Drug metabolism-related eight-gene signature can predict the prognosis of gastric adenocarcinoma

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Abstract

Background: Metabolic abnormalities in patients with gastric adenocarcinoma lead to drug resistance and poor prognosis. Therefore, this study aimed to explore biomarkers that can predict the prognostic risk of gastric adenocarcinoma by analyzing drug metabolism-related genes.

Methods: The RNA-seq and clinical information on gastric adenocarcinoma were downloaded from the UCSC and gene expression omnibus databases. Univariate and least absolute shrinkage and selection operator regression analyses were used to identify the prognostic gene signature of gastric adenocarcinoma. The relationships between gastric adenocarcinoma prognostic risk and tumor microenvironment were assessed using CIBERSORT, EPIC, QUANTISEQ, MCPCounter, xCell, and TIMER algorithms. The potential drugs that could target the gene signatures were predicted in WebGestalt, and molecular docking analysis verified their binding stabilities.

Results: Combined with clinical information, an eight-gene signature, including GPX3, ABCA1, NNMT, NOS3, SLCO4A1, ADH4, DHRS7, and TAP1, was identified from the drug metabolism-related gene set. Based on their expressions, risk scores were calculated, and patients were divided into high- and low-risk groups, which had significant differences in survival status and immune infiltrations. Risk group was also identified as an independent prognostic factor of gastric adenocarcinoma, and the established prognostic and nomogram models exhibited excellent capacities for predicting prognosis. Finally, miconazole and niacin were predicted as potential therapeutic drugs for gastric adenocarcinoma that bond stably with NOS3 and NNMT through hydrogen interactions.

Conclusions: This study proposed a drug metabolism-related eight-gene signature as a potential biomarker to predict the gastric adenocarcinoma prognosis risks.

Keywords
drug metabolism, gastric adenocarcinoma, immune microenvironment, molecular docking, prognostic model
1 | INTRODUCTION

Gastric adenocarcinoma is a life-threatening malignancy of the gastrointestinal tract and has become the third leading cause of cancer death globally. Of all the gastric cancers, approximately 90%–95% are gastric adenocarcinomas. In 2018, more than one million new cases were confirmed, the majority of which were locally advanced at the time of diagnosis. The 5-year survival rate of advanced or metastatic gastric adenocarcinoma is less than 30%. The incidence of local recurrence or distant metastasis of gastric cancer after surgery remains at 40%–70%, even with surgical intervention, radiotherapy, chemotherapy, and other treatment strategies, along with a certain degree of side effects after radiotherapy and chemotherapy. Therefore, numerous studies have been conducted to explore prognostic biomarkers in an attempt to improve the clinical outcome of patients with gastric adenocarcinoma. Among them, Yao and Ren et al. evaluated the importance of immune microenvironment-related genes in gastric adenocarcinoma prognosis by mining public databases. However, a more comprehensive understanding of tumorigenesis mechanisms and the exploration of potential prognostic biomarkers from multiple perspectives are still required.

Metabolic abnormalities are the primary cause of drug resistance in patients with gastric adenocarcinoma, and current studies have provided profound insights into the metabolic changes of gastric adenocarcinoma and discussed their possible regulatory mechanisms. A related genome-wide profile analysis identified several important gastric cancer biomarkers that were significantly associated with drug metabolism pathways. The long non-coding RNA MACC1-AS1, a biomarker related to gastric cancer prognosis, was reported to regulate disease metabolism through enhanced glycolysis and antioxidant capacity. Furthermore, ectopic expression of S100P has been found to correlate with proliferation and increased drug resistance in gastric cancer cells. A bioinformatics study found that the differentially expressed genes (DEGs), including ASPN, COL1A1, FN1, VCAN, and MUC5AC, in gastric cancer were significantly associated with survival prognoses of patients and were predominantly enriched in drug metabolism pathways. Although these genes were found to be involved in drug metabolism pathways, few studies have systematically reported drug metabolism-related drugs and thoroughly explored their prognostic values.

