ORIGINAL RESEARCH

Transcriptome analysis constructed the necroptosis associated prognostic signature in endometrial cancer and identified EZH2 as a potential biomarker

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Abstract

Endometrial cancer (EC) is one of the most common malignancies of the female reproductive system, but our understanding of the tumor microenvironment of EC remains unclear. Programmed cell death (PCD) plays an important role in the genesis and progression of tumors. Necroptosis is a novel form of PCD that does not rely on the caspase system. However, the role of necroptosis in EC is unclear. Transcriptome data of endometrial cancer were downloaded from The Cancer Genome Atlas (TCGA) database and log2 conversion was performed. Expression analysis and correlation analysis were performed to explore necroptosis gene expression and interaction in EC. Lasso regression was used to construct necroptosis-related prognostic signature. Finally, immunocorrelation analysis and single cell sequencing analysis were used to explore the significance of this signature in EC tumor microenvironment. A total of 15 of the 17 necroptosis genes were differentially expressed in EC. Subsequently, necroptosis related prognostic signature was constructed through Lasso regression. Riskscore = $(-0.0999) \times$ *Toll-likereceptor4 (TLR4)* + $(-0.0528) \times$ *tumor necrosis factor receptor superfamily member 1A (TNFRSF1A)* + $(0.1208) \times$ *Enhancer of Zeste Homolog 2 (EZH2)* + $(-0.004) \times$ *N-myc Down-stream Regulated Gene 2 (NDRG2)*. EC patients can be divided into high-risk group and low-risk group based on the median riskscore and the high-risk group has a worse prognosis. Survival analysis showed a worse prognosis for patients in the high-risk group ($p < 0.05$). Immunomicroenvironment analysis showed a significant negative correlation between risk score and infiltration levels of B cells, CD4+ T cells, CD8+ T cells, Endothelial cells, macrophages, and NK cells. Subsequent cell experiments showed that knockdown of the key gene *EZH2* in signature significantly reduced the invasion, migration and healing abilities of EC cell lines, proving that *EZH2* is a promising marker of EC.

Keywords

Endometrial cancer; Programmed cell death; Necroptosis; *EZH2*; Bioinformatics

1. Background

Endometrial cancer (EC) is one of the most common types of cancer in gynecology [1, 2]. It is estimated that the number of new EC cases in the United States will exceed 60,000 in 2022 [3]. Obesity, lack of exercise, and high estrogen levels are considered risk factors for EC [4]. Surgical resection, chemotherapy, radiotherapy, endocrine therapy, and targeted therapy are commonly used for EC [5]. The main treatment options for endometrial cancer include surgery, radiation therapy, chemotherapy, and hormone therapy. The mainstay of treatment for endometrial cancer is surgery, which may involve a hysterectomy (removal of the uterus) and removal of the ovaries and fallopian tubes [6]. In some cases, lymph nodes in the pelvis and abdomen may also be removed [6]. The extent of surgery depends on the stage and grade of the cancer

[6]. Radiation therapy uses high-energy x-rays or other types of radiation to kill cancer cells [7]. It may be used before or after surgery, or as the primary treatment for some early-stage cancers [7]. Radiation therapy can be delivered externally, using a machine outside the body, or internally, using radioactive materials placed inside the body [7]. Chemotherapy involves the use of drugs to kill cancer cells [8]. It may be used alone or in combination with surgery and/or radiation therapy, depending on the stage and grade of the cancer. Chemotherapy is often used for more advanced or aggressive cancers [8]. Hormone therapy is used for certain types of endometrial cancer that are hormone-sensitive. It involves the use of medications that block the effects of estrogen on the cancer cells, such as tamoxifen or aromatase inhibitors. In some cases, a combination of treatments may be used to achieve the best possible outcome. Recently, immunotherapy,

which has been used in a variety of tumor types, has also been approved by the Food and Drug Administration (FDA) for the treatment of EC [8]. The prognosis of early EC is good but progressive or metastatic EC is often accompanied by poor prognosis, with a 5-year survival rate of less than 20% [9–11]. For advanced EC, there is often a lack of effective systematic treatment options, and drug resistance is common in patients, posing challenges for clinical treatment [12]. Complex tumor microenvironment and crosstalk of multiple tumor-related signals are thought to be involved in the progression of EC [13]. However, our understanding of the pathogenesis and tumor microenvironment of EC is limited. It is urgent to find a new entry point to explore the tumor microenvironment of EC, so as to provide new ideas for its diagnosis and treatment.

Programmed cell death (PCD) opens up a new field of cancer research [14]. It is well known that tumor cells' resistance to apoptosis is one of their hallmarks [15]. Inducing tumor cell death is a promising therapeutic direction [16]. Methods of programmed cell death include apoptosis, ferroptosis, pyroptosis, necroptosis, *etc.* [17]. Although the current anticancer agents based on PCD have achieved initial benefits, many patients still develop resistance to drugs targeting caspase [18]. However, necroptosis is a non-caspase-dependent form of PCD, making it the next potential tumor treatment line [19]. Understanding the role of necroptosis in cancer can provide insights into the treatment, prognosis, and risk stratification of patients with cancer.

Bioinformatics is playing an increasingly important role in cancer research [20]. Bioinformatics combines computer algorithms with cancer sequencing data to mine potential biomarkers and build relevant clinical models [21]. TCGA database and Gene Expression Omnibus (GEO) database are currently the most commonly used databases in the field of bioinformatics, containing a large amount of data on a variety of cancers [22].

In this study, we explored the role of necroptosis genes in endometrial cancer using bioinformatics analyses including correlation analysis, survival analysis, regression analysis, immune microenvironment analysis, and single-cell sequencing analysis. Our study may provide some new ideas for the diagnosis and treatment of endometrial cancer.

2. Materials and methods

2.1 Cell culture and transfections of cell lines

The Ishikawa (ISK) and Human endometrial cancer cell 1-A (HEC-1-A) cell lines was purchased from the Institute of the Chinese Academy of Sciences (Shanghai, China). The ISK and HEC-1-A cells were cultured in Dulbecco's modification of Eagle's medium (DMEM) (GIBCO, Nanjing, China) supplemented with 10% fetal bovine serum (C11995500BT, GIBCO, BRL, Invitrogen, Carisbad, CA, USA), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C under 5% carbon dioxide (CO_2) humidified air.

For on-plate transfection of HEC, and ISK cells, this assay were performed as previously reported by Xu *et al.* [23] with minor revision. Briefly, *EZH2*-specific siRNAs

were transfected into cell-lines. These cell lines were cultivated on six-well plates and then treated through using Lipofectamine-3000 according to the manual. After 48 hours transfection, cells were harvested for conducting further experiments. Gene-specific siRNA sequences were as follows: 5′-GAGGUUCAGACGAGCUGAUUU-3′.

