Integrative analysis of miRNA and mRNA expression profiles reveals a novel mRNA/miRNA signature to improve risk classification for patients with gastric cancer

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Abstract. Gastric cancer (GC) is one of the most common types of malignant cancer and is associated with poor prognosis. Although the prognosis of patients with GC is associated with grade, stage and lymph node metastases, these traditional clinical features are inadequate to predict the outcome of GC. Therefore, there has been an increased focus on identifying novel molecular biomarkers for early diagnosis and prognosis, in order to improve outcomes in GC. In the present study, an integrative analysis of microRNA (miRNA) expression profiles, mRNA expression profiles and clinical characteristics was performed in a large cohort of patients with GC in order to identify an integrative prognostic model for improving postoperative risk classification. An integrative mRNA/miRNA signature (IMMIS), comprised of three miRNAs and one mRNA, was identified from a large number of differentially expressed miRNAs and mRNAs using univariate and multivariate Cox regression analysis. The prognostic value of the IMMIS was validated in the discovery cohort, testing cohort and The Cancer Genome Atlas (TCGA) cohort. The present results suggested that the identified signature had a reliable predictive performance and could classify the patients into high- and low-risk groups with significantly different overall survival times. In the discovery cohort, the hazard ratio (HR) was 2.805 with a 95% CI=1.722-4.567 (P<0.001). The median overall survival time as 1.49 vs. 3.85 years. In the testing cohort, the HR was 1.625 with a 95% CI=1.004-2.638 (P=0.039) and the median overall survival time was 2.17 vs. 4.62 years. In the TCGA cohort, the HR was 2.139 with a 95% CI=1.519-3.012 (P<0.001) and the median overall survival time was 1.53 vs. 4.62 years. The IMMIS constituted a reliable independent prognostic factor compared with clinical covariates, including age, sex, grade and stage, as indicated by multivariate and stratified analyses. Furthermore, comparative analysis revealed that the predictive value of the IMMIS was superior to the mRNA-based signature alone. The present results suggested the potential value of the IMMIS as a promising novel biomarker for improving the clinical management of patients with GC.

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Introduction

Gastric cancer (GC), additionally referred to as stomach cancer, is the one of most common types of digestive cancer worldwide and is the third leading cause of cancer-related death (1). China is one of the high-risk areas for digestive cancer, and GC is ranked the second most common cancer behind lung cancer in China (2). Notably, an estimated 477.7 individuals per 100,000 newly diagnosed cases and 158.7 per 100,000 mortalities were reported in China in 2015 (2). Gastric adenocarcinomas are the most common histological type of GC, which account for ~90% of GC cases (3). Although the survival rate of patients with GC has improved over the past years, the prognosis remains unsatisfactory, with a 5-year survival rate of 10% for patients with localized gastric adenocarcinoma and 50% for patients with early resectable gastric adenocarcinoma (4). Although the prognosis of patients with GC is associated with grade, stage and lymph node metastases, these traditional clinical features are inadequate to predict the outcome of GC. Therefore, there is an increasing focus on identifying novel molecular biomarkers for early diagnosis and prognosis to improve outcomes in patients with GC.

With the development of high throughput sequencing technologies for transcriptional analysis, including microarray and RNA sequencing (-Seq), researchers have increased their efforts in trying to identify molecular biomarkers for predicting the outcome of patients with GC at the transcriptional levels. Several mRNA or micro RNA (miRNA) expression signatures have been proposed to predict the survival of patients with GC. For example, Chen *et al* (5) developed a three-mRNA survival prediction model using cDNA microarray data from 18 pairs of cancerous and

noncancerous gastric tissues. A previous study performed by Cho *et al* (6) constructed a six-gene expression-based prognostic risk score to predict the likelihood of relapse after curative resection. For miRNAs, Ding *et al* (7) identified an eight-miRNA signature for predicting the overall survival in 380 patients with gastric adenocarcinoma. However, previous studies on other types of cancer have shown that, notwithstanding the importance of individual RNA, intrinsic multi-RNA-based expression signatures have greater prognostic value (8-10). However, to the best of our knowledge, no integrated studies concerning their prognostic significance have been reported yet.

The aim of the present study was to develop and assess the predictive value of an integrated multi-gene signature by analyzing mRNA and miRNA expression profiles, and clinical information in a large cohort of patients with GC.

