Biochemical and Biophysical Research Communications 439 (2013) 315–320

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Romo1 expression contributes to oxidative stress-induced death of lung epithelial cells

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ARTICLE INFO

Article history:
Received 2 July 2013
Available online 15 July 2013

Keywords:
Idiopathic pulmonary fibrosis
Lung injury
Oxidative stress
Reactive oxygen species
Romo1

ABSTRACT

Oxidant-mediated death of lung epithelial cells due to cigarette smoking plays an important role in pathogenesis in lung diseases such as idiopathic pulmonary fibrosis (IPF). However, the exact mechanism by which oxidants induce epithelial cell death is not fully understood. Reactive oxygen species (ROS) modulator 1 (Romo1) is localized in the mitochondria and mediates mitochondrial ROS production through complex III of the mitochondrial electron transport chain. Here, we show that Romo1 mediates mitochondrial ROS production and apoptosis induced by oxidative stress in lung epithelial cells. Hydrogen peroxide (H_2O_2) treatment increased Romo1 expression, and Romo1 knockdown suppressed the cellular ROS levels and cell death triggered by H_2O_2 treatment. In immunohistochemical staining of lung tissues from patients with IPF, Romo1 was mainly localized in hyperplastic alveolar and bronchial epithelial cells. Romo1 overexpression was detected in 14 of 18 patients with IPF. TUNEL-positive alveolar epithelial cells were also detected in most patients with IPF but not in normal controls. These findings suggest that Romo1 mediates apoptosis induced by oxidative stress in lung epithelial cells.

1. Introduction

Oxidative damage occurs when exogenous and/or endogenous reactive oxygen species (ROS) overwhelm the antioxidant capacity of cells and extracellular spaces, disrupting the redox balance of cells. Exogenous oxidants originate from air pollutants such as ozone, nitric dioxide, sulfur dioxide and especially cigarette smoke [1]. The respiratory tract is especially susceptible to exogenous and endogenous oxidants [2]. ROS generated in the airway are a large cause of cell damage and contribute to lung injury and many chronic inflammatory diseases [3]. Increased apoptosis of lung epithelial and endothelial cells that is not compensated for proliferation results in tissue destruction [4]. Oxidative stress acts as a pathogen in lung diseases associated with lung injury such as acute lung injury (ALI), chronic obstructive pulmonary disease (COPD), lung fibrosis and lung cancer [5]. Oxidant-mediated injury plays an important role in ALI pathogenesis [6,7]. Several studies have suggested that an oxidant–antioxidant imbalance in the airway plays a critical role in the pathogenesis of lung injury in the case of fibrosis [8,9]. Idiopathic pulmonary fibrosis (IPF) is a representative fibrotic lung disease and has a chronic clinical course without proven treatment. The IPF pathogenesis is not well defined, but an association between smoking and IPF incidence or decreased lung function has been shown in epidemiological studies [10,11]. Chronic inflammation induced by stresses such as smoking plays an important role in the onset and progression of IPF.

ROS modulator 1 (Romo1) was first identified in the context of enhanced ROS production [12]. Romo1 is localized in the mitochondria and increases cellular ROS levels via mitochondrial ROS production through complex III of the mitochondrial electron transport chain [13]. In a previous study, ROS levels were downregulated by Romo1 knockdown with Romo1 small interfering RNA (siRNA) in lung fibroblasts [13]. However, the role of Romo1-mediated mitochondrial ROS production in normal apoptosis and lung injury has not been identified. In the present study, we investigated the role of Romo1 as a mediator of mitochondrial ROS production and apoptosis induced by oxidative stress, and the protective effect of Romo1 inhibition in lung injury.

2. Materials and methods

2.1. Patients

This study was performed on lung specimens obtained by open lung biopsy or lung resection from 18 patients with IPF treated at Gangnam Severance Hospital, a tertiary referral medical center in Seoul, Korea. A diagnosis of IPF was based on the American

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http://dx.doi.org/10.1016/j.bbrc.2013.07.012

