions. In contrast, higher levels of PGE2 produced in response to pro-inflammatory cytokines depend upon the coordinated activity of sPLA2, COX-2, and microsomal (m) PGES, which may participate in pathogenic tissue injury.

A second level of regulation, which determines the specific eicosanoid produced following PGH2 production, is via the expression of intermediate and terminal enzymes, which include cPGES and mPGES, prostacyclin synthase, and brain or hematopoietic isoforms of prostaglandin D synthase (bPGDS, hPGDS). Terminal enzyme expression varies with cell type and state of cell activation. Prostanoid end products, formed as described above, act through families of discrete G protein-linked surface receptors and transporters and exert diverse effects on cellular processes both in an autocrine and paracrine fashion.

The role of prostanoid overproduction in joint tissues remains uncertain, and the available literature has focused on actions of PGE2. Potential beneficial effects have been described; for example, PGE2 reverses proteoglycan degradation induced by IL-1 in cartilage explants and enhances collagen type II and proteoglycan synthesis. Conversely, PGE2 may exert catabolic effects via its capacity to stimulate production of matrix metalloproteinases. These observations suggest a pleiotropic role of PGE2 in cartilage homeostasis. Since OA cartilage is a site of significant COX-2 expression and prostanoid overproduction, and the chronic use of COX inhibitors is highly prevalent in OA, the insufficiency of data regarding the expression and actions of eicosanoids in cartilage is noteworthy.

We have utilized genomic approaches to examine gene expression in cartilage and demonstrated over 1,000 genes that are differentially expressed in human OA cartilage compared to normal. A substantial number of these transcripts (an estimated 30%) were either inflammatory mediators or known to be modulated by cytokines. In the current study, we have utilized functional genomic and lipidomic approaches to examine arachidonate pathway related enzymes, as well as to assess the impact of COX-2 derived eicosanoids on OA cartilage gene expression. Our studies demonstrate that OA cartilage produce a remarkably broad array of eicosanoid end products. COX-2 inhibition results in increasing the activity of the lipoxygenase pathway and significant modulation of cytokine and NFkB-related gene expression. These data begin to dissect the discrete eicosanoid pathways that regulate function and gene expression in cartilage, which could lead to better understanding of the role of eicosanoids with respect to NSAIDs currently used in the clinic for treatment of OA symptoms.

**Methods and Materials**

**Reagents**

R&D Microarray was purchased from R&D Membrane Systems, (Minneapolis, MN). ELISA kits for PGE2, PGF2α, PG12, PGD2, TXB2, and LT4 were purchased from Cayman Chemicals Co. (Ann Arbor, MI).

**Procurement of Human Cartilage**

Cartilage slices were taken from the knees of patients with the diagnosis of advanced OA who were undergoing knee replacement surgery and from non-arthritic knees under the guidelines of the Institution Review Board for use of human tissue. Non-arthritic knee cartilage was mostly obtained from patients with fractures or from accident victims after knee amputation. Selected normal tissue was also obtained from National Development and Research Institute (NDRI, Philadelphia, PA, USA). OA patients were free of steroidal and non-steroidal anti-inflammatory drugs for at least 2 weeks before surgery.

**Human OA-Cartilage Explant Assay**

Knee articular cartilage from OA patients was cut into 3 mm discs and placed in a 24 well plate. Ham’s F-12 medium (2 mL) (± 0.1% human albumin), with and without modulators, such as celecoxib, was added in each well. The plates were incubated at 37° C with 21% O2 and 5% CO2, and test samples were withdrawn at different time intervals. After 24 to 120 hours of total incubation time (varied with experiments detailed in specific results), medium from each well was removed, cartilage harvested and weighed to normalize the values of the eicosanoids or mRNA expression. Unless otherwise noted, each experiment represented an individual patient, and a minimum of three to five experiments were performed using cartilage explants from different individual patients.

