Functional analysis of the TMPRSS2:ERG fusion gene in cisplatin-induced cell death

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Abstract. The TMPRSS2:E-twenty-six (ETS) gene fusion occurs frequently in a high proportion of patients with prostate cancer (PCa) in Western countries, and the aberrant expression of TMPRSS2: v-ETS avian erythroblastosis virus E26 oncogene homolog (ERG), the most common form of the corresponding protein, can regulate cell migration and contribute to tumor invasion and metastasis. However, its association with other cellular events, and in particular, cell death, remain unknown. To examine the function of such fusion genes, an expression plasmid containing the TMPRSS2:ERG (ΔERG) sequence from a patient sample was constructed and transiently transfected into DU145 cells, which do not express the fusion gene. It was found that the overexpression of ΔERG significantly inhibited the ability of cisplatin to induce apoptosis in DU145 cells. By contrast, VCaP cells, which do contain TMPRSS2:ERG, were sensitized to cisplatin-induced apoptosis through siRNA inhibition of the fusion gene. To elucidate the underlying mechanism, a stable cell line expressing the ΔERG gene was constructed. Expression of ΔERG did not affect cell migration, but did protect cells from DNA damage and apoptosis induced by cisplatin. Furthermore, knockdown of ΔERG by short interfering RNA resulted in cells regaining their sensitivity to cisplatin. Finally, the gene coding for activating transcription factor 5, which is important for cell survival, may be upregulated by ΔERG. Taken together, these data point to a new function of the TMPRSS2:ERG fusion gene in regulating the apoptotic pathway.

Introduction

Prostate cancer (PCa) is the most common male malignancy, and one of the leading causes of cancer-associated mortality among men in Western countries (1). Extensive studies have been conducted in an effort to understand the genetic mechanisms underlying PCa initiation and progression. Using a systems biology approach, Tomlins et al (2) reported that the androgen response gene transmembrane serine 2 (TMPRSS2) was fused to the E-twenty-six (ETS) family gene v-ETS avian erythroblastosis virus E26 oncogene homolog (ERG) and ETS variant 1 (ETV1) in certain patients with PCa. Among the various types of fusion genes, the TMPRSS2:ERG fusion is the most common, and has been identified in 40-70% of samples from patients with PCa (3).

Functional studies of TMPRSS2:ERG have suggested that it is involved in cellular proliferation, differentiation and invasion (4-7), while its association with additional cellular functions, and in particular, apoptosis, remains unknown. Shao et al (8) reported that celastrol, a nuclear factor-κB inhibitor, can suppress TMPRSS2:ERG-expressing PCa cell growth by inducing apoptosis, implying a possible anti-apoptotic function for the TMPRSS2:ERG fusion gene.

As an effective anticancer drug, cisplatin is widely used in the treatment of many types of solid tumors, including ovarian,
testicular, bladder, cervical, head and neck, and small-cell lung cancer (9,10). Cisplatin is able to interact with DNA, and results in the formation of interstrand and/or intrastrand cross-links (11), with such severe DNA damage considered a major factor in triggering cancer cell apoptosis. However, cisplatin treatment of PCa has been a subject of debate, as while earlier studies suggested a lack of activity, more recent studies using palliation and prostate specific antigen (PSA) endpoints have suggested a greater degree of clinical benefit (12). Therefore, it is of interest to understand why PCa cells have a low response to cisplatin-induced apoptosis, and whether the TMPRSS2:ERG fusion gene may serve a role in this response.

In the present study, the role of TMPRSS2:ERG in cell death, specifically, apoptosis induced by cisplatin was investigated. The TMPRSS2:ERG fusion gene was demonstrated to serve an important role in the regulation of apoptosis induced by cisplatin, and activating transcription factor 5 (ATF5) was suggested to be involved in this process.

Materials and methods

Cell culture and treatment. Human embryonic kidney 293 (HEK293) cells and the prostate cancer cell lines DU145 and VCaP cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and the cells were grown in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.,), cultured in a humidified atmosphere containing 5% CO₂ at 37°C. Cells were plated in 6-well plates and treated at the indicated times or doses with cisplatin (Sigma-Aldrich, St. Louis, MO, USA).