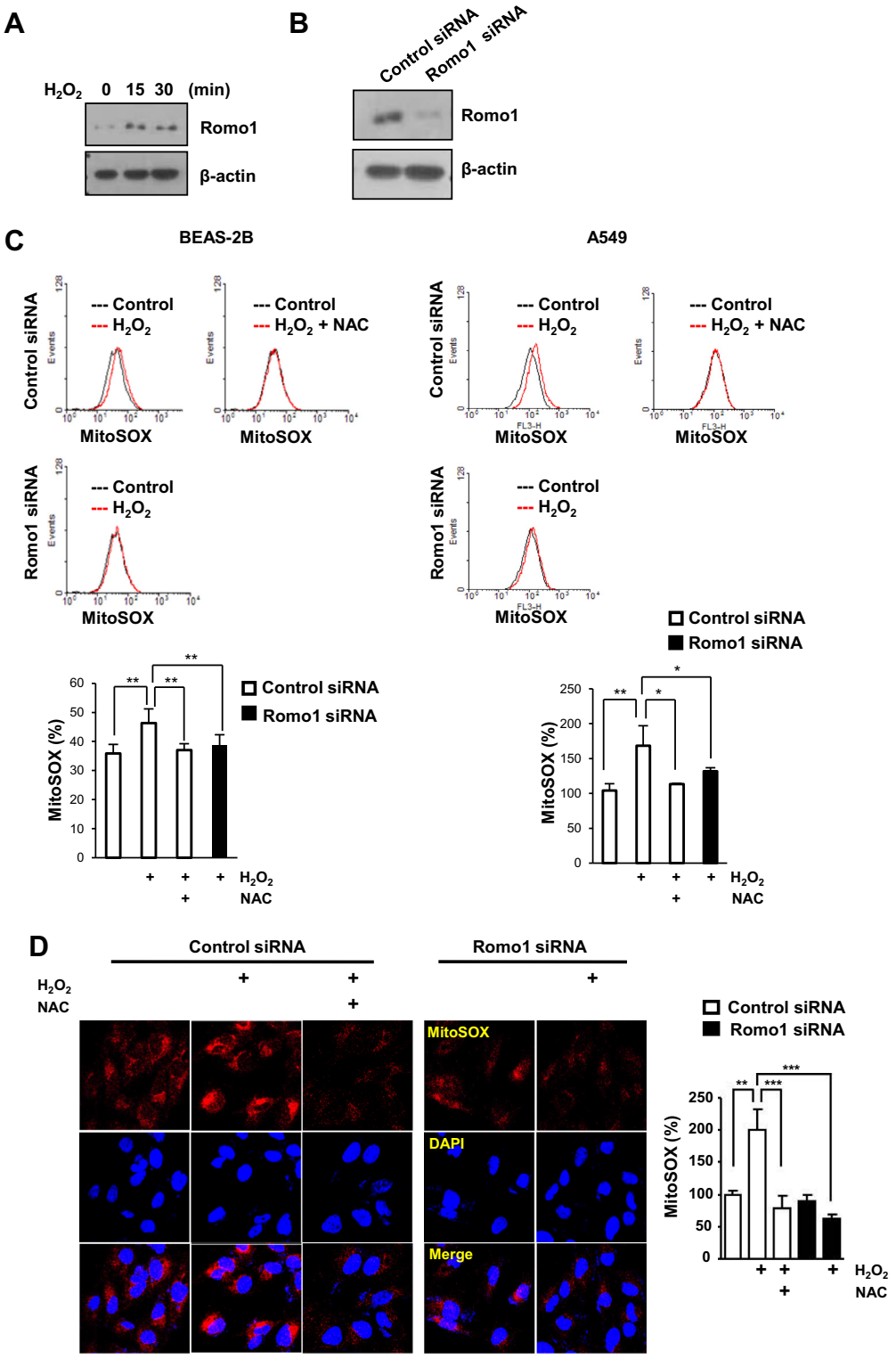

Fig. 1. H_2O_2-induced mitochondrial ROS production through Romo1. (A) After BEAS-2B cells were treated with 200 μM H_2O_2 for the indicated times, Romo1 expression was observed by Western blotting. (B) The cells were transfected with 100 nM Romo1 siRNA and Romo1 expression was observed by Western blotting. (C) After cells were transfected with Romo1 siRNA, BEAS-2B and A549 cells were treated with 200 μM H_2O_2 for 2 h followed by incubation after media change. Cells were stained with MitoSOX and ROS levels were measured by flow cytometry. Data shown are the means of at least three separate experiments. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. control siRNA with H_2O_2 by 2-way ANOVA. (D) BEAS-2B cells were transfected with Romo1 siRNA or NAC, then treated with 200 μM H_2O_2 for 2 h followed by incubation after media change. Cells were stained with MitoSOX and mitochondrial ROS production was observed by confocal microscopy. Images were quantified by MetaMorph software. Data shown are the means of at least three separate experiments. $^{**}p < 0.01$; $^{***}p < 0.001$ vs. control siRNA with H_2O_2 by 2-way ANOVA.

Thoracic Society/European Respiratory Society criteria [14]. The histologic diagnosis of all specimens was compatible with UIP. Normal appearing lung parenchyma specimens were obtained from 18 lung transplant donors at the same center without pathological evidence of any parenchymal disease, including pneumonia, as controls. This study was approved by the Institutional Review

Board, Gangnam Severance Hospital, Yonsei University College of Medicine (IRB No.: 3-2012-0146), and the need for written informed consent was waived as this was a retrospective study and patients were anonymized.

