Free Radical Research, November 2006; 40(11): 1182–1189

informa
healthcare

Inhibitory effect of peroxiredoxin II (Prx II) on Ras–ERK–NFκB pathway in mouse embryonic fibroblast (MEF) senescence

YING-HAO HAN, JEONG-HOON KWON, DAE-YEUL YU, & EUN-YI MOON

Department of Human Genomics, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejeon 305-806, South Korea

Accepted by Professor J. Yodoi

(Received 27 March 2006)

Abstract
Intracellular reactive oxygen species (ROS) were attenuated by the expression of peroxiredoxin II (Prx II). Cellular senescence as judged by senescence-associated (SA)-β-galactosidase (Gal) positive cell formation was increased in Prx II-deficient mouse embryonic fibroblast (MEF). Ras expression was increased following passages. The level of Ras expression was higher in Prx $II^{-/-}$ MEF than wild type MEF. ERK activity was also augmented by the deletion of Prx II. SA-β-Gal-positive cell formation was reduced by PD98059, ERK inhibitor. Activated nuclear transcription factor, nuclear factor-kappaB (NFκB) by the deletion of Prx II was inhibited by the treatment with PD98059. In contrast, no changes in SA-β-Gal-positive cell formation were detected by NFκB inhibitor, *N*-alpha-tosyl-L-phenylalanyl chloromethyl ketone (TPCK). Collectively, results suggest that Prx II deletion activate Ras–ERK–NFκB pathways and cellular senescence in Prx $II^{-/-}$ MEF cells was mediated by ERK activation but not by NFκB activation.

Keywords: *Ras, ERK, NFκB, Peroxiredoxin II, MEF, ROS*

Abbreviations: *ROS, reactive oxygen species; ERK, extracellular receptor kinase; MEF, mouse embryonic fibroblast; Prx II, peroxiredoxin II; SA-β-Gal, senescence-associated-β-glactosidase; SOD, superoxide dismutase; SEAP, secreted alkaline phosphatase; MBP, myelin basic protein; PMA, phorbol 12-myristate 13-acetate; TPCK,* N-*alpha-tosyl-L-phenylalanyl chloromethyl ketone; NFκB, nuclear factor kappaB; EMSA, electrophoretic mobility shift assay; IOM, ionomycin*

Introduction

Cellular senescence is defined as the process of cell cycle which accompanies the exhaustion of replicative potential [1]. Senescent cells remain metabolically active, display characteristic changes in cell morphology, physiology and gene expression, and typically exhibit an up-regulation in the activity of senescence-associated (SA)-β-galactosidase (Gal) [2–4]. Senescent cells are unable to express genes required for proliferation but express late *G1* genes due to repression of E2F-1, leading to a deficiency of E2F activity [5,6].

Reactive oxygen species (ROS) induce cellular senescence, and senescent cells are known to have higher levels of ROS than normal cells [7,8]. ROS is reduced by enzymatic antioxidants such as glutathione, glutathione peroxidase (GPx), catalase, thioredoxins (Trx), superoxide dismutase (SOD) and peroxiredoxins (Prxs). SOD1 knock-down induces senescence in human fibroblasts [9], and $GPx1^{-/-}$ mouse embryonic fibroblast (MEF) display senescent-like features [10]. X-Gal-reactive senescent cells are vigorously formed in Prx $II^{-/-}$ MEF cells, which is strongly correlated with G2/M arrest and extracellular signal regulated kinase (ERK) activation [11].

It has been reported that ERK signal might activate nuclear factor-kappaB (NFκB) in various cellular system [12–14]. NFκB and ERK contribute to the

Correspondence: E. Y. Moon, Department of Human Genomics, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Taejeon 305-806, South Korea. Tel: 82 42 860 4347. Fax: 82 42 860 4608. E-mail: eunyi@kribb.re.kr; eunyimoon@yahoo.com

ISSN 1071-5762 print/ISSN 1029-2470 online © 2006 Informa UK Ltd.
DOI: 10.1080/10715760600868552

survival of the irradiated cell. NFκB and ERK as anti-apoptotic factors could lead to the inhibition of apoptosis and, consequently, to increased mutagenicity. Both NFκB and ERK show a fluctuation in their levels with time [12]. Glycated albumin activates RAW cell ERK and promotes ERK-dependent increases in TGF-β production, oxidative stress and NFκB activation [13]. In JB6 cells, both ERK/Fra-1 and NFκB activity is essential for the transformation response. Inhibition of NFκB and AP-1 activity abrogates transformation in JB6 cells as well as in transgenic mice and human keratinocytes [14]. However, little has been known about whether ERK activate NFκB in Prx II-deleted MEF cells.

NFκB family consists of five members: NFκB1 (p105/p50), NFκB2 (p100/p52), RelA (p65), RelB and c-Rel. The activity of NFκB is primarily controlled at the post-translational level [15,16]. In unstimulated cells, NFκB exists in an inactive state in the cytoplasm complexed with the inhibitory protein called inhibitory factor kappa B (IκB). Upon activation, IκB undergoes phosphorylation and degradation. NFκB-heterodimer is translocated into the nucleus where it binds to DNA and activates transcription [17]. De novo synthesis of new IκB proteins occurs after its phosphorylation and degradation by IκB kinase (IKK) and then IκB enters the nucleus, dissociates NFκB from DNA, and again inactivates NFκB [18].

