Caspase-3-mediated Cleavage of the NF-κB Subunit p65 at the NH2 Terminus Potentiates Naphthoquinone Analog-induced Apoptosis*

Received for publication, February 8, 2001, and in revised form, April 2, 2001  Published, JBC Papers in Press, April 24, 2001, DOI 10.1074/jbc.M101291200

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The transcription factor nuclear factor κB (NF-κB) plays a crucial role in immune and inflammatory response, and protects cells from apoptosis. In this report, we investigate whether the NF-κB signaling pathway is blocked during apoptosis induced by 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (NA), an analog of naphthoquinone. It is observed that NA triggers apoptotic cell death in HeLa cells and destroys resistance to apoptosis caused by tumor necrosis factor-α. Data presented in this study establish that p65/RelA, a subunit of NF-κB, is cleaved at Asp97 by caspase-3 during apoptosis. Caspase-3-cleaved p65 loses transcriptional activity and potentiates NA-induced apoptosis, in contrast to an uncleavable mutant of p65, which protects the cell from apoptosis. Caspase-3, which is responsible for the cleavage of p65, is activated via the cytochrome c/caspase-9 signaling pathway rather than Fas/caspase-8 pathway during NA-induced apoptosis. Our results suggest that NA induces apoptosis by the negative regulation of cell survival through caspase-3-mediated cleavage of p65.

Apoptosis is initiated by a wide variety of stimuli, including developmental signals, cellular stress, and disruption of the cell cycle. Although the cellular mechanisms underlying apoptosis vary according to the type of stimuli involved, it is generally believed that apoptosis can be induced by the activation of pro-apoptotic signaling or inhibition of survival signaling. A well known survival signal pathway is mediated by the transcription factor, nuclear factor κB (NF-κB) (1–3).

NF-κB exists as a dimeric complex, comprising different members of the Rel family: p50, p52, p65, c-Rel, and RelB; all of which contain a well conserved amino-terminal 300 amino acid region. This part of the sequence, known as the "Rel homology region," is responsible for DNA binding, dimerization, and nuclear localization (1, 2). In unstimulated cells, NF-κB is located in the cytoplasm as an inactive complex through interactions with inhibitory proteins, IκBα and IκBβ. Upon cellular stimulation processes, such as cytokine induction or ionizing radiation, IκBs are rapidly phosphorylated and degraded. This in turn releases NF-κB, allowing translocation into the nucleus, binding to specific κB sites, and subsequently inducing the expression of target genes responsible for cell survival (4–8).

Previous studies have shown that activation of NF-κB is inhibited by a variety of mechanisms. The prevention of degradation or induction of IκB synthesis leads to the inhibition of NF-κB activation (9–11). Inhibition of p65 phosphorylation, which is important for the activation of p65, has also been known to suppress transcriptional activity of NF-κB (12). Moreover, the cleavage of NF-κB by caspases inhibits its activity during apoptosis (13, 14). These reports indicate that inactivation of NF-κB activity plays a crucial role in the apoptotic pathway.

Vitamin K3 (menadione) induces growth arrest and apoptosis in various cancer cell lines (15). Despite a broad-range effect on growth suppression of cancer cells, its hydrophobicity presents difficulties for use as an anticancer drug. Earlier experiments focusing on a search for new naphthoquinone analogs with polar groups led to the discovery of 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (NA) by our group (16). NA induced growth inhibition more potently than other analogs and natural VK3, without producing ROS in human hepatocarcinoma cells (15, 16). In view of the fact that, as yet, little is known about the mechanism of action of NA, this study involves a detailed investigation into the molecular function of NA in apoptosis.

In this report, we demonstrate that NA inhibits tumor necrosis factor α (TNF-α) induced activation of NF-κB and induces apoptosis of HeLa cells. NA potently inhibits transcriptional activity of NF-κB through caspase-3-mediated proteolytic cleavage of p65 at Asp97. Truncated p65 leads to the inhibition of transcriptional activity of NF-κB, and therefore the promotion of NA-induced apoptosis, while in contrast, uncleavable mutant p65 protein protects cells from apoptosis. These observations suggest that the activation of NF-κB is inhibited by caspase-3-mediated cleavage of p65, indicating that the survival pathway mediated by NF-κB might be abolished during NA-induced apoptosis.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—Human cervical epithelial carcinoma (HeLa) cells were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Inc.) at 37 °C, in a humidified incubator with an atmosphere of 5% CO2. Unless specified otherwise, all reagents were purchased from Sigma.

