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CREB-Mediated Bcl-2 Expression Contributes to RCAN1 Protection From Hydrogen Peroxide-Induced Neuronal Death

Seon Sook Kim, Shin Ah Jang, and Su Ryeon Seo*

Department of Molecular Bioscience, College of Biomedical Science, Institute of Bioscience & Biotechnology, Kangwon National University, Hyoja-dong, Chuncheon 200-701, Republic of Korea

ABSTRACT

Regulator of calcineurin 1 (RCAN1) is located on the Down syndrome critical region (DSCR) locus in human chromosome 21. In this study, we investigated the functional role of RCAN1 in the reactive oxygen species (ROS)-mediated neuronal death signaling. We found that RCAN1 was able to protect the cells from H_2O_2-induced cytotoxicity. The expression of RCAN1 caused an inhibition of the H_2O_2-induced activation of mitogen-activated protein kinases (MAPKs) and AP-1. In contrast, RCAN1 significantly enhanced the activity of cAMP response element-binding protein (CREB). Furthermore, RCAN1 induced the expression of the CREB target gene, Bcl-2. Consistently, knockdown of endogenous RCAN1 using shRNA down regulated the phosphorylation of CREB and the expression of Bcl-2, which protects the cells from H_2O_2-induced cytotoxicity. Our data provide a new mechanism for the cytoprotective function of RCAN1 in response to oxidant-induced apoptosis. J. Cell. Biochem. 114: 1115–1123, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: RCAN1/DSCR1/ADAPT78; CREB; MAPKs; Bcl-2; HYDROGEN PEROXIDE

The regulator of calcineurin 1 (RCAN1) gene is present in the Down Syndrome Critical Region (DSCR) on human chromosome 21, which is responsible for the phenotypic characteristics of Down syndrome (DS) [Epstein et al., 1991; Fuentes et al., 1995]. The RCAN1 gene consists of seven exons and can be alternatively spliced [Harris et al., 2005]. Among the isoforms, RCAN1-1 encoded by exon 1 and RCAN1-4 encoded by exon 4 are the major isoforms detected in many types of tissues and cells, such as the central nervous system (CNS), heart, and skeletal muscles [Fuentes et al., 1995, 1997]. The RCAN1-1 and RCAN1-4 isoforms differ in their initial exon, but share exons 5, 6, and 7. RCAN1 associates with calcineurin, a Ca^{2+}/calmodulin-dependent phosphatase by inhibiting or activating its phosphatase activity [Fuentes et al., 2000; Rothermel et al., 2000; Chan et al., 2005].

The elevated expression of RCAN1 has been associated with Alzheimer's disease (AD) and Down syndrome (DS), and reduced RCAN1 expression was observed in Huntington's disease [Fuentes et al., 2000; Ermak et al., 2001, 2009]. RCAN1 has been shown to be induced in the peri-infarct cortex following experimental stroke [Cho et al., 2008]. In addition, diverse stimuli, including mitogens, depolarization, and oxidative stress, induce the expression of RCAN1 [Harris et al., 2005]. However, whether the elevated expression of RCAN1 is directly implicated in pathological phenomena remains unclear. The upregulation of RCAN1 expression has been shown to be associated with death protection from thapsigargin-mediated calcium overloading [Zhao et al., 2008]. T helper type I cells of RCAN1$^{-/-}$ mice showed enhanced apoptosis [Ryeom et al., 2003], and the knockdown of RCAN1-4 increased the susceptibility to FAS-mediated and etoposide-induced apoptosis [Kim et al., 2009]. Similarly, the deletion of RCAN1-1 and RCAN1-4 isoforms has been shown to lead to apoptosis in endothelial cells [Ryeom et al., 2008].

In contrast to these reports, the upregulation of RCAN1-1 by glucocorticoid in leukemic cells increased the susceptibility to apoptosis [Hirakawa et al., 2009]. Moreover, the elevated expression of RCAN1 by the stress hormone dexamethasone facilitated neuronal apoptosis through caspase-3 activation [Sun et al., 2012]. Therefore, these conflicting reports suggest a complex role of RCAN1 under stress conditions.

In the present study, we investigated the functional role of RCAN1 in the oxidative stress-mediated cell death-signaling pathway that is

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*Correspondence to: Dr. Su Ryeon Seo, Department of Molecular Bioscience, College of Biomedical Science, Institute of Bioscience & Biotechnology, Kangwon National University, Hyoja-dong, Chuncheon 200-701, Republic of Korea. E-mail: suryeonseo@kangwon.ac.kr

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associated with many neurodegenerative diseases. We found that the expression of RCAN1 was able to protect against the cell death induced by hydrogen peroxide (H_2O_2). We also demonstrated that the ability of RCAN1 to confer protection against H_2O_2-induced death resulted from the upregulation of CREB activity. In addition, we found that RCAN1 induced the expression of the CREB target gene, Bcl-2. Our data provide a new mechanism for the cytoprotective function of RCAN1 via the activation of CREB signaling.

MATERIALS AND METHODS

MATERIALS

Anti-HA, anti-c-fos, and anti-GAPDH antibodies were purchased from Santa Cruz Biotechnology. Anti-RCAN1 antibody was purchased from Sigma–Aldrich. Anti-phospho ERK, anti-ERK, anti-phospho JNK, anti-JNK, anti-phospho p38MAPK, anti-p38 MAPK, anti-phospho CREB, anti-CREB and anti-Bcl-2 antibodies were purchased from cell signaling technology. The pRS-RCAN1 shRNA expression vector was purchased from OriGene Technologies (Rockville, MD). The expression vector for triple HA-RCAN1 (1-1) was kindly provided by S. de la Luna. The expression vector for CREB (S133A) was a gift from K. Saeki. The pCRE-Luciferase reporter vector was purchased from Stratagene.