Materials and methods

Patient datasets. The Cancer Genome Atlas (TCGA) database (cancergenome.nih.gov/) was used to identify the following data: i) Normalized level-3 RNA-Seq data of 375 GC samples and 32 normal tissue samples obtained with HTSeq (version 0.6.1; cancergenome.nih.gov/); ii) normalized level-3 miRNA-Seq data of 436 GC samples; and iii) 41 normal tissue samples obtained with Illumina HiSeq (Illumina, Inc.). For all datasets, corresponding clinical data were collected and analyzed. The Ensembl gene ID of protein-coding genes was retrieved from the HUGO Gene Nomenclature Committee database (www.genenames.org/). Cross-referencing of the Ensembl gene ID with tumor barcodes was performed. Furthermore, data of patients with incomplete expression data and follow-up information were removed from the analysis. A total of 18,528 mRNAs in 407 samples, 1,573 miRNAs in 477 patients and 438 samples with survival information were retained for further analysis in the present study.

Development of an integrative mRNA/miRNA signature (IMMIS). Differentially expressed mRNAs and miRNAs in GC and normal tissue samples were detected using the DEGseq2 package (11). An adjusted $P<0.05$ was used with Benjamini-Hochberg (B-H) correction and a $|\log2 \text{ (fold change)}| >1$ applied. Subsequently, univariate Cox proportional hazards analyses were used to identify prognostic mRNAs and miRNAs from the differentially expressed mRNAs and miRNAs. Finally, an IMMIS was constructed as the linear combination of expression values of each prognostic mRNA and miRNA, which was weighted by their estimated regression coefficients in the multivariate Cox regression analysis (12-18).

Statistical analysis. The differences in overall survival time between the high- and low-risk groups were assessed using Kaplan-Meier survival plots and log-rank tests. Univariate and multivariate Cox regression analyses for overall survival were performed on the individual clinical variables with and without the IMMIS in each cohort. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. All statistical analyses were performed using R-version3.5.2 (http://www.R-project.org/) (19).

Results

Identification of differentially expressed mRNAs and miRNAs between GC samples and normal tissue samples. By comparing the expression profiles of mRNAs and miRNAs between GC samples and normal tissue samples, a total of 4,221 mRNAs and 201 miRNAs were identified to be differentially expressed in GC samples and normal tissue samples according to the DEGseq2 package method with $|\log2 \text{ (fold change)}| >1$ and an adjusted $P<0.05$ after B-H adjustment. These profiles were used for subsequent survival analyses. Among the differentially expressed autophagy-associated mRNAs and miRNAs, 2,055 mRNAs and 138 miRNAs were upregulated, and 2,166 mRNAs and 63 miRNAs were downregulated in GC samples compared with normal tissue samples.

Identification of independent prognostic mRNAs and miRNAs. In order to identify and validate an IMMIS, samples, corresponding clinical data, and mRNA and miRNA expression profiles were initially cross-referenced with tumor barcodes. A total of 361 samples were retained for subsequent survival analyses. All 361 samples with survival information were randomly divided into two equal patient cohorts: i) The discovery cohort (n=181); and ii) testing cohort (n=180). The detailed clinical information of the discovery cohort and testing cohort are provided in Table I. Statistical analysis indicated that the clinical characteristics were similar in the discovery and testing cohorts (Table I). The association between the expression level of differentially expressed mRNAs and miRNAs with the overall survival of patients with GC using the univariate Cox analyses was then examined. A total of 12 RNAs (including 11 mRNAs and 1 miRNA; FDR<0.05) were observed to be significantly associated with overall survival, and were considered candidate prognostic RNAs. All 12 prognostic RNAs were identified as risk factors with positive coefficients (Table II), and high expression of these RNAs was associated with a poor outcome.

Establishment of an IMMIS for survival prediction in the discovery cohort. To construct an IMMIS for survival prediction, the 12 RNAs were fitted into a multivariate Cox regression model in the discovery cohort. Notably, the IMMIS was constructed using the determined expression of 12 prognostic RNAs and multivariate Cox regression coefficient as follows: Risk score=(0.5677x RasGEF domain family member 1C) + [0.0035x dual specificity phosphatase 1 (DUSP1)] + (0.0807x Rap associating with DIL domain) + [0.0281x ADAM metallopeptidase with thrombospondin type 1 motif 8 (ADAMTS8)] + [(-0.0095x atypical chemokine receptor 3 (ACKR3)] + (0.0096x guanidinoacetate N-methyltransferase) + [(-0.1882x collagen and calcium binding EGF domains 1 (CCBE1)] + (0.0024x serpin family E member 1) + (0.4322x doublecortin like kinase 3) + (0.6609x developmental pluripotency associated 2) + (0.1356x integrin binding sialoprotein) + (0.0110x miRNA-184). With the identified IMMIS, 181 patients with GC from the discovery cohort were divided into a high-risk group (n=91) and a low-risk group (n=90) according to the

Table I. Clinical features of the patients with gastric cancer in the discovery cohort, testing cohort and the entire TCGA cohort.

