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ORIGINAL RESEARCH

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Overexpression of *LINC01234* in uterus corpus endometrial cancer correlated with poor clinicopathological characteristics: a study based on TCGA data

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Abstract

Uterus corpus endometrial cancer (UCEC) is associated with a high mortality rate. In this study, we examined the impact of long intergenic non-protein coding RNA 01234 (*LINC01234*) in the diagnosis and survival of UCEC using an open high-throughput sequencing database. The association between *LINC01234* expression and UCEC clinical features was determined using the Wilcoxon rank sum test, logistic regression and Cox regression. To assess the classification effectiveness of *LINC01234* in UCEC, the area under the receiver operating characteristic (ROC) curve (AUC) was performed. Then, Kaplan-Meier analysis was performed to determine the prognostic significance of *LINC01234* in UCEC. The underlying regulatory mechanisms of *LIN01234* were assessed using the Gene set enrichment analysis (GSEA), and the influence on immune infiltration cells was tested by the Spearman correlation method. Datebases showed *LINC01234* was up-regulated in UCEC and associated with poor clinicopathologic characteristics. What's more quantitative real time polymerase chain reaction (qRT-PCR) analyse of clinical tissue specimens, *LINC01234* was actually high expression in UCEC. The results showed an AUC of 0.726, indicating that *LINC01234* had a significant diagnostic value. Further, Kaplan-Meier analysis showed that high *LINC01234* expression was associated with poorer progress free interval (PFI) (hazard ratio (HR): 1.68, 95% confidence interval (CI): 1.18–2.39, p = 0.004), disease-specific survival (DSS) (HR: 2.17, 95% CI: 1.29–3.67, p = 0.004), overall survival (OS) (HR: 1.81, 95% CI: 1.19–2.75, p = 0.005). Cox regression analysis showed *LINC01234* expression was an independent factor for DSS. Pathway enrichment and immune infiltration analysis showed the most likely mechanisms that *LINC01234* promoted tumor progression. *LINC01234* demonstrated diagnostic and prognostic potential in UCEC and was shown to exert its effects via various mechanisms, including cell proliferation, spermatogenesis, angiogenesis and immune response in the tumor microenvironment, to promote tumor progression; thus, indicating that it could be a target for treating UCEC.

Keywords

LINC01234; UCEC; Diagnostic value; Prognostic value; Biomarker

1. Introduction

Uterus corpus endometrial cancer (UCEC) remains one of the most common gynecological cancers with a high incidence and an increasing mortality rate, which has increased from 0.3% to 1.9% per year in the recent ten years [1]. This UCEC burden is believed to be attributed to poor developments in its treatment and diagnosis, thus urging the need for new diagnostic and prognostic biomarkers to improve its early diagnostic rate, provide better prognostic estimation of the patients' underlying disease conditions, and allow timely treatment.

A growing number of studies have indicated that lncRNAs (long non-coding RNAs; made up of ~100 to 200 kb nucleotides) play key roles and are crucial biomarkers in various cancers [2]. Ouyang *et al.* [3] reported seven lncRNAs (AC110491.1, AL451137.1, AC005381.1, AC103563.2, AC007422.2, AC108025.2, and MIR7-3 host gene) were associated with UCEC prognosis. Recent studies investigated the functions of *LINC01234* and showed that it was up-regulated in several cancers [4]. For instance, it was observed to have significantly higher expression in gastric cancer tissues than in normal gastric tissues. It was also reported that the expression of *LINC01234* in esophageal squamous cell carci-

noma and abnormal lung tissues was significantly different compared with normal tissues [4, 5] and was a potential therapeutic target for esophageal cancer due to its ability to regulate cancer cells proliferation, invasion and apoptosis. In multiple myeloma, *LINC01234* overexpression was shown to promote cancer progression by decreasing miR-124-3p expression and up-regulating its GRB2 (growth factor receptor-bound protein 2) targets [6]; thereby regulating the miR-124-3p/GRB2 axis and demonstrating that the *LINC01234*/miR-124-3p/GRB2 axis could be a promising target for treating multiple myeloma.

Using bioinformatics tools and databases, researchers have constructed the *LINC01234* regulatory network, including regulated transcription factors, miRNA and RBP (RNA-binding protein) interactions, and found that *LINC01234* participated in cell cycle and mismatch repair, which regulated cancer-associated genes [7]. In this regard, Chen *et al*. [8] reported that knocking down *LINC01234* induced gastric cancer cells apoptosis and G1 arrest. By analyzing the TCGA (The Cancer Genome Atlas) database, it was found that *LINC01234* was highly expressed in gastric cancer and was associated with worse pathological characteristics, such as more advanced TNM (tumor, node, and metastasis) stage, greater tumor size, and higher risk of lymph node metastasis, leading to shorter survival time. Also, it was reported that the *LINC01234-miR-204-5p-CBFB* axis was related to gastric cancer tumorigenesis and that *LINC01234* served as a competing endogenous RNA (ceRNA) to derepress its endogenous target core-binding factor β (CBFB) [8]. *In vivo* experiments showed that the knocked down of *LINC01234* reduced gastric cancer tumorigenesis. Recent investigations on *LINC01234* showed that it also worked as a ceRNA in the *LINC01234*/miR-433-3p/GRB2 network to accelerate non-small cell lung cancer proliferation [9]. In female-related cancers, including ovarian and triple-negative breast cancer, researchers found that *LINC01234* regulated microRNA, and its targets promoted tumor progression [10, 11]. However, there is currently no research on the role of *LINC01234* in UCEC.

2. Material and methods

2.1 *LINC01234* expression data and UCEC bioinformatics analysis

RNA-sequencing data and clinical information of 552 UCEC cases, including 35 cases with matched adjacent tissues, were collected from the TCGA database (https://genome-cancer.ucsc.edu/). Initially, the downloaded format of RNA-seq data was of level 3 HTSep-fragments per kilobase per million (FPKM) and was transformed into a transcript per million (TPM) format after subsequent analysis. We also assessed *LINC01234* expression using the UCSC (University of California, Santa Cruz) Xena-Brower (https://xenabrowser.net/datapages/), a web-based tool based on the RNA-seq data from TCGA (181 UCEC samples and 23 adjacent samples) and Genotype-Tissue Expression (GETx) (78 normal samples). In addition, we also downloaded the dataset GSE106191 from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), which contained 64 cases of endometrial cancer tissues and 33 cases of endometrial hyperplasia [12]. Further, the web tool Lnc2Cancer 3.0 was also used to analyze *LINC01234* expression in several cancers [13]. This study was performed following the ICMJE requirements.

