Ku Autoantigen Affects the Susceptibility to Anticancer Drugs

Sun Hee Kim, Dooha Kim, Jeong Sil Han, Choon Sik Jeong, Byung Seon Chung, Chi Dug Kang, and Gloria C. Li

ABSTRACT

The Ku70/80 autoantigens (Ku) are the DNA-binding components of a DNA-dependent protein kinase (PK) involved in DNA double strand breaks repairing a V(D)J recombination. Because apoptosis is associated with DNA fragmentation and, consequently, creation of double strand breaks, and a variety of DNA-damaging drugs kill tumor cells by apoptosis, we tested the impact of Ku deficiency on the sensitivity of anticancer drugs. Ku-null mutant cell lines Ku70−/− and Ku80−/− were highly sensitive to anticancer drugs, compared with their wild-type cells. Ku-deficient cells were more sensitive to bleomycin-induced DNA fragmentation and exhibited a higher level of c-jun NH2-kinase-stress-activated PK activity than wild-type cells, whereas R7080-6 cells overexpressing both human Ku70 and Ku80 were resistant to bleomycin-induced apoptosis and exhibited a lower level of c-jun NH2-kinase-stress-activated PK activity. The Ku-protein level and Ku DNA binding activity were decreased after treatment with bleomycin, adriamycin, or vincristine, and the decreases were blocked by the treatment of z-DEVD-fmk, a specific inhibitor of caspase-3, suggesting that loss of Ku DNA binding is, in part, due to a caspase-mediated decrease in Ku protein levels. By contrast, HSF1 DNA-binding activity was increased by the treatment of these anticancer drugs and, simultaneously, mitochondrial heat shock protein HSP70 was specifically induced. Our data suggest that Ku can affect the susceptibility to anticancer drug-induced apoptosis.

INTRODUCTION

Apoptosis is a major form of cell death, characterized by a series of distinct morphological changes in cell structure, and can be activated by a wide variety of extra- and intracellular signals (1–4). Resistance to apoptosis is a frequent characteristic of cancer cells and participates in the initial phase of carcinogenesis and in the development of chemotherapy resistance (5). On the basis of this concept, control of apoptosis has emerged as an important strategy for clinical cancer therapy (6).

DNA DSBs3 may be the most disruptive form of DNA damage and are generated by ionizing radiation and certain anticancer drugs (7, 8). For example, bleomycin promotes oxidative-free radicals, which induce strand breaks, and etoposide and adriamycin inhibit topoisomerase II to create protein-bridged DSBs. DNA-PK has a well-established role in the repair of DSBs (9–12). DNA-PK consists of two components, a 460-kDa catalytic subunit (DNA-PKcs) and a 70-kDa (Ku70) and 86-kDa (Ku80) heterodimeric regulatory component. Ku is involved in the repair of DNA DSBs produced by ionizing radiation, V(D)J recombination, or etoposide (13). It has been proposed that Ku, which is identical to constitutive HSE binding factor, exhibits inverse correlation with the HSE binding activity of HSF1, known as transcription factor of HS genes, indicating that Ku may be involved in the regulation of HS gene expression (14).

In mammalian cells, members of HSP70 family include the cognate form HSC70 (73-kDa) and heat-inducible HSP70 (72-kDa) localized in cytosol along with organelles-resident members, GRP78 in the endoplasmic reticulum lumen and HSP75 in mitochondrial matrix. GRP78 and HSP75 are induced by non-HS stressors, such as accumulation of unfolded or misfolded proteins and disruption in calcium homeostasis (15). Although there are some evidences that HSFs prevent apoptosis, the mechanism of HSF-mediated protection from apoptosis has not been fully evaluated. HSP70 has been suggested to exert its protective action by interfering with the stress-induced apoptotic program (16–18). Overexpression of HSP70 was shown to inhibit the apoptotic process, and antisense inhibition of HSP70 expression induces apoptosis in the acute T-lymphocytic leukemia cell line (19). Targeted gene disruption of HSP70 also results in germ cell apoptosis (20). The ability of HSP70 to prevent stress-induced apoptosis could limit the efficacy of cancer therapy. Expression of HSP70 has enhanced resistance to several anticancer drugs, many of which induce apoptotic cell death (21). HSP70 interferes with the activation of JNK/SAPK, which can be stimulated by hyperthermia, UV, ionizing radiation, oxidative stress, and cytotoxic drugs and mediates the apoptotic process, thus preventing apoptotic signaling (22–25).

