Int J Clin Exp Pathol 2015;8(9):10061-10071
www.ijcep.com /ISSN:1936-2625/IJCEP0009402

Original Article

Anticancer activity of NOB1-targeted shRNA combination with TRAIL in epithelial ovarian cancer cells

Yang Lin, Tianmin Xu, Hong Teng, Manhua Cui

Department of Obstetrics and Gynecology, The Second Hospital, Jilin University, #218 Ziqiagn Street, Nanguan District, Changchun 130041, China

Received April 20, 2015; Accepted June 26, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) based strategy is a promising targeted therapeutic approach for the treatment of ovarian cancer. However, the effectiveness of the treatment remains limited due to the inherent or acquired resistance of tumor cells to TRAIL. Our previously study demonstrated that downregulation of NOB1 (NIN1/RPN12 binding protein 1 homolog) expression by a lentiviral short hairpin RNA (shRNA) delivery system (Lv/sh-NOB1) suppressed ovarian cancer growth. Here, Lv/sh-NOB1 and TRAIL were combined and tested the effects of this combination on ovarian cancer cells to identify more effective therapeutics against ovarian cancer by several *in vitro* experiments. Tumor growth ability in SKVO3 xenograft nude mice was also determined to define this combination treatment effect in tumorigenesis *in vivo*. *In vitro* assay showed that Lv/sh-NOB1 in combination with TRAIL treatment in ovarian cancer cell synergistically suppressed the proliferation and colony formation, as well as induced cell apoptosis and increased the activity of caspase-3, -8 and -9. *In vivo* assay showed that Lv/sh-NOB1 combination with TRAIL synergistically suppressed tumor growth of nude mice model. Importantly, we found that downregulation of NOB1 could upregulate DR5 expression and active MAPK pathway, which might contribute to increase sensitivity TRAIL to ovarian cancer cells. These findings suggested that Lv/sh-NOB1 combination with TRAIL treatment may be a potential treatment approach for ovarian cancer.

Keywords: Ovarian cancer, epithelial ovarian cancer, NOB1

Introduction

Epithelial ovarian cancer (EOC) is one of the most common gynecologic malignancies worldwide, accounting for 85-90% ovarian cancer [1, 2]. It has the highest mortality rate among malignant tumors in female reproductive system [3]. Despite there has been great improvement on traditional treatments for EOC, such as surgery, combination with radiotherapy and chemotherapy, the 5-year survival rate for advanced EOC patients is below 40% [4]. Chemotherapy drugs, such as doxorubicin (DOX), topotecan, gemcitabine, or bevacizumab, have been widely used for patients with advanced EOC, but these chemotherapy drugs did not effective improve patient overall survival due to inhere and acquire resistance to chemotherapeutic agents to ovarian cancer cells [5, 6]. Thus, it is crucial need to develop novel effective therapy for patients with EOC.

Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) has been widely used in a variety of tumor cell types due to its ability to selectively induce apoptosis in variety tumor cells without harm to normal cells [7, 8]. TRAIL can lead to recruitment of the adapter protein Fas-associated death domain (FADD) and procaspase-8 in the death inducing signaling complex (DISC) by binding to two death receptors (DR), DR4 and DR5, which contain a cytoplasmic functional death domain [9, 10]. TRAIL has been used for the treatment of ovarian cancer [11-14]. But many patients with EOC acquire resistance to chemotherapeutic agents, which limits efficacy in ovarian cancers [15, 16]. Therefore, overcoming TRAIL resistance is of great significance in ovarian cancer therapy.

NIN1/RPN12 binding protein 1 homolog (*NOB1*), located on chromosome 16q22.1, encodeing a 50 KDa protein consisting of a PIN (PilT amino

terminus) domain and a zinc ribbon domain, plays a role in maintaining cellular homeostasis by controlling protein degradation [9]. NOB1 is highly expressed in various malignancies such as prostate carcinoma, thyroid cancer, breast infiltrating ductal carcinoma and non-small lung cancer [17-20], but its expression is very low or absent in normal cells, which makes it an attractive target for cancer therapeutics. In addition, recently several studies domonstrated that downregulation of endogenous NOB1 could inhibit tumor growth in several tumor by inhibiting cell proliferation and colony formation, and inducing cell apoptosis and cell arrest G0/G1 stage [17, 19-22]. Especially, recently study has showed that downregulation expression of NOB1 by Ad/sh-NOB1 could enhance radiosensitivity and chemosensitivity in thyroid cancer cells [23, 24]. Our previous study demonstrated that the expression level of NOB1 protein was increased in patients with ovarian cancer and that down-regulation expression of NOB1 by lentiviral-mediated shRNA targeting NOB1 (Ad/sh-NOB1) suppressed ovarian cancer cell proliferation and colony formation [25]. However, to our knowledge, no prior studies have elucidated the downregulation of NOB1 decreased TRAIL resistance to ovarian cancer cells.

We hypothesized that downregulation of NOB1 could increase TRAIL sensitivity to ovarian cancer cell, and that combination Lv/sh-NOB1 and TRAIL may be more effective than either agents administered alone. Therefore, in the present study, we evaluate the potency of Lv/sh-NOB1 in combination with TRAIL in inhibiting ovarian cancer cell growth *in vitro* and *in vivo*.

Materials and method

Reagents and antibody

NOB1 shRNA lentivirus (Lv/sh-NOB1) and scramble shRNA lentivirus (Lv/sh-Scramble) were constructed and stored by our experiments [25]. Human TRAIL was obtained from PeproTech (Rocky Hill, NJ). Medium (RPMI 1640) and fetal bovine serum (FBS) were brought from Gibco (Grand Island, NY, USA). All other chemicals and solvents were obtained from Sigma (St. Louis, MO, USA).

For the western blot analysis, the following antibodies were used: Antibody to NOB1 was brought from Abcam (Cambridge, UK), antibody to GAPDH, Survivin, DR4 and DR5 were brought from Cell Signaling Technology (Danvers, Massachusetts, USA), antibody to Bcl-2, p38MAPK, phosphorylated (p-) p38MAPK, p-ERK, ERK, JNK, p-JNK and Secondary antibodies HRP-conjugated goat anti-mouse IgG was brought from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell culture

A human ovarian surface epithelial cell line (HOSEpiC) and two human ovarian cancer cell lines (SKOV3 and HEY) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere consisting of 5% CO_2.

Cell viability

Ovarian cancer cells infected with Lv/sh-NOB1 or Lv/sh-Scramble (MOI of 50 each), along with untreated cells were seeded in a 96-well plates at a density of 5×10^3 cells per well. After infected for 24 h, different concentration TRAIL (0-100 ng/ml) was added to plates and cultured for 1-3 days, cell viability was determined by MTT assay as previous described [25]. The IC_{50} values were calculated from at least three independent experiments.

