

Original Article

Carboplatin-docetaxel-induced activity against ovarian cancer is dependent on up-regulated lncRNA PVT1

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Abstract: Ovarian cancer is the fourth most ordinary cause of cancer-related deaths in women. In recent, combination chemotherapy with carboplatin and docetaxel was developed as first-line drug to treat ovarian carcinoma. However, the detailed molecular mechanism, which accounts for the cells to apoptosis induced by administration of carboplatin and docetaxel, was unrecognized. In present study, we provide the mechanistic link between mixture of carboplatin plus docetaxel and its anticancer activity. Primarily, a majority of 30 cancer-related long non-coding RNA (lncRNA) showed differential alteration in carboplatin-docetaxel-treated 3AO cells. Among six up-regulating lncRNAs, we screened out carboplatin-docetaxel-induced lncRNA PVT1 which may be a central downstream target of carboplatin plus docetaxel because expression of PVT1 positively correlates with anticancer action of carboplatin plus docetaxel. Besides, p53 and tissue inhibitor of matrix metalloproteinases-1 (TIMP1) were mediated by lncRNA PVT1, which may explain partially the anticancer activity of lncRNA PVT1. Collectively, we have identified a potential mechanism by which PVT1 regulated by carboplatin plus docetaxel contributes to the carboplatin-docetaxel-induced anticancer action in ovarian cancer. These discoveries also give proof of the potential of PVT1 as significant downstream targets for therapeutic intervention in ovarian cancer.

Keywords: Ovarian cancer, lncRNA PVT1, carboplatin, docetaxel, p53

Introduction

Ovarian cancer, an aggressive and deadly epithelial cancer, is the fourth most common cause of cancer-related deaths in women [1]. On an annual basis, ovarian cancer occurred in 200,000 women and led to 125,000 deaths worldwide [1]. Currently, four considerable histological types including serous, endometrioid, clear cell, and mucinous in epithelial ovarian cancer represent the different clinical behavior and molecular characteristics [2]. Unfortunately, there is no accurate non-invasive diagnostic test for ovarian cancer at present, thereby resulting in that most patients are diagnosed at an advanced stage. So far, surgery and drug-based chemotherapy is still the standard treatment for women with advanced ovarian cancer [3]. Although overall survival can be extended due to the use of above therapy, the treatment of patients with ovarian cancer is still a therapeutic challenge because of its unresponsiveness to conventional chemotherapeutic and

biological reagents, which has been attributed to development of resistance to apoptosis [4]. Therefore, a becoming possibility is that understanding and overcoming resistance mechanisms of ovarian cancer to apoptosis would promote recognition of new medicinal targets and exploitation of new treatments.

As we known, carboplatin and docetaxel are two standard chemotherapeutics used to treat ovarian cancer [5]. Given that patients often show resistance to single agent therapy, combination chemotherapy with carboplatin and docetaxel was developed to overcome this resistance [6]. Generally, apoptosis induced by chemotherapeutic drugs are associated with the intrinsic mitochondrial pathway through releasing the apoptogenic proteins such as cytochrome c and second mitochondrial-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low isoelectric point [7, 8]. However, the detailed molecular mechanisms besides intrinsic mitochondrial pathway,

which bear responsibility for the cells to apoptosis induced by carboplatin and docetaxel, especially by combination chemotherapy with carboplatin and docetaxel, are poorly understood.

A line of evidences has revealed that mammalian genomes contain thousands of long non-coding RNA (lncRNA) genes [9, 10], which have been exhibited to be critical in diverse cell development [11, 12] and identified as mediators of disease [13]. Recent report of dysregulated lncRNA expression across numerous cancer types suggests that aberrant lncRNA expression may be a major contributor to tumorigenesis [14]. Two lncRNAs termed HULC and MEG3 are positively and negatively correlated to hepatocellular carcinoma, respectively [15, 16]. Other lncRNAs such as HOTAIR shows enhanced expression in primary breast tumors and metastases, and HOTAIR expression level in primary tumors is a powerful indicator for predicting the eventual metastasis and death [17]. However, to date, only one report introduces the correlation between lncRNA and ovarian cancer [18]. The overall pathophysiological contributions of lncRNAs to women ovarian cancer remain to be studied further. Moreover, the inter-relationship between lncRNA and carboplatin-docetaxel-induced apoptosis in ovarian cancer has not yet been defined.

