European Review for Medical and Pharmacological Sciences 2018; 22: 7183-7188

Long non-coding RNA PVT1 functions as an oncogene in ovarian cancer via upregulating SOX2

M.-F. ZOU[1], J. LING[2], Q.-Y. WU[2], C.-X. ZHANG[2]

[1]Department of Obstetrics and Gynecology, Wujin Hospital of Tradition[al] Chinese [M]edicine, Affiliated to Nanjing University of Chinese Medicine, Changzhou, China
[2]Department of Obstetrics and Gynecology, Affiliated Jiangyin Hos[pital] of [S]outheast U[niver]sity, Jiangyin, China

Abstract. – OBJECTIVE: The aim of this study was to identify the role of long non-coding RNA PVT1 (lnc-PVT1) in the progression of ovarian cancer.

PATIENTS AND METHODS: The expression of lnc-PVT1 in ovarian cancer cells and 50 paired tissue samples were detected by quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR). Moreover, cell proliferation assay and transwell assay were performed to identify the function of lnc-PVT1 *in vitro*. Q[RT-PCR] and Western blot were utilized to explore the possible underlying mechanism.

RESULTS: Compared with normal tissues, expression of lnc-PVT1 T1 was remarkably upregulated in tumor tissues. Moreover, the proliferation and invasion of ovarian cancer cells were promoted after knockdown of lnc-PVT1 *in vitro*. Moreover, both the mRNA and protein levels of SOX2 were suppressed after knockdown of lnc-PVT1. Besides, the expression of lnc-PVT1 in ovarian tissues was positively correlated to lymph node metastasis.

CONCLUSIONS: lnc-PVT1 could enhance the invasion and proliferation of ovarian cancer cells through upregulating SOX2, which might serve as a novel therapeutic target for the treatment of ovarian cancer.

Key Words:
Long non-coding RNA, Lnc-PVT1, Ovarian cancer, SOX2.

Introduction

Ovarian cancer ranks the third common cancer among malignant tumors[1]. The incidence of ovarian cancer in females remains high in the world[2]. Although treatments of ovarian cancer including chemotherapy and surgery have developed rapidly in recent decades, the 5-year survival rate of these patients remains lower than 30%. Therefore, it is necessary to explore the genetic changes in ovarian cancer and the underlying mechanism.

Evidence has proved that long non-coding RNA (lncRNA) may participate in the occurrence and development of malignant tumors. For example, lnc-ANRIL is upregulated in serous ovarian cancer, which is also related to metastasis and prognosis[4]. By regulating epithelial-mesenchymal transition, lnc-TUG1 can regulate the metastasis and proliferation of ovarian cancer[5]. Meanwhile, lnc-HOST2 may promote the proliferation, invasion, and metastasis of epithelial ovarian cancer[6]. Studies have also indicated that overexpression of lnc-HOTAIR may promote tumor metastasis and is closely associated with poor prognosis in epithelial ovarian cancer[7]. However, the role of lnc-PVT1 in ovarian cancer has not been fully elucidated.

Our present study revealed that lnc-PVT1 was significantly upregulated in ovarian cancer samples. Meanwhile, lnc-PVT1 facilitated the invasion and proliferation of ovarian cancer cells *in vitro*. Furthermore, we discovered that lnc-PVT1 exerted its function *via* regulating SOX2 in ovarian cancer.

Patients and Methods

Cell Culture

Totally 5 ovarian cancer cell lines (A2780, TOV112D, HO-8910, OVCAR-3, and SKOV3), 1 normal ovarian cell line (ISOE80) and the 293T cell line (Chinese Type Culture Collection, Chinese

Corresponding Authors: Qunying Wu, MD; e-mail: lingjing_112@163.com
Chenxia Zhang, MD; e-mail: npui36@163.com

Academy of Sciences, Shanghai, China) were used in this study. All cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Hyclone, South Logan, UT, USA) complemented with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) and penicillin. Besides, cells were grown in a 37°C, 5% CO_2 humidified incubator.

Clinical Samples

Human tissue samples were collected from 50 ovarian cancer patients who underwent surgery at the Affiliated Jiangyin Hospital of South-East University. All the collected tissues were kept at –80°C. The written informed consent was obtained from each patient before the surgery. Our study was approved by the Ethics Committee of Affiliated Jiangyin Hospital of South-East University.

