Differentiation Status-dependent Regulation of Cyclooxygenase-2 Expression and Prostaglandin E₂ Production by Epidermal Growth Factor via Mitogen-activated Protein Kinase in Articular Chondrocytes

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Although large amounts of epidermal growth factor (EGF) are found in the synovial fluids of arthritic cartilage, the role of EGF in arthritis is not clearly understood. This study investigated the effect of EGF on differentiation and on inflammatory responses such as cyclooxygenase-2 (COX-2) expression and prostaglandin E₂ (PGE₂) production in articular chondrocytes. EGF caused a loss of differentiated chondrocyte phenotype as demonstrated by inhibition of type II collagen expression and proteoglycan synthesis. EGF also induced COX-2 expression and PGE₂ production. EGF-induced dedifferentiation was caused by EGF receptor-mediated activation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) but not p38 kinase, whereas the activation of both ERK1/2 and p38 kinase was necessary for COX-2 expression and PGE₂ production. Neither the inhibition of COX-2 expression and PGE₂ production nor the addition of exogenous PGE₂ affected EGF-induced dedifferentiation. However, COX-2 expression and PGE₂ production were significantly enhanced in chondrocytes that were dedifferentiated by serial subculture, and EGF also potentiated COX-2 expression and PGE₂ production, although these cells were less sensitive to EGF. Dedifferentiation-induced COX-2 expression and PGE₂ production were mediated by ERK1/2 and p38 kinase signaling. Our results indicate that EGF in articular chondrocytes stimulates COX-2 expression and PGE₂ production via ERK and p38 kinase signaling in association with differentiation status.

Although large amounts of epidermal growth factor (EGF) are found in the synovial fluid of rheumatoid arthritic cartilage, the role of EGF in the pathophysiology of arthritic disease is not fully understood. EGF is known to inhibit chondrocyte differentiation in Meckel’s cartilage and chondrogenesis in chick limb bud mesenchymal cells (4, 5). EGF is also known to inhibit accumulation of sulfated proteoglycan in articular chondrocytes (6, 7), suggesting that EGF not only inhibits chondrocyte differentiation but also causes loss of differentiated chondrocyte phenotype. Articular chondrocytes are characterized by their synthesis of cartilage-specific structural macromolecules such as type II collagen and aggrecan, and loss of differentiated chondrocyte phenotype or dedifferentiation causes cessation of type II collagen expression and induction of fibroblastic types I and III collagens (8). Although the contribution of dedifferentiation in the pathophysiology of arthritis is poorly understood, chondrocyte dedifferentiation is observed in arthritic cartilage, suggesting that EGF contribute to arthritis.

EGF stimulates expression of inflammatory molecules such as cyclooxygenase-2 (COX-2) and prostaglandin E₂ (PGE₂) in various cell types including cell lines originating from chondrocytes (9). However, there are no published data regarding EGF effects on COX-2 expression and PGE₂ production in primary culture articular chondrocytes. Although the role of EGF in PGE₂ production is not clear, pro-inflammatory cytokines such as interleukin-1β (IL-1β) cause PGE₂ production, which is found in arthritic joints in large amounts. PGE₂ is a major mediator of cartilage inflammation, cartilage and juxta-articular bone erosion, and angiogenesis (10–15). The rate-limiting steps in PG synthesis are hydrolysis of phospholipids to produce free arachidonic acid (catalyzed by phospholipase A₂) and conversion of arachidonic acid to PGE₂, which is catalyzed by the two isoforms of COX (16, 17). COX-1 is constitutively expressed, whereas COX-2 is rapidly induced in response to a wide variety of cytokines such as IL-1β and growth factors including EGF. COX-2 expression is regulated at both transcription and post-transcription levels (18–21). Although the molecular mechanism underlying COX-2 expression is not fully understood, several studies indicate that its expression is regulated by mitogen-activated protein kinase (MAPK) subtypes including extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), p38 kinase, and c-Jun N-terminal kinase, depending on the types of extracellular stimuli and cells (20–24). In addition to regulation of COX-2 expression and/or activity, ERK and p38 MAPK are involved in the regulation of chondrocyte dedifferentiation caused by serial subculture (25) or nitric oxide production (26, 27), suggesting these MAPKs regulate both dedifferentiation and inflammatory responses such as COX expression and PGE₂ production.

