Submitted: 06 November, 2023 Accepted: 20 December, 2023 Published: 15 October, 2024 DOI:10.22514/ejgo.2024.095

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Exploring the potential of LNCRNA-MEG-3 as a diagnostic and prognostic marker in epithelial ovarian cancer

Abdulraheem A. Almalki[1], Amal F. Gharib[1], Saad S. Al-Shehri[1], Ahmed Alghamdi[1], Amani A. Alrehaili[1], Hamsa Jameel Banjer[1], Fouzeyyah Ali Alsaeedi[1], Rasha L. Etewa[2], Wael H. Elsawy[3,*]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Taif University, P.O. Box 11099, 21944 Taif, Kingdom of Saudi Arabia
[2]Pathology Department, College of Medicine, Jouf University, 2014 Sakaka, Kingdom of Saudi Arabia
[3]Department of Clinical Oncology, Faculty of Medicine, Zagazig University, 44111 Zagazig, Egypt

***Correspondence**
whelsawy@zu.edu.eg
(Wael H. Elsawy)

Abstract

We investigated the potential diagnostic and prognostic role of maternally expressed 3 (MEG-3), a type of long non-coding RNA, in epithelial ovarian carcinoma (EOC) by comparing long non-coding RNA (LNCRNA-MEG-3) expression in EOC and normal ovarian tissues and exploring clinical correlations. The study included 126 patients diagnosed with stage III EOC, confirmed by histopathological examination. We used quantitative polymerase chain reaction (qPCR) to quantify levels of the long non-coding RNA MEG-3. The long non-coding *RNA MEG-3* expression in EOC tissues was significantly lower than in normal ovarian tissues, indicating its potential as a diagnostic marker. Receiver operating characteristic curve (ROC) analysis demonstrated an Area under the ROC Curve (AUC) of 0.831, signifying its high sensitivity (100%) and specificity (97.04%) in distinguishing malignant ovarian tissues from normal ones. *LNCRNA-MEG-3* expression varied significantly across different EOC stages ($p < 0.0001$) and tumor grades ($p < 0.0001$), correlating with aggressive behavior and serous tumor types. Low *LNCRNA-MEG-3* expression associated with adverse prognostic factors such as ascites ($p < 0.0001$) and poor treatment response ($p < 0.0001$). High *LNCRNA-MEG-3* expression predicted better treatment response. It also correlates with the size of residual disease after debulking surgery, which is an important prognostic factor. Compared to those with low *LNCRNA-MEG-3* expression, patients with high expression had significantly longer overall and disease-free survival ($p = 0.0004$ and $p = 0.0002$, respectively). The study highlights *LNCRNA-MEG-3* as a valuable diagnostic and prognostic marker for EOC. Low expression of the long non-coding *RNA MEG-3* is linked to aggressive tumor features and unfavorable clinical outcomes, stressing the importance of *MEG-3* in managing EOC and tailoring the treatment to the individual patient.

Keywords

Epithelial ovarian cancer; *LNC-MEG-3*; Diagnostic marker; Prognostic marker; Survival

1. Introduction

The most prevalent form of ovarian cancer is EOC, and it accounts for the majority of cases. Its incidence tends to increase with age and is most prevalent among women over 50, with the highest occurrence observed in women between 60 and 79. However, it is essential to note that this type of cancer can affect women of all age groups [1]. EOC is a serious medical condition with high mortality rates. Early detection is crucial to improve outcomes and reduce mortality rates associated with EOC. However, since there are no clear manifestations and reliable detection procedures available, cases are often diagnosed at advanced stages with metastasis beyond the ovaries [2]. These challenges significantly impact the prognosis and treatment outcomes for individuals affected by this condition [3]. Biomarkers are crucial for the early detection and diagnosis of EOC.

Long non-coding RNAs (*LNCRNA*) are *RNA* molecules that do not encode proteins but have essential cell regulatory functions. Research has revealed that long non-coding *RNAs* (*LNCRNA*) are not just "junk" molecules but also perform vital functions and help in regulating gene expression in cells [4]. LNCRNA interact with a wide range of molecules, including *DNA*, *RNA* and proteins, as well as their complicated assemblies. Consequently, *LNCRNA* can govern gene expression at diverse stages, encompassing transcription, post-transcription, and chromatin remodeling [5]. Aberrant expression of long non-coding *RNA* (*LNCRNA*) actively contributes to various biological processes associated with the development and progression of cancer [6].

The discovery of *MEG-3*, a maternally expressed gene, is

a significant breakthrough in *LNCRNA* research. *MEG-3* is a primary *LNCRNA* that exhibits tumor suppressor capabilities. Its expression is widespread in various normal human tissues, emphasizing its importance [7]. It has been discovered that the *MEG-3* gene is consistently suppressed in many types of human cancers, including bladder carcinoma [8], carcinoma of the cervix [9], hepatomas [10], brain gliomas [11], and squamous cell carcinoma of the esophagus [12]. *MEG-3* regulates the *miR-376a/RASA1* (*RAS P21* Protein Activator 1) pathway, which is essential for angiogenesis [13]. Exposure to heavy metallic materials, like hexavalent forms of chromium, nickel and cadmium, reduces the expression levels of the gene, which is also involved in metal carcinogenesis [14]. Additionally, *MEG-3* acts as a competitive endogenous *RNA* (*ceRNA*), engaging with the *miR-21/PTEN* (Phosphatase and tensin homolog) pathway to increase cisplatin sensitivity [15].

