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Expression of Ku70 and Ku80 Mediated by NF-κB and Cyclooxygenase-2 Is Related to Proliferation of Human Gastric Cancer Cells*

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Cyclooxygenase-2 (COX-2) expression is mediated by constitutive NF-κB and regulates human gastric cancer cell growth and proliferation. Inactivating Ku70 or Ku80 suppresses cell growth and induces apoptosis. It has been hypothesized that Ku70 and Ku80 expression may be associated with NF-κB activation and COX-2 expression and is involved in cell proliferation. In this study, we found that inhibition of constitutive NF-κB (by transfecting a mutated IκBα gene) and of COX-2 (by treatment with indomethacin and NS-398) suppressed Ku70 and Ku80 expression in cells. Treatment with prostaglandin E_2 adenocarcinoma gastric (AGS) increased expression of these Ku proteins in cells with low constitutive NF-κB levels. Inhibition of the Ku DNA end-binding activity by transfection with the C-terminal Ku80 expression gene suppressed cell proliferation. Ku70 or Ku80 overexpression by transfection with the Ku70 or Ku80 expression gene, respectively, enhanced proliferation of cells with low NF-κB levels. These results demonstrate that Ku70 and Ku80 expression is mediated by constitutively activated NF-κB and constitutively expressed COX-2 in gastric cancer cells and that the high Ku DNA end-binding activity contributes to cell proliferation. Ku70 and Ku80 expression may be related to gastric cell proliferation and carcinogenesis.

NF-κB is an inducible transcription factor that regulates the activation of a wide variety of genes that respond to immune or inflammatory signals (1). In resting cells, NF-κB is localized to the cytoplasm as a hetero- or homodimer, which is noncovalently associated with the cytoplasmic inhibitory protein IκBα. Upon stimulation with a variety of pathogenic inducers such as viruses, mitogens, bacteria, agents providing oxygen radicals, and inflammatory cytokines, IκBα is phosphorylated, ubiquitinated, and degraded in the cytoplasm, and the NF-κB complex migrates into the nucleus and binds the DNA recognition sites in the regulatory regions of the target genes (2). However, constitutive NF-κB is aberrantly activated in lymphomas and human breast and gastric cancer cells in the resting state (3–6). There are several reports on the role of NF-κB gene products in cell proliferation, transformation, and tumor development (7, 8). Cyclooxygenase-2 (COX-2),[1] an inducible isoform of cyclooxygenase, is also constitutively expressed in certain groups of cancers (9–11) and is related to cell proliferation (12, 13). COX-2 inhibition by specific COX-2 inhibitors suppresses cell proliferation, induces apoptosis, and down-regulates the expression of the anti-apoptotic protein Bcl-2 in pancreatic and colorectal cancer cells (14–19). Prostaglandins that are produced via COX-2 include prostaglandin E_2 (PGE_2) (20, 21) and prostaglandins A_1, A_2, and D_2 (22). They are believed to be the major contributors to cell proliferation and the inflammatory process (23, 24). A previous study demonstrated that COX-2 and prostaglandin syntheses are regulated by constitutive NF-κB, which is related to gastric cancer AGS cell proliferation (11).

The DNA repair protein Ku acts as a heterodimer of the two 70-kDa (Ku70) and 80-kDa (Ku80) subunits and binds to DNA ends, nicks, or single- to double-strand transition (25, 26). It serves as a DNA-binding component of a DNA-dependent protein kinase (DNA-PK) that phosphorylates certain chromatin-bound proteins *in vitro* (27, 28). Both Ku and the catalytic subunit of DNA-PK have been shown to be crucial for DNA double-strand break repair and V(D)J recombination (29–31). The Ku heterodimer binds to the double-strand DNA break and appears to stabilize the binding of the DNA-PK catalytic subunit to the DNA (32–35). Once bound, this complex stimulates DNA repair and signals the damage/stress responses, which might affect apoptosis and cell proliferation (36, 37). In addition, Um *et al.* (38) showed that Ku activity positively correlates with NF-κB activity in multidrug-resistant leukemia cells. Therefore, Ku activity can be regulated by NF-κB activity and affect cell growth and proliferation. Recent studies revealed growth retardation in both Ku70 and Ku80 knockout mice. Nussenzweig *et al.* (39, 40) demonstrated that the $Ku80^{-/-}$ embryonic stem cell line and $Ku80^{-/-}$ mutant primary embryonic fibroblasts display a reduction in cell growth and induction of cell apoptosis compared with $Ku80^{+/-}$ and $Ku80^{+/+}$ control cells. Sadji *et al.* (41) and Li *et al.* (42) showed that human Ku80 knockout colon cells exhibit slower growth than the corresponding control cells. The growth rate of murine embryonic fibroblasts derived from $Ku70^{-/-}$ embryos is lower than that of control murine embryonic fibroblasts (43). These studies show that inactivation of Ku70 or Ku80 drastically reduces the expression of other Ku subunits, resulting in inactivation of Ku DNA end-binding and DNA-PK activities. Moreover, the loss of one subunit destabilizes the other. Therefore, growth inhibition of Ku70- or Ku80-deficient cells would result

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[1] The abbreviations used are: COX-2, cyclooxygenase-2; PGE_2, prostaglandin E_2; DNA-PK, DNA-dependent protein kinase; RT, reverse transcription; DOTAP, *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate; EMSA, electrophoretic mobility shift assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; AGS, adenocarcinoma gastric.

from inactivation of Ku70 or Ku80 and inhibition of Ku DNA end-binding and DNA-PK activities. However, the phenotype of Ku70 knockout mice is somewhat different from that of Ku80 knockout mice (41, 43, 44). These studies suggest that either Ku70 or Ku80 might have a unique function that is independent of the other Ku subunit.

As described above, the expression of the COX-2 and Ku proteins (Ku70 and Ku80) is related to cell proliferation. Therefore, COX-2 expression mediated by constitutive NF-κB might be associated with the expression of both Ku70 and Ku80. This aim of this study was to investigate the role of Ku70 and Ku80 in cell proliferation, which may be mediated by constitutively activated NF-κB and constitutively expressed COX-2 in gastric cancer cells. This study examined whether or not constitutive NF-κB would be inhibited by transfection of the mutated IκBα gene and whether constitutively expressed COX-2 inhibited by treatment with the COX-2 inhibitors indomethacin and NS-398 would suppress Ku70 and Ku80 expression in gastric cancer AGS cells. To clarify the roles of the Ku DNA end-binding activity and Ku70 and Ku80 in cell proliferation, either AGS cells were transfected with the Ku dominant-negative gene to inactivate the Ku DNA end-binding activity, or the cells were transfected with either the Ku70 or Ku80 expression gene to overexpress Ku70 and Ku80, respectively. Cell proliferation was determined in the transfected cells. In addition, cells with low constitutive NF-κB levels were treated with PGE_2 (a COX-2 product), and the expression of Ku70 and Ku80 in the cells was determined. A low constitutive NF-κB level was confirmed by Western blotting for NF-κB p65 in cytoplasmic extracts and nuclear extracts of the cells.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture Conditions—Human gastric cancer AGS cells (gastric adenocarcinoma, ATCC CRL1739) were obtained from American Type Culture Collection (Manassas, VA) and cultured in RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum (Invitrogen) and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin). As described previously (11), IW-6 and IW-10 cells and pcN-3 cells were derived from the AGS cell line stably transfected with the IκBα expression vector mutated at serines 32 and 36 (mutated IκBα gene) to inhibit NF-κB activation and from AGS cells transfected with the control pcDNA3 vector (Invitrogen), respectively. The IW-6, IW-10, and pcN-3 cells were cultured and maintained in medium containing 200 μg/ml G418 (Invitrogen).

Preparation of Extracts—The cells, which were stably transfected with the control pcDNA3 vector or the mutated IκBα gene, were harvested with trypsin, washed with ice-cold phosphate-buffered saline, and lysed by adding SDS buffer (125 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 1% SDS). The lysates were then boiled for 5 min and centrifuged at 15,000 × *g* for 20 min. The supernatants were collected and used as a whole cell extract. To prepare the cytoplasmic and nuclear extracts, the cells were harvested with trypsin, resuspended in 100 μl of hypotonic buffer (10 mM Hepes (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5% Nonidet P-40, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride), and placed on ice for 20 min. The extracts were centrifuged at 15,000 × *g* for 20 min at 4 °C. The supernatants were then collected as the cytoplasmic extracts. The pellets were washed once with hypotonic buffer, resuspended in 50 μl of extraction buffer (20 mM Hepes (pH 7.9), 420 mM NaCl, 0.5 mM EDTA, 1.5 mM $MgCl_2$, 25% glycerol, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride), and placed on ice for 20 min. The extracts were subsequently centrifuged at 15,000 × *g* for 20 min at 4 °C, and the supernatants were used as the nuclear extracts. The protein concentration was determined using the method reported by Bradford (45). For one set of experiments, the cells were treated with various concentrations of the COX-2 inhibitors indomethacin (20, 100, and 200 μM; Sigma) and NS-398 (5, 25, and 50 μM; Alexis Biochemicals, San Diego, CA) or PGE_2 (14, 140, and 1400 nM; Sigma) and cultured for 48 h. Indomethacin was dissolved in ethanol, whereas NS-398 and PGE_2 were dissolved in dimethyl sulfoxide. The final concentration of each vehicle was <0.1%. The vehicle-treated cells were used as the control. The drug concentrations used in this study were adapted from a previous study (11). The whole cell, cytoplasmic, and nuclear extracts from the cells treated with either the COX-2 inhibitors or PGE_2 were prepared as described above.

