INHBA is A Novel Prognostic Biomarker And Correlated With Immune Infiltrates In Gastric Cancer

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Research

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Abstract

Background: Inhibin subunit beta A (INHBA) is reportedly a potential prognostic biomarker for a variety of cancers. However, its role in gastric cancer (GC) remains elusive.

Methods: The expression of INHBA in GC and healthy tissues based on the data obtained from the UCSC Xena database. Logistic regression and Cox regression was performed to explore the correlation between clinical indicators and INHBA expression. Kaplan–Meier curve analysis was performed to assess the impact of INHBA expression on overall survival(OS). In addition, Received operating characteristic curve analysis was implied to clarify the diagnostic role of INHBA in GC. Functional analyses were conducted to explain the potential functions and enrichment pathways for INHBA. TIMER and GEPIA databases were used to calculate the confidence between INHBA and immune cell infiltration in GC.

Results: INHBA was upregulated in GC($P < 0.001$) and associated with a poor prognosis($P = 0.037$). INHBA expression was an independent risk factor for OS($P = 0.004$). Additionally, INHBA was a potential diagnostic marker in GC(AUC=0.961) and it was associated with extracellular matrix organization, response to growth factor, and cell-substrate adhesion. Tumor-associated signaling pathways, such as Wnt, Hippo, and p53, were associated with INHBA. Reactome pathways, such as collagen formation and extracellular matrix organization, were significantly enriched. Moreover, high INHBA expression displayed a strong correlation with immune cell infiltration, especially with macrophage infiltration in GC.

Conclusions: INHBA could be a potential prognostic biomarker for GC and may drive the abnormal activity of critical cancer-associated pathways, potentially contributing to immune cell infiltration to promote GC development and becoming a new drug target for targeted GC therapies.

Introduction

Gastric cancer is a malignant tumor originating from the epithelium of the gastric mucosa, ranking fifth in incidence and third in mortality among all tumors, with nearly 800,000 patients dying of gastric cancer in 2018[1]. GC-inducing factors are numerous, such as smoke, alcohol drinking, high salt intake, and iron deficiency[2-5], but Helicobacter pylori infection is the most prominent risk factor[6, 7]. Therefore, complete H. pylori eradication was recommended by the World Health Organization for preventing the development of gastric cancer [8]. Meanwhile, the development of gastrointestinal endoscopy technology would enable more patients to be effectively diagnosed and treated at the early stage of GC. However, due to the occult development and non-specific clinical symptoms at the early stage, most patients with gastric cancer lose the early-stage treatment opportunities and have a poor prognosis[9]. In addition, successful H. pylori eradication can't completely prevent GC development. Therefore, it would be pivotal to find reliable biomarkers for prognosis to promote GC patient survival.

INHBA, consisting of an alpha and a beta subunit, is a member of the transforming growth factor β (TGF-β) superfamily[10-13]. INHBA comprises a disulfide-linked homodimer, Activin A, which could also promote tumor development[14]. In ovarian cancer, the role of INHBA is closely related to the expression

level of Activin A[15]. A previous study [16] reported high INHBA expression in gastric cancer. However, the diagnostic and prognostic value of INHBA has not been well studied. In the present study, we aimed to explore these aspects, as well as the potential role of INHBA during GC development and progression to find the potential molecular target in GC treatment.

Materials And Methods

Data collection and analysis

INHBA expression data of the GTEx and TCGA data and were obtained from the UCSC Xena database(https://xenabrowser.net/datapages/) and have been uniformly processed through the Toil process [36]. Meanwhile, the GC data in TCGA and the corresponding healthy stomach tissue data in GTEx were extracted for further analysis. Scatter plot was used to show the difference expression of INHBA in tumor and healthy tissues.

Functional enrichment

We conducted enrichment analysis using top 300 positive INHBA-associated genes to evaluated the potential functions of INHBA. The Metascape database [37] was used to perform Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) analyses. Gene Set Enrichment Analysis(GSEA) was performed using the R package clusterProfiler (v.3.6.3) [38]. The nominal P-value, false discovery rate (FDR) q-value and normalized enrichment score(NES) indicated the importance of the association between gene sets and pathways, Gene sets with FDR <0.25 and nom P value <0.05 were significantly enriched.

Immune infiltration analysis

The association between the INHBA expression and immune infiltration was analyzed using the TIMER(http://cistrome. org/TIMER/) and GEPIA(http://gepia.cancer-pku.cn/) databases. TIMER, including the data of 10,897 tumors from 32 cancer types, is a comprehensive analytical web tool to explore the molecular interactions of tumor immune infiltration and gene expression data [39]. GEPIA is an online platform application for gene expression analysis based on the data from the TCGA and the GTEx databases [40]. We thus investigated the relationship between INHBA and tumor-infiltrating immune cells. Moreover, we visualized six types especially macrophage-associated infiltration via TIMER and uncovered the prognostic impact of immune infiltrates in GC. Meanwhile, we further explored the correlation between INHBA expression and immune cell markers, using GEPIA to validate parts of these results.

Statistical Analysis

All data analyses were performed using R (v.3.6.3). A median threshold was employed to distinguish high and low expression of INHBA. The association between INHBA expression and clinical pathologic variables was analyzed by Logistic regression. The Kaplan-Meier curve was used to estimate OS rates between the high and low INHBA gene expression groups. COX regression analysis were subject to

identify independent prognostic factors and assess the impact of INHBA expression on survival as well as other clinicopathologic features. The diagnostic value of INHBA was assessed using ROC curves form pROC package in R. Based on the results of multivariate Cox regression, a nomogram was then constructed to predict the overall GC patient survival in different time periods. A P-values less than 0.05 was considered statistically significant.

Results

GC patient baseline clinical characteristics

The clinical characteristics, including gender, age, race, TNM stage, histologic grade, primary therapy outcome, pathologic stage , and H. pylori infection, were collected (Table 1). In this study, 134 female and 241 male patients were analyzed in total, including 18 H. pylori infection patients and 145 non-H. pylori infection patients. Concerning the GC stage, 53(15%), 111(31.5%), 150(42.7%), and 38(10.8%) patients were in stages I, II, III, and IV, respectively.

High INHBA expression in cancer patients

First, we assessed INHBA expression in pan-cancer data from The Cancer Genome Atlas(TCGA) and Genotype-Tissue Expression(GTEx). The analysis showed that INHBA expression was higher and lower in 17 and 7 tumors, respectively(Figure 1A). In order to clarify the INHBA expression differences between GC and healthy tissues, the INHBA expression level in 375 and 32 GC and adjacent GC tissues were examined, respectively, by scatter plot and a potentially high INHBA expression was verified in the GC tissues($P < 0.001$, Figure 1B). In addition, the INHBA expression in 32 GC tissues and their matched adjacent tissues were also analyzed. The results indicated that GC tissues highly expressed INHBA($P < 0.001$, Figure 1C). Moreover, the INHBA expression of healthy samples from GTEx combined with data from the TCGA database and GC samples from the TCGA database were compared, showing the same results as the aforementioned analysis, indicating that INHBA was significantly overexpressed in the GC samples ($P < 0.001$, Figure 1D).