2.2 RNA extraction and qPCR analysis

These experiments were performed as previously described by Xu *et al.* [23]. Gene-specific primers sequences were as follows: *EZH2* Forward 5′-TGCACATCCTGACTTCTGTG-3′, Reverse 5′-AAGGGCATTCACCAACTCC-3′; *GAPDH* Forward 5′-GGGAGCCAAAAGGGTCAT-3′, Reverse 5′-GAGTCCTTCCACGATACCAA-3′.

2.3 Wound-healing assay

The ISK and HEC-1-A cells were seeded in six-well plates, incubated overnight and then transfected. At 90% confluency, the cell monolayer was scratched with a 200 μL pipette. After washed with Phosphate Buffered Saline (PBS) to remove the debris for three times, the scratched plates were cultured in DMEM without FBS. Images of cell migration were captured at the indicated time points under a microscope at ×100 magnification.

2.4 Cell migration assays

This assay was performed as previously reported by Xu *et al.* [24] with minor revision. Briefly, after 48 h of treated HEC, and ISK cells with *EZH2* specific siRNAs, then perform cell migration assays. $3-5 \times 10^4$ cells were cultivated on the top of a membrane precoated with Matrigel (BD Biosciences) for cell invasion assays (without Matrigel for cell migration assays). Cells inside the upper chamber were removed after incubation for 24 h, 36 h, 48 h, 60 h. While cells on the lower membrane surface were fixed with methanol and then stained with 0.5% Crystal violet solution. Three-five randomly selected fields were counted in per well.

2.5 Data download

Endometrial cancer transcriptome data from TCGA database (https://www.tcga.org) and download in the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Single-cell sequencing dataset GSE139555 for endometrial carcinoma was downloaded from GEO database. All transcriptome data were log2 transformed.

2.6 Extraction and analysis of 17 necroptosis genes

Through a review of previously published literature, we identified 17 genes most associated with necroptosis. Subsequently, we analyzed the expression of these 17 genes in endometrial cancer and normal controls using the "Limma" R package. The "Corrplot" R package was used to analyze the correlation of these 17 genes in endometrial cancer.

2.7 Lasso regression was used to construct the necroptosis related prognostic signature

The Least absolute Shrinkage and Selection Operator (Lasso) regression is a compressed estimation. It produces a more refined model by constructing a penalty function, which compresses some of the coefficients and sets some of the coefficients to zero. Therefore, it retains the advantage of subset contraction and is a biased estimator for complex collinear data. We performed Lasso regression on the above 17 genes to construct prognostic signatures in endometrial cancer. The "Lars" R package was used for the above analysis. A risk score could then be calculated for each patient. Based on the median score, patients in the endometrial cancer cohort can be divided into both high-risk and low-risk groups. The "ggrisk" R package is used to show the patient's risk score. Survival analysis was performed between the two groups. The "survival" R package was used for survival analysis, and $p < 0.05$ was considered statistically significant. The "timeROC" R package is used to build the signature's Receiver Operating Characteristic (ROC) curve to evaluate its area under curve (AUC) value.

2.8 COX regression was performed to explore the independent prognostic value of signature

In order to reduce the influence of bias on the results, univariate COX regression and multivariate COX regression were performed to explore the independent prognostic value of genes in signature in endometrial cancer. Survival R package was used for COX regression analysis. The analysis objects included each gene in signature, age, race, Tumor Node Metastasis (TNM) stage, grade, and new tumor. Finally, a necroptosis-related nomogram was constructed in endometrial cancer using the "rms" R package.

2.9 Correlation analysis of immune cell infiltration

Immune microenvironment plays an important role in tumor genesis and progression. To explore the role of necroptosis related signature in the immune microenvironment, we performed immune correlation analysis. We downloaded the data of immune cell infiltration from TCGA and explored the relationship between risk score and immune cell infiltration through Spearman correlation test. $p < 0.05$ was considered statistically significant.

## 2.10 Expression analysis of model genes in different grades

R software (V4.0.3, The R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis to explore the expression of genes in signature in different endometrial cancers. A p value < 0.05 was considered statistically significant.

2.11 Single-cell sequencing analysis

Tumor Immune Single-cell Hub (TISCH) database contains data from 79 databases for the construction of single-cell sequencing maps in tumors. The database contains 2,045,746 cells from cancer patients and healthy donors. We used TISCH database to analyze the GSE139555 dataset in GEO database and explore the expression of genes in signature at single cell level.

3. Results

Our flow chart was shown in Fig. 1.
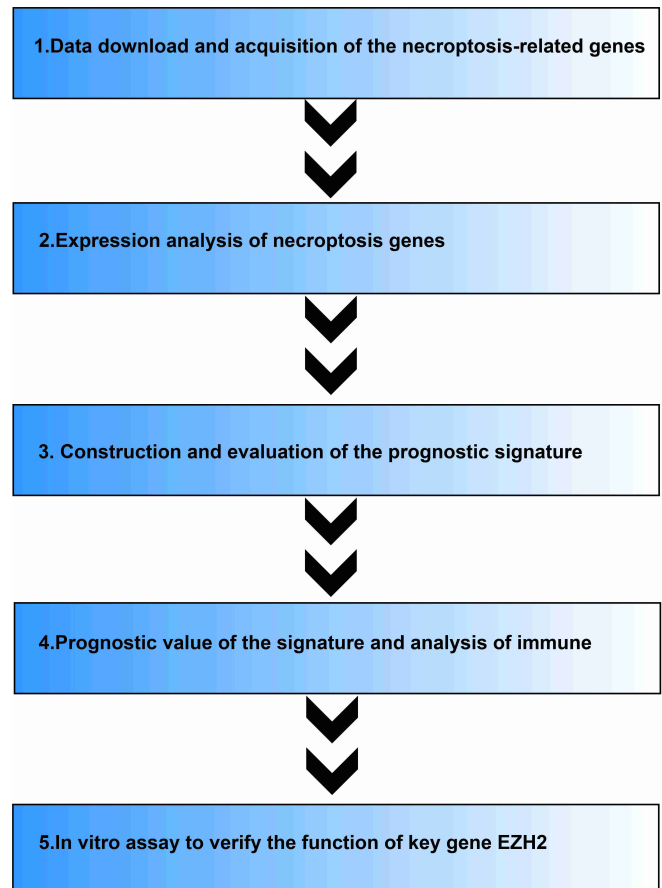
F I G U R E 1. The workflow of our study. *EZH2*: *Enhancer of Zeste Homolog 2*.

