A risk score model with five long non-coding RNAs for predicting prognosis in gastric cancer: an integrated analysis combing TCGA and GEO dataset

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Background. Gastric cancer (GC) is one of the most common carcinomas of the digestive tract, and the prognosis for these patients may be poor. There is evidence that some long non-coding RNAs (lncRNAs) could predict the prognosis of gastric cancer. However, few lncRNA signatures have been used to predict the prognosis of the cancer. We herein aimed at constructing a risk score model based on 5-lncRNA to predict the prognosis of gastric cancer and providing some new potential therapeutic targets in the future. **Methods.** We performed bayesian analysis and survival analysis to identify differential expressed lncRNAs that had significantly different survival times by using gastric cancer patient expression profile data from The Cancer Genome Atlas (TCGA). We then established a formula including five lncRNAs to predict prognosis of GC patients. In addition, to verified the prognostic value of this risk score model, two independent Gene Expression Omnibus (GEO) datasets (GSE62254 (N=300) and GSE 15459 (N=200)) were employed to act as validation groups. **Results.** Based on the character of five-lncRNA, high or low risk subgroups can be divided among GC patients. The prognostic value of the risk score model with five-lncRNA was confirmed in both TCGA dataset and the other two independent GEO datasets. Furthermore, stratification analysis found that the prognostic value of this risk model was independent in GC patients with Ⅱ-Ⅳ stage. Moreover, we constructed a nomogram model combining the clinical factors and five lncRNAs to heighten the accuracy of prognostic prediction. Enrichment analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) suggested that five lncRNAs may be associated with multiple cancer occurrence and progress-related pathways. **Conclusion.** Our results showed that the risk score model with five-lncRNA predicts prognosis of GC patients well especially in stage II-IV and may provide potential therapeutic targets in future.

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Abstract

Background. Gastric cancer (GC) is one of the most common carcinomas of the digestive tract, and the prognosis for these patients may be poor. There is evidence that some long non-coding RNAs (lncRNAs) could predict the prognosis of gastric cancer. However, few lncRNA signatures have been used to predict the prognosis of the cancer. We herein aimed at constructing a risk score model based on 5-lncRNA to predict the prognosis of gastric cancer and providing some new potential therapeutic targets in the future.

Methods. We performed bayesian analysis and survival analysis to identify differential expressed lncRNAs that had significantly different survival times by using gastric cancer patient expression profile data from The Cancer Genome Atlas (TCGA). We then established a formula including five lncRNAs to predict prognosis of GC patients. In addition, to verified the prognostic value of this risk score model, two independent Gene Expression Omnibus (GEO) datasets (GSE62254 (*N*=300) and GSE15459 (*N*=200)) were employed to act as validation groups.

Results. Based on the character of five-lncRNA, high or low risk subgroups can be divided among GC patients. The prognostic value of the risk score model with five-lncRNA was confirmed in both TCGA dataset and the other two independent GEO datasets. Furthermore, stratification analysis found that the prognostic value of this risk model was independent in GC patients with Ⅱ-Ⅳ stage. Moreover, we constructed a nomogram model combining the clinical factors and five lncRNAs to heighten the accuracy of prognostic prediction. Enrichment analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) suggested that five lncRNAs may be associated with multiple cancer occurrence and progress-related pathways.

Conclusion. Our results showed that the risk score model with five-lncRNA predicts prognosis of GC patients well especially in stage II-IV and may provide potential therapeutic targets in future.

Introduction

Gastric cancer (GC) is one of the most common carcinomas of the gastrointestinal (GI) tract and is particularly prevalent in the Asian countries. It is estimated that approximately 679,100 individuals were diagnosed with GC in 2015 in China and approximately 498,000 of them died that same year (Saka et al., 2011; Chen et al., 2016). The standard therapies for GC are surgery and chemotherapy. However, most patients with advanced GC suffer from recurrence of the malignancy and metastasis after treatment, resulting in poor prognosis. Despite considerable research in therapies for gastric cancer, the prospects of survival of patients with GC remain bleak (Saka et al., 2011). The identification of gastric cancer patients with poor survival prognoses and the administration of effective treatment as early as possible are the keys to improving survival. The investigation of potential therapeutic and prognostic biomarkers for gastric cancer is of considerable importance.

Long non-coding RNAs (lncRNAs) are RNAs of ≥200 nucleotides with no or limited protein-coding potential. There is considerable evidence that lncRNAs play crucial roles in the initiation and developments of cancers. For example, lncRNA-ATB disorders contribute to cancer cell proliferation, migration, and invasion, and drug-resistance as well as induce epithelial-mesenchymal transition by competitively binding to microRNAs(Li et al., 2017; Balas & Johnson, 2018). Some researchers have suggested that lncRNAs could act as new prognostic biomarkers in various cancers, including CCAT2 (Yu et al., 2017), HOXB-AS3 (Huang et al., 2017), and ASLNC07322 (Li et al., 2019) in colon cancer. Many lncRNAs closely related to the prognosis of GC have been identified, including MEG3(Wei & Wang, 2017), SNHG7(Wang et al., 2017), and DANCR(Mao et al., 2017)[10]. Risk score models have also been constructed to predict the prognosis of human cancers. In non-small cell lung cancer, differences in prognosis can be identified by its 8-lncRNA signature (Miao et al., 2019). However, the identification of lncRNAs related to prognosis in patients with GC is still in its early stages and additional research is warranted.

In this study, we analyzed the data of 450 patients with GC from The Cancer Genome Atlas (TCGA) database, to identify differentially expressed lncRNAs for the prognostic prediction. We used two independent Gene Expression Omnibus (GEO)(Barrett et al., 2013) datasets to validate the selected-lncRNAs. In addition, we analyzed the accuracy of the prediction of five lncRNAs in different clinical subgroups using lncRNA data in combination with the clinical characteristics of the patients. Furthermore, we constructed a nomogram model by combining clinical factors and five lncRNAs to increase the accuracy of prognostic prediction. Finally, we performed pathway enrichment analysis to determine the potential functions of these lncRNAs in GC.

Materials & Methods

Preparation of GC datasets

We acquired a training dataset of GC samples from TCGA, comprised of 450 samples and 14147 lncRNAs (case: normal = 414:36). We used these 450 samples to perform differential expression analysis. After excluding six cases with missing overall survival (OS) prognostic information, a total of 408 cases were recruited for further univariate Cox proportional hazard regression analysis and subsequent analysis in the training group. The microarray data for the validation group and the survival data of the patients are publicly available at GEO with

accession numbers GSE62254 (N = 300; 1397 lncRNAs) and GSE15459 (N = 200; 1397 lncRNAs).

Normalization of GEO data

Because the two GEO datasets (GSE62254 and GSE15459) were differential expression profiles, we performed quantile normalization on the original data and downloaded it as a probe-level CEL file. Affymetrix U133 Plus 2.0 was used as the probe matching platform. Data were downloaded from the Affymetrix website (http://www.affymetrix.com), and a total of 2986 lncRNA-specific probes were included.

Creation of an lncRNA-based risk model from the training group

The lncRNAs that were differentially expressed between GC and normal gastric tissue in the TCGA dataset were identified via Bayesian analysis using the "limma" R package of the R statistical computing environment (log2|fold change| >1 and adjusted $P < 0.05$), and the adjusted P was used to reduce false positives(Deng et al., 2019; Zeng et al., 2019). The candidate lncRNAs were analyzed using univariate Cox proportional hazard regression analysis ($P < 0.05$). The cutoff values of lncRNA expression were determined as the median of all expression values in Cox survival analysis. In total, we identified 278 lncRNAs with statistically significant differences. After identifying the lncRNAs common to both the TCGA and GEO (GSE62254) datasets, we performed multivariate Cox proportional hazards analysis to identify independent prognostic lncRNAs. Finally, we created an lncRNA-based risk model from a linear combination of the expression levels of these lncRNAs, multiplied by the regression coefficients obtained from multivariate Cox proportional hazards regression analysis.

Validation of the lncRNA-based model for prognostic prediction

We calculated the risk scores of each patient and used the corresponding median score as the cutoff value to classify them into two groups: high risk and low risk subgroups. We used Kaplan-Meier analysis to differentiate the survival of the two groups and time-dependent receiver operating characteristic (ROC) curves to assess our lncRNA-based risk model. We used two GEO datasets to validate the model for prognostic prediction. Cox proportional hazards regression analysis was used to estimate the hazard ratio (HR) of the model with 95% confidence interval to further evaluate the predictive value of the model for each clinical subgroup. Clinical subgroups were determined based on gender, tumor–node–metastases (TNM) stage, histologic grade, race, and age. Finally, we constructed a nomogram combining the model with clinical factors using the "rms" package. We also calculated the concordance index (C-index) and plotted a calibration curve to determine its predictive accuracy and discriminatory capacity.

