The Role of Mitogen-Activated Protein Kinases and Their Relationship with NF-κB and PPARγ in Indomethacin-Induced Apoptosis of Colon Cancer Cells

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ABSTRACT: We have evaluated the role of mitogen-activated protein kinases (MAPKs) and their relationship with nuclear factor κB (NF-κB) and peroxisome proliferator–activated receptor γ (PPARγ) in indomethacin-induced apoptosis of colon cancer cells. We demonstrated that indomethacin can induce the prolonged activation of MAPKs in colon cancer cells; and that of these MAPKs, p38 MAPK may play a partial but significant role in indomethacin-induced apoptosis. Furthermore, indomethacin-induced p38 MAPK-mediated apoptosis of colon cancer cell lines is independent of caspase activation and not associated with indomethacin-induced NF-κB suppression and PPARγ activation.

KEYWORDS: indomethacin; apoptosis; mitogen-activated protein kinases

INTRODUCTION

The induction of apoptosis is a possible mechanism of the antineoplastic effects of nonsteroidal antiinflammatory drugs (NSAIDs).[1] We attempted to demonstrate the role of mitogen-activated protein kinases (MAPKs), which are considered important mediators of proliferative and apoptotic signals,[2,3] in indomethacin-induced apoptosis of colon cancer cells. Furthermore, we evaluated their relationship with nuclear factor κB (NF-κB) and peroxisome proliferator-activated receptor γ (PPARγ), which are associated with NSAID-induced cellular responses.[4,5]

METHODS

HT-29 or DLD-1 colon cancer cells were used. Cell death was assessed by the trypan blue dye exclusion method, and apoptosis was detected by DNA fragmentation

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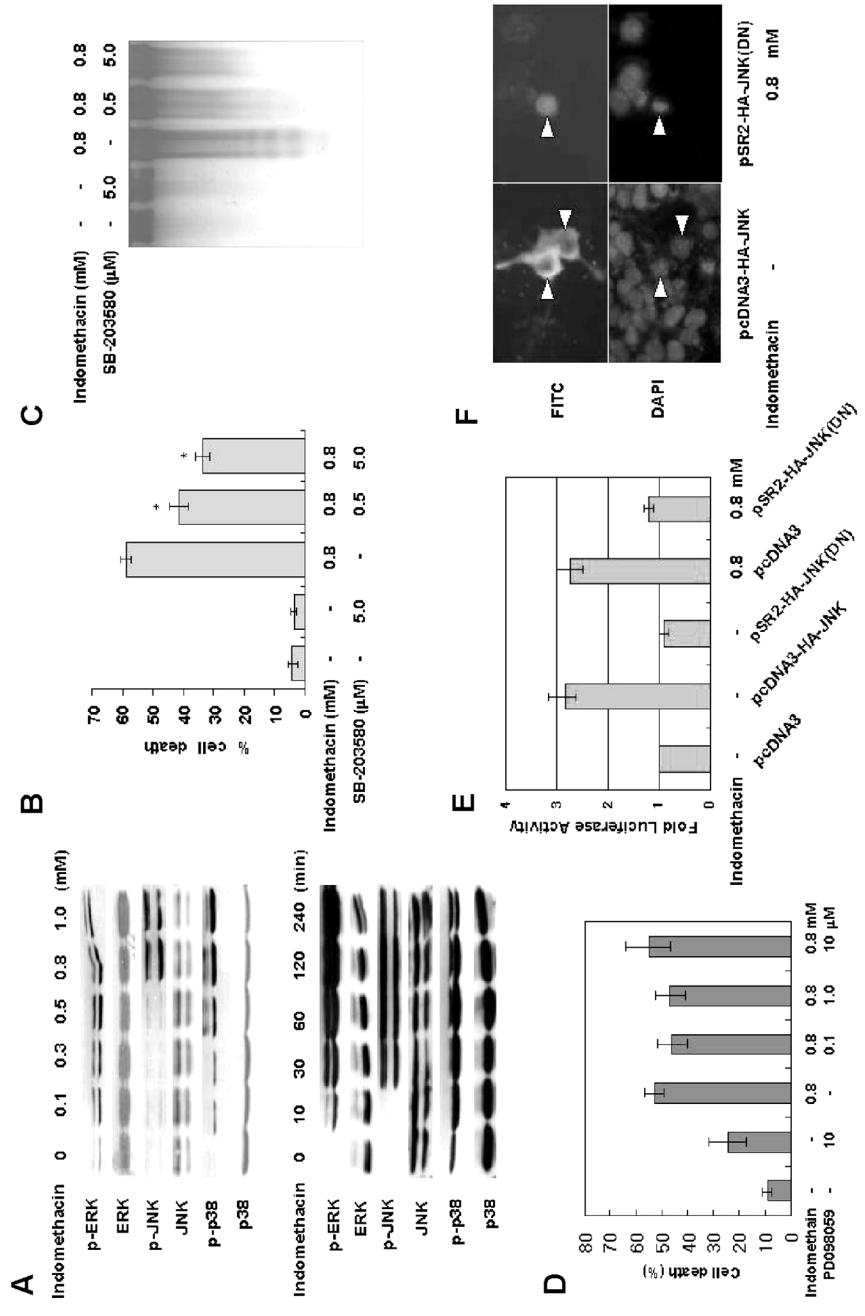

