Biochemical and Biophysical Research Communications 457 (2015) 300–306

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

DNA damage and ER stress contribute to oblongifolin C-induced cell killing in Bax/Bak-deficient cells

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ARTICLE INFO

Article history:
Received 19 December 2014
Available online 7 January 2015

Keywords:
Oblongifolin C
BAX
BAK
Apoptosis
DNA damage
ER stress

ABSTRACT

A key clinical problem in oncology is the treatment of apoptosis-resistant tumors. Tumor cells deficient in both of the proapoptotic proteins Bax and Bak are protected against most chemotherapeutic drug-induced apoptosis. We report here that a natural compound, oblongifolin C (OC), effectively eliminates Bax/Bak-deficient murine embryonic fibroblasts and colon carcinoma HCT116 cells. OC not only triggers DNA double-strand breaks and DNA damage response, but also inhibits repair of DNA damage. In addition, OC induces ER stress through upregulation of the transcription factor CHOP and activation of JNK kinases. Upon treatment with OC, cells undergo Bax/Bak-independent, caspase-mediated apoptosis. Taken together, our data establish a rationale for the broad use of OC to treat apoptosis deficient tumors.

1. Introduction

It is well-established that mitochondria play a key role in regulating apoptosis. In vertebrates, most apoptotic stimuli require MOMP (Mitochondria Outer Membrane Permeabilization) for caspase activation and apoptosis [1]. MOMP is controlled by the Bcl-2 family proteins, which include the proapoptotic Bax-like proteins (e.g., Bax, Bak). Bax and Bak are crucial for inducing MOMP and for the subsequent release of cytochrome c. Tumor cells deficient in both Bax and Bak are resistant to most chemotherapeutic drugs-induced mitochondria-mediated apoptosis [2]. In a murine embryonic fibroblast (MEF) model, Bax and Bak double knockout (DKO) cells failed to undergo apoptosis in response to a wide range of apoptotic signals [3,4].

The endoplasmic reticulum (ER) is also recognized as an important cell death regulator [5,6]. Disturbances in ER function trigger the unfolded protein response (UPR), a process also known as ER stress. Severe ER stress often triggers cellular dysfunction and cell death. Several mediators have been implicated in ER stress induced death and it has been shown that prolonged activation of IRE1 (inositol-requiring enzyme 1) and CHOP (C/EBP-homologous protein, also known as GADD153) can trigger apoptosis through the pro-apoptotic IRE1-TRAF2-JNK pathway or via CHOP-mediated transcriptional regulation of Bcl-2 family [7,8]. The ER-resident caspase-12 has been shown to mediate ER-induced apoptosis [9,10] and calpains, along with other caspases, may also be involved in the processing and activation of caspase-12 and leading to the ER-specific apoptosis pathway [11].

Many anticancer drugs target DNA and trigger cell death signaling. Double-strand DNA breaks (DSBs) are the most serious lesions involved in chromosomal instability and cell death. Cellular responses to DNA damage are orchestrated by two distinct kinase signaling cascades, the ATM-Chk2 and ATR-Chk1 pathways, which are activated by DSBs and single-stranded DNA respectively. In response to DSBs, ATM is required both for ATR-Chk1 activation and to initiate DNA repair pathways via homologous recombination, ATM promotes formation of single-strand DNA at sites of damage through nucleolytic resection [12]. Several lines of evidence support the view that apoptosis provoked by genotoxins is predominantly due to DNA damage. DSBs can trigger either the exogenous death-receptor or endogenous mitochondrial apoptotic pathway, through p53-dependent transcriptional activation of pro-apoptotic

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http://dx.doi.org/10.1016/j.bbrc.2014.12.104
0006-291X/

factors, such as Bax, Puma and Fas, or p53-independent apoptosis *via* other systems [13–15].

We have recently shown that oblongifolin C (OC), a polycyclic polyprenylated acylphloroglucinol derived from *Garcinia yunnanenis* Hu, is an effective autophagic flux inhibitor and sensitizes nutrient-deprived cancer cells to apoptosis [16,17]. However, sensitization to apoptosis and inhibition of autophagic flux can be two independent mechanisms of OC action, because autophagy deficient cells are effectively eliminated by OC. To explore the molecular mechanisms of OC-induced cell death, we employed MEF and human colon carcinoma HCT116 cells with or without expressing functional Bax and Bak. We show that OC triggers DNA damage response, inhibits DNA repair and activates ER stress in both types of cells. As a result, cells undergo Bax/Bak-independent, caspase-mediated apoptosis. Our results thus establish a rationale for the use of the natural compound OC to treat apoptosis-resistant tumors.

2. Materials and methods

2.1. Cell culture and treatments

Mouse embryonic fibroblasts wild-type (*WT*), *atg7*$^{-/-}$, *bax/bak*$^{-/-}$ cells (gift of Kevin M Ryan, Beatson Institute for Cancer Research, Glasgow, UK) were grown in Dulbecco modified Eagle medium (Gibco/Invitrogen, 12800-017) containing 10% fetal bovine serum (PAA, A15-101), 10 U/ml penicillin–streptomycin (Gibco/Invitrogen, 15140-122). HCT116 *WT* and *bax/baka*$^{-/-}$ cells (gift of Stephen Tait, Beatson Institute for Cancer Research, Glasgow, UK) were cultured in RPMI1640 (Gibco/Invitrogen, 11875-093) supplemented with 10% fetal bovine serum and 10 U/ml penicillin–streptomycin. Cells were incubated at 37 °C in 5% CO_2. The cell permeable pan caspase inhibitor Z-VAD-FMK was obtained from Promega (G7231). Cells were treated with OC (15 μM) in the presence or absence of zVAD-fmk (50 μM) for 24 h.