2.2. Cell culture and reagents

Bronchial epithelial cells (BEAS-2B) and cancerous alveolar epithelial cells (A549) were cultured in Ham's F12 and RPMI 1640 medium (Gibco–Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated FBS (Gibco–Invitrogen), sodium bicarbonate (2 mg/ml; Sigma–Aldrich, St. Louis, MO, USA), penicillin (100 units/ml), and streptomycin (100 mg/ml; Gibco–Invitrogen) and grown in 5% CO_2 at 37 °C. 2′,7′-Dichlorofluorescein diacetate (DCF-DA), DAPI and H_2O_2 were obtained from Sigma–Aldrich. MitoSOX was obtained from Molecular Probes (Eugene, OR, USA). Lipofectamine™ was purchased from Gibco–Invitrogen. The mouse, monoclonal antibody (mAb) against Romo1 was described previously [15]. Polyclonal anti-caspase-3 and anti-PARP antibodies were from Cell Signaling Technology (Danvers, MA, USA). Mouse anti-cytosol-specific-β-actin antibodies were from Sigma–Aldrich.

2.3. Measurement of cell growth and TUNEL assay

Double-stranded, siRNA oligonucleotide that targets Romo1 was synthesized by Bioneer (Taejon, Republic of Korea) [16]. Cells (8×10^4) were plated in 60 mm dishes. After 24 h, the cells were transfected with 100 nM control siRNA or Romo1 siRNA using Lipofectamine™ according to the manufacturer's instructions for 24 h or 48 h and then treated with hydrogen peroxide (H_2O_2) for 2 h. After culture, the cells were counted under an inverted light microscope (Olympus IX50, Olympus, Tokyo, Japan). For DAPI staining, cells were fixed with 3.7% paraformaldehyde in PBS at 4 °C for 10 min. After washing three times, the cells were incubated with 0.5 μg/ml DAPI solution in PBS for 20 min. After incubation, the cells were washed with PBS twice and examined by fluorescence microscopy (Olympus LX71 microscope). To quantify apoptosis, 100–200 cells were monitored in each experiment. To detect apoptotic cells in situ in lung tissue sections, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was performed using the TUNEL assay kit (Takara Bio Inc., Otsu, Japan) according to the protocol outlined in the online supplement.

2.4. ROS assay and Western blotting

ROS assay and Western blotting were performed as described previously [15].

2.5. Immunohistochemistry

Immunohistochemical staining for mouse anti-human Romo1 was performed on formalin-fixed, paraffin-embedded specimens from humans with lung injury disease. Immunohistochemistry was performed by using the Vectastain Elite ABC kit (Vector Laboratories, Peterborough, UK) according to the manufacturer's instructions. Four-micron-thick sections were deparaffinized in xylene and rehydrated in a graded alcohol. Romo1 antigen retrieval was accomplished by heating the sections in a steamer with 10 mM citrate buffer (pH 6.0) at 100 °C for 20 min. Endogenous peroxidases were blocked with 0.3% H_2O_2 in methanol for 5 min. After washing with PBS for 5 min, slides were blocked with blocking serum, and the sections were incubated with primary Romo1 antibody at a 1:100 dilution for 1 h at room temperature. After incubating with the primary antibody, sections were treated with biotinylated secondary antibody for 30 min at room temperature, followed by incubation with the avidin-biotinylated HRP complex for 1 h. The sections were again incubated with the avidin biotin complex for 30 min, and the signal was detected using a diaminobenzidine tetrahydrochloride substrate. The developed sections were counterstained with hematoxylin for 10 s.

2.6. Statistical analysis

Data were analyzed with GraphPad PRISM version 4.02 for Windows (GraphPad Software, San Diego, CA, USA) and SPSS version 18.0 statistical software (SPSS, Chicago, IL, USA). Each assay was performed in triplicate and was independently repeated at least three times. Statistical analyses were conducted using a 2-way ANOVA (analysis of variance). The relationship between Romo1 immunoreactivity and TUNEL-positivity was evaluated by calculating Spearman's correlation coefficient. p-values <0.05 were considered statistically significant. Means/medians and S.E.s were also calculated.

3. Results

3.1. Romo1 is a key mediator of mitochondrial ROS production induced by oxidative stress

Oxidative stress has been reported to increase cellular ROS levels and Romo1 expression [15–17]. Therefore, we investigated the relationship between increased cellular ROS levels and Romo1 expression. Cigarette smoke contains many free radicals including H_2O_2, hydroxyl and organic radicals, and exposure to it can generate up to 800 μM H_2O_2 [18,19]. Therefore, we used 200–300 μM H_2O_2 in this study. We treated BEAS-2B cells with H_2O_2 and measured Romo1 expression. As shown in Fig. 1A, H_2O_2 treatment increased Romo1 expression in BEAS-2B cells. Because these results