In the present study, we examined lncRNA-inducing potential of combination with carboplatin and docetaxel in cultured ovarian cancer cell line. The results indicate that carboplatin-docetaxel-induced lncRNA facilitated the ovarian cancer cell apoptosis *in vitro* and inhibit the tumor growth *in vivo*, which may mediated by p53 and tissue inhibitor of matrix metalloproteinases-1 (TIMP1).

Materials and methods

Cell culture

Human 3AO ovarian cancer cell line was maintained and propagated in a humidified 5% CO₂ incubator at 37°C in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% (V/V) FBS (Hyclone), 100 U/ml penicillin G and 100 U/mL streptomycin. Cells grown in 60 mm cell culture dish were allowed approximately to reach 80% confluence. Then cells were rinsed and removed from the dishes by incubating them with a trypsin-EDTA solution (Hyclone), and harvested in a 15 mL centrifuge tube for subsequent study.

lncRNA profiling

To identify the involved lncRNAs, Human Disease-related lncRNA Profiler (System Biosciences) was utilized and in which 30 lncRNAs were selected from the RNA database (<http://research.imb.uq.edu.au/rnadb/Default.aspx>) or lncRNA database (www.lncRNADB.org). Total RNA was extracted from 3AO cells pretreated with or without carboplatin plus docetaxel. Reverse transcription was carried out by using RevertAid™ Reverse Transcriptase (Fermentas) and random primer mix (New England BioLabs). The values for the cells without treatment after normalization by the internal controls served as a basal level of expression of indicated lncRNAs; delta-delta Ct values (no treatment versus carboplatin plus docetaxel treatment) were used to determine their relative expression as fold changes.

Cell proliferation assay

After digesting, 3AO cells were directly cultured at a density of 1×10^5 cells/well in six well plates. Prior to incubation, the cells were cultured overnight. Cell proliferation was also analyzed by using CCK-8 assay kit (Dojindo) according to manufacturer indications. In particular, 3AO cells were respectively incubated in the medium containing carboplatin and docetaxel in the absence or presence of siRNA against indicated lncRNAs in 96-well plates for indicated times. After that, 5 µl CCK-8 reagent was added to each well and incubated at 37°C for 1 h. The cell numbers were assessed by measurement of absorbance at 450 nm. All the experiments were performed in triplicate.

RNA interference

The sequences of small interference RNA (siRNA) corresponding to six lncRNAs consisting of PVT1, TDRG1, PCA3, GAS5, HOTAIR and SRA1 were synthesized (Qiagen). 3AO cells were transfected with above siRNAs (100 nM) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's specifications. Scrambled siRNA was used as negative control. 0, 24, 48 and 72 h post-transfection later, cells were respectively collected for CCK-8 assay.

Over-expressed lncRNA PVT1

The plasmid construction of over-expressed lncRNA PVT1 was carried out as described previously [19]. Briefly, the DNA sequence of PVT1

obtained from Gene Bank (ID: 5820) was used to design the specific primer (forward primer, 5'-TGCTCTAGAATCTGATGCACGTTCCACC-3'; reverse primer, 5'-CCGGAATTCCTTAATTCTCAA-TCTCAAATAC-3') and then whole amplified product PVT1 was acquired. For construction of PVT1 expression vector, amplified product was cut by *Bam*HI/*Xba*I restriction and ligated into pWPXL (Addgene), forming pWPXL-PVT1, which then transfected into 3AO cells. The control plasmid was constructed by ligating any oligonucleotide into pWPXL. The transfer plasmid pWPXL-PVT1 and control plasmid were applied for packaging lentiviral vectors.