DNA constructs and transfection. cDNA corresponding to the most common truncated ERG (ΔERG), the TMPRSS2:ERG fusion gene (T1/E5), was constructed using pEGP-C1 (Clontech Laboratories, Inc., Mountainview, CA, USA) with PstI and BglII sites to generate a green fluorescent protein (GFP)-ΔERG over-expressing vector. Kanamycin resistant colonies were selected and verified by sequencing. Cells were transfected with FugeneHD (Roche Diagnostics, Basel, Switzerland) following the manufacturer’s instructions. In brief, 5 µl FugeneHD was diluted with 50 µl Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.), mixed and incubated at room temperature for 5 min. Subsequently, 1.5 µg plasmid DNA was added to the diluted FugeneHD and mixed, followed by incubation for 20 min at room temperature, following which the FugeneHD-DNA mixture was added to the cells.

To establish the stable cell line, the HEK293 cells were transfected as described above. Following selection with 0.8 mg/ml G418 (Gibco, Thermo Fisher Scientific, Inc.) for 14 days, resistant clones were tested for the expression of ΔERG. The selected stable cell line was designated as Δ293, and the cells were cultured further in the medium supplemented with 300 µg/ml G418 for maintenance. Cells transfected with the GFP-empty vector were designated as E293 and were used as a control.

Scratch assay. Cells were seeded at 1x10⁵ cells/well in triplicate in collagen IV coated 6-well plates (Corning Incorporated, Corning, NY, USA). After 24 h, a scratch through the central axis of the plate was gently made using a pipette tip. The migration of Δ293 and E293 cells into the scratched region was assessed quantitatively at 12 h following the introduction of the scratch in the monolayer.

Cell proliferation assay. Cells were seeded at 5x10³ cells/well into a 96-well plate. Cell proliferation was examined using a Cell Counting Kit-8 (Dojinko Molecular Technologies, Inc., Kumamoto, Japan) following the manufacturer's instructions. Absorbance at 450 nm was measured in quintuplicate using a Tecan Infinite M200 plate reader (Tecan Trading AG, Männedorf, Switzerland) at the indicated time points.

ΔERG knock down. Knockdown of ΔERG expression was achieved by the transfection of ΔERG short interfering RNA (siRNA). The ΔERG siRNA was sequenced by Shanghai GenePharma Co., Ltd., (Shanghai, China), the sequences were as follows: Sense, 5’-CCAGACGUCAACUUUGUTT-3’ and antisense, 5’-ACAGAUUGAGCGUCUGTTT-3’, which were synthesized by. A nonspecific control strand (UUCC GAAGUGUCAGUTT) was used as the negative control (NC).

The siRNA was transfected into the cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's instructions.

Immunoblot analysis. Cells were harvested at the indicated times and washed three times in phosphate-buffered saline (PBS) prior to lysis. Protein concentrations were measured using a Bicinchoninic Acid Protein Assay Reagent kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Equal amounts of the protein (20 µg) were subjected to 12 or 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (Bio-Rad Laboratories, Inc., Hercules, CA, USA), then transferred to polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA), which were incubated first with the primary antibody at 4°C overnight, followed incubation with IR Dye-conjugated secondary antibodies for 1 h at room temperature. The primary antibodies used were as follows: Rabbit polyclonal anti-ERG (1:500; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; cat. no. sc-376293); rabbit polyclonal anti-ATF5 (1:500; Santa Cruz Biotechnology, Inc.; sc-377168); rabbit monoclonal anti-cleaved-caspase-3 (1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA; cat. no. 9661); rabbit polyclonal anti-poly ADP ribose polymerase (PARP-1; 1:1,000; Cell Signaling Technology, Inc.; cat. no. 9542); mouse monoclonal anti-β-actin (1:1,000; Cell Signaling Technology, Inc.; cat. no. 3700); rabbit monoclonal anti-H2AX (1:1,000; EMD Millipore; cat. no. 05-636). Alexa Fluor 488-conjugated goat anti-mouse (1:5,000; cat. no. A-11001) and goat anti-rabbit (1:5,000; cat. no. A11008) IgG were obtained from Thermo Fisher Scientific, Inc.. The results were observed using an Odyssey Infrared Imaging System (Li-Cor, Inc., Lincoln, NB, USA).