Therefore, the current study aimed to identify genes that are significantly associated with gastric adenocarcinoma prognosis from drug metabolism-related gene sets. Based on the expression of these genes, we constructed a prognostic model and explored the predictive performance of the model through internal and external validation. Furthermore, we predicted the drugs that could target these genes and performed a molecular docking analysis to verify their binding. Additionally, multiple databases were used to investigate the relationship between the prognostic risk of gastric adenocarcinoma and the immune microenvironment. The workflow of the study is shown in Figure S1. The feature genes proposed in this study may be potential therapeutic markers, resulting in improved clinical outcomes in patients.

2 | MATERIALS AND METHODS

2.1 | Data acquisition

RNA-seq data of gastric adenocarcinoma and related clinical information, including tumor stage, family history, lymph node examined count, neoplasm histologic grade, primary diagnosis, resection or biopsy site, disease type, overall survival (OS), and OS duration were downloaded from the UCSC Xena (https://xenahubs.net) platform. A total of 32 paracancerous samples and 375 tumor samples were included in this study, and 348 gastric adenocarcinoma samples with prognostic information were enrolled to develop a prognostic model. Furthermore, expression data and clinical information of 65 gastric adenocarcinoma samples in the microarray dataset GSE13861 were obtained from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. GSE13861 was detected using the GPL6884 Illumina HumanWG-6 v3.0 Expression BeadChip and was used as the validation set.

2.2 | Analysis of drug metabolism-related genes

Based on published articles and The Cancer Genome Atlas (TCGA) database, a total of 228 drug metabolism-related genes were matched. Using the empirical Bayes method provided by the limma package (v3.10.3, http://www.bioconductor.org/packages/2.9/bioc/html/lmma.html), drug metabolism-related DEGs between tumor and paracancerous samples were identified with p-values adjusted by Benjamini & Hochberg method < 0.05, and |log fold-change| >0.5 as thresholds.

2.3 | Enrichment analysis and protein-protein-interaction (PPI) network construction

Gene ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of drug metabolism-related DEGs were enriched in DAVID v6.8 (http://david.ncifcrf.gov/). GO and KEGG terms with p-value < 0.05, and gene count ≥2 were selected with significant correlation. Moreover, the STRING database (v11.0, http://string-db.org/) was used to analyze the relationship between proteins coded by drug metabolism-related DEGs, and the PPI network was visualized using Cytoscape (v3.6.1, http://cytoscape.org/).

2.4 | Screening of drug metabolism-related prognostic DEGs

Based on the drug metabolism-related DEGs obtained above and the survival information of gastric adenocarcinoma samples, univariate Cox regression analysis was performed to select drug
metabolism-related prognostic DEGs using the survival package (v2.41–1, http://bioconductor.org/packages/surivalr/) in R3.6.1. DEGs with a p-value < 0.05 were determined to be significantly correlated with prognosis.

### 2.5 Construction and validation of the prognostic model

By utilizing the prognostic information of gastric adenocarcinoma samples in the training set and the expression values of drug metabolism-related prognostic DEGs in each sample, genes were further selected as the optimized gene set using the least absolute shrinkage and selection operator (LASSO) regression analysis in the glmnet package (v2.0-18, http://cran.r-project.org/web/packages/glmnet/index.html) . The risk score of each sample was then calculated as follows:

\[
\text{Risk score} = \sum \beta_{\text{gene}} \times \text{Exp}_{\text{gene}}
\]

where \( \beta_{\text{gene}} \) indicates the LASSO regression coefficient of gene signature, and \( \text{Exp}_{\text{gene}} \) indicates their expression levels in gastric adenocarcinoma samples. To verify the effectiveness of the prognostic model, the risk scores of the samples in GSE13861 were calculated. The samples were then grouped into high- and low-risk groups based on the median risk score. Kaplan-Meier (KM) curves were created to analyze the difference in survival status between the two groups.

### 2.6 Feature analysis of gene signature

The expression cutoff value of each gene signature in the training set was obtained to determine the optimal cutoff point calculated by the Survminer package of R3.6.1 (v0.4.3). KM analysis was then performed to evaluate the difference in survival prognosis between samples in the high- and low-expression groups. A heatmap was created to observe the relationships between the expression level, risk score, and clinical characteristics of each gastric adenocarcinoma sample.