CELL CULTURE AND TRANSFECTION

PC12 cells were maintained in DMEM supplemented with 5% fetal bovine serum (FBS), 10% horse serum, penicillin, and streptomycin. Cells were plated in 60 mm dishes and were transfected with 1 μg of the each expression vector using the Lipofectamine 2000 method (Invitrogen). To obtain stable cell lines, transfected cells were selected using either G418- or puromycin-containing growth medium.

WESTERN BLOT ANALYSIS

The cells were lysed in lysis buffer (20 mM Tris–Cl, pH 7.9, 1% Nonidet P-40, 150 mM NaCl, 1 mM EGTA, 10% glycerol, 1 mM Na_3VO_4, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 10 mM NaF, and 0.2 mM phenylmethylsulfonyl fluoride). A total 50 μg of proteins was separated by 10% SDS–PAGE, and these proteins were transferred to nitrocellulose membranes. The membranes were blocked in TBST buffer (20 mM Tris–Cl, pH 7.6, 137 mM NaCl, 0.05% Tween-20, and 3% nonfat dry milk) for 30 min and then incubated overnight at 4°C in TBST buffer containing the antibodies. The membranes were washed three times with TBST and then incubated with the corresponding secondary horseradish peroxidase-coupled IgG antibody for 1 h at room temperature. The membranes were washed with TBST and then the signals were visualized with ECL reagent.

3-(4,5-DIMETHYLTHIAZOL-2-YL)-2,5-DIPHENYL-TETRAZOLIUM BROMIDE (MTT) ASSAY

After each treatment, the cells were incubated with MTT at a final concentration of 1 mg/ml for 1 h at 37°C, followed by lysis buffering in solubilizing solution (50% dimethylformamide and 20% sodium dodecyl sulfate, pH 4.8) for 24 h. The absorption value was measured at 570 nm. The viability of the PC12 cells cultured in the presence of hydrogen peroxide was calculated as the percent survival relative to the untreated control cells.

BISBENZIMIDE (HOECHST 33258) STAINING

Cells were cultured on poly-L-lysine-coated cover slips and were then treated with H_2O_2 (250 μM) for 12 h. The cells were then fixed with 4% paraformaldehyde for 10 min, followed by incubation with 1 μg/ml Hoechst 33258 in PBS for 20 min. After several washes, the cover-slips were mounted and analyzed using fluorescence microscopy. The condensed nuclei were then scored and quantified. The data are presented as the means ± SD of three independent experiments.

SEMIQUANTITATIVE RT-PCR

Total cellular RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instruction (Invitrogen). Next, 1 μg of total RNA was converted into cDNA using the M-MLV reverse transcriptase (Promega). PCR amplifications of target DNA fragments were performed in duplicate with Top-Taq DNA polymerase (CoreBio, Korea) and specific primers. The primer sequence were as follows: β-actin, 5′-CATGTTTGAGACCTTCAACACCCC-3′ (forward) and 5′-GCCATCTCTTGCTCGAAGTCTAG-3′ (reverse), *c-fos*, 5′-TGGGTGAAACCCTACGCTAC-3′ (forward) and 5′-TTCCTTTCCCTTC GGATTCT-3′ (reverse), *c-jun*, 5′-ACCGACGAGCAGGAGGGCTTC-3′ (forward) and 5′-CAGCGCACCCGGGTTGAAGTTG-3′ (reverse) and Bcl-2, 5′-GCCCGACTTTGCAGAGATGTCCAG-3′ (forward) and 5′-GCCATGCCGGTTCAGGTACTCAG-3′ (reverse). PCR amplifications were performed at 95°C for 10 min, and then 30 cycles at 94°C for 30 s, at 57°C for 45 s and at 72°C for 1 min, followed by extension at 72°C for 10 min. The PCR products were resolved electrophoretically on a 1.2% agarose gel containing 0.05 μg/ml of ethidium bromide. Band intensities were quantified using Gel Logic 100 imaging system (Kodak). For each experiment, the ratio between the optical density of the bands corresponding to the target and β-actin was calculated to quantify the level of the target transcripts.

REPORTER GENE ASSAYS

PC12 cells were plated in 6-well plates and were transfected with 0.5 μg of pGL3-CRE-luc reporter and indicated combinations of HA-RCAN1 and pCMV-CREB (S133A) expression vectors using the Lipofectamine 2000 method (Invitrogen). After 24 h, luciferase activity was measured using the commercial luciferase assay system (Promega). Additionally, all cells were transfected with the β-galactosidase expression plasmids (0.1 μg) as an internal control for measuring transfection efficiency. The level of β-galactosidase activity was determined using the Galacto-star system (PerkinElmer Life Sciences).

STATISTICS

Autographic films for the Western blot analyses were scanned and quantified using ImageJ software. The ratios between the intensity of the target bands and GAPDH were calculated and graphed. The values are expressed as the mean ± standard deviation (SD) of three independent experiments. Statistical significance was evaluated

using Student's t-test; the differences between the two means were considered significant at $P < 0.05$.

