Overexpression of RGPR-p117 suppresses apoptotic cell death and its related gene expression in cloned normal rat kidney proximal tubular epithelial NRK52E cells

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Abstract. The novel protein RGPR-p117 was discovered as a regucalcin gene promoter region-related protein that binds to the TTGGC motif using a yeast one-hybrid system. The role of RGPR-p117 in cell function has not been fully clarified. This study was undertaken to determine whether overexpression of RGPR-p117 regulates various types of signaling factor-induced apoptotic cell death in the cloned normal rat kidney proximal tubular epithelial NRK52E cells. NRK52E cells (wild-type) or stable RGPR-p117/phCMV2-transfected cells (transfectant) were cultured in Dulbecco's modified Eagle's medium containing 5% bovine serum (BS). NRK52E cells with subconfluent monolayers were cultured for 24-72 h in a medium without BS. The presence of tumor necrosis factor-α (TNF-α; 1.0 or 10 ng/ml of medium), lipopolysaccharide (LPS; 0.1 or 1.0 μg/ml), Bay K 8644 (10^{-6} or 10^{-5} M), or thapsigargin (10^{-8} or 10^{-7} M) caused a significant decrease in the number of NRK52E wild-type cells or phCMV2-transfected (mock-type) cells. The effect of TNF-α, LPS, Bay K 8644, or thapsigargin in decreasing cell number was significantly suppressed in the presence of the caspase-3 inhibitor (10^{-8} M) in wild-type cells cultured for 48 h. The effect of TNF-α, LPS, or Bay K 8644 in decreasing cell number was significantly inhibited in the transfectants, while the effect of thapsigargin on cell death was not inhibited in the transfectants. Culture with TNF-α or LPS caused DNA fragmentation in wild-type cells. These effects were significantly suppressed in the transfectants. The result of reverse transcription-polymerase chain reaction analysis using specific primers for the genes of apoptotic cell death-related proteins showed that IAP-1, FADD, caspase-8, caspase-9, and caspase-3 mRNA levels were significantly decreased in the transfectants, while Akt-1, Bid, Apaf-1, and glyceroaldehyde-3-phosphate dehydrogenase mRNA levels were not significantly altered in the transfectants. Culture with TNF-α, LPS, Bay K 8644, or thapsigargin caused a significant increase in Apaf-1 or caspase-3 mRNA levels. Such an effect was not seen in the transfectants. This study demonstrates that overexpression of RGPR-p117 has a suppressive effect on cell death and apoptosis induced by TNF-α, LPS, or Bay K 8644 whose actions are mediated through intracellular signaling pathways. This study also demonstrates that RGPR-p117 regulates the gene expression of apoptosis-related proteins.

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Introduction

RGPR-p117 was discovered as a regucalcin gene promoter region-related protein (1,2). Regucalcin has been demonstrated to play a multifunctional role as a regulatory protein in the intracellular signaling system of many cell types (reviewed in refs. 3,4). RGPR-p117 is conserved in various vertebrate species, including the human, rat, mouse, bovine, rabbit, and chicken (1,2), indicating a high conservation of RGPR-p117 genes throughout evolution. The entire human RGPR-p117 cDNA consists of 3,989 bp and contains an open reading frame (ORF) of 3,180 bp, encoding a protein of 1,060 amino acid residues (1). A comparison of the human RGPR-p117 cDNA sequence with the genomic sequence database indicates that the gene consists of at least 26 exons, spanning ~42 kb and is localized on human chromosome 1q25.2 (1). RGPR-p117 mRNA is ubiquitously expressed in many tissues in the rat (5), and it is stably expressed for physiological changes in the rat liver (6).

Mammalian RGPR-p117 conserves a leucine zipper motif (2). The computer analysis of subcellular localization of RGPR-p117 from six vertebrates shows the probability of nuclear localization at >52.2%; the nuclear localization in the rat and mouse is 78.3% (2). RGPR-p117 is suggested to have a role in nuclear function. RGPR-p117 was shown to localize in the nuclei of cloned normal rat kidney proximal tubular epithelial NRK52E cells (7). Overexpression of RGPR-p117 enhanced the regucalcin promoter activity which is related to the NF-1 consensus sequences including the TTGGC motif, and its enhancing effect was partly mediated through

phosphorylation and dephosphorylation of proteins in NRK52E cells (2,8,9). RGPR-p117 was shown to enhance the expression of regucalcin gene in the nucleus of kidney cells, indicating its role as a transcription factor.

The transfectants which stably overexpress RGPR-p117 in the cloned normal rat kidney proximal tubular epithelial NRK52E cells were generated to elucidate its role in the regulation of cellular function (9,10). Overexpression of RGPR-p117 was shown to induce a decrease in protein and DNA contents in NRK52E cells (10).

