

RESEARCH ARTICLE

XIAP Associated Factor 1 (XAF1) Represses Expression of X-linked Inhibitor of Apoptosis Protein (XIAP) and Regulates Invasion, Cell Cycle, Apoptosis, and Cisplatin Sensitivity of Ovarian Carcinoma Cells

Wen-Jing Zhao¹, Bo-Ya Deng², Xue-Mei Wang^{1*}, Yuan Miao³, Jian-Nan Wang¹

Abstract

Background: X-linked inhibitor of apoptosis protein (XIAP) associated factor 1 (XAF1) exhibits aberrantly low or absent expression in various human malignancies, closely associated with anti-apoptosis and overgrowth of cancer cells. However, limited attention has been directed towards the contribution of XAF1 to invasion, apoptosis, and cisplatin (DDP)-resistance of epithelial ovarian cancer (EOC) cells. This study aimed to evaluate the potential effects of XAF1 on invasion, cell cycle, apoptosis, and cisplatin-resistance by overexpressing XAF1 in SKOV-3 and SKOV-3/DDP cells. **Methods and Results:** The pEGFP-C1-XAF1 plasmid was transfected into SKOV-3 and SKOV-3/DDP cells, and the expression of XAF1 at both mRNA and protein levels was analyzed by reverse transcription-PCR and Western blotting. Overexpression of XAF1 suppressed XIAP expression in both SKOV-3 and SKOV-3/DDP cells. Transwell invasion assays demonstrated that XAF1 exerted a strong anti-invasive effect in XAF1-overexpressing cells. Moreover, flow cytometry analysis revealed that XAF1 overexpression arrested the cell cycle at G0/G1 phase, and cell apoptosis analysis showed that overexpression of XAF1 enhanced apoptosis of SKOV-3 and SKOV-3/DDP cells apparently by activating caspase-9 and caspase-3. Furthermore, MTT assay confirmed a dose-dependent inhibitory effect of cisplatin in the tested tumor cells, and overexpression of XAF1 increased the sensitivity of SKOV-3 and SKOV-3/DDP cells to cisplatin-mediated anti-proliferative effects. **Conclusions:** In summary, our data indicated that overexpression of XAF1 could suppress XIAP expression, inhibit invasion, arrest cell cycle, promote apoptosis, and confer cisplatin-sensitivity in SKOV-3 and SKOV-3/DDP cells. Therefore, XAF1 may be further assessed as a potential target for the treatment of both cisplatin-resistant and non-resistant EOCs

Keywords: Epithelial ovarian cancer cells - XAF1 - invasion - apoptosis - cisplatin sensitivity

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Introduction

Epithelial ovarian cancer (EOC) accounts for 80-90% in all ovarian cancers and is one of the most lethal gynecologic malignancies (Arikan et al., 2014; Mhawech-Fauceglia et al., 2014). An estimated 1 out of 70 women is under life-threatening risk of EOC worldwide (Siegel et al., 2013). EOC is considered to be a silent killer because of its insidious onset and nonspecific early symptoms, thus it is always undiagnosable until advanced stages (Russell et al., 2014). Currently, cisplatin (DDP)-based chemotherapy combined with cytoreduction is the main clinical strategy for EOC, but is limited by high recurrence with a rate of approximately 70% and chemoresistance especially in platinum-resistant tumors (Rai et al., 2014). Hence, effective therapeutic approaches to control recurrence and cisplatin-resistance of EOC are expected

to reduce mortality of this disease.

X-linked inhibitor of apoptosis protein (XIAP) is the most potent member in the family of inhibitors of apoptosis (IAP) and is well known as a repressor of apoptosis by binding to caspase-3, -7, -9 to prevent their activities (LeBleu et al., 2013; Zhu et al., 2014a). Thus, downregulation of XIAP is recognized as an efficient anti-cancer approach (Li et al., 2012; Li et al., 2013). XIAP associated factor 1 (XAF1) is a critical pro-apoptotic molecule and a natural IAP antagonist capable of antagonizing XIAP-mediated anti-apoptotic actions through binding to XIAP. Clinical researches reported that XAF1 was ubiquitously expressed in healthy human tissues, but was at undetectable or extremely low levels and associated with poor prognosis and survival in multiple malignant tumors, such as gastric carcinoma (Byun et al., 2003; Shibata et al., 2007; Kim et al., 2011),