RESULTS

EXPRESSION OF RCAN1 SUPPRESSES H_2O_2-INDUCED DEATH IN PC12 CELLS

To investigate whether the expression of RCAN1 could affect the vulnerability to H_2O_2-induced oxidative stress, neuronal PC12 cells were stably transfected with a HA-tagged RCAN1 expression vector (Fig. 1A). The effects of RCAN1 overexpression on cell survival following exposure to 250 μM H_2O_2 were then measured using an MTT assay (Fig. 1B). As shown in Figure 1B, the expression of RCAN1 significantly protected the cells from H_2O_2-induced cytotoxicity for up to 12 h of treatment. Furthermore, the protective effect of RCAN1 was observed at various concentrations of H_2O_2, ranging from 50 to 400 μM (Fig. 1C). Consistent with these results, the expression of RCAN1 decreased the rate of H_2O_2-induced nuclear condensation, a hallmark of apoptotic cells (Fig. 1D,E). To address whether the protective effect of RCAN1 was due to the clonal variation in cell lines, we selected two other independently derived clones, and the cytotoxicity in response to H_2O_2 was measured (Fig. 1F). These clones showed similar inhibition from the H_2O_2-treatment, indicating that the cytoprotective effect of RCAN1 in

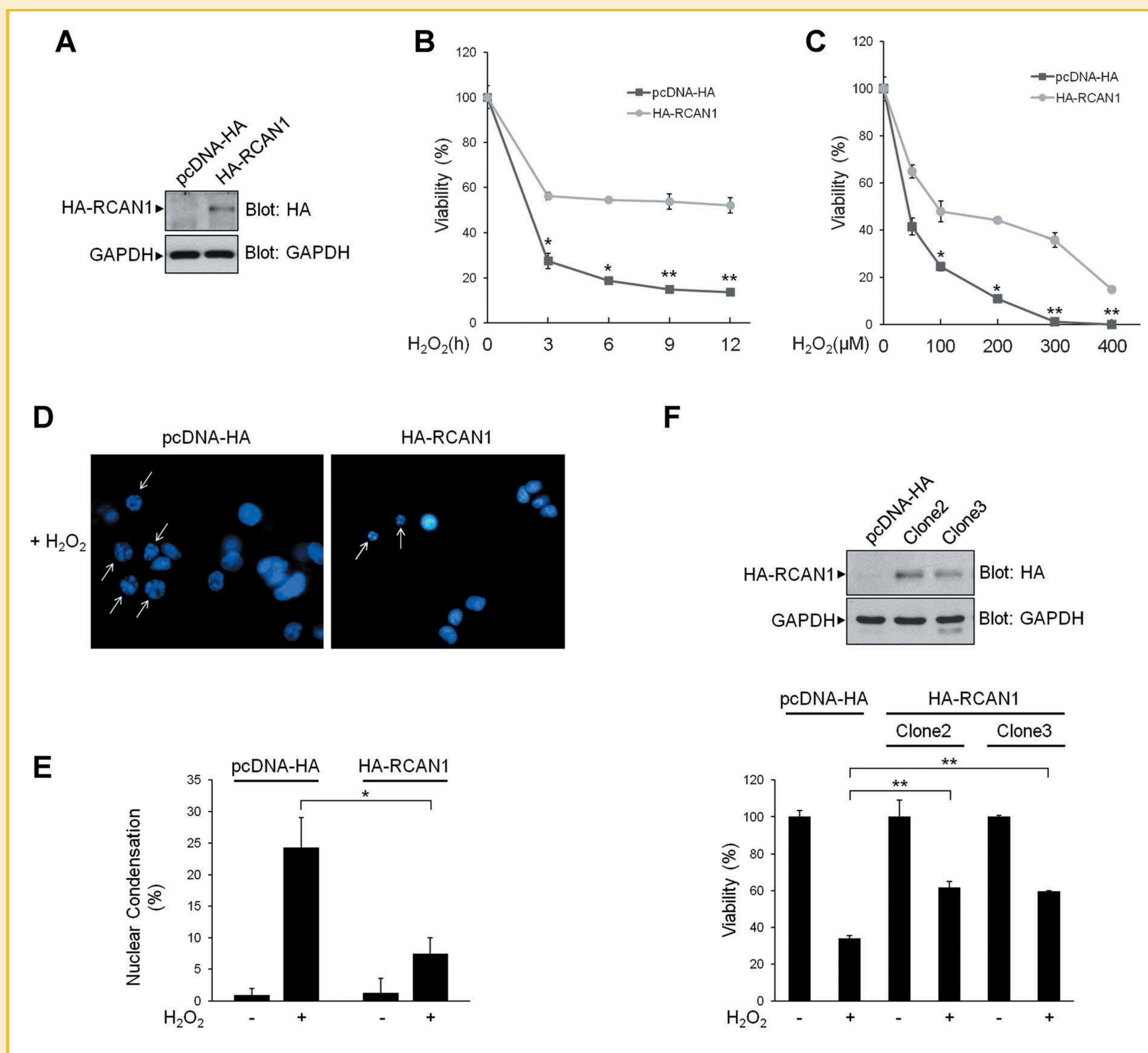

Fig. 1. Overexpression of RCAN1 suppresses H_2O_2-induced death in PC12 cells. A: PC12 cells were transfected with the empty or HA-RCAN1 expression vector and were selected in G418-containing growth medium. The stable RCAN1-overexpressing clone was identified by immunoblotting using an anti-HA antibody. B and C: Cells were treated with H_2O_2 (250 μM) for the indicated times or treated with the indicated concentrations of H_2O_2 for 12 h. The viability of the cells was determined using an MTT assay. The results are presented as the means ± SD of three independent experiments. D: The cells were treated with H_2O_2 (250 μM) for 12 h and the nuclear morphology was determined using Hoechst 33258 staining. An arrowhead indicates a condensed nucleus. E: The condensed nuclei were scored and then quantified. F: The stable RCAN1-overexpressing clones were treated with H_2O_2 (250 μM) for 12 h, and the viability of the cells was determined using an MTT assay. The data are presented as the means ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$

response to oxidative stress was not due to clonal variations. These results suggest that the expression of RCAN1 protects cells from H_2O_2 toxicity.

