Original Article

Risk signature of NETosis-related subtype predicts prognosis and evaluates immunotherapy effectiveness in gastric cancer

Ruyue Chen[1#], Zengwu Yao[2#], Lixin Jiang[1,2,3]

[1]Medical College, Qingdao University, Qingdao, China; [2]Department of Gastrointestinal Surgery, Yantai Yuhuangding Hospital, Shandong University, Yantai, China; [3]Department of General Surgery, Yantai Yeda Hospital, Yantai, China

Contributions: (I) Conception and design: R Chen; (II) Administrative support: Z Yao, L Jiang; (III) Provision of study materials or patients: R Chen; (IV) Collection and assembly of data: R Chen, Z Yao; (V) Data analysis and interpretation: R Chen, Z Yao; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Lixin Jiang, MD. Medical College, Qingdao University, Ningxia Road 308, Qingdao 266071, China; Department of Gastrointestinal Surgery, Yantai Yuhuangding Hospital, Shandong University, Yantai 264001, China; Department of General Surgery, Yantai Yeda Hospital, Yantai 264006, China. Email: jianglixin_1969@163.com.

Background: Gastric cancer (GC) has a high incidence and mortality rate with a poor prognosis, so it is crucial to search for new biomarkers. The role of NETosis, a newly identified type of programmed cell death, in GC and its underlying mechanisms have yet to be explored and still require thorough investigation. Our research seeks to enhance our comprehension of NETosis and may offer novel approaches for treating GC.

Methods: Utilizing The Cancer Genome Atlas-stomach adenocarcinoma (TCGA-STAD) dataset for training and the GSE84433 dataset for validation, our study delved into the associations between NETosis-related genes and the clinical risk of GC. Through comprehensive clustering, enrichment, and immune infiltration analyses, we evaluated the prognostic relevance of these NETosis genes *in vivo*. Furthermore, we devised a NETosis-related risk signature (NRRS) to assess its implications in risk stratification, survival prognosis, immune infiltration, and drug sensitivity. The NRRS's accuracy was validated by immunohistochemical staining.

Results: By applying consensus clustering to data from 62 NETosis-related genes, we categorized patients into two distinct subgroups, C1 and C2. These subgroups demonstrated significant differences. Following this, we developed the NRRS using the least absolute shrinkage and selection operator (LASSO) regression analysis. This process involved the selection of the following genes: *CXCR4*, *NRP1*, *PDCD1*, *CTLA4*, *AKR1B1*, *SERPINE1*, *RGS2*, *SLCO2A1*, *TNFAIP2*, *RNASE1*, *DOC2B*, *APOD*, *ENTPD2*, and *CCL24*. High-risk and low-risk groups can be accurately distinguished. We further verify in the verification set. These results suggest that NETosis is related to the microenvironment of GC. Our designed NRRS can predict the survival of patients with GC.

Conclusions: We emphasized the relationship between NETosis and GC. We built and validated the value of NRRS. This contributes to deepening our view of NETosis and potentially provides new strategies for GC treatment.

Keywords: NETosis; gastric cancer (GC); subtype; prognosis; immunotherapy

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Introduction

Gastric cancer (GC) is a widespread malignant tumor globally, ranking among the top five most common cancers. One of the challenges in managing GC is that its early clinical symptoms are often non-specific, which means patients may not experience significant discomfort or symptoms that clearly point to the disease. As a result, many patients are diagnosed at an advanced stage when the disease is already well-developed, leading to a less favorable prognosis (1-3). The primary treatment for GC continues to be surgical intervention, often supplemented by postoperative immunotherapy, chemotherapy, and other treatment modalities (4-6). Although recent advancements in medical technology have enhanced diagnostic and therapeutic options for GC, the mortality rate among patients remains high and continues to pose a significant challenge (7-9). Consequently, advancing the development of reliable biomarkers and disease models is essential for enhancing the prognosis of GC patients. These tools are vital for better patient outcomes, offering new avenues for therapeutic interventions (10-13).

Programmed cell death encompasses different types of cell death, such as pyroptosis, necroptosis, entosis, as well as ferroptosis and cuprotosis, which have been a hot research topic in recent years (14-18). NETosis is a newly defined mode of programmed cell death. Specifically, reticular DNA structures wrapped by histones, proteases, various antimicrobial proteins and cytoplasmic proteins are released by neutrophils to capture microorganisms and pathogens and are known as neutrophil extracellular traps (NETs) (19-21). NETs may be involved in various types of diseases such as autoimmune diseases, acute lung injury, sepsis and various processes of cancer (22-25).

Currently, NETosis is thought to play a crucial role in tumorigenesis (26), with prognostic features linked to certain malignancies (27-29). However, its specific function and mechanisms in GC remain unexplored, highlighting the need for further comprehensive research.

In our research, we constructed and validated a new NETosis-related risk signature (NRRS), evaluated the prognostic significance of NRRS in gastric cancer, and distinguished patients with varying degrees of sensitivity to immunotherapy, thereby laying the groundwork for enhanced patient-specific treatment strategies. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-377/rc).

Highlight box

Key findings

- NETosis, a recently discovered programmed cell death process, is tightly linked to the immune microenvironment of gastric cancer (GC). The NETosis-related risk signature (NRRS) effectively predicts GC patient survival.

What is known and what is new?

- GC, a highly prevalent cancer with a poor prognosis, is significantly impacted by NETosis in tumor biology.
- We discovered a correlation between NETosis subtypes and the tumor immune microenvironment in GC. We developed a validated NRRS to predict survival, immune infiltration, and drug sensitivity.

What is the implication, and what should change now?

- Our research deepens knowledge of NETosis and proposes fresh therapeutic approaches for GC.

Methods

Data collection

In the training cohort, we obtained transcriptomic data from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) database's stomach adenocarcinoma (STAD) dataset, encompassing 375 tumor samples and 32 normal tissue samples. For validation, we utilized microarray gene chip data from the GSE84433 dataset on the GPL6947 platform from the Gene Expression Omnibus (GEO) database, including data from 357 patients. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Consensus clustering analysis of NETosis-related subtypes

We selected 62 NETosis-related genes from previous studies, including *BST1*, *CD93*, *CEACAM3*, *CREB5*, *CRISPLD2*, *CSF3R*, *CYP4F3*, *DYSF*, *CPPED1*, *G0S2*, *HPSE*, *CXCR1*, *CXCR2*, *KCNJ15*, *LILRB2*, *MGAM*, *PDE4B*, *S100A12*, *SIGLEC5*, *SLC22A4*, *SLC25A37*, *TECPR2*, *TNFRSF10C*, *VNN3*, *AKT1*, *AKT2*, *ATG7*, *CYBB*, *DNASE1*, *ENTPD4*, *HMGB1*, *IL1B*, *ITGAM*, *ITGB2*, *KCNN3*, *MAPK1*, *MAPK3*, *MMP9*, *MTOR*, *PTAFR*, *PIK3CA*, *RIPK1*, *RIPK3*, *SELP*, *SELPLG*, *SIGLEC14*, *TLR2*, *TLR4*, *TLR7*, *TLR8*, *CSF3*, *IL6*, *CTSG*, *MYD88*, *PLA2G7*, *GSDMD*, *CDK6*, *NOX4*, *AGER*, *MME*, *ALPL* and *CLEC6A*.

