Biochemical and Biophysical Research Communications 436 (2013) 467–472

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Autocrine TNF-α-mediated NF-κB activation is a determinant for evasion of CD40-induced cytotoxicity in cancer cells

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ARTICLE INFO

Article history:
Received 28 May 2013
Available online 7 June 2013

Keywords:
CD40/rsCD40L
NF-κB
Autocrine
TNF-α
Cell death
Cancer

ABSTRACT

Activation of CD40 by CD40L results in diverse effects on different malignant cells, causing either promotion of survival, growth and resistance to chemotherapy, or induction of cytostasis and apoptosis. The molecular mechanisms underlying CD40-mediated growth regulation and apoptosis induction in cancer cell are not fully understood. In this study, we investigated the role of NF-κB activation in CD40-mediated cytotoxicity in cancer cells. The results show that activation of CD40 by recombinant soluble CD40 ligand (rsCD40L) readily induced NF-κB activation and blocking NF-κB significantly enhanced rsCD40L-induced apoptosis in cancer cells. Importantly, autocrine of TNF-α induced by rsCD40L was indispensable for both NF-κB activation and cytotoxicity induction, establishing a dual role of autocrine TNF-α that constitutes both pro-apoptotic and anti-apoptotic arms of CD40 signaling. Our results indicate that autocrine TNF-α-mediated NF-κB activation is a determinant for cancer cells' evasion of CD40L-induced cytotoxicity and blocking NF-κB may have potential for improve the value of CD40 as an anticancer agent.

1. Introduction

CD40, a member of the tumor necrosis factor (TNF) receptor (TNFR) superfamily widely expressed in various immune cells, plays critical roles in humoral and cellular immune response [1–3]. CD40 exerts diverse cellular effects after activation by its cognate ligand (CD40L, CD154), a 39-kDa type II integral membrane protein. Engagement of CD40 by CD40L leads to recruitment of several TNFR-associated factors (TRAFs), most notably TRAF2, TRAF3, and TRAF6, to its TRAF-binding site in the cytoplasmic tail. CD40 then mediates activation of multiple signaling pathways including NF-κB, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein (MAP) kinase, extracellular signal-related kinase (ERK), and phosphoinositide 3-kinase (PI3K), resulting in diverse phenotypic consequences in different cell types [4–6].

CD40 is also expressed in nearly all B-cell malignancies and solid tumors such as that from lung, breast, liver, colon, pancreas, kidney, prostate, ovary, and head and neck [7,8]. While the function of CD40–CD40L interaction in immune cells has been the subject of intense investigation, the role of such interaction in malignant cells has not been well clarified. It has been reported that ligation of cell surface CD40 in certain tumor cells contributes to tumor survival, growth, and resistance to chemotherapy [9,10]. In contrast, CD40 ligation results in inhibition of proliferation and activation of apoptosis in other tumor cells, which makes CD40 a potential anti-cancer therapeutic target [7,11,12]. Therefore, it is important to determine the molecular mechanisms underlying CD40-mediated cancer cell growth regulation and apoptosis induction.

The fact that soluble CD40 agonists are growth inhibitory and pro-apoptotic only if RNA or protein synthesis is inhibited suggests that CD40 transduces potent survival signals capable of suppressing its pro-apoptotic effects [12,13]. As a major cell survival signal, NF-κB has been well established as a death brake that restrains the TNFR1-mediated apoptotic pathway. Similar to TNFR1, CD40 mediates activation of both the classical and alternative NF-κB signaling pathways [14,15]. CD40 ligation protected follicular lymphoma from TRAIL-induced apoptosis through NF-κB activation-mediated up-regulation of c-FLIP and Bcl-xL [16]. These observations

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http://dx.doi.org/10.1016/j.bbrc.2013.05.128

prompted us to examine if NF-κB activation also constitutes the anti-apoptotic arm of CD40 signaling that overrides the death-inducing effects of CD40 ligation in cancer cells. The results show that recombinant soluble CD40 ligand (rsCD40L) triggered the activation of NF-κB signaling pathway in cancer cells and blocking NF-κB activation significantly sensitized cancer cells to rsCD40L-induced apoptosis. Autocrine TNF-α induced by CD40 activation substantially contributed to NF-κB activation. These data identify autocrine TNF-α as an important mediator of NF-κB activation in CD40 activated cancer cells and suggest that blocking NF-κB could be an effective therapeutic strategy for improving the anticancer potential of CD40 agonists.