Potential functions of the five lncRNAs

To determine the potential functions of the five lncRNAs, which appeared to be discriminatory, we performed linear regression analysis of the relationship between the lncRNAs and the protein-coding genes in the TCGA dataset. The screening criteria for the protein-coding genes were a positive association with at least one lncRNA (Pearson coefficient > 0.4). After identifying the candidate genes, we screened out aberrantly activated signaling pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis via Web-based Gene Set Analysis Toolkit (http://www.webgestalt.org/), a popular software tool for functional enrichment analysis related to KEGG pathways(Yang et al., 2019; Wang et al., 2013),.

Statistical analysis

We used the R software (version 3.6.1) for statistical analyses. Bayesian analysis was performed using the "limma" R package. Univariate and multivariate Cox proportional hazards regression analyses were conducted to identify prognosis-related lncRNAs. The "survival" and "survminer" package was used for Cox proportional hazards regression analyses, Kaplan–Meier survival analysis and calculation of C-index. A time-dependent ROC curve was constructed to assess the specificity and sensitivity of the risk model by "survivalROC" package. A nomogram

combining the risk score model with the clinical factors was constructed using the "rms" package. The Review Manager software (version 5.3) was used to construct a forest plot. Chi-square tests were used to compare the rates of reoccurrence and death between the high risk and low risk groups. A *P*-value <0.05 was considered statistically significant, and all tests were two sided. Pearson's linear regression analysis was used to determine the relationship between lncRNAs and protein-coding genes.

Results

Identification of five prognostic lncRNAs

We finally included 408 patients with lncRNA expression profile and overall survival information in the training group. We performed Bayesian analysis (log2|fold change| >1 and adjusted $P < 0.05$) and univariate Cox proportional hazard regression analysis ($P < 0.05$) to identify survival-related lncRNAs. A total of 278 lncRNAs were further analyzed. To validate predictive accuracy, we compared the lncRNAs selected from the TCGA database with the GEO validation group. We found that 37 shared lncRNAs were present in both the 278 lncRNAs and the validation dataset (GSE62254) (Table S1). Multivariable Cox proportional hazards regression analyses identified five lncRNAs as independent prognostic factors of GC: LINC00205, TRHDE-AS1, OVAAL, LINC00106, and MIR100HG (Table 1). Fig. 1A-B shows the expression profiles of the five lncRNAs in patients with GC as volcano and heat maps, whereas Fig. 1C-D shows the survival curves based on the OS and disease-free survival (DFS) of the 408 patients. Owing to the lack of clinical data in GSE15459, table 2 shows the clinical features of GC patients in training group and GSE62254.

Creation of an lncRNA-based risk model from the training group

According to the schematic workflow of the present study (Table 3), using the coefficients of the five lncRNAs identified by multivariable Cox hazard analysis, we created a risk-score formula as follows: risk score = (0.249092 × expression level of LINC00205) + (0.182045 × expression level of TRHDE-AS1) + (0.271169 × expression level of OVAAL) + (−0.20794 × expression level of LINC00106) + (0.502539 × expression level of MIR100HG). Among the five lncRNAs, a negative coefficient (LINC00106) indicates a protective factor. The remaining four lncRNAs with positive coefficients, namely LINC00205, TRHDE-AS1, OVAAL, and MIR100HG, were risk factors. The risk score of each patient in the training group were calculated (Table S2), ranged from -2.086959745 to 2.270305234. The patients in the training group were divided into two subgroups: high risk (n = 204) and low risk (n = 204) subgroups, with the median score (−0.001085) as the cutoff value. We performed Kaplan-Meier survival analysis to assess the effect of the lncRNA-based risk model on the OS and DFS of patients with GC in the training group (Fig. 2A-B). Our results indicated that the high-risk group had a significantly worse prognosis than the low risk group for both OS and DFS ($P=1 \times 10^{-6}$ and 6×10^{-6}, respectively). Fig. 2C-F shows the scatter plots for the rates of recurrence incidence and death of patients with GC. The rates of both recurrence and death were significantly higher in the high risk group than in the low risk group ($P < 0.001$). To accurately evaluate the prognostic value of the five-lncRNA signature, we performed time-dependent ROC analysis using the 1-4 years cutoff value of OS and the 1-2 years cutoff of DFS as the ROC ending points (Fig. 2G-H and Fig. S1A-D). The area under the ROC curve (AUC) was 0.734 for the 4-year cutoff value of OS and 0.692 for the 2-year cutoff value of DFS, respectively, and had the highest predictive value among those years, indicating that out model can be used for survival prediction in patients with GC (Fig. 2G-H).

Validation of the lncRNA-based risk model for prognostic prediction in independent groups

To assess the prognostic significance of this novel lncRNA-based risk model involving the five-lncRNA signature in patients with GC, we used the other two independent validation datasets from the GEO database. We calculated risk score using the formula mentioned above (Table S2). The patients with GC in GSE62254 (validation group-1; N = 300) and GSE15459 (validation group-2; N = 200) datasets were divided into high risk and low risk groups according to the median risk score. Owing to the lack of DFS data in GSE15459, we only calculated the OS of the patients. The high risk group had a poorer OS than those in the low risk group (log-rank P = 0.01) (Fig. 3A-B). Fig. 3C–D shows the scatter plots for the death events. The rates of death were significantly higher in the high risk group than in the low risk group ($P < 0.001$). The AUC for the two validation group in four-year cutoff OS was 0.622 and 0.610 for validation group 1 and 2, respectively (Fig. 3E-F). Fig. S2A–F shows the ROC curve for the 1-3 year cutoff OS for the validation groups 1 and 2. Furthermore, we verified the performance of our risk model for the DFS of the GSE62254 dataset (Fig. S3A-D). Our results further confirmed the value and robustness of this risk score model for prognostic prediction in patients with GC.

The lncRNA-based risk model has a favorable prognostic prediction in patients with stage II, III, and IV

To further investigate the performance of our lncRNA-based risk model, we performed stratified Kaplan-Meier survival analysis for the OS in the training group based on the AJCC TNM stage: I, II, III, or IV (Fig. 4A-D). The five-lncRNA signature showed good predictive value for OS of stages II (P = 0.008), III (P = 0.02), and IV (P = 0.01), but not of stage I (P = 0.3).

To estimate the HR of each subgroup of patients as defined by gender, TNM stage, histologic grade, race and age($\geq$ or < 50 years) (Table 4), we used our models to divide the patients into two risk groups on the basis of the median cutoff value. Forest plots are shown in Fig. 5. Table S3 shows the HR of each subgroup of patients in GSE62252. The risk score model had a relatively good prognostic value in the clinical subgroups of gender, histologic grade and age. To improve the prognostic value of this model, we combined the clinical factors with the risk score model to construct a nomogram model for prognostic prediction. The nomogram model and calibration curve are shown in Fig. 6A-B. To evaluate the effect of the nomogram model, we calculated its C-index. The C-index for predicting the 4-year OS of GC patients was 0.69668, indicating that this model is a valuable indicator for prognostic prediction.

Potential functions of the five lncRNAs

To investigate the functions of the five lncRNAs in patients with GC, we calculated Pearson correlations between the five-lncRNA signature and 19605 protein-coding genes in the TCGA dataset. A total of 3069 genes (Table S4) positively correlated with at least one lncRNA (Pearson's coefficient> 0.4) (Fig. 7A). We further selected these genes for KEGG pathway enrichment analysis. By ranking based on –log$P$ value (Q value), we selected the top 10 pathways to draw a bubble plot (Fig. 7B) (Zeng et al., 2019; Deng et al., 2019). For biological processes, the co-expressed genes were mainly enriched in pathways involved in cancer, such as the focal adhesion pathway, cGMP−PKG signaling pathway and calcium signaling pathway. This finding indicates that the five lncRNAs may be involved in the regulation of tumor initiation and progress.

Discussion

In this study, we identified a potential signature involving five lncRNAs that are differentially expressed in tumor and normal tissues, and that might be valuable for prognostic prediction in GC. The prognostic performance of our lncRNA-based risk score model involving these five lncRNAs was verified using both TCGA and GEO datasets. Stratified analysis suggested that the

risk score model is valuable for prognostic prediction in GC patients with stage II to IV. To enhance the predictive accuracy of the model, we combined clinical parameters with the five-lncRNA signature to construct a nomogram model and confirmed its performance using a calibration curve and C index.

GC is a common malignancy of the digestive system (Siegel et al., 2019). Despite continuous improvements in treatment, the 5-year survival rate of patients with advanced GC is still approximately 20% (Min et al., 2019; Misawa et al., 2019). Therefore, early diagnosis, early identification of high-risk patients and the implementation of effective treatment measures as early as possible are warranted to improving survival. It is also important to develop novel prognostic indicators of GC. Over the past few decades, research has shown that protein-coding genes(Ghoorun et al., 2019; Luo et al., 2019) and microRNAs(Li et al., 2020; Zhou et al., 2019), play a vital role in the occurrence and development of various cancers, and can also be used to predict patient prognosis. Several nomogram models involving clinical factors have been constructed to predict the prognosis of GC. For example, Yue(Yu & Zhang, 2019)used tumor size and tumor site, as independent prognostic factors, to construct OS nomograms for predicting outcomes in GC patients, and the C-index of this model indicated that could predict prognosis. Recently, more lncRNAs related to GC prognosis have been discovered; however, prognostic prediction models involving lncRNAs still lack consensus. We present a nomogram including clinical factors and the five-lncRNA signature might be of value for prognostic prediction in GC.

