Biochemical and Biophysical Research Communications 441 (2013) 507–513

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

ICAM-3 endows anticancer drug resistance against microtubule-damaging agents via activation of the ICAM-3-AKT/ERK-CREB-2 pathway and blockage of apoptosis

Kwang-Chul Ahn [a], Jae Yeon Choi [a], Jae-Sung Kim [a], Sang-Gu Hwang [a], Wun-Jae Kim [b], Jong Kuk Park [a,*], Hong-Duck Um [a,*]

[a] Department of Radiation Cancer Research, Korea Institute of Radiological and Medical Sciences, 215-4 Gongneung-Dong, Nowon-Gu, Seoul 139-706, Republic of Korea
[b] Department of Urology, College of Medicine, Chungbuk National University, Chungbuk, Republic of Korea

ARTICLE INFO

Article history:
Received 14 October 2013
Available online 26 October 2013

Keywords:
ICAM-3
Akt
ERK
CREB-2
Drug resistance
Apoptosis
Paclitaxel
Vincristine

ABSTRACT

In a previous study, we showed that induction of ICAM-3 endows radioresistance in cervical cancer [1]. To ascertain whether ICAM-3 also promotes anticancer drug resistance, mock control (H1299/pcDNA3) or ICAM-3-expressing stable transfectants (H1299/ICAM-3) of the non-small cell lung cancer (NSCLC) cell line, NCI-H1299, were generated and treated with the microtubule-damaging agents, paclitaxel (TXL) and vincristine (VCS). TXL-/VCS-treated H1299/ICAM-3 cells showed significantly lower levels of apoptosis, activation of caspases-3, 8 or 9, and decrease in anti-apoptotic protein levels, compared to H1299/pcDNA3 cells. Our data clearly indicate that ICAM-3 promotes drug resistance via inhibition of apoptosis. We additionally showed that Akt, ERK, and CREB-2 are located downstream of ICAM-3, and activation of the ICAM-3-Akt/ERK-CREB-2 pathway induces resistance against TXL and VCS. ICAM-3-expressing stable NCI-H460/ICAM-3 transfectant cells and radioresistant SiHa cells endogenously overexpressing ICAM-3 additionally showed drug resistance against TXL and VCS via activation of the ICAM-3-Akt/ERK-CREB-2 pathway. The finding that ICAM-3 endows drug resistance as well as radioresistance supports its potential utility as a major therapeutic target against cancer.

1. Introduction

Cancer is one of the major causes of death worldwide. Therapeutic options are currently subdivided into three main treatments, specifically, surgical treatment, radiotherapy, and anticancer drugs. Anticancer drugs are divided into several types according to their working target or mechanism, and include a number of families defined by both chemical structure and mechanism of action, including alkylating agents, antibiotics, antimetabolites, topoisomerase I and II inhibitors, microtubule-damaging agents, and platinum compounds [2,3]. In particular, microtubule-damaging agents suppress microtubule dynamics, resulting in blockage of mitosis and cancer cell death. Microtubule-damaging agents are further classified as microtubule-destabilizing agents that inhibit microtubule polymerization (including *Vinca* alkaloids, such as vincristine (VCS)), and microtubule-stabilizing reagents, such as paclitaxel (TXL), that promote microtubule formation [4]. These agents have various target binding sites in microtubules. Binding results in cell death in several blood/solid cancers, including lung cancer. However, resistance against TXL and VCS treatment in cancer is documented. For instance, overexpression of ATP-dependent drug efflux pumps (ABC transporters) has been shown to decrease the intracellular concentrations of these drugs [5]. Additionally, expression of βIII tubulin and Aurora kinase, mutation of the β1-tubulin gene, and alterations in stathmin, Tau, and MCAK proteins have been recently identified as major factors involved in resistance against microtubule-damaging agents [3]. Despite the problems posed by resistance, these agents remain a treatment effective treatment option for various cancers. Therefore, clarification of the associated cell machinery and identification of novel resistance-inducing intracellular targets is essential to optimize their therapeutic efficacy.

The ICAM family of proteins comprises cell surface molecules homologous to the neural cell adhesion molecule (NCAM) and members of the type 1 immunoglobulin superfamily (IgSF) anchored at the cellular membrane [6]. These proteins contain cytoplasmic tail residues, transmembrane residues and extracellular domains, which contribute to the immune response and inflammation through various cell–cell interactions. Interestingly, ICAM proteins appear to have a dual role in cancer. ICAM molecules may target and block tumor progression via stimulation of an immune response, such as leukocyte activation [7,8]. Conversely,

* Corresponding authors.
E-mail addresses: jkpark@kcch.re.kr (J.K. Park), hdum@kcch.re.kr (H.-D. Um).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.10.096

other investigations have shown that overexpression of ICAM molecules is associated with increased cancer malignancy, leading to poor diagnosis, lower survival rates and metastasis in melanoma, breast cancer and leukemia [9–12]. Our group reported that ICAM-3 expression in several cancer cell types and specimens of cervical cancer patients induces enhanced radioresistance via activation of focal adhesion kinase (FAK) [1] and promotes cancer cell proliferation and migration/invasion through activation of Akt and p44/42 extracellular signal-regulated kinases (ERK) [13]. In the current study, we investigated whether ICAM-3 promotes anticancer drug resistance, in view of the finding that this family activates several proteins, such as integrins and IκB kinase (IKK), involved in drug resistance [7,9,10]. Moreover, the ICAM-3-Akt/ERK-CREB-2 pathway was identified as the intracellular machinery involved in ICAM-3-induced anticancer drug resistance. This pathway may be effectively utilized as a target to develop anticancer drugs that simultaneously overcome drug/radioresistance.

2. Materials and methods

2.1. Cell culture and reagents

The human NSCLC cell lines, NCI-H1299 and NCI-H460, and human cervical cancer cell line, SiHa, were purchased from American Type Culture Collection (Rockville, MD). LY294002, PD98052, and z-VAD-fmk were obtained from Calbiochem (La Jolla, CA, USA). Paclitaxel and vincristine were from Sigma (St. Louis, MO). Control (sc-37007; Santa Cruz Biotechnology, Santa Cruz, CA) and CREB-2 siRNA (Santa Cruz Biotechnology, sc-35112) were used for experiments.