2. Materials and methods

2.1. Reagents

rsCD40L and TNF-α neutralizing antibody were from PeproTech (Rocky Hill, NJ). IKK-2 inhibitor IV, IKK inhibitor II, and NF-κB activation inhibitor II were from Calbiochem (La Jolla, CA). 17DMAG and luteolin were from Toris Cookson (Ellisville, Missouri). Small interfering RNA (siRNA) for IKK-2, RelA, TNFR1, and the non-targeting negative control siRNA were obtained from Guangzhou RiboBio (Guangzhou, China). INTERFERin siRNA transfection reagent was from Polyplus transfection (Illkirch, FRANCE). Antibodies against active caspase-3 and poly (ADP-ribose) polymerase (PARP) were from BD bioscience (San Diego, CA). Anti-phospho-IκBα, anti-IκBα, and anti-β-actin were from Cell Signaling (Beverly, MA), Santa Cruz Biotechnology (Santa Cruz, CA) and ProteinTech Group (Chicago, IL), respectively.

2.2. Cell culture

SKOV3 (an ovarian cancer cell line) and A549 (a non-small cell lung cancer cell line) were from American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM or RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone), 1 mmol/L glutamate, 100 units/mL penicillin, and 100 μg/mL streptomycin under standard condition (37 °C, 5% CO2). A549 cells stably transfected with a NF-κB-responsive luciferase reporter plasmids (A549-luc) were cultured in RPMI 1640 with 100 μg/mL hygromycin.

2.3. Lactate dehydrogenase (LDH) release assay and flow cytometric apoptosis analysis

Cell death was quantitively detected based on the release of LDH by using a cytotoxicity detection kit (Promega, Madison, WI) as described previously [17]. All the experiments were repeated three times and the average is shown in each figure. Cell apoptosis was detected by flow cytometric analysis by using an Annexin V-FITC Apoptosis Detection Kit (Nanjing KeyGen Biotech, Nanjing, China) as described previously [18].

2.4. Immunoblotting analysis

Cell extracts were prepared by lysing cells in M_2 buffer (20 mmol/L Tris–HCl (pH 7.6), 0.5% NP40, 250 mmol/L NaCl, 3 mmol/L EGTA, 3 mmol/L EDTA, 2 mmol/L DTT, 0.5 mmol/L phenylmethylsulfonyl fluoride, 20 mmol/L β-glycerophosphate, 1 mmol/L sodium vanadate, 1 μg/mL leupeptin). Protein extracts were resolved in SDS–PAGE and probed by Western blot. The proteins were visualized by enhanced chemiluminescence following the manufacturer's instructions (Millipore, Billerica, MA). Each experiment was repeated three times and representative results are shown in each figure.

2.5. Luciferase assay

SKOV3 cells were cultured in a 24-well plate overnight and then transfected with p5xκB-Luc and pRSV-LacZ using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). Twenty-four hours after transfection, cells were treated as indicted in figure legend. Luciferase activity was detected using a luciferase assay kit (Promega, Madison, WI) and normalized to the β-galactosidase activity of each sample. For the detection of NF-κB-responsive luciferase activity in A549 cells, A549 cells bearing an integrated NF-κB-responsive luciferase reporter were treated with rsCD40L and luciferase activity was detected and normalized to the total protein amount of each sample. All the experiments were repeated three times and the average is shown in each figure.

2.6. Enzyme linked immunosorbent assay (ELISA)

TNF-α level in cell culture supernatant was measured by ELISA by using a human TNF-α ELISA kit (Neobioscience Technology, Shenzhen, China). Briefly, cells were plated onto 48-well plates at 60% to 70% confluence. After culture overnight, cells were treated as described in figure legends. The cell culture supernatants were collected and the concentrations of TNF-α were detected by ELISA following the manufacturer's instructions.

2.7. RNA interference

Small-interfering RNA (siRNA) targeting IKK-2, RelA, TNFR1, or the silencer negative control siRNA was transfected using INTERFERin. Forty-eight hours after transfection, cells were treated as indicated in each figure legend for another 72 h followed by LDH-release based cell death assay.

2.8. Statistical analysis

All numerical data are expressed as mean ± standard deviation (SD) from at least three independent experiments. Statistical significance was examined by paired Student's *t* test using SPSS statistics software package (IBM SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistical significant.

3. Results

3.1. rsCD40L induces NF-κB activation in cancer cells

We first examined the ability of CD40 ligation in activating NF-κB in SKOV3, an ovarian cancer cell line that expresses CD40 [19]. rsCD40L treatment resulted in decreased IκBα expression, which started as early as 5 min after treatment and lasted over 6 h (Fig. 1A). As IκBα is an important component of NF-κB pathway and its degradation is a hallmark of NF-κB activation, the result indicated that CD40 ligation remarkably activated NF-κB in SKOV3 cells. The effect of rsCD40L treatment on NF-κB was further confirmed by luciferase assay. rsCD40L evidently stimulated the NF-κB-driven luciferase reporter activity in SKOV3 cells, which was shown as about three fold increase of NF-κB-responsive activity compared to that of negative control (Fig. 1B). Consistently, the reporter activity was also induced by rsCD40L treatment in a time dependent manner in A549 cells stably transfected with a NF-κB-responsive luciferase reporter, albeit that the dose of rsCD40L used in A549 cells is higher than that used in SKOV3 cells (Fig. 1C).

