Free Radical Research, August 2010; 44(8): 864–870

Knockdown of sensitive to apoptosis gene by small interfering RNA enhances the sensitivity of PC3 cells toward actinomycin D and etoposide

EUN SUN YANG, YUN JEONG HUH & JEEN-WOO PARK

School of Life Sciences and Biotechnology, College of Natural Sciences, Kyungpook National University, Taegu 702-701, Korea

(Received date 5 March 2010; In revised date 9 April 2010)

Abstract

Actinomycin D and etoposide induce the production of reactive oxygen species, which play an important causative role in apoptotic cell death. Sensitive to apoptosis gene (SAG) protein, a redox inducible zinc RING finger protein that protects mammalian cells from apoptosis by redox reagents, is a metal chelator and a potential reactive oxygen species scavenger. The present report show that knockdown of SAG expression in PC3 cells greatly enhances apoptosis induced by actinomycin D and etoposide. Transfection of human prostate cancer PC3 cells with SAG small interfering RNA (siRNA) markedly decreased the expression of SAG, enhancing the susceptibility of actinomycin D- and etoposide-induced apoptosis reflected by DNA fragmentation, cellular redox status and the modulation of apoptotic marker proteins. These results indicate that SAG may play an important role in regulating the apoptosis induced by actinomycin D and etoposide and the sensitizing effect of SAG siRNA on the apoptotic cell death of PC3 cells offers the possibility of developing a modifier of cancer chemotherapy.

Keywords: *Antioxidant protein, siRNA, apoptosis, redox status*

Introduction

Induction of apoptosis in cancer cells has become an indicator of the cancer treatment response and reduction of mortality in cancer patients [1]. Reactive oxygen species (ROS) have been implicated in cell death regulation [2]. Apoptosis can be induced not only by exposing cells to exogenous oxidants [3], but also by many chemical and physical agents capable of inducing cell death which are also known to generate ROS [4]. It has been reported that apoptosis is modulated in human prostate cancer PC3 cells by a variety of anti-cancer drugs which presumably elicit ROS formation, such as doxorubicin, etoposide, antinomycin D and staurosporine [5–7]. In this regard, several anti-cancer drugs can induce apoptosis through modulation of cellular redox status and enhanced apoptosis can be achieved by suppressing the ability of cancer cells to remove ROS.

Sensitive to apoptosis gene (SAG) protein is a redox inducible and evolutionally conserved zinc RING finger protein that protects cells from apoptosis induced by redox agents [8]. SAG is shown to be localized in the cytoplasm and nucleus of the cell and is ubiquitously expressed at high levels in skeletal muscles, heart and testis. SAG encodes a protein that consists of 113 amino acids including 12 cysteine residues with a molecular weight of 12.6 kDa [8]. It has been proposed that on account of its high cysteine content, SAG might play an antioxidant role in the cell through a metal chelator or ROS scavenger [8–10]. Besides being involved in the protection of cells from apoptosis induced by oxidative stress, other biological functions have been considered [11]. Because SAG is overexpressed in several carcinomas [12–14] and plays an important role in the control of cell proliferation

Correspondence: J.-W. Park, School of Life Sciences and Biotechnology, College of Natural Sciences, Kyungpook National University, Taegu 702-701, South Korea. Fax: 82 53 943 2762. Email: parkjw@knu.ac.kr

ISSN 1071-5762 print/ISSN 1029-2470 online © 2010 Informa UK Ltd.
DOI: 10.3109/10715762.2010.485996

[13] SAG appears to be a potential target for anti-cancer therapy.

In the present report, we demonstrate that down-regulation of SAG by a small interfering RNA (siRNA) enhances actinomycin D- and etoposide-induced ROS generation and also significantly increases apoptotic features. The sensitizing effect of SAG siRNA on the apoptotic cell death of PC3 cells offers the possibility of developing a modifier of cancer chemotherapy.

Materials and methods

Materials

Actinomycin D, etoposide, N-acetylcysteine (NAC), xylenol orange, propidium iodide (PI) and anti-rabbit IgG tetramethylrhodamine isothiocyanate (TRITC) conjugated secondary antibody were purchased from Sigma Chemical Co. (St. Louis, MO). Dihydrorhodamine 123, *t*-butoxycarbonyl-Leu-Met-7-amino-4-chloromethylcoumarin (CMAC), 2',7'-dichlorofluoroscin diacetate (DCFH-DA) and rhodamine 123 were obtained from Molecular Probes (Eugene, OR). Antibodies were purchased from Santa Cruz (Santa Cruz, CA) and Cell Signaling (Beverly, MA). Anti-SAG IgG was purchased from Abcam (Cambridge, MA).

