Biochemical and Biophysical Research Communications 453 (2014) 106–111

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Identification of TGF-β-activated kinase 1 as a possible novel target for renal cell carcinoma intervention

Meng Fandong, Li Yan, Tian Xin, Fu Liye, Yin Yuanqin, Sui Chengguang, Ma Ping, Jiang Youhong *

Molecular Oncology Department of Cancer Research Institution, The First Hospital of China Medical University, Shengyang, Liaoning City 110001, China

ARTICLE INFO

Article history:
Received 12 September 2014
Available online 26 September 2014

Keywords:
Renal cell carcinoma (RCC)
TGF-β-activated kinase 1 (TAK1)
NF-κB activation
Apoptosis and chemo-resistance

ABSTRACT

Renal cell carcinoma (RCC) is common renal malignancy within poor prognosis. TGF-β-activated kinase 1 (TAK1) plays vital roles in cell survival, apoptosis-resistance and carcinogenesis through regulating nuclear factor-κB (NF-κB) and other cancer-related pathways. Here we found that TAK1 inhibitors (LYTAK1, 5Z-7-oxozeanol (5Z) and NG-25) suppressed NF-κB activation and RCC cell (786-O and A489 lines) survival. TAK1 inhibitors induced apoptotic cytotoxicity against RCC cells, which was largely inhibited by the broad or specific caspase inhibitors. Further, shRNA-mediated partial depletion of TAK1 reduced 786-O cell viability whiling activating apoptosis. Significantly, TAK1 was over-expressed in human RCC tissues, and its level was correlated with phosphorylated NF-κB. Finally, kinase inhibition or genetic depletion of TAK1 enhanced the activity of vinblastine sulfate (VLB) in RCC cells. Together, these results suggest that TAK1 may be an important oncogene or an effective target for RCC intervention.

1. Introduction

Renal cell carcinoma (RCC) is a common renal malignancy [1–3]. Currently, more than 50% of all RCC patients are found at advanced stages with local or systematic metastasis [1–3]. The prognosis of these advanced/metastatic RCC are poor [1–3]. Surgery remains the only curable therapeutic option for RCC. Combination of chemotherapy could help RCC patient survival, however chemotherapy is considered to be of limited value probably due to pre-existing or acquired chemo-resistance [1,4–6]. Therefore, improvement of treatment efficiency for RCC greatly depends on the identification of key targets (i.e. novel oncogenes), and the search for more efficient agents against these targets [4,5].

One possible target is TGF-β-activated kinase-1 (TAK1), which is a mitogen-activated protein kinase kinase kinase (MAPKKK) [7,8]. It mediates activation of nuclear factor κB (NF-κB) and other signaling pathways to promote cell survival, and to suppress pro-apoptotic signaling [8]. Meanwhile, TAK1 activation also regulates resistance to chemotherapeutic drugs [9–11]. Recent studies have shown that TAK1 might be a key oncogene that is over-expressed and/or over-activated in many tumors. TAK1 inhibition could induce cancer cell apoptotic death [9–12]. Thus, TAK1 may be an effective target for cancer intervention [7,9,10]. However, it is not known the expression of TAK1 in RCC, and if inhibition of TAK1 is effective in killing RCC cells, or at least reducing the chemo-resistance to conventional therapeutic drugs.

In this study, we found that TAK1 is over-expressed in and human RCC tissues. Genetic silencing or inhibition of TAK1 kinase activity induces RCC cell apoptotic death.

2. Materials and methods

2.1. Chemical and reagents

LYTAK1 was obtained from Lilly Co (Indianapolis, IN). NF-25 (Catalog No. HY-15434, MedChem Express) was purchased from Guangzhou Qi-yun Biotechnology Co. Ltd. (Guangzhou, China). Vinblastine sulfate and 5Z-7-Oxozeanol were obtained from Sigma–Aldrich Chemicals (Saint Louis, MO). Z-VAD-fmk and Ac-DEVD-CHO were obtained from Calbiochem (San Diego, CA). All inhibitors were dissolved in dimethyl sulfoxide (DMSO) at a stock concentration. The concentration of DMSO did not exceed 0.1% in any assays. Phospho-TAK1 (Thr-184/187), TAK1, phospho-NF-κB p105 (Ser-933) and tubulin antibodies were purchased form Cell Signaling Tech (Denver MA). Goat anti-rabbit and mouse horseradish peroxidase (HRP)-conjugated IgG were purchased from Santa Cruz biotechnology (Santa Cruz, CA).

Abbreviations: 5Z, 5Z-7-oxozeanol; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NF-κB, nuclear factor-κB; RCC, renal cell carcinoma; shRNA, small hairpin RNA; TAK1, TGF-β-activated kinase 1; VLB, vinblastine sulfate.

* Corresponding author at: 155 Nanjing North Street, He-ping District of Liaoning City, Shenyang Province 110001, China. Fax: +86 2483282311.
E-mail address: youyuanhongyeah@yeah.net (Y. Jiang).

http://dx.doi.org/10.1016/j.bbrc.2014.09.070
0006-291X/

2.2. Cells

Human RCC cell lines 786-O and A489 were purchased from Shanghai Biological Institute (Shanghai, China). RCC cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). HK-2, an immortalized proximal tubule epithelial cell line from normal adult human kidney [13], HK-2 cells were cultured in DMEM/Ham's F12 (Life Technologies, Paisley, UK) supplemented with 5% fetal calf serum (FCS), 2 mM glutamine (Life Technologies Ltd., Paisley, UK), 20 mM HEPES buffer, 0.4 μg/ml hydrocortisone, 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml sodium selenite (Sigma).

