Genomics 112 (2020) 4640–4646

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Tissue-based long non-coding RNAs "*PVT1*, *TUG1* and *MEG3*" signature predicts Cisplatin resistance in ovarian Cancer

Nashwa El-Khazragy[a,b,*], Hayam Fathy Mohammed[c], Mohamed Yassin[d], K.K. Elghoneimy[d], Walid Bayoumy[d], Amr Hewety[d], Hekmat M. El Magdoub[e], Wael Elayat[f], Walid Zaki[f], Gehan Safwat[g], Mai Mosa[g], Khouloud Zedan[g], Salema Salem[g], Azzah M. Bannunah[h], Azza Mansy[i]

[a] Clinical Pathology/Hematology & Biomedical Research Departments, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[b] Biomedical Research Department, Global Research Labs, Cairo, Egypt
[c] Department of Obstetrics and Gynecology Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[d] Department Clinical Oncology, Faculty of Medicine, Ain shams University, Cairo, Egypt
[e] Department of Biochemistry, Faculty of Pharmacy, Misr International University, Cairo, Egypt
[f] Department of Medical Biochemistry, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[g] Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA), Cairo, Egypt
[h] Common First year Deanship, Umm Al-Qura University, Makkah, Saudi Arabia
[i] Department of Clinical Pharmacy, Faculty of Pharmacy, Fayoum University, Egypt

ARTICLE INFO

Keywords:
Ovarian cancer
Long non-coding RNA
Cisplatin resistance
Lnc-PVT1
Lnc-TUG1
Lnc-MEG3

ABSTRACT

Objectives: The current study aimed to investigate the potentiality of three lncRNAs "Plasmacytoma variant translocation 1(*lnc-PVT1*), Taurine upregulated gene type 1(*lnc-TUG1*) and Maternally expressed gene 3 (*lnc-MEG-3*)", to predict Cisplatin resistance in ovarian cancer (OC), in addition, to access their prognostic significance.

Methods: The expression level of lncRNAs were measured in 100 formalin-fixed paraffin-embedded tissue (FFET) samples of OC patients who were treated by Cisplatin-based chemotherapy using qPCR.

Results: The results showed that *lnc_PVT1* was significantly upregulated by 2.3 folds in Cisplatin resistant tissues, while, *lnc-TUG1* and *lnc-MEG3* were downregulated by 1.2 and 3 folds, respectively. In addition, the three lncRNAs exhibited high sensitivity and specificity in predicting chemo-resistance and they were negatively associated with OS and progression-free survival ($p < 0.001$).

Conclusion: The *lnc-PVT1*, *lnc-TUG1*, and *lnc-MEG3* transcriptome signatures could be used for predicting resistance to Cisplatin in OC patients.

1. Introduction

Cisplatin is the key cornerstone chemotherapeutic drug in ovarian cancer (OC) [1], however, chemo-resistance in cancers has contributed to the limitation of its efficacy as anti-cancer therapy [2]. It induces their anti-cancer effect by damaging DNA, which downstream affects cell apoptosis, cell cycle, and DNA synthesis and repair [3] by the formation of DNA adducts that cause cross-linking and steric changes in the DNA, making the cells incompatible with life [1,4]. However, there is an increase in the incidence of resistance to cisplatin, which is associated with worse prognosis [5,6]. Multi molecular mechanisms have been involved in the resistance towards cisplatin [1,7]. It includes: secondary mutations especially in *BRCA1/2* [8], *NF1*, *RAD51B* and *PTEN* [1,7]. Advances in sequencing technology emerge the implication of several species of non-coding RNAs such as *lncRNAs*, *miRNAs* and circular *RNAs* in the development of resistance [3,9]. Furthermore, they can cause invasiveness and resistance to chemotherapy [10].

LncRNA Taurine Upregulated Gene 1 (*lnc-TUG1*), located at chromosome 22q12 [11]. A study of the role of *TUG1* in ovarian cancer revealed that it is upregulated and has an anti-apoptotic function [2,12]. Moreover, the knockdown of *TUG1* resulted in apoptosis of the tumor cells, which is of therapeutic significance [2]. Another studied *lncRNA* is Plasmacytoma Variant Translocation 1 (*PVT1*). It is located in 8q24 'gene desert', which is a well-known region for the oncogenes

* Corresponding author.: Clinical Pathology/Hematology & Biomedical Research Departments, Faculty of Medicine, Ain Shams University, Abbasia, Cairo 11566, Egypt Biomedical Research Department, Global Research Labs, Nasr City, Cairo 11528, Egypt, nashwaelkhazragy@med.asu.edu.eg, nashwa@globalresearchlabs.com.
E-mail address: nashwaelkhazragy@med.asu.edu.eg (N. El-Khazragy).

https://doi.org/10.1016/j.ygeno.2020.08.005
Received 27 June 2020; Received in revised form 22 July 2020; Accepted 4 August 2020
Available online 08 August 2020
0888-7543/

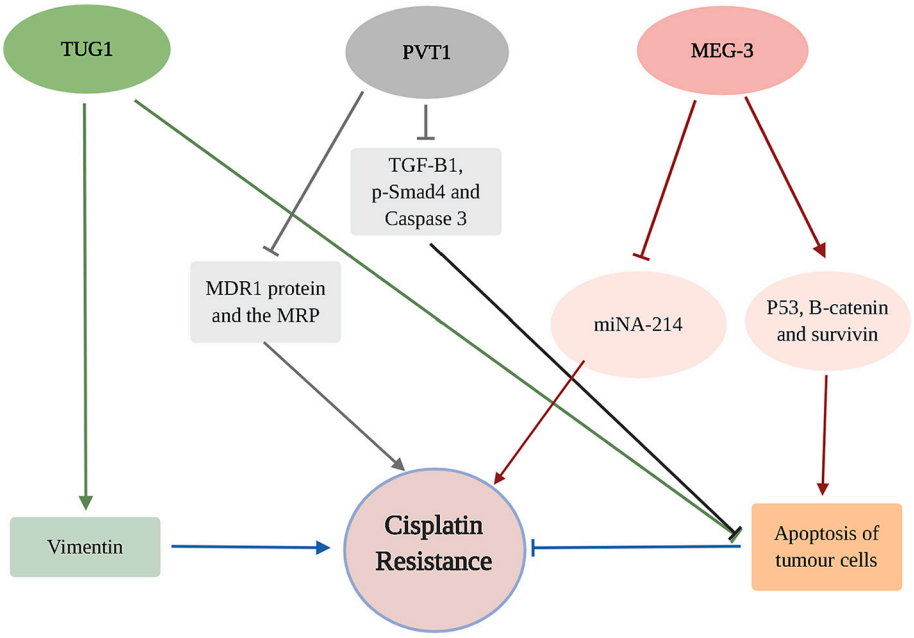

Fig. 1. The mechanism of Cisplatin resistance in ovarian cancer.

