ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Long non-coding RNA MEG3 acts as a tumor inhibitor in human cancer

Yan Li and Yan Wang*

Department of Obstetrics and Gynecology, First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China

Abstract

Introduction: The role of MEG3 in human cancers remains incompletely understood.

Objective: In this study, we elucidate the tumor-suppressive function of MEG3 across multiple cancer types, including ovarian cancer.

Methods: We systematically analyzed MEG3 expression in both tumor and normal tissues using publicly available databases to investigate its regulatory implications for clinical prognosis, pathological stage, tumor purity, chemotherapy resistance, and immune cell infiltration. Furthermore, we explored the underlying regulatory mechanisms of MEG3 by predicting upstream transcription factors and conducting downstream functional enrichment analyses.

Results: Our findings demonstrate that MEG3 expression is significantly downregulated in tumor tissues compared to normal counterparts. Moreover, MEG3 expression is associated with overall survival, relapse-free survival, and tumor, node, metastasis staging across several malignancies. Notably, higher MEG3 expression is negatively correlated with tumor purity and chemotherapy resistance. We identified SPI1 as a potential upstream transcription factor regulating MEG3 expression. Functional enrichment analysis revealed that MEG3 may exert its tumor-suppressive effects through the modulation of the Hippo signaling pathway. In addition, MEG3 methylation was found to regulate the expression of genes involved in the cell cycle of ovarian cancer cells and modulate the ovarian cancer microenvironment by influencing immune cell infiltration.

Conclusion: These results collectively suggest that MEG3 may serve as a tumor suppressor and represent a promising therapeutic target in human cancers.

Keywords: MEG3; Ovarian cancer; Suppressor; Tumor purity; Immune infiltration

***Corresponding author:**
Yan Wang
(docwyan@126.com)

Citation: Li Y, Wang Y. Long non-coding RNA MEG3 acts as a tumor inhibitor in human cancer. *Eurasian J Med Oncol*. 2026;10(5):025510529. doi: 10.36922/EJMO025510529

Received: December 18, 2025

Revised: January 8, 2026

Accepted: January 13, 2026

Published online: February 13, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Long non-coding RNAs (lncRNAs) are RNAs with a length of more than 200 nucleotides and without encoding peptides.[1] In recent years, lncRNAs have become significant regulators in gene expression networks by controlling nuclear structure and transcription, regulating messenger RNA (mRNA) stability, translation, and post-translational modification in the cytoplasm.[2] Recent studies have shown that lncRNAs are related to drug sensitivity and tumor purity, drawing broad consideration in the field of cancer research.[3] An enormous number of studies have shown that the abnormal expression of

lncRNAs produces a marked effect in tumorigenesis and metastasis. The role of lncRNAs acting as oncogenes or tumor suppressors in cancer remains controversial because they assume various roles in different cancers.[4]

The lncRNA maternally expressed gene 3 (MEG3), located on human chromosome 14q32, comprises 10 exons and generates at least 12 distinct splice variants through alternative splicing.[5] Accumulating evidence indicates that MEG3 suppresses tumor cell growth, migration, and invasion while promoting apoptosis through multiple molecular mechanisms.[6,7] Studies have demonstrated that downregulation of MEG3 induces autophagy and enhances cell proliferation in bladder cancer.[8] Conversely, restoration of MEG3 expression inhibits cancer cell proliferation and triggers apoptotic pathways.[9] Moreover, MEG3 overexpression has been shown to significantly reduce tumor proliferation, metastasis, and recurrence. Mechanistically, MEG3 contributes to the stabilization and accumulation of the tumor suppressor p53[10] and modulates genes involved in the transforming growth factor-beta signaling pathway, which play critical roles in cell invasion and immune regulation.[11] In addition, MEG3 interacts with various microRNAs (miRs),[12,13] such as upregulating miR-211 to inhibit the phosphoinositide 3-kinase (PI3K)/Akt and adenosine monophosphate-activated protein kinase (AMPK) signaling pathways, thereby suppressing cell proliferation, migration, and invasion, and inducing apoptosis.[14]

In this study, we systematically investigated the functional role of MEG3 in ovarian cancer by comparing its expression levels between human ovarian cancer tissues and adjacent normal tissues. We further evaluated the association between MEG3 expression and clinical prognosis, tumor purity, and its potential implications for chemotherapy selection. Subsequently, we predicted upstream transcription factors regulating MEG3 expression and performed functional enrichment analysis in the context of ovarian cancer. Finally, we examined the correlation between MEG3 expression and immune cell infiltration within the tumor microenvironment. This research contributes to the understanding of the antitumor impact of MEG3 in human cancer and guides the prevention of tumor development and clinical treatment.

2. Materials and methods

2.1. Gene Expression Profiling Interactive Analysis (GEPIA) database

The GEPIA web server (http://gepia.cancer-pku.cn) is a publicly accessible platform designed for the analysis of gene expression profiles in tumor and normal tissues based on data from The Cancer Genome Atlas (TCGA). In this study, GEPIA was utilized to validate the expression levels of MEG3 across various human cancers and corresponding normal tissues. In addition, the association between MEG3 expression and tumor, node, metastasis (TNM) stage was assessed using this platform.

2.2. Kaplan–Meier plotter database

The Kaplan–Meier plotter (http://kmplot.com/analysis/index.php?p=background) is an online platform for survival analysis that provides comprehensive and well-curated datasets. It was employed in this study to evaluate overall survival (OS) and relapse-free survival (RFS) outcomes. We chose the best cutoff as the threshold to distinguish high-risk and low-risk groups.

2.3. LinkedOmics database

The association between MEG3 expression and tumor purity across multiple cancer types was investigated using the LinkedOmics platform (http://www.linkedomics.org/login.php). This platform was further utilized to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of genes correlated with MEG3 expression in ovarian cancer. The GO analysis encompassed three domains: Biological process (BP), cellular component (CC), and molecular function (MF).

2.4. Cancer Cell Line Encyclopedia (CCLE) database

The CCLE database (https://sites.broadinstitute.org/ccle) is a comprehensive online resource that provides gene expression, methylation, gene mutation, copy number variation, and chemoresistance data for various cancer cell lines. It was utilized in this study to investigate resistance to chemotherapeutic agents and to elucidate the regulatory impact of MEG3 methylation on the expression of associated genes.

2.5. Prediction and verification of MEG3 transcription factor

The JASPAR database (http://jaspar.genereg.net) was employed to predict transcription factors and their corresponding binding sites that may regulate MEG3 expression by analyzing the nucleotide sequence of the MEG3 promoter region. The predicted results were subsequently validated using the Cistrome Data Browser (http://cistrome.org/db/).

2.6. Tumor Immune Estimation Resource (TIMER) database

TIMER (http://timer.cistrome.org) is a comprehensive resource for the systematic analysis of immune infiltration across diverse cancer types. It was applied to analyze the correlation between MEG3 expression, immune infiltration, and immune cell biomarkers in ovarian cancer.

2.7. Statistical analysis

Statistical analyses were performed automatically through the online databases utilized in this study. A $p<0.05$ was considered statistically significant, and results with a false discovery rate <0.05 were regarded as statistically noteworthy.

3. Results

3.1. The expression of MEG3 is downregulated in human pan-cancer and is related to OS, RFS, and TNM stage

Analysis of data from TCGA revealed that MEG3 expression was downregulated in the majority of cancer types compared to corresponding non-tumor tissues, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), kidney chromophobe (KICH), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma, lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma. The difference in MEG3 expression was particularly pronounced in gynecological malignancies (Figure 1). Furthermore, survival analysis demonstrated that MEG3 expression was significantly associated with OS and RFS. Patients with high MEG3 expression exhibited poorer OS in BLCA, KIRP, STAD, and THCA, as well as reduced RFS in CESC and STAD, compared to those with low expression. In contrast, elevated MEG3 expression was linked to improved OS in PAAD and READ, and enhanced RFS in BLCA, LIHC, LUSC, and PAAD (Figure 2). In addition, MEG3 expression in TNM stage II tumors was significantly lower than in stage I tumors in certain cancers,