Several reports have shown that ROS are involved in the activation of NFκB [19,20]. ROS and other free radicals can activate AP-1 and NFκB transcription coordinately [14]. NFκB could take part in the occurrence of senescence by generating an oxidative stress via the induction of MnSOD [21]. The up-regulation of constitutive nuclear NFκB binding activity and increased levels of nuclear p52 and p65 proteins might affect the expression of some NFκB target genes in the aging liver [22]. Little is known about whether NFκB could be activated by ROS produced in Prx II–null MEF cells.

Here, we studied whether ERK signal activates NFκB and whether ERK–NFκB pathway plays a role on inducing cellular senescence in Prx II–null MEF cells. Data suggest that NFκB could be activated by ERK but it does not affect senescent cell formation in MEF cells.

Materials and methods

Mice and chemicals

Wild type and Prx II-deficient mice with 129/SvJ background were maintained in the pathogen-free authorized facility in Korea Research Institute of Bioscience and Biotechnology (KRIBB) where the temperature at 20–22°C, the humidity 50–60% and the 12 h-dark/light cycles were maintained. All animal procedures were conducted in accordance with the guidelines of the institutional Animal Care and Use Committee, KRIBB. The genotyping of animals was performed as described in Ref. [23]. PD98059 and SB203580 were purchased from Calbiochem (La Jolla, CA, USA). Anti-phospho-Erk anti-Ras, anti-cytin Bl, anti-cytin A antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Unless indicated, chemicals were purchased from Sigma Chemical Company (St Louis, MI, USA).

Preparation of mouse embryo fibroblast (MEF)

MEF were prepared with 13.5-day embryos derived from Prx II$^{+/-}$ mice mated each other. Head, tail and viscera were removed, the remain body was minced and dispersed in 0.25% trypsin/EDTA and incubated in a 5% CO_2 at 37°C for 30 min. Then, the large fragments were removed and the cell suspension was plated in 10 cm plates and incubated at 37°C until confluent.

Cell culture

MEF cells were maintained in Dulbecco's modified Eagle medium (DMEM supplemented with 10% fetal bovine serum (FBS), L-glutamine and penicillin/streptomycin. The cells were incubated in a 5% CO_2 at 37°C. For the inhibition of ERK and p38 activity, MEF cells were treated with 5 μM PD98059 and 5 μM SB203580, respectively.

Senescence-associated (SA)-β-galactosidase (Gal) staining

Cells were washed with PBS (pH 7.2–7.4) and fixed with 0.5% glutaraldehyde in PBS for 5 min at room temperature. Then, cells were washed twice with PBS and incubated at 37°C (no CO_2) with fresh SA-β-Gal stain solution [1 mM/ml X-Gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 40 mM sodium phosphate (12.0% Na_2HPO_4 and 88.0% NaH_2PO_4 pH 6.0), 150 mM NaCl, 2 mM $MgCl_2$] [2]. Staining was evident in 2–4 h and maximal in 12–16 h. SA-β-Gal positive cells were detected and counted by light microscopy.

Measurement of NFκB activity

NFκB activation was measured by the transfection with pNFκB–secreted alkaline phosphatase (SEAP) (BD Biosciences, Palo Alto, CA, USA). pNFκB–SEAP is designed for monitoring the activation of NFκB signal transduction pathway. pNFκB–SEAP contains the SEAP reporter gene. This vector also contains four tandem copies of the NFκB consensus sequence fused to a TATA-like promoter region from the Herpes simplex virus thymidine kinase promoter.

After endogenous NFκB proteins bind to the kappa (κ) enhancer element (κB4) transcription is induced and the reporter gene is activated. The secreted SEAP enzyme can be assayed directly from the culture medium using Great EscAPe Chemilumineacence Detection Kits (BD Biosciences Clontech, Palo Alto, CA, USA). Chemilumineacence was measured by luminometer (Perkin Elmer, Wellesely, MA, USA).

RT-PCR

RNA was isolated from MEF cells at each passage using TRIZOL (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from 1 μg of total RNA, using oligo dT_{18} primers and superscript reverse transcriptase in a final volume of 21 μl (Bioneer, Taejeon, Korea). For standard PCR, 1 μl of the first strand cDNA product was then used as a template for PCR amplification with Taq DNA polymerase (Bioneer, Taejeon, Korea). PCR amplification proceeded as follows: 30 thermocycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, using oligonucleotides specific for p16 (sense, atg ggt cgc agg ttc ttg gtc ac; anti-sense, cta tgc ccg tcg gtc tgg gcg a), p21 (sense, gtc caa tcc tgg tga tgt cc; anti-sense, gtt ttc ggc cct gag atg t), p53 (sense, cac agt cgg ata tca gcc tcg a; anti-sense, gcc cca ctt tct tga cca ttg t) and Gapdh (sense, tcc acc acc ctg ttg ctg ta; anti-sense, acc aca gtc cat gcc atc ac).