The current study investigated the role of EGF in the maintenance of chondrocyte phenotype, COX-2 expression, and PGE₂ production. We also examined the functional relationship between EGF-induced dedifferentiation and COX-2 expression.
and characterized signaling pathways involved in EGF action. We report that EGF stimulates dedifferentiation, COX-2 expression, and PGE₂ production in articular chondrocytes via ERK1/2 and p38 kinase signaling and that EGF-induced dedifferentiation further potentiates inflammatory responses in articular chondrocytes.

EXPERIMENTAL PROCEDURES

Isolation and Monolayer Culture of Rabbit Articular Chondrocytes—Articular chondrocytes were isolated from cartilage slices of 2-week-old New Zealand White rabbits by enzymatic digestion as described previously (25). Cartilage slices were dissociated enzymatically in 0.2% collagenase type II (381 units/mg, Sigma) in Dulbecco’s modified Eagle’s medium (Invitrogen). Individual cells were suspended in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) bovine calf serum, 50 μg/ml streptomycin, and 50 units/ml penicillin. Cells were plated on culture dishes at a density of 5 × 10⁵ cells/cm². The medium was replaced every 2 days, and cells were confluent after approximately 5 days. The 2.5-day cell cultures were treated with EGF (Invitrogen). The following pharmacological agents were added 1 h prior to EGF: SB203580 (Calbiochem) to inhibit p38 kinase (29); and tyrphostin AG1478 (Biomol, Plymouth PA) to inhibit MEK1/2 (28); SB203580 (Calbiochem) to inhibit p38 kinase (29); and tyrphostin AG1478 (Biomol, Plymouth Meeting, PA) to antagonize EGF receptors (30). In some experiments, passage (P) 0 cells were subcultured to P3 by plating cells at a density of 5 × 10⁴ cells/cm². Differentiation status of articular chondrocytes was determined by examining the accumulation of sulfated glycosaminoglycan with Alcian blue staining or expression of type II collagen by immunoblot analysis as described previously (25).

Three-dimensional Cultures of Chondrocytes—Dedifferentiated chondrocytes at P4 were three-dimensionally cultured in alginate gel beads prepared in 20 mM HEPES (pH 7.5) containing 0.15 M NaCl, 1% Nonidet P-40, and 0.1% sodium dodecylsulfate. Cells in alginate gel beads were cultured in complete Dulbecco’s modified Eagle’s medium and refed every other day and were recovered by solubilizing alginate with 2 volumes of 50 mM EDTA and 10 mM HEPES (pH 7.4). For pellet culture, dedifferentiated cells were resuspended in 5 × 10⁶ cells/ml and 1 ml of aliquots was pelleted by centrifugation. Cells in alginate gel beads and pellets were incubated for up to 8 days.

Cartilage Explant Culture and Immunohistochemistry—Rabbit joint cartilage explants (∼125 mm²) were fixed in 4% paraformaldehyde for 24 h at 4°C, dehydrated with graded ethanol, embedded in paraffin, and sectioned into 4-μm slices as described previously (31). The sections were stained by standard procedures using Alcian blue or antibody against type II collagen or COX-2 and visualized by developing with a kit purchased from DAKO (Carpinteria, CA).

Immunoblot Analysis—Whole cell lysates were prepared by extracting proteins using a buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, and 0.1% sodium dodecylsulfate supplemented with protease inhibitors (10 μg/ml leupeptin, 10 μg/ml pepstatin A, 10 μg/ml apro tin, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride) and phosphatase inhibitors (1 mM NaF and 1 mM Na₃VO₄). The proteins were size-fractionated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The nitrocellulose sheet was then blocked with 3% nonfat dry milk in Tris-buffered saline. COX-2 was detected using antibody purchased from Cayman Chemical (Ann Arbor, MI), and type II collagen was detected using antibodies purchased from Chemicon International Inc. (Temecula, CA). The bands were visualized using peroxidase-conjugated secondary antibodies and chemiluminescence.