¹Department of Medical Ultrasonics, ²Department of Gynecology, ³Department of Pathology, The First Affiliated Hospital of China Medical University, Shenyang, People's Republic of China *For correspondence: xuemeiwangx@163.com

hepatocellular carcinoma (Zhang et al., 2008; Wu et al., 2010), lung cancer (Chen et al., 2011), clear-cell renal cancer (Kempkensteffen et al., 2009), human melanoma (Ng et al., 2004) and ovarian cancer (Wang et al., 2012). In vitro studies demonstrated that overexpression of XAF1 could promote apoptosis in various cancer cells (Liston et al., 2001; Chen et al., 2011; Zhu et al., 2014b), and XAF1 plays an essential role in the suppression of cell proliferation and tumor growth (Wang et al., 2006; Qi et al., 2007; Qiao et al., 2008; Chen et al., 2011; Zhu et al., 2014b). Wang et al. (2012) reported that XAF1 overexpression could decrease proliferation and microvessel density (MVD) in SKOV-3 cells. However, little is known about the roles of XAF1 in invasion, apoptosis and cisplatin-resistance in normal EOC and cisplatin-resistant EOC cells.

In the present study, we successfully established XAF1-overexpressing SKOV-3 and SKOV-3/DDP cells to explore whether XAF1 could affect invasiveness, apoptosis, and cisplatin-resistance of EOC and cisplatin-resistant EOC cells. Our results showed that overexpression of XAF1 inhibited XIAP expression, suppressed invasion, arrested cell cycle at G0/G1 phase, and promoted apoptosis of SKOV-3 and SKOV-3/DDP cells. Furthermore, XAF1-overexpressing cells were more sensitive to cisplatin.

Materials and Methods

Cell Culture

Human epithelial ovarian cancer cell line SKOV3 and its cisplatin-resistant counterpart SKOV3/DDP cell line were purchased from Cancer Institute and Hospital, Chinese Academy of Medical Sciences (Beijing, China). Cells were grown in Roswell Park Memorial Institute-1640 (RPMI-1640, Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA) and streptomycin/penicillin (100 U/mL) at 37°C in an atmosphere of 5% CO₂.

Plasmid and Transfection

The plasmid pEGFP-C1-XAF1 harboring XAF1-coding sequences was constructed by Wanleibio Co., Ltd. (Shenyang, China) and was transfected into SKOV3 cells and SKOV3/DDP cells respectively using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) strictly according to the manufacturer's instruction. The untransfected control cells (parental SKOV-3 and SKOV-3/DDP respectively) and the cells transfected with the empty vector (pEGFP-C1) were experimented in parallel as control. Cells were harvested at 48h after transfection for further experiments.

Reverse Transcription-PCR

Total RNA in each sample was extracted with an RNA extraction kit (TIANGEN Biotech, Beijing, China) and was reverse-transcribed into cDNA. PCR reactions were carried out with 2× Power Taq PCR MasterMix (Biotek Corporation, Beijing, China), then the products were separated by agarose gel electrophoresis and visualized by Gelview (Solarbio, Beijing, China).

Relative expression was calculated by Quantity One software and β-actin was served as an internal control. The cycling profile was: initial denaturation at 95°C for 5 min, 30 cycles consisting of 95 °C for 20s, 60°C for 20s, and 72°C for 30s. The following primers were used: XAF1, 5'-CGCCCTGTTGAGTGTAAGTT-3' (sense) and 5'-CCTGTTCACTGCGACAG-ACA-3' (antisense); XIAP, 5'-ATGACAGGGCTGGAAGTGACC-3' (sense) and 5'-ACTATGTCCCAGTCAGGGCTCT-3' (antisense); β-actin, 5'-CTTAGTTGC-GTTACACCCTTCTTG-3' (sense) and 5'-CTGTCACCTTCACCGTTCCAGTTT-3' (antisense).

Western Blot

Total proteins were extracted by lysing the cells with NP-40 lysate (Beyotime, Haimen, China) including 1% phenylmethanesulfonyl fluoride (PMSF). The protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Beyotime). 40 μg proteins from each sample was resolved by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were then blocked with 5% non-fat milk overnight and probed with primary antibodies against XAF1 and XIAP (both 1:1000 diluted, Abcam) at 4°C overnight, followed by incubation with a secondary anti-rabbit IgG-HRP antibody (1:5000 dilution, Beyotime) at 37°C for 45 min. The target bands were visualized by enhanced chemiluminescence (ECL) solution (Qihai Biotec, Shanghai, China) and were analyzed by Gel-Pro-Analyzer software (Bethesda, MD, USA). β-actin was served as an internal control.