Flow cytometry. Cells were harvested at 48 h post cisplatin treatment. Cells were centrifuged at 300 x g for 5 min at 4°C and washed with PBS three times, then resuspended in 1X Binding Buffer. Subsequently, Annexin V-phycocyanin
and 7-aminoactinomycin D (EMD Millipore) were added, and cells were incubated at room temperature for 5 min in the dark, following which the apoptotic cells were analyzed by flow cytometry (FC500 MCL; Beckman Coulter, Inc., Brea, CA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA from E293 and Δ293 cells was extracted using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.), and 5 µg of total RNA was reverse-transcribed to cDNA using SuperscriptIII Reverse Transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.). Prior to this, the RNA was treated with DNase I (Sangon Biotech Co., Ltd., Shanghai, China) to remove DNA contamination. The 20 µl PCR reaction was set up using SYBR Green Premix Ex Taq kit (Takara Bio, Inc., Otsu, Japan) on a 96-well plate, and conducted using an ABI Prism Sequence Detection System 7500 (Applied Biosystems, Inc.; Thermo Fisher Scientific, Inc.) with the following PCR conditions: 95°C for 1 min; 95°C for 10 sec, 60°C for 1 min, repeated for 40 cycles. The primers used were as follows: Sense, 5'-AGAGTGCCGCGTGAAGAAGTA-3' and antisense, 5’-AGAGGAAGGCTGGAAGAT-3' for ERG; sense, 5'-TGGCCGTACAAAGGAGAAG-3' and antisense, 5'-AAGGAGCGGCGAGAG-3' for β-actin; sense, 5'-TGGAGAGCTAGAACAAGGAGAT-3' and antisense, 5'-AAGGCTCTAGGTTGCATTCAG-3' for BCL-2; sense, 5'-CTCCATCTACTGCCGCAACGG-3' and antisense, 5'-CTCAGAGCCCGCAGTTGT-3' for ATF5; sense, 5'-GGATTTGTTGCCCCCTCTTTGA-3' and antisense, 5'-TGGCCGCTTCA-3' for ERG; sense, 5'-GGAGGCTCCGGCTGAGTTGA-3' and antisense, 5’-AAGGAACGCCAGAACCAGAT-3' for BIM. Data analysis was performed by the 2-ΔCq method for relative quantification (13), and all samples were normalized to β-actin, which served as an endogenous control.

Chromatin immunoprecipitation (ChIP). ChIP assays were performed using the EZ ChIP kit (EMD Millipore) according to the manufacturer's instructions. Briefly, cross-linked chromatin was sonicated and immunoprecipitated with the indicated antibody. Three independent ChIP analyses were performed to prepare the DNA for future analysis. Immunoprecipitated DNA was purified and quantified by RT-qPCR as described above. The primers were as follows: Sense, 5'-AAGAAGACGCACATCCACCTCG-3' and antisense, 5'-CAAGGGATGGGAAAAGGACG-3' for ATF5.

Statistical analysis. The data are presented as the mean ± standard deviation from a minimum of three independent experiments. The differences between groups were assessed using an unpaired two-tailed Student's t-test or one-way analysis of variance. P<0.05 was considered to indicate a statistically significant difference.

Results

ΔERG inhibits cisplatin-induced apoptosis in PCa cell lines. The present study evaluated the toxicity of cisplatin on DU145 and VcaP cells. It was observed that these two types of cell were relatively resistant to the toxic effect of cisplatin as only at concentrations of 50 µM or higher was significant cell death observed (data not shown). To examine the function of fusion genes in apoptosis, GFP-tagged TMPRSS2:ERG (T1/E5) (ΔERG) was transiently expressed in DU145 cells, which do not express this fusion gene. The cells were then treated with 50 µM cisplatin for 48 h, and the apoptotic cell population was analyzed by flow cytometry. As shown in Fig. 1A, the ΔERG protein was expressed in the transfected DU145 cells as indicated by western blotting. Following cisplatin treatment, the apoptotic cell ratio in the control DU145 cells was 30.59%. However, this ratio was reduced to 14.67% in ΔERG transfected DU145 cells (P<0.01; Fig. 1B). To further investigate the function of ΔERG in apoptosis, VCaP cells, which harbor the TMPRSS2:ERG fusion gene, were transfected with siRNAs to knockdown the expression of the fusion gene (Fig. 1C). Consistent with the idea that the fusion protein inhibits apoptosis, the apoptotic cell ratio increased from 13.59% in control cells to 21.35% in siRNA-transfected cells (P<0.05; Fig. 1D).

