Disruption of Microtubules Sensitizes the DNA Damage-induced Apoptosis Through Inhibiting Nuclear Factor κB (NF-κB) DNA-binding Activity

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INTRODUCTION

The nuclear factor κB (NF-κB) is a rapidly inducible transcription factor involved in regulating the expression of genes relevant in wide range of different cellular processes including apoptosis, cell survival and immune response. In most types of cells, inactive NF-κB is retained in the cytoplasm through its interaction with the inhibitory proteins known as IκBs. Upon activation by various stimuli, such as proinflammatory cytokines, ionizing radiation (IR), DNA damaging agents and cellular stress, IκBs are phosphorylated by the IκB kinase (IKK) complex, and rapidly degraded by the proteasome after polyubiquitination, resulting in NF-κB translocation into the nucleus. Accumulating evidence indicates that NF-κB plays a critical role in cellular protection against to various anticancer drugs- or IR-induced apoptosis, and therefore suggested that the activation of NF-κB may contribute in blocking the efficacy of cancer therapies and radiation. Indeed, inhibition of NF-κB leads to sensitization of apoptotic cell death in response to IR or DNA damaging agents in large number of cancer cells. Furthermore, aberrant dysregulation of NF-κB activity or increased nuclear level of NF-κB subunit (RelA/p50) has been commonly observed in several solid tumors and hematopoietic malignancies, and chemoresistance in selected tumors has been linked to the activated NF-κB status. Therefore, selective suppression of NF-κB will provide a useful strategy for overcoming drug-resistance in patients treated with conventional chemotherapy.

In addition to DNA-damaging agents, it has been previously reported that massive microtubule reorganization by microtubule disrupting agents such as cytochalasins or nocodazole activates NF-κB and induces NF-κB dependent gene expression. So far, although the exact molecular mechanism by which microtubule disrupting agents leads to activation of NF-κB remains to be elucidated, the sensing changes in the state of cytoskeleton may be one of way to regulate NF-κB signaling pathway. Relevant to this issue, microtubule system has recently been identified as an important regulator of translocation of the active NF-κB from cytosol to the nucleus. Thus, it is possible that the disruption of microtubule could be important regul-
lactor for DNA damage-induced NF-κB activation, or acts in synergy to sensitize the apoptotic response against to chemotherapeutic agents.

Here we report that disruption of microtubule inhibits DNA damage-induced NF-κB binding activity without alteration of nuclear translocation of NF-κB subunit, even though it can enhance NF-κB activation in the absence of other signals. Furthermore, such suppressed NF-κB binding activity by combined treatment of microtubule disrupting agent was correlated with the sensitization effect of apoptotic response to DNA damage agents. These results support the idea that, the combined treatment of these compounds could have a synergistic potential use in cancer therapy to overcome NF-κB mediated resistance.

MATERIALS AND METHODS

Cells

Human cervical cancer epithelial cells HeLa (American Type Culture Collection, CCL-2, Bethesda, MD, USA) and mouse embryonic fibroblasts (MEF) cells, including wild-type and p65-/- cells (kindly donated by Dr Zhengang Liu; Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, antibiotics (100 U/mL penicillin G and 100 µg/mL streptomycin), and 10% heat-inactivated FBS and were maintained at 37°C in a humidified incubator containing 5% CO₂.

Reagents

All commercial antibodies were purchased from the following: anti-IKKβ (Upstate Biotech, Waltham, MA, USA); anti-p84N5 (GeneTex, Irvine, CA, USA); anti-actin (Sigma-Aldrich, St. Louis, MO, USA); anti-phospho-IκBα, anti-caspase-3 and anti-caspase-9 (Cell Signaling Technology, Beverly, MA, USA); anti-IκBα, anti-p65, anti-tubulin, anti-SP1 (Santa Cruz Biothenology, Santa Cruz, CA, USA), anti-poly(ADP-ribose) polymerase (PARP) (BD Biosciences Pharmingen, San Diego, CA, USA). Cochicine (Col) were purchased from Sigma-Aldrich. Trypan blue was purchased from Cambrex Bio Science (Walkersville, MD, USA). Adriamycin (Adr), camptothcin (Cpt), nocodazole (Noc), vinblastine (Vin), the NF-κB inhibitor Bay-11, the selective inhibitor of IKK-β 2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide (TPCA-1) and the pancaspase inhibitor Z-VA-D-FMK were purchased from Calbiochem (San Diego, CA, USA). Poly(dI-dC)•poly(dI-dC) and dNTP were purchased from Pharmacia LKB Biotechnology. [α-³²P]dCTP was purchased from Amersham (Piscataway, NJ, USA).