Colony formation assay

Ovarian cancer cells were seeded in six-well culture plates at 1×10^3 cells/well, then were treated with Lv/sh-NOB1 ((MOI of 50 each), Lv/sh-Scramble (MOI of 50 each), TRAIL ($2\times IC_{50}$) or Lv/sh-NOB1 (MOI of 50 each) in combination with TRAIL ($1\times IC_{50}$), respectively. After 14 days, the cells were washed, fixed by paraformaldehyde, and stained with GIEMSA for 10 min, washed three times with ddH_2O to remove extra GIEMSA, and then photographed with a digital camera. The number of colonies (>50 cells/colony) were counted under fluorescence microscopy (MicroPublisher 3.3RTV; Olympus, Tokyo, Japan).

TUNEL assay

To determine the effect of Lv/sh-NOB1 in combination with TRAIL on cell apoptosis, TUNEL assays were performed. In briefly, cells were treated with Lv/sh-NOB1 and TRAIL alone or

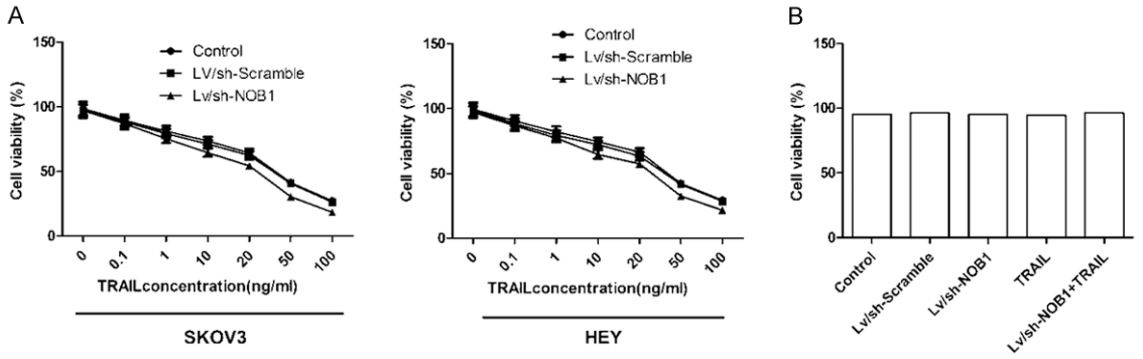

Figure 1. Downregulation of NOB1 enhances TRAIL sensitivity in epithelial ovarian cancer cells. A. Human ovarian cancer cell line, SKOV3 cells and HEY cells were treated with increasing doses of TRAIL alone, Lv/sh-NOB1 alone, or the combination of TRAIL and Lv/sh-NOB1 for 72 h. Cell viability was measured by MTT assay. B. Human ovarian surface epithelial cell line (HOSEpiC) were treated with TRAIL alone, Lv/sh-NOB1 alone, or the combination for 72 h. Cell viability was measured by MTT assay.

both, respectively. 48 h after treatment, apoptotic cells were determined by using the *In Situ* Cell Death Detection Kit (Roche, Mannheim, Germany) following manufacturer's instructions. The cell fluorescence was determined using the flow-cytometry (Becton Dickinson equipped with an UV-argon laser). The number of TUNEL-positive cells was expressed as a percentage of the total number of cells in the sample.

In addition, at the molecular level, we also detected survivin and Bcl-2 protein expression by western blotting as an additional indicator of apoptosis.

Caspase activity

The activity of caspase-3, -8 and -9 were measured with caspases colorimetric protease assay kits (Millipore Corporation, Billerica, MA, USA). In brief, cells were treated with Lv/sh-NOB1 and TRAIL alone or both, respectively. 24 h after treatment, cells were harvested and were lysed in 150 μl buffer provided in the kit (Millipore Corporation, Billerica, MA, USA). 10 μl substrate of each caspase was added to aliquot of lysates, respectively, and then cultured for 2 h. Samples were analyzed at 405 nm in a microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). The relative caspase activity of the control group was taken as 100.

Western blot

Protein was extracted from cells using RIPA lysis buffer (Invitrogen, USA) containing the protease inhibitors cocktail and PMSF in accordance with the manufacturer's protocol. The protein concentration was determined using the Bradford Method using the BCA assay kit (Sigma). Cell extracts (50 μg of protein) were separated on an 8%-15% sodium dodecyl sulfate-polyacrylamide electrophoretic gel (SDS-PAGE) and transferred to nitrocellulose membranes (Bio-Rad, Munich, Germany). The membranes were blocked with 3% non-fat dry milk for 2 h and incubated with primary antibody overnight at 4°C, followed incubated with secondary antibodies HRP-conjugated goat anti-mouse IgG for 2 h at room temperature. Protein bands were visualized with enhanced chemiluminescence reagent (ECL, Amersham, GE Healthcare, Velizy-Villacoublay, France). Blots were stripped and reprobed with anti-GAPDH to control for loading variations.

In vivo tumor growth model

SKVO3 cells (2×10^6) resuspended in 0.1 ml serum-free RPM1640 medium were subcutaneously (s.c.) injected intraperitoneally into 6-week old female Balb/c nu/nu mice (Experimental Animal Center of the Jilin University, Changchun, China). When the tumor volume (TV) reached 120 mm^3, mice were randomly divided into five groups (n=6/group) to receive treatment of an intraperitoneal (i.p.) injection of vehicle control (100 μl of 0.9% NaCl), Lv/sh-Scramble (2×10^8 PFU/dose), Lv/sh-NOB1 (2×10^8 PFU/dose), TRAIL (10 mg/kg body weight), or TRAIL combination Lv/sh-NOB1 (TRAIL: 5 mg/kg body weight, Lv/sh-NOB1: 1×10^8 PFU/dose respectively) on alternative days for 3 weeks. The volume of the tumors and the weight of the mice were measured every

