

Inhibition of tumor necrosis factor- α enhances apoptosis induced by nuclear factor- κ B inhibition in leukemia cells

QIAO-MEI DONG, CHUN LING, XUAN CHEN and LI ZHAO

Central Laboratory, The First Hospital of Lanzhou University, Lanzhou, Gansu 730000, P.R. China

Received July 21, 2014; Accepted September 14, 2015

DOI: 10.3892/ol.2015.3786

Abstract. Inhibition of nuclear factor- κ B (NF- κ B) results in antitumor activity in leukemia cells, and may be a potential therapeutic strategy for the treatment of leukemia. However, a significant limitation of NF- κ B inhibition in the treatment of leukemia is the low efficiency of this technique. NF- κ B inhibitor treatment induces apoptosis in leukemia cells; however, it additionally causes inflammatory molecules to induce increased sensitivity of healthy hematopoietic cells to cell death signals, therefore limiting its clinical applications. Tumor necrosis factor- α (TNF- α) is a key regulator of inflammation, and induces a variety of actions in leukemic and healthy hematopoietic cells. TNF- α induces NF- κ B-dependent and -independent survival signals, promoting the proliferation of leukemia cells. However, in healthy hematopoietic cells, TNF- α induces death signaling, an effect which is enhanced by the inhibition of NF- κ B. Based on these observations, the present study hypothesized that inhibition of TNF- α signaling may be able to protect healthy hematopoietic cells and other tissue cells, while increasing the anti-leukemia effects of NF- κ B inhibition on leukemia cells. The role and underlying molecular mechanisms of TNF- α inhibition in the regulation of NF- κ B inhibition-induced apoptosis in leukemia cells was therefore investigated in the present study. The results indicated that inhibition of TNF- α enhanced NF- κ B inhibition-induced apoptosis in leukemia cells. It was also revealed that protein kinase B was significant in the regulation of TNF- α and NF- κ B inhibition-induced apoptosis. During this process, intrinsic apoptotic pathways were activated. A combination of NF- κ B and TNF- α inhibition may be a potential specific and effective novel therapeutic strategy for the treatment of leukemia.

Introduction

Leukemia is a malignant disease of the hematopoietic system and is one of the ten most common causes of cancer-associated mortality in the Chinese population, particularly amongst individuals aged <35 years old (1). Chemotherapy is the most commonly utilized strategy for the treatment of leukemia (2). Although 50-70% patients achieve remission following treatment with conventional chemotherapy regimens, the majority of patients subsequently relapse and may develop chemoresistance (3-9). An improved understanding of the molecular mechanisms underlying leukemia may aid the identification of novel chemotherapeutic regimens, and improve the efficiency of treatments, therefore prolonging patient survival times. Thus, the mechanisms underlying leukemia require further investigation.

Previously, by comparing the gene expression and molecular activity of leukemic stem cells and healthy hematopoietic stem cells, a number of molecules (interleukin-3R, cluster of differentiation 47, cluster of differentiation 44) and signaling pathways [Nuclear factor- κ B (NF- κ B) (10), Wnt/ β -catenin (11)] have been identified to be highly expressed or activated only in leukemic stem cells. Due to these results, the NF- κ B signaling pathway has attracted the attention of researchers. NF- κ B activity is detectable in the majority of leukemic cells, and its expression is significantly increased in leukemic cells compared with that of healthy bone marrow cells (12). Furthermore, *in vitro* studies have identified that NF- κ B inhibition is capable of inducing apoptosis in leukemic stem cells, and apoptosis levels are significantly increased these cells compared with those of normal hematopoietic stem cells (10). Due to the ability of NF- κ B inhibitor to induce apoptosis of leukemic cells, specifically leukemic stem cells, NF- κ B inhibitors may present a potential anti-leukemic therapy (13-15). However, there are a number of potential side effects of NF- κ B inhibition in the treatment of leukemia, which may limit its clinical application (16). NF- κ B inhibition not only induces apoptosis in leukemia cells, it additionally acts on normal cells, inducing inflammatory molecules, particularly tumor necrosis factor- α (TNF- α), to increase sensitivity to cell death signals (17). It has been reported that the toxicity induced by NF- κ B inhibition was significantly suppressed in TNF- α or TNF- α receptor (Tnfr) knockout mice (18). Therefore, a combination of NF- κ B and TNF- α inhibition may attenuate the side-effects associated with inhibition of NF- κ B alone (18-24).

