Butyric Acid Induces Apoptosis by Up-regulating Bax Expression via Stimulation of the c-Jun N-Terminal Kinase/Activation Protein-1 Pathway in Human Colon Cancer Cells

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Background & Aims: **The colonic epithelial cells near the top of the crypt have been shown to undergo apoptosis. Because butyric acid (BA) is the major short-chain fatty acid produced by fermentation of dietary fiber in the large bowel, it may be an important regulator of apoptosis in colorectal cancer. We investigated which signaling pathway is triggered by BA to undergo apoptosis in human colorectal cancer cells.** ***Methods:*** **Human DiFi and FET colorectal cells were treated with BA to undergo apoptosis and were assayed for activation of c-Jun N-terminal kinase (JNK), transcription factor activation protein 1 (AP1) and NF-κB, and the proapoptotic molecule Bax. The contribution of specific pathways was assessed by examining the effects of dominant-negative mutants of JNK/AP1 or NF-κB on BA-induced Bax expression and apoptosis.** ***Results:*** **BA-mediated DNA fragmentation and Bax induction were preceded by early stimulation of JNK, and the DNA-binding activities of AP1 and NF-κB. BA-induced enhancement of DNA fragmentation and stimulation of Bax promoter activity were blocked by the expression of dominant-negative mutants of JNK1 or AP1 but not NF-κB.** ***Conclusions:*** **These findings suggest that apoptosis triggered by BA involves transcriptional stimulation of the Bax gene via activation of the JNK/AP1 pathway in colonic epithelial cells.**

Several studies suggest that dietary factors are involved in the control of colonic cell growth and differentiation and, therefore, in the etiology of large-bowel cancer.[1] High-fiber diets may protect against development of colorectal cancer,[2] in large part because of production of short-chain fatty acids, particularly butyrate, by anaerobic fermentation of insoluble fiber in the colon.[3] Studies using a rat model of colon cancer have demonstrated that dietary supplementation of fiber leads to increased colonic butyrate levels, reduced cell proliferation, and reduced tumor mass.[4] Butyrate, the preferred oxidative fuel of colonocytes, has a tropic effect on the colonic mucus, and induces cell arrest and terminal differentiation, influences gene expression, alters cell morphology, and induces apoptosis in colon cancer cells (reviewed by Smith et al.[5]). However, the mechanism for its protective effects has not yet been established.

Because colonic epithelial cells near the apex of the crypt can undergo apoptosis,[6,7] and because butyric acid (BA) is locally produced in the large bowel, one potential mechanism for the protective effects of dietary fiber is BA-induced apoptosis. Apoptosis plays an important role in the maintenance of tissue homeostasis and also seems to be important in the normal colon in vivo.[6,8,9] Thus, deregulation of apoptosis may be involved in the survival and development of colorectal cancer.[10] Recent studies suggest that cells derived from a variety of human cancers, including colorectal cancer,[10] have decreased apoptosis in response to various physiologic stimuli.[11,12] In addition, colonic epithelial cells at the apex of crypts display strong immunoreactivity to the proapoptotic protein Bax.[6] Bax forms homodimers and heterodimers with antiapoptotic family members such as Bcl-2, Bcl-X_L, and McL1.[13,14] Current model suggests that the ratio of antiapoptotic to proapoptotic proteins plays a regulatory role in apoptosis.

Activation of the c-Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase family, is involved in mediating apoptosis induced by a variety of agents, including nerve growth factor withdrawal, chemotherapeutic drugs, DNA-damaging agents, and gamma irradiation.[15–17] The activation of JNK involves phosphorylation at Thr-183 and Tyr-185 by JNK kinase (also known as MEKK1).[18] Once activated, JNK induces phosphorylation of c-Jun at Ser-63 and Ser-73, enabling

Abbreviations used in this paper: AP1, activation protein 1; BA, butyric acid; EMSA, electrophoresis mobility shift assay; GST, glutathione *S*-transferase; JNK, c-Jun N-terminal kinase; SDS, sodium dodecyl sulfate.

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c-Jun to induce transcription of genes containing a binding site for the activation protein 1 (AP1) complex, which consists of dimers between members of the Jun and fos families, including c-Jun itself.[18] Overexpression of JNK induces apoptosis, and expression of dominant-negative mutants of the components of the JNK/AP1 pathway inhibit apoptosis induced by growth factor withdrawal or cytotoxic agents,[16] suggesting that stimulation of the AP1 pathway is required for the activation of downstream molecules, such as caspases, that orchestrate apoptosis. Caspase inhibitors can block JNK and p38 mitogen-activated protein kinase activation in response to some agent(s), suggesting that these pathways may be downstream of caspase activation.[19] However, overexpression of upstream regulator of JNK such as MEKK1 can also induce caspase activity and apoptosis.[19–21] Research suggests that the JNK pathway may function both downstream and upstream of caspases in the apoptotic response, depending on the cell type and nature of the apoptotic signal.[19–21] Furthermore, it has been proposed that caspases work both upstream and downstream of JNK and are involved in a positive-feedback pathway that amplifies the apoptotic response.[21] The implication of apoptotic agent-induced stimulation of signaling kinases is that it may lead to activation of downstream transcription factors involved in apoptosis, including c-Jun, c-fos, and NF-κB.[22] Similarly, a number of cytotoxic drugs have been shown to activate NF-κB and induce apoptosis.[17,23] Activated caspases can also cleave IκB, an endogenous inhibitor of NF-κB, to contribute activation of NF-κB.[24]

The molecular mechanism by which butyrate induces apoptosis is not well established. Recent studies suggest that butyrate-mediated apoptosis in colonic epithelial cells involves activation of caspase-3[25] and requires mitochondrial function.[26] We now report that butyrate-mediated DNA fragmentation and Bax induction characteristics of apoptosis were preceded by early stimulation of JNK, AP1, and NF-κB. BA-induced enhancement of DNA fragmentation and stimulation of Bax-promoter activity were blocked by the expression of a dominant-negative mutant of JNK1 or AP1, but not NF-κB. These findings suggest that apoptosis triggered by BA involves transcriptional stimulation of the Bax gene via activation of the JNK/AP1 pathway in colonic epithelial cells.

