

Original Article

Development and validation of a novel disulfidptosis-associated LncRNA model for risk stratification and targeted therapy guidance in gastric adenocarcinoma

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Abstract: Disulfidptosis, as a newly discovered form of cell programmed cell death in recent years, is driving the in-depth understanding of the tumor occurrence mechanism and the exploration of innovative therapeutic approaches. Although the regulatory role of long non-coding RNAs (lncRNAs) in cancer development is well-known, whether they are involved in the disulfidptosis process and their significance in the prognosis of gastric adenocarcinoma remain unclear. This study, using sequencing and clinical data from The Cancer Genome Atlas (TCGA) database, through least absolute shrinkage and selection operator (LASSO) and Cox regression analysis, has for the first time constructed a prognostic evaluation model containing eight disulfidptosis-related lncRNAs (DRlncRNAs). This model covers molecules such as LIMS1-AS1, LINC00460, and AL590681.1, and can clearly distinguish gastric adenocarcinoma patients into high-risk and low-risk groups based on the median risk score. The analysis showed that there were significant differences between the two groups of patients in terms of total survival, relevant signal pathway activity, tumor immune microenvironment characteristics, gene mutation load, drug sensitivity, etc. The prognosis of patients in the low-risk group is obviously better. Moreover, the study further verified through cell experiments, animal models and clinical samples that these 8 types of lncRNA are abnormally expressed in gastric adenocarcinoma, which directly affects the survival of cancer cells. This series of work not only provides a new independent biomarker combination for the prognosis of gastric adenocarcinoma, but also lays an important foundation for an in-depth understanding of the disulfidptosis regulation network and the development of targeted treatment strategies for this pathway.

Keywords: Stomach adenocarcinoma, disulfidptosis, lncRNAs, prognostic model

Introduction

Stomach cancer is the third leading cause of cancer-related deaths worldwide, and its high incidence and mortality put a heavy burden on the health care system and economic resources. More than 95% of gastric cancer cases are histologically classified as adenocarcinoma. According to Lauren's classification system, gastric adenocarcinoma is mainly divided into three subtypes: intestinal type, diffuse type and uncertain type. Diffuse gastric cancer is characterized by the lack of single infiltration cells attached to the wall, while intestinal gastric cancer is characterized by different

degrees of differentiated glandular structure [1, 2]. Early diagnosis is crucial to improve the prognosis of gastric adenocarcinoma patients. However, due to the similar early symptoms to other gastrointestinal diseases, approximately 50% of patients are diagnosed at an advanced stage [3]. Currently, early detection mainly relies on endoscopic examination and imaging assessment. Although CT and endoscopic ultrasound are often used for preoperative evaluation, studies have shown that racial differences may affect the diagnostic accuracy of endoscopic ultrasound [4]. Additionally, these limitations underscore the urgent need for more precise and non-invasive biomarkers for early

detection and prognosis. In recent years, non-coding RNAs (ncRNAs), such as long non-coding RNAs (lncRNAs), have emerged as promising candidates due to their high tissue specificity and regulatory roles in the tumor microenvironment, offering new avenues for precision diagnosis and targeted therapy [5].

A recently discovered phenomenon, disulfidptosis, represents a novel metabolic-related cell death mechanism [6]. Its characteristic is the abnormal accumulation of disulfide bonds in cells with high expression of SLC7A11 under conditions of glucose deprivation. This cell death induced by disulfide bond stress is manifested through the disintegration of the cytoskeleton [7]. lncRNAs, as non-coding RNA transcripts with a length exceeding 200 nucleotides, regulate cellular processes and transcriptional networks by interacting with proteins [8]. Existing studies have confirmed that lncRNAs related to disulfidptosis are associated with the occurrence and development of bladder cancer and lung adenocarcinoma, suggesting that there may be a mechanistic connection between disulfidptosis and malignant tumors [9, 10].

This study will be based on The Cancer Genome Atlas (TCGA)-Stomach Adenocarcinoma (STAD) cohort, using least absolute shrinkage and selection operator (LASSO) regression and multivariate Cox models to construct prognostic features of disulfidptosis-related lncRNAs (DRlncRNAs). It will systematically analyze the expression characteristics, prognostic significance, and possible functional mechanisms of these disulfide reduction-related lncRNAs in gastric adenocarcinoma. By integrating transcriptome, immune infiltration, and drug sensitivity data, we will explore the interaction between DRlncRNAs and the tumor metabolic-immune microenvironment, and verify their regulatory effects on disulfide reduction sensitivity through in vitro experiments. At the same time, we will evaluate the relationship between circulating DRlncRNA levels and clinical pathological features such as differentiation degree, lymph node metastasis, and distant metastasis, providing new biomarkers and theoretical basis for risk stratification and individualized treatment of gastric adenocarcinoma.

Method

Data collection

TCGA supplied RNA-seq reads, clinical sheets, and mutation calls (accessed via <https://cancergenome.nih.gov> and <https://portal.gdc.cancer.gov>). Perl 5.30.0 pulled the raw count tables; R log-transformed and scaled them. Initially, 412 STAD tumours were retrieved. After excluding 5 cases with incomplete clinical follow-up data (missing survival time or vital status) in STAD, 407 tumour samples were retained for prognostic analysis and randomly divided into training (n=204) and test (n=203) cohorts (**Table 1**). Based on the 10 core DRGs identified by Liu et al. [6] through genome-wide CRISPR-Cas9 screening, including SLC7A11 (cystine transporter), SLC3A2 (SLC7A11 chaperone), RPN1 and NCKAP1 (cytoskeleton regulators), mitochondrial oxidative phosphorylation genes (NUBPL, NDUFA11, LRPPRC, OXSM, NDUFS1), and GYS1 (glycogen synthase), we screened for lncRNAs significantly co-expressed with these DRGs in the TCGA-STAD cohort.

Sankey diagram construction

The Sankey diagram was constructed to visualize the co-expression relationships between the 10 core DRGs and prognostic DRlncRNAs. Pearson correlation analysis was performed between each DRG and all lncRNAs in the TCGA-STAD cohort. lncRNAs with $|r| > 0.3$ and $P < 0.05$ were considered significantly co-expressed with DRGs. The width of the lines in the Sankey diagram represents the correlation strength ($|r|$ value), and the color gradient indicates positive (red) or negative (blue) correlations.

Construction of a disulfidptosis-related prognostic signature for STAD

To develop a prognostic model, we employed LASSO regression along with univariate and multivariate Cox regression analyses to systematically examine the association between DRlncRNA expression and survival outcomes in STAD patients. Based on the resulting regression coefficients and prognostic significance, an eight-lncRNA signature was ultimately established.

Disulfidptosis-related lncRNA signature in STAD

Table 1. The clinical characteristics of patients in the TCGA database [n (%)]

Characteristic	Type	Total	Test	Train	P value
n		407	203	204	
Age	≤ 65	183 (44.96)	97 (47.78)	86 (42.16)	0.276
	> 65	221 (54.3)	104 (51.23)	117 (57.35)	
	unknow	3 (0.74)	2 (0.99)	1 (0.49)	
Gender	FEMALE	144 (35.38)	73 (35.96)	71 (34.8)	0.889
	MALE	263 (64.62)	130 (64.04)	133 (65.2)	
Grade	G1	12 (2.95)	6 (2.96)	6 (2.94)	0.575
	G2	144 (35.38)	77 (37.93)	67 (32.84)	
	G3	242 (59.46)	116 (57.14)	126 (61.76)	
	unknow	9 (2.21)	4 (1.97)	5 (2.45)	
Stage	Stage I	55 (13.51)	23 (11.33)	32 (15.69)	0.313
	Stage II	122 (29.98)	55 (27.09)	67 (32.84)	
	Stage III	167 (41.03)	90 (44.33)	77 (37.75)	
	Stage IV	39 (9.58)	20 (9.85)	19 (9.31)	
	unknow	24 (5.9)	15 (7.39)	9 (4.41)	
T	T1	21 (5.16)	7 (3.45)	14 (6.86)	0.428
	T2	86 (21.13)	46 (22.66)	40 (19.61)	
	T3	179 (43.98)	89 (43.84)	90 (44.12)	
	T4	113 (27.76)	55 (27.09)	58 (28.43)	
	unknow	8 (1.97)	6 (2.96)	2 (0.98)	
M	M0	362 (88.94)	182 (89.66)	180 (88.24)	0.561
	M1	26 (6.39)	11 (5.42)	15 (7.35)	
	unknow	19 (4.67)	10 (4.93)	9 (4.41)	
N	N0	121 (29.73)	50 (24.63)	71 (34.80)	0.248
	N1	108 (26.54)	57 (28.08)	51 (25.00)	
	N2	77 (18.92)	40 (19.70)	37 (18.14)	
	N3	82 (20.15)	43 (21.18)	39 (19.12)	
	unknow	19 (4.67)	13 (6.40)	6 (2.94)	

Evaluation of prognostic features of 8-DRlncRNAs

Cases were split at random into training and test arms, tagged high- or low-risk by the median risk score, and Kaplan-Meier curves were drawn. Receiver operating characteristic (ROC) areas for 1-, 3- and 5-year survival were computed. PCA checked whether the risk signature kept its shape, and a handful of R packages were run to confirm it stayed upright under different assumptions.

Analysis of the relationship between risk scores and clinical characteristics

Cox models (univariate and multivariate) tested whether the risk score keeps its punch when age, stage, and grade are already in the equa-

tion. The coefficients in the multivariate table provide a line chart, which can read the survival opportunities of individuals; the calibration curve checks whether the probability of these prints is consistent with the real follow-up data.

Functional pathway enrichment and immune microenvironment assessment

Genes differing between risk arms were flagged at $|FC| > 1$ and $FDR < 0.05$, then fed into GO, KEGG and immune deconvolution to pick out the pathways and microenvironment gaps separating high- from low-risk tumours. Functional interpretation is carried out through KEGG pathway and gene ontology enrichment analysis. According to the established GSEA scheme, the c2.cp.kegg.v7.4.symbols.gmt reference set is used to evaluate the immune infil-

tration pattern through gene set enrichment analysis (GSEA). The CIBERSORT algorithm quantifies the composition of immune cells in tumors. Further comparison based on GSEA revealed the differences in immune response between risk groups, supplemented by the differential expression spectrum of immune-related.

Estimation of tumor mutation burden

The “maftools” R package was used to obtain files from TCGA, and the top 15 genes with the highest mutation frequencies were visualized. The Tumor Immune Dysfunction and Exclusion (TIDE) score was employed to predict immune responses between high- and low-risk groups.

Assessment of drug sensitivity

OncoPredict - together with car, preprocess-Core, genefilter, and sva-estimated IC₅₀ values for standard agents; ggplot2 boxed the high- and low-risk samples for easy comparison.

In vitro validation of key genes

Candidate drug sensitivity assay: Two bioinformatically prioritized compounds - IGF-1R inhibitor BMS-754807 (Selleck Chemicals, Houston, TX, USA, Cat# S2754) and BET inhibitor JQ1 (Selleck Chemicals, Houston, TX, USA, Cat# S7110) - were evaluated for selective cytotoxicity toward high-risk DRlncRNA signatures. AGS cells (American Type Culture Collection, Manassas, VA, USA) stably over-expressing each of the eight DRlncRNAs or empty vector (NC) were seeded at 3×10^3 cells per well in 96-well plates. After attachment, the medium was replaced with glucose-free DMEM (Gibco, Grand Island, NY, USA, Cat# 11966025) supplemented with 10% FBS (Gibco, Grand Island, NY, USA, Cat# 10099141) and serial dilutions of BMS-754807 (0-10 μ M) or JQ1 (0-2 μ M); final DMSO (Sigma-Aldrich, St. Louis, MO, USA, Cat# D2650) $\leq 0.1\%$. Following 72 h continuous exposure, 20 μ L of 5 mg mL⁻¹ MTT (Sigma-Aldrich, St. Louis, MO, USA, Cat# M5655) was added for 4 h, formazan was solubilized with 150 μ L DMSO, and absorbance was read at 570 nm (650 nm background correction) using a microplate reader (BioTek Instruments, Winooski, VT, USA). Viability was expressed as percentage of vehicle-treated controls and IC₅₀ values were calculated by non-linear regres-

sion (GraphPad Prism 9). Each concentration was tested in triplicate and experiments were repeated three times.

DRlncRNA modulation of disulfidptosis sensitivity: AGS cells were transfected to over-express or knock down representative high-risk (LIMS1-AS1) or low-risk (PINK1-AS) DRlncRNAs using Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA, Cat# L3000015); empty vector or scrambled siRNA (RiboBio, Guangzhou, China, Cat# siN05815-122147) served as negative controls (NC). After 48 h, cells were switched to either standard (25 mM glucose) or glucose-free medium. After 24 h of continued deprivation, cellular NADP⁺ and NADPH were extracted using a double-extraction NADP⁺/NADPH assay kit (Abcam, Cambridge, UK, Cat# ab176724) and the NADP⁺/NADPH ratio was determined colorimetrically to reflect redox buffering capacity.

SLC7A11-dependent ¹⁴C-cystine uptake assay: AGS cells stably expressing each DRlncRNA or NC were plated at 1×10^5 per well in 24-well plates. After 6 h in glucose-free DMEM/10% FBS/2 mM glutamine to boost SLC7A11, cells were rinsed twice with KRPH (pH 7.4) and starved 30 min in amino-acid-free KRPH. Uptake started with 100 μ M cystine plus 0.1 μ Ci mL⁻¹ L-[¹⁴C]cystine (PerkinElmer, Boston, MA, USA, Cat# NEC244010UC); 10 min later the reaction was killed with three ice-cold PBS washes. Cells were solubilised in 0.1 M NaOH, 150 μ L lysate mixed with scintillation fluid, and counts read on a β -counter (PerkinElmer, Waltham, MA, USA, Tri-Carb 4910TR). Results (pmol mg⁻¹ protein per 10 min) were normalised to NC and averaged across three independent runs, each in triplicate.