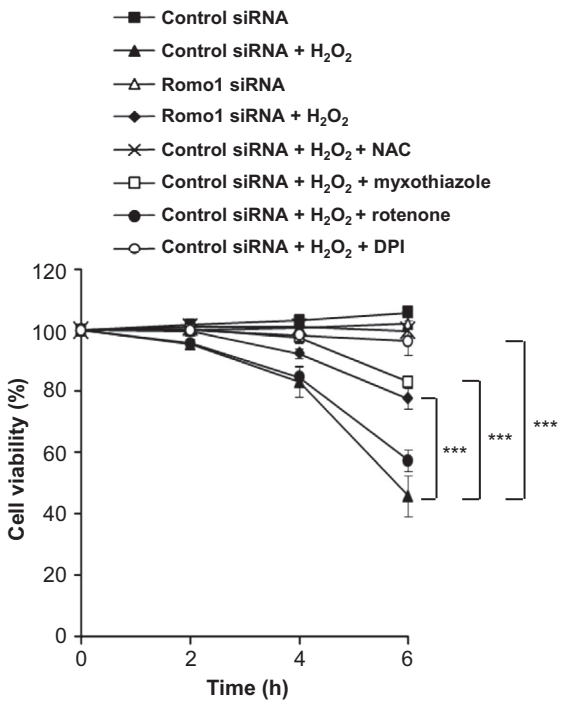

Fig. 2. H_2O_2-induced cell death through Romo1. BEAS-2B cells were transfected with Romo1 siRNA for 48 h and treated with 300 μM H_2O_2 for 2 h in the presence of NAC, DPI, myxothiazol or rotenone. The cells were incubated for indicated times after media change. The results represent the means (±S.E.) of three independent experiments performed in triplicate. $^{***}p < 0.001$ vs. control siRNA with H_2O_2 samples by 2-way ANOVA.

suggest that Romo1 plays a role in increasing mitochondrial ROS levels after H_2O_2 treatment, we evaluated whether Romo1 knockdown suppressed H_2O_2-induced mitochondrial ROS production. Romo1 expression was inhibited by Romo1 siRNA transfection, as observed by Western blotting (Fig. 1B). We examined H_2O_2-induced change in ROS production by staining the cells with MitoSOX, a mitochondrial superoxide indicator. H_2O_2-induced mitochondrial ROS production was suppressed by Romo1 knockdown or NAC pre-treatment in BEAS-2B and A549 cells (Fig. 1C). This finding was also observed by confocal microscopy (Fig. 1D). The decreased in H_2O_2-induced ROS production was quantified by using MetaMorph software (Universal Imaging). These results indicate that Romo1 plays an important role in oxidative stress-induced mitochondrial ROS production.

3.2. Oxidative stress-induced cell death is suppressed by Romo1 knockdown

Because H_2O_2 treatment induces apoptosis in a dose- and time-dependent manner and antioxidants, such as NAC, inhibit oxidative stress-induced apoptosis [20], we determined whether the decreased ROS levels due to Romo1 knockdown inhibited apoptosis of lung epithelial cells. H_2O_2 treatment induced cell death, which was inhibited by antioxidant pre-treatment (Fig. 2). Next, we examined whether Romo1 expression affected H_2O_2-induced cell death. As shown in Fig. 2, transfecting Romo1 siRNA partially suppressed cell death compared with transfecting control siRNA. Treatment with myxothiazole, an electron transport inhibitor at complex III, had a similar effect. In contrast, diphenyleneiodinium (DPI, a flavoprotein inhibitor), which inhibits both NADPH oxidase activity and mitochondrial ROS production, effectively suppressed H_2O_2-induced cell death. However, rotenone, an electron transport inhibitor at complex I, did not block cell death.

To determine if Romo1 knockdown inhibits H_2O_2-induced apoptosis, Western blotting was performed on protein from cells transfected with Romo1 siRNA. PARP cleavage in cells treated with H_2O_2 was blocked by Romo1 knockdown and NAC pre-treatment (Fig. 3A). Caspase 3 cleavage was also blocked in cells transfected with Romo1 siRNA. Apoptosis induced by oxidative stress was also observed by TUNEL and DAPI staining. As shown in Fig. 3B and C, H_2O_2 treatment induced apoptosis, which was abolished by Romo1 knockdown or NAC pre-treatment. These results demonstrate that Romo1 induction by oxidative stress plays an important role in oxidative stress-induced mitochondrial ROS production and apoptosis.

3.3. Romo1 expression is correlated with apoptosis of human tissue in patients with IPF

To determine whether Romo1 expression is increased in tissue from patients with IPF, we examined Romo1 expression on paraffin-embedded specimens by immunohistochemical staining. The alveolar epithelial cells of most patients with IPF (14 of 18) reacted to an antibody against Romo1, whereas those of most patients without IPF (12 of 18) did not react to the Romo1 antibody (Fig. 4A). As expected, because Romo1 localized to the mitochondria, all Romo1-positive cells were stained in the cytoplasm. TUNEL staining was also performed in the IPF tissues. TUNEL-positive