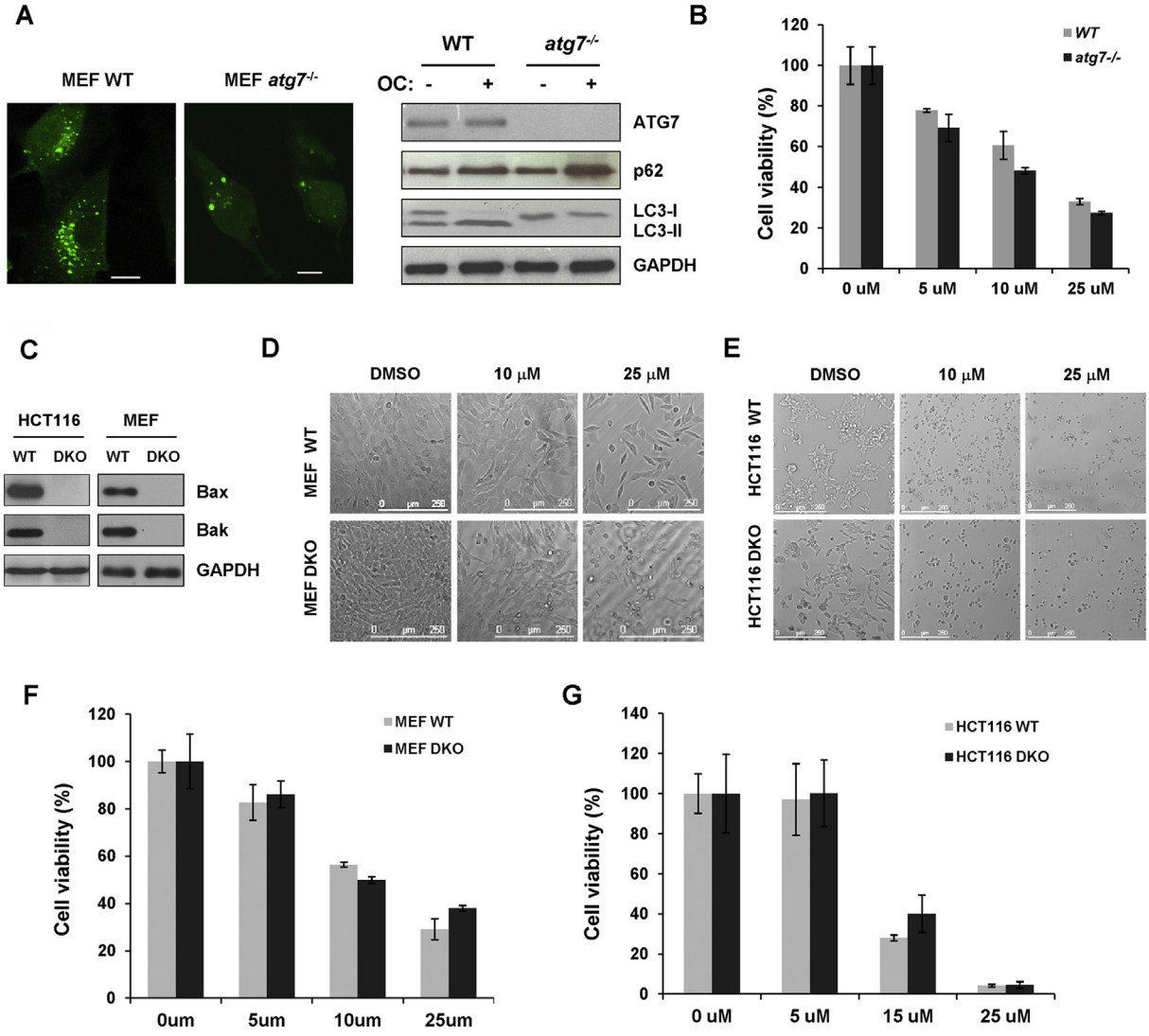

Fig. 1. OC eliminates autophagy and apoptosis deficient cells. (A) WT and *atg7*$^{-/-}$ MEFs transfected with GFP-LC3 were treated with OC (15 μM) for 24 h, OC-induced GFP-LC3 puncta formation was determined by confocal microscopy. Scale bar, 10 μm. Immunoblot of WT and *atg7*$^{-/-}$ MEFs treated with or without OC for 24 h, samples were analyzed for endogenous p62, ATG7, LC3 and GAPDH. (B) Cell viability assay of WT and *atg7*$^{-/-}$ MEFs treated with OC (0, 5, 10, 25 μM) for 24 h. Data shown are means ± SD of 3 independent experiments. (C) Immunoblot analysis of Bax and Bak expression in wild-type (WT) and *bax/bak*$^{-/-}$ (DKO) MEF and HCT116 cells. (D) Phase contrast images of WT and DKO MEFs or (E) HCT116 cells treated with 15 μM OC for 24 h. (F) Cell viability assay of WT and DKO MEFs or (G) HCT116 cells treated with OC (0, 5, 10, 25 μM) for 24 h. Data shown are mean ± SD of three independent experiments.

2.2. Cell viability assay

Cell viability was determined by measuring the conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) to formazan as previously described [18]. The crystal violet or MTT absorbance, corresponding to the number of viable cells was measured in an automated microplate reader at 550 nm. The data represent the means ± SD from four microwells from each of at least three independent experiments.

