NF-κB activation prevents apoptotic oxidative stress via an increase of both thioredoxin and MnSOD levels in TNFα-treated Ewing sarcoma cells

Mojgan Djavaheri-Mergny*, Delphine Javelaud, Juana Wietzerbin, Françoise Besançon

INSERM U365, Institut Curie, Section de recherche, 26 rue d'Ulm, 75248 Paris cedex 05, France

Received 20 July 2004; revised 25 October 2004; accepted 28 October 2004

Available online 11 November 2004

Edited by Frances Shannon

Abstract Repression of activation of c-Jun N-terminal kinase (JNK) participates in the anti-apoptotic effect of nuclear factor-κB (NF-κB) in TNFα-treated Ewing sarcoma cells. As oxidative stress is one of the most prominent activators of JNK, we investigated the relationship between TNFα-induced NF-κB activation and the control of oxidative stress. Inhibition of NF-κB activation resulted in an increase in TNFα-induced ROS production, lipid peroxidation and protein oxidation. Those ROS and lipid peroxides were both involved in TNFα-induced apoptosis, whereas only ROS elevation triggered sustained JNK activation. TNFα increased the level of two antioxidant enzymes, thioredoxin and manganese superoxide dismutase by an NF-κB-dependent mechanism. Inhibition of expression or activity of these enzymes sensitized cells to TNFα-induced apoptosis, indicating their functional role in protection from cell death. Thus, agents that inhibit activities of these enzymes may prove helpful in the treatment of Ewing tumors.

Keywords: Reactive oxygen species; c-Jun N-terminal kinase; Nuclear factor-κB; Tumor necrosis factor α; Apoptosis; Ewing tumor

1. Introduction

Ewing sarcomas are the second most common bone tumors in children and adolescents [1–3]. We have previously demonstrated that activation of the transcription factor nuclear factor-κB (NF-κB) protects Ewing sarcoma cells from death induced by several apoptotic stimuli, thus suggesting that inhibition of this factor may be a way of increasing chemotherapy efficacy [4]. This protection was shown to be at least partly mediated through NF-κB-induced repression of c-Jun N-terminal kinase (JNK) activation [5]. However, the mechanisms of these effects are still incompletely understood, in particular, the link between NF-κB activation and control of oxidative damage-induced apoptosis in Ewing sarcoma cells.

TNFα can induce cell killing in several cell lines through complex signal transduction initiated by its binding to TNF receptors (TNF-R) [6]. This binding is followed by the recruitment of the adapter molecule FADD, which binds pro-caspase-8, resulting in activation of caspase-8. Subsequently, the active form of caspase-8 triggers activation of other caspases, e.g., caspase-3, which execute the terminal phase of apoptosis [7]. Caspase-8 activation can also lead to cleavage of Bid, which may initiate activation of the mitochondrial death pathway by altering several mitochondrial functions [8,9].

Depending on the cell type, it has also been shown that TNFα signaling can lead to production of reactive oxygen species (ROS), mainly superoxide anion $O_2^{\bullet -}$, hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) [10–12]. The level of ROS produced in cells is strictly regulated by enzymatic and non-enzymatic antioxidant effectors that can either directly scavenge ROS or repair oxidative damage [13–15]. When ROS generation exceeds the cell's antioxidant capacity, oxidative damage to cellular components can occur. This situation is termed oxidative stress and can cause cell death [13,14,16,17]. A role of ROS in TNFα-mediated death signaling was suggested by the observation that antioxidants inhibited the apoptotic action of this cytokine in several cell lines [11].

TNFα can also activate the NF-κB transcription factor by recruiting the adapter protein TRADD. Signaling through TRADD culminates in the phosphorylation and degradation of an NF-κB inhibitor of the IκB family, which leads to the activation of the NF-κB transcription factor and promotes the transcription of several anti-apoptotic genes [4,18,19].

In the present study, we questioned whether NF-κB activation modulates TNFα-induced oxidative stress in Ewing sarcoma cells. We report that, in the absence of NF-κB activation, TNFα induces ROS production, lipid peroxidation and protein oxidation. We show that intracellular ROS accumulation participates in TNFα-induced apoptosis and the sustained JNK activation that occurs when NF-κB activation is impaired. We also show that TNFα induces a modulation of the expression of thioredoxin and manganese superoxide dismutase (MnSOD) in an NF-κB dependent manner, and that these two antioxidant enzymes are involved in the protection of Ewing sarcoma cells from TNFα-induced apoptotic oxidative stress.

