Novel Role of Pin1 Induction in Type II Collagen-Mediated Rheumatoid Arthritis

Hye Gwang Jeong, Yuba Raj Pokharel, Sung Chul Lim, Yong Pil Hwang, Eun Hee Han, Jung-Hoon Yoon, Sang-Gun Ahn, Kwang Yeol Lee and Keon Wook Kang

*J Immunol* 2009; 183:6689-6697; Prepublished online 21 October 2009;
doi: 10.4049/jimmunol.0901431
http://www.jimmunol.org/content/183/10/6689

**References**
This article cites 53 articles, 16 of which you can access for free at:
http://www.jimmunol.org/content/183/10/6689.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Novel Role of Pin1 Induction in Type II Collagen-Mediated Rheumatoid Arthritis

Hye Gwang Jeong, Yuba Raj Pokharel, Sung Chul Lim, Yong Pil Hwang, Eun Hee Han, Jung-Hoon Yoon, Sang-Gun Ahn, Kwang Yeol Lee, and Keon Wook Kang

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation in joints and subsequent destruction of cartilage and bone. Inflammation mediators such as proinflammatory cytokines contribute to RA progress. Pin1, a peptidyl prolyl isomerase, plays important pathophysiological roles in several diseases, including cancer and neurodegeneration. In this study, we found that Pin1 and cyclooxygenase-2 (COX-2) were highly expressed in ankle tissues of type II collagen-induced RA mice. HTB-94 cells overexpressing Pin1 and primary cultured human chondrocytes showed increased basal expression of proinflammatory proteins (COX-2, inducible NO synthase, TNF-α, and IL-1β). Site-directed mutagenesis revealed that Pin1-mediated transcriptional activation of COX-2 was coordinately regulated by NF-κB, CREB, and C/EBP. Gel shift, reporter gene, and Western blot analyses confirmed that NF-κB, CREB, and C/EBP were consistently activated in chondrocytes overexpressing Pin1. Treatment of RA mice with juglone, a chemical inhibitor of Pin1, significantly reduced RA progress and COX-2 expression in the ankle tissues. Moreover, juglone dose-dependently decreased the basal COX-2 expression in primary cultured chondrocytes from RA patients. These results demonstrate that Pin1 induction during RA progress stimulates proinflammatory protein expression by activating NF-κB, CREB, and C/EBP, and suggest that Pin1 is a potential therapeutic target of RA.


Received for publication May 6, 2009. Accepted for publication September 5, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 H. G. J. and Y. R. P. contributed equally to this work.

2 H. G. J. and Y. R. P. contributed equally to this work.

3 Address correspondence and reprint requests to Dr. Keon Wook Kang, College of Pharmacy, Chosun University, Gwangju 501-759, South Korea E-mail address: kwkang@chosun.ac.kr

4 Abbreviations used in this paper: RA, rheumatoid arthritis; CII, type II collagen; COX-2, cyclooxygenase-2; CRE, cAMP response element; iNOS, inducible NO synthase; MSCV, murine stem cell virus; siRNA, small interfering RNA.

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0901431

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0901431

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0901431

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00
Cell culture

HTB-94 cells and primary cultured human chondrocytes were obtained from ATCC and Y. Park (College of Medicine, Chonnam National University, Gwangju, South Korea), respectively, and cultured at 37°C in 5% CO₂, 95% air in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin.

CII-induced arthritis and juglone treatment

The institutional animal care and utilization committee of Chosun University approved all the animal procedures used in this study. Male DBA/1J mice (Joong-Ang Experimental Animals), age 8 wk, were used. Bovine CII was dissolved in 0.1 M acetic acid and emulsified in an equal volume of CFA. The mice were immunized intradermally at the base of the tail with 100 µl emulsion containing 150 µg of CII. On day 21, mice were boosted intradermally with 100 µg of CII, and arthritis development was monitored for 10 days. Juglone was dissolved in solubilization solvent (PEG400, Tween 80, ethanol, and sterile water) and i.p. injected from day 22 every other day (4× injection).