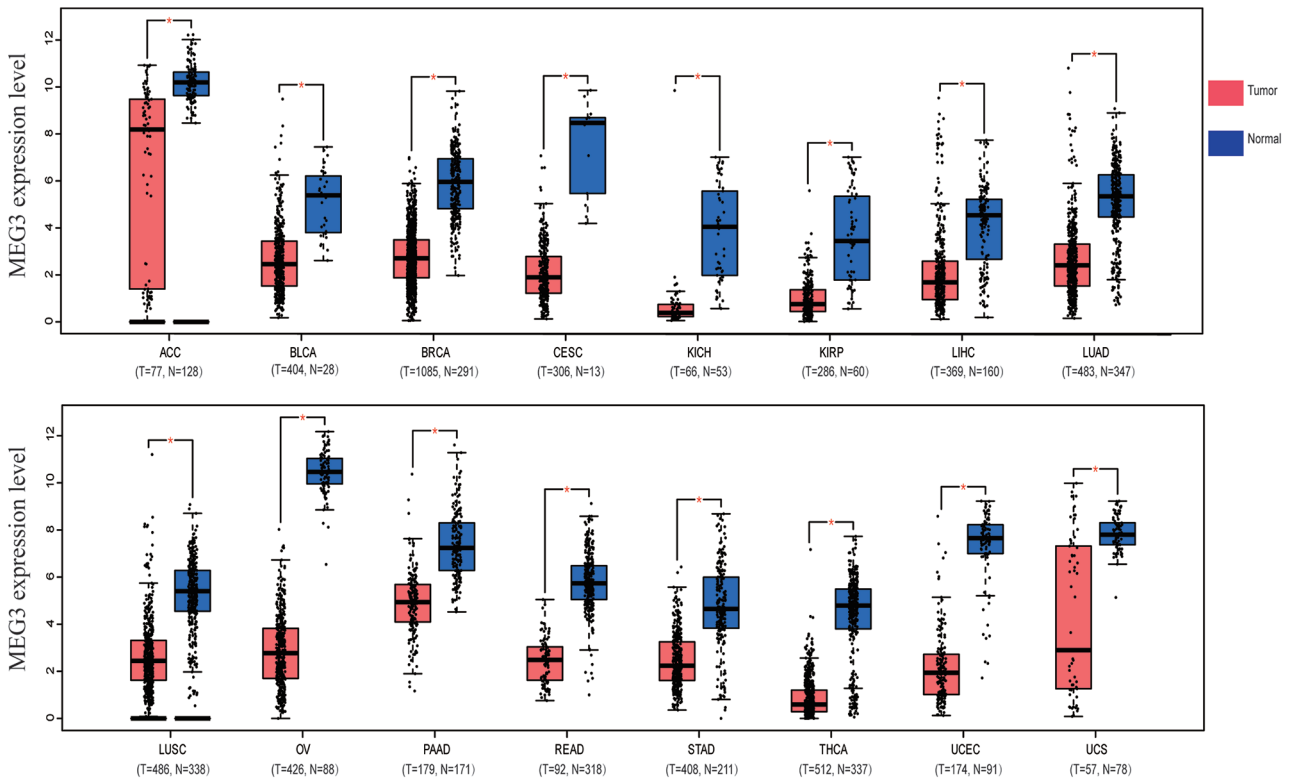


Figure 1. MEG3 expression is downregulated in human pan-cancers, including ACC, BLCA, BRCA, CESC, KICH, KIRP, LIHC, LUAD, LUSC, OV, PAAD, READ, STAD, THCA, UCEC, and UCS. The red columns indicate the expression levels of MEG3 in cancer tissues, and the blue columns represent normal tissues.

Note: *$p<0.05$.

Abbreviations: ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; KICH: Kidney chromophobe; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MEG3: Maternally expressed gene 3; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; READ: Rectum adenocarcinoma; STAD: Stomach adenocarcinoma; THCA: Thyroid carcinoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma.

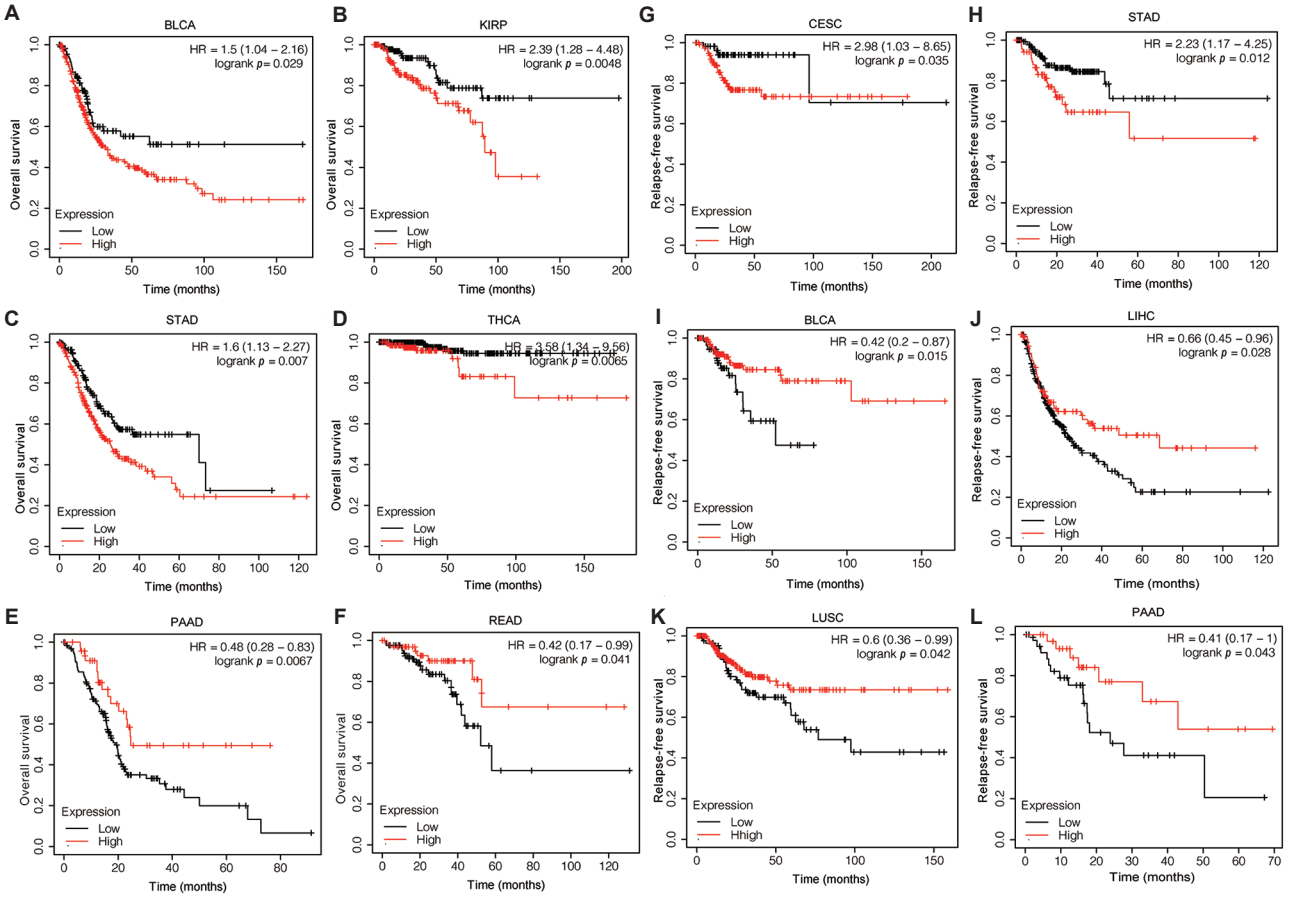


Figure 2. MEG3 expression is associated with OS and RFS. High expression of MEG3 correlates with worse OS in BLCA (A), KIRP (B), STAD (C), THCA (D), as well as better OS in PAAD (E), READ (F) than those with low expression levels. High expression of MEG3 correlates with worse RFS in CESC (G), STAD (H), as well as better RFS in BLCA (I), LIHC (J), LUSC (K), PAAD (L) than those with low expression levels.
Note: All $p<0.05$.
Abbreviations: BLCA: Bladder urothelial carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; HR: Hazard ratio; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUSC: Lung squamous cell carcinoma; MEG3: Maternally expressed gene 3; OS: Overall survival; PAAD: Pancreatic adenocarcinoma; READ: Rectum adenocarcinoma; RFS: Relapse-free survival; STAD: Stomach adenocarcinoma; THCA: Thyroid carcinoma.

including ACC, KICH, THCA, and BRCA. Similarly, in ovarian cancer (e.g., OV), MEG3 levels were lower in TNM stage IIB compared to stage IIA (Figure 3).

3.2. MEG3 negatively affects the purity of tumors

The LinkedOmics platform was further utilized to investigate the association between MEG3 expression and tumor purity. The results revealed that high MEG3 expression was correlated with reduced tumor purity in BLCA (Spearman correlation = −0.35), BRCA (Spearman correlation = −0.37), CESC (Spearman correlation = −0.24), KICH (Spearman correlation = −0.51), OV (Spearman correlation = −0.13), PRAD (Spearman correlation = −0.30), THCA (Spearman correlation = −0.44), and UCEC (Spearman correlation = −0.30). These findings suggest that MEG3 negatively regulates tumor purity (Figure 4).

3.3. MEG3 inhibits chemoresistance in human cancer

The CCLE database was utilized to explore the association between MEG3 expression and chemotherapeutic resistance across pan-cancer types. The results showed that high MEG3 expression was correlated with lower IC50 values for dactinomycin (Pearson correlation = −0.45), tozasertib (Pearson correlation = −0.33), ERK5-IN-1 (Pearson correlation = −0.24), paclitaxel (Pearson correlation = −0.19), YK-4-279 (Pearson correlation = −0.18), APR-246 (Pearson correlation = −0.17), lapatinib (Pearson correlation = −0.14), dinaciclib (Pearson correlation = −0.13), and afatinib (Pearson correlation = −0.13), indicating enhanced drug sensitivity (Figure 5). These findings suggest that MEG3 may exert antitumor effects by reversing resistance to chemotherapeutic agents.