2.3. Immunocytochemistry

For γH2AX staining, cells were fixed in 4% paraformaldehyde in PBS for 15 min, followed by permeabilization with 0.25% Triton X-100 in PBS for 10 min at room temperature. Fixed preparations were blocked with 3% BSA in PBS for 1 h, then incubated with primary antibodies against phosphor serine 139 histone H2AX (Millipore, 0-636) for 1 h. The stained cells were washed and incubated with Alexa Fluor-conjugated secondary antibodies for 1 h. 4′,6-Diamidino-2-phenylindole (DAPI) was used to stain nuclei. All steps were performed at room temperature. Images were captured using an Olympus FV1000 confocal microscope.

2.4. Comet assay

Cells were harvested and mixed with low melting agarose (AB0015, Life technologies). Cell mixture was immediately plated onto center of comet slide and spread using a yellow tip, then solidified, lysed (lysis solution: 1.2 M NaCl, 100 mM Na_2EDTA, 0.1% sodium lauryl sarcosinate, 0.26 M NaOH, pH > 13) and equilibrated. Slides were electrophoresed in alkaline solution (2 mM Na_2EDTA, 0.03 M NaOH, pH ~12.3) for 30 min at 4 °C, fixed and stained with PI. Comet images were captured using a fluorescence microscope.

2.5. Subcellular fractionation

Following induction of apoptosis, cytosolic and mitochondrial fractions were separated using a digitonin-based subcellular fractionation. 1×10^7 cells were harvested by centrifugation at 800 *g*, the pellets were resuspended in cytosolic extraction buffer (250 mM sucrose, 70 mM KCl, 137 mM NaCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4 pH 7.2, 100 μM PMSF, 10 μg/ml leupeptin, 2 μg/ml aprotinin, containing 200 μg/ml digitonin) for 5 min on ice. Cells were then centrifuged at 1000 g for 5 min 4 °C. The supernatants were saved as cytosolic fractions. The pellets were solubilized in mitochondrial lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 0.2% Triton X-100, 0.3% NP-40, 100 μM PMSF, 10 μg/ml leupeptin, 2 μg/ml aprotinin), followed by pelleting at 10 000 *g* for 10 min at 4 °C.

2.6. Western blotting

Cells were lysed in ice-cold whole cell extract buffer (50 mM Tris–HCl, pH 8.0, 4M urea and 1% Triton X-100), supplemented