### 2.7 Statistical analysis of clinical features among risk groups

In the training set, the chi-squared test in R3.6.1 was used for statistical analysis and comparison of categorical variables, including TNM classification, tumor stage, family history, neoplasm histologic grade, primary diagnosis, resection or biopsy site, and disease type, between risk groups. The t test was used for continuous variables, including age and lymph node examined count.

### 2.8 Immune microenvironment analysis between risk groups

In the current study, CIBERSORT (https://cibersort.stanford.edu/index.php) , EPIC (https://gfellerlab.shinyapps.io/EPIC_1-1/), QUANTISEQ, MCP-counter (https://github.com/ebecht/MCPcounter), xCell (https://xcell.ucsf.edu/), and TIMER (https://cistrome.shinyapps.io/timer) were used to estimate immune cell infiltration among risk groups. The Wilcoxon test was used to analyze the difference between the two groups and a heatmap was created accordingly.

### 2.9 Differential pathway analysis between risk groups

Gene Set Enrichment Analysis (GSEA, v3.0) software was used for pathway enrichment with c2.cp.kegg.v7.1.symbols.gmt in MSigDB v7.1 (http://software.broadinstitute.org/gsea/msigdb/index.jsp) as an enrichment background. Then, the differences in enriched pathways were analyzed between risk groups with a false discovery rate (FDR) < 0.05.

### 2.10 Analysis of independent prognostic factors and construction of a nomogram prediction model

To determine whether the prognostic model could be used as an independent prognostic factor, univariate Cox regression analysis was performed on age, sex, tumor stage, family history, neoplasm histologic grade, disease type, and lymph node examined count. Variables with \( p \)-values < 0.05 were selected for multivariate Cox regression analysis, followed by a further selection of statistical significance at \( p < 0.05 \). A nomogram model was created to predict the 1-, 2-, 3-, and 5-year survival probabilities of gastric adenocarcinoma patients according to the multivariate Cox regression analysis results. Calibration curves were generated to verify model accuracy.

### 2.11 Drug enrichment prediction of gene signature

Based on the obtained genes, the WebGestalt database (http://www.webgestalt.org/option.php) was used for drug enrichment prediction using over-representation analysis. Drugs with \( p < 0.05 \) were selected as candidate drugs that could bind with the genes. Based on the prognostic effect of the gene signature, drugs that...
function as inhibitors or inducers were selected. Finally, the binding stabilities between candidate inhibitors or induced drugs and related target genes were validated by molecular docking using AutoDock 4.2.6.

3 | RESULTS

3.1 | Analysis of drug metabolism-related DEGs of gastric adenocarcinoma

Differential analysis was performed between gastric adenocarcinoma samples and paracancerous samples based on 228 matched drug metabolism-related genes. The volcano plot (Figure 1A) shows the 77 obtained drug metabolism-related DEGs. Then, enrichment analysis was performed on these drug metabolism-related DEGs, and 64 biological processes (BP), 13 cellular components (CC), 47 molecular functions (MF), and 21 KEGG pathways were obtained. The bubble charts of Figure 1B display the top 10 GO and KEGG terms, ranked by p-values. The results showed that these DEGs were primarily enriched in GO-BP of several metabolic processes, GO-CC of TAP complex and host cell, GO-MF of aldehyde dehydrogenase activity, and in KEGG pathways of drug metabolism, xenobiotic metabolism, and chemical carcinogenesis. The interactions of these proteins coded by DEGs were then analyzed, and a PPI network was created, as shown in Figure 1C. This PPI network contained 66 drug metabolism-related DEGs and 309 relation pairs. Among these nodes, UGT1A1, CYP2B6, and CYP3A4 had a larger degree.

3.2 | Screening of gene signature of gastric adenocarcinoma

Combined with the prognostic information of gastric adenocarcinoma samples, a univariate Cox regression analysis was
performed, and 11 drug metabolism-related prognostic DEGs were selected with \( p < 0.05 \), as shown in Figure 2A. Then, LASSO regression analysis was performed to select the optimized gene set, and an eight-gene signature was identified, including GPX3, ABCA1, NNMT, NOS3, SLCO4A1, ADH4, DHR57, and TAP1. Their prognostic effects are shown in Figure 2B, and only SLCO4A1 and TAP1 were considered protective factors with hazard ratios <1. Based on the expression cutoff of these genes in the training set, samples were separated into high- and low-expression groups. The KM curves in Figure 2C illustrated that patients with high GPX3, ABCA1, NNMT, NOS3, ADH4, and DHR57 expressions had worse survival status, whereas high SLCO4A1 and TAP1 expressions were significantly associated with better prognoses.