3.2. Blocking NF-κB increases CD40-mediated apoptosis in cancer cells

To investigate the role of CD40-mediated NF-κB activation in regulation of cell death in cancer cells, SKOV3 cells were pre-trea-

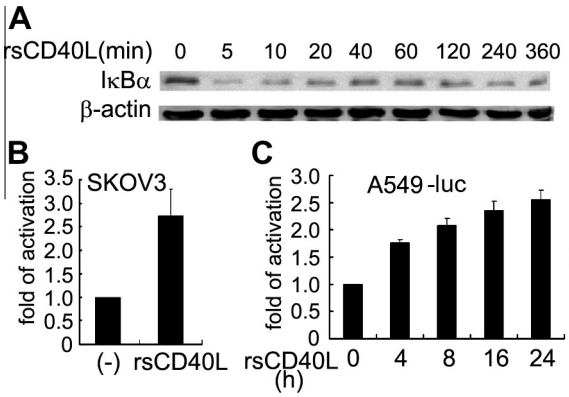

Fig. 1. rsCD40L induces NF-κB activation in cancer cells. (A) SKOV3 cells were treated with rsCD40L (1 μg/mL) for the indicated times. Whole cell lysates were detected for IκBα expression by Western blot. β-actin was measured as input control. (B) SKOV3 cells in 24-well plates were co-transfected with p5xκB-Luc and pRSV-LacZ plasmids. Twenty-four hours post transfection, cells were treated with rsCD40L (1 μg/mL) for another 30 h or left untreated as a control. Luciferase activity was detected and normalized to β-galactosidase activity. Data were expressed as fold increase relative to the untreated control. (C) A549 cells stably transfected with a NF-κB-responsive luciferase reporter (A549-luc) were treated with rsCD40L (3 μg/mL) for the indicated times or left untreated. Luciferase activity was detected and normalized to the total protein amount. Data were expressed as fold increase relative to the untreated control. Columns, mean of three independent experiments; bars, SD.

ted with IKK-2 inhibitor IV to block NF-κB pathway. Consistent with our previous study [19], SKOV3 cells are resistant to rsCD40L-induced cytotoxicity as marginal cell death (less than 5%) was detected in rsCD40L alone treated cells. However, IKK-2 inhibitor IV effectively sensitized SKOV3 cells to rsCD40L-induced cell death in a dose-dependent manner (Fig. 2A). Consistently, IKK inhibitor II, which inhibits the activity of IKK-1 and IKK-2, and NF-κB activation inhibitor II, which selectively blocks nuclear translocation of NF-κB p65 and its transcription activity, also significantly enhanced cell death in rsCD40L treated cells (Fig. 2B). To exclude potential biases of chemical inhibitors, siRNA targeting IKK-2 or RelA/p65, two key components of the NF-κB activation pathway, was employed to block NF-κB activation in SKOV3 cells. As expected, knockdown of either IKK-2 or RelA expression significantly potentiated, while the negative control siRNA had no detectable effect on, rsCD40L-induced cytotoxicity (Fig. 2C). Flow cytometric analysis showed that both early apoptotic and late apoptotic cells were significantly increased when SKOV3 cells were treated with the combination of rsCD40L and IKK-2 inhibitor IV (Fig. 2D). Consistently, the activation of caspase 3 and the cleavage of the caspase-3 substrate PARP (115 kDa) and generation of the small fragment (23-kDa) in the co-treated cells were significantly enhanced (Fig. 2E). A similar enhancing effect of NF-κB blockage on rsCD40L-induced cytotoxicity was also observed in A549 cells (Fig. 2F). Collectively, these results indicate that NF-κB activation overrides CD40-mediated apoptotic effect and substantially contributes to evasion of CD40-mediated apoptosis in cancer cells. Therefore, suppression of NF-κB activation would shift the cell death and survival balance to the side of cell death in rsCD40L-exposed cancer cells.

3.3. CD40-mediated NF-κB activation and cell death depend on autocrine TNF-α

We have previously found that rsCD40L induced rapid and persistent autocrine of TNF-α in SKOV3 cells and A549 cells [19]. As TNF-α by itself is a robust NF-κB activator, we then examined the role of autocrine TNF-α in CD40-mediated NF-κB activation.