[11,13]. A study in ovarian cancer found that it causes cisplatin resistance via inhibition of apoptosis. It was found that it is upregulated in resistant cases and caused decreased expression of *TGF-B1*, which was found to decrease cell growth as well as *caspase-3*, thus, inhibiting apoptosis [14]. On the other hand, Maternally Expressed Gene 3 (*MEG3*), which is present at 14q32 acts by a different mechanism than the previously mentioned *lncRNAs* [10]. *MEG3* acts through multiple mechanisms, which enhance cisplatin sensitivity through targeting miRNA 214 that enhances cisplatin resistance through extracellular vesicles [10]. The role of *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* in Cisplatin resistance in cancer ovary is illustrated in Fig. 1.

Based on the aforementioned observations, it became mandatory to understand the gene expression of these markers and validate them in order to stand on the right treatment for each patient. Based on the previous findings, a recent study referred that lncRNAs can be used as therapeutic targets in ovarian cancer [15]. Therefore, we conducted the current study to investigate the potential value of three *lnc_RNAs* to predict Cisplatin resistance, based on their expression in tissues, in addition, to assess the prognostic value of these markers as regards the overall and progression-free survival in a set of ovarian cancer patients.

2. Materials & methods

2.1. Study population

This retrospective cohort study was conducted among patients with ovarian cancer (the main cohort) and another healthy ovarian tissue represents the control group. A total of 100 ovarian cancer specimens and 30 normal ovarian specimens were collected from the Department of Obstetrics and Gynecology, Ain Shams University Hospitals. The paraffin -embedded tissue for ovarian cancer patient who are eligible for the study, and the healthy ovarian tissue of age matched women was collected from patients who undergo adenexectomy due to uterine prolapse or myoma. Using G program, the sample size was adjusted after setting the alpha error at 5% and power at 80%, the effect size was assumed to 0.8 (Cohen 'sf) between the two groups with taking in consideration 20% dropout rate.

Patients with ovarian cancer, treated with platinum-based chemotherapy in our study setting, along with complete medical records and tissue samples were considered eligible for participation in our study. The enrolled patients were treated with intravenous platinum-based chemotherapy either in the form of "cisplatin or carboplatin" for three to four cycles.

A total of a hundred formalin-fixed, paraffin-embedded (FFPE) ovarian tissue samples of the recruited patients were collected and reviewed. As specimens were retrospectively collected, patients were categorized based on their response to the standard therapeutic regimen of cisplatin-based chemotherapy into two arms: the cisplatin-sensitive arm (n = 68) and the cisplatin-resistant arm (n = 32). Another thirty non-cancerous patients with benign ovarian lesions were included as the control group. Ovarian cancer specimens were categorized according to the clinical and the histopathological features into standard and high-risk groups.

Prior to recruiting the patients, the study protocol was approved by the Institutional Review Board (IRB) Ethics Committee of Ain shams University. Furthermore, this study was conducted in accordance with the World Medical Association Declaration of Helsinki [16]. Written informed consent was obtained from all participants prior; we assured all participants that their information along with their data and medical records remained confidential. A unique identification number was assigned to each patient at the time of enrolment.

2.2. Bioinformatics

We searched for lncRNAs that are highly linked to cisplatin-resistance in cancer. Accordingly, target gene modulation, and drug pharmacogenomics or chemo-resistance pathways were identified using different databases such as NRDT [17] and Pharmaco-miR [18]. With the intention to select three lncRNAs, which are related to cisplatin-resistance and interlink with each other at the same time, we collected all the available information regarding lncRNAs-target gene-drugs. The resultant data were huge and extremely difficult to interpret in a database. Therefore, we decided to study the RNA-drug interactions using a network-based approach. Basically, we searched PubMed/NCBI (National Centre for Biotechnology Information) for all of the recent studies (published from 2015 till the time of conduction of this study) that investigated the lncRNAs involved in drug chemo-resistance.

Based on the aforementioned data, we then built a network of three long non-coding RNAs that are linked to platinum analogues-resistance while interlinking with each other by alternating pathways. We

obtained a fully connected network of three drug/ncRNA interactions, named *lnc-PVT1*, *lnc-TUG1*, and *lnc-MEG3*. A graph theory measures were considered to define the interlink between non-coding RNAs and cisplatin therapeutics' resistance. The selected lncRNAs pathways in the network were based on closeness centrality and shortest path. Eventually, we performed a structural analysis using a cystoscope to identify different clusters of ncRNAs and drugs.

2.3. Total RNA purification and reverse transcription from FFPE tissue sections

Deparaffinization of FFPE tissues was performed using the deparaffinization solution (Cat no: 19093). Then, the total RNA was extracted using the RNeasy FFPE Kit (cat no: 73504). The procedure was conducted according to the manufacturer's protocol (Qiagen, Hilden, Germany). Extracted RNA purity was assessed by measuring the optic density at 260 and 280 nm using an Ultraviolet (UV) spectrophotometer (Eppendorf, Germany). The total RNA was reverse transcribed in a total volume of 20 μl using the QuantiTect RT kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The reaction protocol was adjusted at 37 °C for 60 min, then at 95 °C for 5 min.

2.4. LncRNAs expression analysis by real-time PCR

The cDNA was amplified using specific primers sequences for the *PVT1*, *TUG1*, and *MEG3*. The used primer sequences are [Hs_*PVT1*; ID: LPH17013A, Hs_*TUG1* ID: LPH18394A, and Hs_*MEG3*; ID: LPH02974A] RT2 primer assays cat no: 330701; (Qiagen, Hilden, Germany). The Hs_*GAPDH*_1_SG QuantiTect primer assay (*GAPDH*) cat no: 249900, ID: QT00079247 was used as a housekeeper gene. The reaction mix and cycling protocols were adjusted according to the manufacturer's instruction using the 5 Plex Rotor-Gene PCR Analyzer (Qiagen, Hilden, Germany). The average expression level of all markers was also used to perform data normalization. The relative gene expression (RQ) was determined using the comparative $2^{-\Delta\Delta Ct}$ method.