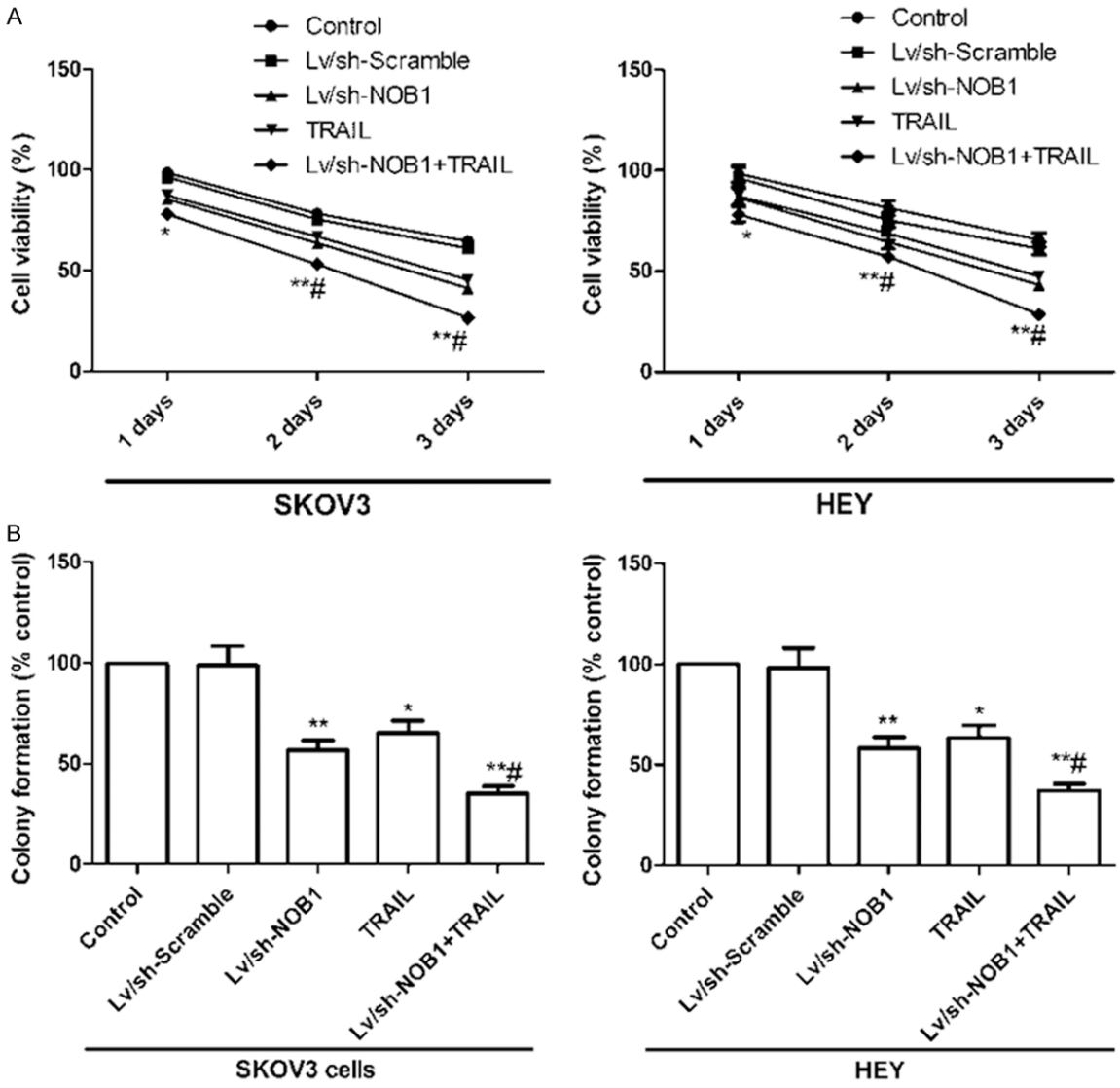

Figure 2. TRAIL and Lv/sh-NOB1 alone and in combination inhibited cell proliferation and colony formation in ovarian cancer cells. A. SKOV3 cells and HEY cells were treated with Lv/sh-NOB1 (MOI of 50 each), Lv/sh-Scramble (MOI of 50 each), TRAIL ($2\times IC_{50}$) or Lv/sh-NOB1 (MOI of 50 each) in combination with TRAIL ($1\times IC_{50}$), cell viability was measured by MTT assay at indicated time. B. Cell colony formation was determined in ovarian cancer cells after treated with TRAIL alone, Lv/sh-NOB1 alone, or the combination for 14 days. *P<0.05, **P<0.01 vs. control, #P<0.05 vs. TRAIL alone.

week. Tumor volume (TV) was measured with a caliper and counted by the following formula: Volume (mm^3) = (length × $width^2$)/2. At the end of *in vivo* experiments, the animals were sacrificed under anesthesia using avertin, tumor tissues were then immediately excised and weighted, then cell apoptosis of tumor tissues were measured using the *In Situ* Cell Death Detection Kit (Roche, Mannheim, Germany) according to manufacturer's instructions. The efficacy of the drug treatment was assessed according to tumor volume inhibition (TVI) percentage in treated vs. control mice, calculated as: TVI = 100-(mean TV treated/mean TV control × 100). This study was approved by the Animal Ethics Committee of Jilin University (Changchun, China).

Statistical analysis

The data shown are presented as the mean ± SD (standard deviation) of at least three inde-

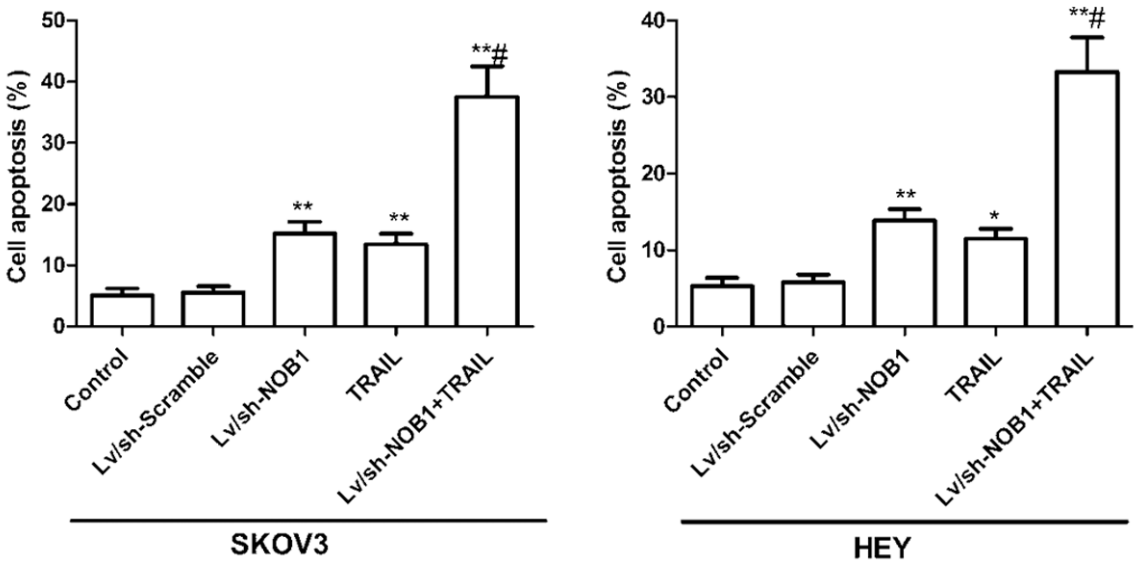

Figure 3. TRAIL and Lv/sh-NOB1 alone and in combination induced apoptosis in ovarian cancer cells. SKOV3 cells and HEY cells were treated with treated with TRAIL alone, Lv/sh-NOB1 alone, or the combination for 48 h, cell apoptosis was determined by TUNEL assay. *P<0.05, **P<0.01 vs. control, #P<0.05 vs. TRAIL alone.

pendent experiments. Differences between groups were analyzed by one-way ANOVA followed by a Tukey post hoc test using Graphpad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). Significant differences among groups were considered at P<0.05.