Western blot analysis

Cells were lysed in ice-cold lysis buffer containing 0.5% Nonidet P-40 (v/v) in 20 mM Tris–HCl (pH 8.3); 150 mM NaCl; protease inhibitors (2 μg/ml aprotinin, pepstatin and chymostatin; 1 μg/ml leupeptin and pepstatin; 1 mM phenylmethyl sulfonyl fluoride (PMSF); and 1 mM Na_4VO_3. Lysates were incubated for 30 min on ice before centrifugation at 14,000 rpm for 5 min at 4°C. Proteins in the supernatant were denatured by boiling for 5 min in sodium dodecyl sulfate (SDS) sample buffer. Proteins were separated by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes. Following transfer, equal loading of protein was verified by Ponceau staining. The membranes were blocked with 5% skim milk in Tris-buffered saline with Tween 20 (TBST) (10 mM Tris–HCl, pH 7.6; 150 mM NaCl; 0.5% Tween 20) and incubated with the indicated antibodies, polyclonal cyclin A (1:5000), and cyclin B1 (1:5000), phospho-ERK (1:1000), Ras (1:1000) and monoclonal tubulin (1:5000). Bound antibodies were visualized with HRP-conjugated secondary antibodies with the use of enhanced chemiluminescence (ECL) (Pierce, Rockford, IL, USA).

ERK activity assay

About 200 μg proteins of cell lysates were incubated with anti-phospho-ERK antibodies (Santa Cruz Biotechnology Inc) at 4°C overnight. Then, phospho-ERK proteins were concentrated with protein A/G agarose (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) at 4°C for 2 h. ERK activity was measured with MAP kinase assay kit (Upstate, Charlottesville, VA, USA) according to the manufacturer's instruction. Briefly, 10 μl of Mg^{2+}/ATP cocktail and 10 μl of Assay Dilution Buffer I (ADBI) including 2 mg/ml dephosphorylated myelin basic protein (MBP) were added to the immunoprecipitated protein then, incubated for 30 min in a 30°C shaking incubator. About 10 μl of the reaction mixture was mixed with 4 μl of 5X sample buffer and 6 μl of Tris-buffered saline (TBS) then, separated in SDS-PAGE. Proteins were transferred to nitrocellulose membrane and immuno-blotted with anti-phospho-MBP antibodies (clone P12).

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as described previously in Ref. [24]. The protein content of the nuclear extracts was determined using a Bio-Rad protein assay kit according to the manufacturer's instruction (Amersham Nioaciences UL, Ltd). The oligonucleotide sequence for NFκB was 5′-gatctcagaggggactttccgagaga-3′ [25]. Double-stranded oligonucleotides were end-labeled with [γ-^{32}P]-ATP. Nuclear extracts (5 μg) were incubated with 2 μg of poly (dI–dC) and a ^{32}P-labeled DNA probe, and DNA binding activity was analyzed using a 5% polyacrylamide gel. After electrophoresis, the gel was dried and subjected to autoradiography. The specificity of binding was examined by competition with an unlabeled oligonucleotide.

Statistical analyses

Experimental differences were tested for statistical significance using ANOVA and Students' *t*-test. *P* value of <0.05 or <0.01 was considered to be significant.

Results

Cellular senescence is enhanced by the deletion of Prx II gene

Prx II gene-deleted mice were generated by the technology of gene recombination. MEF cells prepared from Prx $II^{-/-}$ mice did not express Prx II [23]. The intracellular ROS level was higher in the Prx $II^{-/-}$ MEF cells than in the wild type cells [11]. In comparison with the wild type, the number of SA-β-Gal-positive cells increased due to the deletion