Cell Transfection

Lentiviral small hairpin RNA (shRNA) was synthesized before this study, and pLenti-E-F1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA) was then used fo[r] ning. Next, these viruses, empty vector [(] and the lnc-PVT1 lentiviruses (sh-PVT1[-] re packaged in 293T cells.

Total RNA Extraction and Quantitative Reverse Transcriptase-Pol[ymerase] Chain Reaction (qRT-PCR)

Total RNA was extract[ed] om sam[ples] according to the instructions [of] Izol [re]trogen, Carlsbad, CA [USA], RNA was then reverse-transcri[bed] to comp[lemen]tary DNAs (cDNAs) by using the Reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., D[alian]). QRT-PCR was conducted on [ABI] 7500 stem (Applied Biosystems, Foster[City], CA, USA), and SYBR Green Real-Time P[CR K]as applied. Thermal cycle was as follows: 9[5°C] 30 sec, 95°C for 5 sec for 40 cycle[s a]nd 60°[C] 5 sec. Primer sequences used i[n] s study we[re] lows: PVT1, F: 5'-GT[C]TGAT[C]GTGAG[C] ACG-3', R: 5'-CGAA[C]ATTTC[C]ACACACG-3'; SOX2, F: 5'-TCTTG[C]ATA[C]AGCA[C]C-3', R: 5'-ATCATTT[C]AC[C]GTGT[C]U6: F: 5'-GCTTCGG[C]CACAT[C]TA[C]T-3', R: 5'-CGCTTC[C]ATTTGCGT[C]'; GAPDH: F: 5'-C[C]GCTCCTCC[C]TC-3', R: 5'-ATCC[C]GAC[C]ACCTTCAC-3'.

Cell Proliferation Ass[ay]

According to the instructions of cell counting [K]8 (CCK-8) (D[o]indo Molecular Technologies, [I]Kumamoto, [Ja]pan), treated cells were seeded [in] 6-well plate[s a]nd the proliferation of these c[ells] s monit[ored] every 24 h. Absorbance (OD) valu[e at] elength of 450 nm was detected by a spectrophotometer (Thermo Scientific, Rockford, IL, USA).

Cell Invasion

Totally 5×10^4 cells in 200 µL serum-free Roswell Park Memorial Institute 1640 (RPMI-1640) were transformed to the upper chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA) which was added with Matrigel (50 µg; BD Biosciences, Franklin Lakes, NJ, USA). The lower chamber was added with RPMI-1640 and FBS. After 48 h, a cotton swab was used to wipe

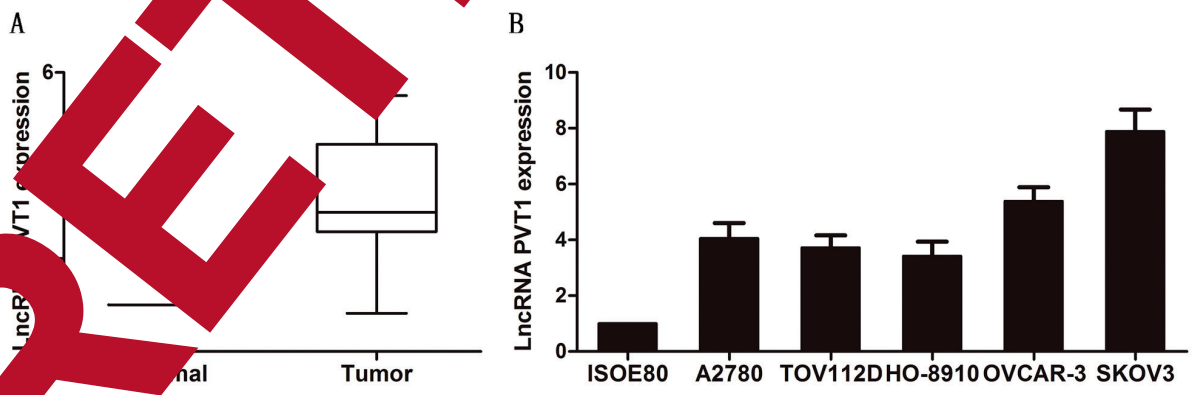


Figure 1. The expression level of lnc-PVT1 was increased in ovarian cancer tissues and cell lines. ***A,*** Lnc-PVT1 expression [was] significantly increased in ovarian cancer tissues compared with normal tissues. ***B,*** The expression level of Lnc-PVT1 re[lative to] GAPDH was determined in human ovarian cancer cell lines and normal ovarian cells (ISOE80) by qRT-PCR. Data were p[resented] as mean ± standard error of the mean. *$p<0.05$.