EXPRESSION OF RCAN1 INHIBITS ROS-MEDIATED MAPKs ACTIVATION

Many reports suggest that mitogen-activated protein kinases (MAPKs), including JNK, ERK, and p38 MAPK, elicit the induction of apoptosis in response to various oxidative stresses [Ruffels et al., 2004; Valencia and Moran, 2004]. Thus, to examine whether the effect of RCAN1 for protecting cells from H_2O_2-mediated toxicity was accompanied by the inhibition of MAPK pathways, we measured the activation of MAPKs using phospho-specific antibodies. As shown in Figure 2A,B, treatment of the cells with 250 μM H_2O_2 caused the activation of JNK, ERK, and p38 MAPKs, and the level of phosphorylated MAPKs in response to H_2O_2 was significantly inhibited in the cells stably overexpressing RCAN1. In contrast, no significant differences regarding the total MAPK levels between the cells stably overexpressing RCAN1 and the empty vector were observed. These results indicate that RCAN1 is responsible for the inhibition of H_2O_2-mediated MAPK activation.

EXPRESSION OF RCAN1 INHIBITS AP-1 ACTIVATION

The activation of MAPKs during apoptosis induces the expression and activation of the immediate early genes, *c-fos* and *c-jun*, which constitute the activator protein-1 (AP-1) family of transcription factors [Jacobs-Helber et al., 1998; Kook et al., 2008]. To address whether the protective ability of RCAN1 in H_2O_2-induced toxicity was associated with AP-1 gene activation, we first examined the levels of the phosphorylated form of c-jun, which is necessary for the transcriptional activation of AP-1. As shown in Figure 3A,B, treatment of the cells with 250 μM H_2O_2 led to significant increases in the activation of c-jun within 30 min of H_2O_2 exposure and the levels of phosphorylated c-jun were completely blocked in the cells that stably overexpressing RCAN1. Consistently, the induction of c-fos protein in response to H_2O_2 was similarly inhibited in the cells overexpressing RCAN1 (Fig. 3A,B).

We then examined the levels of c-fos and c-jun mRNA expression after H_2O_2-treatment using RT-PCR (Fig. 3C,D). As shown in Figure 3C,D, the induction of c-fos and c-jun mRNA in response to H_2O_2 were blocked in the cells that stably overexpressing RCAN1. Taken together, these results indicate that RCAN1 exerts its inhibitory effect on the activation of AP-1 in response to H_2O_2.

