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RESEARCH COMMUNICATION

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Role of a novel circulatory RNA-based biomarker panel expression in ovarian cancer

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Abstract

Ovarian cancer (OC) is considered the sixth commonest cancer affecting women globally. We choose novel integrated specific ovarian cancer RNA biomarker panel; pellino E3 ubiquitin protein ligase family member 3 (PELI3) gene expressions along with its selected epigenetic regulators (microRNA (miR-361-3p) and long noncoding RNA (lncRNA RP5-837J1.2) by bioinformatic methods. Then, differential expressions of the selected panel in the sera of 50 OC patients, 42 cases with benign ovarian lesions, and among 45 controls were determined using real-time polymerase chain reaction quantitative (qRT-PCR). Furthermore, their expression was measured also in malignant ovarian tissues and adjacent nontumor tissues in 23 of 50 OC patients by quantitative qRT-PCR. The current study reported, for the first time, upregulation of serum lncRNA RP5-837J1.2 with concomitant downregulation of miR-361-3p and PELI3 mRNA in malignant group compared with benign and controls groups. There were associations of serum lncRNA RP5-837J1.2 with the affected ovary and worse International Federation of Gynecology and Obstetrics staging; associations of miR-361-3p with tumor size, grade, stage, and presence of metastasis; as well as associations among PELI3 mRNA expression and tumor size, grade, stage, and presence of metastasis among the OC group. In tumor tissues, miR-361-3p and PELI3 mRNA levels were at a higher level than that of nontumor tissues; however, tumor tissue showed lower level of lncRNA RP5-837J1.2 compared to normal tissue. There were positive correlations between serum and tissue level of RNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA, but they did not reach statistical significance. Receiver operating characteristics curve analyses showed that lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA expression levels can discriminate among OC patient, cases with benign

Abbreviations: AUC, Area under the curve; BMI, body mass index; CA125, cancer antigen 125; CEA, carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics; IRAK1, interleukin-1 receptor-associated kinase 1; lncRNA, long noncoding RNA; NSCLC, non-small cell lung cancer; OC, Ovarian cancer; PELI3, Pellino E3 ubiquitin protein ligase family member 3; qRTPCR, polymerase chain reaction; ROC, receiver operating characteristic; RQ, relative quantity; TLRs, Toll-like receptors; TNF, tumor necrosis factor; TRAF6, TNF receptor-associated factor 6 complex.

mass, and controls with an accuracy of 96, 76, and 83%, respectively; which increased if they are combined. This novel diagnostic RNA-based panel biomarker could be helpful for OC diagnosis.

KEYWORDS

bioinformatics, long noncoding RNA, micro-RNA, ovarian cancer, real-time PCR, toll like receptors

1 | INTRODUCTION

Ovarian cancer (OC) is the sixth most common cancer in women worldwide (18 most common cancer overall), with 239,000 new cases diagnosed in 2012.[1,2] The ovary's position is difficult to image and monitor for abnormal growth; thus, over 70% of patients with OC are diagnosed at an advanced stage.[3] Notably, it is the deadliest reproductive cancer in women, accounting for 5% of female cancer deaths all over the world. It is estimated that there was 21,980 new cases and 14,270 deaths from OC in 2014.[4] The overall 5-year relative survival is less than 44%, but when diagnosed in the earliest stages, the 5-year survival is over 90%.[5] Therefore, a better understanding of the biological mechanisms of OC is crucial to create earlier diagnosis and more effective treatment. In Egypt, OC is one of the commonest cancer in females (4.5%) and expected to increase three-fold in incident cancer relative to 2013 by 2050.[6]

Ovarian serous adenocarcinomas are the commonest histotype and account for almost 50% of malignant neoplasms.[7] The majority of cases present in advanced stages and are treated with surgery and systemic chemotherapy. Current treatment is frequently followed by recurrence, which is often resistant to chemotherapy, as demonstrated by 15% long-term survivors.[8] In this context, clarifying molecular mechanisms implicated in the complex process of ovarian carcinogenesis is critical to develop better preventive and diagnostic approaches, as well as more effective treatment methods.

Recent transcriptomic researches revealed that the majority of the transcribed genome is noncoding. Among these, there is a kind of noncoding RNA called long noncoding RNA (lncRNA, 200 bp in length), which has been proven to regulate many biological processes from nuclear organization to epigenetic modification of post-transcriptional regulation and RNA splicing especially in cancers.[9,10]

MicroRNAs (miRNAs) are another class of noncoding RNAs with 18–25 nucleotides, which played important roles in organism by directly binding to the untranslational region of their target genes. miRNAs could regulate cell behaviors, including cell proliferation, migration, invasion, and apoptosis. Based on their functional roles in cell cycles, a number of miRNAs were reported to involve in tumor progression.[11,12]

Notably, growth and progression of cancer are coupled with immune suppression. Tumor cell has the ability to stimulate different checkpoint immune pathways, which results in immunosuppressive functions.[13] Toll-like receptors (TLRs) constitute the basic mechanisms in activation of the innate immune system which subsequently lead to an adaptive immune response. Thus making them crucial key players which modulate tumor dynamics; thus, activation of TLRs on OC cells can lead to a more aggressive tumor phenotype and tumor progression.[14] Ten TLRs were found in humans and classified into two subgroups according to their cellular localization.[15] TLRs 1, 2, 4, 5, 6, and 10 are located on cell surface and they respond primarily to pathogen-associated molecular patterns as lipids and bacterial proteins.[16] On the contrary, TLRs 3, 7, 8, and 9 are located intracellular in endosomes and respond primarily to nucleic acids of both viruses and bacteria.[17] TLRs are classes of type I transmembrane proteins that comprised of an extracellular domain, transmembrane region, and intracellular domain.[17] Recently, researches showed that TLRs expressions can be varied in various tumor cells.[18–21] Overexpression of TLR2, TLR3, and TLR4 has been found in colonic cancer cells majority.[22,23]Furthermore, increased TLR2, TLR3, TLR4, and TLR5 expressions were detected in OC cells.[24,25] TLR dysregulation has been reported also in both normal and neoplastic ovarian tissue.[24] Woo et al. detected different TLR4 and TLR5 expressions in OC which related to angiogenesis.[26] Additionally, an inhibition of the innate antitumor response by TLR4 and TLR5 favoring cancer progression.[27] Although many TLRs promote tumors occurrence and progression through variety mechanisms, some TLRs might have an antitumor effect. Thus, TLRs activation in cancer cells could play complex roles.[28] Emerging evidence has demonstrated that noncoding RNAs (miRNAs and lncRNA) are involved in almost all known cellular processes, including innate and adaptive immune responses, via modulation of gene expression.[29] Putting these data together sheds the light on possible interaction between miRNAs and lncRNA which affects TLR, thus affecting tumor occurrence and OC progression.

Pellino E3 ubiquitin protein ligase family member 3 (PELI3) was initially identified as a scaffolding protein which promotes activation of c-Jun and Elk-1. It is an intermediate signaling protein in the pathway of innate immune response.[30] The subsequent research characterized its ligase activity as well as ubiquitin-protein transferase activity against interleukin-1 receptor-associated kinase 1 (IRAK1).[31]

PELI3 protein has been suggested also to facilitate the immune response signaling transmission from TLRs to IRAK/tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) complex.[10] An autophagy-dependent PELI3 degradation inhibits the expression of proinflammatory interleukin-1 beta (IL-1β) expression.[32] Noteworthy, the PELI3 potential role in diseases including cancer has been barely investigated until now.