**Human OA Cartilage Expression Array for Pharmacogenomics**

Cartilage gene expression was performed as reported. Briefly, knee articular cartilage from OA patients was cut into about 3 mm discs. One gram of each cartilage sample was frozen immediately at -150° C for 0 h control. The remainder of the cartilage sample was placed into 2 mL of
F-12 medium (with 0.1% endotoxin free human albumin) ± 2 µM celecoxib in 24-well plates in triplicates for each data point. The plates were placed in 37°C incubators with CO2. The medium was changed at 48 h and the cartilage was harvested for RNA extraction after 120 h. Total RNA was isolated directly from cartilage without releasing the cells. The cartilage was milled into fine powder in liquid nitrogen and extracted with TRIZOL.21,22

RNA was then extracted with H2O-saturated phenol followed by phenol or chloroform extraction and isopropanol precipitation. The RNA pellet was dissolved in H2O and precipitated with alcohol in the presence of acetic acid. RNA obtained by this method was pure enough for all the applications as previously described.2,21 The total RNA from cartilage samples was used for labeling as described: RT-cDNA labeling reactions were performed using protocol and materials from R&D Systems and [33P]-dATP from NEN Life Sciences Products. Pooled total RNA (2 µg) (N = 5) were used in one set of labeling reactions, using cytokine specific primers (R&D Systems), and 2 µg of pooled total RNA (N = 5) were used in another set of labeling reactions, using cytokine specific primers (R&D Systems), and 2 µg of pooled total RNA (N = 5) were used in another set of labeling reactions, using 16- to 18-mer oligo-dT primers (Gibco-BRL, Rockville, Maryland). Labeled cDNA obtained from both reaction sets were pooled together as recommended by the manufacturer and hybridized to the R&D Systems Cytokine Expression Array blots, using the manufacturer’s hybridization protocol. The array blots were then exposed to PhosphorImager plates (Molecular Dynamics), which were read on a Molecular Dynamics Storm apparatus. Quantitation of the signal was performed using ImageQuaNT software and the data was normalized with β-actin and GAPDH.

**Statistical Analysis**
All experiments using cartilage slices were performed in triplicate or quadruplicate for each parameter studied. The p-value was calculated using Student’s t test between the parameters in the same experiment. Data were expressed as mean ± SD. We applied ANOVA test to derive p-values for all the experiments, where p < 0.05 was considered significant.

**Results**

**Analysis of Eicosanoids Produced by Normal and OA Cartilage Explants**
Gene expression profiling using Affymetrix microarray of normal and OA cartilage showed the presence of various mRNA representing proteins associated with eicosanoids pathway enzymes, such as COX, 5-lipoxygenase, 12-lipoxygenase, and 15-lipoxygenase pathways (Amin and colleagues unpublished data).

Normal and OA cartilage explants were incubated ex vivo to investigate the production of eicosanoids. Figure 1 shows the spontaneous production of multiple eicosanoids in human OA cartilage at 72 h detected in the supernatants by either ELISA or RIA. As reported by us previously, OA cartilage differentially and spontaneously produced PGE2 in normal and OA cartilage.2 Of interest, the explants also generated other bioactive eicosanoids, such as PGF2α, PGF1α, > PGD2, TXB2, and LTB4. We analyzed the data from all five experiments represented by Figure 1 and applied the ANOVA test to derive p-values for each eicosanoid. Further analysis showed that increased production of PGE2 and LTB4 by OA cartilage was significant (p < 0.05), and the increased production of PGF1α and PGD2 approached significance (p < 0.1). The levels PGF2α and TXB2 were not significantly different from normal, consistent with a greater contribution of COX-1 to the production of these eicosanoids. The generation of PGE2, PGF2α, PGF1α, PGD2, TXB2, and LTB4 was further upregulated by addition of IL-1β (1 ng/mL) to OA cartilage (Fig. 2). The increased production of eicosanoids in OA cartilage as compared to normal was consistent with previous observations of elevated expression of COX-2 and IL-1 mRNA in OA cartilage.2,23,24