Mitochondria are one of the targets of anticancer drugs. Some anticancer drugs preferentially attack mitochondrial DNA and lead to mitochondrial dysfunction that contributes to cell damage and death (26, 27). It has been reported that toxic effects induced by anticancer drugs, including cisplatin (28, 29), bleomycin (30), 5-fluorouracil, and methotrexate (31), may be related to damage of mitochondrial DNA, raising the possibility that exposure of tumor cells to some anticancer drugs could modulate mitochondrial HSP75 to protect mitochondria.

In this study, we provided evidences that Ku might oppose anticancer drug induced-apoptosis. Furthermore, during this process, differential regulation of HSP70 and HSP75 expression and activation of JNK/SAPK might play a role in anticancer drug-induced apoptosis.

MATERIALS AND METHODS

Cell Culture.

The human HeLa cervical epithelial carcinoma cells were maintained in DMEM supplemented with 5% (v/v) fetal bovine serum and antibiotics. The mouse embryonic stem ES and Ku-deficient Ku70−/− and Ku80−/− cells were (32, 33) immortalized by SV40 transfection. Rat fibroblast Rat-1 cells were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum and antibiotics. R7080-6 cells, which constitutively overexpress both human Ku70 and Ku80 in Rat-1 cells, were maintained in the medium containing hygromycin (100 μg/ml) and G418 (200 μg/ml; Ref. 34).

Gel Mobility Shift Analysis.

Preparation of cell and the gel mobility-shift assays were performed as described (35). Equal amounts of cellular proteins (20 μg for HeLa cells and 50 μg for ES and Ku70−/− cells) were incubated with a 32P-labeled double-stranded oligonucleotide (5′-GGGGCAAGAATCT-TAGCAGTTTCG-3′) containing the HSE from the rat HSP70 promoter. The protein-bound and free oligonucleotides were electrophoretically separated on 4.5% native polyacrylamide gels in 0.5 × TBE buffer [44.5 mM Tris (pH 8.0), 1 mM EDTA, and 44.5 mM boric acid] for 3 h at 120 V. The gels were dried and autoradiographed. For gel mobility supershift assay, 1 μg of polyclonal antibody specific to HSF1 was incubated with whole cell extracts for 20 min at 21°C before the HSF-HSE binding reaction (36).

Assay of JNK/SAPK Activity. The JNK/SAPK activity was measured with SAPK/JNK Assay Kit (New England Biolabs, Inc.), according to the manufacturer’s protocol. Cells were lysed in 20 mM Tris (pH 7.4), 150 mM...
NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium PPI, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride for 15 min at 4°C. The lysates were centrifuged at 10,000 × g for 15 min at 4°C, and the supernatants were used as cell lysates. Cell lysates (250 μl; ~250 μg total protein) were mixed with 2 μg of glutathione S-transferase-c-Jun fusion protein beads and incubated with gentle rocking overnight at 4°C. The lysates were centrifuged for 30 s at 4°C, and then the pellet was washed twice with 500 μl of lysis buffer and twice with kinase buffer [20 mM Tris (pH 7.5), 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na3VO4, and 10 mM MgCl2] on ice. The pellets were suspended with kinase buffer supplemented with 100 μM ATP and incubated for 30 min at 30°C. The reactions were terminated with 25 μl of 3× sample buffer [62.5 mM Tris (pH 6.8), 2% (w/v) SDS, 10% glycerol, 50 mM DTT, and 0.1% (w/v) bromphenol blue], and the samples were analyzed by 12% SDS PAGE. Phospho (Ser63)-c-jun was detected with specific antibody by Western blot analysis.

Growth Inhibition Assay. Cells (4 × 103 cells/well) were plated in triplicate into 96-well microtiter plates in 200 μl of growth medium containing serial 10-fold dilutions of anticancer drugs. Cells were incubated for 96 h, after which growth inhibition was determined by measuring of the cell number/well. The cell numbers were counted using a Coulter counter. The concentration of each anticancer drug, which reduced cell growth by 50% after 96 h of treatment (IC50), was determined from the growth inhibition plots.