MEG-3 dysregulation in numerous cancers suggests it could be exploited as a diagnostic and prognostic biological marker [16].

The purpose of this study is to investigate the functions of *LNCRNA-MEG-3* in EOC and evaluate its potential as a biological marker for diagnosis and prognosis. The research results will shed light on the clinical importance of *LNCRNA-MEG-3* in EOC. They could help create new ways to diagnose and treat this devastating disease.

2. Patients and methods

The study was conducted on 126 patients who were diagnosed with epithelial ovarian cancer between June 2018 and August 2023.

2.1 Inclusion criteria

- Confirmed diagnosis of stage III epithelial ovarian cancer (EOC) according to FIGO (International Federation of Gynecology and Obstetrics) guidelines [17] and through histological examination.

- All patients underwent debulking surgery within two months before receiving chemotherapy. Tissue samples were obtained from tumor and adjacent normal ovarian tissues for all patients included in the study.

- Patients who were 18 years old or above.

- Patients with normal blood counts, renal function and hepatic function at the start of the study were enrolled.

- No history of chemotherapy or radiation therapy for ovarian cancer.

2.2 Exclusion criteria

- Low-malignant potential ovarian tumors.

- A score of more than two on the Eastern Cooperative Oncology Group (ECOG) functionality criteria [18].

- Glomerular filtration rate (GFR) lower than 60 mL/minute.

- Severe neuropathy.

- History of congestive heart failure.

- History of arrhythmias.

2.3 Management

All participants involved in the study were administered post-operative chemotherapy, which consisted of the intravenous administration of Paclitaxel at a dosage of 175 mg/m^2 over 3 hours. An intravenous infusion of Cisplatin followed this at 75 mg/m^2 after ensuring the patients received sufficient hydration—the treatment regimen comprised six cycles, which were repeated every three weeks. To monitor the patients, regular gynecological examinations, abdominopelvic ultrasonography, and CA-125 (cancer antigen 125) assays were conducted. In addition, further radiographic studies, such as computed tomography (CT) or magnetic resonance imaging of the abdomen and pelvic, were performed before chemotherapy. As required, these investigations were subsequently repeated every two months to assess the clinical response. The assessments used the RECIST revised (Response Evaluation Criteria in Solid Tumors) criteria [19].

After six sessions of chemotherapy, patients who achieved a complete clinical response underwent a laparoscopy. When laparoscopy failed to reveal any signs of disease, a laparotomy was performed to evaluate the pathological response via multiple biopsies. Based on the pathological evaluation of these biopsies, patients were placed into one of three groups: those with a complete response, those with a partial response indicating only microscopic disease, and those with persistent disease.

2.4 Quantitative real-time polymerase chain reaction of *LNCRNA-MEG-3* expression

The *RNA* extraction process was performed on ovarian tissue samples, comprising nearby normal and cancerous tissues, using the RNase Kit QIAamp RNA Blood Mini Kit, Cat. No. 52304 (Qiagen, Germany). A spectrophotometer was used to measure the optical density (OD) at 260 and 280 nm wavelengths to ensure the purity and concentration of the extracted *RNA*. An acceptable range of the A260/A280 ratio is between 1.8 and 2.1, which indicates *RNA* purity. The High-Capacity cDNA Reverse Transcription kit (cat. no. 4368814; Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA, 02451) was utilized for *cDNA* synthesizing. The synthesis reaction was carried out at 42 °C for 50 minutes, following the recommended protocols provided by the kit. The manufacturer's instructions were strictly adhered to during the entire process.

The expression of *LNCRNA-MEG-3* was analyzed through quantitative polymerase chain reaction (qPCR) using an Applied Biosystems Thermocycler ABI7300 (Real-Time PCR System, Applied Biosystems, Foster, CA, 94404, USA) The normalization of *MEG-3* was performed using *β-actin* as the endogenous control. The primers for *β-actin* were purchased from Invitrogen (Thermo Fisher Scientific, Inc.).

Thermo Fisher Scientific, Inc. manufactured the primers used in the research. Invitrogen provided the primer sequences with the following sequences Table 1.

The qPCR amplification procedure began with a 2-minute denaturation step at 94 °C, then involved 45 amplification phases. Each cycle consisted of denaturation at 95 °C for 30 seconds, annealing at 57.2 °C for 30 seconds, and extension at

TABLE 1. Primers used.

	Forward	Reverse
(*LNCRNA-MEG-3*)	5′-ACATGAGGATCACCCATGT-3′	5′-CATGGGTGATCCTCATGT-3′
β-actin	5′-CGGAGTCAACGGATTTGGTC-3′	5′-AGCCTTCTCCATGGTCGTGA-3′

LNCRNA-MEG-3: Long non-coding RNA (LNCRNA) maternally expressed gene 3.

72 °C for 10 minutes. A reaction mixture with a total volume of 20 μL was prepared. The mixture consisted of 5 μL of *cDNA*, 2.5 μL of *TaqDNA* polymerase obtained from Takara Biotechnology Co., Ltd. in Dalian, China, and 5 μL of SYBR-Green qPCR Master Mix, which contained 200 μM *dNTPs*. The specific primer sequences used had a concentration of 200 μM. The remaining volume of the mixture was made up with distilled water. In order to determine the relative *mRNA* expression, the $2^{-\Delta\Delta Ct}$ method was employed. The expression levels of the target gene were normalized to the expression of *β-actin*.