In the present study, we proposed that a RNA-based biomarkers derived from OC could be used as a promising biomarker because lncRNA and miRNA are more useful than mRNA alone. We first detected OC-associated genes and linked to TLR signaling along with their epigenetic regulators via in silico data analysis. Second, to confirm this, we investigated whether lncRNA RP5-837J1.2, miRNA-361-3p (miR-361-3p), and target gene *PELI3* as potential novel biomarkers that could detect reliably discriminate OC patients from benign ovarian mass and healthy females using quantitative real-time polymerase chain reaction (qRT-PCR). Subsequently, verification of the source of selected RNAs by assessing their expression in OC tissues, in a set of 23 primary tumors. Finally, we explored the possible relationship between the chosen RNAs expression with the clinical and pathological features in OC.

2 | PATIENTS AND METHODS

2.1 | Enrollment of study subjects

The present study included 50 Egyptian women (mean age 48.7 ± 9.6 years) with histopathological examination confirmed OC diagnosis of epithelial OC (89%) cancer, 42 cases with benign lesions (serous cyst and cystadenofibroma), and control group included 45 age-matched women with no signs or symptoms of malignancy. Cases with epithelial OC received no radiotherapy, chemotherapy, or immunotherapy or previous surgical treatment. All participants were enrolled from Obstetrics and Gynecology Department, Faculty of Medicine, Benha University Hospital, Egypt. The patients were evaluated according to the staging system of the International Federation of Gynecology and Obstetrics (FIGO), and histopathological diagnosis was performed according to world health organization (WHO).

All included participants completed a questionnaire about their medical and reproductive histories in particular. Data regarding reproductive history (including gravidity, parity, age at first-full term pregnancy, history of breastfeeding, age at menarche and menopause, menstrual cycle regularity, and hormone replacement therapy; use of oral contraceptives was defined as at least 3 months of use), age at first oral contraceptives use, and duration of its use were collected. Anthropometric characteristics (heights, weight, and body mass index [BMI]) were measured. All participants in the current work were nonsmokers. The Ethics Committee of Zagazig, Benha, and Ain Shams, Faculties of Medicine, Egypt, approved our study protocol, and informed consent for the experimental use of specimens was obtained from all participants.

2.2 | Samples collection and processing

All specimens were handled and coded according to ethical standards. Five milliliters of blood was drawn and collected in serum separator tubes. Sera were centrifuged, separated, and divided into five aliquots each and stored at 80°C until use. The OC tissues were snap frozen in liquid nitrogen and stored at 80°C after surgery.

2.3 | Tumor markers analyses

Ovarian tumor markers' levels including carcinoembryonic antigen (CEA) and cancer antigen 125 (CA125) were measured in serum by enzyme-linked immunosorbent assay kit provided by Abcam (Cambridge, MA) according to the manufacturer's instructions.

2.4 | Bioinformatics analysis

Identification of the novel RNA-based biomarker panel consisted of four steps. First, we identified a target gene specific to OC and linked to innate immunity and TLR named PELI3 mRNA. This target gene was downregulated in OC compared with normal ovary ($p < .05$) according to the data from public microarray databases and to decrease the false discovery rate. We used three databases, Protein Atlas database (Available at http://www.proteinatlas.org), Amp Pharmacy Anchorage Database (Available at https://amp.pharm.mssm.edu/archs4/gene/PELI3#tissueexpression), and Gene Card Database (Available at https://www.genecards.org/cgi-bin/carddisp.pl?gene=PELI3) to confirm the gene ontology and expression in cancer (Supporting Information Figures S1–S3). Together, the three databases confirmed the correlation between deregulated PELI3 mRNA in OC and its relation to TLR signaling.

Second, lncRNA RP5-837J1 and miR-361-3p were identified, using an lnCeDB database, available at http://gyanxet-beta.com/lncedb/index.php. We selected them based on their relation to carcinogenesis, competitive endogenous RNA score, and number of miRNA binding sites (Supporting Information Figure S4). Third, we verified that miR-361-3p targeting PELI3 mRNA using TargetScan Predicted Conserved microRNA Targets database (Available at: http://amp.pharm.mssm.edu/Harmonizome/gene-set/miR-361-3p/TargetScan+Predicted+Conserved+micrRNA+Targets; Supporting Information Figure S5). Lastly, we performed pathway enrichment analysis for the chosen miRNA to ensure that it is linked to carcinogenesis, IL signaling, and inflammatory response

using Gene Ontology Annotation Database (Available at https://www.ebi.ac.uk/GOA; Supporting Information Figure S6).

2.5 | Extraction of total RNA and lncRNA and reverse transcription

Total RNA was extracted from serum and tissue samples by QIAzol reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA was eluted using 30 μl of ribonuclease-free water. The quality of total RNA was detected by A260 to A280 ratio and 1.2% agarose gel electrophoresis.[33] A 200 ng of total RNA was transcribed immediately to complementary DNA (cDNA) by QuantiTect Reverse Transcription kit (Quiagen, Germany) following the manufacturer's instructions.

2.6 | Extraction of miRNA

The miRNA in serum and tissues were extracted using miRNeasy isolation kit (Qiagen, Germany) according to the manufacturer's instructions. miRNA was eluted by 30 μl of ribonuclease-free water. The quality of total RNA was detected by A260 to A280 ratio using UV/spectrophotometer and 1.2% agarose gel electrophoresis.[33]The cDNA template was produced by miScript II RT Kit (Qiagen, Germany) following to the manufacturer's instruction.

2.7 | qRT-PCR for selected genes

Expressions of lncRNAs RP5-837J1.2 in serum and OC tissue samples from cases were determined by qRT-PCR assay. The qRT-PCR amplification was done using a cDNA, lncRNA qPCR QuantiFast Primer Assay for Human RP5-837J1.2 (QIAGEN, Valencia, CA, USA; accession ENST00000415412 and ENST00000578488, respectively) and universal SYBR Green qRT-PCR Master Mix according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase housekeeping gene (NM_001256799) was also included in the assay for target gene expression and then used in normalization. This amplification was done on StepOnePlus System (Applied Biosystems).

The expression of miRNA-361-3p in serum and OC tissue samples were assessed in mixture containing total cDNAs, miScript SYBR Green PCR Kit (QIAGEN, Valencia, CA) and provided miScript Universal primer and miRNA-specific forward primer (miR-361-3p miScript Primer assay; accession no: MS00009555) according to the suggested protocol by manufacturer. Housekeeping gene RNU6B (U6 small nuclear RNA) miScript Primer assay (accession no: MS00033740) was also included in the assay for target gene expression and then used in normalization.

Finally, PELI3 mRNA gene expression determination by qRT-PCR in mixture containing cDNA, QuantiFast Probe Assay for Hs_PELI3 mRNA (Qiagen, Germany; NM_001098510, NM_145065), and QuantiTect SYBR Green PCR Master Mix according to manufacturer's protocol and housekeeping gene actin beta (NM NM_001101) were also included in the assay for target gene expression and then used in normalization. PCR cycles were: denaturation at 95°C for 15 min, followed by 40 cycles of denaturation for 10 s at 94°C, annealing for 30 s at 55°C, and extension for 34 s at 70°C. The qPCR was done using Step One Plus System (ABI).