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We used ConsensusClusterPlus for cluster analysis. And the ideal number of clusters was established by combining statistical methods, including the consistent cumulative distribution function (CDF), area under the curve (AUC), and K-value. These techniques were utilized to ensure the stability and reliability of the clustering results.

Differentially expressed genes (DEGs) identification and analysis

Limma, a screening method rooted in generalized linear models, was utilized in this study to identify DEGs between different groups using the R package limma (version 3.40.6). Genes were considered significantly differentially expressed based on an adjusted P value <0.05 and a fold change >1.5. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted to compare the signaling pathways among NETosis clusters, employing statistical significance thresholds of P value and q-value <0.05.

Mutation landscape examination

Genetic mutations among STAD subtypes were explored using somatic mutation data from TCGA, visualized through waterfall plots.

Construction and validation of the NRRS

The least absolute shrinkage and selection operator (LASSO), a popular analysis approach, integrates variable selection and regularization. The non-zero coefficients derived from the LASSO regression represent the weights assigned to each gene in the final risk score model. Furthermore, we developed nomograms to estimate the survival outcomes of GC patients.

Immune cell infiltration analysis

Using the CIBERSORT algorithm, we analyzed the relative abundances of 22 distinct immune cell types. Additionally, we utilized the ESTIMATE algorithm to assess variations in Immune Score, Stromal Score, and ESTIMATE Score. To determine the correlation between the risk score and immune cells, we employed the Spearman correlation test. The Immune Score is a quantification of immune cell infiltration in a tumor microenvironment derived from gene expression data analysis. The Stromal Score assesses the presence of stromal cells within the tumor microenvironment. Lastly, the ESTIMATE Score, an algorithm combining immune and stromal scores, provides an estimation of tumor purity.

Drug sensitivity analysis

The sensitivity to chemotherapy and immunotherapy drugs is assessed by analyzing half-maximal inhibitory concentration (IC50) values. Utilizing the R software "pRRophetic", we determined the IC50 values of various drugs in GC samples, enabling us to evaluate the association between risk levels and drug sensitivity.

Validation of NETosis-associated prognostic genes via immunohistochemical staining

Immunohistochemical staining data of GC and normal tissues were retrieved from the Human Protein Atlas (HPA, http://www.proteinatlas.org/), validating the protein expression of NETosis-related prognostic genes.

Statistical analysis

We identified genes differentially expressed between subtypes using Limma software. Kaplan-Meier curves evaluated overall survival (OS) across patient groups. LASSO regression was applied to pinpoint relevant prognostic genes. Correlation analysis examined the relationship between risk scores and immune cell infiltration, with statistical significance at P<0.05.

Results

Cluster analysis identified NETosis-related subtypes

Figure 1A illustrates that the majority of NETosis genes exhibited elevated expression levels in GC samples relative to normal tissues. Subsequent consistency clustering analysis revealed that the optimal clustering was achieved with K=2, as depicted in *Figure 1B-1D*. Additionally, the two identified subtypes exhibited distinct expression patterns of NETosis genes (*Figure 1E,1F*). Kaplan-Meier survival analysis indicated that patients in the C2 group had a more favorable prognosis (*Figure 1G*), with statistical significance at P=0.03.

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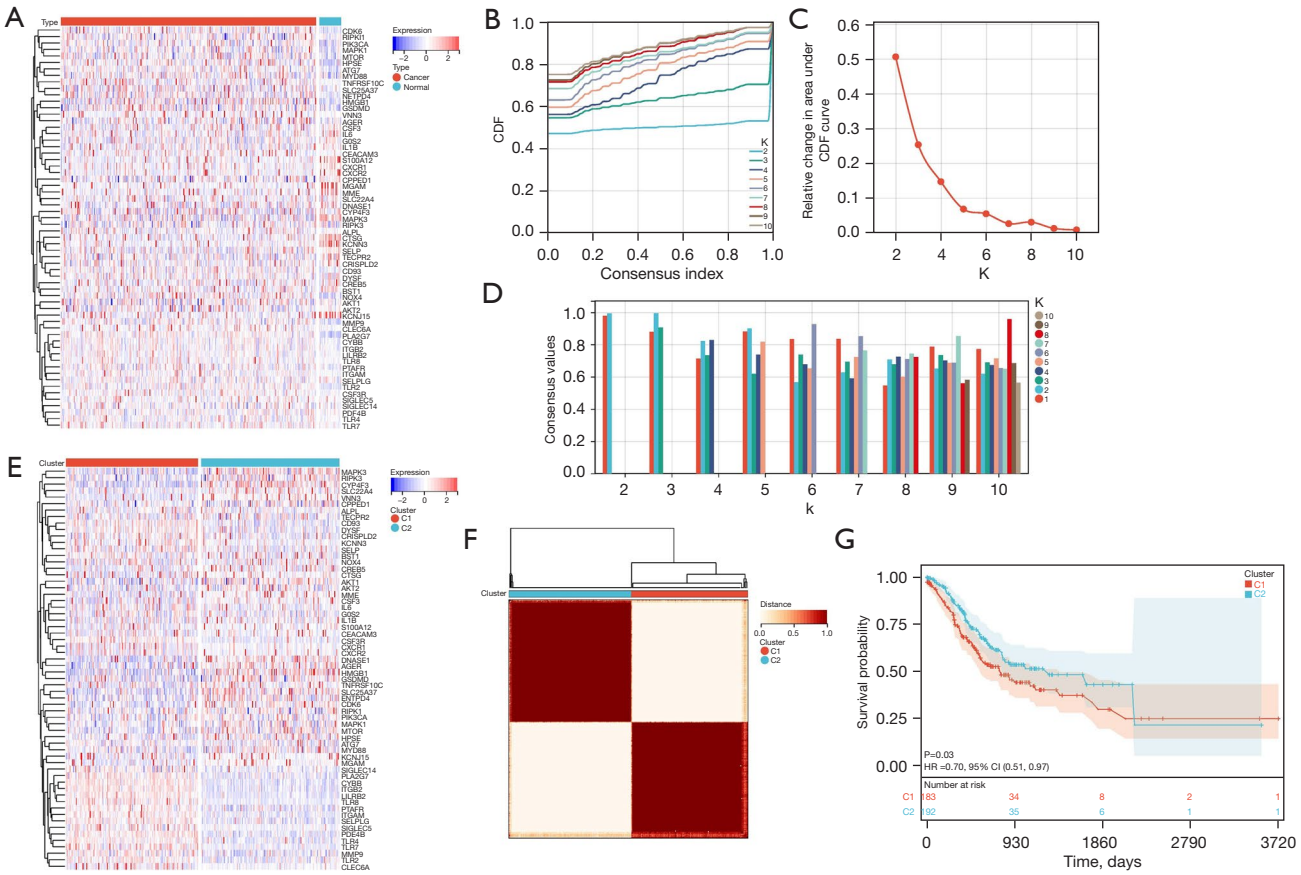

