

Necroptosis-associated LncRNA markers for late survival prediction, immune and gene mutation analysis in ovarian cancer

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Necroptosis-associated LncRNA markers for late survival prediction, immune and gene mutation analysis in ovarian cancer

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Abstract

OBJECTIVE:To investigate the potential prognostic value of necroptosis-associated lncRNAs (NALncRNAs) in patients with ovarian cancer.

METHODS: Gene expression data from ovarian cancer patients with clinical data were downloaded from TCGA database. This is the base data for screening NALncRNAs that represent different survival times. lncRNA risk models were constructed by Cox regression analysis and evaluated for their prognostic value. The link between lncRNA signatures and related pathways, immune cell activity, immunological checkpoints, m6A-related genes, and gene mutations was also investigated.

RESULTS: In this study, eight NALncRNAs including USP30-AS1, MINCR, LINC01096, DNM3OS, LINC02574, DTNB-AS1, ACAP2-IT1, and ILVBL-AS1, all of which were well represented for the outcome of ovarian cancer patients. Nomogram after calibration curve validation has good clinical utility. Risk signatures were shown to be abundant in various pathways related to immunology and cell proliferation, according to gene set enrichment analysis. Patients' late survival outcome corresponds to differences in immune cells, immune checkpoints, immune function, etc. A better immune microenvironment would facilitate late survival, but inexplicably patients with long survival times have a higher immune escape. Patients with high mutations in genes appear to have better survival outcomes.

CONCLUSION: This study identified eight NALncRNAs markers for the first time, providing a valuable basis for more accurate prediction of ovarian cancer prognosis.

Key words: Necroptosis; Ovarian cancer; Survival prediction; LncRNA

Introduction

Because of its more subtle onset, ovarian cancer is still the most difficult of the three major gynecologic cancers to diagnose, and roughly 70% of patients are diagnosed late, resulting in increased mortality and morbidity^{[1][1]}[2]. Even after delicate treatment and care, there is still a possibility of recurrence[3]. Since the etiology and pathogenesis of ovarian cancer are not clear and survival time varies[4], it is essential that we search for new prognosis-related biomarkers for physicians to manage

[1]

ovarian cancer patients at a later stage.

Necroptosis is a programmed form of cellular necrosis that can be initiated by tumor necrosis factor, interferon, and tumor necrosis factor-associated apoptosis-inducing antibodies[5]. One of the main hallmarks of tumors is resistance to apoptosis, so it is important to study apoptosis-related biomarkers for predicting or treating cancer[6]. currently, many studies have shown that the development of various cancers is associated with LncRNAs[7], and several LncRNAs have been identified as possible cancer indicators and targets[8][9].So we used bioinformatics from the perspective of necroptosis to search for LncRNAs that can determine the survival outcome of ovarian cancer.The detailed study route can be seen in Figure 1.

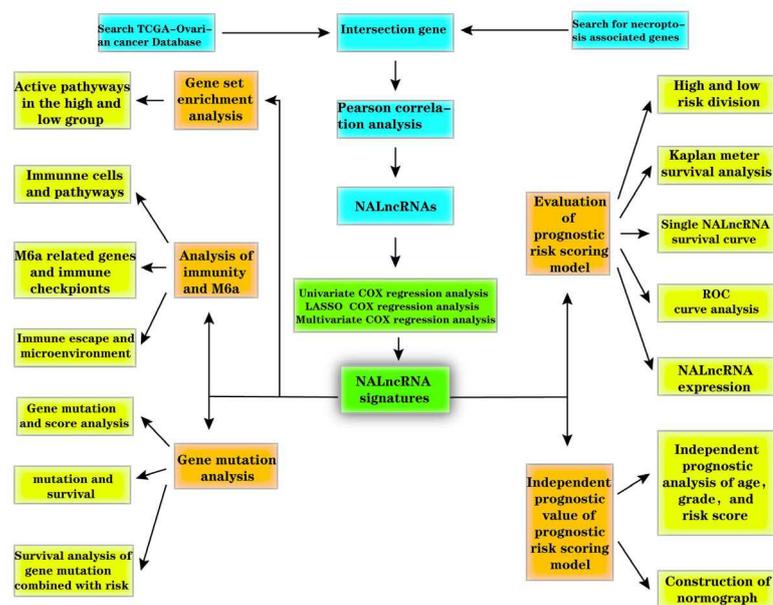


Figure1 flow chart of necrotizing apoptosis-related lncRNAs in predicting ovarian cancer

Methods

Data sources

We obtained tumor tissue RNA sequence data (FPKM values) with clinical information from TCGA database (<https://portal.gdc.cancer.gov/repository>) for 379 ovarian cancer patients, and used R software (4.1.1) was used to extract and normalize the data. Two studies extracted necroptosis-associated genes(NAGs) from published reviews, and a third investigator was sought to decide when disagreement arose. The final result was 104 genes associated with necrotizing apoptosis and is provided in Annex Attachment 1. The intersection of the gene data in the expression matrices with the NAGs yielded the new NALncRNAs expression matrices. Since the TCGA database is an open access resource, this study was exempted from ethics committee approval.