Correspondence to: Ms. Li Zhao, Central Laboratory, The First Hospital of Lanzhou University, 1 Donggang West Road, Lanzhou, Gansu 730000, P.R. China
E-mail: dongqm06@126.com

Key words: nuclear factor- κ B inhibition, tumor necrosis factor α , apoptosis, molecular mechanism, protein kinase B, caspase 9, leukemia

Furthermore, TNF- α induces NF- κ B-dependent and -independent survival signaling, therefore promoting proliferation of leukemia cells (25,26). Based on these previous results, the present study hypothesized that inhibition of TNF- α signaling may be able to protect healthy hematopoietic cells and other healthy tissue cells, while enhancing the anti-leukemic effects of NF- κ B inhibition. A combination of NF- κ B and TNF- α inhibition may be a potential specific and effective novel therapeutic strategy for the treatment of leukemia. The present study aimed to provide insight into the synergistic effects of NF- κ B and TNF- α inhibition on leukemia cells. In addition, the underlying molecular mechanisms were investigated.

Materials and methods

Chemicals. Roswell Park Memorial Institute (RPMI)-1640 and fetal bovine serum (FBS) were purchased from Hyclone (GE Healthcare Life Sciences, Logan, UT, USA). Rabbit monoclonal anti-protein kinase B (Akt) primary antibody used at 1:1,000, and rabbit anti-TNF- α antibody (TNF- α inhibitor) were obtained from Abcam (Cambridge, MA, USA). Rabbit polyclonal anti-caspase 9 primary antibody used at 1:1,000 was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). NF- κ B inhibitor (MG-132) was purchased from Beyotime Institute of Biotechnology (Beijing, China). Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was obtained from Beijing Baosai Biotechnology Co., Ltd. (Beijing, China).

Cell culture and drug treatment. The HL-60, K562 and K562 with doxorubicin resistance (K562-ADM) human leukemia cell lines (Shanghai Institutes for Biological Sciences, Shanghai, China) were grown in RPMI-1640 medium supplemented with 10% FBS, and maintained in humidified 5% CO₂ at 37°C.

For treatment with anti-TNF- α antibody and MG-132, cells were plated at a density of 1.5×10^5 cells/well in 2 ml RPMI-1640 in a 6-well plate. Twenty-four hours later, the medium was replaced and anti-TNF- α antibody (10 ng/ml) and MG-132 (3 μ M) were added, alone or in combination. Cells were incubated at 37°C for 48 h and subsequently used for further experiments.

Detection of apoptosis by flow cytometry (FCM) and microscopy. In order to evaluate the apoptotic rate the various cell types, an Annexin V-FITC apoptosis detection kit was used according to the manufacturer's protocol. Briefly, cells were collected and resuspended in 200 μ l binding buffer (Beijing Baitaike Biology Company (Beijing, China). Subsequently, 10 μ l Annexin V-FITC and 5 μ l propidium iodide (PI) were added to the suspension and allowed to react for 15 min at room temperature. Following incubation, 300 μ l binding buffer was added and the apoptotic rate was determined using a FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). In addition, cell morphology was observed using a U-TV0 phase contrast microscope (Olympus Corporation, Tokyo, Japan).

RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using the TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) method and reverse transcribed using

an Invitrogen SuperScript III First-Strand Synthesis system (Thermo Fisher Scientific, Inc.) to generate complementary DNA, according to the manufacturer's protocol. Transcription of GAPDH was examined using the following primers (Shanghai Sangong Pharmaceutical Co., Ltd., Shanghai, China): F 5'-AGTCAACGGATTTGGTCGTATT-3' and R 5'-AATGAGCCCCAGCCTTCT-3'. Primers for Akt were, F 5'-AAGACGACGAGGACTGCTATG-3' and R 5'-ACCCTTCGCTTATCCACTGA-3'. PCR steps were performed using an Applied Biosystems GeneAmp® PCR System 9700 thermocycler (Thermo Fisher Scientific, Inc.).

RT-PCR was performed using a LightCycler® 480 SYBR Green I Master (Roche Diagnostics, Burgess Hill, UK) according to the manufacturer's instructions. Transcription of β -actin was examined with primers, 5'-TGGCACCACAGCACAATGAA-3' and 5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'. Primers for Akt were, 5'-CTTCGTCCTCCTCCTCACAC-3' and, 5'-GCCTGCTTCTCCAACAACA-3'; and primers for caspase 9 were, 5'-AACAGGCAAGCAGCAAAGTT-3' and, 5'-CCTC-CAGAACCAATGTCCAC-3'. Following an initial denaturation step at 95°C for 5 min, 40 cycles of amplification for each primer pair were performed. Each cycle included a denaturation step, 10 sec at 95°C; an annealing step, 20 sec at 60°C; and an elongation step, 10 sec at 72°C. A final elongation phase was performed at 65°C for 1 min. Relative gene expression levels were measured using a LightCycler 480 (Roche Diagnostics) according to the manufacturer's protocol. Relative changes in the gene expression levels of caspase 9 and Akt were normalized against the levels of β -actin gene expression in each sample. Experiments were performed at least in duplicate for each data point.