### 3.3 Establishment and validation of the prognostic model

Using the LASSO regression coefficients of each gene obtained above, the risk scores of each sample were calculated, and a prognostic model was created. Based on the median value of the risk score, the samples were grouped into high- and low-risk groups. The expression distributions of these genes in the two groups were visualized using a heatmap (Figure 3A). The results suggested that the expression distribution of genes in the risk groups was different. To validate the model efficiency in the training set, we found that the prognostic risk of patients increased along with increased risk scores (Figure 3B). The KM curve (Figure 3C) demonstrated that patients in the high-risk groups had a worse survival status. Receiver operator
characteristic (ROC) curves (Figure 3D) were created to verify model accuracy, and it was observed that the prognostic model had superior performance in predicting 1-, 2-, 3-, and 5-year prognoses with area under the curves (AUCs) of 0.67, 0.679, 0.711, and 0.728, respectively. The prognostic model was further validated using the GSE13861 dataset (Figure 3E-G). Similarly, as the risk score increased, the number of deaths increased. Meanwhile, patients in the high- and low-risk groups had significant differences in survival time, and the model accuracy was proven in the validation set with all AUCs in ROC curves over 0.65. These results indicated a significant association between the risk grouping and actual outcomes.

### 3.4 Difference of clinical characteristics between risk groups

The clinical information of the high- and low-risk groups was compared, as shown in Table 1. The results suggested that the two
TABLE 1  The statistics of clinical features in the high-risk and low-risk groups

| Subgroups                             | Low risk | High risk | p-Value |
|---------------------------------------|----------|-----------|---------|
| Age (mean ± SD)                       | 66.0 ± 10.1 | 64.6 ± 10.6 | 2.058E−01 |
| Lymph node examined count             | 26.5 ± 20.9 | 19.2 ± 15.1 | 4.264E−04 |
| Family history                        |          |           |         |
| Yes                                   | 9        | 6         |         |
| No                                    | 133      | 129       |         |
| NA                                    | 32       | 39        |         |
| Tumor stage                           |          |           | 3.544E−04 |
| I                                     | 1        | 12        |         |
| II                                    | 34       | 54        |         |
| III                                   | 56       | 74        |         |
| IV                                    | 70       | 22        |         |
| NA                                    | 12       | 12        |         |
| Neoplasm histologic grade             |          |           | 3.463E−03 |
| G1                                    | 3        | 6         |         |
| G2                                    | 77       | 46        |         |
| G3                                    | 89       | 118       |         |
| GX                                    | 5        | 4         |         |
| Pathologic M                          |          |           | 8.204E−02 |
| M0                                    | 161      | 150       |         |
| M1                                    | 6        | 16        |         |
| MX                                    | 7        | 8         |         |
| Pathologic N                          |          |           | 3.294E−03 |
| N0                                    | 65       | 38        |         |
| N1                                    | 39       | 53        |         |
| N2                                    | 39       | 32        |         |
| N3                                    | 29       | 42        |         |
| NX                                    | 2        | 7         |         |
| NA                                    | 0        | 2         |         |
| Pathologic T                          |          |           | 6.183E−04 |
| T1                                    | 15       | 1         |         |
| T2                                    | 38       | 36        |         |
| T3                                    | 79       | 80        |         |
| T4                                    | 42       | 53        |         |
| TX                                    | 0        | 4         |         |
| Primary diagnosis                     |          |           | 1.223E−03 |
| Adenocarcinoma                        | 55       | 62        |         |
| Adenocarcinoma with mixed subtypes    | 0        | 1         |         |
| Adenocarcinoma, intestinal type       | 40       | 33        |         |
| Carcinoma, diffuse type               | 20       | 39        |         |
| Mucinous adenocarcinoma               | 6        | 13        |         |
| Papillary adenocarcinoma              | 4        | 1         |         |
| Signet ring cell carcinoma            | 5        | 7         |         |
| Tubular adenocarcinoma                | 44       | 18        |         |