Assessment of arthritis

Mice were sacrificed on day 10 after second CII booster. Before sacrificing, arthritis in each limb of CII-treated mice was clinically assessed by visual edema scoring from 0 to 3, as follows: 0, no swelling; 1, detectable swelling in one joint; 2, nonsevere swelling in two or more joints; 3, severe swelling in two or more joints (19). The left hind limbs, including paws and ankles, were dissected, fixed immediately for 12 h at 10% neutralizing formaldehyde, decalcified in Calci-Clear Rapid (National Diagnostics) for 12 h, and embedded in paraffin. Tissue sections (4 µm) were mounted on common slides for staining with H&E. A certified pathologist scored samples in a blinded fashion. The data are expressed as mean chronic inflam-

Immunohistochemistry

A universal immunoenzyme polymer method was used for immunostain-
ing. Sections (4 µm) were cut from formalin-fixed, paraffin-embedded tissue blocks, mounted on polylysine-coated slides, dewaxed in xylene, and rehydrated through a graded ethanol series. After deparaffinization, Ag retrieval treatment was performed at 121°C for 15 min in 10 mM sodium citrate buffer (pH 6.0), and was then treated with 3% hydrogen peroxide in methanol solution for 20 min to quench endogenous peroxidase activity. To block intrinsic avidin-biotin binding, the tissue slides were treated with avidin-biotin blocking kit reagents (Vectastain Elite ABC kit; Vector Laboratories) for 15 min. Anti-Pin1 and anti-COX-2 Abs were used as the primary Abs. The final products were visualized using the 3-3 diaminobenzidine tetrahydrochloride detection system (DakoCytomation). All experiments were performed in duplicate.

Cell culture

HTB-94 cells and primary cultured human chondrocytes (passage 6) were obtained from ATCC and K. Lee (Chonnam National University, Gwangju, South Korea), respectively. Both the cell types were cultured at 37°C in 5% CO₂, 95% air in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. For all experiments, cells were grown to 80–90% confluency and subjected to no more than 15 passages.

Construction of Pin1 retroviral plasmid and injections

HTB-94 and human chondrocytes stably expressing Pin1 were established using murine stem cell virus (MSCV)-GFP retroviral vector, and Phoenix cells (a packaging cell line) were infected with MSCV-GFP (control) or MSCV-Pin1-GFP (Pin1 overexpression) plasmid. Supernatants containing amphotropic replication-incompetent retroviruses were collected and then stored at −80°C until required. Twenty percent confluent HTB-94 cells and chondrocytes obtained from osteoarthritic patients were multiply infected (12 times) with retrovirus particles. Intensities of infection were monitored by GFP fluorescence and Western blot analysis using Pin1 Ab.

Preparation of nuclear extract and Western blot analysis

Cells were removed using a cell scraper and centrifuged at 2500 × g for 5 min at 4°C. The cells were then swollen with 100 µl of lysis buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.5% Nonidet-P40, 1 mM DTT, and 0.5 mM PMSF). Tubes were vortexed to disrupt cell membranes, and samples were incubated for 10 min on ice and then centrifuged for 5 min at 4°C. Pellets containing crude nuclei were resuspended in 100 µl of extraction buffer (20 mM HEPES (pH 7.9), 400 mM NaCl, 1 mM EDTA, 1 mM DTT, and 1 mM PMSF), incubated for 30 min on ice, and centrifuged at 15,800 × g for 10 min; the supernatants containing the nuclear extracts were collected and stored at −80°C until required. SDS-PAGE and immunoblot analyses were performed, as described previously (21). Cell lysates were fractionated by 10% gel electrophoresis, and electrophoretically transferred to nitrocellulose membranes. The membranes were subsequently incubated with primary Ab, and then with alkaline phosphatase- or HRP-conjugated secondary Abs. Finally, the membranes were developed using either 5-bromo-4-chloro-3-indolylphosphate and NBT or an ECL chemiluminescence detection kit.

Gel shift assay

dsDNA probes (2 pmol/µl) for the consensus sequences of AP-1 (5′-CGCTTGATGAGTCAGCCGGAA-3′) were used for gel shift analyses after end labeling the probe with [γ-32P]ATP and T4 polynucleotide kinase. The reaction mixture contained 2 µl of 5× binding buffer with 20% glycerol, 5 mM MgCl₂, 250 mM NaCl, 2.5 mM EDTA, 2.5 mM DTT, 0.25 mg/ml poly(dI-dC), 50 mM Tris-Cl (pH 7.5), 10 µg of nuclear extracts, and sterile water to a total volume of 10 µl. Incubations were performed at room temperature for 20 min by adding 1 µl of probe (106 cpm) after a 10-min preincubation. The specificity of DNA/protein binding was determined through competition reactions using a 10-fold molar excess of unlabeled oligonucleotides. Samples were loaded onto 5% polyacrylamide gels at 100 V. After electrophoresis, the gels were removed, dried, and autoradiographed.
Construction of a COX-2 promoter-luciferase construct and reporter gene assays

