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Constitutive NF-κB activation and tumor-growth promotion by Romo1-mediated reactive oxygen species production

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ABSTRACT

Deregulation of nuclear factor-κB (NF-κB) and related pathways contribute to tumor cell proliferation and invasion. Mechanisms for constitutive NF-κB activation are not fully explained; however, the underlying defects appear to generate and maintain pro-oxidative conditions. In hepatocellular carcinoma (HCC) tissues, up-regulation of reactive oxygen species modulator 1 (Romo1) correlates positively with tumor size. In the present study, we showed that Romo1 expression is required to maintain constitutive nuclear DNA-binding activity of NF-κB and transcriptional activity through constitutive IκBα phosphorylation. Overexpression of Romo1 promoted p65 nuclear translocation and DNA-binding activity. We also show that Romo1 depletion suppressed anchorage-independent colony formation by HCC cells and suppressed tumor growth *in vivo*. Based on these findings, Romo1 may be a principal regulatory factor in the maintenance of constitutive NF-κB activation in tumor cells. In the interest of anti-proliferative treatments for cancer, Romo1 may also present a productive target for drug development.

1. Introduction

Nuclear factor-kappa B (NF-κB) is a redox-sensitive transcription factor that regulates expression of various genes involved in cell proliferation and inflammation [1,2]. NF-κB is activated in primary tumors induced by carcinogens [3]. Deregulation of NF-κB contributes to the proliferation, resistance to apoptosis, angiogenesis, invasiveness and metastasis of various tumor cell types [4,5]. It may promote tumor development by favoring cell survival rather than by tumor initiation [6]. NF-κB may also inhibit cell death in response to diverse apoptotic stimuli such as tumor necrosis factor (TNF)-α and chemotherapeutic drugs through up-regulation of anti-apoptotic genes [7].

NF-κB activation promotes tumor cell survival by regulating genes such as cyclin D1 that govern cell cycle progression [1]. Inhibition of constitutive NF-κB activity may significantly suppress tumor formation and growth after xenotransplantation of malignant cells into severe combined immunodeficient (SCID) mice [8–10]. Some findings suggest that constitutive activation of NF-κB results from deregulated IκB kinase (IKK) [11,12], which promotes IκBα phosphorylation.

A variety of pro-inflammatory stresses may activate the reactive oxygen species modulator 1 (Romo1), which stimulates cellular reactive oxygen species (ROS) production. Such stressors include phorbol 12-myristate 13-acetate (PMA) and 5-FU [13–15], and intracellular proinflammatory signaling factors such as TNF-α and c-Myc [16,17]. ROS production plays regulatory roles in c-Myc turnover and TNF-α signaling. Romo1 overexpression is observed in most cancer cell lines and hepatic tumor tissues [13,15] and Romo1 up-regulation correlates positively with ROS levels in cancer cells and with tumor progression [14,15]. Mitochondrial ROS may induce NF-κB-dependent gene transactivation [18,19]; however, ROS may either activate or inhibit NF-κB in a cell type-specific manner [20]. Persistent NF-κB activation is found in many cancer cell types [1,3,9,21–23] and ROS concentrations are also relatively high in cancer cells [14,24,25]. Therefore, we investigated the correlation between the expression of Romo1 and NF-κB activation. Our results, presented here, indicate that Romo1 expression leads to an increase in NF-κB activity, which promotes tumor cell growth.

2. Materials and methods

2.1. Cell culture and reagents

Human hepatocarcinoma cells (Huh-7), breast cancer cells (MDA-MB-231, T47D and MCF-7), cervical carcinoma cells (HeLa), prostate cancer cells (DU145), human embryonic kidney (HEK) 293 cells, were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco-Invitrogen, Grand Island, NY). All media contained 10% heat-inactivated FBS (Gibco-Invitrogen), sodium bicarbonate

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(2 mg/ml; Sigma–Aldrich, St Louis, MO), penicillin (100 units/ml), and streptomycin (100 μg/ml; Gibco-Invitrogen) and were grown in 5% CO_2 at 37 °C. N-acetyl-L-cysteine (NAC), myxothiazol and N-carbobenzoxy-1-leucinyl-1-leucinyl-1-norleucinal (MG132) were obtained from Sigma–Aldrich. MitoSOX was obtained from Molecular Probes (Eugene, OR). Rabbit polyclonal anti-phospho-p65 (Ser^{536}) and anti-IκBα, and mouse polyclonal anti-phospho-IκBα ($Ser^{32/36}$) antibodies were purchased from Cell Signaling Technology (Danvers, MA). Mouse polyclonal anti-p65, antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-cytosol-specific-β-actin, anti-FLAG (M2) and anti-HA antibodies were from Sigma–Aldrich. Mouse monoclonal antibody against Romo1 has been described previously [16].