This study was undertaken to determine whether overexpression of RGPR-p117 regulates various types of intracellular signaling factor-induced apoptotic cell death in NRK52E cells. We found that overexpression of RGPR-p117 had a suppressive effect on apoptotic cell death induced by TNF-α, LPS, or Bay K 8644 in NRK52E cells.

Materials and methods

Chemicals. Non-essential amino acid solution, bovine serum (BS), and penicillin-streptomycin solution (5,000 U/ml penicillin; 5,000 μg/ml streptomycin) were obtained from Gibco Laboratories (Grand Island, NY). Dulbecco's modified Eagle's medium (DMEM), tumor necrosis factor-α (TNF-α), lipopolysaccharide (LPS), Bay K 8644, and thapsigargin were obtained from Sigma Chemical Co. (St. Louis, MO). Caspase 3/CPP 32 inhibitor W-1 (caspase inhibitor) and additional chemicals were purchased from Wako Pure Chemical Co. (Osaka, Japan). The reagents used were dissolved in distilled water or ethanol, and some reagents were passed through ion-exchange resin to remove metal ions.

Cell culture. The cloned normal rat kidney proximal tubular epithelial cells (NRK52E; $1x10^5$ cells) (11) were maintained for 72 h in DMEM supplemented with 5% BS, non-essential amino acid, and 50 U/ml, and 50 μg/ml streptomycin in humidified 5% CO_2/95% air at 37˚C to obtain subconfluent monolayers. After culture, cells were washed three times with phosphate-buffered saline (PBS), and the cells were incubated for 24, 48, or 72 h in DMEM without 5% BS in the absence or presence of various factors. Cells were washed three times with PBS after culture. Cells were collected to count cell numbers.

RGPR-p117 transfectants. Expression plasmid for RGPR-p117/phCMV2 was constructed as described previously (7,10). NRK52E cells were grown on 35-mm dishes to ~70% confluence. Each RGPR-p117/phCMV2 and phCMV2 vector alone was transfected into NRK52E cells using the synthetic cationic lipid component, Lipofectamine, according to the manufacturer's instructions (Invitrogen) (7). At 48 h after transfection, cells were harvested and used for subsequent experiments. NRK52E cells were transfected with the RGPR-p117/phCMV2 vector, alone, using Lipofectamine. After 24 h, neomycin (1.0 mg/ml Geneticin G418, Sigma) was added to the cultures for selection, and cells were plated at a limiting dilution. Multiple surviving clones were isolated, transferred to 35-mm dishes, and grown in the medium without neomycin. RGPR-p117 was markedly expressed in the transfectants as estimated using Western blot analysis (10). For the experiments, the transfectants were cultured for 24-72 h in DMEM containing 5% BS.

Cell counting. After trypsinization of each of the culture dishes using 0.2% trpysin plus 0.02% EDTA in Ca^{2+}/Mg^{2+}-free PBS for 2 min at 37˚C, cells were collected and centrifuged in PBS at 100 x g for 5 min. The cells were re-suspended in PBS solution and stained with eosin. Cell numbers were counted under a microscope using a hemocytometer plate. For each dish, we calculated the average of two countings.

Analysis of DNA fragmentation. The cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) and the transfectant of NRK52E cells with subconfluency were cultured for 48 h in DMEM without 5% BS in the presence or absence of various factors. The culture supernatant was removed, and adherent cells were then lysed in 10 mM Tris HCl, pH 7.4, 10 mM EDTA (neutralized), and 0.5% Triton X-100. The DNA content was determined using the method of Ceriotti (12). Low-molecular-weight DNA fragments were separated by electrophoresis in 1.5% agarose gel (13). Gels were visualized by ethidium bromide staining with a UV transilluminator (Funakoshi Co. Ltd., Tokyo, Japan).

Determination of specific mRNA by RT-PCR. Total RNAs were prepared as described previously (14). After culture, cells were washed three times with ice-cold PBS, and then cells were homogenized in buffer solution containing 4 M guanidinium thiocyanate, 24 mM sodium citrate (pH 7.0), 0.5% sarcosyl, and isoamyl alcohol, and the phases were separated by centrifugation at 10,000 x g for 20 min at 4˚C. RNA located in the aqueous phase was precipitated with isopropanol at -20˚C. RNA precipitates were pelleted by centrifugation, and the pellets were redissolved in diethylpyrocarbonate-treated water.

RT-PCR was performed with a Titan™ One Tube RT-PCR kit (Roche Molecular Biochemicals) as recommended by the supplier. The primers were generated based on the published rat sequences. Primers for amplification Bcl-2 cDNA were: 5'-GCTACCGTCGCGACTTTGC-3' (sense strand, positions 545-563 of cDNA sequence) and 5'-GCAG CGTCTTCAGAGACAGC-3' (antisense strand, positions 867-886) (15). The pair of oligonucleotide primers was designed to amplify a 342-bp sequence from the mRNA of rat Bcl-2.