Clinicopathological characteristic	Discovery cohort, n=181	Testing cohort, n=180	Entire TCGA cohort, n=361	P-value	High risk group, n=180	Low risk group, n=181	P-value
Age				0.424[a]			0.492[a]
<68	87	94	181		95	86	
≥68	92	82	174		84	90	
Sex				0.713[a]			0.773[a]
Male	117	112	229		116	113	
Female	64	68	132		64	68	
Stage				0.128[a]			0.133[a]
I	29	20	49		27	22	
II	58	50	108		52	56	
III	62	82	144		80	64	
IV	21	16	37		13	24	
Unknown	11	12	23		8	15	
Grade				0.489[b]			0.404[b]
G1	2	6	8		3	5	
G2	64	67	131		60	71	
G3	109	104	213		112	101	
Unknown	6	3	9		5	4	
Status				0.942[a]			<0.001[a]
Alive	106	111	217		87	130	
Deceased	75	69	144		93	51	
Therapy				0.916[a]			0.193[a]
Chemotherapy	67	72	139		63	76	
Ancillary	8	7	15		10	5	
Unknown	106	112	207		107	100	

[a]χ^2-test; [b]Fisher's exact test. TCGA, The Cancer Genome Atlas.

Table II. Information of mRNAs and microRNAs in the integrative signature.

Ensembl ID	Gene symbol	Genomic location	P-value	Hazard ratio	Coefficient
ENSG00000146090	RASGEF1C	Chr 5: 179,527,795-179,636,153(-)	P<0.001	2.248	0.810
ENSG00000120129	DUSP1	Chr 5: 172,195,093-172,198,198(-)	P<0.001	1.004	0.004
ENSG00000157927	RADIL	Chr 7: 4,836,686-4,923,350(-)	P<0.001	2.358	0.858
ENSG00000134917	ADAMTS8	Chr 11: 130,274,820-130,298,888(-)	P<0.001	1.107	0.102
ENSG00000144476	ACKR3	Chr 2: 237,476,430-237,491,001(+)	P<0.001	1.061	0.059
ENSG00000130005	GAMT	Chr 19: 1,397,091-1,401,569(-)	P<0.001	1.041	0.040
ENSG00000183287	CCBE1	Chr 18: 57,098,172-57,364,612(-)	P<0.001	1.496	0.403
ENSG00000106366	SERPINE1	Chr 7: 100,770,370-100,782,547(+)	P<0.001	1.010	0.010
ENSG00000163673	DCLK3	Chr 3: 36,753,913-36,781,352(-)	P<0.001	7.497	2.014
ENSG00000163530	DPPA2	Chr 3: 109,012,635-109,035,364(-)	P<0.001	1.756	0.563
ENSG00000029559	IBSP	Chr 4: 88,720,733-88,733,074(+)	P<0.001	1.184	0.168
hsa-mir-184	miR-184	Chr 15: 79,209,788-79,209,871(+)	P<0.001	1.010	0.010

RASGEF1C, RasGEF domain family member 1C; DUSP1, dual specificity phosphatase 1; RADIL, Rap associating with DIL domain; ADAMTS8, ADAM metallopeptidase with thrombospondin type 1 motif 8; ACKR3, atypical chemokine receptor 3; GAMT, guanidinoacetate N-methyltransferase; CCBE1, collagen and calcium binding EGF domains 1; SERPINE1, serpin family E member 1; DCLK3, doublecortin like kinase 3; DPPA2, developmental pluripotency associated 2; IBSP, integrin binding sialoprotein; miR-, microRNA.