*Corresponding author. Fax: +33 1 46 77 02 33.
E-mail address: mergny@vjf.inserm.fr (M. Djavaheri-Mergny).

Abbreviations: BNP, *N-t*-butyl-α-phenyl-nitrone; DETC, diethyldithiocarbamic acid; JNK, c-Jun N-terminal kinase; IκB, inhibitor κB; MnSOD, manganese superoxide dismutase; MnTBAP, manganese(III)tetrakis(4-benzoic acid) and porphyrin; NF-κB, nuclear factor-κB; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; TNFα, tumor necrosis factorα

0014-5793/$22.00
doi:10.1016/j.febslet.2004.10.082

2. Materials and methods

2.1. Reagents

DL-α-Tocopherol (vitamin E), Hoechst 33258 dye, Benzon nuclease, 2-thiobarbituric acid, recombinant thioredoxin from *Escherichia coli*, diethyldithiocarbamic acid (DETC), *N*-*t*-butyl-α-phenyl-nitrone (BPN) and *t*-butyl hydroperoxide (*t*-BH) were from Sigma (St. Quentin Fallier, France). Hydroethidium was from Molecular Probes, Inc. (Leiden, The Netherlands). Chariot, a protein delivery reagent, was from Active Motif (Rixensart, Belgium), manganese(III)tetrakis(4-benzoic acid) and porphyrin (MnTBAP) were from Alexis (Illkirch, France). Recombinant human TNFα (specific activity: 5×10^7 U/mg) was from Bender Wien and was used at a concentration of 2000 U/ml.

2.2. Cells

EW7 cells transfected with an empty pc DNA vector (EW7pc cells) or with the IκBα (A32/36)-encoding vector (EW7MAD cells) were previously described [4]. Cells were grown at 37 °C in RPMI medium supplemented with 2 mM L-glutamine and 10% fetal calf serum.

2.3. Detection of apoptosis

Condensed and fragmented nuclei were detected with Hoechst 33258 fluorescent dye. A total of 500 nuclei were counted for each sample. DNA fragmentation was quantified using a cell death detection kit (Roche Diagnostics GmbH, Mannheim, Germany).

2.4. ROS production measurement

Dihydroethidium (HE) was used to monitor intracellular oxidative stress. Briefly, cells were collected following treatment, washed with PBS and then loaded with 2 μM HE for 30 min at 37 °C. The fluorescence of ethidium in cells was measured with a cytofluorometer.

2.5. Determination of protein oxidation and lipid peroxidation

Protein oxidation was determined using an OXYBLOT kit (Intergen Company, Purchase, NY). Briefly, whole cell extracts were analyzed for protein oxidation by derivatization with 2,4-dinitrophenyl hydrazine of protein carbonyls, which were then analyzed by Western blotting using anti-DNP. The lipid peroxidation end-product malondialdehyde (MDA) was measured as previously described by using the TBARS (thiobarbituric acid reactive substances) assay [20].

2.6. Western blot analysis

Equal protein amounts of whole cell extract were analyzed by Western blotting as previously described [21]. Antibodies against the following proteins were used: MnSOD (Upstate Biotechnology, Lake Placid, NY), actin (ICN Biomedicals, Inc., Costa Mesa, CA), thioredoxin, catalase (Sigma, St Quentin Fallier, France), PARP (Alexis, Illkirch, France), heme oxygenase 1 (BD Biosciences, Erembodegem, Belgium), phospho-JNK (New England Biolabs, Beverly, MA, USA) and JNK-1 (Santacruz Biotechnology Inc., Ca, USA).

2.7. Transfections

Antisense phosphorothioate oligonucleotide against thioredoxin 5′-AGTCTTGCTCTCTCGATCTGCTTCACCAT-3′ and the control sense oligonucleotide 5′-ATGGTGAAGCAGATCGAGAGAAAG-ACT-3′ were used [22]. Transfection was carried out with 10 μM of each oligonucleotide by using lipofectamine 2000. Cells were then incubated for 24 h at 37 °C before addition of TNFα. Recombinant thioredoxin (10 μg) was delivered to cells 5 h before TNFα treatment, by using the Chariot kit. The percentages of apoptotic cells and protein expression were determined 16 h after TNFα treatment by staining with Hoechst 33258 dye and Western blot analysis, respectively.