MAPK Assay—ERK1/2 activation was examined using immunoblot analysis as described previously (25) using antibodies specific to activated threonine 202- and tyrosine 204-phosphorylated ERK1/2 (Cell Signaling Technology, Beverly, MA). p38 kinase activity was determined by immune complex kinase assays as described previously (26, 27). Cells were lysed in a buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, and inhibitors of proteases and phosphatases as described above. Total cell lysates were precipitated with polyclonal anti-p38 kinase antibody (Santa Cruz Biotechnology, Santa Cruz, CA), and the immune complexes were collected using protein-A-Sepharose beads. The beads were resuspended in 20 μl of kinase reaction buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 0.1 mM sodium orthovanadate, 10 mM MgCl₂, 5 μCi of [γ-³²P]ATP, and 1 μg of activating transcription factor-2 protein as a substrate (New England Biolabs, Beverly, MA). The kinase reaction was performed for 30 min at 30°C, and phosphorylated activating transcription factor-2 was detected by autoradiography following gel electrophoresis.

PGE₂ Assay—PGE₂ production was determined by measuring the levels of cellular and secreted PGE₂ using an assay kit (Amersham Biosciences). P0 cells or dedifferentiated P4 cells were seeded in standard 96-well microtiter plates at 2 × 10⁴ cells/well. After the indicated treatments, total PGE₂ was quantified according to the manufacturer’s protocol. PGE₂ levels were calculated against a standard curve of PGE₂ and normalized against the amount of genomic DNA.

RESULTS

EGF Causes Dedifferentiation of Articular Chondrocytes—To examine the effects of EGF on articular cartilage chondrocyte differentiation, cartilage explant cultures were treated with 10 ng/ml EGF for 48 h, and expression of cartilage-specific matrix molecules were determined. EGF caused a dramatic loss of type II collagen and sulfated proteoglycan as determined by immunohistochemical staining and Alcian blue staining, respectively (Fig. 1A). EGF also inhibited type II collagen expression in primary culture articular chondrocytes in a dose- and time-dependent manner, and this inhibition was blocked when cells were pretreated with the EGF receptor antagonist AG1478.
EGF-induced COX-2 Expression in Chondrocytes

MAPK Regulates EGF-induced COX-2 Expression, PGE₂ Production, and Dedifferentiation—The effect of EGF on COX-2 expression and PGE₂ production was investigated using both cartilage explant cultures and primary culture chondrocytes. In cartilage explants, EGF stimulated COX-2 expression as determined by immunohistochemical staining (Fig. 2A). In primary culture chondrocytes, EGF increased protein levels of COX-2 in a time- and dose-dependent manner as determined by immunoblot analysis (Fig. 2B). COX-2 was detected 1 h after EGF treatment, and levels peaked at 12 h. The EGF effects on primary culture chondrocytes were completely blocked by AG1478 pretreatment (Fig. 2B). Consistent with the induction of COX-2 expression, EGF stimulated PGE₂ production (Fig. 2C) that was detectable 1 h after EGF addition (Fig. 2D) and was blocked by AG1478 (Fig. 2E). These data indicate that EGF not only causes dedifferentiation of articular chondrocytes but also stimulates COX-2 expression and PGE₂ production.

MAPK Regulates EGF-induced COX-2 Expression, PGE₂ Production, and Dedifferentiation—EGF (5 ng/ml) activated both ERK1/2 and p38 kinase in articular chondrocytes (Fig. 3A, upper panel), and activation of both kinases was inhibited by AG1478 (Fig. 3A, lower panel). To determine whether ERK1/2 activation is associated with dedifferentiation and/or COX-2 expression and PGE₂ production, chondrocytes were treated with EGF in the absence or presence of PD98059, which inhibits ERK1/2 activation (Fig. 3B, upper panel). PD98059 blocked EGF-stimulated dedifferentiation as indicated by the resumption of type II collagen expression (Fig. 3B, upper panel), sulfated proteoglycan accumulation (Fig. 3C), and sulfated proteoglycan production (Fig. 3D). Inhibition of p38 kinase with SB203580 (Fig. 3B, lower panel) completely blocked both EGF-stimulated COX-2 expression (Fig. 3B, upper panel) and PGE₂ production (Fig. 3D). In contrast to the effects of ERK inhibition, the inhibition of p38 kinase with SB203580 did not significantly alter the EGF-induced decrease in type II collagen expression (Fig. 3B, lower panel) and synthesis of proteoglycans (Fig. 3C). Taken together, these results indicate that EGF-induced dedifferentiation is regulated by ERK1/2 activity but not by p38 kinase activity, whereas both ERK1/2 and p38 kinase regulate COX-2 expression and PGE₂ production.

