EJGO European Journal of Gynaecological Oncology

# Effect of siRNA against nerve growth factor (NGF) on growth and invasion of ovarian cancer cell

**Fengjuan Liu[1], Hong Tao[1], Qi Shen[1], Yinglin Zheng[1], Ke Zhang[2], Jinyi Tong[1]**

*[1]Department of Gynecology, First People's Hospital of Hangzhou, Hangzhou*
*[2]Department of Radiation Oncology, Hangzhou Cancer Hospital, Hangzhou (China)*

**Summary**

*Objectives:* To compare the expression of nerve growth factor (NGF), TrkA, p75, and VEGF in normal ovaries and in epithelial ovarian carcinomas. To examine the effects on ovarian cancer cell line (A2780) proliferation, invasion, and apoptosis after NGF down-regulation induced by lentivirus induced RNAi. *Materials and Methods:* The expression and localization of NGF, TrkA, p75, and VEGF in normal ovarian samples and in ovarian cancer samples were analyzed by RT-PCR and immunohistochemistry. NGF knockdown in A2780 was achieved by shRNA and cell proliferation, invasion, and apoptosis was examined by MTT, transwell assay, and flow cytometry, respectively. *Results:* Significantly higher levels of NGF, TrkA, and VEGF were observed in ovarian cancers versus normal ovary, while expression level of p75 maintained stable. In A2780 cells, NGF down-regulation significantly reduced the expression levels of TrkA and VEGF, but did not change that of p75. A significant decrease in proliferation and invasion, as well as an increase in apoptosis was also observed in NGF down-regulation cells. *Conclusions:* The expression of TrkA and VEGF is importantly induced by NGF. Knockdown of NGF promoted apoptosis, thus inhibited cellular proliferation and invasion in A2780 cells. Therefore NGF block may be a potential therapeutic strategy to treat ovarian cancer.

*Key words:* NGF; NGF siRNA; TrkA; p57; VEGF; Ovarian cancer.

## Introduction

Ovarian carcinoma is the most lethal gynecological cancer. Nearly 90% of ovarian cancer cases display epithelial-like histology and are named epithelial ovarian cancer (EOC) [1]. EOC is highly invasive and angiogenic, responds poorly to therapies, and is usually detected at an advanced stage, and thus it remains with a low survival rate [2]. In ovarian cancer, disturbance in the angiogenic process is a key factor in its development and subsequent progression [3]. Therefore, targeting angiogenic process may be a potential therapeutic opportunity [1,4].

Nerve growth factor (NGF), a member of the neurotrophin family, was originally discovered in the nervous system in 1966 [5]. Some authors proved that NGF is overexpressed in many cancers from various tissues such as: lung [6], prostate [7], breast [8], and also in mammalian ovaries [9, 10]. In addition, NGF is involved in angiogenesis, a key feature of tumor development and progression [8].NGF binds to its specific high-affinity tyrosine kinase receptor TrkA and then promotes cell proliferation and migration [11, 12], and significantly stimulates invasion and blood-vessel formation [13]. It has been demonstrated that NGF binding to TrkA receptors activates TrkA autophosphorylation and acts as an indirect angiogenic factor by increasing VEGF expression and also as a direct angiogenic factor by activating TRKA in endothelial cells, therefore increasing angiogenesis in EOC [14]. Furthermore, NGF is also believed to be involved in apoptosis by its low-affinity receptor p75 mediated signals [15]. Therefore, due to its promotion in ovarian cancer development, NGF may be a potential drug target.

In the study, the authors investigated the expression of NGF, TrkA, p75, and VEGF in normal human ovaries, as well as in epithelial ovarian cancers. In addition, the effect of NGF silencing on human ovarian cell proliferation, invasion, and apoptosis was also determined.

## Materials and Methods

Samples from 18 normal ovarian and 29 epithelial ovarian cancer specimens were collected from the Department of Gynecology, Hangzhou First People's Hospital, following informed consent. The normal ovarian samples were collected from total hysterectomies in women 40–78 years-old, undergoing elective pelvic surgery for non-ovarian indications. This work was approved by the Institutional Review Board. After surgery, the pathologist separated a portion of the tissue that was immediately frozen in liquid nitrogen and kept frozen until used for RNA extraction. The remnant tissues were fixed and paraffin-embedded for morphological and immunohistochemical analysis. In all of samples, a third portion was immediately placed in culture media, sent to the laboratory, and used for tissue culture analysis.