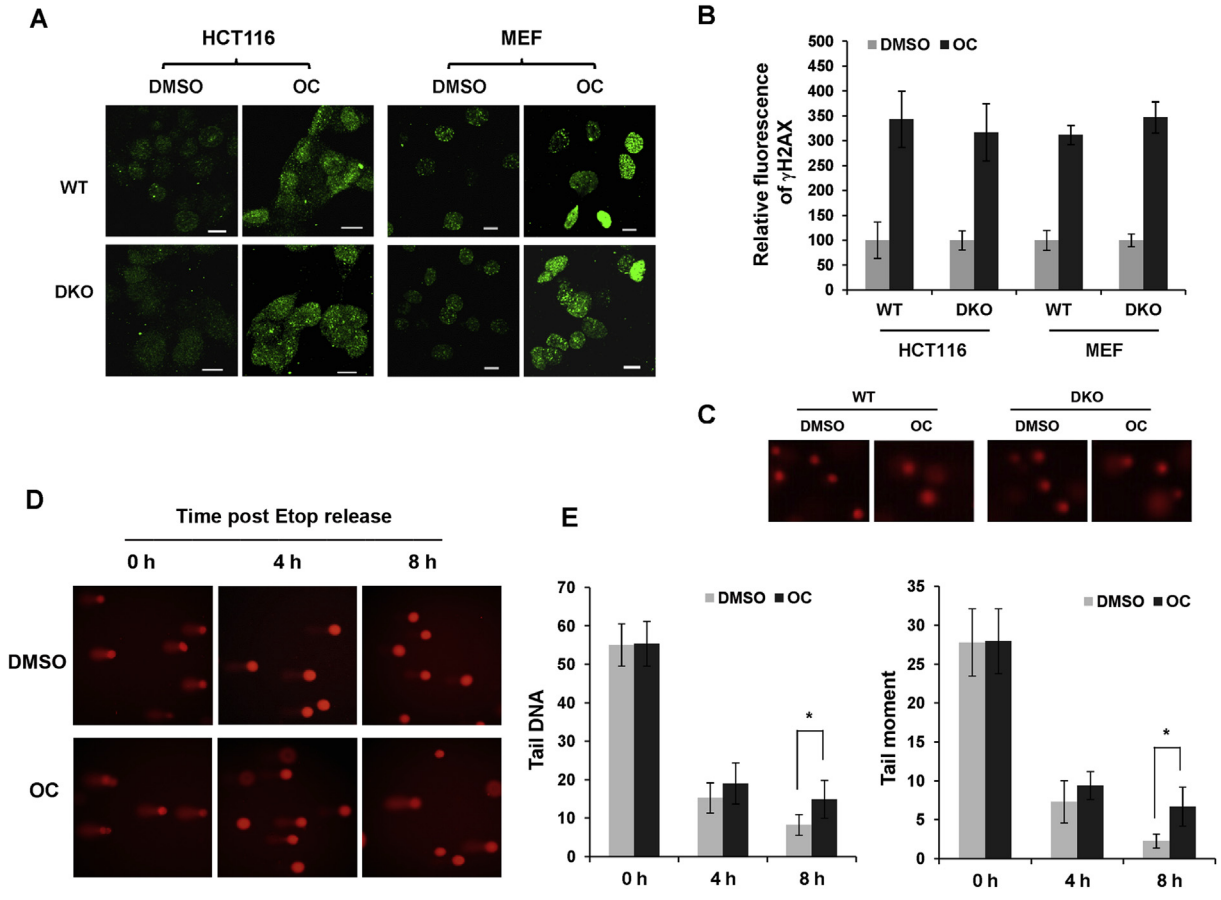

Fig. 2. OC triggers DNA damage and inhibits DNA repair. (A) Confocal microscopy images showing immunofluorescence staining of γH2AX in WT and DKO cells treated with OC (15 μM) for 24 h. Scale bar, 10 μm. (B) Quantification of the fluorescence intensity of endogenous γH2AX. More than 100 cells were counted for each condition and data are presented as mean ± SD from two independent experiments. (C) OC-induced DNA DSBs were evaluated by comet assay in HCT116 cells. (D) HCT116 cells were treated with 10 μM etoposide (Etop) for 12 h. Etop was then removed and cells were cultured in the presence or absence of OC (15 μM). Samples were collected for comet assay at 0, 4 and 8 h after Etop release. (E) Repair of Etop-induced DSBs was determined by measuring tail DNA and tail moment. Data shown are mean ± SD of two independent experiments (*$p < 0.05$).

with complete protease inhibitor mixture (Roche Diagnostics, 04693132001). Lysates were cleared by centrifugation at 10 000 g for 15 min. Cell extracts were resolved by SDS-PAGE and analyzed by western blotting. Antibodies used were as follows. LC3B (Sigma, L7543), p62 (MBL, PM045), GAPDH (Proteintech, 10494-1-AP), cleaved caspase-3 (Cell Signaling Technology, 9664), caspase-3 (Cell signaling Technology, 9662), PARP (Cell Signaling Technology, 5625), cytochrome *c* (Cell Signaling Technology, 4280), BID (Cell Signaling Technology, 2002), Bax (Cell Signaling Technology, 2772), Bak (Cell Signaling Technology, 12015), phospho-Histone H2AX (Ser139) (Millipore, 05-636), phosphor-Chk1 (Ser345) (Cell Signaling Technology, 2348), phosphor-Chk2 (T68) (Abnova, PAB25281), phospho-SAPK/JNK (Thr183/Tyr185) (Cell Signaling Technology, 4671), SAPK/JNK (Cell Signaling Technology, 9252). Following incubation with horseradish peroxidase coupled secondary anti-mouse (KPL, 074-1806) or anti-rabbit antibodies (KPL, 474-1506), protein bands were visualized using ECL blotting detection reagents (KPL, 54-61-00).

2.7. Statistical analysis

All results were expressed as means ± SD of three independent experiments.

Statistical analyses were performed using the student two-tailed *t* test. Values of $^{*}p < 0.05$ were considered to be significant.

3. Results

3.1. OC effectively eliminates Bax/Bak-deficient cells

Previously, we have shown that the natural product OC is a novel autophagic flux inhibitor and sensitizes nutrient-deprived cancer cell to apoptosis [16,17]. To ascertain whether OC-induced cell death is mediated by autophagy inhibition, we used immortalized *atg7*$^{-/-}$ murine embryonic fibroblasts (MEFs) that are deficient in autophagosome formation (Fig. 1A). *Atg7*$^{-/-}$ cells were efficiently killed by OC in a similar manner to wild-type (WT) MEFs (Fig. 1B). These results suggest that autophagy inhibition and sensitization to cell death could be two independent mechanisms of OC action.

To determine the anticancer potential of OC, we employed MEF and HCT116 cells with or without expressing functional proapoptotic proteins Bax and Bak (Fig. 1C). Deficiency of BAX and BAK confers resistance to most conventional cancer therapies. However, OC was found to dramatically suppress cell growth in both WT and *bax/bak*$^{-/-}$ (DKO) cells (Fig. 1D and E). A dose response study revealed that DKO cells were eliminated by OC in a dose-dependent fashion and in a similar manner to WT parental cells (Fig. 1F and G). These data indicate that OC could be therapeutically useful in Bax and Bak-deficient multidrug-resistant cells.

3.2. OC induces DNA damage and inhibits repair of DSBs

The most severe lethal cellular lesions are DNA double-strand breaks (DSBs), which are powerful inducers of cell death. When cells are exposed to chemotherapeutic agents that damage DNA, DSBs are generated, which rapidly results in the phosphorylation of histone H2AX at serine 139 (γH2AX). Therefore, γH2AX is a highly sensitive marker that can be used to examine both DSBs and DNA repair. To explore the molecular mechanisms of OC-induced cell death, we determined the genotoxic potential of OC in MEF and HCT116 cells. As shown in Fig. 2A, OC-treated cells exhibited a marked accumulation of phosphorylated H2AX nuclear foci in all tested cell lines, indicating that OC is able to induce DNA damage that either represent DSBs or will be converted into DSBs (Fig. 2B). Single-cell gel electrophoresis assay also confirmed that treatment