2.5 Statistical analysis

SPSS Statistics 27.0 from IBM Corp., Armonk, NY, USA, was used to analyze the data. GraphPad Prism 9.0 by GraphPad Software, San Diego, CA, USA, was used to present data. Each experiment was repeated three times to ensure reliability, and the data was presented as a mean value with its corresponding standard deviation. Moreover, paired Student's *t*-test and Analysis of Variance (ANOVA) were utilized to compare *LNCRNA-MEG-3* levels across subgroups and investigate the association between *LNCRNA-MEG-3* levels and demographic and biological variables. Furthermore, to investigate the accuracy of *LNCRNA-MEG-3* in diagnosing EOC. This involved using the Receiver Operating Characteristics (ROC) method. The study also used the Kaplan-Meier technique and Cox proportional hazards regression analysis to determine the correlation between *LNCRNA-MEG-3* expression and survival in EOC patients.

3. Results

3.1 The diagnostic potential of *LNCRNA-MEG-3* expression in distinguishing between benign and malignant ovarian tissues

The level of *LNCRNA-MEG-3* was assessed in specimens obtained from patients with EOC and adjacent normal ovarian tissues. The findings revealed that *LNCRNA-MEG-3* levels were substantially reduced in EOC than in neighboring normal ovarian sections. The mean expression level of *LNCRNA-MEG-3* in EOC tissues was 0.78 $\pm$ 0.3, whereas 1.44 $\pm$ 0.55 in normal ovarian tissues. This difference in expression levels between the two groups was statistically significant ($p < 0.0001$). These results indicate that *LNCRNA-MEG-3* is downregulated in EOC, suggesting that *LNCRNA-MEG-3* may play a suppressor role in EOC, Fig. 1A and Table 2.

ROC curve analysis was conducted to evaluate the diagnostic accuracy of *LNCRNA-MEG-3* in differentiating EOC from NAT. The data demonstrated that *LNCRNA-MEG-3* has a robust discriminatory ability, as evidenced by AUC (the area under the curve) of 0.831. This indicates a strong capability to distinguish between the two tissue types. The statistical significance of these results was further supported by the highly significant *p*-value of less than 0.0001. *LNCRNA-MEG-3* performs exceptionally well at a specific cutoff value of 0.405 in this discriminatory task. It achieves a sensitivity of 100%, correctly identifying all cases of malignant ovarian tissues. *LNCRNA-MEG-3* accurately identifies the majority of normal ovarian tissues as negative, demonstrating its exceptional specificity at 97.04% for classifying positive and negative cases, Fig. 1B.

TABLE 2. *LNCRNA-MEG-3* expression in epithelial ovarian cancer (EOC) and normal adjacent ovarian tissues (NAT).

	NAT	EOC
Number of patients	126	126
Minimum	0.460	0.250
Median	1.440	0.780
Maximum	2.460	1.320
Range	2.000	1.070
Mean	1.436	0.784
Std. Deviation	0.553	0.304
Std. Error of Mean	0.049	0.027
Student's *t*-test	11.60	
p-value	<0.0001	

The expression level of *LNCRNA-MEG-3* in EOC patients varies significantly depending on their clinical features, as illustrated in Table 3. The results show significant variations in *LNCRNA-MEG-3* expression across different stages of EOC. Stage IIIA has the highest mean expression of *LNCRNA-MEG-3* (2.225 $\pm$ 0.13), followed by stages IIIB (1.83 $\pm$ 0.1) and IIIC (1.077 $\pm$ 0.35). The *p*-value obtained (<0.0001) highlights a significant difference in *LNCRNA-MEG-3* expression between the various EOC stages. These findings suggest that there might be a correlation between *LNCRNA-MEG-3* expression and the disease's progression or severity. In patients with EOC, the *LNCRNA-MEG-3* expression is significantly associated with tumor histological grade. Patients with moderately to well-differentiated tumors exhibit a higher *LNCRNA-MEG-3* expression level of 2.01 $\pm$ 0.23 compared to patients with poorly differentiated tumors at 1.07 $\pm$ 0.35. The *p*-value, less than 0.0001, confirms that the difference in *LNCRNA-MEG-3* expression across tumor histopathological grades is statistically significant. *LNCRNA-MEG-3* downregulation is associated with aggressive tumor behavior. Our data anal-