Western Blot Analysis—Whole cell extracts (40 μg of protein/lane), cytoplasmic extracts (60 μg of protein/lane), or nuclear extracts (10 μg of protein/lane) were loaded, separated by 8% SDS-PAGE under reducing conditions, and transferred onto nitrocellulose membranes (Amersham Biosciences) by electroblotting. Protein transfer and equality of loading in the lanes were verified using reversible staining with Ponceau S. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and 0.15% Tween 20 (TBS-T) for 3 h at room temperature. The proteins were detected with polyclonal antibodies against Ku70 (1:1000; sc-1487), Ku80 (1:500; sc-1484), actin (1:1000; sc-1615), NF-κB p65 (1:1000; sc-372), aldolase A (1:500; sc-12059), and histone H1 (1:500; sc-8615) (all from Santa Cruz Biotechnology, Santa Cruz, CA) diluted in TBS-T containing 5% dry milk and incubated at 4 °C overnight. After washing with TBS-T, the immunoreactive proteins were visualized using horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies, followed by enhanced chemiluminescence (Amersham Biosciences). Actin was used for the protein loading control, whereas aldolase A and histone H1 were used for the cytoplasmic and nuclear controls, respectively.

Reverse Transcription (RT)-PCR Analysis—Ku70 and Ku80 mRNA expression was assessed by RT-PCR analysis, followed by Southern hybridization, and quantitated using the β-actin housekeeping gene as the internal control. Briefly, the total RNA was extracted, reverse-transcribed into cDNA, and used for PCR with specific human primers for Ku70, Ku80, and β-actin. The sequences of the Ku70 primers were 5′-ATGGCAACTCCAGAGCAGGTG-3′ (forward primer) and 5′-AGTGCTTGGTGAGGGCTTCCA-3′ (reverse primer), giving a 462-bp PCR product (46). For the Ku80 primers, the forward primer was 5′-TGACTTCCTGGATGCACTAATCGT-3′, and the reverse primer was 5′-TTGGAGCCAATGGTCAGTCG-3′, giving a 454-bp PCR product (47). The β-actin primers used were 5′-ACCAACTGGGACGACATGGAG-3′ (forward primer) and 5′-GTGAGGATCTTCATGAG GTAGTC-3′ (reverse primer), giving a 349-bp PCR product (48). Briefly, the PCR products were amplified by 18–19 repeated denaturation cycles at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The 95 °C step was extended to 2 min during the first cycle, and the 72 °C step was extended to 5 min during the final cycle. The PCR products were separated on 1.5% agarose gels containing 0.5 μg/ml ethidium bromide and transferred to a Hybond-N^+ nylon membrane, and the membrane was hybridized overnight at 60 °C with the respective digoxigenin-labeled probe (49). After 20-min washes with 2× and 0.2× SSC buffer containing 0.1% SDS at room temperature and at 60 °C, the digoxigenin label was immunodetected using the digoxigenin detection kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's protocol. Hybridized bands for PCR products were quantified by densitometric analysis. The Ku70 and Ku80 mRNA levels were quantitated using β-actin. The Ku70 and Ku80 mRNA levels in pcN-3 cells were considered as 100%. Each *bar* in the figures represents the mean ± S.E. of three separate experiments. The reverse transcriptase negative control was treated without reverse transcriptase, but following the same procedure as used for the other samples.

Plasmid Construction—The human cDNAs for Ku70 and Ku80 were derived from human pET1a-Ku70 and pET1a-Ku80 (a kind gift from T. Morio, Tokyo Medical and Dental University), respectively. The insert was digested with *Bam*HI and *Eco*RI and subcloned into the *Bam*HI-*Eco*RI sites of the pcDNA3 vector. A C-terminal human Ku80-(427–732) expression vector was generated by PCR using a Ku80 cDNA vector with a specific set of primers to generate artificial *Kpn*I and *Bam*HI sites at the 5′- and 3′-ends, respectively. The sequences of the primers used are as follows: 5′-TGCAGGTACCTATCATGGAAGACTTGCG-3′ and 5′-GGT ACC TAG GTG CTG GAT ATA GTA CAG G-3′. A *Kpn*I-*Bam*HI fragment of the product was subcloned into the *Kpn*I and *Bam*HI sites of the pcDNA3 vector. The PCR-derived part was confirmed by sequencing analysis.

Transfection—Subconfluent AGS cells were plated in 10-cm culture dishes and transfected with 10 μg of the C-terminal Ku80-(427–732) expression plasmid construct using DOTAP (Roche Molecular Biochemicals) for 16 h. After transfection, the cells were trypsinized and plated at 1 × 10^4 cells/10-cm culture dish. The cells were cultured in medium containing 400 μg/ml G418 for 15–17 days, and three to four resistant clones were isolated from each plate. The Ku DNA end-binding activity was determined by an electrophoretic mobility shift assay (EMSA). The positive clones for C-terminal Ku80-(427–732) were maintained in culture medium containing 200 μg/ml G418 for >2 months and are referred to as KuDN-2 and KuDN-7. For the transient transfection of Ku70 or Ku80 into AGS cells, the cells, which were

previously stably transfected with either the mutated IκBα gene or the control pcDNA3 vector, were plated in a 10-cm culture dish and cultured overnight. The cells were transfected with either the Ku70 or Ku80 expression plasmid construct using DOTAP for 16 h. The cells were replated in a 10-cm culture dish and cultured for a further 48 h. Nuclear and cytoplasmic extracts were prepared from the cells. The Ku70 and Ku80 protein levels were determined by Western blot analysis.

EMSA—EMSA was carried out by a slight modification of the method reported by Kim *et al.* (50). Nuclear extracts (10 μg) of the cells transfected with the C-terminal Ku80-(427–732) expression gene were incubated with the ^{32}P-labeled double-stranded oligonucleotide 5′-GGGCCAAGAATCTTAGCAGTTTCGGG-3 in buffer containing 12% glycerol, 12 mM Hepes (pH 7.9), 1 mM EDTA, 1 mM dithiothreitol, 25 mM KCl, 5 mM $MgCl_2$, and 0.04 μg/ml poly[d(I-C)] at room temperature for 30 min. The extracts were then subjected to electrophoretic separation at room temperature on a nondenaturing 5% acrylamide gel at 30 mA using 0.5× Tris borate/EDTA buffer. The gels were dried at 80 °C for 1 h and exposed to radiography film for 6–18 h at −70 °C with intensifying screens (51). For the supershift assay, 10 μg of nuclear extracts were preincubated with 1 μg of polyclonal antibody specific to Ku70 or Ku80 on ice for 30 min prior to the Ku DNA end-binding reaction.

Cell Counting and MTT Assay—Cell number was determined by both direct counting with a hemocytometer using the trypan blue exclusion test (0.2% trypan blue) and an indirect colorimetric immunoassay (MTT assay). MTT was metabolized by NAD-dependent dehydrogenase to form a colored reaction product, and the amount of dye formed directly correlated with the number of cells. For the trypan blue exclusion test, the cells were plated at 2×10^4 cells/well in a 24-well culture plate and incubated for 24, 48, and 72 h. The number of cells was counted with a hemocytometer using 0.2% trypan blue. For the MTT assay, the cells (2×10^3 cells/well) were plated in a 96-well culture plate and cultured for 48 h. MTT (0.5 mg/ml) was added, and the reaction mixture was incubated for 4 h at 37 °C. The cellular formazan was extracted with acidic propan-2-ol, and the absorbance was measured with a dual-wavelength automatic plate reader at 570/630 nm (52). The number of viable cells is expressed as MTT-positive cells, and the number of cells transfected with the control pcDNA3 vector (*pcN-3* in Fig. 5 and *pcDNA* in Fig. 8) was considered to 100%. The relative numbers of MTT-positive cells are expressed as a percentage of the pcN-3 (see Fig. 5) or pcDNA3-transfected (see Fig. 8) cells.