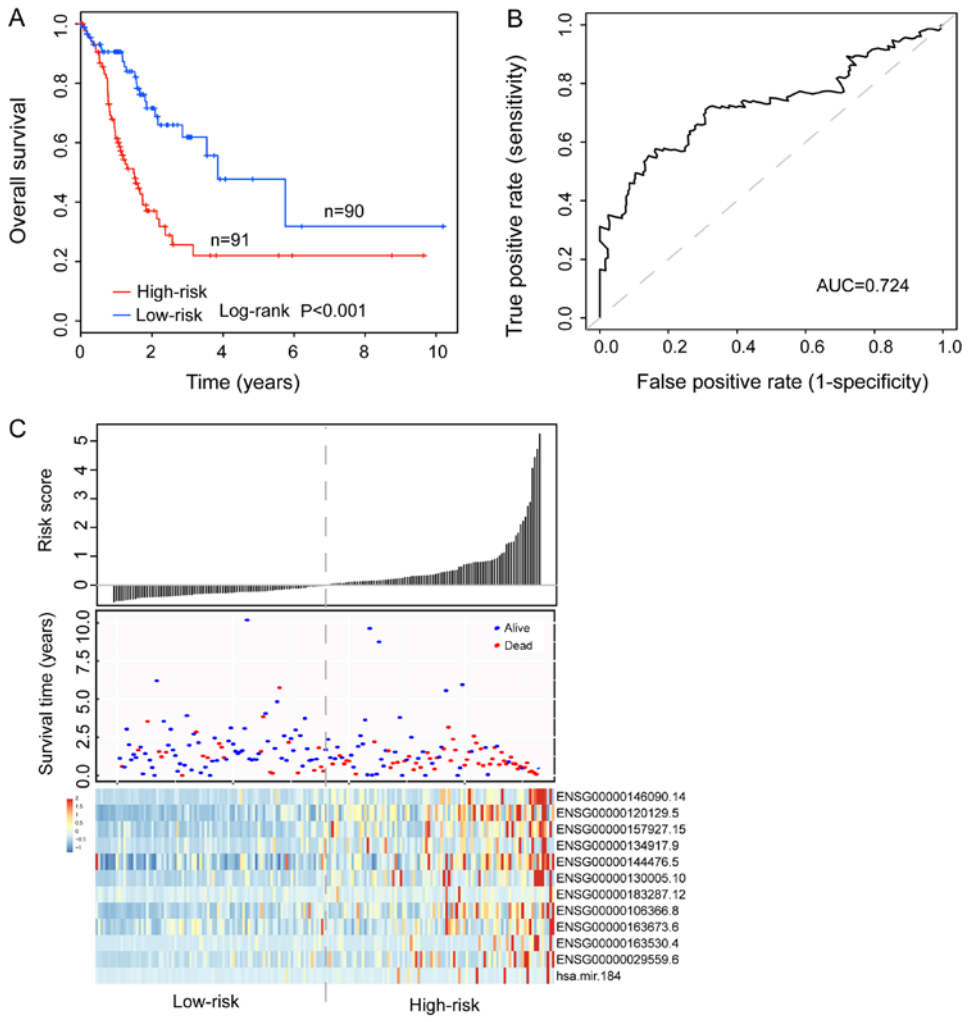

Figure 1. Development of an IMMIS in the discovery cohort. (A) Kaplan-Meier survival curves of overall survival between high- and low-risk groups. Overall survival was significantly increased in the low-risk group compared with the high-risk group. (B) Time-dependent receiver operating characteristic curves at 3 years of overall survival. The AUC for the IMMIS prognostic model was 0.724 for the 3-year overall survival rate. (C) Distribution of risk scores, survival status of patients and expression patterns of the 12 prognostic RNAs in the IMMIS. IMMIS, integrative mRNA/microRNA signature; AUC, area under the curve.

Table III. Univariate and multivariate Cox regression analysis of the IMMIS and other clinicopathological variables. Discovery cohort, n=181

Variables		Univariate model			Multivariate model		
Characteristics	Comparison groups	Hazard ratio	95% Confidence interval	P-value	Hazard Ratio	95% Confidence interval	P-value
IMMIS	High- Vs. Low-risk	2.805	1.722-4.567	<0.001	2.613	1.560-4.375	<0.001
Age	<68 vs. ≥68 years	1.032	1.008-1.056	0.007	1.039	1.011-1.067	0.005
Sex	Male vs. female	1.025	0.634-1.658	0.920	1.007	0.601-1.688	0.979
Stage	I and II vs. III and IV	1.549	0.950-2.527	0.079	1.868	1.096-3.181	0.022
Grade	G1 vs. G2 and G3	0.987	0.612-1.593	0.957	0.937	0.552-1.591	0.811

IMMIS, integrative mRNA/microRNA signature; TCGA, The Cancer Genome Atlas.