Matrigel-Based Invasion Assay

The matrigel-based invasion assay was carried out in a transwell system (Corning, Tewksbury, MA, USA) with a matrigel (BD Biosciences, San Jose, CA, USA) -coated polycarbonate membrane in the upper chamber. Cells were resuspended in FBS-free medium and plated in the upper chamber at a density of 2×10⁴ per well. 800 μl RPMI-1640 supplemented with 20% FBS was added into the lower chamber. After incubation for 24h in a 37°C incubator, the non-migrated cells on the upper surface of the membrane were wiped off with cotton swabs, and the invading cells were fixed with 4% paraformaldehyde for 20 min and stained with hematoxylin for 5 min. The invading cells in each group were counted in five randomly selected fields under an inverted microscope.

Cell cycle analysis by flow cytometry

Cells in each group were fixed with 70% ethanol, and cell cycle analysis was performed with a Cell Cycle Detection Kit (Beyotime) following the manufacturer instructions. Briefly, cells were resuspended in 500 μl binding buffer and mixed sequentially with 25 μl propidium iodide (PI) and 10 μl RNase A. The mixture was incubated for 30 min at 37°C in the dark and then analyzed immediately with a flow cytometer (Becton-Dickinson, Franklin Lakes, USA). The percentage of cells at each stage of cell cycle was obtained using ModFit software.

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Cell apoptosis analysis by flow cytometry

According to the protocol of the Annexin V-FITC/PI apoptosis detection kit (KeyGen Biotech, Nanjing, China), cells were resuspended in 500 μ l binding buffer containing 5 μ l Annexin V-FITC and 5 μ l PI. Then the mixture was incubated for 15 min at room temperature in the dark. Cell apoptosis was detected immediately by flow cytometry and analyzed by CellQuest software.

Cisplatin sensitivity analysis by MTT assay

Cells were seeded in 96-well plates at a density of 2×10^3 per well and cultured to 80% confluence in a 37°C, 5% CO₂ incubator. Cells were then exposed to cisplatin (Meilun, Dalian, China) of an ascending concentration range (0.75, 1.5, 3, 6, 12, 20, 40 μ M) for 48h and 72h respectively, with five replicates for each testing point including untreated controls (0 μ M cisplatin for 48h and 72h) and blank wells. Thereafter, cells were incubated with 8 μ l MTT (0.2 mg/ml) (Sigma-Aldrich, St. Louis, MO, USA) for 4h followed by incubation with 200 μ l DMSO (Sigma-Aldrich, St. Louis, MO, USA) to dissolve the crystals before reading the absorbance at 490 nm in a microplate reader (BIOTEK, Vermont, USA). The results are expressed as inhibitory rates and cisplatin IC₅₀ values (the concentrations resulting in 50% inhibition).

Caspase activity assay

Caspase activity was assessed by Caspase 3 Activity Assay Kit and Caspase 9 Activity Assay Kit (Beyotime). In brief, cells in each group were lysed on ice for 15 min. Bradford method was used to determine protein concentrations in each sample. Then 20 μ g total proteins were added into reaction buffer to a total volume of 90 μ l, and incubated overnight at 37°C with 10 μ l substrates (2 mM) of caspase-3 or caspase-9 (Ac-DEVD-pNA and Ac-LEHD-pNA respectively). The absorbance spectrum of the catalyzed product pNA was determined using a spectrophotometer (Thermo, Waltham, MA, USA), and the values at 405 nm were used as an indicator for caspase activities.

Statistical analysis

Statistical analysis was performed with SPSS 17.0 software. All values are reported as mean \pm standard deviation (SD), and differences between groups were analyzed using one-way analysis of variance (ANOVA). $p < 0.05$ was considered to be statistically significant.

Results

Overexpression of XAF1 in EOC cells suppresses the expression of XIAP

To explore the function of XAF1 in EOC cells, pEGFP-C1-XAF1 plasmid encoding XAF1 was transfected into SKOV-3 and SKOV-3/DDP cells, then the expression levels of XAF1 were examined by reverse transcription-PCR and western blot analysis. The mRNA levels of XAF1 in SKOV-3 and SKOV-3/DDP cells transfected with XAF1 were 1.40-fold and 1.60-fold (Figure 1A, $p < 0.01$) higher than those in the respective control cells transfected with pEGFP-C1 vectors. The protein levels of XAF1 in

SKOV-3 and SKOV-3/DDP were 1.89-fold and 2.57-fold higher than those in the respective control cells (Figure 1B, $p < 0.01$). Thus, the above results indicated that XAF1 was overexpressed in SKOV-3 and SKOV-3/DDP cells transfected with pEGFP-C1-XAF1.