2.3. MTT cell viability assay

Cells were seeded onto 96-well plates (5000 per well) and allowed to attach overnight. Vehicle (0.1% DMSO) or drug was added the following day. Cell viability was tested using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (Sigma, China) according to the manufacturer's instructions. Absorbance was measured at 490 nm through Universal Microplate Reader (Bio-Tek instruments). The OD value of treatment group was always normalized to that of untreated control group.

2.4. The "clonogenicity" assay

Cells were suspended in 1 mL of RPMI containing 0.25% agar (Sigma, Shanghai, China), 10% FBS and indicated treatments. The cell suspension was then added on the top of a pre-solidified 100 mm culture dish. The drug-containing medium was refreshed every two days. After 10 days of incubation, the remaining survival large colonies (>50 μm in diameter) were manually counted, and the number was normalized to that of untreated control group.

2.5. Annexin V FACS analysis of cell apoptosis

Cells (3×10^5) were seeded onto 60-mm dishes and treated the following day with indicated treatment. Floating and attached cells were then collected together and stained with Annexin V-APC (Invitrogen, San Diego, CA) and PI (Invitrogen, San Diego, CA) for flow cytometry measurement of apoptosis levels (BD bioscience). The percentage of Annexin V was recorded as apoptosis intensity.

2.6. Histone-bound DNA ELISA analysis of cell apoptosis

Cytosolic histone-bound DNA fragments caused by apoptosis were detected using a cell apoptosis ELISA kit (Roche Applied Science, Mannheim, Germany) according to manufacturer's protocol [14,15]. Briefly, 1×10^4 cells were seeded in a 96-well microplate and incubated at 37 °C. After treatment, cells were then lysed with lysis buffer provided for 30 min at 25 °C. Supernatant (20 μL) was transferred into the streptavidin-coated multi-plate, anti-histone-biotin (4 μl) and anti-DNA-POD (4 μl) were added to each well, and the plate was shaken for 2 h. After being washed with incubation buffer, ABTS (2,2′-Azino-di[3-ethylbenzthiazolinesulfonate]) solution (100 μl) was pipetted into each well. The absorbance was read immediately in a microplate reader (BIO-RAD, model 3550) at 405 nm. The OD value was utilized as a quantitative indicator of cell apoptosis [14,15].

2.7. Western blots and data analysis

Whole-cell extracts were prepared using a lysis buffer containing 20 mm HEPES (pH 7.4), 150 mm NaCl, 12.5 mm β-glycerophosphate, 1.5 mM $MgCl_2$, 2 mM EGTA, 10 mM NaF, 2 mM DTT, 1 mM Na_3VO_4, 1 mm phenylmethylsulfonyl fluoride, 20 μM aprotinin, 0.5% Triton X-100 (all obtained from Sigma). Cell extracts were resolved on SDS–PAGE and transferred to Hypond-P membranes (GE Healthcare). The membranes were immunoblotted with indicated primary antibodies, and the bound antibodies were visualized with horseradish peroxidase-conjugated antibodies against rabbit or mouse IgG using the ECL western blotting system (GE Healthcare). The band intensity of each blot was quantified by Image J software (NIH) before normalizing with the corresponding loading controls.

2.8. Generation of TAK1 knockdown stable cell lines

Three non-overlapping human TAK1 small hairpin RNA (shRNA) sequences were chosen: 5′-GACACACATGACCAATAACAA-3' (TAK-1 shRNA-1) [16], 5′-GAGGAAAGCGTTTATTGTATT-3′ (TAK1-shRNA-2) [10] and 5′-CCCAATGGCTTATCTTACATT-3′ (TAK-1 shRNA-3) [10]. These sequences were cloned into the pSUPER-puro-retro vector (Promega, Shanghai, China), which was then transfected into HEK-293 cells with plasmids encoding viral packaging proteins VSVG and Hit-60 (Promega) [17] using Lipofectamine 2000 (Invitrogen) reagent with corresponding procedure. The virus-containing supernatants were collected after 48 h of transfection and filtered through a 0.45 μm filter (Corning, Shanghai, China). The RCC cells were then infected by the lentivirus in the presence of the polycation (Sigma) polybrene (Sigma), and the cells were selected by puromycin (0.5 μg/ml) until stable clones can be established. Expressions of TAK1 and equal loading tubulin were tested by Western blots.

2.9. Real-time PCR

Total RNA was isolated using TRIzol reagent (Invitrogen). Contaminated genomic DNA in total RNA was removed by RNase-free DNase I digestion (Invitrogen). Primers used for the amplification of TAK1 or GAPDH cDNA were synthesized as follows: TAK1 5′-primer (5′-GTC GGA AAC CCT TTG ATG AGA TT-3′), TAK1 3′-primer (5′-GCT CTC AAT GGG CTT AGG TAA ATT-3′). GAPDH 5′-primer (5′-CAATGACCCCTTCATTGACC-3′), GAPDH 3′-primer (5′-GACAAGCTTCCCGTTCTCAG-3′) [18]. A typical reaction (50 μl) contained 1/50 of reverse transcription-generated cDNA and 200 nM of primer in 1× SYBR Green RealTime Master Mix buffer (Toyobo, Tokyo, Japan). PCR reactions were carried out on a Bio-Rad IQ5 multicolor detection system by using 3 μg of synthesized cDNA under the following conditions: 95 °C for 5 min, 41 cycles at 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 30 s. One RNA sample of each preparation was processed without RT-reaction to provide a negative control in subsequent PCR. After amplification, melt curve analysis was performed to analyze product melting temperature. The GAPDH gene was chosen as the reference gene for normalization, and the $2^{-\Delta\Delta CT\ (\text{Cycle Threshold})}$ method [19] was applied to quantify TAK1 mRNA change within samples.