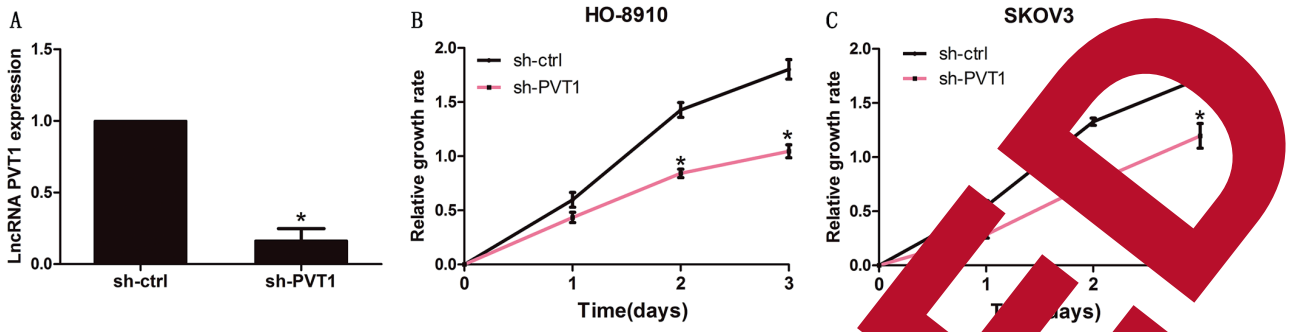


Figure 2. Knockdown of Lnc-PVT1 decreased the proliferation of ovarian cancer c[illegible]. *A*[illegible]sion of [illegible]VT1 in ovarian cancer cells transduced with control shRNA vector (sh-ctrl) or lncRNAPVT1 [illegible]NA (sh[illegible]was de[illegible]d by qRT-PCR. GAPDH was used as an internal control. ***B,*** CCK8 assay showed that knoc[illegible]n of lnc-PV[illegible]if[illegible]y decreased the proliferation of HO-8910 ovarian cancer cells. ***C,*** CCK8 assay showed that kn[illegible]n of lnc-PVT1 [illegible]tly decreased the proliferation of SKOV3 ovarian cancer cells. The results were the average [illegible]pendent experi[illegible]s (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

the top surface of the chambers, and the chambers were immersed with precooling methanol for 10 min, followed by staining in crystal violet for 30 min. The membranes were then dried, inverted, and mounted on microscope slides for analysis. The cells were counted from 3 randomly chosen fields per membrane.

Western Blot Analysis

The radio-immunoprecipitation assay (R[illegible] Beyotime, Shanghai, China) was utilized for [illegible]tal protein extraction. The concentration of e[illegible]tracted proteins was quantifie[illegible]g to the instructions of the bicincho[illegible]c aci[illegible]CA) kit (TaKaRa, Dalian, China[illegible]tal pro[illegible]s were separated by sodium [illegible]l su[illegible]crylamide gel electro[illegible]ore[illegible]AGE) and transferred to poly[illegible]idene [illegible]ide (PVDF) membranes (Mil[illegible]e, Billerica, [illegible]USA). Membranes w[illegible]ed with 5%[illegible]immed milk for 1 h. [illegible]owe[illegible]he incubation of primary antib[illegible]dy overnight[illegible]branes were then incubate[illegible]h the secondar[illegible]ibody at room temper[illegible]e for 1 h. Immun[illegible]reactive bands were [illegible]osed b[illegible]hanced chemiluminescence (E[illegible]etho[illegible]th Im[illegible]J Software. Rabbit anti-[illegible]lycera[illegible]yde 3-phosphate dehydrogenas[illegible]rabbi[illegible]i-SOX2, as well as goat [illegible]bbit s[illegible]antibody, were obtained fro[illegible]ll Signa[illegible]Technology (CST, Danvers, MA[illegible]A).

