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Original Article

Exogenous WWOX enhances apoptosis and weakens metastasis in CNE2 nasopharyngeal carcinoma cells through the intrinsic apoptotic pathway

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Abstract: The WW domain containing oxidoreductase (WWOX) has been postulated to behave as a putative tumor suppressor and that silencing of WWOX expression is linked to the carcinogenesis and progression of various carcinomas. The role of WWOX in nasopharyngeal carcinoma (NPC) remains unclear. Herein, we sought to evaluate the biological feature of WWOX restoration in human CNE2 NPC cells. In vitro experiments manifested that transiently overexpressed WWOX significantly suppressed proliferation as well as invasion and migration of the CNE2 cells. Of note, WWOX-induced apoptosis could be partly reversed by the selective caspase inhibitor, Z-VAD-FMK. Furthermore, immunoblotting analysis indicated that ectopic expression of WWOX could trigger the intrinsic apoptotic signaling pathway characterized by a down-regulation of Bcl-2 and Bcl-xL, and up-regulation of Bax and Cytochrome c along with a remarkable activation of the caspase cascades. Taken together, our data reveal that WWOX behaves as a potent tumor suppressor in CNE2 cells, possibly by enhancing apoptosis and weakening metastasis via the intrinsic apoptotic pathway.

Keywords: WWOX, nasopharyngeal carcinoma, CNE2, metastasis, apoptosis, mechanism

Introduction

Nasopharyngeal carcinoma (NPC) is a kind of malignant tumor occurs in nasopharyngeal mucosa and is characterized by high motility and frequent metastasis [1]. Distant metastasis continues to be the culprit of death among the NPC cases with stage III and IV [2]. In this respect, it is in dire need of finding and developing new diagnostic and therapeutic targets for NPC.

The *WWOX* gene has been proposed as a potential diagnostic or therapeutic target for multiple cancers. In human beings, expression of the WWOX (WW domain containing oxidoreductase) protein is under control by the *WWOX* gene that resides in 16q23.3-24.1 locus, an area designated as the highly affected chromosomal fragile site (FRA16D) [3]. The *WWOX* gene possesses 9 coding exons and encodes the WWOX protein consists of two WW domains in N terminal and one short-chain dehydrogenase (SDR) domain in C terminal [4]. The immunohistochemical analyses have identified the positive expression of WWOX protein in normal human organs and tissues, particularly in hormone related organs, speculating that WWOX may behave as an important regulator in steroid-receptor cell pathways [5]. Since its discovery, WWOX has been reported to function as a putative oxidoreductase and implicate in diverse biological processes, including cell growth, differentiation, metabolism, autophagy, and so forth [6-8]. Particularly, studies in recent years have highlighted the distinct role of WWOX in human cancers and suggest that WWOX is able to serve as a potent tumor suppressor in promoting apoptosis or cell death in various cancers [4, 9-11]. Our previous studies indicated that WWOX behaves as a driver in accelerating apoptosis in leukemia cell lines [12-14]. Notwithstanding, although *WWOX* shows high loss of heterozygosity (LOH) in many

cancer types, it is rare to see that *WWOX* only impairs a single allele instead of both alleles during inactivation [9]. Contradictory results still hold the adverse points regarding the anti-neoplastic effects of WWOX in some cancer types [15]. In NPC, it is speculated that WWOX is not likely to be a classical tumor suppressor, as high percentage of LOH for *WWOX* has been detected in NPC tissues [16]. In order to gain a better understanding of the functional role of WWOX in NPC, we assessed the biological feature of WWOX restoration in human CNE2 NPC cells.

Materials and methods

Reagents

RPMI-1640 medium (Hyclone, Logan, UT, USA); FBS (PAN-Biotech GmbH, Aidenbach, Germany); ZLip2000 (Zoman Biotechnology, Beijing, China); Z-VAD-FMK (SelleckChem, Houston, TX); rabbit anti-human WWOX monoclonal IgG, rabbit anti-human Bcl-2 monoclonal IgG, rabbit anti-human Bax monoclonal IgG, horseradish peroxidase-conjugated goat anti-rabbit IgG and anti-mouse IgG were all purchases from Abcam (Cambridge, MA, USA). Rabbit anti-human cleaved PARP polyclonal IgG (for detection of 85 kDa apoptotic fragment of PARP) and rabbit anti-human caspase-9 polyclonal IgG (Santa Cruz Biotechnology, Inc., Dallas, TX, USA); Annexin V PE/7-AAD apoptosis kit (KeyGEN Biotech, Nanjing, China); RIPA lysis buffer, BCA Protein Quantification kit, CCK-8 assay kit, DNA Fragmentation kit, mouse anti-human Cytochrome c monoclonal IgG2b, mouse anti-human β-actin monoclonal IgG2a, and rabbit anti-human Bcl-xL IgG were all purchased from Beyotime Institute of Biotechnology (Haimen, China).