Determination of [^{3}H]Thymidine Incorporation—The cells (5×10^4/well) were seeded in a 24-well culture plate. After incubating the cells for 24 h, 1 μCi/ml [^{3}H]thymidine (Amersham Biosciences) was added, and incubation was continued for an additional 6 h. The cells were then washed twice with ice-cold phosphate-buffered saline, incubated in 10% trichloroacetic acid for 30 min, and incubated with a solution consisting of 0.3 M NaOH and 1% SDS for 1 h. The cells were lysed by vortexing and analyzed for their radioactivity by liquid scintillation counting. [^{3}H]Thymidine incorporation, which reflected the extent of DNA synthesis, in the cells transfected with the control pcDNA3 vector (*pcN-3* in Fig. 5 and *pcDNA* in Fig. 8) was considered as 100%. The relative amount of [^{3}H]thymidine incorporated is expressed as a percentage of the pcN-3 (see Fig. 5) or pcDNA3-transfected (see Fig. 8) cells.

Statistical Analysis—The results are expressed as means ± S.E. of four separate experiments. Analysis of variance followed by Newman-Keuls test was used for statistical analysis (53). $p < 0.05$ was considered statistically significant.

RESULTS

Decreased Expression of Ku70 and Ku80 in AGS Cells Transfected with the Mutated IκBα Gene—A previous study demonstrated that AGS cells stably transfected with the mutated IκBα gene (IW-6 and IW-10) have lower constitutive NF-κB levels and slower cell proliferation compared with AGS cells transfected with the control pcDNA3 vector (pcN-3) (11). The Ku70 or Ku80 antigen is related to cell growth and proliferation (39–43). It was hypothesized that constitutive NF-κB activation might be associated with Ku80 and Ku70 expression. To confirm this hypothesis, the Ku70 and Ku80 protein and mRNA levels in AGS cells, which were previously transfected with the mutated IκBα gene or the control pcDNA3 vector, were analyzed by Western blot analysis and RT-PCR analysis, respectively (Fig. 1). The Ku70 and Ku80 protein levels in the

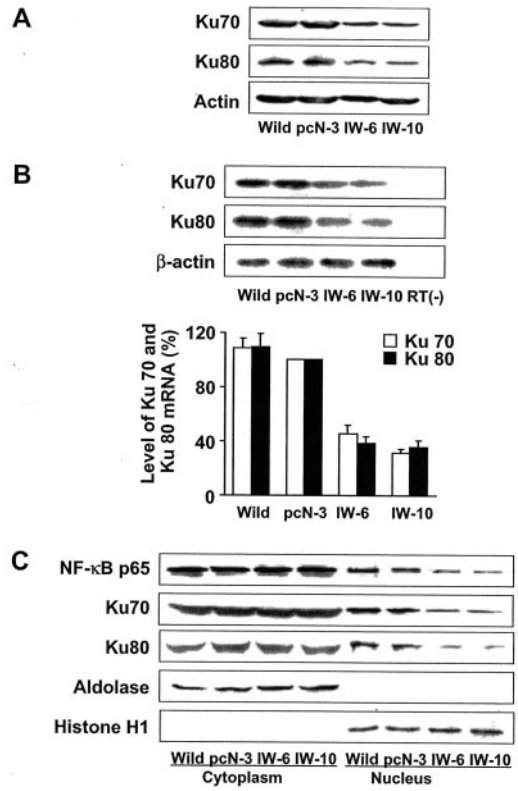

FIG. 1. **The Ku70 and Ku80 protein and mRNA levels in AGS cells transfected with either the control vector or the mutated IκBα gene.** *A*, the whole cell extracts were prepared from wild-type cells, and the cells were transfected with the control pcDNA3 vector (pcN-3) or the mutated IκBα gene (IW-6 and IW-10). The Ku70 and Ku80 protein levels were determined by Western blot analysis. Actin was used for the loading control. *B*, the total RNAs were extracted from the cells. The Ku70 and Ku80 mRNA levels were determined by RT-PCR analysis, followed by Southern hybridization (*upper panel*), and quantitated using the β-actin housekeeping gene (*lower panel*). The Ku70 and Ku80 mRNA levels of pcN-3 cells were considered as 100%. Each *bar* represents the mean ± S.E. of three separate experiments. The reverse transcriptase negative control (*RT*(−)) was treated without reverse transcriptase and was subjected to the same procedure as the other samples. *C*, cytoplasmic and nuclear extracts were prepared from the cells. The Ku70 and Ku80 protein levels as well as the NF-κB p65 protein level were determined by Western blot analysis. Aldolase and histone H1 were used as the cytoplasmic and nuclear controls, respectively.

IW-6 and IW-10 cells were lower than those in the pcN-3 cells (Fig. 1*A*). The Ku70 and Ku80 mRNA levels in the IW-6 and IW-10 cells were lower than those in both the wild-type and pcN-3 cells. The Ku70, Ku80, and β-actin mRNA levels were not detected in the reverse transcriptase negative control, which was treated without reverse transcriptase in the PCR procedure (Fig. 1*B*). Furthermore, to investigate the expression and intracellular localization of Ku70 and Ku80 in cells transfected with the mutated IκBα gene or the control pcDNA3 vector, the Ku protein levels were analyzed in both the nuclear and cytoplasmic extracts by Western blot analysis. A low nuclear level of NF-κB p65 was determined in IW-6 and IW-10 cells. The nuclear levels of Ku70 and Ku80 were lower in the IW-6 and IW-10 cells than in the wild-type and pcN-3 cells. Aldolase was detected only in the cytoplasmic extracts, whereas histone H1 was observed only in the nuclear extracts (Fig. 1*C*). This suggests that the inhibition of constitutive NF-κB activation, which was associated with the suppression of cell growth and proliferation in a previous study (11), may be caused by a reduction in Ku70 and Ku80 expression. Therefore, the effects of COX-2 inhibitors suppressing cell proliferation on Ku70 and Ku80 expression were determined because the con-

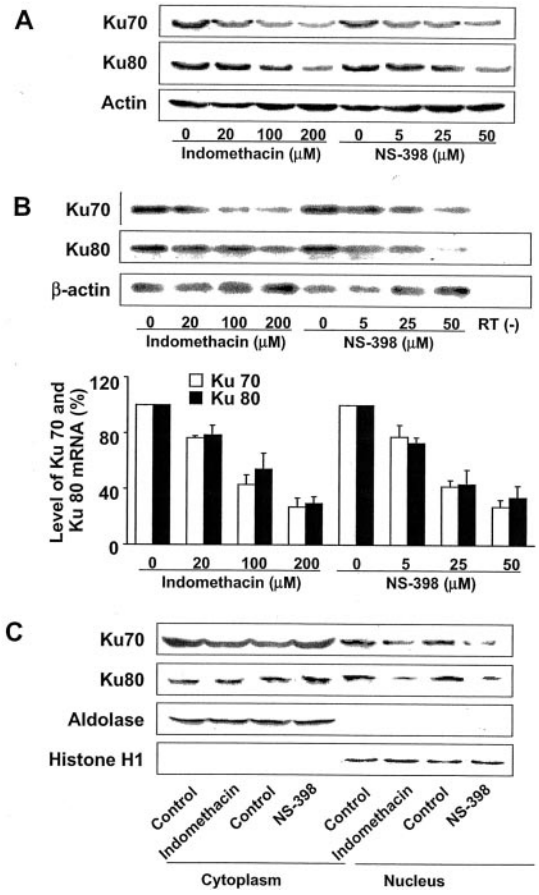

FIG. 2. **Effects of COX-2 inhibitors on the Ku70 and Ku80 protein and mRNA levels in AGS cells.** The cells (plated at 1.5×10^6 cells/dish) were treated with various concentrations of indomethacin or NS-398 for 48 h. *A*, the whole cell extracts were prepared from the cells, and Ku70 and Ku80 protein levels were determined by Western blot analysis. Actin was used for the loading control. *B*, the total RNAs were extracted from the cells. The Ku70 and Ku80 mRNA levels were determined by RT-PCR analysis, followed by Southern hybridization (*upper panel*), and quantitated using the β-actin housekeeping gene (*lower panel*). The Ku70 and Ku80 mRNA levels of pcN-3 cells were considered as 100%. Each *bar* represents the mean ± S.E. of three separate experiments. The reverse transcriptase negative control (*RT*(−)) was treated without reverse transcriptase and was subjected to the same procedure as the other samples. *C*, the cytoplasmic and nuclear extracts were prepared from the cells treated with vehicle (ethanol for indomethacin and dimethyl sulfoxide) for NS-398 (*Control*), 200 μM indomethacin, or 50 μM NS-398 for 48 h. The Ku70 and Ku80 protein levels were determined by Western blot analysis. Aldolase and histone H1 were used as the cytoplasmic and nuclear controls, respectively.