Correlation between INHBA expression and clinical features

The correlation between INHBA expression and clinical features in patients with GC was shown in Table 2. The high INHBA expression in GC patients significantly associated with the T stage ($P < 0.01$), histological type ($P < 0.01$), histologic grade ($P < 0.01$), OS event ($P < 0.01$), pathologic stage ($P < 0.01$, Figure 2). The logistic regression analysis indicated that the expression of INHBA definitely as a dependent variable was associated with poor prognostic clinical characteristics. In GC patients, the high INHBA expression was significantly associated with the T stage(T1 vs. T2&T3&T4: $P = 0.003$), histological type(Diffuse Type &Mucinous Type & Signet Ring Type &Not Otherwise Specified vs. Tubular Type &Papillary Type: $P < 0.001$).

High INHBA expression with poor GC patient prognosis

In order to identify the prognostic value of INHBA in GC, we performed Kaplan–Meier curves analysis and showed that high expression of INHBA correlates with poor prognosis (P = 0.037), similar to pathologic stage Ⅲ (P = 0.014), PR&CR (P = 0.001), T4 (P = 0.03), N2 (P = 0.045),M0 (P = 0.019), Diffuse Type &Mucinous Type &Signet Ring Type &Not Otherwise Specified (P = 0.021), and R0 (P = 0.018) in the subgroup analysis(Figure 3). Univariate Cox analysis confirmed that high INHBA expression was significantly associated with poor OS (P = 0.037). Interestingly, multivariate Cox analysis indicated that the INHBA expression was an independent risk factor for OS in GC patients(P = 0.004, Table 3). Therefore, a nomogram was constructed based on the result of the Cox multivariate analysis to predict the different time periods survival probability of the GC patients by combining the INHBA expression levels with independent clinical variables(Figure 4).

INHBA as a potential new diagnostic biomarker in GC

We conducted a ROC curve analysis to evaluate the diagnostic value of INHBA in GC. The INHBA area under the curve(AUC) was 0.961 (Figure 5A), indicating a high INHBA diagnostic value. The subgroup analysis demonstrated the INHBA gene expression diagnostic value in different clinical features such as T1/T2, T3/T4, tubular type/mucinous type, Barretts esophagus and stage I/II (Figure 5B–5F).

Functional enrichment analyses of INHBA and associated genes

To predict the function of INHBA, including associated pathways, we performed a correlation analysis between INHBA and other GC-related genes using TCGA data and displayed the results as heatmaps (Figure 6). The top 300 genes associated with INHBA were derived and analyzed for enrichment analysis. The GO analysis revealed that INHBA was associated with extracellular matrix organization, response to growth factor, cell-substrate adhesion, and negative regulation of cell differentiation. In addition, the KEGG pathway analysis indicated that protein digestion and absorption, proteoglycans in cancer, Wnt, Hippo as well as p53 signaling pathways comprised the top 300 enriched genes and were involved in crosstalk (Table 4). Meanwhile, INHBA-associated Reactome pathways were screened by GSEA, revealing that IL4 and IL13 signaling, signaling by PDGF, collagen formation and glycosaminoglycan metabolism were significantly enriched (Figure 7). These results suggest that INHBA could be associated with multiple malignancy-related pathways in GC and might promote GC development by altering the cancer microenvironment.

Correlation between INHBA expression and immune cells infiltration in GC

Different immune infiltration levels in the tumor microenvironment were significantly associated with overall patients survival. The above-described findings suggested that INHBA was an independent risk factor and correlated with OS in GC. Therefore, investigating the relationship between INHBA expression and immune infiltration would be reasonable. We used the TIMER database to analyze the correlation between INHBA and the immune infiltration level. The results showed that INHBA was significantly associated with tumor purity, as well as B cells, macrophages, or neutrophil and dendritic cells (Figure 8A). Furthermore, we also performed Kaplan–Meier analysis to assess the association between INHBA

expression and immune cell infiltration in GC. As figure shows, except for the INHBA expression, macrophage infiltration also correlated with GC prognosis (Figure 8B). This indicated that INHBA has a regulatory function on immune cell infiltration in GC, especially on macrophage infiltration.

Association between INHBA expression and immune markers

To further pursue the interplay between INHBA expression and immune cell infiltration in GC, we used the TIMER and GEPIA databases to explore the relationship between INHBA expression and several immune cell markers. Briefly, these included B cells, CD8+ T cells, macrophages, monocytes, dendritic cells, natural killer cells, tumor-associated macrophages, neutrophils, and T cell subsets, such as Th1, Th2, Th17, Treg, Th9, Th22, Tfh, and T cell exhaustion. In TIMER, 35 immune cell markers were significantly associated with INHBA expression before and after tumor purity correction. Most of these markers belong to subsets of T cells, such as Th1/Th2 or Th17/Treg, and macrophages, such as tumor-associated macrophages (TAMs) or M1/M2 macrophages, monocytes, neutrophils and DCs (Table 5 and Figure 9). This predicted that INHBA plays a key role in the tumor microenvironment to affect immune infiltration.

Since macrophages were the most significant immune cells infiltrating in GC (Figure 9), and the macrophage markers clearly correlated with INHBA expression (Table 5), we further pursued the correlation between INHBA expression and macrophage-associated markers in GEPIA(Table 6). As the results show in TIMER, the correlation could be observed between INHBA expression and the markers of M1/M2 macrophages, monocytes, and TAMs. All of this suggested that INHBA might drive tumor-associated macrophage polarization in GC but it needs further experiments to explore the underlying mechanism.

Discussion

Although the rapid development of science and technology has improved tumor diagnosis and treatment strategies, it cannot be ignored that GC prognosis remains poor. In this study, we demonstrated high INHBA expression in GC tissues. During further analysis, we verified that the INHBA expression was associated with the histologic grade, TNM stage, histological type, and primary therapy outcome of GC patients, all of which indicated that increased INHBA expression was closely related to GC development.

Similar findings have been made in other tumor-related studies. A study in patients with breast cancer showed that INHBA was highly expressed in the peripheral circulation of the patients and positively correlated with circulating tumor cell expression [17]. Zhao Z et al [10] demonstrated that INHBA is highly expressed in prostate cancer and can affect the proliferation and invasion of tumor cells through the TGF-β pathway, and Miyamoto Y et al [18] described the same finding in colorectal cancer. Activin A could synergistically influence tumor development through BMP signaling [14], and it was found that Activin A could promote the degradation of muscle proteins by activating the SMAD signaling pathway [19], which could also be responsible for the high INHBA expression to promote the development of tumor malignancy [20].

The relationship between INHBA expression and GC prognosis has been poorly studied. The Kaplan–Meier analysis suggested that GC patients with high INHBA expression displays a worse prognosis. The results of the ROC analysis confirmed the INHBA diagnostic value in GC. Based on the results of the COX multivariate analysis combined with clinical factors we constructed the first Nomogram to predict GC prognosis related to INHBA expression in order to better guide clinical decision making.

Although INHBA has been widely studied in tumors, its effector mechanism in GC remains poorly understood. The GO/KEGG analysis has shown that INHBA is associated with several tumor pathways including Hippo, Wnt, and p53. The Hippo pathway could alter the sensitivity of tumor cells to apoptosis and could affect tumor progression by enhancing drug resistance in the tumor cells [21, 22]. A previous study demonstrated the over-activation of the Wnt pathway in GC cells, affecting metastatic activity [23], as well as the alteration of the Wnt signaling pathway that could cause abnormal Hippo pathway activity [24]. p53 plays a key regulatory role in apoptosis and cell cycle progression. Mutations in the p53 gene have been observed in numerous cancers including GC and are thought to be closely related to cancer prognosis[25]. All these results suggested that INHBA significantly correlated with the cancer-associated pathways.