2.2. Immunoblot assay

Immunoblot assays were performed as described previously [1]. Anti-ICAM-3 antibody was obtained from Abcam (Cambridge, UK), and caspase-3, caspase-8, phospho-Akt, Akt, phospho-CREB, and CREB antibodies from Cell Signaling Technology (Danvers, MA). MMP-2 and MMP-9 antibodies were purchased from Calbiochem (La Jolla, CA).

2.3. Electrophoretic mobility shift assay

The electrophoretic mobility shift assay (EMSA) kit was purchased from Promega Co. (Madison, WI), and the procedures performed as described in the manufacturer's protocol [14]. cAMP response element binding protein (CREB)-2-binding double-stranded oligomer, 5′-*GCG CGG AGC CGA TTA CAT CAG CCC GGG CCT*-3′, was synthesized by Bioneer Co. (Daejeon, Korea), according to a previous report [15]. Relative band density ratios of EMSA results were detected with ImageJ software and calculated.

2.4. RNA isolation, cDNA synthesis and PCR

Total RNA isolation for reverse transcription (RT)-PCR was performed with TRIzol (Invitrogen) using the manufacturer's protocol, and complementary DNA synthesized with the StrataScript reverse transcriptase kit (La Jolla, CA). PCR reactions were performed with the following primers: ICAM-3, 5′-*GTG AAC TGC AGT ACT GAT TGT*-3′ and 5′-*GGT GAT GTT AGA GGA GCC TGT*-3′; β2-microglobulin (β2M), 5′-*GTG GAG CAT TCA GAC TTG TCT TTC AGC A*-3′ and 5′-*TTC ACT CAA TCC AAA TGC GGC ATC TTC*-3′.

2.5. Cell counting assay

Cells were seeded at a density of 2×10^4 cells in 6-well plates, and treated with various doses of TXL or VCS. Cell numbers were determined using a hematocytometer under a microscope.

2.6. PI uptake assay

Propidium iodide (PI, Sigma Co.) was used to detect apoptotic death. Cells were seeded at a density of 1×10^5 cells in 6-well plates and irradiated with various doses of TXL and VCS. Treated cells were trypsinized, washed twice with cold PBS, and resuspended with 200 μL PI (5 μg/mL). Apoptosis was detected and analyzed using a FACSort flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

2.7. Tumor histological section, immunohistochemistry and TUNEL assay

Stable transfectants were injected subcutaneously into the trunks of BALB/cAnNCrj-nu/nu mice (Charles River Japan, Inc.) to construct xenografts, as described in a previous report [16]. NCI-H460/pcDNA3 or NCI-H460/ICAM-3 cells (3×10^6) were injected subcutaneously into the trunks of 6 week-old BALB/cAnNCrj-nu/nu mice (Charles River Japan, Inc.). Mice containing xenografts reaching a volume of more than 100 mm^3 were intraperitoneally injected with TXL (10 mg/kg) or VCS (0.5 mg/kg) twice a week for 2 weeks (four times in total). To perform histological analysis, xenografts were extracted, fixed with formaldehyde, and embedded in a paraffin block. Sliced tissues were stained and analyzed with the Apoptag™ TUNEL assay kit (Millipore Co.). Immunohistochemical detection (IHC) of ICAM-3 in each xenograft was performed with the CAP-PLUS™ Broad Spectrum kit (Zymed Laboratories Inc., South San Francisco, CA), as described in our previous study [17].

2.8. Statistical analysis

Data were calculated with GraphPad Prism software (GraphPad Software, La Jolla, CA), and differences (p values) between experimental groups determined with the t-test. The numbers above each point or bar in every graph indicate the mean percentage of three independent experiments, and error bars signify standard deviations (SD). $^{*}p$ value < 0.05, $^{**}p$ value < 0.001.

3. Results

3.1. ICAM-3 enhances resistance against microtubule-targeting anticancer drugs via inhibition of apoptosis

Although over expression of ICAM-3 has been shown to stimulate radio resistance, the relationship between ICAM-3 and drug resistance is not known. In the current study, we examined whether ICAM-3 additionally endows increased anticancer drug resistance. ICAM-3-expressing stable transfectants of NCI-H1299 (NCI-H1299/ICAM-3) and mock control (NCI-H1299/pcDNA3) cells were generated. High ICAM-3 expression in NCI-H1299/ICAM-3 was confirmed with the immunoblot assay (Fig. 1A). Cell death induction following treatment with microtubule-targeting reagents, TXL and VCS, was detected via PI uptake (Fig. 1B) or cell counting (Fig. S1A), and the cell survival rates calculated. Differences in the mean survival rates between NCI-H1299/ICAM-3 and NCI-H1299/pcDNA3 cells were ~18% in the presence of 20 nM TXL, and ~40% or 34% upon treatment with 10 or 20 nM VCS, respectively, as shown in Fig. S1A. Differences in the mean