Cell Proliferation and Apoptosis Assays—HeLa cells were seeded in 35-mm plates at an initial density of 2 × 104 cells/plate. Cells were cultured for 16–20 h in complete medium and treated with 15 μM NA, 100 ng/ml TNF-α or a combination of NA and TNF-α for 24 h. After staining cells with trypan blue solution (0.4%), cell survival was quantified by measuring dye exclusion. Cell viability was determined as a percentage of control cells. For the apoptosis assay, HeLa cells were
transfected with plasmids encoding p65, p65D97A, or Δp65 with pEGFP-C1 plasmid (CLONTECH) that encodes green fluorescence protein. Apoptotic cells were identified by their rounded morphology, compared with the stand-off morphology of non-apoptotic cells. The number of apoptotic cells were counted and presented as a percentage of the total cell number in transfected cells.

**DNA Fragmentation**—Cells were lysed in 400 μl of lysis buffer (10 mM Tris-HCl (pH 7.9), 100 mM NaCl, 25 mM EDTA (pH 8.0), 0.5% sodium dodecyl sulfate (SDS), 0.1 mg/ml proteinase K), and incubated at 50 °C for 15 h. DNA was prepared using phenol/chloroform extraction and precipitated with ethanol. After treatment with RNase A (0.1 mg/ml) for 2 h, DNA samples were sonicated on ice for 1.5 minutes to generate 70 kbp DNA fragments. DNA samples were transferred to new tubes. Pellets were treated with ice-cold Buffer C (20 mM HEPES (pH 7.9), 0.4 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) for 15 min at 4 °C, followed by centrifugation at 20,000 × g for 5 min. Supernatant fractions were subjected to autoradiography. The results illustrated in Fig. 1 suggest that NA might induce cell death in HeLa cells. However, we next examined the transcriptional activity of NF-κB at the early stages of NA treatment with NA. Apoptotic DNA laddering was faintly observed at 12 h (data not shown), and clearly present at 24 h following treatment with NA. Apoptotic DNA fragmentation (a well known biochemical characteristic) after treatment with NA. We next examined the transcriptional activity of NF-κB.

**RESULTS**

**NA-induced Apoptosis in HeLa Cells**—NA has been shown to induce growth inhibition in human hepatocarcinoma cells (16). The effect of NA on growth in HeLa cells was investigated by treating cells with NA and examining cell viability. We observed a decrease in viability at 12 h, and entire loss of viability after 48 h, indicating that cell death occurs in a time-dependent manner (Fig. 1A). HeLa cells treated with TNF-α demonstrated continuous proliferation, thereby signifying resistance to cell death. However, cells were subjected to autoradiography.

**Western Blot Analysis**—Western blot analysis was carried out on 10 μg of nuclear extract. Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), proteins were electrophoretically transferred to polyvinylidene difluoride (Schleicher and Schuell). Membranes were blocked with TBS containing 0.5% Tween 20 in 5% skimmed milk, and subsequently incubated with the following primary antibodies: anti-p65 COOH terminus (SC-372, Santa Cruz), anti-p65 NH2 terminus (SC-109, Santa Cruz), anti-PARP (N-20, Serotec), anti-Fas (G254–274, PharMingen), anti-FasL (C-178, Santa Cruz), anti-caspase-3 (C31720, Transduction Laboratory), anti-caspase-9 (SC-7885, Santa Cruz), and cytochrome c (65981A, PharMingen). Membranes were washed with TBSST and treated with secondary antibody conjugated with peroxidase. The quantity of protein present was detected using the enhanced chemiluminescence (ECL) detection system (Amerham Pharmacia Biotech), followed by exposure to film.