2.2. siRNA transfection

Romo1 double-stranded small interfering (siRNA) sequences were described previously [17,26]. Control and *Romo1* siRNA sequences were purchased from Bioneer (Taejon, Republic of Korea). The cells (3×10^5) were seeded into 60-mm plates and then transfected with *Romo1* siRNA using Lipofectamine™ (Gibco-Invitrogen).

2.3. shRNA and lentivirus

The shRNA construct in pLKO.1-puro targeting Romo1 sequence was purchased from Sigma–Aldrich. *Romo1* shRNA in the pLKO.1-puro vector was co-transfected with an expression vector containing the *gag*/*pol* and *vsvg* genes into 293 TN cells. Lentivirus was harvested at 48 h after transfection and 8 μg/ml of polybrene was added. Huh-7 and MDA-MB-231 cells were infected with lentivirus and stable transfectant cells were selected in 250 ng/ml of puromycin for 7 days.

2.4. Promoter activity assay

Cells (2×10^5) were seeded into 6-well dishes and then co-transfected with 2 μg of a NF-κB-specific promoter-reporter consisting of three tandem κB sites upstream of a luciferase construct and 0.5 μg of a *β-galactosidase* plasmid using Lipofectamine reagent (Gibco-Invitrogen). After 24 h of transfection, the cells were lysed, and luciferase and β-galactosidase activities were assayed according to the manufacturer's instructions (Promega). Luciferase activity was normalized to the β-galactosidase activity.

2.5. Preparation of cellular extracts and electrophoretic mobility shift assay

The NE-PER® Nuclear and Cytoplasmic Extraction Reagents Kit (Pierce, Rockford, IL) was used to perform nuclear and cytosolic extraction according to the manufacturer's instructions. The electrophoretic mobility shift assay (EMSA) for NF-κB was performed using the Gelshift™ Chemiluminescent EMSA Kit (Active Motif, Carlsbad, CA) following the manufacturer's instructions. The biotin 3′end-labeled double-stranded NF-κB oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′) was obtained from Bioneer (Taejon, Republic of Korea). For suppression of the complex formation, nuclear proteins were pre-incubated with antibody against p65 (1 μg) for 30 min at 37 °C before the addition of labeled oligonucleotide.

2.6. Western blotting and immunofluorescence assay

The Western blotting and immunofluorescence assay were described previously [16].

2.7. In vitro colony-forming assay

Cells (1×10^4) were mixed with medium containing 0.4% agar and seeded onto medium containing 0.6% agar. Complete medium was added onto the top layer every 3 days. Colonies were stained with 0.5 mg/ml nitroblue tetrazolium and counted after 14 days.

2.8. Tumorigenicity assay

Male 5-weeks-old athymic BALB/c nu/nu mice were obtained from Hanlim Animal Center (Suwon, Republic of Korea). Mice ($n = 6$/group) were subcutaneously injected with 5×10^6 cells in a volume of 200 μl PBS. The size of the tumor was measured every 2 days and tumor volumes were estimated as width^2 × length × 0.52.