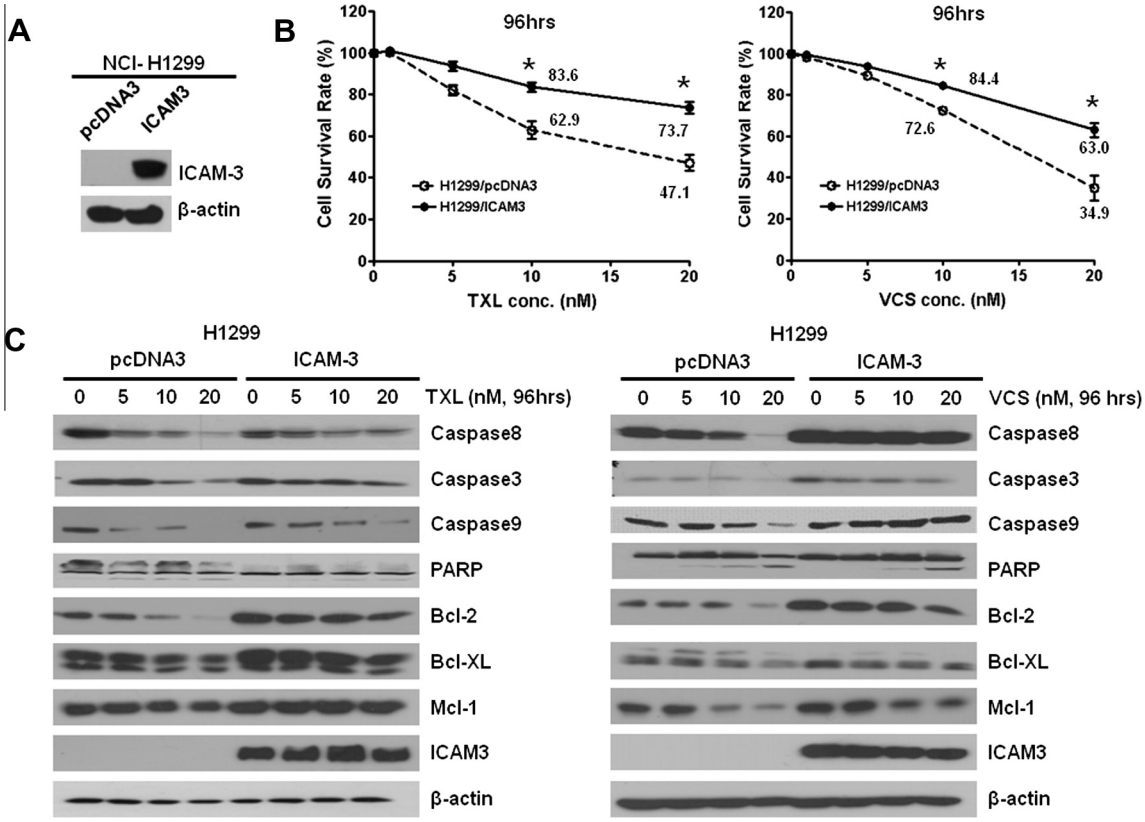

Fig. 1. ICAM-3 inhibits apoptotic cell death of NSCLC by microtubule statistically significant damaging reagents. (A) Immunoblot assay for detection of ICAM-3 in NCI-H1299/pcDNA3 and H1299/ICAM-3 cells. (B) PI uptake assay. Each transfectant was treated with 1, 5, 10, or 20 nM TXL or VCS. (C) Immunoblot assay for apoptotic protein detection. Each transfectant was treated with 1, 5, 10, or 20 nM TXL or VCS.

survival rates of NCI-H1299/ICAM-3 and NCI-H1299/pcDNA3 cells based on PI uptake were ~20% or 27% at 10 or 20 nM TXL and ~18% or 27% at 10 or 20 nM VCS, respectively (Fig. 1B). These survival rate differences between NCI-H1299/pcDNA3 and NCI-H1299/ICAM-3-transfected cells were statistically significant, indicating that ICAM-3 affords drug resistance via inhibition of apoptosis. As apoptosis constitutes the cell death mechanism induced by TXL and VCS, we subsequently focused on confirming whether apoptosis is the major cause of cell death in TXL and VCS-treated transfectants. Pre-treatment of cells with 20 μM z-VAD-fmk, a pan-caspase inhibitor, significantly suppressed apoptosis induced by both drugs (Fig. S1B), implying that apoptosis is the major cellular event in H1299/pcDNA3 and NCI-H1299/ICAM-3 cells treated with TXL and VCS. Treatment with TXL or VCS further induced cleavage of caspases-3, 8, 9, and PARP, which was blocked by ICAM-3 (Fig. 1C). To determine whether Bcl-2 superfamily proteins are involved in ICAM-3-induced drug resistance, levels of Bcl-2, Bcl-X_L and Mcl-1 proteins were examined with the immunoblot assay (Fig. 1D). Notably, activation of caspase, PARP cleavage, and degradation of Bcl-2 superfamily proteins were decreased in a dose-dependent manner upon treatment with TXL or VCS, but to a weaker extent in NCI-H1299/ICAM-3 cells. Our results clearly indicate that ICAM-3 inhibits intracellular apoptotic events induced by TXL and VCS.

3.2. Akt and ERK are located downstream of ICAM-3 and mediate ICAM-3-induced drug resistance

Akt and ERK were further identified as downstream molecules involved in ICAM-3-related intracellular events. We observed increased phosphorylation of Akt or ERK (Fig. 2A), and combinations of chemical inhibitors against Akt or ERK (LY294002 and PD98062, respectively) and TXL/VCS induced synergetic cell death. Cells were treated with TXL, VCS, TXL + LY294002/TXL + PD98062 or VCS + LY294002/VCS + PD98062 combinations, and subjected to the PI uptake assay (Fig. 2B) or cell counting assay (Fig. S2). Treatment with combinations of TXL/VCS and inhibitors led to higher cell death rates, compared to the groups treated with TXL or VCS only. Based on these results, we postulate that enhanced cell survival by ICAM-3 is attributed to activation of Akt or ERK. Our theory was confirmed with the immunoblot assay (Fig. 2C and D). TXL + LY294002/PD98062 and VCS + LY294002/PD98062 combinations promoted activation of caspases-3, 8 and 9, and degradation of Bcl-2 superfamily proteins, including Bcl-2, Bcl-X_L, and Mcl-1. Furthermore, these phenomena in NCI-H1299/ICAM-3 cells were weaker than those of NCI-H1299/pcDNA3 cells. As immunoblot assay data are consistent with quantitative analyses (Fig. S2 and B), we conclude that ICAM-3-induced activation of Akt and ERK directly affects cancer cell survival and apoptotic pathways.

