Rapid Communication

Pancreatology

Pancreatology 2010;10:119–128
DOI: 10.1159/000290656

Received: December 28, 2009
Accepted: February 13, 2010
Published online: May 7, 2010

Dominant Negative p38 Mitogen-Activated Protein Kinase Expression Inhibits NF-κB Activation in AR42J Cells

Erik Twait Deborah E. Williard Isaac Samuel

Surgical Services, Iowa City Veterans Affairs Medical Center, and Department of Surgery, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA

Key Words
AR42J cell · Acinar cell · Acute pancreatitis · p38 MAP kinase · Nuclear factor-κB · Cholecystokinin · Tumor necrosis factor-α · Adenoviral vector

Abstract
Background: The role of the p38 mitogen-activated protein (MAP) kinase in acute pancreatitis pathogenesis is controversial. We hypothesize that p38 plays a role in regulating NF-κB activation in exocrine pancreatic cells. ***Methods:*** AR42J cells incorporating an NF-κB-responsive luciferase reporter, with and without adenoviral transduction of DNp38, were stimulated with cholecystokinin (CCK) or tumor necrosis factor-α (TNF-α) prior to measuring NF-κB activation. ***Results:*** CCK- or TNF-α-stimulated NF-κB-dependent gene transcription (luciferase assay) was substantially subdued by DNp38 expression. These findings were confirmed by electrophoretic mobility shift assay. Nuclear translocation of the p65 NF-κB subunit following agonist stimulation was evident (supershift). Characterization studies showed excellent adenoviral infection efficiency and cell viability in our AR42J cell model. Agonist-stimulated dose- and time-dependent p38 activation, with inhibition by DNp38 expression, was also confirmed. ***Conclusion:*** The p38 MAP kinase regulates NF-κB pathway activation in exocrine pancreatic cells, and thus potentially plays a role in the mechanism of acute pancreatitis pathogenesis.

Introduction

Although mitogen-activated protein (MAP) kinases are established mediators of cellular proinflammatory signaling pathways, the role of the p38 MAP kinase in acute pancreatitis pathogenesis is controversial [1, 2]. Nuclear factor-κB (NF-κB), a transcription factor involved in the induction of many proinflammatory molecules, is reportedly involved in disease pathogenesis [3, 4]. Tumor necrosis factor-α (TNF-α) and cholecystokinin (CCK) activate the NF-κB pathway and induce the expression of proinflammatory mediators in pancreatic acinar cells [5–7]. However, the key signaling mechanisms that underlie NF-κB activation in pancreatic acinar cells are as yet incompletely understood. We hypothesize that the p38 MAP kinase plays an important role in regulating NF-κB activation in exocrine pancreatic cells. To test this hy-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/pan

Isaac Samuel, MD, FRCS, FACS
Department of Surgery, VAMC & UI CCOM
200 Hawkins Drive, 4625 JCP (Surgery)
Iowa City, IA 52242 (USA)
Tel. +1 319 356 7359, Fax +1 319 356 8378, E-Mail Isaac-Samuel@uiowa.edu

pothesis, we expressed a dominant negative form of the p38 MAP kinase (DNp38) and evaluated its effect on NF-κB pathway activation in an exocrine pancreatic malignant cell line (AR42J cells). Our findings indicate that DNp38 expression reduces nuclear translocation and DNA binding of NF-κB and subdues NF-κB-dependent gene transcription following CCK or TNF-α stimulation in AR42J cells. These results provide further evidence for a fundamental role for p38 MAP kinase as a regulator of NF-κB pathway activation, which in turn emphasizes the potential importance of p38 MAP kinase in the mechanism of acute pancreatitis pathogenesis.

Materials and Methods

Materials

Rabbit polyclonal antibodies against total p38 MAPK (Cat. No. 9212) and total NF-κB p65 (Cat. No. 3034), as well as rabbit monoclonal antibody against phospho-p38 MAPK (Cat. No. 4511), were purchased from Cell Signaling (Danvers, Mass., USA). Rabbit polyclonal antibodies against NF-κB p50 (Cat. No. sc-7178x) and NF-κB p65 (Cat. No. sc-109x), and goat anti-rabbit IgG-HRP secondary antibody (Cat. No. sc-2004) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif., USA). Mouse monoclonal antibody against α-tubulin (Cat. No. CP06) was from Calbiochem (San Diego, Calif., USA). Mouse monoclonal antibody against TATA-binding protein (TBP) (Cat. No. ab818) was from Abcam (Cambridge, Mass., USA). AR42J cell line (Cat. No. CRL-1492) was from ATCC (Manassas, Va., USA). F-12K nutrient mixture Kaighn's Modification (Cat. No. 21127-022) was from Invitrogen (Grand Island, N.Y., USA). Protein Assay (Cat. No. 500-0006) was from Bio-Rad Laboratories (Hercules, Calif., USA). Replication-deficient adenoviruses expressing GFP (Ad.GFP), NF-κB-luciferase (Ad.NF-κB-luc) [8], and empty vector (Ad.EV) were purchased from the University of Iowa Vector Core Facility (Iowa City, Iowa, USA). Recombinant adenoviral vector containing the dominant negative form of murine p38α (Cat. No. ADV-105; TGY dual phosphorylation site at Thr^{180}/Tyr^{182} replaced by AGF) was from Cell Biolabs, Inc. (San Diego, Calif., USA). Sulfated CCK-8 (Cat. No. C2175) was from Sigma (St. Louis, Mo., USA). Rat recombinant TNF-α (Cat. No. 510-RT-010) was from R&D Systems, Inc. (Minneapolis, Minn., USA). Oligonucleotides were from Integrated DNA Technologies (Coralville, Iowa, USA). Goat anti-mouse IgG (Cat. No. 31438) was from Pierce Biotechnology (Rockford, Ill., USA).