[illegible]istical Analysis

[illegible]tatistical Product and Service Solutions ([illegible]17.0 (SPSS, Chicago, IL, USA) was used for a[illegible]tatistical analysis. The χ^2-test was applied f[illegible]tegorical variables. The survival of ovarian [illegible]cer patients [illegible]s evaluated by the Kaplan-[illegible]ier method. Student *t*-test was used to [illegible]pare differe[illegible]between groups. Data were r[illegible]nted as [illegible] ± SD. $p<0.05$ was considered stati[illegible]icant.

Results

Expression Level of Lnc-PVT1 In Ovarian Cancer Tissues and Cell Lines

QRT-PCR was first conducted to detect the expression of lnc-PVT1 in 50 ovarian cancer tissues and 5 ovarian cancer cell lines. Results indicated that lnc-PVT1 was significantly upregulated in tumor tissue samples (Figure 1A). Meanwhile, compared with normal epithelial cells, the expression of lnc-PVT1 was significantly higher in ovarian cancer cells (Figure 1B).

Lnc-PVT1 Knockdown Inhibited Cell Invasion and Proliferation In vitro

According to the expression of lnc-PVT1 in the above 5 ovarian cancer cell lines, HO-8910 and SKOV3 were chosen for a subsequent lnc-PVT1 knockdown. Sh-PVT1 and sh-ctrl were synthetized and transfected into these two cell lines. Then, the expression of lnc-PVT1 was confirmed by qRT-PCR (Figure 2A). Furthermore, results of CCK8 assay showed that the proliferation of ovarian cells was significantly inhibited after lnc-PVT1 knockdown (Figure 2B and 2C). Moreover, transwell assay found that silenced lnc-PVT1 suppressed the invasion of ovarian cancer cells (Figure 3A and 3B).

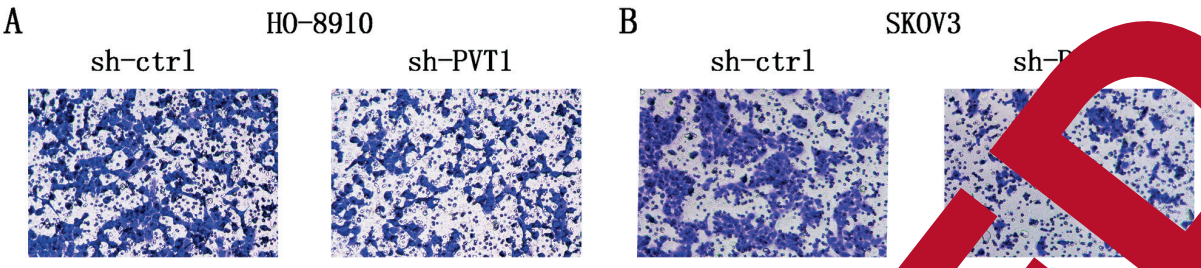
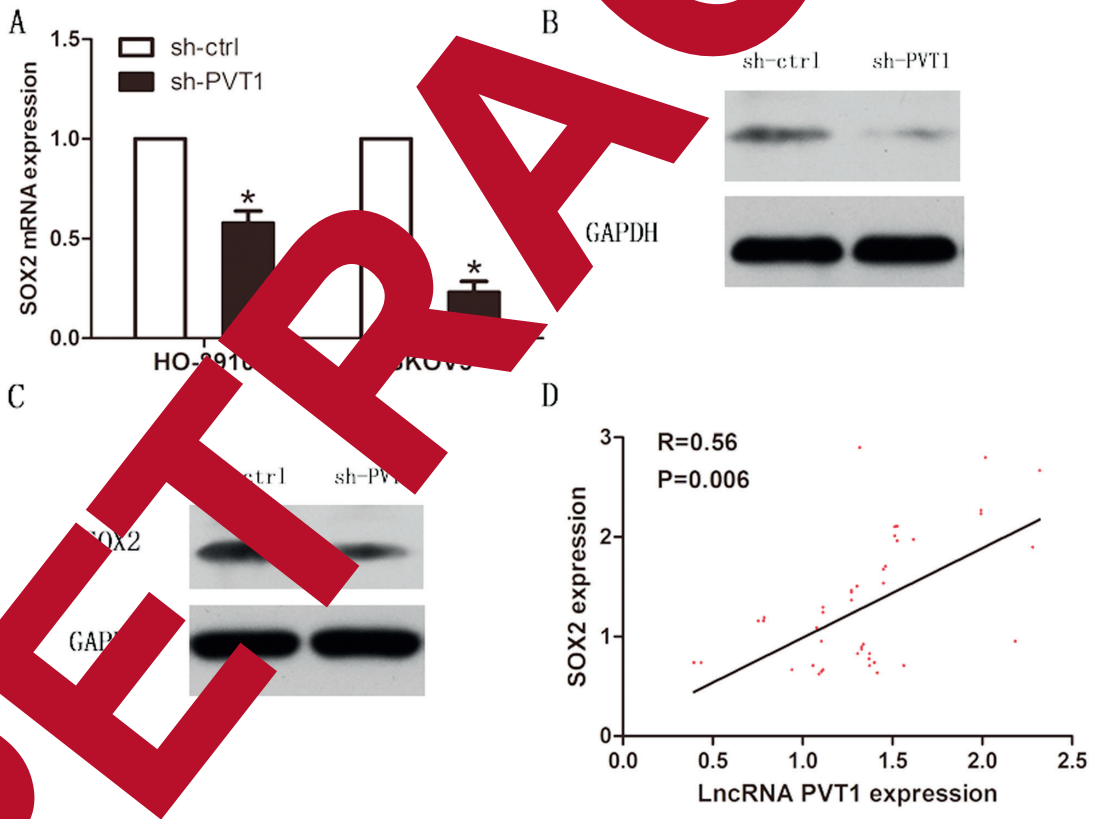