3.3. CREB-2 is involved in ICAM-3-induced drug resistance

To further elucidate the mechanism underlying ICAM-3-induced drug resistance, we examined the involvement of CREB transcriptional factors, in view of the finding that CREB-1 is activated downstream of ICAM-3 [14]. NCI-H1299/ICAM-3 cells contained higher levels of activated CREB-2 than NCI-H1299/pcDNA3 cells (Fig. 3A). Moreover, enhanced activation of CREB-2 was attenuated upon treatment with the chemical inhibitors, LY294002 or PD98065 (Fig. 3B). The results indicate that ICAM-activated Akt or ERK sequentially induces stimulation of CREB-2 activity. Moreover, treatment with CREB-2 siRNA eliminated CREB-2 protein

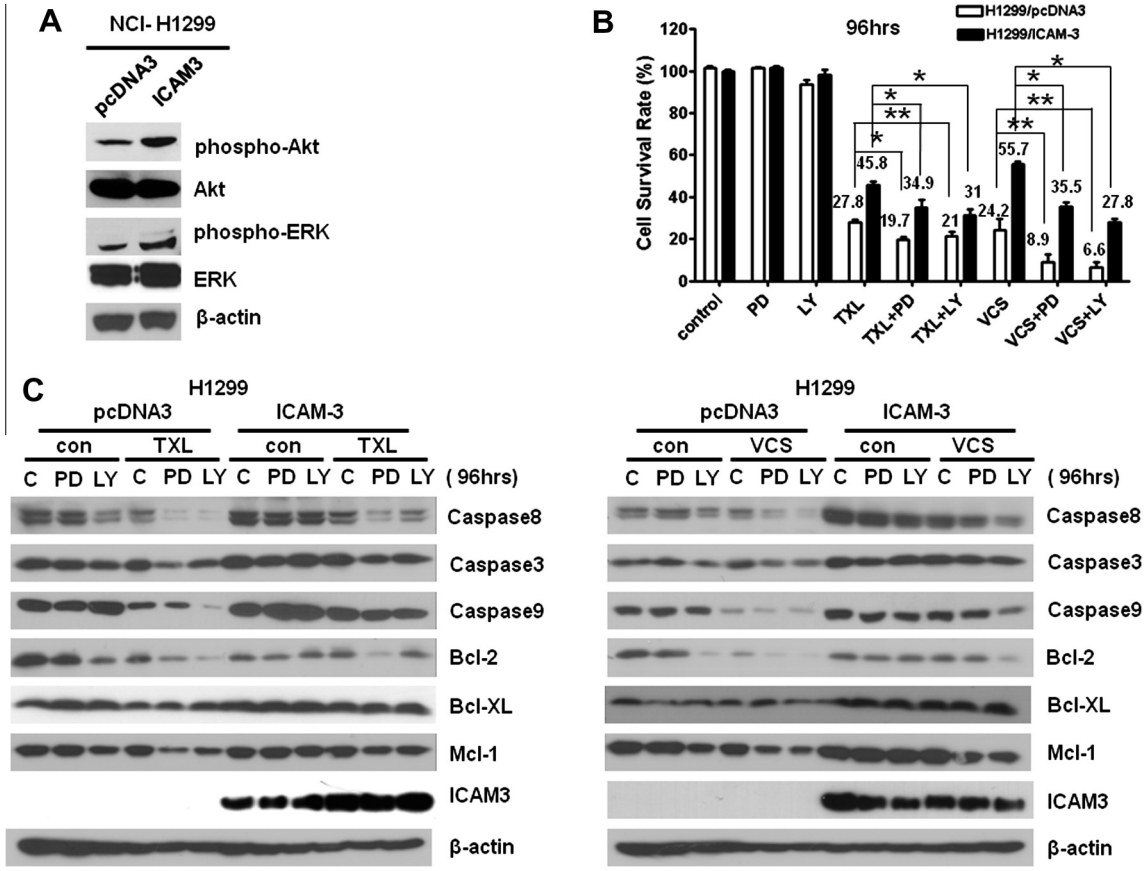

Fig. 2. Akt and the p44/42 ERK pathway may be involved in ICAM3-induced drug resistance against TXL and VCS in NCI-H1299/pcDNA3 and H1299/ICAM-3 cells. (A) Immunoblot assay with detection of Akt and ERK signaling. (B) PI uptake assay confirming that Akt and the p44/42 ERK pathway are located downstream of ICAM-3. Each transfectant was treated with 20 nM TXL/VCS + 20 μM LY294002(LY)/PD98059(PD). (C) Immunoblot assay for detection of apoptotic proteins in cells treated with combinations of 20 nM TXL/VCS + 20 μM LY294002/PD98059. C, PD or LY indicate control (DMSO), PD98059 and LY294002, respectively.

expression (Fig. 3C), and combined treatment with CREB-2 siRNA and TXL/VCS promoted apoptotic cell death (Fig. 3D). An immunoblot assay showed that combined CREB-2 siRNA and TXL/VCS treatment promotes increased activation of several caspases (Fig. 3E). These findings support the theory that CREB-2 promotes ICAM-3-induced resistance against TXL/VCS via inhibition of apoptosis.

3.4. Constitutively increased expression of ICAM-3 in another non-small cell lung cancer and radio resistant cancer cell lines enhances drug resistance