Primers for Akt-1 cDNA were: 5'-GGAGGGCTGGC TGCACAAACG-3' (sense strand, positions 66-88 of cDNA sequence) and 5'-TCGTTCATGGTCACACGGTGCTTG-3' (antisense strand, positions 465-488) (16). The pair of oligonucleotide primers was designed to amplify a 424-bp sequence from the mRNA of rat Akt-1.

Primers for IAP-1 (inhibitor of apoptosis protein 1) cDNA were: 5'-TGGCTACTTCAGTGGCTCCT-3' (sense strand, positions 393-413 of cDNA sequence) and 5'-GCAA AGCAGGCCACTCTATC-3' (antisense strand, positions 620-640 of cDNA sequence) (17). The pair of oligonucleotide primers was designed to amplify a 248-bp sequence from the mRNA of rat IAP-1.

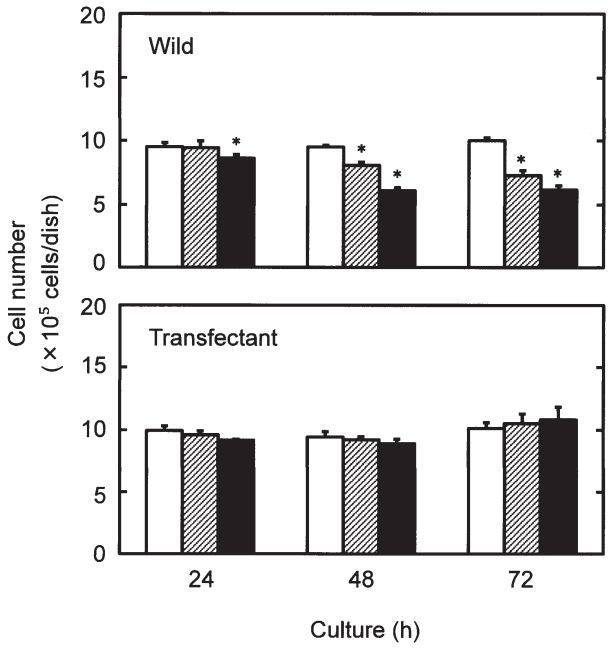

Figure 1. Effect of TNF-α on the number of the cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) or RGPR-p117/phCMV2-transfected cells (transfectant). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to culture medium without BS containing either vehicle or TNF-α (1.0 or 10 ng/ml of medium). After change of medium, cells were incubated for 24, 48, or 72 h, and the number of cells was measured. Each value is the mean ± SEM of six experiments. *P<0.01 compared with the control (none) value. White bars, control (none); hatched bars, TNF-α (1.0 ng/ml); black bars, TNF-α (10 ng/ml).

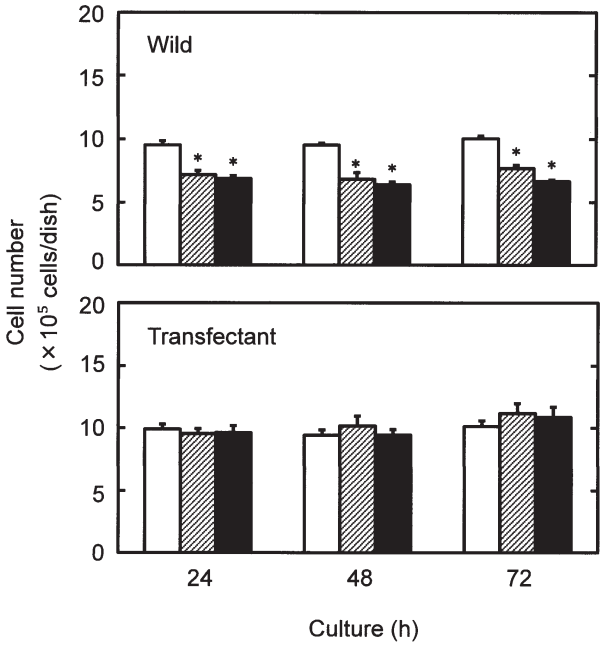

Figure 2. Effect of LPS on the number of the cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) or RGPR-p117/phCMV2-transfected cells (transfectant). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to culture medium without BS containing either vehicle or LPS (0.1 or 1.0 μg/ml of medium). After change of medium, cells were incubated for 24, 48, or 72 h, and the number of cells was measured. Each value is the mean ± SEM of six experiments. *P<0.01 compared with the control (none) value. White bars, control (none); hatched bars, LPS (0.1 μg/ml); black bars, LPS (1.0 μg/ml).