2.2 Clinical tissue specimens and quantitative real-time polymerase chain reaction (qRT-PCR)

We acquired a total of 29 case clinical tissue cDNA which were kindly provived by the Department of Gynecology, Anonymous Hospital and Anonymous university. As described previously [14], these tissue specimens were from the surgery or biopsy in the Department of Gynecology, Anonymous Hospital and Anonymous university from September 2020 to November 2021, and which included two paired EC and adjacent tissues. Entire RNA was reversely transcribed by the reverse transcription kit (Vazyme, Nanjing, China) to be cDNA which was as described previously [14]. The process was conducted on the ABI (Applied Biosystem) StepOne Plus Detector System by SYBR Green I Assay (Takara Shuzo Co. Ltd, Kyoto, Japan). $2^{-\Delta\Delta Ct}$ method was used to calculated RNA transcripts. The house keeping gene *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase) was used for internal control. The primers sequences for qRT-PCR were showed as follows: *LINC01234*-F: 5′-AGCAGGTAGCTACATCCCACAA-3′; *LINC01234*-R: 5′-GGCAGAGCAAGAAGCAAAACAT-3′; *GAPDH*-F: 5′-GAGTCAACGGATTTGGTCGT-3′; *GAPDH*-R: 5′-GACAAGCTTCCCGTTCTCAG-3′.

2.3 Signature pathway enrichment

The GSEA and MSigDBv6.2 (Molecular Signatures Database) were downloaded from the GSEA website (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp). GSEA graph was constructed using the R package clusterProfiler (version 3.6.0, China). We obtained H.all.v6.1.symbols.gmt as the reference gene collection in the MSigDBv6.2 Collections. Hallmark pathway enrichment was performed as previously described [15]. The normalized NS (NES) value > 1, false discovery rate (FDR) q-value < 0.25 and p value < 0.05 was used to select significantly enriched pathways.

2.4 Relationship between immune infiltration and *LINC01234* analysis

The single sample GSEA (ssGSEA) method was used for the immune infiltration analysis as previously described [16]. Briefly, the “GSVA (gene set variation analysis)” function in the R package was used for the ssGSEA method. Then, 24 kinds of immune infiltrated cells from the literature gene expression profile were quantified [17]. The Spearman and Wilcoxon rank sum tests were used to calculate P values to determine the correlation between *LINC01234* and immune infiltrated cells.

2.5 Statistical analysis

Statistical analysis was performed using R (V3.6.3). The Wilcoxon rank sum test, Fisher exact test, Chi-square test and logistic regression were used to determine the relationship between *LINC01234* expression and clinical features. The impact of *LINC01234* on OS was estimated using the Kaplan-Meier method. Cox proportional hazard models were established for evaluating risk factors associated with progression-free survival. p values ≤ 0.05 represented statistical significance.

3. Results

3.1 High *LINC01234* expression was associated with poor clinical characteristics in UCEC

Using the web tools Lnc2Cancer 3.0, we found that *LINC01234* was overexpressed in several tumors (**Supplementary Fig. 1**), including UCEC. Next, we investigated the expression level of *LINC01234* as the first step to understanding its function in UCEC. Our results, based on 552 UCEC tissues and 35 adjacent tissues from the TCGA database (Fig. 1a), showed a higher expression in UCEC tissues than in adjacent normal tissues. However, further comparison only using 35 paired-match tissues from the TCGA database showed no statistical significance between tumoral and adjacent normal tissues (Fig. 1b). We also observed 2 paired-match clinical specimens while it also showed high expression tendency of *LINC01234* in UCEC than adjacent normal tissues (**Supplementary Fig. 2**), which we hypothesized could have resulted due to low tissue quantity for determining statistical significance. Additional analysis comparing 23 normal tissues from TCGA and 78 normal tissues from GTEx, showed that the expression of *LINC01234* in 181 UCEC tissues from TCGA was significantly higher than in normal tissues (Fig. 1c). In the GSE106191 dataset from the GEO database, we analyzed *LINC01234* expression between endometrial cancer (n = 64) and endometrial hyperplasia (n = 33) tissues, and as expected, the results showed that the tumoral tissues had higher expression of *LINC01234* (Fig. 1d). We detected *LINC01234* expression by qRT-PCR in 29 case clinical tissue specimens, which contain 10 normal endometrial tissues and 19 endometrial cancer tissues (Fig. 1e). The overexpression of *LINC01234* in UCEC was confirmed again in clinical specimens. The effectiveness of *LINC01234* that distinguish UCEC from normal was investigated and demonstrated an AUC value of 0.726 (Fig. 1f); indicating that the expression level of *LINC01234* could be used to differentiate UCEC from normal tissues.

The relationship between *LINC01234* and clinicopathological characteristics was determined in UCEC using the TCGA database. A high (n = 276) and low (n = 276) group was stratified based on the mean expression level of *LINC01234*. The baseline characteristics of the 552 patients from the TCGA database are shown in Table 1. Chi-square test or Fisher's exact test showed that *LINC01234* was associated with overall survival (p = 0.009), disease-specific survival (p = 0.007), progression-free interval (p = 0.009), clinical stage ($p < 0.001$), primary therapy outcome (p = 0.042), histological type ($p < 0.001$), residual tumor classification ($p < 0.001$), histologic grade ($p < 0.001$) and tumor invasion (p = 0.004). Further, other subgroup analyses were performed based on different clinical features include age, body mass index (BMI), menopause status, hormones therapy, diabetes, radiation therapy, surgical approach, which had no significant difference.

Further analysis validated that a higher clinical stage was associated with a higher expression of *LINC01234* (Fig. 2a). Association with primary therapy outcomes based on evaluation criteria for solid tumors (WHO), including progressive disease (PD), stable disease (SD), partial response (PR) and complete response (CR), showed that patients who had CR demonstrated the lowest expression of *LINC01234* (Fig. 2b). Association with histological type showed that the mixed and serous histology exhibited higher expression of *LINC01234* than endometrioid histology (Fig. 2c). In regard to residual tumor classification, patients who underwent R1 and R2 resection demonstrated *LINC01234* overexpression compared with R0 resection patients (Fig. 2d). Further, patients with G2 and G3 histological grade demonstrated higher *LINC01234* expression than those of the G1 group (Fig. 2e). The tumor invasion proportion for grouping, more than 50% tumor invasion group was higher *LINC01234* expression than the less than 50% tumo invasion group (Fig. 2f). Overall, these findings showed that higher expression of *LINC01234* was associated with more aggressive clinicopathological characteristics, indicating that it could predict poorer outcomes of UCEC patients.