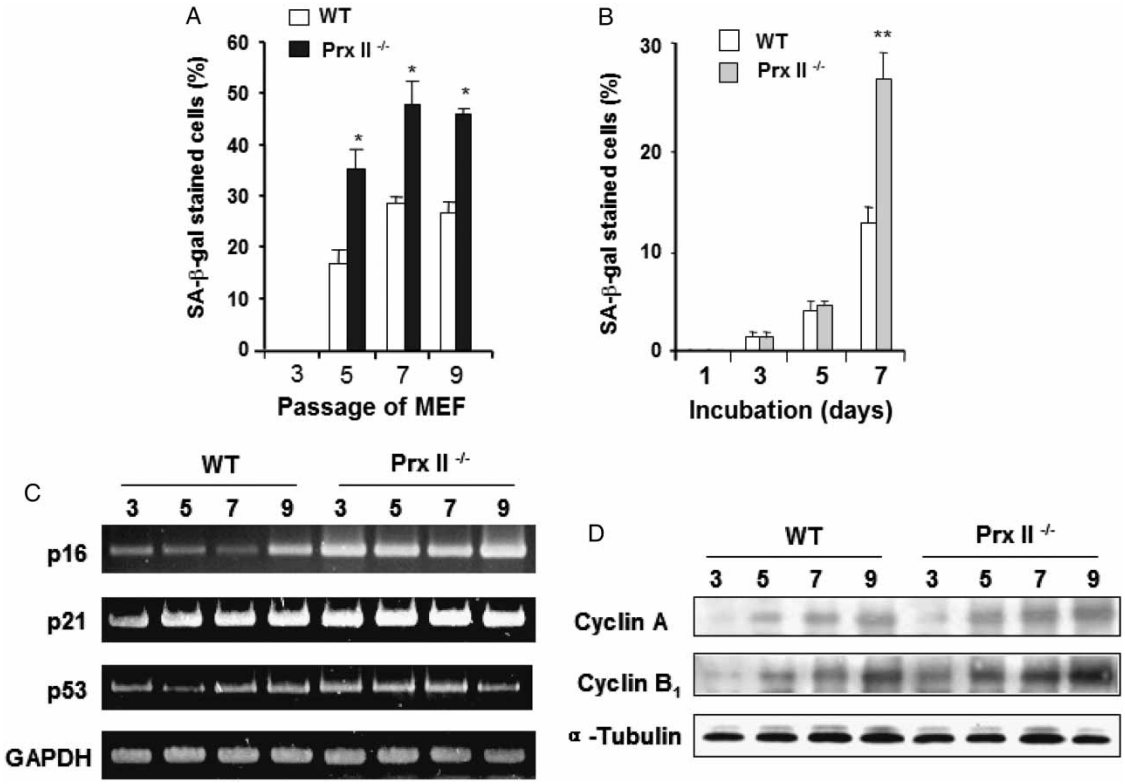

Figure 1. Cellular senescence was enhanced by the deletion of Prx II gene. (A) MEF cells were cultured for the examination of SA-β-Gal activity. SA-β-Gal-positive cells were colored blue by incubating with X-gal, substrate. SA-β-Gal-positive cells were measured every other passage at the third, fifth, seventh or nineth passage. (B) MEF cells at the fourth passage were cultured and SA-β-Gal-positive cells were measured every other day from the first to the seventh. Data were mean of three independent MEF clones. (C) RNA was purified from MEF cells at the third, fifth, seventh and nineth passage with the method described in materials and methods. Transcripts of p16, p21, p53 and GAPDH were detected with oligonucleotides described in materials and methods. (D) Cells lysates were prepared from MEF cells at the third, fifth, seventh and nineth passage. Cell cycle-related proteins were detected by western blot analysis. *, $p < 0.05$; **, $p < 0.01$; significant as compared to wild type control at each passage or at each day.

of Prx II from the fifth passage to the nineth (Figure 1(A)). SA-β-Gal-positive cells were appeared from the third-day after the culture initiation of MEF cells and increased time-dependently by seventh-day (Figure 1(B)). It demonstrates that Prx II$^{-/-}$ MEF cells senesce at a higher rate following passages and incubation days compared to wild type MEF cells.

Cell cycle was arrested and cells are metabolically active but not grow and various molecules were changed in senescent cells [3,26]. To confirm the increased cellular senescence in Prx II-deficient MEF cells, the changes of various senescence-associated molecules were investigated by Western blot analysis and RT-PCR. Previous reports demonstrated p53, p16 and a broad-acting cyclin-dependent kinase inhibitor, p21 increased in senescent cells [3,27,28]. Cell cycle regulators, p53, p16 and p21 were increased in Prx II$^{-/-}$ MEF cells (Figure 1(C)) as compared to wild type MEF cells. Cyclin A and B1 expression was also increased in Prx II$^{-/-}$ MEF cells as compared to wild type MEF (Figure 1(D)). This is the reflection of earlier cell cycle arrest by the deletion of Prx II [11], which is resulted in a higher percentage of SA-β-Gal-positive cells.

Activation of Ras–ERK–NFκB pathways by the deletion of Prx II

We have reported that ERK was activated in Prx II$^{-/-}$ MEF cells and the increased cellular senescence in the Prx II$^{-/-}$ MEF was mediated by p38 and ERK activation [11]. Based on that cellular senescence increased by Ras expression and Ras activates MAPK pathways [29,30], we evaluated Ras expression in wild type and Prx II$^{-/-}$ MEF cells following passages. Figure 2(A) showed that Ras was increased from the third passage to the seventh. Ras level was higher in Prx II$^{-/-}$ MEF than wild type MEF cells. In accordance to this, Higher ERK activity was detected in Prx II$^{-/-}$ MEF cells. ERK activity was measured by immuno-precipitation with anti-phospho-ERK antibodies and incubation with MBP. ERK activity was correlated with the intensity of phospho-MBP band. ERK activity was inhibited by the incubation with PD98059, ERK inhibitor (Figure 2(B)). PD98059 also inhibited SA-β-Gal-positive cell formation in both MEF cells. The percentage of inhibition by PD98059 was much greater in Prx II$^{-/-}$ MEF cells than wild type MEF (Figure 3). Data suggest that

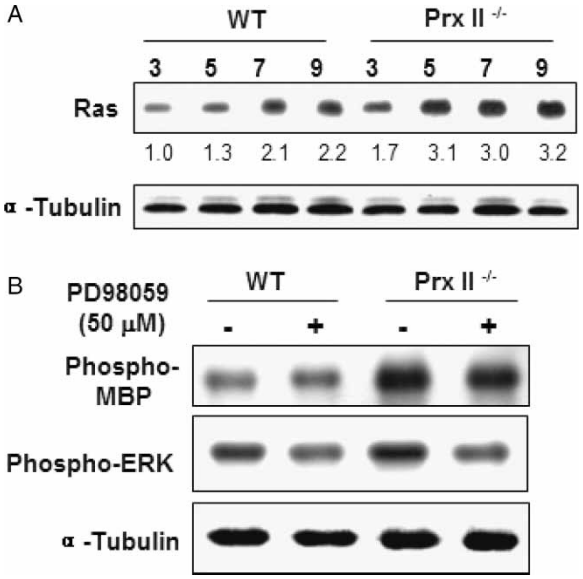

Figure 2. Cellular senescence was accompanied by the increase of Ras protein and ERK activation. (A) MEF cell lysates were prepared from wild type (WT) or Prx II$^{-/-}$ MEF cells at the third, fifth, seventh and nineth passage. Following passages, cell lysates were separated by Western blot for the detection of Ras protein. Fold expression was numerically indicated under each band. (B) MEF cells lysates were prepared and incubated with ERK inhibitor, PD98059 for 30 min at 37°C. Phospho-ERK proteins were concentrated with protein A/G agarose. ERK activity was measured with MAP kinase assay kit described in materials and methods. Phosphorylation of ERK in cell lysate and MBP was detected by Western blot analysis.