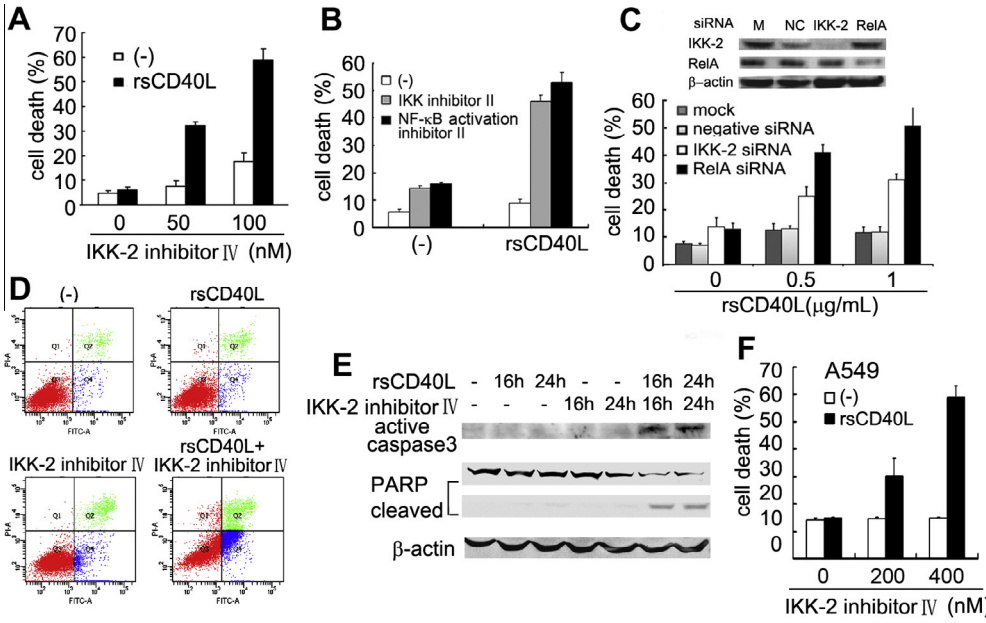

Fig. 2. Blocking NF-κB increases CD40-mediated apoptosis in cancer cells. (A) SKOV3 cells were treated with rsCD40L (1 μg/mL) and increasing concentration of IKK-2 inhibitor IV individually or in combination. Cell death was detected by a LDH release assay 72 h after treatment. Columns are the mean of three experiments. Bars, SD. (B) SKOV3 cells were treated with rsCD40L (1 μg/mL), IKK inhibitor II (20 μM), or NF-κB activation inhibitor II (20 μM) individually or in combination. Cell death was detected as indicated in (A). (C) SKOV3 cells were mock transfected or transfected with IKK-2-, RelA-, or negative control-siRNA. Forty-eight hours after transfection, the cells were treated with the indicated concentrations of rsCD40L for an additional 72 h. Cell death was detected as indicated in (A). Inset, Western blot showed the knockdown of IKK-2 and RelA. (D) SKOV3 cells were treated with rsCD40L (1 μg/mL) or IKK-2 inhibitor IV (50 nM) individually or in combination for 24 h. Cells were then stained with annexin V and PI followed by flow cytometry analysis. Early apoptosis is defined by Annexin V^+/PI^- staining (Q4) and late apoptosis is defined by Annexin V^+/PI^+ staining (Q2). (E) SKOV3 cells were treated with rsCD40L (1 μg/mL) or IKK-2 inhibitor IV (100 nM) individually or in combination for the indicated times. Active-caspase-3 and PARP were examined using Western blot. β-actin was detected as an input control. (F) A549 cells were treated with rsCD40L (3 μg/mL) and increasing concentration of IKK-2 inhibitor IV (200 nM and 400 nM) individually or in combination or left untreated. Cell death was detected as indicated in (A).