Western blotting. Total cells were lysed with buffer (1% sodium dodecyl sulfate, 10 mm Tris-Cl, pH 7.6, 20 g/ml aprotinin, 20 g/ml leupeptin and 1 mm 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride; Shanghai Sangong Pharmaceutical Co., Ltd.). Protein concentrations were determined using the Bradford method. Protein (20 μ g) was separated on 12% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Following blocking with 10% non-fat milk, membranes were incubated with anti-AKT and anti-caspase 9 antibodies (as mentioned in the Chemicals sections, above) at 4°C overnight. Following washing three times with TBS, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG at 1:200 dilution (Abcam, Cambridge, UK) at room temperature for 1 h. The signals were developed using an enhanced chemiluminescence kit (Applygen Technologies, Inc., Beijing, China) and rabbit polyclonal anti- β -actin antibody (dilution, 1:1,000; cat no. ab8227, Abcam) served as an internal control.

Statistical analysis. All statistical comparisons were performed using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Student's t-test was utilized to compare the differences or association between the two groups. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Inhibition of TNF- α enhances apoptosis induced by NF- κ B inhibition in leukemia cells. K562 cells were treated with

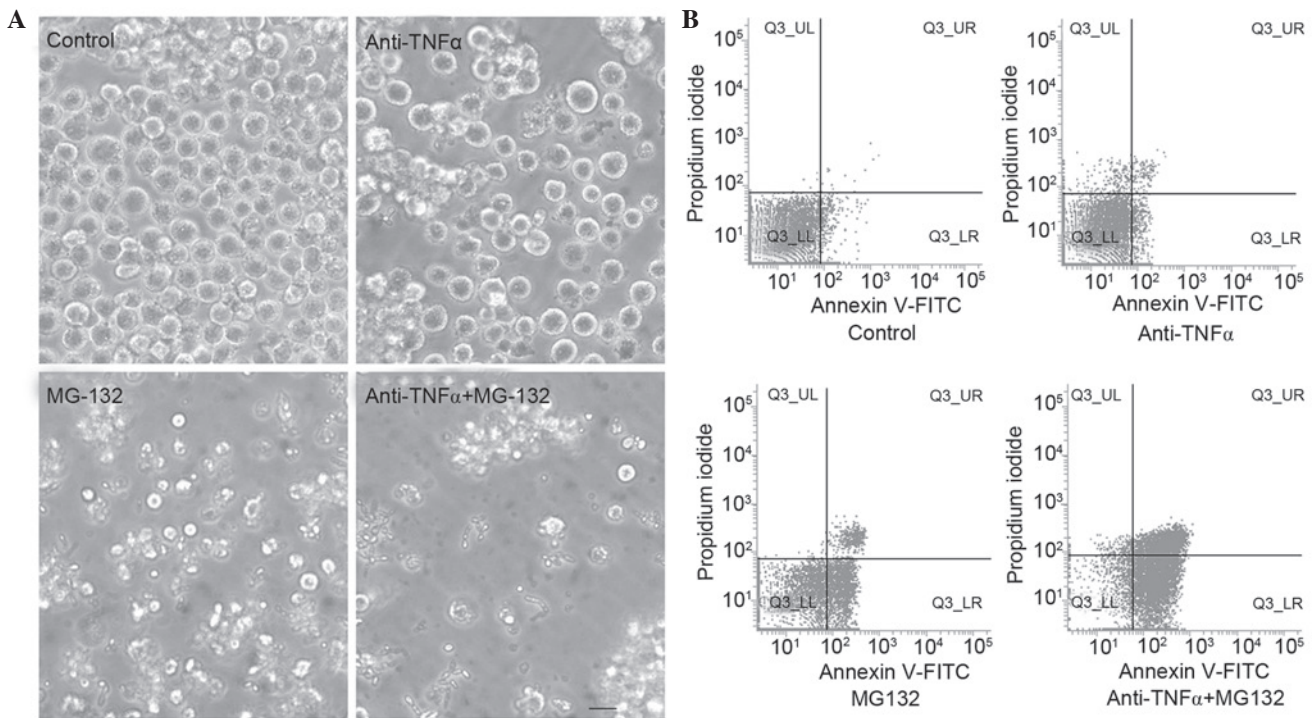


Figure 1. Inhibition of TNF- α enhances apoptosis induced by nuclear factor- κ B inhibition in K562 cells. K562 cells were treated with anti-TNF- α antibody and MG-132, respectively or in combination. Following treatment, the apoptotic morphology and rate were compared. (A) Cell morphology was observed by phase contrast microscopy. Scale bar, 10 μ m; magnification, $\times 400$. (B) Cell apoptosis was detected via flow cytometry and Annexin V-FITC and propidium iodide immunofluorescence. FITC, fluorescein isothiocyanate; TNF- α , tumor necrosis factor α .