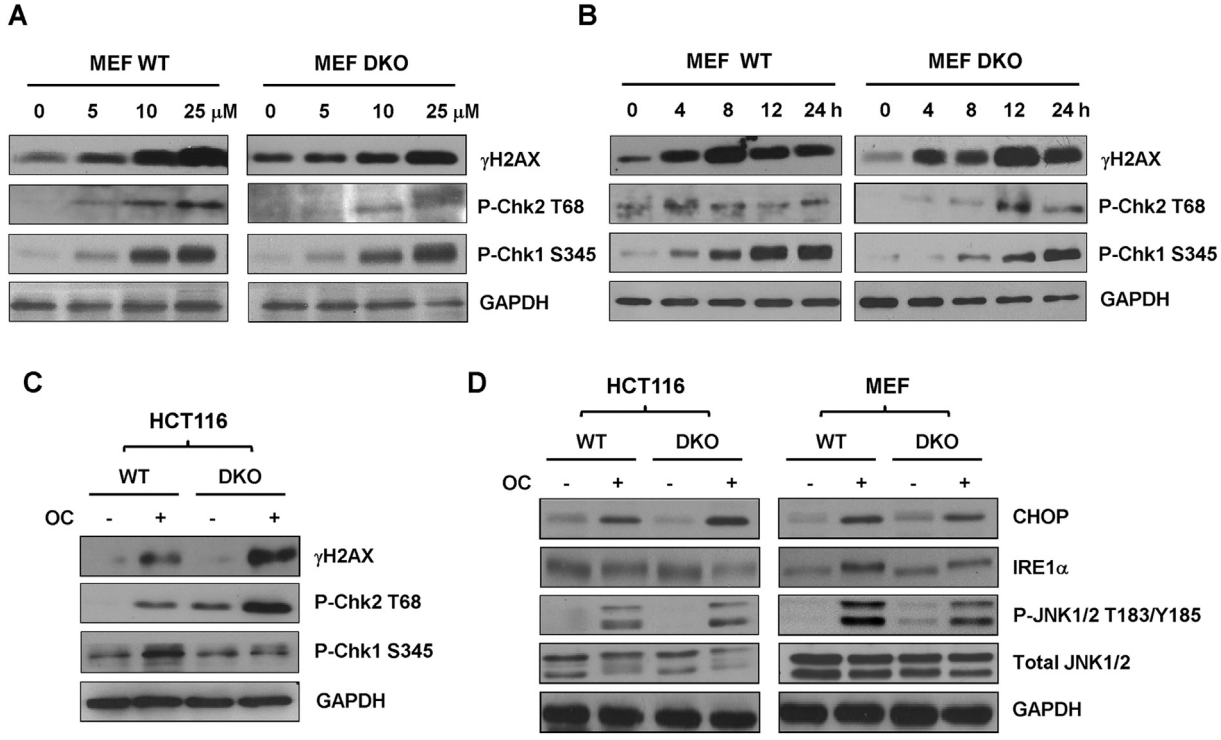

Fig. 3. OC triggers DNA damage response and ER stress. (A) MEF WT and DKO cells were treated with different concentrations of OC (0, 5, 10, 25 μM) for 24 h, or (B) treated with OC at 15 μM over a 24 h time course and were analyzed by western blotting for γH2AX, phosphor Chk1 (ser345), phosphor Chk2 (T68) and GAPDH. (C) Western blot analysis of HCT116 WT and DKO cells treated with 15 μM OC for 24 h. Cells were assayed for γH2AX, phospho-Chk1 (ser345), phospho-Chk2 (T68) and GAPDH. (D) Western blot analysis of WT and DKO cells treated with OC (15 μM) for 24 h for CHOP, IRE1α, phosphor JNK1/2 (T183/Y185), total JNK1/2 and GAPDH.

with OC caused DSBs characterized by obvious "comet tails" (Fig. 2C).

Pharmacological inhibition of DNA damage repair pathways has been explored as a useful strategy to enhance chemo- and radiosensitivity. We performed comet assay to test the effect of OC on DNA repair. At 8 h after releasing from etoposide, control cells were able to repair DNA DSBs, whilst OC caused more comet tails (Fig. 2D). We measured the ability of cells to resolve DSBs, the results showed that OC could inhibit repair of DSBs (Fig. 2E). Therefore, inducing DNA damage and targeting DNA repair pathways may contribute to OC-induced cytotoxicity.

3.3. OC triggers DNA damage response and ER stress

Two main signaling pathways activated by DNA damage consist of the ATM-Chk2 and ATR-Chk1 protein kinases. In response to DSBs, histone H2AX is phosphorylated by two PI3K kinases, ATM and ATR. We assessed phosphorylation of histone H2AX, Chk2 and Chk1 by western blot. OC induced equivalent levels of histone H2AX phosphorylation in a dose- and time-dependent manner in both WT and DKO cells. Chk2 T68 and Chk1 S345, which are widely used as surrogate markers of activation, were phosphorylated to the same extent by OC in these cells (Fig. 3A and B). Also, OC