Knockdown of SAG by siRNA

SAG siRNA and control (scrambled) siRNA were obtained from Samchully Pharm (Seoul, Korea). The sequences of the double-stranded RNAs of SAG and control used in the current experiments are as follows. For SAG, sense and anti-sense siRNAs are 5'-GUCUUA GAUGUCAAGCUGA(dTdT)-3' and 5'-UCAGCUUGACA UCUAAGAC(dTdT)-3', respectively. For scrambled control, sense and anti-sense siRNAs are 5'-CUGAUGACCUGAGU-GAAUGdTdT-3' and 5'-CAUUCACUCAGGU-CAUCAG dTdT-3', respectively. PC3 cells, derived from human prostate cancer, were transfected with 20 nM oligonucleotide by using Lipofectamine 2000 (Invitrogen; Carlsbad, CA, USA) in serum-free conditions according to the manufacturer's protocol. After incubation for 5 h, the cells were washed and supplemented with fresh normal medium containing 10% FBS and 50 units/ml penicillin, 50 μg/ml streptomycin. Cells were grown at 37°C in a 90% humidified atmosphere containing 5% CO_2. Cytotoxicity was determined by trypan blue exclusion assay.

Immunoblot analysis

Proteins were resolved on a SDS-polyacrylamide gel, transferred to nitrocellulose membranes and subsequently subjected to immunoblot analysis using appropriate antibodies. Immunoreactive antigen was then recognized by using horseradish peroxidase-labelled anti-rabbit IgG and the enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech; Buckinghamshire, UK).

Cellular redox status

Generation of ROS was measured using the oxidant-sensitive fluorescent probe DCFH-DA with fluorescence microscopy. Briefly, cells were grown at 2×10^6 cells per 100-mm plate containing slide glass coated with poly-L-lysine and maintained in the growth medium for 24 h. Cells were treated with 10 μM DCFH-DA for 15 min and cells on the slide glass were washed with PBS and a cover glass was put on the slide glass. Intracellular peroxide concentrations were determined using a ferric sensitive dye, xylenol orange [15]. The intracellular GSH level was determined by using a GSH-sensitive fluorescence dye CMAC. PC3 cells were incubated with 5 μM CMAC cell tracker for 30 min. The images of CMAC cell tracker fluorescence by GSH were analysed by the Zeiss Axiovert 40 CFL inverted microscope at fluorescence DAPI region (excitation, 351 nm; emission, 380 nm) [16]. 8-Hydroxy-2'-deoxyguanosine (8-OH-dG) levels of PC3 cells were estimated by using a fluorescent binding assay, as described by Struthers et al. [17].

Mitochondrial redox status and damage

To determine the levels of mitochondrial ROS cells in PBS were incubated for 20 min at 37°C with 5 μM dihydrorhodamine 123 and cells loaded with the fluorescent probes were imaged with a fluorescence microscope. Mitochondrial membrane permeability transition (MPT) was measured by the incorporation of rhodamine 123 dye into the mitochondria, as previously described [18].

DNA fragmentation

To determine the apoptotic DNA fragmentation, a 500 μl aliquot of the lysis buffer (100 mM Tris-HCl, pH 8.5, EDTA, 0.2 M NaCl, 0.2% SDS and 0.2 mg/ml proteinase K) was added to the cell pellet (2×10^5 cells) and incubated at 37°C overnight. DNA was then obtained by ethanol precipitation, separated in a 0.8% agarose gel and visualized under UV light. DNA fragmentation was also determined using the diphenylamine assay as previously described [19].

FACS

Cells were collected at 2000 × g for 5 min and washed once with cold PBS, fixed in 70% ethanol, decant ethanol by centrifuge and stained with 1 ml of solution

containing 50 mg/ml PI, 1 mg/ml RNase A, 1.5% Triton X-100 for at least 1 h in the dark at 4°C. Labelled nuclei were subjected to flow cytometric analysis and then gated on light scatter to remove debris and the percentage of nuclei with a sub-G_1 content was considered apoptotic cells.

Caspase activity assay

Cells were washed three times with chilled PBS, then incubated with 75 µl of lysis buffer (50 mM Tris-Cl, pH 7.4, 1 mM EDTA, 10 mM EGTA, 10 µM digitonin, 0.5 mM PMSF) for 30 min at 37°C. Thereafter, the contents from three wells were pooled and centrifuged at 20 000 × g for 20 min at 4°C. The supernatant was mixed (1:1) with reaction buffer (100 mM HEPES, 1 mM EDTA, 10 mM DTT, 0.5 mM PMSF, 10% glycerol). Caspase-3 activity was measured by a colourimetric caspase-3 assay using Ac-DEVD-pNA and caspase activity was measured as the absorbance at 405 nm for 1 h after incubation of the mixture at 37°C. Caspase activity was calculated as (absorbance/mg of protein in treated sample)/(absorbance/mg of protein in control sample).

Quantitation of relative fluorescence

The averages of fluorescence intensity from fluorescence images were calculated as described [20].