Therefore, it is necessary to explore new biomarkers to improve the assessment of diagnosis and prognosis of GC because of the limitations of the TNM staging and some related scoring systems. Many lncRNAs have been identified, of which only few have been functionally annotated. However, there is evidence to indicate that lncRNAs, acting either as oncogenes or tumor suppressors, participate in the tumorigenesis and development of various cancers by regulating chromatin remodeling, transcription and post-transcriptional modification(Bartonicek et al., 2016; Iyer et al., 2015), and therefore might be valuable for cancer diagnosis and prognosis. Some studies have found that GC-related lncRNAs are involved in biological behaviors including the proliferation, migration, invasion, and autophagy of GC cells, thereby affecting the initiation and prognosis of GC (Mao et al., 2017; Wei & Wang, 2017). For example, the lncRNA MEG3 inhibits the proliferation, metastasis, and prognosis of GC cells by upregulating the expression p53—a key tumor suppressor (Wei & Wang, 2017). We identified five lncRNAs lncRNAs—LINC00205, TRHDE-AS1, OVAAL, LINC00106, and MIR100HG—as predictors of GC prognosis, and developed a risk-score model. Kaplan–Meier analysis suggested that our lncRNA-based risk model is valuable for predicting GC prognosis. We used two independent GEO datasets as validation datasets. Our results confirmed that our risk score model is stable and performs well in the prognostic prediction of GC.

Of the five lncRNAs, LINC00205, TRHDE-AS1, OVAAL, and MIR100HG, act as risk factors of GC, whereas LINC00106 is a protective factor. Apart from LINC00205 and MIR100HG, the other three lncRNAs have not been reported much in the literature. Our study identified LINC00205, TRHDE-AS1, OVAAL, and MIR100HG as potential prognostic biomarkers of GC for the first time. Consistent with our result, it has previously been reported that high expression of LINC00106 indicates prolonged OS in GC (Qi et al., 2020). Nevertheless, the role of this lncRNA in GC as well as its specific mechanism need to be further investigated. Interestingly, in hepatocellular carcinoma (HCC), comprehensive genome-wide analysis has revealed that the expression of LINC00205, a tumor suppressor, is positively associated with OS and recurrence-free survival (Cui et al., 2017). A study has showed that, as a competing endogenous RNA with lower expression levels in tumor tissues, LINC00205 may negatively regulate HCC progression via the miR-184/EPHX1 axis (Long et al., 2019), While

another study has indicated that LINC00205, can act as an oncogene, and can promote the proliferation, migration and invasion of HCC cells by targeting miR-122-5p(Zhang et al., 2019). In addition, LINC00205 can act as a protective factor in pancreatic cancer survival [HR = 0.58, *P* (log rank) = 0.0091](Giulietti et al., 2018). The reported role and therefore prognostic prediction value of LINC00205 in various cancers shows significant discrepancies. These discrepancies might be associated with the specificities of different cancers. It has been reported that the upregulation of TRHDE-AS1 inhibits the growth of lung carcinoma through competitive combination with the miRNA-103-KLF4 axis(Zhuan et al., 2019). One study has found that OVVAL is highly expressed in colon cancer and melanoma, and further experimental results showed that OVAAL promotes the proliferation of cancer cells via dual mechanisms controlling RAF/MEK/ERK signaling and p27-mediated cell senescence(Sang et al., 2018). The lncRNA MIR100HG has been studied as an oncogene in acute megakaryoblastic leukemia(Emmrich et al., 2014), laryngeal squamous cell carcinoma(Huang et al., 2019), and for its role in mediating cetuximab resistance via Wnt/β-catenin signaling(Lu et al., 2017) in colorectal cancer. Although the roles of these lncRNAs in cancer need to be further investigated, our results may provide a novel approach to study GC.

To further investigate the functions of the five lncRNAs in GC, we performed pathway enrichment analysis. These genes are enriched in cancer regulation, including cGMP−PKG signaling pathway, calcium signaling pathway, and focal adhesion pathway etc. This finding suggests that the five lncRNAs may play an important role in the occurrence and development of GC. There is evidence that lncRNAs can promote tumorigenesis through the cGMP−PKG signaling pathway. For example, the overexpression of SRRM2-AS accelerates angiogenesis in nasopharyngeal carcinoma via the cGMP−PKG signaling pathway(Chen et al., 2019). It has been reported the calcium signaling pathway mainly involved in metabolic diseases and heart diseases over the past years(Berridge, 2016; Dewenter et al., 2017). A research showed that calcium signaling pathway was associated with cancer cell survival, but more details on how it affects are still to be studied(Reczek & Chandel, 2018). Focal adhesion are special sites where integrin receptors aggregated in cells interact with extracellular matrix and intracellular actin skeleton(Burridge, 2017), and it plays a critical role in tumor invasion and migration(Shen et al., 2018). There is evidence that knock down of the Linc01060 could promote the pancreatic cancer progression via Vinculin-Mediated Focal Adhesion pathway Turnover(Shi et al., 2018). However, whether the lncRNA can mediate the progress of GC through Focal Adhesion pathway is less reported. In short, lncRNA may participate in the genesis and development of various tumors through the above pathways.

Risk score model is a common and widely available method to predict the prognosis of patients with multiple diseases(Lemke et al., 2017; Li et al., 2018; Yang et al., 2017; Sobotka et al., 2018). Our risk score was determined by the expression of independent survival-lncRNAs obtained after Cox hazard analyses and its corresponding coefficients. It was calculated by using the binary lncRNA expression values according to the medians of original lncRNA expression values. This adjustment would help to improve the clinical application of prognostic model in other study population(Zhang et al., 2018). In general, the higher the risk score, the worse the prognosis, which is consistent with our analysis. Our Kaplan–Meier survival analysis showed that the patients in high risk group significantly had a poorer prognosis than in low risk group. Our risk score model based on lncRNAs has several advantages. This model based on the expression of 5-lncRNA provides a novel non-invasive method for predicting the prognosis of GC patients before surgery. Compared with traditional invasive pathological examinations, it reduces unnecessary pain for patients. Secondly, this 5-lncRNA risk model can provide preoperative risk predictive probability of individual mortality and recurrence in different clinical endpoints. It is simple and convenient for clinicians and patients to understand. Thirdly, our model used the

median of 5-lncRNA-based risk score as the cutoff value to divide patients into high risk and low risk groups. It can timely identify those patients at high risk of death or recurrence, and prompt clinicians take clinical interventions as early as possible to improve their prognoses.

There have been several reports on the lncRNA signatures for GC. A previous study has reported a 24-lncRNA signature can predict outcomes in GC patients by applying the random survival forest-variable hunting (RSF-VH) algorithm using the GEO datasets (Zhu et al., 2016). However, due to the limited amount of data in the GEO datasets, the lncRNAs identified in this study might not represent the complete population of lncRNAs involved in GC. In our study, we integrated a total of 950 samples from the TCGA and GEO databases to comprehensively investigate the potentially prognostic lncRNAs. This greatly improves the accuracy, reliability and robustness of our model. A 6-lncRNA prognostic signature was established by Robust likelihood-based survival and LASSO model(Ma et al., 2019). Whether the 6-lncRNA combined with other clinical features can promote the predictive power remains blank. To improve the accuracy of the five-lncRNA prognosis model, we combined it with clinical factors to develop a nomogram model which could predict the OS of GC patients. Zhu(Zhu et al., 2018) et al constructed a 11-lncRNA signature by univariate and multivariate Cox regression analyses. Although an internal validation was validated by using the bootstrap resampling method, external validation studies are needed to further evaluate the value of this model. We not only included two external verification datasets, but also performed survival analysis, ROC curve, and forest plot for predictive verification, indicating a favorable effectiveness of our model.

Of course, there are some limitations of the present study. We integrated the data from the TCGA and GEO databases to improve the amount of the cases, reducing bias from a small sample size. Integrated analysis has been proved to be an effective approach for multiple datasets with different platforms using R package (Zhang et al., 2019), thus promoting reliability of our conclusion (Ma et al., 2017). However, TCGA dataset has more lncRNAs than the GEO (14147:1397) because of different sequencing technology: TCGA used RNA sequencing technology, while GEO used microarray chip technology. Intersection of three datasets has inevitably omitted potential prognostic lncRNAs. Moreover, the clinical characteristics of patients in three datasets are heterogeneous. This might have inevitably led to a bias. Besides, owing to the lack of DFS and clinical data in GSE15459, we used only one external validation group to verify the prognostic value of the five-lncRNA signature for the DFS of patients. In addition, many important variables affecting the prognosis of patients with GC are not provided in TCGA and GEO datasets, such as dietary habit, previous disease, history of chemotherapy or radiation, and family history of cancer. Thus, on the one hand, it is necessary to perform a large-scale multi-center prospective clinical study based on the same sequencing technology to decrease the bias mentioned above. On the other hand, based on existing data, it is beneficial to develop innovative statistical algorithms to reduce the heterogeneity of different data sources. Last, due to the limitated studies regarding these lncRNAs so far, experimental research on these lncRNAs is highly warranted to further understand their functions in GC in the future.