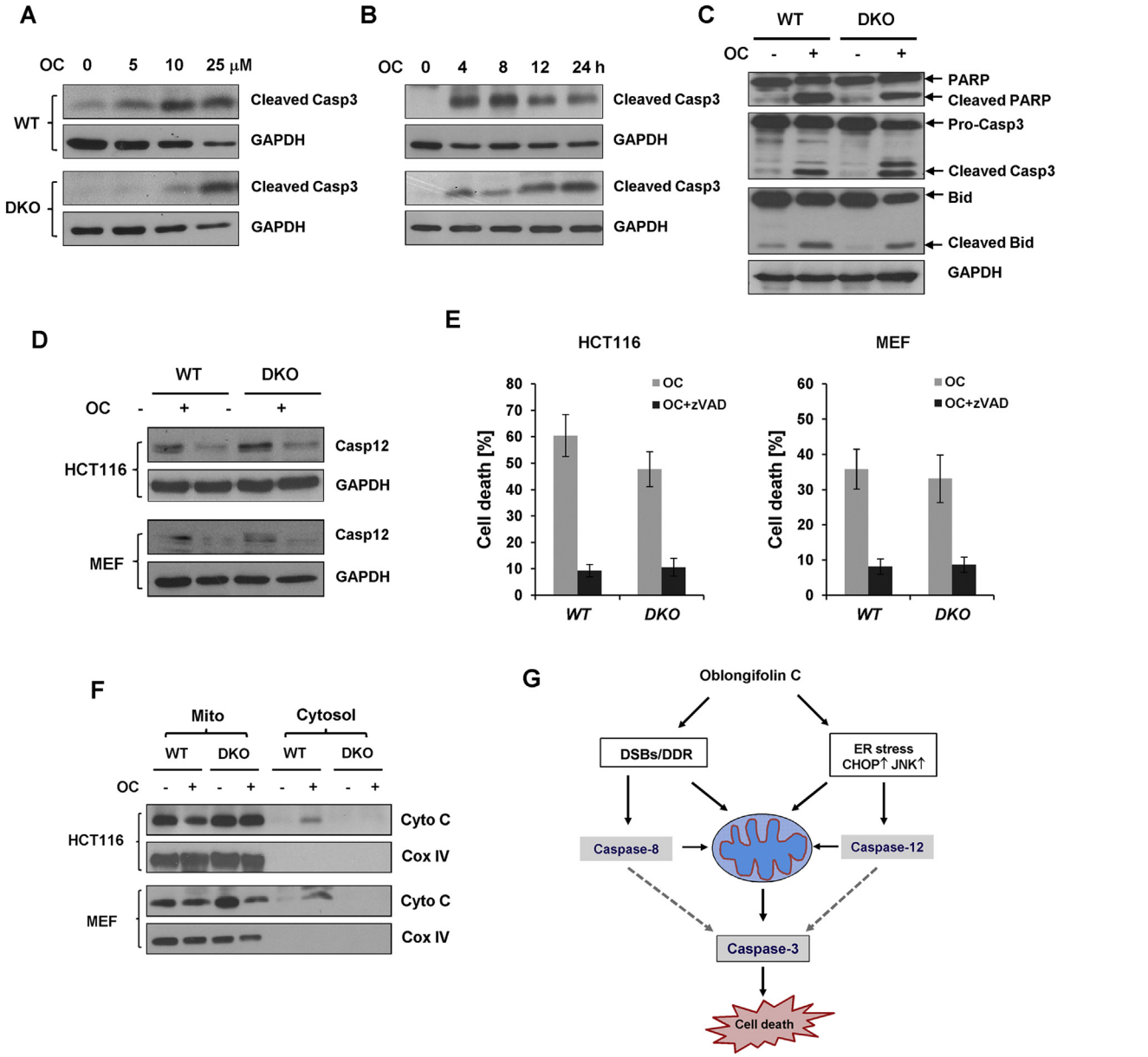

Fig. 4. OC triggers caspase-dependent apoptosis in WT and DKO cells. (A) MEF WT and DKO cells treated with OC (0, 5, 10, 25 μM) for 24 h, or (B) treated with 15 μM OC over a time course (0, 4, 8, 12 and 24 h) were analyzed by western blotting for cleaved caspase-3 and GAPDH. (C) HCT116 WT and DKO cells were treated with 15 μM OC for 24 h and samples were analyzed by western blotting for PARP, caspase-3, Bid and GAPDH. (D) Western blot analysis of WT and DKO cells treated with OC for 24 h for caspase-12 and GAPDH. (E) MEF or HCT116 cells were treated with OC (15 μM) in the presence or absence of zVAD-fmk (50 μM) for 24 h. Cell death was determined by FACS analysis of propidium iodide (PI) uptake. Data shown are mean ± SD of three independent experiments. (F) OC induces cytochrome c release in WT but not DKO cells. The mitochondrial and cytosolic fractions of WT and DKO cells treated with OC (15 μM) for 24 h were analyzed by western blotting for cytochrome *c* and CoxIV. (G) Proposed model of OC-induced cell death pathways. Both DNA damage and ER stress contribute to OC-induced apoptosis. In Bax/Bak-expressing cells, OC predominantly triggers the endogenous mitochondria-dependent pathway of apoptosis. In Bax/Bak-deficient cells, OC induces mitochondria-independent apoptosis, caspase-8 or ER stress-specific caspase-12 may contribute directly to the activation of downstream effector caspases.

consistently induced phosphorylation of histone H2AX, Chk2 T68 and Chk1 S345 in WT and DKO HCT116 cell lines (Fig. 3C).

Endoplasmic reticulum (ER) stress is known to be an important inducer of cell death. Distinct components of the ER stress signaling pathway, such as CHOP, PERK and ATF6, have been implicated in apoptosis resulting from ER stress [10,19–21]. Interestingly, we found that in all cell lines tested, OC induced significant upregulation of the transcription factor CHOP. In addition, we observed enhanced phosphorylation of IRE1α and the IRE1α substrate JNK1/2 in OC-treated WT and DKO cells (Fig. 3D). These data suggest that ER stress may also contribute to OC-induced cell death.