Expression of ΔERG renders HEK293 cells resistant to cisplatin-induced apoptosis. The toxic effects of cisplatin on HEK293 cells were also examined. It was demonstrated that compared with PCa cells, HEK293 cells were more sensitive to the toxic effect of cisplatin, as at a concentration of 1 µM marked cell death was observed and at 100 µM, almost all the cells had undergone cell death (data not shown). To determine whether ΔERG only has the anti-apoptosis function in PCa cells but not in other types of cell, a stable cell line expressing the ΔERG gene in HEK293 cells was established using HEK 293 cells (Δ293 cells), with cells transfected with the empty plasmid serving as controls (E293 cells). GFP fluorescence demonstrated that the majority of the empty plasmid serving as controls (E293 cells). GFP fluorescence demonstrated that the majority of the ΔERG fusion protein was localized within the nucleus, and western blot analysis using a commercial anti-ERG antibody confirmed the expression of ΔERG (Fig. 2A). The effect of ΔERG on cell growth was investigated and the results indicated that the expression of the ΔERG gene in HEK293 cells did not affect cell growth (data not shown). To further examine whether ΔERG does indeed serve a role in protecting cells from cisplatin-induced apoptosis, Δ293 cells were treated with 50 µM cisplatin for 48 h. The cell viability of E293 cells was 10% with cisplatin treatment, while in Δ293 cells, the viability was ~46%, suggesting that ΔERG can protect Δ293 cells from cell death induced by cisplatin treatment (Fig. 2D). Further analysis revealed that the apoptotic cell ratio in E293 cells was 67.26%, while in Δ293 cells it was 25.89% (P<0.01; Fig. 2E). To verify that apoptosis had indeed occurred, a number of apoptosis-associated proteins, including caspase 3 and PARP-1, were examined by western blotting following cisplatin treatment. As shown in Fig. 2F, caspase 3 and PARP-1 cleavage was apparent in E293 cells following cisplatin treatment, however, was less abundant in Δ293 cells under the same conditions. To further demonstrate that ΔERG was responsible for the protective effect, ΔERG was knocked down in Δ293 cells using specific siRNA (Fig. 2G), which indicated that loss of ΔERG can restore the sensitivity of Δ293 cells to cisplatin treatment (Fig. 2H). Taken together, these data demonstrate that ΔERG is able to protect cells from apoptosis induced by cisplatin.
Figure 1. Effects of ΔERG on cisplatin-induced apoptosis in DU145 and VCaP cells. At 24 h following transfection, cells were treated with 50 µM cisplatin for 48 h, and apoptosis was detected by 7-aminoactinomycin D and annexin-V phycoerythrin double staining. (A) Western blotting of ΔERG protein expression in DU145 cells transfected with GFP-vector or GFP-ΔERG. (B) Quantitative results of cisplatin-induced apoptosis, as measured by flow cytometry analysis in DU145 cells. (C) Western blotting of ΔERG protein expression in VCaP cells transfected with control or specific siRNA directed against ΔERG. (D) Quantitative results of cisplatin-induced apoptosis as measured by flow cytometry analysis in VCaP cells. ΔERG, truncated v-E-twenty-six avian erythroblastosis virus E26 oncogene homolog; GFP, green fluorescent protein; siRNA, short interfering RNA; PBS, phosphate-buffered saline; Cis, cisplatin; siNC, si-negative control; Con, control.

Figure 2. Effects of ΔERG overexpression on cisplatin-induced apoptosis in HEK293 cells. (A) Fluorescent images of the expression of ΔERG in E293 or Δ293 cells (magnification, x200). (B) Western blotting for ΔERG expression. (C) Representative phase contrast microscopy images of E293 and Δ293 cell density and morphological alterations following 50 µM cisplatin treatment for 48 h (magnification, x200). (D) Cell viability as measured with a Cell Counting Kit-8 kit. ΔERG, truncated v-E-twenty-six avian erythroblastosis virus E26 oncogene homolog; E293, p-enhanced green fluorescent vector-transfected HEK293 cells; Δ293, ΔERG-transfected HEK293 cells.
It has been previously reported that a major function of \( \text{TMPRSS2:ERG} \) is to promote PCa cell migration and invasion (1,14-18). Therefore, the effect of \( \Delta \text{ERG} \) on cell migration was investigated using a scratch assay. Of note, no significant difference was observed in the scratch assay results between \( \Delta \text{293} \) and E293 cells (Fig. 3), suggesting that overexpressing \( \Delta \text{ERG} \) did not affect cell motility, at least in HEK293 cells.