Figure 1 Identification of NETosis-related subtypes by consensus clustering. (A) Heatmap of expression of 62 NETosis-related genes in GC samples, including tumor and normal samples. (B,C) Cluster analysis of CDF indicating area under the curve at k=2–10 and delta decreasing trend. (D) Example cluster consistency plot showing the optimal consensus value when k=2. (E) Heatmap of NETosis-related gene expression in the 2 subtypes. (F) Consensus matrix for optimal k=2. (G) Kaplan-Meier curve of OS for the 2 subtypes (P=0.03). GC, gastric cancer; CDF, cumulative distribution function; HR, hazard ratio; CI, confidence interval; OS, overall survival.

Differential expression analysis and enrichment profiling of NETosis-related subtypes

A comprehensive analysis of DEGs between the two NETosis subtypes yielded a total of 1,210 upregulated and 47 downregulated genes (*Figure 2A*). These genes displayed distinct expression patterns between the subtypes (*Figure 2B*). Further GO and KEGG enrichment analyses revealed that the majority of DEGs were involved in immune-related processes, including immune system regulation, cell migration, adaptive immune response, leukocyte migration, cytokine-cytokine receptor interaction, cancer-related pathways, and human papillomavirus infection (*Figure 2C,2D*).

Comparison of somatic mutations, tumor microenvironment, and immune checkpoint across NETosis-related subtypes

Somatic mutations in patients from each category were examined, and a waterfall chart was generated (*Figure 3A,3B*). Within category C1, the genes most commonly affected by mutations were *TTN*, *TP53*, *ARID1A*, *MUC16*, and *LRP1B*. While in group C2, *SYNE1* was the 5th most frequently mutated gene with a mutation frequency of 30.7%, which was higher than the mutation frequency of *SYNE1* in group C1 (23.8%). In addition, *TTN* and *TP53* also had much higher mutation frequencies in the C2 group (63.7%,

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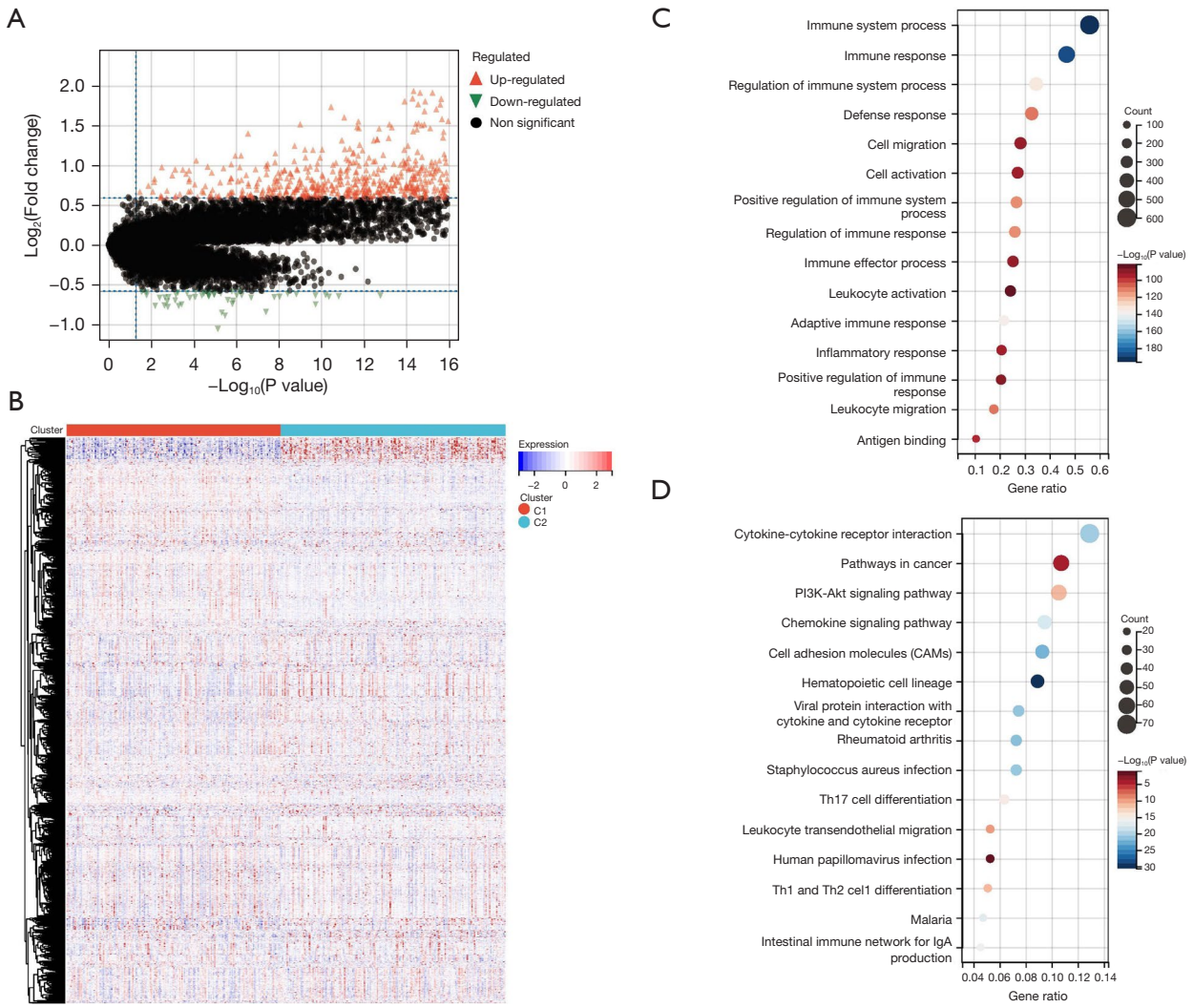

Figure 2 Identification of DEGs and biological pathways between the 2 NETosis subtypes. (A) Volcano plot showing NETosis-related DEGs in both groups. (B) Heatmap showing the expression of 1,257 overlapping DEGs between the 2 isoforms. (C,D) Bubble plots showing the enrichment of potential signaling pathways for GO and KEGG. DEG, differentially expressed gene; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

59.2%) than in the C1 group (47.6%, 40.2%).