**In Vitro Cleavage Assay**—In vitro transcription and translation were performed with the TNT-coupled reticulocyte system (Promega) and [35S]methionine ([1,000 Ci/mmol, Amerham Pharmacia Biotech] according to the manufacturer’s instructions. Aliquots of 3 μl of each translation reaction mixture were incubated with 2 μl of recombinant caspases (caspase-3, caspase-1, or caspase-8) in the presence or absence of 10 μM YVAD-fmk or Ac-DEVD-CHO in reaction buffer (20 mM HEPES (pH 7.4), 100 mM NaCl, 0.5% Nonidet P-40, 10 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) for 90 min at 30 °C. [35S]Methionine-labeled p65 and mutants (p65D97A or p65Δ577) were incubated with 90 μg of cell lysates prepared from cells treated with NA for 24 h at 30 °C. Samples were subsequently subjected to 10% SDS-PAGE and autoradiography.
Fig. 1. Effect of NA on the induction of apoptosis in HeLa cells. A, HeLa cells were treated with 15 μM NA or 100 ng/ml TNF-α for 24 h, TNF-α for 1 h before treatment with NA (TNF-α + NA), or NA for 1 h prior to treatment with TNF-α (NA + TNF-α). Cell survival was quantified at the indicated intervals by trypan blue dye exclusion. The percentage of cell survival was defined as the relative number of surviving untreated cells. B, apoptotic DNA fragmentation of the same samples was visualized by ethidium bromide staining, following 1.5% agarose gel electrophoresis.

Results suggest that inhibition of NF-κB activity may be part of the mechanism responsible for the apoptotic effect of NA.

NA Does Not Prevent Nuclear Translocation of p65—It is known that NF-κB sequesters in the cytoplasm due to interactions with IκB, and translocates to the nucleus in response to extracellular stimuli (2, 17). With a view to understanding the molecular mechanism of NA, we examined whether NA-mediated NF-κB suppression is achieved by perturbation of nuclear translocation of NF-κB. HeLa cells were exposed to NA or TNF-α for 30 min, and the level of p65, a subunit of NF-κB, in nuclear fractions, was assessed. As shown in Fig. 3A, exposure of cells to TNF-α results in an increase in the level of p65 protein in cell nuclei, in contrast to nuclear p65 in NA-treated cells, where no increase is observed. However, the amount of TNF-α-induced nuclear p65 was not reduced by pretreatment with NA, indicating that NA does not interrupt nuclear translocation of p65 induced by TNF-α. From the above data, we conclude that inhibition of TNF-α-induced NF-κB activation by NA is due to prevention of nuclear translocation of p65.

p65 Is Cleaved during NA-induced Apoptosis—The next step was to investigate whether there are any changes in the amount of nuclear p65 at 24 h when cells undergo apoptosis induced by NA treatment. It was found that the protein level of nuclear p65 in NA-treated cells is not altered, compared with that of control or TNF-α-treated cells (Fig. 3B). The cleavage of poly(ADP-ribose) polymerase (PARP), a predominant biochemical hallmark of apoptosis, was further observed in cells. Interestingly, the smaller band of p65 (M<sub>r</sub> ~ 55,000) (Δp65) was recognized in NA-treated cells, but not in the control or TNF-α-treated cells (Fig. 3B). The truncated p65 (Δp65) is assumed to be a cleavage product generated during the apoptotic process. Experiments were conducted to analyze whether the appearance of Δp65 is correlated with NA-induced apoptosis. As shown in Fig. 4A, the Δp65 band firstly appears in cells treated with 10 μM NA for 24 h. In a time course experiment, this band was observed at 12 h after the addition of 15 μM NA (Fig. 4B). In contrast to the results obtained with p65, we could not observe any cleavage of p50, which is another component of NF-κB. Since PARP cleavage was observed at 24 h after treatment with NA (Fig. 4B, last panel), cleavage of p65 should be an earlier event. Our observations suggest that Δp65 is the cleavage product of p65 produced during NA-induced apoptosis.

p65 Is Cleaved at Asp<sup>97</sup> by Caspase-3—It is known that various proteins are cleaved by caspase-3 during apoptosis (18–21). To evaluate whether caspases are responsible for p65
HeLa cells were treated with 0–20 μM Thr-Asp-Asp\(^294\)-Arg\(^295\), Ala-Ser-Val-Asp\(^469\)-Asn\(^470\)). In order to determine the Western blot of p65 indicates nonspecific bands. A, anti-p65 antibody. Separated by 10% SDS-PAGE and Western blotting was performed with antibodies for p65 or PARP. Note that the asterisk on the right side of the Western blot of p65 indicates nonspecific bands.