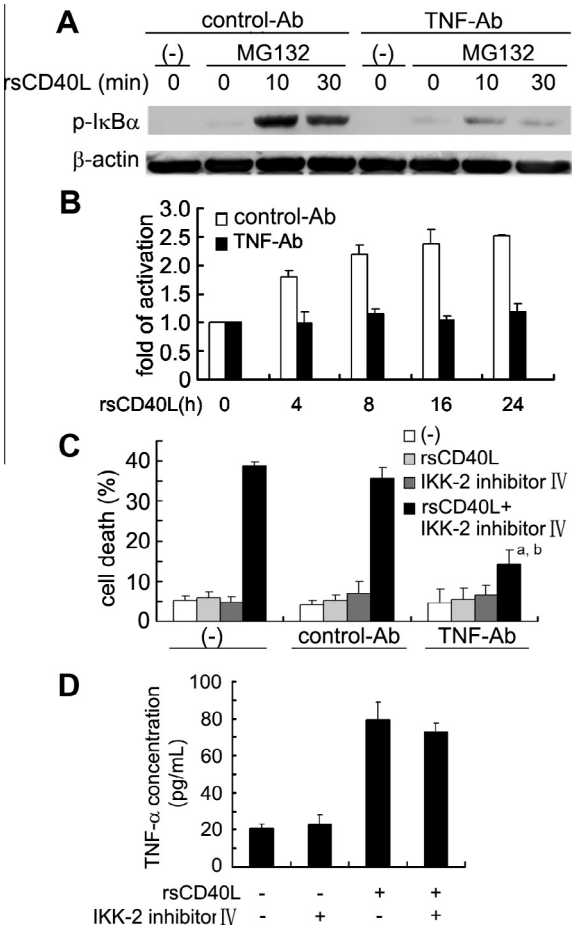

Fig. 3. CD40L-mediated NF-κB activation and cell death depend on autocrine TNF-α. (A) SKOV3 cells were pre-treated with MG132 for 30 min or left untreated. Then the cells were treated with TNF-α neutralizing antibody (1 μg/mL) or control antibody (1 μg/mL) for another 30 min, followed by treatment with rsCD40L (1 μg/mL) for the indicated times. Phospho-IκBα was detected by Western blot. β-actin was measured as input control. (B) A549 cells stably transfected with a NF-κB–responsive luciferase reporter (A549-luc) were pre-treated with TNF-α neutralizing antibody (1 μg/mL) or control antibody (1 μg/mL) for 30 min followed by rsCD40L (3 μg/mL) treatment for the indicated times or left untreated. Luciferase activity was detected as indicated in Fig. 1(C). (C) SKOV3 cells were pre-treated with TNF-α neutralizing antibody (1 μg/mL) or control antibody (1 μg/mL) for 30 min or left untreated, followed by rsCD40L (1 μg/mL) and IKK-2 inhibitor IV (50 nM) treatment. Cell death was detected as indicated in Fig. 2(A). a, $P < 0.01$ compared with cells without antibody treatment; b, $P < 0.01$ compared with cells treated with control antibody. (D) SKOV3 cells were treated with rsCD40L (1 μg/mL) and IKK-2 inhibitor IV (50 nM) individually or in combination for 8 h. TNF-α level in the culture supernatant was measured using ELISA. Columns, mean of three independent experiments; bars, SD.

A TNF-α neutralizing antibody that blocks TNF-α binding to its receptor effectively suppressed rsCD40L-induced phosphorylation of IκBα, a hallmark of NF-κB activation, in SKOV3 cells (Fig. 3A). The role of autocrine TNF-α in rsCD40L-mediated NF-κB activation was further confirmed in A549 cells stably transfected with a NF-κB-responsive luciferase reporter. rsCD40L-induced luciferase activity was abrogated when cells were pretreated with TNF-α neutralizing antibody (Fig. 3B). As it is well known that TNF-α activates apoptotic pathway, we examined if autocrine TNF-α may be involved in cell death induced by rsCD40L treatment under the condition of NF-κB blockage. Indeed, the enhanced cytotoxicity induced by rsCD40L and IKK-2 inhibitor IV co-treatment was significantly inhibited by pre-treatment with TNF-α neutralizing antibody (Fig. 3C). CD40 ligation triggered TNF-α secretion was unlikely to depend on NF-κB activation, because IKK inhibitor IV exerted no obvious effect on basal or rsCD40L induced TNF-α secretion (Fig. 3D).

3.4. NF-κB blockers 17DMAG and luteolin sensitize CD40-induced cell death

17DMAG and luteolin are two wildly studied compounds with clinical application potential in anticancer therapy. It is well accepted that these two compounds block NF-κB through distinct mechanisms. Therefor, we examined if 17DMAG and luteolin can sensitize CD40-mediated cytotoxicity. 17DMAG and luteolin caused a synergistic cytotoxicity in rsCD40L-treated SKOV3 cells in a dose-dependent manner (Fig. 4A and B). As expected, both 17DMAG and luteolin effectively inhibited rsCD40L-induced NF-κB activation (Fig. 4C) and rsCD40L-induced TNF-α autocrine was not affected by either 17DMAG or luteolin treatment (Fig. 4D). Blocking TNF-α signaling by siRNA targeting TNFR1 significantly inhibited the cytotoxicity induced by rsCD40L and 17DMAG co-treatment (Fig. 4E). These results strongly suggest that the combination of NF-κB blocking agents and CD40 agonists has potential clinical significance for cancer therapy.