Primers for FADD (Fas-associating death domain protein) cDNA were: 5'-CTGGGCAGACACGACCTACT-3' (sense strand, positions 208-228 of cDNA sequence) and 5'-CTCCCTTACCCGATCACTCA-3' (antisense strand, positions 429-449 of cDNA sequence) (18). The pair of oligonucleotide primers was designed to amplify a 242-bp sequence from the mRNA of rat FADD.

Primers for caspase-8 cDNA were: 5'-TGAAGGAGCTG CTTTTCCAT-3' (sense strand, positions 185-205 of cDNA sequence) and 5'-ATCAAGCAGGCTCGAGTTGT-3' (antisense strand, positions 423-443 of cDNA sequence) (19). The pair of oligonucleotide primers was designed to amplify a 259-bp sequence from the mRNA of rat caspase-8.

Primers for caspase-9 cDNA were: 5'-AAGACCATG GCTTTGAGGTG-3' (sense strand, positions 989-1009 of cDNA sequence) and 5'-CAGGAACCGCTCTTCTTGTC-3' (antisense strand, positions 1129-1219 of cDNA sequence) (20). The pair of oligonucleotide primers was designed to amplify a 231-bp sequence from the mRNA of rat caspase-9.

Primers for Bid cDNA were: 5'-AGCACATCACAAAC CTGCTG-3' (sense strand, positions 41-61 of cDNA sequence) and 5'-CTGGCAATGTTGTGGATGAC-3' (antisense strand, positions 266-286 of cDNA sequence) (21). The pair of oligonucleotide primers was designed to amplify a 246-bp sequence from the mRNA of rat Bid.

Primers for Apaf-1 cDNA were: 5'-TCCAGCGGCAA GGACACAGACG-3' (sense strand, positions 906-927 of cDNA sequence) and 5'-CAACCGCGTGCAAAGATTCT GCA-3' (antisense strand, positions 1213-1235 of cDNA sequence). The pair of oligonucleotide primers was designed to amplify a 330-bp sequence from the mRNA of rat Apaf-1 (22).

Primers for caspase-3 were: 5'-CGCAATGGTACCGA TGTCGATGC-3' (sense strand, positions 246-268 of cDNA sequence) and 5'-GCAGTCCAGCTCTGTACCTCGGCA-3' (antisense strand, positions 543-566) (22). The pair of oligonucleotide primers was designed to amplify a 321-bp sequence from the mRNA of rat caspase-3. Glyceroaldehyde-3-phosphase dehydrogenase (G3PDH) was used as an internal control to evaluate total RNA input. G3PDH cDNA were 5'-GATTGGCCGTATCGGACGC-3' (sense strand) and 5'-CTCCTTGGAGGCCATGTAGG-3' (antisense strand). The pair of oligonucleotide primers was designed to amplify a 977-bp sequence from the mRNA of rat G3PDH.

RT-PCR was performed using a reaction mixture (20 μl) containing 2 or 4 μg of total RNAs, the supplied RT-PCR buffer, Titan™ enzyme mix (AMV and Expand™ High Fidelity), 0.2 mM dNTP, 5 mM dithiothreitol, 5 U RNase inhibitor, and 0.3 μM primers. Samples were incubated at 50˚C for 30 min and then amplified for 30 cycles under the following conditions: denaturation for 30 sec at 94˚C, annealing for 30 sec at 56˚C, and extension for 60 sec at 62.0-63.4˚C. The amplified products were separated by electrophoresis on a 1.5% agarose gel and visualized by ethidium bromide staining. Image density was quantified with a FluoroImager SI (Amersham Phamacia Biotech).

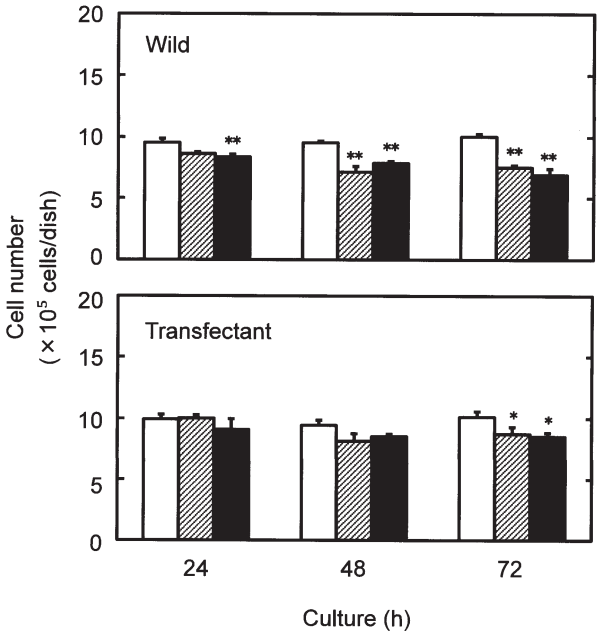

Figure 3. Effect of Bay K 8644 on the number of the cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) or RGPR-p117/phCMV2-transfected cells (transfectant). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to culture medium without BS containing either vehicle or Bay K 8644 (10^{-6} or 10^{-5} M). After change of medium, cells were incubated for 24, 48, or 72 h, and the number of cells was measured. Each value is the mean ± SEM of six experiments. *P<0.05 or **P<0.01 compared with the control (none) value. White bars, control (none); hatched bars, Bay K 8644 (10^{-6} M); black bars, Bay K 8644 (10^{-5} M).