Quantitative RT-PCR assay

For quantitative RT-PCR assay, RNA was isolated from 3AO cells using the RNeasy RNA Isolation kit (Qiagen) according to the manufacturer's instructions. First-strand cDNAs were generated from 3 µg of total RNA using commercially available kits (Applied Biosystems). All subsequent PCR reactions were performed using the 7 Universal PCR Master Mix (Applied Biosystems).

Primers of p53 used for amplification were CCCCTCCTGGCCCTGTCATCTTC (forward) and GCAGCGCTCACAACTCCGTCAT (reverse); Primers of TIMP1 used for amplification were 5'-TATCCGGTACGCTACACCC-3' (forward) and 5'-TGGGCATATCCACAGAGGCT-3' (reverse); Primers of lncRNA PVT1 used for amplification were 3'-CATCCGGCGCTCAGCT-5' (forward) and 3'-TCATGATGGCTGTATGTGCCA-5' (reverse).

Amplification and detection of mRNA were analyzed by 7500 real-time PCR System (Applied Biosystems). To normalize mRNA concentrations, transcriptional levels of β-actin mRNA were identified in parallel for each sample, and relative transcriptional level of Notch1 was adjusted by standardization based on the β-actin mRNA levels. Samples for each experimental condition were run in triplicate.

Inhibition of tumor growth in vivo

To create an experimental tumor model, 1×10^7 of 3AO cells transfected with either scrambled short hairpin RNA (shRNA) or two PVT1 shRNAs were suspended in 100 µl of PBS and were subcutaneously injected into the flank of female CD1 nude mice (9-12 weeks old). The tumor size was measured every six days using a stan-

dard calipers measuring tumor length and width in a blinded fashion and the tumor volume was calculated using the formula: length \times width² \times 0.52. After 31 days, animals were killed for determination of tumor weights. All work was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) to assess the treatment effects by using DPS 3.2 software. The Student *T*-test was used to determine the statistically significant differences in numbers with two significant levels (0.05 and 0.01). The results are shown as mean \pm standard deviation (SD).

Results

Carboplatin-docetaxel induces differential expression of lncRNA

We firstly explored the influence of carboplatin plus docetaxel pretreatment on the expression of lncRNAs in human ovarian cancer cell line, 3AO cell. Thirty vital tumor-related lncRNAs were selected to analysis and ultimately, as expected, a majority of these lncRNAs expression had been changed in 3AO cells in response to carboplatin plus docetaxel incubation (**Figure 1A**). When using cutoff value of 1.8-fold up-regulation or 3-fold down-regulation, ten lncRNAs showed significant differences in carboplatin-docetaxel-treated cells in contrast to untreated cells (**Figure 1B**). These ten lncRNAs termed PVT1, TDRG1, PCAT1, GAS5, HOTAIR, SRA1, BCYRN1, CASC2, HI9 and MEG3. Out of them, six were up-regulated in carboplatin-docetaxel-treated cells while four were down-regulated. Collectively, these data suggest that combination chemotherapy with carboplatin and docetaxel has an ability to alter the lncRNA expression in 3AO cell.

lncRNA PVT1 contributes to carboplatin-docetaxel-induced inhibition of cell proliferation

In general, abundance of above cancer-related lncRNAs was differentially increased in the carboplatin-docetaxel-treated cells that may avail anti-cancer (or vice versa). Given that, we primarily focus on the six lncRNAs, PVT1, TDRG1,