Phenotypic analysis following DRlncRNA modulation

Total RNA was isolated from the human gastric epithelial line GES-1 and the gastric cancer line AGS using TRIzol reagent (Invitrogen, Carlsbad, CA, USA, Cat# 15596026), reverse transcribed using PrimeScript RT Master Mix (Takara Bio, Shiga, Japan, Cat# RR036A), and subjected to quantitative real-time PCR using TB Green Premix Ex Taq II (Takara Bio, Shiga, Japan, Cat# RR820A) on a CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). Relative DRlncRNA expression was calculated using the 2^{- $\Delta\Delta$ Ct}

method. For proliferation analysis, AGS cells transfected to overexpress or silence individual DRlncRNAs were plated in 96-well plates at 1×10^3 cells per well. Cell viability was measured daily over 5 consecutive days using the MTT assay. Growth curves were generated to evaluate the effect of DRlncRNA alteration on cellular proliferation.

In vivo validation of DRlncRNAs

Animals and ethics: Male Wistar rats (4-6 weeks old, 45-55 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China; License No. SCXK (Beijing) 2021-0006). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of First People's Hospital of Yunnan Province (Approval No.: SL202203001), in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals (NIH).

Euthanasia method: At the end of the experimental period (12 months), rats were euthanized by intraperitoneal injection of sodium pentobarbital (150 mg/kg) followed by exsanguination via cardiac puncture. Death was confirmed by cessation of heartbeat and respiration. Stomach tissues were immediately excised, rinsed with sterile PBS, snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction.

Construction of a gastric adenocarcinoma rat model: 4- to 6-week-old male Wistar rats (45-55 g, Charles River Laboratories, Beijing, China) were split 10 per group. The test group drank water laced with 80 mg L^{-1} N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, Sigma-Aldrich, St. Louis, MO, USA, Cat# N5255) for 9 months, then plain water until month 12; controls drank tap water throughout. At the end of the 12-month experimental period, all rats were humanely euthanized by intraperitoneal injection of sodium pentobarbital at a dose of 150 mg/kg body weight (Sigma-Aldrich, St. Louis, MO, USA, Cat# P3761). Following confirmation of deep anesthesia (absence of pedal withdrawal reflex), exsanguination was performed via cardiac puncture as a secondary method to ensure death. Death was confirmed by complete cessation of heartbeat and respiration for a minimum of 5 minutes. Stomach tissues were immediately excised, rinsed with sterile

phosphate-buffered saline (PBS), snap-frozen in liquid nitrogen, and stored at -80°C until RNA extraction. All procedures were conducted in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020 Edition).

qRT-PCR for DRlncRNA expression in animal tissues: At sacrifice, stomachs were removed, and total RNA was extracted from gastric tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA, Cat# 15596026). RNA quality and concentration were assessed using a Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). After reverse transcription using PrimeScript RT Master Mix (Takara Bio, Shiga, Japan, Cat# RR036A), DRlncRNA levels were quantified by qRT-PCR using TB Green Premix Ex Taq II (Takara Bio, Shiga, Japan, Cat# RR820A) on a CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). Relative expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method with GAPDH as the internal control.

Clinical validation of DRlncRNAs

Clinical samples and ethics: This study was approved by the Ethics Committee of the First People's Hospital of Yunnan Province (Approval No.: KHLL2025-KY345). Serum samples were collected from 40 patients with gastric adenocarcinoma and 40 age-matched healthy volunteers.

qRT-PCR for DRlncRNA expression in human serum: This study was conducted with the approval of the Ethics Committee of the First People's Hospital of Yunnan Province (Approval No.: KHLL2025-KY345). Serum samples were obtained from 40 gastric adenocarcinoma patients and 40 age-matched healthy volunteers. The gastric cancer cohort consisted of 22 males and 18 females, while the control group included 25 males and 15 females. Inclusion criteria required patients to have complete clinical documentation, diagnosis consistent with gastric adenocarcinoma, no prior history of radiotherapy or chemotherapy, and the ability to provide informed consent. Patients who experienced perioperative mortality or were unable to undergo surgical treatment were excluded. Total RNA was isolated using a commercial blood RNA extraction kit (Thermo Fisher Scientific, Waltham, MA, USA, Cat# 12183018A) and quantified using a

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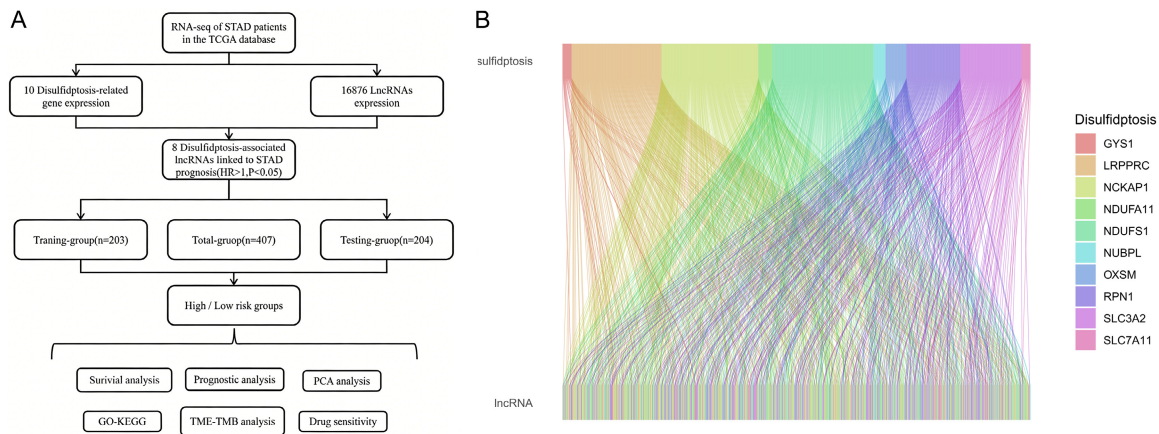


Figure 1. Study workflow and co-expression analysis of DRGs and DRlncRNAs. A: Schematic overview of the study design, including data collection, model construction, multi-omics analysis, and experimental validation. B: Sankey diagram showing co-expression relationships between 10 DRGs and prognostic lncRNAs. Line width represents Pearson correlation coefficient strength ($|r| > 0.3, P < 0.05$); red lines indicate positive correlation, blue lines indicate negative correlation. DRG, disulfidptosis-related gene; lncRNA, long non-coding RNA.

NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Reverse transcription was performed with a cDNA synthesis kit (Takara Bio, Shiga, Japan, Cat# RR036A), followed by real-time quantitative PCR analysis using TB Green Premix Ex Taq II (Takara Bio, Shiga, Japan, Cat# RR820A) on a CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). U6 was used as the internal control for normalization.

Correlation analysis between clinical features and serum mRNA expression: Patients with gastric adenocarcinoma were stratified into stages I-IV according to the TNM classification of the American Joint Committee on Cancer (AJCC, 8th edition). The association between AJCC stage and serum mRNA abundance was first evaluated. We next investigated whether serum DRlncRNA levels reflected biological aggressiveness independent of stage. Post-operative histology was independently reviewed by two pathologists. According to WHO standards, tumor differentiation is divided into medium differentiation (G1), medium differentiation (G2) or low differentiation (G3). The node status is recorded as N0, N1, N2 or N3, and further divided into N0 and N1-N3 for statistical analysis. According to the results of enhanced CT/MRI and intraoperative examination, the distant transfer is divided into M0 or M1. In general, these clinical pathological variables are related to the serum mRNA expression system to clarify their potential role in the progression of gastric adenocarcinoma.

Statistical analysis

R software (version 4.2.1; <https://www.r-project.org>) and Perl (version 5.30.0; <https://strawberryperl.com>) were used for Pearson correlation, Cox regression and Kaplan-Meier survival analysis. SPSS 26.0 and GraphPad Prism 9.5 were used for statistical analysis and graphing. Continuous variables are expressed as mean $\pm$ standard deviation (SD). Comparisons between two groups were performed using Student's t-test or Wilcoxon rank-sum test, as appropriate. For comparisons among multiple groups, one-way ANOVA followed by Tukey's post-hoc test was used. For repeated-measures data, two-way repeated measures ANOVA with Bonferroni correction was applied. $P < 0.05$ indicates statistical significance.

Results

Clinical data

In this study, a total of 407 STAD tumor samples with complete clinical follow-up data were analyzed. The 407 tumor samples were randomly divided into training cohorts ($n=204$) and test cohorts ($n=203$) (Figure 1A). There was no significant difference in the clinical or pathological characteristics of the two groups ($P > 0.05$). From the TCGA database, we identified 16,876 lncRNAs, of which 1,078 were significantly co-expressed with the 10 core DRGs (SLC7A11, SLC3A2, RPN1, NCKAP1, NUBPL, NDUFA11, LRPPRC, OXSM, NDUFS1, and GYS1) defined by Liu et al. [6]. The Sankey diagram

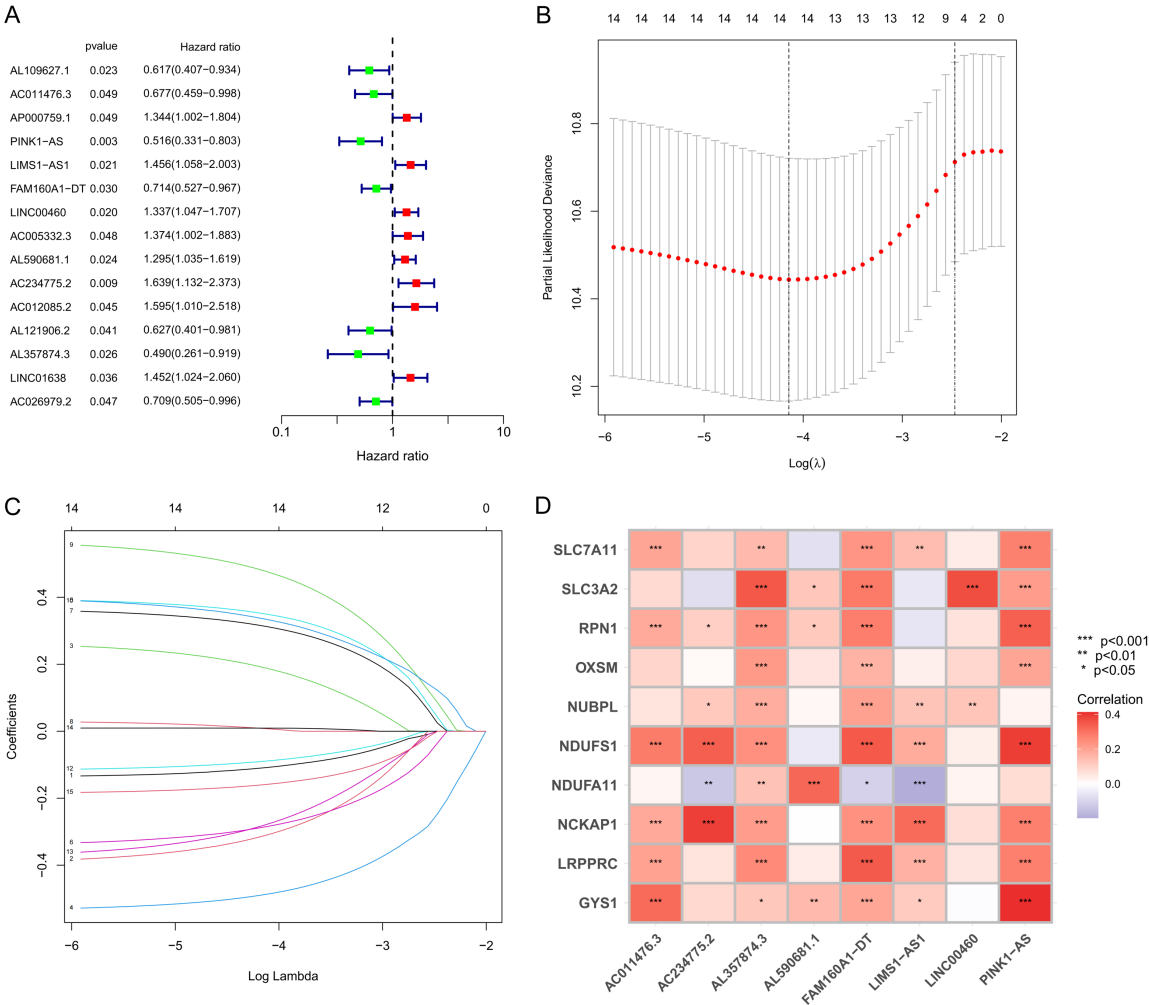


Figure 2. Identification of the disulfidptosis-related lncRNA signature. A: Forest plot showing univariate Cox regression analysis results for identifying prognostic DRlncRNAs. HR with 95% confidence intervals and P values are displayed. B, C: LASSO-Cox regression analysis for model construction. B: Partial likelihood deviance versus log(λ) for selecting the optimal penalty parameter. C: Coefficient profiles of the lncRNAs as a function of log(λ). D: Heatmap showing Pearson correlation coefficients between the eight signature lncRNAs and ten DRGs. Red indicates positive correlation, blue indicates negative correlation. HR, Hazard ratios; DRG, disulfidptosis-related gene; lncRNA, long non-coding RNA.

(Figure 1B) illustrates the co-expression relationships between the 10 core DRGs and the prognostic lncRNAs identified. Wider lines indicate stronger correlations ($|r| > 0.3$, $P < 0.05$), with red representing positive correlation and blue representing negative correlation.