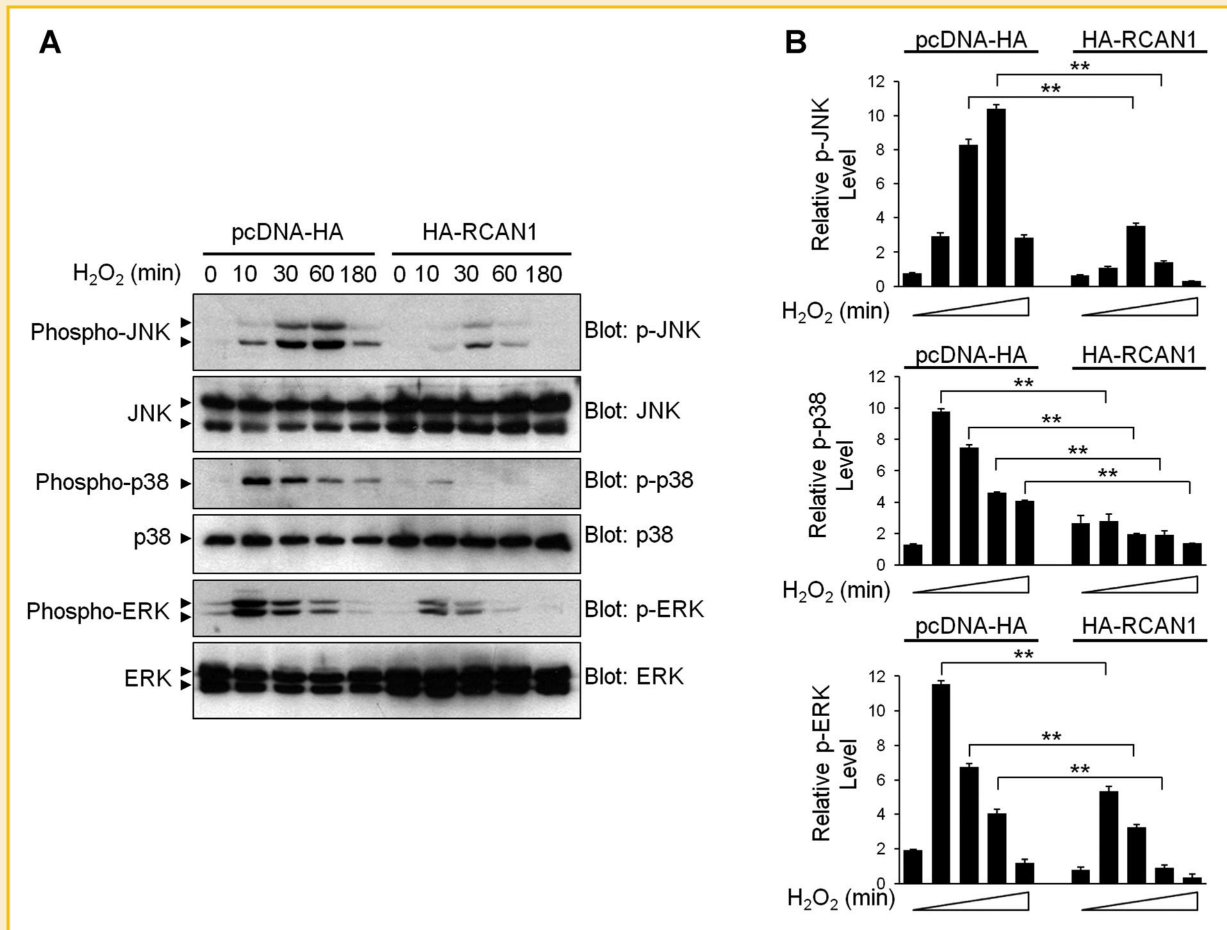

Fig. 2. Overexpression of RCAN1 inhibits ROS-mediated MAPKs activation. PC12 cells that were stably transfected with either the empty or HA-RCAN1 expression vector were treated with H_2O_2 (250 μM) for the indicated times. The cell extracts were immunoblotted using the indicated antibodies (A) and then quantified (B). The graphs are presented as the means ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$

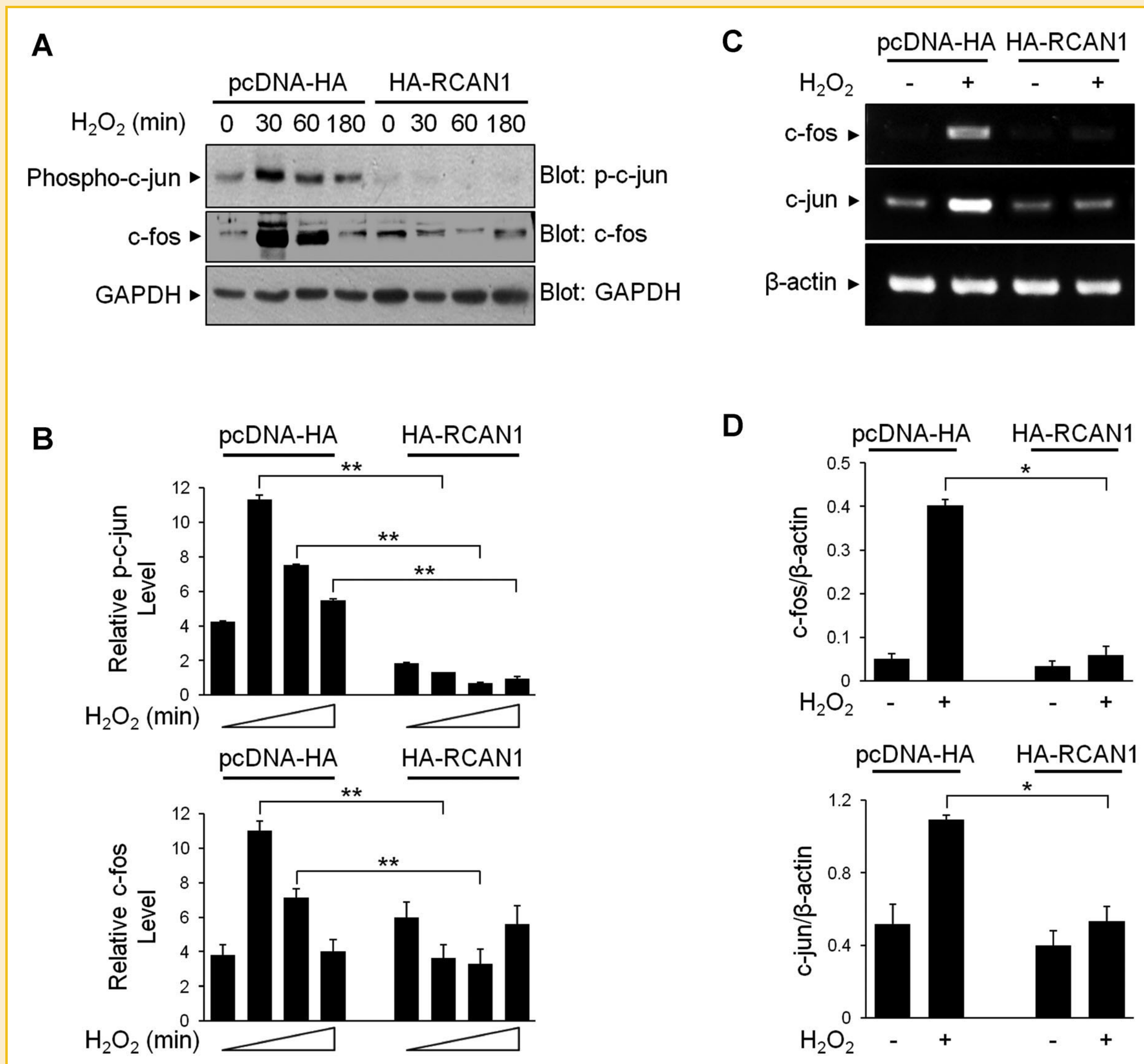

Fig. 3. Overexpression of RCAN1 inhibits AP-1 activation. A and B: PC12 cells that were stably transfected with either the empty or HA-RCAN1 expression vector were treated with H_2O_2 (250 μM) for the indicated times. The cell extracts were immunoblotted using the anti-phospho-c-jun, c-fos, and anti-GAPDH antibodies and then quantified. C and D: The cells were treated with H_2O_2 (250 μM) for 3 h, and mRNA levels of *c-fos*, *c-jun*, and *β-actin* were measured by RT-PCR and then quantified. The graphs are presented as the means ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$