Cleavage, cells were incubated with a caspase-3-specific inhibitor (Ac-DEVD-CHO), and an inhibitor for a broad spectrum of caspases (zVAD-fmk) for 1 h prior to treatment with NA. Cleavage of p65 was monitored by Western blot analysis. Upon treatment with Ac-DEVD-CHO, we failed to observe the appearance of both the cleavage product (Fig. 5A) and DNA fragmentation (Fig. 5B), which indicates that the cleavage of p65 is catalyzed by caspase-3. To provide direct evidence of this theory, in vitro-translated \(^{35}\)S-labeled p65 was incubated with recombinant caspase-1, caspase-3, and caspase-8. It was noted that while p65 was cleaved by caspase-3 and inhibited by Ac-DEVD-CHO (Fig. 6), caspase-1 and caspase-8 had no effect on the cleavage of p65. In view of the data obtained, we conclude that p65 is a substrate of caspase-3 in vitro.

Caspase-3 has been shown to recognize and cleave specific substrates containing a conserved DXD consensus tetrapeptide motif (22). Putative recognition sites for caspase-3 exist in the amino acid sequence of p65 (Asp-Cys-Arg-Asn\(^{97}\)-Gly\(^{98}\), Asp-Thr-Asp-Asp\(^{294}\)-Arg\(^{295}\), Ala-Ser-Val-Asp\(^{469}\)-Asn\(^{470}\)). In order to identify the cleavage site of p65 recognized by caspase-3, in vitro labeled \(^{35}\)S-labeled p65 was incubated with caspase-3, and Western blot analysis was performed using antibodies against the COOH and NH\(_2\) termini of p65. The \(^{35}\)S-labeled p65 band was recognized by an antibody against the COOH terminus, but not by an antibody against the COOH terminus, but not by an antibody against the NOH\(_2\) terminus antibody (Fig. 7A). Moreover, the \(^{35}\)S-labeled p65 generated during NA-induced apoptosis was not recognized by the NH\(_2\) terminus-specific antibody (Fig. 7B). Taken together, the results clearly demonstrate that p65 is cleaved by caspase-3 at the NH\(_2\) terminus. To determine the exact cleavage site, we introduced point mutations at Asp97 (p65\(^{D97A}\)) and Asp\(^{469}\) (p65\(^{D469A}\)). Wild-type (wt) p65 and the two p65 mutants were translated in vitro and incubated with cell lysates prepared from NA-treated cells. As shown in Fig. 7C, p65\(^{D97A}\) was resistant to caspase-3 in cell lysates, while wt p65 and p65\(^{D469A}\) mutant proteins were cleaved. Our results confirm that caspase-3 cleaves the Asp-Cys-Arg-Asp\(^{97}\)-Gly\(^{98}\) motif of p65 during NA-induced apoptosis. The findings so far imply that NF-κB might be inactivated through caspase-3-mediated cleavage of p65 after the Asp\(^{97}\) residue during NA-induced apoptosis.

**Effect of Caspase-3-mediated Cleavage of p65 on Transcriptional Activity of NF-κB and NA-induced Apoptosis**—The role of caspase-3-mediated p65 cleavage on NF-κB activity and apoptosis was further investigated. The NH\(_2\) terminus of p65 contains a 300-amino acid Rel homology domain, which includes a DNA-binding domain, dimerization domain, and the nuclear translocation signal. In order to examine whether the transcriptional activity of NF-κB is disrupted by cleavage of p65, HeLa cells were co-transfected with NF-κB-luciferase reporter plasmid together with wild-type (wt) p65, caspase-3 resistant mutant (p65\(^{D97A}\)), and caspase-3-cleaved COOH-terminal fragment of p65 (Δp65), respectively, following which luciferase activity was monitored. NF-κB activity was reduced to half that of control in the Δp65-transfected cells, while a 2-fold increase was observed in cells transfected with p65 or...
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Fig. 7. Caspase 3-mediated cleavage of p65 occurs at the NH₂ terminus. A, in vitro translated [35S]-labeled p65 was incubated with recombinant caspase-3 alone, or in the presence of 10 µM caspase inhibitors, Ac-DEVD-CHO or zVAD-fmk. The reaction mixture was separated by 10% SDS-PAGE and subjected to Western blot analysis with specific antibodies against the COOH and NH₂ termini of p65, respectively. B, HeLa cells were treated with NA for the indicated time period. Cell lysates were subjected to SDS-PAGE (10%), followed by Western blot analysis using polyclonal antibody against the NH₂ terminus of p65. C, HeLa cells were treated with 15 µM NA for 24 h and cell lysates were prepared. Aliquots of 5 µl of in vitro translated [35S]Met-p65 and mutants ([35S]Met-p65D97A or [35S]Met-p65D469A) were incubated with 200 µg of cell lysate in the absence or presence of Ac-DEVD-CHO for 90 min at 30 °C. Reaction mixtures were further subjected to 10% SDS-PAGE, and autoradiography was performed on the dried gel.