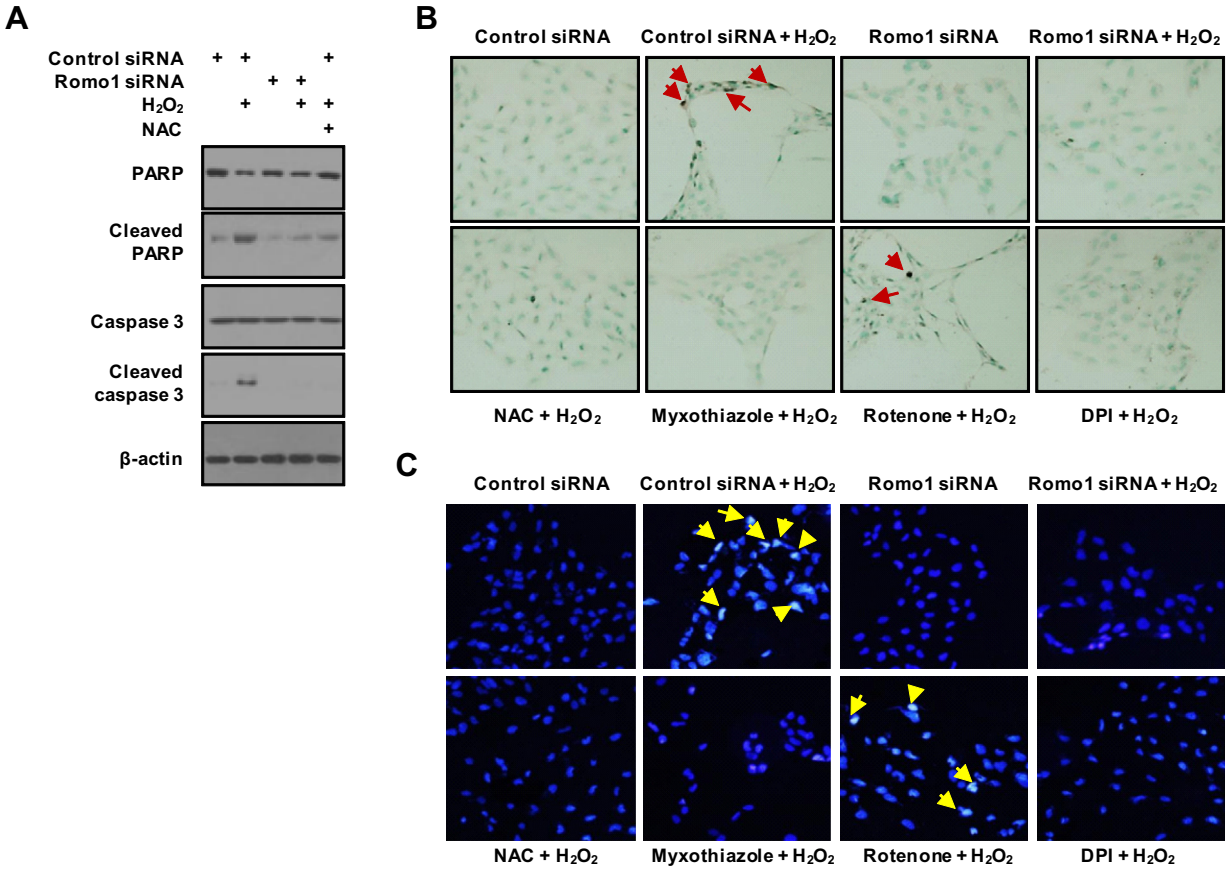

Fig. 3. H_2O_2-induced apoptotic cell death through Romo1. (A) After transfecting BEAS-2B cells with Romo1 siRNA and incubating for 48 h, the cells were treated with 300 μM H_2O_2 for 2 h and incubated for 6 h after media change for Western blotting. (B) TUNEL staining of H_2O_2-induced apoptotic cell death. After transfecting BEAS-2B cells with Romo1 siRNA for 48 h, the cells were treated with 300 μM H_2O_2 for 2 h and incubated for 6 h for TUNEL staining. Apoptotic cells were observed under inverted light microscopy (arrows, 200×). (C) Representative DAPI staining image. Apoptotic cells were observed under inverted light microscopy (arrows, 200×).

epithelial cells, which are apoptotic, were seen in most IPF tissues (13 of 18 patients) but not in most controls (12 of 18 patients). The quantified immunoreactivity score of Romo1 in patients with IPF was significantly higher than controls ($p < 0.005$, Fig. 4B). The number of TUNEL-positive cells in patients with IPF was also significantly higher than controls ($p < 0.001$, Fig. 4B). The Romo1 immunoreactivity score corresponded to TUNEL-positivity in all patients with 18 IPF with a Spearman's correlation of 0.679 ($p < 0.01$) and in 36 all patients with a correlation of 0.598 ($p < 0.01$). These results indicate that Romo1 overexpression correlates with epithelial cell death in patients with IPF.