Differentiation Status-dependent COX-2 Expression and PGE₂ Production—Because EGF in articular chondrocytes causes both dedifferentiation and COX-2 expression, we next examined the functional relationship between EGF-induced dedifferentiation and inflammatory responses. Differentiation status-dependent COX-2 expression and PGE₂ production were examined first. Serial subculturing of chondrocytes caused dedifferentiation as demonstrated by cessation of type II collagen expression (Fig. 4A). This chondrocyte phenotype loss was accompanied by significantly increased levels of COX-2 protein (Fig. 4A) and PGE₂ production (Fig. 4B). When these dedifferentiated P3 cells were redifferentiated into chondrocytes by three-dimensional culture in alginate gel (Fig. 4C) or by pellet culture (Fig. 4D), COX-2 protein levels dropped to those observed in differentiated P0 cells. Therefore, COX-2 expression and PGE₂ production were inversely related to the differentiation status of articular chondrocytes.

COX-2 Expression and PGE₂ Production Do Not Mediate EGF-induced Dedifferentiation—Because PGE₂ production is known to modulate chondrocyte differentiation (32–34), we next examined whether COX-2 expression and the resulting PGE₂ production in EGF-treated cells are involved in dedifferentiation of chondrocytes. The COX-2 inhibitors indomethacin or NS398 (35) significantly blocked EGF-induced PGE₂ production as expected (Fig. 5A). However, EGF-induced inhibition of sulfated proteoglycan accumulation (Fig. 5B) and type II collagen expression (Fig. 5C) were not affected by indomethacin or NS398. In addition, treatment of P0 cells with exogenous PGE₂ did not significantly affect proteoglycan synthesis (Fig. 5B) or type II collagen expression (Fig. 5C). These data indicate that COX-2 expression and PGE₂ production are not involved in EGF-induced dedifferentiation of chondrocytes.

Differentiation of Chondrocytes Potentiates COX-2 Expression and PGE₂ Production—We examined the effect of EGF on COX-2 expression and PGE₂ production in dedifferentiated cells. We found that EGF further increased COX-2 expression (Fig. 6A) and PGE₂ production in dedifferentiated P3 cells (Fig. 6B). Although the sensitivity of dedifferentiated chondrocytes
to EGF was much less than that of P0 cells, the levels of COX-2 and PGE$_2$ were much higher in dedifferentiated chondrocytes. In an attempt to reveal the mechanisms underlying increased expression of COX-2 and PGE$_2$ production in dedifferentiated cells, the differentiation status-dependent activity of MAPK subtypes was examined. Serial subculture of chondrocytes to P3 caused sustained activation of both ERK1/2 and p38 kinase (Fig. 7A). The inhibition of ERK activity with PD98059 but not p38 kinase activity with SB203580 partially maintained type II collagen expression in P3 cells (Fig. 7B). Blocking ERK activity did not significantly affect dedifferentiation-induced increase of COX-2 protein level (Fig. 7B), but it did inhibit COX-2 activity as demonstrated by the inhibition of PGE$_2$ production (Fig. 7C). The inhibition of p38 kinase with SB203580 blocked both dedifferentiation and EGF-induced potentiation of COX-2 expression (Fig. 7B) and PGE$_2$ production (Fig. 7C). The above results suggest that dedifferentiation-induced activation of ERK1/2 and p38 kinase causes increased COX-2 expression and PGE$_2$ production in articular chondrocytes.

**DISCUSSION**

Chondrocytes in normal articular cartilage are a unique cell type in that their differentiated phenotype is reversible. Chondrocyte phenotype is regulated by a balance of anabolic and catabolic molecular reactions that are involved in maintaining homeostasis of cartilage tissue (8). Differentiated chondrocytes lose their phenotype and transform into fibroblast-like cells upon exposure to soluble factors such as IL-1$\beta$ (36), retinoic acid (31, 37), and nitric oxide (26, 38) or during serial subculture in vitro (25, 39). Such a destruction of homeostasis is believed to be involved in the pathophysiology of arthritis (8, 40). Because large amounts of EGF are found in the synovial fluid of arthritic cartilage (1, 2), it is likely that EGF contributes to the disease, although the role of EGF is poorly understood. In this study, we demonstrated that EGF caused both dedifferentiation and inflammatory responses such as COX-2 expression and PGE$_2$ production. We also demonstrated that dedifferentiation of chondrocytes by serial subculture caused significant increases in COX-2 expression and PGE$_2$ produc-
tion, even though COX-2 expression and PGE$_2$ production did not affect dedifferentiation. Therefore, our results indicate that EGF-induced inflammatory responses and suggest that dedifferentiation of chondrocytes may worsen arthritic cartilage inflammation.