2.5. Statistical analysis

The data were presented as mean ± standard deviation for normally-distributed continuous variables, and as the median and interquartile range (IQR) for not normally-distributed variables. Categorical variables were presented as frequencies and percentages. We used the *Mann Whitney U* test for non-parametric comparisons between the two groups. We used the *Kaplan Meier* Survival analysis for comparison of survival between the analyzed groups. The cut-off values were determined using the median level of long non-coding RNAs. Receiver operator curves were used to evaluate the prognostic accuracy. The survival between different groups was compared using the log-rank test. All of the statistical analyses were performed using the Statistical Package for Social Science (*SPSS-IBM, Version 23*). A *p*-value of < 0.05 was considered the cut-off point for statistical significance. All the presented graphs are plotted by *GraphPad Prism version 8.0*.

3. Results

3.1. Patients' characteristics

In this study, we have included a total of hundred ovarian cancer cases with a mean age of 47.7 ± 9.1), range: 27–59 years. Baseline patients' characteristics are presented in Table 1. Ovarian cancer patients were then divided into two groups based on their response to cisplatin: cisplatin-sensitive group ($n = 68$) and cisplatin-resistant group ($n = 32$). Endometrioid carcinoma was detected only among sensitive groups (44%). Meanwhile, approximately 50% of cases in both groups were of high grade tumors (III-IV). Surprisingly, the majority of Cisplatin resistant patients (41%) developed local relapse, and 100% of patients who relapsed at distant sites belong to the Cisplatin resistant group (Table 1).

Table 1
Demographic characteristics of the ovarian cancer patients.

Variable	Cisplatin sensitive $n = 68$	Cisplatin Resistant $n = 32$	Statistics
Age (years)	mean ± SD: 50.0 ± 11.5 range: 17 _ 66	mean ± SD:46 ± 5.0 range: 38 _ 59	χ^2:0.5 $p = 0.477$
CEA subgroups			
≤100 (U/ml)	42(62)	13(41)	χ^2 : 3.9
> 100 (U/ml)	26(38)	19(59)	$p = 0.05$
CA125 subgroups			
≤280 (U/ml)	9(13)	0	χ^2: 4.7
> 280 (U/ml)	59(89)	32(100)	$p = 0.477$
Pathological type			
Adenocarcinoma	16(24)	8(25)	χ^2: 26.3
Serous	14(21)	9(28)	$p = 0.001$
Endometroid	30(44)	0	
Others[Papillary, Mucinous]	8(12)	15(47)	
Grade			
I-II	28(41)	27(84)	χ^2: 16.4
III-IV	40(59)	5(16)	$p = 0.001$
Local Metastases			
No	59(87)	19(59)	χ^2: 9.5
Yes	9(13)	13(41)	$p = 0.002$
Distant Metastases			
No	68(100)	8(25)	χ^2: 67.0
Yes	0	24(75)	$p = 0.001$

SD = standard deviation; n (%) = number (percentage), CEA = carcinoembryonic antigen; CA-125 = Cancer antigen 125

3.2. Tissue expression levels of lnc-PVT1, lnc-TUG1 and lnc-MEG3 in ovarian cancer

We noted a significant increase in the expression levels of *lnc-PVT1* (Fig. 2a), meanwhile, the *lnc-TUG1* (Fig. 2b), and *lnc-MEG3* (Fig. 2c) were downregulated in cases of OC compared to healthy controls ($p < 0.01$) (Table 2). Upon comparing the levels of *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* between the cisplatin-sensitive and cisplatin-resistant arms, we noted a significant increase in the log level of *lnc-PVT1* by 2.3 folds in resistant cases compared to sensitive cases (Median = 49 vs 21; $p = 0.001$) (Fig. 2e).On the other hand, a lower median expression levels of the *lnc-TUG1*(Fig. 2f) and *lnc-MEG3* (Fig. 2g) were significantly associated with Cisplatin resistant ovarian tissues ($p < 0.05$).

3.3. Determinants of lncRNAs (PVT1, TUG1, and MEG3) levels in ovarian cancer tissue

Our analysis revealed that the expression level of lncRNAs differed based on a certain set of variables (Table 3). The *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* expression in OC tissues were significantly varied based on histopathological grade and metastatic tumors, in this regard, the median expression level of *lnc-PVT1* was significantly higher in higher grade tumors (III-IV) (median = 38) and metastatic tumors (median = 48), $p < 0.001$. Meanwhile, lower expression levels of the *lnc-TUG1* and *lnc-MEG3* were significantly detected in these groups. In addition, no significant determinants of the three *lncRNAs* expression were significantly associated with tumor histopathological type, serum CEA and CA125 levels subgroups ($p > 0.05$) (Table 3).

3.4. Relationship between cisplatin-resistance and lncRNAs (PVT1, TUG1, and MEG3) in ovarian cancer

Based on our analysis, we found that *lnc-PVT1*, *lnc-TUG1*, and *lnc-MEG3* had accurately predicted the resistance to cisplatin (Fig. 2h). The