Materials and Methods

Cell Cultures and Extracts

Human DiFi and FET colorectal carcinoma cells were maintained in Dulbecco's modified Eagle medium–F12 (1:1) supplemented with 10% fetal calf serum.[27–29] Cells were lysed, and lysates (30 μg protein) were resolved by 7% or 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), followed by transfer to the membranes and probing with the desired antibodies, and immune complexes were detected by using an enhanced chemiluminescent system. All antibodies, including the anti-Bax Ab (N-20) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Sodium butyrate was purchased from Sigma Chemical Co. (St. Louis, MO).

Apoptotic Assays

For DNA fragmentation assay, low-molecular-weight DNA was isolated as described previously.[29,30] Briefly, cells (3×10^6/plate) were seeded in 100-mm plates and treated with or without BA. Both floating and attached cells were scraped and collected in medium, washed 3 times with phosphate-buffered saline (PBS), and resuspended in 1 mL of lysis buffer (20 mmol/L Tris-HCl [pH 8], 10 mmol/L EDTA [pH 8], and 0.5% Triton X-100). Low-molecular-weight DNA was extracted from the supernatant and visualized by 1.5% agarose gel electrophoresis and ethidium bromide staining.[30] The percentages of cells in different phases of the cell cycle were determined by staining with propidium iodide (Sigma) and analyzing cells on a FACScan (Becton Dickinson, Franklin Lakes, NJ) at 514 nm as described.[30]

JNK Assay

For the JNK assay, cell lysates containing 500 μg protein were incubated with a glutathione *S*-transferase (GST)–fusion protein containing N-terminal amino acids 1–89 of c-Jun. JNK was precipitated with GST beads, and the in vitro kinase assay was performed as described previously.[31]

Electrophoresis Mobility Shift Assay

Cells were washed with PBS and collected by centrifugation. Cytosolic and nuclear extracts were prepared. The electrophoresis mobility shift assay (EMSA) was performed by incubating the nuclear extract (5 μg) with a ^{32}P-radiolabeled, high-affinity, double-stranded AP1 oligonucleotide (Santa Cruz Biotechnology). The DNA-protein complexes were resolved on native 5% polyacrylamide gels. For supershift assays, 1 mL of antibody against each of the AP1 subunits was added to the EMSA reaction 10 minutes before electrophoresis.[30]

Metabolic Labeling

An equal number of cells were treated with or without BA for 12 hours. Cells were metabolically labeled with 100 μCi/mL [^{35}S]methionine in methionine-free medium containing 2% fetal bovine serum during the last 6 hours of incubation. Cells were then washed twice with PBS, and lysates were prepared. Lysates containing equal amounts of perceptible trichloroacetic acid counts were immunoprecipitated with the desired monoclonal antibodies, resolved on an SDS–polyacrylamide gel, and analyzed by autoradiography.[28]

Luciferase Assays

Colonic cells were serum starved in low-serum medium (Dulbecco's modified Eagle medium containing 0.1% serum) for 28 hours. Cells were then transiently cotransfected with a luciferase plasmid and a control pSVb-Gal vector (Promega, Madison, WI) using Lipofectamine (GIBCO, Rockville, MD). Luciferase activity was measured 36 hours after transfection using a luciferase assay kit (Promega) as described.[32] Cells were lysed in Reporter Assay Buffer (Promega). An aliquot (20 μL) of the lysate was used to determine the luciferase assay by means of a luciferase assay kit from Promega. Another aliquot (10 μL) of the same lysate was used to determine β-galactosidase activity using the Galacto-light system and luminometer. β-Galactosidase activity was used to normalize transfection efficiencies. Where indicated, cells were treated with 3 mmol/L BA before lysis.

Construction of a Mutated Bax Promoter

A 370–base pair (bp) Bax-luciferase construct encoding the −680 to −317 region of the Bax promoter[33,34] was used to generate AP1 wild-type or mutated constructs by polymerase chain reaction. The 370-bp PGL3-bax plasmid was used as a template with the following oligonucleotides: forward primer Ap1wt, 5′-GCAAGGTACCAAACCACTCAGT-3, and Ap1wt, 5-TACGGTACCACCTCGTTTTTAGTCATC-3′; and reverse primer: 5′-CTTTATGTTTTTGGCGTCTTC-3′. Amplified products were digested with *Kpn*I and *Xho*I enzymes and cloned into the PGL3 vector. To verify the AP1 mutation, constructs were sequenced.

Results and Discussion

BA Induces Apoptosis in Human Tumor Cells

We and other investigators have shown that BA induces apoptosis in human colorectal carcinoma cells.[5,27–29] In the present study, we set out to delineate the signaling pathway that leads to this induction of apoptosis in response to BA. We show that treatment of FET colorectal cancer cells with BA was accompanied by induction of apoptosis in a time-dependent manner, as determined by the appearance of DNA fragmentation (Figure 1*A*), enhancement in the percentage of cells in the pre-G_0 apoptotic population (Figure 1*B*), proteolytic cleavage of lamin B1 and PARP (poly[ADP-ribose] polymerase), and activation of caspase-3 (Figure 1*C*).