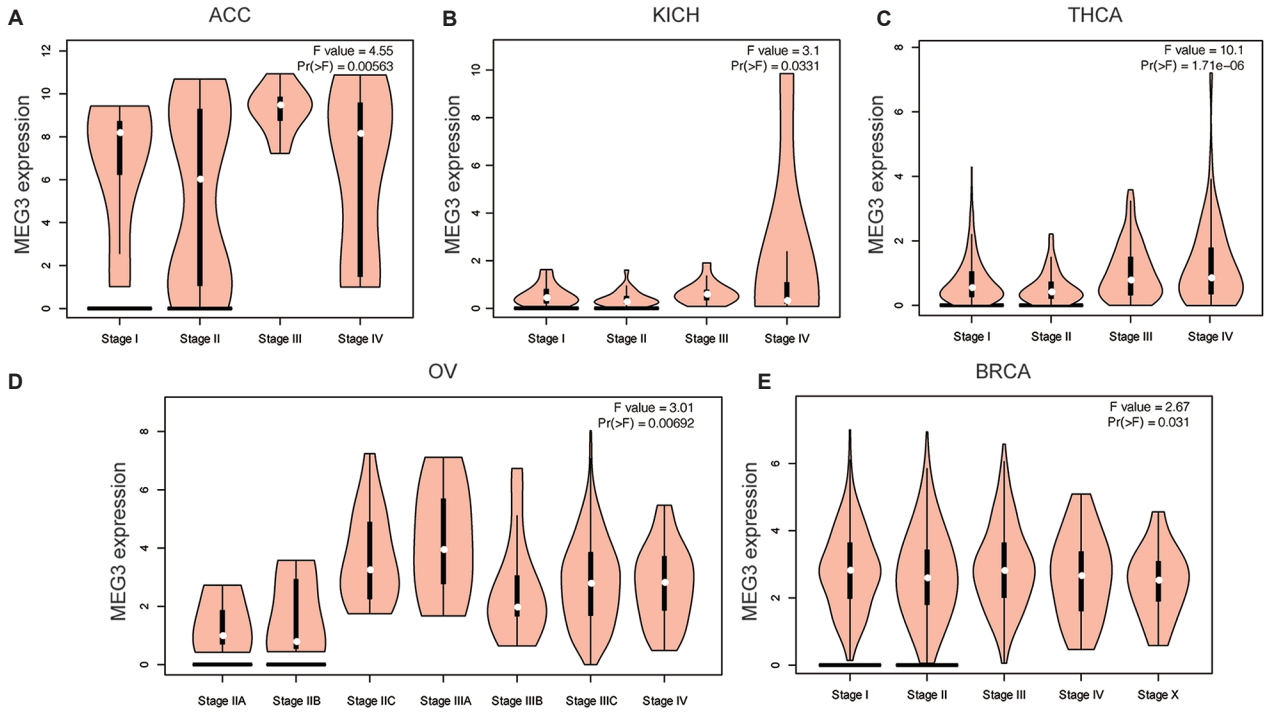


Figure 3. High expression of MEG3 correlates with low tumor, node, metastasis (TNM) stage in human cancer. Expression of MEG3 is lower in more advanced TNM stage in ACC (A), KICH (B), THCA (C), OV (D), and BRCA (E) than their low-TNM-stage counterparts.
Note: All $p<0.05$.
Abbreviations: ACC: Adrenocortical carcinoma; BRCA: Breast invasive carcinoma; KICH: Kidney chromophobe; MEG3: Maternally expressed gene 3; OV: Ovarian serous cystadenocarcinoma; THCA: Thyroid carcinoma.

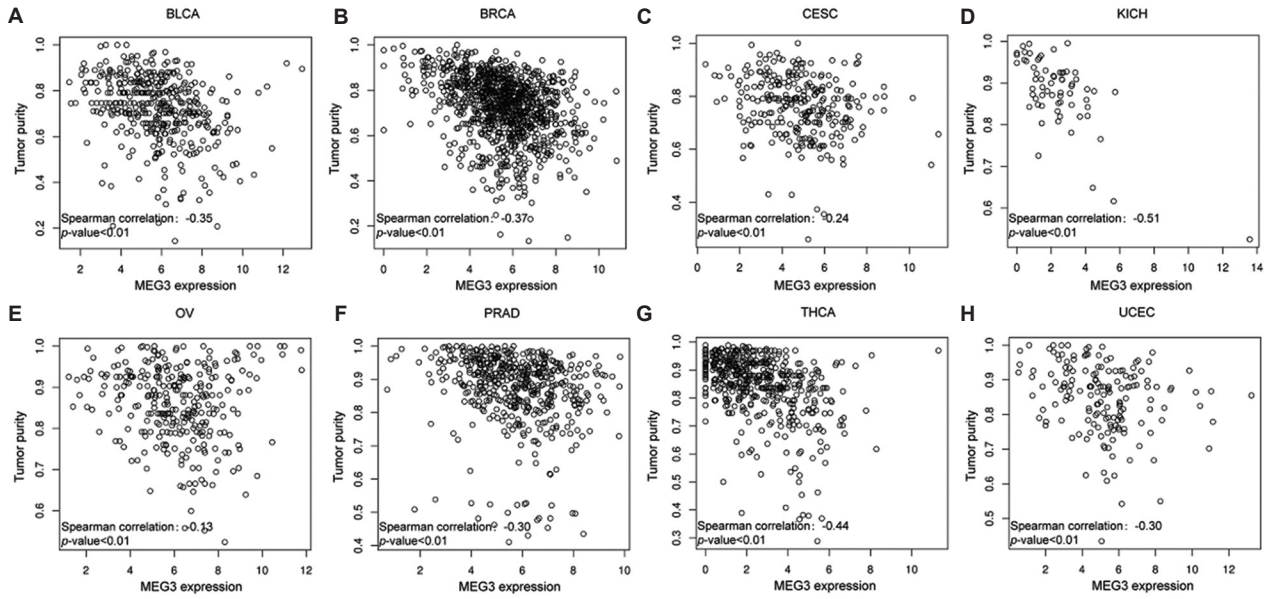


Figure 4. High expression of MEG3 leads to a decrease in tumor purity in human cancers. MEG3 reversely controls tumor purity in BLAC (A), BRCA (B), CESC (C), KICH (D), PRAD (E), OV (F), THCA (G), and UCEC (H).
Note: All $p<0.01$.
Abbreviations: BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; KICH: Kidney chromophobe; MEG3: Maternally expressed gene 3; OV: Ovarian serous cystadenocarcinoma; PRAD: Prostate adenocarcinoma; THCA: Thyroid carcinoma; UCEC: Uterine corpus endometrial carcinoma.