All samples were fixed in 10% buffered formalin. Formalin-

Revised manuscript accepted for publication November 24, 2016

Table 1. — *Sequences of q-RT-PCR primers.*

| Gene name | | Sequence (5'-3') | Length |
|---|---|---|---|
| hs NGF | F | CAACAGGACTCACAGGAGCA | 150 bp |
| | R | ACCTCTCCCAACACCATCAC | |
| hs NGFR | F | AGGTGACCTTCTGGGAAATG | 146 bp |
| | R | CCCAAACCTGACTCCATCAT | |
| hstrkA | F | GAGTGGTTAGCCGGAATACT | 154 bp |
| | R | CTTGCCTAGAGAAGCAGGAA | |
| hs VEGF | F | GACATCTTCCAGGAGTACC | 197 bp |
| | R | TGCTGTAGGAAGCTCATCTC | |
| hs GAPDH | F | GAAGGTGAAGGTCGGAGTC | 225 bp |
| | R | GAAGATGGTGATGGGATTTC | |

Table 2. — *Sequences of NGF targets siRNAs.*

| siRNAs | | Sequences (5'-3') |
|---|---|---|
| si-1 | Sense: | GAGGUGAACAUUAACAACAdTdT |
| | Antisense: | UGUUGUUAAUGUUCACCUCdTdT |
| si-2 | Sense: | CACUGGAACUCAUAUUGUAdTdT |
| | Antisense: | UACAAUAUGAGUUCCAGUGdTdT |
| si-3 | Sense: | CUGGACUAAACUUCAGCAUdTdT |
| | Antisense: | AUGCUGAAGUUUAGUCCAGdTdT |
| si-4 | Sense: | GACCACCGCCACAGACAUCdTdT |
| | Antisense: | GAUGUCUGUGGCGGUGGUCdTdT |
| si-5 | Sense: | GGCAAGGAGGUGAUGGUGUdTdT |
| | Antisense: | ACACCAUCACCUCCUUGCCdTdT |
| si-6 | Sense: | GGACUAAACUUCAGCAUUCdTdT |
| | Antisense: | GAAUGCUGAAGUUUAGUCCdTdT |
| si-7 | Sense: | GCAGGAAGGCUGUGAGAAGdTdT |
| | Antisense: | CUUCUCACAGCCUUCCUGCdTdT |
| si-8 | Sense: | GACACUCAGGAUCUGGACUdTdT |
| | Antisense: | AGUCCAGAUCCUGAGUGUCdTdT |
| si-9 | Sense: | CGACUCACACCUUUGUCAAdTdT |
| | Antisense: | UUGACAAAGGUGUGAGUCGdTdT |
| si-10 | Sense: | GUUGGGAGAGGUGAACAUUdTdT |
| | Antisense: | AAUGUUCACCUCUCCCAACdTdT |
| si-NC | Sense: | UUCUCCGAACGUGUCACGUdTdT |
| | Antisense: | ACGUGACACGUUCGGAGAAdTdT |

fixed ovarian tissues were cut in 4 to 6 μm sections. Immunohistochemical analysis was carried out as described by Campos *et al.* [16]. The primary antibodies were mouse monoclonal primary antibody to human NGF or p75 and rabbit monoclonal primary antibody to human TrkA or VEGF, respectively, with a 1:100 dilution. Sections were examined under a 55i microscope. Each slide had its respective negative control (without primary or secondary antibodies). Slide counts were performed by three independent technicians blinded to sample classification; 250 epithelial cells were counted. Qualitative stain intensities were 0 = no staining, 1 = weak, 2 = moderate, and 3 = strong.

Total RNA was extracted from tissues (or cells) using RISO reagent according to the manufacturer's protocol. The cDNA was produced by reverse transcription and expression levels of target mRNAs were determined by a quantitative RT-PCR method using a one-step qPCR kit according to the manufacturer's instructions. Specific primers for each gene are summarized in Table 1.

Ten siRNAs targeting human NGF (accession no. NM_002506.2) gene were designed and synthesized. The nucleotide sequences are listed in Table 2. si-NC targeting non-sense sequence served as the negative control.