In addition to the study of the tumors themselves, studying the tumor microenvironment, e.g., the extracellular matrix, is of increasing importance. The GSEA results showed that the IL4- and IL13-, PDGF-, collagen formation-, glycosaminoglycan metabolism-, extracellular matrix organization-, and ECM proteoglycan-related signaling pathways were significantly enriched, most of which are involved in the ECM-associated Reactome. The microenvironment formed by the alterations in the arrangement and orientation of the ECM components has a crucial impact on tumor development[26]. As a fundamental component of the ECM, collagen could influence cancer progression by altering the cellular transduction pathways [27], while mutated oncogenes such as the previously mentioned p53 could, in turn, affect collagen content and structure within the tumor cells, thereby affecting the endostasis of the ECM and might further promote tumor progression[28]. ECM proteoglycans are proteins covalently bound to glycosaminoglycans, which are important for maintaining the ECM structure and function due to their negative charge [29]. The above-described discussion suggests that INHBA might influence the development of GC through the alterations of the extracellular matrix microenvironment. Moreover, Th2 cells drive type 2 immune responses to produce cytokines IL4 and IL13, regulating functional TAM polarization [30]. This could imply that INHBA might be related to immune infiltration in GC progression.

Immune cell infiltration is an important component of the tumor microenvironment and plays a critical role in cancer initiation and progression. Different immune cells largely contribute to and are also functionally affected by the ECM changes [31]. Our findings suggested that INHBA functions through the ECM-associated Reactome, it would thus be meaningful to identify the association between INHBA expression and the immune cell infiltration level. We analyzed INHBA expression and immune cell infiltration in GC using TIMER as a tool and found that macrophage and B cell infiltration positively correlated with INHBA expression. Meanwhile, the Kaplan–Meier analysis demonstrated high macrophage infiltration, indicating a poor GC prognosis. Moreover, we further confirmed that the

macrophage markers positively correlated with INHBA expression in TIMER and GEPIA, including those of M1/M2 macrophages, monocytes, and TAMs. All these results suggested a key role of INHBA in modulating TAM polarization. It is well known that macrophages originate from two different sources, the major being blood monocytes. One of the key characteristics of macrophages is their high-level plasticity and that they are called TAM when accumulated in tumors [32, 33]. TAM is the essential component of the tumor microenvironment in solid tumors and contributes to tumor growth, cell proliferation, and dissemination [34, 35]. These functions might be supported by TAM polarization into M2 macrophages. In this study, the upregulated INHBA with the elevated trend of monocytes, TAMs, and M2 macrophages suggested that INHBA might promote TAM polarization to M2 macrophages in GC. Meanwhile, INHBA negatively correlated with tumor purity, implying that INHBA also largely existed in the tumor microenvironment. These findings indirectly proved that INHBA has a close relationship with immune infiltration. Interestingly, we also found that Treg, Th17, DC, and M1 macrophage elevation trend was followed by upregulated INHBA, suggesting that INHBA could exert a dual, "yin-yang" influence on the GC immune infiltration, and the landscape of immune infiltration is dynamic and complex. Future experiments are needed to test these hypotheses.

However, this study also has some limitations. First, the data were obtained originated only from an online database, which makes it difficult to ensure the integrity of the clinical information. Future experiments using big sample clinical trial data might overcome this limitation. Second, we used multiple databases for the necessary information bioinformatics analysis but the different algorithms in the several databases might lead to conflicting results. Future in vitro and in vivo experiments could further clarify the role of INHBA in GC. Third, although we found that high INHBA expression and immune infiltration are associated with GC prognosis, we cannot confirm whether this regulation is direct or indirect through other mediators. Therefore, further studies would be required to spell out the exact mechanisms underlying these associations. Last but not the least, all results we analyzed were based on tissue-derived data but negative to explore the expression of INHBA at the cellular level. Therefore, more comprehensive data would be required for future studies to better understand the subject.

Conclusion

In summary, we found that INHBA was upregulated in GC and could be a potential diagnostic and independent prognostic biomarker for GC. In addition, INHBA might regulate tumor-associated pathways in GC, the alteration of the tumor microenvironment such as that of the ECM, and INHBA-related immune cell infiltration might promote GC development. At the same time, INHBA might become a potential therapeutic target in GC.

Abbreviations

Inhibin subunit beta A :INHBA; gastric cancer :GC; overall survival :OS; Received operating characteristic :ROC; The Cancer Genome Atlas :TCGA; Genotype-Tissue Expression :GTEx; transforming growth factor β :TGF-β; CR: complete response; PD :progressive disease; SD :stable disease; PR :partial response; area under the curve :AUC; Gene Ontology :GO; Kyoto Encyclopedia of Genes and Genomes :KEGG; Gene Set Enrichment Analysis :GSEA; normalized enrichment score :NES; false discovery rate :FDR; Tfh :Follicular helper T cell; Th :T helper cell; Treg :Regulatory T cell; TAM :Tumor-associated macrophage; NK :natural killer cell; DC :dendritic cell; None :Correlation without adjustment; Purity :Correlation adjusted by purity; Cor :R value of Spearman's correlation.

Declarations

Acknowledgments

Not applicable.

Author Contributions

H.S.P and Y.W.F conceived and designed the research, Z.Z.S contributed to the data collection, H.G.H and Y.Z.H analyzed data and wrote the paper, Z.W and W.J revised the paper. All authors read and approved the final manuscript.

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Availability of data and material

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Clinical Characteristics of patients with GC based on TCGA

Clinical Characteristics		N	(%)
Age (years)	<=65	164	44.2
	>65	207	55.8
Gender	Female	134	35.7
	Male	241	64.3
Race	Asian	74	22.9
	Non-Asian	249	77.1
T stage	T1	19	5.1
	T2	80	21.8
	T3	168	45.8
	T4	100	27.3
N stage	N0	111	31.1
	N1	97	27.2
	N2	75	21
	N3	74	20.7
M stage	M0	330	93
	M1	25	7
Primary therapy outcome	PD+SD	82	25.9
	PR+CR	235	74.1
Stage	I	53	15
	II	111	31.5
	III	150	42.7
	IV	38	10.8
Histologic grade	G1	10	2.7
	G2	137	37.5
	G3	219	59.8
H pylori infection	No	145	89
	Yes	18	11

CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response

Table 2. Logistic analysis of the association between INHBA expression and clinical characteristics

Characteristics	Total	INHBA expression		
	(N)	OR	95%CI	*p* value
T stage (T1vs.T2&T3&T4)	367	9.107	2.56-57.992	**0.003**
M stage (M0 vs. M1)	355	1.097	0.483-2.507	0.824
N stage (N0 vs. N1&N2&N3)	357	0.826	0.526-1.293	0.403
Pathologic stage (Stage I vs. Stage II &Stage III &Stage IV)	352	1.477	0.821-2.695	0.197
Gender (Male vs. Female)	375	0.919	0.602-1.402	0.695
Age (>65 vs. <=65)	371	1.029	0.683-1.552	0.890
Primary therapy outcome (PR&CR vs. PD&SD)	317	1.603	0.966-2.687	0.070
H pylori infection (Yes vs. No)	163	1.209	0.422-3.265	0.712
Residual tumor (R2 vs. R1&R0)	329	0.644	0.214-1.776	0.405
Histologic grade (G1 vs. G2&G3)	366	1.569	0.441-6.225	0.491
Histological type (Diffuse Type &Mucinous Type &Signet Ring Type &Not Otherwise Specified vs. Tubular Type &Papillary Type)	374	2.674	1.572-4.668	**<0.001**

CR, complete response; PD, progressive disease; SD, stable disease; PR, partial response

Table 3. Univariate and multivariate Cox regression analyses of clinical characteristics associated with overall survival.