**Regulation of Eicosanoid Production by COX Inhibitors**
We next performed experiments that examined the effects of cyclooxygenase inhibitors (commonly used in

![Figure 1](image-url) **Figure 1** Analysis of eicosanoids in normal and OA cartilage. Normal and OA cartilage explants were incubated in ex vivo conditions and the spontaneous production of eicosanoids was estimated at 72 h by ELISA or RIA. The data are representative of one of five experiments. Note that the concentration units of panel A (ng/g of cartilage) differs from panel B (pg/g of cartilage). *p < 0.05 expression of PGE2 and LTB4 by OA cartilage versus control; †p < 0.1, expression of PGF1α and PGD2 versus control were derived by ANOVA test.
the treatment of OA) on the spontaneous production of eicosanoids by cartilage explants. We developed an experimental protocol to simultaneously perform ex vivo functional and pharmacogenomic studies. The cartilage explants were incubated in the presence and absence of the selective COX-2 inhibitor, celecoxib (2 µM), or the non-selective inhibitor, indomethacin at concentrations (5 µM) that were sufficient to inhibit PGE2 production by at least 50%. Supernatants were assessed for effects on individual eicosanoid production, while total RNA was isolated for gene expression studies.

Figure 3 shows regulation of spontaneous (and IL-1β-induced) production of selected eicosanoids in OA cartilage by celecoxib and indomethacin. The selective COX-2 inhibitor celecoxib was as effective as indomethacin in inhibiting the generation of PGE2, PGF1α, and PGF2α; suggesting that in OA cartilage, these prostanoids are predominantly synthesized by COX-2. In contrast, indomethacin significantly inhibited PGD2 and TXB2 production (p < 0.05), whereas the inhibition of these eicosanoids by celecoxib did not reach statistical significance. The data suggest that there is significant contribution by COX-1 to the production of TXB2 and PGD2 in OA cartilage.

The most striking effect of COX inhibitors on OA cartilage explants was to increase the production of LTB4 by 2 to 4 folds. These data indicate that the inhibition of COX in OA cartilage results in the increased utilization of arachidonic acid substrate by the lipoxygenase pathway. Of interest, the enhanced production of LTB4 was greater in the presence of indomethacin than celecoxib, suggesting that blocking of both COX pathways results in shunting of more substrate arachidonic acid for LTB4 production. Surprisingly, similar observations could not be reproduced in primary cultures of human chondrocytes in the presence...
or absence of IL-1 or COX inhibitors. (Amin and colleagues unpublished data).

**Regulation of Gene Expression by COX Inhibitors**

Having demonstrated profound changes in the eicosanoid profile affected by COX inhibitors, we examined the effect of COX inhibition on global mRNA expression in OA cartilage. We utilized the nylon membrane microarray blots for this study to identify changes in selected low abundance transcripts known to influence cartilage homeostasis.\(^9\) We focused on the regulation of mRNAs for TNFα superfamily, TGFβ superfamily, proteases, neurotropic factors, cytokines and chemokines and their receptors, integrins, fibroblast growth factors, EGF factor family, and insulin growth factor family.

The data was normalized using levels of mRNAs for housekeeping genes such as β-actin and GAPDH. Addition of celecoxib (2 µM) resulted in more than 50% inhibition of spontaneous production of PGE2, as shown in Figure 4. Inhibition of COX-2 by celecoxib resulted in significant effects (defined as changes of 50%) on various transcripts. Figure 4A depicts those genes upregulated in the presence of celecoxib, while Figure 4B shows those that were downregulated. The data indicate that celecoxib, and by inference COX-2 derived prostanoids, modulates expression of numerous cytokine and growth factor dependent genes. These include the TNF, FGF and TGFβ superfamilies, proteases, neurotropic factors, interleukins and cytokines and their receptors, angiogenic factors, and adhesion molecules. Among the celecoxib modulated genes, approximately 50% are known to be modulated by NF-kB and AP-1 transcription.