3. Results

3.1. TNFα-induced oxidative stress in Ewing sarcoma cells occurs only in the absence of NF-κB activation

To investigate the relationship between the status of NF-κB activation and ROS production in Ewing sarcoma cells, we compared ROS production of TNFα-treated EW7MAD cells, in which NF-κB activation is inhibited by stable expression of a super repressor form of IκBα, IκBα (A32/36), with that of TNFα-treated control cells (EW7pc cells) [4]. Intracellular ROS production was measured by using the non-fluorescent hydroethidium compound which, when oxidized, yields fluorescent ethidium. As shown in Fig. 1A, TNFα treatment of EW7MAD cells resulted in an increase in ethidium fluorescence intensity as compared to control cells. This effect was detectable after 8 h of treatment and was markedly increased after 16 h, indicating that TNFα-induced intracellular accumulation of ROS. Inversely, we found that treatment of EW7pc cells with TNFα did not result in the accumulation of ROS

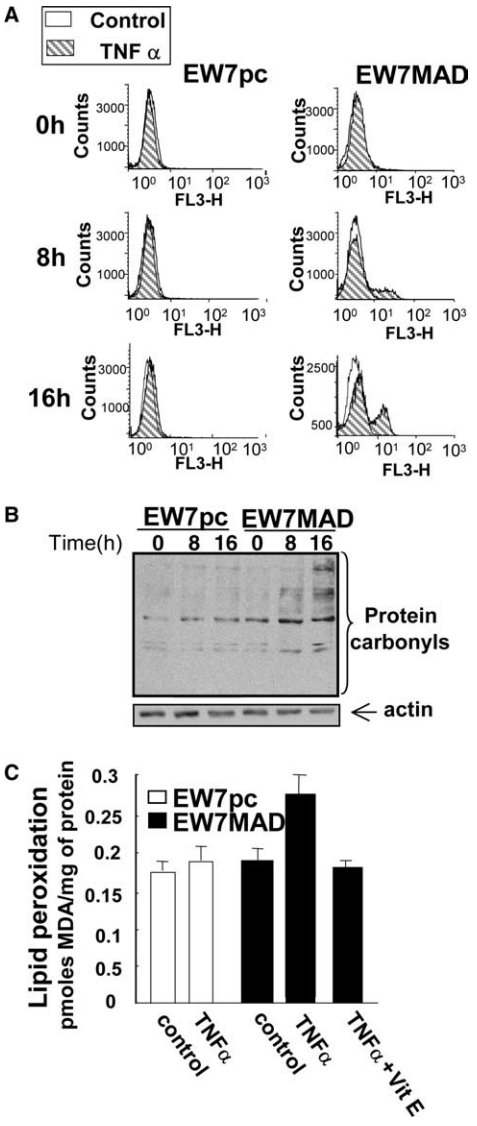

Fig. 1. Inhibition of NF-κB activation results in oxidative stress in TNFα-treated Ewing sarcoma cells. EW7pc cells and EW7MAD cells were treated with TNFα for the indicated times. (A) Cells were then labeled with 2 μM hydroethidium for 30 min at 37 °C. Ethidium fluorescence was analyzed by FACS. One representative experiment out of three is shown. (B) Whole cell extracts were analyzed for protein oxidation with the Oxyblot kit. (C) EW7pc cells and EW7MAD cells were incubated with or without 50 μM vitamin E (Vit E) for 16 h and then treated with 2000 U/ml TNFα for 16 h. The lipid peroxidation end-products were measured and the results are expressed as pmol of malondialdehyde (MDA) equivalents per μg of protein. Results represent means ± S.E.M. of three independent experiments.

in these cells. These results indicate that lack of NF-κB activation in TNFα-treated EW7MAD cells allows intracellular ROS production.