Since NCI-H1299 is a *p53*- and *PTEN* -null cell line, we hypothesized that enhancement of drug resistance by ICAM-3 in NCI-H1299 is independent of *p53* and *PTEN*. To confirm this theory, we examined whether ICAM-3-induced drug resistance is related to *p53* or *PTEN* expression. As the NCI-H460 cell line contains both wild-type *p53* and *PTEN* [18], we constructed ICAM-3-overexpressing stable transfectants (NCI-H460/ICAM-3) and mock control (NCI-H460/pcDNA3) cells. Data from an immunoblot assay (Fig. 4A) revealed increased expression of phospho-Akt and ERK (Fig. 4A), and elevated CREB-2 activation (Fig. 4B). Treatment of both cell lines with TXL/VCS led to 20% higher survival of NCI-H460/ICAM-3, compared to NCI-H460/pcDNA3 cells (Fig. 4C). This protective role of ICAM-3 was attributable to inhibition of apoptosis, as confirmed with detection of caspases-3, 8, 9 and anti-apoptotic Bcl-2 in an immunoblot assay (Fig. 4D). We additionally used SiHa cells and its radio resistant cell line (SiHa/R) to establish whether endogenous elevated ICAM-3 induces drug resistance against TXL/VCS with the same apoptotic machinery (Fig. 4E–H). SiHa/R cells contained elevated ICAM-3, phospho-Akt/ERK and activated CREB-2, compared to original parent SiHa cells (Fig. 4E and F). SiHa/R cells additionally showed resistance to TXL/VCS (Fig. 4G), reduced activation or cleavage of caspases and PARP, and lower decrease in Mcl-1 than SiHa cells treated with TXL/VCS (Fig. 4H). Since the SiHa cell line does not express Bcl-2, we observed expression of Mcl-1 instead of Bcl-2 [19]. To determine whether ICAM-3 induces similar drug resistance *in vivo*, NCI-H460/pcDNA3 and NCI-H460/ICAM-3 cells were injected into mice, and xenografts of each cell line formed. Extraction of tumor tissue after injection of TXL and VCS were performed, as described in Section 2. Using the TUNEL assay, apoptotic cells were counted and graphed as a percentage on the total cell population. The number of apoptotic NCI-H460/pcDNA3 cells was about 2.59-fold higher than that of NCI-H460/ICAM-3 cells following TXL treatment, and 2.16-fold higher following VCS treatment, respectively (Fig. S3). The results clearly suggest that ICAM-3 enhances drug resistance via blockage of apoptosis *in vivo* as well as *in vitro*.

4. Discussion

Several new functions of ICAM-3 have been identified in this study. Overexpression of ICAM-3 induced resistance against anti-microtubule reagents (Figs. 1 and S1A). Occurrence of drug resistance leads to failure of cancer treatment and consequent patient death in most cases, and therefore, further investigations are warranted to minimize drug resistance. Investigators have put forward diverse theories on anticancer drug resistance mechanisms.

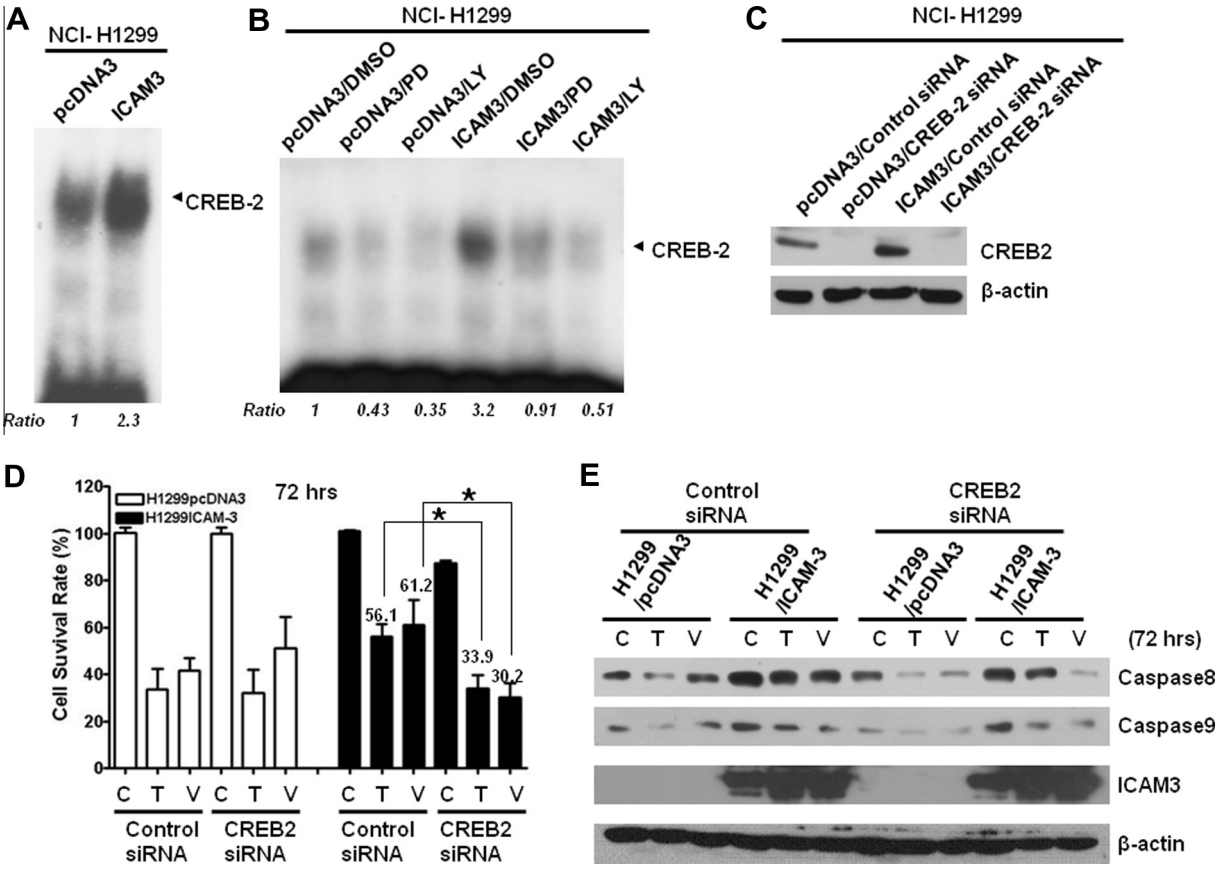

Fig. 3. CREB-2 is involved in ICAM-3-induced drug resistance. (A) EMSA assay for detection of CREB-2 activity. (B) EMSA assay for CREB-2 detected using samples pretreated with DMSO, PD98059 (20 nM, PD) or LY294002 (20 nM, LY). (C) Immunoblot assay to detect the effects of CREB-2 siRNA. (D) PI uptake assay. Each transfectant cell line was pre-treated with control siRNA or CREB-2 siRNA, followed by 20 nM TXL or VCS. (E) Immunoblot assay for detection of apoptotic proteins. Cells were treated with combinations of CREB-2 siRNA + 20 nM TXL/VCS. C, T and V for each immunoblot band indicate control (DMSO), TXL and VCS-treated samples, respectively.