Characteristics	Univariate analysis		Multivariate analysis	
	HR(95% CI)	P value	HR (95% CI)	P value
T stage (T3&T4&T2 vs. T1)	8.829 (1.234-63.151)	**0.030**	12964342.319 (0.000-Inf)	0.995
N stage (N1&N2&N3 vs. N0)	1.925 (1.264-2.931)	**0.002**	1.804 (0.841-3.869)	0.130
M stage (M1 vs. M0)	2.254 (1.295-3.924)	**0.004**	1.199 (0.487-2.953)	0.692
Primary therapy outcome (PR&CR vs. PD&SD)	0.244 (0.168-0.354)	**<0.001**	0.225 (0.144-0.350)	**<0.001**
Pathologic stage (Stage III &Stage IV vs. Stage I &Stage II)	1.947 (1.358-2.793)	**<0.001**	1.102 (0.590-2.060)	0.760
Gender (Male vs. Female)	1.267 (0.891-1.804)	0.188		
Age (>65 vs. <=65)	1.620 (1.154-2.276)	**0.005**	1.832 (1.172-2.864)	**0.008**
Histological type (Diffuse Type &Mucinous Type &Not Otherwise Specified &Signet Ring Type vs. Papillary Type &Tubular Type)	1.094 (0.727-1.646)	0.668		
Residual tumor (R1&R2 vs. R0)	3.445 (2.160-5.494)	**<0.001**	1.461 (0.788-2.709)	0.228
H pylori infection (Yes vs. No)	0.650 (0.279-1.513)	0.317		
Histologic grade (G3 vs. G1&G2)	1.353 (0.957-1.914)	0.087	1.532 (0.973-2.411)	0.065
INHBA (High vs. Low)	1.422 (1.022-1.978)	**0.037**	1.894 (1.233-2.907)	**0.004**
TP53 (High vs. Low)	0.761 (0.548-1.057)	0.103		

Table 4. Enrichment analyses of high expression of INHBA and associated gene

Category	Description	Count(%)	Log10(P)	Log10(q)
GO:BP	Extracellular matrix organization	74(25.78)	-71.40	-67.21
GO:BP	response to growth factor	48(16.72)	-24.07	-20.83
GO:BP	negative regulation of cell differentiation	29(10.10)	-9.11	-6.62
GO:BP	vasculature development	52(18.12)	-26.42	-23.07
GO:BP	cell-substrate adhesion	31(10.80)	-18.97	-15.92
GO:MF	extracellular matrix structural constituent	46(16.03)	-51.89	-48.22
GO:MF	glycosaminoglycan binding	31(10.80)	-24.84	-21.78
GO:MF	collagen binding	24(8.36)	-30.21	-26.85
GO:MF	growth factor binding	21(7.32)	-18.07	-15.30
GO:MF	receptor regulator activity	21(7.32)	-6.72	-4.43
GO:CC	extracellular matrix	96(33.45)	-89.91	-86.63
GO:CC	endoplasmic reticulum lumen	42(14.63)	-34.01	-31.20
GO:CC	collagen trimer	21(7.32)	-22.52	-19.84
GO:CC	basement membrane	15(5.23)	-13.37	-10.91
GO:CC	focal adhesion	19(6.62)	-7.12	-4.92
KEGG	Protein digestion and absorption	13(4.53)	-11.09	-8.24
KEGG	Proteoglycans in cancer	15(5.23)	-8.09	-6.02
KEGG	Hippo signaling pathway	9(3.14)	-4.51	-2.75
KEGG	Wnt signaling pathway	7(2.44)	-2.74	-1.22
KEGG	p53 signaling pathway	4(1.39)	-2.06	-0.68

Table 5. Correlation analysis between INHBA and markers of immune cells in TIMER

Cell type	Gene marker	None		Purity	
		Cor	p	Cor	p
B cell	CD19	-0.023	0.634	-0.041	0.428
	KRT20	-0.096	*	-0.122	*
	CD38	0.136	*	0.091	0.08
CD8+T cell	CD8A	0.12	*	0.083	0.109
	CD8B	0.037	0.45	0.017	0.747
Tfh	CXCR5	0.032	0.515	0.001	0.981
	ICOS	0.136	**	0.108	*
	BCL-6	0.279	***	0.251	***
Th1	IL12RB2	0.05	0.309	0.035	0.496
	WSX-1	0.108	*	0.097	0.06
	STAT4	0.112	*	0.079	0.124
	IFNG	0.083	0.09	0.056	0.278
	TBX21	0.116	*	0.09	0.08
	STAT1	0.113	*	0.103	*
	TNF-α	0.189	***	0.168	**
Th2	CCR3	0.044	0.375	0.044	0.389
	STAT6	-0.016	0.744	-0.014	0.786
	GATA3	0.209	***	0.189	***
	STAT5A	0.206	***	0.207	***
Th9	TGFBR2	0.099	*	0.092	0.07
	IRF4	0.07	0.156	0.031	0.543
	SPI1	0.327	***	0.302	***
Th17	IL-21R	0.179	***	0.16	**
	IL-23R	-0.042	0.389	-0.055	0.284
	STAT3	0.246	***	0.243	***
Th22	CCR10	0.189	***	0.174	***
	AHR	0.085	0.08	0.084	0.103

Treg	FOXP3	0.2	***	0.173	***
	CCR8	0.276	***	0.268	***
	IL2RA	0.26	***	0.242	***
T cell exhaustion	PDCD1	0.125	*	0.105	*
	CTLA4	0.135	**	0.111	*
	HAVCR2	0.371	***	0.354	***
Macrophage	CD68	0.209	***	0.196	***
	ITGAM	0.269	***	0.264	***
M1	NOS2	0.032	0.52	0.023	0.651
	ROS	0.154	**	0.173	***
	IRF5	0.174	***	0.181	***
	PTGS2	0.347	***	0.35	***
M2	ARG1	-0.021	0.667	-0.01	0.839
	MRC1	0.276	***	0.259	***
TAM	CCL2	0.415	***	0.397	***
	CCR5	0.216	***	0.188	***
	CD80	0.282	***	0.265	***
	CD86	0.347	***	0.321	***
Monocyte	CD14	0.409	***	0.379	***
	CD16	0.464	***	0.436	***
	CD115	0.309	***	0.289	***
NK	XCL1	-0.005	0.913	-0.006	0.914
	KIR3DL1	-0.006	0.905	-0.055	0.289
	CD7	0.148	**	0.117	*
Neutrophil	FUT4	-0.043	0.385	-0.036	0.483
	MPO	0.223	***	0.215	***
	CEACAM8	-0.026	0.595	-0.017	0.74
	ITGAM	0.269	***	0.264	***
DC	BDCA1	0.005	0.913	-0.018	0.721