High metastatic human ovarian carcinoma cell line A2780 was provided by Chinese Academy of Medical Sciences, and cultivated with RPMI1640 media and supplemented with 10% fetal calf serum. The cells were maintained at 37 in a humidified atmosphere with 5% CO2. The day before transfection, cells were seeded into 24-well plates. When the confluence reached 50%, 50 nM of siRNA were transfected into cells using lipofectamine 2000 reagent according to the manufacturer's instructions.

Efficient shRNA against NGF was cloned into pLV-shRNA-zsGreen1, and named lenti-shRNA-NGF. shRNA against non-sense sequence was used as control. Infectious virus was produced by co-transfection of HEK293T cells ($5\times10^6$ cells in 10-cm disc) using lipofectamine 2000. Nine ug of ViraPower Packaging Mix and 3 ug of lenti-shRNA-NGF were transfected into HEK293T cells, and expression of zsGreen was monitored by fluorescence microscopy. Viral supernatant was collected after culturing for 72 hours, and stored in -80°C refrigerator for later use. Quantitative PCR method was used to determine the number of vector copies associated with genomic DNA extracted from transduced cells [17].

Total proteins were extracted using mammalian cell total protein lysis buffer. Denatured proteins were separated by 8% SDS polyacrylamide gel electrophoresis and then electroblotted onto PVDF membrane. After blocking for two hours with PBST containing 5% skim milk powder, the membrane were, respectively, incubated overnight at 4°C with a 1/100 dilution of mouse monoclonal primary antibody to human NGF or p75, a 1/100 dilution of rabbit monoclonal primary antibody to human TrkA and VEGF , or a 1/400 dilution of mouse monoclonal antibody to human β-actin. The membranes were then washed in PBST and incubated with a 1/1000 dilution of goat anti-mouse HRP-conjugated secondary antibody or goat anti-rabbit HRP-conjugated secondary antibody at room temperature for two hours. Detection of β-actin at 1:500 dilution was used as a loading control. All bands were detected using ECL chemiluminescence reagent.

Cells were seeded in 96-well plates at a density of $5\times10^4$ cells/ml. At 24, 48, 72, and 96 hours, cell proliferation capacity was examined in quadruplicate using an MTT assay.

Apoptosis was measured using an Annexin V/PE 7-AADApoptosis detection Kit and analyzed by FACScan flow cytometry. Cell cycle was measured by flow cytometry using cell cycle and apoptosis analysis kit according to the manufacturer's instructions.

Cell invasion was detected by a transwell assay. 100 ul of cells with a density of $1*10^6$cells/ml were added to the upper chamber with serum-free DMEM medium, and a chemoattractant (10% FBS/DMEM medium) was added to the lower chamber. To determine the amount of invasion, cells were incubated for 24 hours, and the cells on the upper surface of membrane were cleared. After fixation by 10% formaldehyde, the cells on the underside of membrane were stained with 0.5% crystal violet for five minutes. The migrated cells of random five fields of each transwell membrane were counted under a microscope at ×100 magnification field.

Each assay was performed at least three times. The data were expressed as means ± SD. Statistical analysis was performed using a student's *t*-test and a one-way ANOVA. $P$ value < 0.05 was considered to be statistically significant.

## Results

Q-RT-PCR analysis of NGF, TrkA, p75, and VEGF mRNA levels were performed in the normal ovary tissues

Table 3. — *NGF, TrkA, p75, and VEGF protein expression the entire samples.*

| | Semi-quantitative scores | | | | |
|---|---|---|---|---|---|
| | No staining (0) | Weak (1) | Moderate (2) | Strong (3) | Total |
| | | NGF | | | |
| Ovarian cancer | 0 (0%) | 18 (62%) | 10 (34%) | 1 (3%) | 29 |
| Normal ovary tissue | 5 (26%) | 11 (58%) | 3 (16%) | 0 (0%) | 19 |
| | | TrkA | | | |
| Ovarian cancer | 1 (3%) | 4 (14%) | 12 (41%) | 12 (41%) | 29 |
| Normal ovary tissue | 2 (11%) | 9 (47%) | 6 (32%) | 2 (11%) | 19 |
| | | p75 | | | |
| Ovarian cancer | 21 (72%) | 8 (28%) | 0 (0%) | 0 (0%) | 29 |
| Normal ovary tissue | 7 (37%) | 9 (47%) | 2 (11%) | 1 (5%) | 19 |
| | | VEGF | | | |
| Ovarian cancer | 0 (0%) | 6 (21%) | 10 (34%) | 13 (45%) | 29 |
| Normal ovary tissue | 2 (11%) | 11 (58%) | 5 (26%) | 1 (5%) | 19 |