Our ESTIMATE analysis demonstrated higher scores in all three categories for group C1 compared to group C2 (*Figure 3C*). The evaluation of immune checkpoints (*Figure 3D*) showed increased expression in group C1 for most immune checkpoints. Furthermore, using CIBERSORT, we visualized the differences in immune cell infiltration between the two groups via a box-and-line plot (*Figure 3E*). The findings indicated greater infiltration of cells like activated CD4 memory T cells, resting dendritic cells, and neutrophils in the C1 group, while the C2 group exhibited higher infiltration of resting CD4 memory T cells and activated NK cells.

Development and verification of the NETosis-associated risk signature (NRRS)

We used the LASSO Cox method for regression analysis. Specifically, we integrate survival time, status, and gene expression data together (*Figure 4A,4B*). We set the lambda value to 0.055467178709963 and obtained 14 prognostic genes related to NETosis: *CXCR4*, *NRP1*, *PDCD1*, *CTLA4*,

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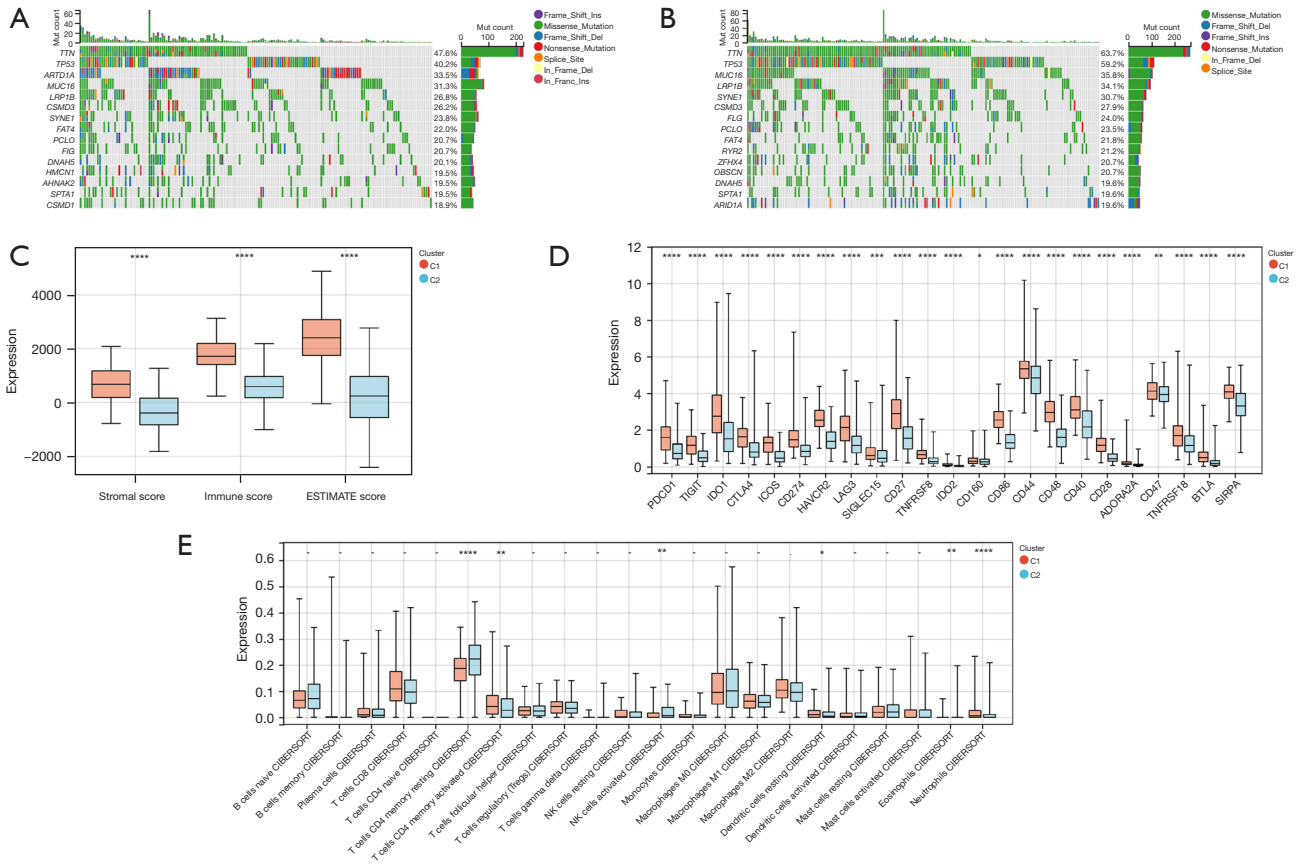

Figure 3 Somatic mutations and immune infiltration in 2 NETosis subtypes. (A,B) Mutation map waterfall plot showing the 15 most common mutated genes in gastric cancer differing between groups C1 and C2. (C) ESTIMATE box-and-line plot showing the Immune Score, Stromal Score and ESTIMATE Score of the different subtypes of the infiltration. (D) Box-and-line plot showing the expression of some of the immune checkpoints in the 2 NETosis subtypes. (E) CIBERSORT box-and-line plot showing the differences in the infiltration of 22 immune cells between the 2 NETosis subtypes. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; -, not significant. Immune Score: the immune score is a measure derived from the analysis of gene expression data that quantifies the infiltration of immune cells in a tumor microenvironment. Stromal Score: the stromal score evaluates the presence of stromal cells within the tumor microenvironment. ESTIMATE Score: ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) is an algorithm that combines the immune and stromal scores to estimate the tumor purity.