Excess intracellular ROS production and/or impairment of the antioxidant defense system may result in oxidative damage leading to cell death [14]. We therefore investigated whether TNFα induced such damage in EW7MAD cells. As shown in Fig. 1B, TNFα markedly increased the level of oxidized proteins in EW7MAD cells as compared to EW7pc cells. As excessive ROS production in cells may induce damage of cellular lipids, we determined the level of lipid peroxidation end-product, malondialdehyde, in EW7pc cells and EW7MAD after treatment with TNFα. As shown in Fig. 1C, TNFα induced a significantly greater increase in lipid peroxidation in EW7MAD as compared to EW7pc cells. As expected, vitamin E, a known lipid peroxidation chain-breaking antioxidant, completely inhibited lipid peroxidation induced by TNFα.

3.2. ROS scavengers inhibit both TNFα-induced apoptosis and activation of JNK and caspase-3 in EW7MAD cells

In order to determine the role of oxidative stress in apoptosis, EW7MAD cells were pre-treated with the ROS scavenger, BPN or vitamin E before addition of TNFα and apoptosis was evaluated by Hoechst staining. We found that both treatment with BPN and vitamin E reduced TNFα-induced cell death in EW7MAD cells, indicating the participation of ROS and lipid peroxidation in TNFα-induced apoptosis (Fig. 2A). As we have previously shown that TNFα-induced apoptosis in EW7-MAD cells requires both sustained activation of JNK and caspase 3 activation [4,5], we examined the effects of BPN and vitamin E on these apoptotic events. As shown in Fig. 2B, pre-treatment of EW7MAD cells with BPN completely abrogated the sustained activation of JNK triggered by TNFα when NF-κB activation was impaired, while vitamin E had no effect. These results show that ROS production, but not lipid peroxidation is involved in the prolongation of JNK activation. We also examined the effect of antioxidant supplementation on TNFα-induced caspase-3 activation which was assessed by the appearance of cleavage products of PARP, a well-known substrate of this caspase. As shown in Fig. 2C, addition of BPN or vitamin E reduced TNFα-induced PARP cleavage in EW7MAD cells. These results suggest that TNFα-induced ROS production and lipid peroxidation are both involved in caspase-3 activation, whereas sustained JNK activation is only mediated by the elevation of ROS concentration.

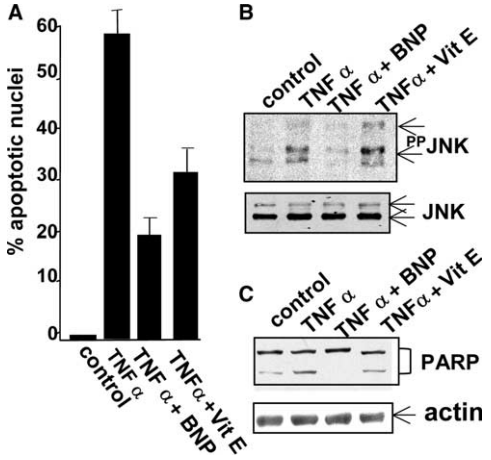

Fig. 2. Antioxidants inhibit both TNFα-induced apoptosis and activation of JNK and caspase-3. EW7MAD cells were first treated with either 50 μM *N-t*-butyl-α phenyl-nitone (BPN) for 2 h or with 50 μM vitamin E (Vit E) for 16 h prior to treatment with TNFα for 16 h. (A) The percentage of apoptotic cells was determined by staining with Hoechst. (B, C) Cellular extracts were prepared and analyzed by Western blot using antibodies directed against either phosphoJNK (^{PP}JNK), anti-JNK-1 (B) or PARP (C). One representative experiment out of two is shown.

3.3. Treatment with TNFα results in increased expression of antioxidant enzymes in EW7pc cells as compared to EW7MAD cells

The redox status of cells is regulated by the intracellular antioxidant defense system [15]. Our results showing that TNFα induced production of ROS in EW7MAD cells but not in EW7pc cells suggest an impairment of the antioxidant defense system in EW7MAD cells in comparison to EW7pc cells. The level of expression of antioxidant enzymes was therefore compared in these two cell types upon treatment with TNFα. Several distinct cellular antioxidant enzymes were examined: manganese superoxide dismutase, a mitochondrial antioxidant which scavenges superoxide anion, thioredoxin which by its oxido-reductase activity scavenges ROS and