Logistic regression method showed that *LINC01234* was significantly related to clinical stage ($p < 0.001$), primary therapy outcome (p = 0.006), histological type ($p < 0.001$), residual tumor classification ($p < 0.001$), histologic grade ($p < 0.001$) and tumor invasion (p = 0.003) (Table 2).

Next, we performed Kaplan-Meier analysis to determine the prognostic value of LIN01234 for progress-free interval (PFI, Fig. 3a), disease-specific survival (DSS, Fig. 3b), overall survival (OS, Fig. 3c) and disease-free survival (DFS, **Supplementary Fig. 3**) in 551 UCEC cases grouped into low and high groups based on the mean expression level of LIN01234. The results showed that the high LIN01234 expression group had worse prognoses.

3.2 Prognostic factor based on Cox regression model in UCEC

To further investigate the prognostic value of *LINC01234*, its association with progress free interval (PFI) (Table 3), disease-specific survival (DSS) (Table 4), overall survival (OS) (Table 5), were determined via Cox univariate and multivariate analysis. The results showed that clinical stage, primary therapy outcome, residual tumor classification and *LINC01234* expression ($p < 0.05$) were significantly associated with PFI, DSS and OS. Multivariate analysis identified clinical stage and primary therapy outcome as independent factors for PFI, DSS and OS. Residual tumor classification and *LINC01234*expression were an independent factor for DSS.