BA-Induced Apoptosis Is Accompanied by Increased Expression of Bax

Because apoptosis is regulated by the ratio between proapoptotic and antiapoptotic gene products, and because Bcl-2 overexpression leads to inhibition of BA-induced apoptosis,[27] we hypothesized that BA-regulated apoptosis involves Bax and thus modulates the ratio of Bax to Bcl-2 in favor of cell death. Therefore, we examined the effect of BA on Bax expression in colorectal cancer cells. As shown in Figure 2*A*, treatment of FET cells with BA stimulated Bax protein expression in a time-dependent manner starting at 6 hours after treatment. As an internal control, the upper portion of the Bax blot was probed with an unrelated antibody against vinculin. To examine FET cells for newly synthesized Bax protein, we showed that BA treatment resulted in increased expression of [^{35}S]methionine-labeled Bax protein (Figure 2*B*) and Bax messenger RNA (Figure 2*C*).

BA-Induced Apoptosis Is Accompanied by Increased Stimulation of JNK Activity

To examine the role of the JNK pathway in BA-induced apoptosis in colorectal cancer cells, we examined FET cells for JNK activity after treatment with BA. Cells were treated with BA for various times, and

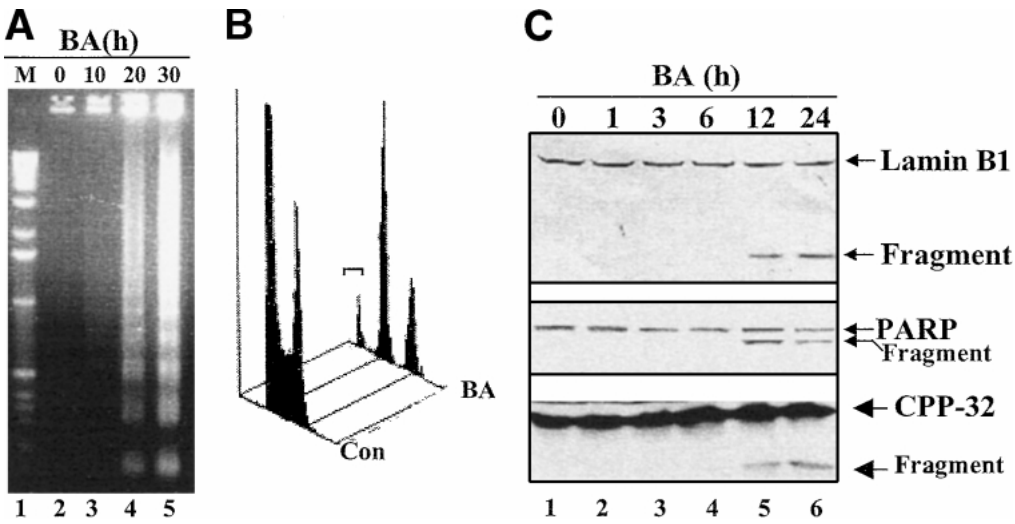

Figure 1. BA induces apoptosis in FET colon cancer cells. (*A*) Cells were cultured with 3 mmol/L sodium butyrate for different times, and DNA fragmentation was measured by ethidium bromide staining. (*B*) Fluorescence-activated cell sorter profile of cells treated with 3 mmol/L BA for 24 hours. *Bar* shows apoptotic cells. (*C*) Cells were treated with 3 mmol/L BA for different times, and cell extracts were immunoblotted with the indicated antibodies.

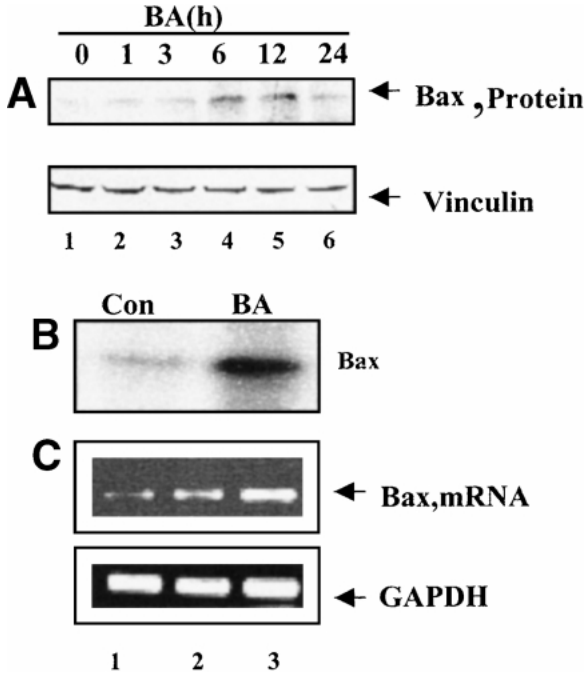

Figure 2. BA induces expression of Bax. (*A*) FET cells were treated with 3 mmol/L BA for indicated times. Cell extracts were analyzed by SDS-PAGE and immunoblotted with an anti-Bax antibody (*upper panel*). As an internal control, the upper portion of the Bax blot was blotted with an unrelated antivinculin antibody (*lower panel*). (*B*) Cells were treated with or without 3 mmol/L BA for indicated times and metabolically labeled with [^{35}S]methionine for the final 3 hours. Lysates containing equal amounts of perceptible trichloroacetic acid counts (cpm) were immunoprecipitated with an anti-Bax antibody and analyzed by SDS-PAGE. (*C*) FET cells were treated without (*lane 1*) or with 3 mmol/L BA for 3 hours (*lane 2*) or 6 hours (*lane 3*). One hundred nanograms of RNA from each condition was analyzed by reverse-transcription polymerase chain reaction for the expression of Bax or glyceraldehyde-3-phosphate-dehydrogenase messenger RNA. Results shown are representative of 3 independent experiments.