Blagosklonny [20] presented two types of mechanisms underlying resistance to TXL, specifically, non-oncogenic and oncogenic. Detoxification of cytotoxic agents via pumping to the outside of cancer cells with the ABC transporter represents non-oncogenic resistance machinery. Occurrence of non-oncogenic resistance does not induce physiological changes in cancer cells. Oncogenic resistance stems from overexpression and activation of various oncogenes, such as Akt or Bcl-X_L. Our data indicate that oncogenic resistance is the major cause of ICAM-3-induced resistance against TXL or VCS, since overexpression of ICAM-3 activates oncogenes, including Akt or ERK, in various cancer cell lines (Figs. 2 and 4).

Apoptosis is mediated through two major pathways, specifically, intrinsic and extrinsic (or death receptor) mechanisms. The intrinsic pathway commences with external stress, followed by mitochondrial permeability (MPT) changes and caspase-9 activation. The extrinsic pathway involves death receptor/ligand binding and caspase-8 activation. Caspase activation is involved in every apoptotic pathway, whereby caspases-8 and -9 are 'initiators' of the extrinsic and intrinsic pathway, respectively. Caspase-3 is a common 'executioner' caspase in all apoptotic pathways [21]. Anti-apoptotic molecules, such as the Bcl-2 family, inhibit cytochrome *c* release from mitochondria via regulation of mitochondrial permeability. As ICAM-3 inhibited activation of both the intrinsic and extrinsic apoptotic cell death pathways and blocked degradation in our study, we postulated that ICAM-3-induced drug resistance results from regulation of caspase activation and mitochondrial permeability. Experiments performed to identify the downstream molecules involved in ICAM-3 activity revealed that drug resistance is evoked via activation of ICAM-3-Akt or p44/42 ERK-CREB-2 pathway (Fig. 2). Akt is activated/overexpressed, depending on the *PTEN* gene status in various cancers, including NSCLC, breast cancer and glioma, and known to induce drug resistance via inhibition of pro-apoptotic molecules, such as BAD, caspases, or activation of anti-apoptotic proteins, IKK and CREB [22]. ERK is part of the Ras/Raf/MEK/ERK pathway that has also significant effects on the regulation of apoptosis via post-translational phosphorylation of regulatory molecules, including Bad, Bim, Mcl-1, caspase 9 and Bcl-2 [23]. Therefore, our data are consistent with previous reports that activation of Akt or ERK induces drug resistance via inhibition of caspase activation and degradation of anti-apoptotic molecules (Fig. 2). We further demonstrated that CREB-2 is activated downstream of Akt and ERK in NCI-H1299/ICAM-3 cells (Fig. 3A and B). Elimination of CREB protein promoted cell death via increased caspase activation and Bcl-2 degradation (Fig. 3C–E). These results are in keeping with the previous finding that CREB proteins are located downstream of Akt and ERK, which induce CREB phosphorylation at Ser-133 and stimulate recruitment of CREB-binding protein (CBP) [24,25]. Thus, Akt stimulates expression of cell survival target genes statistically significant, including Mcl-1 and Bcl-2. Overexpression of ICAM-3 in another NSCLC cell line, NCI-H460, similarly endowed drug resistance via activation of the ICAM-3-Akt/ERK-CREB-2 pathway (Fig. 4A–D). Our results imply that enhancement of drug resistance by ICAM-3 in NSCLC cells is not related to *p53* and *PTEN*, since NCI-H1299 cells are devoid of *p53* and *PTEN* while the NCI-H460 cell line contains these molecules [18]. Several previous reports have shown