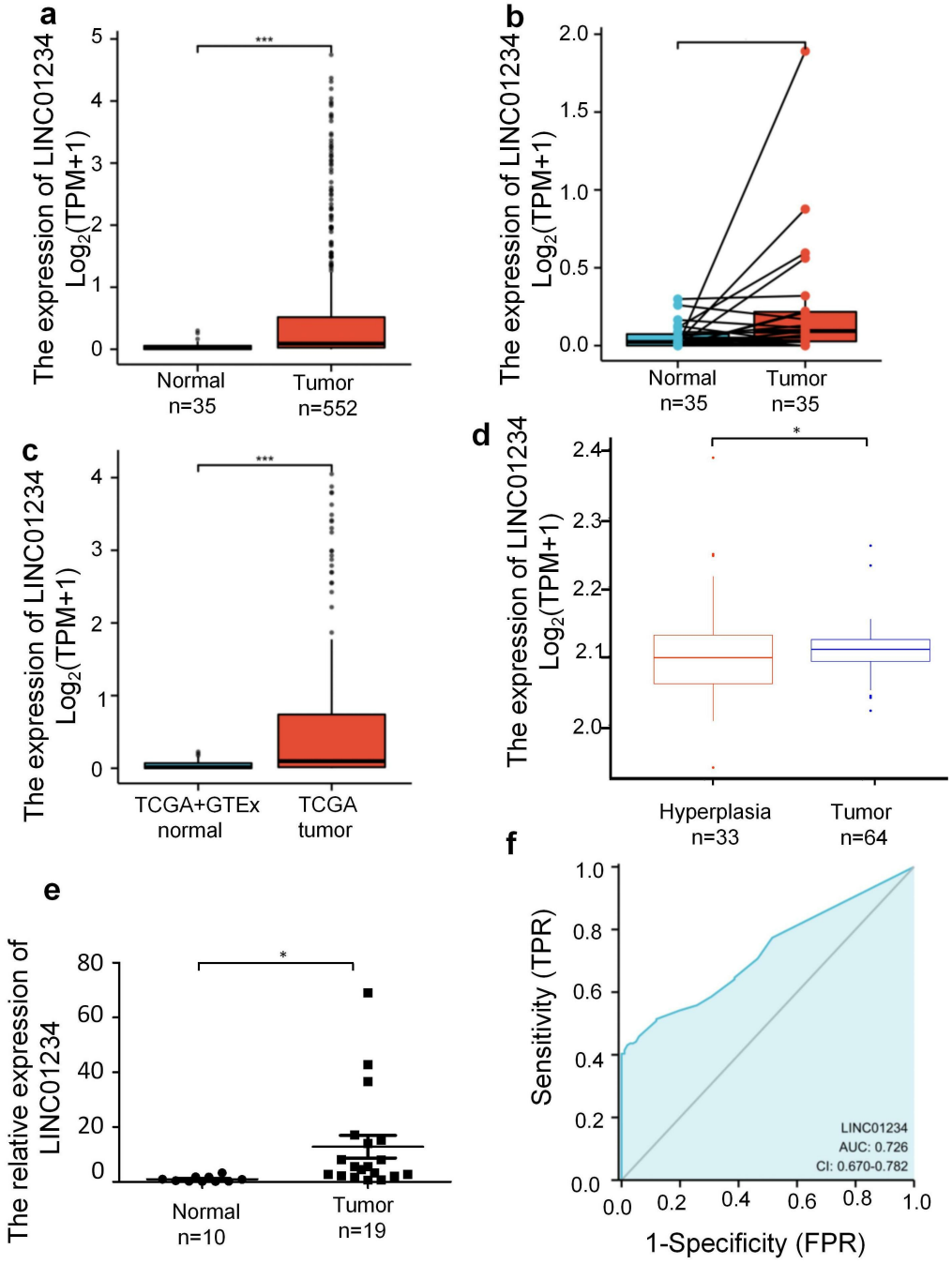

FIGURE 1. Difference in *LINC01234* expression level between normal and UCEC tissues. (a) The expression of *LINC01234* in 552 UCEC tissues and 35 adjacent normal tissues from the TCGA database was significantly different, based on the Wilcoxon rank sum test. (b) The expression of *LINC01234* in paired 35 UCEC tissues and adjacent tissues from the TCGA database showed no statistical difference, based on the Wilcoxon signed rank sum test. (c) The difference in *LINC01234* expression in normal tissues from the TCGA and GTEx was statistically different from the UCEC tissues of the TCGA, based on the Wilcoxon rank sum test. (d) The difference of *LINC01234* expression in endometrial hyperplasia tissues (n = 33) and endometrial cancer tissues (n = 64) from the GEO database was based on the Wilcoxon rank sum test. (e) The difference of *LINC01234* expression in normal endometrial tissues (n = 10) and UCEC tissues (n = 19) from clinical specimens, was normalized with the mean value of normal endometrial tissues based on qRT-PCR results. (f) The *LINC01234* can distinguish normal and UCEC tissues with an AUC of 0.726. The FPR is represented by the X-axis, and the corresponding TPR is represented by the Y-axis. *, p-value $<$ 0.05. ***, p-value $<$ 0.001. Abbreviations: FPR: false positive rate; TPR: true positive rate; UCEC: uterus corpus endometrial cancer; TPM: transcript per million; TCGA: The Cancer Genome Atlas; GTEx: Genotype-Tissue Expression; AUC: area under the curve; CI: confidence interval.

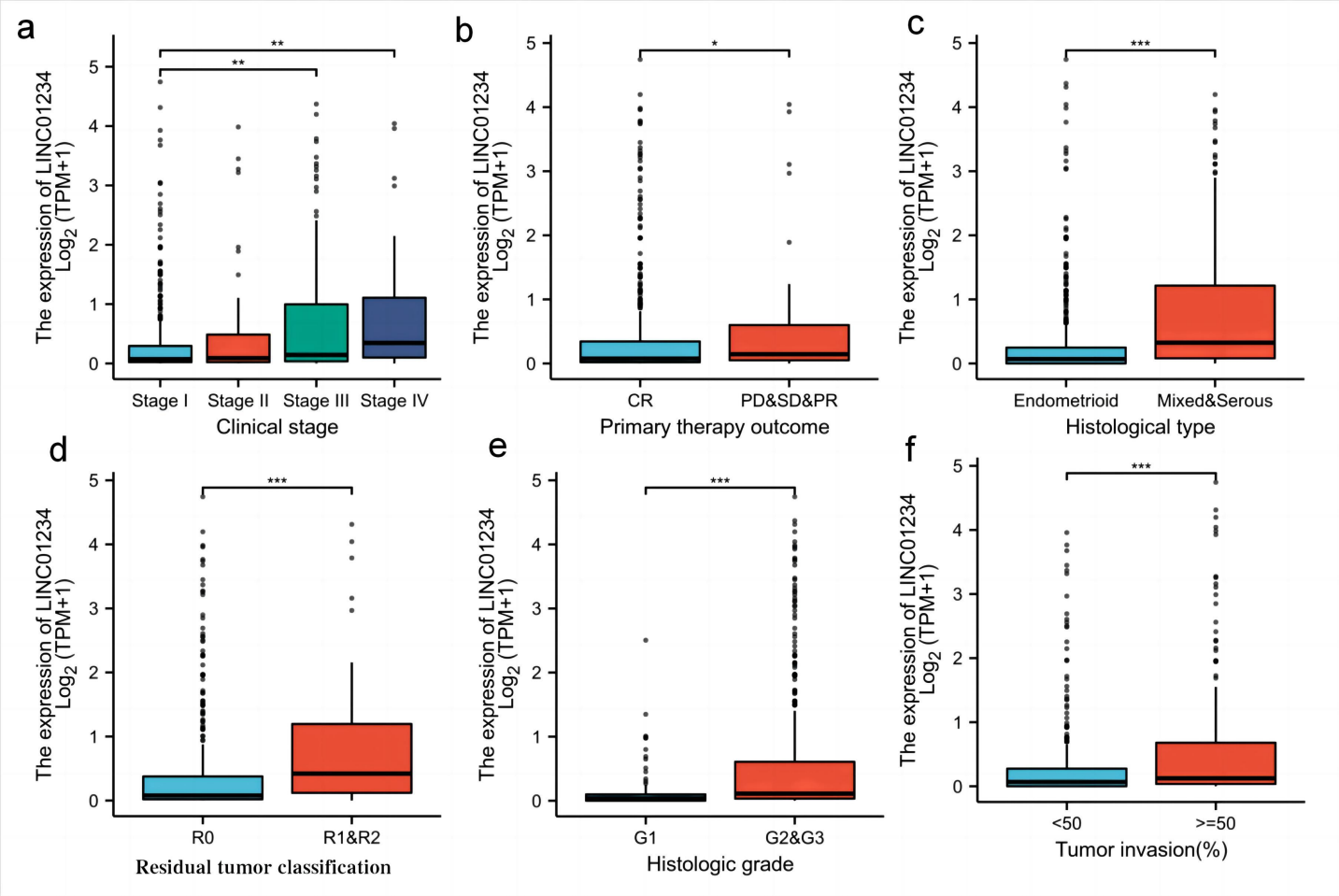

FIGURE 2. Association between *LINC01234* expression and the clinicopathological characteristics of UCEC patients from the TCGA database. The Wilcoxon rank sum test was used to detect the relationship between the expression of *LINC01234* and clinical stage (a), primary therapy outcome (b), histological type (c), residual tumor classification (d), histologic grade, and (e), tumor invasion (f). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. CR: complete response; PD: progressive disease; SD: stable disease; PR: partial response. TPM: transcript per million.