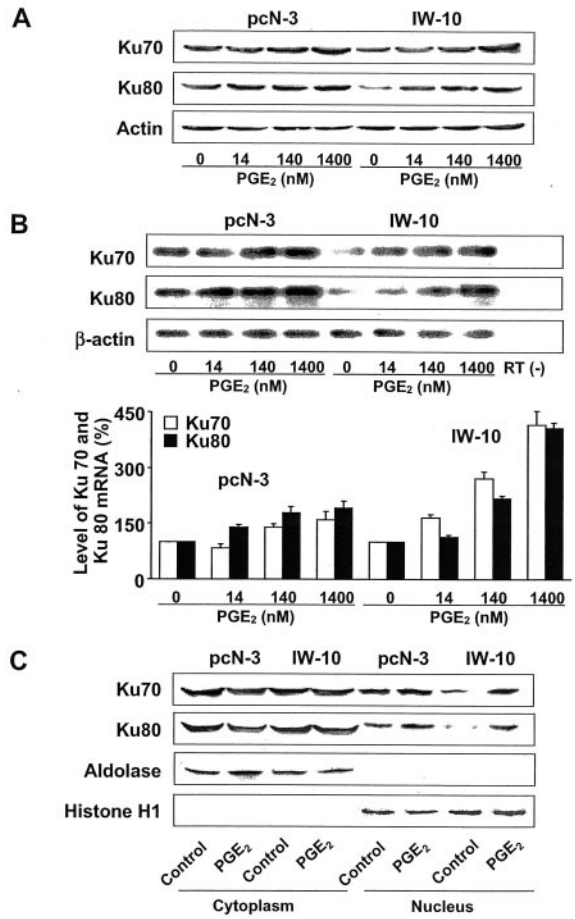

FIG. 3. **Effects of PGE_2 on the Ku70 and Ku80 protein and mRNA levels in AGS cells transfected with the control vector or the mutated IκBα gene.** pcN-3 and IW-10 cells were plated at of 1.5×10^6 cells/dish. The cells were treated with various concentrations of PGE_2 for 48 h. *A*, the whole cell extracts were prepared from the cells, and the Ku70 and Ku80 protein levels were determined by Western blot analysis. Actin was used for the loading control. *B*, the total RNAs were extracted from the cells. The Ku70 and Ku80 mRNA levels were determined by RT-PCR analysis, followed by Southern hybridization (*upper panel*), and quantitated using the β-actin housekeeping gene (*lower panel*). The Ku70 and Ku80 mRNA levels in pcN-3 cells were considered as 100%. Each *bar* represents the mean ± S.E. of three separate experiments. The reverse transcriptase negative control (*RT*(−)) was treated without reverse transcriptase and subjected to the same procedure as the other samples. *C*, the cytoplasmic and nuclear extracts were prepared from cells treated with the vehicle dimethyl sulfoxide (*Control*) or 1400 nM PGE_2 for 48 h. The Ku70 and Ku80 protein levels were determined by Western blot analysis. Aldolase and histone H1 were used as the cytoplasmic and nuclear controls, respectively.

stitutive expression of COX-2 is mediated by constitutive NF-κB in AGS cells.

Effects of COX-2 Inhibitors on the Expression of Ku70 and Ku80—Several reports have shown that constitutive COX-2 is related to the proliferation of certain cancer cells (14–18). It was previously demonstrated that inhibiting COX-2 expression by specific COX-2 inhibitors suppresses AGS cell growth and proliferation (11). Therefore, COX-2 inhibition was examined to determine whether it could reduce the Ku70 and Ku80 expression levels. Treatment with a nonspecific COX-2 inhibitor (indomethacin) resulted in a dose-dependent suppression of the Ku70 and Ku80 protein and mRNA levels after 48 h of treatment (Fig. 2, *A* and *B*). Similar results were observed after treatment with a specific COX-2 inhibitor (NS-398). The inhibitory effect of NS-398 on Ku70 and Ku80 expression was more potent than that of indomethacin. The Ku70 and Ku80 protein levels in the nuclear extracts were significantly lower in the cells treated with indomethacin (200 μM) and NS-398 (50 μM) than in the control cells treated with each vehicle (Fig. 2*C*). These results demonstrate that COX-2 expression is positively related to Ku70 and Ku80 expression in gastric cancer AGS cells.

Effects of PGE_2 on the Expression of Ku70 and Ku80—Prostaglandins that are produced by COX-2 have diverse bioactive activities, including growth-promoting actions in colon and gastric cancer cells (23–24). PGE_2 was investigated to determine whether it could enhance Ku70 and Ku80 expression. The effects of PGE_2 on the Ku70 and Ku80 protein and mRNA levels were determined in AGS cells transfected with either the mutated IκBα gene or the control pcDNA3 vector after 48 h of treatment (Fig. 3, *A* and *B*). Treatment with PGE_2 dose-dependently increased the Ku70 and Ku80 protein and mRNA levels in the IW-10 cells. PGE_2 did not affect the Ku70 and Ku80 protein and mRNA levels in the pcN-3 cells. These results suggest that Ku70 and Ku80 expression is not induced in cells

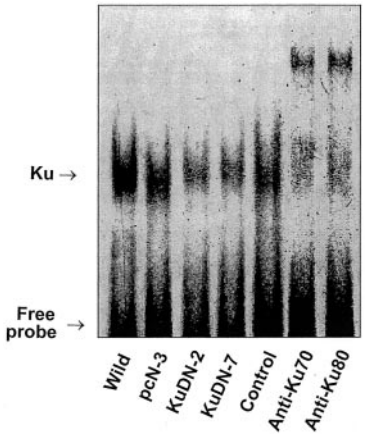

FIG. 4. **Ku DNA end-binding activity in a nuclear extract of AGS cells transfected with the C-terminal Ku80-(427–732) expression gene.** The nuclear extracts (10 μg) were prepared from wild-type cells, pcN-3 cells, and cells transfected with the C-terminal Ku80-(427–732) expression gene (KuDN-2 and KuDN-7). The Ku DNA end-binding activity was determined by EMSA. For the supershift assay, the nuclear extracts were preincubated with anti-Ku70 or anti-Ku80 antibody and subjected to EMSA. *Control* represents the Ku DNA end-binding activity from the nuclear extract preincubated without antibody.

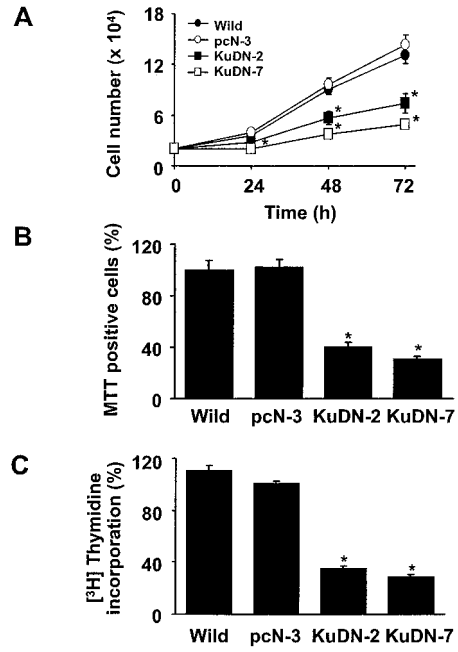

FIG. 5. **Proliferation of AGS cells transfected with the control vector or with the C-terminal Ku80-(427–732) expression gene as determined by cell counting, MTT-positive cells, and [^{3}H]thymidine incorporation.** *A*, for viable cell counting, wild-type cells, pcN-3 cells, and cells transfected with the C-terminal Ku80-(427–732) expression gene (KuDN-2 and KuDN-7) were plated at 2×10^4 cells/well. The cells were incubated for the indicated time periods, and cell number was counted using a trypan blue exclusion test. Each *point* represents the mean ± S.E. of four separate experiments. *, $p < 0.01$ *versus* pcN-3 at each time point. *B*, for the analysis of MTT-positive cells, cells (2×10^3 cells/well) were plated and cultured for 48 h. Viable cells were assessed by the MTT assay, and MTT-positive cells are expressed as a percentage of the pcN-3 cells. The relative MTT-positive cells are expressed as a percentage of the pcN-3 cells. Each *bar* represents the mean ± S.E. of four separate experiments. *, $p < 0.01$ *versus* pcN-3 cells. *C*, for the [^{3}H]thymidine incorporation assay, the cells were plated at 5×10^4 cells/well and cultured for 24 h. The cells were cultured for 6 h after adding 1 μCi/ml [^{3}H]thymidine. The amount of [^{3}H]thymidine incorporated into the pcN-3 cells was considered as 100%. The relative [^{3}H]thymidine incorporation is expressed as a percentage of the pcN-3 cells. Each *bar* represents the mean ± S.E. of four separate experiments. *, $p < 0.01$ *versus* pcN-3 cells.

where COX-2 is highly constitutively expressed. Treatment with PGE_2 (1400 nM) prevented the decrease in the Ku70 and Ku80 protein levels in the IW-10 cells (Fig. 3*C*). This shows that prostaglandins produced by COX-2 might induce Ku70 and Ku80 expression in cells with a low COX-2 expression level.