3.4. OC induces caspase-dependent cell death in Bax/Bak-deficient cells

Previous work has shown that OC triggers mitochondria-dependent apoptosis. To determine whether Bax/Bak-deficient cells also follow the apoptotic pathway, we investigated the activation of caspases. Cleavage of caspase-3 in response to OC was easily observed in WT and DKO MEFs, in a time- and dose-dependent manner (Fig. 4A and B). These results were confirmed in WT and *bax/bak*$^{-/-}$ HCT116 cells that were exposed to OC. The caspase-3 specific 89 kDa fragment of poly (ADP-ribose) polymerase (PARP) and cleavage of caspase-8 substrate Bid were also detected in DKO cells (Fig. 4C). Caspase-12 is localized within the ER and activated by ER stress. We detected a marked decrease of pro caspase-12, indicating that this caspase-12 might be involved in OC mediated ER-specific apoptosis (Fig. 4D). Pharmacological caspase inhibition by z-VAD-fmk resulted in marked reduction of cell death in DKO cells after 24 h of treatment with OC (Fig. 4E), a phenomenon that was also seen in WT cells, indicating that OC triggers caspase-dependent cell death in Bax/Bak-deficient cells.

It has been shown previously that OC treatment causes BAX translocation and cytochrome *c* release in tumor cells. We assessed the release of cytochrome *c* in Bax/Bak-deficient MEFs and HCT116 cells. As shown in Fig. 4F, cytochrome *c* release was clearly detected in the cytosolic fractions of WT but not DKO cells exposed to OC. Therefore, OC induces caspase-3 activation independent of mitochondrial cytochrome c release in Bax/Bak-deficient cells, caspase-8 or ER-specific caspase-12 may directly activate downstream effector caspase.

4. Discussion

Previously, we have shown that OC is a potent autophagic flux inhibitor and sensitizes tumor cells to caspase-3 mediated apoptosis. However, sensitization to apoptosis and inhibition of autophagic flux might be two independent mechanisms of OC action. In this study, we explored the molecular mechanisms of OC-induced cell death. We demonstrate that both DNA damage and ER stress contribute to OC-mediated cell killing. Since cytochrome c is not released in Bax/Bak-deficient cells, OC may trigger both mitochondria-dependent and independent pathways of apoptosis (Fig. 4G).

Many anticancer agents target cancer cells by directly or indirectly inducing DNA damage [22–24]. DNA double-stand breaks (DSBs) are the most severe form of DNA damage and many anticancer therapies (e.g. etoposide, cisplatin, doxorubicin, camptothecin, *etc.*) and radiation therapy introduce toxic DSBs, which trigger death signals in tumors. Upon recognizing DNA damage, cells initiate the DNA damage response, which is a crucial signaling pathway that serves to coordinate important biochemical and cellular events, such as DNA repair, cell cycle arrest and eventually cell survival or death [12,24]. Targeting cancer cells with genotoxic agents has demonstrated clinical utility and natural herbs represent an important source of these anticancer compounds. For example, DNA damage induced by artesunate, a semisynthetic derivative of the natural product artemisinin, contributes to its therapeutic effect against cancer cells [25]. Salvicine, a novel diterpenoid quinone compound synthesized by structural modification of a *Salvia prionitis lance* natural product, triggers DSBs and apoptosis by ROS generation and toposiomerase II inhibition [26,27]. The data presented herein suggest that OC is not only a potent DNA damage inducer, but it may also target DNA repair pathways. Inhibition of DNA repair pathways has been explored as a useful strategy in anticancer treatment, further study will be required to exploit the detailed mechanisms of OC in DNA repair.

ER stress inducers trigger UPR, and distinct components of the UPR, such as CHOP, PERK and ATF6, have been implicated in apoptosis resulting from ER stress [10,19–21]. A widely cited mechanism of CHOP-induced apoptosis is the suppression of the pro-apoptotic protein Bcl-2 [28]. We found that in OC-treated cells, induction of CHOP occurred in line with the downregulation of Bcl-2, indicating OC-treated cells may undergo CHOP-mediated apoptosis through the downregulation of Bcl-2 (data now shown). Taken together, our study demonstrates that the natural compound OC efficiently eliminates Bax/Bak-deficient cells, both DNA damage and ER-stress contribute to OC-mediated cell killing. Our results thus suggest that OC might be an attractive anticancer compound for the treatment of drug-resistant tumors.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 21272135, to NH.Xu), the Overseas High-caliber Personnel Foundation of Shenzhen (No. KQC201109050084A, to NH.Xu).

Transparency document

The transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrc.2014.12.104.