Figure 4 Regulation of COX-2 Inhibition by Celecoxib on Gene Expression of Cytokine and Growth Factors in OA-Cartilage. OA cartilage explants were cultured for five days in the presence or absence of celecoxib (2 µM). PGE2 levels were estimated on day 5. RNA was extracted on day 5 from five individual patient cartilage specimens. Equal amounts of RNA from the five patients were pooled for expression study using an R&D nylon blot. The values were normalized against housekeeping gene GAPDH and plotted. A, Shows down regulation of spontaneous production of PGE2 by celecoxib and 50% up regulation of multiple specific transcripts. (Figure continued on next page.)
factors in various cell types, consistent with observations that prostanoids exert direct effects on these regulatory transcription factors.  

**Discussion**

We and others have previously reported that the human osteoarthritis cartilage express COX-2, and spontaneously produce PGE2 ex vivo. The present studies provide the first description of the broad spectrum of cyclooxygenase and lipoxygenase-derived eicosanoids, in addition to PGE2, that are also produced by OA cartilage. These include other prostanoids (PGF1α, PGF2α, TXB2, and PGD2) and lipoxygenase-derived products such as LTB4. Some of the end products of eicosanoids were identified and characterized by various ELISAs suggesting activation of numerous pathways. The identification of PG-like compounds and various expected metabolic intermediates of eicosanoid biosynthesis (such as 15-HETE, 12-HETE and 5-HETE) was further confirmed by LC-MS-MS analysis (Amin and colleagues unpublished data). To date, no other human diseased tissue has been demonstrated to spontaneously produce such a diversity of eicosanoid end products.

The regulation of end-product generation by a given cell type requires the coordinate expression of specific enzymes of arachidonate metabolism. Given the broad spectrum of eicosanoids secreted by OA cartilage, it is not surprising to note that there is existence of coordinated regulation of various eicosanoids. Coordinate regulation of COX-2 and mPGES levels has previously been reported in human rheumatoid synovial cells and in an immortalized chondrocyte cell line. Recently, mPGES1-deficient (mPGES1-/-) mice have been reported to display a marked reduction in inflammatory responses compared with mPGES1+/+ mice as assessed by multiple assays, including the development of multiple specific transcripts in the same experiment. Abbreviations: PIN-Protein Inhibitor of Neuronal Nitric Oxide, CCR-9-Receptor for Chemokine CCL25, LAMP-Limbic System Associated Membrane Protein, DNAM- DNAX Accessory Molecule 1, CAD5-VE cadherin, FLK1-vascular endothelial growth factor receptor 2, Bob-G protein coupled receptor 2, GPR3-G-protein coupled receptor-3, MMP-matrix metalloproteinases, BCAM-B-lymphocyte cell adhesion molecule, ALCAM-activated leukocyte cell adhesion molecule, CD166, ENA78- epithelial derived neutrophil activity 78, BMP-3-bone morphogenic protein-3, LT beta R-lymphotoxin beta receptor, TIMP-3-tissue inhibitor of metalloproteinases 3, NGFR- nerve growth factor receptor, TrkC-tyrosine kinase receptor, MPLR-C-myeoloproliferative leukemia virus type P, FGFRI-fibroblast growth factor receptor 1, Epo-erythropoietin, CCR5-Burkitt’s lymphoma receptor, CCR4-C-C chemokine receptor-4.
of collagen-induced arthritis. Furthermore, chondrocyte isolated from mPGES1-/- mice expressed lower levels of MMP-3 and -13 in IL-1 stimulated condition. In these studies, mPGES-1 was also shown to be responsible for the production of PGE2 that mediates acute pain during an inflammatory response and could be a potential target in cartilage. We and others have shown that blocking (EP4 receptor) or induction of PGE2 receptor, EP2 can block MMP13 expression in OA chondrocytes. PG2E also induces apoptosis in chondrocytes via caspase activation and mitochondrial dysfunction. All the above studies further confirm that blocking of PGE2 in OA cartilage could improve pain and cartilage function.