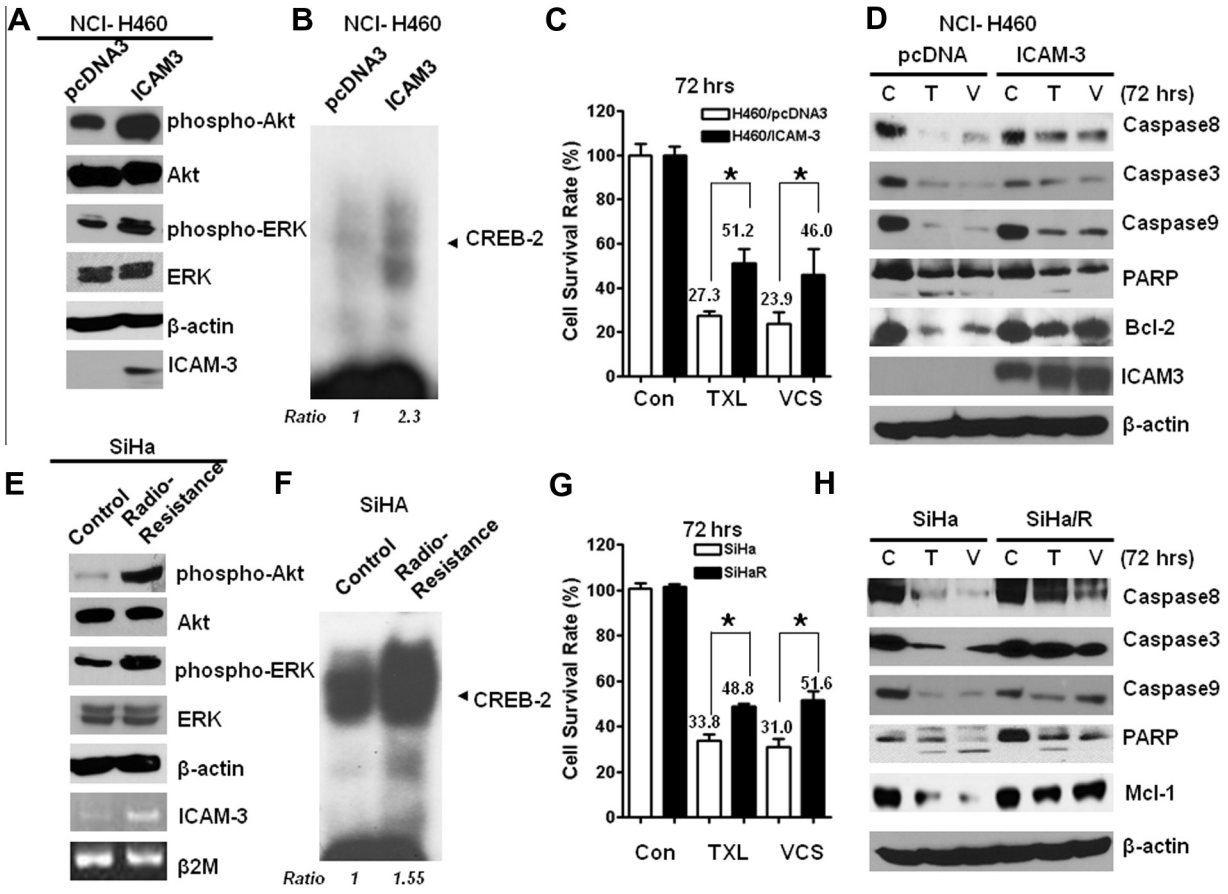

Fig. 4. Increased ICAM-3 expression in NCI-H460 and radioresistant cancer cells (SiHa-R) enhances drug resistance. (A–D) NCI-H460/pcDNA3 and H460/ICAM-3. (A) Immunoblot assay for detection of Akt and ERK signaling. (B) EMSA assay for detection of CREB-2. (C) PI uptake assay. Each transfectant cell line was treated with 20 nM TXL or VCS. (D) Immunoblot assay with apoptotic proteins. (E and F) SiHa cells and the radioresistant cell line, SiHa/R. (E) Immunoblot assay for detection of Akt and ERK signaling and RT-RCR assay for ICAM-3 or β2M. (F) EMSA assay for detection of CREB-2. (G) PI uptake assay for apoptotic cell death in SiHa and SiHa/R cells treated with 20 nM TXL or VCS. (H) Immunoblot assay with apoptotic proteins.

that both *p53* and *PTEN* are major tumor suppressors that regulate drug resistance [3,22]. However, our results indicate that enhancement of drug resistance by ICAM-3 is independent of intracellular *p53* and *PTEN* status (Fig. 4A–D). Additionally, overexpression of ICAM-3 in radioresistant SiHa (SiHa/R) cells increased drug resistance via the ICAM-3-Akt/ERK-CREB-2 pathway (Fig. 4E–H). These findings highlight the possibility that radioresistance and drug resistance in SiHa cells share common machinery starting with overexpression of ICAM-3. Interestingly, ICAM-3 overexpression in cancer patient specimens *in vivo* was detected in stromal tissues around cancer tissue, but not within the cancer [1,26]. Earlier investigators monitored a stroma-tumor coalition in cancer, and suggested environment-mediated drug resistance (EMDR). As one of the EMDR categories, cell adhesion-mediated drug resistance (CAM-DR) is mediated by adhesion of tumor cell integrins to stromal fibroblasts or components of the extracellular matrix (ECM). ICAM-1, one of the ICAM superfamily proteins, is also known to be involved in CAM-DR. CAM-RR is analogous term of CAM-DR referring to cell adhesion-mediated resistance to radiotherapy [27].

Our results collectively suggest a novel role of ICAM-3 in increasing anticancer drug resistance, which is mediated via ICAM-3-Akt or ERK-CREB-2 protein pathways, independent of *p53* and *PTEN*. Increased drug resistance shares a common intracellular signaling pathway with promotion of cell proliferation in ICAM-3 overexpressing cells [13]. In view of our previous results showing that radioresistance induced by ICAM-3 is dependent on FAK activation, we postulate that FAK is one of the signaling components of ICAM-3-induced drug resistance [1]. However, we have not investigated the involvement of FAK in this study. A number of investigators have reported that interactions between FAK and Akt are required for various processes, such as cell adhesion and survival, drug resistance and radioresistance [28–30]. A comprehensive set of studies on the effects of ICAM-3 signaling and related mechanisms, especially with the use of *in vivo* systems, is required to clarify the role of ICAM-3 in cancer, with a view to facilitating cancer treatment. Several monoclonal antibodies, antisense oligonucleotides and small molecules abolishing the action of ICAM-1 have been generated for the treatment of inflammation and rheumatoid arthritis [31]. Our findings on ICAM-3 and associated signaling pathways present novel therapeutic targets for immune diseases and cancer.

Acknowledgments

This work was supported by the Nuclear Research & Development Program of the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (2012M2A2A7010459) and, in part, by the Basic Science Research Program through the NRF (2008-0062611).

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.2013.10.096.

References

[1] Y.M. Chung, B.G. Kim, C.S. Park, S.J. Huh, J. Kim, J.K. Park, S.M. Cho, B.S. Kim, J.S. Kim, Y.D. Yoo, D.S. Bae, Increased expression of ICAM-3 is associated with radiation resistance in cervical cancer, Int. J. Cancer 117 (2005) 194–201.

[2] E. Espinosa, P. Zamora, J. Feliu, M. González Barón, Classification of anticancer drugs – a new system based on therapeutic targets, Cancer Treat. Rev. 29 (2003) 515–523.

[3] M. Rebucci, C. Michiels, Molecular aspects of cancer cell resistance to chemotherapy, Biochem. Pharmacol. 85 (2013) 1219–1226.

[4] M.A. Jordan, L. Wilson, Microtubules as a target for anticancer drugs, Nat. Rev. Cancer 4 (2004) 253–265.

[5] S.V. Ambudkar, C. Kimchi-Sarfaty, Z.E. Sauna, M.M. Gottesman, P-glycoprotein: from genomics to mechanism, Oncogene 22 (2003) 7468–7485.

[6] D. Simmons, M.W. Makgoba, B. Seed, ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM, Nature 331 (1998) 624–627.