Identification of NALncRNAs

To identify NALncRNAs in ovarian cancer patients, we used Pearson correlation to assess the degree of association between LncRNAs and NAGs. Typically, we used a

threshold of $p < 0.001$ and a correlation coefficient |Correlation Coefficient| > 0.4 to screen for NALncRNAs.

Validation and construction of a prognostic risk model for NALncRNAs

We used perl language (perl 5.28.1) to merge differentially expressed NALncRNAs expression with survival time, removing the mutilated and uninformative data. First, univariate Cox regression analysis was completed using the "survival" package to screen for necrotrophic apoptosis-related lncRNAs that could distinguish survival outcomes; We utilized the R package "glmnet" to limit the range screened by univariate and develop prognostic models using the least absolute shrinkage and selection operator (LASSO) Cox regression model to eliminate the influence of overfitting. Multivariate Cox regression analysis was used to determine which NALncRNAs predict survival in ovarian cancer patients.

Next from the already obtained necroptosis-related lncRNAs, the prognostic gene-lncRNA correlation network was drawn and visualized with Cytoscape software (3.7.2) to facilitate the observation of possible relationships between lncRNA and genes. Construct a scoring model based on survival outcomes of ovarian cancer patients and output risk score coefficients (coef) for genes included in the model. The constructed prognostic risk score model was calculated as: risk score = prognostic NALncRNAs gene expression 1 \times coef1 + prognostic NALncRNAs gene expression 2 \times coef 2 + ... + prognostic NALncRNAs gene expression n \times coef n. The median determined by the above method is used to distinguish between high and low patient risk.

Kaplan-Meier analysis was used to compare the overall survival time (os) of the two groups, and HRs forest plots and heat maps of NALncRNAs prognostic models with survival curves for each prognostic gene were shown. Evaluate the relationship between each variable and risk value and prognosis using single-factor and multi-factor independent prognostic analysis to determine whether the constructed risk model can meet the criteria for independent prognosis. Evaluation of survival outcome prediction models using ROC curves with the "timeROC" package function of R software.

The samples were divided into groups based on their risk scores, and the risk score distribution and survival status were mapped using the R package "pheatmap." The Hosmer-Lemeshow test was also employed to see if the anticipated outcome was congruent with the actual outcome.

Gene Set Enrichment Analysis(GSEA)

Expressed gene sets from low- or high-risk populations and marker gene sets collected in the kegg database V7.4 were analyzed using GSEA (4.1.0) software. Setting FDR < 0.05 is considered valuable.

Immunocorrelation analysis

We used CiberSort[10], TIMER[11], MCP-COUNTER[12], QUANTISEQ[13], XCell[14], and estimation of the ratio of immune cells to cancer cells (EPIC)[15] to assess the activity of immune cells in both populations based on FIRLS characteristics. Simultaneously, ssGSEA analysis was carried out using the R software package "gsva" to analyze the immune cell infiltration percentage and immune pathway activity in

the high-risk and low-risk ovarian cancer groups. Immune checkpoints may be useful in guiding ovarian cancer treatment[16][17].The relationship between survival outcomes and immune checkpoints can be understood by analyzing gene expression levels in two groups of ovarian cancer patients with different survival times. Immune escape in ovarian cancer affects the effectiveness of immunotherapy[18], and the microenvironment of ovarian cancer cells interacts with tumor cells through secretory intercommunication, which can promote cancer cell invasion and metastasis[19][20]; therefore, we analyzed and compared the scores of immune escape and immune microenvironment in both groups.

M6a gene correlation analysis

M6A and LncRNA are linked to the development of ovarian cancer, particularly m6A-regulated CACNA1G-AS1, which has been identified as a cancerous LncRNA[21]. Because M6A-related genes are important regulators of tumor growth, we looked into the link between survival risk and m6a-related genes by comparing gene expression levels in two groups with varied survival outcomes.

Gene mutation analysis

Gene mutations play a role in the development of ovarian cancer[22] and its antagonistic therapy, and suitable regimens can be designed based on the tumor mutation profile. Different gene mutations have different effects[23] on the late stage of tumor patients, so we looked at gene mutations in high- and low-risk groups to see if there was a link between high and low mutation levels and ovarian cancer patients' survival times.

Results

Identification of differentially expressed NALncRNAs

Based on the most recent lncRNA annotation file, we found 4668 lncRNAs in the RNA-SEQ data of ovarian cancer patients (see Appendix S2), and then performed Pearson correlation analysis of these lncRNAs with 104 NAGs, resulting in 164 NALncRNAs, and finally identified 8 differentially expressed NALncRNAs.