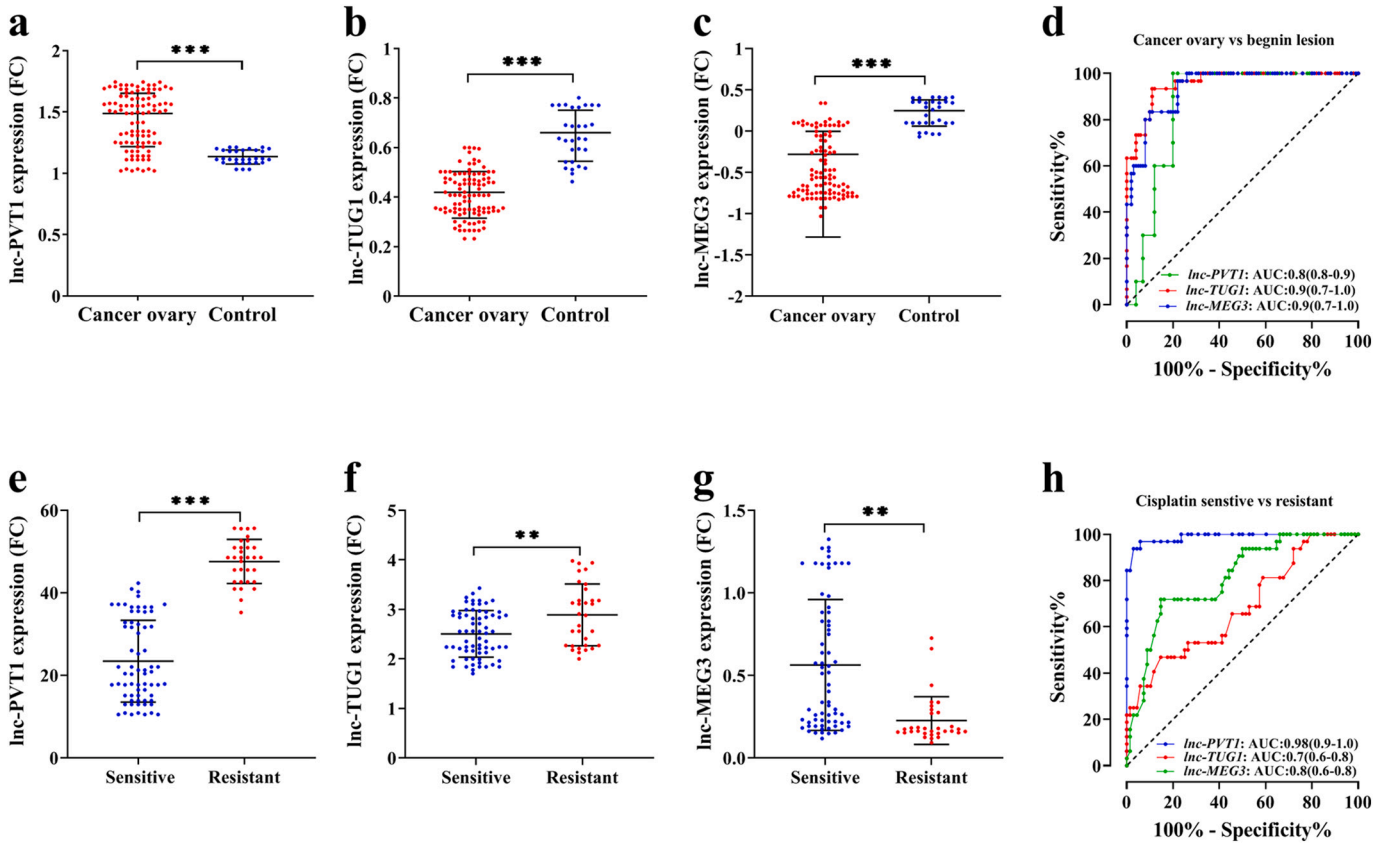

Fig. 2. Boxplot graphs demonstrating a significant difference for the expression of *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* ($p < 0.001$), (a): higher expression levels of *lnc-PVT1* was detected in ovarian cancer tissue, whereas; the *lnc-TUG1* (b) and *lnc-MEG3* (c) were downregulated, when compared with healthy lesions. (d): a ROC curve analysis illustrated the diagnostic potential of *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* in discriminating ovarian cancer from healthy tissue, at a calculated cut-off value > 16, the calculated sensitivities and specificities for *lnc-PVT1* are 100% and 80%, respectively, for *lnc-TUG1*, the biomarker sensitivity was 80% and 92% specificity at a cut-off value < 3.4. A biomarker sensitivity and specificity of 80% and 90% were detected for the lnc-*MEG3* expression at cut-off value < 1.3. The AUC and 95% CI for each *lncRNA* is presented on the figure. Similarly, significant association was detected for the expression of *lnc-PVT1* (e), *lnc-TUG1* (f) and *lnc-MEG3* (g) between Cisplatin sensitive and resistant ovarian cancer tissue ($p < 0.01$). (***): $p < 0.001$, (**): $p < 0.01$, (*): $p < 0.05$. Furthermore, the predictive potential of the three *lncRNAs* to predict resistance to Cisplatin in ovarian cancer are presented in Fig. 2h, the calculated cut-off values at which the biomarker could predict Cisplatin resistance is > 40 for *lnc-PVT1*, < 2.8 for *lnc-TUG1* and < 0.2 for *lnc-MEG3*, the AUC and 95% CI for each biomarker is presented on the figure. *Lnc-PVT1*: long noncoding RNA Plasmacytoma variant translocation 1, *lnc-TUG1*: long noncoding RNA Taurine upregulated gene type 1, *lnc-MEG-3:* long noncoding RNA Maternally expressed gene 3, AUC: area under the curve, CI: confidence interval, ROC: Receiving operating characteristics.

accurate cut-off value was determined based on ROC, which was > 40 for *lnc-PVT1*, < 2.8 for *lnc-TUG1*, and < 0.2 for *lnc-MEG3* (Supplementary Table S2). The calculated biomarkers sensitivities and specificities were [(*lnc-PVT1*: 94% and 96%, $p < 0.001$), (*lnc-TUG1*: 53% and 74%, $p < 0.003$) and (*lnc-MEG3*: 72% and 84%, $p < 0.002$)]; respectively. Furthermore, a ROC analysis revealed significant diagnostic potential for the three *lncRNAs* to discriminate cancer ovary from healthy tissue (p < 0.001) Fig. 2d and Supplementary Table S1.

3.5. Lnc-PVT1, lnc-TUG1 and lnc-MEG3 tissue expression values predict survival outcome ovarian cancer patients treated by cisplatin-based chemotherapy

We conduct a survival analysis, and the Kaplan Meier graph was plotted. The OC patients were grouped according to a calculated cut-off value which represents the median values of the *lncRNA* expression in ovarian cancer tissues. The expression levels of *lncRNAs* in ovarian cancer tissue above and below the median value represent the

Table 2
Comparative analysis between different studied groups for the *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* in different studied groups.