Results

Downregulation of NOB1 enhances TRAIL sensitivity in human ovarian cancer cells but not in normal cells

It was well known that platinum drugs are highly effective at initial treatment and are therefore used as standard first-line therapy in various cancers. However, the platinum resistance limits effective for patients with advanced EOC. To investigate whether downregulation of NOB1 expression has the potential to sensitize ovarian cancer cells to TRAIL, ovarian cancer cells were infected with Lv/sh-NOB1 or Lv/sh-Scramble, and then were incubated with TRAIL (0-100 ng/ml). MTT assays were performed following treatment and the IC_{50} for TRAIL was calculated for each of the groups treated. It was found that downregulation of NOB1 by Lv/sh-NOB1 enhanced the growth inhibitory effect of TRAIL in SKOV3 cells and HEY cells (**Figure 1A**). The IC_{50} values for TRAIL combined with or without Lv/sh-NOB1 in SKOV3 and HEY were found to be 25.48 ± 3.08 ng/ml versus 37.78 ± 4.12 ng/ml, and 27.78 ± 3.08 ng/ml versus 39.89 ± 4.53 ng/ml, respectively, suggesting that downregulation of NOB1 expression sensitized ovarian cancer cells to TRAIL. Of note, we did not observe a significant cytotoxic effect in human ovarian surface epithelial cells (HOSEpiC) following single agent or combination treatment (**Figure 1B**), indicating a tumor cell-selective effect.

TRAIL and Lv/sh-NOB1 alone or combination inhibited cell proliferation and colony formation of ovarian cancer cells

To evaluate the effect of TRAIL and Lv/sh-NOB1 alone and both on the cells proliferation of ovarian cells, MTT assay was performed after cells were treated with Lv/sh-NOB1 (MOI of 50 each), Lv/sh-Scramble (MOI of 50 each), TRAIL ($2\times IC_{50}$) or Lv/sh-NOB1 (MOI of 50 each) in combination with TRAIL ($1\times IC_{50}$), respectively. The results showed that the TRAIL and Lv/sh-NOB1 alone or combination significantly decreased cell viability compared to control group and Lv/sh-Scramble treatment group (**Figure 2A**, P<0.05). TRAIL in combination with Lv/sh-NOB1 resulted in greatest reduction of cell viability in both SKOV3 cells and HEY cells (**Figure 2A**, P<0.05).

To assess the effects of TRAIL and Lv/sh-NOB1 alone or combination on cell colony formation, SKVO3 and HEY cells were treated with TRAIL and Lv/sh-NOB1 alone or combination. After 15

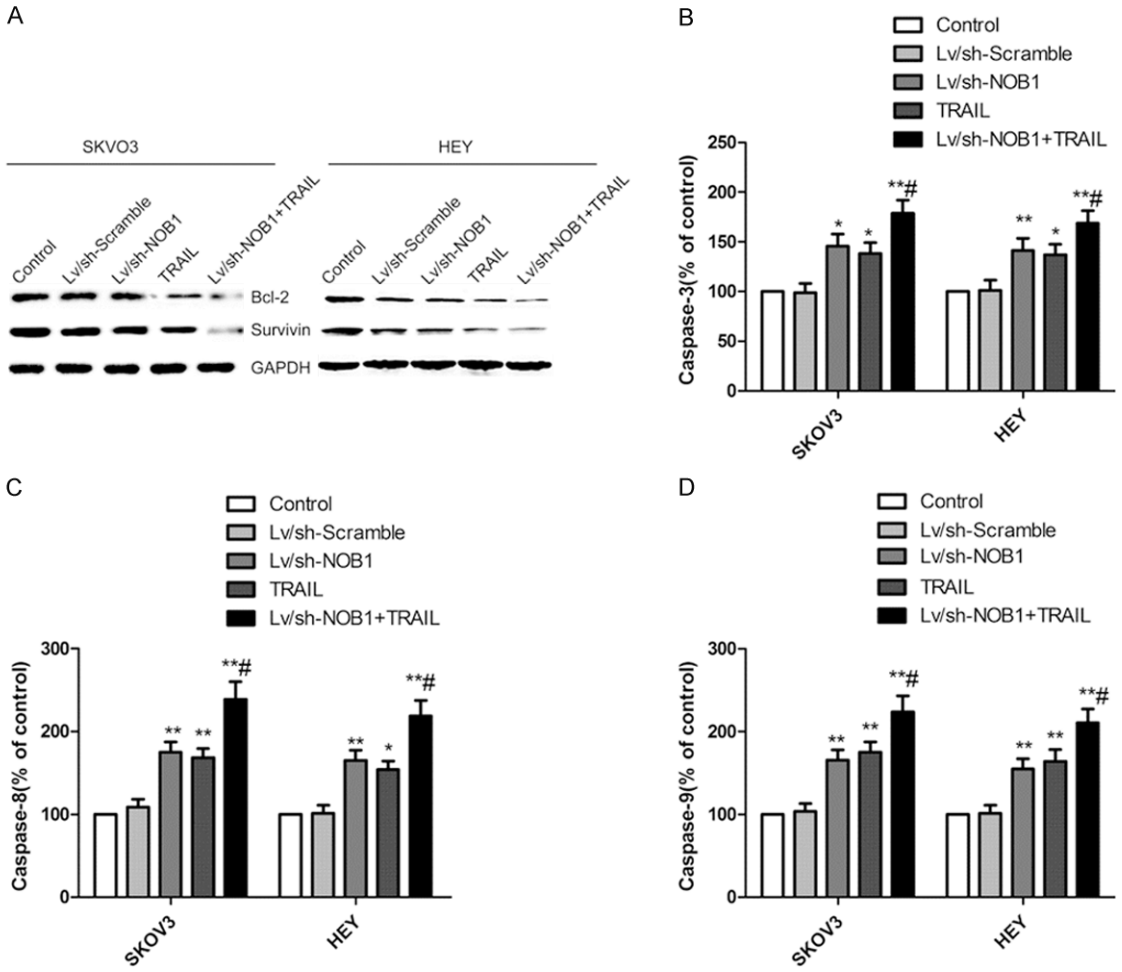

Figure 4. TRAIL and Lv/sh-NOB1 alone and in combination inhibited anti-apoptosis protein expression, increased caspase activity. A. SKOV3 cells and HEY cells were treated with TRAIL alone, Lv/sh-NOB1 alone, or the combination for 24 h. The anti-apoptosis protein, survivin and Bcl-2 protein expression were examined by Western blot using corresponding antibodies. GAPDH was used as a loading control. B-D. The activity of caspase-3, -8 and -9 were detected in ovarian cancer cells after treated with TRAIL alone, Lv/sh-NOB1 alone, or the combination. *P<0.05, **P<0.01 vs. control, #P<0.05 vs. TRAIL alone.