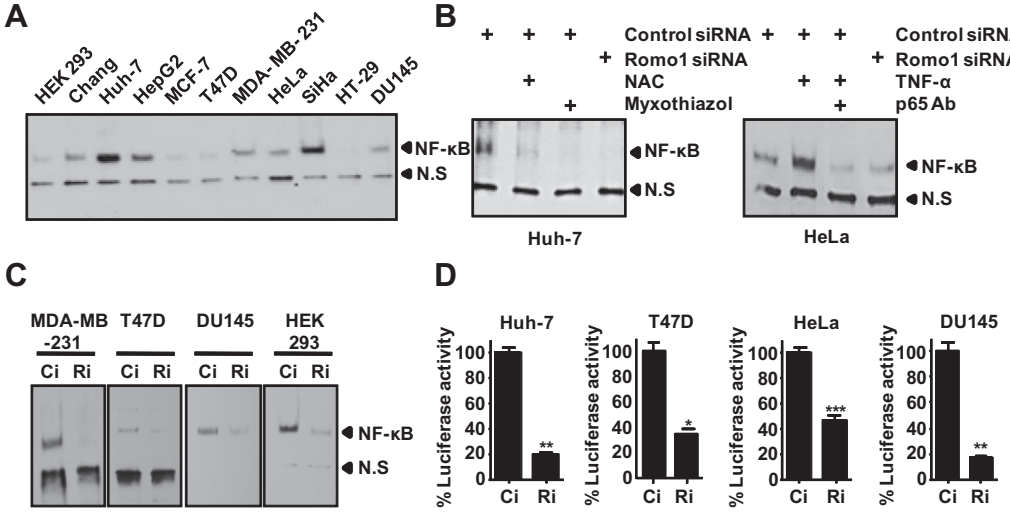

Fig. 1. Constitutive activation of NF-κB by Romo1 expression. (A) EMSA was performed using nuclear extracts from various cell lines. (B) Huh-7 cells were transfected with *Romo1* siRNA for 48 h and then incubated with NAC (20 mM) or myxothiazol (1 μM) for 4 h. HeLa cells were transfected with *Romo1* siRNA and then incubated with TNF-α (20 ng/ml) or anti-p65 antibody; EMSA was performed using nuclear extracts from these cells. (C) EMSA was performed using nuclear extracts from MDA-MB-231, T47D, DU145 and HEK 293 cells transfected with *Romo1* siRNA. (D) Huh-7, T47D, HeLa and DU145 cells were co-transfected with *Romo1* siRNA, NF-κB luciferase reporter gene and *β-galactosidase* gene. Cells were lysed and luciferase activity was measured. Data represent the mean (SE) of at least three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control siRNA by two-way analysis of variance (ANOVA). Ci, control siRNA; Ri, *Romo1* siRNA; N.S, non-specific signal.