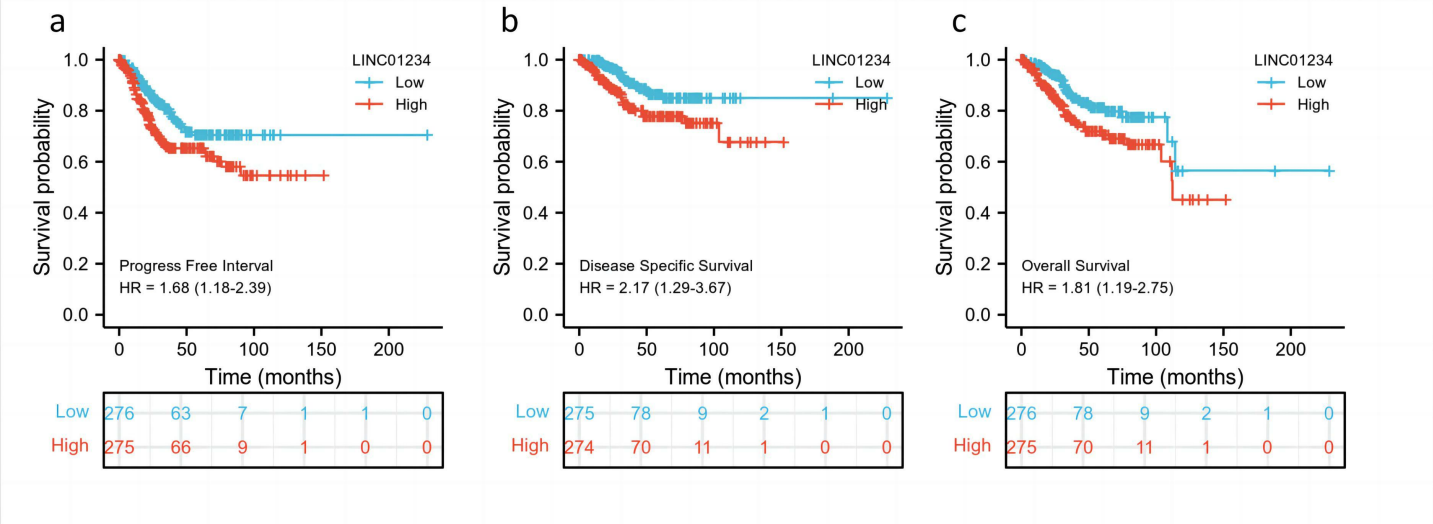

FIGURE 3. *LINC01234* expression influenced the prognosis of UCEC. (a) High *LINC01234* expression group have lower PFI ($p = 0.004$) than low *LINC01234* expression group. (b) High *LINC01234* expression group also have lower DSS ($p = 0.004$). (c) High *LINC01234* expression group have lower OS ($p = 0.005$). Abbreviations: PFI: progress-free interval; DSS: disease-specific survival; OS: overall survival; UCEC: uterus corpus endometrial cancer; HR: hazard ratio.

TABLE 1. Correlation between *LINC01234* expression and the clinicopathological characteristics of UCEC patients.

Characteristic	Low expression of *LINC01234*	High expression of *LINC01234*	*p*
n	276	276	
OS event, n (%)			
Alive	241 (43.7%)	217 (39.3%)	0.009
Dead	35 (6.3%)	59 (10.7%)	
DSS event, n (%)			
Alive	254 (46.2%)	233 (42.4%)	0.007
Dead	21 (3.8%)	42 (7.6%)	
PFI event, n (%)			
Alive	225 (40.8%)	198 (35.9%)	0.009
Dead	51 (9.2%)	78 (14.1%)	
Clinical stage, n (%)			
Stage I	191 (34.6%)	151 (27.4%)	<0.001
Stage II	25 (4.5%)	26 (4.7%)	
Stage III	53 (9.6%)	77 (13.9%)	
Stage IV	7 (1.3%)	22 (4.0%)	
Primary therapy outcome, n (%)			
PD	6 (1.2%)	14 (2.9%)	0.042
SD	2 (0.4%)	4 (0.8%)	
PR	4 (0.8%)	8 (1.7%)	
CR	245 (51.0%)	197 (41.0%)	
Age, n (%)			
≤60	108 (19.7%)	98 (17.9%)	0.488
>60	168 (30.6%)	175 (31.9%)	
BMI, n (%)			
≤30	107 (20.6%)	105 (20.2%)	1.000
>30	155 (29.9%)	152 (29.3%)	
Histological type, n (%)			
Endometrioid	234 (42.4%)	176 (31.9%)	<0.001
Mixed	9 (1.6%)	15 (2.7%)	
Serous	33 (6.0%)	85 (15.4%)	
Residual tumor classification, n (%)			
R0	200 (48.4%)	175 (42.4%)	<0.001
R1	6 (1.5%)	16 (3.9%)	
R2	2 (0.5%)	14 (3.4%)	
Histologic grade, n (%)			
G1	71 (13.1%)	27 (5.1%)	<0.001
G2	76 (14.0%)	44 (8.1%)	
G3	124 (22.9%)	199 (36.8%)	
Tumor invasion (%), n (%)			
<50	149 (31.4%)	110 (23.2%)	0.004
≥50	94 (19.8%)	121 (25.5%)	
Menopause status, n (%)			
Pre	20 (3.9%)	15 (3.0%)	0.536
Peri	10 (1.9%)	7 (1.4%)	
Post	225 (44.5%)	229 (45.3%)	

TABLE 1. Continued.

Characteristic	Low expression of *LINC01234*	High expression of *LINC01234*	*p*
n	276	276	
Hormones therapy, n (%)			
No	158 (45.9%)	139 (40.4%)	0.700
Yes	27 (7.8%)	20 (5.8%)	
Diabetes, n (%)			
No	169 (37.5%)	159 (35.3%)	0.885
Yes	65 (14.4%)	58 (12.9%)	
Radiation therapy, n (%)			
No	144 (27.3%)	135 (25.6%)	0.849
Yes	125 (23.7%)	123 (23.3%)	
Surgical approach, n (%)			
Minimally Invasive	115 (21.7%)	93 (17.5%)	0.053
Open	149 (28.1%)	173 (32.6%)	

OS: overall survival; DSS: disease-specific survival; PFI: progress free interval; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response; BMI: body mass index.

TABLE 2. Logistic regression analysis between *LINC01234* expression and clinicopathologic characteristics.

Characteristics	Total (N)	Odds Ratio (OR)	*p* value
Clinical stage (Stage II & Stage III & Stage IV *vs.* Stage I)	552	1.860 (1.315–2.641)	<0.001
Primary therapy outcome (PD & SD & PR *vs.* CR)	480	2.695 (1.354–5.664)	0.006
Histological type (Mixed & Serous *vs.* Endometrioid)	552	3.166 (2.114–4.808)	<0.001
Residual tumor classification (R0 *vs.* R1&R2)	413	0.233 (0.098–0.499)	<0.001
Histologic grade (G2 & G3 *vs.* G1)	541	3.195 (1.997–5.243)	<0.001
Tumor invasion (%) (≥50 *vs.* <50)	474	1.744 (1.212–2.517)	0.003

Abbreviations: PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

TABLE 3. Cox regression analysis between PFI and clinicopathologic characteristics using the TCGA database.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
Clinical stage	551				
Stage I	341	Reference			
Stage II & Stage III & Stage IV	210	2.527 (1.780–3.587)	<0.001	2.403 (1.458–3.960)	<0.001
Primary therapy outcome	480				
PD & SD & PR	38	Reference			
CR	442	0.120 (0.078–0.184)	<0.001	0.185 (0.105–0.325)	<0.001
Residual tumor classification	412				
R0	374	Reference			
R1 & R2	38	2.724 (1.623–4.571)	<0.001	1.386 (0.733–2.621)	0.315
LINC01234	551				
Low	276	Reference			
High	275	1.681 (1.181–2.393)	0.004	1.372 (0.859–2.193)	0.185

CI: confidence interval; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response.