median risk score value (0.646), which was considered the cut-off value. Survival analysis showed that patients in the high-risk group had significantly shorter overall survival compared with those in the low-risk group, and median survival was 1.49 vs. 3.85 years, respectively. (P<0.001; Fig. 1A). The result of univariate Cox regression analysis indicated that the HR of the high-risk group compared with the low-risk group regarding overall survival was 2.805 (P<0.001; 95% CI=1.722-4.567; Table III). The 3- and 5-year overall survival rates of patients in the high-risk group were 25.7 and 22%, respectively, which were significantly lower compared with those of patients in the low-risk group. Specifically, he 3- and 5- year overall survival rates of patients in the low-risk group were 61.8 and 47.7%, respectively. Notably, the area under the curve for the IMMIS prognostic model was 0.724 for the 3-year overall survival rate (Fig. 1B). In addition, the distribution of risk scores, the survival status of patients and expression patterns of the 12 prognostic RNAs in the IMMIS are presented in Fig. 1C.

Validation of the IMMIS for survival prediction in the testing cohort and entire TCGA cohort. To confirm the predictive value of the IMMIS for survival prediction, the predictive ability of the IMMIS in the testing cohort and entire TCGA cohort was analyzed. With the same risk score model and cut-off derived from the discovery cohort, the IMMIS classified 89 and 91 patients of the testing cohort into high- and low-risk groups, respectively. Consistent with the findings in the discovery cohort, the overall survival time of the high-risk group patients was significantly shorter than that of the patients in the low-risk group. Specifically, the median survival time was 2.17 and. 4.62 years in patients in the high- and low-risk groups, respectively. (P=0.047; Fig. 2A). The 3- and 5-year overall survival rates of patients in the high-risk group were 44.5 and 26.7%, whereas the corresponding rates were 53 and 42.4% in the low-risk group, respectively. In the entire TCGA cohort, 361 patients were classified in either the high-risk (n=180) and low-risk (n=181) groups. Notably, overall survival was significantly different between the high- years and low-risk groups according to the IMMIS and the cut-off value derived from the discovery cohort. The median survival was 1.53 and 4.62 years in the high- and low-risk groups, respectively. (P<0.001; Fig. 2B). The 3- and 5-year overall survival rates of patients in the high-risk group were 35.6 and 26.1%, whereas the corresponding rates were 57.6 and 45.3% in the low-risk group, respectively. The HR of the high-risk group compared with the low-risk group regarding overall survival was 1.625 (P=0.039, 95% CI=1.004-2.638) in the testing cohort and 2.139 (P<0.001, 95% CI=1.519-3.012) in the entire TCGA cohort (Table IV).

Independence of the IMMIS from other clinicopathological factors. To examine whether the predictive value of the IMMIS was independent of other clinicopathological factors associated with patients with GC, multivariate Cox regression analysis was used to compare the performance of the IMMIS and other clinicopathological factors, including age,

Table IV. Univariate and multivariate Cox regression analysis of the IMMIS and other clinicopathological variables.

A, Testing cohort, n=180

Variables		Univariate model			Multivariate model		
Characteristics	Comparison groups	Hazard ratio	IMMIS	High- vs. Low-risk	Hazard ratio	IMMIS	High- vs. Low-risk
IMMIS	High vs. Low	1.625	1.004-2.638	0.039	1.269	1.001-2.112	0.049
Age	<68 vs. ≥68 years	1.016	0.992-0.402	0.191	1.026	1.001-1.052	0.045
Sex	Male vs. female	1.607	0.955-2.704	0.074	1.696	0.971-2.962	0.063
Stage	I and II vs. III and IV	2.157	1.259-3.694	0.005	1.804	1.033-3.150	0.038
Grade	G1 vs. G2 and G3	1.757	1.057-2.921	0.030	1.851	1.050-3.265	0.033

B, Entire TCGA cohort, n=361

Variables		Univariate model			Multivariate model		
Characteristics	Comparison groups	Hazard ratio	IMMIS	High- vs. Low-risk	Hazard ratio	IMMIS	High- vs. Low-risk
IMMIS	High vs. Low	2.139	1.519-3.012	<0.001	1.844	1.288-2.638	0.001
Age, n=355	<68 vs. ≥68 years	1.024	1.007-1.041	0.004	1.029	1.011-1.047	0.001
Sex	Male vs. female	1.282	0.901-1.825	0.167	1.269	0.876-1.840	0.208
Stage, n=338	I and II vs. III and IV	1.751	1.228-1.866	0.002	1.723	1.195-2.485	0.004
Grade	G1 vs. G2 and G3	1.319	0.932-1.866	0.118	1.250	0.862-1.814	0.239

IMMIS, integrative mRNA/microRNA signature; TCGA, The Cancer Genome Atlas.