4. Discussion

Oxidative stress in excess of the capacity of cells and tissues to detoxify and scavenge causes imbalanced ROS production and leads to cell dysfunction. Recent studies showed that oxidative stress is important in the pathogenesis of lung injury and fibrosis [21,22]. The respiratory tract is susceptible to endogenous and exogenous oxidants, especially cigarette smoke [1,2], which contains numerous free radicals such H_2O_2, hydroxyl and organic radicals [18]. Exposure to cigarette smoke can generate up to 800 μM H_2O_2 [19]. The free radicals trigger lung injury, contributing to lung diseases such as COPD, IPF and cancer. Previously, we reported that Romo1 expression was increased in response to external stresses such as H_2O_2 exposure, TPA treatment and serum deprivation [23,24]. This Romo1 induction facilitates ROS production. Therefore, we determined whether Romo1 expression is involved in oxidative stress that induces mitochondrial ROS production and lung epithelial cell death. We observed that Romo1 knockdown suppressed the increased mitochondrial ROS production caused by H_2O_2 treatment in lung epithelial cells. In addition, H_2O_2 treatment increased Romo1 expression as confirmed by Western blotting and immunofluorescence. Previous reports demonstrated that Romo1 is located in the mitochondrial membrane and has a role in ROS production from the mitochondrial electron transport chain [13,15]. A majority of mitochondrial ROS is produced at complexes I and III [25]. However because Romo1-derived ROS is inhibited by complex III inhibitor, Romo1-derived ROS mainly originates from complex III of the mitochondrial respiratory chain.

Repeated damage and impaired repair of lung epithelial cells caused by oxidative stress trigger lung injury, which contributes to diseases such as COPD and pulmonary fibrosis, which have been linked to lung cancer [26,27]. Increased proliferation and function of epithelial cells caused by lung injury seem to contribute to lung cancer development [27]. However, the mechanism by which lung epithelial cell death in IPF is involved in cancer development has not been identified. In the present study, we suggest that Romo1 mediates oxidative stress-induced lung injury. Recent reports