Proliferation of AGS Cells Transfected with the C-terminal Ku80-(427–732) Expression Gene—To inhibit the Ku DNA end-binding activity, AGS cells were transfected with the vector containing the terminal 926 bp of the Ku80 cDNA, corresponding to the C-terminal 305 amino acids of the Ku80 protein, referred to the C-terminal Ku80-(427–732) expression gene. This fragment could interact with Ku70, but the heterodimer of Ku70 and C-terminal Ku80 could not bind to the double-stranded DNA ends, resulting in decreased Ku-dependent DNA end-binding activity in the cells (54–57). In this study, AGS cells were stably transfected with the C-terminal Ku80-(427–732) expression gene. The stably transfected clones of the C-terminal Ku80-(427–732) expression gene (KuDN-2 and KuDN-7) were then selected and analyzed for Ku DNA end-binding activity by EMSA (Fig. 4). A single slowly migrating band appeared in the gel that was supershifted in the presence of anti-Ku70 and anti-Ku80 antibodies. This retarded band can be confidently ascribed to a Ku·DNA complex. The intensity of this band was lower in the cells transfected with the C-terminal Ku80-(427–732) expression gene (KuDN-2 and KuDN-7) than in both the wild-type and pcN-3 cells. Because there was no evidence of smaller DNA end-binding product(s) in the cells transfected with the C-terminal Ku80-(427–732) expression gene, neither C-terminal Ku80-(427–732) alone nor a putative heterodimer of Ku70 and C-terminal Ku80-(427–732) exhibited DNA end-binding activity. To evaluate the relationship between inhibition of the Ku DNA end-binding activity and cell proliferation, the cells were transfected with either the C-terminal Ku80-(427–732) expression gene (KuDN-2 and KuDN-7) or with the control pcDNA3 vector (pcN-3) and cultured. The number of viable cells was determined by the trypan blue exclusion test (Fig. 5*A*). Cell proliferation was lower in the cells transfected with the C-terminal Ku80-(427–732) expression gene (KuDN-2 and KuDN-7) than in the wild-type cells and the cells transfected with the control pcDNA3 vector (pcN-3) for a 72-h culture period. A similar phenomenon was shown by other methods determining the MTT-positive cells (Fig. 5*B*) and [^{3}H]thymidine incorporation (Fig. 5*C*). The number of MTT-positive cells and the extent of [^{3}H]thymidine incorporation were lower in the cells transfected with the C-terminal Ku80-(427–732) expression gene than in the wild-type and pcN-3 cells. This shows that the inhibition of the Ku DNA end-binding activity results in a strong down-regulation of cell proliferation.

Proliferation of AGS Cells Transfected with the Ku70 or Ku80 Expression Gene—pcN-3 and IW-10 cells were transiently transfected with the cDNA for p70 (Ku70) or p80 (Ku80). The Ku70 and Ku80 protein expression levels in these cells were determined by Western blot analysis (Fig. 6). The nuclear level of Ku70 in the IW-10 cells transfected with the cDNA for Ku70 (*pcDNA-Ku70*) was significantly higher than in the wild-type IW-10 cells and in the IW-10 cells transfected with the control vector (*pcDNA*). Correspondingly, the nuclear level of Ku80 in the IW-10 cells transfected with the cDNA for Ku80 (*pcDNA-Ku80*) was significantly higher than in the wild-type IW-10 cells and the IW-10 cells transfected with the control pcDNA3 vector (Fig. 6*B*). These effects were similar to those shown in pcN-3 cells (Fig. 6*A*). However, increases in the nuclear levels of both Ku70 by the transfection of the Ku70 ex-

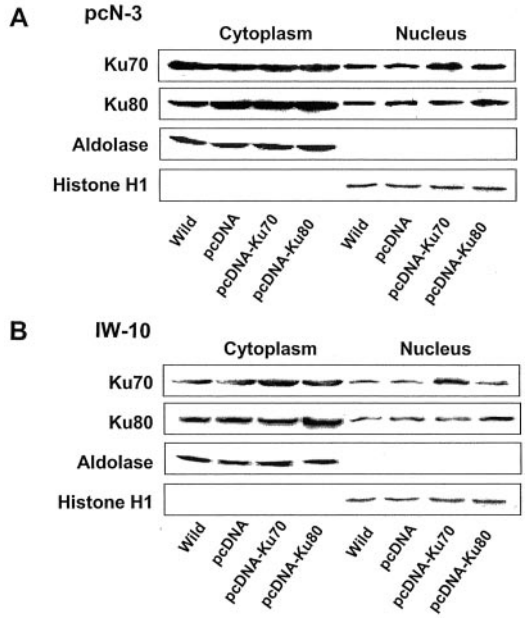

FIG. 6. **Ku70 and Ku80 protein levels in the cytoplasmic and nuclear extracts of pcN-3 and IW-10 cells transfected with the Ku70 or Ku80 expression gene.** pcN-3 and IW-10 cells were transiently transfected with the Ku70 or Ku80 expression gene (*pcDNA-Ku70* and *pcDNA-Ku80*, respectively). The cytoplasmic and nuclear extracts were prepared from pcN-3 (*A*) and IW-10 (*B*) cells transfected with or without the Ku70 or Ku80 expression gene. The Ku70 and Ku80 protein levels were determined by Western blot analysis. *Wild*, non-transfected cells; *pcDNA*, cells transfected with the control pcDNA3 vector. Aldolase and histone H1 were used as the cytoplasmic and nuclear controls, respectively.

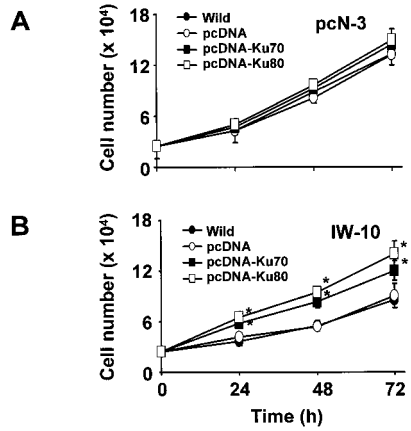

FIG. 7. **Effect of transfection with the Ku70 or Ku80 expression gene on the proliferation of pcN-3 and IW-10 cells as determined by cell counting.** pcN-3 (*A*) and IW-10 (*B*) cells were transiently transfected with the control pcDNA3 vector (*pcDNA*) or with the Ku70 or Ku80 expression gene (*pcDNA-Ku70* and *pcDNA-Ku80*, respectively). The cells were plated at 2×10^4 cells/well and then incubated for the indicated time periods. The number of cells was counted by the trypan blue exclusion test. Each *point* represents the mean ± S.E. of four separate experiments. *, $p < 0.01$ *versus* pcDNA3-transfected cells at each time point. *Wild*, non-transfected cells.

pression gene and Ku80 by the transfection of the Ku80 expression gene in the pcN-3 cells were relatively lower than those in the IW-10 cells. This may have been caused by the relatively low expression levels of the Ku proteins in the IW-10 cells compared with those in the pcN-3 cells. The cytoplasmic levels of Ku70 and Ku80 were unchanged by transfection with the Ku70 or Ku80 expression gene. In addition, transfection with the Ku70 expression gene did not affect the nuclear level of Ku80, and transfection with the Ku80 expression gene had no effect on the nuclear level of Ku70 in either the pcN-3 or IW-10 cells.