JNK1 activity was measured in cell extracts by immunoprecipitation with a c-Jun–specific antibody and an immunocomplex kinase assay using GST-Jun as a substrate. As shown in Figure 3*A*, BA treatment induced stimulation of JNK1 activity with a maximal activation between 3 and 6 hours after treatment. BA has no effect on ERK activity (data not shown). In brief, data to this point suggested that the activation of JNK preceded the appearance of DNA laddering in BA-treated cells.

BA Stimulates Activation of AP1

Recent studies suggest that the apoptosis-inducing function of JNK is associated with JNK's ability to induce expression of the AP1 transcription factor.[35] Therefore, we examined the effects of BA on activation of DNA-binding activity of AP1 in FET cells using EMSA with nuclear extracts. As shown in Figure 3*B*, BA treatment resulted in a transient increase in AP1 DNA-binding activity at 3 hours. The observed AP1-DNA complex was competed by a 100-fold molar excess of unlabeled AP1 probe and thus resulted from specific DNA-protein interaction (data not shown). We next determined which AP1 subunits were in the major AP1-DNA complex. Antibody supershift experiments demonstrated that incubation of nuclear extracts with a c-Jun–specific (and not a c-fos–specific) antiserum resulted in supershift of the AP1-DNA binding complex (Figure 3*C*). To examine whether BA-induced AP1 activity could stimulate the transcriptional activation of its target genes in vivo, we used a reporter construct with a 137-bp collagenase-gene promoter containing a single AP1 site (TGAGTCA) fused with the luciferase gene. In this assay, activation of luciferase transcription would indicate functional Jun/Jun dimers binding to the AP1 consensus sequence. As shown in Figure 3*D*, BA treatment stimulated activation of AP1-driven transcription in FET cells. Together, these results established that BA stimulation of JNK1 was accompanied by activation of the AP1 transcription factor.

Studies have shown that NF-κB can also modulate apoptosis[17,23]; we next explored whether BA could induce NF-κB in colorectal cancer cells. As shown in Figure 3*D*, BA stimulated the DNA-binding activity of NF-κB in FET cells.

BA-Triggered Apoptosis Requires a Functional AP1 Pathway

To assess the involvement of AP1 activation during BA-induced apoptosis, we examined the effects of transient expression of dominant-negative c-Jun mutant TAM 67 (lacking its transactivation domain) or TAM-fos (leucine-zipper domain of TAM 67 replaced with leucine zipper of c-fos so that it can heterodimerize only with Jun family members)[36] or a dominant-negative JNK1 mutant on BA-induced AP1 activation, DNA fragmentation, and lamin B1 cleavage. Expression of TAM67 or JNK1 mutants in FET cells significantly inhibited BA-triggered stimulation of AP1 DNA-binding activity (Figure 4*A*), DNA fragmentation (Figure 4*B*), and generation of lamin B1 fragments (Figure 4*C*). Quantitation of cell viability indicated that dominant-negative JNK1 mutants reduced the percentage of dead cells in BA-treated FET cells (Figure 4*D*). Although BA stimulated the DNA-binding activity of NF-κB (Figure 3*D*), BA-mediated DNA fragmentation remained unaffected by expression of an IκB-S12 mutant (Figure 4*E*) that effectively blocks stimulation of NF-κB activity.[37] Similar results were obtained with DiFi cells. Because BA-induced apoptosis was blocked by dominant-negative mutant of JNK1 but not by NF-κB, these results suggested that BA-induced apoptosis may involve stimulation of