Analysis of Internucleosomal DNA Fragmentation. After drug treatments, cells were harvested and DNA was isolated, as described (37). Briefly, 1 × 106 cells were lysed in 100 μl of lysis buffer containing 0.5% SDS and 1.2 mg/ml proteinase K and incubated at 50°C for 3 h. RNase A (0.2 mg/ml) was added and incubated at 37°C overnight. DNA was extracted with phenol/ chloroform (1:1, v/v) and precipitated with ethanol. The dried pellet was resuspended in TE buffer [10 mM Tris (pH 8.0) and 1 mM EDTA]. DNA concentration was determined spectrophotometrically. DNA (20 μg) was subjected to electrophoresis in 1.5% agarose and visualized by staining with ethidium bromide.

Western Blot Analysis. Protein samples (20 μg for HeLa cells and 50 μg for other cell lines) were separated by SDS-PAGE and blotted to nitrocellulose membrane. The membrane was incubated with antibody as specified, followed by secondary antibody conjugated with horseradish peroxidase. Specific antigen-antibody complexes were detected by enhanced chemiluminescence (Pierce Chemical Co.). The following antibodies were used in these studies. The affinity purified polyclonal antibody specific to the 70- and 86-kDa Ku protein and affinity purified rabbit serum for HSF1 were prepared, as described previously (34). The antibodies specific to HSP70 and HSC70 were from Stress Gen, and anti-HSP75 antibody was provided by Dr. T. Li (Northwestern University, Evanston, IL). The anti-PARP antibody and anticaspase-3 (CPP32) antibody were from Santa Cruz Biotechnology. Secondary antibodies were from Boehringer Mannheim.

RESULTS

Effects of Ku on the Anticancer Drug Sensitivity and Antican-
cer Drug-induced Apoptosis. Absence of Ku autoantigen (Ku) has been reported to confer radiation hypersensitivity and deficiency in DNA DSB repair (10–12). Therefore, to test the impact of Ku deficiency on the sensitivity against anticancer drugs, Ku-deficient and wild-type cells were treated with various concentrations of anticancer drugs for 96 h, and growth inhibition was determined by measuring the number of cells/well. Table 1 demonstrates the IC50 of anticancer drugs and drug sensitization ratio in Ku70−/−, Ku80−/−, and wild-type cells. Ku-deficient Ku70−/− and Ku80−/− cells were 25.6- and 13.9-fold more sensitive to bleomycin and 13.8- and 9-fold more sensitive to adriamycin than wild-type cells, respectively. On the other hand, drug sensitization effects against vincristine, Taxol, or VP-16 in Ku-deficient cells were about 3–6-fold higher than the wild-type cells. By contrast, R7080-6 cells overexpressing both human Ku70 and Ku80 showed 3.6- or 2.2-fold more resistance to bleomycin or vincristine than their parental Rat-1 cells (data not shown). These findings are of particular interest because absence of Ku results in cellular hypersensitivity to mechanistically distinct anticancer drugs that damage either DNA or microtubules and suggest the possibility that Ku plays an important role in modulation of anticancer drug sensitivity.

It has been known that many anticancer drugs could induce apoptosis in various cells (6, 20–22). To investigate, we compared DNA fragmentation in Ku-deficient or Ku-overexpressing cells after exposure to bleomycin in doses ranging from 1–10 μg/ml. The Ku70−/− cells seemed to be more susceptible to bleomycin-induced DNA fragmentation compared with wild-type cells (Fig. 1A). In contrast, R7080-6 cells were more resistant to bleomycin-induced DNA fragmentation than their parental Rat-1 cells (Fig. 1B). These findings indicate that Ku might play a protective role against anticancer drug-induced apoptosis.

Table 1 Comparison of IC50 values of anticancer drugs in Ku-deficient and wild type (wt) cells.

The concentration of each anticancer drug, which reduced cell growth by 50% after 96 h of treatment (IC50) was determined from growth inhibition plots. Values are the average of (at least) two independent experiments and triplicate determinants in each experiment.