A DRlncRNA prognostic marker for STAD: development and validation

Univariate Cox regression analysis initially identified 15 prognostic DRlncRNAs significantly associated with overall survival ($P < 0.05$) (Figure 2A). Among these, 8 lncRNAs showed risk

ratios greater than 1, indicating that they may be potential risk factors: AP000759.1, LIMS1-AS1, LINC00460, AC005332.3, AL590681.1, AC234775.2, A C012085.2 and LINC01638. On the contrary, 7 lncRNAs (AL109627.1, AC011476.3, PINK1-AS, FAM160A1-DT, AL12-1906.2, AL357874.3 and AC026979.2) are related to protective effects. Finally, eight key lncRNAs are selected as variables and a prediction model is established using LASSO-Cox regression (Figure 2B, 2C). The corresponding heat map provides an intuitive summary of the expression correlation between these 8 lncRNAs and 10 key disulfur bond-related

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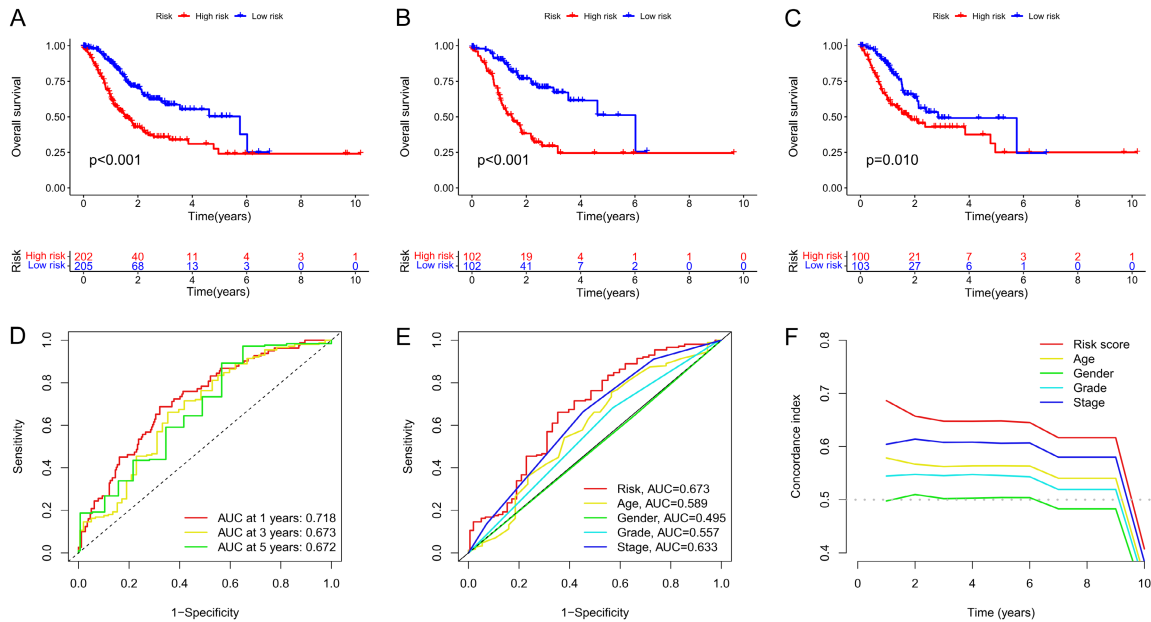


Figure 3. Validation of the prognostic signature. (A-C) Kaplan-Meier survival curves for OS comparing high-risk and low-risk groups in the entire cohort (A, n=407), training cohort (B, n=204), and testing cohort (C, n=203). *P* values were calculated using the log-rank test. (D) Time-dependent ROC curves showing the predictive accuracy of the signature for 1-year, 3-year, and 5-year OS. AUC, area under the curve. (E) ROC curves comparing the predictive performance of the risk model with other clinical parameters for 5-year OS. (F) C-index curves showing the time-dependent predictive performance of the risk model and clinical parameters. OS, overall survival; AUC, area under the curve; ROC, receiver operating characteristic.

genes. The heatmap in **Figure 2D** illustrates the expression correlation between the eight prognostic lncRNAs and the ten core DRGs, revealing distinct co-expression patterns between risk-associated and protective lncRNAs.

Patients are divided into high-risk subgroups and low-risk subgroups according to the median risk score. Kaplan-Meier survival analysis reveals the consistent trend of the whole cohort (**Figure 3A**), training set (**Figure 3B**) and test set (**Figure 3C**): the overall survival of the high-risk group is significantly shortened. We gauged how well the signature forecasts outcome by running ROC analysis; the curve areas came in at 0.72, 0.67, and 0.67 for 1-, 3-, and 5-year survival, respectively (**Figure 3D**). While gender did not significantly influence risk classification (AUC=0.495), variables including age (AUC=0.589), tumor grade (AUC=0.557), and clinical stage (AUC=0.633) showed clear discriminatory capacity (**Figure 3E**). Time-dependent concordance indices further indicated that the prognostic impact of age, grade, and stage strengthened over time (**Figure 3F**). Risk scores and the matching survival snapshot are

plotted for the whole group, the training fraction, and the hold-out fraction (**Figure 4A-F**). Additionally, expression heatmaps of the eight disulfidptosis-associated lncRNAs were generated across these three groups (**Figure 4G-I**).

Independent prognostic marker for DRlncRNAs in predicting clinical outcomes

Univariate and multivariate Cox runs were carried out to see whether the DRlncRNA score adds anything to STAD prognosis. Age, stage, and the risk score itself survived both rounds (**Figure 5A, 5B**). Kaplan-Meier lines pulled apart early and stayed separate, giving a progression-free gap with a *P*-value of 0.009 (**Figure 5C**); the upper-risk band consistently did worse. PCA was performed to evaluate the distribution patterns of patient samples. While PCA based on all expressed genes showed no distinct separation between high- and low-risk groups, PCA using the eight risk lncRNAs revealed clear clustering that effectively distinguished the two risk groups (**Figure 5D, 5E**). Furthermore, PCA based on the 10 DRGs (**Figure 5F**) and 15 initial DRlncRNAs (**Figure 5G**) also showed differential clustering pat-

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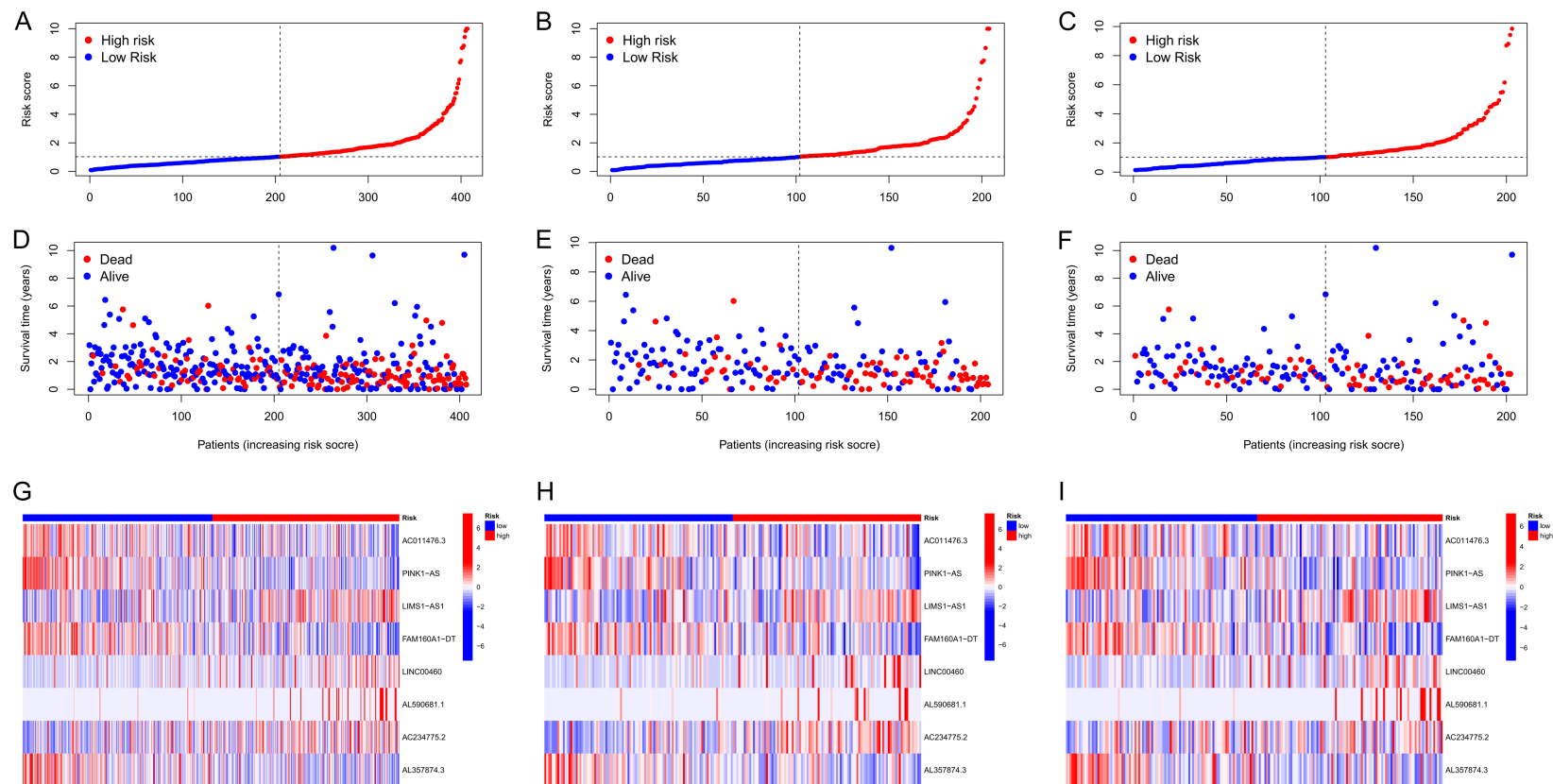


Figure 4. Risk score distribution and lncRNA expression profiles. (A-C) Risk score distribution in the entire cohort (A), training cohort (B), and testing cohort (C). The dashed line indicates the median risk score cutoff. (D-F) Survival status of patients in the three cohorts. Red dots indicate deceased patients, green dots indicate alive patients. (G-I) Heatmaps showing expression levels of the eight DRlncRNAs in the entire cohort (G), training cohort (H), and testing cohort (I). Red represents high expression, blue represents low expression. DRlncRNA, disulfidptosis-related long non-coding RNA.

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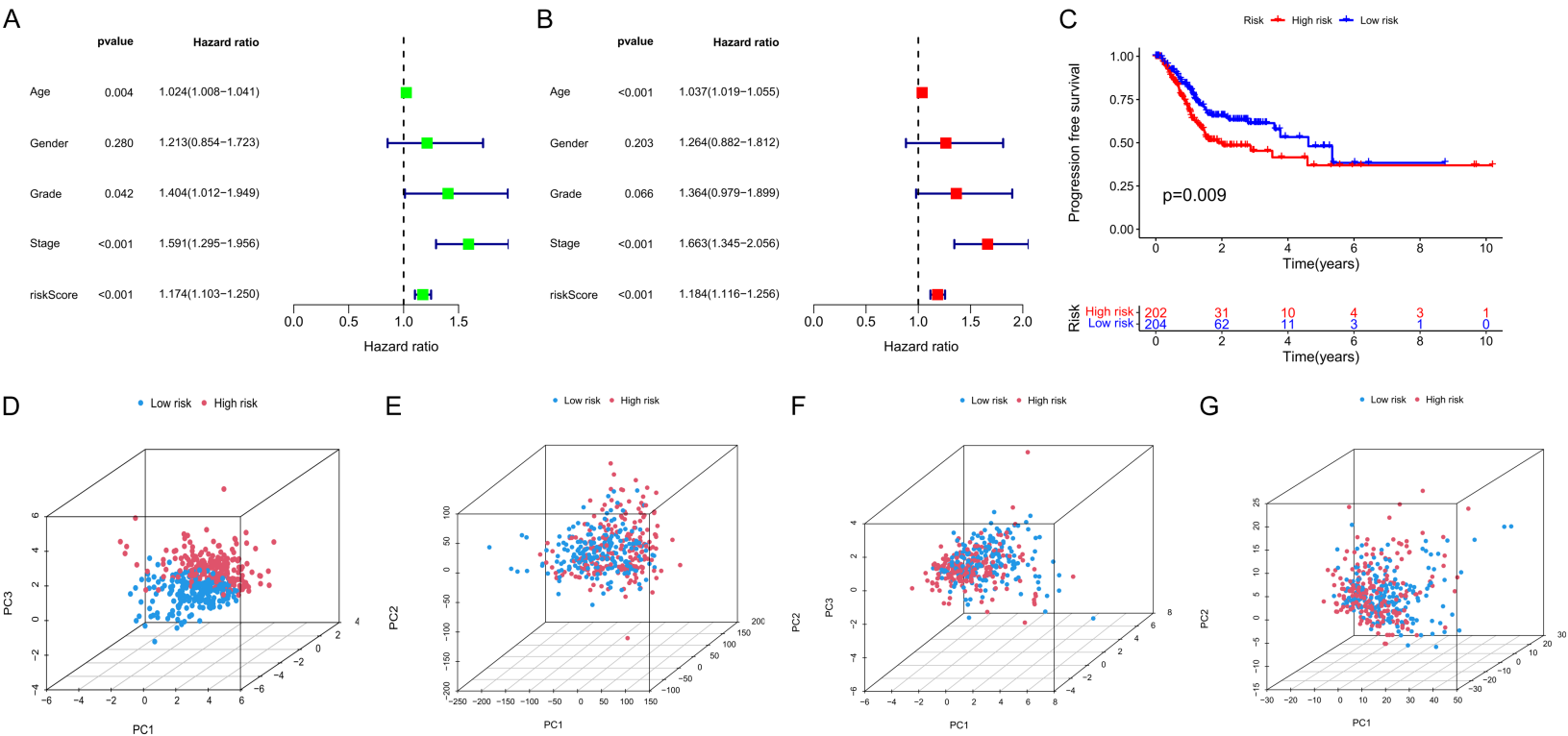


Figure 5. Independent prognostic value of the risk signature. (A, B) Forest plots showing univariate (A) and multivariate (B) Cox regression analyses for OS. HR with 95% confidence intervals and *P* values are displayed. (C) Kaplan-Meier curves for PFS comparing high-risk and low-risk groups. *P* value was calculated using the log-rank test. (D-G) PCA plots showing patient distribution based on the eight signature lncRNAs (D), all expressed genes (E), ten DRGs (F), and fifteen DRlncRNAs (G). Each dot represents a patient; red indicates high-risk, green indicates low-risk. OS, overall survival; HR, Hazard ratios; PFS, progression-free survival; DRG, disulfidptosis-related gene; PCA, Principal component analysis; DRlncRNA, disulfidptosis-related long non-coding RNA.