2.10. Human RCC specimens

Specimens were obtained from three RCC patients who underwent total nephroureterectomy. All patients were treated at the First Hospital of China Medical University (Shengyang, China). No patients had received irradiation or chemotherapy prior to surgery. In each fresh-isolated specimen, tumor tissues and the surrounding normal tissues were separated. Tissues were thoroughly washed in PBS containing antibiotics and DTT (2.5 mM), and then minced by scalpel into small pieces into DMEM plus 10% FBS containing 100 units/ml penicillin–streptomycin. Tissues were then analyzed by Western blots and real-time PCR. All patients enrolled provided individual informed consent with institutional review board approval of all protocols. These experiments were conducted

by the principles set out in the Declaration of Helsinki and the NIH Belmont Report.

2.11. Statistical analysis

Experimental results shown were repeated at least three times with similar results obtained. Results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using Student's *t*-test and one-way ANOVA using SPSS 12.0 (SPSS Inc, Chicago, IL). Significance was set at $p < 0.05$. The concentration of each drug used as well as the duration of each treatment were chosen based on literatures and pre-experiment results.

3. Results

3.1. Inhibition of TAK1 kinase activity suppresses NF-κB activation and RCC cell survival

We first investigated the effect of TAK1 inhibitors on RCC cell survival. Results showed that LYTAK1, a specific TAK1 inhibitor [10], reduced the viability of 786-O RCC cells (Fig. 1A and B). The effect of LYTAK1 was both time- (Fig. 1A) and dose-dependent (Fig. 1B). Cell viability decrease started 48 h after LYTAK1 (100 nM) stimulation (Fig 1A), and only relatively high concentrations of LYTAK1 (10–100 nM) inhibited 786-O cell survival (Fig. 1B). Similar activity by LYTAK1 was also observed in another RCC cell line (A489) (Fig. 1C and D). Two other TAK1 inhibitors, including 5Z-7-oxozeanol (5Z) [11] and NG-25 (NG) [20,21], also inhibited the viability of 786-O/A489 RCC cells (Fig. 1E and F). Thus, inhibition of TAK1 activity suppresses the survival of RCC cells. Notably, as shown in Fig. 1G, LYTAK1 (50–100 nM) showed limited effect on the survival of non-cancerous proximal tubule epithelial cells (HK-2) [13], indicating the selective activity of LYTAK1 against cancer cells. TAK1 is a known upstream kinase for NF-κB activation, which is important for RCC progression and cell survival [22–24]. We then tested the effect of TAK1 inhibitors on NF-κB phosphorylation at Ser-933 (the indicator of NF-κB activation) in RCC cells, and results demonstrated that TAK1 inhibitors (LYTAK1, 5Z and NG-25) significantly decreased NF-κB activation in both 786-O cells and A489 cells (Fig. 1H and I). As expected,