4. Discussion

Given the diverse cellular outcomes of CD40 activation in cancer cells, it is important to elucidate the molecular mechanisms underlying CD40-mediated cell growth regulation and apoptosis induction. In this study, we found that activation of CD40 by rsCD40L readily induces NF-κB activation in cancer cells and blocking NF-κB significantly enhanced rsCD40L-induced apoptosis. Importantly, autocrine TNF-α is crucial for NF-κB activation and cytotoxicity induced by CD40 ligation. These results strongly suggest that autocrine TNF-α plays a major role in CD40 signaling, and NF-κB activation is a determinant for evasion of CD40-induced cytotoxicity in cancer cells. Thus, blocking NF-κB may have potential clinical implication in CD40-targeting anticancer therapy.

Unlike that of other members of the TNFR family such as TNFR1, Fas, DR4, and DR5, the intracellular domain of CD40 lacks death domain and cannot recruit and activate caspases directly. Nevertheless, CD40 is able to activate apoptosis, which was shown to be associated with stabilization of TRAF3 and activation of JNK [20], or transactivation of other TNFR members that have death domain [11]. Our previous study has shown that activation of CD40 by rsCD40L induces rapid and persistent autocrine of TNF-α in SKOV3 cells and A549 cells, and autocrine TNF-α plays a critical role in potentiation of cisplatin-induced cytotoxicity in cancer cells [19]. In this study, we found that autocrine TNF-α is indispensable for CD40-induced cancer cell death when NF-κB is blocked, which was shown as blocking TNF-α signaling with either neutralizing antibody or siRNA targeting TNFR1 readily suppressed cell death. Thus, our result indicate that autocrine TNF-α substantially contributes to apoptotic response of CD40 signaling in these cancer cells.

CD40 ligation has been reported to induce activation of cell survival pathways NF-κB and PI3 kinase/Akt [13,21]. Consistently, our results showed that CD40 activation induced NF-κB activation in SKOV3 cells and A549 cells. Autocrine TNF-α is likely the main mediator of CD40L-induced NF-κB activation, as the TNF-α neutralizing antibody dramatically suppressed rsCD40L-induced NF-κB activation. It is interesting that IκBα was only partially restored even at 6 h after rsCD40L treatment (Fig. 1A), which is different from the complete recovery within 60 min after a single dose TNF-α treatment. As degradation initiates NF-κB activation and IκBα recovery is triggered by NF-κB, this is likely due to continuous NF-κB induction by sustained TNF-α autocrine in CD40-treated