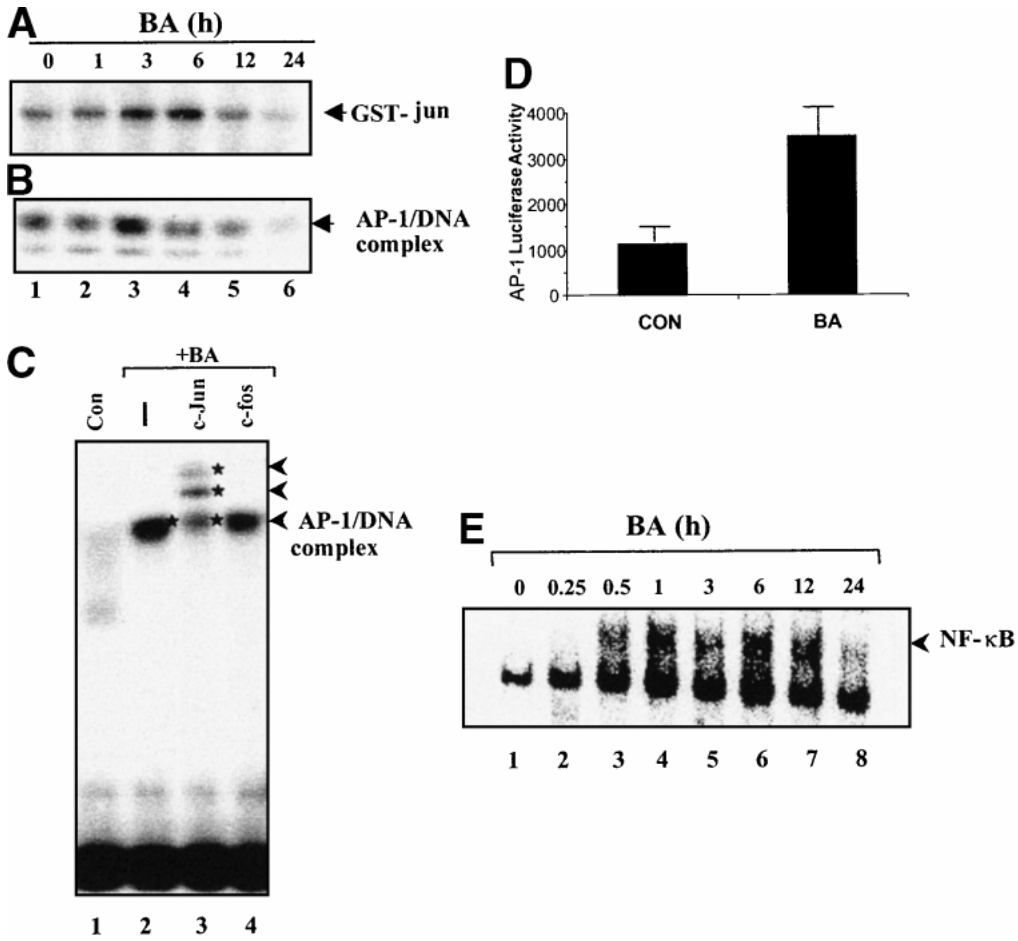

Figure 3. BA-induced apoptosis is accompanied by stimulation of the JNK/AP1 pathway. (*A*) FET cells were treated with or without 3 mmol/L BA for different times, and cell extracts were assayed for JNK activity, using GST-Jun as a substrate. The kinase reaction products were resolved on a 10% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and visualized by phosphoimaging. (*B*) FET cells were treated with or without 3 mmol/L BA, and nuclear extracts were assayed for AP1 DNA-binding activity. *Arrowhead* shows AP1-DNA complex. (*C*) Nuclear extracts from control and BA-treated FET cells were incubated with a c-Jun–specific or c-fos–specific antiserum. *Top 2 arrowheads* show supershift of the AP1-DNA. (*D*) FET cells were transiently transfected with a reporter gene construct containing an AP1 site, treated with or without 3 mmol/L BA for 24 hours, and measured for luciferase activity. (*E*) FET cells were treated with or without 3 mmol/L BA, and nuclear extracts were assayed for NF-κB DNA binding activity. Similar results were obtained in 3 or more independent experiments.

AP1 DNA-binding activity and not NF-κB DNA-binding activity.

BA Up-regulates Bax Promoter Activity via the JNK Pathway

Next, we hypothesized that BA-mediated Bax expression involves AP1. To examine whether BA could stimulate the transcription of Bax in vivo, we used a reporter construct in which a 972-bp Bax promoter[33,34] containing several AP1 sites was fused with the luciferase gene. As shown in Figure 5*A*, BA treatment stimulated the activation of Bax transcription in FET cells. This activity was suppressed in FET and DiFi cells by the expression of dominant-negative JNK1 or TAM67 mutants (Figure 5*B* and *C*).

To further define the role of AP1 sites in BA-mediated Bax transcription, we examined a series of Bax-promoter deletions to identify the region responsive to BA. BA treatment resulted in a 4-fold increase in induction from a 370-bp construct encoding the −680 to −317 region of the Bax promoter,[33,34] suggesting the presence of the BA-responsive element. Analysis of this sequence in the Transcription Factor Database V1.3[38] revealed the presence of an AP1 site in the −416 to −410 region. To evaluate the contribution of this AP1 site to the regulation of Bax-promoter activity by BA, we constructed 2 luciferase constructs containing the −420 to 317 region of the promoter, either wild-type (AP1wt; ACTCAGT) or mutated (AP1mt; ACCTCGT), in the AP1 site. Treatment with BA caused a 4-fold stimulation of luciferase activity from the AP1wt construct. However, mutation of the AP1 site reduced the stimulation in luciferase