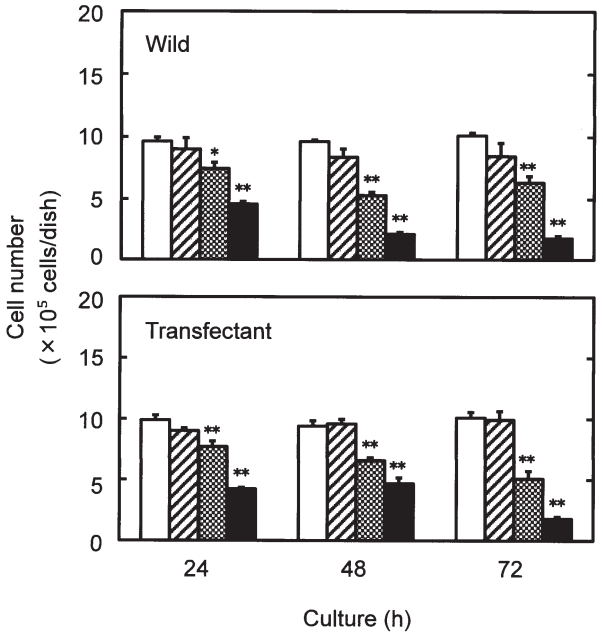

Figure 4. Effect of thapsigargin (TPG) on the number of the cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) or RGPR-p117/phCMV2-transfected cells (transfectant). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to culture medium without BS containing either vehicle or TPG (10^{-9}-10^{-7} M). After change of medium, cells were incubated for 24, 48, or 72 h, and the number of cells was measured. Each value is the mean ± SEM of six experiments. *P<0.05 or **P<0.01 compared with the control (none) value. White bars, control (none); hatched bars, TPG (10^{-9} M); double-hatched bars, TPG (10^{-8} M); black bars, TPG (10^{-7} M).

Statistical analysis. Data were expressed as the mean ± SEM. The significance of differences between the values was estimated by the Student's t-test or by analysis of variance (ANOVA) for comparing multiple groups. A P-value of <0.05 was considered to indicate a statistically significant difference.

Results

Effect of TNF-a, LPS, Bay K 8644, or thapsigargin on kidney NRK52E cells overexpressing RGPR-p117. The cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type), phCMV2-transfected cells (mock-type), or stable RGPR-p117/phCMV2-transfected cells (transfectant) were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers. The proliferation of the cells was not significantly changed in the transfectants or mock-type cells (10). After culture for 72 h, cells were changed to a medium not containing BS in the presence or absence of various factors that induce cell death and apoptosis and were further cultured for 24-72 h. The expression of RGPR-p117 in the cells was prominent in the transfectants (data not shown), as previously reported (10).

NRK52E cells with subconfluent monolayers were cultured for 24, 48, or 72 h in a medium without BS containing either vehicle, TNF-α (0.1 or 1.0 ng/ml) (Fig. 1), LPS (0.1 or 1.0 μg/ml) (Fig. 2), Bay K 8644 (10^{-6} or 10^{-5} M), or thapsigargin (10^{-8} or 10^{-7} M). The number of wild-type cells was significantly decreased after culture for 24-72 h in the