<table>
<thead>
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<th>Drugs</th>
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<th>IC50 (ng/ml)</th>
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<td></td>
<td>Ku80−/−</td>
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<td>13.8</td>
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wt, wild type; BLM, bleomycin; ADR, adriamycin; VCR, vincristine.
Fig. 1. Effect of Ku on bleomycin-induced internucleosomal DNA fragmentation. Ku70−/− and its wild-type (WT) cells (A) or R7080-6 and parental Rat-1 cells (B) were exposed to the indicated doses of bleomycin (BLM) for 24 h. Subsequently, cells were harvested and lysed. Purified DNA fragmentation from the supernatant of lysed cells were electrophoresed in agarose gels and detected by ethidium bromide staining.

Fig. 2. Effects of bleomycin on HSF- and Ku-DNA binding activity, level of HSPs. A, HeLa cells (1 × 10⁶ cells/ml) were treated with the indicated doses of bleomycin (BLM) for 6 h. Whole-cell extracts were prepared and subjected to gel mobility shift assay (top) or Western blot analysis with anti-HSF75 or HSC70 antibody (bottom). B, electromobility supershift assay of HSF-HSE complexes from bleomycin-treated HeLa cells and bleomycin-induced phosphorylation. The cells (1 × 10⁶ cells/ml) were untreated (C; control) or treated with bleomycin (BLM; 10 μg/ml, 6 h), or HS (45°C for 15 min). Whole cell extracts were preincubated with anti-HSF1 polyclonal antibody, and gel mobility super-shift assay was performed (top). Phosphorylation states of HSF1 after exposure to BLM or HS were determined by Western blot analysis with anti-HSF1 antibody (bottom).

Fig. 3. Modulation of HSF- or Ku-DNA binding activity by anticancer drugs and recovery of Ku-DNA binding activity after removal of vincristine (VCR), adriamycin (ADR), or bleomycin (BLM). HeLa cells (1 × 10⁶ cells/ml) were exposed to 4 μg/ml ADR (A, left), 0.4 μM VCR (A, right), or 20 μg/ml BLM (B) for 6 h and recovered in fresh medium without drug for indicated periods. Whole cell extracts prepared from drug-treated cells were subjected to gel mobility shift assay (top) or Western blot analysis by using anti-HSF75 or HSP70 antibody (bottom).

Fig. 4. Effect of vincristine on HSF-DNA binding activity in Ku-deficient cells and modulation of HSP75 level by Ku. Wild-type (WT) and Ku-deficient cells (1 × 10⁶ cells/ml) were treated with 0.2 μM vincristine for the indicated time periods. Whole cell extracts were prepared and subjected to gel mobility shift assay (A) or Western blot analysis by using anti-HSF75 or HSP75 antibody (B, left). Rat-1 and R7080-6 cells were treated with 0.4 μM vincristine for the indicated time periods, and the HSP75 or HSC70 level was determined by the Western blot analysis with anti-HSF75 or HSP75 antibody (B, right). HSC70 levels are shown as reference control.