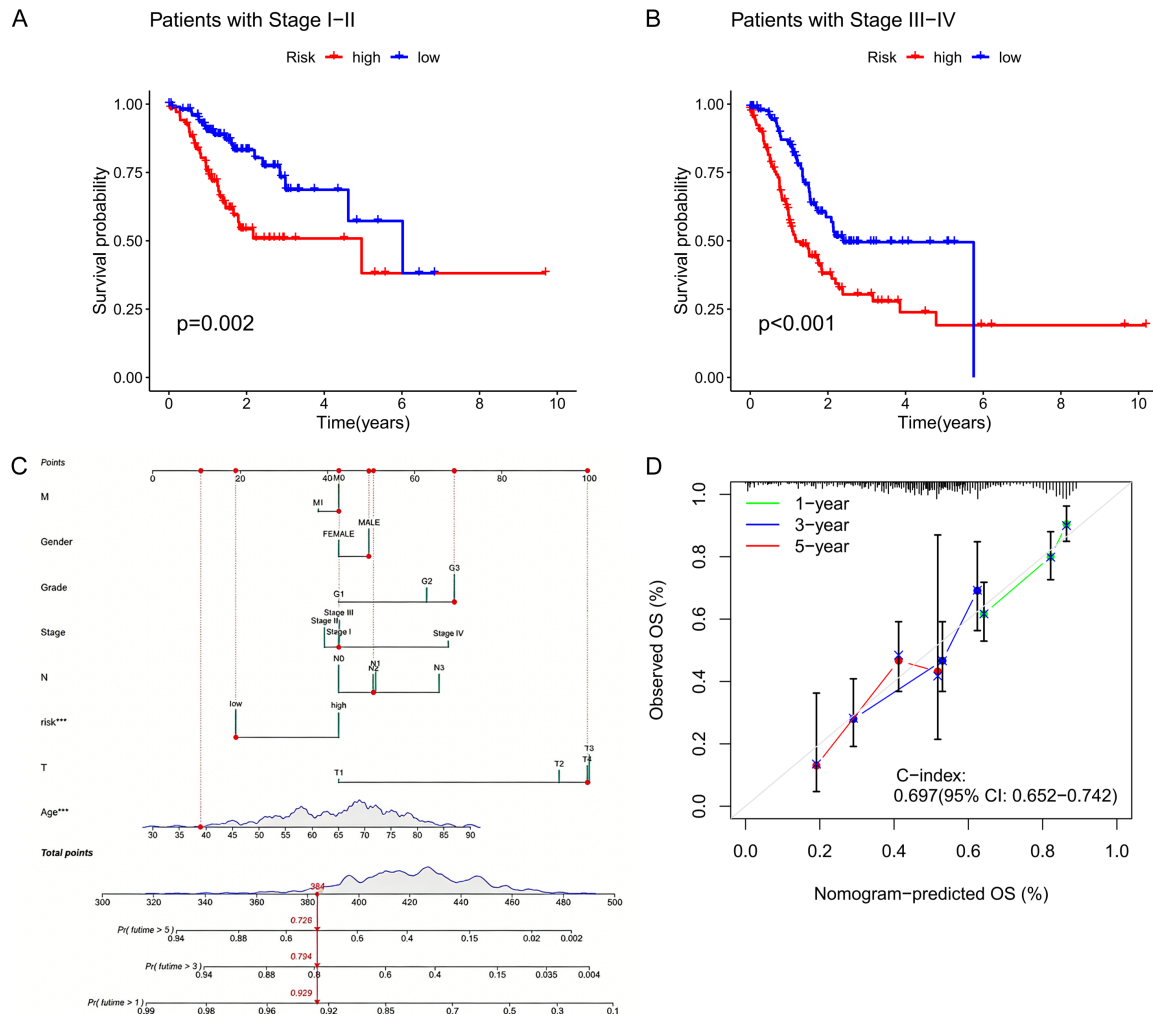


Figure 6. Subgroup analysis and nomogram. (A, B) Kaplan-Meier survival curves for overall survival (OS) comparing high-risk and low-risk patients in stage I-II (A) and stage III-IV (B) subgroups. *P* values were calculated using the log-rank test. (C) Nomogram integrating the eight DRlncRNAs for predicting 1-year, 3-year, and 5-year OS. Points are assigned to each variable based on their contribution, and total points correspond to predicted survival probabilities. (D) Calibration curves showing the agreement between predicted and observed survival probabilities at 1 year, 3 years, and 5 years. The diagonal dashed line represents perfect prediction. OS, overall survival; DRlncRNA, disulfidptosis-related long non-coding RNA.

terns. The subgroup analysis according to clinical staging further confirms the robustness of the risk model. Compared with the low-risk group, the survival results of patients in the high-risk group were always poor, whether in stage I-II (Figure 6A) or stage III-IV subgroup (Figure 6B). A nomogram integrating the eight DRlncRNA variables was constructed to facilitate individualized survival prediction (Figure 6C). Calibration curves indicated strong concordance between predicted and observed survival probabilities (Figure 6D), supporting the model's reliability in prognostic assessment for STAD patients.

Functional enrichment analysis

GO enrichment pinned the shifted genes to muscle mechanics and contraction. At the cellular level they clustered around extracellular matrix shelves and the secretory-granule lumen, while their molecular jobs centred on ligand-receptor hook-ups and agonist activity (Figure 7A-D). KEGG added neuroactive ligand-receptor chatter, cAMP and calcium signalling to the list. Splitting the cohort by risk gave different colour on the pathway map: the high-risk arm leaned toward complement/coagulation cascades and both dilated and hypertro-

Disulfidptosis-related lncRNA signature in STAD

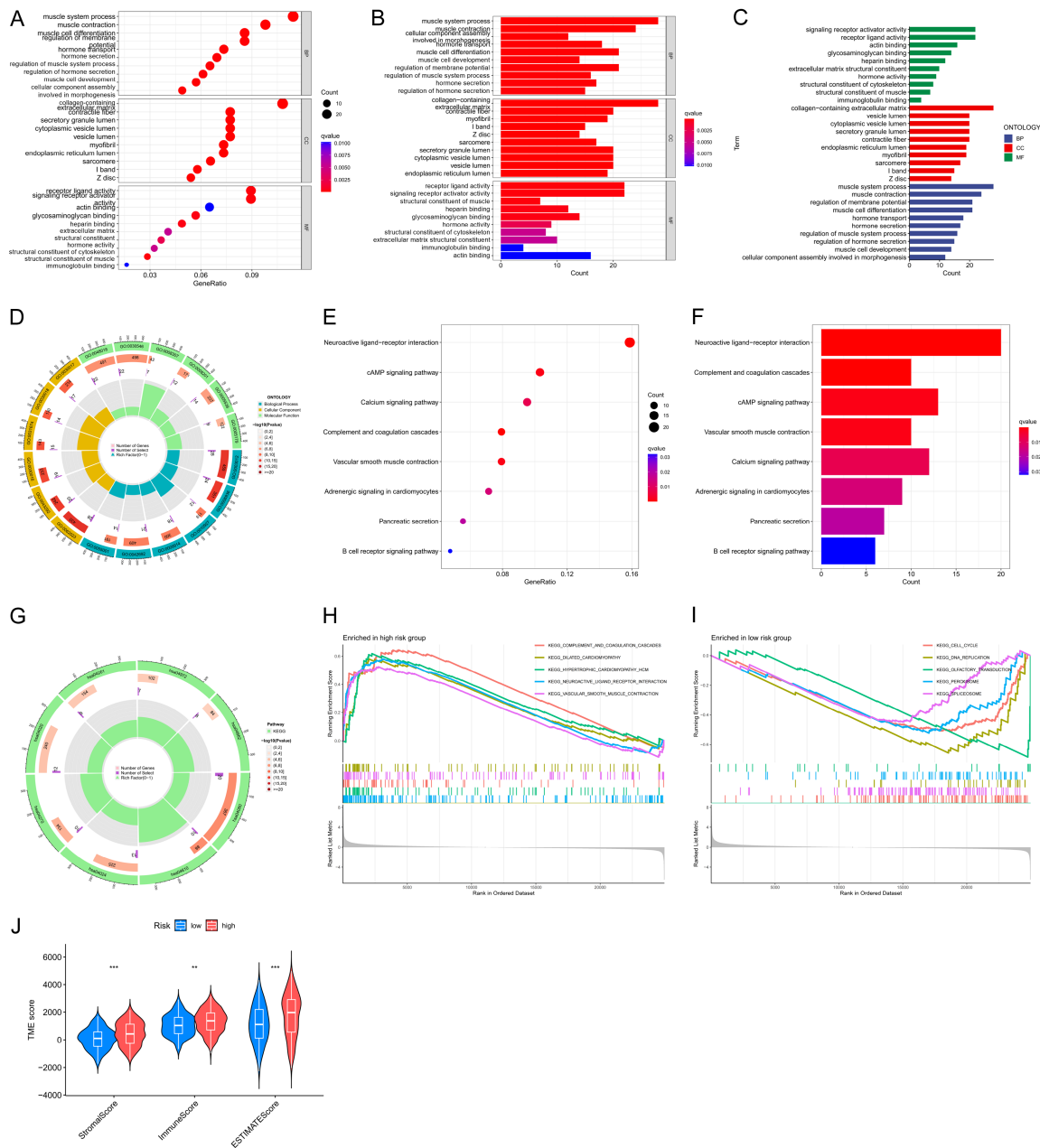


Figure 7. Functional enrichment analysis. (A-D) GO enrichment analysis of differentially expressed genes between high-risk and low-risk groups. (A) BP, (B) CC, (C) MF. (D) Bubble plot showing the top enriched GO terms with corresponding gene ratios and adjusted *P* values. (E-G) KEGG pathway enrichment analysis. (E) Bar plot showing top enriched pathways with gene counts and adjusted *P* values. (F, G) Dot plots showing enriched pathways in high-risk (F) and low-risk (G) groups. (H, I) GSEA showing significantly enriched pathways in high-risk (H) and low-risk (I) groups. NES and FDR are indicated. (J) Comparison of StromalScore, ImmuneScore, and ESTIMATEScore between high-risk and low-risk groups. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene Set Enrichment Analysis; NES, normalized enrichment score; FDR, false discovery rate.

phic cardiomyopathy, whereas the low-risk arm tilted toward cell-cycle control, DNA replication and olfactory transduction (**Figure 7E-G**). GSEA further validated these findings, revealing that high-risk patients showed significant enrich-

ment in complement and coagulation cascades, as well as dilated and hypertrophic cardiomyopathy pathways (**Figure 7H**), whereas low-risk patients exhibited distinct enrichment in cell cycle regulation, DNA replication, and

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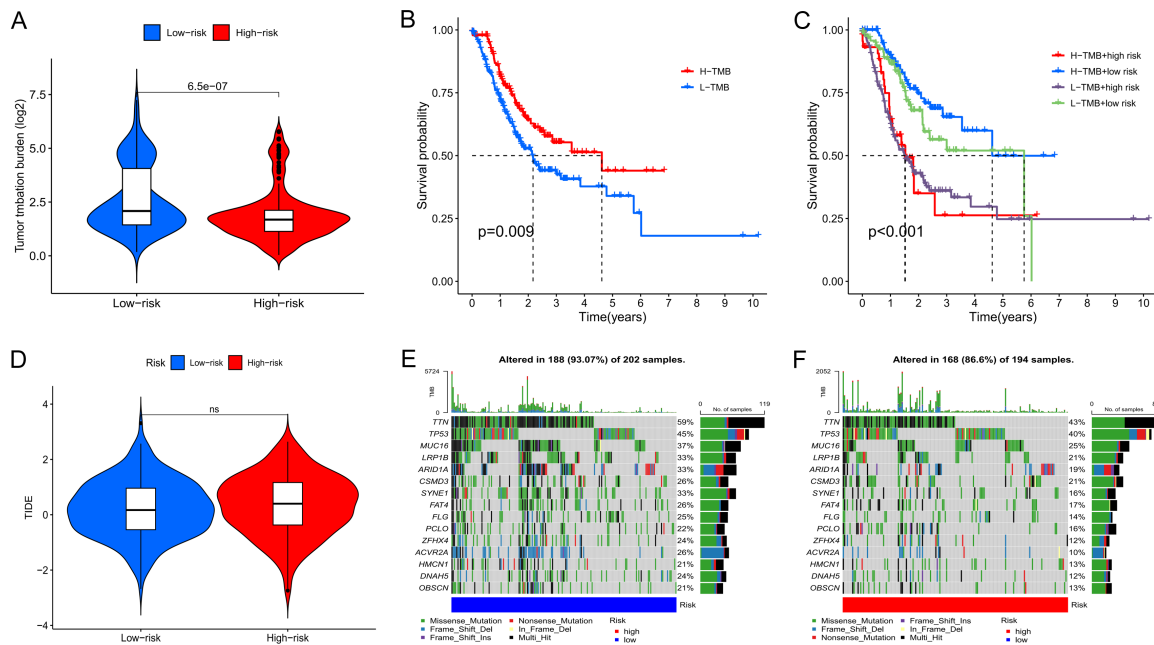


Figure 9. TMB analysis. (A) Box plot comparing TMB levels between high-risk and low-risk groups. (B) Kaplan-Meier survival curves for OS comparing patients with high TMB and low TMB. The median TMB was used as the cutoff. (C) Kaplan-Meier survival curves for patients stratified by both risk group and TMB level. Patients were divided into four subgroups: high-risk/high-TMB, high-risk/low-TMB, low-risk/high-TMB, and low-risk/low-TMB. (D) Box plot comparing TIDE scores between high-risk and low-risk groups. (E, F) Waterfall plots showing the top 15 mutated genes in high-risk (E) and low-risk (F) groups. Different colors represent different mutation types. TMB, tumor mutation burden; OS, overall survival; TIDE, Tumor Immune Dysfunction and Exclusion.

Drug-susceptibility validation

MTT assays showed that AGS cells over-expressing high-risk lncRNAs (LIMS1-AS1, LINC00460, AL590681.1 and AC234775.2) exhibited a sharp increase in sensitivity to both BMS-754807 and JQ1, reflected by a marked reduction in IC_{50} values relative to NC controls. Conversely, forced expression of protective lncRNAs (AC011476.3, PINK1-AS, FAM160A1-DT and AL357874.3) conferred clear drug resistance, with IC_{50} levels significantly elevated (Figure 11A, 11B).