Conclusions

We established a risk score model including five lncRNAs to predict GC patients' OS and DFS, particularly in those with Ⅱ-Ⅳ stage. Our findings also provided evidence of developing effective prognostic biomarkers for GC patients and potential therapeutic targets in the future.

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Figure 1

The expression information of five lncRNAs, overall survival and disease free survival in gastric cancer patients in the TCGA dataset.

(A) Volcano plot with blue dots indicating five lncRNAs expression levels which is significantly different between tumor and normal tissue based on the criteria of an absolute log2 fold change (FC)>1 and adjusted $P < 0.05$. (B) Heatmap of the five-lncRNA expression profile of the 414 patients in the TCGA dataset. Among five lncRNAs, MIR100HG and TRHDE-AS1 have a similar expression in 414 patients in the TCGA dataset, otherwise the other three lncRNAs do as well. (C-D) The survival curves based on the OS and DFS of the 408 patients in TCGA dataset.

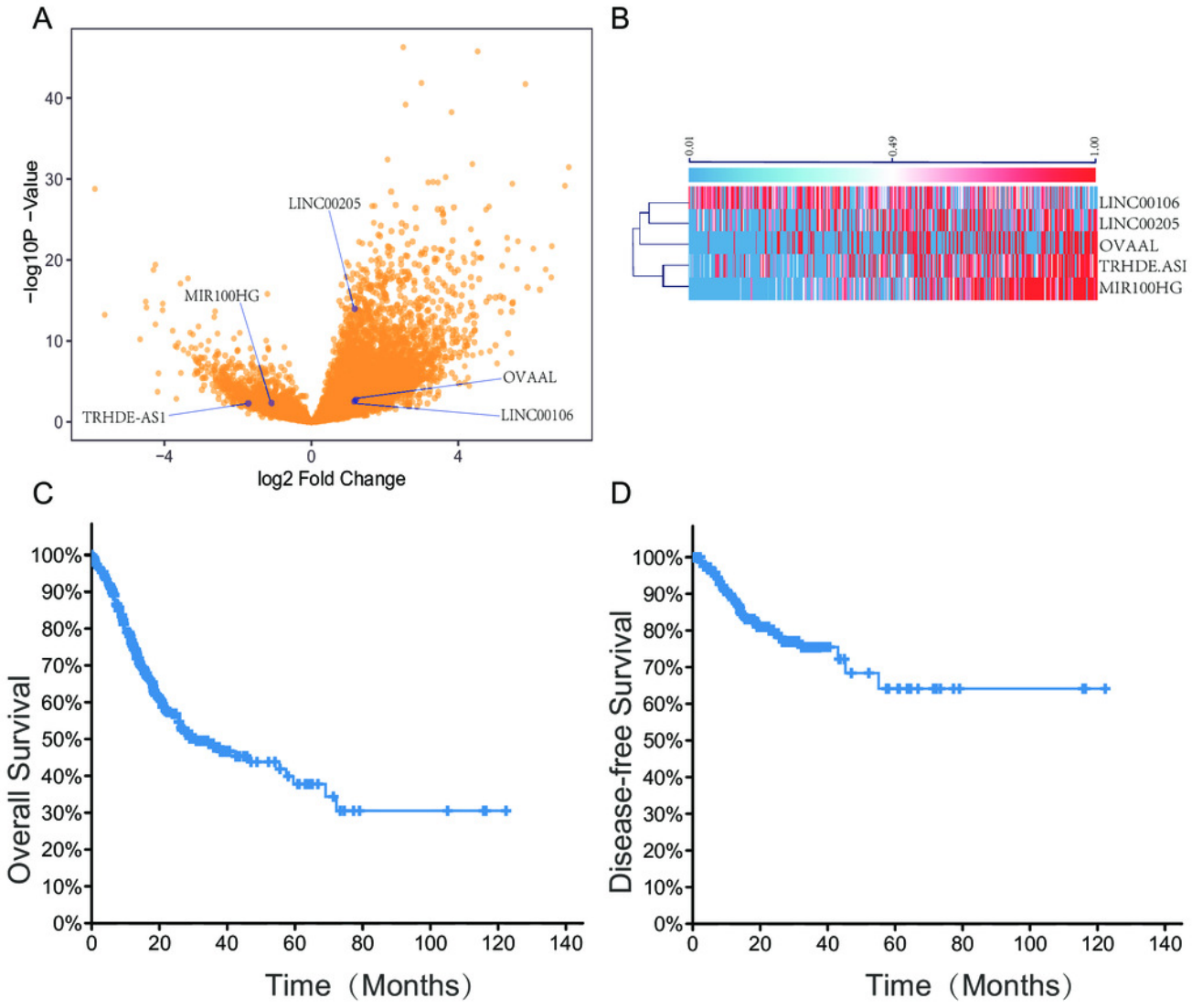

A
−log10P −Value
log2 Fold Change
LINC00205
MIR100HG
OVAAL
TRHDE-AS1
LINC00106
B
LINC00106
LINC00205
OVAAL
TRHDE.ASI
MIR100HG
C
Overall Survival
Time（Months）
D
Disease-free Survival
Time（Months）

Figure 2

The prognostic value of lncRNA-based risk model in training group.

(A-B) Kaplan-Meier analysis of patients' OS and DFS in the high risk (n = 204) and low risk (n = 204) subgroups of the training group. (C) The scatter plot of lncRNA-based risk model distribution for patient survival status. (D) The percentage of patient survival status in the high risk and low risk subgroups of the training group. (E) The lncRNA-based risk model distribution for patient recurrence. (F) The percentage of patient recurrence in the high risk and low risk subgroups of the training group. (G-H) The time-dependent ROC analysis of the risk score for prediction the 4-year cutoff OS and 2-year cutoff DFS of the training group. The area under the curve was calculated for ROC curve. ***P<0.001.