Expression levels in tissue and serum samples for lncRNAs RP5-837J1.2, miRNA-361-3p, and PELI3 were assessed using the comparative Ct method. The expression level of each specimen was the relative value to the average expression of healthy control. Fold differences were obtained using the following equation: $2^{-\Delta\Delta C}$, where Ct is the threshold cycle, $\Delta\Delta C$ equals ΔCt of the sample target gene minus the mean ΔCt of the control group.[34]

2.8 | Statistical analysis

Data were expressed as median, mean rank, or numbers (percentages) when appropriate. Clinical features of three studied groups were compared using one way analysis of variance or chi-square (χ^2) test. The nonparametric Mann–Whitney or Kruskal–Wallis tests were used in comparison of three investigated biomarkers from independent samples with skewed data. Evaluation of potential associations of clinicopathological features of OC with novel biomarkers (lnc-RNAs RP5-837J1.2, miRNA-361-3p, and PELI3) genes expression were done using Kruskal–Wallis, chi-square, or Mann–Whitney *U* test. A receiver-operating characteristic (ROC) curve was performed to detect the optimal cutoff which maximizes the sum of sensitivity and specificity of each biomarker.[35] Spearman's rank correlations were done to investigate the inter-relation between the three biomarkers.[36] A statistical significance was set at ($p < .05$), and all tests were two sided. Statistical Package for the Social Sciences software (SPSS Version 21) was used in all statistical analyses.

3 | RESULTS

3.1 | Clinicopathological features of study population

The demographic and clinical characteristics of study subjects were summarized in Table 1. The distributions of the risk factors were nonsignificantly different between study groups ($p > .05$). According to FIGO and WHO standards, these patients were classified as: 32 cases in early stage (64%) (Stages I and II) and 18 patients (36%) in late stage

TABLE 1 Study population demographic and clinical characteristics ($N = 137$)

	Malignant ($n = 50$)	Benign ($n = 42$)	Controls ($n = 45$)	p value	$\chi 2$ (a), F (b)
Age					
≥52.4 years ($n = 23$)	27 (54%)	24 (57.1%)	23 (51.1%)	.567	$\chi 2$ (a) = 2.292
<52.4 years ($n = 27$)	23 (46%)	18 (42.9%)	22 (48.9%)		
BMI (kg/m^2)					
Overweight (24–29.5)	12 (24%)	9 (21.4%)	12 (26.7%)	.849	$\chi 2$ (a) = 4.253
Obese (>29.5)	38 (76%)	33 (78.6%)	33 (73.3%)		
CA125					
Positive (≥117.5 U/ml)	45 (90%)	5 (11.9%)	—		
Negative (<117.5 U/ml)	5 (10%)	37 (88.1%)	45 (100%)	<.001**	$\chi 2$ (a) = 70.900
CEA					
Positive (≥4.45 ng/ml)	43 (86%)	13 (31%)	2 (4.4%)		
Negative (<4.45 ng/ml)	7 (14%)	29 (69%)	43 (95.6%)	<.001**	$\chi 2$ (a) = 45.540
CA125/CEA ratio					
Positive (≥25.3)	47 (94%)	3 (7.1%)	—		
Negative (<25.3)	3 (6%)	39 (92.9%)	45 (100%)	<.001**	$\chi 2$ (a) = 85.541
Histological grade					
Grade 1	6 (12%)				
Grade 2	23 (46%)				
Grade 3	21 (42%)				
Pathology					
Stage 1	28 (56%)				
Stage 2	22 (44%)				
Tumor size					
T1	3 (6%)				
T2	21 (42%)				
T3	17 (34%)				
T4	9 (18%)				
FIGO					
Stage 1	20 (40%)				
Stage 2	12 (24%)				
Stage 3	8 (16%)				
Stage 4	10 (20%)				
Affected ovary					
Left	28 (56%)				
Right	22(44%)				
Metastasis					
Metastatic	9 (18%)				
Nonmetastatic	41 (82%)				
Histopathological types					
Serous adenocarcinoma	31(62%)				
Others	19(38%)				

Abbreviations: BMI, body mass index; CA125, cancer antigen 125; CEA, carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics.

*Significant correlation was detected between investigated groups at $p < .05$.

**Highly significant correlation was detected between investigated groups at $p < .01$.

[a]Chi-square test.

[b]One way Anova test.

(Stages III and IV). Additionally, 31 (62%) of cases had serous adenocarcinoma and 19 (38%) OC patients suffered from other OC types: 29 cases of grades G1 and G2 and 21 cases of grade G3 OC.

3.2 | Expression levels of serum lncRNA RP5-837J1, miR-361-3p, and PELI3 mRNA among three studied groups

There was statistical significant upregulation of lncRNA RP5-837J1.2 with concomitant downregulation of miR-361-3p and PELI3 mRNA in malignant group compared with benign and healthy control groups (Figures 1, 2 and 3). The expression levels of lncRNA RP5-837J1.2 were 6.56 vs. 3.8, 1.9-fold higher in patients with OC compared with those with benign ovarian masses and healthy controls respectively ($p < .001$ for each) (Figure 1). In contrast, expression levels of miR-361-3p and PELI3 mRNA were 2.67, 2.9-fold higher in healthy control as compared with malignant group ($p < .001$ for each; Figures 2, 3).

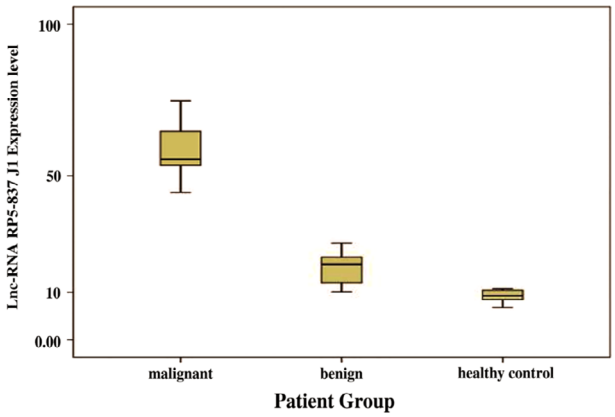

FIGURE 1 lncRNA RP5-837J1.2 expression levels in the three studied groups. lncRNA, long noncoding RNA

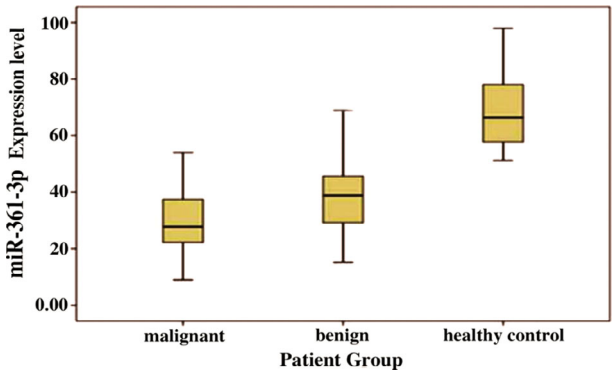

FIGURE 2 MicroRNA-361-3p expression levels in the three studied groups of patients