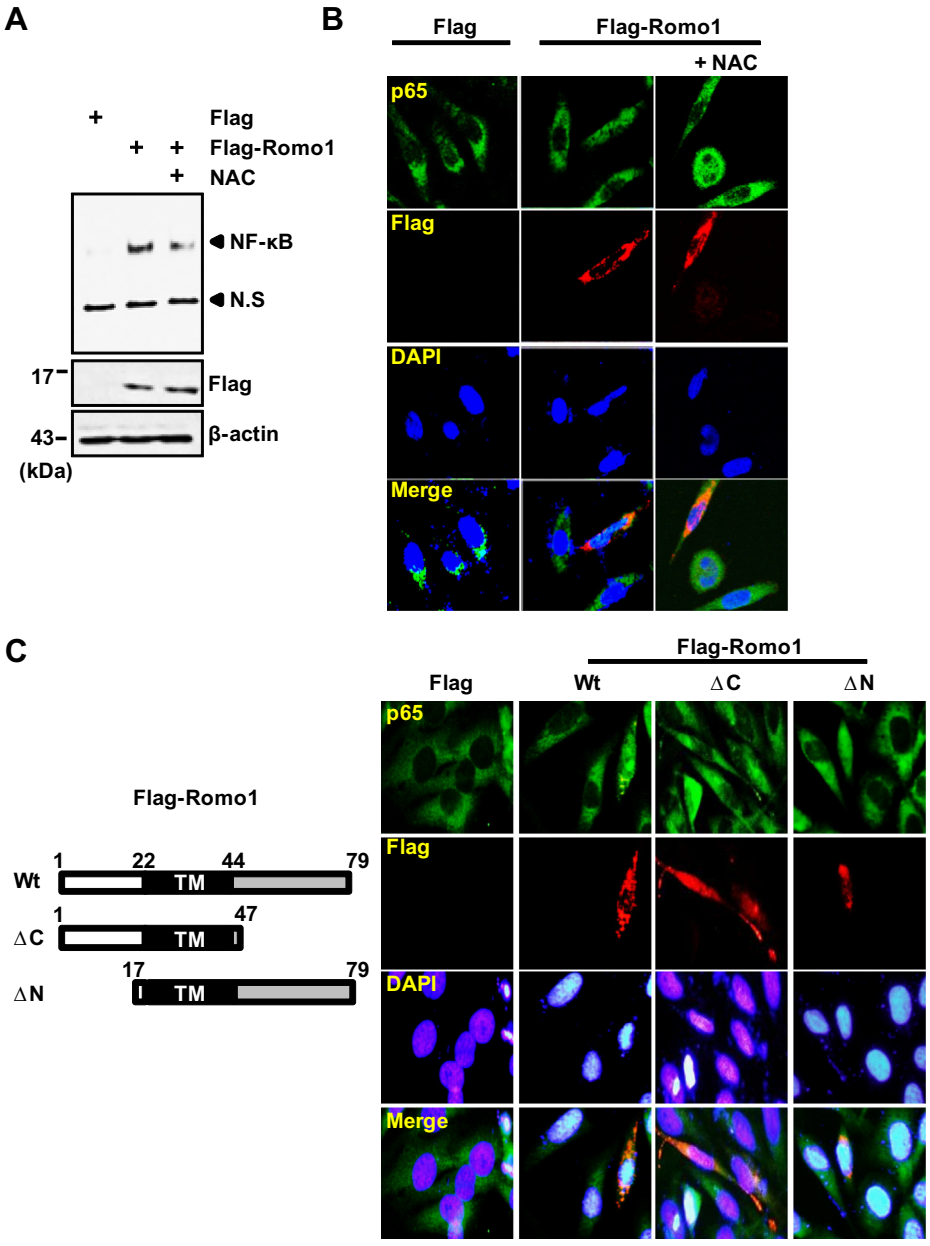

Fig. 2. Romo1-induced NF-κB activation. (A) HEK 293 cells were transfected with Flag-tagged *Romo1* and incubated with NAC for 2 h. EMSA was performed using nuclear extracts from the cells. (B) HeLa cells were transfected with Flag-tagged *Romo1* and incubated with NAC. Subcellular localization of p65 was observed by confocal microscopy. The cells were stained with anti-p65 antibody (green) or anti-Flag antibody (red). (C) HeLa cells were transfected with Flag-tagged *Romo1* deletion constructs, and subcellular localization of p65 was observed by confocal microscopy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.9. Statistical analysis

Each assay was performed in triplicate and independently repeated at least three times. Statistical significance was defined as $P < 0.05$. Means, SEs and *P*s were calculated using GraphPad PRISM version 4.02 for Windows (GraphPad Software, San Diego, CA).

3. Results

3.1. Romo1 expression increases intracellular NF-κB activity

Studies demonstrate constitutive NF-κB activation in cancer cells, and show that it is associated with oxidative stress [1,27]. To detect constitutive NF-κB activation in various cell lines, we performed an electrophoretic mobility shift assay (EMSA). As shown in Fig. 1A, NF-κB displayed high DNA-binding activity in most cancer cell lines tested. The hepatoma (Huh-7 and HepG2) and SiHa cells expressed particularly high levels of NF-κB activity. Since we also observed Romo1 overexpression in these cells [15], we tested the correlation of Romo1 expression with constitutive NF-κB activation. Huh-7 cells, which show high constitutive DNA-binding activity of NF-κB, were transfected with *Romo1* small-interfering RNA (siRNA) and DNA-binding activity of NF-κB was observed by EMSA. As shown in Fig. 1B, Romo1 depletion led to a significant decrease in the DNA-binding activity of NF-κB. Romo1 enhances cellular ROS levels through a mitochondrial respiratory chain complex III [13]. The DNA-binding activity of NF-κB was also reduced by treatment with either N-acetyl-L-cysteine (NAC), an antioxidant, or myxothiazol, a mitochondrial respiratory

chain complex III inhibitor (Fig. 1B). This result was confirmed in HeLa cells. TNF-α treatment led to increased NF-κB DNA-binding activity, but this effect was abolished by incubation with antibody to p65, a component of NF-κB. The same result was observed in various cell lines (Fig. 1C). Using an NF-κB-luciferase reporter plasmid, we also measured NF-κB-promoter activity and found that Romo1 knockdown suppressed the constitutive transcriptional activity of NF-κB in various cancer cell lines (Fig. 1D).

3.2. Romo1 induced the constitutive activation of NF-κB

Next, we tested the effects of Romo1 expression on NF-κB DNA-binding activity and nuclear translocation. A high level of DNA-binding by NF-κB was detected in HEK 293 cells transfected with *Romo1* and NAC suppressed this activity (Fig. 2A). As shown in Fig. 2B and Supplementary Fig. S1, Romo1 transfection also promoted nuclear translocation of p65 and NAC treatment blocked this action. Previously, we reported that the C-terminal domain of Romo1 is essential for Romo1-induced ROS generation. To test the effect of specific sequence deletions from Romo1 on nuclear translocation of p65, two deletion constructs of *Romo1*, designated Flag-Romo1-ΔC and Flag-Romo1-ΔN, were transfected into HeLa cells and p65 nuclear translocation was observed. As shown in Fig. 2C, nuclear translocation of p65 proceeded following transfection of either wild-type Romo1 or Romo1-ΔN. In contrast, Romo1-ΔC failed to support this nuclear translocation. These results identify Romo1 as an upstream regulator of NF-κB.

Since the constitutive activation of NF-κB is associated with IKK activation [11], we investigated the correlation of Romo1-induced NF-κB activation with IKK activation. First, we found that Romo1 knockdown led to a decrease in constitutive IκBα phosphorylation although expression levels of IκBα phosphorylation are not directly correlated with Romo1 expression (Fig. 3A). It may be cell-line specific. To test the effect of Romo1 knockdown on IκBα degradation, we performed Western blot analysis of IκBα-immunoprecipitates with anti-HA antibody as the probe. HA-tagged ubiquitylated IκBα was apparent on these blots (Fig. 3B). By this analysis, however, Romo1 depletion reduced the amount of IκBα ubiquitylation. Consistent with the effect of Romo1 depletion on IκBα phosphorylation, we found that Romo1 overexpression stimulated IκBα phosphorylation; moreover, NAC treatment suppressed this effect (Fig. 3C).

