Supplementary

MATERIAL AND METHODS

Materials

Dulbecco's Modified Eagle Medium, Nutrient Mixture F-12 medium, Biotin-14-ATP, Alexa Fluor 488 goat anti-rabbit immunoglobulin G and Alexa Fluor 488-conjugated streptavidin were obtained from Life Technologies Corporation (Carlsbad, CA). Paraformaldehyde, PGE$_2$, aspirin, etodolac, diclofenac, meloxicam and pranoprofen were obtained from Wako Co (Osaka, Japan). Celecoxib was from LKT Laboratories Inc (St Paul, MN). Peptides for the assay of caspase-3-like activity were from Peptide Institute Inc (Osaka, Japan). Fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bromfenac, ibuprofen, glucose, sorbitol and indomethacin were obtained from Sigma Aldrich (St. Louis, MO). Terminal nucleotidyl transferase (TdTase) and the 5-bromo-2'-deoxyuridine (BrdU) enzyme immunoassay (EIA) kit were obtained from
Roche (Mannheim, Germany). VECTASHIELD was from Vector Laboratories (Burlingame, CA). 4’,6-diamidino-2-phenylindole (DAPI) was from Dojindo Laboratories (Kumamoto, Japan). The RNeasy kit was obtained from Qiagen Inc (Valencia, CA). The first-strand cDNA synthesis kit was from Takara Bio (Ohtsu, Japan) and the SsoFast™ EvaGreen® Supermix was from Bio-Rad (Hercules, CA). Antibodies against actin and lamin B were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). An antibody against NFAT5 was from Thermo Fischer Scientific (Waltham, MA, USA). Diclofenac eye drops and control eye drops were a gift from Wakamoto Co (Tokyo, Japan). The PGE$_2$ EIA kit and SC-560 were purchased from Cayman Chemical (Ann Arbor, MI).

**Assay for Caspase Activity**

Caspase-3-like activity was determined as described previously.\textsuperscript{1} Briefly, cells were collected by centrifugation and suspended in extraction buffer (50 mM PIPES/KOH (pH 7.0), 50 mM KCl, 5 mM EGTA, 2 mM MgCl$_2$ and 1 mM dithiothreitol). Samples were sonicated and centrifuged, after which the supernatants were incubated with fluorogenic
peptide substrates (acetyl-DEVD-methylcoumarin amide) in reaction buffer (100 mM HEPES/KOH (pH 7.5), 10% sucrose, 0.1% CHAPS and 1 mg/ml bovine serum albumin) for 2 h at 37°C. The release of aminomethylcoumarin (AMC) was determined using a fluorescence spectrophotometer. One unit of protease activity was defined as the amount of enzyme required to release 1 pmol of AMC/min.

**Real-time RT-PCR Analysis**

Total RNA was extracted using the RNeasy kit according to the manufacturer’s protocol. Samples (1 µg of RNA) were reverse-transcribed using the first-strand cDNA synthesis kit according to the manufacturer’s instructions. Synthesized cDNA was used in real-time RT-PCR experiments (CFX96™ Real time system, Bio-Rad) using SsoFast™ EvaGreen Supermix, and analyzed with Opticon Monitor software according to the manufacturer’s instructions. To normalize the amount of total RNA present in each reaction, *actin* was used as an internal standard. Primers were designed using the Primer3 website (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The primers used were (name, forward primer and reverse primer): *bgtl*,


5′-cccgaggagggagagaagtt-3′ and 5′-tccatcttggtgctgattg-3′; actin, 5′-tgcctttctctggacct-3′ and 5′-tgtgacctctgctgattg-3′; tnf-α, 5′-aggccaagccctggtatgac-3′ and 5′-cacagggcaatgatcccaaagtag-3′; mmp-9, 5′-tgacagcgacaagaagt-3′ and 5′-cagtaaaggtacagtt-3′.

**BrdU Incorporation Assay**

Cell proliferation was estimated based on the incorporation of BrdU.² Cells were incubated with BrdU (10 μM) for 2 h and fixed. BrdU incorporation was determined with the BrdU EIA kit according to the manufacturer’s instructions.

**Immunoblotting Analysis**

Whole cell and nuclear extracts were prepared as described previously.¹,³ The protein concentration of each sample was determined by the Bradford method.⁴ Samples were applied to 8% (NFAT5 and lamin B) or 12% (actin) SDS polyacrylamide gel electrophoresis, after which the proteins were immunoblotted with each antibody.
TdT-mediated Biotinylated UTP Nick End Labelling (TUNEL) and Immunohistochemical Analyses

Eyes were fixed in 4% buffered paraformaldehyde and embedded in paraffin before being cut into 4 µm-thick sections.

For TUNEL analysis, sections were incubated first with proteinase K for 15 min at 37°C, then with TdT and biotin 14-ATP for 1 h at 37°C, and finally for 2 h with Alexa Fluor 488 conjugated with streptavidin. Samples were mounted with VECTASHIELD and inspected using fluorescence microscopy (Olympus IX73).

For immunohistochemical analysis to detect NFAT5, sections were incubated with proteinase K for 20 min for antigen activation before blocking with 3% bovine serum albumin for 30 min, incubated for 12 h with antibody to NFAT5 (1:100 dilution) in the presence of 3% bovine serum albumin. Sections were then incubated with Alexa Fluor 488 goat anti-rabbit immunoglobulin G. Samples were mounted with VECTASHIELD and inspected with the aid of a fluorescence microscope (Olympus IX73). Fluorescence intensity was determined using LuminaVision software.