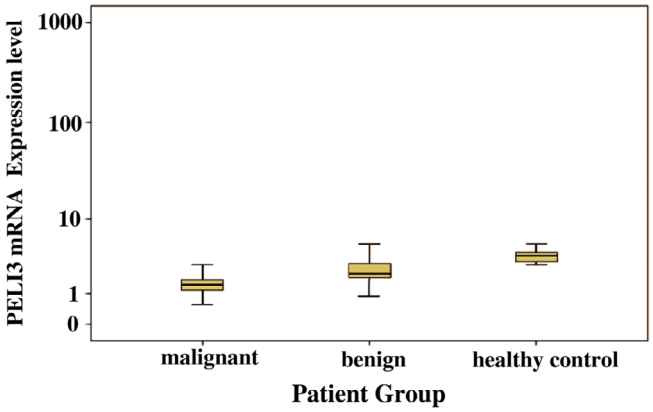

FIGURE 3 *PELI3* gene expression levels in the three studied groups of patients

3.3 | Correlations between the serum expression levels of lncRNA RP5-837J1.2, miR-361-3p, and target gene with ovarian tumor markers parameters in OC patients group

Our findings revealed a significant negative correlation between target gene *PELI3* expression and classical OC marker CEA ($p = .003$). Notably, there were negative correlations of lncRNA RP5-837J1.2 with its targets (PELI3) mRNA ($p = .01$) and with miR-361-3p ($p = .04$) expressions levels; whereas it is positively correlated with CA125/CEA ratio ($p = .02$). Additionally, the miR-361-3p expression levels were negatively correlated with PELI3 mRNA concentrations ($p < .001$) (Table 2).

3.4 | Diagnostic utility of serum expression level of RNA as potential diagnostic biomarkers for OC

ROC curve analysis showed that lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA expression levels can discriminate between patient and control serum with an accuracy of 96, 76, and 83%, respectively. Area under the curve was 0.996, 0.839, and 0.842, respectively, $p < .001$ for each (Figures 4a,c and 5a), sensitivity of 97.3, 83.5, and 88.6%, and a specificity of 94.6, 70.1, and 79.3%, respectively (Table 3). The cutoff values of serum expression of lncRNA RP5-837J1.2 was ≥2.75 (49 of 50 OC cases), ≤1.93 for miR-361-3p expression (48 of 50 OC cases), and ≤2 for PELI3 mRNA expression (46 of 50 of OC cases) as calculated by ROC analyses. Lastly, lncRNA RP5-837J1.2, miRNA-361-3p, combined lncRNA-miRNA, and PELI3 mRNA were positive in (50 malignant; 42 benign cases; Figure 4). Noteworthy, based on the cutoff values previously calculated according to ROC curves, the diagnostic value of combined serum-based RNA biomarkers panel was significantly better

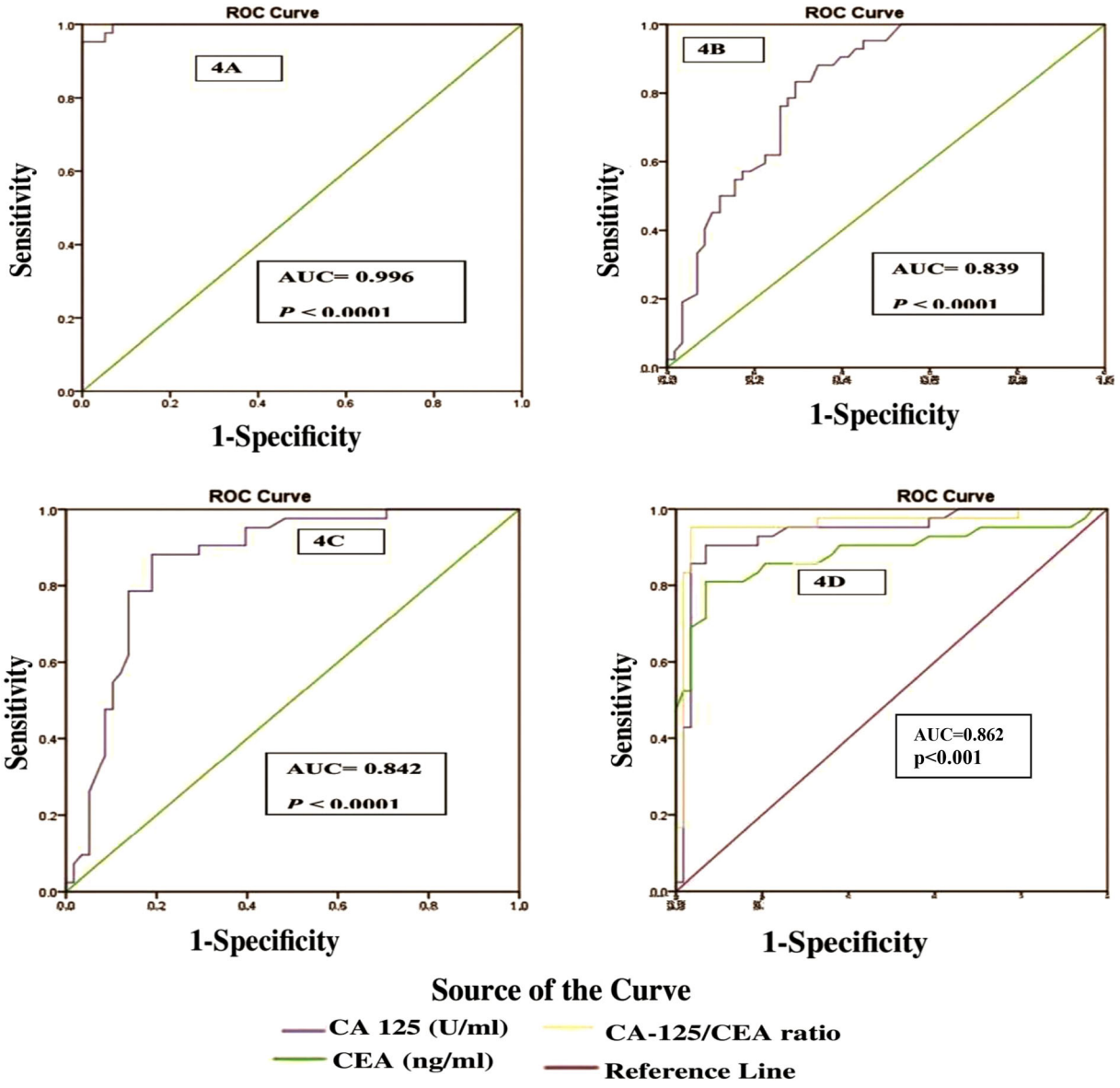

FIGURE 4 (a) Receiver–operator characteristic curve for lncRNA RP5-837J1.2 as a discriminate between OC patients vs. control group and patients with benign mass. (b) Receiver–operator characteristic curve for microRNA-361-3p as a discriminate between OC patients vs. control group and patients with benign mass. (c) Receiver operator characteristic curve for autophagy gene (*PELI3*) as a discriminate between OC patients vs. control group and patients with benign mass. (d) Receiver–operator characteristic curve for CA125, CEA, and CA125-CEA ratio as a discriminate between OC patients vs. control group and patients with benign mass. CEA, carcinoembryonic antigen; lncRNA, long noncoding RNA; OC, ovarian cancer

than that of the individual miRNA reaching 100% sensitivity and 77% specificity in diagnosing OC (Table 3).