Variable	Studied groups (cancer ovary vs control)			Cisplatin sensitivity (sensitive vs resistant)		
	Median (range)		Statistics	Median (range)		Statistics
	Ovarian cancer $n = 100$	Heathy control $n = 30$		Cisplatin sensitive $n = 68$	Cisplatin resistant $n = 32$	
***lnc-PVT1*(log$_{10}$)**	32.0(11 _ 57)	13.0(11 _ 16)	*U*: 402, $p = 0.001$*	21(11 _ 42)	49(35 _ 56)	*U*:24, $p = 0.001$*
***lnc-TUG1*log$_{10}$)**	2.6(1.7 _4.0)	4.0(3.0 _ 6.0)	*U*; 124, $p = 0.001$*	2.5(1.7 _ 3.4)	2.9(2.0 _ 4.0)	*U*:688, $p = 0.005$*
***lnc-MEG3* (log$_{10}$)**	0.2(0.1 _ 2.2)	2.3(0.9 _ 2.6)	*U*:183, $p = 0.001$*	0.2(0.1 _ 1.0)	0.6(0.13 _ 1.33)	*U*:99, $p = 0.002$*

U = Mann-Whitney test value;* = test is significant at level < 0.01;, *lnc-PVT1*: long noncoding RNA plasmacytoma variant translocation 1, *lnc-TUG1*: long noncoding RNA Taurine upregulated gene type 1, *lnc-MEG-3*: long noncoding RNA Maternally expressed gene 3.

Table 3
Comparative analysis between different Ovarian Cancer risk groups for the tissue expression levels of *lnc-PVT1*, *lnc-TUG1*and *lnc_MEG3* genes.

Variable	*lnc-PVT1* [log_{10}]		*lnc-TUG1* [log_{10}]		*lnc-MEG3* [log_{10}]	
	Median range	*P* value	Median range	*P* value	Median range	*P* value
CEA subgroups						
≤100(U/ml)	32(10 _ 56)	0.229	2.6(1.7_ 4.0)	0.221	0.3(0.1 _ 2.2)	0.411
> 100(U/ml)	37(11_56)		2.4(1.7_3.4)		0.2(0.09 _2.2)	
CA_125 subgroups						
≤280 (U/ml)	32(11 _ 36)	0.430	2.6(1.8_ 3.0)	0.217	0.3(0.1 _ 2.2)	0.369
> 280 (U/ml)	33(10 _ 56)		2.4(1.7 _ 2.8)		0.2(0.09 _ 2.2)	
Pathological type						
Adenocarcinoma	36 (11 _ 52)	0.821	2.2(1.7 _ 4.0)	0.852	0.4 (0.1 _ 2.4)	0.715
Serous	38 (10–56)		2.4 (1.8–4.0)		0.3 (0.1–2.6)	
Endometroid	33 (10–42)		2.6 (1.7–3.2)		0.2 (0.1–2.3)	
Others	37 (12–52)		2.2 (2.0–3.5)		0.2 (0.2–2.4)	
Histopathological Grade						
I - II	22 (10 _ 46)	0.001	2.6 (1.8 _ 4.0)	0.032	0.3 (0.2 _ 2.2)	0.042
III - IV	38 (10 _ 49)		2.0 (1.7 _ 3.0)		0.2(0.09 _ 2.0)	
Distant metastases						
No	22(10 _ 52)	0.001	2.7 (1.8 _ 4.0)	0.002	0.3 (0.2 _ 2.2)	0.036
Yes	48(35 _ 56)		1.6 (1.2 _ 2.8)		0.1 (0.1 _ 2.0)	

PVT1: Plasmacytoma Variant Transcription1gene; *TUG*: Taurine upregulated gene 1; *MEG3*: Maternity expressed gene 3, CEA: Carcinoembryonic antigen; CA-125: Cancer antigen 125

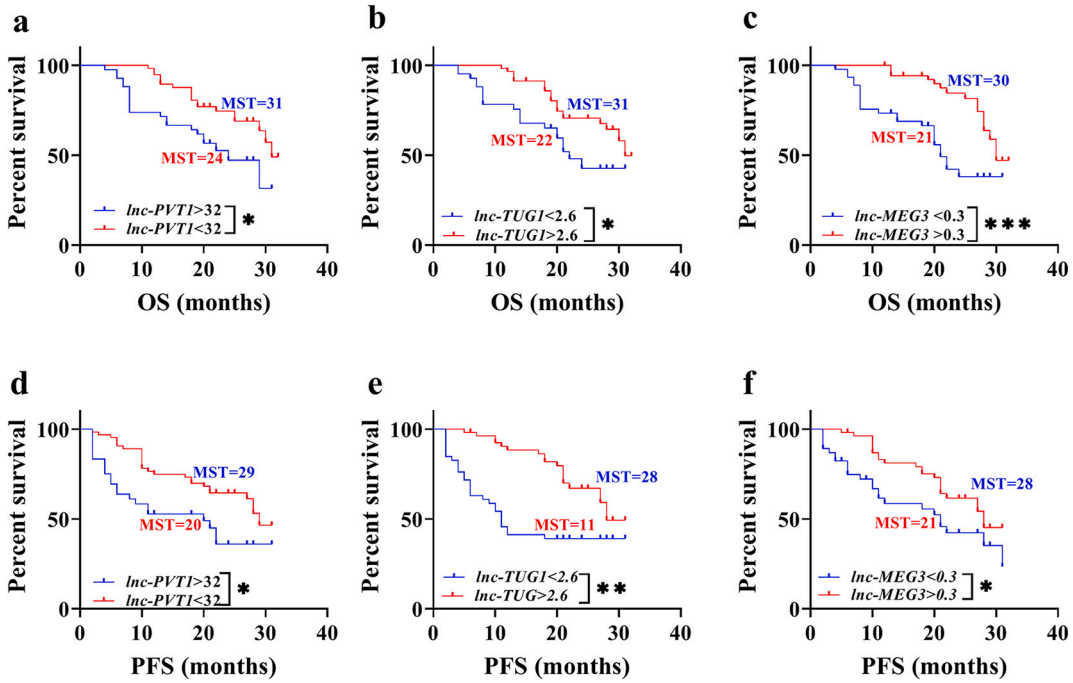

Fig. 3. A Kaplan Meier survival curves for OS and PFS in the validation cohort based on the level of expression of *lncRNAs* in ovarian cancer tissue, higher expression levels of *lnc-PVT1* > 32 is significantly associated with short OS, $p = 0.001$ (Fig. 3a) and PFS, $p = 0.01$ (Fig. 3d). For *lnc-TUG1* (Fig. 3b and Fig. 3e) and *lnc-MEG3* (Fig. 3c and Fig. 3f), a tissue expression levels < 2.6 and < 0.3, respectively are associated with poor OS and PFS probability scores. OS: overall survival, PFS: progression free survival, (***): p < 0.001, (**): p < 0.01, (*): p < 0.05.

comparing factor. Higher expression levels than the aforementioned cut-off values resulted in significantly poorer overall survival as regards *lnc-PVT1* (24 vs 31 months, $p = 0.001$) (Fig. 3a). Meanwhile, lower expression values for *lnc-TUG1* (MST: 22 vs 31, $p = 0.02$) and *lnc-MEG3* in ovarian cancer tissues (MST: 21 vs 30, $p = 0.004$) are significantly associated with poor survival in cisplatin-treated cases. (Table 4), the optimum cut-off value was > 32, < 2.6 and < 0.3 for *lnc-PVT1(*Figure 3a), *lnc-TUG1* (Fig. 3b) and *lnc-MEG3* (Fig. 3c), respectively.