3.3. Romo1 expression is essential for tumor cell growth

Constitutive activation of NF-κB is reported to play an important role in tumor cell growth [1,28]. To demonstrate the correlation between Romo1 expression and tumor cell growth, we prepared stable transfectant cell lines expressing the *Romo1* short hairpin (sh) RNA. As shown in Fig. 4A, Romo1 depletion in these transfectants retarded the growth of Huh-7 and MDA-MB-231 cells, and similar results were obtained using soft agar assays (Fig. 4B). These results confirmed our previous findings [14,29]. We further examined whether Romo1 depletion inhibited tumor growth in immunodeficient mice. Huh-7 cells stably transfected with *Romo1* shRNA were subcutaneously injected into athymic nude mice and tumor formation was monitored for 40 days. As shown in Fig. 4C, tumor growth was detectible by 18 days following the injection of control cells, and tumor size increased continuously thereafter. Following injection of cells transfected with *Romo1* shRNA, tumor growth became detectible at 38 days and only in three mice. Romo1 knockdown by *Romo1* shRNA was tested by RT-PCR (Supplementary Fig. S2). These results are consistent with a role for Romo1 expression in the constitutive activation of NF-κB and in tumor-growth promotion.

4. Discussion

Persistent NF-κB activation, observed in many types of cancer cells, may be attributable to chromosomal amplification,

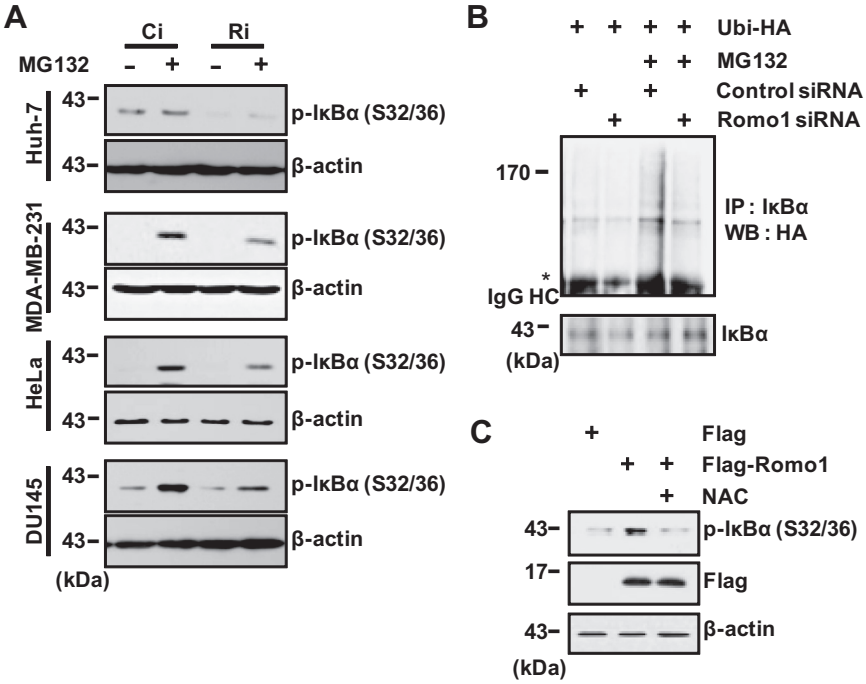

Fig. 3. Constitutive phosphorylation of IκBα by Romo1 expression. (A) Huh-7, MDA-MB-231, HeLa and DU145 cells were transfected with *Romo1* siRNA, and then treated with MG132 (10 μM) for 6 h. Cytosol extracts were prepared and p-IκBα ($Ser^{32/36}$) expression was detected by Western blotting. Ci, control siRNA; Ri, *Romo1* siRNA. (B) After HEK 293 cells were co-transfected with *Romo1* siRNA and ubiquitin-HA, the cells were treated with MG132. Cell extracts were immunoprecipitated with anti-IκB antibody and Western blotting was performed with anti-HA antibody. IgG HC, immunoglobulin heavy chain. (C) After HEK 293 cells were transfected with Flag-tagged *Romo1*, the cells were incubated with NAC for 2 h and cytosol extracts were isolated for Western blotting.