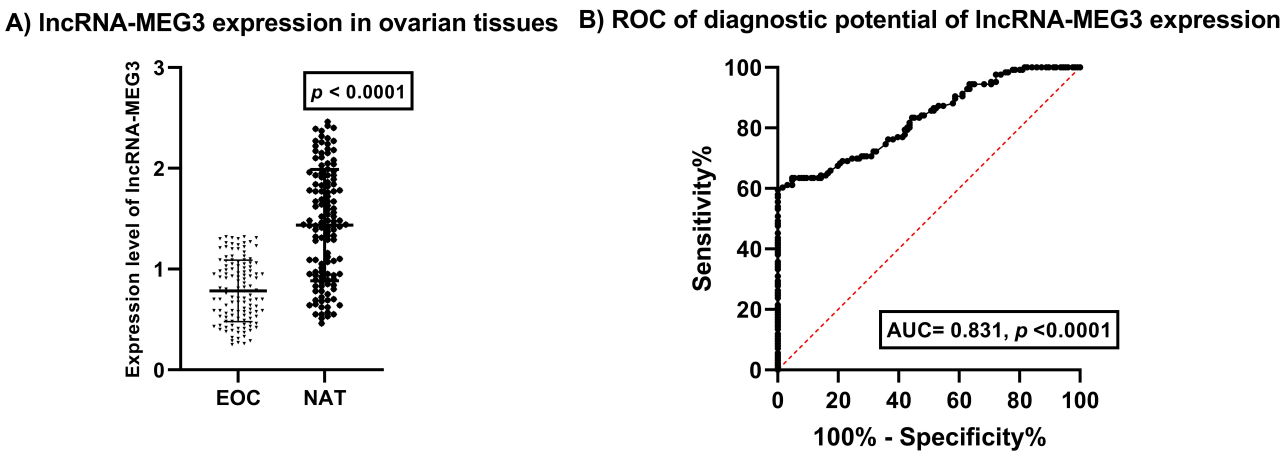

FIGURE 1. ***LNCRNA-MEG-3*** **expression and diagnostic precision in epithelial ovarian cancer tissues.** ROC: Receiver Operating Characteristics; EOC: epithelial ovarian cancer; NAT: normal adjacent ovarian tissues; AUC: area under the curve; *LNCRNA-MEG-3*: Long non-coding *RNA* (*LNCRNA*) maternally expressed gene 3.

TABLE 3. Relationship between *LNCRNA-MEG-3* expression and clinical characteristics and prognostic factors in epithelial ovarian cancer (EOC).

	No	%	LNCRNA-MEG-3		*t*-test	*p*
			Mean	SD		
Stage						
IIIA	23	18.3	2.225	0.128		
IIIB	25	19.8	1.828	0.101	173.2*	<0.0001
IIIC	78	61.9	1.077	0.349		
Grade						
Moderate + Well differentiated	49	38.9	2.01	0.234	16.74	<0.0001
Poorly differentiated	77	61.1	1.07	0.346		
Pathology						
Serous	81	64.3	1.782	0.344	17.48	<0.0001
Non-serous	45	35.7	0.813	0.187		
Ascites						
Absent	47	37.3	2.026	0.227	16.34	<0.0001
Present	79	62.6	1.085	0.353		
Response						
CR, Microscopic disease, PR	65	51.6	1.889	0.2971	17.92	<0.0001
No response or progressive disease	61	48.4	0.953	0.2884		
Residual Disease						
>2 cm	47	37.3	0.830	0.201	17.85	<0.0001
<2 cm	79	62.6	1.796	0.337		

**Analysis of variance (ANOVA); CR: Complete response; PR: partial response; SD: Standard Deviation; LNCRNA-MEG-3: Long non-coding RNA maternally expressed gene 3.*

ysis revealed a strong relationship between *LNCRNA-MEG-3* level and the different tumor types identified in EOC patients. Notably, patients with serous tumor types exhibit a significantly elevated mean *LNCRNA-MEG-3* expression level (1.78 ± 0.34) compared to non-serous tumor types (0.813 ± 0.19). The remarkably low *p*-value (<0.0001) underscores the statistical significance of this disparity. This data shows that *LNCRNA-MEG-3* expression could be used to predict tumor aggressiveness in EOC patients.

Our study found a correlation between *LNCRNA-MEG-3* levels and ascites in EOC patients. Ascites is commonly known as an adverse prognostic factor for EOC. Patients without ascites display higher levels of *LNCRNA-MEG-3* expression (2.03 ± 0.23) compared to those with ascites (1.09 ± 0.35). The low *p*-value (less than 0.0001) further highlights the statistical importance of this correlation. These findings imply that low *LNCRNA-MEG-3* expression levels may be linked to unfavorable prognostic indicators in EOC, such as the presence of ascites. We identified a strong relationship between *LNCRNA-MEG-3* expression and treatment response in patients with EOC. Patients who responded well to treatment had higher *LNCRNA-MEG-3* expression levels (1.89 ± 0.3) than non-responders (0.95 ± 0.29). The statistical significance of the difference in *LNCRNA-MEG-3* expression between patients who responded to treatment and those who did not was confirmed by the *p*-value (<0.0001). Based on the results, the expression of *LNCRNA-MEG-3* in EOC may correlate with the response to treatment. Higher *LNCRNA-MEG-3* expression levels appear to correlate with a more favorable response to treatment. According to this data, *LNCRNA-MEG-3* could be a prognostic EOC biomarker.

Our findings indicate a strong link between the level of *LNCRNA-MEG-3* and the size of residual disease in EOC patients who have undergone debulking surgery. According to our study, patients with a residual disease size exceeding 2 cm display a lower level of *LNCRNA-MEG-3* expression (0.83 ± 0.2) compared to those with a residual disease size below 2 cm (1.8 ± 0.34). The *p*-value, which is less than 0.0001, confirms the statistical significance of this difference. The extent of residual disease post-debulking surgery is a crucial prognostic factor in EOC.