Construction of a NALncRNAs risk model with prognostic value

In this study, data information from 379 ovarian cancer patients were selected for preliminary screening of prognosis-related lncRNAs using one-way Cox regression analysis, and a total of 12 necrotizing apoptosis-related lncRNAs associated with prognosis were identified (Figure .2A). Then, Lasso regression with multivariate Cox regression analysis was performed, and the results showed that 8 lncRNAs were included in the model (Figure 2B-D). These 8 prognostic NALncRNAs were USP30-AS1, MINCR, LINC01096, DN3OS, LINC02574, DTNB-AS1, ACAP2-IT1, and ILVBL-AS1. Based on the best λ value, a prognostic risk score model for NALncRNAs was constructed: risk value (risk score) = (-0.0784×PCAT19 expression value) + (1.0279×CDKN2B-AS1 expression value) + (0.3622×LINC01936 expression value) + (0.0099×LINC02178 expression value) + (-0.0065×BMPR1B-DT expression value) + (-0.1754× LINC00237 expression value) + (-0.0801×TRPM2-AS expression value). The final resulting gene and lncRNA correlation network showed that 8 lncRNAs were associated with 14 genes. (Figure .2E)

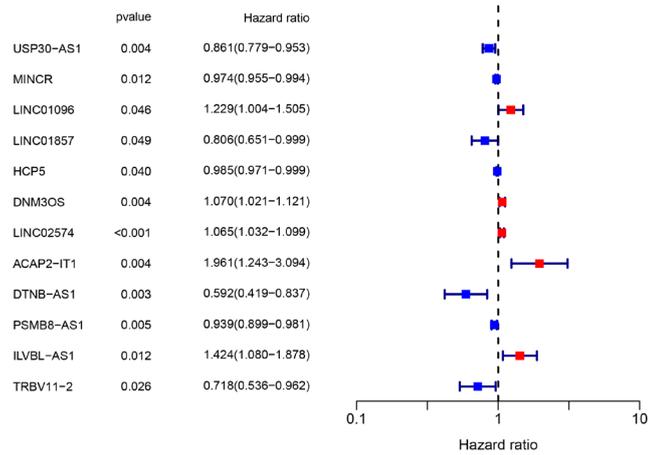


Figure .2A Univariate Cox regression analysis of 12 NALncRNAs initially screened for association with ovarian cancer prognosis

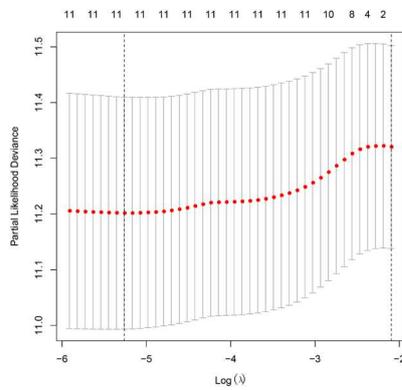


Figure .2B Multivariate cox regression forest plot shows that USP30-AS1, MINCR, LINC01096, DNM3OS, LINC02574, DTNB-AS1, ACAP2-IT1 and ILVBL-AS1 were prognosis-associated NALncRNAs and were involved in forming the prognostic model.

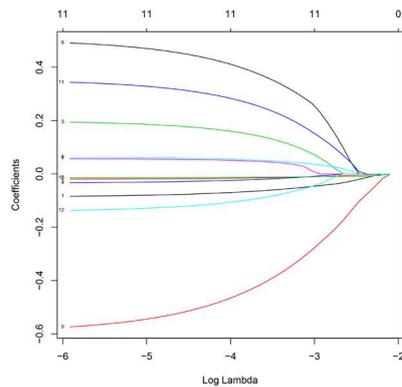


Figure .2C Lasso-Cox regression analysis shows that 12 NALncRNAs are good candidates for constructing prognostic characteristics.

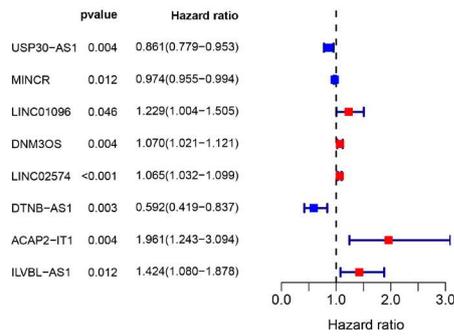


Figure .2D Multivariate cox regression forest plot shows that USP30-AS1、MINCR、LINC01096、DNM3OS、LINC02574、DTNB-AS1、ACAP2-IT1and ILVBL-AS1 were prognosis-associated NALncRNAs and were involved in forming the prognostic model.

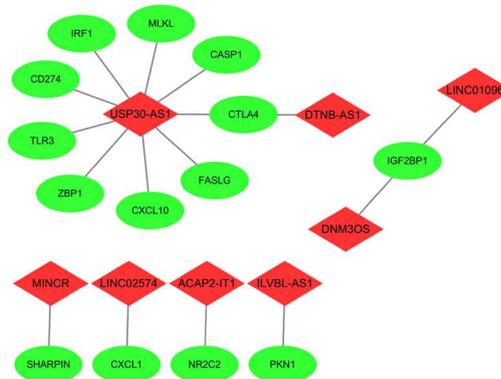


Figure .2E Gene-LncRNA co-expression network of prognosis-associated NALncRNAs and NAGs.