EXPRESSION OF RCAN1 ENHANCES CREB ACTIVITY AND INCREASES Bcl-2 EXPRESSION

It has been shown that the activation of CREB is important for maintaining cell survival under many pathological conditions [Jean et al., 1998; Ciani et al., 2002]. In an attempt to elucidate the molecular mechanism underlying the RCAN1-mediated protection of cells from H_2O_2-induced cytotoxicity, we investigated whether RCAN1 affected CREB phosphorylation (Fig. 4A). Interestingly, we observed increases in the basal level of CREB phosphorylation in the cells that stably overexpressing RCAN1 compared to control. Furthermore, the level of phosphorylated CREB in response to H_2O_2 was significantly enhanced and sustained in the RCAN1 over-expressing cells (Fig. 4A,B).

We next investigated whether the ability of RCAN1 to increase CREB activity contributed to an increase in the expression of an endogenous CREB-target gene. We examined the changes in the expression of the anti-apoptotic Bcl-2 protein, which is known to have a CREB-binding site in its promoter and to protect various cell types from injury [Merry and Korsmeyer, 1997; Pugazhenthi et al., 1999; Perianayagam et al., 2006]. As shown in Figure 4C,D, the expression of Bcl-2 was moderately but significantly enhanced in the cells stably overexpressing RCAN1, suggesting that the stable RCAN1 overexpression could enhance the expression of Bcl-2.

To provide further evidence that the ability of RCAN1 to increase CREB activation, we examined whether RCAN1 exerted an effect on CRE-containing gene transcription (Fig. 4E). As shown in Figure 4E, the expression of RCAN1 induced CRE-containing reporter activity in a dose-dependent manner. The induced CRE-containing gene transcription by RCAN1 was blocked by the expression of the dominant-negative CREB mutant (S133A) [Gonzalez and Montminy,

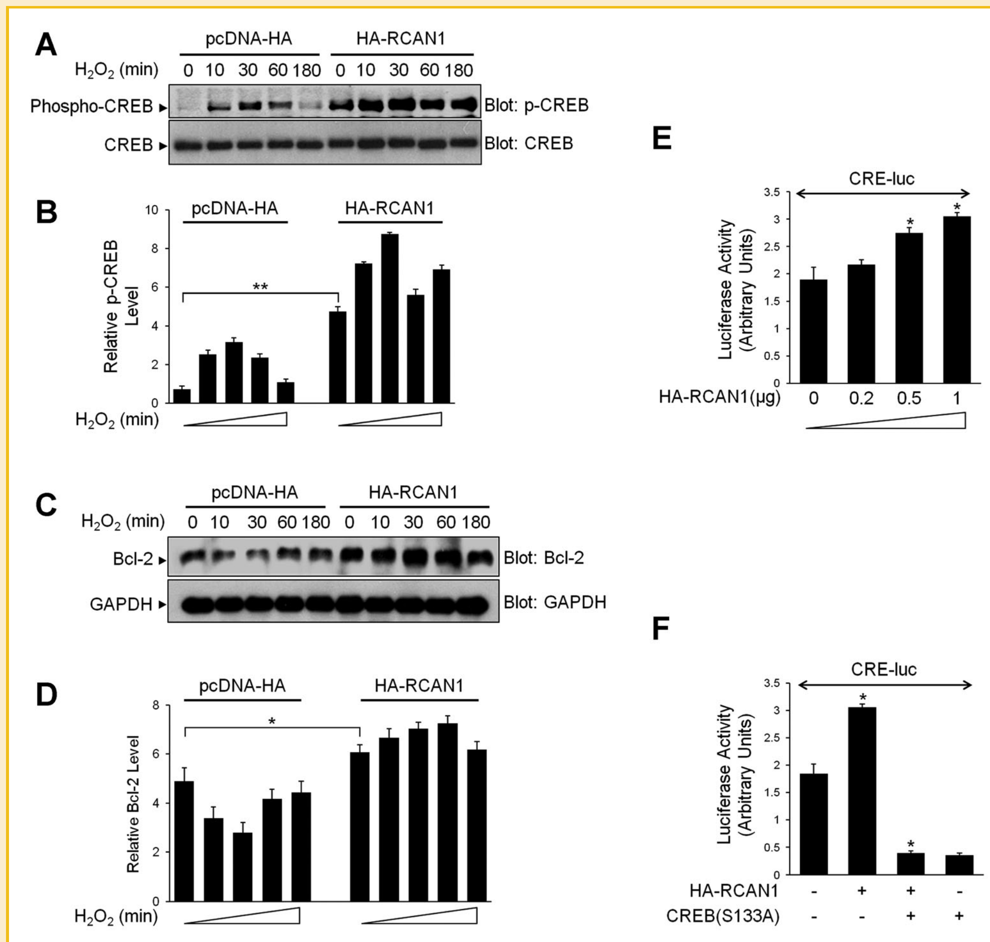

Fig. 4. Overexpression of RCAN1 enhances CREB activity and increases Bcl-2 expression. A–D: PC12 cells that were stably transfected with either the empty or HA-RCAN1 expression vector were treated with H_2O_2 (250 μM) for the indicated times. The cell extracts were immunoblotted using the anti-phospho-CREB, anti-CREB, anti-Bcl-2 and anti-GAPDH antibodies (A and C) and were then quantified (B and D). E and F: PC12 cells were transfected with either a CRE-luciferase reporter construct alone or together with the indicated expression vectors. After 24 h, the cells were analyzed for luciferase activity. The results were normalized with β-galactosidase activity. The graphs are presented as the means ± SD of three independent experiments. $^*P < 0.05$; $^{**}P < 0.01$

1989], indicating that the ability of RCAN1 to induce CRE-containing gene expression was mediated by CREB activation (Fig. 4F).