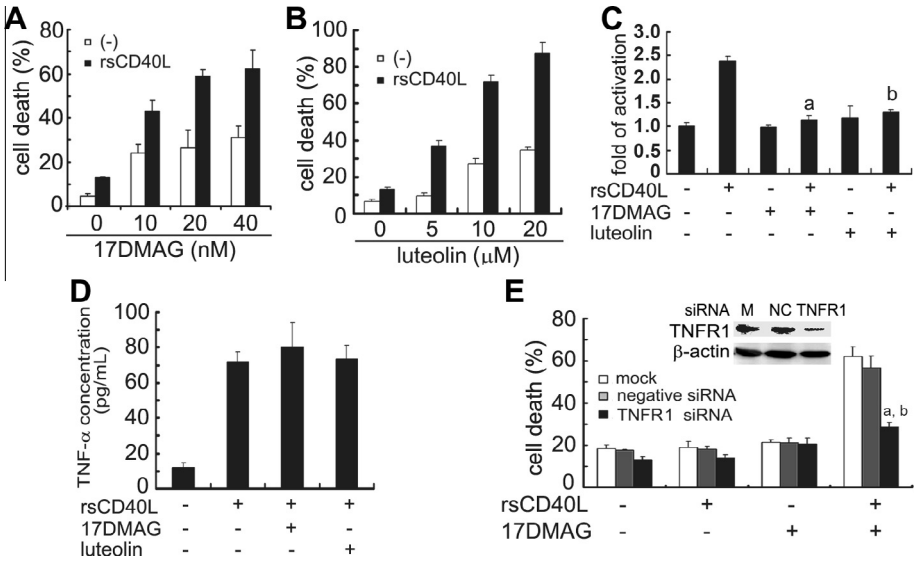

Fig. 4. NF-κB blockers luteolin and 17DMAG sensitize CD40-induced cell death. (A) and (B) SKOV3 cells were pretreated with increasing concentrations of 17DMAG for 10 h (A) or increasing concentrations of luteolin for 1 h (B) followed by rsCD40L treatment (1 μg/mL) or left untreated. Seventy-two hours later, cell death was detected as indicated in Fig. 2(A). (C) A549 cells stably transfected with a NF-κB-responsive luciferase reporter (A549-luc) were pretreated with 17DMAG (20 nM) for 10 h or luteolin (10 μM) for 1 h followed by rsCD40L treatment (3 μg/mL) for another 24 h or left untreated. Luciferase activity was detected as indicated in Fig. 1(C). a and b, $P < 0.01$ compared with rsCD40L alone treatment. (D) SKOV3 cells were pretreated with 17DMAG (20 nM) for 10 h or luteolin (10 μM) for 1 h followed by rsCD40L treatment (1 μg/mL) for another 8 h. TNF-α level in the culture supernatant was measured using ELISA. (E) SKOV3 cells were mock transfected or transfected with TNFR1- or negative control-siRNA. Forty-eight hours after transfection, cells were treated with rsCD40L (1 μg/mL) and 17DMAG (20 nM) individually or in combination for another 72 h and cell death was detected as indicated in Fig. 2(A). Inset, Western blot showing the knockdown of TNFR1. a, $P < 0.01$ compared with mock transfection; b, $P < 0.01$ compared with negative siRNA transfection. Columns, mean of three independent experiments; bars, SD.

cells [19]. The exact mechanism is worthy further study. Importantly, we found that blocking NF-κB sensitized both SKOV3 and A549 cells to rsCD40L-induced cell death. These results are inconsistent with previous report that CD40 utilizes only the PI3 kinase cascade for antiapoptotic responses [21]. The discrepancy is currently unknown but may be due to cell type specificity and different dose of rsCD40L used. Indeed, different cell lines have different sensitivity to rsCD40L. For example, although treatment with 1 μg/mL of rsCD40L efficiently caused cell death in SKOV3 cells under the condition of NF-κB blockage, this dosage of rsCD40L exerted no obvious effect on A549 cells in our study.

To the best of our knowledge, this is the first report that establishes a dual role of autocrine TNF-α in both pro-apoptotic and anti-apoptotic signaling in cell's response to rsCD40L. As NF-κB inhibition does not affect basal or rsCD40L induced TNF-α secretion but significantly sensitizes cancer cells to rsCD40L induced cytotoxicity, blocking NF-κB could be an effective therapeutic strategy for improving the anticancer potential of CD40-targeting therapy. In this regard, potential anticancer agents having a NF-κB-suppressing activity such as 17DMAG and luteolin would be candidates for combination therapy with CD40 agonists.

Acknowledgments

This study was supported by grants from National Natural Science Foundation of China (81172111) and by Program for New Century Excellent Talents in University from Chinese Ministry of Education (NCET-11-0349).

References

[1] C. Ahonen, E. Manning, L.D. Erickson, B. O'Connor, E.F. Lind, S.S. Pullen, M.R. Kehry, R.J. Noelle, The CD40-TRAF6 axis controls affinity maturation and the generation of long-lived plasma cells, Nat. Immunol. 3 (2002) 451–456.

[2] H. Jabara, D. Laouini, E. Tsitsikov, E. Mizoguchi, A. Bhan, E. Castigli, F. Dedeoglu, V. Pivniouk, S. Brodeur, R. Geha, The binding site for TRAF2 and TRAF3 but not for TRAF6 is essential for CD40-mediated immunoglobulin class switching, Immunity 17 (2002) 265–276.

[3] L.F. Lu, C.L. Ahonen, E.F. Lind, V.S. Raman, W.J. Cook, L.L. Lin, R.J. Noelle, The in vivo function of a noncanonical TRAF2-binding domain in the C-terminus of CD40 in driving B-cell growth and differentiation, Blood 110 (2007) 193–200.

[4] S. Vallabhapurapu, A. Matsuzawa, W. Zhang, P.H. Tseng, J.J. Keats, H. Wang, D.A. Vignali, P.L. Bergsagel, M. Karin, Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling, Nat. Immunol. 9 (2008) 1364–1370.

[5] A.C. Grammer, J.L. Swantek, R.D. McFarland, Y. Miura, T. Geppert, P.E. Lipsky, TNF receptor-associated factor-3 signaling mediates activation of p38 and Jun N-terminal kinase, cytokine secretion, and Ig production following ligation of CD40 on human B cells, J. Immunol. 161 (1998) 1183–1193.

[6] C.C. Davies, T.W. Mak, L.S. Young, A.G. Eliopoulos, TRAF6 is required for TRAF2-dependent CD40 signal transduction in nonhemopoietic cells, Mol. Cell. Biol. 25 (2005) 9806–9819.