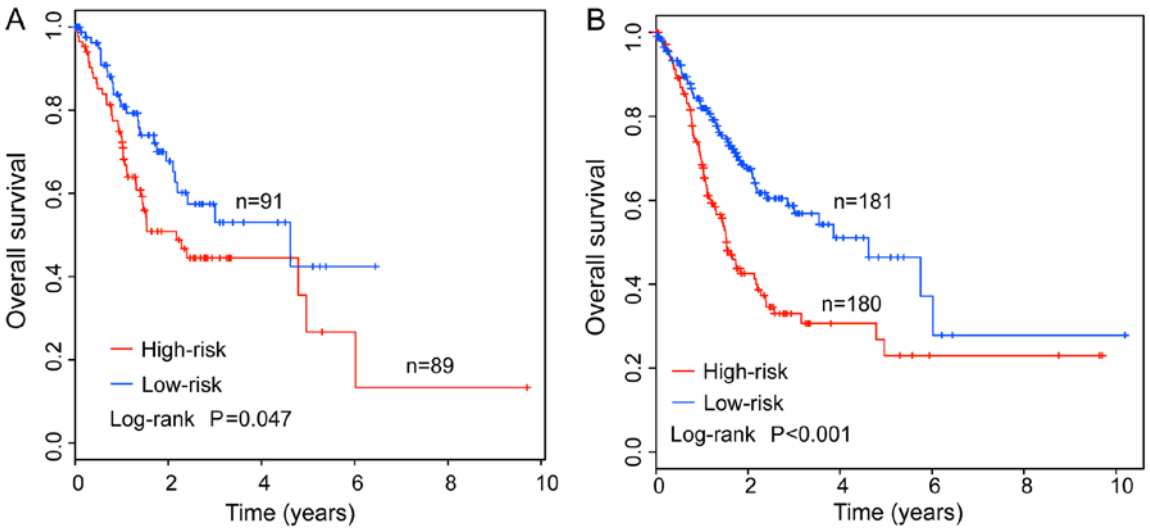

Figure 2. Validation of an integrative mRNA/microRNA signature. Kaplan-Meier survival curves of overall survival between high- and low-risk groups in the (A) testing cohort and (B) entire The Cancer Genome Atlas cohort.