Statistical analysis

The difference between two mean values was analysed by Student's *t*-test and was considered to be statistically significant when $p < 0.05$.

Replicates

Unless otherwise indicated, each result described in this paper is representative of at least three separate experiments.

Results

To inhibit the expression of SAG, an *in vitro*-transcribed siRNA designed to target SAG was synthesized. PC3 cells were transfected with a control scrambled siRNA or SAG siRNA and then protein levels were analysed by Western blotting. SAG protein levels were significantly decreased in SAG siRNA-transfected cells as compared to control cells (Figure 1A). Notably, in the presence of actinomycin D or etoposide, there was a significant decrease in PC3 cell viability and SAG siRNA-transfected cells were more sensitive to these agents than control cells (Figure 1B).

To determine whether SAG knockdown resulted in increased apoptosis of PC3 cells, control and SAG siRNA-transfected cells were exposed to actinomycin D or etoposide and apoptotic cells were quantified by calculating the number of sub-diploid cells in cell cycle histograms. In the presence of actinomycin D or etoposide, the number of apoptotic cells was higher for SAG siRNA-transfected cells as compared to control cells (Figure 2A). As shown in Figure 2B, DNA fragmentation was more apparent in SAG siRNA-transfected PC3 cells as compared to control cells following exposure to actinomycin D or etoposide. Analysis of the cells by diphenylamine assay confirmed that DNA fragmentation was significantly increased in SAG siRNA-transfected PC3 cells exposed to etoposide (Figure 2C). Caspases have

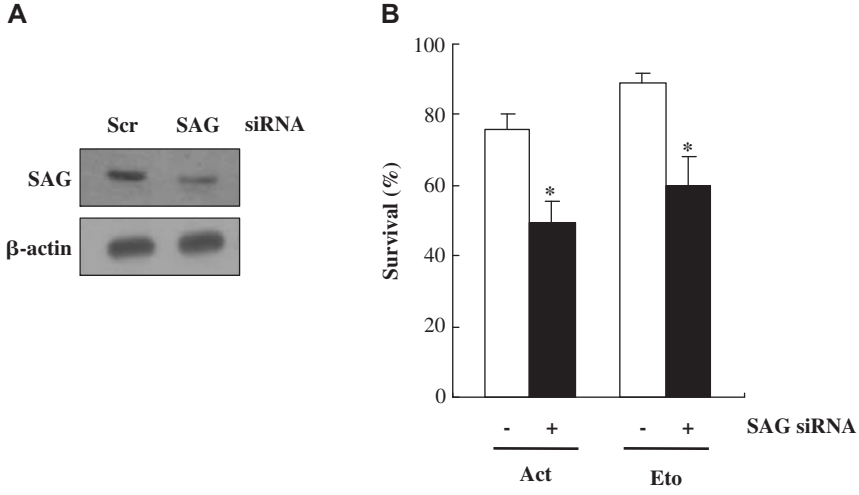

Figure 1. Knockdown of SAG by siRNA in PC3 cells. (A) PC3 cells were transfected with scrambled siRNA (Scr) or SAG siRNA. After 48 h, the transfected cells were disrupted by sonication and then protein levels of SAG were determined. β-Actin was run as an internal control. (B) Viability of transfectant cancer cell exposed to actinomycin D or etoposide. After SAG siRNA- and control scrambled siRNA-transfected PC3 cells were exposed to 200 nM actinomycin D or 100 µM etoposide for 1 day at 37°C, viability of cells was determined by trypan blue exclusion assay. Data are presented as means ± SD of three separate experiments. $^*p < 0.01$ vs control scrambled siRNA-transfected cells exposed to actinomycin D or etoposide.