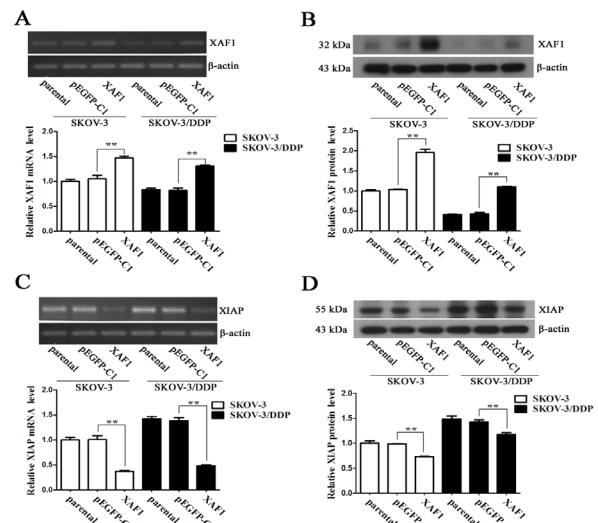


Figure 1. Overexpression of XAF1 in EOC Cells Suppresses the Expression of XIAP. (A) Reverse transcription-PCR analysis of XAF1 mRNA expression levels; (B) Representative photographs of western blot analysis on XAF1 protein expression levels; (C) Reverse transcription-PCR analysis of XIAP mRNA expression levels; (D) Representative photographs of western blot analysis on XIAP protein expression levels. Data in each group are expressed as mean \pm SD from three independent experiments. β -actin was used as an internal control. ** $p < 0.01$

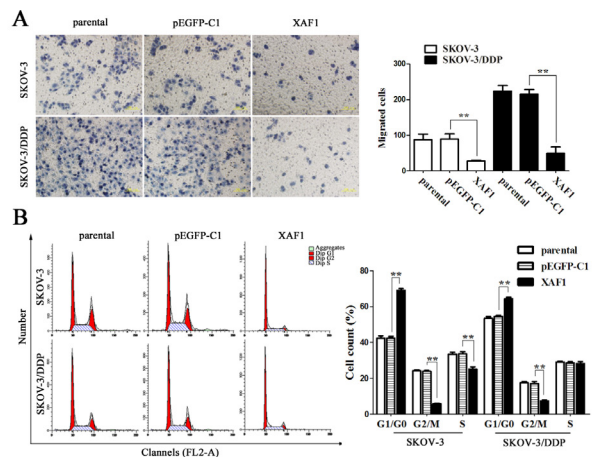


Figure 2. XAF1 Inhibits Invasion and Arrests Cell-cycle of SKOV-3 and SKOV-3/DDP Cells. (A) Cellular invasion was assessed with a Matrigel-coated Transwell system. After incubated for 24h, invading cells were fixed and stained with hematoxylin. Quantitation of invading cells was carried out with five randomly selected microscopic fields. Scale bars indicate 100 μ m at 200 \times magnification. (B) Cell cycle distribution by flow cytometry analysis. Cells in each group were fixed and stained with propidium iodide, followed by flow cytometry analysis for the percentages of cells at each cell cycle stage. The above two experiments were repeated three times, and the representative images are shown. Data are represented as mean \pm SD, ** $p < 0.01$

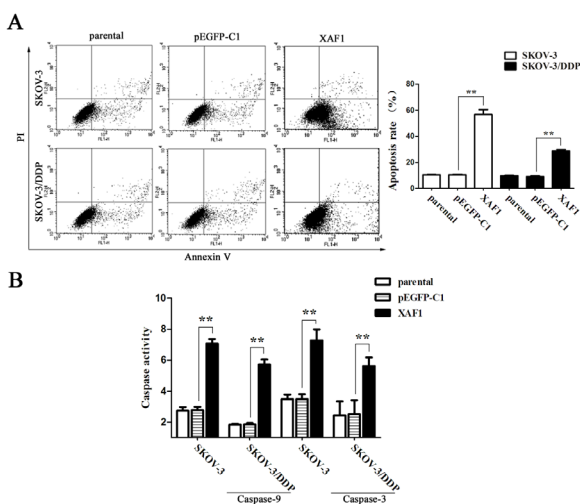


Figure 3. XAF1 Promotes Apoptosis of SKOV-3 and SKOV-3/DDP Cells. (A) Cell apoptosis was examined by Annexin V-FITC and propidium iodide staining followed by flow cytometry. Representative flow cytometry results from repeated experiments are shown on the left. The cells are characterized as early apoptotic (bottom right quadrant), late apoptotic (top right quadrant), necrotic (top left quadrant), and healthy cells (bottom left quadrant) based on the flow cytometry results, and the total apoptosis rate is summarized in a bar chart on the right. (B) Activation of caspase-9 and caspase-3 was determined with the respective Caspase Activity Assay Kits. The absorbance of the caspase-catalyzed product at 405 nm indicates caspase activity and the values are expressed as mean±SD of three independent experiments, ***p*<0.01