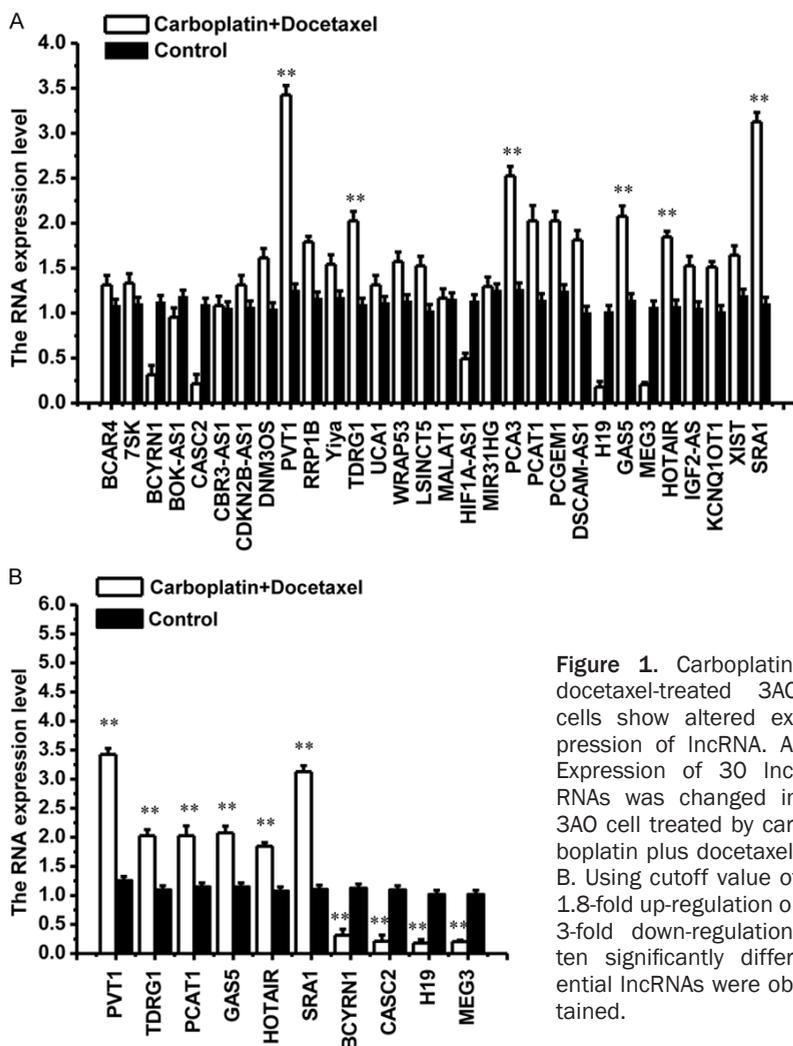


Figure 1. Carboplatin-docetaxel-treated 3AO cells show altered expression of lncRNA. A. Expression of 30 lncRNAs was changed in 3AO cell treated by carboplatin plus docetaxel. B. Using cutoff value of 1.8-fold up-regulation or 3-fold down-regulation, ten significantly differential lncRNAs were obtained.

PCAT1, GAS5, HOTAIR, and SRA1, which were all boosted by carboplatin plus docetaxel treatment. To examine whether these up-regulated lncRNAs contributes to anti-cancer effect of carboplatin plus docetaxel treatment, we knocked down the expression level of these lncRNAs using RNAi technique. The siRNA corresponding to these six lncRNAs were transfected and their function was analysis. Endogenous lncRNAs mRNA level was analyzed by RT-PCR and detected to be lower in lncRNA siRNA-transfected cells as compared to cells transfected with scrambled siRNA control constructs, demonstrating the effectiveness of lncRNA siRNA (data not shown). Because administration of carboplatin and docetaxel enhances the expression of these six lncRNAs, the effect of lncRNAs inhibition on anticancer action of carboplatin and docetaxel was analyzed. Interestingly, the cell proliferation was

only obviously increased in carboplatin-docetaxel-treated cells in treatment with PVT1 siRNA, except for treatment with other lncRNA siRNAs, when compared with the carboplatin-docetaxel-treated cells in the presence of scrambled siRNA (Figure 2). These observations indicate that a carboplatin-docetaxel-regulated lncRNA, PVT1, have an anticancer activity, which may be responsible for carboplatin-docetaxel-induced inhibition of cell proliferation.