DRlncRNAs control disulfidptosis sensitivity via NADPH

Under high-glucose (25 mM) conditions, the $NADP^+/NADPH$ ratio remained uniform across all groups, showing no significant deviation from the empty-vector control, indicating that metabolic redox homeostasis is insensitive to DRlncRNA expression in nutrient-rich settings. Upon glucose deprivation, the disulfidptosis pathway was activated and the regulatory ca-

capacity of key DRlncRNAs became immediately apparent. The overexpression of the high-risk factor LIMS1-AS1 significantly increased the $NADP^+/NADPH$ ratio, and its knock significantly inhibited the ratio; LINC00460, AL590681.1 and AC234775.2 observed the same directional trend, Confirm that the dangerous lncRNA cooperates to amplify NADPH consumption. On the contrary, the overexpression of the protective factor PINK1-AS significantly reduces the $NADP^+/NADPH$ ratio under hunger stress, and its knockdown leads to a significant increase; FAM160A1-DT and AL357874.3 show the same pattern (Figure 12A-H).

Cystine-influx functional assay

Cystine-uptake assays revealed that, under glucose-deprived conditions, overexpression of high-risk DRlncRNAs (LIMS1-AS1, LINC00460, AL590681.1 and AC234775.2) significantly enhanced L-[^{14}C]-cystine influx, whereas their knockdown reduced uptake to approximately 60% of the baseline level. Conversely, overexpression of protective DRlncRNAs (AC01-1476.3, PINK1-AS, FAM160A1-DT and AL35-

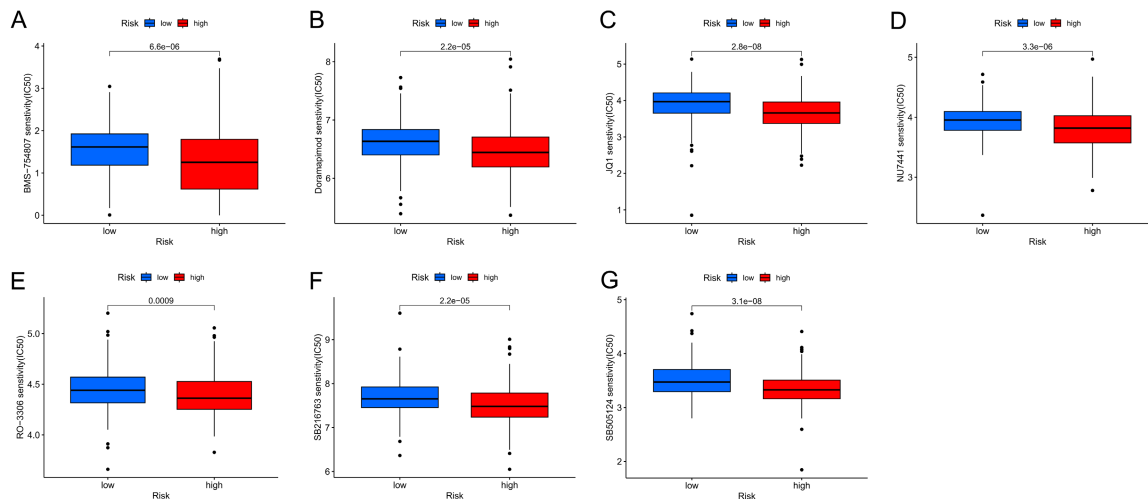


Figure 10. Drug sensitivity prediction. (A-G) Box plots showing estimated IC_{50} values of seven anti-cancer drugs in high-risk and low-risk groups: (A) BMS-754807, (B) Doramapimod, (C) JQ1, (D) NU7441, (E) RO-3306, (F) SB216763, (G) SB505124. Lower IC_{50} values indicate higher drug sensitivity. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. IC_{50} , half-maximal inhibitory concentration.

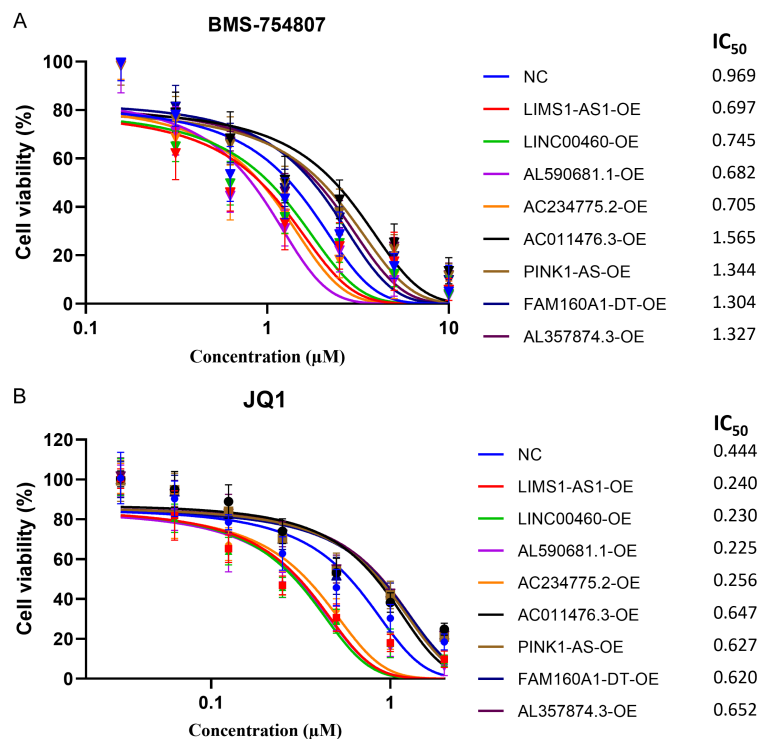


Figure 11. Validation of drug sensitivity in vitro. (A, B) IC_{50} values of BMS-754807 (A) and JQ1 (B) in AGS cells OE or KD the eight DRlncRNAs. NC, negative control. Data are presented as mean $\pm$ SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$ compared with NC group. IC_{50} , half-maximal inhibitory concentration; OE, overexpression; KD, knockdown; NC, negative control; SD, standard deviation.

Expression levels of DRlncRNAs and cell viability in cells

Compared with the expression levels of DRlncRNAs in GES-1 cells, the mRNA expression levels of LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2 were significantly increased in AGS cells, whereas the mRNA expression levels of AC011476.3, PINK1-AS, FAM160A1-DT, and AL357874.3 were significantly decreased (Figure 14A-H). Compared with the DRlncRNA knockdown groups, the cell viability of the overexpression groups of LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2 significantly increased over time, while the cell viability of the overexpression groups of AC011476.3, PINK1-AS, FAM160A1-DT, and AL357874.3 significantly decreased over time (Figure 14I-P).

Expression levels of DRlncRNAs in animal tissues

7874.3) suppressed cystine uptake to 0.6-fold ($P < 0.01$), and their knockdown markedly restored it (Figure 13A-H).

To further validate the expression patterns of the eight DRlncRNAs in vivo, we established a gastric adenocarcinoma rat model using

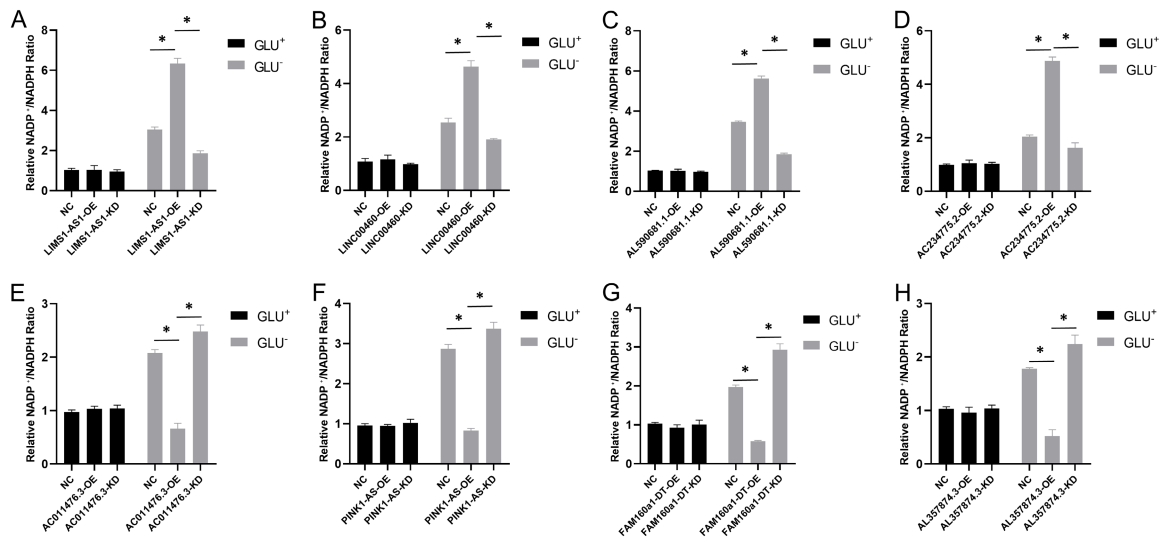


Figure 12. Effects of DRlncRNAs on NADP⁺/NADPH ratio. (A-H) NADP⁺/NADPH ratio in AGS cells after OE or KD of each of the eight DRlncRNAs under high-glucose (HG, 25 mM) or glucose-free (GF) conditions for 24 hours: (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. NC, negative control. Data are presented as mean $\pm$ SD from three independent experiments, each performed in triplicate. * P < 0.05, ** P < 0.01, *** P < 0.001 compared with NC under the same culture condition; ns, not significant. DRlncRNA, disulfidptosis-related long non-coding RNA; OE, overexpression; KD, knockdown; NC, negative control; SD, standard deviation.

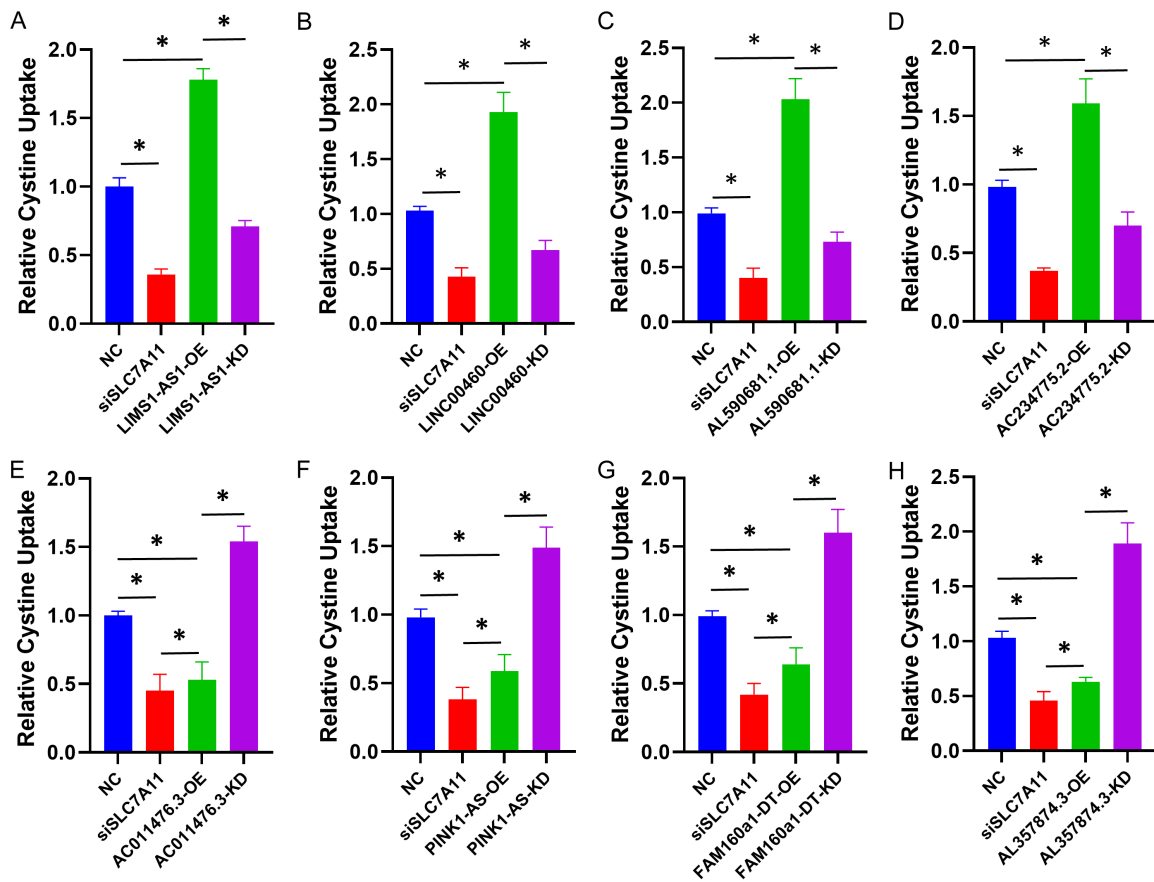


Figure 13. Effects of DRlncRNAs on cystine uptake. (A-H) L-[¹⁴C]-cystine uptake in AGS cells after OE or KD of each of the eight DRlncRNAs under glucose-free conditions: (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) ACO11476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. NC, negative control. Uptake values were normalized to NC and presented as mean $\pm$ SD from three independent experiments, each performed in triplicate. * P < 0.05, ** P < 0.01, *** P < 0.001 compared with NC. DRlncRNA, disulfidptosis-related long non-coding RNA; OE, overexpression; KD, knockdown; NC, negative control; SD, standard deviation.