(Continues)
TABLE 1 (Continued)

| Subgroups                        | Low risk | High risk | p-Value |
|----------------------------------|----------|-----------|---------|
| Site of resection or biopsy      |          |           |         |
| Body of stomach                  | 46       | 38        | 7.366E−01 |
| Cardia                           | 44       | 40        |         |
| Fundus of stomach                | 18       | 22        |         |
| Gastric antrum                   | 59       | 68        |         |
| Lesser curvature of stomach      | 1        | 0         |         |
| Stomach                          | 6        | 6         |         |
| Disease type                     |          |           | 1.941E−01 |
| Adenomas and Adenocarcinomas     | 162      | 154       |         |
| Cystic, Mucinous and Serous Neoplasms | 12     | 20        |         |

Note: Bold p-value < 0.05 indicates statistical significance.
Abbreviations: NA, not available; SD, standard division.

FIGURE 4 Estimation of immune cell infiltration abundance in high- and low-risk groups. (A) The infiltration abundances of immune and stromal cells estimated by CIBERSORT, EPIC, QUANTISEQ, MCPCounter, xCell, and TIMER algorithms; (B) the heatmap shows 59 immune microenvironment-related cells with p < 0.05; (C) the difference in immune scores between high- and low-risk groups; (D) the difference in stromal scores between high- and low-risk groups.
groups had significant differences in lymph node examined count, tumor stage, neoplasm histologic grade, pathologic N, pathologic T, and primary diagnosis.

3.5 | Difference of immune microenvironment between risk groups

Based on CIBERSORT, EPIC, QUANTISEQ, MCPCounter, xCell, and TIMER algorithms, the relative infiltration abundances of immune and stromal cells were estimated. The infiltration abundance of immune cells is shown in Figure 4A. Then, by comparing the differences between risk groups, 59 immune microenvironment-related immune cells is shown in Figure 4A. Then, by comparing the differences between risk groups, 59 immune microenvironment-related cells (p < 0.05) were screened out, as shown in Figure 4B. Immune and stromal scores were calculated for each sample, and significant differences between risk groups were visualized using box plots (Figure 4C,D).

3.6 | GSEA pathway enrichment analysis among risk groups

The KEGG pathways of high- and low-risk groups were analyzed using GSEA. By setting the threshold of FDR < 0.05, the high-risk group significantly enriched 25 KEGG pathways with a normalized enrichment score (NES) >0. Furthermore, 11 significant pathways were enriched in the low-risk group, with an NES < 0. The enriched pathways of these two groups are shown in Table 2.

3.7 | Analysis of independent prognostic factors and establishment of a nomogram model

To further identify the independent prognostic factors, age, sex, tumor stage, family history, neoplasm histologic grade, TNM classification, disease type, and lymph node examined count were incorporated into the univariate Cox regression analysis (Figure 5A). Variables with p-values < 0.05 were then selected for multivariate Cox regression analysis. Finally, the risk group, pathologic N, and pathologic M were identified as independent prognostic factors (Figure 5B). A nomogram model was established to predict 1-, 2-, 3-, and 5-year survival probabilities (Figure 5C), and calibration curves were created to verify model accuracy. Figure 5D shows that the predicted probabilities of 2-, 3-, and 5-year OS were similar to the actual OS, thereby suggesting an excellent prediction accuracy of the nomogram model.

3.8 | Molecular docking analysis of predicted drugs and related targets

Drug prediction enrichment analysis was performed on an eight-gene signature, and 30 drugs were predicted to target 7 genes. Among them, high ABCA1, NNMT, and NOS3 expression levels were correlated with poor prognoses, and their related inhibitors or binders’ glyburide, niacin, and miconazole, respectively, were selected for molecular docking analyses. The binding results of glyburide-ABCA1, miconazole-NOS3, and niacin-NNMT complexes are shown in Figure 6A–C, respectively. As a result, miconazole bonded with the PHE-473 residue of NOS3 by a hydrogen bond with a length of 2.5 Å. Moreover, the small molecular ligand niacin bonded with ARG-30 and SER-32 residues of the receptor NNMT through hydrogen bond interactions. These findings illustrated that these ligand-receptor complexes were in a stable state of binding.