As regards progression-free survival (PFS), the *lnc-TUG1* showed the highest prognostic potential and prediction to disease outcome in ovarian cancer tissues (Fig. 3d-3f), the median PFS was 11 vs 28 months in patients who express *lnc-TUG1* below the cut-off value of 2.6 compared with those > 2.6, p = 0.001 (Table 4, Fig. 3e). Similar finding was detected for *lnc-MEG3* expression < 0.3 (median PFS: 21 vs 28, $p = 0.03$) (Table 4, Fig. 3f). On the other hand, higher expression levels of *lnc-PVT1* than the reported cut-off (32) values resulted in significantly poorer PFS (20 vs 29) months; $p = 0.01$) (Table 4, Fig. 3d).

4. Discussion

We conducted the current study design to investigate the potential of three lncRNAs "*PVT1*, *TUG1*, and *MEG3*" in prediction for Cisplatin resistance in OC patients. Although, all the conducted studies were concerned to the mechanistic pathway and conducted experimentally

Table 4
Predictive and prognostic value of "*lnc_PVT1*, *lnc_TUG1* and *lnc_MEG3*" for disease outcome in Ovarian Cancer patients treated by Cisplatin based therapy (Log Mantel test).

Parameter	Overall survival (months)			Progression Free Survival (PFS) (months)		
	MST	X^2 (95% CI)	*P* value	MST	X^2 (95% CI)	*P* value
***lnc_PVT1*(log_{10})**						
Low(≤32)	31.0	6.0(0.2 _ 0.9)	0.001	29.0	6.6(0.3 _ 0.9)	0.01
High (> 32)	24.0			20.0		
***lnc_TUG1*(log_{10})**						
High (> 2.6)	31.0	5.0(0.3 _ 1.0)	0.02	28.0	11(0.2 _ 0.7)	0.001
Low (< 2.6)	22.0			11.0		
***lnc_MEG3*(log_{10})**						
High (> 0.3)	30.0	12.5(0.2 _ 0.7)	0.004	28	4.7(0.3 _ 1.0)	0.03
High (≤ 0.3)	21.0			21		

MST: median survival time, X^2: Chi-square, CI: confidence interval.

by *in vitro* and *in vivo* studies, up to date, no litreatures have demonstrated the prediction potential of these lncRNAs on clinical samples. Based on the current analysis, the overexpression of *lnc-PVT1* and downregulation of *lnc-TUG1* and *lnc-MEG3*, in ovarian cancer tissues, are significantly associated with Cisplatin resistance and advanced histopathological grade. Furthermore, In terms of overall survival, we noted that higher expression levels (above the cut-off points) of *lnc-PVT1* were correlated with poorer OS and PFS. In contrast, the lower expression levels below the calculated cut-off value of *lnc-TUG1* and *lnc-MEG3* expression levels are significantly associated with the worse patient's survival.

In the previous literatures, there were no reports regarding the prevalence of cisplatin-resistant ovarian tumors. However, the worse prognosis and high rate of tumor recurrence in these cases caught the attention of several researchers to comprehend the causes of this problem [11,19]. The overexpression of *lnc-PVT1* in the tumor cells was evident in many tumors not only in ovarian cancer [13], as it is considered as an oncogene [20]. A study in ovarian cell tumors found that this *lncRNA* enhanced tumor progression and invasion [20,21]. Moreover, it caused cisplatin resistance through inhibition of apoptosis [22,23]. In agreement with our results, previous studies have reported that the level of *lnc-PVT* is correlated with tumor staging and invasion [23], and thus, it can be used as a prognostic biomarker in human cancers [21,24]. Moreover, it was found to be associated with shorter overall survival and progression-free survival in epithelial ovarian carcinoma, which is inconsistent with our results [20]. This goes in line with the findings of other studies [25]. Furthermore, it was noted that *lnc-PVT1* could predict the relapse in ovarian cancer, where patients could be categorized into three risk groups: high, mixed, and low risk [20].

As regards the *lnc-TUG1*, it was also noted that it promotes cell proliferation and invasion in ovarian cancer by affecting the epithelial-mesenchymal transition, and it promotes proliferation and inhibits cells apoptosis through regulating *AURKA* in epithelial ovarian cancer cells [12,26]. Moreover, it was found that it mediated the resistance to cisplatin [27], in non-small cell lung cancer [28] and in oesophageal squamous cell carcinoma [29], which is consistent with our study. However, research on the correlation between the expression of this *lncRNA* and ovarian cancer survival in the literature is very limited. Based on its mechanism in inducing cell proliferation and resistance, our results seem consistent with the reported mechanisms as the high *lnc-TUG1* levels were associated with better survival. In other tumors, the downregulation of *lnc-TUG1* was associated with shorter survival and cisplatin resistance through its effect on *EZH2* and apoptotic pathways [26,29–31].

In the present study, the downregulation of *lnc-MEG3* in ovarian cancer tissue was associated with poor survival in our study which is consistent with the reported mechanisms in literature [32,33]. The *lnc-MEG3* was used as a target in ovarian cancer for decreasing cisplatin resistance, it contributes to cisplatin-induced apoptosis via inhibition of autophagy in human glioma cells [32]. In lung cancer cells, it was found that downregulation of *lnc-MEG3* enhances cisplatin resistance through activation of the *WNT/β*-catenin signalling pathway [34], moreover, it was found to be significantly correlated with chemo-sensitive response This goes in line with our findings as we found significantly lower expression levels of *lnc-MEG3* in cisplatin-resistant cases compared to sensitive ones. In contrast, the expression level of *lnc-MEG3* was upregulated in another study, it upregulated *p53*, *β-catenin*, and *survivin*, causing apoptosis of tumor cells, and thus, enhancing survival [35], and it inhibits cell migration and invasion and enhances cisplatin chemosensitivity in bladder cancer cells [36]. The conflict and contradiction of the expression and behave of *lnc-MEG3* in cancers, supports the fact that it not being established at the present as a tumor marker in ovarian carcinoma [34], however, further researches are needed to confirm its prognostic value in ovarian cancer.