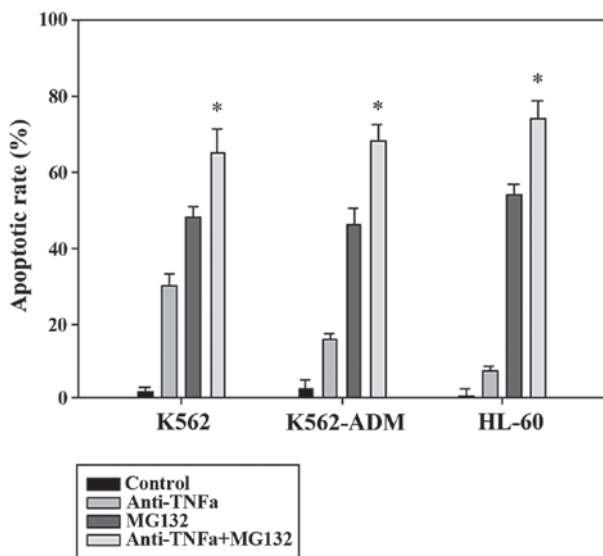


Figure 2. Combined effects of anti-TNF- α antibody and MG-132 treatment in various leukemia cell types. K562, K562-ADM and HL-60 cells were treated with anti-TNF- α antibody and MG-132, respectively or in combination. The control cells were cultured in unsupplemented RPMI-1640 medium. Following treatment, apoptotic rates were detected via flow cytometry. TNF- α , tumor necrosis factor- α . * $P < 0.05$ vs. control.

anti-TNF- α antibody (TNF- α inhibitor) and MG-132 (NF- κ B inhibitor) (27), respectively or in combination. Following treatment, the apoptotic morphology and rate were compared with those of the control group. Cell morphology was observed by phase contrast microscopy. Compared with the control group, anti-TNF- α antibody or MG-132 treatment

induced a typical apoptotic cell morphology (intense staining, fragmented and condensed nuclei) in K562 cells, and anti-TNF- α antibody and MG-132 co-treated cells displayed more marked apoptotic morphology compared with that of the groups treated with anti-TNF- α antibody or MG-132 separately (Fig. 1A).

Subsequently, the apoptotic rates of the anti-TNF- α antibody and MG-132 combined treatment group, and the separately treated groups, were detected using FCM and Annexin V-FITC and PI immunofluorescence (Fig. 1B). The results indicated that the apoptotic rate of the combined treatment group ($68.31 \pm 4.15\%$) was increased compared with that of the groups that were treated separately (anti-TNF- α antibody treatment, $30.5 \pm 3.11\%$; MG-132 treatment, $50.53 \pm 2.85\%$) and the control group ($2.23 \pm 1.23\%$; $P < 0.05$).

In order to confirm that the effects of co-treatment with TNF- α inhibitor and NF- κ B inhibitor were not restricted to K562 cells, additional types of leukemia cell, including K562-ADM and HL-60, were investigated (Fig. 2). These cells were also treated with anti-TNF- α antibody and MG-132, respectively or in combination. Following treatment, the apoptotic rate was detected via FCM. The apoptotic rates of the combined treatment group in K562-ADM cells ($72.38 \pm 2.57\%$) were increased compared with those of the separately treated groups (anti-TNF- α antibody treatment, $18.37 \pm 1.35\%$; MG-132 treatment, $46.35 \pm 3.65\%$) and the control group ($3.25 \pm 2.33\%$; $P < 0.05$). The apoptotic rate of the combined treatment group in HL-60 cells ($78.65 \pm 3.72\%$) was also increased compared with the separately treated groups (anti-TNF- α antibody treatment, $6.85 \pm 1.42\%$; MG-132 treatment, $57.44 \pm 2.46\%$) and the control group ($1.46 \pm 2.28\%$; $P < 0.05$).