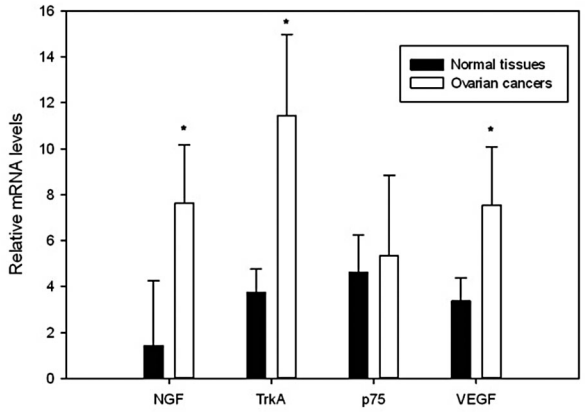


Figure 1. — mRNA levels of NGF, TrkA, p75, and VEGF in ovarian carcinoma and ovary tissues. *indicates $p < 0.05$

and the ovarian tumors. The results are summarized in Figure 1. The mRNA levels of NGF, TrkA, and VEGF were significantly higher in ovarian tumors when compared to normal ovarian tissues, while p75 mRNA showed no significant difference between normal ovary tissues and ovarian samples.

To verify expression levels of NGF, TrkA, p75, and VEGF, immunohistochemical analysis was also carried out. The distribution of NGF, TrkA, p75 and VEGF protein expression in primary tumors and normal tissues is detailed in Table 3, and the representative microphotographs are shown in Figure 2. 100% ovarian cancers showed NGF expression, whereas 74% samples were detected to be NGF positive in ovary samples. The expression of TrkA protein was detected in carcinoma cells in 28 of 29 (97%), and 24 of 29 (82%) samples showed moderate to strong TrkA expression. In normal ovary samples, 17 of 19 (89%) samples were TrkA positive, and eight of 19 (43%) were moderate to strong TrkA expression. The expression of VEGF in ovarian cancer was also higher than in normal tissues. The expression of p75 protein was not significant between ovarian tissues and ovary tissues. The results showed that staining and intensity of NGF, TrkA, and VEGF were remarkably higher in carcinoma tissues compared with the normal surface epithelium.

Table 4. — *Invasion cell numbers per field and corresponding migrated cell rate.*

| Group | Numbers of invasion cells/field (±SD) | Migrated cell rate (%) |
|---|---|---|
| Lenti-shRNA-NGF | 231±7.21 | 59.0±7.21 |
| Lenti-shRNA-NC | 532±15.57 | 6.0±15.57 |
| Normal cells | 566±24.11 | / |

The knockdown efficiency of siRNA against NGF was evaluated in A2780 cells. As shown in Figure 3, si-9 proved to be the most efficient target, which significantly reduced the mRNA level of NGF gene to 22.6% compared to si-NC treated cells. The sequence of si-9 was then cloned into pLV-shRNA-zsGreen1, and corresponding recombinant lentivirus was produced. The titer of lenti-shRNA-NGF was $1.18\times10^7$v.p./ml, detected by Q-RT-PCR method.

Stably suppression of NGF gene in A2780 cells was achieved by a lentivirus-mediated shRNA method. NGF mRNA and protein expression in lenti-shRNA-NGF treated cells was significantly reduced to 34% and 60% (Figure 4), respectively, compared to the negative control. The cells with down-regulation of NGF were used for the following detection.

Using NGF down-regulation cells, the authors detected the expression levels of p75, trkA, and VEGF. As shown in Figure 5, the mRNA levels of trkA and VEGF, as well as protein levels in NGF down-regulation cells were respectively suppressed by 60% and 43% compared to normal cells. The expression level of p75, however, showed no significant change between NGF down-regulation cells and normal cells.