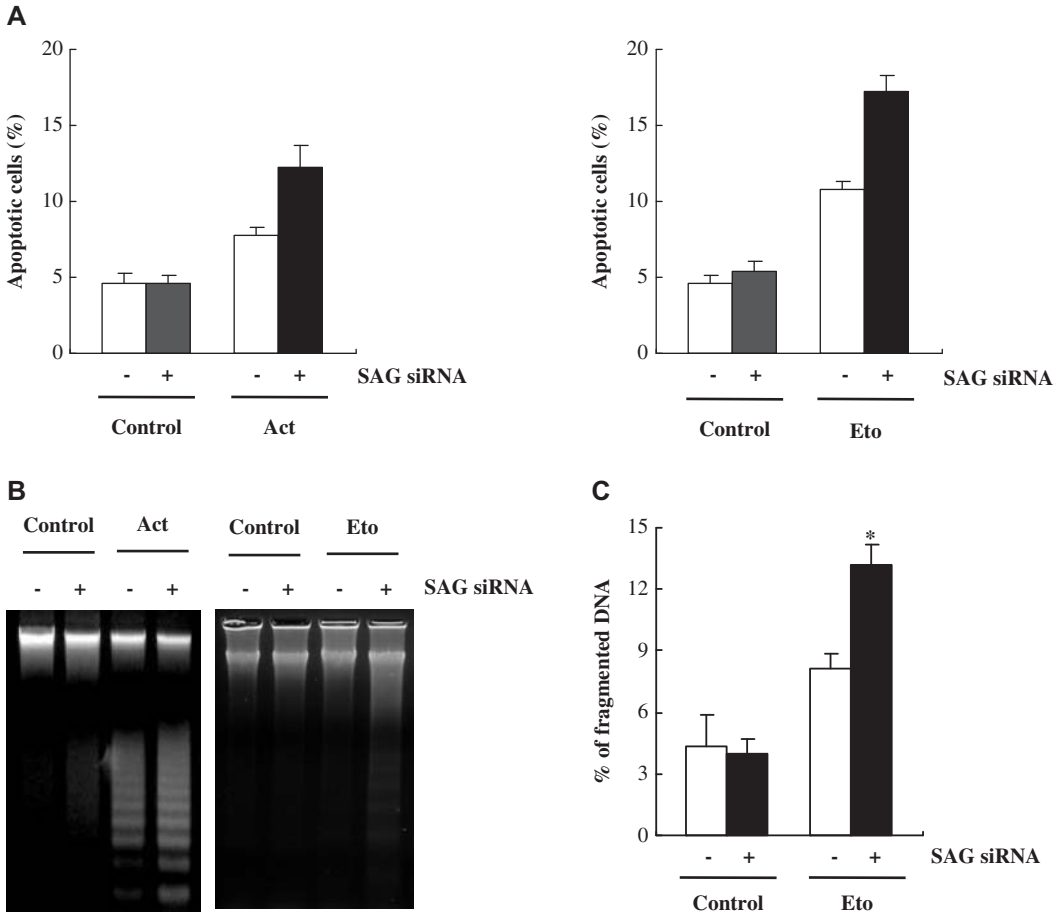

Figure 2. Actinomycin D- or etoposide-induced apoptosis in SAG siRNA transfectant PC3 cells. (A) Cell cycle analysis with cellular DNA content was examined by flow cytometry. The sub-G_1 region (presented as 'M1') includes cells undergoing apoptosis and the percentage of apoptotic cells was calculated from cell cycle histogram. Data are presented as means ± SD of three separate experiments. (B) Agarose gel electrophoresis of nuclear DNA fragments of PC3 transfectant cells exposed 200 nM actinomycin D or 100 μM etoposide for 1 day. (C) DNA fragmentation was determined using diphenylamine assay. Data are presented as means ± SD of three separate experiments. $^*p < 0.01$ vs control cells exposed to etoposide. Act, Actinomycin D; Eto, etoposide.

been identified as important mediators of apoptosis induced by various apoptotic stimuli. Caspase-3 activity was significantly increased in SAG siRNA-transfected cells as compared to control cells upon exposure to actinomycin D or etoposide (Figure 3A). Activation of caspase-3 and cleavage of PARP, a caspase-3 substrate, were also measured by immunoblot analysis. Cleavage of procaspase-3 and accumulation of the PARP cleavage fragment were markedly increased in SAG siRNA-transfected cells as compared to control cells upon exposure to actinomycin D or etoposide. Evaluation of the effect of SAG knockdown on the mitochondrial pathway of apoptosis revealed that the abundance of the anti-apoptotic protein Bcl-X_L was significantly decreased, and Bax, a pro-apoptotic protein, exhibited a more pronounced increase in SAG siRNA-transfected cells as compared to control cells following exposure to actinomycin D or etoposide (Figure 3B).

To determine the effect of SAG knockdown on mitochondrial integrity and function, both of which play a critical role in apoptosis, we examined changes in the MPT. The MPT reflects the opening of large pores in the mitochondrial membrane and can be measured using the fluorescent lipophilic cationic dye rhodamine 123. Significantly less rhodamine 123 dye was taken up by the mitochondria of SAG siRNA-transfected cells as compared to control cells following exposure to actinomycin D or etoposide (Figure 4A). To determine whether these changes in the MPT were accompanied by an increase in ROS, the levels of mitochondrial ROS in PC3 cells were probed with the oxidant-sensitive probe dihydrorhodamine 123 using fluorescence microscopy. Dihydrorhodamine 123 fluorescence was significantly enhanced in SAG siRNA-transfected cells as compared to control cells upon exposure to actinomycin D or etoposide (Figure 4B).