A significant finding of this study is that EGF caused both dedifferentiation and COX-2 expression/PGE$_2$ response. Previous reports (41, 42) indicate that dedifferentiation of chondrocytes caused reduction in responsiveness to the inflammatory cytokines such as IL-1$\beta$ with respect to COX-2 expression and PGE$_2$. 

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**Fig. 5.** EGF-induced COX-2 expression and/or PGE$_2$ production does not affect dedifferentiation. A and B, chondrocytes were treated with indomethacin (Indo, 25 $\mu$g/ml) or NS398 (2.5 $\mu$M) and then with EGF (5 ng/ml) for 24 h. Alternatively, 200 ng/ml PGE$_2$ was added to chondrocytes for 48 h. PGE$_2$ was measured using an assay kit (A). Accumulation of sulfated proteoglycan was quantified using Alcian blue staining (B). C, chondrocytes were treated with the indicated concentrations of indomethacin (upper panel) or NS398 (middle panel) for 1 h prior to EGF (5 ng/ml) addition. Alternatively, chondrocytes were treated with the indicated concentrations of exogenous PGE$_2$ for 48 h. Type II collagen expression was determined by immunoblot analysis. The data in A and B represent mean values ± S.D., and the data in C represent a typical experiment ($n = 4$).

**Fig. 6.** EGF potentiates dedifferentiation-induced COX-2 expression and PGE$_2$ production. A and B, differentiated P0 chondrocytes and dedifferentiated P3 cells were treated with 5 ng/ml EGF for 48 h. Levels of type II collagen and COX-2 were determined by immunoblot analysis (A). PGE$_2$ levels were determined using an assay kit (B). The data represent results of a typical experiment (A) or mean values ± S.D. (B) from at least four independent experiments.

**Fig. 7.** ERK1/2 and p38 kinase mediates dedifferentiation-induced increase of COX-2 expression and PGE$_2$ production. A, chondrocytes were subcultured to P3, and ERK1/2 and p38 kinase activity were determined by immunoblot analysis and immune complex kinase assay, respectively. B and C, differentiated P0 chondrocytes or dedifferentiated P3 cells were either untreated (Con) or treated with 20 $\mu$M PD98059 (PD) or 20 $\mu$M SB203580 (SB) for 1 h and then exposed to vehicle alone or 5 ng/ml EGF for 24 h. Type II collagen and COX-2 were detected using immunoblot analysis (B). PGE$_2$ production was measured using an assay kit and normalized by determining the amount of total genomic DNA. The data represent results of a typical experiment or mean value ± S.D. from at least four independent experiments.
production. Our current results also indicate that dedifferentiated cells are much less sensitive to EGF-induced increases in PGE$_2$ production. For instance, PGE$_2$ production increased >5-fold in differentiated P0 cells following EGF treatment, whereas only a ~2-fold increase was observed in dedifferentiated P3 cells (Fig. 6). The amount of PGE$_2$ produced per well by dedifferentiated cells either in the absence or presence of EGF was much less than that observed in differentiated P0 cells. However, when the amount of PGE$_2$ produced was normalized against genomic DNA to quantify PGE$_2$ production per cell, significantly higher amounts of PGE$_2$ were produced by differentiated cells (Fig. 6). Similar results were observed when chondrocytes were treated with IL-1β (data not shown). Therefore, our observations clearly indicate that dedifferentiated chondrocytes produce more PGE$_2$, although sensitivity to EGF or IL-1β is much lower.