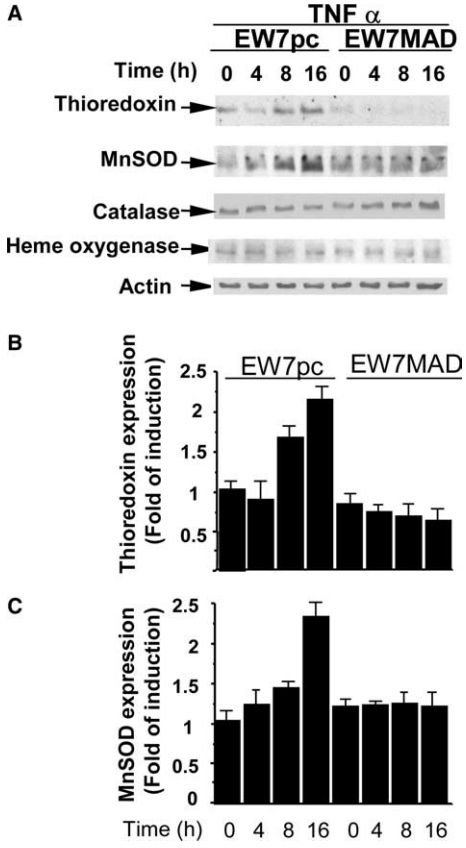

Fig. 3. Comparison of the antioxidant enzyme status in Ewing sarcoma cells treated with TNFα in the presence and absence of a repressor of NF-κB activation. (A) EW7pc cells and EW7MAD cells were treated with TNFα for the indicated times before cell lysis. Equal protein amounts of whole cell extract were analyzed by Western blotting using antibodies directed against thioredoxin, MnSOD, heme oxygenase-1, and catalase. One representative experiment out of three is shown. Bands corresponding to thioredoxin (B) and MnSOD (C) were quantified using NIH Image software and were normalized to the actin. Values represent means ± S.E.M. of three independent experiments.

catalyzes reduction of thiol-oxidized proteins, and catalase which neutralizes hydrogen peroxide production by its conversion to water [15]. Heme oxygenase-1 was also examined as it is known to act as an antioxidant by leading to the formation of bilirubin, which possesses ROS scavenger properties [23]. As shown in Fig. 3A, TNFα upregulated both thioredoxin and manganese superoxide dismutase expression in EW7pc cells but not in EW7MAD cells. Expression of catalase and heme oxygenase-1 remained unchanged in both cells after treatment with TNFα.

3.4. *Thioredoxin and manganese superoxide dismutase are both involved in protection against apoptosis induced by TNFα in EW7pc*

The higher level of expression of thioredoxin and MnSOD in response to TNFα in EW7pc cells compared to that observed in EW7MAD cells suggests a role of these antioxidant enzymes in protection against TNFα-induced apoptosis in EW7pc cells. To test this hypothesis, we examined, on the one hand, the effect of antioxidant supplementation on TNFα-induced apoptosis in EW7MAD and, on the other hand, the effect of inhibition of thioredoxin and MnSOD activities on the sensitivity of EW7pc cells to TNFα-induced cell death. As shown in Fig. 4A, addition of the MnTBAP compound that mimics MnSOD activity partially protected against TNFα-induced cell death in EW7-MAD cells, as evaluated by Hoechst staining. In contrast, in EW7pc cells inhibition of superoxide dismutase activity by DETC by itself induced cell death, which was enhanced in the presence of TNFα treatment (Fig. 4B). Similar experiments have been done to determine the role of thioredoxin in the control of apoptosis. As shown in Fig. 4C, pretreatment of EW7-MAD cells with recombinant thioredoxin partially inhibited TNFα-induced apoptosis. On the other hand, inhibition of thioredoxin expression in EW7pc by an antisense oligonucleotide sensitized these cells to TNFα treatment (Fig. 4D). These results demonstrate the functional role of both thioredoxin and MnSOD in protection against apoptosis in EW7pc cells.