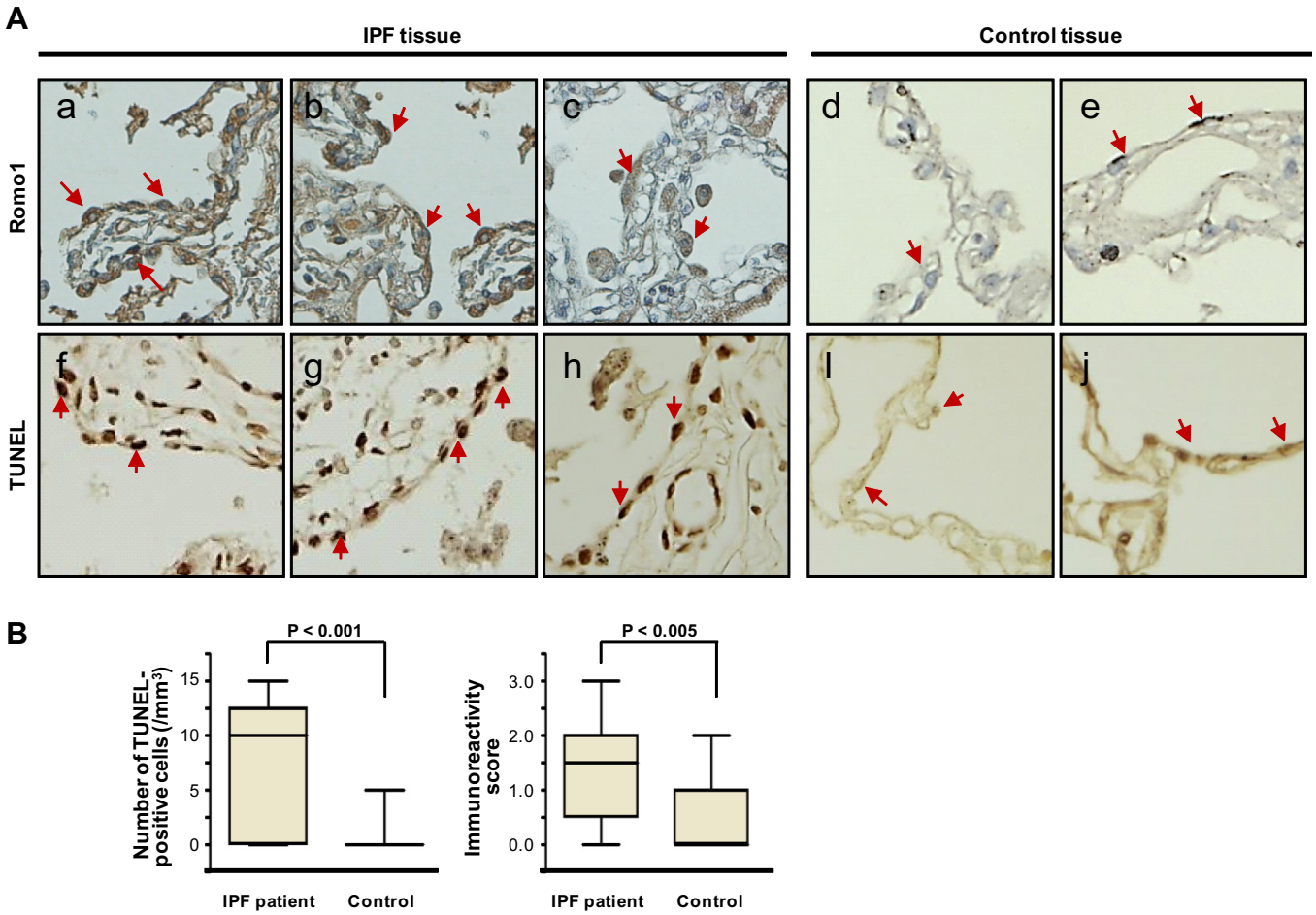

Fig. 4. TUNEL staining and Romo1 expression in alveolar epithelial cells from IPF tissues. (A) Romo1 immunohistochemical staining and TUNEL staining of paraffin-embedded lung tissues from patients with IPF and control patients (200×). Romo1 overexpression was strongly detected in alveolar epithelial cells from patients with IPF (a–c) but not in controls (d, e). TUNEL staining was predominantly detected in alveolar epithelial cells of patients with IPF (f–h) but not in controls (i, j). (B) Quantitative results from patients with IPF and control patients. The immunoreactivity score of Romo1 (left) and the number of TUNEL-positive cells (right) in each field under light microscopy at 200× magnification are presented. The box plot shows the median (central thick lines), 25% and 75% quartiles (box height), and range.

demonstrate that Romo1 is overexpressed in a variety of cancer cell lines and in HCC tissues [12,23]. Here, we showed that Romo1 overexpression was detected in many IPF tissues and it correlated with epithelial cell death in patients with IPF as observed by TUNEL staining. However, there is no direct evidence that Romo1 is involved in lung carcinogenesis. Since lung carcinogenesis is a long and complicated process, further extensive studies are required to elucidate the role of Romo1 in lung carcinogenesis.

Chronic oxidative damage is usually observed in the lung tissue and bronchoalveolar lavage fluid of patients with IPF [8]. In previous studies using lung tissues from patients with IPF, TUNEL-positive cells, which are apoptotic, were mainly alveolar epithelial cells [28]. Apoptosis was also detected in fibrotic lesions to a moderate degree and was decreased in severe lesions. Epithelial cell apoptosis was promoted by a paracrine mechanism and matrix crosslinking reactions [29]. These changes caused by oxidative stress mediate fibrogenic effects in lung tissues. In the present study, Romo1 expression was highly correlated with epithelial cell apoptosis in lung tissue from patients with IPF. These findings suggest that Romo1 mediates oxidative stress-induced apoptosis in lung injury. To investigate the exact role of Romo1 in lung fibrosis, further studies are required, but our findings suggest a therapeutic potential to block lung injury and fibrosis by modulating oxidative stress-induced apoptosis of lung epithelial cells.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A2003274 and NRF-2013R1A1A2009792) and by the National Research Foundation of Korea (NRF) Grant funded by the Korea Government (NRF-2012R1A2A2A01045800).