Cell culture, transfection and caspase activity blocking

Human CNE2 poorly differentiated nasopharyngeal carcinoma cells (Chinese Academy of Medical Sciences) were cultured using RPMI-1640 medium plus 10% FBS without penicillin/streptomycin. The CNE2 cells were transfected using a recombinant expression vector, pCMV-WWOX-EGFP that we previously constructed [14]. A commercial transfection reagent, ZLip-2000 was utilized according to its protocol: 2.5 × 10^5 cells/well were seeded and cultivated in the 6-well plates till to reach a density of 60% to 70% confluence before transfection. The transfection mixture was prepared by adding 4 μg pCMV-WWOX-EGFP in 200 μl antibiotics-free RPMI-1640, and 10 μl ZLip2000 in another 200 μl antibiotics-free RPMI-1640, followed by mixing the mixture together to obtain the ZLip2000-DNA complex. The ZLip2000-DNA complex were subsequently supplemented to the seeded cells (400 μl/well) with a total volume of 2 ml per well. The culture medium was replaced with new RPMI-1640 media after transfection for 4 to 6 h. Expression of the tagged EGFP was observed via fluorescence microscopy (Olympus) at 24 h after transfection. Additionally, cell caspase activity was blocked with or without a broad caspase inhibitor, Z-VAD-FMK (20 μM).

Proliferation assay

Evaluation of cell proliferation was enabled by using a CCK-8 assay kit. Briefly, 2.0 × 10^3 cells/well was seeded in 96-well plates with a total volume of 100 μl. Cell transfection followed the procedures above (0.2 μg vector and 0.5 μl ZLip2000 were utilized per well). Before testing, 10 μl CCK-8 was supplemented to each well followed by incubating at 37°C for 3 h. The OD value for each well was monitored using a microplate reader (BioTek Instruments, USA) with double wavelength of 450 nm and 630 nm. The inhibition ratio (%) was calculated according to the formula reported by our previously study [13]. Each test was repeated for 3 times.

Wound healing migration assay

The wound healing experiments were conducted utilizing the 6-well culture plates: monolayer CNE2 cells (5 × 10^5/well) were raised in serum-free RPMI-1640 media, and culturing to a density of 90% confluence. A 200 μl volume pipette tip (Eppendorf Lab Technologies) was applied to introduce a scratch through the cell monolayer. Images were captured at 48 h following wounding.

Invasion assay

In vitro matrigel experiments were enabled by using the cell invasion chambers with 8 μM pore filters (Millipore Corp., Billerica, MA, USA) and followed the procedures we recently described [17].

Flow cytometry analysis

The apoptotic ratio (%) was measured using Annexin V PE/7-AAD double colored fluorescent