	THBD	0.27	***	0.257	***
	ITGAX	0.367	***	0.354	***

Tfh :Follicular helper T cell, Th :T helper cell, Treg :Regulatory T cell, TAM :Tumor-associated macrophage, NK: natural killer cell, DC :dendritic cell, None :Correlation without adjustment, Purity :Correlation adjusted by purity, Cor :R value of Spearman's correlation. *P < 0.05; **P < 0.01; ***P < 0.001

Table 6. Correlation analysis between INHBA and macrophage markers in GEPIA

Cell type	Gene marker	Tumor		Normal	
		Cor	p	Cor	p
M1	NOS2	0.042	0.4	0.2	0.25
	ROS	0.2	***	-0.22	0.19
	IRF5	0.22	***	-0.16	0.34
	PTGS2	0.38	***	0.23	0.17
M2	ARG1	-0.017	0.74	-0.1	0.54
	MRC1	0.32	***	0.62	***
TAM	CCL2	0.43	***	0.62	***
	CCR5	0.24	***	-0.39	*
	CD80	0.34	***	-0.26	0.13
	CD86	0.36	***	-0.22	0.19
Monocyte	CD14	0.44	***	0.2	0.25
	CD16	0.5	***	0.024	0.89
	CD115	0.35	***	0.089	0.6

Tfh :Follicular helper T cell, Th :T helper cell, Treg :Regulatory T cell, TAM :Tumor-associated macrophage, NK: natural killer cell, DC :dendritic cell, None :Correlation without adjustment, Purity :Correlation adjusted by purity, Cor :R value of Spearman's correlation. *P < 0.05; **P < 0.01; ***P < 0.001

Figures

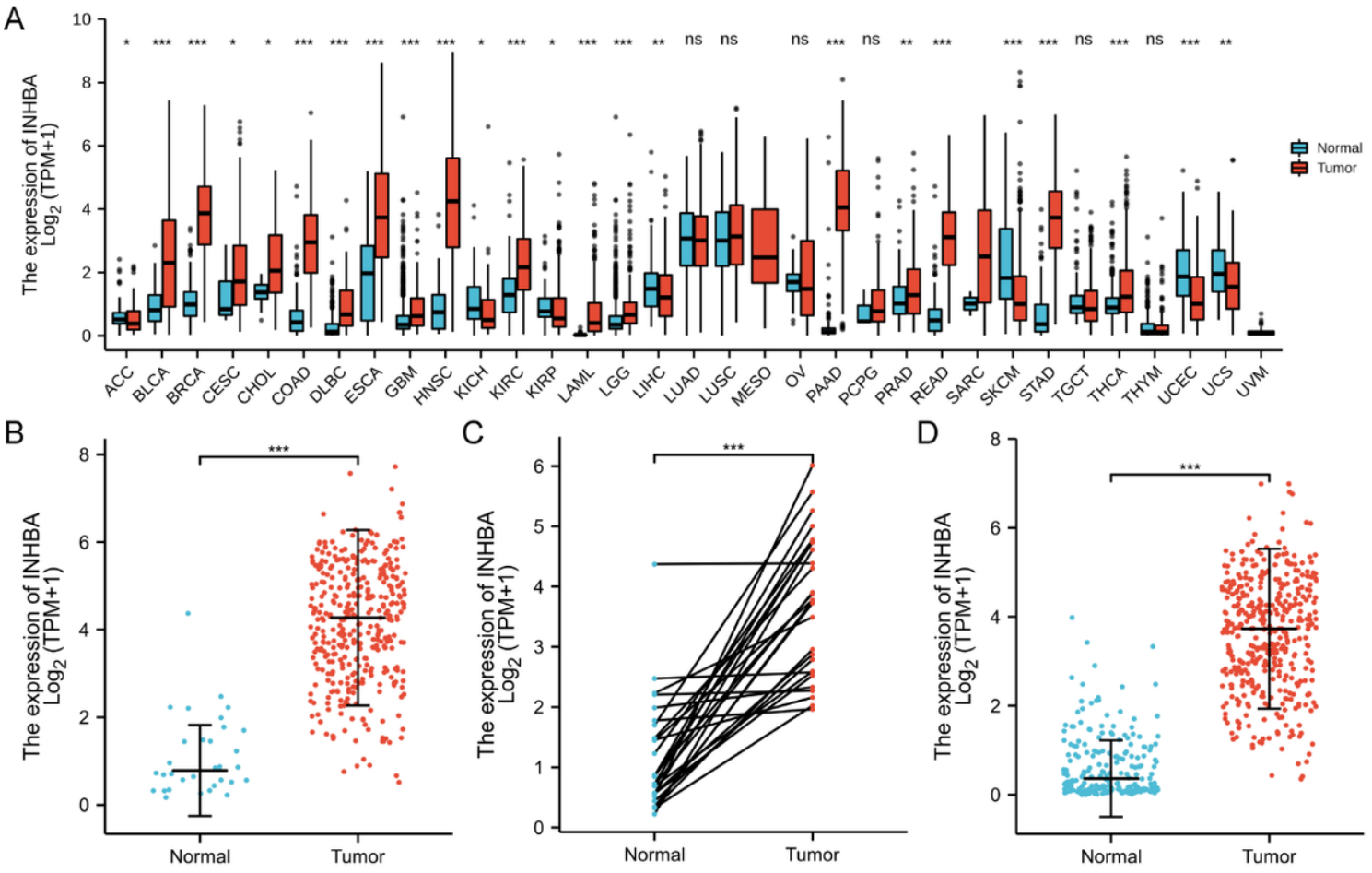

Figure 1

High INHAB expression in Pan-cancer and GC. (A)INHBA expression in TCGA and GTEx pan-cancer data. (B-C)INHBA expression in tumor and normal tissues in gastric cancer from TCGA database. (D)INHBA expression in tumor and normal tissues in GC from TCGA and GTEx database. * P<0.05,** P <0.01,*** P <0.001