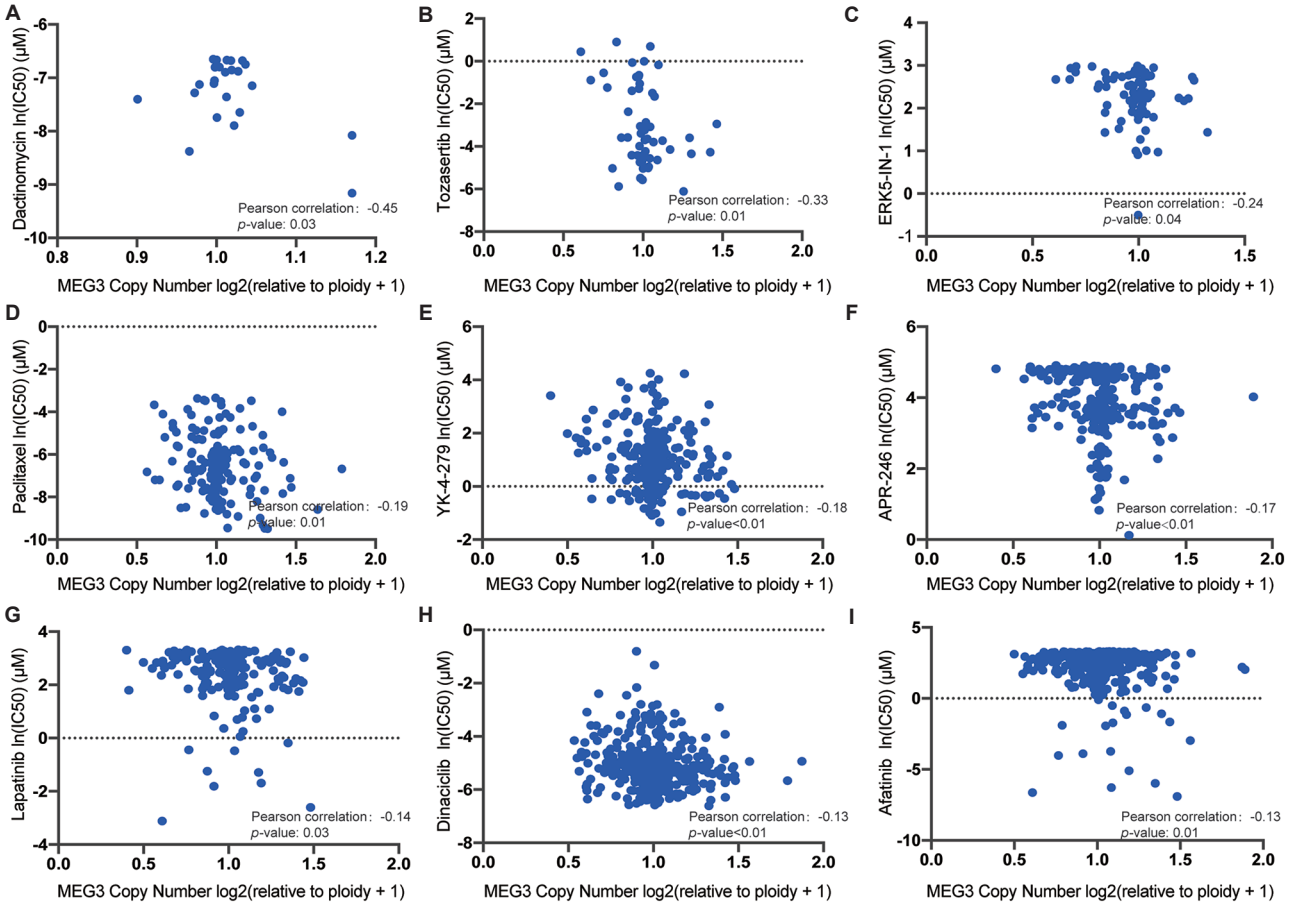


Figure 5. MEG3 reverses chemoresistance in human cancer. High expression of MEG3 correlates with low IC50 of chemotherapeutic drugs, including dactinomycin (A), tozasertib (B), ERK5-IN-1 (C), paclitaxel (D), YK-4-279 (E), APR-246 (F), lapatinib (G), dinaciclib (H), and afatinib (I).
Note: All $p<0.05$.
Abbreviation: MEG3: Maternally expressed gene 3.

3.4. SPI1 acts as a transcription factor of MEG3

The JASPAR database was employed to predict potential transcription factors that may regulate MEG3 expression. The results revealed the top 10 transcription factors and their corresponding binding sites with the strongest predicted associations (Figure 6A). Among these, SPI1 exhibited the highest correlation, and its putative binding site within the MEG3 promoter region is presented in Figure 6B. To validate these predictions, the Cistrome Data Browser database was utilized. A DNA-binding peak between SPI1 and the MEG3 promoter region was observed across multiple cell lines (Figure 6C), further supporting the role of SPI1 as a transcription factor that binds to and regulates MEG3 expression.

3.5. MEG3 plays important biological functions in ovarian cancer

The differential expression of MEG3 between tumor and normal tissues is most pronounced in ovarian cancer compared to other cancer types. Therefore, we focused on investigating the potential biological functions of MEG3 in ovarian cancer. GO functional enrichment analysis was performed to explore the biological roles of MEG3 co-expressed genes. The results revealed that enriched functions were primarily associated with translational elongation (GO:0006414), nicotinamide adenine dinucleotide (NADH) dehydrogenase complex assembly (GO:0010257), and mitochondrial respiratory chain complex assembly (GO:0033108) for BP; respiratory chain (GO:0070469), oxidoreductase complex (GO:1990204), and NADH dehydrogenase complex (GO:0030964) for CC; and structural constituent of ribosome (GO:0003735), ribosomal RNA binding (GO:0019843), and oxidoreductase activity, acting on NAD(P)H (GO:0016651) for MF (Table 1). Subsequent KEGG pathway enrichment analysis indicated that mRNAs positively co-expressed with MEG3 were predominantly enriched in glycosaminoglycan biosynthesis, extracellular matrix (ECM)–receptor interaction, and nicotine addiction

A

Name	Score	Relative score	Start	End	Predicted sequence
MA0080.2.SPI1	11.54	1.00	1301	1307	AGGAAGT
MA0152.1.NFATC2	11.36	1.00	341	347	TTTTCCA
MA0152.1.NFATC2	11.36	1.00	577	583	TTTTCCA
MA0104.1.Mycn	10.53	1.00	1560	1565	CACGTG
MA0081.1.SPIB	10.47	1.00	510	516	AGAGGAA
MA0081.1.SPIB	10.47	1.00	1299	1305	AGAGGAA
MA0498.2.MEIS1	10.35	1.00	472	478	TTGACAG
MA0004.1.Arnt	10.35	1.00	1560	1565	CACGTG
MA0151.1.Arid3a	9.84	1.00	1517	1522	ATTAAA
MA0130.1.ZNF354C	8.92	1.00	1661	1666	ATCCAC

B

Name	Score	Relative score	Start	End	Strand	Predicted sequence
MA0080.2.SPI1	11.54	1.00	1301	1307	+	AGGAAGT
MA0080.2.SPI1	8.328	0.89	82	88	–	AGGAAGG
MA0080.2.SPI1	7.819	0.88	517	523	–	AGGAATT
MA0080.2.SPI1	7.015	0.85	369	375	+	GGGAAGC
MA0080.2.SPI1	6.972	0.85	219	225	–	GGGAAAT
MA0080.2.SPI1	6.972	0.85	1832	1838	+	GGGAAAT
MA0080.2.SPI1	6.792	0.84	768	774	+	AGGCAGT
MA0080.2.SPI1	6.792	0.84	1592	1598	+	AGGGAGT
MA0080.2.SPI1	6.496	0.83	20	26	–	GGGAAGA
MA0080.2.SPI1	6.496	0.83	721	727	–	GGGAAGA

C

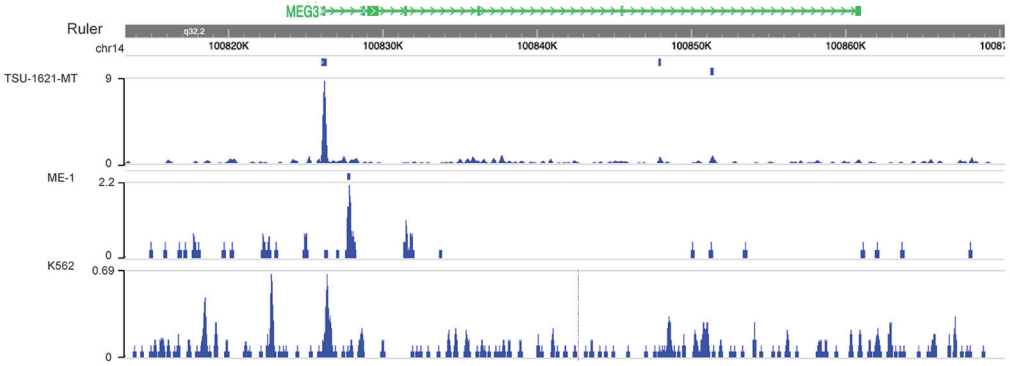


Figure 6. SPI1 acts as a transcription factor of MEG3. (A) The top 10 transcription factors of MEG3 and the binding sites with the strongest correlation. (B) SPI1 binding site prediction provided by the JASPAR database. (C) SPI1 binds in the MEG3 promoter locus in TSU-1621-MT, ME-1, and K562 from the Cistrome Data Browser databases.
Abbreviation: MEG3: Maternally expressed gene 3.

Table 1. Enriched Gene Oncology (GO) items

Enriched category	Description	Count	NES	*p*-value	FDR
Biological process					
GO: 0006414	Translational elongation	123	−3.359	<0.0001	<0.0001
GO: 0010257	NADH dehydrogenase complex assembly	49	−3.184	<0.0001	<0.0001
GO: 0033108	Mitochondrial respiratory chain complex assembly	68	−3.118	<0.0001	<0.0001
Cellular components					
GO: 0070469	Respiratory chain	84	−3.271	<0.0001	<0.0001
GO: 1990204	Oxidoreductase complex	95	−3.260	<0.0001	<0.0001
GO: 0030964	NADH dehydrogenase complex	43	−3.098	<0.0001	<0.0001
Molecular function					
GO: 0003735	Structural constituent of the ribosome	153	−3.828	<0.0001	<0.0001
GO: 0019843	rRNA binding	59	−2.726	<0.0001	<0.0001
GO: 0016651	Oxidoreductase activity, acting on NAD (P) H	96	−2.720	<0.0001	<0.0001

Abbreviations: FDR: False discovery rate; NADH: Nicotinamide adenine dinucleotide; NES: Normalized enrichment score; rRNA: Ribosomal RNA.