To investigate in more detail the association between enhanced actinomycin D- and etoposide-induced apoptotic cell death and increased ROS formation in SAG siRNA-transfected cells, ROS levels in PC3 cells were evaluated by fluorescence microscopy using the specific oxidant-sensitive dye

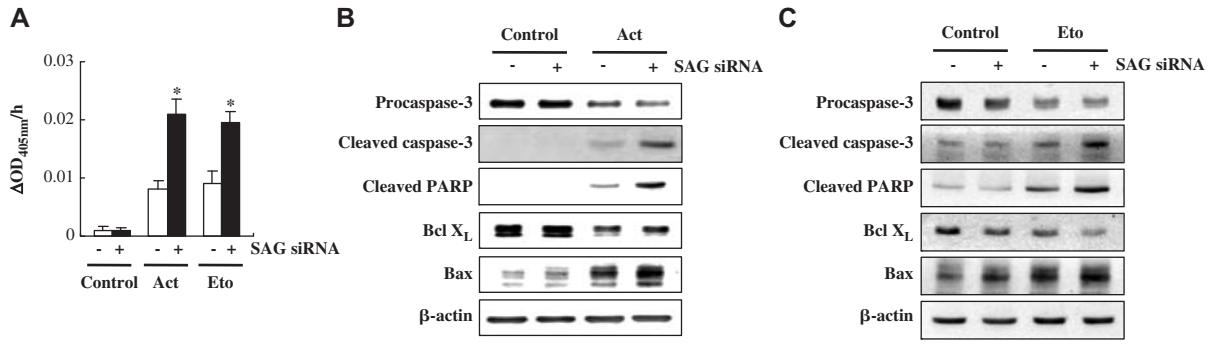

Figure 3. Modulation of apoptotic marker proteins in SAG siRNA transfectant PC3 cells. (A) Activation of caspase-3 in PC3 transfectant cells. Cells were exposed to 200 nM actinomycin D or 100 μM etoposide for 1 day and then PC3 cells were lysed and centrifuged. The supernatant was then added to Ac-DEVD-*p*NA and subjected to caspase colourimetric activity. Protease activity of caspase-3 was calculated by monitoring the absorbance at 405 nm. Data are presented as means ± SD of three separate experiments. $^{*}p < 0.01$ vs control scrambled siRNA-transfected cells exposed to actinomycin D or etoposide. (B) Immunoblot analysis of various apoptosis-related proteins in PC3 transfectant cells unexposed or exposed to actinomycin D or etoposide. Cell extracts were subjected to 10–12.5% SDS-PAGE and immunoblotted with antibodies against cleaved caspase-3, procaspase-3, cleaved PARP, Bcl-X_L and Bax. β-Actin was run as an internal control.

DCFH-DA. As shown in Figure 5A, DCF fluorescence increased in PC3 cells upon exposure to actinomycin D or etoposide and this increase was more pronounced in SAG siRNA-transfected cells. The level of cellular peroxides was also significantly enhanced in SAG siRNA-transfected cells as compared to control cells (Figure 5B). GSH is a major antioxidant that protects cells from oxidative stress by scavenging peroxides in the cytosol and mitochondria [21]. Upon exposure to actinomycin D or etoposide, in SAG siRNA-transfected cells there was a decrease in the fluorescence intensity of the GSH-sensitive fluorescent dye CMAC, which reflected the depletion of intracellular GSH, as compared to control cells (Figure 5C). In addition, the fluorescent intensity reflects endogenous levels of 8-OH-dG, the major DNA lesion induced by oxidative stress, was significantly increased in SAG siRNA-transfected cells as compared to control cells upon exposure to actinomycin D or etoposide (Figure 5D).

Discussion

Growing evidence suggests that cancer cells produce high levels of ROS and are constantly under oxidative stress [22]. The balance of ROS formation and anti-oxidative defense level is crucial in maintenance of an appropriate level of intracellular ROS and signalling cellular proliferation [23]. Over-production of ROS or an inability to suppress ROS results in a significant increase in intracellular oxidative stress, which in turn leads to cellular damage and, ultimately, cell death [24]. The results of recent *in vitro* studies have suggested that many anti-cancer drugs, as well as ionizing radiation, induce cancer cell death through the accumulation of cellular ROS [25,26].