days' cultivation, colonies were fixed and stained with Giemsa. It was found that colony formation number have significantly reduction in TRAIL and Lv/sh-NOB1 alone or combination groups relative to control group and Lv/sh-Scramble group in SKVO3 and HEY cells (P<0.05, **Figure 2B**). The combination treatment resulted in a greater reduction of colony formation number (P<0.05, **Figure 2B**).

TRAIL and Lv/sh-NOB1 alone or combination induced cell apoptosis in ovarian cancer cells

The effects of TRAIL and Lv/sh-NOB1 alone or combination on cell apoptosis in ovarian cancer cells were analyzed by TUNEL.TUNEL assay showed that apoptosis in SKOV-3 and HEY cells was induced at 15.26 ± 1.84% and 13.88 ± 1.52% by Lv/sh-NOB1, at 13.42 ± 1.76% and 11.53 ± 1.21% by TRAIL, and at 37.48 ± 5.06% and 33.28 ± 4.54% by the TRAIL combination with Lv/sh-NOB1 (**Figure 3**), suggesting that combination of both TRAIL and Lv/sh-NOB1 could dramatically enhance cell apoptosis.

TRAIL and Lv/sh-NOB1 alone or combination inhibited anti-apoptotic molecules expression, and increased caspase activity in ovarian cancer cells

To explore the possible mechanism of induction cell apoptosis of TRAIL and Lv/sh-NOB1 alone or combination in ovarian cancer cells, anti-apoptotic molecules, such as survivin and

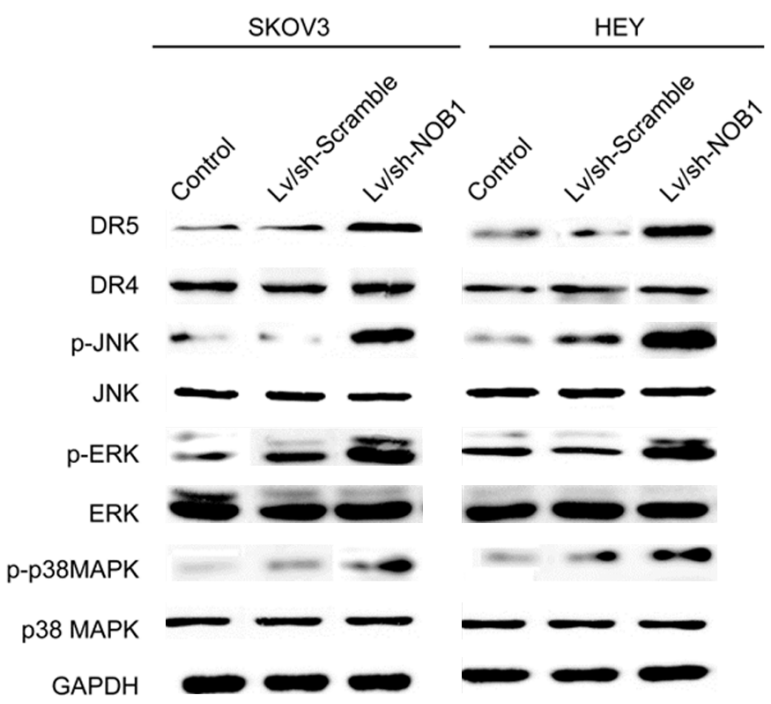

Figure 5. Lv/sh-NOB1 upregulation of DR5 expression and activation MAPK signal pathway. Cells were treated with TRAIL for 12 h, followed by treated with Lv/sh-NOB1 or Lv/sh-Scramble for 24 h, and the expression of DR4, DR5, ERK, p-ERK, JNK, p-JNK, p38MAPK, p-p38MAPK were determined by western blot. GAPDH was used as loading control.

Bcl-2 were determined by western blot. Western blot assay showed that TRAIL and Lv/sh-NOB1 alone or combination could significantly decrease survivin and Bcl-2 in ovarian cancer cells compared to control group and Lv/sh-scramble group (**Figure 4A**). The combination treatment led to survivin and Bcl-2 protein expression greatest reduction (**Figure 4A**).

Additionally, activity of caspase-3, -8, and -9 also were determined in ovarian cells after treated with TRAIL and Lv/sh-NOB1 alone or combination. The result showed that activity of caspase-3, -8, and -9 were significantly increased when the ovarian cancer cells were exposed to both TRAIL and Lv/sh-NOB1 alone or combination (**Figure 4B-D**), and that the combination treatment caused the greatest addition activity of caspase-3, 8, and 9 in ovarian cancer cells (**Figure 4B-D**).

Lv/sh-NOB1 upregulation of DR5 expression and activation MAPK signal pathway

It has been reported that several therapeutic agents can potentiate TRAIL efficacy through the upregulation of DR5 [26-30], we examined the expression of both DR4 and DR5 when ovarian cancer cells were treated with TRAIL for 12 h, followed treated with Lv/sh-NOB1 or Lv/sh-Scramble for 24 h. The result of Western blot showed that Lv/sh-NOB1 enhanced the expression of DR5, without modifying that of DR4, suggesting that Lv/sh-NOB1 can potentiate TRAIL efficacy by upregulation of DR5 (**Figure 5**).

Because several studies demonstrated that the induction of DR5 by different agents is mediated by MAPKs [27-30], we investigated the effects of Lv/sh-NOB1 on the activation of ERK, JNK and p38 MAPK in ovarian cells when cells were treated with TRAIL for 12 h, followed treated with Lv/sh-NOB1 or Lv/sh-Scramble for 24 h. Results from western blots indicated that Lv/sh-NOB1 resulted in a remarked addition of phosphorylated p38-MAPK, ERK and JNK in ovarian cancer cells, without modifying that of the total protein levels of p38MAPK, ERK and JNK in each group (**Figure 5**).