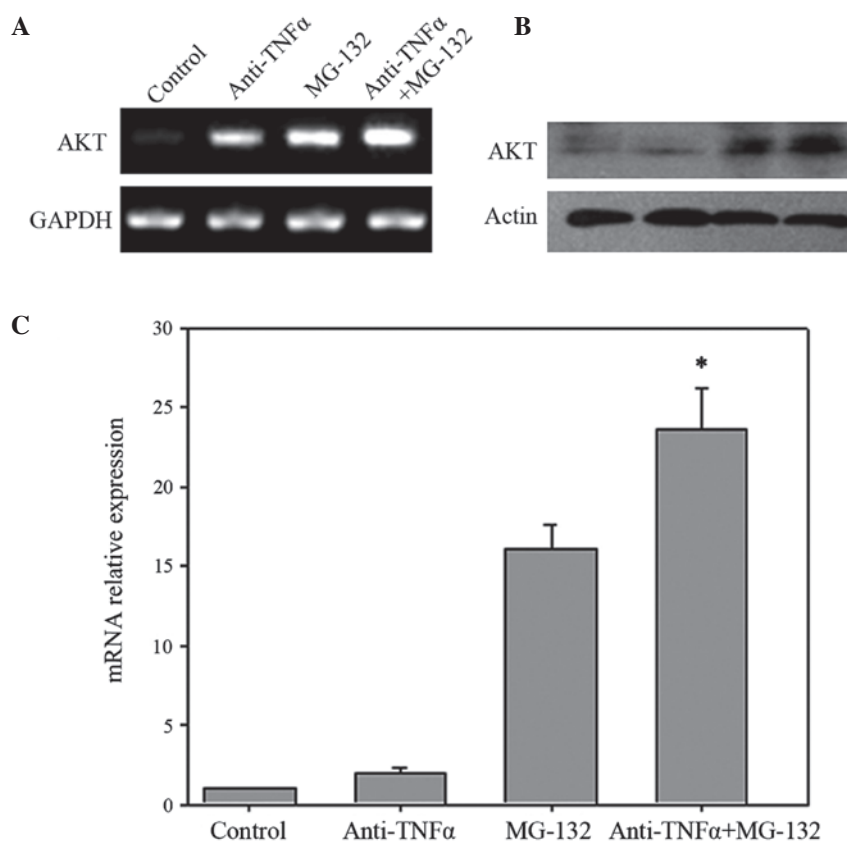


Figure 3. Combined treatment with anti-TNF- α antibody and MG-132 promotes the expression of Akt. K562 cells were treated with anti-TNF- α antibody and MG-132, respectively or in combination. Following treatment, mRNA and protein expression of Akt were detected by (A) RT-PCR, (B) western blotting and (C) RT-qPCR. *P<0.05 vs. control. mRNA, messenger RNA; TNF- α , tumor necrosis factor α ; RT-PCR, reverse transcription-polymerase chain reaction; q, quantitative.

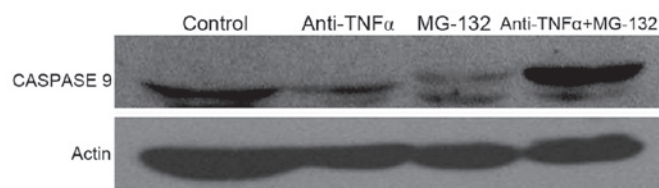


Figure 4. Combined treatment with anti-TNF- α antibody and MG-132 promotes activation of caspase 9. K562 cells were treated with anti-TNF- α antibody and MG-132, respectively or in combination. Following treatment, caspase 9 protein expression was detected via western blotting. TNF- α , tumor necrosis factor α .

Treatment with a combination of anti-TNF- α antibody and MG-132 promotes Akt expression. Akt is located downstream of TNF- α and NF- κ B signaling (28). The messenger RNA (mRNA) and protein expression levels of Akt in anti-TNF- α antibody and MG-132 co-treated cells, separately treated cells and control cells were therefore detected by RT-PCR (Fig. 3A), western blotting (Fig. 3B) and RT-quantitative (q) PCR (Fig. 3C). The results of the present study revealed that the expression levels of Akt were increased in anti-TNF- α antibody or MG-132 treated cells, while co-treated cells expressed increased levels of Akt compared with those of the separately treated cells or control cells, in terms of mRNA and protein.

Combined treatment with anti-TNF- α antibody and MG-132 promotes caspase 9 activation. Caspase 9 is activated in the

intrinsic apoptotic pathway (29), therefore in the present study, expression and activation of caspase 9 in anti-TNF- α antibody and MG-132 co-treated, separately-treated and control cells, was investigated using western blotting. Caspase 9 was identified to be highly expressed and activated in co-treated cells, compared with control cells and separately treated cells (Fig. 4). Caspase 9 activation was promoted following TNF- α and NF- κ B inhibitor co-treatment.