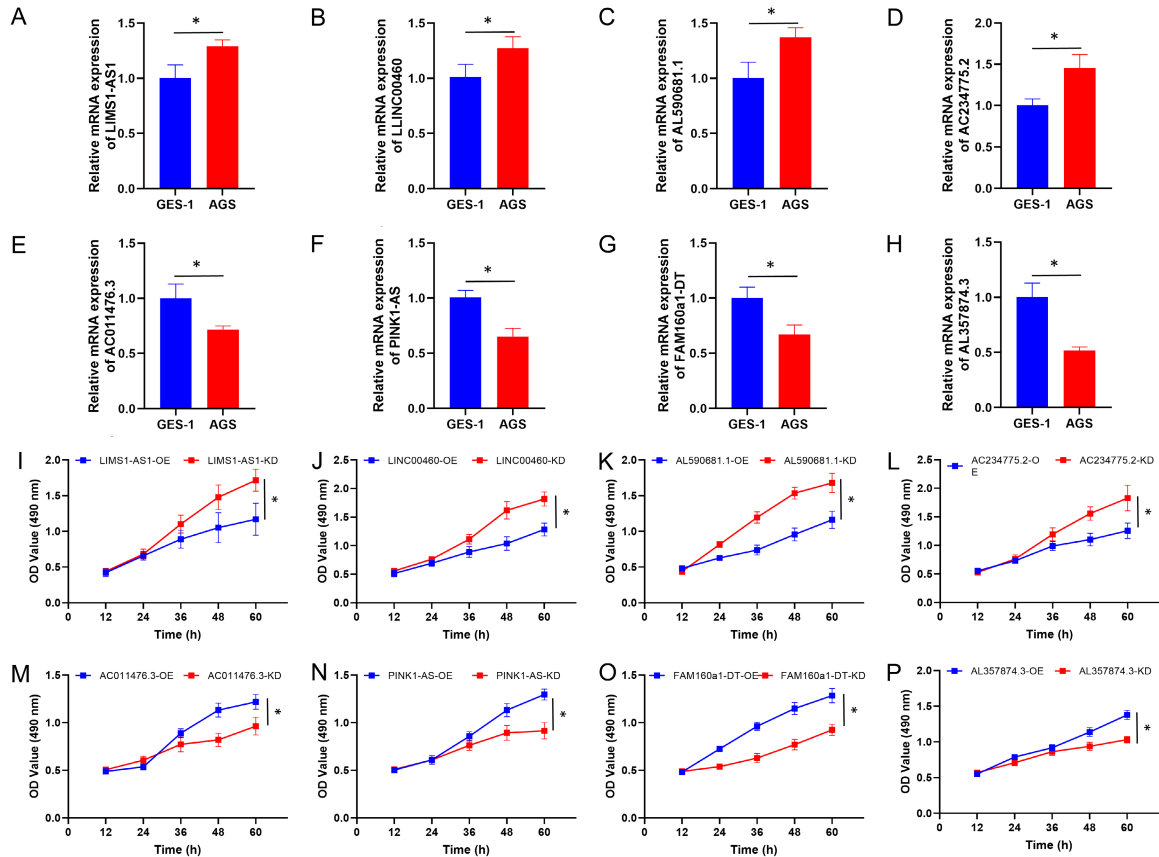


Figure 14. DRlncRNA expression and cell viability in vitro. (A-H) Relative expression levels of eight DRlncRNAs in GES-1 (normal gastric epithelial cell line) and AGS (gastric cancer cell line) measured by qRT-PCR: (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) ACO11476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. Expression levels were normalized to GAPDH. (I-P) Cell viability of AGS cells after OE or KD of the eight DRlncRNAs over 5 days as measured by MTT assay: (I) LIMS1-AS1, (J) LINC00460, (K) AL590681.1, (L) AC234775.2, (M) ACO11476.3, (N) PINK1-AS, (O) FAM160A1-DT, (P) AL357874.3. NC, negative control. Data are presented as mean $\pm$ SD from three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001 compared with NC at the same time point. DRlncRNA, disulfidptosis-related long non-coding RNA; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; OE, overexpression; KD, knockdown; NC, negative control; SD, standard deviation; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

MNNG induction. qRT-PCR analysis revealed that the expression levels of the four high-risk DRlncRNAs (LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2) were significantly upregulated in the gastric tissues of model rats compared with control rats. Conversely, the expression levels of the four low-risk DRlncRNAs (ACO11476.3, PINK1-AS, FAM160A1-DT,

and AL357874.3) were significantly downregulated (Figure 15A-H, all P < 0.05).

Expression levels of DRlncRNAs in human serum

To evaluate the clinical translational potential of the eight DRlncRNAs, we measured their expression levels in serum samples from 40

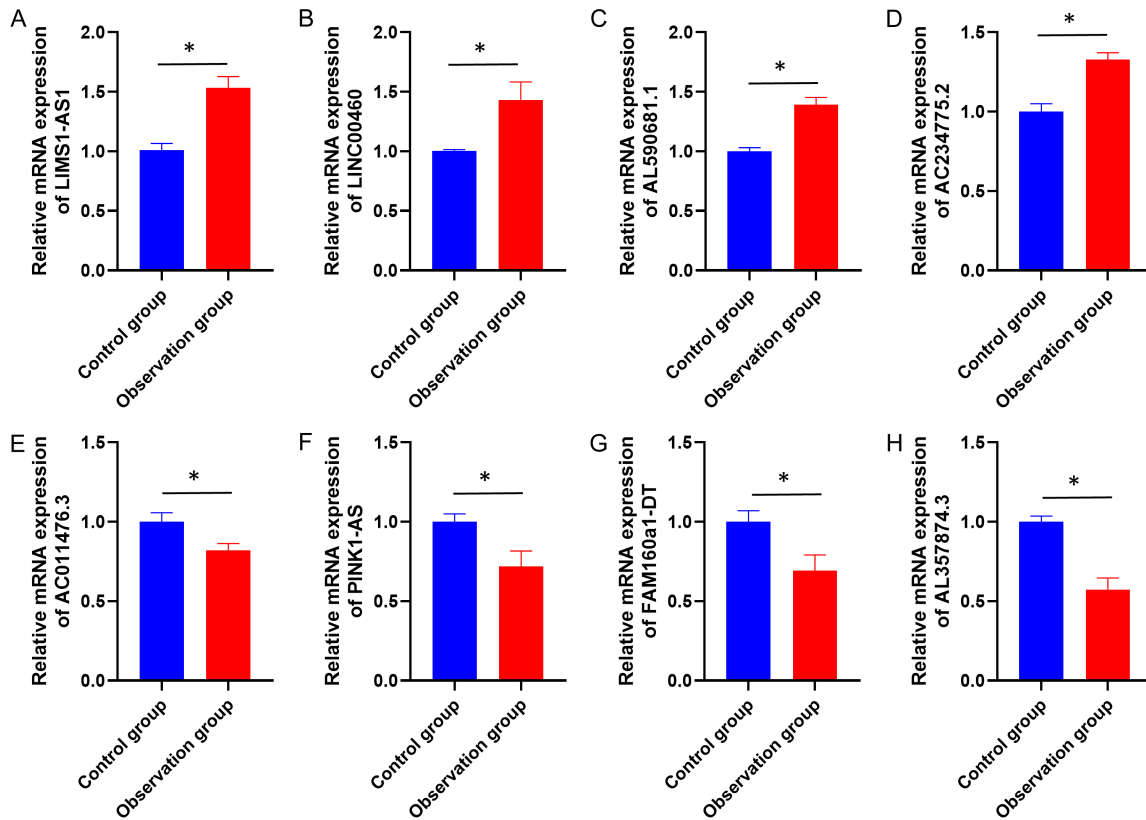


Figure 15. Expression levels of eight DRlncRNAs in animal tissues. (A-H) Relative expression levels of eight DRlncRNAs in gastric tissues from control rats (n=10) and MNNG-induced gastric adenocarcinoma model rats (n=10) measured by qRT-PCR: (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. Expression levels were normalized to GAPDH. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control group. DRlncRNA, disulfidptosis-related long non-coding RNA; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SD, standard deviation.

gastric adenocarcinoma patients and 40 age-matched healthy controls using qRT-PCR. Consistent with the findings in cell lines and animal tissues, the four high-risk DRlncRNAs (LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2) exhibited significantly elevated expression in the serum of STAD patients compared with healthy controls. In contrast, the four low-risk DRlncRNAs (AC011476.3, PINK1-AS, FAM160A1-DT, and AL357874.3) showed significantly reduced expression levels (**Figure 16A-H**, all $P < 0.05$).

Correlation analysis between clinical characteristics and DRlncRNA expression levels

Correlation between DRlncRNAs and clinicopathological features. We systematically evaluated the relationship between serum DRlncRNA levels and key clinicopathological parameters to assess their clinical relevance.

Correlation analysis revealed a stepwise increase in risk-associated lncRNAs from stage I to IV: LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2. Conversely, protective lncRNAs showed progressive decline: AC011476.3, PINK1-AS, FAM160A1-DT, and AL357874.3. Stage IV patients exhibited 2.8- to 4.2-fold higher levels of risk lncRNAs compared to stage I, while protective lncRNAs decreased by 60-75% (**Figure 17**).

In poorly differentiated tumors (G3), risk lncRNAs were significantly elevated compared to well-differentiated (G1) and moderately differentiated (G2) tumors: LIMS1-AS1 (G3 vs. G1: 3.6-fold, $P < 0.001$), LINC00460 (3.2-fold, $P < 0.001$), AL590681.1 (2.9-fold, $P < 0.01$), and AC234775.2 (2.7-fold, $P < 0.01$). Protective lncRNAs showed the opposite trend, with G3 levels 45-60% lower than G1 ($P < 0.01$) (**Figure 18**).

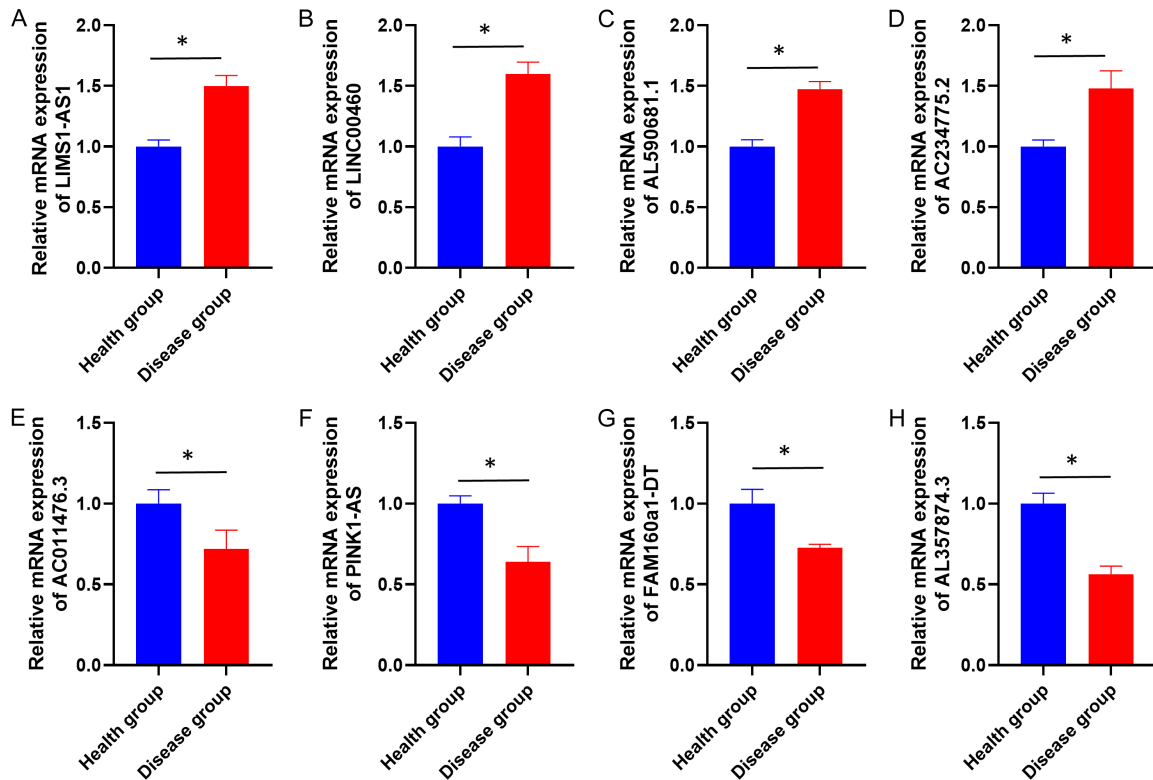


Figure 16. Expression levels of eight DRlncRNAs in human serum. (A-H) Relative expression levels of eight DRlncRNAs in serum samples from healthy controls (n=40) and gastric adenocarcinoma patients (n=40) measured by qRT-PCR: (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. Expression levels were normalized to U6. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control group. DRlncRNA, disulfidptosis-related long non-coding RNA; qRT-PCR, quantitative real-time polymerase chain reaction; SD, standard deviation.

Risk lncRNAs were significantly higher in N1-N3 (node-positive) patients compared to N0 (node-negative). Positive correlations were observed between lncRNA levels and nodal burden (N0 < N1 < N2 < N3). Protective lncRNAs decreased progressively with increasing nodal involvement ($P < 0.01$) (Figure 19).

M1 patients (distant metastasis) displayed the highest levels of risk lncRNAs and lowest levels of protective lncRNAs. Risk lncRNAs in M1 patients were elevated 4.5- to 6.2-fold compared to M0 ($P < 0.001$), while protective lncRNAs were reduced by 65-78% ($P < 0.001$). The combined stratification of nodal and distant metastatic status revealed that patients with both N⁺ and M⁺ showed the most aggressive DRlncRNA expression profile (Figure 20).

Collectively, these findings demonstrate that DRlncRNA abundance mirrors tumor aggressiveness across multiple clinical dimensions,

with the four risk lncRNAs consistently elevated and the four protective lncRNAs consistently suppressed in advanced disease stages, high-grade tumors, and metastatic lesions.