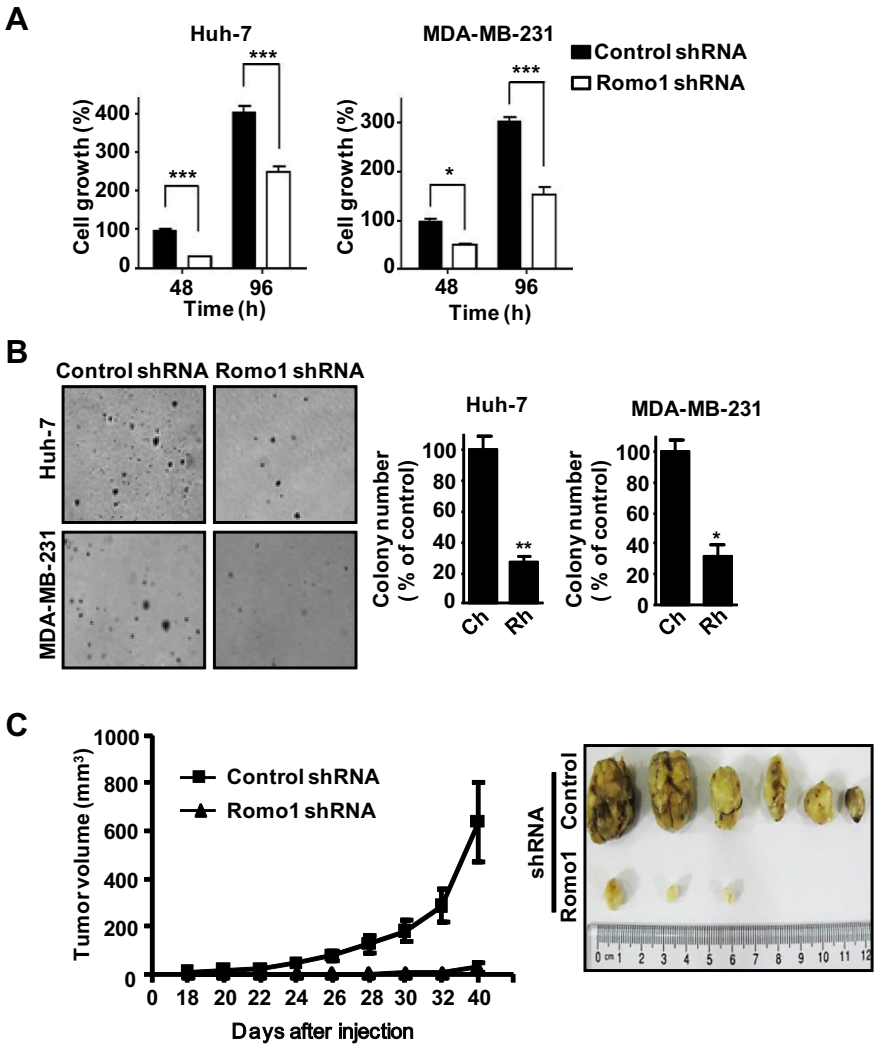

Fig. 4. Effect of Romo1 knockdown on tumor cell growth. (A) Huh-7 and MDA-MB-231 cells were infected with lentivirus containing control shRNA or *Romo1* shRNA, selected by puromycin and then cultured. At the indicated times, cells were stained with trypan blue and counted. (B) Soft agar colony assays were performed with the cells that stably expressed control shRNA or *Romo1* shRNA. Colonies were counted after 14 days. Ch, control shRNA; Rh, *Romo1* shRNA. (C) Huh-7 cells stably expressing control shRNA or *Romo1* shRNA were subcutaneously injected into 5-weeks-old nude mice and tumor size was measured at the indicated times ($n = 6$). Data are presented as the mean and SE of at least 3 independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control shRNA by 2-way analysis of variance (ANOVA).

overexpression and rearrangement of genes coding for Rel/NF-κB factors [1,23]. Sustained NF-κB activation may also occur through constitutive activation of an upstream mediator such as IKK11 or an increase in the rate of IκB degradation [30]. Many intermediate signaling factors in NF-κB activation in transformed cells remain to be identified. In the present study, we showed that ROS induction related to Romo1 expression correlated with NF-κB activity. Conversely, suppression of Romo1 expression was associated with reduction in NF-κB activity. These results point to Romo1 as a major regulator of constitutive NF-κB activation in tumor cells.

The effect of H_2O_2 treatment on NF-κB activation is cell type-specific and some cells are activated by ROS treatment positively and other cells are affected negatively [31]. However, abundant evidence supports a role for ROS in NF-κB activation. NAC suppresses TNF-α-induced NF-κB activation [32] and TNF-α-induced phosphorylation of NIK, IKKα and IKKβ [33]. Although ROS-dependent NF-κB activation is cell type-specific, constitutive NF-κB activation by Romo1 expression is not. We found that Romo1 depletion down-regulated constitutive NF-κB activity in all of the cancer cell lines that we tested in this study (Fig. 1).