[7] A.W. Tong, M.J. Stone, Prospects for CD40-directed experimental therapy of human cancer, Cancer Gene Ther. 10 (2003) 1–13.

[8] N.M. Bereznaya, V.F. Chekhun, Expression of CD40 and CD40L on tumor cells: the role of their interaction and new approach to immunotherapy, Exp. Oncol. 29 (2007) 2–12.

[9] N. Voorzanger-Rousselot, L. Alberti, J.Y. Blay, CD40L induces multidrug resistance to apoptosis in breast carcinoma and lymphoma cells through caspase independent and dependent pathways, BMC Cancer 6 (2006) 75.

[10] B. Zheng, P. Fiumara, Y.V. Li, G. Georgakis, V. Snell, M. Younes, J.N. Vauthey, A. Carbone, A. Younes, MEK/ERK pathway is aberrantly active in Hodgkin disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell proliferation and survival, Blood 102 (2003) 1019–1027.

[11] A.G. Eliopoulos, C. Davies, P.G. Knox, N.J. Gallagher, S.C. Afford, D.H. Adams, L.S. Young, CD40 induces apoptosis in carcinoma cells through activation of cytotoxic ligands of the tumor necrosis factor superfamily, Mol. Cell. Biol. 20 (2000) 5503–5515.

[12] N.T. Georgopoulos, A. Merrick, N. Scott, P.J. Selby, A. Melcher, L.K. Trejdosiewicz, CD40-mediated death and cytokine secretion in colorectal cancer: a potential target for inflammatory tumour cell killing, Int. J. Cancer 121 (2007) 1373–1381.

[13] C.C. Davies, J. Mason, M.J. Wakelam, L.S. Young, A.G. Eliopoulos, Inhibition of phosphatidylinositol 3-kinase- and ERK MAPK-regulated protein synthesis reveals the pro-apoptotic properties of CD40 ligation in carcinoma cells, J. Biol. Chem. 279 (2004) 1010–1019.

[14] H.J. Coope, P.G. Atkinson, B. Huhse, M. Belich, J. Janzen, M.J. Holman, G.G. Klaus, L.H. Johnston, S.C. Ley, CD40 regulates the processing of NF-kappaB2 p100 to p52, Embo J. 21 (2002) 5375–5385.

[15] I. Berberich, G.L. Shu, E.A. Clark, Cross-linking CD40 on B cells rapidly activates nuclear factor-kappa B, J. Immunol. 153 (1994) 4357–4366.

[16] M. Travert, P. Ame-Thomas, C. Pangault, A. Morizot, O. Micheau, G. Semana, T. Lamy, T. Fest, K. Tarte, T. Guillaudeux, CD40 ligand protects from TRAIL-induced apoptosis in follicular lymphomas through NF-kappaB activation and up-regulation of c-FLIP and Bcl-xL, J. Immunol. 181 (2008) 1001–1011.
[17] X. Wang, W. Ju, J. Renouard, J. Aden, S.A. Belinsky, Y. Lin, 17-allylamino-17-demethoxygeldanamycin synergistically potentiates tumor necrosis factor-induced lung cancer cell death by blocking the nuclear factor-kappaB pathway, Cancer Res. 66 (2006) 1089–1095.
[18] Q. Wang, X.L. Zheng, L. Yang, F. Shi, L.B. Gao, Y.J. Zhong, H. Sun, F. He, Y. Lin, X. Wang, Reactive oxygen species-mediated apoptosis contributes to chemosensitization effect of saikosaponins on cisplatin-induced cytotoxicity in cancer cells, J. Exp. Clin. Cancer Res. 29 (2010) 159–166.
[19] H. Sun, Y.J. Zhong, X.L. Zheng, Q. Wang, L. Yang, F. Shi, J.Q. Yan, F. He, L.C. Liao, Y. Lin, L. Zhang, X. Wang, Critical role of CD40-mediated autocrine tumor necrosis factor-alpha in potentiation of cisplatin-induced cytotoxicity in cancer cells, Cancer Sci. 103 (2012) 197–202.
[20] N.T. Georgopoulos, L.P. Steele, M.J. Thomson, P.J. Selby, J. Southgate, L.K. Trejdosiewicz, A novel mechanism of CD40-induced apoptosis of carcinoma cells involving TRAF3 and JNK/AP-1 activation, Cell Death Differ. 13 (2006) 1789–1801.
[21] C.C. Davies, D. Bem, L.S. Young, A.G. Eliopoulos, NF-kappaB overrides the apoptotic program of TNF receptor 1 but not CD40 in carcinoma cells, Cell. Signal. 17 (2005) 729–738.