AKR1B1, *SERPINE1*, *RGS2*, *SLCO2A1*, *TNFAIP2*, *RNASE1*, *DOC2B*, *APOD*, *ENTPD2*, and *CCL24*. The non-zero coefficients from the LASSO regression represent the weights assigned to each gene in the final risk score model. The model formula for RiskScore is as follows: RiskScore = 0.028993043781327 × *CXCR4* + 0.0314706454214018 × *NRP1* – 0.0941878557499203 × *PDCD1* – 0.0610832396989102 × *CTLA4* + 0.149627851386361 × *AKR1B1* + 0.123843546915026 × *SERPINE1* + 0.008120183886935 × *RGS2* + 0.00732963996657683 × *SLCO2A1* – 0.00734132828114282 × *TNFAIP2* + 0.0387613971398363 × *RNASE1* – 0.0548303763103828 × *DOC2B* + 0.0194583010341922 × *APOD* – 0.040210942582457 × *ENTPD2* – 0.0107916247408867 × *CCL24*.

We were surprised to find that as the risk score increased, there was a significant decrease in the survival of patients. We found that *CXCR4*, *NRP1*, *AKR1B1*, *SERPINE1*, *RGS2*, *SLCO2A1*, *RNASE1*, *APOD*, and *CCL24* were risk factors, their expression rose as the risk score increased. On the contrary, *PDCD1*, *CTLA4*, *TNFAIP2*, *DOC2B*, and *ENTPD2* were protective factors, showing their expression decreased as the risk score increased (*Figure 4C*).

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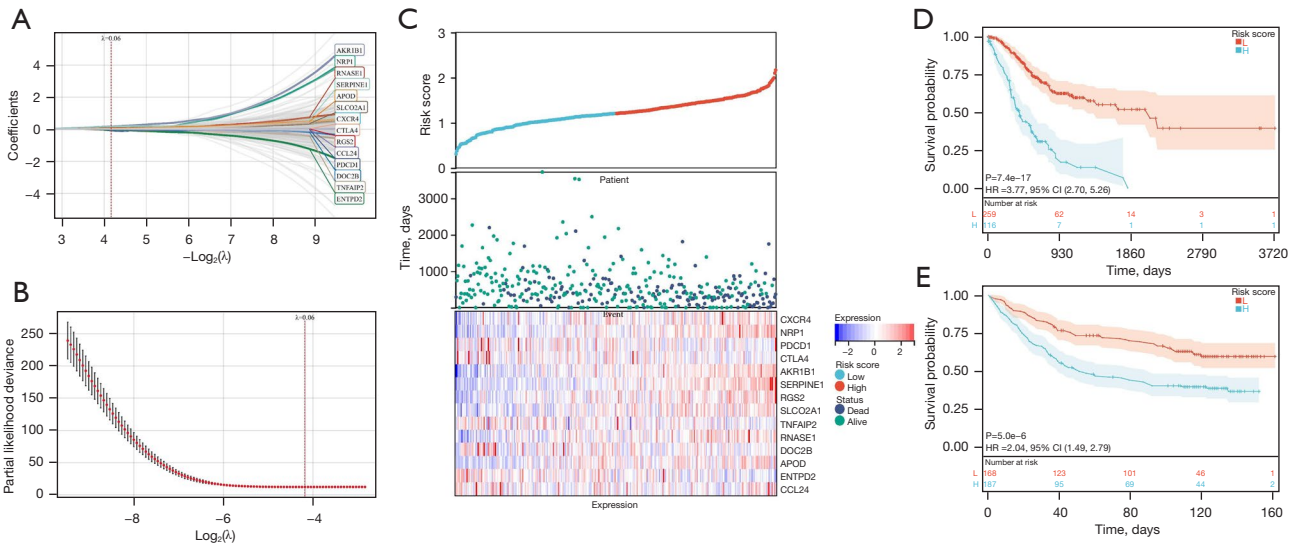

Figure 4 Construction and validation of the NRRS. (A,B) LASSO analysis of 14 NETosis-related prognostic genes extracted. (C) Prognostic heatmap showing the relationship between different risk scores, patient survival, and gene expression changes in the NETosis-related risk model. (D,E) Kaplan-Meier curves for NRRS in the training dataset TCGA-STAD and validation dataset GSE84433. NRRS, NETosis-related risk signature; LASSO, least absolute shrinkage and selection operator; TCGA-STAD, The Cancer Genome Atlas-stomach adenocarcinoma; L, low risk score group; H, high risk score group; HR, hazard ratio; CI, confidence interval.

Furthermore, we employed Kaplan-Meier analysis to ascertain the prognostic significance of the NRRS in GC patients. In the training set, the low-risk group had a better prognosis ($P=7.4\times^{-17}$) (*Figure 4D*), GSE84433 survival of the validation set showed a similar result ($P=5.0\times10^{-6}$) (*Figure 4E*).

The association of NRRS with prognosis

Our multivariate Cox regression analysis identified the NRRS as an independent prognostic factor for OS in GC patients (*Figure 5A*). We constructed nomograms and to improve survival predictions (*Figure 5B,5C*). In the receiver operating characteristic ROC analysis, the AUC values for the one-, three-, and five-year periods were 0.76, 0.81, and 0.85 (*Figure 5D*). Therefore, these results indicate that the NRRS is an effective tool for predicting the survival rate of GC patients.

Exploration of NRRS correlation with drug sensitivity and tumor microenvironment

The risk score positively correlated with infiltrating monocytes and M2 macrophages while negatively correlating with activated CD4 memory T cells and T follicular helper cells in both the training (*Figure 6A-6D*) and validation sets (*Figure 6E-6H*).

We screened compounds using predicted IC50 values, revealing drug sensitivities among patient groups (*Figure 7*). Low-risk patients exhibited greater sensitivity to Bcl-2 inhibitors (ABT-263), veliparib (ABT-888), Chk inhibitors (AZD7762), camptothecin, lestaurtinib (CEP-701), ATM inhibitors (KU-55933), methotrexate, B-RafV600E inhibitors (PLX4720), sunitinib, and vinblastine. Conversely, high-risk patients were more responsive to Akt inhibitors such as A-443654, AKT inhibitor VIII, saracatinib (AZD-0530), afatinib (BIBW2992), dasatinib, and elesclomol. These findings suggest that individualized drug selection may be beneficial for different groups of patients.

Validation of NETosis-related prognostic genes via protein levels reveals promising prognostic indicators

We examined the levels of crucial genes associated with NETosis in both GC and healthy tissues by utilizing data from HPA immunohistochemical staining (*Figure 8*). Our analysis revealed a notable increase in *APOD* levels in GC tissues, while *ENTPD2* and *PDCD1* showed a decrease in expression. Conversely, *NRP1* and *SERPINE1* displayed minimal variance between tumor and normal tissues.