3.1 Expression and correlation analysis of necroptosis gene in endometrial carcinoma

As shown in Fig. 2A, 15 members of the 17 necroptosis genes were differentially expressed in endometrial cancer compared to normal tissue. *Receptor-Interacting Protein Kinase 1* (RIPK1), *Receptor-Interacting Protein Kinase 3* (RIPK3), *Mixed Lineage Kinase Domain-Like* (MLKL), *TLR4, TNFRSF1A, nuclear receptor subfamily 2, group C, member 2* (NR2C2), *High Mobility Group Box 1* (HMGB1), *Ubiquitin specific protease* (USP22), *aldehyde dehydrogenase 2 family* (ALDH2) and *N-myc Down-stream Regulated Gene 2* (NDRG2) were significantly down-regulated in endometrial cancer (Fig. 2A, **$p < 0.01$, ***$p < 0.001$). However,

phosphoglycerate mutase family member 5 (*PGAM5*), *Z-DNA binding protein 1* (*ZBP1*), *C-X-C ligand motif 1* (*CXCL1*), *TRAF2* and *EZH2* were significantly up-regulated in endometrial cancer (Fig. 2A, **$p < 0.01$, ***$p < 0.001$). Next, correlation analysis revealed necroptosis gene interactions in endometrial cancer. *HMGB1* and *EZH2* had the strongest positive correlation, with a correlation value of 0.51 (Fig. 2B).

3.2 Construction of the necroptosis-related prognostic signature

As shown in Fig. 3A,B, Lasso regression was used to construct prognostic signature. We can see that the optimal λ value is 4. Riskscore = $(-0.0999) \times TLR4 + (-0.0528) \times TNFRSF1A + (0.1208) \times EZH2 + (-0.004) \times NDRG2$. Each endometrial cancer patient in the TCGA database could be calculated to obtain a risk score. Patients were divided into high-risk and low-risk groups based on median risk score values as truncations (Fig. 3C). Survival analysis showed that the high-risk group had significantly worse prognosis (Fig. 3D, $p < 0.01$). ROC curve showed that the AUC values of this signature at 1, 3 and 5 years were 0.597, 0.633 and 0.659, respectively (Fig. 3E). Thus, the prognostic risk of EC patients can be quantified by this formula, and their 1, 3 and 5-year survival rates can be assessed.

3.3 COX regression was performed to explore the independent prognostic value of signature

To investigate the independent prognostic value of this signature and other clinical features, univariate and multivariate COX regressions were performed. First, univariate COX regression showed that EZH2, TLR4, TNFRSF1A, Age, TNM stage and grade were independent prognostic indicators of endometrial cancer (Fig. 4A). Multivariate COX regression showed that TLR4 and TNM stages were independent prognostic indicators of endometrial cancer (Fig. 4B). Subsequently, we constructed a Nomogram to evaluate the 1, 3 and 5-year survival rate of the patients (Fig. 4C). ROC curve showed that the 1, 3 and 5 years AUC values of nomogram were 0.80, 0.79 and 0.81, respectively (Fig. 4D). Subsequently, the clinical decision curve showed that patients with EC could benefit greatly from a clinical intervention based on nomogram (Fig. 4E). It can be seen that the signature constructed by us has high accuracy in the prognosis assessment of EC patients.

3.4 Correlation analysis of immune cell infiltration

To investigate the role of signature in the immune microenvironment of endometrial cancer, immune-correlation analysis was performed. The results showed a significant negative correlation between risk score and infiltration levels of B cells, CD4+ T cells, CD8+ T cells, Endothelial cells, macrophages, and NK cells (Fig. 5A–F). This may be the underlying cause of the difference in prognosis between the two groups and provide reference for us to understand the mechanism.

3.5 Expression analysis of model genes in different grades

As shown in Fig. 6A, TLR4 gene expression in the G2 and G3 groups was significantly up-regulated compared with endometrial cancer in the G1 group. As shown in Fig. 6B, the expression level of NDRG2 increased gradually with the increase of grade. However, the expression level of EZH2 decreased gradually with the increase of grade (Fig. 6C). The expression level of TNFRSF1A increased gradually with the increase of grade (Fig. 6D).

3.6 Single-cell sequencing analysis

Firstly, dimensionality reduction cluster analysis showed that endometrial cancer cells in dataset GSE139555 could be divided into CD4T conv cells, CD8T cells, CD8Tex cells, fibroblasts, Tprolif cells, and Treg cells (Fig. 7A). Subsequently, we explored the expression profiles of EZH2, TLR4, TNFRSF1A and NDRG2 genes in Signature at the single-cell level (Fig. 7B–E).

## 3.7 Cell experiments to verify the function of the key gene: EZH2

First, PCR experiments showed that EZH2 expression was significantly knocked down in HEC and ISK cell lines after siRNA transfection (Fig. 8A). Wound healing experiment showed that the healing ability of HEC cell line and ISK cell line decreased significantly after EZH2 knockdown (Fig. 8B). Transwell experiment showed that the migration ability of HEC cell lines and ISK cell lines was significantly reduced after EZH2 knockdown (Fig. 8C–D).

4. Discussion

Endometrial cancer (EC) is the most common malignant tumor of the female reproductive tract globally, posing a significant threat to women's health [25]. Although more prevalent in postmenopausal women, EC can also occur in younger patients [26, 27]. Currently, EC is divided into four molecular subtypes: DNA Polymerase Epsilon (POLE) Ultra-mutated, Microsatellite Instability (MSI), Copy Number Low (CNL), and Copy Number High (CNH) [28]. Vaginal bleeding is a common symptom of EC, enabling early diagnosis in many cases [29]. However, late diagnosis is common, especially in underdeveloped areas, leading to tumor progression or metastasis [30]. Metastatic EC is challenging to treat, with a 5-year productivity of less than 20% [31]. Consequently, there is an urgent need to explore the tumor microenvironment of EC for potential biomarkers that can aid its management.