Prognostic risk score model evaluation

The computed survival risk values were used to define the high- and low-risk groups of ovarian cancer patients included in the study. The risk score was proportional to the mortality rate(Figure 3A-B). The high-risk group had a statistically significant lower survival rate than the low-risk group, according to k-M survival analysis, and the survival rate dropped year by year over time, with the high-risk group having a significantly lower survival rate than the low-risk group. (Figure 3C). Single-gene survival analysis was performed based on the expression profiles of eight prognostic genes, in which MINCR and USP30-AS1 were significantly different (Figure 3D). ROC curves were used to determine the sensitivity and specificity of the prognostic model, and the area under the curve at all four time points was more than 0.6, indicating that the risk model had good predictive power(Figure 3E).The heat map also clearly depicts the differences in expression of the eight NALncRNAs between the two risk groups. (Figure 4F)

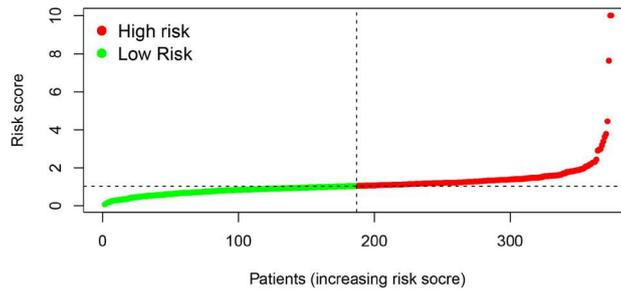


Figure .3A Differentiation of ovarian cancer patients based on risk score.

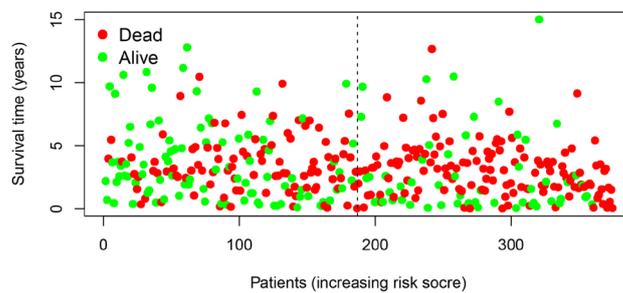


Figure .3B Patient's Survival Status Chart of ovarian cancer.

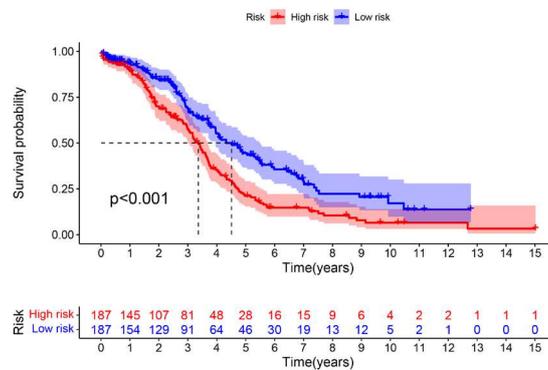


Figure .3C Patients with varying ovarian cancer risks had dramatically variable survival durations, according to a Kaplan-Meier survival analysis.

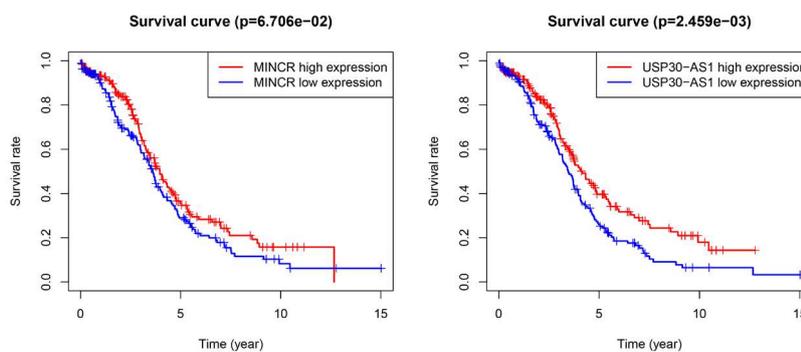


Figure .3D Prognostic survival curves of MINCR and USP30-AS1

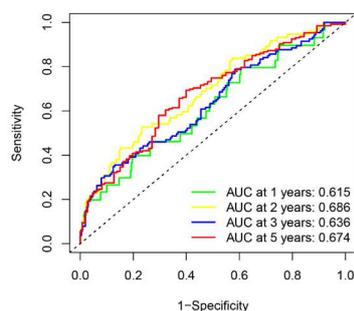


Figure .3E The prognostic accuracy of the risk score was verified by the AUC values at four different time points.



Figure.3F Eight selected lncRNAs are significantly differentially expressed in ovarian cancer patients with different risks.