5. Conclusion

Based on our results and proposed mechanisms in the literature, the tissue expression levels of *lnc-PVT1*, *lnc-TUG1* and *lnc-MEG3* can be used as biomarkers to predict cisplatin-resistance in ovarian cancer. Their accuracy as predictors for resistance and survival is significant. However, the limitation of this study was linked to small sample size, therefore, more studies are warranted to thoroughly investigate the clinical value of the three *lncRNAs* on a large scale of ovarian cancer samples with attention to baseline patients' characteristics and tumor status.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions.

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MY, EKK, HM, WB and AH. The first draft of the manuscript was written by NE and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

The research was self -funded by the authors.

Declaration of Competing Interest

All Authors declare that they have no conflict of interest.

ORCID No: 0000–0001–6646-4674.

References

[1] S. Dasari, Tchounwou PBJEjop, Cisplatin in cancer therapy: molecular mechanisms of action, Eur. J. Pharmacol. 740 (2014) 364–378, https://doi.org/10.1016/j.ejphar.2014.07.025.

[2] E.L. Christie, D.D.L. Bowtell, Acquired chemotherapy resistance in ovarian cancer, Ann. Oncol. 28 (2017) 13–15, https://doi.org/10.1093/annonc/mdx446.

[3] C. Abildgaard, L.M. Do Canto, K.D. Steffensen, S.R. Rogatto, Long non-coding RNAs involved in resistance to chemotherapy in ovarian Cancer, Front. Oncol. 9 (2020), https://doi.org/10.3389/fonc.2019.01549.

[4] T. Thigpen, M.F. Brady, G.A. Omura, W.T. Creasman, W.P. Mcguire, W.J. Hoskins, et al., Age as a prognostic factor in ovarian carcinoma: the gynecologic oncology group experience, Cancer. 71 (1993) 606–614, https://doi.org/10.1002/cncr.2820710218 (S2.

[5] D.W. Cramer, WrJJotNCI Welch, Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis, J. Natl. Cancer Inst. 71 (4) (1983) 717–721, https://doi.org/10.1093/jnci/71.4.717.

[6] W.J. Hoskins, W.P. McGuire, M.F. Brady, H.D. Homesley, W.T. Creasman, M. Berman, et al., The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma, Am. J. Obstet. Gynecol. 170 (4) (1994) 974–980, https://doi.org/10.1016/s0002-9378(94)70090-7.

[7] N. Abu, K.W. Hon, S. Jeyaraman, R.J.F.O. Jamal, Long noncoding RNAs as biotargets in cisplatin-based drug resistance, Future Oncol. 14 (29) (2018) 3085–3095, https://doi.org/10.2217/fon-2018-0303.

[8] J.J.V.A. Prat, Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features, Virchows Arch. 460 (3) (2012) 237–249, https://doi.org/10.1007/s00428-012-1203-5.

[9] A. Wambecke, M. Ahmad, B. Lambert, F. Joly, L. Poulain, C. Denoyelle, et al., The influence of long non-coding RNAs on the response to chemotherapy in ovarian cancer, Gynecol. Oncol. 156 (3) (2020) 726–733, https://doi.org/10.1016/j.ygyno.2019.12.020.

[10] L.P. Martin, T.C. Hamilton, Schilder RJJCcr, Platinum resistance: the role of DNA repair pathways, Clin. Cancer Res. 14 (5) (2008) 1291–1295, https://doi.org/10.1158/1078-0432.CCR-07-2238.

[11] R.J. Parker, A. Eastman, F. Bostick-Bruton, Reed EJTJoci, Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation, J. Clin. Investig. 87 (3) (1991) 772–777, https://doi.org/10.1172/jci115080.

[12] D. Kuang, X. Zhang, S. Hua, W. Dong, Z.J.E. Li, Pathology m. long non-coding RNA TUG1 regulates ovarian cancer proliferation and metastasis via affecting epithelial-mesenchymal transition, Exp. Mol. Pathol. 101 (2) (2016) 267–273, https://doi.org/10.1016/j.yexmp.2016.09.008.

[13] M. Cui, L. You, X. Ren, W. Zhao, Q. Liao, Y.J.B. Zhao, et al., Long non-coding RNA PVT1 and cancer, Biochem. Biophys. Res. Commun. 471 (1) (2016) 10–14, https://doi.org/10.1016/j.bbrc.2015.12.101.

[14] A.M. Patch, E.L. Christie, D. Etemadmoghadam, D.W. Garsed, J. George, S. Fereday, et al., Whole-genome characterization of chemoresistant ovarian cancer (vol 521, pg 489, 2015), Nature. 527 (7578) (2015) 398, https://doi.org/10.1038/nature15716.

[15] J. Song, W.F. Zhang, S. Wang, K. Liu, F.Z. Song, Ran L. a panel of 7 prognosis-related long non-coding RNAs to improve platinum-based chemoresistance prediction in ovarian cancer, Int. J. Oncol. 53 (2) (2018) 866–876, https://doi.org/10.3892/ijo.2018.4403.

[16] H.J.R.J. Declaration, World Medical Association Declaration of Helsinki, 2014 (2014), p. 22.

[17] X. Chen, Y.-Z. Sun, D.-H. Zhang, J.-Q. Li, G.-Y. Yan, J.-Y. An, et al., NRDTD: a database for clinically or experimentally supported non-coding RNAs and drug targets associations, Database (Oxford). 2017 (2017), https://doi.org/10.1093/database/bax057.

[18] J.L. Rukov, R. Wilentzik, I. Jaffe, J. Vinther, Shomron NJBib, Pharmaco-miR: linking microRNAs and drug effects, Brief. Bioinform. 15 (4) (2014) 648–659, https://doi.org/10.1093/bib/bbs082.