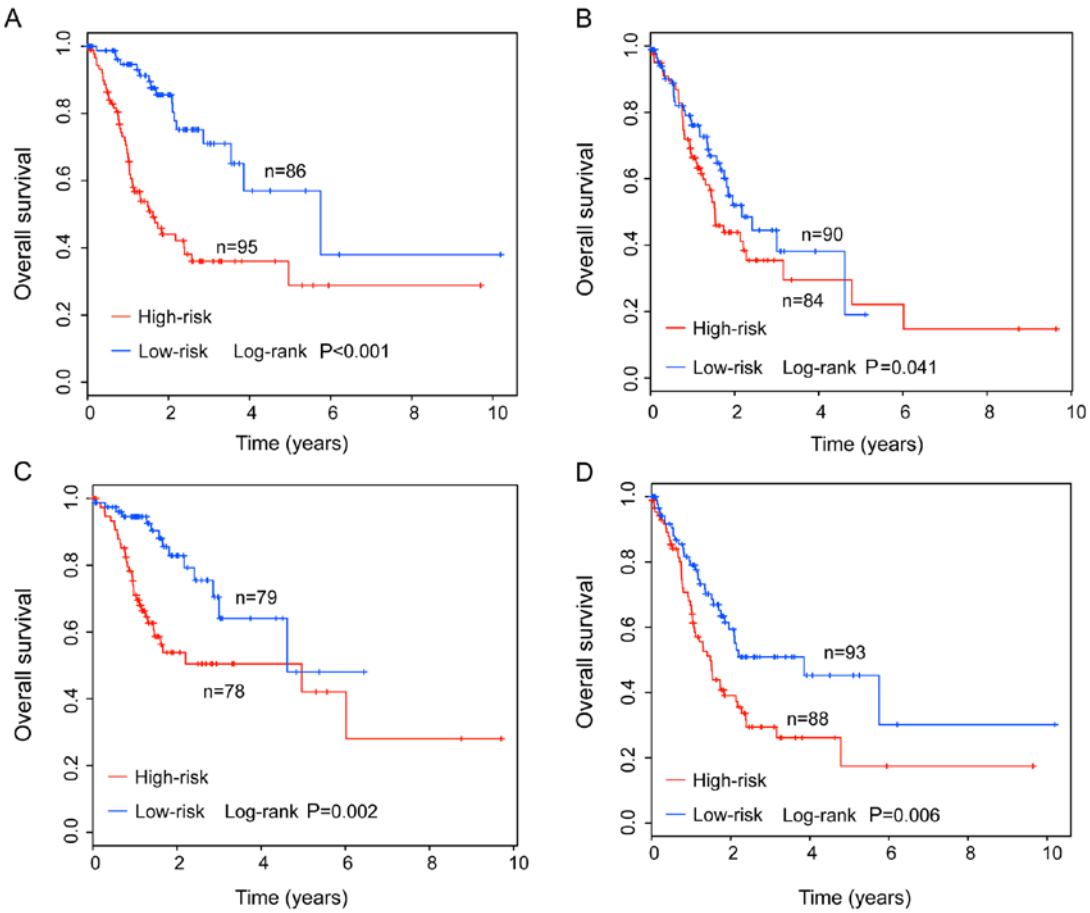

Figure 3. Stratified analysis by age and stage. Kaplan-Meier survival curves of overall survival rates between high- and low-risk groups in (A) younger, <68 years, and (B) older, ≥68 years, patients. Kaplan-Meier survival curves of overall survival between high- and low-risk groups in (C) early-stage, I/II, and (D) advanced-stage, III/IV, patients.

sex, stage and grade. The results from the multivariate Cox regression analysis indicated that the IMMIS, stage and age were significantly associated with overall survival of patients with GC (Table III). Therefore, stratification analysis on age and stage to determine whether the IMMIS was independent of age and stage was also performed. First, 355 patients (excluding 6 patients without age data) were classified into a younger stratum (n=181) and an elder stratum (n=174) according to the median age (68 years). Results of stratification analysis for age revealed that, within each age stratum, the IMMIS could further

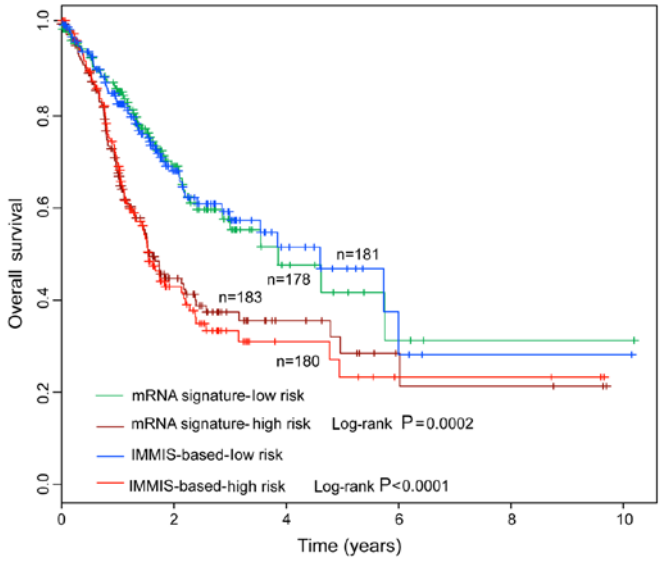

Figure 4. Comparison of the prognostic value of the IMMIS and mRNA-based signature for survival prediction. IMMIS, integrative mRNA/microRNA signature.

subdivide the patients into the high- and low-risk groups with significantly different overall survival. The median survival was 1.61 and 5.75 years in the high- and low-risk groups, respectively (P<0.001), for the younger group. The median survival was 1.53 and 2.17 years in the high- and low-risk groups, respectively, (P=0.041) for the elder group (Fig. 3A and B). Subsequently, 338 patients (excluding 23 patients without stage data) were classified into an early-stage stratum (stage I/II; n=157) and an advanced-stage stratum (stage III/IV; n=181) according to the GC stage (20). Results of stratification analysis based on stage indicated that, within each stage stratum, the IMMIS could further subdivide the patients into the high- and low-risk groups with significantly different overall survival. The median survival was 4.62 and 4.96 years in the high- and low-risk groups, respectively (P=0.002), for the early-stage group, and 1.49 and 3.85 years in the high- and low-risk groups, respectively (P=0.006) for the advanced-stage group (Fig. 3C and D). Multivariate and stratification analysis demonstrated that the survival prediction ability of the IMMIS was independent of other clinicopathological factors associated with survival prediction in patients with GC.