Discussion

NF- κ B is a nuclear transcription factor, which may mediate survival pathways in a number of types of cancer, including leukemia (30). NF- κ B induces the expression of genes involved in cell proliferation, angiogenesis and metastasis, and possesses significant roles in carcinogenesis and chemoresistance (31). NF- κ B activity is detectable in the majority of leukemic cells, and its expression has been observed to be significantly increased in leukemic cells compared with that of healthy bone marrow cells (32,33). NF- κ B inhibition exerts antitumor activity in leukemia cells (27). Cosimo *et al* (34) reported that inhibition of NF- κ B was able to induce apoptosis in chronic lymphocytic leukemia cells. Therefore, inhibition of the activity of NF- κ B may be a potential novel therapeutic strategy for the treatment of leukemia. However, a significant limitation of NF- κ B inhibition in the treatment of leukemia is the low efficiency. NF- κ B inhibition induces apoptosis in leukemia cells, however additionally causes inflammatory molecules (particularly TNF- α)

to increase the sensitivity of normal hematopoietic cells to cell death signals (18), which limit its clinical application.

TNF- α is a central regulator of inflammation, which exerts various functions in leukemia and normal hematopoietic cells (35). TNF- α is continually expressed in a number of types of leukemia cell (36), and has been demonstrated to upregulate certain molecules involved in cell growth and proliferation via the NF- κ B-dependent or -independent pathway in leukemia (37). TNF- α is critical for the growth and survival of leukemic cells. However, in healthy hematopoietic cells, TNF- α induces death signaling (20). TNF- α -induced death signaling has been observed to be enhanced by NF- κ B inhibition in healthy hematopoietic cells. The toxicity induced by NF- κ B inhibition was observed to be significantly suppressed in TNF- α or TnfR knockout mice (25). In the present study, it was suggested that inhibition of TNF- α may be capable of enhancing NF- κ B inhibitor-induced apoptosis in leukemia cells. Combined treatment with NF- κ B and TNF- α inhibitors may be a specific and effective novel therapeutic strategy for the treatment of leukemia. Leukemia cells were treated with anti-TNF- α antibody and MG-132, respectively or in combination, and the apoptotic rates of these various treatment groups were compared. It was identified that combination treatment with anti-TNF- α antibody and MG-132 enhanced apoptosis in a number of leukemia cell types, including K562 and HL-60 cells. K562-ADM is a doxorubicin-resistant leukemia cell line. In K562-ADM cells, which are chemoresistant, a significant increase in apoptosis was also observed following co-treatment with MG-132 and anti-TNF- α antibody.

The molecular mechanism by which inhibition of TNF- α and NF- κ B promotes apoptosis in leukemia cells was additionally elucidated. Previously, it was demonstrated that Akt is a downstream molecule influenced by NF- κ B signaling (38). In addition, TNF- α induced activation of the phosphoinositide 3-kinase/Akt pathway, and therefore the transcriptional activation of NF- κ B, in C4HD murine mammary tumor cells (28). In the present study, the expression of Akt in the presence of anti-TNF- α antibody and MG-132, respectively or in combination, was detected. The present study identified that the expression of Akt was significantly increased, at the mRNA and protein levels, when leukemia cells were treated with anti-TNF- α antibody and MG-132 in combination. This result further confirmed the involvement of Akt in the regulation of TNF- α and NF- κ B inhibition-induced apoptosis in K562 cells.

The results of the present study also revealed that caspase 9 was activated following anti-TNF- α antibody and MG-132 co-treatment. However, low levels of cleavage of caspase 9 were identified in cells treated with anti-TNF- α antibody or MG-132 alone. Caspase 9 is activated during the intrinsic apoptotic pathway (29). In K562 cells, inhibition of NF- κ B and TNF- α signaling induced activation of the intrinsic apoptotic pathway, during which caspase 9 was activated.

In conclusion, the present study revealed that inhibition of TNF- α enhanced NF- κ B inhibition-induced apoptosis in leukemia cells. Akt was significant in the regulation of TNF- α and NF- κ B inhibition-induced apoptosis. During this process, the intrinsic apoptotic pathway was activated. A combination of treatment with NF- κ B inhibitor and TNF- α inhibitor may therefore present a specific and effective novel therapeutic strategy for the treatment of leukemia.

Acknowledgements

The present study was supported by the Fundamental Research Fund for the Central Universities (grant no. lzujbky-2013-150), the Science and Technology Plan Project in Gansu Province (grant no. 1308RJYA071) and the Research Program of the First Hospital of Lanzhou University (grant no. ldyyylnlc201101).