Effects of TRAIL and Lv/sh-NOB1 alone or combination on tumor growth in vivo

Finally, we investigated the therapeutic potential of TRAIL in combination with Lv/sh-NOB1 in SKOV3 cells following xenotransplantation into Balb/c nude mice. Tumor bearing mice were treated with TRAIL and Lv/sh-NOB1 alone or in combination. Individual treatments moderately inhibited tumor growth, with 45.96% and 48.24% TVI for TRAIL and Lv/sh-NOB1, respectively, at the end of the experiment (day 21; **Figure 6A-C**). Interestingly, treatment with TRAIL and Lv/sh-NOB1 in combination resulted in a significantly enhanced antitumor activity (70.34 % TVI at day 21). In addition, we did not observe any gross signs of toxicity and/or possible side effects/mortality during the treat-

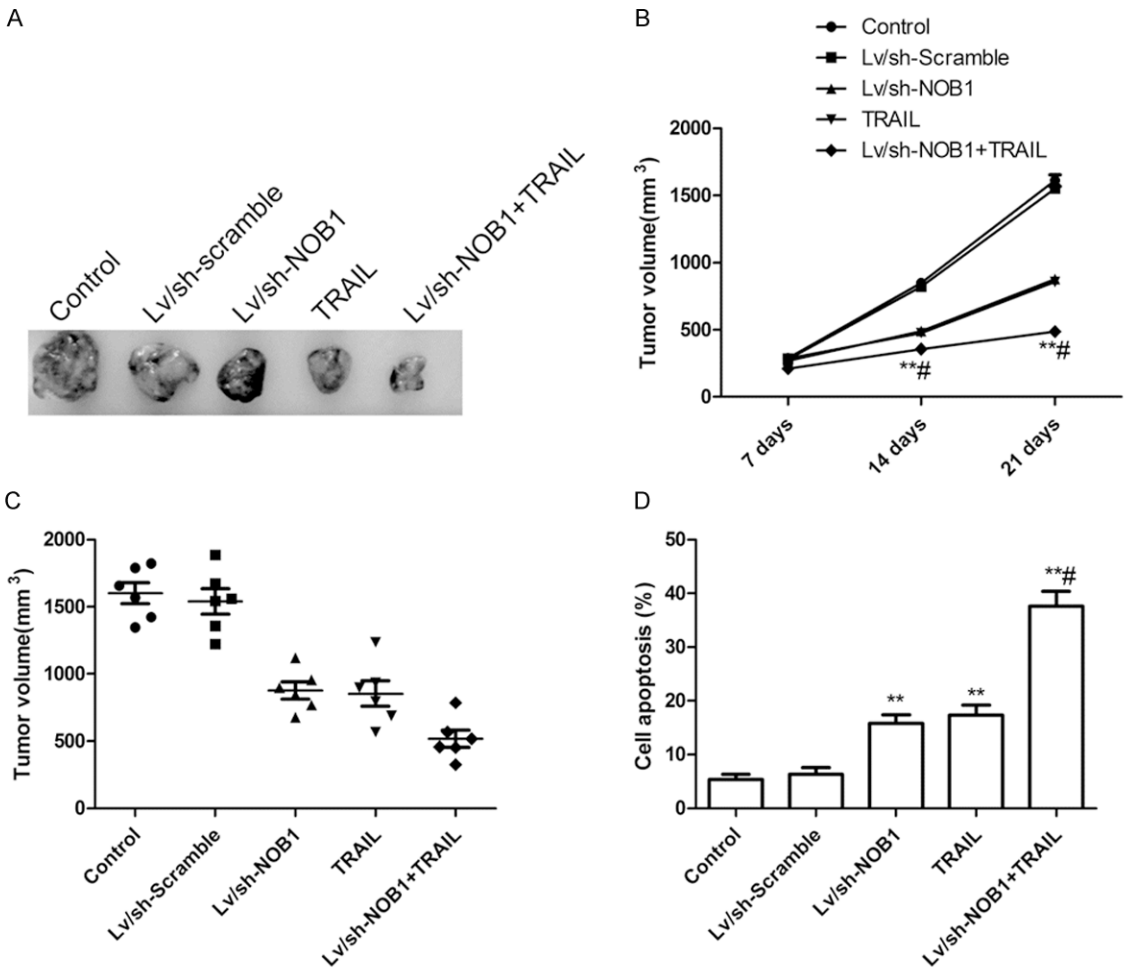

Figure 6. Antitumor activity of TRAIL and Lv/sh-NOB1 on SKOV3 cells xenotransplanted into nude mice. A. Graphs of the tumor tissue from different experimental groups. B. Tumor growth curves of different experimental groups. C. Tumor volume distribution in the different experimental groups at the end of the experiment (day 21). D. Cell apoptosis of tumor tissue from different experimental groups were determined by TUNEL assay. *P<0.05, **P<0.01 vs. control, #P<0.05 vs. TRAIL alone.

ment of TRAIL and Lv/sh-NOB1 alone or combination relative to the control group. Cell apoptosis of tumor tissue were determined by TUNNL. The results from TUNNL demonstrated that TRAIL and Lv/sh-NOB1 alone or combination could obviously induce cell apoptosis of tumor tissue compared to control group and Lv/sh-Scramble group (P<0.05, **Figure 6D**). The combination treatment resulted in greatest addition compared to single agent treatment. These data suggested that that combination of TRAIL and Lv/sh-NOB1 could suppress tumor growth of ovarian cancer *in vivo*.

Discussion

Platinum drugs are highly effective at initial treatment and more than 80% of the ovarian cancer patients initially respond to first-line chemotherapy [31], thus platinum drugs used as standard first-line therapy for ovarian cancer. However, most patients with advanced cancer could cause acquired drug resistant, leading to incurable disease [15, 16]. Additionally, conventional platinum drugs have high toxicity to normal tissues, which also limit the administration of the treatment [15, 16]. Accordingly, there remains an ongoing need to develop more effective strategies to decrease platinum drugs resistant. In the present study, to our knowledge, we have found that the combination of TRAIL and Lv/sh-NOB1 inhibited proliferation and colony formation, and enhanced apoptosis, and increased caspase-3, -8 and -9 activities in ovarian cancer cells, but not in normal cells, suggesting that TRAIL in combination with Lv/sh-NOB1 may be a potential effective

combination strategy for the treatment of ovarian cancer.