4 | DISCUSSION

Gastric adenocarcinoma ranks fifth among the most prevalent malignancies worldwide, and the development of drug resistance because of metabolic disorders is one of the reasons for poor prognosis. Therefore, by mining TCGA and GEO, we identified eight prognostic genes, namely GPX3, ABCA1, NNMT, NOS3, SLCO4A1, ADH4, DHR57, and TAP1 from the drug metabolism-related gene set using univariate and LASSO regression analyses. Then, a prognostic model was constructed, and patients were grouped into high- and low-risk groups. Survival analyses showed that patients in the high-risk group had worse prognoses, while the ROC curves showed that the prognostic model had good predictive performance in both the training and validation sets with AUCs > 0.65. Furthermore, the risk group was identified as an independent prognostic factor, and the established nomogram model exhibited good accuracy in predicting 1-, 2-, 3-, and 5-year survival probabilities.

Among the eight genes, we found that SLCO4A1 and TAP1 were protective factors for gastric adenocarcinoma prognosis, whereas high GPX3, ABCA1, NNMT, NOS3, ADH4, and DHR57 expressions were significantly associated with poor prognoses. It has been reported that in gastric cancer patients over 60 years of age, GPX3 hypermethylation was significantly correlated with a shorter time to tumor recurrence. Meanwhile, Wang et al. believed that GPX3 is a risk factor for gastric cancer, and the intron single nucleotide polymorphism of GPX3 may alter gastric cancer risk by affecting gene expression levels. Therefore, we speculated that the risk role of GPX3 in gastric adenocarcinoma prognosis may be directly related to changes in gene epigenetics. As for NNMT, related studies found that high NNMT expression in stromal cells may predict an unfavorable postoperative prognosis for gastric carcinoma. NNMT is known to act as a negative predictor for gastric carcinoma prognosis and is correlated with immune infiltrates. These findings confirmed our results, and upregulated NNMT in gastric cancer cells may promote the occurrence of epithelial-mesenchymal transition by activating TGF-β1/SMAD signaling, thereby leading to tumor recurrence and metastasis. A pan-cancer analysis found that increased NOS3 expression in gastric adenocarcinoma may lead to poor prognosis through several typical cancer-related pathways.
This finding is consistent with our results; however, how NOS3 influences gastric adenocarcinoma prognosis through cancer-related pathways requires investigation.

The tumor microenvironment significantly contributes to the occurrence, progression, prognosis, and immunotherapy response of gastric adenocarcinoma. Gastric adenocarcinoma is a chronic gastritis caused by Helicobacter pylori and is often characterized by the infiltration of immune cells, including granulocytes, macrophages, and T lymphocytes. Therefore, the current study explored the relationship between prognostic risk and immune cell infiltration. Significant differences in the infiltration of 59 immune microenvironment-related cells, such as B cells, T cells, neutrophils, and macrophages were found between the risk groups. Relevant studies have reported that high infiltration of B lymphocytes is beneficial for gastric cancer prognosis. Fristedt and Knief et al. suggested that increased infiltration density of B cells

| Terms (High-risk)                                      | NES   | FDR p-Value |
|--------------------------------------------------------|-------|-------------|
| Focal adhesion                                          | 2.068 | 0.040       |
| Regulation of actin cytoskeleton                        | 2.054 | 0.024       |
| Hypertrophic cardiomyopathy HCM                         | 2.031 | 0.021       |
| Complement and coagulation cascades                     | 2.023 | 0.018       |
| Vascular smooth muscle contraction                      | 2.004 | 0.018       |
| Glycosphingolipid biosynthesis ganglio series           | 1.992 | 0.018       |
| Calcium signaling pathway                               | 1.976 | 0.018       |
| KEGG dilated cardiomyopathy                             | 1.965 | 0.019       |
| Arrhythmogenic right ventricular cardiomyopathy ARVC    | 1.964 | 0.017       |
| ECM receptor interaction                                | 1.934 | 0.023       |
| GAP junction                                            | 1.923 | 0.025       |
| Leukocyte transendothelial migration                    | 1.920 | 0.024       |
| Vasopressin regulated water reabsorption                | 1.861 | 0.040       |
| TGF-β signaling pathway                                 | 1.851 | 0.041       |
| Neuroactive ligand-receptor interaction                 | 1.851 | 0.039       |
| Melanogenesis                                           | 1.846 | 0.038       |
| Pathogenic escherichia coli infection                   | 1.833 | 0.041       |
| Cell adhesion molecules cams                            | 1.829 | 0.040       |
| MAPK signaling pathway                                  | 1.821 | 0.041       |
| Prion diseases                                          | 1.817 | 0.041       |
| Adherens junction                                       | 1.802 | 0.045       |
| Glycosaminoglycan biosynthesis chondroitin sulfate      | 1.801 | 0.043       |
| Melanoma                                                | 1.788 | 0.046       |
| Hematopoietic cell lineage                              | 1.775 | 0.049       |
| Axon guidance                                           | 1.770 | 0.049       |