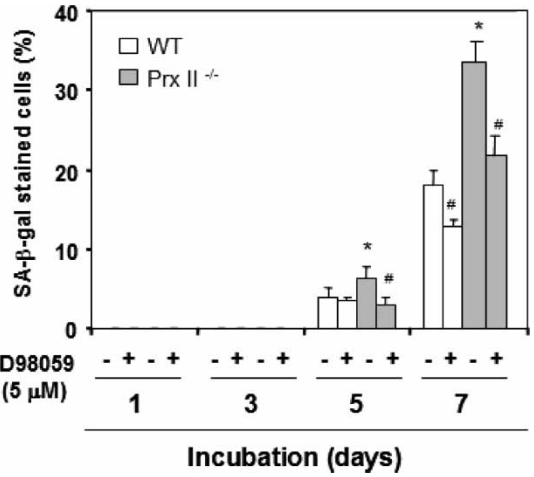

Figure 3. Cellular senescence was inhibited by the treatment with PD98059. Wild type (WT) and Prx II$^{-/-}$ MEF cells were treated with PD98059 (2 μM). Medium containing PD98059 was freshly changed everyday. SA-β-Gal-positive cells were measured every other day from first to seventh. Data were mean of three independent MEF clones. *, $p < 0.05$; significant as compared to wild type control at each day. #, $p < 0.05$; significant as compared to PD98059-untreated control at each day.

Ras/ERK pathway was activated in Prx II$^{-/-}$ MEF cells.

ERK-mediated NFκB activation

NFκB plays multiple functions in various cell types including cell survival [15,17,31]. In a certain cell, ERK activated NFκB [12–14]. We evaluated whether ERK activation leads to nuclear translocation of NFκB in Prx II$^{-/-}$ MEF cells. NFκB activation was measured by SEAP assay in pNFκB–SEAP-transfected MEF cells in the presence or absence of PMA/ionomycin (IOM). Higher SEAP activity was detected in Prx II$^{-/-}$ MEF cells compared to wild type MEF (Figure 4(A)). This was correlated with the increased degradation of IκBα (Figure 4(B)). NFκB activation was also examined by DNA binding activity using electrophoretic mobility shift assay (EMSA). We showed our experimental control data to EMSA using unlabeled NFκB probe and mutant NFκB probe in our previous report [32]. DNA binding activity of NFκB was higher in Prx II$^{-/-}$ MEF compared to wild type MEF cells (Figure 4(C)). PD98059 inhibited DNA binding activity of NFκB in Prx II$^{-/-}$ MEF. PD98059 also inhibited the degradation of IκBα and nuclear translocation of p65 (Figure 5). The p38 kinase-mediated NFkB activation is more often in various cell systems than ERK-mediated NFκB activation [33–35]. Our previous report showed p38 kinase activation in Prx II$^{-/-}$ MEF cells [11]. So, we also used SB203580, p38 kinase inhibitor to see whether p38 kinase activates NFκB in Prx II$^{-/-}$ MEF cells. Results showed that SB203580 reduced NFkB activation in Prx II$^{-/-}$ MEF cells. However, although MEF cells were treated with *N*-alpha-tosyl-L-phenylalanyl chloromethyl ketone (TPCK), which has been known as an inhibitor to IκB phosphorylation [31], no inhibition was found in SA-β-Gal-positive cell formation (Figure 6). Data suggest that cellular senescence is, in large part, reflected through ERK activation but not by NFκB activation.

Discussion

The senescence of MEF cells was induced by high oxygen concentrations [36]. Several antioxidant enzymes were reported to be involved in cellular senescence. SOD1 or Gpx1 protects cells from cellular senescence [9,10]. Oxidative stress via the induction of MnSOD occurred senescence [21]. Prx II-deletion augmented cellular senescence in MEF cells through ERK and p38 activation. The deletion of Prx II increased ROS production and SA-β-Gal-positive cell formation. Results suggest that ERK is an important common enzyme in the cellular senescence and skin aging [11].

It is well-known that ROS activate NFκB [19,37–41]. In addition, NFκB activation is associated with cellular senescence and aging [42]. Phosphorylation of ERK and IκB in T cells lead to the up-modulation of c-Jun, c-Fos, Egr-1 and NFκB transcription factors