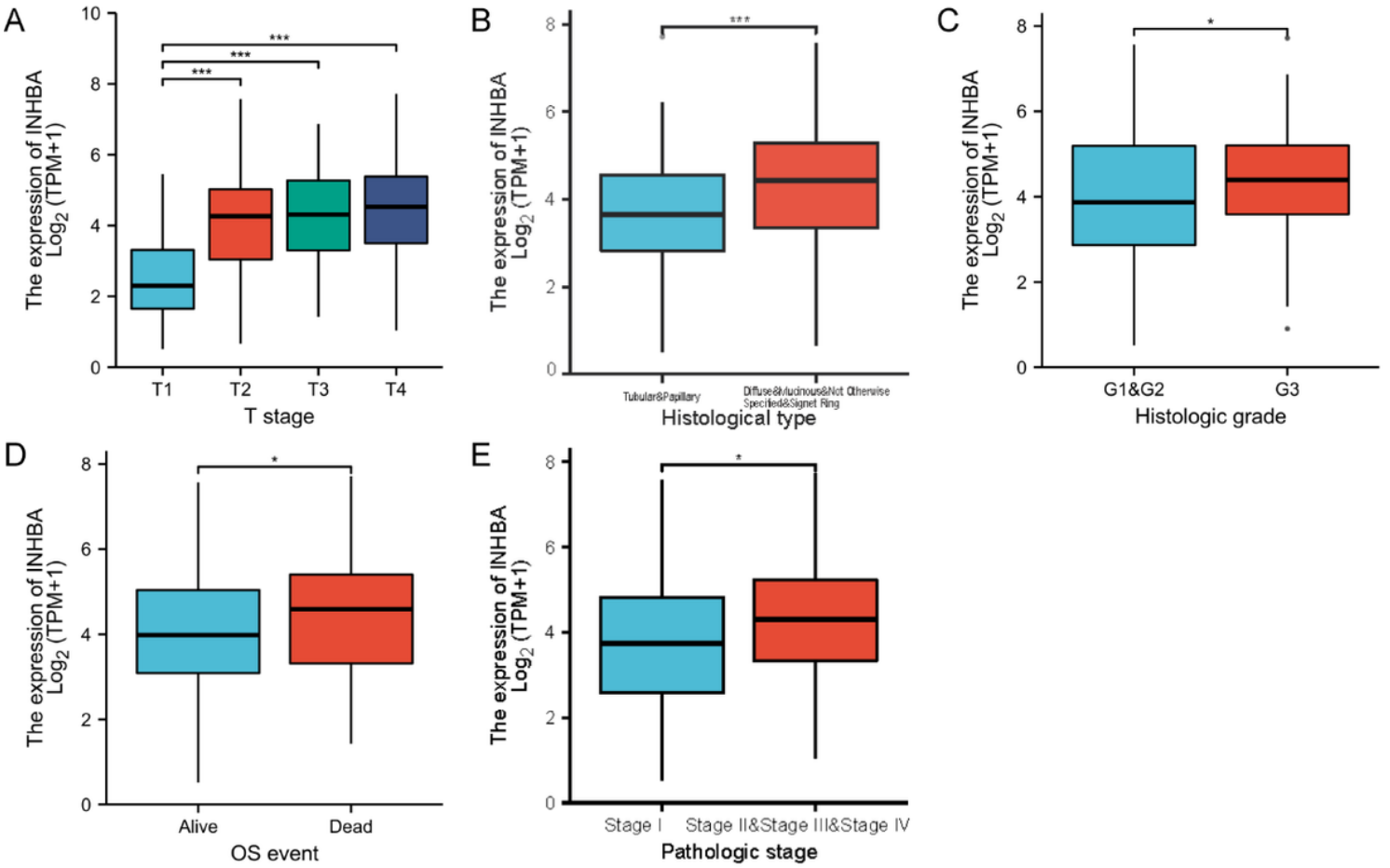

Figure 2

Box plot evaluating the association of INHBA expression and other clinical characteristics in GC patients. (A) T stage; (B) Histological type; (C) Histologic grade; (D) OS event; (E)Pathologic stage.

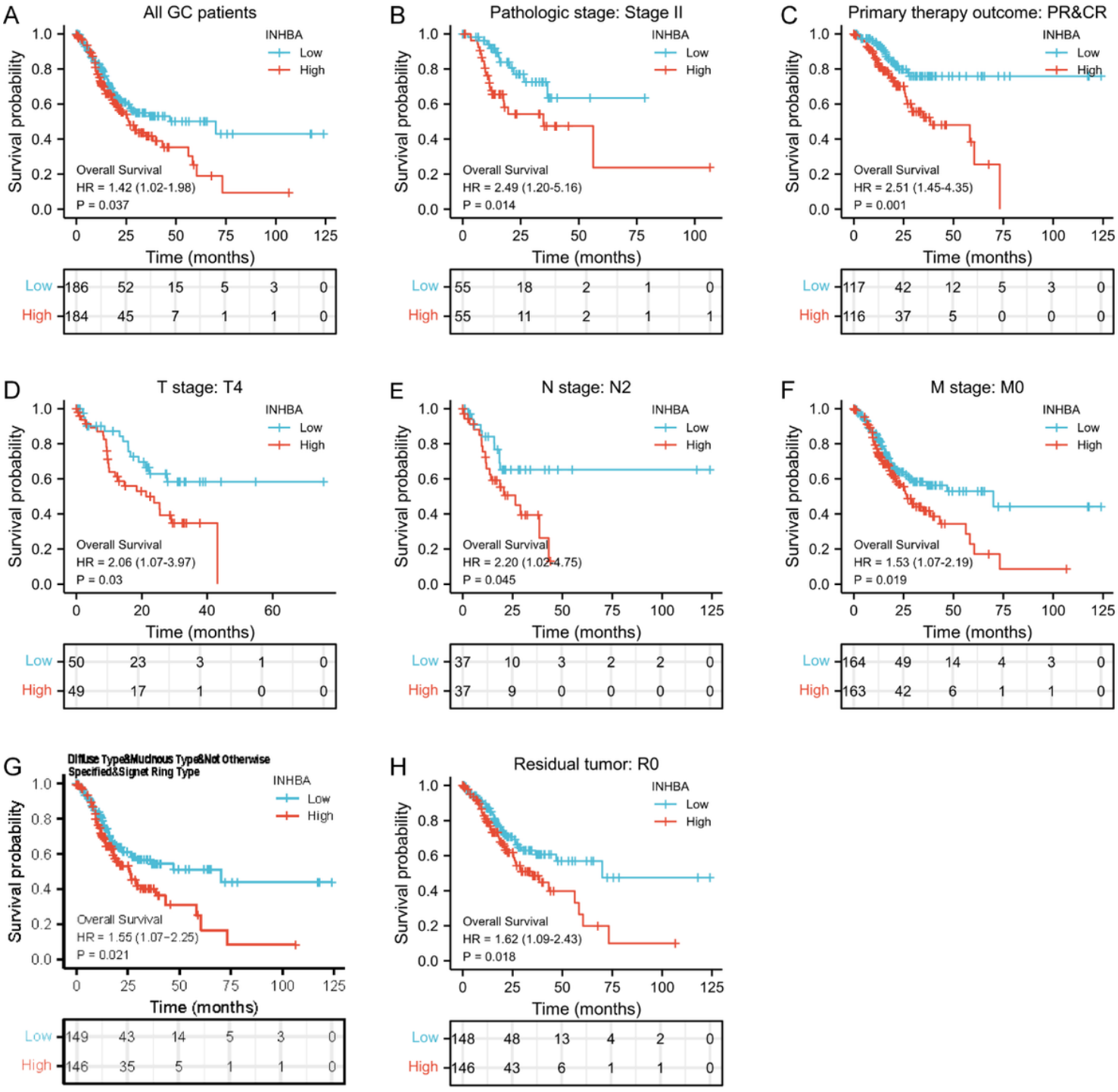

Figure 3

Kaplan-Meier analysis for OS in GC. (A) Kaplan-Meier curve analysis for INHBA in all gastric cancer patients; (B–H) Subgroup analysis for pathologic stage Ⅱ, PR&CR, T4, N2,M0, Diffuse Type &Mucinous Type &Signet Ring Type &Not Otherwise Specified, and R0.