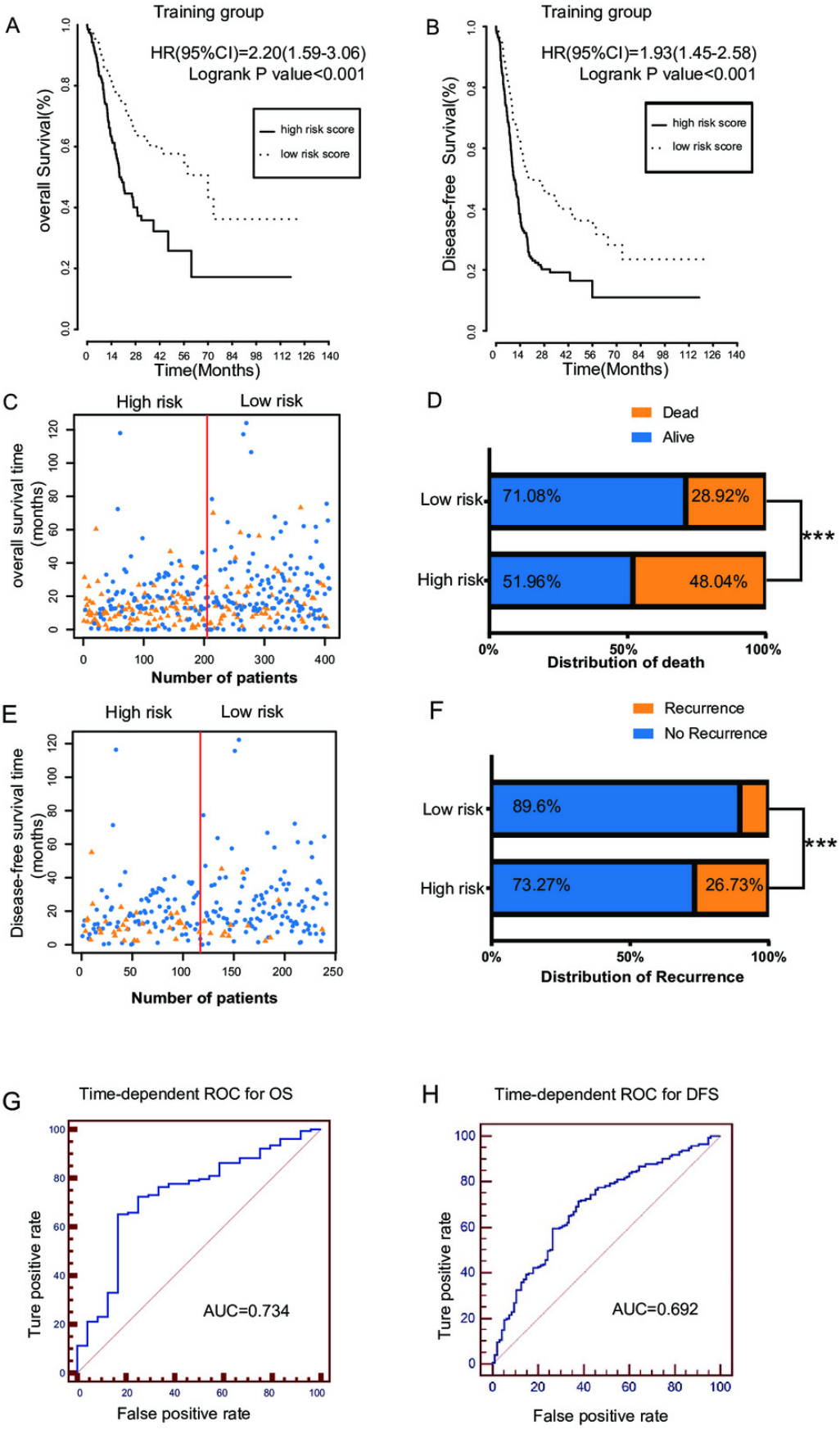

A
Training group
HR(95%CI)=2.20(1.59-3.06)
Logrank P value<0.001
high risk score
low risk score
overall Survival(%)
Time(Months)
B
Training group
HR(95%CI)=1.93(1.45-2.58)
Logrank P value<0.001
high risk score
low risk score
Disease-free Survival(%)
Time(Months)
C
High risk
Low risk
overall survival time (months)
Number of patients
D
Dead
Alive
Low risk
71.08%
28.92%
High risk
51.96%
48.04%
***
0%
50%
100%
Distribution of death
E
High risk
Low risk
Disease-free survival time (months)
Number of patients
F
Recurrence
No Recurrence
Low risk
89.6%
High risk
73.27%
26.73%
***
0%
50%
100%
Distribution of Recurrence
G
Time-dependent ROC for OS
Ture positive rate
AUC=0.734
False positive rate
H
Time-dependent ROC for DFS
Ture positive rate
AUC=0.692
False positive rate

Figure 3

The prognostic value of lncRNA-based risk model in two independent GEO validation groups.

(A-B) Kaplan-Meier analysis of predicting OS of GC patients based on the high risk and low risk subgroups in two independent validation groups (GSE62254 and GSE15459). (C-D) The scatter plot of five-lncRNA-based risk score distribution for patient survival status in two independent validation groups.(E-F) The time-dependent ROC analysis of the risk score for prediction the 4-year cutoff OS of the two independent validation groups. The area under the curve was calculated for ROC curve.

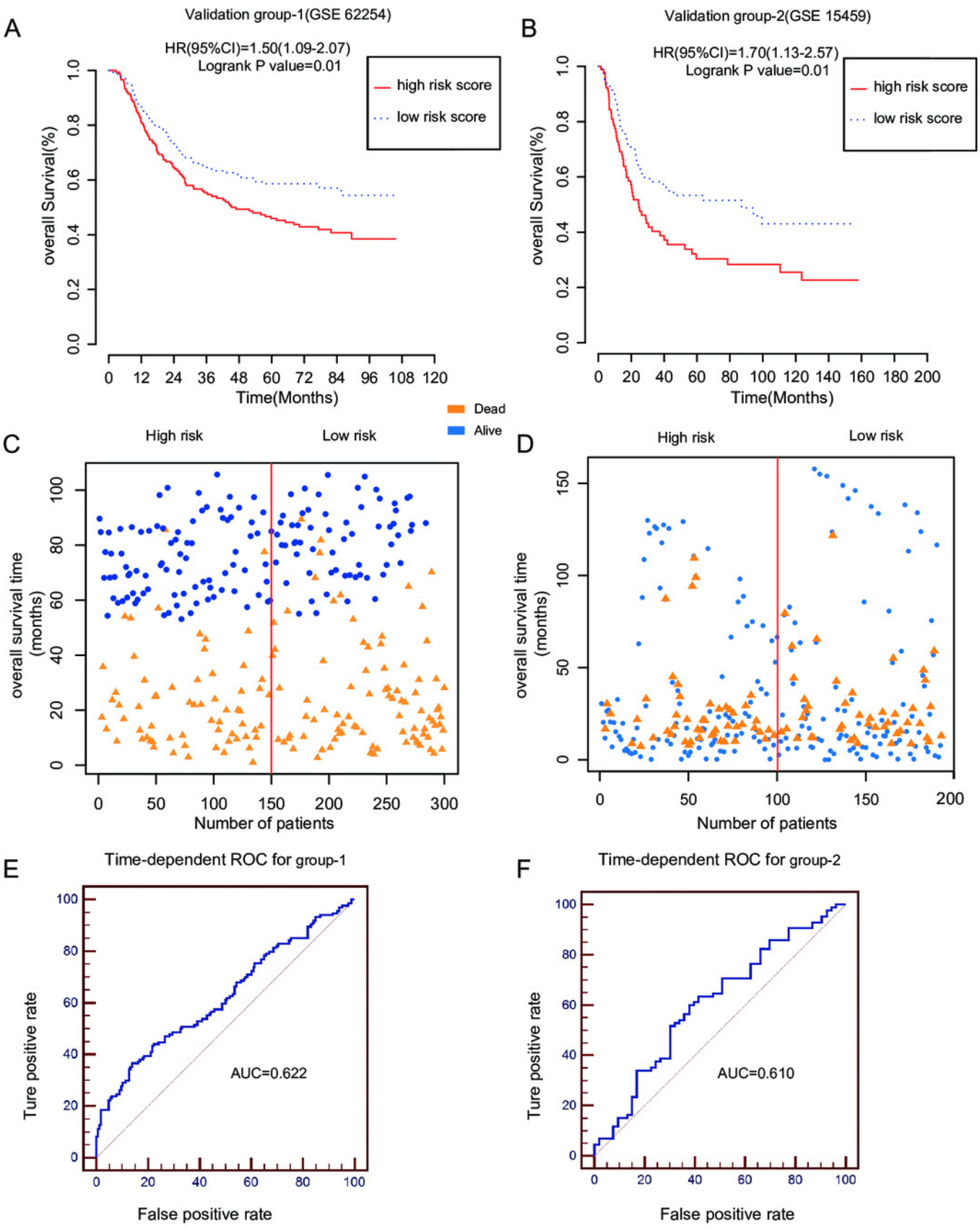

A
Validation group-1(GSE 62254)
HR(95%CI)=1.50(1.09-2.07)
Logrank P value=0.01
high risk score
low risk score
overall Survival(%)
Time(Months)
B
Validation group-2(GSE 15459)
HR(95%CI)=1.70(1.13-2.57)
Logrank P value=0.01
high risk score
low risk score
overall Survival(%)
Time(Months)
C
Dead
Alive
High risk
Low risk
overall survival time (months)
Number of patients
D
High risk
Low risk
overall survival time (months)
Number of patients
E
Time-dependent ROC for group-1
AUC=0.622
Ture positive rate
False positive rate
F
Time-dependent ROC for group-2
AUC=0.610
Ture positive rate
False positive rate

Figure 4

The prognostic value of lncRNA-based risk model in subgroups according to the TNM stage.

(A-D) Kaplan-Meier analysis of the OS of GC patients with stageⅠ, Ⅱ, Ⅲ and Ⅳ, respectively.