KNOCKDOWN OF RCAN1 SUPPRESSES CREB ACTIVITY AND DECREASES Bcl-2 EXPRESSION

To investigate the physiological relevance of RCAN1 in CREB-mediated Bcl-2 expression, we generated RCAN1 stable knockdown cells. PC12 cells were stably transfected with pRS-RCAN1 shRNA expression vectors to knock down endogenous RCAN1, and the knockdown efficiency of these cells was confirmed by Western blot analysis (Fig. 5A).

As shown in Figure 5B,C, the knockdown of endogenous RCAN1 decreased the CREB phosphorylation in response to H_2O_2 (Fig. 5B,C). Consistently, the knockdown of RCAN1 decreased the expression of Bcl-2, supporting the notion that the expression of RCAN1 can exert an anti-apoptotic activity by activating CREB phosphorylation and its downstream target gene, the anti-apoptotic protein Bcl-2 (Fig. 5D,E).

To provide further evidence that the ability of RCAN1 to increase Bcl-2 expression, we measured Bcl-2 mRNA levels using RT-PCR (Fig. 5F,G). As shown in Figure 5F,G, the level of Bcl-2 mRNA was significantly decreased by the knockdown of endogenous RCAN1. Furthermore, the decreased level of Bcl-2 was compensated by the overexpression of RCAN1, and the compensatory effect of RCAN1 was blocked by the coexpression of the inactive CREB mutant (S133A), indicating that the ability of RCAN1 to induce Bcl-2 expression was dependent on CREB activation (Fig. 5F,G).

We next examined whether the knockdown of endogenous RCAN1 could exacerbate the cytotoxicity to H_2O_2 (Fig. 5H). As shown in Figure 5H, the knockdown of endogenous RCAN1 caused an enhanced vulnerability to H_2O_2. Consistently, this effect was compensated by the overexpression of RCAN1, and the compensatory effect was abrogated by the coexpression of CREB mutant (S133A), indicating that the protective effect of RCAN1 from H_2O_2 was mediated by CREB activation.

Taken together, these results indicate that activation of CREB by RCAN1 contributes to the protective effect of RCAN1 from H_2O_2-induced oxidative stress by increasing Bcl-2 expression.