Discussion

STAD, as a digestive system malignancy with a high mortality rate worldwide, has long relied on the traditional treatment model of surgical resection combined with adjuvant radiotherapy and chemotherapy. However, the prognosis of patients with advanced disease remains unsatisfactory [11]. This dilemma highlights the inadequacy of the current treatment system in elucidating the molecular mechanisms of the disease and propels researchers to explore more precise targeted therapies and prognostic assessments. In recent years, disulfidptosis, as a novel mode of metabolic cell death, has been proven to have clinical relevance in regulating the fate of tumor cells in various cancers

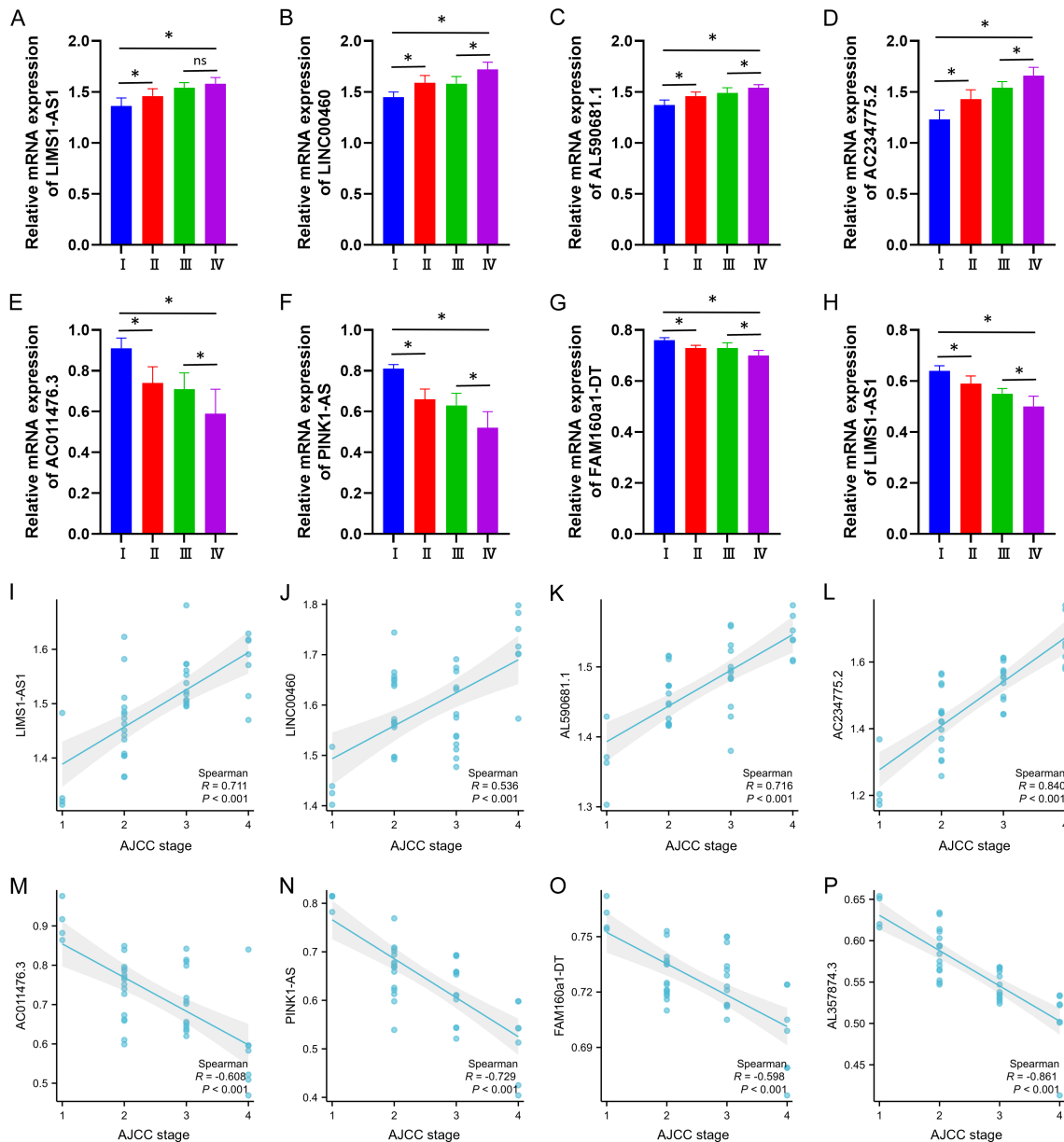


Figure 17. Correlation between DRlncRNA expression and AJCC stage. (A-H) Serum expression levels of eight DRlncRNAs in gastric adenocarcinoma patients stratified by AJCC stage (I, II, III, IV): (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. (I-P) Correlation analyses between DRlncRNA expression levels and AJCC stage: (I) LIMS1-AS1, (J) LINC00460, (K) AL590681.1, (L) AC234775.2, (M) AC011476.3, (N) PINK1-AS, (O) FAM160A1-DT, (P) AL357874.3. Data are presented as mean $\pm$ SD. ρ , Spearman correlation coefficient. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DRlncRNA, disulfidptosis-related long non-coding RNA; AJCC, American Joint Committee on Cancer; SD, standard deviation.

[12]. lncRNAs have become an important molecular bridge connecting specific cell death pathways to tumor progression due to their key role in gene expression regulation [13, 14]. Among them, DRlncRNAs have shown clear prognostic value in tumors such as cervical cancer and rectal adenocarcinoma. This back-

ground prompted us to propose the core hypothesis that DRlncRNAs may serve as novel biomarkers for prognostic evaluation of STAD and represent potential therapeutic targets.

Using transcriptome profiles from STAD tissue and raw measurements held in the TCGA re-

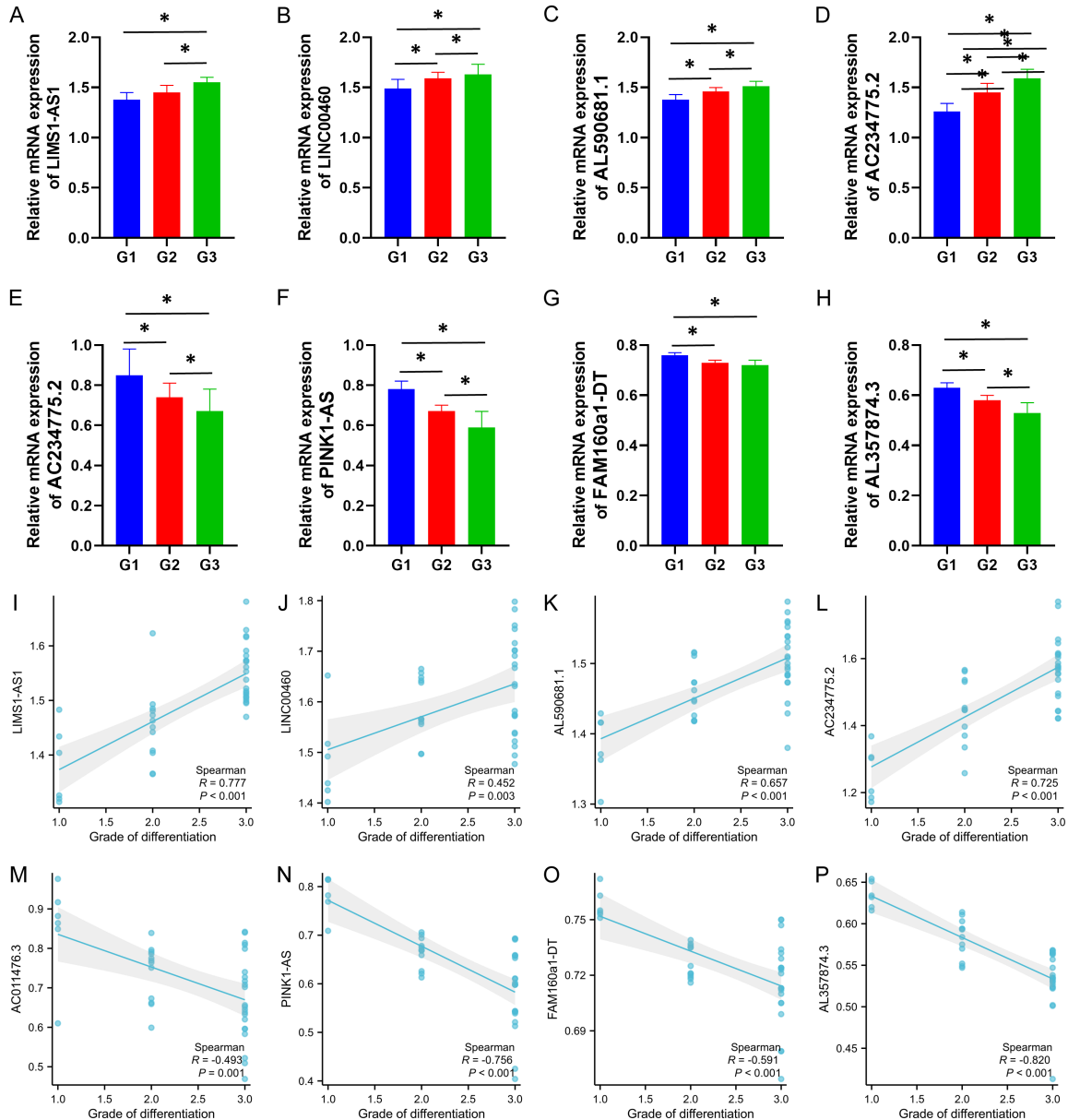


Figure 18. Correlation between DRlncRNA expression and differentiation grade. (A-H) Serum expression levels of eight DRlncRNAs in gastric adenocarcinoma patients stratified by differentiation grade (G1, G2, G3): (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. (I-P) Correlation analyses between DRlncRNA expression levels and differentiation grade: (I) LIMS1-AS1, (J) LINC00460, (K) AL590681.1, (L) AC234775.2, (M) AC011476.3, (N) PINK1-AS, (O) FAM160A1-DT, (P) AL357874.3. Data are presented as mean ± SD. ρ , Spearman correlation coefficient. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DRlncRNA, disulfidptosis-related long non-coding RNA; SD, standard deviation.

pository, we assembled and cross-checked an eight-DRlncRNA signature (LIMS1-AS1, LINC00460, AL590681.1, AC234775.2, AC011476.3, PINK1-AS, FAM160A1-DT, AL357874.3). The classifier achieved robust risk separation in both training and testing cohorts, clearly stratifying patients into high- and low-

risk groups, and revealed marked multi-omic divergence between the two subgroups, laying the groundwork for downstream mechanistic investigation.

LIMS1-AS1, as a key component of this model, regulates the expression of the LIMS1 gene

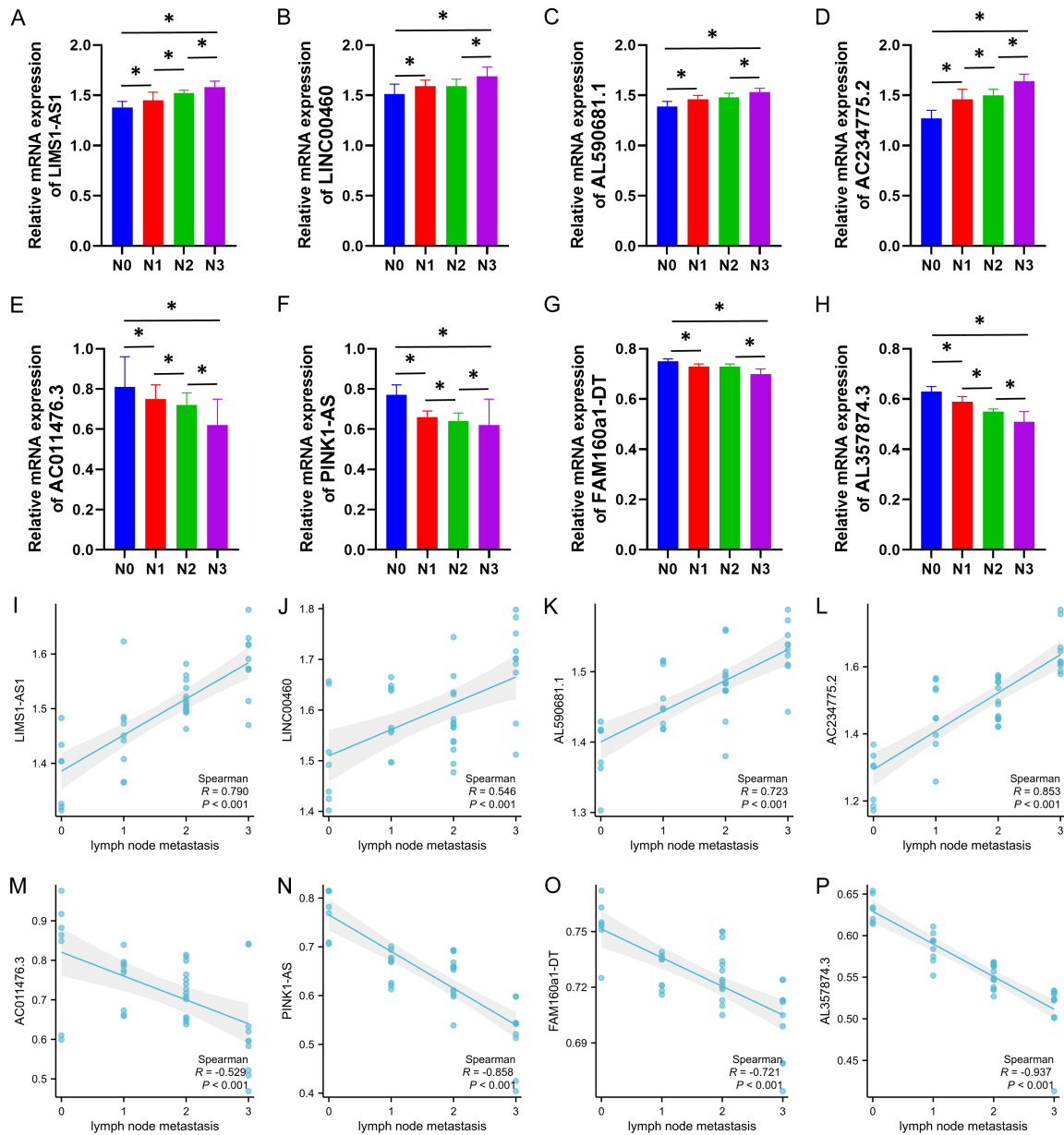


Figure 19. Correlation between DRlncRNA expression and lymph node metastasis. (A-H) Serum expression levels of eight DRlncRNAs in gastric adenocarcinoma patients stratified by lymph node metastasis status (N0, N1, N2, N3): (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. (I-P) Correlation analyses between DRlncRNA expression levels and nodal involvement: (I) LIMS1-AS1, (J) LINC00460, (K) AL590681.1, (L) AC234775.2, (M) AC011476.3, (N) PINK1-AS, (O) FAM160A1-DT, (P) AL357874.3. Data are presented as mean $\pm$ SD. p, Spearman correlation coefficient. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DRlncRNA, disulfidptosis-related long non-coding RNA; SD, standard deviation.

and participates in cytoskeleton organization and signal transduction. It is highly correlated with the regulation of STAD cell infiltration ability [15]. Functional in vitro assays demonstrated that LIMS1-AS1 overexpression markedly reduced the IC_{50} of the IGF-1R inhibitor BMS-754807 in AGS cells, whereas its knockdown elevated the IC_{50} , indicating that this risk-asso-

ciated DRlncRNA directly modulates drug sensitivity and promotes cell survival under metabolic stress. Qu C et al. also determined that LIMS1-AS1 is one of the prognostic lncRNAs of gastric adenocarcinoma [15]. LINC00460 promotes tumor development through the PI3K/AKT signaling pathway and acts as a miRNA sponge in various cancers. This observation