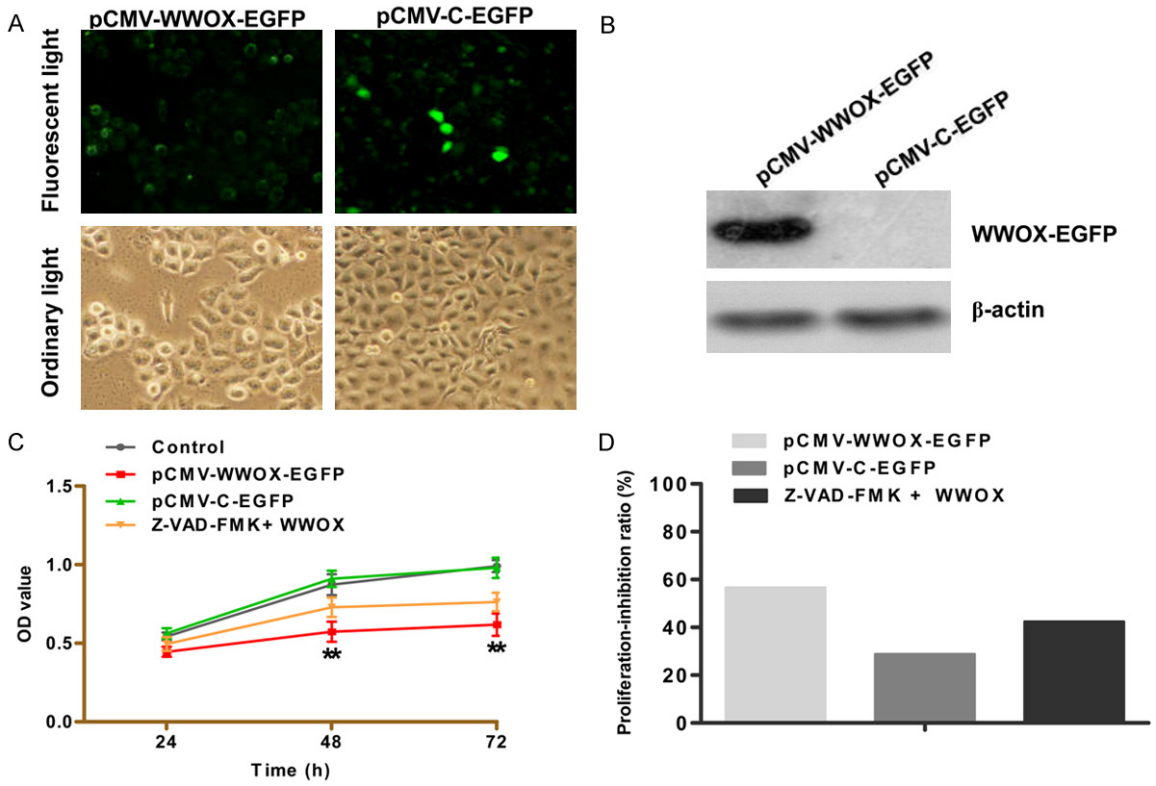

Figure 1. Effects of WWOX restoration on CNE2 cell proliferation. A, B: Expression of WWOX-EGFP fusion protein were observed at 12 h after transfection (× 200) and measured by immunoblotting. C, D: Cell growth curve and inhibition ration were plotted and calculated with or without a broader caspase inhibitor. **P<0.01 vs. pCMV-C-EGFP group at 48 h and 72 h during transfection.

by flow cytometry [12]. Treated CNE2 cells were harvested at 72 h after transfection and washed 2 times with PBS buffer. Subsequently, 1-5 × 10^5 cells were suspended utilizing 500 μl Binding Buffer, followed by adding 1 μl Annexin V-PE and incubating at room temperature for 15 min away from light. Later, 5 μl of 7-AAD was supplemented and placed in dark at room temperature for 15 min. Stained cells were finally analyzed using a Becton Dickinson FACSCalibur.

DNA ladder analysis

The "DNA ladder" analysis was performed with a DNA Fragmentation kit according to its instructions. A total of 2 μg extracted DNA for each sample was subjected to the agarose gel electrophoresis (1.0%) with a constant voltage of 20 V for 4 h.

Western blot analysis

Cells were lysed using the RIPA buffer and spun at 12,000 × g for 15 min. The protein lysates were further quantified utilizing a commercial BCA Protein Quantification kit. Proteins were separated and transferred onto PVDF membranes (Millipore Corp., Billerica, MA, USA), followed by incubating in TBS plus 5% blocking serum. The blocked membranes were then subjected to a serious of primary antibodies: rabbit anti-human WWOX (1:1000), rabbit anti-Bcl-2 (1:500), anti-Bcl-xL (1:300), anti-Bax (1:150), anti-caspase-9 (1:500), anti-cleaved PARP (1:300), and mouse anti-human Cytochrome c (1:300), and β-actin (1:1000). All the primary antibodies were incubating at 4°C overnight, and washed 3 times with TBS plus 0.2% Tween-20. The secondary antibodies were all diluted at 1:10,000 and incubating at room temperature for 2 h. Images of the protein bands were quantified using Image J software (version 1.43b).

Statistical analysis

Data were documented as the mean ± standard deviation (SD), and group differences were

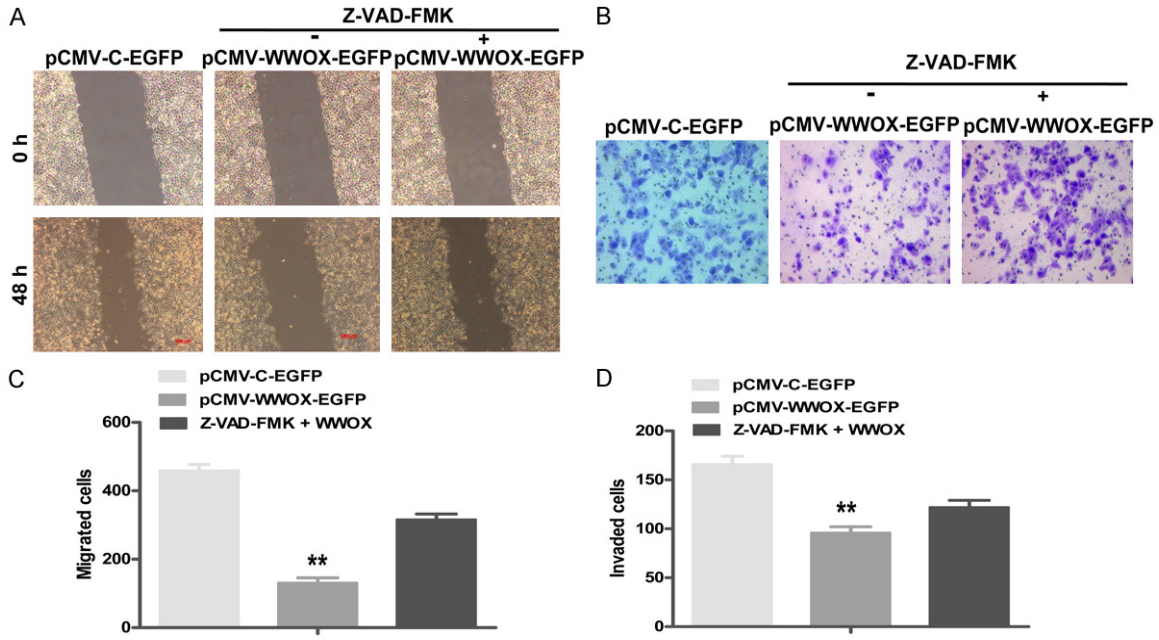

Figure 2. Effects of WWOX restoration on CNE2 cell migration and invasion. A, C: Wound healing migration assay. B, D: Migration and invasion ability of the CNE2 cells were assessed by Transwell assay, × 200. **P<0.01 vs. pCMV-C-EGFP group.