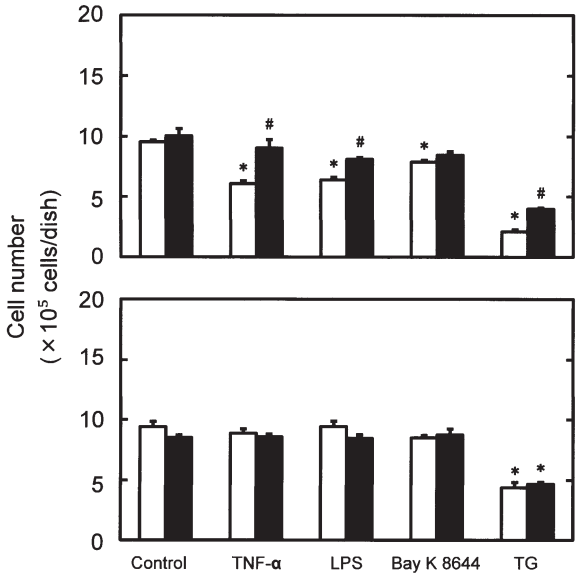

Figure 5. Effect of caspase inhibitor on various factor-induced decreases in the number of cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) or RGPR-p117/phCMV2-transfected cells (transfectant). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to culture medium without BS containing either vehicle, TNF-α (10 ng/ml of medium), LPS (1.0 μg/ml), Bay K 8644 (10^{-7} M), or thapsigargin (TG) (10^{-8} M) in the presence or absence of caspase-3 inhibitor (10^{-8} M). After change of medium, cells were incubated for 48 h, and the number of cells was measured. Each value is the mean ± SEM of six experiments. *P<0.01 compared to the control (none) value. $^{\#}$P<0.01 compared to the value without caspase-3 inhibitor. White bars, without caspase-3 inhibitor; black bars, with caspase-3 inhibitor.

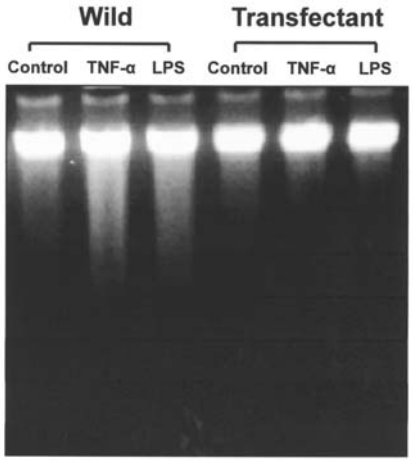

Figure 6. Effect of TNF-α or LPS on DNA fragmentation in the cloned normal rat proximal tubular epithelial NRK52E cells (wild-type) or RGPR-p117/phCMV2-transfected cells (transfectant). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to culture medium without BS containing either vehicle, TNF-α (10 ng/ml), or LPS (1.0 μg/ml). After change of medium, cells were incubated for 48 h, and the lysate (containing 1.0 μg of DNA) of adherent cells was applied to agarose gel. The figure shows one of four experiments with separate samples.

presence of TNF-α (10 ng/ml), LPS (0.1 or 1.0 μg/ml), Bay K 8644 (10^{-5} M), or thapsigargin (10^{-8} or 10^{-7} M). These decreases were also observed in mock-type cells (data not shown). The effect of TNF-α (10 ng/ml) or LPS (0.1 or 1.0 μg/ml) in decreasing the number of NRK52E cells (wild-type) was not observed in the stable RGPR-p117/phCMV2-transfected cells cultured for 24, 48, or 72 h (Fig. 1). The effect of LPS- (0.1 or 1.0 μg/ml) inducing cell death was not observed in the transfectants (Fig. 2). The effect of Bay K 8644 (10^{-6} or 10^{-5} M) in decreasing the number of wild-type cells was significantly suppressed in the transfectants cultured for 24 or 48 h (Fig. 3). Culture with Bay K 8644 (10^{-6} or 10^{-5} M) for 72 h caused a significant decrease in the cell number of the transfectants. The effect of thapsigargin (10^{-8} or 10^{-7} M) in inducing cell death was also observed in the transfectants cultured for 24-72 h (Fig. 4).

NRK52E cells with subconfluent monolayers were cultured for 48 h in a medium without BS containing either vehicle, TNF-α (10 ng/ml), LPS (1.0 μg/ml), Bay K 8644 (10^{-5} M), or thapsigargin (10^{-7} M) in the presence or absence of the caspase-3 inhibitor (10^{-8} M) with an effective concentration on the enzyme activity (Fig. 5). The effect of TNF-α, LPS, Bay K 8644, or thapsigargin in decreasing the cell number of NRK52E wild-type cells was significantly inhibited in the presence of the caspase-3 inhibitor. The preventive effect of the caspase-3 inhibitor on thapsigargin-induced cell death of NRK52E wild-type cells was not observed in the transfectants.

NRK52E wild-type cells and transfectants with subconfluent monolayers were cultured for 48 h in a medium without BS containing either vehicle, TNF-α (10 ng/ml), or LPS (1.0 μg/ml) (Fig. 6). Adherent cells were lysed, and then