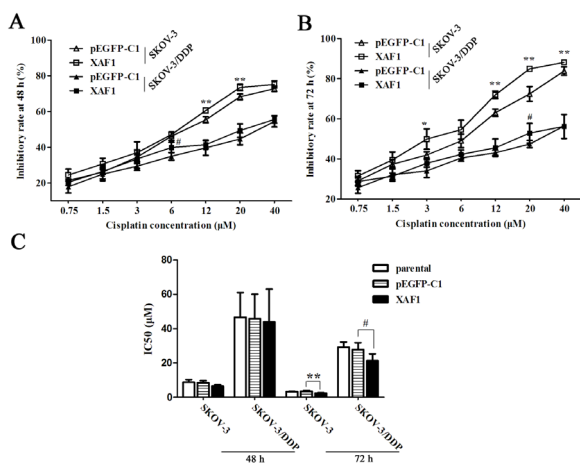


Figure 4. XAF1 Increases the Sensitivity to Cisplatin-Induced Suppression of Proliferation in SKOV-3 and SKOV-3/DDP Cells. Cells were seeded in 96-well plates and exposed to increased concentrations of cisplatin (0.75, 1.5, 3, 6, 12, 20, 40 μM) for the indicated time, followed by MTT assay. (A) The curve of inhibitory rates with 48h incubation. (B) The curve of inhibitory rates with 72h incubation. (C) IC₅₀ values of cisplatin in the indicated cells. Experiments were performed for three times with 5 replicates in each testing point, and data are expressed as mean±SD. Compared with SKOV-3 pEGFP-C1 control, ***p*<0.01; compared with SKOV-3/DDP pEGFP-C1 control, #*p*<0.05

Further study revealed that the mRNA and protein levels of XIAP in XAF1-overexpressing SKOV-3 and SKOV-3/DDP cells were decreased significantly compared with the respective pEGFP-C1 control cells (Figure 1C, 1D, *p*<0.01), suggesting that XAF1 overexpression in SKOV-3 and SKOV-3/DDP cells could suppress the expression of XIAP.

XAF1 inhibits invasion and arrests cell-cycle of SKOV-3 and SKOV-3/DDP cells

The matrigel-based invasion assay was employed to detect the effect of XAF1 on the invasiveness of EOC cells. The numbers of invading XAF1-overexpressing SKOV-3 and SKOV-3/DDP cells was 27.80±2.40, 49.60±17.68 respectively, and were significantly decreased compared with the respective pEGFP-C1 control cells (89.67±14.23, 215.60±12.81, respectively) (Figure 2A, *p*<0.01), indicating that overexpression of XAF1 could reduce the invasive capacity of both SKOV-3 and SKOV-3/DDP cells.

Next, we used PI staining-based flow cytometry analysis to investigate if XAF1 could affect the cell cycle. The results showed that XAF1-overexpressing SKOV-3 cells exhibited a substantial proportion of cells arrested in the G0/G1 phase compared with pEGFP-C1 control cells (*p*<0.01), resulting in the sharply decline of cell numbers in G2/M phase (*p*<0.01) (Figure 2B). A similar trend of cell cycle distribution was observed in XAF1-overexpressing SKOV-3/DDP cells, suggesting a cytostatic effect of XAF1 by arresting cell cycle at G0/G1 phase in both SKOV-3 and SKOV-3/DDP cells.

XAF1 promotes apoptosis of SKOV-3 and SKOV-3/DDP cells

To address the effect of XAF1 on apoptosis of EOC cells, Annexin V-FITC/PI Apoptosis Detection Kits and Caspase-3/Caspase-9 Activity Assay Kits were used to assess apoptosis rate and caspase activity in XAF1-overexpressing cells. Flow cytometry results showed that the total apoptosis rate was stimulated by 5.40 folds and 3.12 folds respectively in XAF1-overexpressing SKOV-3 and SKOV-3/DDP cells compare with the respective pEGFP-C1 controls (Figure 3A, *p*<0.01). Meanwhile, the activities of caspase-9 and caspase-3 in XAF1-overexpressing cells were significantly elevated compared with the respective pEGFP-C1 control cells (Figure 3B, *p*<0.01). Taken together, overexpressing of XAF1 could promote apoptosis of SKOV-3 and SKOV-3/DDP cells by activating the classic caspase pathway.