References

[1] I. Edirisinghe, I. Rahman, Cigarette smoke-mediated oxidative stress, shear stress, and endothelial dysfunction: role of VEGFR2, Ann. N.Y. Acad. Sci. 1203 (2010) 66–72.
[2] S. Rajendrasozhan, S.R. Yang, I. Edirisinghe, H. Yao, D. Adenuga, I. Rahman, Deacetylases and NF-kappaB in redox regulation of cigarette smoke-induced lung inflammation: epigenetics in pathogenesis of COPD, Antioxid. Redox Signal. 10 (2008) 799–811.
[3] W.A. Pryor, K. Stone, Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite, Ann. N.Y. Acad. Sci. 686 (1993) 12–27. discussion 27–28.
[4] I.K. Demedts, T. Demoor, K.R. Bracke, G.F. Joos, G.G. Brusselle, Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema, Respir. Res. 7 (2006) 53.
[5] I.T. Lee, C.M. Yang, Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases, Biochem. Pharmacol. 84 (2012) 581–590.
[6] C.W. Chow, M.T. Herrera Abreu, T. Suzuki, G.P. Downey, Oxidative stress and acute lung injury, Am. J. Respir. Cell Mol. Biol. 29 (2003) 427–431.
[7] P.A. Ward, Oxidative stress: acute and progressive lung injury, Ann. N.Y. Acad. Sci. 1203 (2010) 53–59.
[8] M. Bocchino, S. Agnese, E. Fagone, S. Svegliati, D. Grieco, C. Vancheri, A. Gabrielli, A. Sanduzzi, E.V. Avvedimento, Reactive oxygen species are required for maintenance and differentiation of primary lung fibroblasts in idiopathic pulmonary fibrosis, PLoS One 5 (2010) e14003.
[9] F. Gao, V.L. Kinnula, M. Myllarniemi, T.D. Oury, Extracellular superoxide dismutase in pulmonary fibrosis, Antioxid. Redox Signal. 10 (2008) 343–354.
[10] A.L. Olson, J.J. Swigris, Idiopathic pulmonary fibrosis: diagnosis and epidemiology, Clin. Chest Med. 33 (2012) 41–50.
[11] H. Tanjore, T.S. Blackwell, W.E. Lawson, Emerging evidence for endoplasmic reticulum stress in the pathogenesis of idiopathic pulmonary fibrosis, Am. J. Physiol. Lung Cell Mol. Physiol. 302 (2012) L721–729.
[12] Y.M. Chung, J.S. Kim, Y.D. Yoo, A novel protein, Romo1, induces ROS production in the mitochondria, Biochem. Biophys. Res. Commun. 347 (2006) 649–655.
[13] Y.M. Chung, S.B. Lee, H.J. Kim, S.H. Park, J.J. Kim, J.S. Chung, Y.D. Yoo, Replicative senescence induced by Romo1-derived reactive oxygen species, J. Biol. Chem. 283 (2008) 33763–33771.
[14] American Thoracic Society, European Respiratory Society, American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee June 2001, Am. J. Respir. Crit. Care Med. 165 (2002) 277–304.
[15] J.J. Kim, S.B. Lee, J.K. Park, Y.D. Yoo, TNF-alpha-induced ROS production triggering apoptosis is directly linked to Romo1 and Bcl-X(L), Cell Death Differ. 17 (2010) 1420–1434.
[16] I.T. Hwang, Y.M. Chung, J.J. Kim, J.S. Chung, B.S. Kim, H.J. Kim, J.S. Kim, Y.D. Yoo, Drug resistance to 5-FU linked to reactive oxygen species modulator 1, Biochem. Biophys. Res. Commun. 359 (2007) 304–310.
[17] D.B. Zorov, C.R. Filburn, L.O. Klotz, J.L. Zweier, S.J. Sollott, Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes, J. Exp. Med. 192 (2000) 1001–1014.
[18] T. Goldkorn, S. Filosto, Lung injury and cancer: mechanistic insights into ceramide and EGFR signaling under cigarette smoke, Am. J. Respir. Cell Mol. Biol. 43 (2010) 259–268.
[19] M. Levy, E. Khan, M. Careaga, T. Goldkorn, Neutral sphingomyelinase 2 is activated by cigarette smoke to augment ceramide-induced apoptosis in lung cell death, Am. J. Physiol. Lung Cell Mol. Physiol. 297 (2009) L125–133.
[20] A. Dumont, S.P. Hehner, T.G. Hofmann, M. Ueffing, W. Droge, M.L. Schmitz, Hydrogen peroxide-induced apoptosis is CD95-independent, requires the release of mitochondria-derived reactive oxygen species and the activation of NF-kappaB, Oncogene 18 (1999) 747–757.
[21] J. Araya, S.L. Nishimura, Fibrogenic reactions in lung disease, Annu. Rev. Pathol. 5 (2010) 77–98.
[22] N.K. Burki, M. Alam, L.Y. Lee, The pulmonary effects of intravenous adenosine in asthmatic subjects, Respir. Res. 7 (2006) 139.
[23] J.S. Chung, S. Park, S.H. Park, E.R. Park, P.H. Cha, B.Y. Kim, Y.M. Chung, S.R. Woo, C.J. Han, S.B. Kim, K.S. Suh, J.J. Jang, K. Lee, D.W. Choi, S. Lee, G.Y. Lee, K.B. Hahm, J.A. Shin, B.S. Kim, K.H. Noh, T.W. Kim, K.H. Lee, Y.D. Yoo, Overexpression of Romo1 promotes production of reactive oxygen species and invasiveness of hepatic tumor cells, Gastroenterology 143 (2012) 1084–1094 e1087.
[24] S.B. Lee, J.J. Kim, T.W. Kim, B.S. Kim, M.S. Lee, Y.D. Yoo, Serum deprivation-induced reactive oxygen species production is mediated by Romo1, Apoptosis 15 (2010) 204–218.
[25] F.L. Muller, Y. Liu, H. Van Remmen, Complex III releases superoxide to both sides of the inner mitochondrial membrane, J. Biol. Chem. 279 (2004) 49064–49073.
[26] M. Konigshoff, Lung cancer in pulmonary fibrosis: tales of epithelial cell plasticity, Respiration 81 (2011) 353–358.
[27] M. Konigshoff, M. Kramer, N. Balsara, J. Wilhelm, O.V. Amarie, A. Jahn, F. Rose, L. Fink, W. Seeger, L. Schaefer, A. Gunther, O. Eickelberg, WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis, J. Clin. Invest. 119 (2009) 772–787.
[28] K. Yoshida, K. Kuwano, N. Hagimoto, K. Watanabe, T. Matsuba, M. Fujita, I. Inoshima, N. Hara, MAP kinase activation and apoptosis in lung tissues from patients with idiopathic pulmonary fibrosis, J. Pathol. 198 (2002) 388–396.
[29] M. Waghray, Z. Cui, J.C. Horowitz, I.M. Subramanian, F.J. Martinez, G.B. Toews, V.J. Thannickal, Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts, FASEB J. 19 (2005) 854–856.