Disulfidptosis-related lncRNA signature in STAD

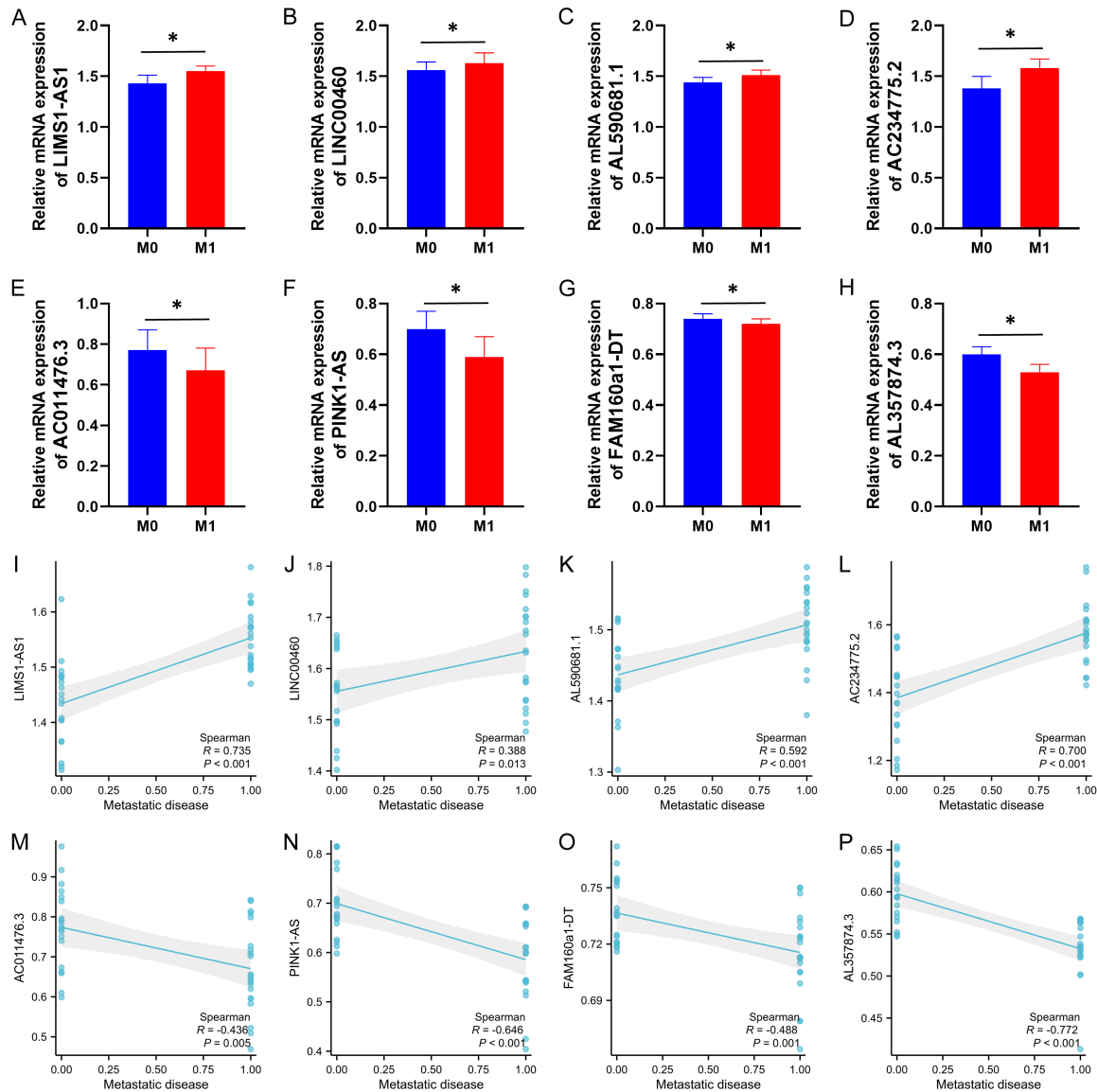


Figure 20. Correlation between DRlncRNA expression and distant metastasis. (A-H) Serum expression levels of eight DRlncRNAs in gastric adenocarcinoma patients with (M1) or without (M0) distant metastasis: (A) LIMS1-AS1, (B) LINC00460, (C) AL590681.1, (D) AC234775.2, (E) AC011476.3, (F) PINK1-AS, (G) FAM160A1-DT, (H) AL357874.3. (I-P) Correlation analyses between DRlncRNA expression levels and metastatic status: (I) LIMS1-AS1, (J) LINC00460, (K) AL590681.1, (L) AC234775.2, (M) AC011476.3, (N) PINK1-AS, (O) FAM160A1-DT, (P) AL357874.3. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DRlncRNA, disulfidptosis-related long non-coding RNA; SD, standard deviation.

highlights its potential role as a STAD oncogene [16]. In addition, the correlation between AC234775.2 and immune cell infiltration and immune function provides important insights for understanding its role in regulating the immune microenvironment of STAD [17].

For protective DRlncRNAs, AC011476.3, PINK1-AS, and FAM160A1-DT have demonstrated prognostic significance in other malignan-

cies, suggesting functional consistency across cancer types [18-20]. Notably, FAM160A1-DT is upregulated in normal tissues compared with cancer tissues and has been incorporated into disulfidptosis-related prognostic models for colorectal adenocarcinoma. The roles of AL590681.1 and AL357874.3 remain underexplored, presenting new directions for investigating regulatory molecules in STAD. Consistent with our bioinformatics analysis, in vitro

cell experiments, in vivo animal models, and clinical sample validation collectively confirmed that the eight DRlncRNAs exhibit distinct expression patterns: the four risk-associated lncRNAs (LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2) were significantly upregulated, whereas the four protective lncRNAs (AC011476.3, PINK1-AS, FAM160A1-DT, and AL357874.3) were significantly downregulated in STAD tissues compared with controls.

Our experimental findings provide direct evidence linking the eight-DRlncRNA signature to disulfidptosis regulation and clinical outcomes. Under glucose deprivation - a critical trigger for disulfidptosis - we observed striking differences between high- and low-risk DRlncRNAs. Specifically, overexpression of high-risk DRlncRNAs (LIMS1-AS1, LINC00460, AL590681.1, and AC234775.2) led to significantly enhanced cystine uptake and elevated NADP⁺/NADPH ratios (**Figures 12, 13**), indicating increased NADPH consumption and reduced cellular reducing capacity. This metabolic state renders cells more vulnerable to disulfide stress, as NADPH is essential for maintaining intracellular disulfide balance [8]. Consequently, high-risk tumors exhibit heightened sensitivity to disulfidptosis, which paradoxically may drive clonal selection for aggressive, therapy-resistant variants under the metabolic pressures of the tumor microenvironment. In contrast, low-risk DRlncRNAs (AC011476.3, PINK1-AS, FAM160A1-DT, and AL357874.3) exerted the opposite effects: they suppressed cystine uptake and preserved NADPH pools, maintaining redox homeostasis and conferring resistance to disulfidptosis. This protective metabolic phenotype is associated with less aggressive disease and improved survival outcomes. Thus, the prognostic stratification of our eight-lncRNA signature directly reflects differential disulfidptosis susceptibility. High-risk tumors exhibit a metabolic state primed for disulfide stress, which through evolutionary adaptation selects for more malignant clones, whereas low-risk tumors maintain metabolic stability, favoring indolent phenotypes and better clinical outcomes.

To further characterize the biological processes underlying the divergent risk phenotypes, we performed functional enrichment analysis.

Differentially expressed genes in high-risk STAD patients were significantly enriched in biological processes related to muscle system function, extracellular matrix (ECM) remodeling, and receptor-ligand interactions (**Figure 7A-D**). These functions collectively form a collaborative network driving malignant progression, consistent with previous reports emphasizing the role of actin cytoskeletal remodeling in gastric cancer EMT [21]. Enrichment of muscle system-related genes suggests that tumor cells may acquire enhanced migration and invasiveness through upregulation of contractile proteins, promoting deep gastric wall invasion. Active ECM remodeling reflects accelerated matrix degradation and turnover in the tumor microenvironment, which not only provides a physical pathway for tumor cell migration but also activates oncogenic signals through release of stored growth factors [22]. Notably, these ECM alterations may synergize with disulfidptosis: our mechanistic data show that high-risk DRlncRNAs enhance cystine uptake and NADPH consumption, and the resulting disulfide stress could further activate matrix metalloproteinases, creating a positive feedback loop that promotes both metabolic vulnerability and invasive capacity.

At the pathway level, KEGG analysis showed that high-risk STAD patients commonly exhibit enrichment in neuroactive ligand-receptor interactions, cAMP signaling, and calcium signaling pathways. This suggests a broad dysregulation of the GPCR signaling network. This may lead to the abnormal release of proto-secreted hormones such as gastrin, which in turn continuously activates proliferation signals and regulates the expression of Bcl-2 family proteins, and mediates cell apoptosis resistance [23]. Additionally, activation of the complement and coagulation cascade was associated with poor prognosis. This pathway recruits immunosuppressive cell populations such as myeloid-derived suppressor cells, while its inflammatory mediators (e.g., C5a) promote angiogenesis and tumor migration, creating a pro-tumorigenic inflammatory environment [24]. Recent studies have shown interplay between complement signaling and immune checkpoints; for instance, combining C5a blockade with PD-1 inhibitors enhances CD8⁺ T cell responses in lung cancer models [25]. In contrast, low-risk STAD patients exhibited enrichment of cell

cycle and DNA replication pathways, indicating more stable proliferation regulation and greater genomic integrity, consistent with their better prognosis.

In addition, stromal, immune, and ESTIMATE scores were significantly higher in the high-risk group, indicating a more complex tumor micro-environment. Increased matrix components may promote EMT through the TGF- β signaling pathway, while the presence of both activated and inhibitory immune cells within the infiltrate can further facilitate immune escape [26]. These features not only clarify the mechanisms underlying the poor prognosis of high-risk STAD patients but also suggest that combined therapies targeting matrix remodeling and immune checkpoints may represent a promising strategy to improve outcomes in this subgroup.

The TME and TMB are key factors influencing immune escape and treatment response in STAD. In the TME of high-risk patients, we observed increased infiltration of resting NK cells and eosinophils, alongside activation of various immune cell subsets and type I/II interferon response pathways. This unique “strong immune response but dysfunctional” phenotype is characterized by inhibited cytotoxicity of resting NK cells, eosinophil secretion of tumor-promoting factors (e.g., VEGF and MMPs), Th2 polarization creating an immunosuppressive microenvironment, PD-L1-mediated T cell exhaustion, and upregulation of MHC class I molecules. These factors collectively contribute to the paradoxical phenomenon of “strong immune infiltration yet poor prognosis” in high-risk patients. This finding has important implications for immunotherapy selection in STAD: single-agent PD-1/PD-L1 inhibitors may have limited efficacy, whereas combination strategies targeting Th2 polarization, NK cell activation, or eosinophil chemotaxis may be more effective at reversing immunosuppression [27].

Regarding TMB, the low-risk group exhibited higher TMB levels, consistent with increased neoantigen production and stronger anti-tumor immune responses. Survival outcome differences between the high-TMB/high-risk and low-TMB/high-risk subgroups further underscore the modulatory role of TMB on prognosis in high-risk patients. Additionally, the lower TIDE scores observed in the high-risk group, together with frequent TP53 and TTN mutations, pro-

vide insights into the molecular mechanisms underlying poor prognosis, highlighting aspects of immune escape potential and driver gene mutations. TP53-mutated tumors may exhibit resistance not only to immune checkpoint inhibitors but also to other immunotherapies such as CAR-T cell therapy and hematopoietic stem cell transplantation [28]. TTN mutations enhance TTN protein stability, reduce intracellular ferrous ion levels, and significantly decrease sensitivity to 5-FU in liver cancer [29]. These findings suggest potential roles for TP53 and TTN mutations in treatment resistance in STAD, offering new targets for developing more effective therapeutic strategies.

Chemotherapy drug sensitivity analysis provides a direct reference for precision treatment of high-risk STAD patients. Drugs with high sensitivity in this subgroup target core dependency pathways, including IGF-1R, p38 MAPK, and BET protein-mediated transcriptional regulation [30-32]. Previous studies have confirmed the key roles of these pathways in tumor cell proliferation and maintenance of anti-apoptotic phenotypes. Our research further validated the inhibitory effects of BMS-754807, doramapimod, and JQ1 on these pathways, indicating that these agents can effectively suppress malignant activity in high-risk patients. This finding aligns with conclusions from Hassan MS et al. in preclinical pancreatic cancer models [33]. Moreover, drug screening results targeting DNA damage repair, cell cycle, GSK-3 β , and TGF- β pathways address gaps in existing research. The high sensitivity to NU7441, R0-3306, SB216763, and SB505124 suggests that high-risk STAD patients exhibit “treatment vulnerabilities” in pathways associated with genomic instability and invasive potential. This result supports the concept of “multitargeted therapy for STAD” proposed by Sundar R et al. [34], providing a foundation for designing combination therapy regimens.

In summary, the eight-DRlncRNA prognostic model established in this study provides a novel biomarker for STAD prognosis assessment. By establishing a direct mechanistic link between the risk signature and disulfidptosis - wherein high-risk DRlncRNAs enhance cystine uptake and deplete NADPH under glucose deprivation, sensitizing cells to disulfide stress -

our multi-dimensional analysis clarifies the molecular characteristics and potential therapeutic targets in high-risk patients, offering an important reference for precision diagnosis and treatment of STAD.

Limitations

However, this study has several limitations. First, model validation relies on public databases and a limited set of clinical samples; multicenter studies with larger cohorts are needed to confirm its clinical value. Second, the specific mechanisms of AL590681.1 and AL357874.3 remain unclear and require further investigation through in vitro and in vivo experiments. Finally, the feasibility of DRlncRNAs as therapeutic targets and the efficacy of combination regimens warrant validation in additional preclinical and clinical studies. Future research will focus on these directions to facilitate clinical translation of DRlncRNAs in STAD.

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Disclosure of conflict of interest

None.

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