TABLE 4. Cox regression analysis between DSS and clinicopathologic characteristics using the TCGA database.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
Clinical stage	549				
Stage I	340	Reference			
Stage II & Stage III & Stage IV	209	6.034 (3.372–10.797)	<0.001	5.255 (2.173–12.708)	<0.001
Primary therapy outcome	480				
PD & SD & PR	38	Reference			
CR	442	0.074 (0.042–0.129)	<0.001	0.182 (0.086–0.387)	<0.001
Residual tumor classification	412				
R0	374	Reference			
R1 & R2	38	5.310 (2.894–9.745)	<0.001	2.283 (1.067–4.886)	0.033
LINC01234	549				
Low	275	Reference			
High	274	2.175 (1.288–3.672)	0.004	2.116 (1.032–4.338)	0.041

CI: confidence interval; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response.

TABLE 5. Cox regression analysis between OS and clinicopathologic characteristics using the TCGA database.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p* value	Hazard ratio (95% CI)	*p* value
Clinical stage	551				
Stage I	341	Reference			
Stage II & Stage III & Stage IV	210	3.270 (2.145–4.984)	<0.001	2.790 (1.550–5.021)	<0.001
Primary therapy outcome	480				
PD & SD & PR	38	Reference			
CR	442	0.129 (0.078–0.215)	<0.001	0.272 (0.139–0.533)	<0.001
Residual tumor classification	412				
R0	374	Reference			
R1 & R2	38	3.101 (1.768–5.440)	<0.001	1.841 (0.927–3.654)	0.081
LINC01234	551				
Low	276	Reference			
High	275	1.812 (1.193–2.753)	0.005	1.659 (0.962–2.861)	0.068

CI: confidence interval; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response.

3.3 Signaling pathways of *LINC01234* via GSEA

Here, we explored *LINC01234*-related pathways related to tumor progression. GSEA showed that *LINC01234* expression was correlated with hallmark terms *E2F* targets, pancreas-β-cells, notch signaling, wnt-β-catenin-signaling, spermatogenesis, TGF (transforming growth factor)-β-signaling, angiogenesis and reactive oxygen species pathways (Fig. 4). These pathways were related with cell proliferation, spermatogenesis or angiogenesis and contributed to tumorigenesis. The *p* value < 0.05 and FDR <0.25 represent significant enrichment. The corresponding NES, P, and FDR q values are shown in Table 6.

3.4 Relationship between *LINC01234* expression and immune infiltration cell

It is well-known that the immune cells of the tumor microenvironment constitution are among the key factors determining tumor progression. Thus, here, we investigated the relationship between immune cell infiltration and *LINC01234* expression using the Spearman correlation method, based on the absolute Spearman r value. (Fig. 5a). The results showed that the expression of *LINC01234* was negatively correlated with neutrophils infiltration (Fig. 5b) and the NK CD56bright cells infiltration (Fig. 5d). The Spearman Correlation Coefficient between the infiltrative neutrophils or NK CD56bright cells and the expression of *LINC01234* respectively were −0.260