Western blot analysis

After treatment with different reagents as described in the Figure legends, cells were collected and lysed in M2 buffer (20 mM Tris at pH 7.6, 0.5% NP-40, 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 2 mM DTT; 0.5 mM PMSE 20 mM β-glycerol phosphate, 1 mM sodium vanadate, 1 µg/mL leupeptin). Fifty micrograms of the cell lysates were subjected to SDS-polyacrylamide gel and blotted onto PVDF membrane. After blocking with 5% skim milk in PBS/T, the membrane was probed with the relevant antibody and visualized by enhanced chemiluminescence (ECL), according to the manufacturer’s instruction (Amersham).

Preparation of nuclear extracts

Nuclear extracts from whole cell extracts were prepared as described elsewhere (18), with minor modifications. In brief, after treatment with different reagents as described in the figure legends, cells were centrifuged (1,500 rpm, 5 min, 4°C) and then suspended in ice-cold buffer C (20 mM HEPES, pH 7.9; 0 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1 mM DTT; 0.5 mM phenylmethylsulfonyl fluoride [PMSF]) and 0.5% NP-40 for 30 min, and centrifuged (12,000 rpm, 10 min, 4°C) to separate the cytosolic fraction (supernatant) and nuclear fraction (pellet). The nuclear fraction was re-suspended in ice-cold buffer C (20 mM HEPES, pH 7.9; 400 mM NaCl; 1 mM EDTA; 1 mM EGTA; 1 mM DTT; 1 mM PMSE; 20% glycerol) for 30 min, and centrifuged at 12,000 rpm for 10 min, 4°C). Protein concentration was estimated using the Bio-Rad protein assay reagent and an equal amount of proteins per sample of nuclear extract was further analyzed by SDS-PAGE and EMSA.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed as described previously (18). Briefly, 5 µg of nuclear extract was incubated at room temperature for 20 min with reaction buffer containing 20 mM HEPES, pH 7.9, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT; 5% glycerol, 200 µg/mL BSA, and 2 µg of poly(dI-dC)•poly(dI-dC). Then the ³²P-labeled double-stranded oligonucleotide (1 ng, ≥1×10⁷ cpm) containing the NF-κB binding consensus sequence (5´-GGCAACCTGGGG-CAGGCCCCCTT-3´) was added to the reaction mixture for an additional 10 min at room temperature. The reaction products were fractionated on a nondenaturating 6% polyacrylamide gel, which was then dried and subjected to autoradiography. Western blot analysis with antibody against SP1 was also performed in the same nuclear extract as a control protein input.

Determination of cell death

Cells were seeded in six-well plates and treated with the indicated concentrations of reagents as described in the figure legends. Cell death, as observed by microscopy, was characterized by rounding up and detaching of the cells from the plates. Cell death was quantified by trypsinization, followed by staining with trypan blue and counting with a hemocytometer (American Optical, New York, NY, USA). The stained cells (blue) were counted as dead cells and were expressed as a percentage of cells. For
Statistical analysis
Data are expressed as the mean±S.E. from at least three separate experiments performed. The differences between groups were analyzed using a Student’s t test, and \( P<0.05 \) is considered statistically significant. Statistical analyses were carried out using SPSS software (ver. 11.0; SPSS Inc., Chicago, IL, USA).