To examine the effect of NGF knockdown on ovarian cancer cell proliferation, lenti-shRNA-NGF, lenti-shRNA-

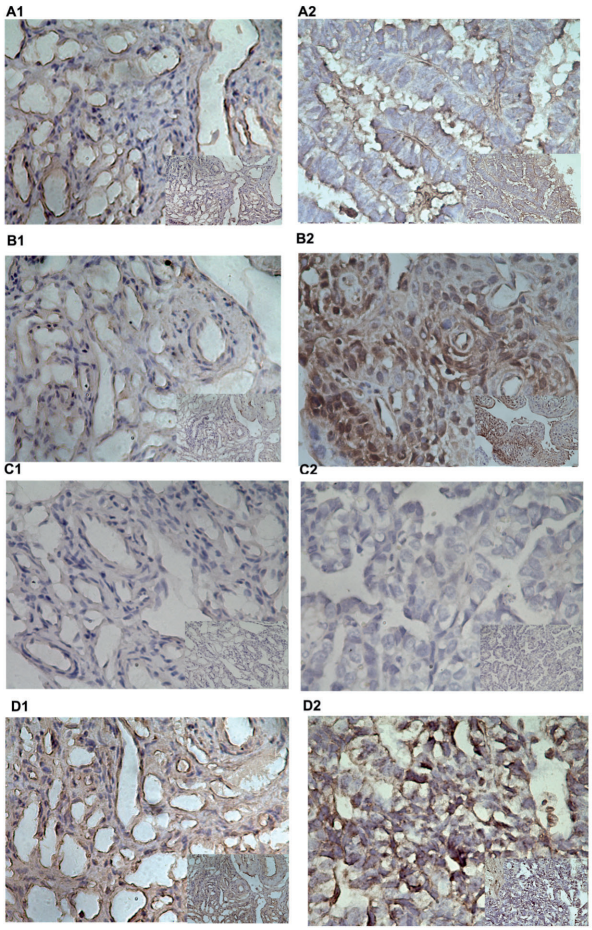


Figure 2. — NGF, TrkA, p75 and VEGF protein expression in ovarian carcinoma and ovary tissues. Panel A: Immunodetection of NGF. Panel B: Immunodetection of TrkA. Panel C: Immunodetection of VEGF. Panel D: Immunodetection of p75. A1, B1, C1, D1: Normal ovary. A2, B2, C2, D2: Ovarian carcinoma tissues. Panels A-D are magnified at ×400. Inner panels are magnified at ×200.

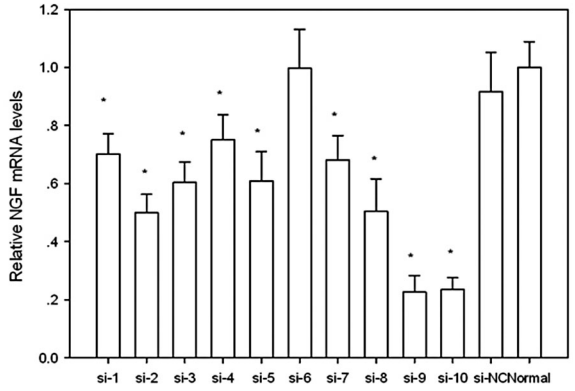


Figure 3. — siRNAs suppressed NGF expression in A2780 cells Quantitative real-time PCR was performed for siRNAs against NGF 48 hours after transfection. Values were averaged from three independent experiments. *indicates significant difference compared to si-NC treated cells ($p < 0.01$).

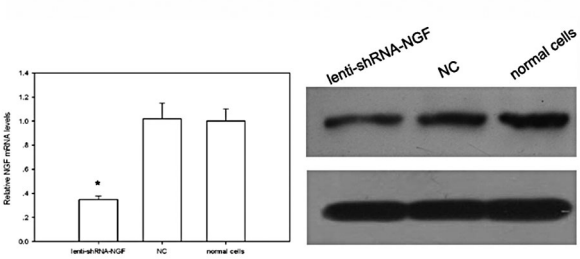


Figure 4. — NGF mRNA and protein expression is reduced by lentivirus-mediated shRNA treatment. *indicates $p < 0.05$ compared to NC group.

NC infected A2780 cells, and normal A2780 cells were subjected to MTT assay. The growth curves are shown in Figure 6. Cell growth in NGF down-regulation cells was significantly inhibited compared with that in lenti-shRNA-NC cells or normal cells.