\( \Delta \text{ERG} \) upregulates expression of the anti-apoptotic gene \( \text{ATF5} \). Considering the potential anti-apoptotic function of \( \Delta \text{ERG} \), and given that the protein functions as a transcription factor, whether it was able to influence the expression of key regulators in apoptosis, such as members of the BCL-2 family, was of interest. Therefore, RT-qPCR was conducted to examine the expression of the BCL-2 family members BCL-2, BCL-XL, BIM and BAX in the presence and absence of \( \Delta \text{ERG} \). Notably,
none of these genes showed differential expression between E293 and Δ293 cells (Fig. 4A). However, ATF5 (also known as ATFx), a member of the basic zipper protein ATF/cAMP response element-binding protein family which can suppress apoptosis (19), was observed to be up-regulated in Δ293 cells (Fig. 4A). To further examine the possibility that ATF5 expression was regulated by ΔERG, the protein levels of ATF5 were examined by western blotting in Δ293 cells, transfected with either control siRNA or with siRNA designed to knockdown ΔERG. As shown in Fig. 4B, the ATF5 protein levels were reduced when ΔERG was knocked down, suggesting that ΔERG serves a role in the regulation of ATF5 expression.

As ERG is a transcription factor, it was then investigated whether ΔERG is able to bind directly to the ATF5 gene to regulate ATF5 expression. To investigate this, a ChIP assay was performed in Δ293 cells using a specific anti-ERG antibody, which indicated that ΔERG was able to bind to the ATF5 promoter region in Δ293 cells (Fig. 4C).

Expression of ΔERG protects cells from cisplatin-induced DNA damage. As a classical DNA damage agent, cisplatin is thought to induce apoptosis, at least in part, through the generation of DNA damage. Therefore, the effect of ΔERG expression on cisplatin-induced DNA damage was assessed. E293 and Δ293 cells were treated with cisplatin (50 µM) for 24 h, and the level of γH2AX, a sensitive indicator for DNA damage, was examined by western blotting. As shown in Fig. 5, cisplatin treatment increased the level of γH2AX in the Δ293 and E293 cells. However, the increase was much smaller in the Δ293 cells (lanes 3, 4 and 5) compared with the E293 cells (lanes 8 and 9), indicating that the expression
of ΔERG may partially protect cells from cisplatin-induced DNA damage.

Discussion

The potential role of TMPRSS2:ERG fusion in prostate tumorigenesis and tumor progression has been under intensive investigation. Enhancing cell invasion and metastasis has been regarded as the major function of this protein, and although overexpression of ERG was observed to induce a neoplastic phenotype in prostate cells in certain studies (5,6), it was thought that the protein may require the cooperation of additional genes, such as phosphatase and tensin homolog or members of the phosphatidylinositol 3-kinase pathway (14,15), to result in transformation. However, its association with apoptosis/cell death, a major mechanism for the majority of cancer chemotherapeutic drugs, was relatively unexplored prior to the current study. Platinum chemotherapeutic drugs, such as cisplatin and carboplatin, have moderate single-agent activity in PCa (12). However, when combined with a taxane and estramustine, high response rates were observed in several clinical trials (20,21). Considering the high prevalence of TMPRSS2:ERG fusion in patients with PCa, its potential role in the relatively low response rate of PCa to cisplatin was explored.

Indeed, PCa cells were observed to be relatively resistant to the cytotoxic effects of cisplatin. For example, only at doses of 50 µM or above were significant levels of cell death induced by cisplatin in either DU145 or VCaP cells (data not shown). By contrast, significant cell death was not induced in HEK293 cells by cisplatin at concentrations as low as 1 µM, and high levels of cell death was observed at 100 µM (data not shown). Therefore, 50 µM was selected as the test concentration for all subsequent experiments. The response of the PCa cell lines to cisplatin treatment was investigated in DU145 cells, which do not express the fusion gene and VCaP cells, which harbor the fusion gene. This indicated that cisplatin is able to induce up to 30% apoptosis in DU145 cells, while the drug induces ~13% apoptosis in VCaP cells (Fig. 1). However, when ΔERG was expressed in DU145 cells, the apoptotic cell ratio was reduced to ~15%. In comparison, knocking down the endogenous fusion gene expressed in the VCaP cell line led to an increased apoptotic cell ratio. Thus, this indicates that ΔERG may influence the response of these cells to cisplatin. To discover whether or not the anti-apoptotic effect of ΔERG was limited to PCa cells, ΔERG was introduced into the HEK293 cells. Notably, no significant effect of ΔERG on HEK293 cell growth or migration was observed (data not shown and Fig. 3). These observations are in contradiction to the report by Tian et al (7) in which it was shown that the overexpression of TMPRSS2:ERG in PCa cells markedly increased invasion. A possible explanation may be that different types of cells were used, with HEK293 cells originating from the kidney rather than the prostate, and thus may have a different genetic background. Alternatively, an effect of ΔERG on HEK293 cells was observed with respect to apoptosis. Although cisplatin induced apoptosis in ~70% of HEK293 cells, which only expressed the GFP-tag, the apoptotic cell ratio was around 30% in the Δ293 cells that expressed the ΔERG (Fig. 2). An additional piece of evidence for the anti-apoptotic function of ΔERG comes from the observation that knocking down ΔERG in Δ293 cells rendered the cells sensitive to cisplatin (Fig. 2). Taken together, these data indicate an anti-apoptotic effect of ΔERG. Therefore, future treatment planning for patients with PCa may be enhanced by assessing the status of the TMPRSS2:ERG gene fusion.