Modulation of Anticancer Drug-Induced Apoptosis by Ku

The Decrease of Ku Protein Level and Ku-DNA Binding Activity during the Anticancer Drug-Induced Apoptosis. Because Ku is involved in repairing DNA DSBs, it has been suggested that Ku might regulate the apoptotic cell death, acting as an inhibitor of phenomena are general responses to anticancer drugs, we tested the effect of other drugs on Ku- and HSF1-DNA binding activity and HSP75 level. When HeLa cells were treated with 4 μg/ml adriamycin or 0.4 μM vincristine for 4–6 h, the decrease of Ku-DNA binding activity and the concurrent increase of HSF-DNA binding activity were found. At this time, the level of HSP75 was increased, but the level of HSP70 was decreased. (Fig. 3A), and these were followed by the delayed onset of decrease of Ku proteins (data not shown). The decreased Ku-DNA binding activity and the concomitantly increased HSF-DNA binding activity by the treatment of adriamycin or vincristine were gradually recovered (starting at 4 h after removal of the drug), and the up-regulated HSP75 level was turned down dramatically (Fig. 3B). Therefore, these results suggest that the anticancer drug-induced apoptotic cell death could be accompanied by the decreased Ku-DNA binding activity and the concurrently increased HSF-DNA binding activity, and subsequent induction of HSP75 was closely correlated with the Ku- and HSF-DNA binding activity. To confirm the correlation between inactivation of Ku-DNA binding activity and induction of HSP75, the modulation of HSP75 level by Ku was examined in Ku-deficient and Ku-overexpressing cells after treatment with vincristine. Ku70−/− cells exhibited deficiency of Ku-DNA binding activity, but the basal and vincristine-induced HSF-DNA binding activities and HSP75 levels were higher in Ku-deficient cells than in wild-type cells. (Fig. 4, A and B). In contrast, the basal and vincristine-induced HSP75 levels were down-regulated in Ku-overexpressing R7080-6 cells as compared with those in Rat-1 cells (Fig. 4B), indicating that the expression level of HSP75 is correlated negatively to the Ku-DNA binding activity.
apoptosis, and, by contrast, degradation of Ku may help to prevent the inappropriate repair of fragmented nuclear DNA during apoptosis (42, 43). However, other studies have shown that DNA-PKcs, the catalytic subunit of DNA-PK, is preferentially degraded during apoptosis, but Ku is neither cleaved nor decreased during apoptosis (44, 45). Here, we examined whether the decrease of Ku-DNA binding activity by anticancer drug is due to the decreased level of Ku protein. The vinblastine-induced decrease of Ku-DNA binding activity was associated with decreased levels of Ku proteins in HeLa cells (Fig. 5A). We also tested the effect of other anticancer drugs on the level of Ku protein and PARP degradation. In vincristine- or adriamycin-treated HeLa cells, the level of Ku protein was decreased in dose-dependent manner, and this was associated with proteolytic cleavage of PARP, which is a substrate of caspase-3 and consequently cleaved during apoptosis (Fig. 5B). To examine whether decrease in Ku proteins by anticancer drug could be prevented by caspase-3 inhibitor, HeLa or R7080-6 cells were treated with bleomycin (1.25–10 μg/ml) for 24 h in the absence or presence of z-DEVD-fmk, a specific caspase-3 inhibitor. HeLa and R7080-6 cells treated with bleomycin (10 μg/ml) exhibited decreased levels of Ku proteins, but the bleomycin-induced decrease in Ku proteins was blocked by pretreatment of z-DEVD-fmk in a dose-dependent manner (Fig. 6, A and B). We also tested the effect of z-DEVD-fmk on the decrease of Ku-DNA binding activity by anticancer drugs. z-DEVD-fmk pretreatment prevented the bleomycin-induced decrease of Ku-DNA binding activity in HeLa cells (Fig. 7, top). Similar results were obtained in vincristine- or adriamycin-treated HeLa cells (Fig. 7, middle and bottom). These results suggest that the Ku protein level is decreased due to proteolytic cleavage after treatment of anticancer drugs, and thereby the appropriate repair of fragmented nuclear DNA would be prevented during apoptosis, leading to apoptotic cell death.

Comparison of JNK/SAPK Activity in Ku-deficient or Ku-overexpressing Cells. It is well known that the activation of JNK/SAPK mediates the apoptotic process after various stimuli, including cytotoxic drugs (22–24). To elucidate whether the altered susceptibility to apoptosis in Ku-deficient or Ku-overexpressing cells is associated with altered activity of JNK/SAPK, we examined JNK/SAPK activity in vincristine-treated cells by immunocomplex kinase assay using GST-c-Jun as substrate. The basal and anticancer drug-stimulated activities of JNK/SAPK in Ku-deficient cells were significantly higher than those in wild-type cells, even though JNK/SAPK protein levels were not altered in these cells (Fig. 8A). Conversely, R7080-6 cells, which were more resistant to drug-induced apoptosis, exhibited lower level of basal and anticancer drug-stimulated JNK/SAPK activity than Rat-1 cells (Fig. 8B). These findings suggest that Ku might affect the susceptibility to anticancer drug-induced apoptosis, in part, via modulation of JNK/SAPK pathway.

Fig. 5. Effects of anticancer drugs on Ku proteins and PARP degradation. A, HeLa cells (1 × 10⁶ cells/ml) were treated with the indicated doses of vinblastine (VBL) for 6 h. Whole cell extracts from drug-treated cells were subjected to gel mobility shift assay (top) or Western blot analysis by using anti-Ku70 and Ku80 antibodies (middle) and HSC70 antibody (bottom). B, whole cell extracts from HeLa cells treated with the indicated doses of vincristine (VCR; left) or adriamycin (ADR; right) for 16 h were subjected to western blot analysis for determination of the levels of Ku proteins and cleavage of PARP.