Oxidative stress may promote tumorigenesis through signaling related to the epithelial–mesenchymal transition, cell migration, invasiveness, angiogenesis and metastasis [34]. Molecular mechanisms of these pathways in progression are not yet well-defined. In our previous reports, we showed that Romo1 expression was responsible for high ROS levels in tumor cells and that Romo1 depletion reduced tumor cell growth in vitro [14,29]. The constitutive activation of NF-κB is also shown to be critical for tumor formation and growth in SCID mice [8]. The suppression of constitutive NF-κB activity may significantly inhibit tumor formation and growth in SCID mice [8–10]. In a previous study, we found a significant correlation of Romo1 expression with tumor size in HCC [15]. In the present study, ROS induced by Romo1 expression and tumor cell growth were correlated. These findings suggest that constitutive activation of NF-κB is a downstream mediator of Romo1-promoted tumor growth.

Conflicts of interest

None declared.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.059.

References

[1] A.S. Baldwin Jr., The NF-kappa B and I kappa B proteins: new discoveries and insights, Annu. Rev. Immunol. 14 (1996) 649–683.
[2] S. Ghosh, M.S. Hayden, New regulators of NF-kappaB in inflammation, Nat. Rev. Immunol. 8 (2008) 837–848.
[3] M.A. Sovak, R.E. Bellas, D.W. Kim, G.J. Zanieski, A.E. Rogers, A.M. Traish, G.E. Sonenshein, Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer, J. Clin. Invest. 100 (1997) 2952–2960.
[4] H.J. Kim, N. Hawke, A.S. Baldwin, NF-kappaB and IKK as therapeutic targets in cancer, Cell Death Differ. 13 (2006) 738–747.
[5] N. Sasaki, T. Morisaki, K. Hashizume, T. Yao, M. Tsuneyoshi, H. Noshiro, K. Nakamura, T. Yamanaka, A. Uchiyama, M. Tanaka, M. Katano, Nuclear factor-kappaB p65 (RelA) transcription factor is constitutively activated in human gastric carcinoma tissue, Clin. Cancer Res. 7 (2001) 4136–4142.
[6] E. Pikarsky, R.M. Porat, I. Stein, R. Abramovitch, S. Amit, S. Kasem, E. Gutkovich-Pyest, S. Urieli-Shoval, E. Galun, Y. Ben-Neriah, NF-kappaB functions as a tumour promoter in inflammation-associated cancer, Nature 431 (2004) 461–466.
[7] M. Barkett, T.D. Gilmore, Control of apoptosis by Rel/NF-kappaB transcription factors, Oncogene 18 (1999) 6910–6924.
[8] R.C. Bargou, F. Emmerich, D. Krappmann, K. Bommert, M.Y. Mapara, W. Arnold, H.D. Royer, E. Grinstein, A. Greiner, C. Scheidereit, B. Dorken, Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells, J. Clin. Invest. 100 (1997) 2961–2969.
[9] R. Visconti, J. Cerutti, S. Battista, M. Fedele, F. Trapasso, K. Zeki, M.P. Miano, F. de Nigris, L. Casalino, F. Curcio, M. Santoro, A. Fusco, Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NFkappaB p65 protein expression, Oncogene 15 (1997) 1987–1994.
[10] D.C. Duffey, Z. Chen, G. Dong, F.G. Ondrey, J.S. Wolf, K. Brown, U. Siebenlist, C. Van Waes, Expression of a dominant-negative mutant inhibitor-kappaBalpha of nuclear factor-kappaB in human head and neck squamous cell carcinoma inhibits survival, proinflammatory cytokine expression, and tumor growth in vivo, Cancer Res. 59 (1999) 3468–3474.
[11] A.V. Gasparian, Y.J. Yao, D. Kowalczyk, L.A. Lyakh, A. Karseladze, T.J. Slaga, I.V. Budunova, The role of IKK in constitutive activation of NF-kappaB transcription factor in prostate carcinoma cells, J. Cell Sci. 115 (2002) 141–151.
[12] W. Wilson 3rd, A.S. Baldwin, Maintenance of constitutive IkappaB kinase activity by glycogen synthase kinase-3alpha/beta in pancreatic cancer, Cancer Res. 68 (2008) 8156–8163.
[13] 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.
[14] A.R. Na, Y.M. Chung, S.B. Lee, S.H. Park, M.S. Lee, Y.D. Yoo, A critical role for Romo1-derived ROS in cell proliferation, Biochem. Biophys. Res. Commun. 369 (2008) 672–678.