In the past, apoptosis was considered to be the only form of programmed cell death, but more and more studies have confirmed that the forms of programmed cell death are diverse, including apoptosis, ferroptosis, pyroptosis, necroptosis and other forms [32]. And the role of these programmed cell death patterns in cancer seems paradoxical and double-sided [33]. On the one hand, programmed death of cancer cells can reduce their resistance to apoptosis [34]. On the other hand, programmed death can produce many reactive oxygen species
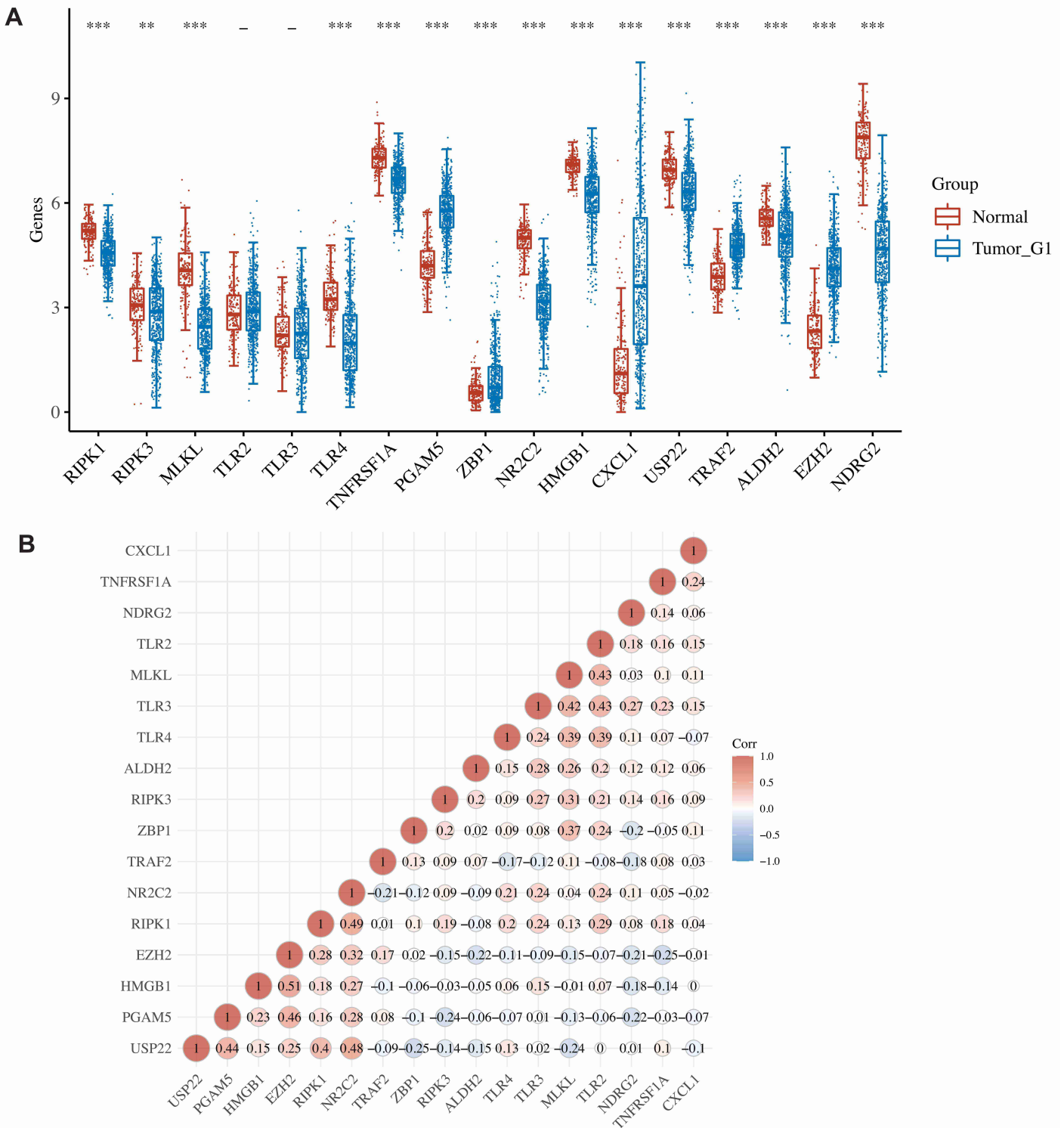
F I G U R E 2. Expression and correlation analysis of necroptosis gene in endometrial carcinoma. (A) *Receptor-Interacting Protein Kinase 1* (*RIPK1*), *Receptor-Interacting Protein Kinase 3* (*RIPK3*), *Mixed Lineage Kinase Domain-Like* (*MLKL*), *Toll-likereceptor4* (*TLR4*), *tumor necrosis factor receptor superfamily, member 1A* (*TNFRSF1A*), *nuclear receptor subfamily 2, group C, member 2* (*NR2C2*), *High Mobility Group Box 1* (*HMGB1*), *Ubiquitin specific protease* (*USP22*), *aldehyde dehydrogenase 2 family* (*ALDH2*) and *N-myc Down-stream Regulated Gene 2* (*NDRG2*) were significantly down-regulated in endometrial cancer (**$p < 0.01$, ***$p < 0.001$). However, *phosphoglycerate mutase family member 5* (*PGAM5*), *Z-DNA binding protein 1* (*ZBP1*), *C-X-C ligand motif 1* (*CXCL1*), *TNF receptor-associated factor 2* (*TRAF2*) and *Enhancer of Zeste Homolog* (*EZH2*) were significantly up-regulated in endometrial cancer (**$p < 0.01$, ***$p < 0.001$). (B) Correlation analysis of necroptosis gene interactions in endometrial cancer. *HMGB1* and *EZH2* had the strongest positive correlation, with a correlation value of 0.51.
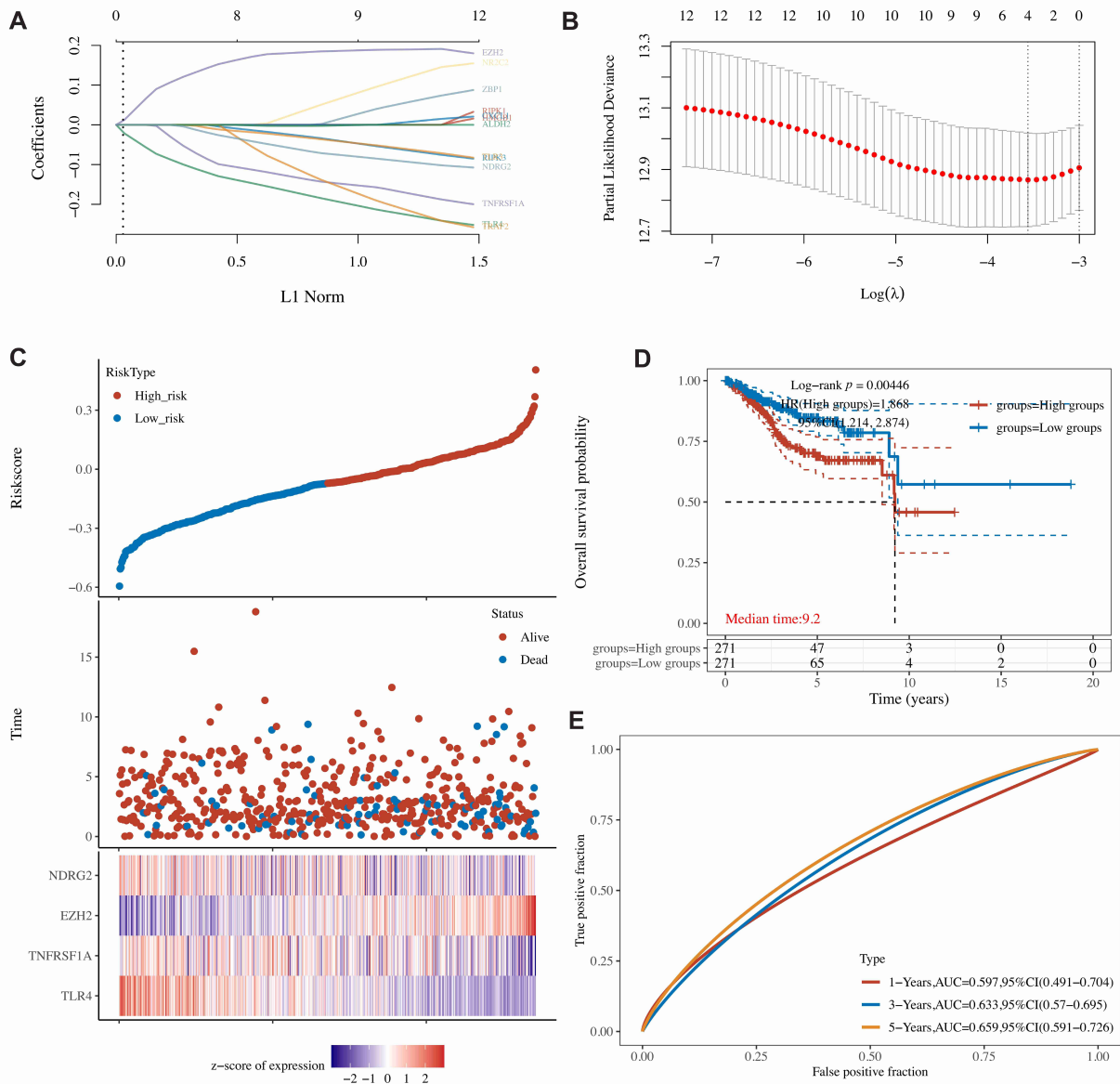
FIGURE 3. Construction of the necroptosis-related prognostic signature. (A,B) Lasso regression analysis. (C) Risk score composition of endometrial cancer patients in TCGA database. (D) Survival analysis showed that the high-risk group had significantly worse prognosis ($p < 0.01$). (E) ROC curve of the signature. NDRG: N-myc Down-stream Regulated Gene 2; EZH: Enhancer of Zeste Homolog; TNFRSF1A: tumor necrosis factor receptor superfamily, member 1A; TLR: Toll-like receptor; AUC: area under curve; CI: Confidence interval.