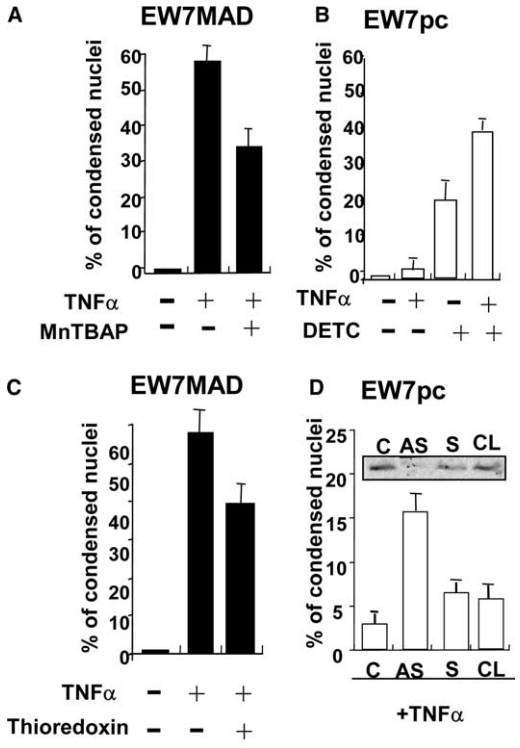

Fig. 4. Thioredoxin and MnSOD are both involved in protection against apoptosis induced by TNFα in EW7pc cells. (A) EW7MAD cells and (B) EW7pc cells were first incubated for 2 h with MnTBAP (100 μM) and DETC (5 mM), respectively, and then treated for 16 h with TNFα before evaluation of apoptosis by Hoechst staining. (C) Thioredoxin recombinant proteins were delivered to EW7MAD cells by using the Chariot kit 5 h before TNFα treatment. (D) The sense and antisense thioredoxin oligonucleotides were introduced into EW7pc cells by using lipofectamine 2000, 24 h before the beginning of TNFα treatment. The percentages of apoptotic cells and thioredoxin protein expression were determined by staining with Hoechst staining and Western blot analysis, respectively, after 16 h of TNF treatment. C: untreated, AS: antisense treated, S: sense treated, CL: lipofectamine alone treated. Values represent means ± S.E.M. of three independent experiments.

4. Discussion

In the present study, we provide evidence that NF-κB activation prevents apoptotic oxidative stress induced by TNFα in Ewing sarcoma cells. This interpretation is based on results showing that (1) TNFα promotes accumulation of ROS and induces protein and lipid oxidation only in cells in which NF-κB activation is impaired. (2) In the latter cells, ROS scavengers inhibit TNFα-induced apoptosis.

Cellular antioxidants can control the redox status of cells in response to an oxidant injury [13,14]. Depending on cell type, NF-κB activation can regulate the expression of several antioxidant enzymes that may be involved in protection against cell injury [24–27]. The role of thioredoxin in the control of oxidative stress induced by TNFα has also been pointed out [28], but there are no data concerning the involvement of NF-κB activation in the regulation of its expression. In the present study, we show for the first time that, in Ewing sarcoma cells, NF-κB-dependent thioredoxin expression is involved in protection against TNFα-induced apoptosis. The effective role of thioredoxin in the protection against TNFα-induced apoptosis was demonstrated by experiments showing that specific inhibition of thioredoxin expression sensitized Ewing sarcoma cells to TNFα-induced apoptosis. Furthermore, the addition of recombinant thioredoxin inhibited apoptosis of Ewing sarcoma cells in which NF-κB activation was impaired. In agreement with our results, it has been shown that cancer cell lines with elevated expression of thioredoxin are resistant to a variety of apoptosis-inducing agents, including ROS [29,30]. This protective effect of thioredoxin is assumed to be the result of its antioxidant function [15,31]. We found that NF-κB activation also increased the expression level of MnSOD in TNFα-treated Ewing sarcoma cells. In accordance with our results, it has been shown that overexpression of MnSOD prevents apoptosis induced by several oxidative stress inducers, including TNFα [32–34]. In contrast, upregulation of MnSOD can induce apoptosis in some other model systems [35]. Moreover, it has also been reported that upregulation of MnSOD by the c-Rel transcription factor first induces resistance to the apoptotic burst of $O_2^{\cdot-}$ produced by TNFα and later increases H_2O_2 accumulation, which results in apoptosis of HeLa cells [15,36]. These different results may be explained by the fact that MnSOD is an enzyme which catalyzes the dismutation of superoxide anion to hydrogen peroxide, which still has to be neutralized by several antioxidant peroxidases [37]. However, if the latter antioxi-

dant system is impaired, hydrogen peroxide accumulates in cells and may in turn induce oxidative stress. Thus, the balance between MnSOD and peroxidase activities in cells could determine the anti- or pro-apoptotic effect of MnSOD upregulation in cells. Our results suggest that in Ewing sarcoma cells, thioredoxin may contribute to the neutralization of the H_2O_2 produced by TNFα-enhanced MnSOD activity.