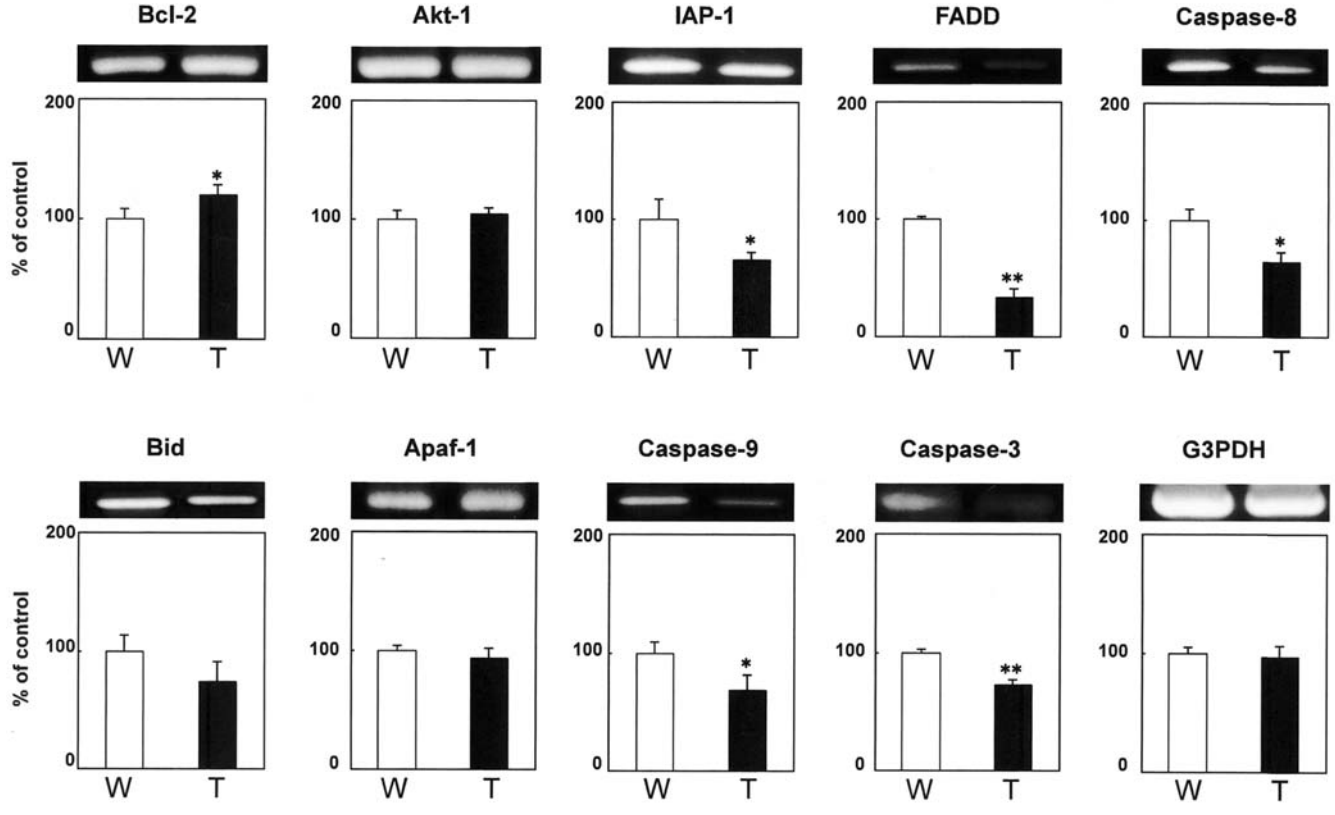

Figure 7. Change in mRNA expression of various proteins which are related to cell death and apoptosis in the cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type; W) or RGPR-p117/phCMV2-transfected cells (T). Cells were cultured for 72 h in a medium containing 5% BS to obtain subconfluent monolayers and then exchanged to a culture medium without BS. After change of medium, cells were cultured for 48 h. Total RNAs (2 μg) extracted from the cells were analyzed by RT-PCR using specific primers. The figure shows one of four experiments with separate samples. The densitometric data for each mRNA level were indicated as % of control (mean ± SEM of five experiments). $^{*}P<0.05$ or $^{**}P<0.01$ compared with the control (wild-type) value.

the lysate was separated using electrophoresis in agarose gel. Culture with TNF-α or LPS caused DNA fragmentation in wild-type cells. The effect of these factors on DNA fragmentation was significantly suppressed in the transfectants.

Change in gene expression of apoptosis-related proteins in kidney NRK52E cells overexpressing RGPR-p117. The cloned normal rat kidney proximal tubular epithelial NRK52E cells (wild-type) or stable RGPR-p117/phCMV2-transfected cells with subconfluent monolayers were cultured for 48 h in a medium without BS containing either vehicle, TNF-α (1.0 ng/ml), LPS (1.0 μg/ml), Bay K 8644 (10^{-7} M), or thapsigargin (10^{-8} M). Total RNAs were extracted from adherent cells. The result of RT-PCR analysis using specific primers showed that the mRNA expression of apoptosis-related proteins IAP-1, FADD, caspase-8, caspase-9, or caspase-3 was significantly decreased in transfectants as compared with that of wild-type cells (Fig. 7). Akt-1, Bid, Apaf-1, or G3PDH mRNA levels were not significantly changed in the transfectants.