analyzed via one-way analysis of variance (ANOVA) based on the platform of SPSS 16.0 software. P<0.05 was regarded as data with statistical differences.

Results

WWOX restoration suppresses proliferation in CNE2 cells

Firstly, we examined whether the combined plasmid was successfully transfected into CNE2 cells (a kind of NPC cell line reveals undetectable levels of endogenous WWOX) through fluorescence microscope and immunoblotting. Expression of WWOX-EGFP fusion protein was observed and detected at 12 h after transfection (**Figure 1A** and **1B**). As shown in **Figure 1C** and **1D**, proliferation was significantly inhibited in pCMV-WWOX-EGFP-transfected CNE2 cells as compared to the pCMV-C-EGFP contrast, all with P<0.01 at 48 and 72 h during transfection, whereas in Z-VAD-FMK supplemented pCMV-WWOX-EGFP group, the inhibiting effects could be partly inversed.

WWOX restoration reduces the migration and invasion of CNE2 cells

To dissect the character of WWOX in the metastasis of CNE2 cells, we performed *in vitro* cell migration and invasion experiments. The migration and invasion ability were markedly decreased in WWOX restored CNE2 cells when versus the paired control transfected with empty plasmid (**Figure 2**, P<0.01). Furthermore, Z-VAD-FMK could partially rescue the inhibition of migration and invasion in WWOX overexpression cells.

WWOX overexpression enhances apoptosis in CNE2 cells

In order to validate whether the growth inhibiting effects of WWOX restoration is due to apoptosis, we employed flow cytometric analysis and DNA degradative fragments assay. As the transfecting plasmid was EGFP tagged, we stained cells using Annexin V PE/7-AAD (orange red fluorescence) [12]. In comparison to pCMV-C-EGFP control, pCMV-WWOX-EGFP transfected CNE2 cells exhibited higher apoptosis ratios (%) (**Figure 3A** and **3B**, P<0.01, when vs. pCMV-C-EGFP group) as well as increased apoptotic 'DNA ladders' with time lapse (**Figure 3C**). Importantly, WWOX re-expression cells revealed decreased apoptosis ratios (%) and inconspicuous 'DNA degradative fragments' after treated by Z-VAD-FMK (**Figure 3**). These results strongly suggested that the caspase cascade is linked to WWOX-induced apoptosis in CNE2 cells.