XAF1 increases the sensitivity to cisplatin-induced suppression of proliferation in SKOV-3 and SKOV-3/DDP cells

The inhibitory curves were plotted according to MTT assay results to visualize the effect of XAF1 on cisplatin sensitivity in EOC cells. The results of 48h and 72h both showed that the growth inhibitory rate of cisplatin in SKOV-3 and SKOV-3/DDP cells was significantly elevated in a dose-dependent manner upon cisplatin treatment. The anti-proliferative effect of cisplatin in control SKOV-3/DDP cells transfected with empty

pEGFP-C1 vector was remarkably reduced compared with control SKOV-3-pEGFP-C1 cells at both 48h and 72h ($p < 0.01$), confirming the cisplatin-resistant property in SKOV-3/DDP cells (Figure 4A, 4B). In addition, compared with control SKOV-3 cells, overexpression of XAF1 in SKOV-3 cells boosted cisplatin-induced growth inhibition at concentrations above 12 μM at 48h and at concentrations above 3 μM at 72h. Meanwhile, overexpression of XAF1 in cisplatin-resistant SKOV-3/DDP cells slightly increased the sensitivity to cisplatin-mediated growth inhibition compared with control SKOV-3/DDP cells, but the boosting effect was not as prominent as in SKOV-3 cells. Furthermore, as shown in Figure 4C, the IC_{50} values of cisplatin at 72h in both cell types overexpressing XAF1 were notably lower than the respective control cells transfected with pEGFP-C1 vector alone. These results demonstrated that overexpression of XAF1 could enhance the sensitivity to cisplatin and boosted its anti-proliferative function in SKOV-3 and SKOV-3/DDP cells.

Discussion

XAF1 has been recognized as a tumor suppressor concerning the pro-apoptotic and anti-proliferative properties in cancer cells, and it is frequently silent or down-regulated in numerous malignancies. However, the roles of XAF1 in cell invasion, apoptosis, and cisplatin-resistance in epithelial ovarian cancer (EOC) cells have rarely been reported. Here, we demonstrated that overexpression of XAF1 in SKOV-3 and SKOV-3/DDP cells down-regulated XIAP expression, reduced invasiveness, promoted apoptosis, and increased sensitivity to cisplatin in the cells. Overall, these results preliminarily identified the roles of XAF1 in EOC and cisplatin-resistant EOC cells.

It is a well-established fact that transcriptional inactivation of XAF1 expression in tumors is a result of aberrant DNA methylation in the promoter region (Kempkensteffen et al., 2007; Murphy et al., 2008; Chen et al., 2012; Yin et al., 2012; Ling et al., 2013). Transcriptional repression of XAF1 disturbs intracellular homeostasis of XIAP and XAF1, leading to up-regulation of XIAP, and vice versa (Shibata et al., 2008). Our study demonstrated that overexpression of XAF1 down-regulated XIAP in SKOV-3 and SKOV-3/DDP cells, which was consistent with previous studies.

XAF1 has been shown to be expressed at much lower levels in poorly differentiated tumors than in high or moderate high differentiated tumors (Ma et al., 2005; Sakemi et al., 2007; Chen et al., 2011), and poor differentiation is often associated with high invasiveness of cancer cells. In the present study, we demonstrated that overexpression of XAF1 could suppress invasion not only in normal EOC cells, but also in the cisplatin-resistant EOC cells, suggesting that XAF1 plays an universal suppressive role in tumor cell invasion in EOC. In addition, prior studies reported that XAF1 could reduce proliferation of lung cancer cells and hepatocellular carcinoma cells (Yang et al., 2014; Zhu et al., 2014b),

and a similar study also demonstrated that the viability of SKOV-3 cells with XAF1 overexpression was notably decreased compared with cells transfected with empty vectors (Wang et al., 2012). However, the mechanism for the anti-proliferative function of XAF1 remains unclear. Our results showed that overexpression of XAF1 arrested the cell cycle at G0/G1 phase in both SKOV-3 and SKOV-3/DDP cells, suggesting a cytostatic effect which might lead to cell apoptosis.

Caspases are key enzymes responsible for executing apoptosis or programmed cell death. Cell apoptosis is mainly implemented through caspases-mediated cascade amplification reactions. Caspase-9 and caspase-3 are the major initiators and effectors in the intrinsic apoptotic pathway. Liston et al. found out that XAF1 triggers the re-localization of XIAP from the cytoplasm to the nucleus to sequester the interaction between XIAP and caspase-3, therefore antagonizing XIAP-mediated anti-apoptotic activities (Liston et al., 2001). Currently, the pro-apoptotic effect of XAF1 as evidenced by activating Caspase3/9 pathway has been reported in hepatocellular carcinoma cells and lung cancer cells (Ma et al., 2014; Yang et al., 2014). In this study, we observed that overexpression of XAF1 raised the apoptosis rate and enhanced activity of caspase-3 and caspase-9 in SKOV-3 and SKOV-3/DDP cells, suggesting that XAF1 may promote cell apoptosis via activating caspase-3 and caspase-9 in EOC.