and inflammatory substances to promote the proliferation and metastasis of residual cancer cells and the change of immune microenvironment [35]. Necroptosis is a form of programmed death that does not rely on the caspase system and may be a promising future approach for treating cancer or improving resistance to caspase-dependent programmed death [36].

In this study, transcriptome analysis and single-cell analysis were performed to explore the role of necroptosis genes in endometrial cancer. First, we analyzed the expression and correlation of these genes in endometrial cancer. Subsequently, necroptosis related prognostic signature was constructed through Lasso regression. With this signature, endometrial cancer patients can be well grouped at risk, with patients at high risk having a significantly worse prognosis. Subsequent COX regression and nomogram construction can be

used to evaluate the prognosis of endometrial cancer patients. Analysis of the immune microenvironment of the necroptosis can provide reference for necroptosis immunotherapy.

Numerous studies have investigated the role of necroptosis in cancer. For instance, Baik *et al.* [37] reported that ZBP1 inhibits breast cancer metastasis by blocking necroptosis, while Xie *et al.* [38] found that the Mechanistic Target of Rapamycin (mTOR)/RIPK3/Necroptosis axis drives intestinal tumor progression. Furthermore, Xie *et al.* [39] used transcriptome and single-cell sequencing analysis to construct a prognostic stratification method for Uveal melanoma. However, the role of necroptosis in endometrial cancer is still unclear. Therefore, we constructed a necroptosis prognostic signature in endometrial cancer and investigated its role in the tumor microenvironment. This could aid in early prognostic
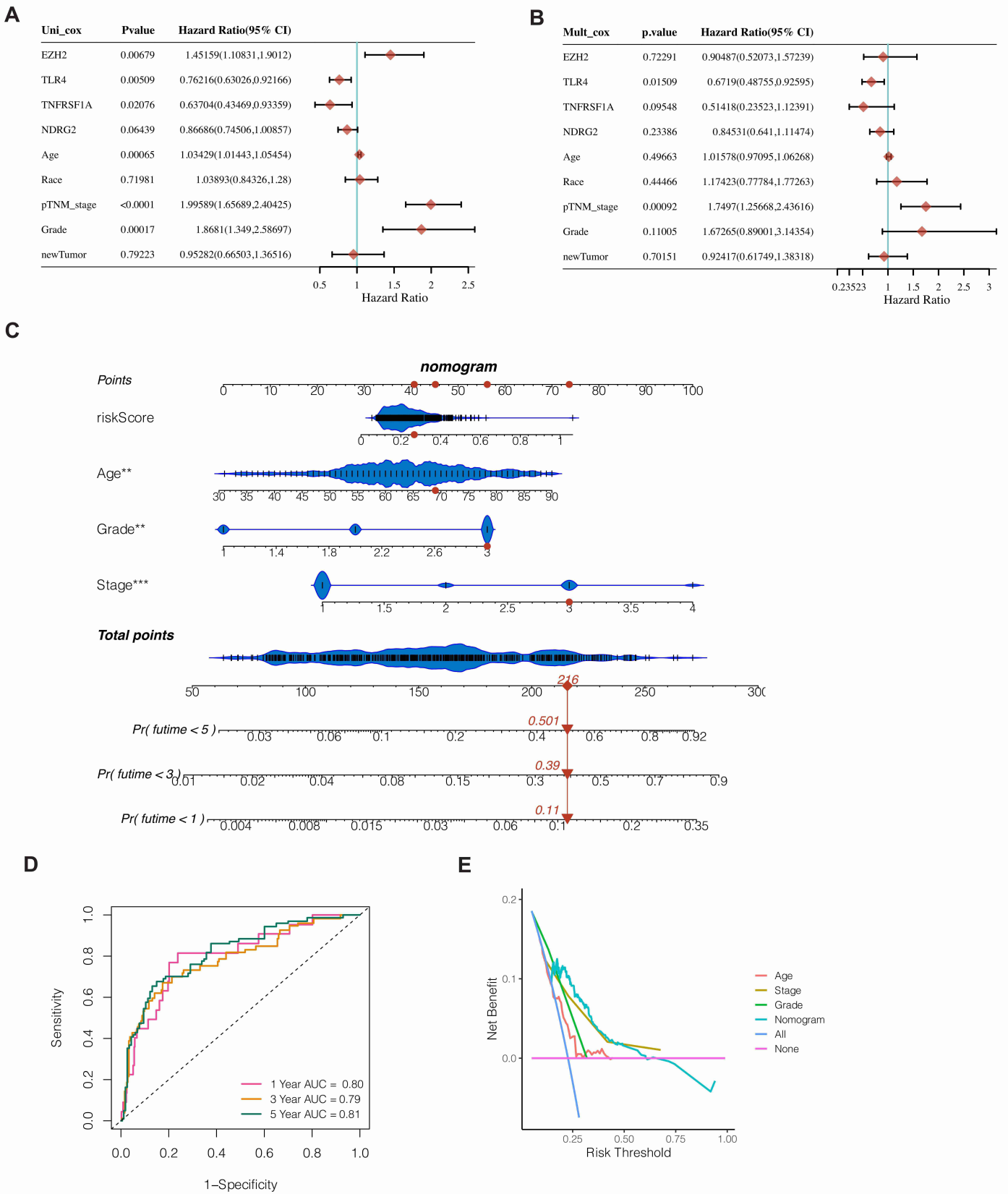
FIGURE 4. COX regression was performed to explore the independent prognostic value of signature. (A) Univariate COX regression. (B) Multivariate COX regression. (C) Nomogram of the signature. (D) Receiver Operating Characteristic Curve (ROC) curve of the nomogram. (E) Clinical decision curve.
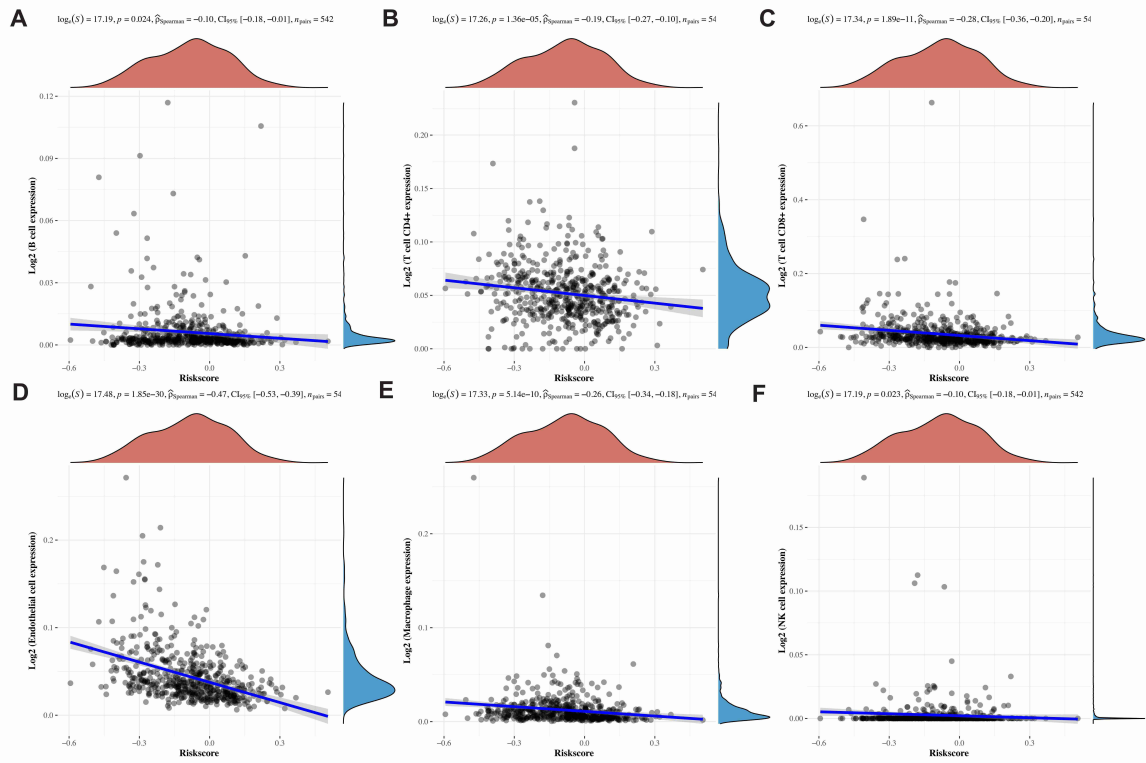
F I G U R E 5. Correlation analysis of immune cell infiltration. (A–F) Risk score was significantly correlated with B cells, CD4+ T cells, CD8+ T cells, macrophages, and NK cells.
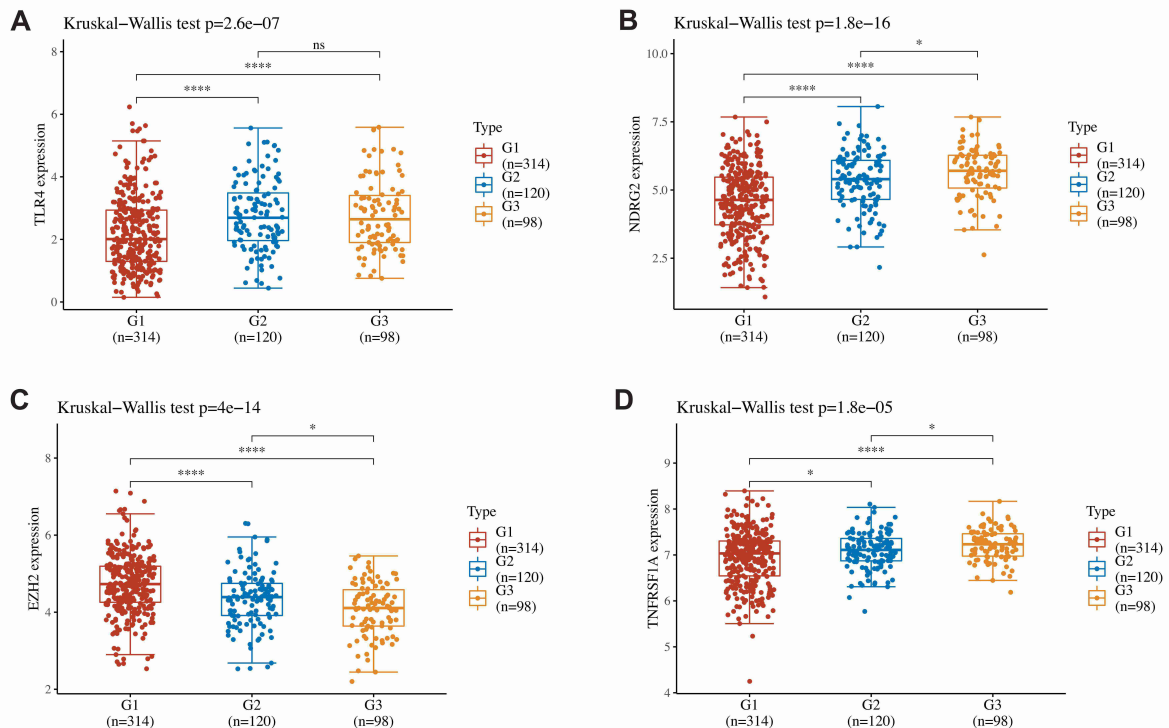