The prognostic risk score model's independent prognostic value

Univariate and multivariate Cox regression analyses were used to see if the prognostic risk score model could be used as an independent prognostic predictor. Both univariate and multivariate Cox regression analyses revealed that age and risk score were significantly associated with OS in ovarian cancer patients. (Figure 4A-B). As a result, age and risk score can be employed as independent prognostic indicators in the evaluation of ovarian cancer patients. The ROC curve illustrates the accuracy of these indicators as independent prognostic factors for patients (Figure 4F), where grading is less accurate as an independent prognostic factor. Nomograms predict late survival outcomes based on patient age, classification, risk score and high and low risk (Figure 4C). 1-year, 3-year, and 5-year corrected plots demonstrate the consistency of the predictions of the nomograms with the observed outcomes. (Figure 4D).

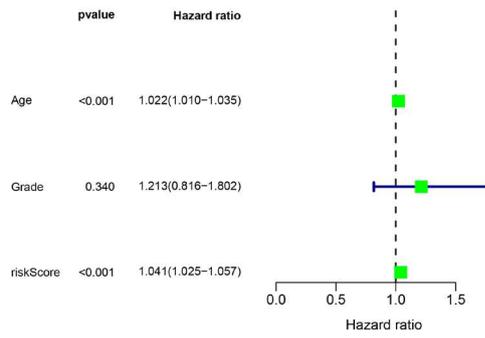


Figure.4A Age, grading, and risk score were used in a univariate independent prognostic analysis.

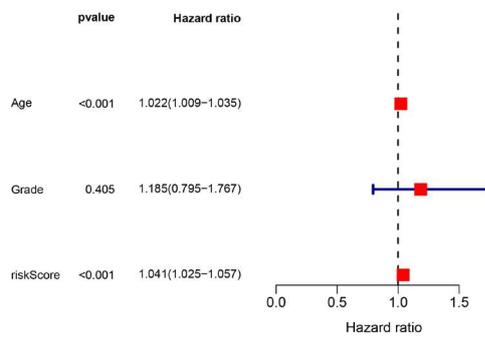


Figure.4B Age, grading, and risk score are all used in a multivariate independent prognostic analysis.