3.2 *LNCRNA-MEG-3* expression and its influence on survival in epithelial ovarian cancer patients

The long non-coding *RNA MEG-3* expression significantly impacts the overall survival of patients with EOC. The Log-rank (Mantel-Cox) test resulted in a highly significant chi-square value of 12.56 and a *p*-value of 0.0004, indicating a significant disparity in survival between the two groups (low *LNCRNA-MEG-3* and high *LNCRNA-MEG-3*).

Furthermore, patients with high *LNCRNA-MEG-3* expression have a notably higher median survival of 58.00 compared to 38.00 for those with low *LNCRNA-MEG-3* expression.

Patients with higher *LNCRNA-MEG-3* expression have a substantially lower risk of mortality, as indicated by the Mantel-Haenszel Hazard Ratio of 3.889, in comparison to those with lower *LNCRNA-MEG-3* expression. The Log-rank Hazard Ratio of 3.054 shows a similar trend.

Patients with EOC express *LNCRNA-MEG-3*, which significantly impacts their disease-free survival. The Log-rank (Mantel-Cox) test indicates a highly significant chi-square value of 13.81 and a *p*-value of 0.0002, implying a notable difference in disease-free survival between low- *LNCRNA-MEG-3* and high- *LNCRNA-MEG-3* groups Fig. 2A.

The median survival comparison found that patients having higher *LNCRNA-MEG-3* expression have a considerably longer median disease-free survival of 64.00, compared to 36.00 for those with low *LNCRNA-MEG-3* expression. The Hazard Ratios further confirm the differences in disease-free survival. The Mantel-Haenszel Hazard Ratio is 4.227 (A/B), indicating that high *LNCRNA-MEG-3* expression patients have a considerably lower risk of disease recurrence than low *LNCRNA-MEG-3* expression patients. The Log-rank Hazard Ratio is 3.187, confirming a similar trend in Fig. 2B.

High *LNCRNA-MEG-3* expression is a valuable predictor of survival in EOC, associated with improved outcomes.

4. Discussion

EOC is often diagnosed in advanced stages due to the lack of early signs and effective detection methods, making it a significant threat [1]. The aggressive nature of this cancer, limited treatment options, and high mortality rate only add to the risks. Recurrence is a common issue, and EOC can have significant physical and psychological effects on individuals [20]. To reduce these risks, early detection and proactive healthcare management are crucial for improving outcomes [21].

The aim of this study is to investigate the role of *LNCRNA-MEG-3* in epithelial ovarian cancer (EOC) and assess its potential as a diagnostic and prognostic biomarker. Furthermore, the study examined the correlation between *LNCRNA-MEG-3* and the clinical outcomes as well as clinicopathological characteristics of EOC.

The aim of this study was to assess the levels of *LNCRNA-MEG-3* in both EOC samples and corresponding healthy ovarian epithelial samples from the same subjects. Our results showed a significant reduction in *LNCRNA-MEG-3* levels in malignant tissue compared to healthy cells. This significant downregulation of *LNCRNA MEG-3* suggests its potential tumor-suppressor role, contributing to ovarian cancer pathogenesis. Downregulation of *LNCRNA-MEG-3* has been noted in various cancers, including squamous cell carcinoma of the head and neck [22], gall bladder adenocarcinoma [23], colorectal adenocarcinomas [24], lung adenocarcinoma [25], cervical cancer [26], breast cancer [27], brain gliomas [28] and Ovarian carcinoma [29].

As part of our research, we analyzed the effectiveness of *LNCRNA-MEG-3* in identifying malignant and normal ovarian tissue. Our results have identified *LNCRNA-MEG-3* as a dependable biomarker with high discriminatory ability. These findings highlight the importance of *LNCRNA-MEG3* as a valuable diagnostic marker for detecting EOC. *LNCRNA-MEG-3* expression levels were markedly decreased in colorectal carcinoma (CRC) tissue and serum. The sensitivity and specificity of serum *LNCRNA-MEG-3* levels in CRC

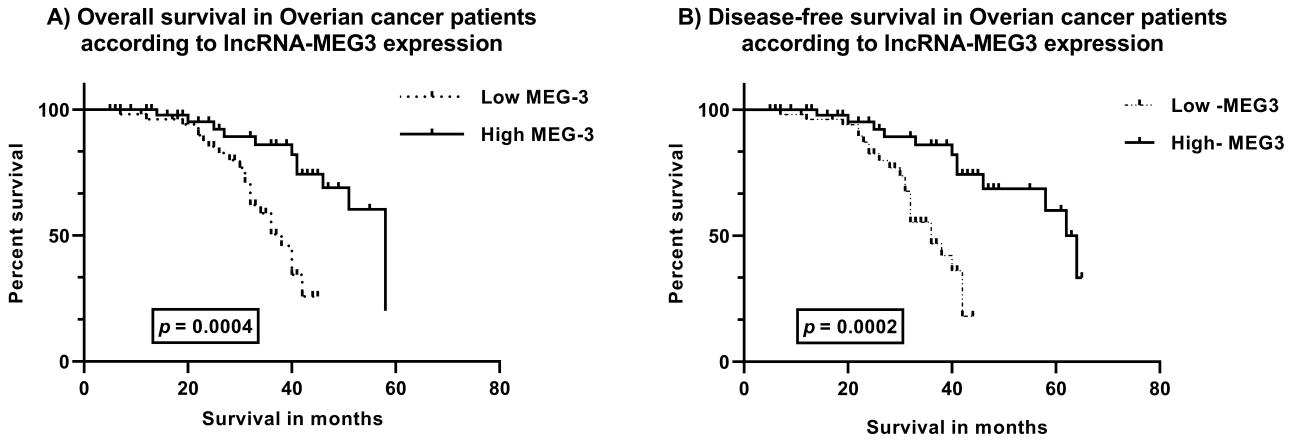

FIGURE 2. ***LNCRNA-MEG-3*** **expression and its influence on survival in epithelial ovarian cancer patients.** *LNCRNA-MEG-3*: Long non-coding RNA maternally expressed gene 3.