Our data indicate that the predominant cyclooxygenase products generated by OA cartilage are COX-2 derived. In particular, the production of PGE2, PGF1α, and PGF2α can be inhibited by 50% by celecoxib (2 µM), and this inhibition was not increased by non-selective COX inhibitor, indomethacin. It is likely, however, that COX-1 products are also generated. For example, celecoxib did not inhibit the production of TXB2 and PGD2 as effectively as indomethacin, suggesting a COX-1 source for synthesis of these products.

Our data also demonstrate that the inflammatory eicosanoid LTBA4 is spontaneously produced by human OA chondrocytes. LTBA4 was detected by ELISA. In this study, 5- and 12-LO but not 15-LO mRNA could be detected by Real-Time PCR (unpublished data). Furthermore, 5-LO protein constitutively expressed by OA chondrocytes could be further induced by IL-1β treatment. Interestingly, Fernor and coworkers reported that explants of porcine articular cartilage subjected to mechanical compression significantly increased LTBA4 and 5-LO protein production. Their findings provide a direct link between mechanical stress and eicosanoid production in cartilage.

An additional significant finding of current study, which may have clinical implications, is that cyclooxygenase inhibitors augment LTBA4 production by OA explant cultures. While specific inhibition of COX-2 with celecoxib alone was sufficient to cause increased utilization of arachidonate by the lipoxygenase pathway, increases of LTBA4 production were even greater in the presence of indomethacin, suggesting that the inhibition of both COX-1 and COX-2 provided more arachidonate substrate for metabolism by the lipoxygenase pathway. Shunting of arachidonic acid by COX-2 inhibitors may be due to inhibition of PGE2, which is known to inhibit 5-LO activity thorough phosphorylation mediated by protein kinase A (PKA). It was of interest that enhanced LTBA4 production caused by COX inhibitors was a characteristic of cartilage explants but not monolayer chondrocytes cultures (Amin and colleagues unpublished data).

Similar to our studies, Chen and associates have shown that blocking of COX-2 expression and PGE2 production led to increased LTBA4 in human OA chondrocytes. The reasons for this are not clear but could be due to the activation of phospholipases that regulate arachidonate pools available for the LO system in explants but not monolayer cultures. It is also worth noting that IL-1 is one of many diverse stimuli acting upon the chondrocytes in OA explants (e.g., fragments of fibronectin, collagen, and TNFα) that are likely to account for functional differences compared to cartilage and chondrocytes studied in monolayer cultures. Furthermore, we note that, as is common in the field, the OA cartilage specimens used for functional studies are from patients with late disease who are undergoing knee replacement surgery. The non-OA control cartilage samples were obtained from accident victims (trauma) patients. The relative expression levels of inflammatory mediator(s) production in response to IL-1 was similar in both OA and control specimens. However, spontaneous production of eicosanoids that we observed in OA cartilage was negligible or absent in normal cartilage. This could be due to active release of IL-1 by fibronectin and collagen fragments by proteases in OA cartilage, which in turn activates many other signaling pathways involved in lipid and other inflammatory mediatory production.

It should be noted that the experiments reported here were performed in normoxia (21% O2, 5% CO2) conditions employed for studies of human cartilage explant cultures. While there are multiple reports that cartilage obtained from OA patients express COX-2, and spontaneously produce PGE2 ex vivo, there are no studies to confirm robust production of PGE2 by human OA cartilage in vivo. However, Cernanec and coworkers indicated that porcine cartilage explants maintained in hypoxic conditions do not produce PGE2 in response to IL-1, unless nitric oxide production is inhibited. It is of interest, however, that these explants are capable of producing PGE2 under hypoxic conditions in the absence of NO.