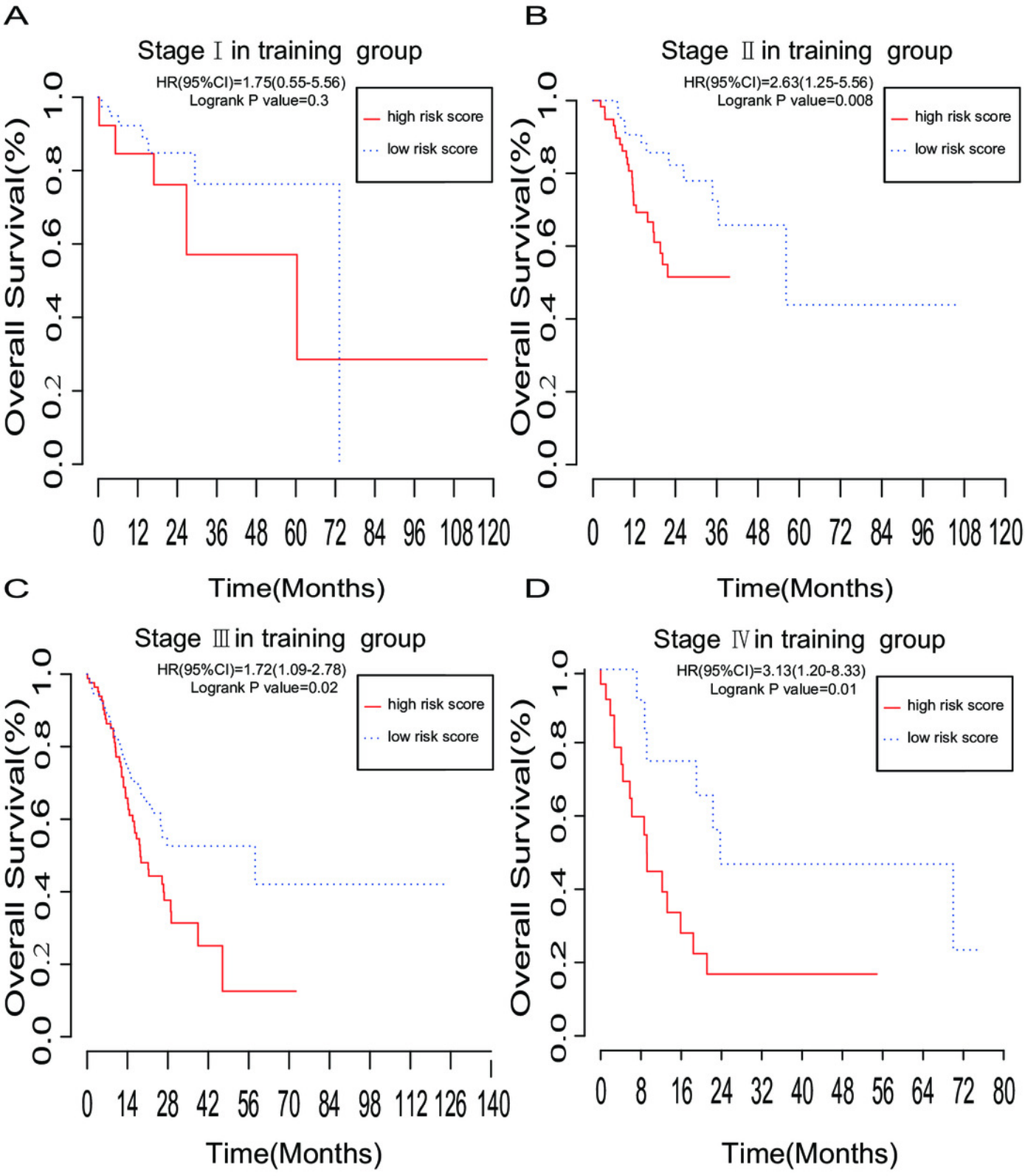

A
Stage Ⅰ in training group
HR(95%CI)=1.75(0.55-5.56)
Logrank P value=0.3
high risk score
low risk score
Overall Survival(%)
0.0 0.2 0.4 0.6 0.8 1.0
0 12 24 36 48 60 72 84 96 108 120
Time(Months)
B
Stage Ⅱ in training group
HR(95%CI)=2.63(1.25-5.56)
Logrank P value=0.008
high risk score
low risk score
Overall Survival(%)
0.0 0.2 0.4 0.6 0.8 1.0
0 12 24 36 48 60 72 84 96 108 120
Time(Months)
C
Stage Ⅲ in training group
HR(95%CI)=1.72(1.09-2.78)
Logrank P value=0.02
high risk score
low risk score
Overall Survival(%)
0.0 0.2 0.4 0.6 0.8 1.0
0 14 28 42 56 70 84 98 112 126 140
Time(Months)
D
Stage Ⅳ in training group
HR(95%CI)=3.13(1.20-8.33)
Logrank P value=0.01
high risk score
low risk score
Overall Survival(%)
0.0 0.2 0.4 0.6 0.8 1.0
0 8 16 24 32 40 48 56 64 72 80
Time(Months)

Figure 5

Forest plot to evaluate prognostic value of lncRNA-based risk model in subgroups divided by clinical factors.

Forest plot for clinic subgroup

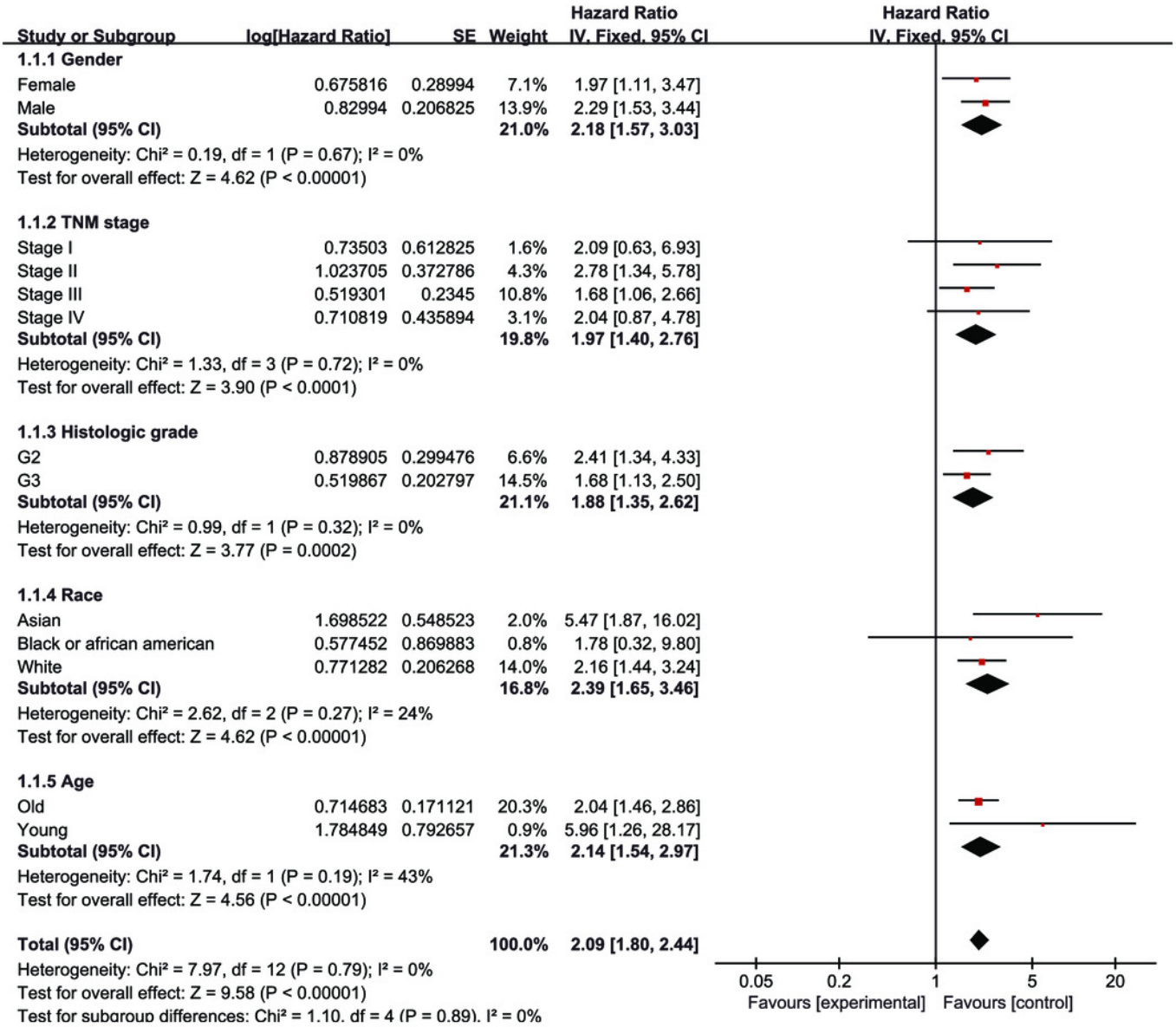
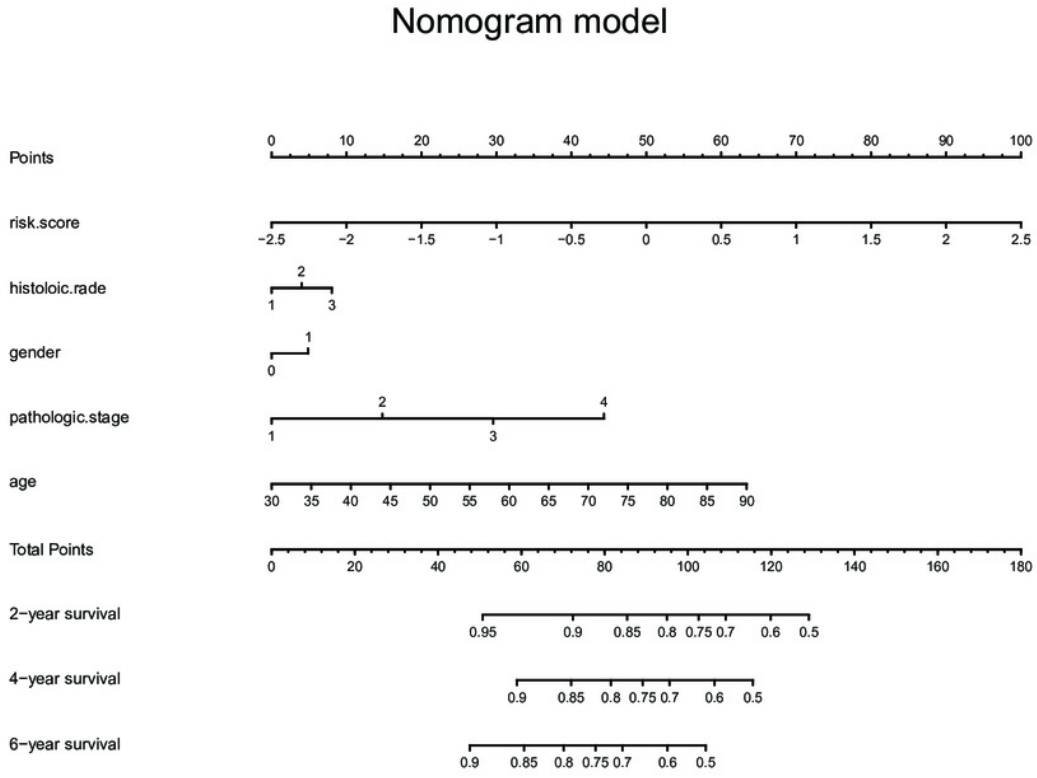


Figure 6

The prognostic value of a nomogram model combining five-lncRNA signature with the clinical factors.