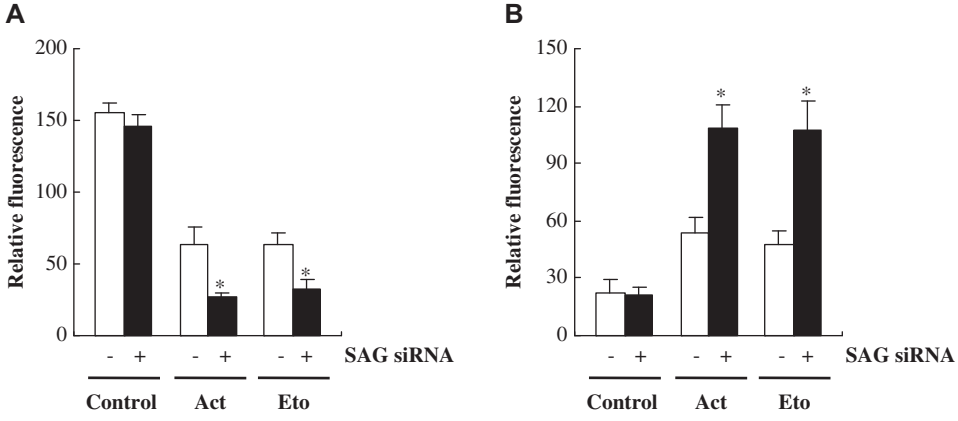

Figure 4. Effects of SAG siRNA on mitochondrial dysfunction and mitochondrial redox status of PC3 transfectant cells exposed to actinomycin D or etoposide. (A) Effect of SAG siRNA on MPT. MPT of PC3 transfectant cells was measured by the incorporation of rhodamine 123 dye into the mitochondria. (B) Effect of SAG siRNA on mitochondrial ROS generation. Dihydrorhodamine 123 was employed to detect mitochondrial ROS. Fluorescence images were obtained under microscopy. (A, B) The averages of fluorescence intensity were calculated as described [20]. Data are presented as means ± SD of three separate experiments. $^{*}p < 0.01$ vs control scrambled siRNA-transfected cells exposed to actinomycin D or etoposide.

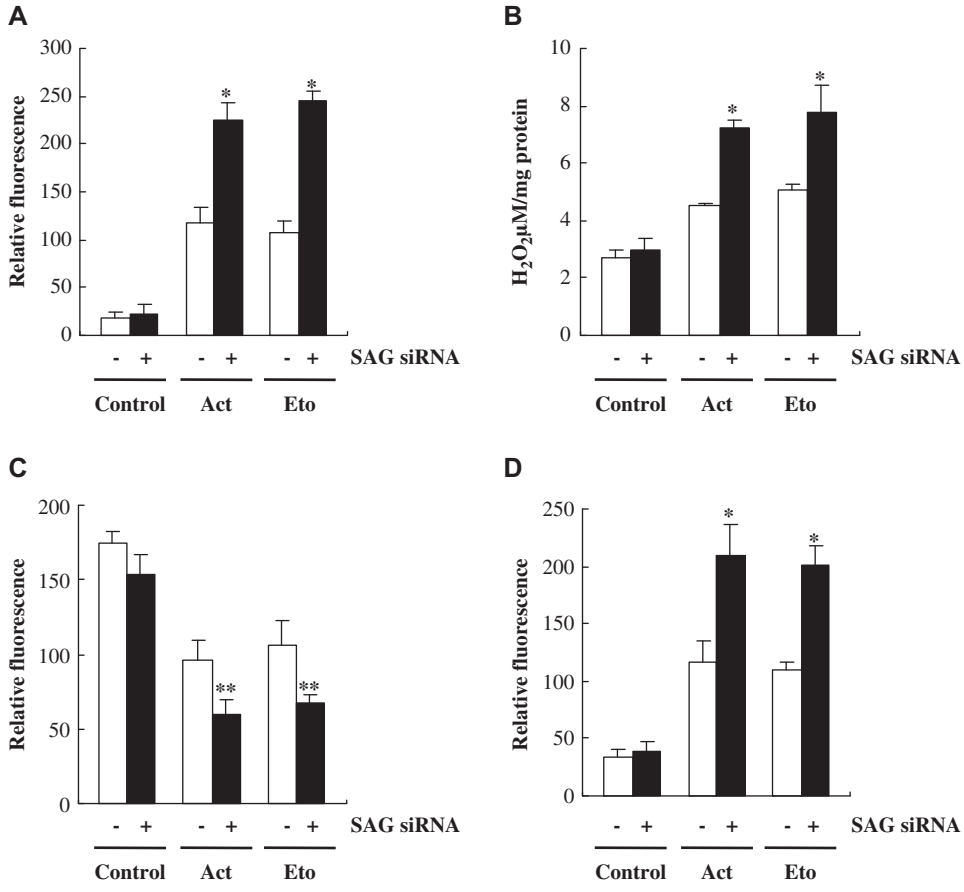

Figure 5. Cellular redox status and oxidative DNA damage in SAG siRNA transfectant PC3 cells. (A) DCF fluorescence was measured in PC3 transfectant cells exposed to actinomycin D or etoposide by fluorescence microscope. (B) Production of peroxides in PC3 cells exposed to actinomycin D or etoposide was determined by the method described under Materials and methods. (C) Effect of SAG siRNA transfection on GSH levels. Fluorescence image of CMAC-loaded cells was obtained under microscopy. (D) 8-OH-dG levels reflected by the binding of avidin-TRITC were visualized by a fluorescence microscope with 488 nm excitation and 580 nm emission. (A, C, D) The averages of fluorescence intensity were calculated as described [20]. Data are presented as means ± SD of three separate experiments. $^{*}p < 0.01$, $^{**}p < 0.05$ vs control scrambled siRNA-transfected cells exposed to actinomycin D or etoposide.