Figure 3. Knockdown of lnc-PVT1 decreased the invasion of ovarian cancer cells. ***A,*** Tra[illegible] assay s[illegible]d that the n[illegible]h-ber of invaded cells in the sh-PVT1 group was obviously reduced when compared with t[illegible]trl gro[illegible]HO-891[illegible]ovarian cancer cells. ***B,*** Transwell assay showed that the number of invaded cells in the sh-PVT1 gro[illegible]antly re[illegible] when compared with the sh-ctrl group in SKOV3 ovarian cancer cells. The results were the [illegible]ge o[illegible]epende[illegible]erimen-ts Data were presented as mean ± standard error of the mean. *$p<0.05$.

Lnc-PVT1 Promoted Ovarian Cancer Tumorigenesis Via SOX2

Various studies have confirmed that SOX2, as a novel oncogene discovered recently, plays a crucial role in tumor proliferation and metastasis and can be regulated by several noncoding RNAs.

QRT-PC[illegible]esults[illegible]nstrated that the mRNA expression of SOX2[illegible]ownregulated in ovarian[illegible] cells transi[illegible]with sh-PVT1 (Fig[illegible]A). Western blot analysis further verified the protein ex[illegible]ession level of SOX2 was also [illegible]wnregulated i[illegible]arian cancer cells after tran-



Fig[illegible][illegible]on between SOX2 and lnc-*PVT1* in ovarian cancer. ***A,*** The mRNA expression level of SOX2 in the sh-[illegible] group was significantly decreased when compared with the sh-ctrl group in HO-8910 and SKOV3cells. ***B,*** The protein [illegible]ession of SOX2 was repressed after knockdown of lnc-PVT1 in HO-8910 cells. ***C,*** The protein expression of SOX2 was [illegible]ed after knockdown of lnc-PVT1 in SKOV3 cells. ***D,*** Linear correlation between the expression level of SOX2 and lnc-P[illegible]arian cancer tissues. The results represented the average of three independent experiments. Data were presented as mean[illegible]andard error of the mean. *$p<0.05$.

sfecting sh-PVT1 (Figure 4B and 4C). To explore the interaction between lnc-PVT1 and SOX2, the expression level of SOX2 was detected in tissues. Linear correlation analysis revealed that the SOX2 expression was positively correlated with lnc-PVT1 expression in ovarian cancer tissues (Figure 4D).