Performance comparison of IMMIS with mRNA-based signature. The predictive value of the IMMIS compared with an mRNA-based signature was also evaluated. A total of 11 mRNAs of the IMMIS were used to construct an mRNA-based signature. When applied to the entire TCGA cohort, the IMMIS and mRNA-based signatures reliably classified the patients into high-risk group and low-risk group with significantly different overall survival (Fig. 4). However, Kaplan-Meier survival curves revealed that patients in the high-risk group predicted by the IMMIS had a poorer prognosis compared with those in the high-risk group predicted by the mRNA-based signature, and patients in low-risk group predicted by the IMMIS had an improved prognosis compared with those in the low-risk group predicted by the mRNA-based signature (Fig. 4). The present results suggested that the IMMIS may have improved prognosis prediction ability compared with the mRNA-based signature.

Discussion

The Tumor-Node-Metastasis (TNM) classification of malignant tumors is widely used for the staging and risk stratification of patients with GC (20). However, patients with similar clinical features and those who belong to the same TNM stage may have different clinical outcomes (20). Advances in molecular biology and particularly in the omics sciences have recently demonstrated the complex heterogeneity of GC characterized by genetic and epigenetic changes (21). The findings imply the potential of molecular aberrations as alternative biomarkers for aiding TNM staging and improving prognosis predictions of patients with GC (13,14). Accumulating evidence has revealed that the dysregulated expression of mRNAs and miRNAs has critical roles in the development and progression of GC (20-29), highlighting the applications of miRNAs and mRNAs as molecular biomarkers for predicting the prognoses of patients with GC in clinical practice. Although increasing efforts have been made to discover novel mRNA- or miRNA-based expression signatures for improving prognosis prediction, the predictive signatures previously developed mainly consider a single type of RNA. Several studies have investigated and revealed the prospect of the combination of multiple RNA types for risk stratification in several types of cancer, including colon cancer, breast cancer, glioblastoma multiforme and ovarian cancer (8,9,30). However, it is still unknown whether multi-RNA-based signature could substantially increase the prognostic value in GC.

In the present study, an integrative analysis of mRNA expression profiles, miRNA expression profiles and clinical information in a large cohort of patients with GC was performed. After dividing the entire TCGA cohort into a discovery cohort and a testing cohort, a novel multi-RNA-based signature, consisting of 11 mRNAs and one miRNA, was identified. The predictive value of the identified signature was successfully validated in the testing cohort, which reflects the reproducibility of the integrative signature. Moreover, the integrative signature could act as an independent factor for survival prediction in patients with GC. Notably, comparative analysis revealed that the predictive value of the IMMIS was superior compared with an mRNA-based signature alone. Therefore, the present results suggested that the IMMIS may be a promising signature to effectively identify patients with GC who are at high risk of mortality and guide individualized therapy choices.

The majority of genes included in the integrative signature have been experimentally demonstrated to be associated with GC. For example, DUSP1, a member of the threonine-tyrosine dual-specificity phosphatase family, is involved in cellular proliferation, differentiation and apoptosis, and is additionally associated with tumor carcinogenesis progression (31). Teng *et al* (32) demonstrated that DUSP1 can induce apatinib resistance by activating the mitogen-activated protein kinase-signaling pathway in patients with GC. The expression status of ADAMTS8 has been investigated in GC, and the expression levels are significantly increased in patients with GC (33). In addition, ADAMTS8 is associated with grade and tumor size (34). Kim *et al* (35) investigated the expression

of ACKR3 and its clinical relevance in GC, and found that ACKR3 expression is associated with aggressive behavior and poor prognosis in GC. A recent study has suggested that CCBE1 may have an important role in the progression of gastrointestinal stromal tumors by enhancing angiogenesis and mediating resistance to imatinib (36). Notably, miRNA-184 has been reported to inhibit cell proliferation and invasion, and functions as a potential oncogene in several types of cancer (37,38). A recent study has indicated that overexpression of miRNA-184 is associated with poor outcome in GC (7).

In conclusion, in the present study, a novel IMMIS was constructed, which could effectively stratify patients into low- and high-risk groups of mortality. With further prospective study, the IMMIS may add more information to the current TNM staging system and could improve prognosis prediction. However, there were some limitations in the present study. Firstly, the integrative signature was only based on available TCGA datasets, and no other independent patient datasets were tested. Secondly, the biological functions of the mRNAs and miRNA incorporated in the integrated signature require further experimental studies.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YYW conceived and designed the experiments. XY, FMZ, ZWG and WYK performed the experiments and analyzed the data. YYW wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Only publicly available datasets were used and ethical approval was not received in the present study, as it was not necessary.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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