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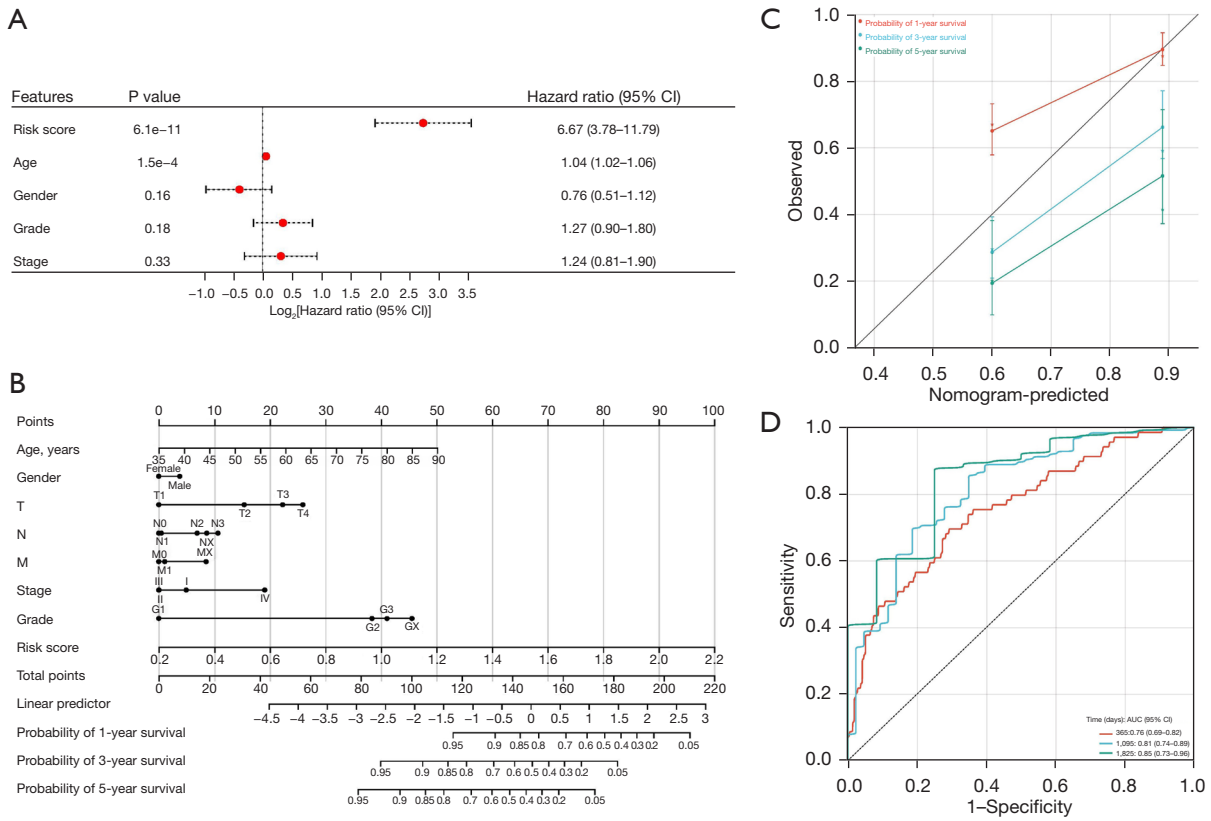

Figure 5 Association of NRRS with GC prognosis. (A) Multivariate Cox analysis to assess the independent prognostic value of NRRS in patients with GC. (B-D) Nomo plots, calibration plots, and time-dependent ROC curve analyses used to predict 1-, 3-, and 5-year survival probabilities of patients. NRRS, NETosis-related risk signature; GC, gastric cancer; CI, confidence interval; ROC, receiver operating characteristic; AUC, area under the curve.

Regrettably, data for additional genes was unavailable for our study. Collectively, our findings suggest that these specific markers could potentially serve as significant indicators in the context of GC progression.

Discussion

GC remains a major threat to human health worldwide, especially in Asian countries, where morbidity and mortality remain high. Researchers are also constantly working to find tools that can help predict or improve outcomes for patients with GC. Encouragingly, various gene models related to programmed death have been developed, such as pyroptosis, necroptosis, entosis, as well as the popular ferroptosis and cuprotosis, etc. (14-18), which effectively accelerates the process of GC biological targets and potential therapies.

NETosis is a newly discovered novel mode of cell death that has been less studied. NETosis is morphologically different from apoptosis and autophagy, NETosis exhibits reticular DNA structures released by neutrophils to capture microorganisms and pathogens. There are fewer studies in this area, but the emergence of this death mechanism has provided new ideas for cancer treatment (25-29). The pathway and mechanism of its involvement in GC have not been reported. Researchers still need to study NETosis in depth.

In our research, we have identified two distinct subtypes of NETosis by analyzing the expression of 62 NETosis-related genes. Using advanced statistical methods such as limma and Cox-LASSO, we were able to pinpoint 14 genes that play a crucial role in these subtypes and constructed the NRRS. These genes are believed to be involved in cancer development and inflammation by affecting the body's immune response. One of the key genes we identified, *NRP1*, has been shown to have contrasting effects on different types of cancer. Overexpression of *NRP1* can enhance

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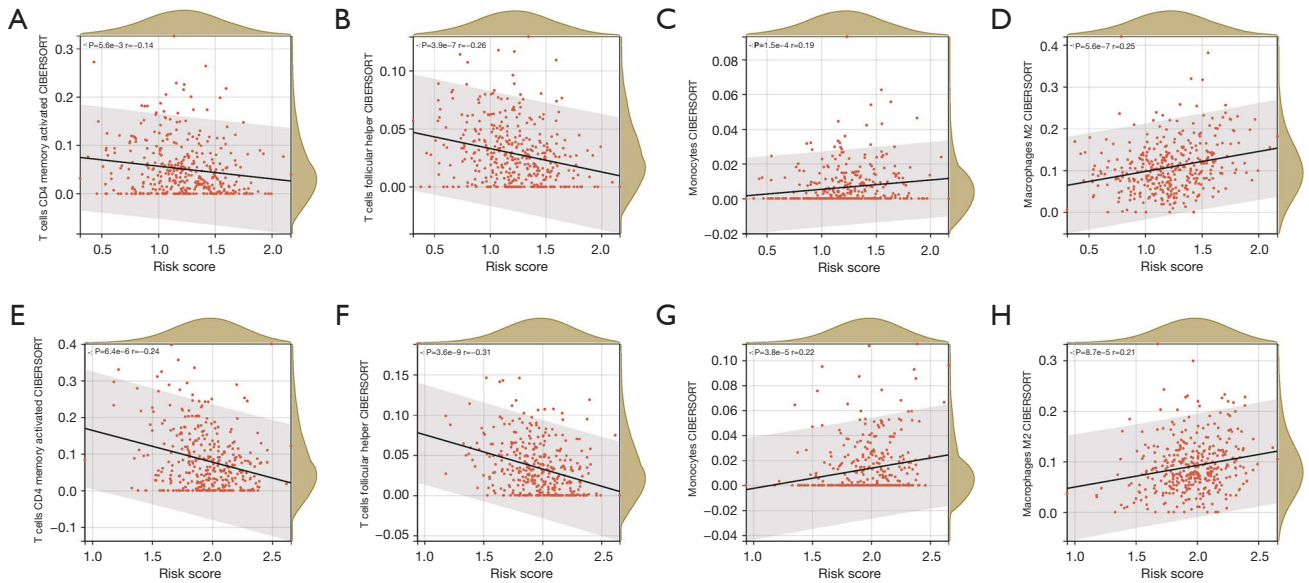