| Terms (Low-risk)                                        | NES   | FDR p-Value |
|--------------------------------------------------------|-------|-------------|
| Spliceosome                                            | −2.117| 0.015       |
| DNA replication                                        | −1.966| 0.039       |
| RNA degradation                                        | −1.946| 0.032       |
| Base excision repair                                   | −1.896| 0.040       |
| Homologous recombination                               | −1.891| 0.035       |
| Aminocyl tRNA biosynthesis                             | −1.886| 0.031       |
| One carbon pool by folate                              | −1.876| 0.029       |
| Pyrimidine metabolism                                  | −1.870| 0.027       |
| Proteasome                                             | −1.868| 0.025       |
| Cell cycle                                             | −1.862| 0.023       |
| Nucleotide excision repair                             | −1.800| 0.039       |

Abbreviations: FDR, false discovery rate; GSEA, gene set enrichment analysis; NES, normalized enrichment score.
or plasma cells was associated with improved prognosis and prolonged OS of esophageal and gastric cancers.\textsuperscript{42,43} As for T cells, increased CD8\textsuperscript{+} T cell infiltration was associated with impaired OS, and PD-L1 expression was higher in patients with a higher CD8\textsuperscript{+} T cell density, suggesting a possible mechanism of adaptive immune resistance.\textsuperscript{44} Christina et al.\textsuperscript{45} reported that the high CD8\textsuperscript{+} T cell density was an adverse prognostic factor for gastric adenocarcinoma patients. However, certain contrasting results suggested that increased CD3\textsuperscript{+} and CD8\textsuperscript{+} T lymphocyte infiltrations are associated with improved survival status.\textsuperscript{46} The upregulation of
PD-1 and PD-L1 is believed to promote T-cell apoptosis in gastric adenocarcinoma. The association between T cell infiltration and the prognostic risk of gastric adenocarcinoma has been extensively introduced; however, the specific prognostic effects of T cells remain controversial, possibly because of the differences in identified T cell subtype markers and gastric cancer molecular subtypes among different studies.

At the end of this study, we predicted several inhibitors or binders based on prognostic risk genes as potential drugs for gastric adenocarcinoma treatment, and the molecular docking analysis showed that the drug ligand and the protein receptor bond stably in the form of hydrogen bonds. However, our understanding of the therapeutic potential of drugs for gastric adenocarcinoma is limited. In future studies, we will conduct animal experiments to explore whether the molecular mechanism of niacin and miconazole for gastric adenocarcinoma treatment is related to the expression of target genes.

5 | CONCLUSIONS

In summary, this study proposed an eight-gene signature related to drug metabolism as a potential biomarker to predict the prognostic risk of gastric adenocarcinoma patients. The predicted drugs (niacin and miconazole) can stably bind to target genes and have therapeutic potential for gastric adenocarcinoma patients. Additionally, we found a significant correlation between the tumor immune microenvironment and gastric adenocarcinoma prognosis. Our study helps to better understand the relationship between gastric adenocarcinoma prognosis, drug metabolism, and the immune microenvironment.

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Hongmei Yin and Qiong He conceived and designed the research, and Zhen Li and Jia Chen participated in the acquisition of data. Wanli Yang performed the analysis and interpretation of data. Jia Chen and Wanli Yang participated in the design of the study and performed the statistical analyses. Hongmei Yin, Qiong He, and Xiaobo Hu conceived the study, participated in its design and coordination, and helped draft the manuscript and revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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