[7] K. Maeda, S.M. Kang, T. Sawada, Y. Nishiguchi, M. Yashiro, Y. Ogawa, M. Ohira, T. Ishikawa, Y.S. Hirakawa, K. Chung, Expression of intercellular adhesion molecule-1 and prognosis in colorectal cancer, Oncol. Rep. 9 (2002) 511–514.

[8] A. Tachimori, N. Yamada, Y. Sakate, M. Yashiro, K. Maeda, M. Ohira, H. Nishino, K. Hirakawa, Up regulation of ICAM-1 gene expression inhibits tumour growth and liver metastasis in colorectal carcinoma, Eur. J. Cancer 41 (2005) 1802–1810.

[9] J.P. Johnson, Cell adhesion molecules in the development and progression of malignant melanoma, Cancer Metastasis Rev. 18 (1999) 345–357.

[10] H. Kobayashi, K.C. Boelte, P.C. Lin, Endothelial cell adhesion molecules and cancer progression, Curr. Med. Chem. 14 (2007) 377–386.

[11] S. Molica, A. Dattilo, A. Mannella, D. Levato, Intercellular adhesion molecules (ICAMs) 2 and 3 are frequently expressed in B cell chronic lymphocytic leukemia, Leukemia 10 (1996) 907–908.

[12] S.A. Mousa, Cell adhesion molecules: potential therapeutic & diagnostic implications, Mol. Biotechnol. 38 (2008) 33–40.

[13] Y.G. Kim, M.J. Kim, J.S. Lim, M.S. Lee, J.S. Kim, Y.D. Yoo, ICAM-3-induced cancer cell proliferation through the PI3K/Akt pathway, Cancer Lett. 239 (2006) 103–110.

[14] J.K. Park, S.H. Park, K. So, I.H. Bae, Y.D. Yoo, H.D. Um, ICAM-3 enhances the migratory and invasive potential of human non-small cell lung cancer cells by inducing MMP-2 and MMP-9 via Akt and CREB, Int. J. Oncol. 36 (2010) 181–192.

[15] C.N. Roybal, L.A. Hunsaker, O. Barbash, D.L. Vander Jagt, S.F. Abcouwer, The oxidative stressor arsenite activates vascular endothelial growth factor mRNA transcription by an ATF4-dependent mechanism, J. Biol. Chem. 280 (2005) 20331–20339.

[16] J.K. Park, Y.M. Chung, B.G. Kim, Y.A. Yoo, B.S. Yang, J.S. Kim, Y.D. Yoo, N'-(phenyl-pyridin-2-yl-methylene)-hydrazine carbodithioic acid methyl ester enhances adiation-induced cell death by targeting Bcl-2 against human lung carcinoma cells, Mol. Cancer Ther. 3 (2004) 403–407.

[17] J.K. Park, S.J. Jang, S.W. Kang, S. Park, S.G. Hwang, W.J. Kim, J.H. Kang, H.D. Um, Establishment of animal model for the analysis of cancer cell metastasis during radiotherapy, Radiat. Oncol. 7 (2012) 153.

[18] J.K. Park, H.Y. Jung, S.H. Park, S.Y. Kang, M.R. Yi, H.D. Um, S.H. Hong, Combination of PTEN and gamma-ionizing radiation enhances cell death and G(2)/M arrest through regulation of AKT activity and p21 induction in non-small-cell lung cancer cells, Int. J. Radiat. Oncol. Biol. Phys. 70 (2008) 1552–1560.

[19] X.H. Liang, S. Mungal, A. Ayscue, J.D. Meissner, P. Wodnicki, D. Hockenbery, S. Lockett, B. Herman, Bcl-2 protooncogene expression in cervical carcinoma cell lines containing inactive p53, J. Cell. Biochem. 57 (1995) 509–521.

[20] M.V. Blagosklonny, Oncogenic resistance to growth-limiting conditions, Nat. Rev. Cancer 2 (2002) 221–225.

[21] S. Elmore, Apoptosis: a review of programmed cell death, Toxicol. Pathol. 35 (2007) 495–516.

[22] G. Song, G. Ouyang, S. Bao, The activation of Akt/PKB signaling pathway and cell survival, J. Cell. Mol. Med. 9 (2005) 59–71.

[23] K. Balmanno, S.J. Cook, Tumour cell survival signalling by the ERK1/2 pathway, Cell Death Differ. 16 (2009) 368–377.

[24] K. Du, M. Montminy, CREB is a regulatory target for the protein kinase Akt/PKB, J. Biol. Chem. 273 (1998) 32377–32379.

[25] A. Bonni, A. Brunet, A.E. West, S. Robert Datta, M.A. Takasu, M.E. Greenberg, Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms, Science 12 (1999) 1358–1362.

[26] S.B. Fox, G.D. Turner, K.C. Gatter, A.L. Harris, The increased expression of adhesion molecules ICAM-3, E- and P-selectins on breast cancer endothelium, J. Pathol. 177 (1995) 369–376.

[27] M.B. Meads, R.A. Gatenby, W.S. Dalton, Environment-mediated drug resistance: a major contributor to minimal residual disease, Nat. Rev. Cancer 9 (2009) 665–674.

[28] M.D. Basson, An intracellular signal pathway that regulates cancer cell adhesion in response to extracellular forces, Cancer Res. 68 (2008) 2–4.

[29] H. Xia, R.S. Nho, J. Kahm, J. Kleidon, C.A. Henke, Focal adhesion kinase is upstream of phosphatidylinositol 3-kinase/Akt in regulating fibroblast survival in response to contraction of type I collagen matrices via a beta 1 integrin viability signaling pathway, J. Biol. Chem 279 (2004) 33024–33034.

[30] M. Fraser, B. Leung, A. Jahani-Asl, X. Yan, W.E. Thompson, B.K. Tsang, Chemoresistance in human ovarian cancer: the role of apoptotic regulators, Reprod. Biol. Endocrinol. 1 (2003) 66.

[31] T. Hideshima, D. Chauhan, K. Podar, R.L. Schlossman, P. Richardson, K.C. Anderson, Novel therapies targeting the myeloma cell and its bone marrow microenvironment, Semin. Oncol. 28 (2001) 607–612.