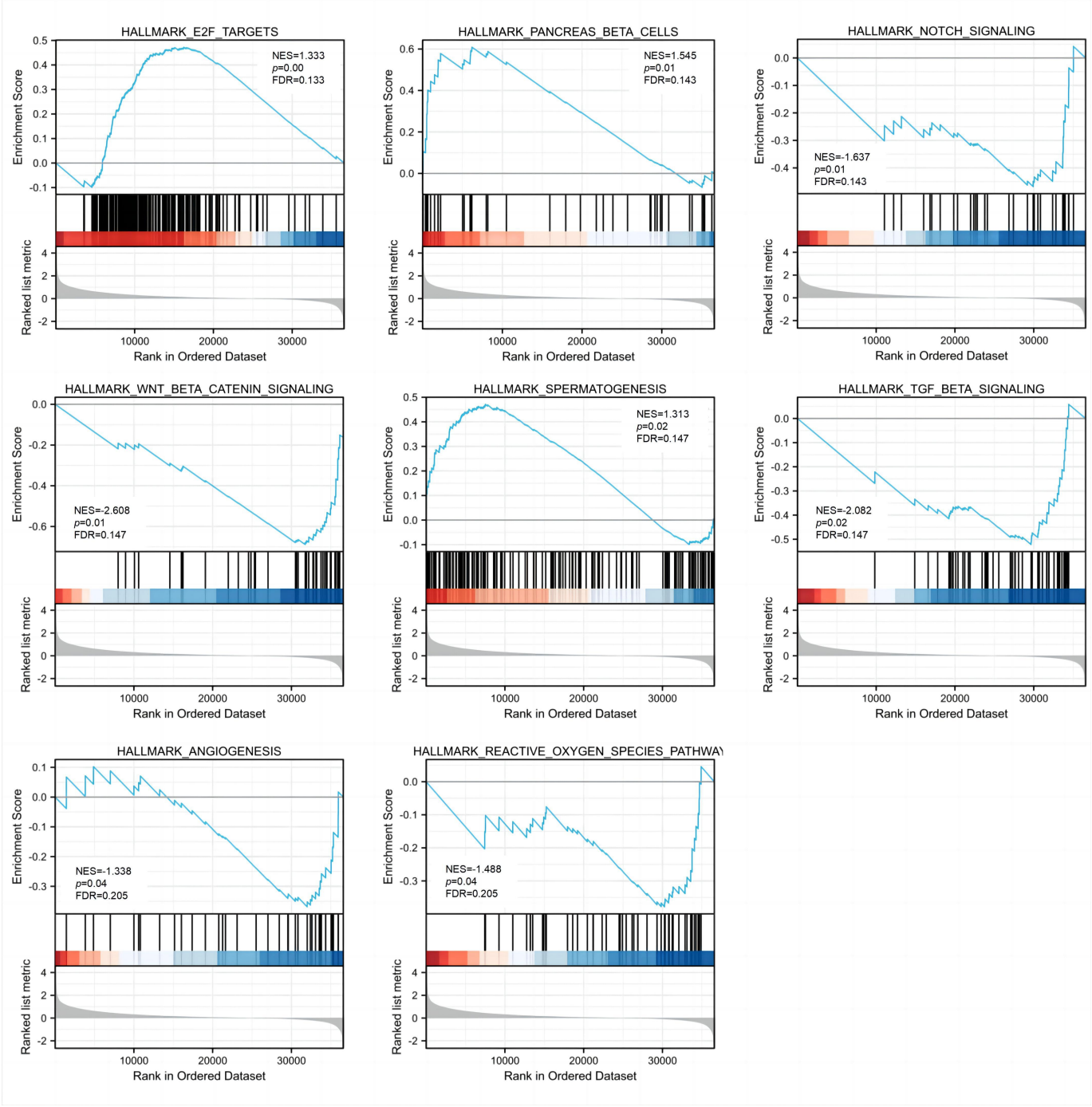

FIGURE 4. GSEA was used to draw the enrichment plot. The *LINC01234* high expression group is significantly enriched in the left red area. Abbreviations: NES: normalized NS; FDR: false discovery rate.

(Fig. 5c, $p < 0.001$) and −0.210 ($p < 0.001$, Fig. 5e). Collectively, these results showed that *LINC01234* was negatively correlated with infiltrative neutrophils and NK CD56bright cells which influenced immune response in the tumor microenvironment.

4. Discussion

Endometrial cancer is the sixth most common gynecological malignant tumor worldwide and has incremental morbidity and mortality, which urges the need for more precise biomarkers that could reflect the tumor microenvironment and predict the effectiveness of therapy to guide treatment decisions [18]. In this study, we investigated the association between *LINC01234* expression and the diagnosis and prognosis of UCEC. Dysregulation in *LINC01234* was shown to play an important role in the initiation and progression of several cancers [6–11]. For instance, the expression of *LINC01234* was found to be upregulated in triple-negative breast cancer (TNBC) cell lines or tissues [11]. *LINC01234* was also reported to be closely related to TNBC cell growth and differentiation via GSEA analysis. Further, *in vivo* and *in vitro* analysis showed that *LINC01234* promoted tumor growth and invasion [11]. In this study, our results showed that *LINC01234* expression was

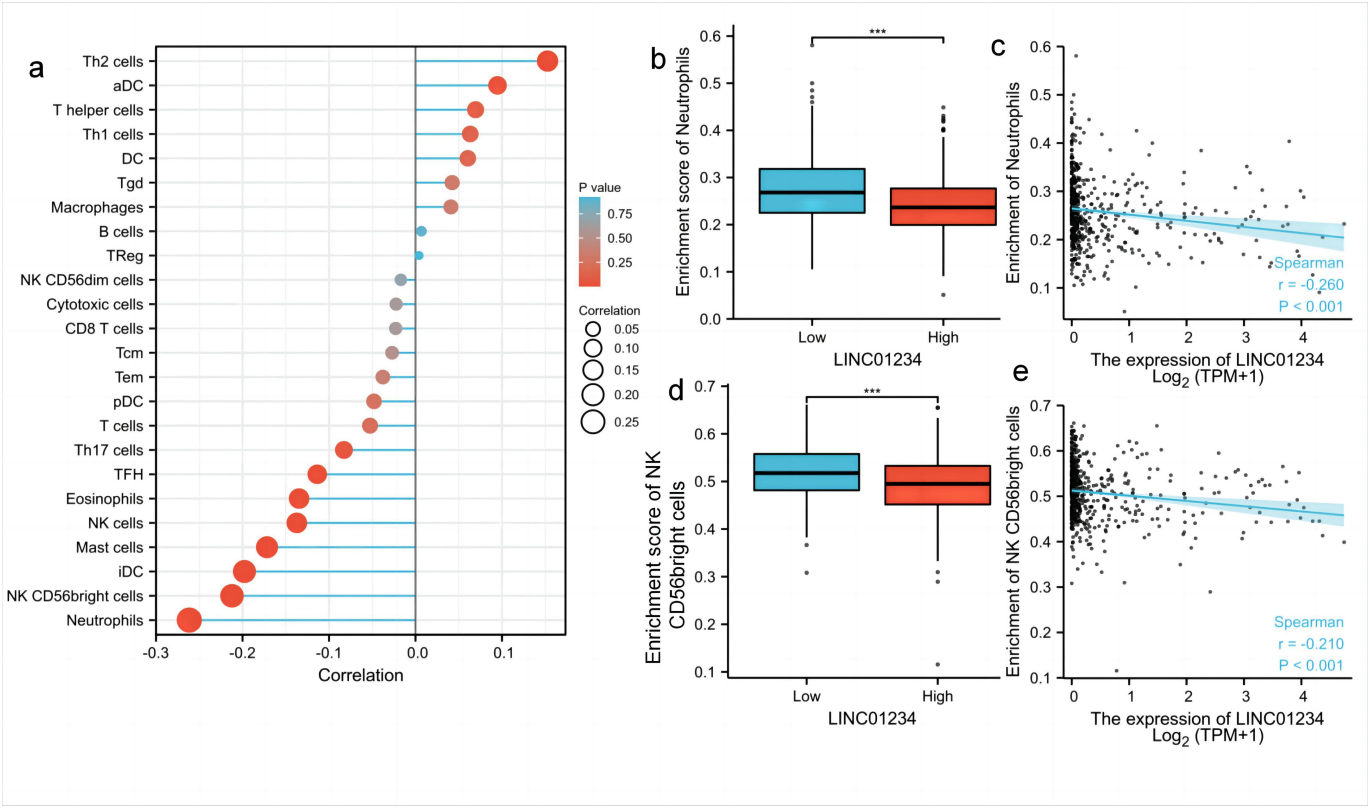

FIGURE 5. Relationship between the expression of *LINC01234* and immune infiltration. (a) The forest plot shows the correlation between *LINC01234* expression and 24 types of immune cells. The dot color represents *P* values, and the dot size represents the Spearman correlation r value. (b) The difference in neutrophils between *LINC01234* low and high expression groups was determined using the Wilcoxon rank sum test. (c) The correlation between *LINC01234* expression and neutrophils was shown by the Spearman correlation method. (d) The difference of NK CD56bright cells between *LINC01234* low and high expression groups was identified using the Wilcoxon rank sum test. (e) The correlation between *LINC01234* expression and neutrophils is shown by the Spearman correlation method. Abbreviations: aDC: activated dendritic cell; Tgd: T gamma delta; Treg: T regulatory cell; Tcm: T central memory; Tem: T effector memory; pDC: plasmacytoid dendritic cell; TFH: T follicular helper cell; NK cells: natural killer cells; iDC: interdigitating cell; CD: cluster of differentiation; TPM: transcript per million.