FIGURE 1. *See following page for legnd.*

in agarose gel electrophoresis. MAPK activation was assessed by western blot, using phosphospecific antibodies against MAPKs. For the inhibition of ERK and p38 MAPK, PD098059 and SB203580, respectively, were utilized. To demonstrate the role of JNK, hemagglutinin (HA) epitope-tagged JNK expression plasmid or dominant negative JNK plasmid was transiently transfected, and the change of nuclear shape due to altered JNK activity with or without indomethacin treatment was estimated by immunofluorescence study, using the FITC-conjugated anti-HA monoclonal antibody and DAPI. Caspase-3 activity was measured using Ac-DEVD-AMC. To evaluate the transcriptional activities of NFκB and PPARγ, HIV-1 long terminal repeat luciferase construct containing two NF-κB binding sites or PPRE3-tk-luciferase construct were transfected, and luciferase activities were measured.

RESULTS AND DISCUSSION

Treatment of HT-29 and DLD-1 cells with indomethacin results in the induction of apoptosis accompanied by sustained activation of all three MAPK subfamilies (FIG. 1A). SB203580 reduced indomethacin-induced apoptosis by 43% (FIG. 1B and C), wherease PD098059 did not affect cell death (FIG. 1D). Overexpressed or suppressed JNK activity did not affect cell survival or indomethacin-induced apoptosis (FIG. 1E and F). P38 MAPK and caspase-3 activation were not significantly interlinked in indomethacin-induced apoptosis (FIG. 2A and B). Indomethacin suppressed NF-κB transcriptional activation and induced PPARγ transcriptional activation (FIG. 2C-F). However, indomethacin-induced NF-κB suppression and PPARγ activation were not affected by pretreatment of p38 MAPK inhibitor (FIG. 2D-F).

From these results, we conclude that indomethacin can induce the prolonged activation of MAPKs in colon cancer cells and that of these MAPKs, p38 MAPK may play a partial but significant role in indomethacin-induced apoptosis. Furthermore, indomethacin-induced p38 MAPK-mediated apoptosis of colon cancer cells is independent of caspase activation and is not associated with indomethacin-induced NF-κB suppression and PPARγ activation.

FIGURE 1. (A) Dose- and time-dependent phosphorylation of ERK, JNK/SAPK, and p38 MAPK in indomethacin-treated HT-29 colon cancer cells (Western blot analyses using phosphospecific antibodies against ERK, JNK/SAPK, and p38 MAPK). **(B and C)** Partial inhibition of indomethacin-induced apoptosis by p38 MAPK inhibitor SB203580. **(B)** HT-29 cells were exposed to indomethacin for 24 hours with or without pretreatment of SB203580 for 30 minutes (cell count by trypan blue dye exclusion method). **(C)** DNA fragmentation detected by agarose gel electrophoresis was reduced by SB203580 pretreatment in a dose-dependent manner. **(D)** Effect of MEK1 inhibitor PD98059 pretreatment on indomethacin-induced HT-29 cell death (cell count by trypan blue dye exclusion method). **(E and F)** Effect of JNK overexpression or inhibition on cell survival or indomethacin-induced apoptosis. **(E)** Using PathDetect c-Jun *trans*-reporting system, DLD-1 cells were transfected with 0.7 μg of empty vector (pcDNA3), JNK expression plasmid (pcDNA3-HA-JNK), or dominant-negative JNK plasmid [pSR2-HA-JNK(DN)]. **(F)** After staining with FITC-conjugated anti-HA mouse monoclonal antibody and DAPI, nuclear shapes (DAPI) of cells that expressed transfected plasmid (FITC) were observed by the fluorescent microscope (magnification ×400).