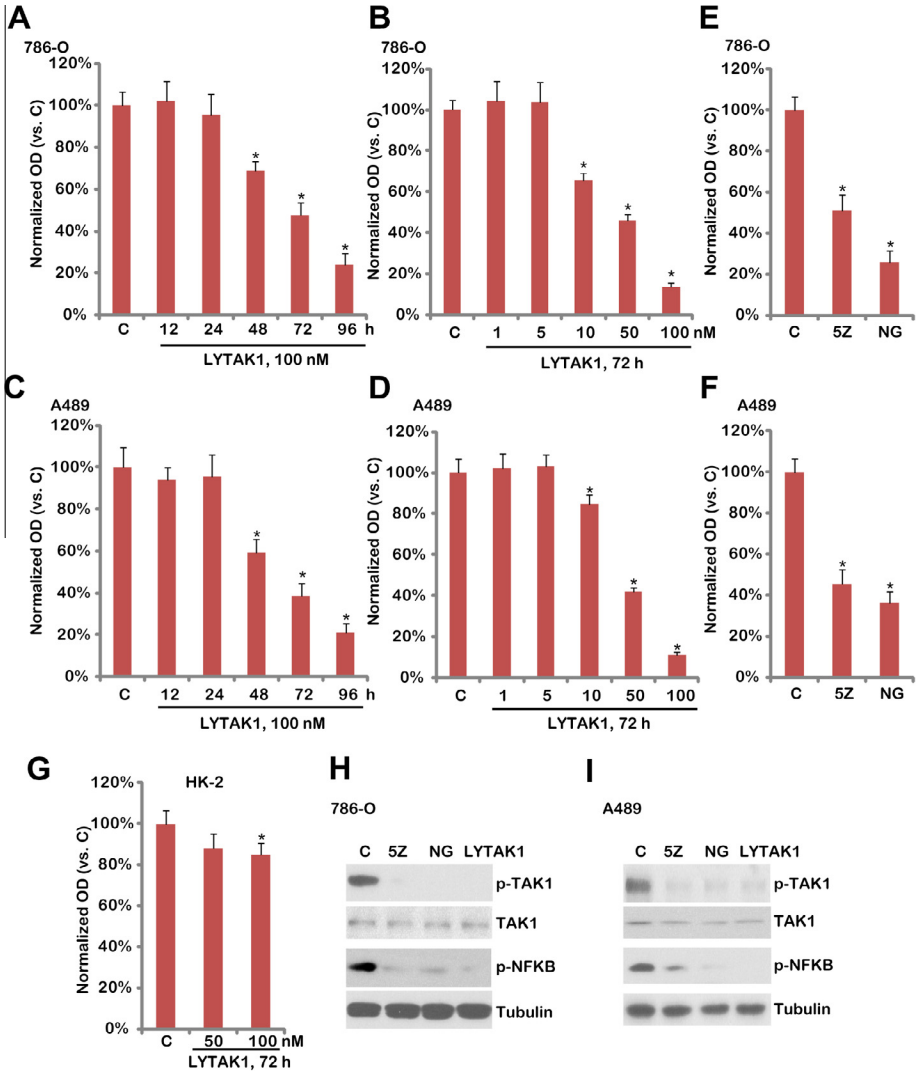

Fig. 1. Inhibition of TAK1 kinase activity suppresses NF-κB activation and RCC cell survival. The cell viability of 786-O/A489 cells treated with LYTAK1 (100 nM) for indicated time (12–96 h), or treated with indicated concentrations of LYTAK1 (1–100 nM) for 72 h, was tested by MTT assay (A–D). The cell viability of 786-O/A489 cells treated with 5Z-7-oxozeanol (5Z, 0.25 μM) or NG-25 (NG, 2.5 μM) for 72 h was tested (E and F). The cell viability of HK-2 cells after indicated LYTAK1 stimulation was tested (G). 786-O/A489 RCC cells were treated with LYTAK1 (100 nM), 5Z-7-oxozeanol (5Z, 0.25 μM) or NG-25 (NG-25, 2.5 μM) for 24 h, Western blots were applied to tested list proteins (H and I). "C" stands for untreated control group. Experiments in this figure were repeated three times. Data were normalized to group "C", and were presented as mean ± SD. *$p < 0.05$ vs. group "C".

TAK1 inhibitors blocked TAK1 phosphorylation in RCC cells (Fig. 1H and I). Thus, inhibition of TAK1 kinase activity suppresses NF-κB phosphorylation and RCC cell survival.

3.2. Inhibition of TAK1 kinase activity induces apoptotic cytotoxicity against RCC cells

We then focused on the effect of TAK1 inhibitors on RCC cell apoptosis, which was detected by Annexin V FACS assay and histone-DNA apoptosis ELISA assay [14,25]. Both assays showed that LYTAK1 induced significant cell apoptosis in 786-O cells, which was inhibited by the broad caspase inhibitor z-VAD-fmk ("ZVAD") and the caspase-3 specific inhibitor DEVD-CHO ("DVED") (Fig. 2A and B). Both z-VAD-fmk and DEVD-CHO inhibited LYTAK1-induced 786-O viability decrease as well (Fig. 2C). The results from clonogenicity assay illustrated that LYTAK1 dramatically downregulated the number of viable 786-O colonies, and such an effect was inhibited by both caspase inhibitors (Fig. 2D). Further, two other TAK1 inhibitors (5Z and NG) also decreased the number of viable 786-O colonies, which was again alleviated by z-VAD-fmk (Fig. 2E). In A489 cells, TAK1 inhibitors-mediated viability reduction was also suppressed by z-VAD-fmk (Fig. 2F). Thus, these results suggest that TAK1 inhibitors induce apoptotic cytotoxicity against RCC cells.

3.3. Partial depletion of TAK1 by target shRNAs inhibits RCC cell survival while activating apoptosis

To rule out the possible off-target toxicity of the TAK1 inhibitors, shRNA strategy was applied to selectively knockdown TAK1 in 786-O cells. Western blot and real-time PCR results in Fig. 3A demonstrated that TAK1 protein and mRNA expressions were significantly downregulated in stable 786-O cells expressing TAK1 shRNAs. We applied three non-overlapping shRNAs against non-overlapping sequence of human TAK1 mRNA, and all three of them dramatically inhibited TAK1 expression independently (Fig. 3A). The scramble control (SC) shRNA had no significant effect on TAK1 expression (Fig. 3A). Further, p-NF-κB was inhibited by TAK1 depletion, once again confirming that TAK1 acts as the upstream kinase for NF-κB activation in RCC cells (Fig. 3A, upper panel). The stable 786-O cells with TAK1 shRNAs showed reduced cell viability (Fig. 3B) and spontaneous cell apoptosis (Fig. 3C). These similar results were also seen in A489 cells (Data not shown). Thus, TAK1 is important for RCC cell survival, and genetic depletion of TAK1 could induce RCC cell apoptosis.

3.4. TAK1 and p-NF-κB are both over-expressed in human RCC tissues

We also tested expression of TAK1 in both human RCC tissues ("T") and surrounding normal kidney tissues ("N"). Western blot data showed that TAK1 and p-NF-κB were both over-expressed in three tested human RCC tissues, as compared to the surrounding normal tissues (Fig. 3D). TAK1 and p-NF-κB expressions were two–three times higher in cancer tissues (Fig. 3E), and their expressions were correlated (Fig. 3E). Real-time PCR results confirmed upregulation of TAK1 mRNA in RCC tissues (Fig. 3F).