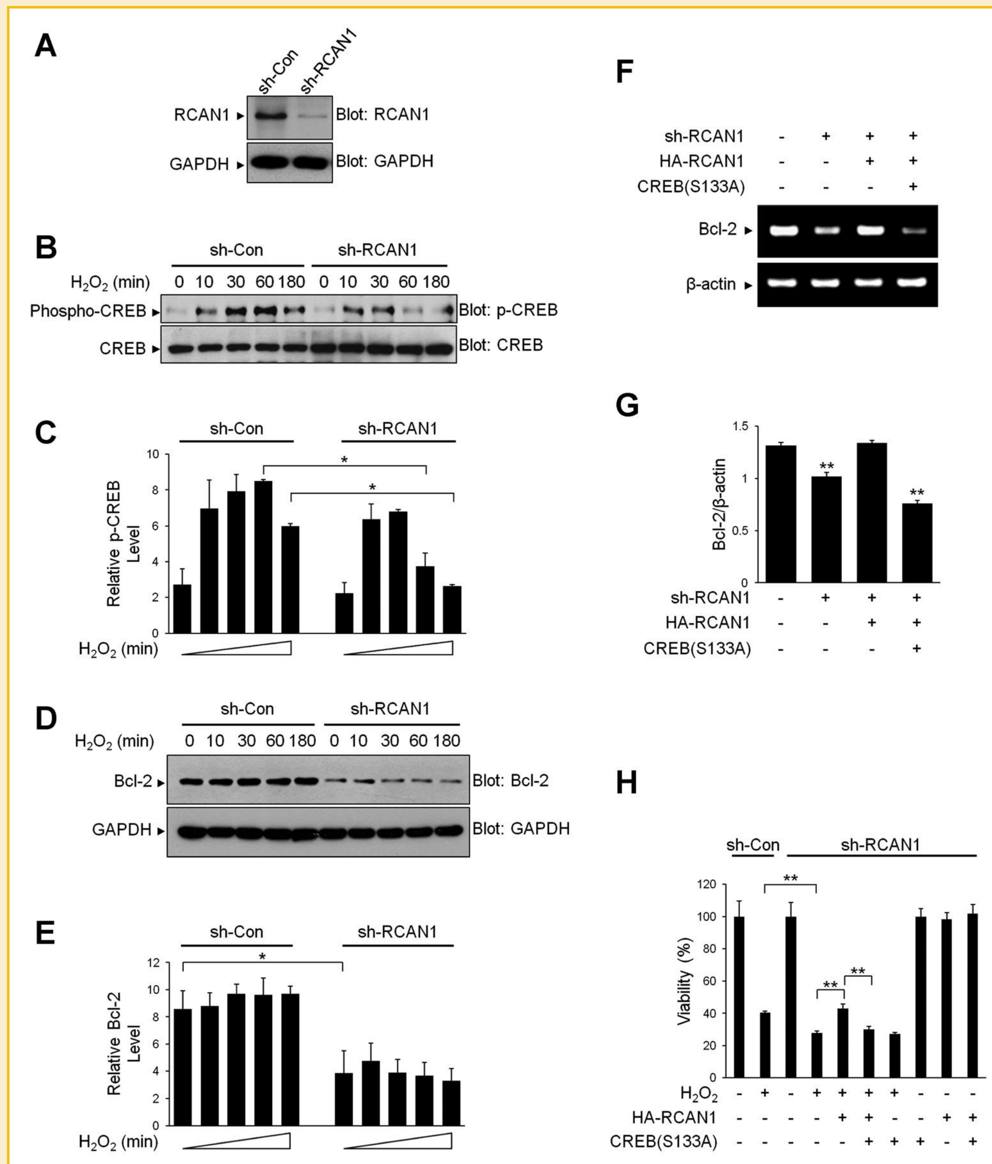

Fig. 5. Knockdown of RCAN1 suppresses CREB activity and decreases Bcl-2 expression. A: PC12 cells were transfected with the empty or pRS-RCAN1 shRNA expression vector and were selected in puromycin-containing growth medium. Stable RCAN1-knockdown clone was identified by immunoblotting using an anti-RCAN1 antibody. B and D: Cells were treated with H_2O_2 (250 μM) for the indicated times. The cell extracts were immunoblotted using anti-phospho-CREB, anti-CREB, anti-Bcl-2 and anti-GAPDH antibodies (B and D) and then quantified (C and E). F and G: PC12 cells were transfected with the indicated expression vectors and were expressed for 24 h. mRNA levels of *Bcl-2* and *β-actin* were measured by RT-PCR (F) and then quantified (G). H: Stable RCAN1-knockdown cells were expressed with the indicated expression vectors. After 24 h, the cells were treated with H_2O_2 (250 μM) for 12 h. The viability of these cells was determined using an MTT assay. The graphs are presented as the means ± SD of three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$

DISCUSSION

Although oxidative stress has been shown to induce the expressions of RCAN1, the functional role of RCAN1 has remained unclear to date. In this study, we analyzed the role of RCAN1 in the ROS-mediated cell death-signaling pathway and provided evidence that the stable expression of RCAN1 was able to protect against the hydrogen peroxide (H_2O_2)-induced toxicity via the upregulation of CREB activity.

Our results indicating that the cytoprotective effect of RCAN1 are in agreement with a previous report observing that the forced expression of *adapt78*, another name for RCAN1, in Chinese hamster HA-1 fibroblasts resulted in decreased cytotoxicity with hydrogen peroxide and calcium treatment [Leahy and Crawford, 2000; Ermak et al., 2002]. Consistent with these results, McKeon and colleagues reported that $CD4^{+}$ T cells from RCAN-null mice displayed increased apoptosis after CD3 stimulation, and they proposed that the elevated expression of the Fas ligand underlies the mechanism for the death-promoting effect in RCAN1-deficient T cells [Ryeom et al., 2003]. In support of this finding, the knockdown of RCAN1-4 resulted in enhanced etoposide and Fas-mediated apoptosis in U87-MG cells [Kim et al., 2009]. However, $RCAN1^{-/-}$

neurons showed an increased resistance to H_2O_2 toxicity [Porta et al., 2007]. Thus, changes in the amount of RCAN1 could represent an important effect in modulating cell survival and death pathways.

Regarding the molecular mechanisms underlying the protective role of RCAN1 in the ROS-mediated cell death pathway, our data provide evidence that the enhanced and prolonged activation of CREB by RCAN1 was directly correlated with the protection against ROS-induced apoptotic commitment in cells. Consistent with the actions of RCAN1 for enhancing CREB activity, we found that the stable expression of RCAN1 increased the expression of Bcl-2, an anti-apoptotic member of the Bcl-2 family of proteins. The Bcl-2 gene contains a CREB-binding site in its upstream promoter region [Pugazhenthi et al., 1999; Perianayagam et al., 2006]. It has been reported that the induction of Bcl-2 expression due to the activation of CREB during B-cell activation rescued cells from apoptosis [Wilson et al., 1996]. Furthermore, the knockdown of CREB decreased Bcl-2 levels and sensitized cells to TRAIL, suggesting the CREB-mediated anti-apoptotic effects of Bcl-2 [Shankar et al., 2010]. In support of our observations, the disturbance of CREB activity in overexpression mutants of Nebula, the *Drosophila* homolog of human RCAN1, has been reported [Chang et al., 2003]. Moreover, elevated levels of CREB activity have been reported in DS brains [Yuan et al., 2003]. It is unclear whether the enhanced effect of RCAN1 on the activation of CREB by oxidative stress is direct or indirect. However, RCAN1 was identified as an enzymatic inhibitor of calcineurin, it is likely that the inhibition of the calcineurin pathway might play a role in the activation of CREB signaling. Similarly, it has been reported that RCAN1 overexpression can protect against mutant huntingtin toxicity, which is caused by the inhibition of calcineurin [Ermak et al., 2009].

The results presented here support our previous assertion that RCAN1 increases the activation of CREB in response to the activation of the intracellular cAMP pathway [Kim and Seo, 2011]. Furthermore, we have also reported that the forced expression of CREB facilitates the proteasomal degradation of RCAN1 [Seo and Chung, 2008]. Taken together, these results indicate an important role for RCAN1 in the fine-tuning of the CREB-regulatory circuit, which is important in neuronal activity.

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