Fig. 6. Suppression of Ku protein level by bleomycin and its reversion by z-DEVD-fmk. HeLa (A) and R7080-6 (B) cells (1 × 10⁶ cells/ml) were treated with the indicated doses of bleomycin (BLM) for 24 h (left) or were preincubated in the absence or presence of the indicated doses of z-DEVD-fmk for 6 h, and then were treated with or without 10 μg/ml BLM for an additional 24 h (A, middle and right, and B, right). Whole cell extracts were subjected to Western blot analysis by using anti-Ku protein, anti-HSC70 antibody, or anti-caspase-3 antibody. A, right, demonstrated that z-DEVD-fmk inhibit effectively the cleavage of procaspase-3 (CPP32).

Fig. 7. Suppression of Ku-DNA binding activity by anticancer drugs and its reversion by z-DEVD-fmk. HeLa cells (1 × 10⁶ cells/ml) were preincubated in the absence or presence of the indicated doses of z-DEVD-fmk for 6 h before treatment with 2 μg/ml bleomycin (BLM), 0.4 μg vincristine (VCR), or 4 μg/ml adriamycin (ADR) for an additional hour. Whole cell extracts were prepared and were subjected to gel mobility shift assay.

Fig. 8. Comparison of JNK/SAPK activity in Ku-deficient or Ku-overexpressing cells. Ku70⁻/⁻, Ku80⁻/⁻, and wild-type (W) cells (A) or R7080-6 and parental Rat-1 cells (B; 1 × 10⁶ cells/ml) were treated with 0.4 μM vincristine (VCR) for 30 min. Activity and amount JNK/SAPK were determined with assay of JNK/SAPK activity as described in “Materials and Methods” (top) and Western blot analysis using anti-JNK antibody (bottom), respectively.
DISCUSSION

The Ku autoantigen is a heterodimer of 70 kDa (Ku70) and ~80 kDa (Ku80) subunits that are the DNA-binding component of the DNA-PK complex involved in DNA repair (9). The Ku70−/− embryonic stem cells have markedly increased sensitivity to γ-irradiation relative to Ku70+/+ or wild-type embryonic stem cells (10). In addition, defects in DNA-PK subunits have been shown to result in a reduced capacity to repair DNA DSBs and consequently confer sensitivity to X-rays (11). Because apoptosis could be induced by DNA damages, including DNA DSBs after exposure to genotoxic agents such as ionizing radiation and DNA-damaging anticancer drugs, and is associated with internucleosomal chromatin fragmentation and creation of dsDNA breaks, the suppression of DNA-PK activity during the initiation and execution phase of apoptosis will lead to augmentation of apoptotic process. Therefore, in the present study, we studied the role of Ku during the apoptotic cell death induced by anticancer drugs, including DNA-damaging and antimicrotubule agents.

Our results showed that Ku-deficient Ku70−/− and Ku80−/− cells were more sensitive to anticancer drugs than their wild-type cells in the growth inhibition and DNA fragmentation assays, and conversely R7080-6 cells overexpressing both Ku70 and Ku80 were less sensitive to drug-induced DNA fragmentation than the parental Rat-1 cells. These findings indicated that the sensitivity against DNA-damaging and antimicrotubule agents could be modulated by Ku expression level. However, it is obscure how Ku modulate the drug sensitivity against non-DNA-damaging agents, as well as DNA-damaging agents.