3.5. Inhibition or depletion of TAK1 enhances the activity of vinblastine sulfate in RCC cells

Vinblastine sulfate (VLB)-based chemotherapy is currently used to reduce tumor recurrence and prolong postoperative survival in RCC [4,5,26]. The limited response rate for VLB as a single agent in metastasis RCC treatment has led to the alternative modulation strategies [4,5,26]. Above results show that TAK1 is important for

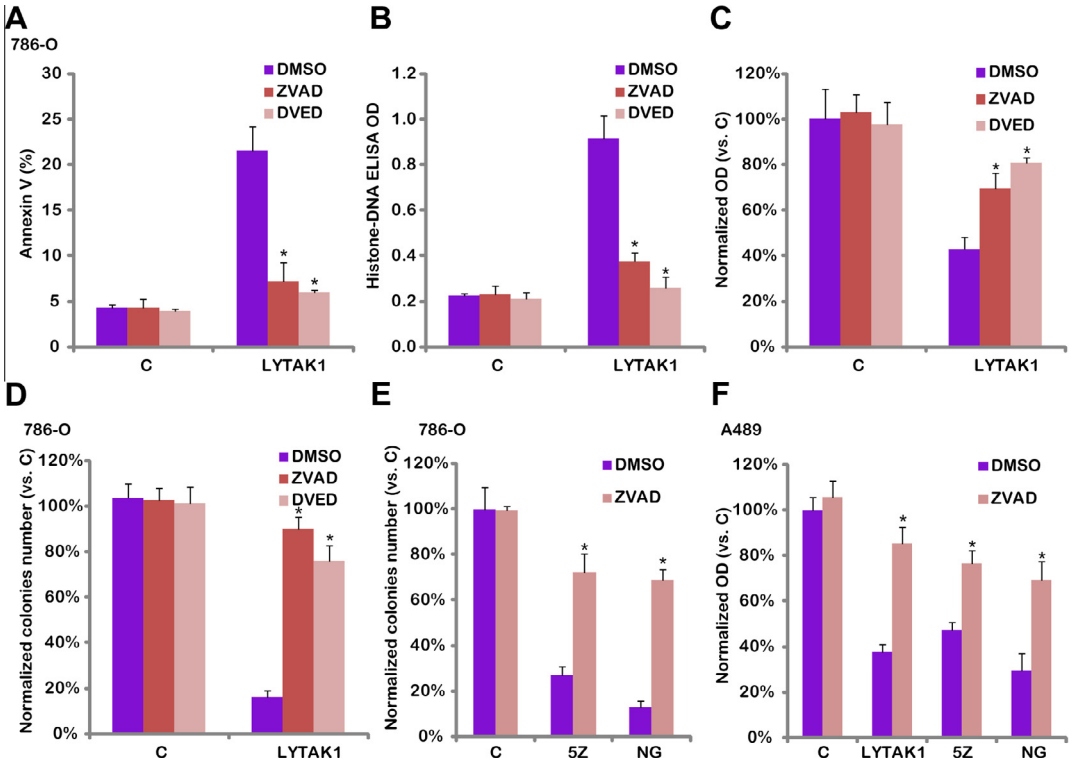

Fig. 2. Inhibition of TAK1 kinase activity induces apoptotic RCC cell death. 786-O cells, pretreated with z-VAD-fmk (ZVAD, 50 μM) or DEVD-CHO (DVED, 50 μM) for 1 h, were stimulated with LYTAK1 (100 nM), cells were then cultured, cell apoptosis was analyzed by Annexin V FACS assay (A) and histone-DNA apoptosis ELISA assay (B) after 48 h; cell viability was tested by MTT assay after 72 h (C). The effect of z-VAD-fmk (ZVAD, 50 μM) or DEVD-CHO (DVED, 50 μM) on LYTAK1 (100 nM), 5Z-7-oxozeanol (5Z, 0.25 μM) or NG-25 (NG, 2.5 μM)-induced 786-O colonies formation was tested by "clonogenicity" assay (D and E). The cell viability of A489 cells treated with 72 h of LYTAK1 (100 nM), 5Z-7-oxozeanol (5Z, 0.25 μM) or NG-25 (NG, 2.5 μM), co-stimulated with z-VAD-fmk (50 μM), was tested by MTT assay (F). Experiments in this figure were repeated three times. "C" stands for untreated control group. Data were normalized to group "C" and were presented as mean ± SD. $^*p < 0.05$ vs. DMSO (0.1%) group.