References

[1] G. Kroemer, L. Galluzzi, C. Brenner, Mitochondrial membrane permeabilization in cell death, Physiol. Rev. 87 (2007) 99–163.
[2] U. Fischer, K. Janssen, K. Schulze-Osthoff, Cutting-edge apoptosis-based therapeutics: a panacea for cancer? BioDrugs 21 (2007) 273–297.
[3] M.C. Wei, W.X. Zong, E.H. Cheng, T. Lindsten, V. Panoutsakopoulou, A.J. Ross, K.A. Roth, G.R. MacGregor, C.B. Thompson, S.J. Korsmeyer, Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death, Science 292 (2001) 727–730.
[4] W.X. Zong, T. Lindsten, A.J. Ross, G.R. MacGregor, C.B. Thompson, BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak, Genes Dev. 15 (2001) 1481–1486.
[5] M. Boyce, J. Yuan, Cellular response to endoplasmic reticulum stress: a matter of life or death, Cell Death Differ. 13 (2006) 363–373.
[6] C. Xu, B. Bailly-Maitre, J.C. Reed, Endoplasmic reticulum stress: cell life and death decisions, J. Clin. Invest. 115 (2005) 2656–2664.
[7] E. Szegezdi, U. Fitzgerald, A. Samali, Caspase-12 and ER-stress-mediated apoptosis: the story so far, Ann. N. Y. Acad. Sci. 1010 (2003) 186–194.
[8] I. Tabas, D. Ron, Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress, Nat. Cell Biol. 13 (2011) 184–190.
[9] T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner, J. Yuan, Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, Nature 403 (2000) 98–103.
[10] E. Szegezdi, S.E. Logue, A.M. Gorman, A. Samali, Mediators of endoplasmic reticulum stress-induced apoptosis, EMBO Rep. 7 (2006) 880–885.
[11] J.A. Martinez, Z. Zhang, S.I. Svetlov, R.L. Hayes, K.K. Wang, S.F. Larner, Calpain and caspase processing of caspase-12 contribute to the ER stress-induced cell death pathway in differentiated PC12 cells, Apoptosis 15 (2010) 1480–1493.

[12] J. Smith, L.M. Tho, N. Xu, D.A. Gillespie, The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer, Adv. Cancer Res. 108 (2010) 73–112.
[13] G. Melino, F. Bernassola, M. Ranalli, K. Yee, W.X. Zong, M. Corazzari, R.A. Knight, D.R. Green, C. Thompson, K.H. Vousden, p73 induces apoptosis via PUMA transactivation and Bax mitochondrial translocation, J. Biol. Chem. 279 (2004) 8076–8083.
[14] M. Urist, T. Tanaka, M.V. Poyurovsky, C. Prives, p73 induction after DNA damage is regulated by checkpoint kinases Chk1 and Chk2, Genes Dev. 18 (2004) 3041–3054.
[15] J.W. Rocco, C.O. Leong, N. Kuperwasser, M.P. DeYoung, L.W. Ellisen, p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis, Cancer Cell 9 (2006) 45–56.
[16] Y. Lao, G. Wan, Z. Liu, X. Wang, P. Ruan, W. Xu, D. Xu, W. Xie, Y. Zhang, H. Xu, N. Xu, The natural compound oblongifolin C inhibits autophagic flux and enhances antitumor efficacy of nutrient deprivation, Autophagy 10 (2014) 736–749.
[17] C. Feng, L.Y. Zhou, T. Yu, G. Xu, H.L. Tian, J.J. Xu, H.X. Xu, K.Q. Luo, A new anticancer compound, oblongifolin C, inhibits tumor growth and promotes apoptosis in HeLa cells through Bax activation, Int. J. Cancer 131 (2012) 1445–1454.
[18] I. Ciechomska, M. Legat, J. Golab, A. Wesolowska, Z. Kurzaj, A. Mackiewicz, B. Kaminska, Cyclosporine A and its non-immunosuppressive derivative NIM811 induce apoptosis of malignant melanoma cells in in vitro and in vivo studies, Int. J. Cancer 117 (2005) 59–67.
[19] H.P. Harding, Y. Zhang, A. Bertolotti, H. Zeng, D. Ron, Perk is essential for translational regulation and cell survival during the unfolded protein response, Mol. Cell 5 (2000) 897–904.
[20] K. Yamamoto, T. Sato, T. Matsui, M. Sato, T. Okada, H. Yoshida, A. Harada, K. Mori, Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1, Dev. Cell 13 (2007) 365–376.
[21] H. Zinszner, M. Kuroda, X. Wang, N. Batchvarova, R.T. Lightfoot, H. Remotti, J.L. Stevens, D. Ron, CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum, Genes Dev. 12 (1998) 982–995.
[22] R.W. Johnstone, A.A. Ruefli, S.W. Lowe, Apoptosis: a link between cancer genetics and chemotherapy, Cell 108 (2002) 153–164.
[23] M.S. Ricci, W.X. Zong, Chemotherapeutic approaches for targeting cell death pathways, Oncologist 11 (2006) 342–357.
[24] D. Woods, J.J. Turchi, Chemotherapy induced DNA damage response: convergence of drugs and pathways, Cancer Biol. Ther. 14 (2013) 379–389.
[25] P.C. Li, E. Lam, W.P. Roos, M.Z. Zdzienicka, B. Kaina, T. Efferth, Artesunate derived from traditional Chinese medicine induces DNA damage and repair, Cancer Res. 68 (2008) 4347–4351.
[26] Y. Cai, J. Lu, Z. Miao, L. Lin, J. Ding, Reactive oxygen species contribute to cell killing and P-glycoprotein downregulation by salvicine in multidrug resistant K562/A02 cells, Cancer Biol. Ther. 6 (2007) 1794–1799.
[27] Y.J. Cai, J.J. Lu, H. Zhu, H. Xie, M. Huang, L.P. Lin, X.W. Zhang, J. Ding, Salvicine triggers DNA double-strand breaks and apoptosis by GSH-depletion-driven H2O2 generation and topoisomerase II inhibition, Free Radical Biol. Med. 45 (2008) 627–635.
[28] K.D. McCullough, J.L. Martindale, L.O. Klotz, T.Y. Aw, N.J. Holbrook, Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state, Mol. Cell Biol. 21 (2001) 1249–1259.