(A) A nomogram model combining five-lncRNA signature with the clinical factors for predicting the 4-year OS of GC patients. (B) The nomogram calibration curve to evaluate the prediction of 4-year OS of GC patients. The C index of this model was also calculated.

A


Nomogram model
Points
0 10 20 30 40 50 60 70 80 90 100
risk.score
−2.5 −2 −1.5 −1 −0.5 0 0.5 1 1.5 2 2.5
histoloic.rade
2
1 3
gender
1
0
pathologic.stage
2 4
1 3
age
30 35 40 45 50 55 60 65 70 75 80 85 90
Total Points
0 20 40 60 80 100 120 140 160 180
2−year survival
0.95 0.9 0.85 0.8 0.75 0.7 0.6 0.5
4−year survival
0.9 0.85 0.8 0.75 0.7 0.6 0.5
6−year survival
0.9 0.85 0.8 0.75 0.7 0.6 0.5

B

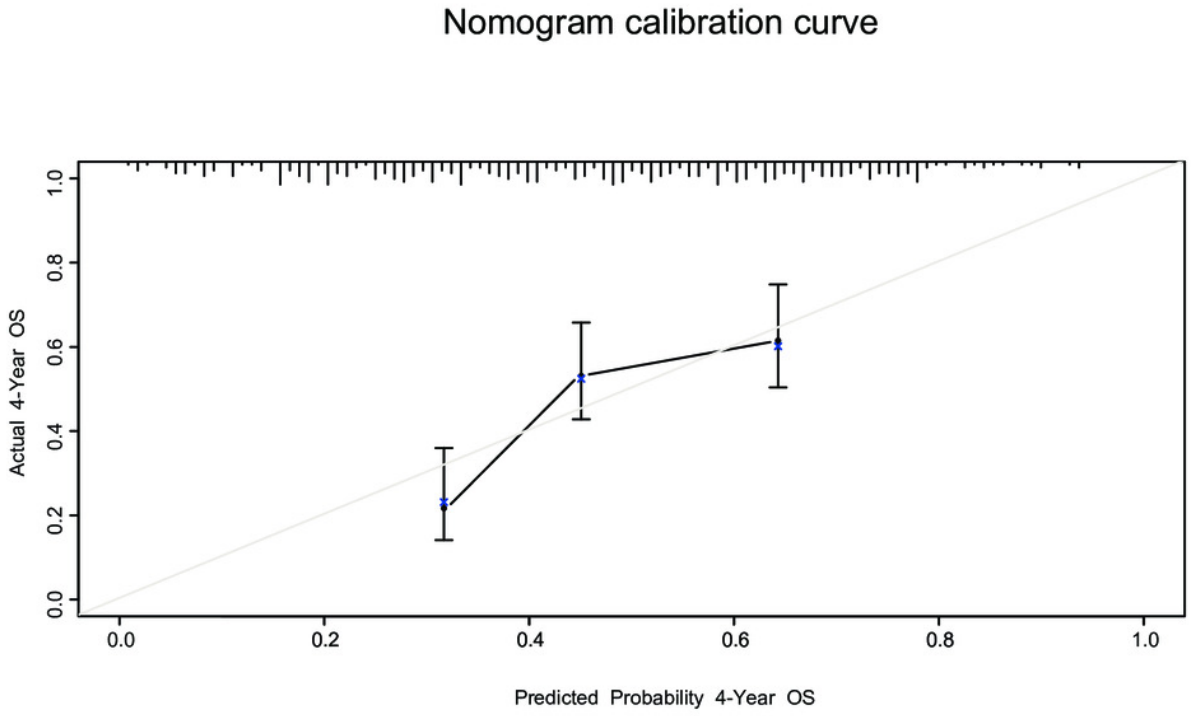
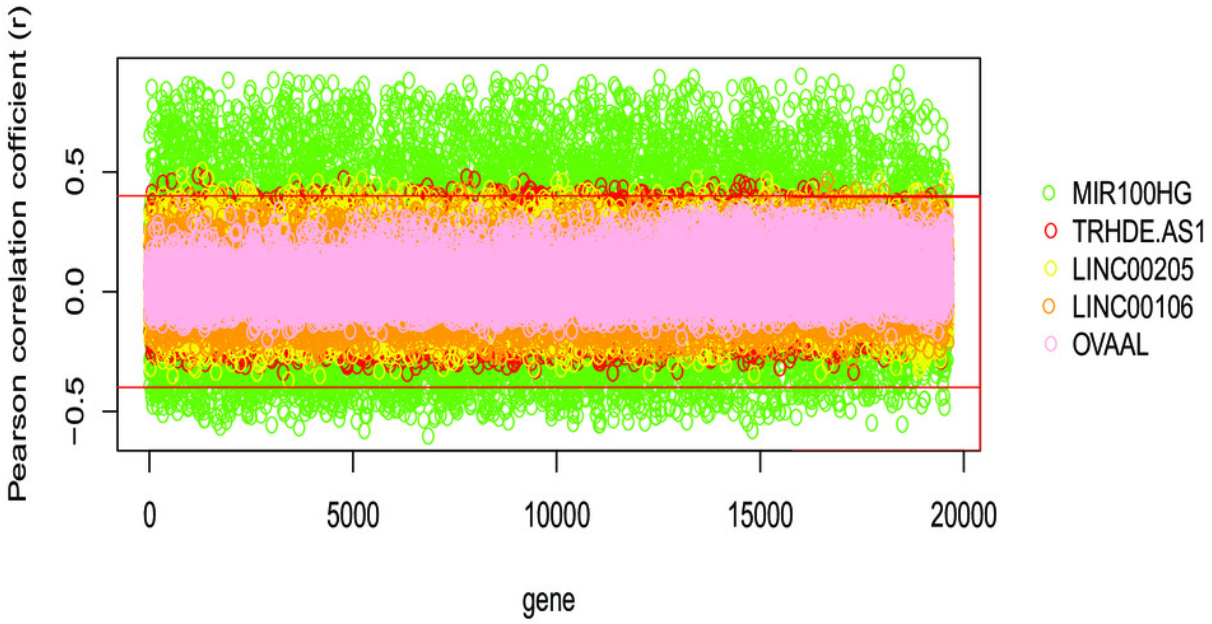

Nomogram calibration curve
Actual 4-Year OS
0.0 0.2 0.4 0.6 0.8 1.0
Predicted Probability 4-Year OS
0.0 0.2 0.4 0.6 0.8 1.0

Figure 7

Potential functions of the five lncRNAs

(A) The Pearson correlation coefficient between 19605 protein-coding genes and five lncRNAs in TCGA dataset. (B) The functional enrichment bubble map of pathways by KEGG pathway analysis. Bubble size represents the number of gene enriched in the pathway.

A


Pearson correlation cofficient (r)
0.5
0.0
−0.5
0
5000
10000
15000
20000
gene
MIR100HG
TRHDE.AS1
LINC00205
LINC00106
OVAAL

B

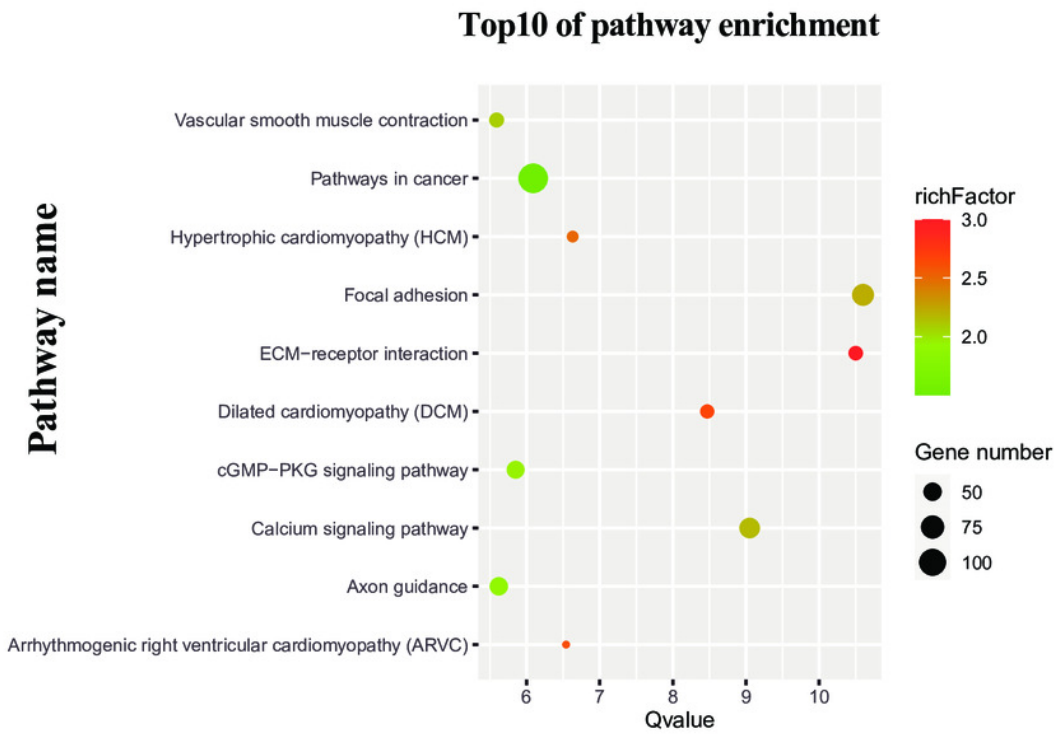

Top10 of pathway enrichment
Pathway name
Vascular smooth muscle contraction
Pathways in cancer
Hypertrophic cardiomyopathy (HCM)
Focal adhesion
ECM−receptor interaction
Dilated cardiomyopathy (DCM)
cGMP−PKG signaling pathway
Calcium signaling pathway
Axon guidance
Arrhythmogenic right ventricular cardiomyopathy (ARVC)
6
7
8
9
10
Qvalue
richFactor
3.0
2.5
2.0
Gene number
50
75
100