(Figure 7A). In contrast, mRNAs negatively co-expressed with MEG3 were significantly enriched in ribosome, proteasome, and oxidative phosphorylation, as determined by the over-representation analysis (ORA) method (Figure 7B). Similarly, pathways such as ECM–receptor interaction, basal cell carcinoma, focal adhesion, ribosome, proteasome, oxidative phosphorylation, DNA replication, and graft-versus-host disease were also identified in the Gene Set Enrichment Analysis (GSEA) results (Figure 7C). Notably, the Hippo signaling pathway emerged as a significant pathway in the KEGG enrichment outcomes. The detailed pathway activation pattern is illustrated in Figure 7D.

3.6. MEG3 methylation directs the expression of cell cycle-related genes in ovarian cancer

Data on MEG3 methylation and expression profiles of related genes in 18 high-grade serous ovarian cancer cell

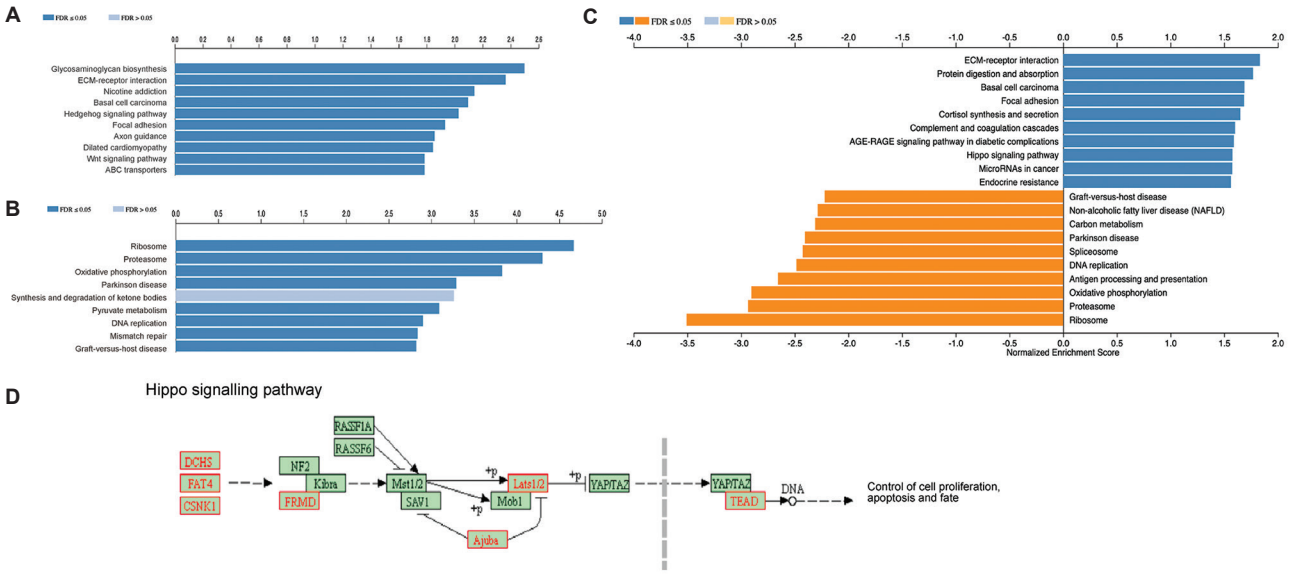


Figure 7. MEG3 plays important biological functions in ovarian cancer. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment results of messenger RNAs positively (A) and negatively (B) co-expressed with MEG3 by the ORA method and Gene Set Enrichment Analysis (GSEA) method (C). (D) Hippo signaling pathway diagram.
Abbreviations: ORA: Over-representation analysis; MEG3: Maternally expressed gene 3.

lines were obtained from the CCLE database. Analysis revealed that increased MEG3 methylation was positively correlated with elevated expression of cell cycle-associated genes in ovarian cancer cell lines, including cyclin E2 (*CCNE2*; Pearson correlation = 0.54), cyclin O (*CCNO*; Pearson correlation = 0.49), cell division cycle 14A (*CDC14A*; Pearson correlation = 0.62), cell division cycle 73 (*CDC73*; Pearson correlation = 0.48), cyclin-dependent kinase-like 1 (*CDKL1*; Pearson correlation = 0.64), and cell growth regulator with ring finger domain 1 (*CGRRF1*; Pearson correlation = 0.54) (Figure 8).

3.7. MEG3 regulates immune cell infiltration and immune biomarkers in ovarian cancer

Tumor microenvironment and immune infiltration are important factors influencing the prognosis of patients. To explore the interaction between MEG3 and immune infiltration, the expression of MEG3 and the level of immune cell infiltration were downloaded from the TIMER database. MEG3 expression was positively associated with natural killer (NK) cells (Spearman correlation = 0.29), neutrophil cells (Spearman correlation = 0.28), macrophages (Spearman correlation = 0.21), and monocytes (Spearman correlation = 0.13). Conversely, MEG3 expression was negatively associated with B cells (Spearman correlation = −0.27), $CD4^+$ T cells (Spearman correlation = −0.25), $CD8^+$ T cells (Spearman correlation = −0.22), and myeloid dendritic cells (Spearman correlation = −0.22) in OV (Figure 9). Next, for further researching the role of MEG3 in tumor immunity, we probed into the connection between MEG3 expression and immune cell biomarkers. As displayed in Table 2, MEG3 was significantly positively correlated with biomarkers of neutrophil cells (*CD66b*), macrophage cells (*INOS*), and Th17 cells (*STAT3*), and negatively correlated with biomarkers of T cells (*CD2*, *CD3D*, *CD3E*), CD8+ T cells (*CD8A*), Th1 cells (*TBET*), dendritic cells (*HLA-DPA1*, *HLA-DPB1*, *HLA-DQB1*, *HLA-DRA*), and immune checkpoint (*CTLA4*, *GZMB*, *TIM3*).

4. Discussion

Cancer is a major global public health problem[15] with complicated pathogenesis, which is associated with multiple gene mutations, epigenetic changes, and chromosome translocation, deletions, and amplifications.[16] Although numerous studies have confirmed that lncRNAs are involved in a series of processes regulating oncogenes, tumor suppressor genes, tumor biology, tumor proliferation, chemotherapy resistance, and metastasis,[17] understanding the functions and detailed characteristics of a large number of lncRNAs in various cancers is a challenging task.

As an antitumor agent or tumor suppressor, MEG3 is downregulated in various tumor tissues. Our results demonstrated that MEG3 expression was significantly lower in tumor tissues compared to normal tissues—findings consistent with previous reports.[18] Notably, in gynecological malignancies—including cervical cancer,

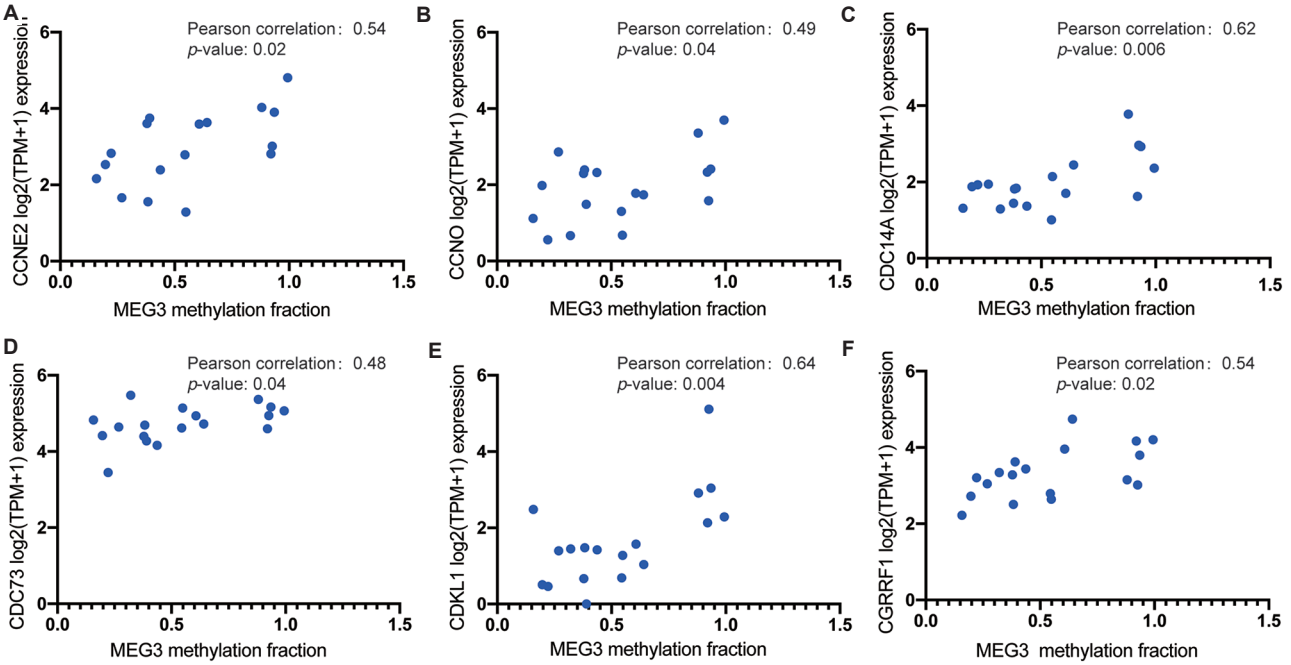


Figure 8. The expansion of MEG3 methylation upregulates the expression of genes related to the cell cycle in ovarian cancer cell lines, including *CCNE2* (A), *CCNO* (B), *CDC14A* (C), *CDC73* (D), *CDKL1* (E), and *CGRRF1* (F).
Note: All $p<0.05$.
Abbreviations: *CCNE2*: Cyclin E2; *CCNO*: Cyclin O; *CDC14A*: Cell division cycle 14A; *CDC73*: Cell division cycle 73; *CDKL1*: Cyclin-dependent kinase-like 1; *CGRRF1*: Cell growth regulator with ring finger domain 1; MEG3: Maternally expressed gene 3.