TABLE 6. GSEA analysis showing the enriched hallmark terms in low and high *LINC01234* expression groups.

ID	ES	NES	*p* value	FDR
HALLMARK E2F TARGETS	0.47	1.33	0.00	0.13
HALLMARK PANCREAS BETA CELLS	0.61	1.55	0.01	0.14
HALLMARK NOTCH SIGNALING	−0.47	−1.64	0.01	0.14
HALLMARK WNT BETA CATENIN SIGNALING	−0.69	−2.61	0.01	0.15
HALLMARK SPERMATOGENESIS	0.47	1.31	0.02	0.15
HALLMARK TGF BETA SIGNALING	−0.52	−2.08	0.02	0.15
HALLMARK ANGIOGENESIS	−0.37	−1.34	0.04	0.20
HALLMARK REACTIVE OXYGEN SPECIES PATHWAY	−0.38	−1.49	0.04	0.20

Abbreviations: ES, enrichment score. NES, normalized enrichment score; FDR, false discovery rate.

negatively associated with the Wnt-β-catenin signal pathway based on GSEA analysis, opposite to a previous study which showed that *LINC01234* activated the Wnt-β-catenin signaling pathway to enhance TNBC progression [19]. This finding may indicate that *LINC01234* has different functions in different tumors. Thus, the signaling mechanisms observed in this study should be further validated via experimental research. We also observed that high *LINC01234* expression in UCEC was associated with worse clinical outcomes, which was concordant with the findings in colorectal cancer, indicating that it was associated with reduced OS and more aggrevssive clinicopathological factors [20], thereby indicating *LINC01234* as a predictive biomarker of UCEC.

Recently, the influence of immune cell infiltration on cancers was reported [21–23]. In UCEC, immune infiltration was a research hotspot and was reported to facilitate tumor

progression [24]. In gastric cancer, *LINC01234* was shown to be involved in immune-related pathways such as the B-cell receptor signaling pathway [7]. However, to the best of our knowledge, there is no research on the relationship between the expression of *LINC01234* and infiltrated immune cells of the tumor microenvironment. Our results showed that *LINC01234* was negatively correlated with neutrophils and NK CD56bright cells. High-risk breast cancer was associated with a low abundance of tumor-infiltrated neutrophils and worse overall outcomes [25]. Similarly, in this research, a low abundance of tumor-infiltrated neutrophils was associated with high expression of *LINC01234*, and when high expression of *LINC01234* in UCEC was associatied with poorer survival outcomes. The literature [26] showed in newly diagnosed multiple myeloma NK cells are shifted towards CD56bright NK cells which exhibits selective loss of cytokine production. What's more, CD56bright NK cells secreted interferon-γ, tumor necrosis factor-α to regulate immune system [27]. So, we inferred that *LINC01234* depressed NK CD56bright cells, leading to faster progression to a more advanced UCEC clinical stage. Overall, high expression of *LINC01234* could impair immune infiltration, including neutrophils and NK CD56bright cells, and lead to worse clinical features. This correlation indicates a potential mechanism for tumor cells to escape the immune system and could thus be a new direction for immune target therapy.

To the best of our knowledge, we are the first to explore the significance of *LINC01234* in UCEC and immune infiltration. However, despite the important findings reported in this study, there were some limitations that should be addressed. The conclusions were mainly based on bioinformatic analysis of the TCGA database only with clinical tissue specimens expression validation by qRT-PCR in experimental research. In addition, the number of normal and tumor tissues might have been limited, which might have contributed to the lack of statistical significance for the expression of *LINC01234* compared between two paired groups in Fig. 1b and **Supplementary Fig. 2**. Further, although we evaluated several pathways with *LINC01234* in UCEC, the network in the tumor microenvironment of *LINC01234* was quite complex and deserved more research. Thus, the findings of this study should be validated to fully elucidate the role of *LINC01234* in UCEC and immune infiltration.

5. Conclusions

Based on existing literature, we hypothesized that *LINC01234* could modulate the occurrence and development of UCEC. Thus, in this study, we investigated the diagnostic and prognostic significance of *LINC01234* in UCEC. Information on *LINC01234* expression was retrieved from the TCGA database, which was verified in clinical tissue specimens. We explored the difference in *LINC01234* expression between UCEC and corresponding normal tissues, the relationship between *LINC01234* and the clinical features of UCEC patients, and the association of high and low *LINC01234* expression with the survival of UCEC patients. GSEA analysis was also performed to determine the functions of *LINC01234*, and Spearman correlation analysis to investigate its influence on immune infiltration to promote tumor progression.

This present study showed that an increased *LINC01234* expression in UCEC was associated with worse clinical characteristics and significantly shorter PFI, DSS and OS. The related pathways and immune infiltration cells with *LINC01234* expression in UCEC were also explored. Collectively, we revealed the potential functions of *LINC01234* in UCEC and indicated that it could be a potential biomarker for the diagnosis, prognostic estimation and guiding the treatment of UCEC.

AVAILABILITY OF DATA AND MATERIALS

All the datasets analysed during the current study are available in the TCGA database (https://genome-cancer.ucsc.edu/) and the dataset GSE106191 is from the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

AUTHOR CONTRIBUTIONS

PM and AQX—designed the research study. AQX—performed the research. PM, AQX and LZ—analyzed the data. PM and AQX—wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (No. 2022-S017). All patients have signed the written informed consent forms before surgical resection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.ejgo.net/files/article/1691341350587252736/attachment/Supplementary%20material.docx.

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