To evaluate the mechanism of NGF knockdown mediated cell growth inhibition, the cell cycle distribution was detected in NGF knockdown cells and control cells by flow cytometry. As shown in Figure 7, in NGF suppression cells, the cell cycle progression was blocked with a significant increase in the percentage of cells in the G2/M phase (24.4% in lenti-shRNA-NGF cells versus 17.6% in lenti-shRNA-NC cells). The results indicated that NGF knockdown induced cell cycle arrest at G2/M phase.

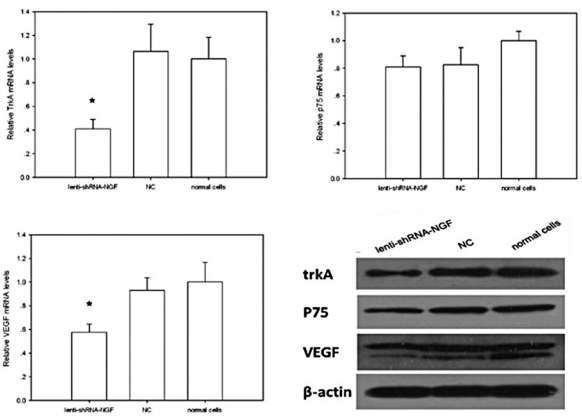


Figure 5. — The expression levels of TrkA, p75, and VEGF in NGF knock-down cells.
* indicates $p < 0.05$ compared to NC group.

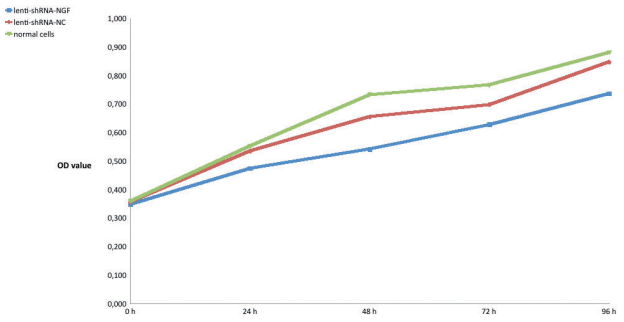


Figure 6. — Effects of NGF down-regulation on the growth of A2780 cells. MTT assay was used to determine the growth of A2780 (sample size n=3). * indicates that $p < 0.05$.

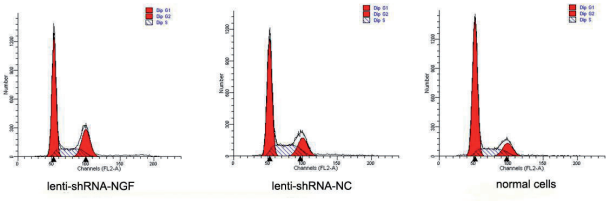


Figure 7. — NGF down-regulation blocked cell cycle in the G2/M phase.

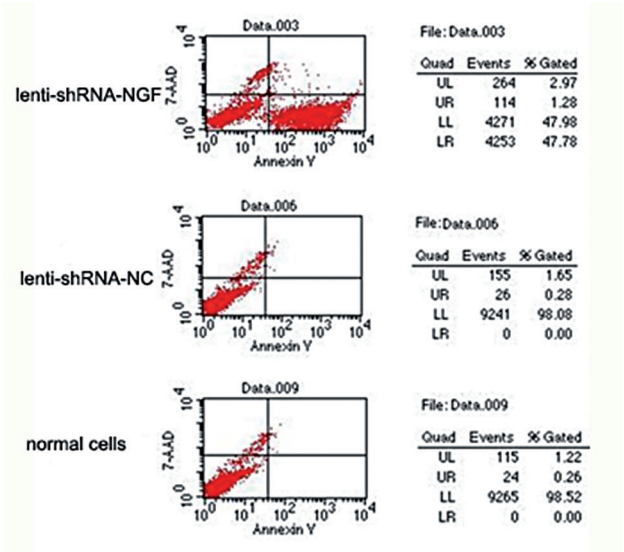


Figure 8. — Apoptosis in A2780 cells. Apoptosis rates were measured by FACS analysis after Annexin V-PE/7-AAD staining. Percentage of apoptotic cells include both early- and late-stage apoptosis (UR and LR).