In addition to PGE$_2$ production, the levels of COX-2 protein were much higher in serially subcultured dedifferentiated cells compared with differentiated P0 cells (Fig. 4). We also observed that dedifferentiation of chondrocytes by exposure to phorbol ester, nitric oxide, or retinoic acid caused significantly elevated expression of COX-2 (data not shown). The results of this study are different to those reported by Thomas et al. (42) in which IL-1β-treated differentiated and dedifferentiated immortalized chondrocytes showed similar COX-2 mRNA levels. This discrepancy may be the result of different cell origins and culture systems. Those studies compared responses of an immortalized human articular chondrocyte cell line with responses of cells cultured as monolayers and three-dimensionally. This study used primary rabbit articular chondrocytes cultured in monolayers. Indeed, although EGF is known to induce COX-2 expression with MAPK subtypes including ERK1/2, p38 kinase, and c-Jun N-terminal kinase (20–24). Therefore, it is likely that EGF-induced differentiation further elevates COX-2 expression and PGE$_2$ production and that EGF found in arthritic cartilage synovial fluid contributes to inflammation and cartilage destruction during arthritic disease.

REFERENCES

1. Sato, K., Kikuchi, S., Sekimoto, M., Kabuyama, Y., Homma, M. K., and Homma, Y. (2001) Arthritis Rheum. 44, 260–265
2. Lui, K. P., Panach, A. S., Santhanagopal, S., Dixon, S. J., and Bernier, S. M. (2002) J. Cell Physiol. 202, 103–112
3. Ishizeki, K., Takahashi, N., and Nawa, T. (2001) Cell Tissue Res. 304, 67–80
4. Dealy, C. N., Scranton, V., and Cheng, H. C. (1998) Dev. Biol. 202, 43–55
5. Yoon, Y. M., Oh, C.-D., Kim, D.-Y., Lee, Y.-S., Park, W.-H., Huh, T.-L., Kang, S.-S., and Chun, J.-S. (2000) J. Biol. Chem. 275, 12353–12359
6. Prins, A. P. A., Lipman, J. M., McDevitt, C. A., and Sokoloff, L. (1982) Arthritis Rheum. 25, 1228–1238
7. Verschueren, P. J., Jousten, L. A., Van der Kraan, P. M., and Van den Berg, W. B. (1994) Ann. Rheum. Dis. 53, 455–460
8. Sandell, L. J., and Aigner, I. (2001) Arthritis Res. 3, 107–113
9. Sakai, T., Kanbe, P., Mistuyma, H., Ishiguro, N., Kureokuchi, T., Takigawa, M., Iwata, H., and Hao, S. (2001) J. Bone Miner. Res. 16, 1272–1278
10. Robinson, D. R., Tashjian, A. H. J., and Levine, L. (1975) J. Clin. Invest. 56, 1181–1186
11. Ben-Av, P., Crofford, L. J., Wilder, R. L., and Hla, T. (1995) FEBS Lett. 372, 83–87
12. Amin, A. R., Attur, M., Patel, R. N., Thacker, G. D., Marshall, P. J., Rediske, J., Stuchin, S. A., Patel, I. R., and Abramson, S. B. (1997) J. Clin. Invest. 99, 1231–1237
13. Amin, A. R., Attur, M., and Abramson, S. B. (1999) Curr. Opin. Rheumatol. 11, 202–209
14. Amin, A. R., Dave, M., Attur, M., and Abramson, S. B. (2000) Curr. Rimmune Pharmacol. Rep. 2, 447–453
15. Abramson, S. B. (1999) Osteoarthritis Cartilage 7, 380–381
16. Smith, W., Garavito, R., and DeWitt, D. (1996) Curr. Opin. Rheumatol. 8, 331–336
17. Smith, W., Garavito, R., and DeWitt, D. (1996) J. Biol. Chem. 271, 33157–33160
18. Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S. Van De, R., He, Q. W., de Ladurantaye, M., Quintero, M., Mancini, J., Stuchin, S. A., Patel, I. R., and Abramson, S. B. (1997) J. Cell. Biochem. 69, 392–413
19. Newton, R., Seybold, J., Kautter, L. M., Bergmann, M., and Barnes, P. J. (1998) J. Biol. Chem. 273, 32312–32321
20. Lasa, M., Mahtani, K. R., Finch, A., Brewer, G., Sakkavala, J., and Clark, A. R. (2000) Mol. Cell. Biol. 20, 4365–4374
21. Fauser, W. H., He, Y., He, Q., de Ladurantaye, M., Quintero, M., Mancini, A., and Di Battista, J. A. (2001) J. Biol. Chem. 276, 31720–31731
22. Sheng, H., Williams, C. S., Shao, L., Liang, P., Doxois, R. N., and Beauchamp, R. D. (1996) J. Biol. Chem. 271, 22120–22127
23. Matsuzawa, H., Sakane, M., Subbarauamia, K., Kamitani, H., Eling, T. E., Dannenberg, A. J., Tanabe, T., Inoue, H., Arata, J., and Jetten, A. M. (1999) J. Biol. Chem. 274, 29138–29148
24. Guan, Z., Buckman, S. Y., Miller, B. W., Springer, L. D., and Morrison, A. R. (1998) J. Biol. Chem. 273, 28670–28678
25. Yoon, Y.-M., Kim, S.-J., Oh, C.-D., Ju, J.-W., Song, W.-K., Yoo, Y.-J., Huh, T.-L., and Chun, J.-S. (2000) J. Biol. Chem. 277, 8412–8420
26. Kim, S.-J., Ju, J.-W., Oh, C.-D., Yoon, Y.-M., Song, W.-K., Kim, J.-H., Yoo, Y.-J., Bang, O.-S., Kang, S.-S., and Chun, J.-S. (2002) J. Biol. Chem. 277, 1332–1339
27. Kim, S.-J., Hwang, S.-G., Shin, D.-Y., Kang, S.-S., and Chun, J.-S. (2002) J. Biol. Chem. 277, 33501–33508
28. Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 27489–27494
29. Cuenda, A., Rous, J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P. R., and Lee, J. C. (1995) FEBS Lett. 364, 229–233
30. Leviandier, A., and G oat, A. (1985) Science 227, 1782–1784
31. Ryu, J.-H., Kim, S.-J., Kim, S.-H., Oh, C.-D., Hwang, S.-G., Chun, C.-H., Oh, S.-H., Seong, J.-K., Huh, T.-L., and Chun, J.-S. (2002) Development 129, 5541–5550