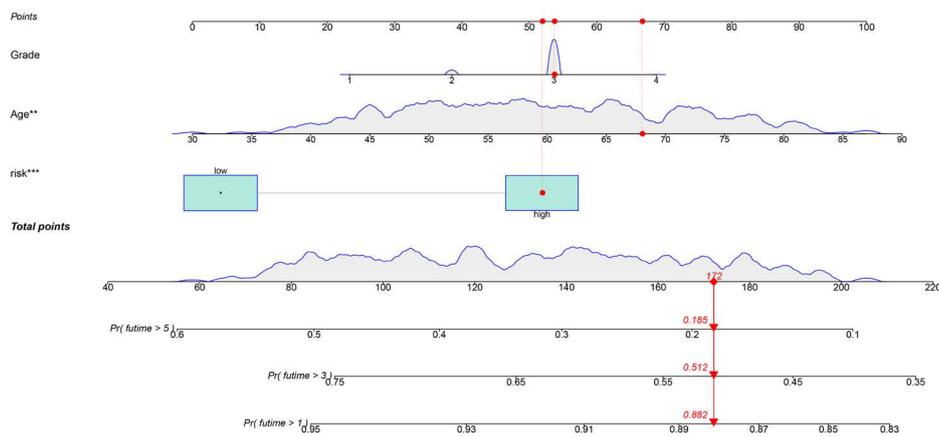


Figure.4C Nomogram combining risk scores with clinical factors

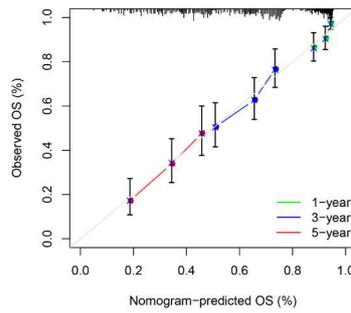


Figure.4D 1-year, 3-year and 5-year Nomogram correction curves

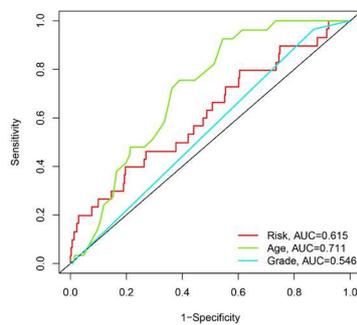
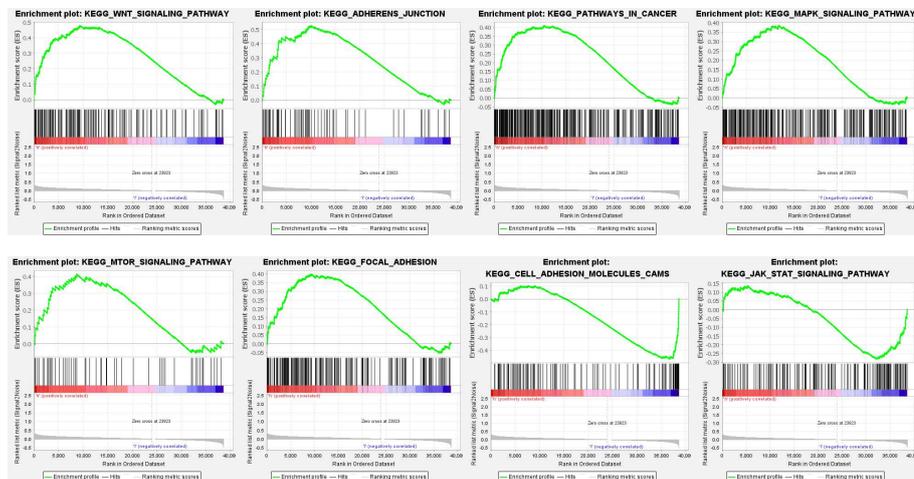


Figure.4F The AUC of the ROC curve validated the prognostic accuracy of prognostic-related clinical factors.

Signaling pathway analysis

A gene set enrichment analysis (GSEA) was done using differentially expressed genes between the high-risk and low-risk groups to investigate the biological activities and signaling pathways of NAIncRNAs. Endometrial cancer, MAPK signaling, ECM receptor, Endocytosis, and other related pathways were found to be more active in the high-risk ovarian cancer group. In the low-risk group, Peroxisome, Steroid hormone biosynthesis, Oxidative phosphorylation, Jak stat signaling, Arginine and proline metabolism, Alpha linolenic acid metabolism were more active. (Figure 5)



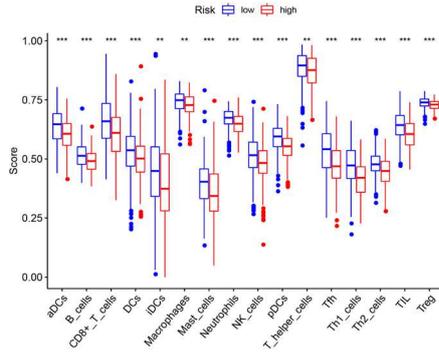


Figure.6B B-C Between low- and high-risk groups, ssGSEA scores of 16 immune cell types were compared to enrichment scores of 13 immune-related pathways.

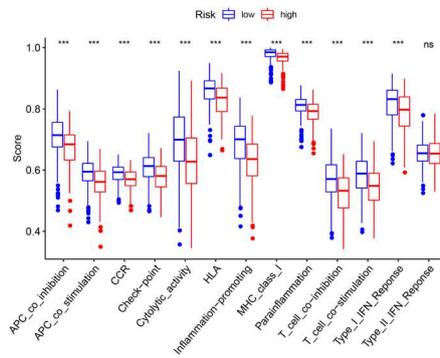


Figure 6C

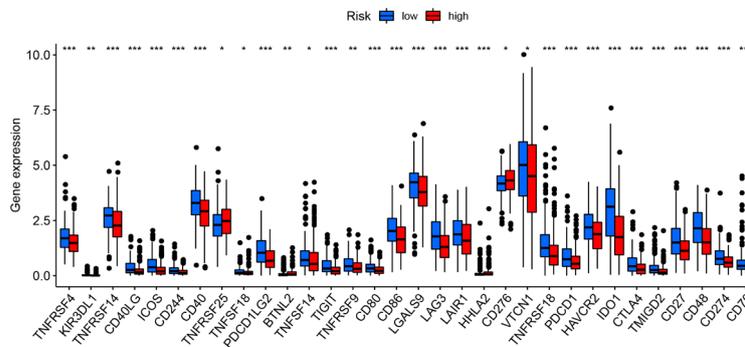


Figure.6D The difference in immune checkpoint expression between the two groups was investigated.

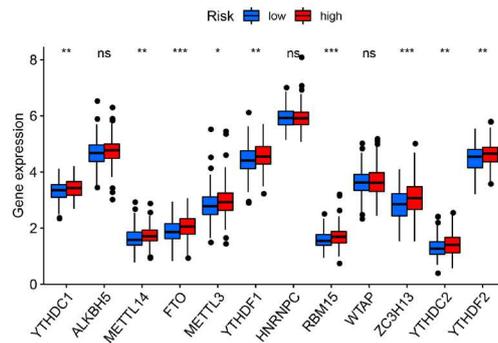


Figure.6E Associations between the risk signature and m6A-related genes.

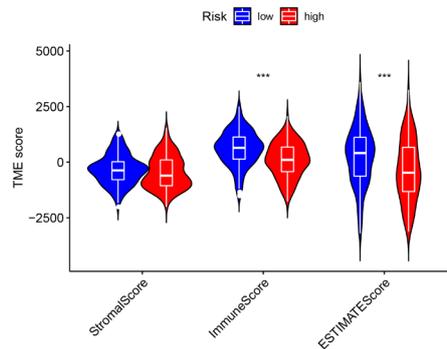


Figure.6F The high-risk and low-risk groups' stromal, immune, and ESTIMATE scores were plotted, and the low-risk group's immune and ESTIMATE scores were greater than the high-risk group's.