We found that impairment of NF-κB activity resulted in protein and lipid oxidation in TNFα-treated Ewing sarcoma cells. Thioredoxin is a well-known example of a protein that is negatively regulated in its function upon oxidation. Oxidation of thioredoxin leads to the dissociation of ASK-1 from thioredoxin and to its activation. Subsequently, the activated form of ASK-1 can trigger JNK activation [38–40]. Although both pro- and anti-apoptotic effects of JNK have been reported [41], activation of this MAP kinase has been clearly shown to induce apoptosis in Ewing sarcoma cells [5,21]. In these cells, induction of apoptosis by TNFα required a sustained activation of JNK which occurred only in the absence of NF-κB activation [5]. In the present study, we identified TNFα-induced ROS production as the cause of the sustained activation of JNK observed in EW7 cells carrying a repressor of NF-κB activation as compared to control cells. Thus, the modulation of the JNK pathway by ROS is one of the mechanisms by which TNFα-induces apoptosis in these cells. In accordance with our results, a recent report showed that NF-κB inhibits TNFα-induced accumulation of ROS, which mediates prolonged MAPK activation and necrotic cell death in murine embryonic fibroblasts (MEFs) [42]. Also, Chen et al. [43] demonstrated that the increased accumulation of ROS is responsible for the prolonged JNK activation in arsenic-stimulated IKKβ−/− MEFs.

We also found that TNFα induced lipid peroxidation in cells expressing the inhibitor of NF-κB activation. Addition of vitamin E, a well-known inhibitor of lipid peroxidation, protected these cells against TNFα-induced apoptosis and caspase-3 activation without any effect on JNK activation, indicating that lipid peroxidation triggered a death signal downstream of JNK activation. In accordance with these results, it has been shown that agents that induce lipid peroxidation activate cell death in several cell lines [44]. One of the mechanisms that has been proposed to explain this effect is the dissociation of cytochrome *c* from mitochondria upon peroxidation of the mitochondrial membrane [45].

In summary, we have identified mechanisms by which NF-κB controls intracellular ROS production and protein or lipid oxidation induced by TNFα in Ewing sarcoma cells. Our findings also suggest that agents that decrease thioredoxin and MnSOD activities might be beneficial adjuvants in the treatment of Ewing tumors.

Acknowledgement: We thank Dr. O. Delattre and Prof. G. Lenoir for providing us with the Ewing sarcoma cells. We are grateful to C. Silvestri for professional technical assistance and D. Rouillard for FACS analysis. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM) and by a grant from the Association pour la Recherche sur le Cancer (ARC) no. 4214.