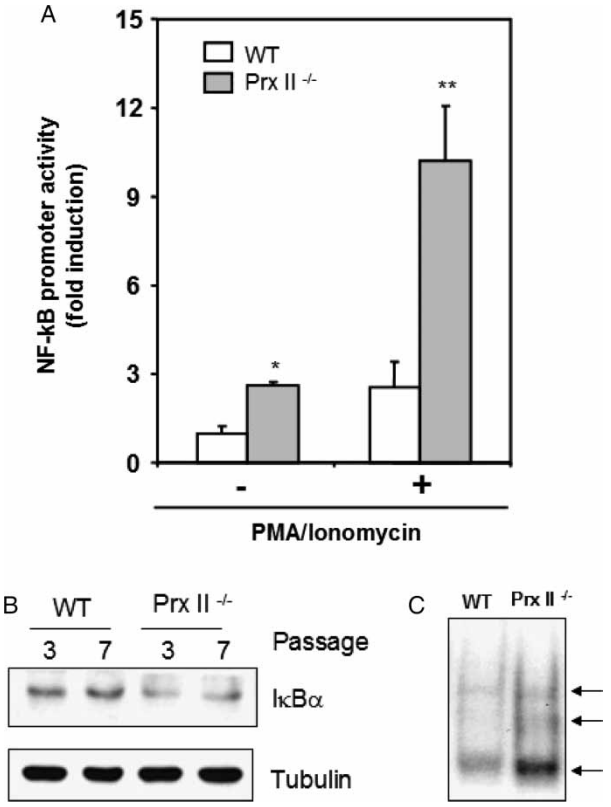

Figure 4. NFκB was activated by the deletion of Prx II gene. (A) Wild type (WT) and Prx II$^{-/-}$ MEF cells were transfected with pNFκB–SEAP. Secreted SEAP activity in the presence or absence of PMA (500 nM)/IOM (1 μM) was measured with Great EscAPe Chemilumineacence Detection Kits described in materials and methods. (B) and (C) Cytosol and nuclear extract were prepared from MEF cells at the third and seventh passage. IκBα degradation was detected by Western blot analysis. EMSA described in materials and methods was performed to detect NFκB activation. Arrows indicate NF-kB bound ^{32}P radio-labeled NFκB probe. *, $p < 0.05$; significant as compared to wild type control.

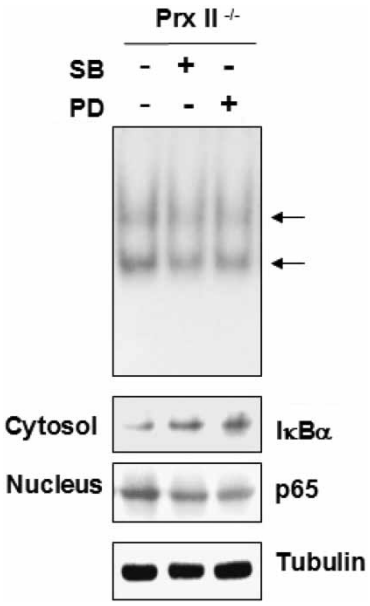

Figure 5. PD98059 inhibit NFκB activation in Prx II$^{-/-}$ cells. Cytosol and nuclear extracts were prepared from Prx II$^{-/-}$ MEF cells treated or untreated with inhibitors, SB203580 (SB) or PD98059 (PD). IκBα degradation and nuclear translocation of p65 were detected by Western blot analysis. EMSA was performed by the method described in materials and methods to detect the changes of NFκB activation. Arrows indicate NFκB bound ^{32}P radio-labeled NFκB probe.

[43]. However, a little is known regarding ERK–NFκB activation on cellular senescence. Here, we investigated whether ERK activation affects ROS-induced NFκB activation then, cellular senescence. We prepared MEF cells with 13.5-day embryos. Cellular senescence of each MEF passage was assessed by measuring senescence-associated (SA)-β-Gal activity with a substrate, X-Gal. The result of this evaluation indicated that the number of SA-β-Gal-positive cells was passage-dependently and day-dependently increased in both Prx II$^{-/-}$ and wild type MEF. SA-β-Gal-positive cell formation was increased by the deletion of Prx II. Prx II-deletion also augmented ERK activity, resulting in NFκB activation. However, no changes in SA-β-Gal-positive cell formation were detected by NFκB inhibitor, TPCK.

A few possibilities are to explain no effect on SA-β-Gal-positive cell formation by TPCK. ERK can activate other transcription factor, such as AP-1 [43,44]. ROS and other free radicals can activate AP-1 and NFκB transcription coordinately [14]. It implicates that the inhibition of only NFκB is not sufficient to suppress SA-β-Gal-positive cell formation. IKK contains IKKα, IKKβ and IKKγ. TPCK may not inhibit the phosphorylation by all IKK but by one of IKK, IKKα or IKKβ. So, it is possible for NFkB activation by different IKK to show the different effect on cellular senescence. This is required to be cleared in the next study.

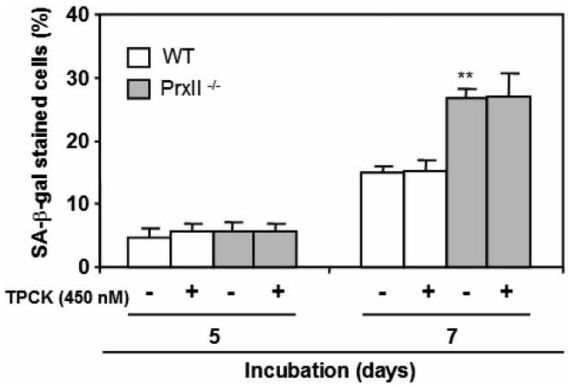

Figure 6. No changes on cellular senescence were detected by NFκB inhibition. Wild type (WT) and Prx II$^{-/-}$ MEF cells were treated with *N*-alpha-tosyl-L-phenylalanyl chloromethyl ketone (TPCK, 450 nM) for 5 or 7 days. Medium containing TPCK was freshly changed everyday. SA-β-Gal-positive cells were measured every other day from the first passage to the seventh. Data were mean of three independent MEF clones. *, $p < 0.05$; significant as compared to wild type control.