p65D97A (Fig. 8A). This suggests that cleavage of p65 at Asp⁹⁷ impairs its DNA binding ability, and therefore leads to a reduction in the transcriptional activity of NF-κB.

Experiments were subsequently conducted to analyze the effect of p65 cleavage on NA-induced apoptosis. Cells were expressed with different concentrations of p65, p65D97A, and ∆p65, together with GFP. NA-induced apoptosis was monitored by counting the number of dead GFP-positive cells after treatment with NA for 12 h (see Fig. 8B). Following exposure of cells to NA, apoptosis was significantly enhanced in ∆p65-transfected cells. In contrast, p65 and p65D97A inhibited apoptosis in a dose-dependent manner. However, p65D97A was more effective at apoptotic inhibition than wild-type p65, suggesting the requirement of intact p65 for the inhibition of apoptosis. We conclude from the data that NA induces caspase-3-mediated cleavage of p65, leading to the disruption of anti-apoptotic function of NF-κB, and consequently to cell death.

Caspase-3 Is Activated through Caspase-9 Signaling, Rather than the Caspase-8 Pathway during NA-induced Apoptosis—The molecular mechanism linked to NA-induced activation of caspase-3 was further investigated in this study. Caspase-3 is activated by two main pathways. Ligation of Fas ligand (FasL) to Fas activates caspase-8, which in turn cleaves and activates other caspases including caspase-3 (23, 24). The second pathway involves the activation of caspase-3 by caspase-9. Caspase-9 is activated by cytochrome c released from mitochondria in the presence of Apaf-1 and dATP during apoptosis (25, 26). Previous reports demonstrated that ligation of Fas induces caspase-3-mediated cleavage of p65 at the COOH terminus during apoptosis in Jurkat T cells (13). This leads to the query as to whether caspase-3 is activated by an increase in Fas or FasL expression during NA-induced apoptosis (Fig. 9A). No change in the protein levels of Fas and FasL were observed following treatment with NA, indicating that Fas-stimulated caspase-8 signaling pathway is possibly not related to the activation of caspase-3 in our system.

This leaves the possibility of the second pathway being pref-
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The transcription factor, NF-κB, is activated by various stimuli, and protects against apoptosis (3, 27, 28). TNF-α can trigger two different pathways with opposite effects simultaneously, the apoptotic pathway mediated by caspase-8, and survival signaling mediated by NF-κB. It is known that TNF-α does not induce apoptosis unless NF-κB pathway is blocked (29). Previous studies have led to the discovery of the activation mechanism of NF-κB upon various apoptotic stimuli (1, 2, 4, 5). However, at present, it is currently unclear how apoptotic pathways overcome survival signaling mediated by NF-κB, and consequently induce cell death. In this article, we investigate whether the anti-apoptotic signaling mediated by NF-κB is inhibited during apoptosis induced by NA. We show that NA triggers apoptotic cell death in HeLa cells and destroys resistance to apoptosis caused by TNF-α. Furthermore, NA inhibits NF-κB activation through caspase-3-mediated cleavage of p65 at the NH2 terminus during apoptosis in HeLa cells.