[19] H. Itamochi, J. Kigawa, R. Akeshima, S. Sato, S. Kamazawa, M. Takahashi, et al., Mechanisms of cisplatin resistance in clear cell carcinoma of the ovary, Oncology. 62 (4) (2002) 349–353, https://doi.org/10.1159/000065067.

[20] Y. Guan, W.-L. Kuo, J.L. Stilwell, H. Takano, A.V. Lapuk, J. Fridlyand, et al., Amplification of PVT1 contributes to the pathophysiology of ovarian and breast cancer, Clin. Cancer Res. 13 (19) (2007) 5745–5755, https://doi.org/10.1158/1078-0432.CCR-06-2882.

[21] R.-q. He, Qin M-j, P. Lin, Y.-H. Luo, J. Ma, H. Yang, et al., Prognostic significance of LncRNA PVT1 and its potential target gene network in human cancers: a comprehensive inquiry based upon 21 cancer types and 9972 cases, Cell. Physiol. Biochem. 46 (2) (2018) 591–608, https://doi.org/10.1159/000488627.

[22] E. Liu, Z. Liu, Y. Zhou, R. Mi, Wang DJIjoc, Medicine e. overexpression of long non-coding RNA PVT1 in ovarian cancer cells promotes cisplatin resistance by regulating apoptotic pathways, Int. J. Clin. Exp. Med. 8 (11) (2015) 20565.

[23] F. Wang, X. Ji, J.J. Wang, X.R. Ma, Y. Yang, J.H. Zuo, et al., LncRNA PVT1 enhances proliferation and Cisplatin resistance via regulating miR-194-5p/HIF1a Axis in Oral squamous cell carcinoma, Oncotargets Ther. 13 (2020) 243–252, https://doi.org/10.2147/ott.S232405.

[24] Q. Yang, Y. Yu, Z.Q. Sun, Y. Pan, Long non-coding RNA PVT1 promotes cell proliferation and invasion through regulating miR-133a in ovarian cancer, Biomed. Pharmacother. 106 (2018) 61–67, https://doi.org/10.1016/j.biopha.2018.06.112.

[25] P. Martini, L. Paracchini, G. Caratti, M. Mello-Grand, R. Fruscio, L. Beltrame, et al., lncRNAs as novel indicators of patients' prognosis in stage I epithelial ovarian cancer: a retrospective and multicentric study, Clin. Cancer Res. 23 (9) (2017) 2356–2366, https://doi.org/10.1158/1078-0432.ccr-16-1402.

[26] T. Li, Y. Chen, J. Zhang, S.J.M. Liu, LncRNA TUG1 promotes cells proliferation and inhibits cells apoptosis through regulating AURKA in epithelial ovarian cancer cells, Medicine (Baltimore) 97 (36) (2018) e12131, , https://doi.org/10.1097/md.0000000000012131.

[27] J.Y. Qu, M.A. Kamal, C.F. Yuan, The regulatory roles of long non-coding RNA in the Chemoresistance process of ovarian cancer, Curr. Pharm. Des. 25 (8) (2019) 856–861, https://doi.org/10.2174/1381612825666190404122154.

[28] S.H. Guo, L. Zhang, Y.H. Zhang, Z. Wu, D.W. He, X. Li, et al., Long non-coding RNA TUG1 enhances chemosensitivity in non-small cell lung cancer by impairing microRNA-221-dependent PTEN inhibition, Aging-Us. 11 (18) (2019) 7553–7569, https://doi.org/10.18632/aging.102271.

[29] L. Jiang, W. Wang, G. Li, C. Sun, Z. Ren, H. Sheng, et al., High TUG1 expression is associated with chemotherapy resistance and poor prognosis in esophageal squamous cell carcinoma, Cancer Chemother. Pharmacol. 78 (2) (2016) 333–339, https://doi.org/10.1007/s00280-016-3066-y.

[30] C.-H. Xie, Y.-M. Cao, Y. Huang, Q.-W. Shi, J.-H. Guo, Z.-W. Fan, et al., Long non-coding RNA TUG1 contributes to tumorigenesis of human osteosarcoma by sponging miR-9-5p and regulating POU2F1 expression, Tumour Biol. 37 (11) (2016) 15031–15041, https://doi.org/10.1007/s13277-016-5391-5.

[31] C. Xu, Y. Guo, H. Liu, G. Chen, Y. Yan, T.J.C. Liu, et al., TUG1 confers cisplatin resistance in esophageal squamous cell carcinoma by epigenetically suppressing PDCD4 expression via EZH2, Cell Biosci. 8 (1) (2018) 1–11, https://doi.org/10.1186/s13578-018-0260-0.

[32] B.B. Ma, Z.B. Gao, J.C. Lou, H.Q. Zhang, Z.B. Yuan, Q. Wu, et al., Long non-coding RNA MEG3 contributes to cisplatin-induced apoptosis via inhibition of autophagy in human glioma cells, Mol. Med. Rep. 16 (3) (2017) 2946–2952, https://doi.org/10.3892/mmr.2017.6897.

[33] F.Y. Fan, R. Deng, H. Yi, H.P. Sun, G.C. He, Y. Su, The inhibitory effect of MEG3/miR-214/AIFM2 axis on the growth of T-cell lymphoblastic lymphoma, Int. J. Oncol. 51 (1) (2017) 316–326, https://doi.org/10.3892/ijo.2017.4006.

[34] Y. Xia, Z. He, B. Liu, P. Wang, Chen YJMmr, Downregulation of Meg3 enhances cisplatin resistance of lung cancer cells through activation of the WNT/β-catenin signaling pathway, Mol. Med. Rep. 12 (3) (2015) 4530–4537, https://doi.org/10.3892/mmr.2015.3897.

[35] Xiu Y-l, Sun K-x, X. Chen, S. Chen, Y. Zhao, Guo Q-g, et al., Upregulation of the lncRNA Meg3 induces autophagy to inhibit tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3, Oncotarget. 8 (19) (2017) 31714, , https://doi.org/10.18632/oncotarget.15955.

[36] S.Q. Feng, X.Y. Zhang, H.T. Fan, Q.J. Sun, M. Zhang, Up-regulation of LncRNA MEG3 inhibits cell migration and invasion and enhances cisplatin chemosensitivity in bladder cancer cells, Neoplasma. 65 (6) (2018) 925–932, https://doi.org/10.4149/neo_2018_180125N55.