diagnosis were remarkably high, indicating its reliability as a diagnostic marker for CRC [30]. Wan *et al.* [31] discovered that patients with cervical carcinoma had significantly lower levels of *MEG-3* than the normal group. *LNCRNA-MEG-3* demonstrated a strong ability to differentiate between cervical cancer cases and controls, suggesting that it could be an accurate detection marker for cervical cancer. These findings highlight the specificity and sensitivity of *LNCRNA-MEG-3*, which make it a promising candidate for cancer diagnosis [31]. Our study, along with previous research, provides valuable insights for further investigation and underscores the reliable potential of *LNCRNA-MEG-3* in cancer diagnosis.

Our findings show that levels of *LNCRNA-MEG-3* differ significantly based on clinical characteristics among EOC patients. We discovered that *LNCRNA-MEG-3* downregulation was strongly linked to advanced clinical stage, poorly differentiated histopathology, and aggressive tumor types. Additionally, we found that its downregulation correlated with unfavorable prognostic factors such as non-responsiveness or disease progression during treatment, residual disease exceeding 2 cm after debulking surgery, and the presence of ascites. Our study has shown that patients with high levels of *LNCRNA-MEG-3* responded well to the treatment. In breast cancer, Li *et al.* [32] reported that low levels of *MEG-3* were associated with an increase in chemoresistance and a decrease in response to neoadjuvant therapy. Similarly, low levels of *MEG-3* are linked with increased chemoresistance in pancreatic and small-cell lung cancer [33, 34]. These results demonstrate the potential of *LNCRNA-MEG-3* as a valuable prognostic biomarker in EOC. Wan *et al.* [31] found a significant link between low *LNCRNA-MEG-3* levels and adverse clinical characteristics in cervical cancer. The study included 84 patients and revealed that decreased *LNCRNA-MEG-3* expression was linked to lymph node spread, vaginal wall invasion, and progressed FIGO staging [31]. In CRC patients, a strong correlation exists between serum *LNCRNA-MEG-3* levels, tumor size and clinical stage. According to the study findings, patients with lower serum *LNCRNA-MEG3* levels are more likely to develop extensive malignancies and stages of CRC [30]. *MEG-3* is strongly related to unfavorable outcome in hepatocellular carcinoma (HCC) by Hussein *et al.* [35] This discovery suggests that *MEG-3* levels could be a prognostic biomarker for predicting unfavorable clinical outcomes in HCC patients. Moreover, in breast carcinoma, low *LNCRNA-MEG-3* level was linked to poor prognostic features as high FIGO stage and large tumor size >5 m [36]. *LNCRNA-MEG-3* is a useful diagnostic and prognostic marker in OEC cancer. Its identification can facilitate the development of more effective treatment strategies. Further research in this field can help devise better diagnostic and treatment approaches for patients with EOC.

Our study has shown a significant association between the downregulation of *LNCRNA-MEG-3* and short overall and disease-free survival in EOC. Specifically, we found that patients with reduced levels of *LNCRNA-MEG-3* had a median overall survival of 38 months, which is lower than the 58 months observed in those with high *LNCRNA-MEG-3* expression. Similarly, patients with higher *LNCRNA-MEG-3* had a disease-free survival of 64 months, while those with lower *LNCRNA-MEG-3* had a survival of 36 months. Our findings highlight the importance of *LNCRNA-MEG-3* expression levels as a prognostic factor in EOC, where lower expression indicates poorer survival outcomes. Buttarelli *et al.* [37] reported that higher *LNCRNA-MEG-3* expression was independently related to improved disease-free survival and overall survival in high-grade serous ovarian cancer (HGSOC) patients. A study on cervical cancer survival rates showed significant variation in patients with higher and lower *LNCRNA-MEG-3* expression. Patients with lower *LNCRNA-MEG-3* expression lived significantly shorter than those with higher *LNCRNA-MEG-3* expression [31]. Moreover, increased levels of *LNCRNA-MEG-3* were associated with significantly higher overall survival rates in colorectal cancer patients [30].

Our findings and previous research emphasize the importance of *LNCRNA-MEG-3* expression as a biological marker, showing its potential as a valuable biomarker for the prediction

of patient outcomes. These findings suggest that *LNCRNA-MEG-3* can be used as a prognostic marker.

5. Conclusions

The study reveals that *LNCRNA-MEG-3* has the potential to differentiate between EOC and normal ovarian tissues. Its expression is notably reduced in EOC, indicating its potential application as a diagnostic marker. Additionally, low levels of *LNCRNA-MEG-3* have a strong correlation with adverse prognostic factors and shorter survival in patients with EOC. These data emphasize the significance of *LNCRNA-MEG-3* as a promising marker for identifying EOC and predicting the outcome.