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32. Goldring, M. B., Suen, L. F., Yamin, R., and Lai, W. F. (1996) Am. J. Ther. 3, 9–16
33. Schwartz, Z., Gilley, R. M., Sylviya, V. L., Dean, D. D., and Boyan, B. D. (1998) Endocrinology 139, 1825–1834
34. Goldring, M. B., and Berenbaum, F. (1999) Osteoarthritis Cartilage 7, 386–388
35. Futaki, N., Takahashi, S., Yokoyama, M., Arai, I., Higuchi, S., and Otomo, S. (1994) Prostaglandins 47, 55–59
36. Goldring, M. B., Birkhead, J., Sandell, L. J., Kimura, T., and Krane, S. M. (1988) J. Clin. Invest. 82, 2026–2037
37. Hein, R., Krieg, T., Mueller, P. K., and Braun-Falco, O. (1984) Biochem. Pharmacol. 33, 3263–3267
38. Taskiran, D., Stefanovic-Racić, M., Georgescu, H. I., and Evans, C. H. (1994) Biochem. Biophys. Res. Commun. 200, 142–148
39. Benya, P. D., Padilla, S. R., and Nimni, M. E. (1978) Cell 15, 1313–1321
40. Gay, S., Gay, R. E., and Koppman, W. J. (1993) Ann. Rheum. Dis. 52, 39–47
41. Lemare, F., Steinberg, N., Griel, C. L., Demignot, S., and Adolphe, M. (1998) J. Cell. Physiol. 176, 303–313
42. Thomas, B., Thirion, S., Humbert, L., Tan, L., Goldring, M. B., Bereziat, G., and Berenbaum, F. (2002) Biochem. J. 362, 367–373
43. Kinoshita, A., Takigawa, M., and Suzuki, F. (1992) Biochem. Biophys. Res. Commun. 183, 14–20
44. Gilroy, D. W., Tomlison, A., Greenslade, K., Seed, M. P., and Willoughby, D. A. (1998) Inflammation 22, 509–519
45. Myers, L. K., Kang, A. H., Postlethwaite, A. E., Rosloniec, E. F., Morham, S. G., Goorha, S. S., and Ballou, L. R. (2000) Arthritis Rheum. 43, 2687–2693