The actions of eicosanoids in cartilage are not well characterized. Most studies have examined the effects of PGE2, which has been demonstrated in vitro to enhance synthesis or degradation of collagen type II and proteoglycan synthesis in chondrocytes and to regulate the production of matrix metalloproteinases in synoviocytes and other cell types. Our study clearly demonstrates that PGE2 is one of multiple lipid mediators produced by diseased cartilage, including molecules that have been associated with important roles in other tissues, such as PGD2, PGI2, and LTBA4.

Prostacyclin, in addition to its essential role in maintaining the homeostasis of the microvasculature, has been reported to serve as an endogenous ligand for PPARγ and to regulate functions such as tumor progression, apoptosis, and fertility. The potential effects of prostacyclin in cartilage have not been examined, although it is interesting to note that mice with a targeted deletion of the prostacyclin receptor experience reduced pain and inflammation.

We also observed increased production of PGD2 by OA explants, which was accompanied by the expression of both the isoforms of PGD2 synthase as reported by Urade. Preliminary experiments indicate that PGD2, via mechanisms...
independent from PGJ2 (unpublished data), augments the production of PGE2 and thereby plays an autocrine amplification role in cartilage. Additionally, Zayed and associates have also shown that PGD2 via DP1 receptor inhibited IL-1 induced collagenase expression in human chondrocytes.44 In addition, PGD2 can be non-enzymatically converted to PGJ2, a cyclopentenone ligand of PPARγ.45 Treatment of chondrocytes with the stable metabolite 15-deoxy-delta12, 14-prostaglandin J2 (15d- PGJ2) decreased nitric oxide and MMP-13 production following stimulation with IL-1β, TNFα, or IL-17.45 It has also been suggested that PGJ2 exerts these “chondroprotective” effects by interfering with the activation of AP-1 and NFκB.46

Individual eicosanoids are known to act via discrete G-protein coupled receptors to signal through diverse intracellular pathways, including those regulated by cAMP, protein kinases C and A, PPARγ, NFκB, and AP-1. It was therefore not surprising to observe in our pharmacogenomic studies that COX-2 inhibition, by altering the profile of eicosanoids spontaneously generated by diseased OA cartilage, resulted in profound effects on the transcription of multiple genes that could alter cartilage homeostasis. The data show that more than 90 cytokine and growth factor transcripts are modulated (i.e., 50% up or down regulated) by the COX-2 specific inhibitor, celecoxib.

Of the modulated genes, the majority were regulated NFκB and AP-1 transcription factors. While we cannot exclude some contribution to these results directly by the compound celecoxib, it is likely that the predominant effects are due to the profound shifts in eicosanoid end-products (including the increased activity of the 5-LO pathway) observed in the presence and absence of COX inhibitors. Previous studies have shown modulation of NFκB and AP-1 by a high concentration (> 20 µM) of NSAIDs.47 However, our studies showed modulation of NFκB and AP-1-dependent transcripts at low concentrations of celecoxib, which is therefore likely to predominantly reflect the balance of eicosanoids acting in an autocoid fashion to regulate gene expression in chondrocytes, rather than direct effect of the drug itself.

In summary, our studies demonstrate that OA cartilage is the site of active eicosanoid production. It seems OA chondrocytes, much like activated macrophage and monocytes produce a broad spectrum of products derived from both the cyclooxygenase and lipoxygenase pathways consistent with our hypothesis.1 Treatment of OA cartilage with commonly utilized cyclooxygenase inhibitors increases LTB4 production, which is associated with a marked change in the transcription of multiple cytokine and growth factor dependent genes which are prerequisite for normal cartilage homeostasis. Recent literatures also support that eicosanoids (PGE2, PGD2, and LTB4) along with other factors upregulate cartilage degrading factors such as MMPs, which are involved in the pathophysiology of OA progression. These observations may explain the grounds as to why this group of NSAIDs other than inhibiting the pain associated with the disease also exerts effects on cartilage metabolism in OA.

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