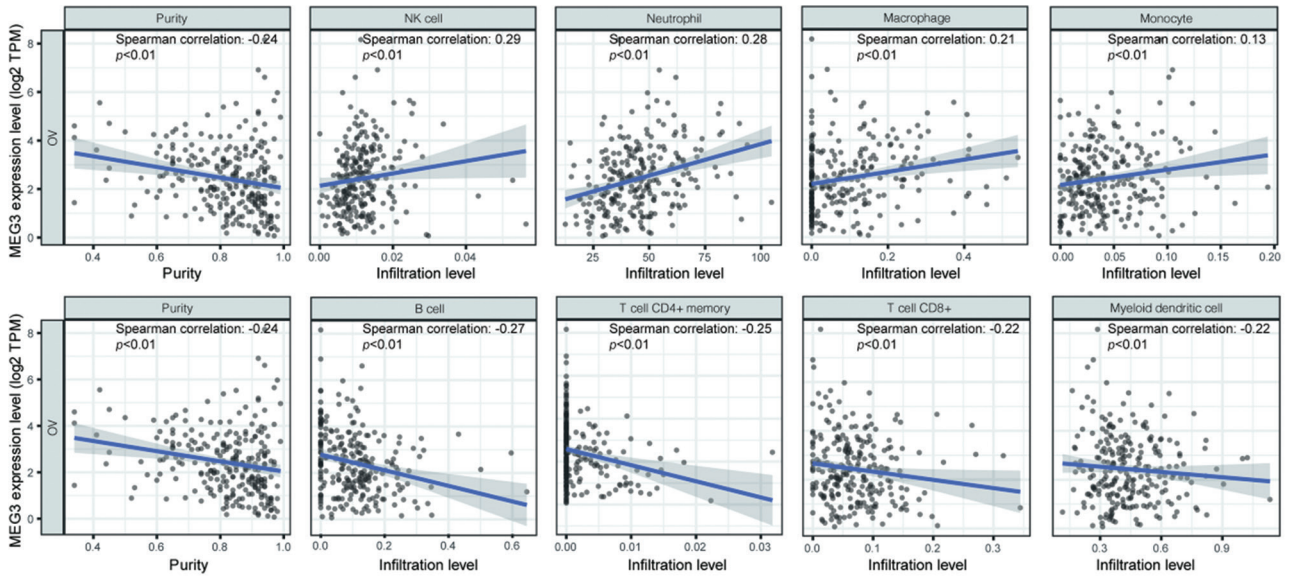


Figure 9. The expression of MEG3 and the level of immune cell infiltration in OV. The correlation of MEG3 expression with NK cell, neutrophil, macrophage, monocyte, B cell, $CD4^+$ T cell, $CD8^+$ T cell, and dendritic cell infiltration levels.
Note: All $p<0.01$.
Abbreviations: MEG3: Maternally expressed gene 3; NK: Natural killer; OV: Ovarian serous cystadenocarcinoma.

ovarian cancer, endometrial cancer, and uterine sarcoma—the differential expression of MEG3 is more pronounced. Dong *et al.*[19,20] and Guo *et al.*[19,20] demonstrated that MEG3 induces apoptosis by modulating the Notch signaling

Table 2. Correlation analysis between MEG3 and biomarkers of immune cells in ovarian serous cystadenocarcinoma determined by the TIMER database

Immune cell	Biomarker	*R* value	*p*-value
Neutrophil	*CD66b*	0.26	3.39e−05
M1 macrophage	*INOS*	0.21	1.12e−03
Th17	*STAT3*	0.16	1.18e−02
T cell (general)	*CD2*	−0.19	2.42e−03
	CD3D	−0.25	5.28e−05
	CD3E	−0.18	4.28e−03
$CD8^+$T cell	*CD8A*	−0.18	5.43e−03
Th1	*TBET*	−0.15	1.52e−02
Dendritic cell	*HLA-DPA1*	−0.20	1.25e−03
	HLA-DPB1	−0.22	5.74e−04
	HLA-DQB1	−0.16	9.61e−03
	HLA-DRA	−0.26	3.55e−05
Immune checkpoint	*CTLA4*	−0.15	1.62e−02
	GZMB	−0.24	1.51e−04
	TIM3	−0.14	2.68e−02

Abbreviations: MEG3: Maternally expressed gene 3; TIMER: Tumor Immune Estimation Resource.

pathway, PI3K, and its downstream proteins in endometrial carcinoma. Wang *et al.*[21] showed that MEG3 inhibits cancer cell proliferation and instigates their apoptosis by regulating the expression of suppressor gene *PTEN* in ovarian cancer. Similarly, overexpression of MEG3 inhibits cell proliferation and epithelial to mesenchymal transition in ovarian cancer by regulating miR-219a-5p/epidermal growth factor receptor (EGFR) axis and miR-30e-3p.[22,23] The difference in MEG3 expression affects the prognosis in different types of cancer. There is significant heterogeneity in the metabolic profiles, signaling pathway activities, differentiation degrees, and genomic stability across different tumor types. This diversity arises from variations in the tumor microenvironment, the specificity of epigenetic modifications and regulatory networks, as well as dynamic alterations in protein function. Decrease in MEG3 expression is also considered to be a marker of tumor progression in ACC, KICH, THCA, OV, and BRCA.

Chemotherapeutic agents are widely used in the clinical management of malignant tumors, alongside surgery and radiotherapy. Resistance to these agents represents one of the primary factors contributing to poor patient prognosis. Therefore, we focused on the role of MEG3 in chemotherapeutic drug resistance. Our findings revealed a negative correlation between MEG3 expression and the IC50 values of multiple chemotherapeutic drugs, indicating that higher MEG3 expression levels are associated with increased drug sensitivity. Dactinomycin showed the strongest association with MEG3 and is clinically employed in the treatment of certain malignancies. It is well-established that dactinomycin exhibits antitumor, antibacterial, and antiviral activities. Its antitumor mechanism involves interference with nucleic acid synthesis and inhibition of DNA replication. In addition, dactinomycin can induce cell differentiation and apoptosis, suppress the activity of specific proteases, and modulate the cell cycle.[24] Dactinomycin is effective in the treatment of neuroblastoma,[25] gestational trophoblastic tumor,[26] Hodgkin's disease,[27] testicular tumors,[28] and Wilms' tumor.[29] Moreover, it is often used in combination with other drugs in tumor treatment; for example, the etoposide, methotrexate, actinomycin D–cyclophosphamide, vincristine (Oncovin) (EMA–CO) therapeutic schedule is the most widely used multiagent regimen for gestational trophoblastic tumor.[30] MEG3 can enhance the sensitivity and lessen the resistance of chemotherapeutic drugs, improving the therapeutic effect and assuming a part in repressing tumor growth.

Subsequently, SPI1 is considered the most potent transcription factor regulating MEG3 expression. Functional enrichment analysis revealed that MEG3-associated genes were enriched in the Hippo signaling pathway in ovarian cancer. The Hippo signaling pathway regulates organ size by controlling cell proliferation, apoptosis, and stem cell self-renewal.[31] In addition, numerous studies have confirmed that this pathway plays an important part in carcinostatic activities.[32] It consists of a conserved kinase cascade. In mammals, upon sensing extracellular growth-inhibitory signals through upstream membrane receptors, the pathway transmits signals through a series of phosphorylation events that ultimately inactivate the downstream effectors Yes-associated protein and transcriptional coactivator with PDZ-binding motif (TAZ),[33] thereby functioning as a key inhibitor of cell growth. MEG3 is downregulated in ovarian tumor tissues, concurrent with suppression of the Hippo signaling pathway, suggesting that MEG3 may exert its antitumor effects through the activation of this pathway.