However, in addition to enhanced ROS production, defects in cellular antioxidant systems would also result in excess ROS that could trigger cancer cell death. In this regard, several antioxidant enzymes, including superoxide dismutase (SOD), the thioredoxin system, glutathione peroxidase, $NADP^+$-dependent isocitrate dehydrogenases, peroxiredoxin and catalase, have emerged as important targets in anti-cancer drug development [27–30].

SAG was originally identified as a gene that is induced during 1,10-phenanthroline-induced apoptosis [8]. SAG is a cysteine-rich metal-binding protein that is evolutionarily conserved among humans, yeast and *C. elegans* [8]. Although the biological roles of SAG have yet to be fully elucidated, SAG is believed to serve as an important line of defence against oxidative stress-induced damage and redox-induced apoptosis [8,11,31]. Several mechanisms of anti-apoptotic function of SAG have been proposed. SAG-induced apoptosis protection may be achieved by its metal ion binding and ROS scavenging activity to protect mitochondria from ROS-induced damage [11]. We previously showed that heat shock-induced apoptosis is suppressed in SAG-over-expressing cells, presumably due to maintenance of cellular redox status [31]. Alternatively, SAG indirectly affects the cellular redox status by regulating antioxidant defenses by other, yet unknown, mechanisms. Another potential mechanism by which SAG protects apoptosis is through the ubiquitination and degradation of IκB [32]. SAG has been known as a dual-function protein with antioxidant activity when acting alone or E3 ubiquitin ligase activity when complexed with other ligase components [8,9].

In summary, the results of the current study demonstrated that RNAi-mediated SAG knockdown sensitizes PC3 cells to actinomycin D- or etoposide-induced apoptosis through increased generation of ROS. Sensitization to anti-cancer agents by treatment with SAG siRNA has significant ramifications for cancer treatment. Although anti-cancer drugs are widely used as a first-line treatment for most cancers, chemotherapy is often compromised by chemoresistance induced by consecutive administration of drug

and systemic toxicity. Therefore, methods of sensitizing tumour cells to anti-cancer agents are urgently needed. Our results suggest that SAG siRNA in combination with anti-cancer drugs offers the possibility of improved cancer therapy.