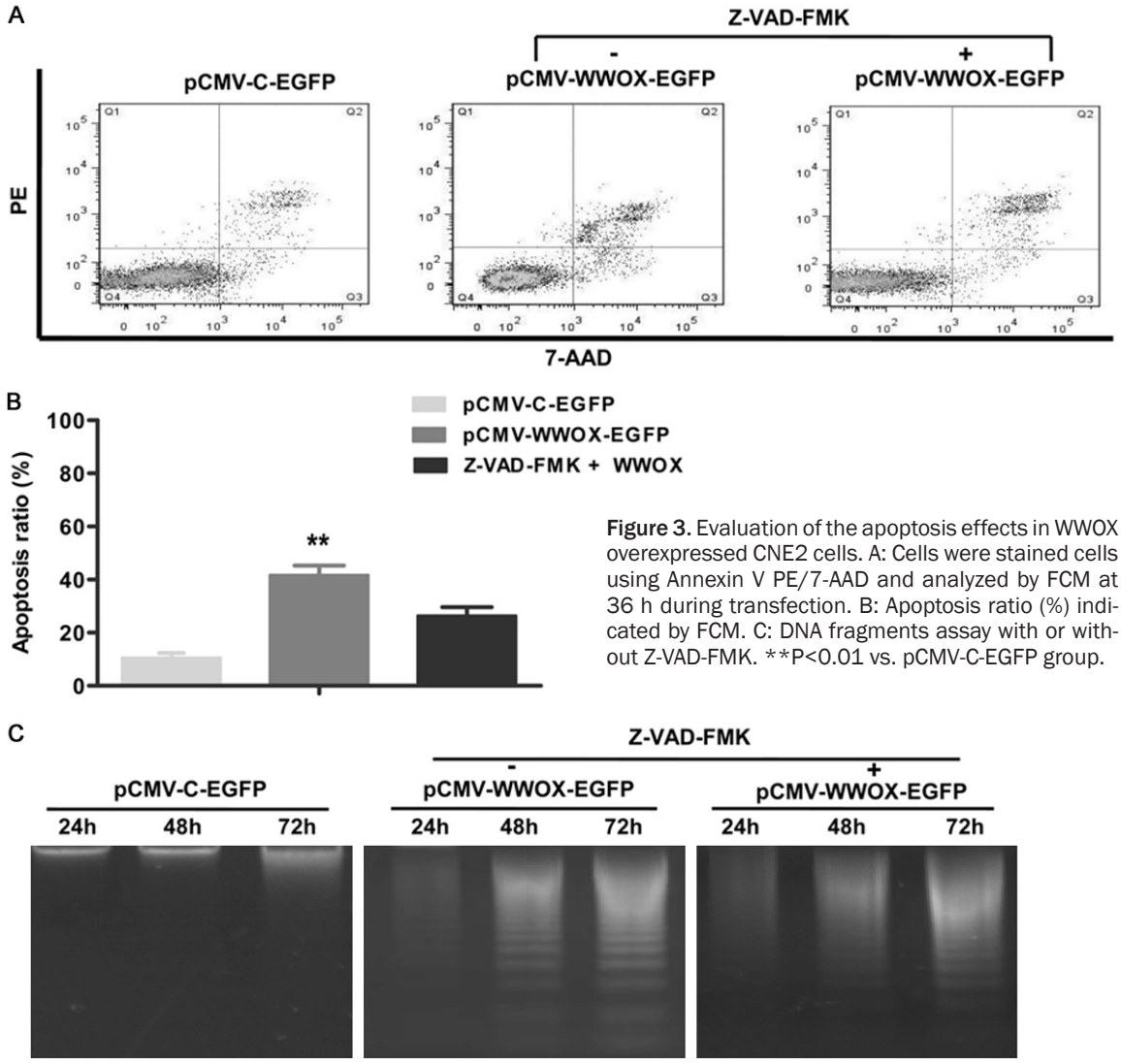

Figure 3. Evaluation of the apoptosis effects in WWOX overexpressed CNE2 cells. A: Cells were stained cells using Annexin V PE/7-AAD and analyzed by FCM at 36 h during transfection. B: Apoptosis ratio (%) indicated by FCM. C: DNA fragments assay with or without Z-VAD-FMK. **P<0.01 vs. pCMV-C-EGFP group.

WWOX re-expression triggers the mitochondrial pathway in CNE2 cells

Lastly, in order to address whether exogenous WWOX promotes cell apoptosis via the mitochondrial signaling pathway, we assessed the levels of apoptosis-associated proteins by immunoblotting. Endogenous levels of Bcl-2 and Bcl-xL were down-regulated, whereas Bax and Cytochrome c were elevated after re-expression of WWOX in CNE2 cells (**Figure 4A**). As expected, WWOX restoration promoted the cleave effects of Caspase-9, Caspase-3 and PARP in CNE2 cells (**Figure 4A**), whereas blocking caspase activity using Z-VAD-FMK displayed contradict effects to apoptosis by preventing the alteration of Bcl-2, Bcl-xL, Bax and Cytochrome c expression levels as well as the activation of Caspase-9 and Caspase-3 proteins in the caspase cascade (**Figure 4A**).

Discussion

Inactivation of the tumor suppressor genes is a hallmark of most of malignant neoplasms [18], including nasopharyngeal carcinoma (NPC). Reduced or loss of WWOX expression is linked to the tumorigenesis and development of tumors of various origins (reviewed in [7, 8, 11]. Additionally, several studies have documented that exogenous WWOX could inhibit cancer cell growth in vitro and reduce tumor burden in vivo, revealing a more aggressive tumor behavior in diverse carcinomas [4, 19, 20]. We have previously shown that WWOX expression is reduced or absent in the majority of leukemia cases and