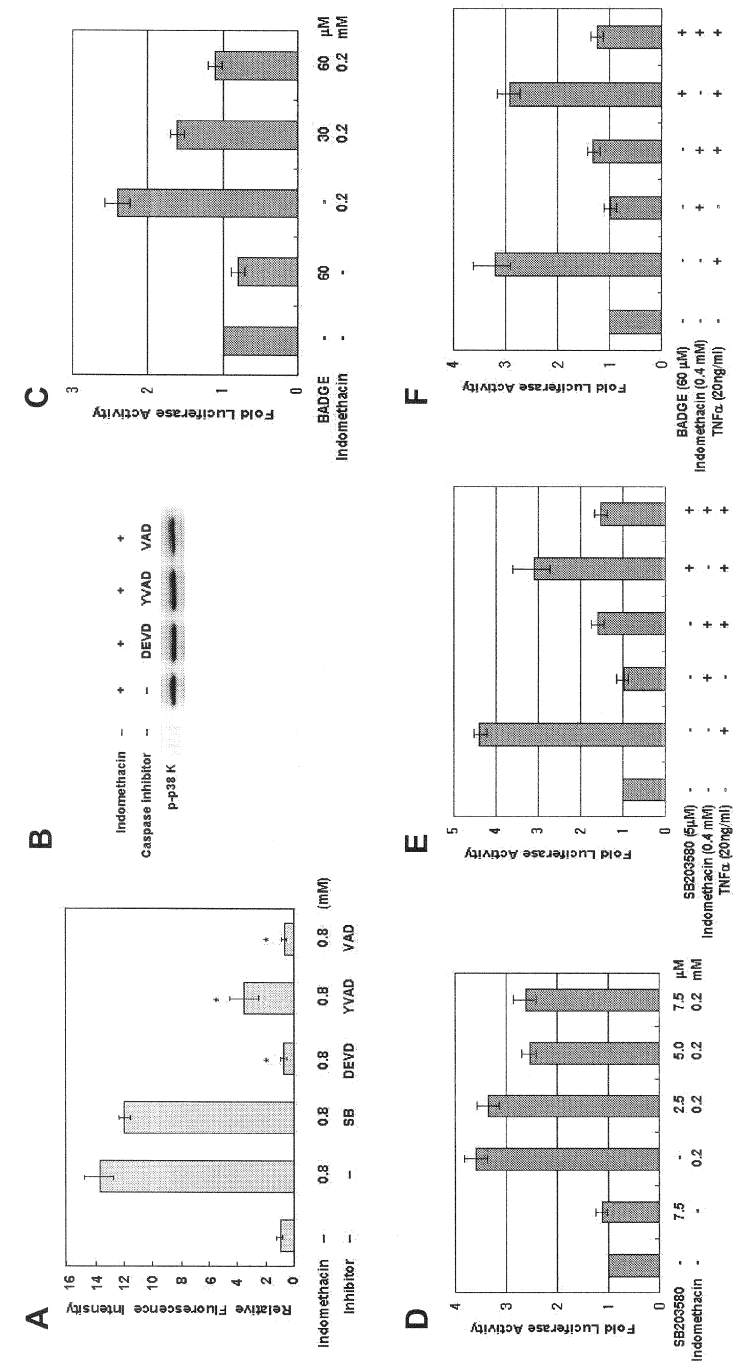

FIGURE 2. *See following page for legnd.*

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FIGURE 2. (A) Effect of p38 MAPK inhibitor SB203580 on caspase-3 activity in indomethacin-treated HT-29 cells. HT-29 cells were treated with indomethacin for 4 hours in the presence or absence of SB203580 (5 mM) or caspase inhibitors (10 mM) (caspase-3-like protease activity by a fluorogenic assay). **(B)** Effect of caspase inhibitors on indomethacin-induced p38 MAPK activation. HT-29 cells were treated with indomethacin for 1 hour in the presence or absence of caspase inhibitors (10 mM) (Western blot using phosphospecific antibody against p38 MAPK). **(C and D)** Effect of indomethacin acting as PPARγ ligand and its relationship with p38 MAPK activation. **(C)** HT-29 cells were transfected with PPRE3-tk-luciferase construct (0.7 μg) and pCMV-β-gal plasmid (0.2 μg) and exposed to indomethacin with or without pretreatment of the PPARγ antagonist BADGE. **(D)** HT-29 cells that were transfected, as in **C**, were pretreated with SB203580 and then treated with indomethacin. **(E and F)** Inhibitory effect of indomethacin on NF-κB activity and its relationship with p38 MAPK or PPARγ activation. **(E)** HT-29 cells were transfected with HIV-1 long terminal repeat luciferase construct containing two NF-κB binding sites (0.7 μg) and pCMV-β-gal plasmid (0.2 μg) and exposed to tumor necrosis factor (TNF)–α with or without pretreatment of SB203580 and/or indomethacin. **(F)** HT-29 cells that were transfected, as in **E**, were treated with TNF-α with or without pretreatment of BADGE and/or indomethacin. Luciferase activity represents data that have been normalized with galactosidase activity. SB, SB203580; DEVD, Ac-DEVD-CHO; YVAD, Ac-YVAD-CHO; VAD, Z-VAD-FMK.