Table 1(on next page)

Five lncRNAs significantly associated with prognosis of GC patients in the training group.

Derived from the multivariable Cox proportional hazards regression analysis in the training group.

LncRNA name	Ensemble ID	Chr.	Coordinate	Coefficient	Hazard ratio	P value
LINC00205	ENSG00000223768.1	21	45288052-45297354	0.249092	1.373451497	0.047216345
TRHDE-AS1	ENSG00000236333.3	12	72253507-72273509	0.182045	1.846654514	0.000109193
OVAAL	ENSG00000236719.2	1	180558974-180566518	0.271169	1.880897277	0.0000744
LINC00106	ENSG00000236871.6	X&Y	1397025-1399412	-0.207942	0.624972486	0.003469142
MIR100HG	ENSG00000255248.6	11	122028329-122422871	0.502539	1.396343319	0.036829012

1
2

Table 2(on next page)

The clinical features of GC patients in training group and GSE62254.

	Training group		Validation group-1 (GSE62254)	
Variables	**n=408**	**%**	**n=300**	**%**
Gender				
Male	263	64.46	199	66.33
Female	145	35.54	101	33.67
Age				
Old (>=50 years old)	377	92.40	262	87.33
Young (<50 years old)	31	7.60	38	12.67
TNM stage				
Stage Ⅰ	55	13.48	30	10.00
Stage Ⅱ	120	29.41	96	32.00
Stage Ⅲ	167	40.93	95	31.67
Stage Ⅳ	41	10.05	79	26.33
Not Available	25	6.13	0	
T stage				
T1	20	4.90	2	0.67
T2	87	21.32	186	62.00
T3	178	43.63	91	30.33
T4	114	27.94	21	7.00
TX	9	2.21	0	
N stage				
N0	120	29.41	38	12.67
N1	110	26.96	131	43.67
N2	77	18.87	80	26.67
N3	82	20.10	51	17.00
NX	17	4.17	0	
Not Available	2	0.49	0	
M stage				
M0	362	88.73	273	91.00
M1	27	6.62	27	9.00
MX	19	4.66	0	
Survival status				
Alive	251	61.52	148	49.33
Dead	157	38.48	152	50.67

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Table 3(on next page)

The schematic workflow of the present study.

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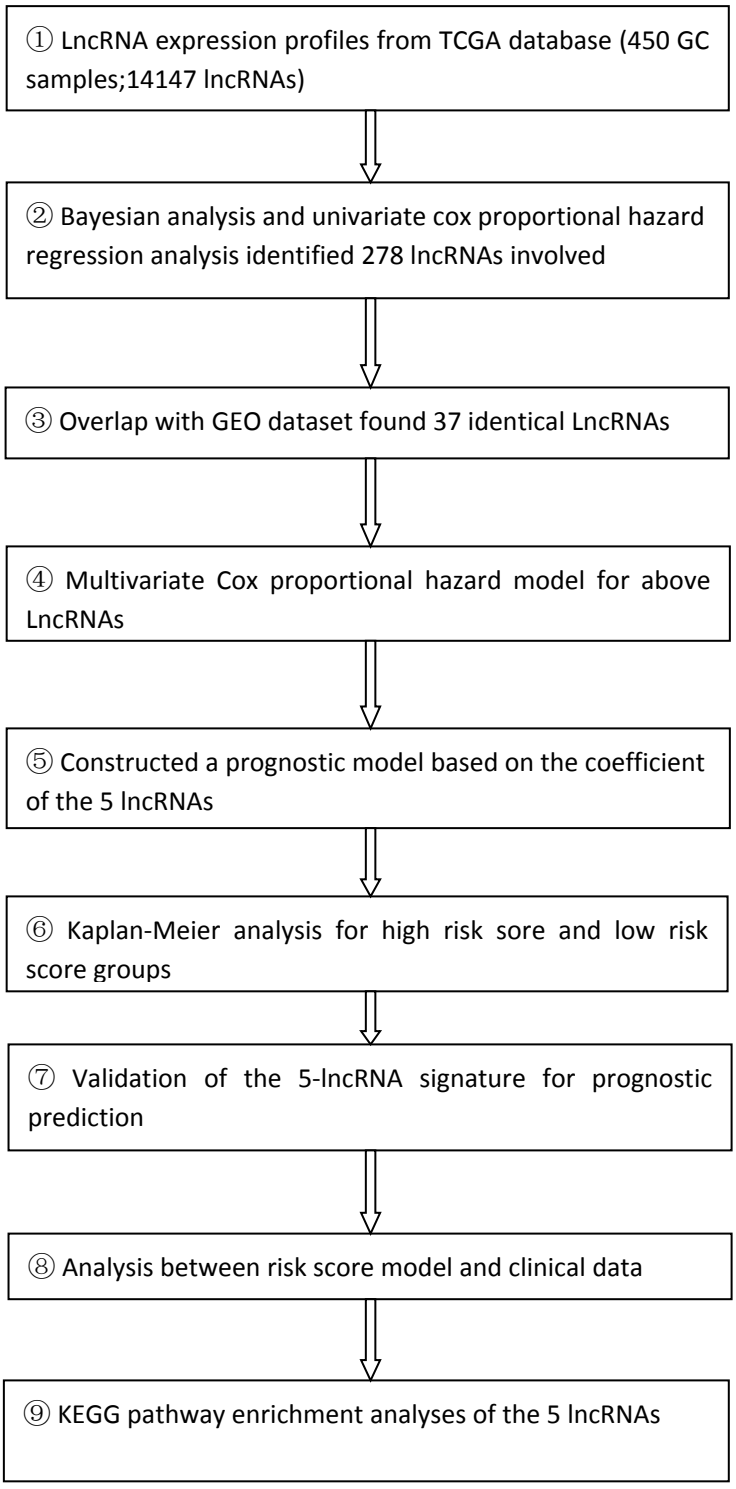
① LncRNA expression profiles from TCGA database (450 GC samples;14147 lncRNAs)
② Bayesian analysis and univariate cox proportional hazard regression analysis identified 278 lncRNAs involved
③ Overlap with GEO dataset found 37 identical LncRNAs
④ Multivariate Cox proportional hazard model for above LncRNAs
⑤ Constructed a prognostic model based on the coefficient of the 5 lncRNAs
⑥ Kaplan-Meier analysis for high risk sore and low risk score groups
⑦ Validation of the 5-lncRNA signature for prognostic prediction
⑧ Analysis between risk score model and clinical data
⑨ KEGG pathway enrichment analyses of the 5 lncRNAs

Table 4(on next page)

The association between five-lncRNA signature and OS of GC patients in training group.

Abbreviations: HR, Hazard ratio; 95%CI, 95% confidence interval.

	Number (High Risk score/Low Risk score)	HR (95%CI)	P value
Total	204/204	2.09 (1.80, 2.44)	0.000001
Gender			
Male	129/134	2.29 (1.53, 3.44)	0.00002
Female	75/70	1.97 (1.11, 3.47)	0.01
Histologic grade			
G2	47/97	2.41 (1.34, 4.33)	0.0006
G3	146/97	1.68 (1.13, 2.50)	0.02
Race			
Asian	44/41	5.47 (1.87, 16.02)	0.001
Black or african american	4/8	1.78 (0.32, 9.80)	0.6
White	138/120	2.16 (1.44, 3.24)	0.0003
Age			
Old (>=50 years old)	186/191	2.04 (1.46, 2.86)	0.00001
Young (<50 years old)	18/13	5.96 (1.26, 28.17)	0.008
TNM stage			
Stage I	14/41	2.09 (0.63, 6.93)	0.3
Stage II	62/58	2.78 (1.34, 5.78)	0.008
Stage III	87/77	1.68 (1.06, 2.66)	0.02
Stage IV	25/16	2.04 (0.87, 4.78)	0.01

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