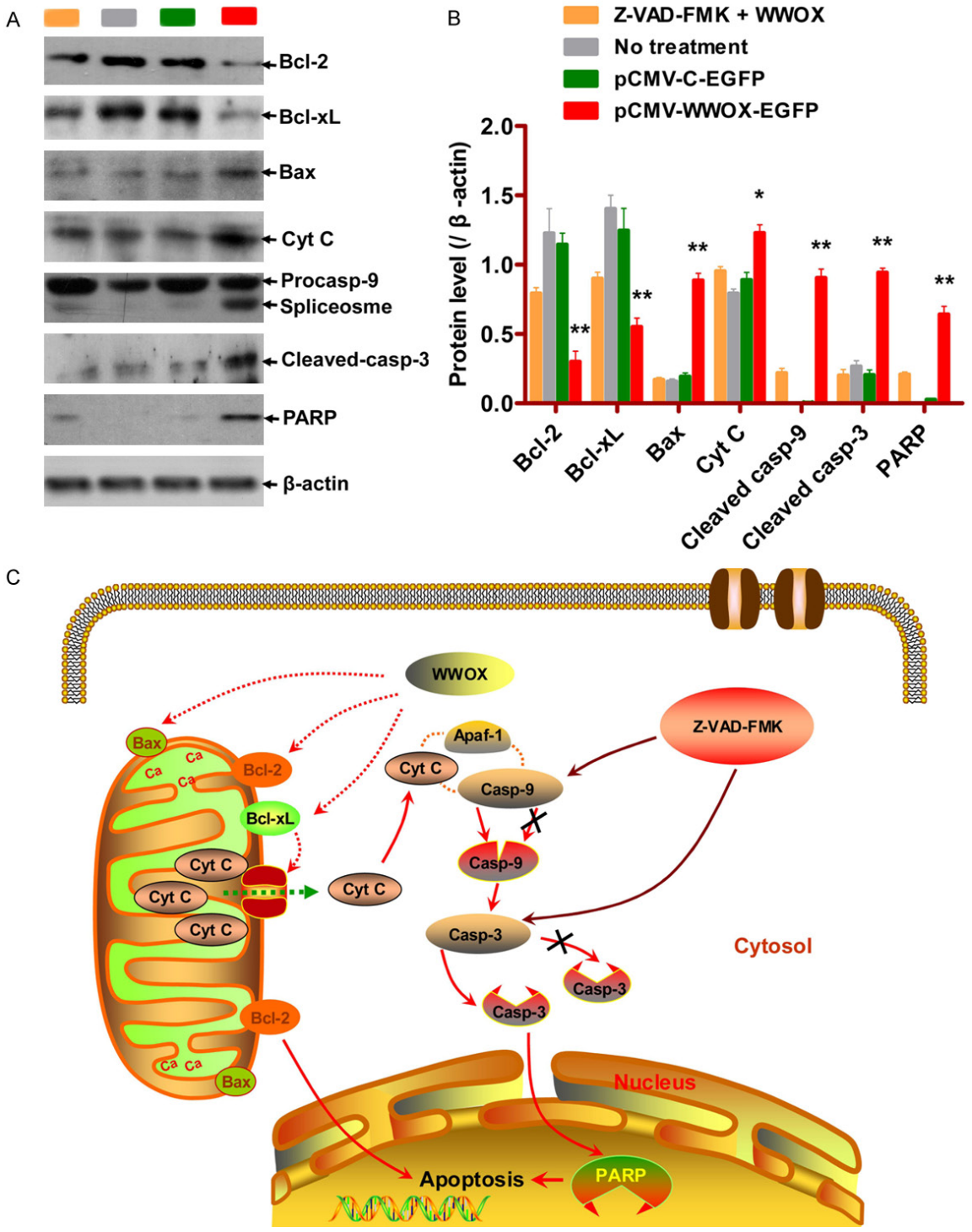

Figure 4. Expression level of apoptosis related proteins in WWOX-mediated apoptosis in CNE2 cells. A: The apoptotic proteins were analyzed by immunoblotting. B: Expression of apoptotic proteins were quantified using ImageJ software. C: Schematic diagram of WWOX-mediated apoptosis in CNE2 cells via the mitochondrial pathway. Casp-9: caspase-9; Casp-3: caspase-3; Cyt C: Cytochrome c. *P<0.05, **P<0.01 vs. pCMV-C-EGFP group.

that restoring WWOX levels resulted in a blockage of tumorigenicity in leukemia cell lines [12, 13, 21]. An interesting finding that emerged from our recent study was that some NPC cases express little even no *WWOX*/WWOX in blood or tumor lesions (unpublished data), which further

prompted us to hypothesize that WWOX might implicate in the tumorigenesis of NPC. Herein, we restored WWOX expression in CNE2 NPC cells (a cell line lacks endogenous WWOX) by transfecting with the pCMV-WWOX-EGFP plasmid [14]. In agreement with most of the published studies supporting the antineoplastic effects of WWOX in tumors [13, 14, 20, 22, 23], we observed distinct suppression effects of WWOX in CNE2 cell proliferation and metastasis.

An important finding from our study is that WWOX is likely to enhance apoptosis and weaken metastasis in CNE2 through the caspase-dependent mechanism. Before this, several studies have provided evidence upholding that WWOX is a caspase-linked tumor suppressor which suppresses cancer cell viability and promotes apoptosis by triggering the caspase cascade [13, 14, 22, 23]. In order to verify whether the caspase-dependent mechanism is involved in WWOX-mediated cell death, we blocked the caspase activity in WWOX restored CNE2 cell utilizing a broader caspase inhibitor (Z-VAD-FMK). Our data showed that inhibiting effects of WWOX on cell viability could be partly inversed by the supplement of Z-VAD-FMK. It is therefore convincible that WWOX suppresses CNE2 proliferation likely through the regulation of the caspase cascade.