References

1. Elbahesh E, Patel N and Tabbara IA: Treatment of acute promyelocytic leukemia. *Anticancer Res* 34: 1507-1517, 2014.
2. Lynch RC and Medeiros BC: Chemotherapy options for previously untreated acute myeloid leukemia. *Expert Opin Pharmacother* 16: 2149-2162, 2015.
3. Tallman MS, Gilliland DG and Rowe JM: Drug therapy for acute myeloid leukemia. *Blood* 106: 1154-1163, 2005.
4. Chomel JC, Bonnet ML, Sorel N, Bertrand A, Meunier MC, Fichelson S, Melkus M, Bennaceur-Griscelli A, Guilhot F and Turhan AG: Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. *Blood* 118: 3657-3660, 2011.
5. Schrappe M, Valsecchi MG, Bartram CR, Schrauder A, Panzer-Grümayer R, Möricke A, Parasole R, Zimmermann M, Dworzak M, Buldini B, *et al*: Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: Results of the AIEOP-BFM-ALL 2000 study. *Blood* 118: 2077-2084, 2011.
6. Oran B and de Lima M: Prevention and treatment of acute myeloid leukemia relapse after allogeneic stem cell transplantation. *Curr Opin Hematol* 18: 388-394, 2011.
7. Campana D: Progress of minimal residual disease studies in childhood acute leukemia. *Curr Hematol Malig Rep* 5: 169-176, 2010.
8. Ravindranath Y: Recent advances in pediatric acute lymphoblastic and myeloid leukemia. *Curr Opin Oncol* 15: 23-35, 2003.
9. Rowe JM and Goldstone AH: How I treat acute lymphocytic leukemia in adults. *Blood* 110: 2268-2275, 2007.
10. Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, Luger SM and Jordan CT: Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. *Blood* 98: 2301-2307, 2001.
11. Wang Y, Krivtsov AV, Sinha AU, North TE, Goessling W, Feng Z, Zon LI and Armstrong SA: The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. *Science* 327: 1650-1653, 2010.
12. Bellavia D, Campese AF, Alesse E, Vacca A, Felli MP, Balestri A, Stoppacciaro A, Tiverson C, Tatangelo L, Giovarelli M, *et al*: Constitutive activation of NF-kappaB and T-cell leukemia/lymphoma in Notch3 transgenic mice. *Embo J* 19: 3337-3348, 2000.
13. Hadian K and Krappmann D: Signals from the nucleus: Activation of NF-kappaB by cytosolic ATM in the DNA damage response. *Sci Signal* 4: pe2, 2011.
14. Miyamoto S: Nuclear initiated NF-kappaB signaling: NEMO and ATM take center stage. *Cell Res* 21: 116-130, 2011.
15. Wu ZH and Miyamoto S: Induction of a pro-apoptotic ATM-NF-kappaB pathway and its repression by ATR in response to replication stress. *EMBO J* 27: 1963-1973, 2008.
16. Sakurai T, Maeda S, Chang L and Karin M: Loss of hepatic NF-kappa B activity enhances chemical hepatocarcinogenesis through sustained c-Jun N-terminal kinase 1 activation. *Proc Natl Acad Sci USA* 103: 10544-10551, 2006.
17. Igarashi H, Baba Y, Nagai Y, Jimi E, Ghosh S and Kincade PW: NF-kappaB is dispensable for normal lymphocyte development in bone marrow but required for protection of progenitors from TNFalpha. *Int Immunol* 18: 653-659, 2006.
18. Wullaert A, van Loo G, Heyninck K and Beyaert R: Hepatic tumor necrosis factor signaling and nuclear factor-kappaB: Effects on liver homeostasis and beyond. *Endocr Rev* 28: 365-386, 2007.
19. Oguma K, Oshima H and Oshima M: Inflammation, tumor necrosis factor and Wnt promotion in gastric cancer development. *Future Oncol* 6: 515-526, 2010.
20. Balkwill F: Tumour necrosis factor and cancer. *Nat Rev Cancer* 9: 361-371, 2009.