There is ample evidence in earlier literature to illustrate that the activity of NF-κB is inhibited by various drugs via a number of pathways. Glucocorticoids inhibit NF-κB activity by increasing levels of IκBα, and consequently sequestering NF-κB in the cytoplasm (9). Moreover, NF-κB activation is prevented by anti-inflammatory drugs like sodium salicylate and aspirin (10), which prevent degradation of IκBα by inhibiting the activity of IκBα kinase β (IKKβ). Proteosome inhibitors additionally block degradation of IκBα (11). It was reported that 3-deazaadenosine, which is known to induce apoptosis in human and mouse leukemia cells, inhibits the transcriptional activity of NF-κB through obstruction of p65 phosphorylation without reducing DNA binding activity (12). Our present analyses demonstrate that activation of NF-κB is inhibited by proteolytic cleavage of p65 during NA-induced apoptosis. The cleavage, induced by caspase-3, occurs at a site in the NH2 terminus of the protein. Another recent study showed that ligation of Fas repressed NF-κB activity in Jurkat T cells by inducing proteolytic cleavage of p65 and p50 during apoptosis (13). Data presented in this study indicate that p65 cleavage occurs at the COOH terminus, although also mediated by caspase-3. The cleavage at the COOH terminus of p65 was further examined during apoptosis induced by deprivation of growth factor (14), where caspase-6 and -7 are available for the COOH-terminal cleavage of p65, as well as caspase-3. Note that our results on the cleavage site of p65 at the NH2 terminus are significantly different from previous reports, although the fact that caspase-3 is responsible for the cleavage is universally established. With a view to explaining the dissimilar findings, we cannot exclude the possibility that another caspase is involved in the cleavage at this site, because the cell lysate had a higher activity of caspase than recombinant caspase-3 on the cleavage of p65 (Fig. 7C). The identity of these caspases is still somewhat ambiguous. It is difficult to explain why activated caspase-3 targets only the NH2 terminus of p65 during NA-induced apoptosis. One possible explanation for this discrepancy is that the activation mechanism of caspase-3 or the environment in which caspase-3 targets p65 in our system might be different from that in the other studies conducted so far.

It has been demonstrated that caspase-3 activation is regulated by at least two mechanisms. One involves a direct pathway from caspase-8 (23, 24), and the other is mediated by caspase-9, following the release of cytochrome c from mitochondria (25, 26). NA induces apoptosis via cytochrome c release into the cytoplasm and activation of caspase-9 (Fig. 9). However, the expression profiles of Fas and FasL do not change during NA-induced apoptosis, suggesting that caspase-3 is activated by caspase-9 rather than caspase-8. It must therefore be concluded that two caspase-3 activation pathways exist differently that both ultimately lead to the cleavage of p65. One might mediate the NH2-terminal cleavage of p65 in NA-induced apoptosis, while the other mediates the COOH-terminal cleavage of p65 in Fas-induced apoptosis.

During analyses on NF-κB transcriptional activity using the p65 mutants, it was noted that the p65 mutant truncated at the NH2 terminus (Δp65) inhibits NF-κB-dependent transcription by half that of the control protein following overexpression (Fig. 8A). This data can be explained by the functional structure of p65. The NH2 terminus of p65 contains a Rel homology domain within which lies a DNA-binding domain, dimerization domain, and nuclear localization signal. Proteolytic cleavage at this site might impair the DNA binding ability of p65, therefore leading to a decrease in the transcriptional activity of NF-κB.

Overexpression of the deletion mutant Δp65 significantly increases NA-induced apoptosis, while uncleavable p65 mutant (p65D97A) blocks apoptosis (Fig. 8B). Several genes induced by NF-κB are known to down-regulate apoptosis, including manganese superoxide dismutase (30), zinc finger protein A20 (31), and cellular inhibitor for apoptosis (CIAP) (32). Thus, the abrogation of transcriptional activity of NF-κB by caspase-3-mediated cleavage of p65 might diminish the anti-apoptotic effect of NF-κB during apoptosis, which in turn explains why cells are more sensitive to apoptosis by NA.

In conclusion, we suggest that NA represses NF-κB activity via caspase-3-mediated cleavage of p65, therefore sensitizing cells to apoptosis. Although the apoptotic mechanism of NA is currently unclear, down-regulation of NF-κB activity is evidently one of the active processes in NA-induced apoptosis. Several anticancer agents are less effective in the induction of apoptosis because of their concomitant activation of NF-κB. In this regard, NA might be a promising future candidate for cancer therapy.

Acknowledgments—We thank Dr. Y. K. Jung for generously providing recombinant caspase-1, -3, and -8, and numerous helpful discussions, Dr. I. S. Kim for the human p65 and p50 cDNA, and Dr. J. W. Kim for the NF-κB-luciferase reporter plasmid.

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