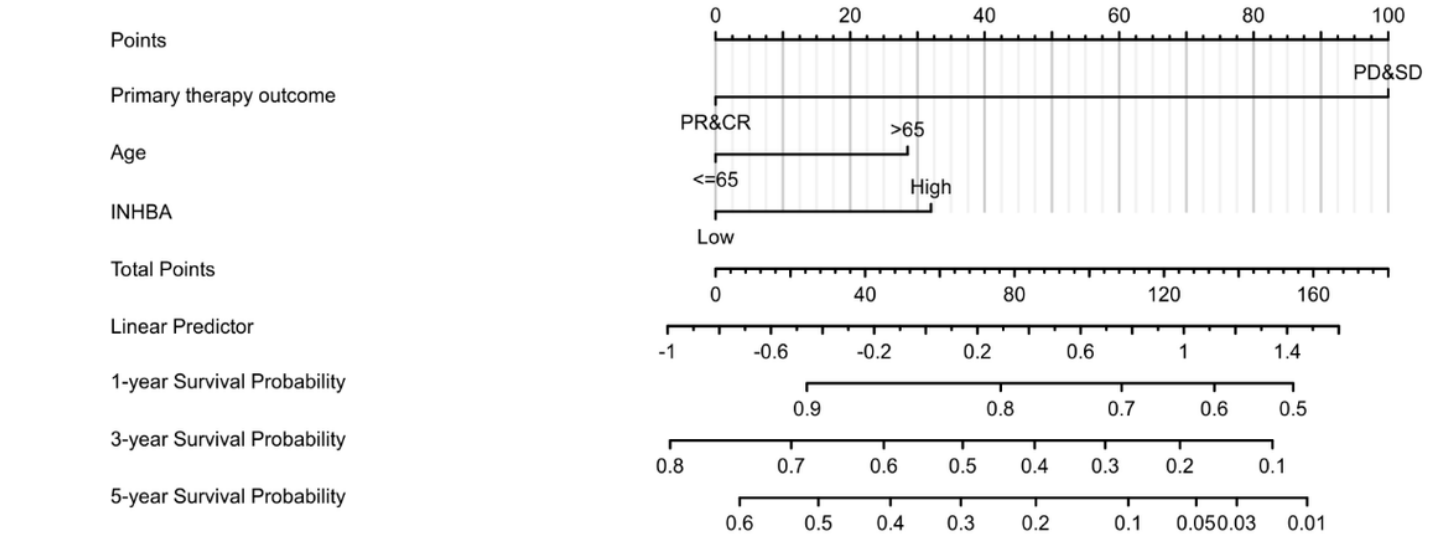

Figure 4

Nomogram for GC patients with 1-, 3- and 5-year OS. According to the positive variables in Cox multivariate analysis, three variables (primary therapy outcome, age , INHBA) were selected to construct the nomogram for risk estimation.

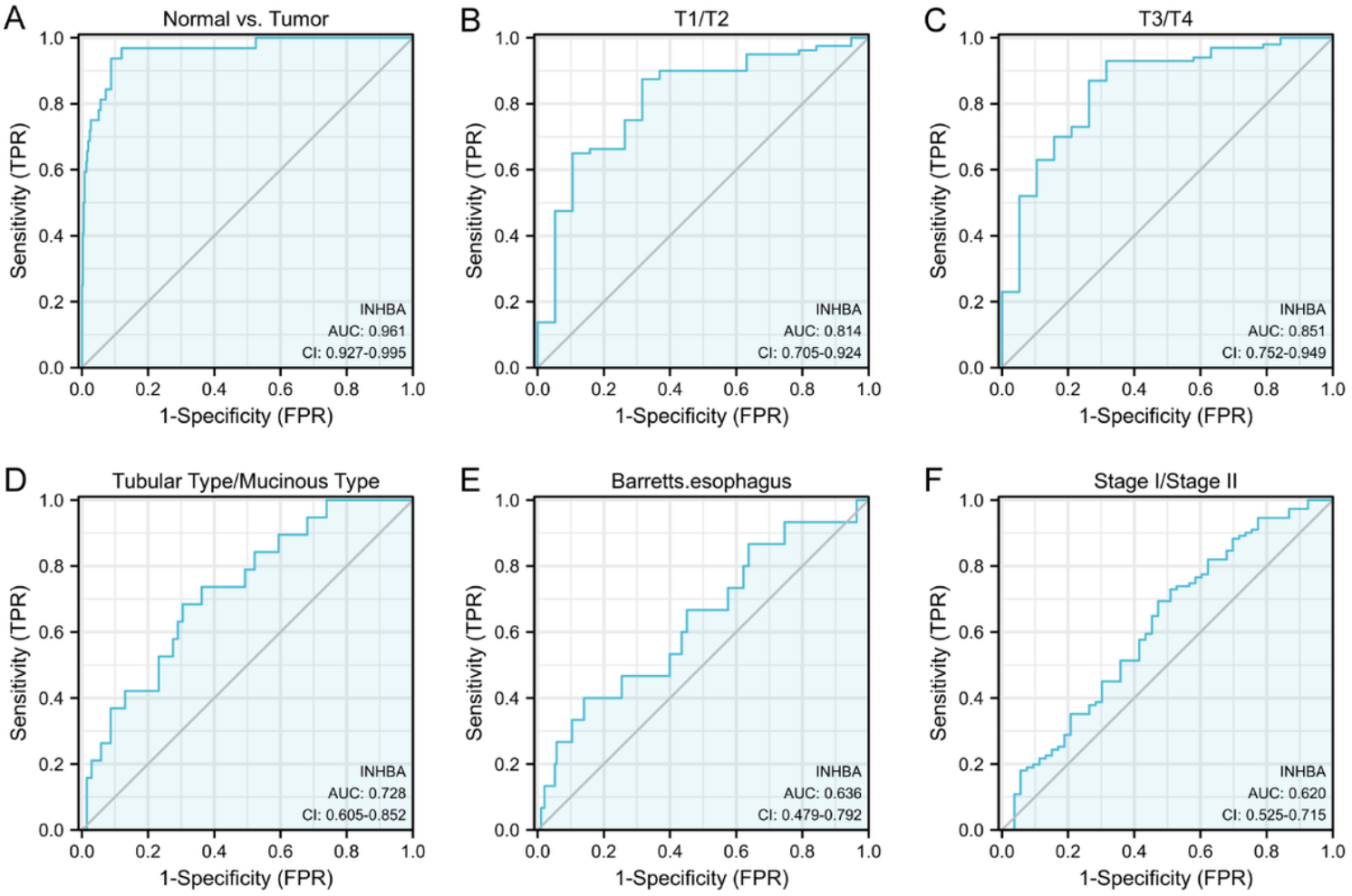

Figure 5

Diagnostic value of INHBA expression in GC. (A) ROC curve for INHBA in normal lung tissue and GC; (B–F) Subgroup analysis for T1/T2, T3/T4, tubular type/mucinous type, Barretts esophagus, stage I/II.