References

[1] Delattre, O., et al. (1992) Nature 359, 162–165.
[2] Sorensen, P.H., Lessnick, S.L., Lopez-Terrada, D., Liu, X.F., Triche, T.J. and Denny, C.T. (1994) Nat. Genet. 6, 146–151.
[3] Kovar, H. (1998) Curr. Opin. Oncol. 10, 334–342.
[4] Javelaud, D., Wietzerbin, J., Delattre, O. and Besancon, F. (2000) Oncogene 19, 61–68.
[5] Javelaud, D. and Besancon, F. (2001) Oncogene 20, 4365–4372.
[6] Reed, J.C. (2000) Am. J. Pathol. 157, 1415–1430.
[7] Debatin, K.M. and Krammer, P.H. (2004) Oncogene 23, 2950–2966.
[8] Mignotte, B. and Vayssiere, J.L. (1998) Eur. J. Biochem. 252, 1–15.
[9] Ferri, K.F. and Kroemer, G. (2000) Bioessays 23, 111–115.
[10] Sidoti-de Fraisse, C., Rincheval, V., Risler, Y., Mignotte, B. and Vayssiere, J.L. (1998) Oncogene 17, 1639–1651.
[11] Garg, A.K. and Aggarwal, B.B. (2002) Mol. Immunol. 39, 509–517.
[12] Finkel, T. (2003) Curr. Opin. Cell Biol. 15, 247–254.
[13] Sies, H. (1997) Exp. Physiol. 82, 291–295.
[14] Martindale, J.L. and Holbrook, N.J. (2002) J. Cell Physiol. 192, 1–15.
[15] Nordberg, J. and Arner, E.S. (2001) Free Radic. Biol. Med. 31, 1287–1312.
[16] Halliwell, B. and Gutteridge, J.M. (1984) Lancet 2, 1095.
[17] Benhar, M., Engelberg, D. and Levitzki, A. (2001) EMBO Rep. 3, 420–425.
[18] Stancovski, I. and Baltimore, D. (1997) Cell 91, 299–302.
[19] Lin, A. and Karin, M. (2003) Semin. Cancer Biol. 13, 107–114.
[20] Yagi, K. (1987) Chem. Phys. Lipids 45, 337–351.
[21] Djavaheri-Mergny, M., Wietzerbin, J. and Besancon, F. (2003) Oncogene 22, 2558–2567.
[22] Haendeler, J., Hoffmann, J., Tischler, V., Berk, B.C., Zeiher, A.M. and Dimmeler, S. (2002) Nat. Cell Biol. 4, 743–749.
[23] Maines, M.D. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 517–554.
[24] Delhalle, S., Deregowski, V., Benoit, V., Merville, M.P. and Bours, V. (2002) Oncogene 21, 3917–3924.
[25] Brouard, S., Berberat, P.O., Tobiasch, E., Seldon, M.P., Bach, F.H. and Soares, M.P. (2002) J. Biol. Chem. 277, 17950–17961.
[26] Pahl, H.L. (1999) Oncogene 18, 6853–6866.
[27] Iwanaga, M., Mori, K., Iida, T., Urata, Y., Matsuo, T., Yasunaga, A., Shibata, S. and Kondo, T. (1998) Free Radic. Biol. Med. 24, 1256–1268.
[28] Matsuda, M., et al. (1991) J. Immunol. 147, 3837–3841.
[29] Patenaude, A., Murthy, M.R. and Mirault, M.E. (2004) J. Biol. Chem..
[30] Kwon, Y.W., Masutani, H., Nakamura, H., Ishii, Y. and Yodoi, J. (2003) Biol. Chem. 384, 991–996.
[31] Masutani, H. and Yodoi, J. (2002) Methods Enzymol. 347, 279–286.
[32] Manna, S.K., Zhang, H.J., Yan, T., Oberley, L.W. and Aggarwal, B.B. (1998) J. Biol. Chem. 273, 13245–13254.
[33] Motoori, S., et al. (2001) Cancer Res. 61, 5382–5388.
[34] Zhao, Y., Kiningham, K.K., Lin, S.M. and St Clair, D.K. (2001) Antioxid. Redox Signal 3, 375–386.
[35] Zhong, W., Oberley, L.W., Oberley, T.D., Yan, T., Domann, F.E. and St Clair, D.K. (1996) Cell Growth Differ. 7, 1175–1186.
[36] Bernard, D., Monte, D., Vandenbunder, B. and Abbadie, C. (2002) Oncogene 21, 4392–4402.
[37] Halliwell, B. (1999) Free Radic. Res. 31, 261–272.
[38] Saitoh, M., et al. (1998) Embo J. 17, 2596–2606.
[39] Adler, V., Yin, Z., Tew, K.D. and Ronai, Z. (1999) Oncogene 18, 6104–6111.
[40] Tobiume, K., et al. (2001) EMBO Rep. 2, 222–228.
[41] Varfolomeev, E.E. and Ashkenasi, A. (2004) cell 116, 491–497.
[42] Sakon, S., et al. (2003) Embo J. 22, 3898–3909.
[43] Chen, F., Castranova, V., Li, Z., Karin, M. and Shi, X. (2003) Cancer Res. 63, 7689–7693.
[44] Tang, D.G., La, E., Kern, J. and Kehrer, J.P. (2002) Biol. Chem. 383, 425–442.
[45] Petrosillo, G., Ruggiero, F.M., Pistolese, M. and Paradies, G. (2001) FEBS Lett. 509, 435–438.