RESULTS
Activation of NF-κB by microtubule disrupting agents
To characterize the NF-κB signaling pathway in HeLa cells affected by the disruption of the microtubule network, we compared the kinetics of the degradation of IκB-α at various time points after tumor necrosis factor (TNF) or tubulin polymerization inhibitors (colchicine, vinblastine and nocodazole). As shown in the bottom panel of Fig. 1A, when cells were treated...
with TNF, the expression of IκB-α started to be degraded at 15 min after treatment, and was recovered by 60 min. In contrast to the cells treated with TNF, the IκB-α degradation and its recovery were slower and weaker upon treatment with the microtubule disrupting agents (top three panels of Fig. 1A), which suggest that disruption of the microtubule network affects NF-κB activity in a different manner with a classical pathway. Since the nuclear translocation of NF-κB subunit, relA (p65) is essential for the NF-κB signaling pathway, we next examined the effect of the microtubule disrupting agents on the nuclear translocation of p65. As expected, the treatment of microtubule disrupting agents resulted in enhanced translocation of p65 into the nucleus, even though the extent of accumulated p65 into nucleus was lower than that of TNF treatment (Fig. 1B). As previously reported (19), the expression of nuclear protein encoded by N5 gene (p84N5) occurred principally in the nuclear fraction (Fig. 1B, bottom panels). However, the expression levels of p65 on the total cell extract were unaffected by treatment with microtubule disrupting agents (Fig. 1A). To confirm these observations, nuclear extracts were examined for NF-κB binding activity by EMSA using a palindromic NF-κB binding site. Consistent with above results, the binding activity of NF-κB subunit complex was gradually increased in response to microtubule agents, indicating that microtubule disruption directly induce NF-κB binding activity through nuclear translocation of NF-κB subunit, p65 without the expressional change of NF-κB. As a control of nuclear protein contents, the SP-1 protein level was examined by immunoblotting with an anti-SP-1 antibody and no difference was observed (Fig. 2B, bottom panels).

**Microtubule disrupting agents suppress the DNA damage-induced NF-κB binding activity**

Previous studies have demonstrated that the DNA damage trigger a cytoplasmic signaling that leads to the activation of IKK and NF-κB, which is associated predominantly with protecting cells from apoptosis (6, 7). Therefore it is a possibility that NF-κB activation by DNA damaging agents could be responsible for the development for the chemoresistance. We next examined whether the DNA damage-induced NF-κB activation is affected by the disruption of microtubule network. Two DNA damaging agents...
agents, adriamycin (Adr) and comptothecin (Cpt), were used to
detect the degradation of IκB-α and NF-κB binding activity in
total cell extract and nuclear extract respectively. As similar to
the effect of microtubule disrupting agents, Adr and Cpt in-
duced a slow and sustained degradation of IκB-α protein until
after 9 h of treatment (Fig. 2A). Consistently, an increase in NF-
κB binding activity in response to Adr or Cpt treatment was evi-
dent, and the kinetics of NF-κB binding activity were correlated
well with the IκB-α degradation (Fig. 2B, top panels).

Recent reports (16, 17) support the notion that microtubule
network may play an important role for nuclear translocation of
NF-κB subunits even though the microtubule disruption para-
doxically induced NF-κB activation (Fig. 1). To examine the issue
whether microtubule integrity affect the DNA damage-induced
NF-κB activation, we evaluated the NF-κB binding activity at dif-
ferent time points under the condition of microtubule disrupt-
ion. Interestingly, pretreatment with Col, Vin or Noc dramati-
cally suppressed either Adr- or Cpt-induced NF-κB binding ac-
tivities (Fig. 3), indicating that functional microtubule network
is required for the DNA binding activity. We also confirmed that
TNF-induced NF-κB DNA binding activity was suppressed by pre-
treatment with these microtubule disrupting agents (Data not shown).

Because the above results clearly demonstrated that micro-
tubule disrupting agents suppressed the DNA damage-induced
NF-κB DNA binding activity, we subsequently examined wheth-
er these agents affect the IκB-α degradation and nuclear trans-
location of p65 induced by DNA damage. Surprisingly, there was
detectable degradation of IκB-α protein in response to Adr- or
Cpt treatment under the condition of microtubule disruption
(Fig. 4A, B). Furthermore pretreatment with Col, Vin or Noc dramati-
cally suppressed either Adr- or Cpt-induced IκB-α binding ac-
tivity at the level of nucleus but not at the cytoplasmic event.

Microtubule disrupting agents sensitzes DNA damage-
induced apoptotic cell death

Previous study has reported that DNA damaging agents such as
Adr or Cpt can directly induce apoptosis in human cancer cells
(20). The nature of DNA damage-induced cell death was as-
sewed by the activation of the caspase cascade, including ca-
pase-9 and -3 and resultant cleavage of PARP. Caspase-9, -3 and
PARP were processed after Adr treatment, as shown by the de-
gradation of inactive pro-enzymes in a time-dependent manner
(Fig. 5A, top three panels). Moreover, the increased processing
of caspase cascade by Adr was completely abolished by pretreat-
ment with Z-VAD-FMK, a membrane permeable pancaspase
inhibitor (Fig. 5A, fifth row), indicating that the activation of ca-
pase cascade plays an important role in DNA damage-induced
apoptotic cell death in HeLa cell. Because NF-κB has been im-
plicated in apoptosis, we further examined whether the activa-
tion of NF-κB plays a role in DNA damage-induced cell death by
comparing the responses of wild-type (WT) and p65-null
(p65-/-) MEF cells. As shown in Fig. 5B, the extent of cell death
was much higher in p65-/MEF cells than in WT MEF cells mea-
sured by trypan blue exclusion assays. To ensure that p65 expres-
sion is indeed defective in p65-/MEF cells, the expression lev-
els of p65 and IKK-β in WT and p65-/MEF cells are examined
(Fig. 5C). Furthermore, pretreatment of HeLa cells with NF-κB-
specific inhibitor Bay-11 and TPCA-1 before Adr exposure dra-
matically enhanced Adr-induced cell death (Fig. 5D). These re-
results clearly indicate that NF-κB activation provides an impor-
tant anti-apoptotic role in DNA damage-induced cell death.