Discussion

Latest studies have discovered that lnc-PVT1 is upregulated in many cancers and may participate in tumor progression. For example, overexpression of lnc-PVT1 has been found to be closely related to the development and poor prognosis of pancreatic cancer[8]. Lnc-PVT1 is remarkably upregulated in non-small cell lung cancer, eventually promoting the tumorigenesis of NSCLC[9]. Meanwhile, high lnc-PVT1 expression level promotes cell invasion in esophageal cancer *via* inducing epithelial-to-mesenchymal transition[10]. Besides, strong evidence has shown the oncogenic role of lnc-PVT1 in cervical cancer as well as its role in poor prognosis[11]. Moreover, several e[illegible]ments have found that lnc-PVT1 express[illegible] promote multidrug resistance in the treati[illegible] of cancers, including gastric carcinoma, colo[illegible] cancer, and others[12,13]. However, the exact rol[illegible] lnc-PVT1 in ovarian cancer remain[illegible] unclear.

In the present study, we fo[illegible]nc-PVT1 was upregulated in ovarian c[illegible]r tiss[illegible]and cell lines. Furthermore, lnc-P[illegible] knock[illegible] signi-ficantly inhibited the p[illegible]tion ability of ovarian can[illegible]r c[illegible] data indicated that lnc-PVT1 [illegible]ght ser[illegible]n oncogene and promote the t[illegible]igenesis of [illegible] cancer.

SOX2 is a n[illegible]gene that dis[illegible]red recently. It's re[illegible]d th[illegible]X2 plays a role in the proliferatio[illegible]and metasta[illegible]umors, which can be regul[illegible] by several no[illegible]g RNAs. For examp[illegible]OX2 regulates the in[illegible]ation and development of prim[illegible]kin tumor through regulating fu[illegible] of s[illegible]umor-i[illegible]ating cells and cancer stem-[illegible]esopha[illegible]squamous cells, SOX2 i[illegible]overex[illegible]d, wh[illegible] may promote the metastasis by act[illegible]e STAT3/HIF-1α signaling pathway[illegible][15,16]. M[illegible]hile, SOX2 overexpression has [illegible]found to be associated with migration, epithelial-[illegible]chymal transition, and invasion in laryngeal cancer Hep-2 cells[17]. Moreover, in a high-grade oligodendroglioma in a mouse model, S[illegible] found to be the key step for tumor initiation [illegible]esides, studies have also suggested that SOX2 participates in the development of tamoxifen resistance in breast cancer cells[19]. I[illegible] Western blot analysis demonstrate[illegible]at SO[illegible] was suppressed after the lnc-PVT[illegible]ockdown *in vitro*. In addition, we discovere[illegible]ositive correlation between SOX2 and lnc-P[illegible]pression in tumor tissues. The abov[illegible]sults a[illegible]ested that lnc-PVT1 might rea[illegible]its functio[illegible] geting SOX2.

Conclusions

We sugge[illegible]ew biomarker[illegible] progression of ovarian [illegible]anwhile, our [illegible]ults also indicate tha[illegible]PVT[illegible]l to the carcinogenesis of ovarian cancer, which[illegible] served as a promising bio[illegible]or ovarian ca[illegible]

Conflict of Interest

The authors declare[illegible] they have no conflict of interest.

References

1) [illegible]ANI N, PRASAD SR, VIKRAM R, SHANBHOGUE AK, [illegible]NER PC, FASIH N. Histologic, molecular, and cytogenetic features of ovarian cancers: implications for diagnosis and treatment. Radiographics 2011; 31: 625-646.
2) HOLMES D. Ovarian cancer: beyond resistance. Nature 2015; 527: S217.
3) XI QP, PU DH, LU WN. Research on application value of combined detection of serum CA125, HE4 and TK1 in the diagnosis of ovarian cancer. Eur Rev Med Pharmacol Sci 2017; 21: 4536-4541.
4) QIU JJ, LIN YY, DING JX, FENG WW, JIN HY, HUA KQ. Long non-coding RNA ANRIL predicts poor prognosis and promotes invasion/metastasis in serous ovarian cancer. Int J Oncol 2015; 46: 2497-2505.
5) KUANG D, ZHANG X, HUA S, DONG W, LI Z. Long non-coding RNA TUG1 regulates ovarian cancer proliferation and metastasis via affecting epithelial-mesenchymal transition. Exp Mol Pathol 2016; 101: 267-273.
6) GAO Y, MENG H, LIU S, HU J, ZHANG Y, JIAO T, LIU Y, OU J, WANG D, YAO L, LIU S, HUI N. LncRNA-HOST2 regulates cell biological behaviors in epithelial ovarian cancer through a mechanism involving microRNA let-7b. Hum Mol Genet 2015; 24: 841-852.
7) QIU JJ, LIN YY, YE LC, DING JX, FENG WW, JIN HY, ZHANG Y, LI Q, HUA KQ. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. Gynecol Oncol 2014; 134: 121-128.