Discussion

Overexpression of regucalcin was found to have a suppressive effect on cell death induced by TNF-α, LPS, or Bay K 8644 in the cloned normal rat kidney proximal epithelial NRK52E cells. Cell death induced by these factors was significantly inhibited in the presence of the caspase-3 inhibitor in NRK52E cells. TNF-α- or LPS-induced DNA fragmentation in NRK52E wild-type cells was significantly inhibited in the transfectants, suggesting that overexpression of RGPR-p117 has a suppressive effect on apoptotic cell death. It is thus speculated that RGPR-p117 regulates the intracellular signaling pathway related to apoptotic cell death induced by TNF-α, LPS, or Bay K 8644, an agonist of Ca^{2+} entry in cells.

Culture with thapsigargin, an inhibitor of Ca^{2+} pump (Ca^{2+}-ATPase) activity in endoplasmic reticulum (Ca^{2+} store), caused a significant increase in the cell death of NRK52E wild-type cells. Thapsigargin-induced cell death was partly suppressed in the presence of the caspase-3 inhibitor. Overexpression of RGPR-p117 did not have a suppressive effect on thapsigargin-induced cell death in NRK52E cells. RGPR-p117 may not regulate the signaling mechanism of cell death induced by thapsigargin.

Overexpression of RGPR-p117 was found to induce a decrease in mRNA levels of FADD, caspase-8, caspase-9, or caspase-3 involved in the stimulation of apoptotic cell death in NRK52E cells. This finding suggests that overexpression of RGPR-p117 has a suppressive effect on apoptotic cell death due to a decrease in the gene expression of various proteins which induce apoptosis. Meanwhile, overexpression of RGPR-p117 caused a significant increase in the gene expression of Bcl-2 related to the stimulation of apoptosis and a significant decrease in the gene expression of IAP-1 involved in the inhibition of apoptotic cell death. However, overexpression of RGPR-p117 caused a significant decrease in cell death in NRK52E cells. The presence of the caspase-3 inhibitor caused a significant inhibition in the apoptotic cell death induced by TNF-α, LPS, or Bay K 8644. It is speculated that the decrease in caspase-3 is important in the suppression of apoptotic cell death in transfectants. The suppression of caspase-3 mRNA expression caused by overexpression of RGPR-p117 may contribute to RGPR-p117-induced apoptotic cell death.

Overexpression of RGPR-p117 did not have a suppressive effect on cell death induced by thapsigargin. An increase in the expression of Apaf-1 and caspase-3 mRNAs after culture with thapsigargin was not observed in the transfectants. Thapsigargin is an inhibitor of Ca^{2+}-ATPase in the endoplasmic reticulum (Ca^{2+} store) in cells. Treatment with thapsigargin causes an elevation in sustained Ca^{2+} concentration in cells and induces apoptosis in hepatoma cells (24,25). Experiments on nuclei isolated from thymocytes clearly demonstrated the induction of Ca^{2+}-dependent endonuclease activity during the triggering of apoptotic events (26). A nuclear Ca^{2+}/Mg^{2+}-dependent endonuclease, which is able to digest chromatin *in situ* into mononucleosomal and oligonucleosomal fragments, has been purified from human spleen cells (27). Increases in intracellular Ca^{2+} concentration are believed to activate this nuclease and to mediate DNA cleavages into oligonucleosome fragments (28,29). Presumably, RGPR-p117 does not directly inhibit the endonuclease in the nucleus.

RGPR-p117 is known to enhance regucalcin mRNA expression and its protein level in NRK52E cells (8,9). Regucalcin has a suppressive effect on various types of factor-induced cell death in NRK52E cells (30). It is possible that overexpression of RGPR-p117 enhances regucalcin expression, and this increase partly contributes to the exhibition of the suppressive effect of RGPR-p117 overexpression on cell death induced by various factors.

RGPR-p117 has been shown to localize in the nucleus in NRK52E cells (7), and it may bind to the TTGGC motif in the promoter region of the rat regucalcin gene (9). The TTGGC motif is present in the promoter region of the genes of caspase-3, caspase-8, or FADD as shown in the Databases. The expression of these genes was suppressed in the transfectants. It is thus speculated that RGPR-p117 binds to the TTGGC motif in the promoter region of the genes of caspase-3, caspase-8, or FADD in the nucleus of NRK52E cells and that it suppresses the expression levels of these genes.

RGPR-p117 may play a role as a transcription factor for regucalcin gene expression in NRK52E cells (9). In addition, it is possible that RGPR-p117 regulates the gene expression of caspase-3, caspase-8, or FADD in NRK52E cells. This remains to be elucidated.

In conclusion, we demonstrated that overexpression of RGPR-p117 has a suppressive effect on apoptotic cell death induced by TNF-α, LPS, or Bay K 8644 in the cloned normal rat kidney proximal tubular epithelial NRK52E cells, suggesting that this effect is partly related to the regulation of the gene expression of proteins which are involved in apoptotic cell death.

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