To determine whether inhibition of NF-κB binding activities
by microtubule disruption leads to sensitization of cytotoxic re-
sponse against DNA damaging agent, HeLa cells were pretreat-
ed with microtubule disrupting agents for 30 min followed by
Adr, and cell viability was observed with an inverted light micro-
scope and trypan blue exclusion assay. As shown in Fig. 5E,
pretreatment with Col, Vin and Noc markedly enhanced Adr-
induced cytotoxicity, whereas only a limited cells death was
observed in Z-VAD-FMK pretreated cells. The quantification of time-dependent cell death was summarized in Fig. 5F: Data suggested that microtubule disruption greatly sensitized DNA damage-induced apoptotic cell death. Furthermore, the combined treatment of microtubule disrupting agents and Adr significantly enhanced the cleavage of both caspase-9 and caspase-3 as evidenced by the decrease of procaspase-9 and increase of the active forms of caspase-3 (p21 fragment) (Fig. 5G, top two panels). A time-dependent enhancement of PARP cleavage was also detected in cells co-treated with microtubule dis-
rupting agents and Adr (Fig. 5G, third panel). Thus these results indicate that microtubule disrupting agents are able to sensitize DNA damage-induced apoptosis via promoting the caspase cascade.

DISCUSSION

It has been well established that the activity of NF-κB can be induced by IR or some conventional chemotherapeutic agents including Adr, etoposide, Cpt, vinca alkaloids and taxol (4, 15). Once activated by those stress, NF-κB has been shown to suppress the apoptotic potential of the chemotherapy in several cancer cells and in vivo cancer model (21, 22). Thus it has been widely proposed that activation of NF-κB in response to chemotherapy could be a potential mechanism of inducible chemoresistance which protects cancer cells from apoptotic stress. As documented previously, anti-carcinogenic property of DNA damaging agents such as Adr and Cpt is closely associated with an activation of tumor suppressor gene p53 which results in limited cell proliferation and mitochondrial dependent apoptosis through transcriptionally activating p53 target genes (23, 24). In accordance with previous observations in a variety of tumor cell lines, we found that Adr-induced apoptosis in HeLa cells appeared to accompany the activation of caspase-9 and -3, and subsequent PARP cleavage (Fig. 5A), which indicate a prominent role for mitochondria in Adr-induced cell death. In the intrinsic mitochondrial apoptotic pathway, p53-target gene products including Bax and Bak are involved in the release of cytochrome c, resulting in activation of caspase-9, which in turn activates execution-type caspase-3 (25, 26). In contrast to p53-mediated apoptotic response, DNA damaging agents also activates NF-κB signaling pathway which can suppress mitochondrial-dependent apoptotic pathway through transcriptional induction of anti-apoptotic genes including Bcl2. In addition, a number of factors including changes in cellular levels of P-glycoprotein family of multi- drug resistance (MDR), detoxification enzymes (e.g., glutathione S-transferase), DNA repair enzymes (e.g., DNA topoisomerase II), various oncogenes and mutated p53 have associated with development of cancer chemoresistance (27, 28). In this study, NF-κB subunit (p65) null MEF cells were found to be much more resistant to cell death as compared with wild-type cells, which suggest that NF-κB plays a role in blocking the efficacy of chemotherapy with DNA damaging agents (Fig. 5B). This interpretation is further supported by our observation that the inhibition of NF-κB using Bay-11 and TPCA-1 led to a dramatic enhanced cell death induced by Adr in HeLa cells (Fig. 5D). Although the further studies will be required to elucidate the exact molecular mechanisms of cancer chemoresistance, our study clearly raises the possibility that NF-κB could functions as an anti-apoptotic mediator in response to DNA damage. Consistent with this conclusion, there are some evidences that the combinational chemotherapies to inhibit NF-κB activation, including the administration of proteasome-specific inhibitors such as velcade, MG132 or some antioxidants such as N-acetylcystein provide a chemosensitization against DNA damage-induced killing (5).