Once the anti-apoptotic function of the fusion gene had been determined in the current study, the underlying molecular mechanism was further probed. The BCL-2 family of proteins include both anti-apoptotic (BCL-2, BCL-XL and MCL-1) and apoptotic (BAK, BAX, BID and BIM) members (22). The regulation and balance of the BCL-2 family proteins in a particular cell results in either the inhibition or induction of the apoptotic signaling pathways (23). Therefore, the expression of several BCL-2 family members was examined. Notably, none of the BCL-2 family members examined exhibited differential expression between the E293 and Δ293 cells (Fig. 4), suggesting that they may not be involved in the anti-apoptotic activity of ΔERG. However, it was observed that the expression of ATF5, a transcriptional activator with anti-apoptotic activity, was increased in Δ293 cells. It has been reported that when ATF5 was stably expressed in an IL-3-dependent cell line, apoptosis was suppressed through cytokine deprivation (24). Additionally, overexpression of ATF5 suppressed apoptosis induced by serum withdrawal in HeLa cells (25). By contrast, inhibition of endogenous ATF5 activity by the introduction of a dominant-negative form of ATF5 led to apoptosis in asynchronously growing cells (24,25). To determine whether ΔERG was involved in the regulation of ATF5 expression, ΔERG was knocked down in Δ293 cells, and the protein levels of ATF5 were examined by western blotting. As shown in Fig. 4, knock down ΔERG resulted in a reduction in ATF5 expression. Subsequently, ChIP analysis was performed, and the results indicated that ΔERG is able to bind directly to the promoter region of ATF5 (Fig. 4). Based on these observations, ΔERG is proposed to potentially upregulate the expression of ATF5, which in turn exerts anti-apoptotic activities through the activation of downstream signaling pathways.

Finally, since cisplatin-induced DNA damage is considered to be a major factor in triggering apoptosis, it was examined whether ΔERG is able to influence cisplatin-induced DNA damage. γH2AX is regarded to be a sensitive indicator for DNA damage (26). The results of the present study indicated that expression of the γH2AX protein was significantly reduced in Δ293 cells, suggesting that overexpression of ΔERG may protect cells from cisplatin-induced DNA damage (Fig. 5). The role of TMPRSS2:ERG in the DNA damage process remains controversial. TMPRSS2:ERG has been previously shown to interact with the enzyme PARP1 and the catalytic subunit of DNA protein kinase in a DNA-dependent manner, and overexpression of similar fusion genes induces DNA double-strand breaks (27). In addition, Han et al (28) observed that overexpression of TMPRSS2:ERG increases PARP1 activity and clonogenic survival (28). Haffner et al (29) reported that androgen signaling promotes co-recruitment of the androgen receptor and topoisomerase II beta (TOP2B) to sites of TMPRSS2:ERG genomic breakpoints, triggering recombminogenic TOP2B-mediated DNA double-strand breaks (DSBs). Furthermore, androgen stimulation resulted in de novo production of TMPRSS2:ERG fusion transcripts in a process that required TOP2B and components of
the DSB repair machinery (29). Thus, how ΔERG may interfere with the DNA damage process remains clear, and the potential link between ΔERG and DNA damage is of great interest for future studies.

In summary, the current study indicated that the ΔERG protein is able to inhibit cisplatin-induced DNA damage and cell death, and that the ATF5 gene may participate in such events.

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