Figure 6 Association of NETosis-related risk signature with tumor microenvironment. Scatter plots (A-D) show the correlation of risk scores with training-focused activated CD4 memory T cells, T follicular helper cells, monocytes, and M2 macrophages infiltration, as further validated by the GSE84433 cohort (E-H).

the growth and movement of prostate cancer cells (30), while its downregulation may contribute to bladder cancer progression (31). Another important finding is the role of arachidonic acid (ARA) when bound to *APOD* in regulating inflammation and oxidative stress (32). The administration of antiplatelet therapy has been observed to elevate the levels of *SERPINE1* in cancer cells, which in turn has been linked to an increase in cell motility and an enhanced propensity for colon cancer metastasis (33). Based on our analysis of these 14 genes, we found that the NRRS we developed showed a better prognosis for survival. In conclusion, our research suggests that NETosis could serve as a promising therapeutic target and potentially function as a diagnostic and prognostic biomarker for GC. By understanding the intricate mechanisms of these genes and their impact on cancer development, we may pave the way for more effective treatments and personalized medicine approaches in the future.

The GO and KEGG analyses highlighted distinct immune-related processes that the different groups were engaged in. These included the regulation of immune system processes, cell migration, leukocyte activation, adaptive immune responses, leukocyte migration, and cytokine-cytokine receptor interactions. Furthermore, pathways in cancer, human papillomavirus infection, Th17 cell differentiation, and Th1 and Th2 cell differentiation were also identified. These findings shed light on the reasons behind the improved survival rates of GC patients in group C2, showcasing the importance of immune responses in patient outcomes.

The analysis of CIBERSORT results revealed a notable disparity in immune cell infiltration between group C1 and group C2. Specifically, group C1 exhibited increased levels of activated CD4 memory T cells, resting dendritic cells, and neutrophils, while group C2 showcased higher amounts of resting CD4 memory T cells and activated NK cells. Natural killer cells, known for their potent anti-cancer function in immunotherapy (34,35), were found to be more prevalent in the C2 group, emphasizing their significance in targeting cancerous cells. Memory CD4 T cells, on the other hand, play a crucial role in mounting a swift and efficient secondary immune response, thus accelerating the systemic immune reaction (36). Additionally, dendritic cells, renowned for their capacity to activate naïve T cells and facilitate effector differentiation (37,38), were also more abundant in the C2 group. This disparity in immune cell composition likely contributes to the superior survival outcomes observed in group C2. Further analysis uncovered higher expression levels of immune checkpoints in the C1 group compared to the C2 group. This suggests that immunotherapy may yield more favorable results for patients in the C1 group, aligning with previous research findings. These insights underscore

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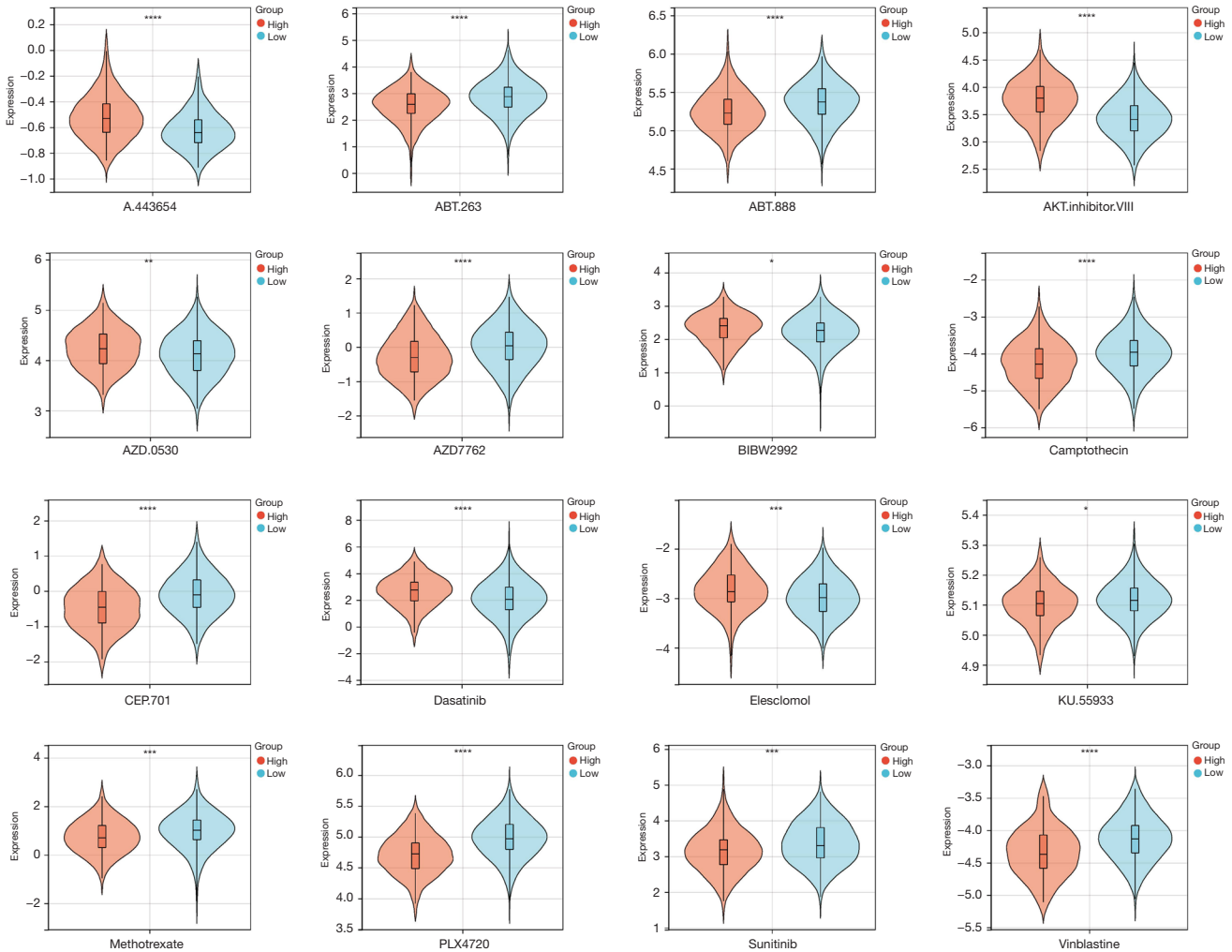