To determine the transcriptional activity of the COX-2 gene, we used the pGL-COX-2-574 luciferase reporter gene. To construct the luciferase (LUC) reporter gene plasmid, COX-2-LUC(H11002574), a DNA fragment containing 574 bp of 5'-flanking sequences and 113 bp of 5'-untranslated region from the human COX-2 gene, was first amplified by PCR using a human genomic clone as the template. The PCR fragment was then cloned into pGL3-Basic (Promega). Site-directed mutagenesis of NF-κB, cAMP response element (CRE)/AP-1, and NF-IL-6/CEBP binding sites was performed using a LAPCR in vitro mutagenesis kit (Takara Shuzo) (22). A total of 1 μg of the plasmid was transfected into the cells using Lipo-ffectAMINE2000 (Invitrogen) or Hilymax reagent (Dojindo Molecular Technologies), according to the manufacturer’s instructions. After 6 h, the transfection medium was replaced with the basal culture medium without serum and the cells were further incubated for 18 h. The luciferase activities in the cell lysates were then measured using a luminometer. The relative luciferase activity was calculated by normalizing the promoter-driven luciferase activity vs human Renilla luciferase or β-galactosidase.

RT-PCR

The total RNA was isolated using a total RNA isolation kit (RNAgents; Promega). The total RNA (1.0 μg) obtained from the cells was reverse transcribed using an oligo(dT) 18 mer as a primer and Moloney murine leukemia virus reverse transcriptase (Bioneer) to produce the cDNAs. PCR was performed using the selective primers for human TNF-α, IL-1β, IFN-γ, and S16 ribosomal protein genes. The PCRs were performed for 42 cycles using the following conditions: denaturation at 98°C for 10 s, annealing at 62°C and 50°C for IL-1β and TNF-α, respectively, for 0.5 min, and elongation at 72°C for 1 min. The band intensities of the amplified DNAs were compared after visualization using FLA-7000 (Fuji film).

ELISA

Commercial ELISA kits (Cayman Chemical and BD Biosciences) were used to determine PGE2 and IL-4 concentrations in culture medium, according to the manufacturer’s protocols.

Statistics

Paired Student’s t test was used to determine the significance of differences between treatment groups. Statistical significance was accepted for p values of <0.05.

Results

Pin1 induction in arthritic tissues

Pin1 contributes to degenerative disease in brain (13). To determine whether Pin1 is chronically expressed in arthritic lesions, we
determined Pin1 levels by immunohistochemistry in the ankle tissues from CII-induced RA mice. Hind paw swelling and erythema were increased in all mice injected with CII (data not shown). RA tissues showed higher levels of Pin1 induction, mainly distributed in chondrocytes, lymphocytes, and fibroblasts (Fig. 1A, upper). COX-2 is highly expressed in human and animal arthritic tissues (6), and COX-2 staining showed similar patterns to Pin1 staining (Fig. 1A, lower). Western blot analysis also showed concomitant expression of COX-2 and Pin1 in RA tissue homogenates, but not in control (Fig. 1B).

Role of Pin1 in proinflammatory protein expression
To clarify the phenotypes of Pin1 overexpression in RA tissues, we established stably Pin1-overexpressing HTB-94 cells, a human chondrocyte cell line (Pin1-HTB-94) using retroviral infections. Pin1-HTB-94 cells had higher Pin1 levels than GFP-HTB-94 (GFP-overexpressing) cells (Fig. 2A). Western blot analysis showed that COX-2 expression was up-regulated in Pin1-HTB-94 cells (Fig. 2A). Production of PGE2, a stable autacoid produced by COX-2, was 4.3-fold higher in Pin1-HTB-94 cells (Fig. 2B). Moreover, Pin1-HTB-94 cells showed higher protein or mRNA levels of iNOS, TNF-α, and IL-1β, representative proinflammatory enzymes and cytokines (Fig. 2C). It has been shown that Pin1 knockout mice show both Th1 and Th2 abnormalities (23). Hence, we additionally determined the mRNA expression of IFN-γ (Th1 dependent) and IL-4 production (Th2 dependent) in both GFP-HTB-94 and Pin1-HTB-94 cells. Although IL-4 protein levels were slightly reduced (Fig. 2B), IFN-γ mRNA levels were enhanced in Pin1-overexpressing cells (Fig. 2C). These results were consistent with the previous reports showing Pin1 inhibition selectively suppressed IFN-γ expression in bronchoalveolar lavage cells (23, 24). We then established Pin1-overexpressing human primary chondrocytes using ankle tissues obtained during surgery in an osteoarthritic patient. Although primary chondrocytes showed basal levels of Pin1 and COX-2, stable Pin1 overexpression increased COX-2 expression as well (Fig. 2D).