3.5 | Positivity rate of serum RNA-based biomarker panel expression among OC patients in relation to different clinicopathological parameters

Further deep data analyses concerning the positive values which calculated by ROC revealed that there was no significant association of serum expression cutoff of lncRNA RP5-837J1.2≥2.75 (49 of 50 OC cases) with any of the clinicopathological characteristics except affected ovary ($p = .005$) and FIGO staging ($p = .03$; Table 4). On the other side, there was significant correlation between miR-361-3p expression cutoff ≤1.93 (48 of 50 OC cases) with tumor size, grade, stage, and presence of metastasis ($p < .05$; Table 5). Lastly, there was significant correlation between PELI3 mRNA expression with cutoff ≤2 (46 of 50 of OC cases) and tumor size, grade, stage, and presence of

TABLE 2 Correlations between expressions of RNA-based biomarker panel and laboratory parameters in the ovarian cancer group

Correlations								
			lncRNA RP5-837J1.2	miR-361-3p	*PELI3* gene	CA125 (U/ml)	CEA (ng/ml)	CA125/CEA ratio
Spearman's rho	lncRNA RP5-837J1.2	Correlation coefficient	1.000	−0.280	−0.369*	0.266	0.165	0.045
		Significance (two tailed)	—	.04*	.016	.089	.297	.796
		N	50	50	50	50	50	50
	miR-361-3p	Correlation coefficient	−0.280	1.000	0.778**	0.084	−0.133	0.183
		Significance (two tailed)	.072	—	<.001	.596	.401	.287
		N	50	50	50	50	50	50
	PELI3 gene	Correlation coefficient	−0.369*	0.778**	1.000	−0.035	−0.447**	0.217
		Significance (two tailed)	.016	<.001	—	.825	.003	.171
		N	50	50	50	50	50	50
	CA125 (U/ml)	Correlation coefficient	0.266	0.084	−0.035	1.000	0.350*	0.757**
		Significance (two tailed)	.089	.596	.825	—	.023	<.001
		N	50	50	50	50	50	50
	CEA (ng/ml)	Correlation coefficient	0.165	−0.133	−.0447**	0.350*	1.000	−0.222
		Significance (two tailed)	.297	.401	.003	.023	—	0.168
		N	50	50	50	50	50	50
	CA125/CEA ratio	Correlation coefficient	0.045	0.183	0.217	0.757**	−0.222	1.000
		Significance (two tailed)	.02*	.287	.171	<.001	.168	—

Abbreviations: CA125, cancer antigen 125; CEA, carcinoembryonic antigen.
*Correlation is significant at the .05 level (two tailed).
**Correlation is significant at the .01 level (two tailed).

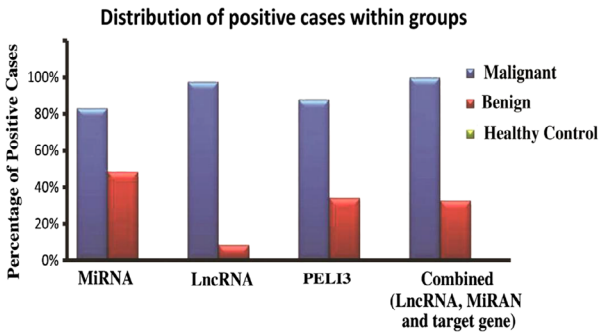

FIGURE 5 Distribution of positive cases within three groups

metastasis in the malignant group (Table 6). Putting these findings together, these RNA-based biomarkers can be novel promising biomarkers detecting metastasis and reflecting the clinicopathological features of tumor. Interestingly, the impact of two potential influencing factors on the levels of lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA was analyzed in women without malignant disease including controls and those with benign ovarian lesions. Neither age nor the BMI affects their levels in human serum. Of note, there was a significant negative correlation among serum lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA levels among all the study groups ($p = .01$ for each).

3.6 | Expression of RNA-based biomarker panel in matched OC tissues and adjacent-cancer free tissues

We examined RNA-based biomarker panel in 23 malignant ovarian tissues and adjacent nontumor tissues out of 42 OC patients by qRT-PCR. In tumor tissues, miR-361-3p and PELI3 mRNA levels were at a higher level than that of non-tumor tissues, with the median of 2.1, 1.78 and 2, 1.6, respectively; however, tumor tissue showed lower level of lncRNA RP5-837J1.2 compared with normal tissue with a median of 1.15 vs. 21. These data indicated that aberrant expression of the chosen RNA-based biomarker panel may be related to OC development. Of note, there was weak positive correlations between serum and tissue expression levels of lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA, but they did not reach statistical significance ($p > .05$; Table 7; Figure 6).

3.7 | Logistic regression analysis for prediction OC risk factors

Finally it was so essential to find the risk factors for OC and this was achieved by logistic regression analysis, which revealed that classical markers of OC including CA125/CEA

TABLE 3 Performance characteristics of the investigated serum parameters among different groups of the study

Biomarker	Sensitivity	Specificity	PPV	NPV	Accuracy
lncRNA	97.3%	94.6%	93.2%	98.8%	96%
miRNA	83.5%	70.1%	67.3%	85.4%	76%
PELI3 gene	88.6%	79.3%	75.5%	90.2%	83%
Combined (lncRNA RP5-837J1, miR-361-3p, and PELI3 mRNA)	100%	77%	75%	100%	88%
CA125	90.5%	93.1%	90.5%	93.1%	92%
CEA	85.7%	79.3	75%	88.5%	82%
CA125:CEA ratio	95.2%	96.6%	95.2%	96.6%	96%

Abbreviations: CA125, cancer antigen 125; CEA, carcinoembryonic antigen; PPV, positive predictive value; NPV, negative predictive value.

ratio ($p = .04$), CEA levels ($p = .004$), serum miR-361-3p expression ($p = .03$), and lncRNA RP5-837J1.2 expression in serum ($p < .001$) were independent predictor for OC development (Table 8). Thus, these data could be very useful in early predication of malignant changes in ovary.

4 | DISCUSSION

OC considered one of the most common cancer affecting women worldwide with a highest mortality rate compared with other gynecologic malignancies in the world.[37] Despite several biomarkers have been used in OC, reliable diagnostic and prognostic biomarkers are still absent. In addition, most patients diagnosed at late stages (III and IV), and the relapse is common with resistance to subsequent therapy.[38,39] Increased CA125 levels are found in 82% of patients with OC, 28% of cases with nongynecological cancers (including pancreatic cancer, breast cancer, and colon cancer), 6% of cases with benign gynecological diseases (including endometriosis, leiomyomas, and pelvic inflammatory disease), or other medical disease (including hepatic cirrhosis and heart failure), and in 1% of the normal population.[40,41] Taken together, discovering novel diagnostic reliable biomarkers for early diagnosis of OC is urgently needed by clinicians dealing with these patients.