LncRNA PVT1 mediates the expression of p53 and TIMP1

Next, we investigate whether lncRNA PVT1 have a capability to regulate the expression of p53 and TIMP1 in 3AO cells. To this end, we established lncRNA PVT1 recombinant plasmid and then transferred it into 3AO cells. After that, the lncRNA PVT1 expression was markedly increased and this promoting effect

represented a time-dependent manner (Figure 3A), confirming the effectiveness of over-expression of lncRNA PVT1. Besides, we also found that expression of p53 and TIMP1 was obviously promoted in lncRNA PVT1-transfected cells in contrast to control and this increasing effect also presented a time-dependent manner (Figure 3A).

To further estimate the effect of lncRNA PVT1 on expression of p53 and TIMP1, a direct knockdown of expression level of lncRNA PVT1 was performed. In this experiment, the down-regulated expression of p53 and TIMP1 was caused by the suppressing expression of lncRNA PVT1 (Figure 3B). Taken together, these findings reveal that lncRNA PVT1 is able to facilitate the expression of p53 and TIMP1 in ovarian cancer cell, showing the positive regulation of PVT1 on expression of p53 and TIMP1.

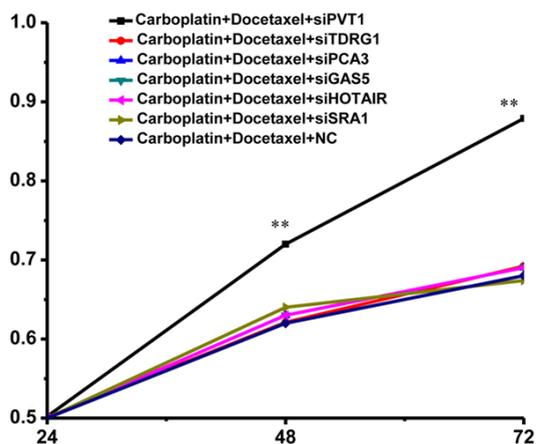


Figure 2. One up-regulated lncRNA, PVT1, contributes to anti-cancer effect of carboplatin and docetaxel. After transfecting the indicated siRNA, we found that cell proliferation was obviously increased in carboplatin-docetaxel-treated cells in treatment with PVT1 siRNA, except for treatment with other lncRNA siRNAs.

Knockdown of lncRNA PVT1 promotes the tumor growth in vivo

Obviously, it would be more relevant to develop a therapeutic intervention if we could demonstrate that knockdown of lncRNA PVT1 gene could result in tumor growth acceleration in vivo. To this end, we knocked down the expression of lncRNA PVT1 utilizing vector encoding PVT1 shRNA and then examined the growth of PVT1 shRNA-treated human ovarian cancer cell xenografts in nude animals. In this experiment, two PVT1 shRNAs were used to transfer into 3AO cells, respectively. Result revealed that knockdown of lncRNA PVT1 cause a marked reduction in tumor size in a human ovarian cancer xenograft model (Figure 4). Among thirty days' growth, the higher growth velocity was detected in nude mice subcutaneously injected with PVT1 shRNA-transfected 3AO cells in comparison with mice injected with scrambled shRNA-transfected 3AO cells ($P < 0.01$, Figure 4). Besides that, mean tumor volumes of PVT1 shRNA-treated mice were induced by 50% at day 30 in contrast to the scrambled shRNA-treated mice (Figure 4), exhibiting a potent anti-tumor activity of lncRNA PVT1.