Pin1-dependent, simultaneous activation of NF-κB, CREB, and C/EBP is required for COX-2 expression
Because COX-2-mediated PG production is a representative inflammation index in RA, we chose COX-2 gene expression as a model system for additional experiments. COX-2 expression is transcriptionally regulated by C/EBP, CREB, and NF-κB, either synergistically or independently (25, 26). We therefore transfected GFP-HTB-94 and Pin1-HTB-94 cells with either a wild-type COX-2 promoter-luciferase chimeric construct that contained the 574-bp 5′-flanking region of human COX-2 gene, a C/EBP mutant with an NF-IL-6 site (−132 to −124) mutation, an NF-κB mutant (−223 to −214), or a CRE/AP-1 mutant (−59 to −53) (27). Wild-type COX-2 promoter activity was ~5-fold higher in Pin1-HTB-94 cells than GFP-HTB-94 cells (Fig. 3A). Mutants in each binding site (C/EBP, NF-κB, or CRE/AP-1) showed significantly lower Pin1-inducible reporter activity (Fig. 3A), particularly for NF-κB and CRE/AP-1 mutants (83 and 97% inhibition, respectively) (Fig. 3A). These results demonstrate that C/EBP, NF-κB, and CRE/AP-1 elements are all required for Pin1-mediated trans activation of COX-2.