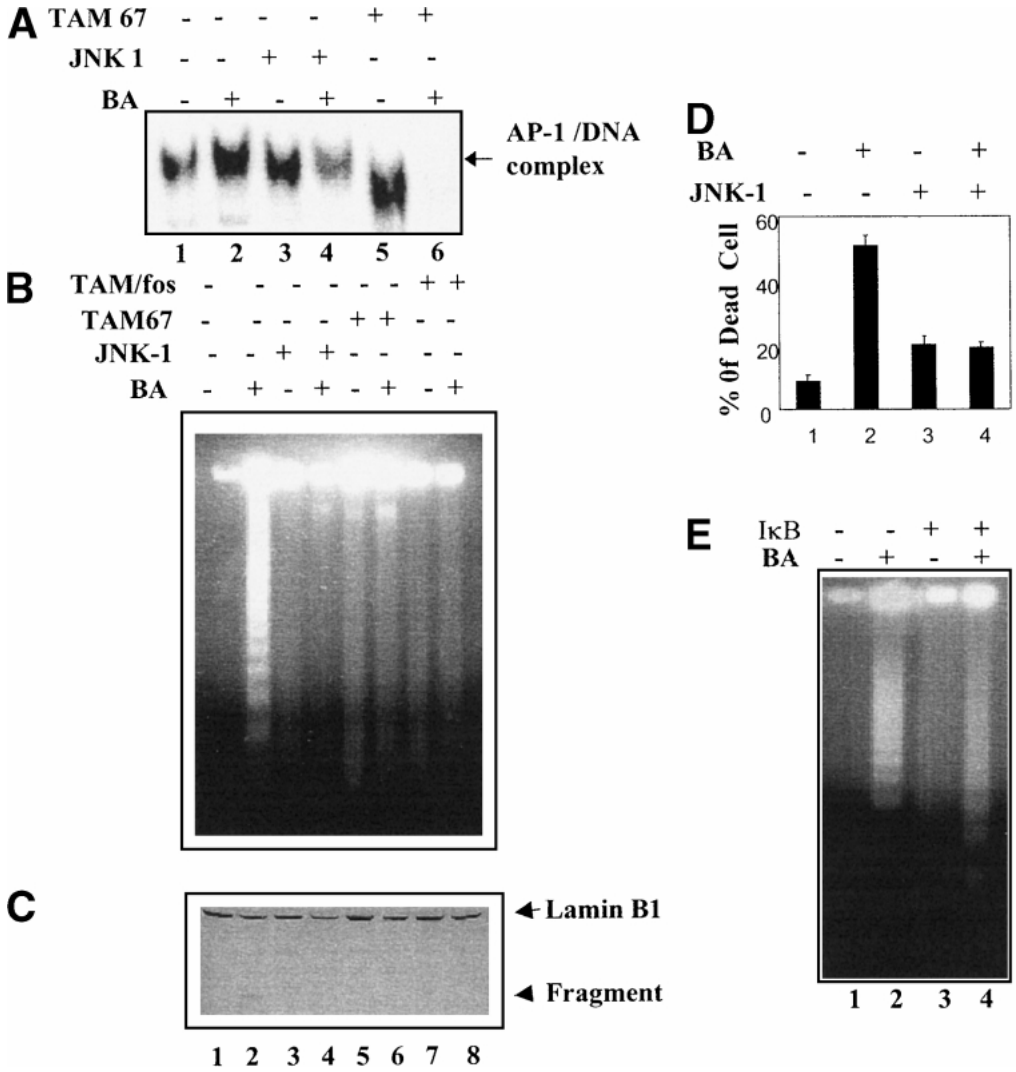

Figure 4. BA-induced apoptosis requires the JNK/AP1 pathway. (*A*) FET cells were transiently transfected with dominant-negative JNK1 (*lanes 3* and *4*) or TAM 67 (*lanes 5* and *6*) mutants and treated with or without 3 mmol/L BA for 3 hours. Nuclear extracts were assayed for AP1 DNA-binding activity. *Arrowhead* shows AP1-DNA complex. (*B*) FET cells were transiently transfected with dominant-negative JNK1 (*lanes 3* and *4*), or TAM 67 (*lanes 5* and *6*), or TAM/Fos (*lanes 7* and *8*) mutants and treated with or without 3 mmol/L BA for 20 hours. DNA fragmentation was measured by ethidium bromide staining. (*C*) Cell lysates from duplicate culture plates from the panel *B* experiment were analyzed by SDS-PAGE and immunoblotted with an anti–lamin B1 monoclonal antibody. (*D*) FET cells were transfected with dominant-negative JNK1 and treated with or without 3 nmol/L BA for 20 hours. Cellular viability was determined by trypan blue staining. Results are representative of 2 separate experiments. (*E*) FET cells were transiently transfected with a caspase-3 inhibitor (*lanes 1* and *2*) or a dominant-negative IκB mutant (*lanes 3* and *4*) and treated with or without 3 mmol/L BA for 20 hours. DNA fragmentation was measured by ethidium bromide staining. Results are representative of 3 experiments.

activity to 1.5-fold. These results suggested a potential role of the AP1 site located in the −416 to −410 region of the Bax promoter in the promoter's BA-mediated stimulation. These results suggested that BA-induced apoptosis involved stimulation of AP1 DNA-binding activity.

This study demonstrates that treatment with BA can activate a signaling pathway that includes the stimulation of JNK1 and AP1, and may be involved in apoptotic cell death of colorectal cancer cells. It is well accepted that JNK is the only enzyme that can phosphorylate c-Jun at Ser-63 and Ser-73, and induce the transcription of genes containing AP1 consensus sites.[22] On the other hand, phosphorylation of the carboxyl domain of c-Jun by ERK has been shown to inhibit its DNA-binding function.[37] In this context, our observation that BA induced AP1 DNA-binding activity without any influence on ERK is consistent with the involvement of JNK in activating AP1. Furthermore, we demonstrated an essential role for the JNK/AP1 pathway in BA-induced apoptosis (by directly blocking the JNK1 function). In addition, we demonstrated that BA-stimulated AP1 ac-