On the other hand, deregulation of WWOX is connected with carcinogenesis and cancer metastasis, as WWOX is barely detectable in nearly all of metastatic tumor tissues [4]. Some study had highlighted that reduced protein level of WWOX contributes to lymph node metastasis [24, 25]. Our findings now further show that WWOX also plays a critical role in NPC cell metastasis: the transiently overexpressed WWOX could weaken migration and invasion of the CNE2 cells. As reported, recent study has demonstrated that WWOX suppresses osteosarcoma metastasis by modulating RUNX2 transactivation function [26]. Here, we further blocked the caspase activity in WWOX restored CNE2 cells utilizing Z-VAD-FMK, and observed that Z-VAD-FMK could partially rescue the inhibition of migration and invasion of the WWOX overexpressed cells. Consistent with the above-mentioned notion, our results suggest that WWOX suppresses the metastasis of CNE2 also through the caspase-dependent mechanism.

Nevertheless, the classic apoptosis pathways all converge on the activation of the caspase protease family [27]. Indeed, emergence evidence has revealed that WWOX exerts its antineoplastic effects through the intrinsic apoptotic pathways [7, 13, 14, 22]. We therefore deeply assessed the expression levels of apoptosis-associated factors involved in mitochondrial signaling pathway. Bcl-2 is one of the critical regulators of the apoptotic process and regulates apoptosis by controlling the mitochondrial membrane permeability [28], yet Bcl-xL controls outer mitochondrial membrane channel (VDAC) opening and thus regulates the mitochondrial Cytochrome c released from mitochondria to cytosol [29, 30]. Bax accelerates programmed cell death by antagonizing the apoptosis repressor Bcl-2 and forms heterodimers with Bcl-xL or Bcl-2 [31]. The released Cytochrome c induces the binding effects of Caspase-9 to the apoptosis-activating factor (Apalf-1) into Caspase-9-Apalf-1 complex, which further induces the cleaving procedure and activate Caspase-3 (**Figure 4C**) [30]. Subsequently, the spliced Caspase-3 sequentially cleaves PARP between Asp124 and Gly215 and finally leads to apoptosis. Our study demonstrated that WWOX overexpression resulted in a down-regulation of Bcl-2 and Bcl-xL, and up-regulation of Bax and Cytochrome c. Furthermore, both procaspase-3 and -9 were cleaved and activated by presenting their spliceosomes in WWOX restored CNE2 cells. Notably, Z-VAD-FMK partly blocked the alteration of Bcl-2, Bcl-xL, Bax and Cytochrome c as well as the activation of Caspase-9 and Caspase-3 proteins during WWOX re-expression. These data provide evidence that the intrinsic apoptotic pathway is involved in the WWOX-mediated apoptosis in CNE2 cells.

In summary, re-expression of WWOX is a potent inducer of apoptosis in CNE2 NPC cells and restored WWOX promotes apoptosis in CNE2 cells possibly by triggering the mitochondrial signaling pathway. Our results may offer better understanding of the biological features of WWOX in NPC, thereby paving the way for finding and developing novel biomarkers and therapeutic targets for NPC. Despite the promising results, the current dose has many flaws. For example, we only assessed the functional role of WWOX in vitro and only one kind of cell line was evaluated. Additionally, the clinical charac-

terization of WWOX in NPC also warranted further investigation.

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Disclosure of conflict of interest

None.

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References

[1] Huang J, Li Q, Zheng Y, Shen J, Li B, Zou R, Wang J and Yuan Y. Partial hepatectomy for liver metastases from nasopharyngeal carcinoma: a comparative study and review of the literature. BMC Cancer 2014; 14: 818.

[2] Chiang AK, Mak NK and Ng WT. Translational research in nasopharyngeal carcinoma. Oral Oncol 2014; 50: 345-352.

[3] Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA and Aldaz CM. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. Cancer Res 2000; 60: 2140-2145.

[4] Li J, Liu J, Ren Y, Yang J and Liu P. Common Chromosomal Fragile Site Gene WWOX in Metabolic Disorders and Tumors. Int J Biol Sci 2014; 10: 142-148.

[5] Nunez MI, Ludes-Meyers J and Aldaz CM. WWOX protein expression in normal human tissues. J Mol Histol 2006; 37: 115-125.

[6] Del Mare S, Salah Z and Aqeilan RI. WWOX: its genomics, partners, and functions. J Cell Biochem 2009; 108: 737-745.

[7] Lo JY, Chou YT, Lai FJ and Hsu LJ. Regulation of cell signaling and apoptosis by tumor suppressor WWOX. Exp Biol Med (Maywood) 2015; 240: 383-391.

[8] Richards RI, Choo A, Lee CS, Dayan S and O'Keefe L. WWOX, the chromosomal fragile site FRA16D spanning gene: its role in metabolism and contribution to cancer. Exp Biol Med (Maywood) 2015; 240: 338-344.

[9] Baryla I, Styczen-Binkowska E and Bednarek AK. Alteration of WWOX in human cancer: a clinical view. Exp Biol Med (Maywood) 2015; 240: 305-314.