The COX-2 promoter contains two NF-κB consensus sequences, and Pin1-inducible proinflammatory genes, including iNOS, IL-1β, and TNF-α, require NF-κB activation (28–30). The NF-κB minimal reporter activity and nuclear p65 levels were higher in Pin1 cells (Fig. 3B). Tosyl phenylalanyl chloromethyl ketone a specific NF-κB inhibitor, suppressed Pin1-mediated COX-2 expression (Fig. 3C). Pin1 selectively increases nuclear p65 sequestration by inhibiting p65 binding to I-κBα (31). Hence, Pin1-mediated induction of proinflammatory cytokines could result from p65/NF-κB activation.
FIGURE 4. Role of CREB and C/EBP activation in Pin1-inducible COX-2 expression. A, CREB activation in Pin1-overexpressed chondrocytes. Upper panel, Nuclear levels of CREB. Each lane represents different sample. Lower panel, CRE reporter gene analysis. HTB-94 cells were transfected with pCRE-Luc plasmid, and reporter gene analysis was performed, as described in the legend of Fig. 3A. Data represent the means ± SD of four separate samples (significant as compared with GFP-HTB-94 cells; **, p < 0.01). The results were confirmed by two separate experiments. B, C/EBP activation in Pin1-overexpressed chondrocytes. Upper panel, Nuclear levels of C/EBPα and C/EBPβ. Lower panel, C/EBP reporter gene analysis. HTB-94 cells were transfected with pC/EBP-Luc plasmid, and reporter gene analysis was performed, as described in the legend of Fig. 3A. Data represent the means ± SD of 10 separate samples (significant as compared with GFP-HTB-94 cells; **, p < 0.01). The results were confirmed by two separate experiments. C, AP-1 activation in Pin1-overexpressed chondrocytes. Left upper panel, Nuclear levels of c-Jun, JunB, JunD, c-Fos, and Fra1. Left lower panel, AP-1 reporter gene analysis. HTB-94 cells were transfected with pAP-1-Luc plasmid, and reporter gene analysis was performed, as described in the legend of Fig. 3A. Data represent the means ± SD of eight separate samples (significant as compared with GFP-HTB-94 cells; **, p < 0.01). The results were confirmed by two separate experiments. Right panel, AP-1 DNA-binding activity. Nuclear fractions were isolated from GFP-HTB-94 and Pin1-HTB-94 cells serum deprived for 18 h. All lanes contained 10 μg of nuclear extracts and radiolabeled putative AP-1 consensus sequence. D, Effects of c-Jun siRNA on the reporter activities of AP-1 and COX-2 promoter in Pin1-overexpressed chondrocytes. GFP-HTB-94 and Pin1-HTB-94 cells were cotransfected with pAP-1-luc or pGL-COX-2-574 reporter plasmid in combination with c-Jun siRNA (c-Jun siR, 20 pmol) or control siRNA (Con siR, 20 pmol). Data represent the means ± SD of three separate samples (significant as compared with GFP-HTB-94 cells, **, p < 0.01; significant as compared with control siRNA-transfected Pin1-HTB-94 cells, #, p < 0.05). The results were confirmed by two separate experiments. E, MAPK phosphorylation in Pin1-overexpressed chondrocytes. The activations of ERK, p38 kinase, and JNK were assessed by immunoblotting the phosphorylated forms of three MAPKs.
Because C/EBP and CRE/AP-1 elements regulate cytokine- or UV B-inducible COX-2 gene transcription (32), we determined the activity of each transcription factor using minimal reporter genes. pCRE-Luc and pC/EBP-Luc activities were enhanced by 13.7- and 4.2-fold in Pin1-HTB-94 cells (Fig. 4, A and B). Nuclear levels of CREB were also sharply increased in Pin1-HTB-94 cells (Fig. 4, A and B), but C/EBP/H9251 and C/EBP/H9252 increased only marginally (Fig. 4 B). c-Jun and c-Fos are cis/trans isomerized by Pin1 to activate AP-1-dependent gene transcription after phosphorylation by MAPK (33, 34). c-Jun activation by platelet-derived growth factor or serum increases COX-2 protein levels via CRE/AP-1 (35). In this study, Pin1 increased AP-1 minimal reporter activity, AP-1-binding activity, and the nuclear distribution of c-Jun, c-Fos, and Fra1, but not nuclear levels of JunB and JunD (Fig. 4C). AP-1 reporter activity was almost completely suppressed by c-Jun small interfering RNA (siRNA). However, c-Jun siRNA failed to reduce wild-type COX-2 promoter activity, indicating that increased COX-2 expression by Pin1 may not be related to AP-1 (Fig. 4D). c-Jun/AP-1 phosphorylation and expression are controlled by MAPK (36), which also regulates COX-2 expression (37, 38). To study whether MAPK pathways are activated in Pin1 cells, we measured the phosphorylated form of each MAPK. Pin1 cells had higher levels of phosphorylated ERK, but not JNK or p38 kinase (Fig. 4E). However, incubation of Pin1 cells with specific MAPK inhibitors (PD98059, ERK inhibitor; SB203580, p38 kinase inhibitor; SP600125, JNK inhibitor) for 36 h did not change COX-2 protein levels (Fig. 3C). Thus,
NF-κB, C/EBP, and CREB, but not AP-1, affect Pin1-dependent COX-2 expression in chondrocytes.

**Juglone inhibits RA progress in CII-inducible DBA/1J mice and suppresses COX-2 expression in human primary cultured RA chondrocytes**

Next, we tested the effect of the Pin1 inhibitor, juglone, on CII-induced RA in DBA/1J mice. The compound covalently inactivates a cysteine residue in the active site of Pin1 isomerase (39). Intraperitoneal juglone injection was done every other day after a booster injection of CII and continued for 9 days (total four times). As shown in Fig. 5A, Softex x-ray images showed that CII-induced hind paw swelling was almost completely blocked in juglone-injected mice. Ankle edema score was also reduced in juglone-treated group (Table I). We further histopathologically evaluated ankle joints for RA grades by the severity of inflammation, fibrosis, damage to the articular cartilage and bone, and ankylosis after sacrificing the mice (Fig. 5B and Table I). Juglone treatment (1, 5, and 10 mg/kg) also significantly inhibited the histological damage and cumulative arthritis injury scores compared with the vehicle-treated CII-RA group (Table I). Moreover, juglone treatment reduced COX-2 expression in the RA tissues (Fig. 5C).