TRAIL could trigger rapid apoptosis *in vitro* and *in vivo* in various tumor cell types without affecting normal cells, thus it has been considered as a promising candidate in cancer therapy [7, 8]. However, the clinical activity of TRAIL is limited due to the inherent or acquired resistance of tumors cells [15, 16]. It has been showed that alteration of gene expression may occur and influence cellular sensitivity to chemotherapeutic drugs during tumorigenesis or the course of chemotherapy [32]. For example, Xu *et al* reported that knockdown of Protein phosphatase 2A (PP2A) expression by siRNA or pharmacological inhibition of PP2A activity increases TRAIL-induced apoptosis [33]. Chandrasekaran et al showed that siRNA mediated knockdown of COX-2 expression in breast cancer cells render them sensitive to TRAIL by increasing the expression of DR4 and DR5 [34]. Garimella et al demonstrated that downregulation of SRC or BCL2L1 expression by small-molecule inhibition of, sensitizes breast cancer cells to TRAIL-induced apoptosis, including cell lines resistant to TRAIL-induced cytotoxicity [35]. In the present study, we found that downregulation expression of NOB1 by Lv/sh-NOB1 increased sensitivity of ovarian cancer cells to TRAIL; and that Lv/sh-NOB1 in combination with lower dose of TRAIL synergistically suppress the proliferation and colony formation, and induce cell apoptosis in vitro, and suppressed tumor growth *in vivo*, suggests a potential new therapeutic option for TRAIL-based combination therapy for ovarian cancer.

Death receptors 5 (DR5), a member of the TRAIL family of receptors, is able to interact with its endogenous ligand Apo2L/TRAIL, induces apoptosis through caspases in transformed cell lines while normal cells are unaffected, has been considered as a promising anti-cancer target [36, 37]. A growing body of evidence has shown that the DR5 plays a crucial role in sensitizing cancer cells to apoptosis induced by TRAIL and chemotherapeutic agents [26-30, 36, 37]. In this study, our result showed that Lv/sh-NOB1 increased the expression of DR5, without modifying that of DR4, which suggesting that Lv/sh-NOB1 can potentiate TRAIL efficacy by upregulation expression of DR5.

It has been found that several mechanisms involved in the induction of DR5 expression, such as ROS generation [38, 39], TP53 upregulation [39], CHOP upregulation [40], and MAPK activation [27-30, 40]. In the present study showed that lv/sh-NOB1 induced the activation of MAPK signal pathway and the upregulation of DR5, which contribute to sensitive TRAIL to ovarian cancer cells. In agreement with our results, recently study showed that downregulation of NOB1 expression by Ad/sh-NOB1 sensitized thyroid cancer cells to doxorubicin through MAPK signal pathway [24]. These studies implied that downregulation of NOB1 could sensitive chemotherapy drugs to cancer cells through activation of MAPK signal pathway.

In summary, the findings we reported here present evidence that Lv/sh-NOB1 in combination with lower dose of TRAIL synergistic inhibited cell proliferation and colony formation, induced cell apoptosis and increased the activity of caspase-3, -8 and -9 in ovarian cancer cells; as well as suppressed tumor growth in nude mice model. We also provided evidence that downregulation expression of NOB1 by Lv/sh-NOB1 sensitizing effect to TRAIL to ovarian cancer cells involves the upregulation of the death receptor possibly through MAPK signal pathway. Therefore, it may be worthwhile to consider combination with Lv/sh-NOB1 and TRAIL as novel therapeutic strategy for patients with advanced ovarian cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Manhua Cui, Department of Obstetrics and Gynecology, The Second Hospital, Jilin University, 218 Ziqiagn Street, Nanguan District, Changchun 130041, China. E-mail: cuimanhua1326@163.com

References

[1] Ozols RF, Bookman MA, Connolly DC, Daly MB, Godwin AK, Schilder RJ, Xu X and Hamilton TC. Focus on epithelial ovarian cancer. Cancer Cell 2004; 5: 19-24.

[2] Desai A, Xu J, Aysola K, Qin Y, Okoli C, Hariprasad R, Chinemerem U, Gates C, Reddy A, Danner O, Franklin G, Ngozi A, Cantuaria G, Singh K, Grizzle W, Landen C, Partridge EE, Rice VM, Reddy ES and Rao VN. Epithelial ovarian cancer: An overview. World J Transl Med 2014; 3: 1-8.

[3] Pliarchopoulou K and Pectasides D. Epithelial ovarian cancer: focus on targeted therapy. Crit Rev Oncol Hematol 2011; 79: 17-23.