Our study found that MEG3 methylation can affect the expression of cell cycle and division-related genes in ovarian cancer. *CCNE2* is a member of the cyclin family and a key regulator of the cell cycle. Its primary function is to facilitate the transition of cells from the G0/G1 phase to the S phase through binding to cyclin-dependent kinase 2 (CDK2) and promoting phosphorylation of the retinoblastoma protein. This protein plays a critical role in cancer progression. It has been reported that *CCNE2* is overexpressed in various human malignancies, and consequently, inhibitors targeting *CCNE2* have been approved for the treatment of several cancer types.[34]

CDKL1, a serine-threonine protein kinase, functions as an important regulator of tumor proliferation and invasion. It has been reported that *CDKL1* is upregulated in melanoma, breast cancer, gastric cancer, and colorectal cancer.[35,36] We may regulate the proliferation and development of ovarian cancer by managing the degree of MEG3 methylation.

The tumor microenvironment is a complex system, comprising tumor cells, endothelial cells, ECM, fibroblasts, and immune cells. All these components interact with tumor cells as they grow. This mixture is considered to play a considerable role in tumor growth, disease progression, and drug resistance.[37,38] Tumor purity is defined as the proportion of tumor cells in tumor tissues.[39] Our data showed that the expression of MEG3 was contrarily associated with tumor purity, implying that we can reduce the tumor purity and regulate immune cell infiltration in the tumor microenvironment by increasing the expression of MEG3. A variety of immune cells play essential roles in regulating tumor growth. NK cells were viewed as connected with immune surveillance in 1980. A few investigations have confirmed that patients with NK cell dysfunction have a higher incidence rate of cancer. Subsequently, many studies have found that the function of NK cells in cancer patients was weakened. The reduction of human NK cell activity increases the risk of suffering from a variety of malignant tumors.[40] NK cells primarily execute the antitumor effect by secreting cytotoxic perforin and granzyme upon activation through cell-surface receptor–ligand interactions.[41] Macrophages usually represent a double-sided function of accelerating and restraining tumors. For example, M1 macrophages mainly produce proinflammatory cytokines to enhance the antitumor effect. However, M2 macrophages promote tumor progression by inducing proliferation, angiogenesis, ECM deposition, and cell migration.[42] Tumor-infiltrating B cells exert antitumor activity through antigen presentation and the release of granzyme. Regulatory B cells inhibit immunity, promoting angiogenesis and tumor development by releasing vascular endothelial growth factor (VEGF) and interleukin (IL)-10.[43] The typical antitumor immune cells are $CD8^+$ T lymphocytes, which can recognize tumor cells in an antigen-specific way and secrete cytotoxic molecules to kill them immediately.[44] In the tumor microenvironment, dendritic cells absorb and present antigens, resulting in the immune response of T cells, which can affect the tumor-killing effect of T cells.[45] Importantly, the entire composition of immune cells is not a binary antitumor or tumor-promoting environment, but a mixture of these cell types. The eventual phenotype is decided by the interaction between immune cells and non-immune cells. These cells communicate with each other through direct contact or the production of cytokines and chemokines, and control tumor growth in the way of autocrine and paracrine.[46]

We further found that MEG3 was associated with immune checkpoints in ovarian cancer. In recent years, immune checkpoint blocking therapy has shown significant efficacy in the treatment of various types of tumors.[47] Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is expressed on regulatory T (Treg) and activated T cells, sharing the B7 molecular ligand with CD28. The combination of CTLA-4 and B7 can inhibit the combination of B7 and CD28, interrupting T cell activation and participating in the negative regulation of immune response. Inhibitors targeting CTLA-4 can block the combination of CTLA-4 and B7, thereby preventing the generation of T cell inhibitory signals and enhancing the specific antitumor immune response.[48] Meanwhile, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is a kind of T cell surface inhibitory molecule, which can cause T cell exhaustion. Similar to CTLA-4, TIM-3 is also one of the most studied immunotherapeutic targets at present. It is selectively expressed in secreting T helper cells (Th1 and Th17), Treg cells, dendritic cells, monocytes, mast cells, NK cells, and tumor-infiltrating lymphocytes. It is also expressed on tumor cells, such as melanoma, gastric cancer, and B-cell lymphoma.[49] As a promising inhibitory receptor in many emerging immune checkpoints, TIM-3 has achieved initial results in clinical trials as a monotherapy or combined with anti-programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) drugs. The drug types have been expanded from monoclonal antibodies to double antibodies, and the indications cover blood tumors and solid tumors. The future trend remains to be further studied.[50]

One of the key strengths of this study lies in its comprehensive pan-cancer analysis combined with an in-depth investigation of ovarian cancer. Systematic comparisons of MEG3 expression levels between tumor and adjacent normal tissues across multiple malignancies provide robust evidence that MEG3 is consistently downregulated in various cancers, particularly in gynecological tumors. Furthermore, the association between MEG3 expression and critical clinical parameters—such as OS, RFS, and TNM staging—highlights its potential prognostic significance. The integration of multiple molecular and cellular factors—including tumor purity, chemotherapy resistance, DNA methylation patterns, and immune cell infiltration—enhances the analytical depth and extends beyond conventional descriptive expression profiling. In addition, the examination of upstream transcriptional regulation, notably the identification of SPI1 as a putative transcription factor modulating

MEG3 expression, along with downstream functional enrichment analyses implicating pathways such as Hippo signaling, constitutes a significant strength and offers a plausible mechanistic framework for understanding MEG3's role in tumorigenesis. This study provides the first multidimensional evidence of the antitumor effects of MEG3 in ovarian cancer, offering novel insights into strategies for tumor prevention and clinical management.

5. Conclusion

This study revealed the antitumor effect of MEG3 as a potential therapeutic target or tumor marker, potentially contributing to the comprehensive prevention and treatment of ovarian cancer.

Acknowledgments

None.

Funding

This work was supported by The Foundation of First Affiliated Hospital of Harbin Medical University (2023M19) and Heilongjiang Province Jizhong Medical Aid Charity Foundation (2020HX029).

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Yan Wang
Formal analysis: Yan Li
Investigation: Yan Li
Methodology: Yan Li
Writing – original draft: Yan Li
Writing – review & editing: Yan Li

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable, as no new data were created or analyzed in this study.

References

1. Liu SJ, Dang HX, Lim DA, Feng FY, Maher CA. Long noncoding RNAs in cancer metastasis. *Nat Rev Cancer*. 2021;21(7):446-460.

   doi: 10.1038/s41568-021-00353-1

2. Qian X, Yang J, Qiu Q, *et al*. LCAT3, a novel m6A-regulated long non-coding RNA, plays an oncogenic role in lung cancer via binding with FUBP1 to activate c-MYC. *J Hematol Oncol*. 2021;14(1):112.

   doi: 10.1186/s13045-021-01123-0

3. Liu K, Gao L, Ma X, *et al*. Long non-coding RNAs regulate drug resistance in cancer. *Mol Cancer*. 2020;19(1):54.

   doi: 10.1186/s12943-020-01162-0

4. Hu Q, Egranov SD, Lin C, Yang L. Long noncoding RNA loss in immune suppression in cancer. *Pharmacol Ther*. 2020;213:107591.

   doi: 10.1016/j.pharmthera.2020.107591

5. Sherpa C, Rausch JW, Le Grice SF. Structural characterization of maternally expressed gene 3 RNA reveals conserved motifs and potential sites of interaction with polycomb repressive complex 2. *Nucleic Acids Res*. 2018;46(19):10432-10447.

   doi: 10.1093/nar/gky722

6. Dong S, Ma M, Li M, *et al*. LncRNA MEG3 regulates breast cancer proliferation and apoptosis through miR-141-3p/RBMS3 axis. *Genomics*. 2021;113(4):1689-1704.

   doi: 10.1016/j.ygeno.2021.04.015

7. Buttarelli M, De Donato M, Raspaglio G, *et al.* Clinical value of lncRNA MEG3 in high-grade serous ovarian cancer. *Cancers (Basel)*. 2020;12(4):966.

   doi: 10.3390/cancers12040966

8. Ying L, Huang Y, Chen H, *et al.* Downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer. *Mol Biosyst*. 2013;9(3):407-411.

   doi: 10.1039/c2mb25386k

9. Xiu YL, Sun KX, Chen X, *et al.* Upregulation of the lncRNA Meg3 induces autophagy to inhibit tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3. *Oncotarget*. 2017;8(19):31714-31725.

   doi: 10.18632/oncotarget.15955

10. Uroda T, Anastasakou E, Rossi A, *et al.* Conserved pseudoknots in lncRNA MEG3 are essential for stimulation of the p53 pathway. *Mol Cell*. 2019;75(5):982-995.e9.

    doi: 10.1016/j.molcel.2019.07.025

11. Mondal T, Subhash S, Vaid R, *et al*. MEG3 long noncoding RNA regulates the TGF-β pathway genes through formation of RNA-DNA triplex structures. *Nat Commun*. 2015;6:7743.

    doi: 10.1038/ncomms8743

12. Shan G, Tang T, Xia Y, Qian HJ. MEG3 interacted with miR-494 to repress bladder cancer progression through targeting PTEN. *J Cell Physiol*. 2020;235(2):1120-1128.