To determine whether Pin1 inhibition decreases proinflammatory mediators in human chondrocytes from RA patients, we determined COX-2 protein levels in primary cultured chondrocytes (Fig. 5D). These results imply that Pin1 could be a pharmacological target for RA.

**Discussion**

Although RA is a common inflammatory disease, the molecular pathogenesis of this disease is still unclear. Data presented in this study indicate that Pin1 expression increases in the lesion area of CII-mediated arthritis and plays a crucial role in the excess production of proinflammatory mediators, including PGs, NO, TNF-α, and IL-1β.

NF-κB, which forms a homo- or heterodimer complex, regulates inflammatory genes such as COX-2, iNOS, TNF-α, and IL-1β (40), and many phytochemicals that inhibit NF-κB can reduce RA in animal and human studies (41, 42). In this study, stable Pin1 overexpression caused both the sustained nuclear translocation of p65 and increased NF-κB transcription. Pin1 binds to the phosphorylated Thr286-Pro of p65 and subsequently inhibits I-κBα binding, which increases the nuclear localization and the activity of NF-κB (31). We also revealed that COX-2 gene expression and its promoter activity were dependent on NF-κB activity. Thus, NF-κB activation in Pin1-overexpressed chondrocytes is obviously associated with the overwhelmed production of proinflammatory mediators during RA progress.

COX-2 and iNOS expression are also transcriptionally regulated by C/EBP and CREB as well as NF-κB, and these transcription factors may be synergistically or independently involved in the expression of this gene expression (26, 43, 44). In this study, CRE and C/EBPs were consistently activated by Pin1, and these transcription factors are active to increase COX-2 expression in Pin1-overexpressed chondrocytes. In silico analysis revealed that CREB variants contain Ser/Thr-Pro-rich segments and speculated that consensus phosphorylation sequences for Pin1 or Polo-like kinase were located in a highly conserved region of trans activation domains of CREB (45). Although there is still no report showing that transcriptional activity of CREB is dependent on Pin1, the isomerase may control the transcriptional activity of CREB.

Pin1 isomerizes phosphorylated c-Jun/c-Fos to activate AP-1 (33, 34). Pin1-overexpressed chondrocyte cell line showed higher nuclear levels of c-Jun, c-Fos, and Fra1, but not JunB and JunD. c-Jun siRNA blocked Pin1-induced AP-1 reporter activity, but not COX-2 promoter activity, indicating that c-Jun/AP-1 activation is not essential for COX-2 expression. Several reports have shown that MAPKs, ERK, JNK, and p38 kinase regulate COX-2 expression through the regulation of NF-κB, C/EBP, or CREB (21, 37, 46).
38). In this study, we found only ERK pathway was consistently activated in Pin1-overexpressed chondrocytes, but inhibition of JNK, p38 kinase, and ERK did not reduce COX-2 expression. Thus, MAPKs are not required for Pin1-mediated COX-2 expression.

Abnormal Pin1 activity contributes to the pathogenesis of diverse diseases, including cancer, Alzheimer’s disease, asthma, and allergies (24, 46, 47). We show in this study for the first time that Pin1 may also be a suitable target for RA. In fact, anti-cancer agents such as methotrexate and azathioprine are used for the treatment of RA (48). Pin1 inhibition has anti-cancer effects in experimental studies (49, 50), and recently, diverse chemical-based or peptide-based Pin1 inhibitors have been synthesized (51, 52). We found that multiple treatments of 10 mg/kg juglone caused severe toxic effects in mice, such as paralysis and even death (three among seven mice). A recent study also revealed that juglone inhibited postmitotic dephosphorylation and mitotic exit in a Pin1-independent manner, and consequently caused failure of mitotic spindle assembly (53). Because higher concentration ranges of juglone are cytotoxic, as evidenced by our animal study, we believe that juglone cannot be clinically used for RA. However, if more safe Pin1 inhibitors are available, the compounds could be applied for RA treatment.

Taken together, Pin1 is up-regulated in chondrocytes, lymphocytes, and fibroblasts of RA lesions in CIA-injected RA mice, and Pin1 overexpression induces proinflammatory proteins, including COX-2, iNOS, TNF-α, and IL-1β. Pin1-dependent COX-2 expression requires the simultaneous activation of NF-κB, B/Rel in induction of nitric oxide synthase.

References