Figure 7 Box line plot showing the results of drug sensitivity analysis for gastric cancer (GC) patients in the high/low risk score group. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.

the importance of understanding and leveraging the distinct immune cell profiles in guiding personalized treatment strategies for cancer patients.

Moreover, we also selected a variety of drugs based on the IC50 prediction, and the high- and low-risk groups had different sensitivities to some of these drugs, which may help patients in different groups to choose more effective and personalised drug regimens. Finally, we queried the HPA database and in turn assessed the immunohistochemical staining of NETosis-associated prognostic genes in normal tissues and GCs. It is clear that these markers are likely to play a role in GC. Taken together, the NRRS we developed can more accurately predict the survival prognosis of GC patients in advance, and has a better guiding role for diagnostic and therapeutic markers of GC.

It is acknowledged that this study is not without its limitations. Firstly, Bioinformatics analysis methods have a wide range of applications, including but not limited to drug design and screening, protein structure prediction, genome-wide chain analysis, and so on. It plays an important role in various aspects of the medical field. Nevertheless, it is our hope that in the future, we will be able to apply western blot, polymerase chain reaction (PCR), and other experiments to validate the results in this paper. Secondly, our findings are in need of further validation in an external GC queue. In conclusion, this paper presents the findings of a retrospective study. To confirm the results, further prospective, multicentre studies are required.

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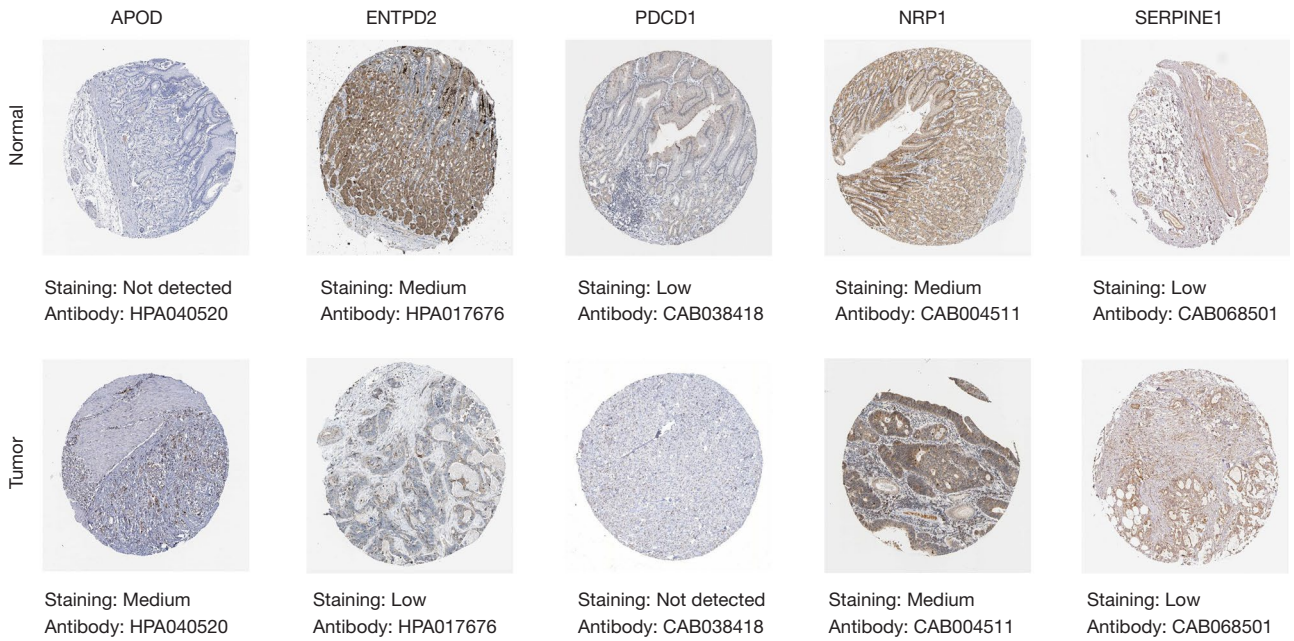

Figure 8 Validation of NETosis-associated prognostic genes at the protein expression level. Representative immunohistochemistry images of and gastric cancer tissues sourced from the Human Protein Atlas database (https://www.proteinatlas.org/). Image credit goes to the Human Protein Atlas. The links to the individual normal and tumor tissues of each protein are provided for *APOD* (https://www.proteinatlas.org/ENSG00000189058-APOD/tissue/stomach; https://www.proteinatlas.org/ENSG00000189058-APOD/pathology/stomach+cancer), *ENTPD2* (https://www.proteinatlas.org/ENSG00000054179-ENTPD2/tissue/stomach; https://www.proteinatlas.org/ENSG00000054179-ENTPD2/pathology/stomach+cancer), *PDCD1* (https://www.proteinatlas.org/ENSG00000188389-PDCD1/tissue/stomach; https://www.proteinatlas.org/ENSG00000188389-PDCD1/pathology/stomach+cancer), *NRP1* (https://www.proteinatlas.org/ENSG00000099250-NRP1/tissue/stomach; https://www.proteinatlas.org/ENSG00000099250-NRP1/pathology/stomach+cancer), *SERPINE1* (https://www.proteinatlas.org/ENSG00000106366-SERPINE1/tissue/stomach; https://www.proteinatlas.org/ENSG00000106366-SERPINE1/pathology/stomach+cancer), respectively. Scale bar, 200 µm. Staining degree: high, medium, low, and not detected.

Conclusions

In conclusion, our study highlights the association of NETosis subtypes with GC. NRRS were also established and their effects were validated in other cohorts. This model is of great significance with GC. The aforementioned results may facilitate a more profound comprehension of NETosis and offer novel avenues for tailored therapeutic interventions.

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Footnote

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appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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