Discussion

Despite numerous attempts, the mortality rate for ovarian cancer is approximately 62.5 % annually [1]. In part, this is because therapy tar-

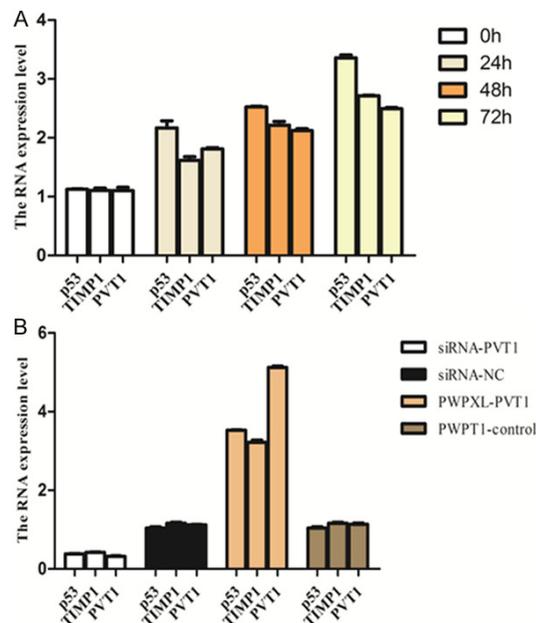


Figure 3. The expression of p53 and TIMP1 may be mediated by lncRNA PVT1. A. Over-expression of lncRNA PVT1 up-regulates the mRNA level of p53 and TIMP1. B. Knockdown of expression level of lncRNA PVT1 inhibits the transcriptional level of p53 and TIMP1.

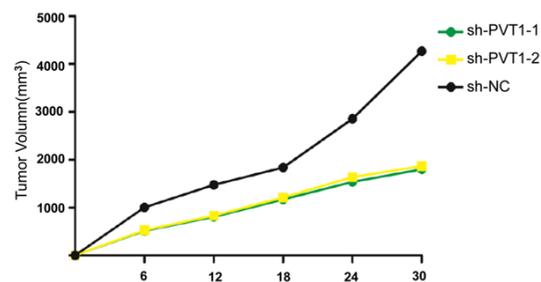


Figure 4. Tumor growth in vivo was boosted by knock-down of lncRNA PVT1.

getting tumor cells have largely failed. Recently, combination chemotherapy with carboplatin and docetaxel was developed as first-line drug to treat ovarian carcinoma [5, 20]. Although the anticancer mechanism induced by single chemotherapeutic drug such as carboplatin or docetaxel was exploited preliminarily [21, 22], the detailed molecular mechanism, which accounts for the cells to apoptosis induced by combination chemotherapy with carboplatin and docetaxel, was non-elucidated. In present study, we found that mixture of carboplatin and docetaxel is an inducer of lncRNA PVT1. lncRNA PVT1 contributes to anticancer activity of combination chemotherapy with carboplatin

and docetaxel. Moreover, in subsequent experiment, we provide the probably mechanistic link between lncRNA PVT1 and its anticancer activity, and then demonstrate the functional significance of lncRNA PVT1 *in vivo*.

As a newly arisen class of non-coding genes, lncRNAs have been recently discovered to be universally transcribed in the mammalian genome. Alterations in the primary structure, secondary structure and expression levels of lncRNAs as well as their cognate RNA-binding proteins are often involved in human diseases, particularly in cancer [23]. However, very little was realized on expression patterns and biological meaning of lncRNAs in human ovarian cancer. Also, very little was reported on the relationship between chemotherapeutic drug and lncRNA. The first important discovery in our study is that administration of exogenous carboplatin plus docetaxel differentially alters the expression of lncRNAs, which is supposed to be a novel finding because previous studies did not reveal how any lncRNA might be responsible for carboplatin-docetaxel-induced anticancer activity. Currently, we reveal that carboplatin-docetaxel-induced anticancer activity may partly due to the up-regulating lncRNA PVT1 at least, demonstrating a plausible lncRNA-regulated mechanism. Although the lncRNA involved in ovarian cancer and this cancer cured by combination chemotherapy with carboplatin and docetaxel have been extensively documented in the literature [20-23], the ability of mixture of carboplatin plus docetaxel to promote lncRNA PVT1 signaling and against ovarian cancer was previously unknown.