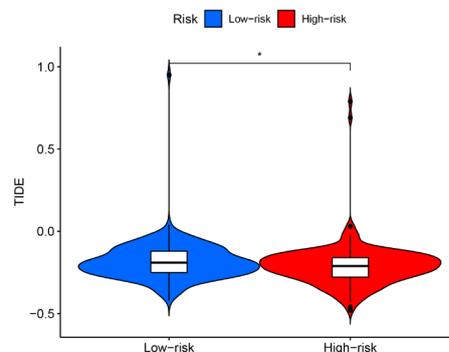


Figure.6G High and low risk groups had different immune escape scores, with the low risk group having higher TIDE scores than the high risk group.

Gene mutation and survival prediction

The analysis of the top 20 mutations in patients in the high and low risk groups showed that the mutated genes were the same in both groups and there was no difference in the mutation scores. (Figure 7A-C) Survival was higher in the high-mutation group than in the low-mutation group, with the best survival in the high-mutation-low-risk group and the worst survival in the low-mutation-high-risk group. (Figure 7D-E)

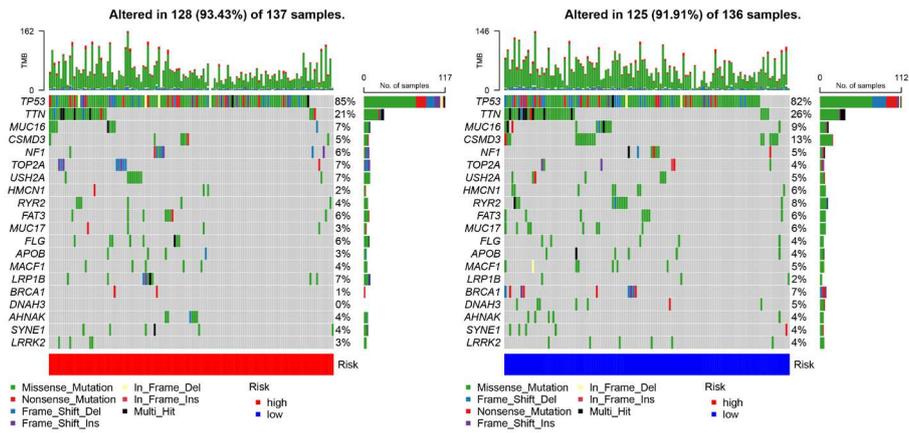


Figure 7A-B The percentages on the right in the waterfall plot of the top 20 mutations in the high and low risk groups represent the mutation frequency of the genes, and different colors represent different mutation types.

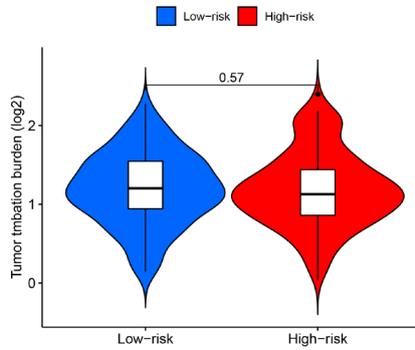


Figure.7C Analysis of gene mutation score, no difference between high and low risk groups.

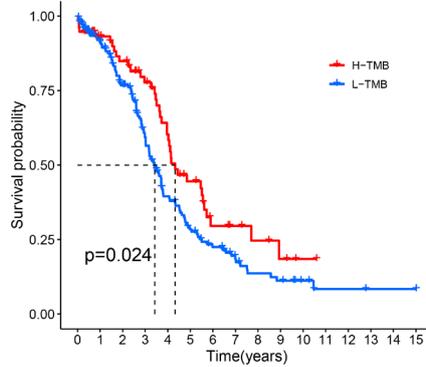


Figure.7D The graph of survival status difference between high and low mutation groups shows good survival status difference between high and low mutation groups.

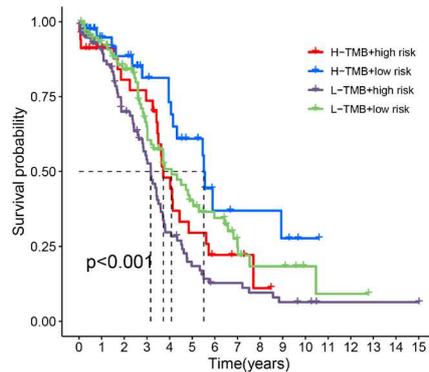


Figure.7E When the combined survival status of the high and low mutation groups, as well as the high and low risk groups, was analyzed, it was discovered that the high mutation low risk group had the greatest survival status, while the low mutation high risk group had the poorest.