Cellular senescence accompanies cell cycle arrest and the reduced apoptosis [11]. Apoptosis rate was lower in Prx $II^{-/-}$ MEF than wild type MEF. These are consistent with other reports on Prx II-deleted cells. RBC in Prx II-deleted mice has been reported to contain Heinz bodies, which is resulted in splenomegaly [23]. Prx II gene-deleted thymus was notably enlarged and exhibited a higher level of thymocytes, which are resulted from the reduced apoptosis [45].

In conclusion, although the mechanisms have yet to be fully defined, data indicate that Prx II is one of the major regulators in the control of cellular senescence. Results for the first time suggest that Prx II might inhibit the activation of Ras–ERK–NFκB signaling pathways. It also suggests that cellular senescence in Prx $II^{-/-}$ MEF cells was mediated by Erk activation but not by NFκB activation.

Acknowledgements

This work was supported by grants from Molecular and Cellular BioDiscovery Research Program, Ministry of Science and Technology, Korea, National Cancer Control Program, Ministry of Health and Welfare, Korea, and KRIBB Initiative Program, Korea.

References

[1] Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. Exp Cell Res 1965;37:614–636.

[2] Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, Peacocke M, Campisi J. A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. Proc Natl Acad Sci USA 1995;92:9363–9367.

[3] Campisi J. Cellular senescence as a tumor-suppressor mechanism. Trends Cell Biol 2001;11:S27–S31.

[4] Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. Curr Biol 1999;9:939–945.

[5] Dimri GP, Testori A, Acosta M, Campisi J. Replicative senescence, aging and growth-regulatory transcription factors. Biol Signals 1996;5:154–162.

[6] Dimri GP, Hara E, Campisi J. Regulation of two E2F-related genes in presenescent and senescent human fibroblasts. J Biol Chem 1994;269:16180–16186.

[7] Macip S, Igarashi M, Fang L, Chen A, Pan ZQ, Lee SW, Aaronson SA. Inhibition of p21-mediated ROS accumulation can rescue p21-induced senescence. Embo J 2002;21: 2180–2188.

[8] Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, Ames BN. Mitochondrial decay in hepatocytes from old rats: Membrane potential declines, heterogeneity and oxidants increase. Proc Natl Acad Sci USA 1997;94: 3064–3069.

[9] Blander G, de Oliveira RM, Conboy CM, Haigis M, Guarente L. Superoxide dismutase 1 knock-down induces senescence in human fibroblasts. J Biol Chem 2003;278:38966–38969.

[10] de Haan JB, Bladier C, Lotfi-Miri M, Taylor J, Hutchinson P, Crack PJ, Hertzog P, Kola I. Fibroblasts derived from Gpx1 knockout mice display senescent-like features and are susceptible to H2O2-mediated cell death. Free Radic Biol Med 2004;36:53–64.

[11] Han YH, Kim HS, Kim JM, Kim SK, Yu DY, Moon EY. Inhibitory role of peroxiredoxin II (Prx II) on cellular senescence. FEBS Lett 2005;579:4897–4902.

[12] Mitra AK, Sarma A, Krishna M, Verma NC. Expression of NF-kappaB and ERK following heavy ion irradiation. J Environ Pathol Toxicol Oncol 2004;23:53–59.

[13] Cohen MP, Shea E, Chen S, Shearman CW. Glycated albumin increases oxidative stress, activates NF-kappaB and extracellular signal-regulated kinase (ERK), and stimulates ERK-dependent transforming growth factor-beta 1 production in macrophage RAW cells. J Lab Clin Med 2003;141:242–249.

[14] Dhar A, Young MR, Colburn NH. The role of AP-1, NF-kappaB and ROS/NOS in skin carcinogenesis: The JB6 model is predictive. Mol Cell Biochem 2002;234–235:185–193.

[15] Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 2004;25:280–288.

[16] Finco TS, Baldwin AS. Mechanistic aspects of NF-kappaB regulation: The emerging role of phosphorylation and proteolysis. Immunity 1995;3:263–272.

[17] Shishodia S, Aggarwal BB. Nuclear factor-kappaB: A friend or a foe in cancer? Biochem Pharmacol 2004;68:1071–1080.

[18] Huang TT, Kudo N, Yoshida M, Miyamoto S. A nuclear export signal in the N-terminal regulatory domain of IkappaBalpha controls cytoplasmic localization of inactive NF-kappaB/IkappaBalpha complexes. Proc Natl Acad Sci USA 2000;97:1014–1019.

[19] Asehnoune K, Strassheim D, Mitra S, Kim JY, Abraham E. Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-kappaB. J Immunol 2004;172: 2522–2529.

[20] Sanlioglu S, Williams CM, Samavati L, Butler NS, Wang G, McCray PB Jr, Ritchie TC, Hunninghake GW, Zandi E, Engelhardt JF. Lipopolysaccharide induces Rac1-dependent reactive oxygen species formation and coordinates tumor necrosis factor-alpha secretion through IKK regulation of NF-kappaB. J Biol Chem 2001;276:30188–30198.

[21] Bernard D, Gosselin K, Monte D, Vercamer C, Bouali F, Pourtier A, Vandenbunder B, Abbadie C. Involvement of Rel/nuclear factor-kappaB transcription factors in keratinocyte senescence. Cancer Res 2004;64:472–481.

[22] Helenius M, Kyrylenko S, Vehvilainen P, Salminen A. Characterization of aging-associated up-regulation of constitutive nuclear factor-kappaB binding activity. Antioxid Redox Signal 2001;3:147–156.