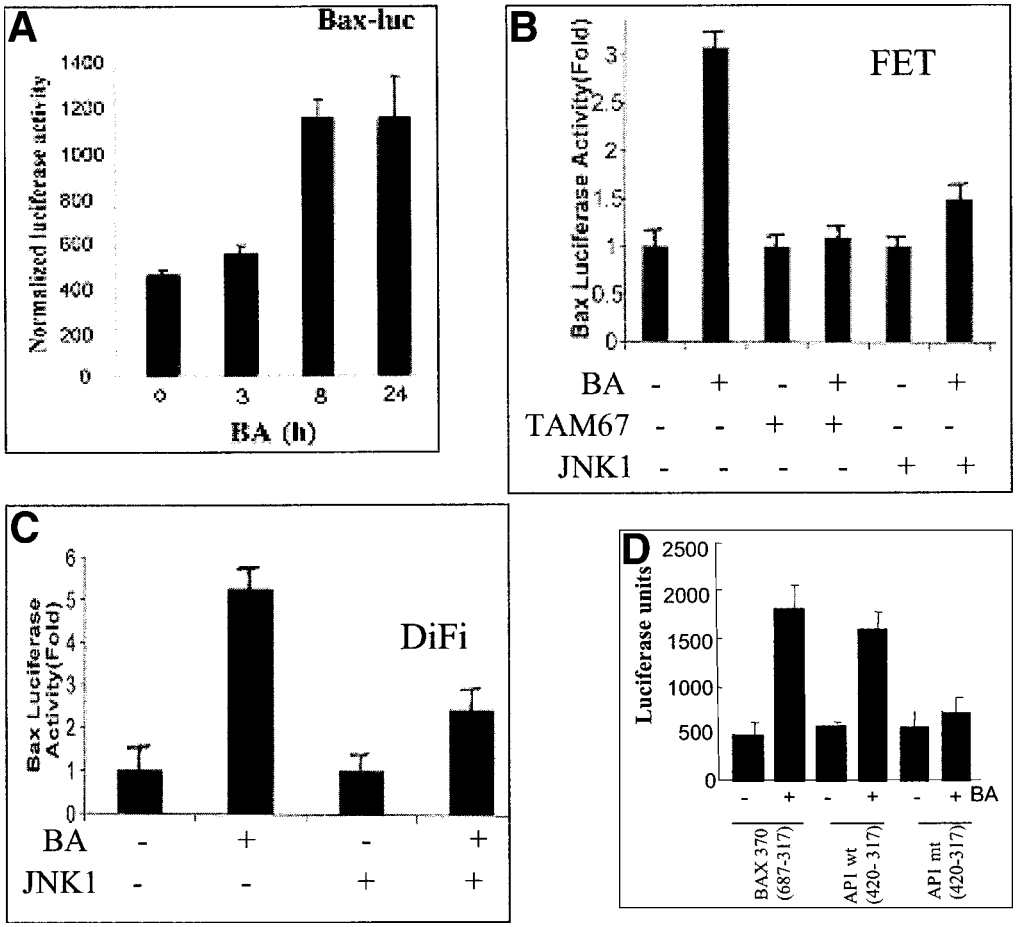

Figure 5. Regulation of Bax-promoter activity by BA. (*A*) FET cells were transiently transfected with a Bax-luciferase reporter construct and treated with or without 3 mmol/L BA for different times. Luciferase activity was measured. (*B*) FET cells were transiently transfected with dominant-negative JNK1 or TAM 67 mutants and treated with or without 3 mmol/L BA for 20 hours. Luciferase activity was measured. (*C*) DiFi cells were transiently transfected with the dominant-negative JNK1 mutant and treated with or without 3 mmol/L BA for 20 hours, and luciferase activity was measured. Results are representative of 2 independent experiments. (*D*) FET cells were transfected with the indicated Bax promoter constructs and a β-galactosidase control plasmid. After 24 hours, cells were treated with 3 mmol/L BA for 16 hours. Results are representative of 2 experiments.

tivity may regulate the transcription of the proapoptotic gene Bax, whose promoter contains an AP1 consensus sequence. Our findings raise questions about the intracellular events that result from BA treatment and lead to stimulation of JNK1 activity. It is possible that inhibition or activation of certain signal-transduction pathways modulated by BA alters the status of different proteins that can activate JNK. Alternatively, BA treatment may alter the intracellular pH and activate JNK. Because BA is a very potent activator of a number of cellular genes, we cannot rule out the potential role of BA in up-regulating signal-transduction components that regulate JNK activity. The possible role of upstream regulators of JNK activation and the manner in which these putative regulators are activated by BA remain to be explored. In summary, we provide evidence to suggest that activation of the JNK/AP1 pathway, and not the NF-κB pathway, regulates short-chain fatty acid–induced apoptosis, possibly by up-regulating the expression of the proapoptotic gene Bax.

References

1. Ames BN. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science 1983;221:1256–1264.
2. Harris PJ, Ferguson LR. Dietary fibre: its composition and role in protection against colorectal cancer. Mutat Res 1993;290:97–110.
3. McIntyre A, Gibson PR, Young GP. Butyrate production from dietary fiber and protection against large bowel cancer in a rat model. Gut 1993;34:386–391.
4. Boffia LC, Lupton JR, Mariani MR, Ceppi M, Newmark HL, Scalmati A, Lipkin M. Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short chain fatty acids by variation of dietary fiber (wheat bran) in rats. Cancer Res 1992;52:5906–5912.
5. Smith JG, Yokoyama WH, German JB. Butyric acid from the diet: actions at the level of gene expression. Crit Rev Food Sci Nutr 1998;8:259–297.
6. Krajewski S, Krajewska M, Shabaik A, Miyashita T, Wang HG,