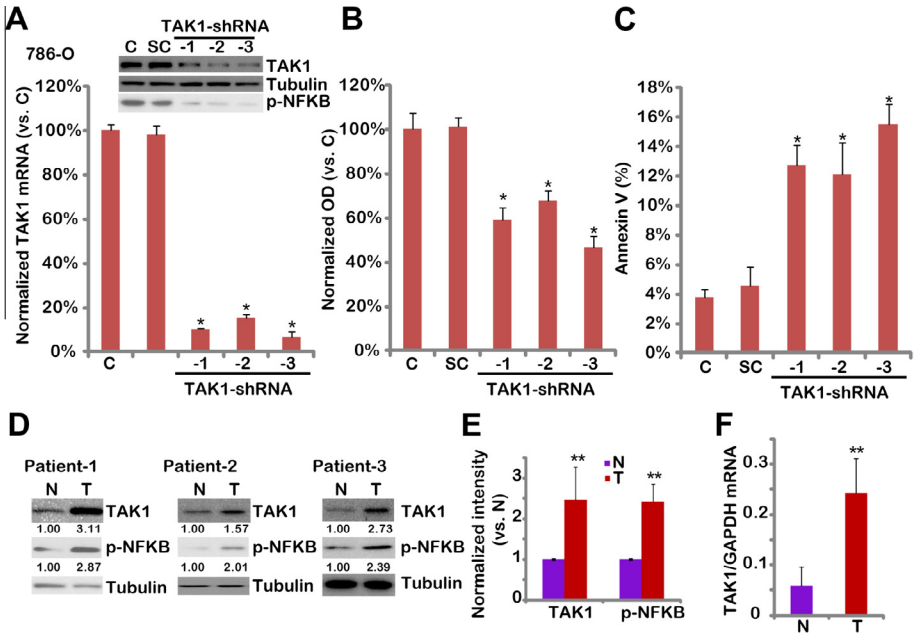

Fig. 3. RCC cells with TAK1 shRNA show reduced cell viability and increased cell apoptosis. The exactly same number of stable 786-O cells expressing scramble control (SC) shRNA or TAK1-shRNAs (−1/−2/−3) were cultured, TAK1 protein and mRNA expressions were tested by Western blots and real-time PCR respectively (A), p-NF-κB and tubulin in these lines were tested by Western blots (A); cell viability was assayed after 72 h of culture (B); cell apoptosis was examined after 48 h of culture (C). Expressions of TAK1, p-NF-κB and tubulin in human RCC tissues ("T") and corresponding surrounding normal tissues ("N") were tested by Western blots (D), TAK1 and p-NF-κB expressions in "T" tissues were normalized to "N" tissues (E). TAK1/GAPDH mRNA expression was examined by real-time PCR (F). Experiments in this figure were repeated three times. Data were presented as mean ± SD. "C" stands for non-infection control. $^{*}p < 0.05$ vs. "C" group. $^{**}p < 0.05$ vs. "N" group.

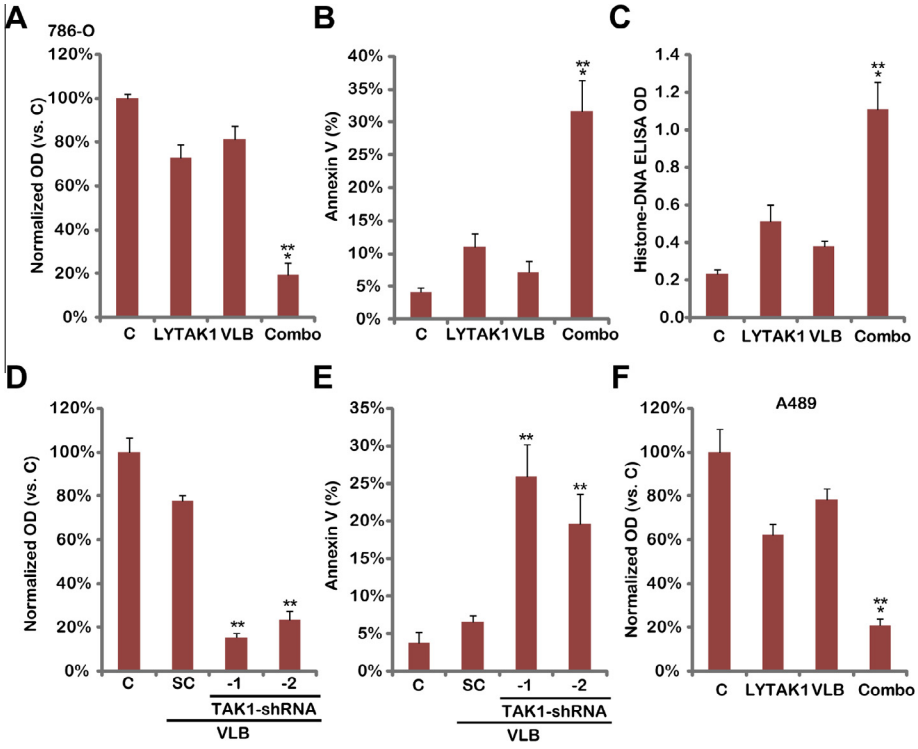

Fig. 4. Inhibition or depletion of TAK1 enhances the activity of VLB in RCC cells. 786-O cells were treated with LYTAK1 (100 nM), or in combination with vinblastine sulfate (VLB, 0.25 μM), cells were further cultured, cell viability was tested by MTT assay after 72 h (A), cell apoptosis was tested by Annexin V FACS assay (B) and histone-DNA apoptosis ELISA assay (C) after 48 h. Stable 786-O cells expressing scramble control (SC) shRNA or TAK1-shRNAs (−1/−2) were treated with VLB (0.3 μg/ml), cell viability (D) and cell apoptosis (E) were tested similarly. A489 cells were treated with LYTAK1 (100 nM), or in combination with VLB (0.3 μg/ml), for 72 h, cell viability was tested (F). Experiments in this figure were repeated three times. Data were normalized to group "C", and were presented as mean ± SD. $^{*}p < 0.05$ vs. LYTAK1 group. $^{**}p < 0.05$ vs. VLB group.