[23] Lee TH, Kim SU, Yu SL, Kim SH, Park do S, Moon HB, Dho SH, Kwon KS, Kwon HJ, Han YH, Jeong S, Kang SW, Shin HS, Lee KK, Rhee SG, Yu DY. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. Blood 2003;101:5033–5038.

[24] Jeon YJ, Kim YK, Lee M, Park SM, Han SB, Kim HM. Radicicol suppresses expression of inducible nitric-oxide synthase by blocking p38 kinase and nuclear factor-kappaB/Rel in lipopolysaccharide-stimulated macrophages. J Pharmacol Exp Ther 2000;294:548–554.

[25] Pierce JW, Lenardo M, Baltimore D. Oligonucleotide that binds nuclear factor NF-kappaB acts as a lymphoid-specific and inducible enhancer element. Proc Natl Acad Sci USA 1988;85:1482–1486.

[26] Campisi J. Cancer and ageing: Rival demons? Nat Rev Cancer 2003;3:339–349.

[27] Sayama K, Shirakata Y, Midorikawa K, Hanakawa Y, Hashimoto K. Possible involvement of p21 but not of p16 or p53 in keratinocyte senescence. J Cell Physiol 1999;179: 40–44.

[28] Weinberg WC, Denning MF. P21Waf1 control of epithelial cell cycle and cell fate. Crit Rev Oral Biol Med 2002;13:453–464.

[29] Yoon S, Seger R. The extracellular signal-regulated kinase: Multiple substrates regulate diverse cellular functions. Growth Factors 2006;24:21–44.

[30] Hutter E, Unterluggauer H, Uberall F, Schramek H, Jansen-Durr P. Replicative senescence of human fibroblasts: The role of Ras-dependent signaling and oxidative stress. Exp Gerontol 2002;37:1165–1174.

[31] Ballif BA, Shimamura A, Pae E, Blenis J. Disruption of 3-phosphoinositide-dependent kinase 1 (PDK1) signaling by the anti-tumorigenic and anti-proliferative agent *N*-alpha-tosyl-L-phenylalanyl chloromethyl ketone. J Biol Chem 2001;276:12466–12475.

[32] Kang JS, Yoon YD, Han MH, Han SB, Lee K, Kang MR, Moon EY, Jeon YJ, Park SK, Kim HM. Estrogen receptor-independent inhibition of tumor necrosis factor-alpha gene expression by phytoestrogen equol is mediated by blocking nuclear factor-kappaB activation in mouse macrophages. Biochem Pharmacol 2005;71:136–143.

[33] N'Guessan PD, Hippenstiel S, Etouem MO, Zahlten J, Beermann W, Lindner D, Opitz B, Witzenrath M, Rosseau S, Suttorp N, Schmeck B. *Streptococcus pneumoniae*-induced p38 MAPK- and NF-{kappa}B-dependent COX-2 expression in human lung epithelium. Am J Physiol Lung Cell Mol Physiol 2006.

[34] Ohno S, Im HJ, Knudson CB, Knudson W. Hyaluronan oligosaccharides induce MMP-13 via transcriptional activation of NFkB and p38 MAP kinase in articular chondrocytes. J Biol Chem 2006;281:17952–17960.

[35] Popov Y, Patsenker E, Bauer M, Niedobitek E, Schulze-Krebs A, Schuppan D. Halofuginone induces matrix metalloproteinases in rat hepatic stellate cells via activation of p38 and NFKB. J Biol Chem 2006;281:15090–15098.

[36] Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat Cell Biol 2003;5:741–747.

[37] Baeuerle PA, Henkel T. Function and activation of NF-kappaB in the immune system. Annu Rev Immunol 1994;12:141–179.

[38] Flohe L, Brigelius-Flohe R, Saliou C, Traber MG, Packer L. Redox regulation of NF-kappaB activation. Free Radic Biol Med 1997;22:1115–1126.

[39] Toledano MB, Leonard WJ. Modulation of transcription factor NF-kappaB binding activity by oxidation–reduction *in vitro*. Proc Natl Acad Sci USA 1991;88:4328–4332.

[40] Zhang H, Spapen H, Nguyen DN, Benlabed M, Buurman WA, Vincent JL. Protective effects of *N*-acetyl-L-cysteine in endotoxemia. Am J Physiol 1994;266:H1746–H1754.

[41] Ginn-Pease ME, Whisler RL. Redox signals and NF-kappaB activation in T cells. Free Radic Biol Med 1998;25:346–361.

[42] Lavrovsky Y, Chatterjee B, Clark RA, Roy AK. Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. Exp Gerontol 2000;35:521–532.

[43] Li-Weber M, Treiber MK, Giaisi M, Palfi K, Stephan N, Parg S, Krammer PH. Ultraviolet irradiation suppresses T cell activation via blocking TCR-mediated ERK and NF-kappaB signaling pathways. J Immunol 2005;175:2132–2143.

[44] Karin M, Takahashi T, Kapahi P, Delhase M, Chen Y, Makris C, Rothwarf D, Baud V, Natoli G, Guido F, Li N. Oxidative stress and gene expression: The AP-1 and NF-kappaB connections. Biofactors 2001;15:87–89.

[45] Moon EY, Han YH, Lee DS, Han YM, Yu DY. Reactive oxygen species induced by the deletion of peroxiredoxin II (Prx II) increases the number of thymocytes resulting in the enlargement of Prx II-null thymus. Eur J Immunol 2004;34:2119–2128.