Cell Culture

AR42J cells were maintained in F-12K medium supplemented with 20% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml), in poly-D-lysine-coated culture dishes. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Immunoblotting

For immunoblots using whole cell protein extracts, AR42J cells were lysed following stimulation in 100 μl of a modified RIPA buffer (50 mM Tris-Cl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% deoxycholate) on ice for 10 min. The soluble protein was collected by centrifugation at 15,000 *g* for 10 min at 4°C. Nuclear protein extracts were obtained following stimulation of cells using a commercial extraction kit (NE-PER Nuclear and Cytoplasmic Extraction Reagents, Cat. No. 78833, Pierce Biotechnology). Lysate protein concentrations were measured by Bradford assay (Bio-Rad), then aliquots containing either 40 μg (whole cell) or 10 μg (nuclear) of lysate were denatured in SDS sample buffer and boiled for 3 min. Proteins were subjected to SDS-PAGE on 12% gels and transferred to PVDF membranes. Blots were probed with primary antibody followed by HRP-conjugated secondary antibody. Proteins were visualized using chemiluminescent detection (ECL Plus Western blotting detection reagent, Cat. No. RPN2132, Amersham Pharmacia Biotech, Piscataway, N.J., USA). Membranes were then stripped for 20 min at 50°C with a mild stripping buffer (69.3 mM SDS, 125 mM Tris pH 6.8, 243 mM β-mercaptoethanol), and re-probed for sample loading control (total p38, α-tubulin, or TBP, as appropriate). Densitometry analysis of immunoblots was then performed using ImageJ software (Version 1.4, National Institutes of Health, Bethesda, Md., USA).

Time-Course and Dose-Response Studies

AR42J cells were seeded at a density of $1E^6$ cells/well in 6-well culture dishes and incubated for 24 h. To determine the optimal response time for p38 MAP kinase activation, cells were stimulated with 10 μM CCK or 10 ng/ml TNF-α for 0, 1, 3, 5, 10 or 20 min prior to harvest. For dose-response studies, the cells were stimulated with various doses of CCK or TNF-α for 5 min. Cells were then lysed in 100 μl modified RIPA buffer, and protein concentration was determined by Bradford assay. Immunoblotting using primary antibody against dually phosphorylated p38 (1:1,000 v/v) and HRP-conjugated secondary antibody was performed, followed by stripping and probing for total p38 (1:1,000 v/v).

Adenovirus Infection of AR42J Cells

To evaluate infection efficiency, AR42J cells were incubated with Ad.GFP (5 MOI) and imaged with an Olympus IX51 Inverted Florescent Microscope (Leeds Precision Instruments, Inc., Minneapolis, Minn., USA). To assess cellular injury and viability, AR42J cells were plated at a density of $5E^5$ cells/well in 12-well culture dishes and incubated with Ad.EV (5 MOI) or medium alone. After 24 and 48 h, cell viability was evaluated by ATP assay while cell injury was assessed by LDH assay (CellTiter-Glo Luminescent Cell Viability Assay, Cat. No. G7571; CytoTox 96 Non-Radioactive Cytotoxicity Assay, Cat. No. G1780; Promega, Madison, Wisc., USA).

Immunoblot Analysis of Cells Expressing DNp38

To test the effect of DNp38 expression on p38 MAP kinase activation, AR42J cells were plated at a density of $1E^6$ cells/well in 6-well culture dishes and infected with 5 MOI Ad.EV or Ad.DNp38. At 48 h post-infection, cells were stimulated with 10 μM CCK or 10 ng/ml TNF-α for 5 min and lysed in 100 μl of modified RIPA buffer. Whole cell lysate protein concentration was determined by Bradford assay and immunoblotting using primary antibody against dually phosphorylated p38 (1:1,000 v/v) and HRP-conjugated secondary antibody was performed, followed by stripping and re-probing for α-tubulin (1:2,000 v/v). To