Recently, reports have identified and detected aberrant expression of miRNAs in ovarian tumorigenesis leading to OC, which represents them as novel target and biomarkers for early detection, diagnosis, and prognosis of OC.[2] In addition, another level of gene regulation was done by lncRNAs within the human body.[42] However, molecular mechanisms and biofunctions of lncRNAs could play an important role in human diseases, such as cancers tumorigenesis, growth, and metastasis, which is still considered a mystery.[43] Furthermore, several researches highlighted the important role of TLR in modulation of immune response in OC.[27] Noteworthy, autophagy-dependent processes are linked also to TLRs signaling by recruiting BECN1/Beclin1 (Beclin 1, autophagy related), one of the key factors in autophagosome formation.[44] On the contrary, a study by Zhan et al. revealed that TLR4- and TLR3-induced autophagy increases the production of several cytokines, chemokines, and immunosuppressive factors leading to enhanced migration and invasion of lung cancer.[45]

Putting these data together, identification and validation of novel RNA-based biomarker panel could be helpful for early diagnosis of this aggressive cancer which almost diagnosed at late stages with high degree of mortality early. In the current research, we hypothesized, for the first time, that (circulating PELI3 mRNA) by bioinformatics analysis could be risk for OC. Then, we search for its epigenetic regulators through specific database (lncRNAs RP5-837J1.2 and miR-361-3p) that could give an idea about their interactions and their impact on OC risk. Thus can help in clarifying complex interactions of RNAs (coding and noncoding) which could be potentially dysregulated in OC. Finally, validation of this miRNA/lncRNA and their target can be possible novel biomarkers.[46]

In the current work, the majority of OC patients had serous adenocarcinoma 31 (62%) and 19 (38%) suffered from other OC types. Previous reports confirmed this finding.[47,48]

For the first time, this study demonstrated a statistically significant upregulation lncRNA RP5-837J1.2 in serum and OC tissues with concomitant downregulation of serum miR-361-3p and PELI3 mRNA compared with benign ovarian masses and healthy control groups. Furthermore, ROC curve analyses showed that lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA serum expression levels can discriminate between patient and healthy controls with an accuracy of 96, 76, and 83%; sensitivity of 97.3, 83.5, and 88.6%; and a specificity of 94.6, 70.1, and 79.3%, respectively. Also, ROC curve analyses demonstrated that the diagnostic value of the combined serum-based RNA biomarker panel was significantly better than that of the individual miRNA reaching 100% sensitivity and 77% specificity among OC patients.

TABLE 4 Positivity rate of serum *lncRNA RP5-837J1.2* expression among OC patients in relation to different clinicopathological characteristics ($N = 50$)

Clinicopathological factors	Median	Mean rank	Statistics	Number of cases/50 ≥ 2.75	p value, $\chi 2$ (c)
Mean age					
≥52.4 years	2.235	51.40	$p = .72$ NS		$p = .34$ NS
<52.4 years	2.410	49.60	U (a) =120.5	28 (56%)	$\chi 2$ (c) = 0.976
BMI					
Underweight (less than 19.5)	—	—	$p = .084$ NS		$p = .279$ NS
Normal (19.5–24)	—	—	$\chi 2$ (b) = 2.990	—	$\chi 2$ (c) = 0.597
Overweight (24–29.5)	1.59	42.04		11 (22%)	
Obese (greater than 29.5)	2.52	53.47		38 (76%)	
Histological grade					
Grade 1	22.4	25.9	$p = .218$ NS	5 (10%)	$p = .538$ NS
Grade 2	12.9	17.92	$\chi 2$ (b) = 3.045	22 (44%)	$\chi 2$ (c) = 1.240
Grade 3	13.05	24.06		22 (44%)	
Pathology					
Stage 1	13.2	22.65	$p = .503$ NS	25 (50%)	$p = .358$ NS
Stage 2	12.9	20.11	U (a) =192.0	24 (48%)	$\chi 2$ (c) = 0.846
Tumor size					
T1	12.45	16.00	$p = .722$ NS	3 (6%)	$p = .681$ NS
T2	12.50	19.85	$\chi 2$ (b) = 1.331	21 (42%)	$\chi 2$ (c) = 1.506
T3	14.75	22.32		16 (32%)	
T4	12.90	24.56		9 (18%)	
FIGO					
Stage 1	12.9	19.79	$p = .03$*	20 (40%)	$p = .351$ NS
Stage 2	15.65	24.3	$\chi 2$ (b) = 185.3	12 (24%)	$\chi 2$ (c) = 3.278
Stage 3	12.3	21.71		8 (14%)	
Stage 4	13.4	21.44		9 (18.0%)	
Affected ovary					
Left	11.9	16.72	$p = .005$*	28 (56%)	$p = .358$ NS
Right	14.9	27.29	U (a) = 108.5	21 (42%)	$\chi 2$ (c) = 0.846
Metastasis					
Metastatic	13.05	24.38	$p = .461$ NS	9 (18.0%)	$p = .623$ NS
Nonmetastatic	13.005	20.82	U (a) = 113.0	40 (80%)	$\chi 2$ (c) = .241

Note: a, Mann–Whitney test; b, Kruskal–Wallis test; c, chi-square test. p—NS, not significant (>.05); **, $p < .01$ is highly significant; *, $p < .05$ is significant. The cutoff was ≥2.5 for RP5-837J1.2.

Abbreviations: BMI, body mass index; CA125, cancer antigen 125; CEA, carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics; OC, ovarian cancer.

No previous study had clarified this serum-based RNA biomarker panel role in OC. Notably, researches revealed differentially expressed lncRNAs clusters in OC cells with distinct metastatic potentials.[49] Liu and his colleagues had investigated 4,956 lncRNAs in a microarray study, 583 and 578 were upregulated or downregulated, respectively, in highly in OC cells especially metastatic one, compared with the parental cells, respectively, which shows a possible role for lncRNAs in epithelial OC and/or presence of metastasis.[49] Another clusters of lncRNA RP or lncRNA RP5 have been investigated in various cancer. For example, a recent report by Ding et al. showed that lncRNA RP5-857K21.3, RP11-1C1.7, and RP11-872J21.3 were upregulated in OC cases compared with healthy control.[50] Furthermore,

TABLE 5 Positivity rate of serum miR-361-3p expression among OC patients in relation to different clinicopathological characteristics ($N = 50$)