8) Huang C, Yu W, Wang Q, Cui H, Wang Y, Zhang L, Han F, Huang T. Increased expression of the lncRNA PVT1 is associated with poor prognosis in pancreatic cancer patients. Minerva Med 2015; 106: 143-149.

9) Yang YR, Zang SZ, Zhong CL, Li YX, Zhao SS, Feng XJ. Increased expression of the lncRNA PVT1 promotes tumorigenesis in non-small cell lung cancer. Int J Clin Exp Pathol 2014; 7: 6929-6935.

10) Zheng X, Hu H, Li S. High expression of lncRNA PVT1 promotes invasion by inducing epithelial-to-mesenchymal transition in esophageal cancer. Oncol Lett 2016; 12: 2357-2362.

11) Iden M, Fye S, Li K, Chowdhury T, Ramchandran R, Rader JS. The lncRNA PVT1 contributes to the cervical cancer phenotype and associates with poor patient prognosis. PLoS One 2016; 11: e156274.

12) Fan H, Zhu JH, Yao XQ. Knockdown of long non-coding RNA PVT1 reverses multidrug resistance in colorectal cancer cells. Mol Med Rep 2018; 17: 8309-8315.

13) Zhang XW, Bu P, Liu L, Zhang XZ, Li J. Overexpression of long non-coding RNA PVT1 in gastric cancer cells promotes the development of multidrug resistance. Biochem Biophys Res Commun 2015; 462: 227-232.

14) Boumahdi S, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, Delatte B, Caauwe A, Siggers S, Nkusi E, Brohee S, Salmon I, Dubois C, Del MV, Fuks F, Beck B, Blanpain C. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. Nature 201[illegible]: 246-25[illegible]

15) Gao H, Teng C, Huang W, Peng [illegible]ang C. SOX2 promotes the epithelial to mes[illegible]al transition of esophageal squamous cells by [illegible]ting slug expression through the a[illegible]tion of [illegible]F-alpha signaling. Int J Mol [illegible]015; 16: 21[illegible]

16) Rassouli FB, Matin M, [illegible]Bahrami R, Ghaffa[illegible]gan K, Sisakhtnezha[illegible]Cheshom[illegible]Abbasi F. SOX2 expression in g[illegible]testin[illegible]ncers o[illegible]anian patients. Int J Biol [illegible]; 30: e[illegible]320.

17) Yang N, Hui [illegible]ng Y, Y[illegible]ang X[illegible]expression of SO[illegible]promotes i[illegible]asion, and epitheli[illegible]nchymal tran[illegible]through the Wnt/b[illegible]pathway in l[illegible]yngeal cancer Hep[illegible]lls. T[illegible]Biol 2014; 35: 7965-7973.

18) Favaro R, Appollon[illegible]gatta S, Sanga AB, Pagel-[illegible]mbini E, Pisati F, [illegible]enghi S, Foti M, Finoc-[illegible]G, Malatesta P, Nic[illegible]lis SK. Sox2 is required to maintain cancer stem cells in a mouse model of high-grade o[illegible]lendroglioma. Cancer Res 2014; 74: 1833-184[illegible]

[illegible]va M, Dom[illegible]G, Iriondo O, Rabano M, Simoes [illegible]Comail[illegible]Barredo I, Lopez-Ruiz JA, Zabalza I, [illegible]o M. Sox2 promotes tamoxifen resistance in breast cancer cells. EMBO Mol Med 2014; 6: 66-79.