Besides, the second core discovery in our study is the capability of lncRNA PVT1 to boost the expression of p53 and TIMP1. As a major regulator for gene expression, p53 protein, also termed as tumor suppressor p53, is able to directly or indirectly regulate numerous protein-coding genes and non-coding genes, thereby indicating the significance in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumor inhibitor, suppressing cancer [24-27]. Another up-regulating protein, TIMP1 also has a role in blocking of tumor growth, invasion and metastasis based on previous studies on experimental models [28-30]. Thus, a suitable possibility is that anticancer activity induced by lncRNA PVT1 is completed by activating the expression of p53 and TIMP1 which may mediate its biological role as

a tumor suppressor, and certainly this inference needs to be further investigated in future.

In conclusion, our work provides strong evidence that carboplatin-docetaxel-induced anticancer activity seems to depend largely on induction of lncRNA PVT1, which boosts up-regulation of p53 and TIMP1. These data highlight the up- and down-stream connection between chemotherapeutic drugs (mixture of carboplatin and docetaxel) and lncRNA, and also highlight the significance and relevance of lncRNA PVT1 as potential downstream biomarkers for therapeutic intervention in ovarian cancer.

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Disclosure of conflict of interest

None.

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References

- [1] Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Kurzeder C du Bois A, Sehouli J, Kimmig R, Stähle A, Collinson F, Essapen S, Gourley C, Lortholary A, Selle F, Mirza MR, Leminen A, Plante M, Stark D, Qian W, Parmar MK, Oza AM; ICON7 Investigators. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* 2011; 365: 2484-2496.
- [2] Faça VM, Ventura AP, Fitzgibbon MP, Pereira-Faça SR, Pitteri SJ, Green AE, Ireton RC, Zhang Q, Wang H and O'Briant KC. Proteomic analysis of ovarian cancer cells reveals dynamic processes of protein secretion and shedding of extra-cellular domains. *PLoS One* 2008; 3: e2425.
- [3] Marcus CS, Maxwell GL, Darcy KM, Hamilton CA and McGuire WP. Current approaches and challenges in managing and monitoring treatment response in ovarian cancer. *J Cancer* 2014; 5: 25.
- [4] Agarwal R and Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nat Rev Cancer* 2003; 3: 502-516.
- [5] Vasey PA, Jayson GC, Gordon A, Gabra H, Coleman R, Atkinson R, Parkin D, Paul J, Hay A