Clinicopathological factors	Median	Mean rank	Statistics	Number of cases/50 ≤ 1.93	p value, $\chi 2$ (c)
Mean age					
≥52.4 years	1.99	49.85	$p = .986$ NS	26 (52%)	$p = .627$ NS
<52.4 years	1.88	50.05	U (a) = 1,222.5	22 (44%)	$\chi 2$ (c) = 0.236
BMI					
Underweight (less than 19.5)	—	—	$p = .123$ NS	—	$p = .674$ NS
Normal (19.5–24)	—	—	$\chi 2$ (b) = 2.373	—	$\chi 2$ (c) = 0.255
Overweight (24–29.5)	2.33	58.04		11 (22%)	
Obese (greater than 29.5)	1.87	47.85		37 (74%)	
Histological grade					
Grade 1	1.89	34.2	$p = .005$**	5 (10%)	$p = .336$ NS
Grade 2	1.45	24.03	$\chi 2$ (b) = 10.765	23 (46%)	$\chi 2$ (c) = 2.371
Grade 3	1.11	15.31		20 (40%)	
Pathology					
Stage 1	1.13	17.07	$p = .01$*	26 (52%)	$p = .147$ NS
Stage 2	1.78	26.87	U (a) = 116.500	22 (44%)	$\chi 2$ (c) = 2.326
Tumor size					
T1	1.91	27.5		1 (2%)	$p = .303$ NS
T2	1.75	24.65	$p = .003$**	21 (42%)	$\chi 2$ (c) = 3.640
T3	1.72	25.5	$\chi 2$ (b) = 13.999	17 (34%)	
T4	0.98	8.0		9 (18%)	
FIGO					
Stage 1	1.75	24.50		19 (38%)	$p = .756$ NS
Stage 2	1.55	21.90	$p = .466$ NS	11 (22%)	$\chi 2$ (c) = 1.188
Stage 3	1.12	19.29	$\chi 2$ (b) = 2.554	8 (16%)	
Stage 4	1.18	16.56		10 (20%)	
Affected ovary					
Left	1.33	20.74	$p = .658$ NS	27 (54%)	$p = .174$ NS
Right	1.45	22.42	U (a) = 201.0	21 (42%)	$\chi 2$ (c) = 1.78
Metastasis					
Metastatic	1.00	8.38	$p = .001$**	9 (18.0%)	$p = .160$ NS
Nonmetastatic	1.71	24.59	U (a) =31.0	39 (78%)	$\chi 2$ (c) =1.976

Note: a, Mann–Whitney test; b, Kruskal–Wallis test; c, chi-square test. p—NS, not significant (>.05); **, $p < .01$ is highly significant; *, $p < .05$ is significant. The cutoff was ≤1.93for miR-361-3p expression.

Abbreviations: BMI, body mass index; CA125, cancer antigen 125; CEA, carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics; OC, ovarian cancer.

previous study showed that RP5-919F19 was highly expressed in gastric carcinoma and RP11-167N4 expression was significantly lower in tumor tissues than adjacent non-tumor tissues.[51] Additionally, lncRNA RP5-1024C24.1 expression was significantly downregulated and related to clinical stage, and lnc-RNA RP11-402L6.1 expression was upregulated and correlated with lymph node metastasis in previous report.[52] Putting these data together points to differential expression profiles of lnc-RNAs, suggesting their role as a center control module for signal communication in tumorigenesis and cancer progression, which makes them promising biomarkers for early detection and screening especially in highly aggressive and lately diagnosed as OC.

TABLE 6 Positivity rate of *PELI3* autophagy gene expression among OC patients in relation to different clinicopathological characteristics ($N = 50$)

Clinicopathological factors	Median	Mean rank	Statistics	Number of cases /50 ≤ 2	*p* value,χ2 (c)
Mean age					
≥52.4 years	2.12	50.72	$p = .801$ NS	26 (52%)	$p = .136$ NS
<52.4 years	1.99	49.27	U (a) = 1,189.000	20 (40%)	χ2 (c) = 2.221
BMI					
Underweight (less than 19.5)	—	—		—	$p = .213$ NS
Normal (18.5–24.9)	—	—	$p = .662$ NS	—	χ2 (c) = 1.548
Overweight (25–29.9)	2.55	54.48	χ2 (b) = 0.416	8 (16%)	
Obese (greater than 30)	2.00	49.1		38 (76%)	
Histological grade					
Grade 1	1.99	29.6	$p = .012$*	5 (10%)	$p = .106$ NS
Grade 2	1.67	25.34	χ2 (b) = 8.812	21(42%)	χ2(c) =4.490
Grade 3	1.23	15.19		20(40%)	
Pathology					
Stage 1	1.33	17.98	$p = .04$*	27 (54%)	$p = .096$ NS
Stage 2	1.63	25.76	U (a) = 137.5	21 (42%)	χ2 (c) = 2.768
Tumor size					
T1	3.39	41.5		—	$p = .001$*
T2	1.56	23.94	$p < .001$**	20 (40%)	χ2 (c) = 16.319
T3	1.55	24.68	χ2 (b) = 18.677	17 (34%)	
T4	0.98	7.50		9 (18%)	
FIGO					
Stage 1	1.56	24.32		18 (36%)	
Stage 2	1.51	22.65	$p = .448$ NS	10 (20%)	$p = .640$ NS
Stage 3	1.23	18.57	χ2 (b) = 2.654	8 (16%)	χ2 (c) = 1.688
Stage 4	1.31	16.63		10 (20%)	
Affected ovary					
Left	1.56	23.43	$p = .260$ NS	26 (52%)	$p = .480$ NS
Right	1.29	19.16	U (a) = 174.0	20 (40%)	χ2 (c) = 0.499
Metastasis					
Metastatic	1.00	8.25	$p = .001$**	6 (12%)	$p = .248$ NS
Nonmetastatic	1.56	24.62	U (a) = 30.000	40 (80%)	χ2 (c) = 1.335

Note: a, Mann–Whitney test; b, Kruskal–Wallis test; c, chi-square test. *p*—NS, not significant (>.05); **, $p < .01$ is highly significant; *, $p < .05$ is significant. The cutoff was ≤2for autophagy gene expression.

Abbreviations: BMI, body mass index; CA125, cancer antigen 125; CEA, carcinoembryonic antigen; FIGO, International Federation of Gynecology and Obstetrics; OC, ovarian cancer.

Interestingly, our finding demonstrated that upregulation of lncRNA RP5-837J1.2 was accompanied with down-regulation of serum miR-361-3p. This could be explained as lncRNAs exert "sponge-like" effects on various miRNAs, which subsequently inhibits miRNA-mediated functions and downstream targets; *PELI3* expression in this study based on bioinformatic analysis. Thus, crosstalk between two ncRNAs could contributes to the disease pathogenesis including cancer.[53,54]

Several reports showed an altered expression of miR-361 in various types of cancer including squamous cell carcinoma,[55] cancer cervix,[56] prostate cancer,[57] colorectal and gastric cancer,[58] and hepatocellular carcinoma.[59] These reports proposed that miRNA-361 could play different role depending on

TABLE 7 Correlation between serum and tissue-based expression of RNA-based biomarker panel in OC cases

Correlations								
			lncRNA RP5-837J1.2 RQ in tissue	**lncRNA RP5-837J1.2 RQ in serum**	**miR-361-3p RQ in tissue**	**miR-361-3p RQ in serum**	***PELI3* gene RQ in tissue**	**PELI3Gene RQ in serum**
Spearman's rho	lncRNA RP5-837J1.2 RQ in tissue	Correlation coefficient	1.000	0.074	−0.257	0.068	−0.264	−0.153
		Significance (two tailed)	—	.758	.274	.774	.261	.518
	lncRNA RP5-837J1.2 RQ in serum	Correlation coefficient	0.074	1.000	0.198	−0.252	0.143	−0.464*
		Significance (two tailed)	.758	—	.404	.284	.548	.040
	miR-361-3p RQ in tissue	Correlation coefficient	−0.257	0.198	1.000	0.226	0.594**	−0.209
		Significance (two tailed)	.274	.404	—	.339	.006	.376
	miR-361-3p RQ in serum	Correlation coefficient	0.068	−0.252	0.226	1.000	0.118	0.569**
		Significance (two tailed)	.774	.284	.339	—	.620	.009
	PELI3 e RQ in tissue	Correlation coefficient	−0.264	0.143	0.594**	0.118	1.000	−0.001
		Significance (two tailed)	.261	.548	.006	.620	—	.997
	PELI3 RQ in serum	Correlation coefficient	−0.153	−0.464*	−0.209	0.569**	−0.001	1.000
		Significance (two tailed)	.518	.040	.376	.009	.997	—

Abbreviations: OC, ovarian cancer; RQ, relative quantity.