Discussion

In recent years, there have been revolutionary advances in sequencing technology, and LncRNA, CircRNA and MiRNA have been detected. These do technology products can not only explain the onset and progression of cancer, but also provide research references for further treatment[24][25]. LncRNA plays a role in cell proliferation, differentiation, migration, invasion, and death via regulating gene transcription rate, translation, and post-translational modifications[26][27]. Because the survival rate of ovarian cancer patients is among the lowest among gynecological cancers, this study looked into NALncRNAs to predict survival and further assessed the immunological and gene mutation status of high and low risk patients.

A total of 8 NALncRNAs were screened in this study, among which LINC01096, DNM3OS, LINC02574, ACAP2-IT1, and ILVBL-AS1 represented shorter survival time for ovarian cancer patients, and USP30-AS1, MINCR, and DTNB-AS1 represented longer survival time for ovarian cancer patients. Many studies have shown that the above LncRNAs are specific in the development of cancer. For example, in the current investigation, LINC01096 was not shown to be predictive for ovarian cancer but was found to be significantly expressed in breast cancer tissue cells, and knocking down LINC01096 prevented[28] cancer cell migration and invasion. DNM3OS was overexpressed in ovarian cancer, and knockdown of it similarly inhibited migration and invasion of cancer cells and reduced mesenchymal to epithelial transformation[29]. USP30-AS1 inhibits apoptosis in acute myeloid leukemia cells and may promote cancer cell survival through cis-regulation of USP30 and ANKRD13A[30]. By serving as a competitive endogenous RNA, MINCR may increase glioma cell proliferation and migration[31]. As seen in Figure.2E, IGF2BP1, LINC01096 and DNM3OS co-expression. It has been shown that IGF2BP1 promotes the invasion of SRC/MAPK-driven ovarian cancer cells[32].

Through GSEA, we found that pathways related to jak stat signaling, MAPK signaling, and endocytosis were more active in the high-risk group. jak stat signaling promotes tumor development and mediates almost all immunomodulatory processes, including immune escape[33]. MAPK signaling plays an important role in

the proliferation and metastasis of ovarian cancer cells, and this pathway is usually interfered with to influence apoptosis of cancer cells[34]. The most important cause of death in ovarian cancer is recurrence and metastasis of tumor cells, and abnormal activation of the TGF- β signaling pathway in active passages in the high-risk group is just able to promote tumor metastasis and recurrence[35]. Oxidative phosphorylation, arginine and proline metabolism, and alpha linolenic acid metabolism are more active pathways in the low-risk group of endometrial cancer. Among them, small molecule inhibitors often act on the multimeric complexes in the Oxidative phosphorylation pathway, thus inhibiting metabolism as well as inducing oxidative damage and cancer cell death[36]. Related investigators have shown that the arginine and proline metabolism pathway can be interfered with to induce apoptosis in ovarian cancer cells and inhibit S-phase growth[37].

The higher B cell enrichment scores in the low-risk group in the immune cell and pathway scores further imply that synergistic interactions between tumor-infiltrating T cells and B cells may be linked to better ovarian cancer patient survival[38]. Upregulated expression of human leukocyte antigen-G (HLA-G) was one of the methods by which ovarian cancer cells avoided immune monitoring, and the HLA enrichment score was greater in the low-risk group. It's perplexing that the low-risk group had a larger immune escape rate and a superior survival outcome deficiency[39]. In the immunological checkpoint, the low-risk group's IDO1 score was higher than the high-risk group's, and previous studies found a link between high IDO1 expression and overall ovarian cancer survival[40]. We found that the expression of M6A-related genes FTO was higher in the high-risk group, and related studies showed that FTO could not only inhibit apoptosis but also promote proliferation of ovarian cancer cells[41]. Both immune scores and microenvironment scores were higher in the low-risk group of ovarian cancer in our findings, which also surface the positive role of better immune and microenvironment in survival outcomes in ovarian cancer patients.

In our mutation analysis study, the highest mutated gene in the high and low risk groups was TP53, which improves the accuracy of cancer survival prognosis[42]. The top 20 mutated genes were the same in both groups, and there was no difference in gene mutation scores. However, our findings suggest that high mutations are protective for ovarian cancer patients, and ovarian cancer patients with high mutations and low risk have the best survival, while those with low mutations and high risk have the worst survival.

Limitations

Although the LncRNA risk model we developed has strong predictive value in terms of LncRNA, immunity and gene mutation in high and low risk groups, there are some shortcomings in our current study. First, our data collection, although retrieving the original dataset used to build the LncRNA-related model from the TCGA database, still requires other databases to collect data to verify the accuracy of our results. Secondly, the exact mechanism by which necroptosis regulates the exact process of ovarian cancer is still unclear and needs to be elucidated by further experimental studies.

Conclusion

In this study, eight NAIncRNA markers were identified for the first time, providing a valuable basis for more accurate prediction of prognosis in ovarian patients. And it provides a reference for further exploring the immune and gene mutation studies in ovarian cancer patients with high mortality.

Conflict of Interest: The author of this article declares that he or she has no conflicts of interest.

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