    doi: 10.1002/jcp.29025

13. Huang C, Liao X, Jin H, *et al.* MEG3, as a competing

endogenous RNA, binds with miR-27a to promote PHLPP2 protein translation and impairs bladder cancer invasion. *Mol Ther Nucleic Acids*. 2019;16:51-62.

doi: 10.1016/j.omtn.2019.01.014

14. Dai Y, Wan Y, Qiu M, *et al*. lncRNA MEG3 suppresses the tumorigenesis of hemangioma by sponging miR-494 and regulating PTEN/PI3K/AKT pathway. *Cell Physiol Biochem*. 2018;51(6):2872-2886.

doi: 10.1159/000496040

15. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30.

doi: 10.3322/caac.21590

16. Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: A new paradigm. *Cancer Res*. 2017;77(15):3965-3981.

doi: 10.1158/0008-5472.CAN-16-2634

17. Huang GQ, Ke ZP, Hu HB, Gu B. Co-expression network analysis of long noncoding RNAs (lncRNAs) and cancer genes revealsSFTA1P and CASC2abnormalities in lung squamous cell carcinoma. *Cancer Biol Ther*. 2017;18(2):115-122.

doi: 10.1080/15384047.2017.1281494

18. Al-Rugeebah A, Alanazi M, Parine NR. MEG3: An oncogenic long non-coding RNA in different cancers. *Pathol Oncol Res*. 2019;25(3):859-874.

doi: 10.1007/s12253-019-00614-3

19. Dong P, Xiong Y, Yue J, *et al*. Exploring lncRNA-mediated regulatory networks in endometrial cancer cells and the tumor microenvironment: Advances and challenges. *Cancers (Basel)*. 2019;11(2):234.

doi: 10.3390/cancers11020234

20. Guo Q, Qian Z, Yan D, Li L, Huang L. LncRNA-MEG3 inhibits cell proliferation of endometrial carcinoma by repressing Notch signaling. *Biomed Pharmacother*. 2016;82:589-594.

doi: 10.1016/j.biopha.2016.02.049

21. Wang J, Xu W, He Y, Xia Q, Liu S. LncRNA MEG3 impacts proliferation, invasion, and migration of ovarian cancer cells through regulating PTEN. *Inflamm Res*. 2018;67(11-12):927-936.

doi: 10.1007/s00011-018-1186-z

22. Wang L, Yu M, Zhao S. lncRNA MEG3 modified epithelial-mesenchymal transition of ovarian cancer cells by sponging miR-219a-5p and regulating EGFR. *J Cell Biochem*. 2019;120(10):17709-17722.

doi: 10.1002/jcb.29037

23. Liu Y, Xu Y, Ding L, Yu L, Zhang B, Wei D. LncRNA MEG3 suppressed the progression of ovarian cancer via sponging miR-30e-3p and regulating LAMA4 expression. *Cancer Cell Int*. 2020;20:181.

doi: 10.1186/s12935-020-01259-y

24. Samuels LD. Actinomycin and its effects. Influence on an effector pathway for hormonal control. *N Engl J Med*. 1964;271:1301-1308.CONTD.

doi: 10.1056/NEJM196412172712507

25. Cortes CL, Veiga SR, Almacellas E, *et al*. Effect of low doses of actinomycin D on neuroblastoma cell lines. *Mol Cancer*. 2016;15:1.

doi: 10.1186/s12943-015-0489-8

26. Even C, Pautier P, Duvillard P, *et al*. Actinomycin D, cisplatin, and etoposide regimen is associated with almost universal cure in patients with high-risk gestational trophoblastic neoplasia. *Eur J Cancer*. 2014;50(12):2082-2089.

doi: 10.1016/j.ejca.2014.05.002

27. Markasz L, Kis LL, Stuber G, *et al*. Hodgkin-lymphoma-derived cells show high sensitivity to dactinomycin and paclitaxel. *Leuk Lymphoma*. 2007;48(9):1835-1845.

doi: 10.1080/10428190701559132

28. Pontes JE, Magoss I, Staubitz W, Murphy GP. The treatment of stages I and II nonseminomatous testicular tumors: The Buffalo experience from 1970 to 1979. *J Urol*. 1982;128(6):1201-1204.

doi: 10.1016/s0022-5347(17)53423-0

29. Farber S, D'Angio G, Evans A, Mitus A. Clinical studies of actinomycin D with special reference to Wilms' tumor in children. 1960. *J Urol*. 2002;168(6):2560-2562; discussion 2563.

doi: 10.1097/01.ju.0000042140.76846.d9

30. Jareemit N, Horowitz NS, Goldstein DP, Berkowitz RS, Elias KM. EMA vs EMACO in the treatment of gestational trophoblastic neoplasia. *Gynecol Oncol*. 2020;158(1):99-104.

doi: 10.1016/j.ygyno.2020.04.699

31. Zhao B, Tumaneng K, Guan KL. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nat Cell Biol*. 2011;13(8):877-883.

doi: 10.1038/ncb2303

32. Li FL, Guan KL. The two sides of Hippo pathway in cancer. *Semin Cancer Biol*. 85:33-42.

doi: 10.1016/j.semcancer.2021.07.006

33. Kulkarni A, Chang MT, Vissers JHA, Dey A, Harvey KF. The hippo pathway as a driver of select human cancers. *Trends Cancer*. 2020;6(9):781-796.

doi: 10.1016/j.trecan.2020.04.004

34. Xie Q, Chen C, Li H, *et al*. miR-3687 overexpression promotes bladder cancer cell growth by inhibiting the negative effect of FOXP1 on cyclin E2 transcription. *Mol Ther*. 2019;27(5):1028-1038.

doi: 10.1016/j.ymthe.2019.03.006

35. Li W, Cao J, Liu J, *et al.* Downregulation of CDKL1 suppresses neuroblastoma cell proliferation, migration and invasion. *Cell Mol Biol Lett*. 2019;24:19.

doi: 10.1186/s11658-019-0139-z

36. Qin C, Ren L, Ji M, *et al*. CDKL1 promotes tumor proliferation and invasion in colorectal cancer. *Onco Targets Ther*. 2017;10:1613-1624.

doi: 10.2147/OTT.S133014

37. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646-674.

doi: 10.1016/j.cell.2011.02.013

38. Junttila MR, de Sauvage FJ. Influence of tumour microenvironment heterogeneity on therapeutic response. *Nature*. 2013;501(7467):346-354.

doi: 10.1038/nature12626

39. Xiong Y, Xiong Z, Cao H, Li C, Wanggou S, Li X. Multi-dimensional omics characterization in glioblastoma identifies the purity-associated pattern and prognostic gene signatures. *Cancer Cell Int*. 2020;20:37.

doi: 10.1186/s12935-020-1116-3

40. Morvan MG, Lanier LL. NK cells and cancer: You can teach innate cells new tricks. *Nat Rev Cancer*. 2016;16(1):7-19.

doi: 10.1038/nrc.2015.5

41. Choucair K, Duff JR, Cassidy CS, *et al.* Natural killer cells: A review of biology, therapeutic potential and challenges in treatment of solid tumors. *Future Oncol*. 2019;15(26):3053-3069.

doi: 10.2217/fon-2019-0116

42. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14(7):399-416.

doi: 10.1038/nrclinonc.2016.217

43. Wang SS, Liu W, Ly D, Xu H, Qu L, Zhang L. Tumor-infiltrating B cells: Their role and application in anti-tumor immunity in lung cancer. *Cell Mol Immunol*. 2019;16(1):6-18.

doi: 10.1038/s41423-018-0027-x

44. Haabeth OA, Lorvik KB, Hammarstrom C, *et al.* Inflammation driven by tumour-specific Th1 cells protects against B-cell cancer. *Nat Commun*. 2011;2:240.

doi: 10.1038/ncomms1239

45. Merad M, Salmon H. Cancer: A dendritic-cell brake on antitumour immunity. *Nature*. 2015;523(7560):294-295.

doi: 10.1038/523294a

46. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;140(6):883-899.

doi: 10.1016/j.cell.2010.01.025

47. Zhang H, Dai Z, Wu W, *et al*. Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. *J Exp Clin Cancer Res*. 2021;40(1):184.

doi: 10.1186/s13046-021-01987-7

48. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: A moving target in immunotherapy. *Blood*. 2018;1 31(1):58-67.

doi: 10.1182/blood-2017-06-741033

49. Solinas C, De Silva P, Bron D, *et al*. Significance of TIM3 expression in cancer: From biology to the clinic. *Semin Oncol*. 2019;46(4-5):372-379.

doi: 10.1053/j.seminoncol.2019.08.005

50. Friedlaender A, Addeo A, Banna G. New emerging targets in cancer immunotherapy: The role of TIM3. *ESMO Open*. 2019;4(Suppl 3):e000497.

doi: 10.1136/esmoopen-2019-000497