AVAILABILITY OF DATA AND MATERIALS

The data will be available on request from the corresponding author.

AUTHOR CONTRIBUTIONS

AbAA, AFG, WHE, FAA, AA—conceived and designed experiments, drafted the manuscript; HJB, SSA, RLE, AmAA—contributed to the analysis and/or interpretation of data; AFG, WHE, FAA, AA, AbAA, AmAA—revised the manuscript for important intellectual content. All authors have read the manuscript and approved the submission.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Committee of Ethics of Research at Zagazig University approved the study (ZU-256/2018), and all participating patients provided written informed consent before enrollment.

ACKNOWLEDGMENT

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project No. TU-DSPP-2024-54.

FUNDING

This research was funded by Taif University, Saudi Arabia, Project No. TU-DSPP-2024-54.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Gaona-Luviano P, Medina-Gaona LA, Magaña-Pérez K. Epidemiology of ovarian cancer. Chinese Clinical Oncology. 2020; 9: 47.

[2] Chakraborty S, Shenoy PS, Mehrotra M, Phadte P, Singh P, Rekhi B, *et al*. Through the looking glass: updated insights on ovarian cancer diagnostics. Diagnostics. 2023; 13: 713.

[3] Leone Roberti Maggiore U, Bogani G, Martinelli F, Signorelli M, Chiappa V, Lopez S, *et al*. Response to treatment and prognostic significance of supradiaphragmatic disease in patients with high-grade serous ovarian cancer. European Journal of Surgical Oncology. 2022; 48: 2551–2557.

[4] Yao Z, Yang Y, Sun M, He Y, Liao L, Chen K, *et al*. New insights into the interplay between long non-coding RNAs and RNA-binding proteins in cancer. Cancer Communications. 2022; 42: 117–140.

[5] Zhang L, Zhao F, Li W, Song G, Kasim V, Wu S. The biological roles and molecular mechanisms of long non-coding RNA MEG3 in the hallmarks of cancer. Cancers. 2022; 14: 6032.

[6] Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. Cancer Research. 2017; 77: 3965–3981.

[7] Chang L, Wang G, Jia T, Zhang L, Li Y, Han Y, *et al*. Armored long non-coding RNA MEG3 targeting EGFR based on recombinant MS2 bacteriophage virus-like particles against hepatocellular carcinoma. Oncotarget. 2016; 7: 23988–24004.

[8] Tantray I, Ojha R, Sharma AP. Non-coding RNA and autophagy: finding novel ways to improve the diagnostic management of bladder cancer. Frontiers in Genetics. 2023; 13: 1051762.

[9] Kumar A, Girisa S, Alqahtani MS, Abbas M, Hegde M, Sethi G, *et al*. Targeting autophagy using long non-coding RNAs (LncRNAs): new landscapes in the arena of cancer therapeutics. Cells. 2023; 12: 810.

[10] Wu J, Zhu Y, Cong Q, Xu Q. Non-coding RNAs: role of miRNAs and lncRNAs in the regulation of autophagy in hepatocellular carcinoma. Oncology Reports. 2023; 49: 1–14.

[11] Lu E, Gareev I, Yuan C, Liang Y, Sun J, Chen X, *et al*. The mechanisms of current platinum anticancer drug resistance in the glioma. Current Pharmaceutical Design. 2022; 28: 1863–1869.

[12] Dong Z, Zhang A, Liu S, Lu F, Guo Y, Zhang G, *et al*. Aberrant methylation-mediated silencing of lncRNA MEG3 functions as a ceRNA in esophageal cancer. Molecular Cancer Research. 2017; 15: 800–810.

[13] Li Y, Zhang L, Zhao Y, Peng H, Bai W, Zhang N. MEG3 Sponges miRNA-376a and YBX1 to regulate angiogenesis in ovarian cancer endothelial cells. Heliyon. 2023; 9: e13204.

[14] Zhang Z, Shi S, Li J, Costa M. Long non-coding RNA MEG3 in metal carcinogenesis. Toxics. 2023; 11: 157.

[15] Du Y, Geng G, Zhao C, Gao T, Wei B. LncRNA MEG3 promotes cisplatin sensitivity of cervical cancer cells by regulating the miR-21/PTEN axis. BMC Cancer. 2022; 22: 1145.

[16] Orafidiya F, Deng L, Bevan CL, Fletcher CE. Crosstalk between Long non coding RNAs, microRNAs and DNA damage repair in prostate cancer: new therapeutic opportunities? Cancers. 2022; 14: 755.

[17] Kehoe S. FIGO staging in ovarian carcinoma and histological subtypes. Journal of Gynecologic Oncology. 2020; 31: e70.

[18] Sehgal K, Gill RR, Widick P, Bindal P, McDonald DC, Shea M, *et al*. Association of performance status with survival in patients with advanced non-small cell lung cancer treated with Pembrolizumab monotherapy. JAMA Network Open. 2021; 4: e2037120.

[19] Armato SG, Nowak AK. Revised modified response evaluation criteria in solid tumors for assessment of response in malignant pleural mesothelioma (Version 1.1). Journal of Thoracic Oncology. 2018; 13: 1012–1021.

[20] Jiang X, Li W, Li X, Bai H, Zhang Z. Current status and future prospects of PARP inhibitor clinical trials in ovarian cancer. Cancer Management and Research. 2019; 11: 4371–4390.