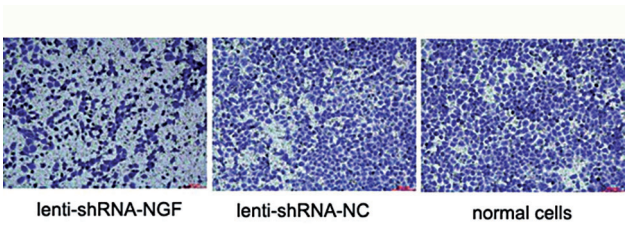


Figure 9. — Effect of NGF down-regulation on invasion ability of A2870 cells by transwell assay. The experiments were repeated three times.

NGF knockdown was proved to induce cell cycle arrest at G2/M phase, which suggested that NGF knockdown could increase apoptosis of cells. Therefore, Annexin V-PE/7-AAD staining was used to detect apoptotic cells induced by NGF silence. As shown in Figure 8, apoptotic cells in NC group or normal group were rare (<1%). However, NGF knockdown significantly promoted apoptosis in 49%.

The results of transwell assay are shown in Figure 9. Cell number of each field was counted, and migrated cell rate was expressed relative to that of normal cell group (Table 4). The knockdown of NGF significantly inhibited cell migration and invasion with an inhibition rate of 59%. There was no significant difference in the invasion ability between Lenti-shRNA-NC cells and normal cells. The results suggested that the knockdown of NGF suppressed the invasion of ovarian cancer cells.

## Discussion

Increased angiogenic activity is a remarkable feature of ovarian cancer [18]. In previous reports, it was shown that epithelial cells of EOC overexpress TRKA and NGF [12, 16]. Similar results were proved in the present study; the authors found a very strong presence of NGF and its receptor TrkA, as well as its effector VEGF in ovarian cancer tissues studied. The results supported the idea that NGF may induce an increase in expression of VEGF through activation of TrkA and p75, the low-affinity receptor of NGF, and showed a sustained expression in ovarian tissues and normal ovary tissues, indicating a less prominent part in NGF signaling pathway. The key role in angiogenesis and significant overexpression ability of NGF in ovarian tumors made it a potential target in diagnosis and therapeutics.

In the present paper, the authors also researched the effects on ovarian cell migration, invasion, and apoptosis after NGF silencing. NGF silencing was achieved by RNAi. The most efficient siRNA targeting NGF gene was screened from 10 siRNA candidates *in vitro*. To enhance transfection efficiency, recombinant lentivirus was produced to express shRNA, and thus stably suppressed NGF

levels in A2780 cells. The expression levels of TrkA, p75, and VEGF were then analyzed in NGF down-regulation cells. The authors found a decrease of TRKA and VEGF in transcripts and proteins in NGF down-regulation cells compared to the normal A2780 cells, while p75 expression level did not change. The results are in accord with the present pathological data.

NGF is reported to promote cell proliferation and migration and significantly stimulate invasion and blood-vessel formation [12, 13]. Therefore, the relevant indexes were further tested in NGF down-regulated A2780 cells. A modest decrease in cell growth and a significant increase in spontaneous cell apoptosis were observed in NGF down-regulated cells compared to the normal control cells. Further studies demonstrated that increased apoptosis is related to the increased G2/M phase block. The increase of apoptosis may be mediated by p75 signals [15]. Furthermore, cell invasion was strongly inhibited after NGF down-regulation. These results firmly suggest that NGF is involved in ovarian cancer proliferation, invasion, and apoptosis, and blocking NGF expression by RNAi could be a therapeutic strategy to treat ovarian cancer.

## Acknowledgement

This work was supported by Zhejiang Provincial Natural Science Foundation of China.

## References

[1] Urzúa U., Tapia V., Geraldo M.P., Selman A., Vega M., Romero C.: "Nerve growth factor stimulates cellular proliferation of human epithelial ovarian cancer". *Horm. Metab. Res.*, 2012, *44*, 656.

[2] Bast R., Hennessy B., Mills G.B.: "The biology of ovarian cancer: new opportunities for translation". *Nat. Rev. Cancer*, 2009, *9*, 415.

[3] Reynolds L., Grazul-Bilska A., Redmer D.: "Angiogenesis in the female reproductive organs: pathological implications". *Int. J. Exp-Pathol.*, 2002, *83*, 151.