RCC cell survival, we thus hypothesized that TAK1 inhibition might increase VLB activity in RCC cells. As demonstrated, a low concentration of LYTAK1 (10 nM) dramatically enhanced VLB-induced viability reduction (Fig. 4A) and apoptosis (Fig. 4B and C) in 786-O cells. LYTAK1 (10 nM) alone only induced minor cytotoxicity against 786-O cells (Fig. 4A–C). Partial depletion of TAK1 by targeted-shRNAs also increased VLB-induced cytotoxicity in 786-O cells (Fig. 4D and E). In A489 RCC cells, LYTAK1 and VLB co-administration similarly caused a dramatic inhibition on cell viability, and its efficiency was much higher than either agent alone (Fig. 4F). Together, these results suggest that TAK1 might be a chemo-resistance factor in RCC, and TAK1 inhibition or depletion could enhance VLB's activity at least *in vitro*.

4. Discussions

RCC, especially the metastatic or advanced forms, remains to be one of the most lethal human malignancies [2,3]. One of the major challenges is to develop effective therapeutic strategies that target the key molecular alterations of RCC, aiming to improve patients' survival [2,3]. We here observed that TAK1 is over-expressed in human RCC tissues. Our study is among the first to demonstrate that genetic silencing or inhibition of the kinase activity of TAK1 could inhibit survival and induce apoptosis of RCC cells. Thus, TAK1 could be a possible novel target for RCC intervention.

TAK1 activates numerous signal transduction pathways associated with cancer progression [7,8]. For example, TAK1 acts as the key upstream kinase in the NF-κB pathway, which promotes cell survival and proliferation, thus becoming the focus of treatment intervention for many cancers [7–11]. In this study, we found that inhibition of TAK1 by its inhibitors (LYTAK1, 5Z or NF-25), or by shRNA-mediated silencing, suppressed NF-κB activation. While in human RCC tissues, high level of p-NF-κB was correlated with over-expressed TAK1. Thus, we propose that NF-κB might be the downstream target of TAK1 to mediate TAK1's activity in promoting cancer survival, apoptosis-resistance and progression in RCC cells.

We hypothesized that TAK1 might be responsible for the resistance of RCC cells to the pro-apoptotic effect of VLB. As TAK1 is important for NF-κB activation, the latter is a known pro-survival and chemo-resistance factor [22–24]. As a matter of fact, we found that inhibition or depletion of TAK1 reversed the resistance of RCC cells to VLB. Considering TAK1 and p-NF-κB were over-expressed in RCC tissues, our data suggest that TAK1-NF-κB pathway might represent a major chemo-resistance signaling in RCC.

In summary, the results of this study suggest that genetic silencing or inhibition of TAK1 kinase activity might be a potential therapeutic approach to inhibit RCC.

Conflict of interests

No conflict of interests by authors.

Acknowledgment

This study was supported by the Scientific Research Project of Liaoning Province Department of Education (ls2010170).