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- and Kaye SB. Phase III randomized trial of docetaxel-carboplatin versus paclitaxel-carboplatin as first-line chemotherapy for ovarian carcinoma. *J Natl Cancer Inst* 2004; 96: 1682-1691.
- [6] Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nature Rev Cancer* 2007; 7: 573-584.
- [7] Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, Moritz RL, Simpson RJ and Vaux DL. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 2000; 102: 43-53.
- [8] Hegde R, Srinivasula SM, Zhang Z, Wassell R, Mukattash R, Cilenti L, DuBois G, Lazebnik Y, Zervos AS and Fernandes-Alnemri T. Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *J Biol Chem* 2002; 277: 432-438.
- [9] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW and Cassady JP. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 2009; 458: 223-227.
- [10] Cabili MN, Trapnell C, Goff L, Koziol M, Tazon-Vega B, Regev A and Rinn JL. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev* 2011; 25: 1915-1927.
- [11] Boumil RM and Lee JT. Forty years of decoding the silence in X-chromosome inactivation. *Hum Mol Genet* 2001; 10: 2225-2232.
- [12] Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, Horlings HM, Shah N, Umbricht C and Wang P. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nat Genet* 2011; 43: 621-629.
- [13] Gomez JA, Wapinski OL, Yang YW, Bureau J-F, Gopinath S, Monack DM, Chang HY, Brahic M and Kirkegaard K. The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon- γ locus. *Cell* 2013; 152: 743-754.
- [14] Huarte M and Rinn JL. Large non-coding RNAs: missing links in cancer? *Hum Mol Genet* 2010; 19: R152-R161.
- [15] Panzitt K, Tschernatsch MM, Guelly C, Moustafa T, Stradner M, Strohmaier HM, Buck CR, Denk H, Schroeder R and Trauner M. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. *Gastroenterology* 2007; 132: 330-342.
- [16] Braconi C, Kogure T, Valeri N, Huang N, Nuovo G, Costinean S, Negrini M, Miotto E, Croce C and Patel T. microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. *Oncogene* 2011; 30: 4750-4756.
- [17] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai M-C, Hung T, Argani P and Rinn JL. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010; 464: 1071-1076.
- [18] Silva JM, Boczek NJ, Berres MW, Ma X and Smith DI. LSINCT5 is over expressed in breast and ovarian cancer and affects cellular proliferation. *RNA Biol* 2011; 8: 496-505.
- [19] Zhu Y, Zhong Z and Liu Z. Lentiviral vector-mediated upregulation of let-7a inhibits gastric carcinoma cell growth in vitro and in vivo. *Scand J Gastroenterol* 2011; 46: 53-59.
- [20] Anwer K, Kelly FJ, Chu C, Fewell JG, Lewis D and Alvarez RD. Phase I trial of a formulated IL-12 plasmid in combination with carboplatin and docetaxel chemotherapy in the treatment of platinum-sensitive recurrent ovarian cancer. *Gynecol Oncol* 2013; 131: 169-173.
- [21] Azarenko O, Smiyun G, Mah J, Wilson L and Jordan MA. Antiproliferative mechanism of action of the novel taxane cabazitaxel as compared with the parent compound docetaxel in MCF7 breast cancer cells. *Mol Cancer Ther* 2014; 13: 2092-2103.
- [22] Turner N, Stewart J, Barnett F and White S. Syndrome of inappropriate anti-diuretic hormone secretion secondary to carboplatin after docetaxel-carboplatin-trastuzumab combination for early stage HER-2 positive breast cancer. *Asia Pac J Clin Oncol* 2012; 8: e9-e11.
- [23] Wapinski O and Chang HY. Long noncoding RNAs and human disease. *Trends Cell Biol* 2011; 21: 354-361.
- [24] Komarov PG, Komarova EA, Kondratov RV, Christov-Tselkov K, Coon JS, Chernov MV and Gudkov AV. A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. *Science* 1999; 285: 1733-1737.
- [25] El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B. *WAF1*, a potential mediator of p53 tumor suppression. *Cell* 1993; 75: 817-825.
- [26] Toshiyuki M and Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human *bax* gene. *Cell* 1995; 80: 293-299.
- [27] Liu Q, Huang J, Zhou N, Zhang Z, Zhang A, Lu Z, Wu F and Mo YY. LncRNA loc285194 is a p53-regulated tumor suppressor. *Nucleic Acids Res* 2013; 41: 4976-4987.
- [28] Krüger A, Sanchez-Sweatman OH, Martin DC, Fata JE, Ho AT, Orr FW, Rütger U and Khokha R. Host TIMP-1 overexpression confers resistance to experimental brain metastasis of a fibrosarcoma cell line. *Oncogene* 1998; 16: 2419-2423.

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- [29] Martin DC, Sanchez-Sweetman OH, Ho A, Inderde DS, Tsao MS and Khokha R. Transgenic TIMP-1 inhibits simian virus 40 T antigen-induced hepatocarcinogenesis by impairment of hepatocellular proliferation and tumor angiogenesis. *Lab Invest* 1999; 79: 225-234.
- [30] Ikenaka Y, Yoshiji H, Kuriyama S, Yoshii J, Noguchi R, Tsujinoue H, Yanase K, Namisaki T, Imazu H and Masaki T. Tissue inhibitor of metalloproteinases-1 (TIMP-1) inhibits tumor growth and angiogenesis in the TIMP-1 transgenic mouse model. *Int J Cancer* 2003; 105: 340-346.