[4] Julio-Pieper M., Lara H., Bravo J., Romero C.: "Effects of nerve growth factor (NGF) on blood vessels area and expression of the angiogenic factors VEGF and TGF beta1 in the rat ovary". *Reprod. Biol. Endocrinol.*, 2006, *4*, 57.

[5] Levi-Montalcini R.: "The nerve growth factor: its mode of action on sensory and sympathetic nerve cells". *Harvey Lect.*, 1966, *60*, 217.

[6] Oelmann E., Srete L., Schuller I., Serve H., Koenigsmann M., Wiedenmann B., *et al.*: "Nerve growth factor stimulates clonal growth of human lung cancer cell lines and a human glioblastoma cell line expressing high-affinity nerve growth factor binding sites involving tyrosine kinase signaling". *Cancer Res.*, 1995, *55*, 2212.

[7] Sortino M.A., Condorelli F., Vancheri C., Chiarenza A., Bernardini R., Consoli U., *et al.*: "Mitogenic effect of nerve growth factor(NGF) in LNCaP prostate adenocarcinoma cells: role of the high and low affinity NGF receptors". *Mol. Endocrinol.*, 2000, *14*, 124.

[8] Tagliabue E., Castiglioni F., Ghirelli C., Modugno M., Asnaghi L., Somenzi G., *et al.*: "Nerve growth factor cooperates with p185 (HER2) in activating growth of human breast carcinoma cells". *J. Biol. Chem.*, 2000, *275*, 5388.

[9] Dissen G., Hirshfield A., Malamed S., Ojeda S.: "Expression of neurotrophins and their receptors in the mammalian ovary is developmentally regulated: changes at the time of folliculogenesis". *Endocrinology*, 1995, *136*, 4681.

[10] Dissen G., Romero C., Hirshfield A., Ojeda S.R.: "Nerve growth factor is required for early follicular development in the mammalian ovary". *Endocrinology*, 2001, *142*, 2078.

[11] Cantarella G., Lempereur L., Presta M., Ribatti D., Lombardo G., Lazarovici P., *et al*.: "Nerve growth factor-endothelial cell interaction leads to angiogenesis in vitro and in vivo". *FASEB J.*, 2002, *16*, 1307.

[12] Tapia V., Gabler F., Munoz M., Yaziqi R., Paredes A., Selman A., *et al*.: "Tyrosine kinase A receptor (trkA): a potencial marker in epithelial ovarian cancer". *Gynecol. Oncol.*, 2011, *121*, 13.

[13] Calza L., Giardino L., Giuliani A., Aloe L., Levi-Montalcini R.: "Nerve growth factor control of neuronal expression of angiogenetic and vasoactive factors". *Proc. Natl. Acad. Sci. USA*, 2001, *98*, 4160.

[14] Vera C., Tapia V., Vega M., Romero C.: "Role of nerve growth factor and its TRKA receptor in normal ovarian and epithelial ovarian cancer angiogenesis". *J. Ovarian Res.*, 2014, *7*, 82.

[15] Kaplan D., Miller F.D.: "Signal transduction by the neurotrophin receptors". *Curr. Opin. Cell. Biol.*, 1997, *9*, 213.

[16] Campos X., Muñoz Y., Selman A., Yazigi R., Moyano L., Weinstein-Oppenheimer C., et al.: "Nerve growth factor and its high-affinity receptor TrkA participate in the control of vascular endothelial growth factor expression in epithelial ovarian cancer". *Gynecol. Oncol.*, 2007, *104*, 168.

[17] Zhang X., LaRussa V., Reiser J.: "Transduction of bone-marrow-derived mesenchymal stem cells by using lentivirus vectors pseudotypedwith modified RD114 envelope glycoproteins". *J.Virol.*, 2004, *78*, 1219.

[18] Gómez-Raposo C., Mendiola M., Barriuso J., Casado E., Hardisson D., Redondo A.: "Angiogenesis and ovarian cancer". *Clin. Transl. Oncol.*, 2009, *11*, 564.

Corresponding Author:
JINYI TONG, M.D.
First People's Hospital of Hangzhou
4 Xueshi Road
Hangzhou 310006 (China)
e-mail: tongjinyi2016@hotmail.com