In addition to DNA damaging agents, previous studies reported that microtubule disrupting agents can enhance the NF-κB dependent transcriptional activity presumably through the increased activity of protein kinase C (14, 29). Consistently we have also confirmed that Col, Vin and Noc have all been shown to activate NF-κB (Fig. 1). However, it has been recently proposed that the state of microtubule polymerization may also be crucial in the translocation of the active NF-κB subunit from cytosol to the nucleus. For instance, disruption of microtubule function by pretreatment with Col or Vin impaired accumulation and subsequent binding activities of NF-κB subunit p65 and p50 in the nucleus following glutamate stimulation (30) or zinc deficient cells which caused deficit to tubulin polymerization (16). Based on these observations, we speculated that the combined treatment of microtubule disrupting agents affected the nuclear targeting of NF-κB by DNA damage. Interestingly, nuclear translocation of NF-κB subunit p65 in response to DNA damage was not affected after pretreatment with microtubule disrupting agents in our experimental conditions (Fig. 4C, D). Similarly, Mikenberg et al. (30) have shown that microtubule perturbing compounds did not affect TNF-induced nuclear accumulation of p65 by using confocal microscopy. These results strongly suggest that NF-κB movement activated by either DNA damage or TNF is independent of cytoskeleton-based transport system in non-neuronal cancer cells. Despite these insights, we found that the microtubule disruption with Col, Vin and Noc led to drastically suppressed DNA damage-induced NF-κB binding activity by EMSA using nuclear extracts (Fig. 3), suggesting that the functional microtubule network is required for the DNA binding activity rather than the nuclear translocation of activated NF-κB subunit p65. Furthermore, we also found a significant increase in caspase-dependent apoptotic cell death after Adr treatment under the condition of microtubule disruption (Fig. 5E-G), suggesting that the suppressed NF-κB binding activity by the combined therapy may contribute to sensitize to trigger apoptotic machinery.

Taken together, data from this study collectively suggest that inhibition of microtubule network significantly chemosensitizes the cancer cells to die by apoptosis through suppressing NF-κB DNA binding activity. However, further study elucidating the molecular mechanism of microtubule disrupting agents on the regulation of NF-κB DNA binding activity in the nucleus will be critical for improving the value in the clinical application of these combined chemotherapies.
REFERENCES

1. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005; 5: 749-59.
2. Lin Y, Bai L, Chen W, Xu S. The NF-kappaB activation pathways, emerging molecular targets for cancer prevention and therapy. Expert Opin Ther Targets 2010; 14: 45-55.
3. Brown K, Gersberenger S, Carlson L, Franzoso G, Siebenlist U. Control of I kappa B-alpha proteinolysis by site-specific, signal-induced phosphorylation. Science 1995; 267: 1485-8.
4. Hur GM, Lewis J, Yang Q, Lin Y, Nakano H, Nedospasov S, Liu ZG. The death domain kinase RIP has an essential role in DNA damage-induced NF-kappaB activation. Genes Dev 2003; 17: 873-82.
5. Waddick KG, Uckun FM. Innovative treatment programs against cancer: II. Nuclear factor-kappaB (NF-kappaB) as a molecular target. Biochem Pharmacol 1999; 57: 9-17.
6. Daroczi B, Kari G, Ren Q, Dicker AP, Rodeck U. Nuclear factor kappaB inhibitors alleviate and the proteasome inhibitor PS-341 exacerbates radiation toxicity in zebrafish embryos. Mol Cancer Ther 2009; 8: 2625-34.
7. Li F, Sethi G. Targeting transcription factor NF-kappaB to overcome chemoresistance and radioresistance in cancer therapy. Biochim Biophys Acta 2010; 1805: 167-80.
8. Egan LJ, Eckmann L, Greten FR, Chae S, Li ZW, Myhre GM, Bobine S, Karin M, Kagnoff MF. IkappaB-alpha-beta-dependent NF-kappaB activation provides radioprotection to the intestinal epithelium. Proc Natl Acad Sci USA 2004; 101: 2452-7.
9. Starenki D, Namba H, Saenko V, Ohtsuru A, Yamashita S. Inhibition of nuclear factor-kappaB caspase potentiates the effect of a combination treatment of anaplastic thyroid cancer cells. J Clin Endocrinol Metab 2004; 89: 410-8.
10. Ahmed KM, Li JJ. ATM-NF-kappaB connection as a target for tumor radiosensitization. Curr Cancer Drug Targets 2007; 7: 335-42.
11. Fan Y, Dutta J, Gupta N, Fan G, Gellinas C. Regulation of programmed cell death by NF-kappaB and its role in tumorigenesis and therapy. Adv Exp Med Biol 2008; 615: 223-30.
12. Nadiminty N, Lou W, Sun M, Chen J, Yue J, Kung HJ, Evans CP, Zhou Q, Gao AC. Aberrant activation of the androgen receptor by NF-kappaB2/ p52 in prostate cancer cells. Cancer Res 2010; 70: 3309-19.
13. Puvvada SD, Funkhouser WK, Greene K, Deal A, Chu H, Baldwin AS, Tepper JE, O’Neil BH. NF-kB and Bcl-3 activation are prognostic in metastatic colorectal cancer. Oncology 2010; 78: 181-8.
14. Rosette C, Karin M. Cytoskeletal control of gene expression: depolymerization of microtubules activates NF-kappaB. J Cell Biol 1995; 128: 1111-9.
15. Das KC, White CW. Activation of NF-kappaB by antineoplastic agents. Role of protein kinase C. J Biol Chem 1997; 272: 14914-20.
16. Mackenzie GG, Keen CL, Oteiza PI. Microtubules are required for NF-kappaB nuclear translocation in neuroblastoma IMR-32 cells: modulation by zinc. J Neurochem 2006; 99: 402-15.
17. Mikenberg I, Widera D, Kaus A, Kaltenschmidt B, Kaltenschmidt C. Transcription factor NF-kappaB is transported to the nucleus via cytoplasmic dynein/dynactin motor complex in hippocampal neurons. PLoS One 2007; 2: e589.
18. Devary Y, Rosette C, DiDonato JA, Karin M. NF-kappaB activation by ultraviolet light not dependent on a nuclear signal. Science 1993; 261: 1442-5.
19. Doostzadeh-Cizeron J, Terry NH, Goodrich DW. The nuclear death domain protein p84NS activates a G2/M cell cycle checkpoint prior to the onset of apoptosis. J Biol Chem 2001; 276: 1127-32.
20. Kim R, Eui M, Tanabe K. Caspase-dependent and -independent cell death pathways after DNA damage (Review). Oncol Rep 2005; 14: 595-9.
21. Berenson JR, Ma HM, Vescio R. The role of nuclear factor-kappaB in the biology and treatment of multiple myeloma. Semin Oncol 2001; 28: 626-33.
22. Um JH, Kang CD, Lee BG, Kim DW, Chung BS, Kim SH. Increased and correlated nuclear factor-kappa B and Ku autoantigen activities are associated with development of multidrug resistance. Oncogene 2001; 20: 6048-56.
23. Meulmeester E, Jochems AG. p53: a guide to apoptosis. Curr Cancer Drug Targets 2008; 8: 87-97.
24. Green DR, Kroemer G. Cytoplasmic functions of the tumour suppressor p53. Nature 2009; 458: 1127-30.
25. Schuler M, Green DR. Mechanisms of p53-dependent apoptosis. Biochem Soc Trans 2001; 29: 684-8.
26. Shen Y, White E. p53-dependent apoptosis pathways. Adv Cancer Res 2001; 82: 55-84.
27. el-Deiry WS. Role of oncogenes in resistance and killing by cancer therapeu- tic agents. Curr Opin Oncol 1997; 9: 79-87.
28. Pérez-Tomás R. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. Curr Med Chem 2006; 13: 1859-76.
29. Németh ZH, Deitch EA, Davidson MT, Szabó C, Vizi ES, Haskó G. Disruption of the actin cytoskeleton results in nuclear factor-kappaB activation and inflammatory mediator production in cultured human intestinal epithelial cells. J Cell Physiol 2004; 200: 71-81.
30. Mikenberg I, Widera D, Kaus A, Kaltenschmidt B, Kaltenschmidt C. TNF-alpha mediated transport of NF-kappaB to the nucleus is independent of the cytoskeleton-based transport system in non-neuronal cells. Eur J Cell Biol 2006; 85: 529-36.