Cisplatin (DDP) is one of the most common chemotherapeutic drugs capable of inhibiting DNA replication in cancer cells and damaging the structure of cell membrane, but is often accompanied by drug resistance which is directly correlated with poor prognosis. Ju and Ma demonstrated that XAF1 could increase the sensitivity to cisplatin-mediated anti-proliferative and pro-apoptotic effects in colon cancer cells and hepatocellular carcinoma cells (Ma et al., 2014; Zhu et al., 2014b), and Wang et al. confirmed that XAF1 could enhance sensitivity of SKOV3 cells to cisplatin-mediated apoptosis (Wang et al., 2012). Here we showed that XAF1 could improve the sensitivity of both SKOV-3 and SKOV-3/DDP cells to cisplatin, and boosted the growth inhibitory effects of cisplatin in SKOV-3 cells to a relatively greater extent. We therefore concluded that XAF1 could enhance the therapeutic effects of cisplatin in both cisplatin-nonresistant and cisplatin-resistant EOC cells.

In summary, the present study demonstrated that overexpression of XAF1 could suppress XIAP expression, induce cell cycle arrest, promote apoptosis, and enhance cisplatin-mediated growth inhibition in both EOC cells and cisplatin-resistant EOC cells. Our data preliminarily identified the significant roles of XAF1 in EOC and cisplatin-resistant EOC cells, and suggest that XAF1 may serve as a potential target for gene therapy for treating cisplatin-resistant and -nonresistant EOCs.