Proliferation of the wild-type pcN-3 cells (Fig. 7*A*) was higher than that of the wild-type IW-10 cells (Fig. 7*B*) containing low nuclear levels of NF-κB and Ku proteins during a 72-h culture period. To evaluate the relationship between overexpression of the Ku70 and Ku80 proteins and cell proliferation, the cells were transfected with the cDNA for either Ku70 or Ku80 (*pcDNA-Ku70* and *pcDNA-Ku80*) or with the control pcDNA3 vector (*pcDNA*) and cultured. Cell proliferation was determined by the relative number of MTT-positive cells and the extent of [^{3}H]thymidine incorporation (Figs. 7 and 8). In the IW-10 cells, proliferation was significantly higher in the cells transfected with the cDNA for either Ku70 or Ku80 than in the wild-type and pcDNA3-transfected cells. The increase in proliferation was slightly higher in the Ku80-overexpressing IW-10 cells than in the Ku70-overexpressing IW-10 cells. However, the effect of Ku70 and Ku80 cDNA transfection on the increase in cell proliferation was lower in the pcN-3 cells. This demonstrates that Ku70 and Ku80 overexpression induces a strong up-regulation of cell proliferation, which is correlated with the nuclear levels of Ku70 and Ku80.

DISCUSSION

NF-κB is constitutively activated in B-cell lymphoma, breast, and gastric cancer cells (3–6, 11). Cell proliferation and tumorigenesis involve the constitutive induction of NF-κB activation (58–62). The NF-κB target genes have been implicated in the prevention of cell death by regulating the expression of genes such as those for the tumor necrosis factor receptor-associated factors TRAF1 and TRAF2, the inhibitor of apoptosis proteins c-IAP1 and c-IAP2, Bcl-x_L, and Bcl-2 (63, 64). COX-2 is constitutively expressed in some cancers (9–11) and is related to cell proliferation (12, 13). Sasaki *et al.* (65) reported that NF-κB is constitutively activated in human gastric carcinoma tissue as opposed to adjacent normal epithelial cells. Premalignant and malignant gastric lesions exhibit strong COX-2 expression, which is not observed in normal lesions (66, 67). A previous study demonstrated that constitutively expressed COX-2, which is mediated by constitutive NF-κB, regulates gastric cancer AGS cell proliferation (11). This suggests that constitutive COX-2 expression via constitutive NF-κB may be a principal mechanism for gastric carcinogenesis and tumorigenesis. Several studies have shown that inhibiting the expression of either Ku70 or Ku80 results in inhibition of cell growth and induction of apoptosis (39–43).

In this study, constitutively activated NF-κB and COX-2 expression were examined to determine whether they could regulate Ku70 or/and Ku80 expression in gastric cancer AGS cells. AGS cells with a low level of constitutive NF-κB had a lower expression level of Ku70 and Ku80, which was reflected in the lower nuclear levels of Ku proteins, than the wild-type cells and the cells transfected with control vector. This finding contrasts with the report by Um *et al.* (38), who showed that PC-12-NF-κB cells overexpressing both p50 and p65 subunits of NF-κB exhibited an increase in Ku70 and Ku80 expression compared with the parental PC-12 cells. COX-2 inhibitors such as indomethacin and NS-398 were found to suppress Ku70 and Ku80 expression in AGS cells. PGE_2, a COX-2 product, enhanced the Ku70 and Ku80 expression levels in the cells with low constitutive NF-κB levels. These results suggest that Ku70 and Ku80 expression may be regulated by COX-2 and COX-2 products (prostaglandins) via an NF-κB-dependent mechanism in AGS cells. Prostaglandins exert their biological action via specific receptors (prostaglandin E receptors 1–4) (68). Pai *et al.* (69) reported that PGE_2 transactivates the epidermal growth factor receptors, which then trigger mitogenic signaling (70–72). Efficient DNA repair in actively growing cells requires

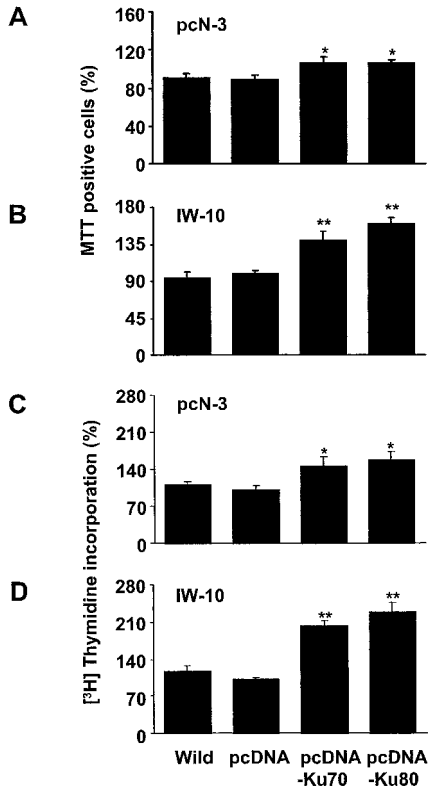

FIG. 8. **Effect of transfection with the Ku70 or Ku80 expression gene on the proliferation of pcN-3 and IW-10 cells as determined by MTT-positive cells and [^{3}H]thymidine incorporation.** For analysis of MTT-positive cells, pcN-3 (*A*) and IW-10 (*B*) cells were transiently transfected with the control pcDNA3 vector (*pcDNA*) or with the Ku70 or Ku80 expression gene (*pcDNA-Ku70* and *pcDNA-Ku80*, respectively). The cells were plated at 2×10^3 cells/well and cultured for 48 h. Viable cells were assessed by the MTT assay, and MTT-positive cells are expressed as a percentage of the cells transfected with the control pcDNA3 vector. The relative MTT-positive cells are expressed as a percentage of the pcDNA3-transfected cells. Each *bar* represents the mean ± S.E. of four separate experiments. *, $p < 0.05$ *versus* pcN-3 cells; **, $p < 0.01$ *versus* pcN-3 cells. For determination of [^{3}H]thymidine incorporation, the pcN-3 (*C*) and IW-10 (*D*) cells were transiently transfected with the control pcDNA3 vector or with the Ku70 or Ku80 expression gene. The cells were plated at 5×10^4 cells/well and cultured for 24 h. After adding 1 μCi/ml [^{3}H]thymidine, the cells were cultured for 6 h. The level of [^{3}H]thymidine incorporation in the cells transfected with the control pcDNA expression vector was considered as 100%. The relative [^{3}H]thymidine incorporation level is expressed as a percentage of the pcDNA3-transfected cells. Each *bar* represents the mean ± S.E. of four separate experiments. *, $p < 0.05$ *versus* pcN-3 cells; **, $p < 0.01$ *versus* pcN-3 cells.

growth factor signaling (73, 74). Epidermal growth factor receptor-mediated signaling is associated with mitogenesis and cell proliferation (75, 76). Because epidermal growth factor receptor signaling requires the maintenance of a nuclear level of DNA-PK and its regulatory heterodimeric complex (Ku70·Ku80) in mammalian cells (77), the role of Ku70·Ku80 in cell proliferation and growth is postulated. Further study should be performed to determine whether the prostaglandins produced by COX-2 activate specific receptors such as epidermal growth factor and other growth factor receptors, triggering specific signaling related to Ku70 and Ku80 expression in gastric cancer cells. In addition, a heterodimer of Ku70 and Ku80 is a regulatory subunit of DNA-PK that phosphorylates many proteins, including transcription factors such as c-Jun, c-Fos, c-Myc, and many more. They appear to be multifunctional proteins that are implicated in cellular processes such as DNA replication, transcriptional regulation, and control of the G_2 and M phases of the cell cycle (78). Therefore, Ku70 and Ku80 may be involved in cell proliferation by regulating cell cycle-associated proteins or growth-related gene expression.

This study demonstrated that the inhibition of the Ku DNA end-binding activity by transfection of the C-terminal Ku80-(427–732) expression gene resulted in the suppression of AGS cell proliferation. Several reports have shown that inactivating Ku80 or Ku70 reduces the expression of the other Ku subunit (Ku70 or Ku80) and then inhibits the Ku DNA end-binding and DNA-PK activities in Ku70- or Ku80-deficient cells (39–43). These results suggest that disruption of either of the Ku subunits would reduce the Ku DNA end-binding activity, which then would inhibit the functional role of the Ku proteins. Therefore, inhibition of the cell growth caused by a reduction in the Ku70 and Ku80 nuclear levels may be related to the loss of the Ku DNA end-binding activity. This was proven by the observation that inhibition of the Ku DNA end-binding activity by transfection with C-terminal Ku80-(427–732) resulted in inhibition of cell proliferation. These results show that Ku70 or Ku80 overexpression in AGS cells with a low constitutive NF-κB level does not induce an increase in the nuclear level of the other Ku subunit (Ku80 or Ku70), which concurs with a previous report (79). Overexpression of either Ku70 or Ku80 without enhancement of the other Ku subunit induced an increase in cell proliferation. Null knockout mice for DNA-PK_{cs} do not exhibit growth retardation, whereas growth retardation has been observed in either Ku70 or Ku80 knockout mice (40, 43, 44, 80). This suggest that Ku70 and Ku80 are associated with growth regulation independent of the function of DNA-PK. Ku70 has been reported to show Ku80-dependent and Ku80-independent DNA binding (81). In recent studies using knockout mice, some differences in the phenotypes of Ku70 and Ku80 knockout mice have been reported (40, 43, 44), suggesting the possibility that Ku70 and Ku80 may have unique functions, including cell proliferation, that are independent of the other Ku subunit. It has been reported that a Ku70 and Ku80 deficiency (but not a DNA-PK_{cs} deficiency) results in a dramatic increase in cell apoptosis (39, 40, 42, 82). However, we found that inhibiting Ku70 and Ku80 expression by the COX-2 inhibitors and transfection of C-terminal Ku80-(427–732) did not induce apoptosis, as determined by the DNA fragmentation assay (data not shown). In addition, Ku70 or/and Ku80 expression may have anti-apoptotic properties because inhibition of Ku70 and Ku80 suppressed cell proliferation. These results are supported by Li *et al.* (42), who demonstrated that Ku80 inactivation results in induction of the tumor suppressor protein p53, which contributes to inhibition of cell growth. However, it was previously determined that the proliferation of AGS cells with low Ku70 and Ku80 expression levels was not associated with p53 expression and that inhibition of Ku expression after treatment with COX-2 inhibitors did not induce p53 expression (data not shown).