Reed JC. Immunohistochemical determination of in vivo distribution of Bax, a dominant inhibitor of Bcl-2. Am J Pathol 1994;145: 1323–1336.
7. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493–501.
8. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. BCL2 protein is topographically restricted in tissue characterized by apoptotic cell death. Proc Natl Acad Sci U S A 1991;88:6961–6965.
9. Gordon JI. Intestinal epithelial differentiation: new insights from chimeric and transgenic mice. J Cell Biol 1989;108:1187–1194.
10. Bedi A, Pasricha PJ, Akhtar AJ, Barber JP, Bedi GC, Giardiello FM, Zehnbauer BA, Hamilton SR, Jones RJ. Inhibition of apoptosis during development of colorectal cancer. Cancer Res 1995;55: 1811–1816.
11. Carson DA, Ribeiro JM. Apoptosis and disease. Lancet 1993; 341:1251–1254.
12. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995;267:1456–1462.
13. Boise LH, Gottschalk AR, Quintans J, Thompson CB. Bcl-2 and Bcl-2–related proteins in apoptosis regulation. Curr Top Microbiol Immunol 1995;200:107–121.
14. Korsmeyer SJ. Regulators of cell death. Trends Genet 1995;11: 101–105.
15. Kanamoto T, Mota M, Takeda K, Rubin LL, Miyazono K, Ichijo H, Bazenet CE. Role of apoptosis signal-regulating kinase in regulation of the c-Jun N-terminal kinase pathway and apoptosis in sympathetic neurons. Mol Cell Biol 2000;20:196–204.
16. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;70:1326–1331.
17. Seimiya H, Mashima T, Toho M, Tsuruo T. c-Jun NH2-terminal kinase–mediated activation of interleukin-1beta converting enzyme/CED-3-like protease during anticancer drug–induced apoptosis. J Biol Chem 1997;272:4631–4636.
18. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 1995;270:16483–16486.
19. Juo P, Kuo CJ, Reynolds SE, Konz RF, Raingeaud J, Davis RJ, Biemann HP, Blenis J. Fas activation of the p38 mitogen-activated protein kinase signalling pathway requires ICE/CED-3 family proteases. Mol Cell Biol 1997;17:24–35.
20. Huang S, Jiang Y, Li Z, Nishida E, Mathias P, Liu S, Ulevitch RJ, Nemerow GR, Han J. Apoptosis signaling pathway in T cells is composed of ICE/Ced-3 family proteases and MAP kinase kinase 6b. Immunity 1997;6:739–749.
21. Graves JD, Gotoh Y, Draves KE, Ambrose D, Han DK, Wright M, Chernoff J, Clark EA. Krebs EG. Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1. EMBO J 1998;17:2224–2234.
22. Devary Y, Gottieb R, Smeal T, Karin M. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell 1992;71:1081–1091.
23. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, Green DR. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. Mol Cell 1998;1:543–551.
24. Barkett M, Xue D, Horvitz HR, Gilmore TD. Phosphorylation of I-kappa B inhibits its cleavage by caspase CPP32 in vitro. J Biol Chem 1997;272:29419–29422.
25. Medina V, Edmonds B, Young GP, James R, Appleton S, Zalewski PD. Induction of Caspase-3 protease activity and apoptosis by butyrate and trichostatin (inhibitors of histone deacetylases): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependence pathway. Cancer Res 1997;57:3697–3707.
26. Heerdt BG, Houston MA, Augenlicht LH. Short-chain fatty acid–initiated cell cycle arrest and apoptosis of colonic epithelial cells linked to mitochondrial function. Cell Growth Differ 1997;8:523–532.
27. Mandal M, Wu X, Kumar R. Bcl-2 deregulation leads to inhibition of sodium butyrate-induced apotosis in human colorectal carcinoma cells. Carcinogenesis 1997;17:229–232.
28. Mandal M, Adam L, Mendelsohn J, Kumar R. Nuclear targeting of Bax during apoptosis in colorectal cancer cells. Oncogene 1998; 17:999–1007.
29. Mandal M, Kumar R. Redistribution of activated caspase-3 to the nucleus during apoptosis. Biochem Biophys Res Commun 1999; 260:775–780.
30. Mandal M, Maggirwar SB, Sharma N, Kaufmann SH, Sun SC, Kumar R. Bcl-2 prevents CD-95 (Fas/APO-1)-induced degradation of lamin B and poly(ADP-ribose) polymerase, and restores the NF-kappa B signaling pathway. J Biol Chem 1996;271:30354–30359.
31. Vadlamudi R, Adam L, Talukder A, Mendelsohn J, Kumar R. Serine modification of paxillin by heregulin: role of p38 mitogen-activated protein kinase. Oncogene 1999;18:7253–7264.
32. Vadlamudi R, Mandal M, Adam L, Bandyopadhyay D, Steinbach G, Mendelsohn J, Kumar R. Regulation of cyclooxygenase-2 pathway by HER2 receptor. Oncogene 1999;18:305–314.
33. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bx gene. Cell 1995;80:293–299.
34. Thornborrow EC, Manfredi JJ. One mechanism for cell type–specific regulation of the bax promoter by the tumor suppressor p53 is dictated by the p53 response element. J Biol Chem 1999;274:33747–33756.
35. Watabe M, Ito K, Masuda Y, Nakajo S, Nakaya K. Activation of AP-1 is required for bufalin-induced apoptosis in human leukemia U937 cells. Oncogene 1998;16:779–787.
36. Brown PH, Chen TK, Birrer MJ. Mechanism of action of a dominant-negative mutants of c-Jun. Oncogene 1994;9:791–799.
37. Harhaj EW, Maggirwar SB, Good L, Sun SC. CD28 mediates a potent costimulatory signal for rapid degradation of Iκ-beta which is associated with accelerated activation of various NF-κB/Rel heterodimers. Mol Cell Biol 1996;16:6736–6743.
38. Akiyama Y. TFSEARCH: searching transcription factor binding sites. http://www.vwcp.or.jp/papia/.

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