*Significant correlation was detected between investigated groups using Spearman's correlation at $p < .05$.

**Highly significant correlation was detected between investigated groups at $p < .01$.

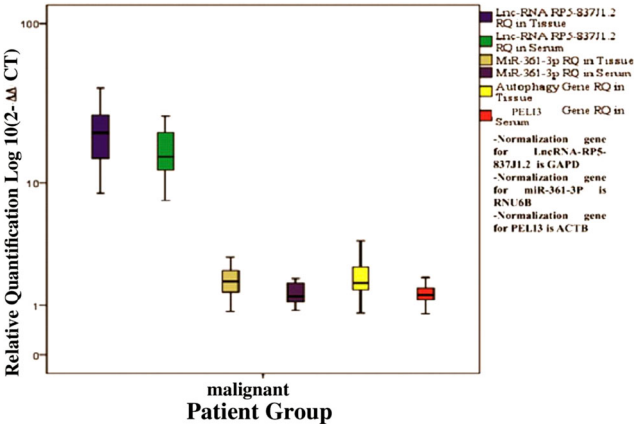

FIGURE 6 Relative expression of three genes among OC group. OC, ovarian cancer

the type of tumor.[55–59] In the current work, serum miR-361-3p expression was significantly correlated with tumor size, grade, stage, and presence of metastasis. In agreement with this data, Chen et al. demonstrated a significant lower expression of miR-361-3p in nonsmall cell lung cancer (NSCLC) tissues compared with corresponding normal lung, and this lower expression was associated with advanced stage and lymph node metastasis. In addition, the enforced miR-361-3p expression leads to NSCLC cell growth, proliferation, formation of clone, migration, and invasion in vitro, as well as tumorigenicity and intrapulmonary metastasis in vivo.[60]

Noteworthy, miR-361-5p significantly inhibited the growth of NSCLC in a nude mouse xenograft model and decreased *signal transducer and activator of transcription 6* (*STAT6*) and Bcl-xL expression, thus modulating cytokine, growth factor expression, and susceptibility to apoptosis.[44]Wang et al. proposed that many miRNAs, including miR-361-3p, can modulate TLRs signaling especially TLR2 and TLR4 in human mesenchymal stem cells.[61] Noteworthy, miR-361-3p mediates recruitment of +T cells in response to various infections such as *Leishmania braziliensis* and cancers, thus to produce inflammatory cytokine TNF family increasing cytotoxicity of tumor cells.[62]

In order to illustrate the role of the investigated RNA panel (lncRNA RP5-837J1.2 and miR-361-3p) through bioinformatics analysis, we explored their target's mRNA (PELI3). We found for the first time that there was statistically significant downregulation of PELI3 mRNA in malignant group compared with benign and healthy control groups. Furthermore, there was significant correlation of PELI3 mRNA expression with tumor size, grade, stage, and presence of metastasis in the OC patients. These findings together shed the light on important role of *PELI3* in cancer and its progression. *PELI3* is suggested to be an activator of several signaling cascades including MAPK14/p38α, MAPK8/JNK1-MAPK9/JNK2, and mitogen-activated protein kinase (MAPK) 1/ERK2-MAPK3/ERK1 signaling cascades of TLRs/IL-1R pathways.[45–48] Additionally, *PELI3's* really interesting new gene (RING) domain, responsible for its E3 ubiquitin ligase activity, is necessary for IRAK1 ubiquitination and therefore promotes signal transduction.[63]Putting these findings together could explain antitumor and immunomodulatory effect of *PELI3*.

5 | CONCLUSION

This study demonstrated a novel approach which enables a reliable integration of *PELI3* gene expression with its selected epigenetic regulators, thus helps in providing an accurate early biomarker panel (lncRNA RP5-837J1.2, miR-361-3p, and PELI3 mRNA) for OC early diagnosis and prognosis.

In our work, significant upregulation lncRNA RP5-837J1.2 in serum and OC tissues was accompanied by concomitant downregulation of serum miR-361-3p, PELI3

TABLE 8 Logistic regression analysis to clarify the main predictors of ovarian cancer

	Unstandardized coefficients			95% confidence interval for *B*		Correlations		
Parameter	*B*	*SE*	Significance	Lower bound	Upper bound	Zero order	Partial	Part
(constant)	2.156	0.120	.000	1.917	2.396			
lncRNA RP5-837J1.2 RQ in serum	−0.033	0.004	<.001	−0.042	−0.024	−0.843	−0.607	−0.360
miR-361-3p RQ in serum	0.029	0.029	.03	−0.029	0.087	0.540	0.104	0.049
PELI3 gene RQ in serum	0.000	0.001	.694	−0.001	0.001	0.100	0.041	0.019
CA125 (U/ml)	5.841	0.000	.900	−0.001	0.001	−0.716	0.013	0.006
CA125/CEA ratio	−0.005	0.003	.042	−0.010	0.000	−0.634	−0.209	−0.101
CEA (ng/ml)	−0.057	0.019	.004	−0.095	−0.018	−0.658	−0.291	−0.144

Note: Significant level at $p < .05$.

Abbreviations: CA125, cancer antigen 125; CEA, carcinoembryonic antigen; RQ, relative quantity.

mRNA, and more compared with benign ovarian masses and healthy control groups. Thus, serum decreases miR-361-3p expression was significantly correlated with worse tumor prognosis as tumor size, grade, stage, and presence of metastasis due to immunosuppression mediated by TLRs, basic mechanisms in activation of immune, and adaptive immune responses. The effect of miR-361-3p on TLRs signaling pathway could be mediated through *PELI3* gene which facilitate immune response signaling transmission from TLRs to IRAK/TRAF6 complex TLRs/IL-1R pathways mainly which ended by cytotoxicity. Therefore, downregulation of serum miR-361-3p and PELI3 mRNA was accompanied by tumor growth and progression coupled with immune suppression mediated by tumor cells stimulating different checkpoint immune pathways and results in antitumor immunosuppression favoring cancer progression. This hot spot panel can be promising serum panel for early detection of such aggressive and lately diagnosed among high-risk OC cases and benign masses. Thus, by this multiple genetic levels panel, the lower reliability of single-gene biomarker assay can be overcome to reach higher accuracy, sensitivity, and specificity. This work opens a new window of RNA–RNA crosstalk characteristics and its impact on target gene which offer new targets for therapy in OC.

The study limitations include the following: it was performed with relatively limited sample size. Moreover, in vitro functional analysis is needed to elucidate more biological mechanisms of RNA–RNA crosstalk in OC and to confirm the possible role of the chosen genes in OC suggested by both bioinformatics analysis and literature review. Furthermore larger multicenter studies are strongly recommended.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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