[21] Chien J, Poole EM. Ovarian cancer prevention, screening, and early detection. International Journal of Gynecological Cancer. 2017; 27: S20–S22.

[22] Ji Y, Feng G, Hou Y, Yu Y, Wang R, Yuan H. Long noncoding RNA MEG3 decreases the growth of head and neck squamous cell carcinoma by regulating the expression of miR-421 and E-cadherin. Cancer medicine. 2020; 9: 3954–3963.

[23] Bao D, Yuan RX, Zhang Y. Effects of lncRNA MEG3 on proliferation and apoptosis of gallbladder cancer cells through regulating NF-κB signaling pathway. European Review for Medical & Pharmacological Sciences. 2020; 24: 6632–6638.

[24] Wang G, Ye Q, Ning S, Yang Z, Chen Y, Zhang L, *et al*. LncRNA MEG3 promotes endoplasmic reticulum stress and suppresses proliferation and invasion of colorectal carcinoma cells through the MEG3/miR-103a-3p/PDHB ceRNA pathway. Neoplasma. 2021; 68: 362–374.

[25] Zhao Y, Zhu Z, Shi S, Wang J, Li N. Long non-coding RNA MEG3 regulates migration and invasion of lung cancer stem cells via miR-650/SLC34a2 axis. Biomedicine & Pharmacotherapy. 2019; 120: 109457.

[26] Pan X, Cao Y, Liu J, Ding J, Xie X, Cao P. MEG3 induces cervical carcinoma cells' apoptosis through endoplasmic reticulum stress by miR-7-5p/STC1 axis. Cancer Biotherapy and Radiopharmaceuticals. 2021; 36: 501–510.

[27] Shaker O, Ayeldeen G, Abdelhamid A. The impact of single nucleotide polymorphism in the long non-coding MEG3 gene on microRNA-182 and microRNA-29 expression levels in the development of breast cancer in Egyptian women. Frontiers in Genetics. 2021; 12: 683809.

[28] Qin WX, Shi Y, Zhu D, Li YP, Chen YH, Cui J, *et al*. EZH2-mediated H3K27me3 enrichment on the lncRNA MEG3 promoter regulates the growth and metastasis of glioma cells by regulating miR-21-3p. European Review for Medical & Pharmacological Sciences. 2020; 24: 3204–3214.

[29] Tao P, Yang B, Zhang H, Sun L, Wang Y, Zheng W. The overexpression of lncRNA MEG3 inhibits cell viability and invasion and promotes apoptosis in ovarian cancer by sponging miR-205-5p. International Journal of Clinical and Experimental Pathology. 2020; 13: 869.

[30] Wang W, Xie Y, Chen F, Liu X, Zhong L, Wang H, *et al*. LncRNA MEG3 acts a biomarker and regulates cell functions by targeting ADAR1 in colorectal cancer. World Journal of Gastroenterology. 2019; 25: 3972–3984.

[31] Wan S, Zhao H. Analysis of diagnostic and prognostic value of lncRNA MEG3 in cervical cancer. Oncology Letters. 2020; 20: 183.

[32] Li H, Wang P, Liu J, Liu W, Wu X, Ding J, *et al*. Hypermethylation of lncRNA MEG3 impairs chemosensitivity of breast cancer cells. Journal of Clinical Laboratory Analysis. 2020; 34: e23369.

[33] Xie W, Chu M, Song G, Zuo Z, Han Z, Chen C, *et al*. Emerging roles of long noncoding RNAs in chemoresistance of pancreatic cancer. Seminars in Cancer Biology. 2022; 83: 303–318.

[34] Sun Y, Hao G, Zhuang M, Lv H, Liu C, Su K. MEG3 LncRNA from exosomes released from cancer-associated fibroblasts enhances cisplatin chemoresistance in SCLC via a MiR-15a-5p/CCNE1 axis. Yonsei Medical Journal. 2022; 63: 229.

[35] Hussein YM, Ghareib AF, Mohamed RH, Radwan MI, Elsawy WH. MAGE-3 and MAGE-4 genes as possible markers for early detection of metastases in hepatitis C virus Egyptian patients complicated by hepatocellular carcinoma. Medical Oncology. 2012; 29: 994–999.

[36] Ali MA, Shaker OG, Alazrak M, AbdelHafez MN, Khalefa AA, Hemeda NF, *et al*. Association analyses of a genetic variant in long non-coding RNA MEG3 with breast cancer susceptibility and serum MEG3 expression level in the Egyptian population. Cancer Biomarkers. 2020; 28: 49–63.

[37] Buttarelli M, De Donato M, Raspaglio G, Babini G, Ciucci A, Martinelli E, *et al*. Clinical value of lncRNA MEG3 in high-grade serous ovarian cancer. Cancers. 2020; 12: 966.

How to cite this article: Abdulraheem A. Almalki, Amal F. Gharib, Saad S. Al-Shehri, Ahmed Alghamdi, Amani A. Alrehaili, Hamsa Jameel Banjer, *et al*. Exploring the potential of LNCRNA-MEG-3 as a diagnostic and prognostic marker in epithelial ovarian cancer. European Journal of Gynaecological Oncology. 2024; 45(5): 83-90. doi: 10.22514/ejgo.2024.095.