It has been proposed that Ku binds competitively with HSF to HSE (40). On HS, a rapid increase in the level of HSFI-DNA binding activity correlates with a rapid decrease in Ku-DNA binding activity with induction of HSP70 (39, 46). These findings suggest that Ku may be involved in the regulation of HS gene expression. There are several evidences that HSPs can modulate the apoptotic cell death processes. Overexpression of human HSP70 can protect cells from apoptotic stimuli (47). Also, in some human cancer cell lines, an elevated level of HSP70 is associated with resistance to apoptosis-inducing anticancer drugs (21). Conversely, down-regulation of HSP70 expression results in apoptosis (19, 20). Therefore, Ku may affect the drug sensitivity through regulation of HS gene expression. In the present study, during the anticancer drug-induced apoptosis, Ku-DNA binding activity was decreased, and concurrently HSE-binding activity and phosphorylation of HSFI were increased, and these results were followed by the up-regulation of HSP75 and down-regulation of HSP70. The anticancer drug-induced form of HSFI does not seem to undergo extensive phosphorylation, as does the heat-inducible form. Treatment of mammalian cells with salicylate or azetidine lead to activation of HSFI trimerization and DNA binding; however, salicylate-induced form of HSFI is not hyperphosphorylated like the heat-induced form, and this is correlated with a decreased level of HSP70 transcription (48, 49). Thus, the decreased level of HSP70 after exposure to anticancer drugs seemed to be associated with the partially phosphorylated HSFI.

HSP75, the function of which is not yet known, is a mitochondrial HSP. Recently, it has been shown that HSP75 is induced by amino acid deprivation (50) and ischemia (51), suggesting that the induction of HSP75 may represent a sensitive marker of stressed tissue. Therefore, anticancer drug-induced up-regulation of HSP75 seemed to be associated with the apoptotic process. However, the mechanism of up-regulation of HSP75 after exposure to anticancer drugs remained to be unraveled. Because HSP75 is not induced by heat stress, regulation of HSP75 expression is different from that of HSP70, which is strongly induced by HS. Our study showed that the induction of HSP75 paralleled the activation of HSFI-DNA binding activity and inhibition of Ku-DNA binding activity, and level of HSP75 in Ku-deficient cells was higher than that in their parental cells, suggesting that inhibition of Ku-DNA binding activity and/or the activation of HSFI-DNA binding activity induced by anticancer drugs are involved in the induction of HSP75.

In view of the role of Ku in double-strand DNA break repair, it has been suggested that Ku might prevent the apoptotic cell death after DNA damage, and, by contrast, degradation of Ku may help to prevent the inappropriate repair of fragmented nuclear DNA during apoptosis (42, 43). However, in other studies, it has been shown that DNA-PKcs, the catalytic subunit of DNA-PK, is preferentially degraded during apoptosis, but Ku level is not changed during apoptosis (44, 45). In the present study, we have shown that Ku protein levels were decreased after treatment of anticancer drugs and this was prevented by the pretreatment of z-DEVD-fmk, a specific caspase-3 inhibitor, suggesting that the Ku protein level is decreased due to proteolytic cleavage during the anticancer drugs-induced apoptosis, and thereby the inappropriate repair of fragmented nuclear DNA would be prevented during apoptosis, and the damaged cells lead to apoptotic cell death.

JNK/SAPK was recently shown as an essential component of a signal transduction pathway that leads to apoptosis in response to various inducers of apoptosis. In fact, overexpression of dominant negative mutant of JNK/SAPK-activating kinase, SEK1, inhibits apoptosis in response to HS, UV irradiation, oxidative stress, and other harmful factors (52, 53). Therefore, it was curious whether the altered susceptibility to apoptosis in Ku-deficient or Ku-overexpressing cells is associated with an altered activity of JNK/SAPK. Here, we showed that the activity of JNK/SAPK was increased in Ku-deficient cells and decreased in Ku-overexpressing cells. However, it is unknown how Ku modulates the activity of JNK/SAPK. Recently, it has been reported that the elevated level of HSP70 inhibits a signal transduction pathway leading to programmed cell death by preventing stress-induced activation of JNK/SAPK (18); in our study, increase of HSP75 and concurrent decrease of HSP70 was observed after treatment with anticancer drugs. Therefore, there are possibilities that after exposure to anticancer drugs the decreased HSP70 level might affect, in part, the activity of JNK/SAPK and consequently the susceptibility to anticancer drug-induced apoptosis, otherwise the increased HSP75 might be responsible.

Taken together, our results suggest that the modulation of the Ku level affects HSFI-DNA binding activity and subsequently the levels of HSP70 and HSP75, which might lead to the change in susceptibility to anticancer drug-induced apoptosis through the JNK/SAPK pathway.

REFERENCES


Unpublished data.
MODULATION OF ANTICANCER DRUG-INDUCED APOPTOSIS BY KU


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