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References

- Arikan SK, Kasap B, Yetimallar H, et al (2014). Impact of prognostic factors on survival rates in patients with ovarian carcinoma. *Asian Pac J Cancer Prev*, **15**, 6087-94.
- Byun DS, Cho K, Ryu BK, et al (2003). Hypermethylation of XIAP-associated factor 1, a putative tumor suppressor gene from the 17p13.2 locus, in human gastric adenocarcinomas. *Cancer Res*, **63**, 7068-75.
- Chen XY, He QY, Guo MZ (2012). XAF1 is frequently methylated in human esophageal cancer. *World J Gastroenterol*, **18**, 2844-9.
- Chen YB, Shu J, Yang WT, et al (2011). XAF1 as a prognostic biomarker and therapeutic target in squamous cell lung cancer. *Chin Med J*, **124**, 3238-43.
- Kempkensteffen C, Fritzsche FR, Johannsen M, et al (2009). Down-regulation of the pro-apoptotic XIAP associated factor-1 (XAF1) during progression of clear-cell renal cancer. *BMC Cancer*, **9**, 276.
- Kempkensteffen C, Hinz S, Schrader M, et al (2007). Gene expression and promoter methylation of the XIAP-associated Factor 1 in renal cell carcinomas: correlations with pathology and outcome. *Cancer Lett*, **254**, 227-35.
- Kim MA, Lee HE, Lee HS, et al (2011). Expression of apoptosis-related proteins and its clinical implication in surgically resected gastric carcinoma. *Virchows Arch*, **459**, 503-10.
- LeBleu VS, Taduri G, O'Connell J, et al (2013). Origin and function of myofibroblasts in kidney fibrosis. *Nat Med*, **19**, 1047-53.
- Li G, Chang H, Zhai YP, et al (2013). Targeted silencing of inhibitors of apoptosis proteins with siRNAs: a potential anti-cancer strategy for hepatocellular carcinoma. *Asian Pac J Cancer Prev*, **14**, 4943-52.
- Li XQ, Ke XZ, Wang YM (2012). Treatment of malignant melanoma by downregulation of XIAP and overexpression of TRAIL with a conditionally replicating oncolytic adenovirus. *Asian Pac J Cancer Prev*, **13**, 1471-6.
- Ling ZQ, Lv P, Lu XX, et al (2013). Circulating methylated DNA Indicates poor prognosis for gastric cancer. *PLoS One*, **8**, 67195.
- Liston P, Fong WG, Kelly NL, et al (2001). Identification of XAF1 as an antagonist of XIAP anti-Caspase activity. *Nat Cell Biol*, **3**, 128-33.
- Ma B, Wang Y, Zhou X, et al (2014). Synergistic suppression effect on tumor growth of hepatocellular carcinoma by combining oncolytic adenovirus carrying XAF1 with cisplatin. *J Cancer Res Clin Oncol*, **141**, 419-29.
- Ma TL, Ni PH, Zhong J, et al (2005). Low expression of XIAP-associated factor 1 in human colorectal cancers. *Chin J Dig Dis*, **6**, 10-4.
- Mhaweck-Fauceglia P, Yan L, Sharifian M, et al (2014). Stromal expression of fibroblast activation protein alpha (FAP) predicts platinum resistance and shorter recurrence in patients with epithelial ovarian cancer. *Cancer Microenviron*. [Epub ahead of print]
- Murphy TM, Perry AS, Lawler M (2008). The emergence of DNA methylation as a key modulator of aberrant cell death in prostate cancer. *Endocr Relat Cancer*, **15**, 11-25.
- Ng KC, Campos EI, Martinka M, et al (2004). XAF1 expression is significantly reduced in human melanoma. *J Invest Dermatol*, **123**, 1127-34.
- Qi R, Gu J, Zhang Z, et al (2007). Potent antitumor efficacy of XAF1 delivered by conditionally replicative adenovirus vector via caspase-independent apoptosis. *Cancer Gene Ther*, **14**, 82-90.
- Qiao L, Gu Q, Dai Y, et al (2008). XIAP-associated factor 1 (XAF1) suppresses angiogenesis in mouse endothelial cells. *Tumour Biol*, **29**, 122-9.
- Rai B, Bansal A, Patel FD, et al (2014). Radiotherapy for ovarian cancers - redefining the role. *Asian Pac J Cancer Prev*, **15**, 4759-63.
- Russell S, Duquette M, Liu J, et al (2014). Combined therapy with thrombospondin-1 type I repeats (3TSR) and chemotherapy induces regression and significantly improves survival in a preclinical model of advanced stage epithelial ovarian cancer. *FASEB J*, **29**, 576-88.
- Sakemi R, Yano H, Ogasawara S, et al (2007). X-linked inhibitor of apoptosis (XIAP) and XIAP-associated factor-1 expressions and their relationship to apoptosis in human hepatocellular carcinoma and non-cancerous liver tissues. *Oncol Rep*, **18**, 65-70.
- Shibata T, Mahotka C, Wethkamp N, et al (2007). Disturbed expression of the apoptosis regulators XIAP, XAF1, and Smac/DIABLO in gastric adenocarcinomas. *Diagn Mol Pathol*, **16**, 1-8.
- Shibata T, Noguchi T, Takeno S, et al (2008). Disturbed XIAP and XAF1 expression balance is an independent prognostic factor in gastric adenocarcinomas. *Ann Surg Oncol*, **15**, 3579-87.
- Siegel R, Naishadham D, Jemal A (2013). Cancer statistics, 2013. *CA Cancer J Clin*, **63**, 11-30.
- Wang J, Peng Y, Sun YW, et al (2006). All-trans retinoic acid induces XAF1 expression through an interferon regulatory factor-1 element in colon cancer. *Gastroenterology*, **130**, 747-58.
- Wang Y, Mao H, Hao Q, et al (2012). Association of expression of XIAP-associated factor 1 (XAF1) with clinicopathologic factors, overall survival, microvessel density and cisplatin-resistance in ovarian cancer. *Regul Pept*, **178**, 36-42.
- Wu LM, Zhang F, Zhou L, et al (2010). Predictive value of CpG island methylator phenotype for tumor recurrence in hepatitis B virus-associated hepatocellular carcinoma following liver transplantation. *BMC Cancer*, **10**, 399.
- Yang WT, Chen DL, Zhang FQ, et al (2014). Experimental study on inhibition effects of the XAF1 gene against lung cancer cell proliferation. *Asian Pac J Cancer Prev*, **15**, 7825-9.
- Yin DT, Chen G, Li HQ, et al (2012). Relationship between methylation status of promoter and expression of XAF1 gene in papillary thyroid carcinoma. *Zhonghua Yi Xue Za Zhi*, **92**, 1967-70.
- Zhang F, Wu LM, Zhou L, et al (2008). Predictive value of expression and promoter hypermethylation of XAF1 in hepatitis B virus-associated hepatocellular carcinoma treated with transplantation. *Ann Surg Oncol*, **15**, 3494-502.
- Zhu L, Cheng X, Ding Y, et al (2014a). Bone marrow-derived myofibroblasts promote colon tumorigenesis through the IL-6/JAK2/STAT3 pathway. *Cancer Lett*, **343**, 80-9.
- Zhu LM, Shi DM, Dai Q, et al (2014b). Tumor suppressor XAF1 induces apoptosis, inhibits angiogenesis and inhibits tumor growth in hepatocellular carcinoma. *Oncotarget*, **5**, 5403-15.