21. Geisler F, Algül H, Paxian S and Schmid RM: Genetic inactivation of RelA/p65 sensitizes adult mouse hepatocytes to TNF-induced apoptosis *in vivo* and *in vitro*. *Gastroenterology* 132: 2489-2503, 2007.
22. Hatano E: Tumor necrosis factor signaling in hepatocyte apoptosis. *J Gastroenterol Hepatol* 22 (Suppl 1): S43-S44, 2007.
23. Quante M and Wang TC: Inflammation and stem cells in gastrointestinal carcinogenesis. *Physiology (Bethesda)* 23: 350-359, 2008.
24. Mantovani A, Allavena P, Sica A and Balkwill F: Cancer-related inflammation. *Nature* 454: 436-444, 2008.
25. Lee YR, Yu HN, Noh EM, Youn HJ, Song EK, Han MK, Park CS, Kim BS, Park YS, Park BK, *et al*: TNF-alpha upregulates PTEN via NF-kappaB signaling pathways in human leukemic cells. *Exp Mol Med* 39: 121-127, 2007.
26. Kagoya Y, Yoshimi A, Kataoka K, Nakagawa M, Kumano K, Arai S, Kobayashi H, Saito T, Iwakura Y and Kurokawa M: Positive feedback between NF- κ B and TNF- α promotes leukemia-initiating cell capacity. *J Clin Invest* 124: 528-542, 2014.
27. Morotti A, Cilloni D, Pautasso M, Messa F, Arruga F, Defilippi I, Carturan S, Catalano R, Rosso V, Chiarenza A, *et al*: NF- κ B inhibition as a strategy to enhance etoposide-induced apoptosis in K562 cell line. *Am J Hematol* 81: 938-945, 2006.
28. Rivas MA, Carnevale RP, Proietti CJ, Rosemblit C, Beguelin W, Salatino M, Charreau EH, Frahm I, Sapia S, Brouckaert P, *et al*: TNF alpha acting on TNFR1 promotes breast cancer growth via p42/P44 MAPK, JNK, Akt and NF-kappa B-dependent pathways. *Exp Cell Res* 314: 509-529, 2008.
29. Mueller T, Voigt W, Simon H, Fruehauf A, Bulankin A, Grothey A and Schmoll HJ: Failure of activation of caspase-9 induces a higher threshold for apoptosis and cisplatin resistance in testicular cancer. *Cancer Res* 63: 513-521, 2003.
30. Giuliani C, Napolitano G, Bucci I, Montani V and Monaco F: NF- κ B transcription factor: Role in the pathogenesis of inflammatory, autoimmune, and neoplastic diseases and therapy implications. *Clin Ter* 152: 249-253, 2001 (In Italian).
31. Baud V and Jacque E: The alternative NF- κ B activation pathway and cancer: Friend or foe? *Med Sci (Paris)* 24: 1083-1088, 2008 (In French).
32. Moitreyee CK, Suraksha A and Swarup AS: Potential role of NF- κ B and RXR beta like proteins in interferon induced HLA class I and beta globin gene transcription in K562 erythroleukaemia cells. *Mol Cell Biochem* 178: 103-112, 1998.
33. Kamińska E, Ociepa T, Wysocki M, Kurylak A, Matysiak M, Urański T, Urańska E and Domagała W: Activation of NF- κ B in leukemic cells in response to initial prednisone therapy in children with acute lymphoblastic leukaemia: Relation to other prognostic factors. *Pol J Pathol* 62: 5-11, 2011.
34. Cosimo E, McCaig AM, Carter-Brzezinski LJ, Wheadon H, Leach MT, Le Ster K, Berthou C, Durieu E, Oumata N, Galons H, *et al*: Inhibition of NF- κ B-mediated signaling by the cyclin-dependent kinase inhibitor CR8 overcomes prosurvival stimuli to induce apoptosis in chronic lymphocytic leukemia cells. *Clin Cancer Res* 19: 2393-2405, 2013.
35. Wu X, Xu W, Feng X, He Y, Liu X, Gao Y, Yang S, Shao Z, Yang C and Ye Z: TNF- α mediated inflammatory macrophage polarization contributes to the pathogenesis of steroid-induced osteonecrosis in mice. *Int J Immunopathol Pharmacol* 28: 351-361, 2015.
36. Schulz U, Munker R, Ertl B, Holler E and Kolb HJ: Different types of human leukemias express the message for TNF-alpha and interleukin-10. *Eur J Med Res* 6: 359-363, 2001.
37. Aggarwal BB, Shishodia S, Ashikawa K and Bharti AC: The role of TNF and its family members in inflammation and cancer: lessons from gene deletion. *Curr Drug Targets Inflamm Allergy* 1: 327-41, 2002.
38. Wang HQ, Li DL, Lu YJ, Cui XX, Zhou XF, Lin WP, Conney AH, Zhang K, Du ZY and Zheng X: Anticancer activity of *Acanthopanax trifoliatum* (L) Merr extracts is associated with inhibition of NF- κ B activity and decreased Erk1/2 and Akt phosphorylation. *Asian Pac J Cancer Prev* 15: 9341-9346, 2014.