[4] Wang M, He Y, Shi L and Shi C. Multivariate analysis by Cox proportional hazard model on prognosis of patient with epithelial ovarian cancer. Eur J Gynaecol Oncol 2011; 32: 171-177.
[5] Walters Haygood CL, Arend RC, Straughn JM and Buchsbaum DJ. Ovarian cancer stem cells: Can targeted therapy lead to improved progression-free survival? World J Stem Cells 2014; 6: 441-447.
[6] Falci C, Dieci MV, Guarneri V, Solda C, Bria E, Tortora G and Conte P. Maintenance therapy in epithelial ovarian cancer: from chemotherapy to targeted agents. Expert Rev Anticancer Ther 2014; 14: 1041-1050.
[7] Fang F, Wang AP and Yang SF. Antitumor activity of a novel recombinant mutant human tumor necrosis factor-related apoptosis-inducing ligand. Acta Pharmacol Sin 2005; 26: 1373-1381.
[8] Qin JZ, Bacon P, Chaturvedi V and Nickoloff BJ. Role of NF-kappaB activity in apoptotic response of keratinocytes mediated by interferon-gamma, tumor necrosis factor-alpha, and tumor-necrosis-factor-related apoptosis-inducing ligand. J Invest Dermatol 2001; 117: 898-907.
[9] Kischkel FC, Lawrence DA, Chuntharapai A, Schow P, Kim KJ and Ashkenazi A. Apo2L/TRAIL-dependent recruitment of endogenous FADD and caspase-8 to death receptors 4 and 5. Immunity 2000; 12: 611-620.
[10] LeBlanc HN and Ashkenazi A. Apo2L/TRAIL and its death and decoy receptors. Cell Death Differ 2003; 10: 66-75.
[11] Almasan A and Ashkenazi A. Apo2L/TRAIL: apoptosis signaling, biology, and potential for cancer therapy. Cytokine Growth Factor Rev 2003; 14: 337-348.
[12] Srivastava RK. TRAIL/Apo-2L: mechanisms and clinical applications in cancer. Neoplasia 2001; 3: 535-546.
[13] Khaider NG, Lane D, Matte I, Rancourt C and Piche A. Targeted ovarian cancer treatment: the TRAILs of resistance. Am J Cancer Res 2012; 2: 75-92.
[14] Duiker EW, de Vries EG, Mahalingam D, Meersma GJ, Boersma-van Ek W, Hollema H, Lub-de Hooge MN, van Dam GM, Cool RH, Quax WJ, Samali A, van der Zee AG and de Jong S. Enhanced antitumor efficacy of a DR5-specific TRAIL variant over recombinant human TRAIL in a bioluminescent ovarian cancer xenograft model. Clin Cancer Res 2009; 15: 2048-2057.
[15] Kendrick JE, Estes JM, Straughn JM Jr, Alvarez RD and Buchsbaum DJ. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its therapeutic potential in breast and gynecologic cancers. Gynecol Oncol 2007; 106: 614-621.
[16] Bevis KS, Buchsbaum DJ and Straughn JM Jr. Overcoming TRAIL resistance in ovarian carcinoma. Gynecol Oncol 2010; 119: 157-163.
[17] Liu K, Chen HL, Gu MM and You QS. Relationship between NOB1 expression and prognosis of resected non-small cell lung cancer. Int J Biol Markers 2015; 30: e43-8.
[18] Lin S, Meng W, Zhang W, Liu J, Wang P, Xue S and Chen G. Expression of the NOB1 gene and its clinical significance in papillary thyroid carcinoma. J Int Med Res 2013; 41: 568-572.
[19] Zhang X, Zhang D, Qu F, Hong Y, Cao J, Pan X, Li L, Huang Y, Huang H, Yin L, Chen L, Ren J, Wang Z, Xu D and Cui X. Knockdown of NOB1 expression inhibits the malignant transformation of human prostate cancer cells. Mol Cell Biochem 2014; 396: 1-8.
[20] Li XY, Luo QF, Li J, Wei CK, Kong XJ, Zhang JF and Fang L. Clinical significance of NOB1 expression in breast infiltrating ductal carcinoma. Int J Clin Exp Pathol 2013; 6: 2137-2144.
[21] Wang H, Li P and Zhao B. Knockdown of NOB1 expression by RNAi inhibits cellular proliferation and migration in human gliomas. Gene 2013; 528: 146-153.
[22] Chen B, Liu J, Wu D, Qin Y, Peng C, Li C and Wang J. Gene silencing of NOB1 by lentivirus suppresses growth and migration of human osteosarcoma cells. Mol Med Rep 2014; 9: 2173-2179.
[23] Meng W, Wang PS, Liu J, Xue S, Wang GM, Meng XY and Chen G. Adenovirus-mediated siRNA targeting NOB1 inhibits tumor growth and enhances radiosensitivity of human papillary thyroid carcinoma in vitro and in vivo. Oncol Rep 2014; 32: 2411-2420.
[24] Liu J, Dong BF, Wang PS, Ren PY, Xue S, Zhang X, Han Z and Chen G. Silencing NOB1 enhances doxorubicin antitumor activity of the papillary thyroid carcinoma in vitro and in vivo. Oncol Rep 2015; 33: 1551-1559.
[25] Lin Y, Peng S, Yu H, Teng H and Cui M. RNAi-mediated downregulation of NOB1 suppresses the growth and colony-formation ability of human ovarian cancer cells. Med Oncol 2012; 29: 311-317.
[26] Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla T and Rizzuto R. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. J Cell Biol 2006; 175: 901-911.
[27] Matsui TA, Sowa Y, Yoshida T, Murata H, Horinaka M, Wakada M, Nakanishi R, Sakabe T, Kubo T and Sakai T. Sulforaphane enhances

TRAIL-induced apoptosis through the induction of DR5 expression in human osteosarcoma cells. Carcinogenesis 2006; 27: 1768-1777.

[28] Do MT, Na M, Kim HG, Khanal T, Choi JH, Jin SW, Oh SH, Hwang IH, Chung YC, Kim HS, Jeong TC and Jeong HG. Ilimaquinone induces death receptor expression and sensitizes human colon cancer cells to TRAIL-induced apoptosis through activation of ROS-ERK/p38 MAPK-CHOP signaling pathways. Food Chem Toxicol 2014; 71: 51-59.

[29] Gupta SC, Reuter S, Phromnoi K, Park B, Hema PS, Nair M and Aggarwal BB. Nimbolide sensitizes human colon cancer cells to TRAIL through reactive oxygen species- and ERK-dependent up-regulation of death receptors, p53, and Bax. J Biol Chem 2011; 286: 1134-1146.

[30] Woo JS, Kim SM, Jeong CH, Ryu CH and Jeun SS. Lipoxygenase inhibitor MK886 potentiates TRAIL-induced apoptosis through CHOP- and p38 MAPK-mediated up-regulation of death receptor 5 in malignant glioma. Biochem Biophys Res Commun 2013; 431: 354-359.

[31] Agarwal R and Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat Rev Cancer 2003; 3: 502-516.

[32] el-Deiry WS. Role of oncogenes in resistance and killing by cancer therapeutic agents. Curr Opin Oncol 1997; 9: 79-87.

[33] Xu J, Zhou JY, Xu Z, Kho DH, Zhuang Z, Raz A and Wu GS. The role of Cullin3-mediated ubiquitination of the catalytic subunit of PP2A in TRAIL signaling. Cell Cycle 2014; 13: 3750-3758.

[34] Chandrasekaran S, Marshall JR, Messing JA, Hsu JW and King MR. TRAIL-mediated apoptosis in breast cancer cells cultured as 3D spheroids. PLoS One 2014; 9: e111487.

[35] Garimella SV, Gehlhaus K, Dine JL, Pitt JJ, Grandin M, Chakka S, Nau MM, Caplen NJ and Lipkowitz S. Identification of novel molecular regulators of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in breast cancer cells by RNAi screening. Breast Cancer Res 2014; 16: R41.

[36] Kelley SK and Ashkenazi A. Targeting death receptors in cancer with Apo2L/TRAIL. Curr Opin Pharmacol 2004; 4: 333-339.

[37] Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrokh Z and Schwall RH. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999; 104: 155-162.

[38] Sung B, Ravindran J, Prasad S, Pandey MK and Aggarwal BB. Gossypol induces death receptor-5 through activation of the ROS-ERK-CHOP pathway and sensitizes colon cancer cells to TRAIL. J Biol Chem 2010; 285: 35418-35427.

[39] Park EJ, Choi KS, Yoo YH and Kwon TK. Nutlin-3, a small-molecule MDM2 inhibitor, sensitizes Caki cells to TRAIL-induced apoptosis through p53-mediated PUMA upregulation and ROS-mediated DR5 upregulation. Anticancer Drugs 2013; 24: 260-269.

[40] Pennati M, Sbarra S, De Cesare M, Lopergolo A, Locatelli SL, Campi E, Daidone MG, Carlo-Stella C, Gianni AM and Zaffaroni N. YM155 sensitizes triple-negative breast cancer to membrane-bound TRAIL through p38 MAPK- and CHOP-mediated DR5 upregulation. Int J Cancer 2015; 136: 299-309.