[10] Teng CC, Yang YT, Chen YC, Kuo YM and Sze CI. Role of WWOX/WOX1 in Alzheimer's disease pathology and in cell death signaling. Front Biosci (Schol Ed) 2013; 5: 72-85.

[11] Schrock MS and Huebner K. WWOX: a fragile tumor suppressor. Exp Biol Med (Maywood) 2015; 240: 296-304.

[12] Lin D, Cui Z, Kong L, Cheng F, Xu J and Lan F. p73 participates in WWOX-mediated apoptosis in leukemia cells. Int J Mol Med 2013; 31: 849-854.

[13] Cui Z, Lin D, Cheng F, Luo L, Kong L, Xu J, Hu J and Lan F. The role of the WWOX gene in leukemia and its mechanisms of action. Oncol Rep 2013; 29: 2154-2162.

[14] Zhang H, Kong L, Cui Z, Du W, He Y, Yang Z, Wang L and Chen X. The WWOX gene inhibits the growth of U266 multiple myeloma cells by triggering the intrinsic apoptotic pathway. Int J Mol Med 2014; 34: 804-809.

[15] Watanabe A, Hippo Y, Taniguchi H, Iwanari H, Yashiro M, Hirakawa K, Kodama T and Aburatani H. An opposing view on WWOX protein function as a tumor suppressor. Cancer Res 2003; 63: 8629-8633.

[16] Yang Z, Lan H, Chen X, Li P, Li S, Mo W and Tang A. Molecular alterations of the WWOX gene in nasopharyngeal carcinoma. Neoplasma 2014; 61: 170-176.

[17] Kong L, Du W, Cui Z, Wang L, Yang Z, Zhang H and Lin D. Expression of lactate dehydrogenase C in MDAMB231 cells and its role in tumor invasion and migration. Mol Med Rep 2016; 13: 3533-3538.

[18] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.

[19] Gao G and Smith DI. WWOX, large common fragile site genes, and cancer. Exp Biol Med (Maywood) 2015; 240: 285-295.

[20] Fabbri M, Iliopoulos D, Trapasso F, Aqeilan RI, Cimmino A, Zanesi N, Yendamuri S, Han SY, Amadori D, Huebner K and Croce CM. WWOX gene restoration prevents lung cancer growth in vitro and in vivo. Proc Natl Acad Sci U S A 2005; 102: 15611-15616.

[21] Chen J, Chen Y, Gu L, Li X, Gao Y, Lyu X, Chen L, Luo G, Wang L, Xie Y, Duan J, Peng C and Ma X. LncRNAs act as prognostic and diagnostic biomarkers in renal cell carcinoma: a systematic review and meta-analysis. Oncotarget 2016; 7: 74325-74336.

[22] Zhang P, Jia R, Ying L, Liu B, Qian G, Fan X and Ge S. WWOX-mediated apoptosis in A549 cells mainly involves the mitochondrial pathway. Mol Med Rep 2012; 6: 121-124.

[23] Hu BS, Tan JW, Zhu GH, Wang DF, Zhou X and Sun ZQ. WWOX induces apoptosis and inhibits

proliferation of human hepatoma cell line SMMC-7721. World J Gastroenterol 2012; 18: 3020-3026.
[24] Lan C, Chenggang W, Yulan B, Xiaohui D, Junhui Z and Xiao W. Aberrant expression of WWOX protein in epithelial ovarian cancer: a clinicopathologic and immunohistochemical study. Int J Gynecol Pathol 2012; 31: 125-132.
[25] Maeda N, Semba S, Nakayama S, Yanagihara K and Yokozaki H. Loss of WW domain-containing oxidoreductase expression in the progression and development of gastric carcinoma: clinical and histopathologic correlations. Virchows Arch 2010; 457: 423-432.
[26] Del Mare S and Aqeilan RI. Tumor Suppressor WWOX inhibits osteosarcoma metastasis by modulating RUNX2 function. Sci Rep 2015; 5: 12959.
[27] Green DR and Llambi F. Cell death signaling. Cold Spring Harb Perspect Biol 2015; 7.
[28] Gross A. BCL-2 family proteins as regulators of mitochondria metabolism. Biochim Biophys Acta 2016; 1857: 1243-1246.
[29] Zheng JH, Viacava Follis A, Kriwacki RW and Moldoveanu T. Discoveries and controversies in BCL-2 protein-mediated apoptosis. FEBS J 2016; 283: 2690-700.
[30] Shoshan-Barmatz V, Ben-Hail D, Admoni L, Krelin Y and Tripathi SS. The mitochondrial voltage-dependent anion channel 1 in tumor cells. Biochim Biophys Acta 2015; 1848: 2547-2575.
[31] Wang K, Gross A, Waksman G and Korsmeyer SJ. Mutagenesis of the BH3 domain of BAX identifies residues critical for dimerization and killing. Mol Cell Biol 1998; 18: 6083-6089.