F I G U R E 6. Expression analysis of model genes in different grades. (A) *Toll-likereceptor4* (*TLR4*) gene expression in the G2 and G3 groups was significantly up-regulated compared with endometrial cancer in the G1 group. (B) The expression level of *N-myc Down-stream Regulated Gene 2* (*NDRG2*) increased gradually with the increase of grade. (C) The expression level of *Enhancer of Zeste Homolog* (*EZH2*) decreased gradually with the increase of grade. (D) The expression level of *tumor necrosis factor receptor superfamily, member 1A* (*TNFRSF1A*) increased gradually with the increase of grade.
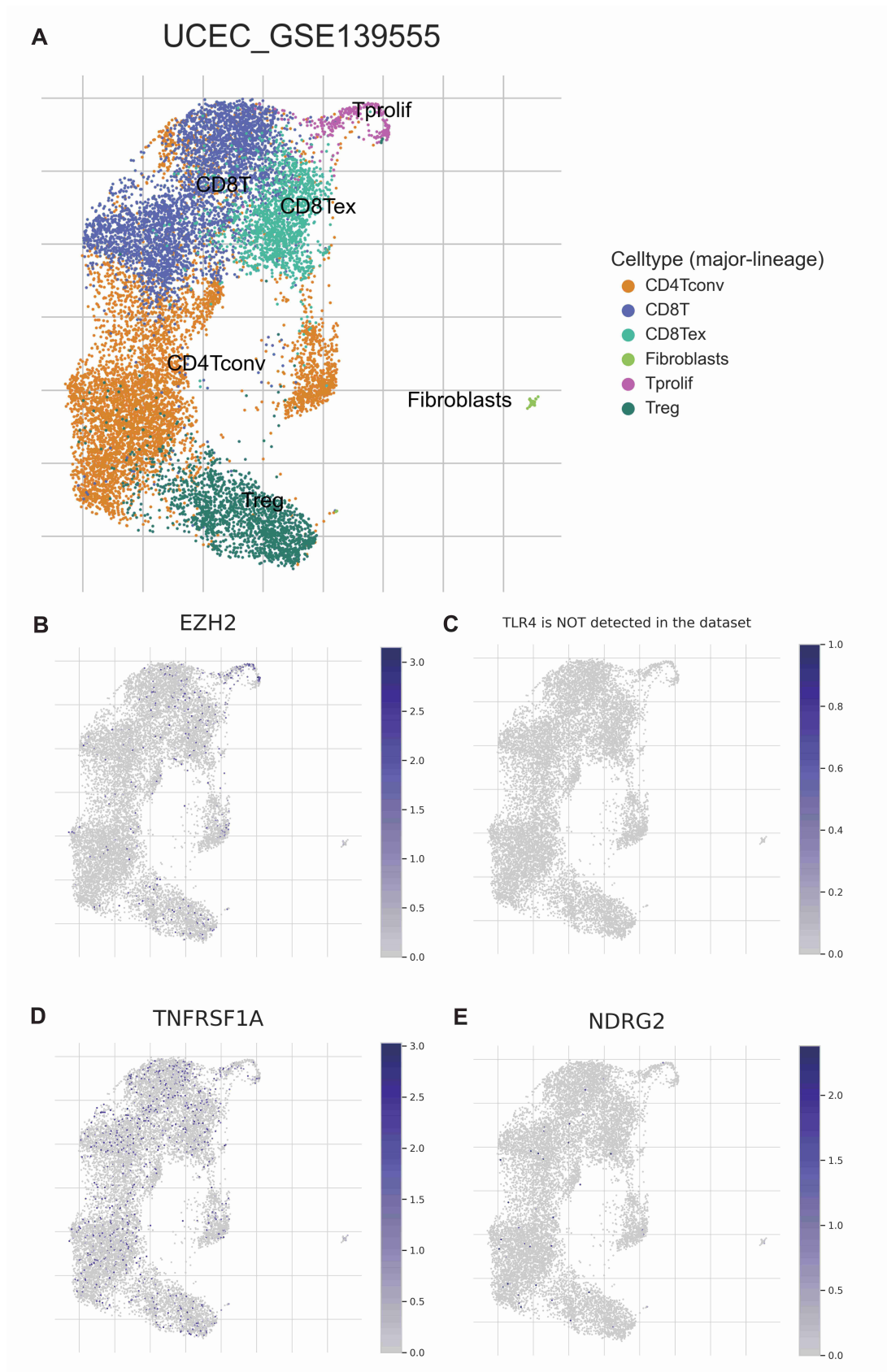
FIGURE 7. Single-cell sequencing analysis. (A) Dimensionality reduction cluster analysis of GSE139555. (B–E) The expression profiles of EZH2, TLR4, TNFRSF1A and NDRG2 genes in Signature at the single-cell level. UCEC: Uterine Corpus Endometrial Carcinoma; EZH: Enhancer of Zeste Homolog; TLR: Toll-like receptor; TNFRSF1A: tumor necrosis factor receptor superfamily, member 1A; NDRG: N-myc Down-stream Regulated Gene.
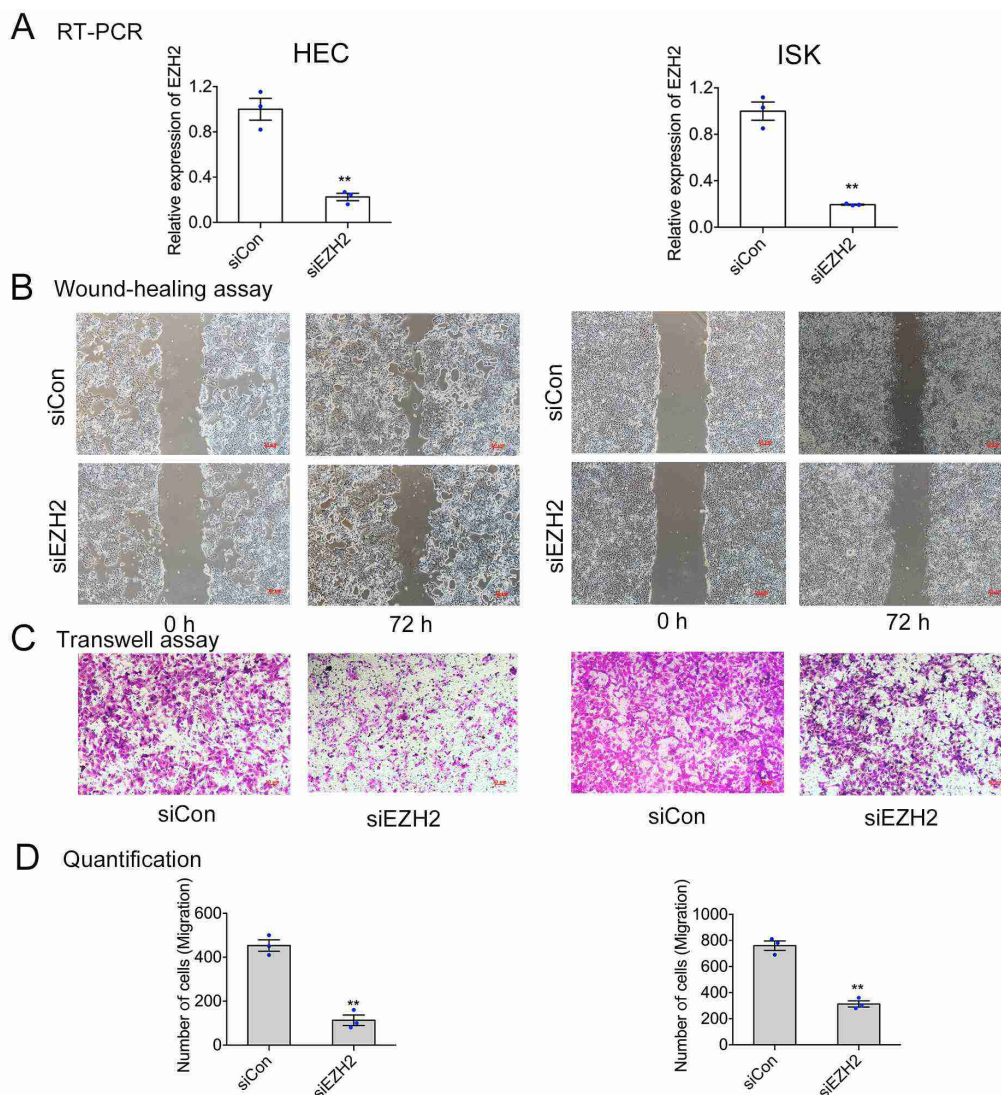
FIGURE 8. Cell experiments to verify the function of a key gene, EZH2. (A) EZH2 gene knockout efficiency of HEC cell line and ISK cell line was verified by PCR. EZH2 gene was significantly knocked down in HEC and ISK cell lines (**$p < 0.01$). (B) Wound healing experiments showed that the healing ability of HEC and ISK cell lines was significantly reduced after EZH2 knockdown. (C) Transwell experiment showed that the migration ability of HEC and ISK cell lines was significantly reduced after EZH2 knockdown. (D) Quantification of the Transwell experiment (**$p < 0.01$). RT-PCR: Reverse Transcription-Polymerase Chain Reaction; HEC: Human endometrial cancer cell; EZH: Enhancer of Zeste Homolog; ISK: Ishikawa.