Declaration of interest: This work was supported by the Basic Research Program (2009-0057934) and the Nuclear Research Program (2009-0074175) of the Korea Science and Engineering Foundation. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Smets LA. Programmed cell death (apoptosis) and response to anti-cancer drugs. Anticancer Drugs 1994;5:3–9.
[2] Buttke TM, Sandstorm PA. Oxidative stress as a mediator of apoptosis. Immunol Today 1994;15:7–10.
[3] Goldkorn T, Balaban N, Shannon M, Chea V, Gould R, Last J, Chan C, Chavez C. H_2O_2 acts on cellular membranes to generate ceramide signaling and initiate apoptosis in tracheobronchial epithelial cells. J Cell Sci 1998;111:3209–3220.
[4] Gouaze V, Mirault ME, Carpentier S, Salvayre R, Levade T, Andrieu-Abadie N. Glutathione peroxidase-1 overexpression prevents ceramide production and partially inhibits apoptosis in doxorubicin-treated human breast carcinoma cells. Mol Pharmacol 2001;60:488–496.
[5] Munshi A, McDonnell TJ, Meyn RE. Chemotherapeutic agents enhance TRAIL-induced apoptosis in prostate cancer cells. Cancer Chemother Pharmacol 2002;50:46–52.
[6] Zisman A. Actinomycin D and gemcitabine synergistically sensitize androgen-independent prostate cancer cells to Apo2L/TRAIL-mediated apoptosis. J Immunother 2001;24:459–471.
[7] Rokhlin OW, Glover RA, Cohen MB. Fas-mediated apoptosis in human prostatic carcinoma cell lines occurs via activation of caspase-8 and caspase-7. Cancer Res 1998;58:5870–5875.
[8] Duan H, Wang Y, Aviram M, Swaroop M, Loo JA, Sun Y. SAG, a novel zinc RING finger protein that protects cells from apoptosis induced by redox agents. Mol Cell Biol 1999;19:3145–3155.
[9] Swaroop M, Bian J, Aviram M, Duan H, Bisgaier CL, Loo JA, Sun Y. Expression, purification, and biochemical characterization of SAG, a ring finger redox-sensitive protein. Free Radic Biol Med 1999;27:193–202.
[10] Kim SY, BaeYS, Park J-W. Thiol-linked peroxidase activity of human sensitive to apoptosis gene (SAG) protein. Free Radic Res 2002;36:73–78.
[11] Sun Y, Tan M, Duan H, Swaroop M. SAG/ROG/Rbx/Hrt, a zinc finger gene family: Molecular cloning, biochemical properties, and biological functions. Antioxid Redox Signal 2001;3:635–650.
[12] Sun Y. Alterations of SAG mRNA in human cancer cell lines: requirement for the RING finger domain for apoptosis protection. Carcinogenesis 1999;20:1899–1903.
[13] Huang Y, Duan H, Sun Y. Elevated expression of SAG/ROC2/Rbx2/Hrt2 in human colon carcinoma: SAG does not induce neoplastic transformation, but antisense SAG transfection inhibits tumor cell growth. Mol Carcinog 2001;30:62–70.
[14] Sasaki H, Yukiue H, Kobayashi Y, Moriyama S, Nakashima Y, Kaji M, Fukai I, Kiriyama M, Yamakawa Y, Fujii Y. Expression of the sensitive to apoptosis gene, SAG, as a prognostic marker in nonsmall cell lung cancer. Int J Cancer 2001;95:375–377.
[15] Lee JH, Park J-W. A manganese porphyrin complex is a novel radiation protector. Free Radic Biol Med 2004;37:272–283.
[16] Tauskela JS, Hewitt K, Kang LP, Comas T, Gendron T, Hakim A, Hogan M, Durkin J, Morley P. Evaluation of glutathione-sensitive fluorescent dyes in conical culture. Glia 2001;30:329–341.
[17] Struthers I, Patel R, Clark J, Thomas S. Direct detection of 8-oxodeoxyguanosine and 8-oxoguanine by avidin and its analogues. Anal Biochem 1998;255:20–31.
[18] Pastorino JG, Simbula G, Yamamoto K, Glascott PA, Jr, Rothman RJ, Farber JL. The cytotoxicity of tumor necrosis factor depends on induction of the mitochondrial permeability transition. J Biol Chem 1996;271:29792–29798.
[19] Williams JM, Lea N, Nord JM, Roberts LM, Milford DV, Taylor CM. Comparison of ribosome-inactivating proteins in the induction of apoptosis. Toxicol Lett 1997;91:121–127.
[20] Sundaresan M, Yu ZX, Ferrans CJ, Irani K, Finkel T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. Science 1995;270:296–299.
[21] Voehringer DW. Bcl-2 and glutathione: alterations in cellular redox state that regulate apoptosis sensitivity. Free Radic Biol Med 1999;27:945–950.
[22] Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett 1995;358:1–3.
[23] Martin KR, Barrett JC. Reactive oxygen species as double-edged swords in cellular processes: low-dose cell signaling versus high-dose toxicity. Human Exp Toxicol 2002;21:71–75.
[24] Javvadi P, Segan AT, Tuttle SW, Koumenis C. The chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase pathway. Mol Pharmcol 2008;73:1491–1501.
[25] Huang P, Feng L, Oldham EA, Keating MJ, Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. Nature 2000;407:390–395.
[26] Pelicano H, Feng L, Zhou Y, Carew JS, Hileman EO, Plunkett W, Keating MJ, Huang P. Inhibition of mitochondrial respiration: a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism. J Biol Chem 2003;278:37832–37839.
[27] Wang J, Yi J. Cancer cell killing via ROS. Cancer Biol Ther 2008;7:1875–1884.
[28] Kil IS, Kim SY, Lee SJ, Park J-W. Small interfering RNA-mediated silencing of mitochondrial $NADP^+$-dependent isocitrate dehydrogenase enhances the sensitivity of HeLa cells toward tumor necrosis factor-α and anticancer drugs. Free Radic Biol Med 2007;43:165–173.
[29] Smith-Pearson PS, Kooshki M, Spitz DR, Poole LB, Zhao W, Robbins ME. Decreasing peroxiredoxin II expression decreases glutathione, alters cell cycle distribution, and sensitizes glioma cells to ionizing radiation and H_2O_2. Free Radic Biol Med 2008;45:1178–1189.
[30] Smith PS, Zhao W, Spitz DR, Robbins ME. Inhibiting catalase activity sensitizes 36B10 rat glioma cells to oxidative stress. Free Radic Biol Med 2006;42:787–797.
[31] Lee SJ, Yang ES, Kim SY, Kim SY, Shin SW, Park J-W. Regulation of heat shock-induced apoptosis by sensitive to apoptosis gene protein. Free Radic Biol Med 2008;45:167–176.
[32] Swaroop M, Gosink M, Sun Y. SAG/ROC2/Rbx/Hrt2, a component of SCF E3 ubiqutin ligase: genomic structure, a splicing variant, and two family pseudogenes. DNA Cell Biol 2001;20:425–434.

This paper was first published online on Early Online on 20 May 2010.