PGE_2 (produced by COX-2) decreases cell death and regulates cultured tumor cell proliferation (83). Inhibition of PGE_2 production by sulindac (84) has a marked inhibitory effect on the development of colon tumors in mice (85). In addition, PGE_2 induces Bcl-2 expression in human colon cancer cells (14). COX-2 inhibition by NS-398 induces apoptosis with a lower Bcl-2 protein level in human prostate cancer cells (18). Gao *et al.* (67) reported that Bcl-2 and COX-2 (but not p53) might play a role in the early genesis/progression of a gastric carcinoma. Therefore, PGE_2 might be involved in cell growth and proliferation and enhance the tumorigenic potential in some cancer cells. In B-cell chronic lymphocytic leukemia, the level of anti-apoptotic Bcl-2 shows a positive correlation with the Ku80 level (86). Ku70 and Ku80 expression is higher in aggressive breast tumors compared with normal tissue (87). These studies sug-

gest a possible relationship among PGE_2 produced by COX-2; the levels of Bcl-2, Ku70, and Ku80; and cancer cell proliferation. It is suggested that the gastric cell hyperproliferation associated with carcinogenesis might be associated with both high expression and high nuclear levels of Ku70 and Ku80 in a COX-2-dependent mechanism, which is mediated by NF-κB activation in gastric cancer cells. Further study should focus on the action mechanism of COX-2 and its products in Ku70 and Ku80 expression and the possible mechanism and mediator(s) that induce cell proliferation by Ku70 and Ku80 in gastric cancer cells.