[15] J.S. Chung, S.H. Park, S. Ho Park, E.R. Park, P.H. Cha, B.Y. Kim, Y.M. Chung, S.R. Woo, C.J. Han, S.B. Kim, Overexpression of Romo1 promotes production of reactive oxygen species and invasiveness of hepatic tumor cells, Gastroenterology (2012).
[16] J. Kim, S. Lee, J. Park, Y. Yoo, TNF-α-induced ROS production triggering apoptosis is directly linked to Romo1 and Bcl-XL, Cell Death Differ. 17 (2010) 1420–1434.
[17] S.B. Lee, J.J. Kim, J.S. Chung, M.S. Lee, K.H. Lee, B.S. Kim, Y. Do Yoo, Romo1 is a negative-feedback regulator of Myc, J. Cell Sci. 124 (2011) 1911–1924.
[18] G. Hughes, M.P. Murphy, E.C. Ledgerwood, Mitochondrial reactive oxygen species regulate the temporal activation of nuclear factor kappaB to modulate tumour necrosis factor-induced apoptosis: evidence from mitochondria-targeted antioxidants, Biochem. J. 389 (2005) 83–89.
[19] J.M. Lluis, F. Buricchi, P. Chiarugi, A. Morales, J.C. Fernandez-Checa, Dual role of mitochondrial reactive oxygen species in hypoxia signaling: activation of nuclear factor-{kappa}B via c-SRC and oxidant-dependent cell death, Cancer Res. 67 (2007) 7368–7377.
[20] V. Oliveira-Marques, H.S. Marinho, L. Cyrne, F. Antunes, Role of hydrogen peroxide in NF-kappaB activation: from inducer to modulator, Antioxid. Redox Signal. 11 (2009) 2223–2243.
[21] R.C. Bargou, C. Leng, D. Krappmann, F. Emmerich, M.Y. Mapara, K. Bommert, H.D. Royer, C. Scheidereit, B. Dorken, High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed–Sternberg cells, Blood 87 (1996) 4340–4347.
[22] H. Nakshatri, P. Bhat-Nakshatri, D.A. Martin, R.J. Goulet Jr., G.W. Sledge Jr., Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth, Mol. Cell. Biol. 17 (1997) 3629–3639.
[23] B. Rayet, C. Gelinas, Aberrant rel/nfkb genes and activity in human cancer, Oncogene 18 (1999) 6938–6947.
[24] A. Laurent, C. Nicco, C. Chereau, C. Goulvestre, J. Alexandre, A. Alves, E. Levy, F. Goldwasser, Y. Panis, O. Soubrane, B. Weill, F. Batteux, Controlling tumor growth by modulating endogenous production of reactive oxygen species, Cancer Res. 65 (2005) 948–956.
[25] T.P. Szatrowski, C.F. Nathan, Production of large amounts of hydrogen peroxide by human tumor cells, Cancer Res. 51 (1991) 794–798.
[26] 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.
[27] S.S. Brar, T.P. Kennedy, A.R. Whorton, A.B. Sturrock, T.P. Huecksteadt, A.J. Ghio, J.R. Hoidal, Reactive oxygen species from NAD(P)H:quinone oxidoreductase constitutively activate NF-kappaB in malignant melanoma cells, Am. J. Physiol. Cell Physiol. 280 (2001) C659–C676.
[28] K.A. Higgins, J.R. Perez, T.A. Coleman, K. Dorshkind, W.A. McComas, U.M. Sarmiento, C.A. Rosen, R. Narayanan, Antisense inhibition of the p65 subunit of NF-kappa B blocks tumorigenicity and causes tumor regression, Proc. Natl. Acad. Sci. USA 90 (1993) 9901–9905.
[29] J.S. Chung, S.B. Lee, S.H. Park, S.T. Kang, A.R. Na, T.S. Chang, H.J. Kim, Y.D. Yoo, Mitochondrial reactive oxygen species originating from Romo1 exert an important role in normal cell cycle progression by regulating p27Kip1 expression, Free Radical Res. 43 (2009) 729–737.
[30] S. Miyamoto, P.J. Chiao, I.M. Verma, Enhanced I kappa B alpha degradation is responsible for constitutive NF-kappa B activity in mature murine B-cell lines, Mol. Cell. Biol. 14 (1994) 3276–3282.
[31] Y. Kabe, K. Ando, S. Hirao, M. Yoshida, H. Handa, Redox regulation of NF-kappaB activation: distinct redox regulation between the cytoplasm and the nucleus, Antioxid. Redox Signal. 7 (2005) 395–403.
[32] F.J. Staal, M. Roederer, L.A. Herzenberg, L.A. Herzenberg, Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus, Proc. Natl. Acad. Sci. USA 87 (1990) 9943–9947.
[33] H. Kamata, T. Manabe, S. Oka, K. Kamata, H. Hirata, Hydrogen peroxide activates IkappaB kinases through phosphorylation of serine residues in the activation loops, FEBS Lett. 519 (2002) 231–237.
[34] W.S. Wu, The signaling mechanism of ROS in tumor progression, Cancer Metastasis Rev. 25 (2006) 695–705.