assessment and intervention for endometrial cancer, as well as in the development of immunotherapy.

Our study identified EZH2, a key gene in the signature we constructed, as a potential marker for endometrial cancer. Gui *et al*. [40] found that EZH2 promotes EC cell proliferation by silencing TCF3 expression, while Ihira *et al*. [41] reported that inhibition of EZH2 activates the Mir-361/Twist axis to inhibit EC progression. In addition, Oki *et al*. [42] found that the EZH2 inhibitor GSK126 significantly inhibits EC cell proliferation. Therefore, the development of treatment regimens targeting EZH2 may hold potential value for EC patients.

Several bioinformatics studies have constructed programmed cell death-related signatures in endometrial cancer, but no necroptosis-related signature has been developed until now. For example, Yin *et al*. [43] constructed a ferroptosis-related prognostic signature in endometrial cancer, with high-risk patients exhibiting worse prognoses. Chen *et al*. [44] developed a 6-gene pyroptosis-related signature to assess prognosis and immune invasion of endometrial cancer, reporting that high-risk EC patients had lower levels of immune infiltration and lower immune function activity. Similarly, Liang *et al*. [45] constructed a pyroptosis-related prognostic signature composed of nine lncRNAs, with high-risk EC patients showing poor prognoses. In our study, the 1-, 3- and 5-year AUC values of the signature were 0.597, 0.633 and 0.659, respectively. Although our AUC values were lower than those of previous studies, we further optimized our model by building a dynamic nomogram. The 1-, 3- and 5-year AUC values of the nomogram were 0.80, 0.79 and 0.81, respectively, enhancing the accuracy of our signature. Furthermore, the clinical decision curve indicated that patients would benefit greatly from clinical intervention based on the nomogram. This compensates for the lower AUC

values of the risk scores.

5. Conclusions

Overall, our study provides the first prognostic signature of the necroptosis gene in endometrial cancer, which is valuable not only for patient prognosis assessment, but also for precise treatment of EC. In addition, we also identified EZH2 as a possible effective marker for EC, which can provide a reference for the diagnosis and treatment of EC to a certain extent.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article (and supplementary material).

AUTHOR CONTRIBUTIONS

ZZD —conducted cell experiments (PCR, cell migration, cell scratch test and so on) *in vitro*, analyzed the data, and made the figures. ZZD—analyzed the data from the database. ZZD, CW and LZS—provided intellectual insights and critical discussion of this study. All funds of the project come from LZS.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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