REFERENCES

1. Baeuerle, P. A., and Baltimore, D. (1996) *Cell* **87,** 13–20
2. Thanos, D., and Maniatis, T. (1995) *Cell* **80,** 529–532
3. Wu, M., Lee, H., Bellas, R. E., Schauer, S. L., Arsura, M., Katz, D., FitzGerald, M. J., Rothstein, T. L., Sherr, D. H., and Sonenshein, G. E. (1996) *EMBO J.* **15,** 4682–4690
4. Sovak, M. A., Arsura, M., Zanieeski, G., Kavanagh, K. T., and Sonenshein, D. (1999) *Cell Growth & Differ.* **10,** 537–544
5. Kim, H., Seo, J. Y., and Kim, K. H. (1999) *Pharmacology* **59,** 201–211
6. Kim, H., Seo, J. Y., and Kim, K. H. (2000) *Dig. Dis. Sci.* **45,** 621–628
7. Wulczyn, F. G., Krappmann, D., and Scheidereit, C. (1996) *J. Mol. Med.* **74,** 749–769
8. Siebenlist, U., Franzoso, G., and Brown, K. (1994) *Annu. Rev. Cell Biol.* **10,** 405–455
9. Battu, S., Chable-Rabinovitch, H., Rigaud, M., and Beneytout, J. L. (1998) *Anticancer Res.* **18,** 2397–2403
10. Sheng, G. G., Shao, J., Sheng, H., Hooton, E. B., Isakson, P. C., Morrow, J. D., Coffey, R. J., Jr., DuBois, R. N., and Beauchamp, R. D. (1997) *Gastroenterology* **113,** 1883–1891
11. Lim, J. W., Kim, H., and Kim, K. H. (2001) *Lab. Invest.* **81,** 349–360
12. Molina, M. A., Sitja-Arnau, M., Lemoine, M. G., Frazier, M. L., and Sinicrope, F. A (1999) *Cancer Res.* **59,** 4356–4362
13. Higashi, Y., Kanekura, T., and Kanzaki, T. (2000) *Int. J. Cancer* **86,** 667–671
14. Sheng, H., Shao, J., Morrow, J. D., Beauchamp, R. D., and DuBois, R. N. (1998) *Cancer Res.* **58,** 362–366
15. Sawaoka, H., Kawano, S., Tsuji, S., Tsujii, M., Gunawan, E. S., Takei, Y., Nagano, K., and Hori, M. (1998) *Am. J. Physiol.* **274,** G1061–G1067
16. Battu, S., Rigaud, M., and Beneytout, J. L. (1998) *Anticancer Res.* **18,** 3579–3583
17. Erickson, B. A., Longo, W. E., Panesar, N., Mazuski, J. E., and Kaminski, D. L. (1999) *J. Surg. Res.* **81,** 101–107
18. Liu, X. H., Yao, S., Kirschenbaum, A., and Levine, A. C. (1998) *Cancer Res.* **58,** 4245–4249
19. Kinoshita, T., Takahashi, Y., Sakashita, T., Inoue, H., Tanabe, T., and Yoshimoto, T. (1999) *Biochim. Biophys. Acta* **1438,** 120–130
20. Tjandrawinata, R. R., Dahiya, R., and Hughes-Fulford, M. (1997) *Br. J. Cancer* **75,** 1111–1118
21. Hida, T., Leyton, J., Makheja, A. N., Ben-Av, P., Hla, T., Martinez, A., Mulshine, J., Malkani, S., Chung, P., and Moody, T. W. (1998) *Anticancer Res.* **18,** 775–782
22. Bhuyan, B. K., Adams, E. G., Badiner, G. J., Li, L. H., and Barden, K. (1986) *Cancer Res.* **46,** 1688–1693
23. Seibert, K., Zhang, Y., and Leahy, K. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 12013–12017
24. Seibert, K., Masferrer, J., Zhang, Y., Gregory, S., Olson, G., Hauser, S., Leahy, K., Perkins, W., and Isakson, P. (1995) *Agents Actions* **46,** 41–50
25. Mimori, T., and Hardin, J. A. (1986) *J. Biol. Chem.* **261,** 10375–10379
26. Rathmell, W. K., and Chu, G. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7623–7627
27. Dvir, A., Peterson, S. R., Knuth, M. W., Lu, H., and Dynan, W. S. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 11920–11924
28. Gottlieb, T. M., and Jackson, S. P. (1993) *Cell* **72,** 131–142
29. Finnie, N. J., Gottlieb, T. M., Blunt, T., Jeggo, P. A., and Jackson, S. P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 320–324
30. Lees-Miller, S. P., Godbout, R., Chan, D. W., Weinfeld, M., Day, R. T. S., Barron, G. M., III, and Allaunits-Turner, T. (1995) *Science* **267,** 1183–1185
31. Peterson, S. R., Kurimasa, A., Oshimura, M., Dynan, W. S., Bradbury, E. M., and Chen, D. J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 3171–3174
32. Blier, P. R., Griffith, A. J., Craft, J., and Hardin, J. A. (1993) *J. Biol. Chem.* **268,** 7594–7601
33. Jin, S., Inoue, S., and Weaver, D. T. (1997) *Cancer Surv.* **29,** 221–261
34. Smith, G. C., and Jackson, S. P. (1999) *Genes Dev.* **3,** 916–934
35. Taccioli, G. E., Gottlieb, T. M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W., Jackson, S. P., and Jeggo, P. A. (1994) *Science* **265,** 1442–1445
36. Woo, R. A., McLure, K. G., Lees-Miller, S. P., Rancourt, D. E., and Lee, P. W. (1998) *Nature* **394,** 700–704
37. Liu, L., Kwak, Y. T., Bex, F., Garcia-Martinez, L. F., Li, X. H., Meek, K., Lane, W. S., and Gaynor, R. B. (1998) *Mol. Cell. Biol.* **18,** 4221–4234
38. Um, J. H., Kang, C. D., Lee, B. G., Kim, D. W., Chung, B. S., and Kim, S. H. (2001) *Oncogene* **20,** 6048–6056
39. Nussenzweig, A., Sokol, K., Burgman, P., Li, L., and Li, G. C. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 13588–13593
40. Nussenzweig, A., Chen, C., da Costa Soares, V., Sanchez, M., Sokol, K., Nussenzweig, M. C., and Li, G. C. (1996) *Nature* **382,** 551–555
41. Sadji, Z., Le Romancer, M., Lewin, M. J., and Reyl-Desmars, F. (2000) *Cell. Signal.* **12,** 745–750
42. Li, G., Nelsen, C., and Hendrickson, E. A. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 832–837
43. Gu, Y., Seidl, K. J., Rathbun, G. A., Zhu, C., Manis, J. P., van der Stoep, N., Davidson, L., Cheng, H. L., Sekiguchi, J. M., Frank, K., Stanhope-Baker, P., Schlissel, M. S., Roth, D. B., and Alt, F. W. (1997) *Immunity* **7,** 653–665
44. Ouyang, H., Nussenzweig, A., Kurimasa, A., da Costa Soares, V., Li, X., Cordon-Cardo, C., Li, W., Cheong, N., Nussenzweig, M., Iliakis, G., Chen, D. J., and Li, G. C. (1997) *J. Exp. Med.* **186,** 921–929
45. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
46. Zhao, Y. L., Piao, C. Q., Wu, L. J., Suzuki, M., and Hei, T. K. (2000) *Carcinogenesis* **21,** 2005–2010
47. Mimori, T., Ohosone, Y., Hama, N., Suwa, A., Akizuki, M., Homma, M., Griffith, A. J., and Hardin, J. A. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 1777–1781
48. Nakajima-Iijima, S., Hamada, H., Reddy, P., and Kakunaga, T. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82,** 6133–6137
49. Lipart, C., and Renault, T. (2002) *J. Virol. Methods* **101,** 1–10
50. Kim, S. H., Um, J. H., Dong-Won, B., Kwon, B. H., Kim, D. W., Chung, B. S., and Kang, C. D. (2000) *Leuk. Res.* **24,** 917–925
51. Li, J. J., Westergaard, C., Ghosh, P., and Colburn, N. H. (1997) *Cancer Res.* **57,** 3569–3576
52. Yang, X., Bialik, S., Jones, B. E., Limuro, Y., Kitsis, R. N., Srinivasan, A., Brenner, D. A., and Czaja, M. J. (1998) *Am. J. Physiol.* **275,** C1058–C1066
53. Tallarida, R. J., and Murray, R. B. (1987) *Manual of Pharmacologic Calculations with Computer Programs*, pp. 121–125, Springer-Verlag New York Inc., New York
54. Marangoni, E., Foray, N., O'Driscoll, M., Douc-Rasy, S., Bernier, J., Bourhis, J., and Jeggo, P. (2000) *Nucleic Acids Res.* **28,** 4778–4782
55. Osipovich, O., Duhe, R. J., Hasty, P., Durum, S. K., and Muegge, K. (1999) *Biochem. Biophys. Res. Commun.* **261,** 802–807
56. Wu, X., and Lieber, M. R. (1996) *Mol. Cell. Biol.* **16,** 5186–5193
57. Cary, R. B., Chen, F., Shen, Z., and Chen, D. (1998) *Nucleic Acids Res.* **26,** 974–979
58. Factor, V., Oliver, A. L., Panta, G. R., Thorgeirsson, S. S., Sonenshein, G. E., and Arsura, M. (2001) *Hepatology* **34,** 32–41
59. Tai, D. I., Tsai, S. L., Chang, Y. H., Huang, S. N., Chen, T. C., Chang, K. S., and Liaw, Y. F. (2000) *Cancer* (*Phila.*) **89,** 2274–2281
60. Jo, H., Zhang, R., Zhang, H., McKinsey, T. A., Shao, J., Beauchamp, R. D., Ballard, D. W., and Liang, P. (2000) *Oncogene* **19,** 841–849
61. Rayet, B., and Gelinas, C. (1999) *Oncogene* **18,** 6938–6947
62. Bargou, R. C., Emmerich, F., Krappmann, D., Bommert, K., Mapara, M. Y., Arnold, W., Royer, H. D., Grinstein, E., Greiner, A., Scheidereit, C., and Dorken, B. (1997) *J. Clin. Invest.* **100,** 2961–2969
63. Barkett, M., and Gilmore, T. D. (1999) *Oncogene* **18,** 6910–6924
64. Bureau, F., Vanderplasschen, A., Jaspar, F., Minner, F., Pastoret, P. P., Merville, M. P., Bours, V., and Lekeux, P. (2002) *Blood* **99,** 3683–3691
65. Sasaki, N., Morisaki, T., Hashizume, K., Yao, T., Tsuneyoshi, M., Noshiro, H., Nakamura, K., Yamanaka, T., Uchiyama, A., Tanaka, M., and Katano, M. (2001) *Clin. Cancer Res.* **7,** 4136–4142
66. Sung, J. J., Leung, W. K., Go, M. Y., To, K. F., Cheng, A. S., Ng, E. K., and Chan, F. K. (2000) *Am. J. Pathol.* **157,** 729–735
67. Gao, H. J., Yu, L. Z., Bai, J. F., Peng, Y. S., Sun, G., Zhao, H. L., Miu, K., Lu, X. Z., Zhang, X. Y., and Zhao, Z. Q. (2000) *World J. Gastroenterol.* **6,** 848–854
68. Narumiya, S., Sugimoto, Y., and Ushikubi, F. (1999) *Physiol. Rev.* **79,** 1193–1226
69. Pai, R., Soreghan, B., Szabo, I. L., Pavelka, M., Baatar, D., and Tarnawski, A. S. (2002) *Nat. Med.* **8,** 289–293
70. Resnick, M. B., Gallinger, S., Wang, H. H., and Odze, R. D. (1995) *Cancer* (*Phila.*) **76,** 187–194
71. Shimada, N., Suzuki, J., Fujita, J., Kawakami, Y., Tsukakoshi, H., Hosokawa, M., and Sato, N. (1998) *J. Gastroenterol. Hepatol.* **13,** 794–800
72. Malecka-Panas, E., Kordek, R., Biernat, W., Tureaud, J., Liberski, P. P., and Majumdar, A. P. (1997) *Hepatogastroenterology* **44,** 435–440
73. Mendelsohn, J., and Fan, Z. (1997) *J. Natl. Cancer Inst.* **89,** 341–343
74. Haimovitz-Friedman, A., Vlodavsky, I., Chaudhuri, A., Witte, L., and Fuks, Z. (1991) *Cancer Res.* **51,** 2552–2558
75. Aaronson, S. A. (1991) *Science* **254,** 1146–1153
76. Kumar, R., and Mendelsohn, R. (1991) *Curr. Opin. Oncol.* **3,** 70–74
77. Bandyopadhyay, D., Mandal, M., Adam, L., Mendelsohn, J., and Kumar, R. (1998) *J. Biol. Chem.* **273,** 1568–1573
78. Tuteja, R., and Tuteja, N. (2000) *Crit. Rev. Biochem. Mol. Biol.* **35,** 1–33
79. Ghoshal, K., Li, Z., and Jacob, S. T. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 10390–10395
80. Gao, Y., Chaudhuri, J., Zhu, C., Davidson, L., Weaver, D. T., and Alt, F. W. (1998) *Immunity* **9,** 367–376
81. Wang, J., Dong, X., Myung, K., Hendrickson, E. A., and Reeves, W. H. (1998) *J. Biol. Chem.* **273,** 842–848
82. Gu, Y., Sekiguchi, J., Gao, Y., Dikkes, P., Frank, K., Ferguson, D., Hasty, P., Chun, J., and Alt, F. W. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 2668–2673
83. Raz, A. (2002) *Biochem. Pharmacol.* **63,** 343–347
84. Taylor, M. T., Lawson, K. R., Ignatenko, N. A., Marek, S. E., Stringer, D. E., Skovan, B. A., and Gerner, E. W. (2000) *Cancer Res.* **60,** 6607–6610
85. Moorghen, M., Orde, M., Finney, K. J., Appleton, D. R., and Watson, A. J. (1998) *Cell Proliferation* **31,** 59–70
86. Klein, A., Miera, O., Bauer, O., Golfier, S., and Schriever, F. (2000) *Leukemia* (*Baltimore*) **14,** 40–46
87. Pucci, S., Mazzarelli, P., Rabitti, C., Giai, M., Gallucci, M., Flammia, G., Alcini, A., Altomare, V., and Fazio, V. M. (2001) *Oncogene* **20,** 739–747

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