References

[1] R.J. Motzer, T.E. Hutson, D. Cella, J. Reeves, R. Hawkins, J. Guo, P. Nathan, M. Staehler, P. de Souza, J.R. Merchan, E. Boleti, K. Fife, J. Jin, R. Jones, H. Uemura, U. De Giorgi, U. Harmenberg, J. Wang, C.N. Sternberg, K. Deen, L. McCann, M.D. Hackshaw, R. Crescenzo, L.N. Pandite, T.K. Choueiri, Pazopanib versus sunitinib in metastatic renal-cell carcinoma, N. Engl. J. Med. 369 (2013) 722–731.
[2] H.T. Cohen, F.J. McGovern, Renal-cell carcinoma, N. Engl. J. Med. 353 (2005) 2477–2490.
[3] R.J. Motzer, N.H. Bander, D.M. Nanus, Renal-cell carcinoma, N. Engl. J. Med. 335 (1996) 865–875.
[4] R.J. Amato, Chemotherapy for renal cell carcinoma, Semin. Oncol. 27 (2000) 177–186.
[5] B. Ljungberg, N.C. Cowan, D.C. Hanbury, M. Hora, M.A. Kuczyk, A.S. Merseburger, J.J. Patard, P.F. Mulders, I.C. Sinescu, EAU guidelines on renal cell carcinoma: the 2010 update, Eur. Urol. 58 (2010) 398–406.
[6] J.P. Dutcher, D. Nanus, Long-term survival of patients with sarcomatoid renal cell cancer treated with chemotherapy, Med. Oncol. 28 (2011) 1530–1533.
[7] Y.S. Roh, J. Song, E. Seki, TAK1 regulates hepatic cell survival and carcinogenesis, J. Gastroenterol. 49 (2014) 185–194.
[8] H. Sakurai, Targeting of TAK1 in inflammatory disorders and cancer, Trends Pharmacol. Sci. 33 (2012) 522–530.
[9] S.E. Martin, Z.H. Wu, K. Gehlhaus, T.L. Jones, Y.W. Zhang, R. Guha, S. Miyamoto, Y. Pommier, N.J. Caplen, RNAi screening identifies TAK1 as a potential target for the enhanced efficacy of topoisomerase inhibitors, Curr. Cancer Drug Targets 11 (2011) 976–986.
[10] D. Melisi, Q. Xia, G. Paradiso, J. Ling, T. Moccia, C. Carbone, A. Budillon, J.L. Abbruzzese, P.J. Chiao, Modulation of pancreatic cancer chemoresistance by inhibition of TAK1, J. Natl. Cancer Inst. 103 (2011) 1190–1204.
[11] A. Singh, M.F. Sweeney, M. Yu, A. Burger, P. Greninger, C. Benes, D.A. Haber, J. Settleman, TAK1 inhibition promotes apoptosis in KRAS-dependent colon cancers, Cell 148 (2012) 639–650.
[12] D.M. Ray, P.H. Myers, J.T. Painter, M.J. Hoenerhoff, K. Olden, J.D. Roberts, Inhibition of transforming growth factor-beta-activated kinase-1 blocks cancer cell adhesion, invasion, and metastasis, Br. J. Cancer 107 (2012) 129–136.
[13] M.J. Ryan, G. Johnson, J. Kirk, S.M. Fuerstenberg, R.A. Zager, B. Torok-Storb, HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney, Kidney Int. 45 (1994) 48–57.
[14] F.G. Yang, Z.W. Zhang, D.Q. Xin, C.J. Shi, J.P. Wu, Y.L. Guo, Y.F. Guan, Peroxisome proliferator-activated receptor gamma ligands induce cell cycle arrest and apoptosis in human renal carcinoma cell lines, Acta Pharmacol. Sin. 26 (2005) 753–761.
[15] Y. Tu, C. Ji, B. Yang, Z. Yang, H. Gu, C.C. Lu, R. Wang, Z.L. Su, B. Chen, W.L. Sun, J.P. Xia, Z.G. Bi, L. He, DNA-dependent protein kinase catalytic subunit (DNA-PKcs)-SIN1 association mediates ultraviolet B (UVB)-induced Akt Ser-473 phosphorylation and skin cell survival, Mol. Cancer 12 (2013) 172.
[16] M. Yu, D.T. Ting, S.L. Stott, B.S. Wittner, F. Ozsolak, S. Paul, J.C. Ciciliano, M.E. Smas, D. Winokur, A.J. Gilman, M.J. Ulman, K. Xega, G. Contino, B. Alagesan, B.W. Brannigan, P.M. Milos, D.P. Ryan, L.V. Sequist, N. Bardeesy, S. Ramaswamy, M. Toner, S. Maheswaran, D.A. Haber, RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis, Nature 487 (2012) 510–513.
[17] A. Zecevic, H. Menard, V. Gurel, E. Hagan, R. DeCaro, A. Zhitkovich, WRN helicase promotes repair of DNA double-strand breaks caused by aberrant mismatch repair of chromium-DNA adducts, Cell Cycle 8 (2009) 2769–2778.
[18] A.R. Klatt, G. Klinger, O. Neumuller, B. Eidenmuller, I. Wagner, T. Achenbach, T. Aigner, E. Bartnik, TAK1 downregulation reduces IL-1beta induced expression of MMP13, MMP1 and TNF-alpha, Biomed. Pharmacother. 60 (2006) 55–61.
[19] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[20] J. Zhang, K. Clark, T. Lawrence, M.W. Peggie, P. Cohen, An unexpected twist to the activation of IKKbeta: TAK1 primes IKKbeta for activation by autophosphorylation, Biochem. J. 461 (2014) 531–537.
[21] E. Pauls, N. Shpiro, M. Peggie, E.R. Young, R.J. Sorcek, L. Tan, H.G. Choi, P. Cohen, Essential role for IKKbeta in production of type 1 interferons by plasmacytoid dendritic cells, J. Biol. Chem. 287 (2012) 19216–19228.
[22] D. Oka, K. Nishimura, M. Shiba, Y. Nakai, Y. Arai, M. Nakayama, H. Takayama, H. Inoue, A. Okuyama, N. Nonomura, Sesquiterpene lactone parthenolide suppresses tumor growth in a xenograft model of renal cell carcinoma by inhibiting the activation of NF-kappaB, Int. J. Cancer 120 (2007) 2576–2581.
[23] K.V. Gurova, J.E. Hill, C. Guo, A. Prokvolit, L.G. Burdelya, E. Samoylova, A.V. Khodyakova, R. Ganapathi, M. Ganapathi, N.D. Tararova, D. Bosykh, D. Lvovskiy, T.R. Webb, G.R. Stark, A.V. Gudkov, Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-kappaB-dependent mechanism of p53 suppression in tumors, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 17448–17453.
[24] M. Oya, A. Takayanagi, A. Horiguchi, R. Mizuno, M. Ohtsubo, K. Marumo, N. Shimizu, M. Murai, Increased nuclear factor-kappa B activation is related to the tumor development of renal cell carcinoma, Carcinogenesis 24 (2003) 377–384.
[25] T. Yu, J. Li, H. Sun, C6 ceramide potentiates curcumin-induced cell death and apoptosis in melanoma cell lines in vitro, Cancer Chemother. Pharmacol. 66 (2010) 999–1003.
[26] S. Buti, M. Bersanelli, A. Sikokis, F. Maines, F. Facchinetti, E. Bria, A. Ardizzoni, G. Tortora, F. Massari, Chemotherapy in metastatic renal cell carcinoma today? A systematic review, Anticancer Drugs 24 (2013) 535–554.