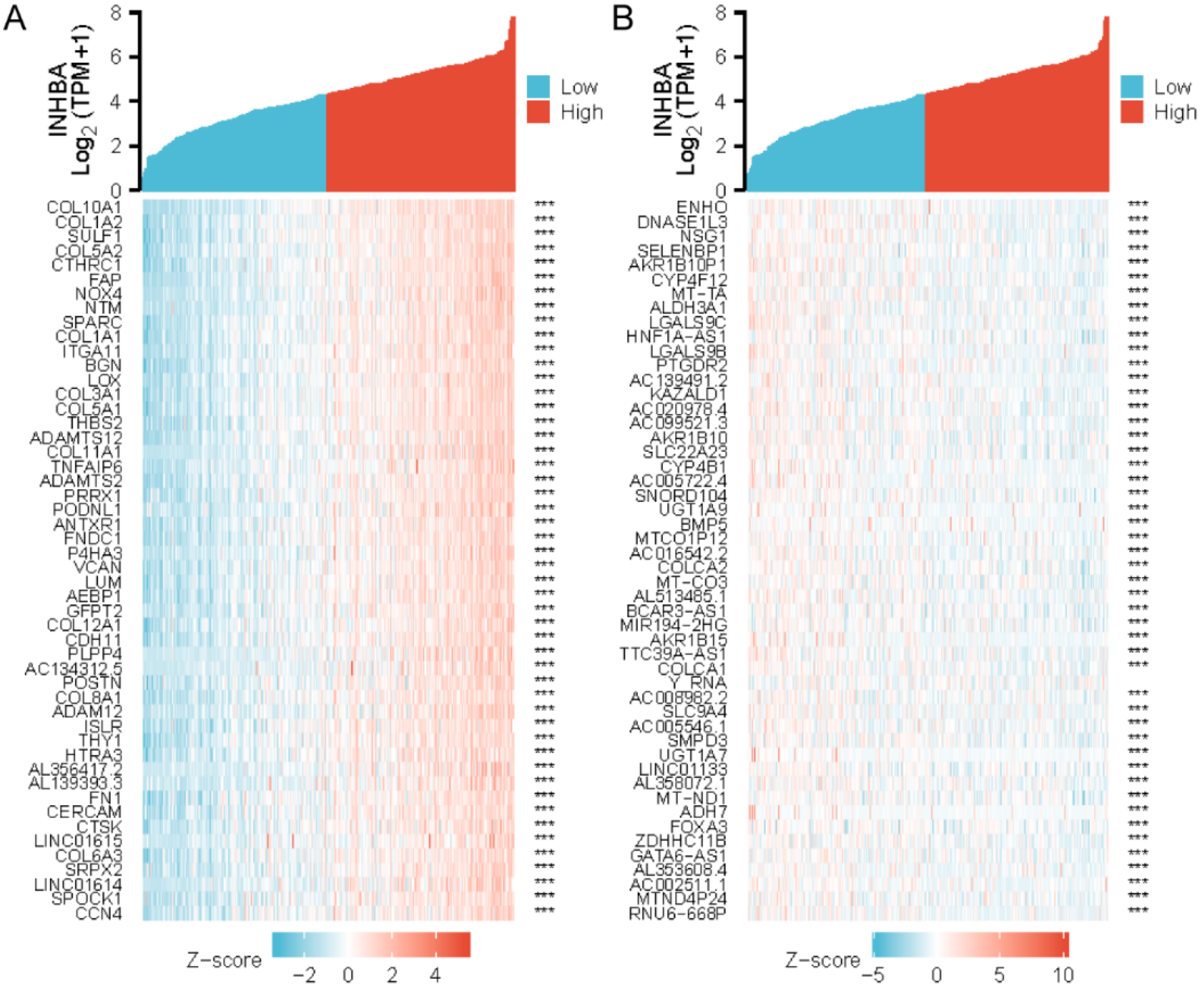

Figure 6

Association between INHBA expression and (A) Top 50 genes most positively associated with INHBA are shown in a heatmap. (B) Top 50 genes most negatively associated with INHBA are shown in a heatmap.

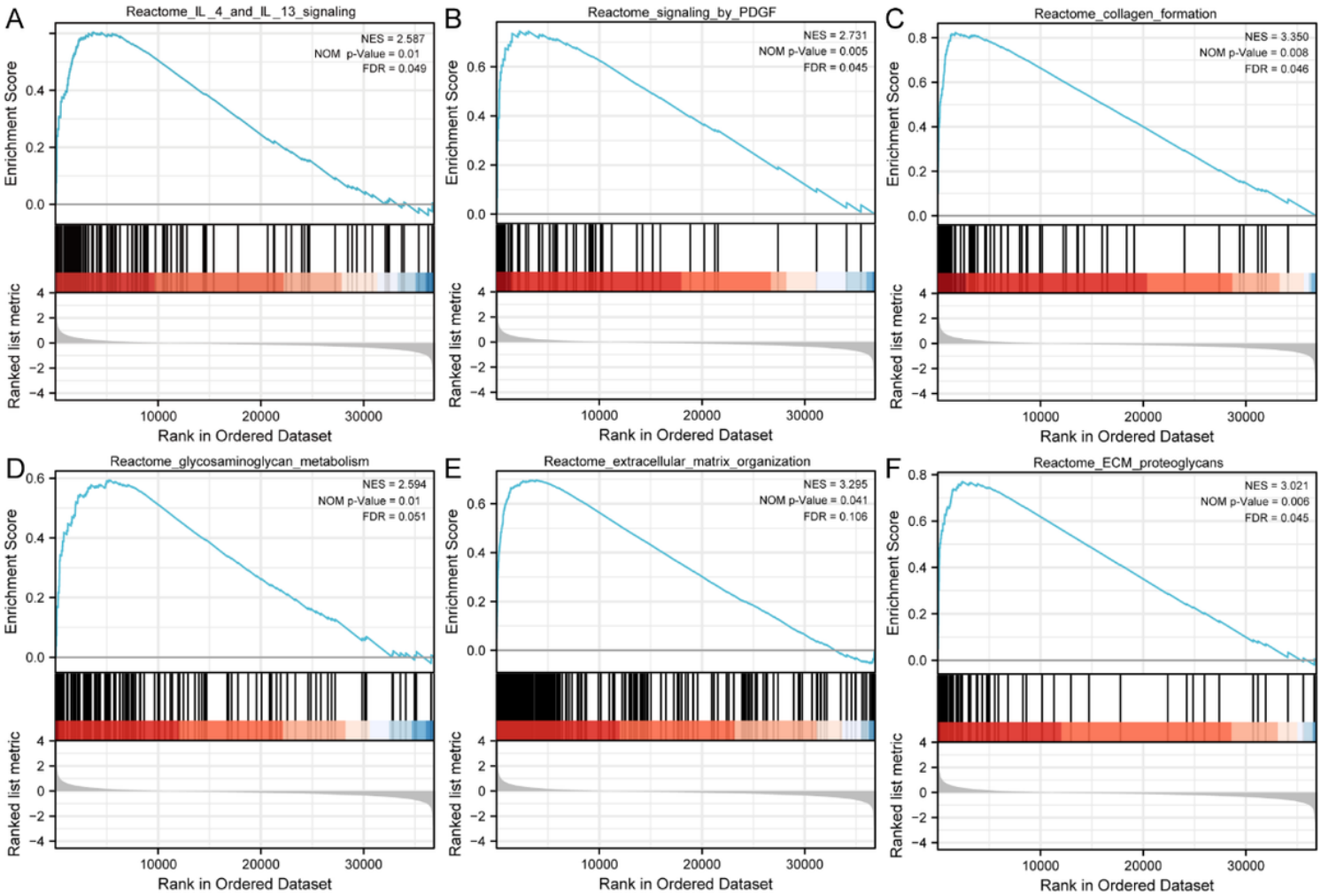

Figure 7

GSEA gene set reactome of (A) IL4 and IL13 signaling, (B) signaling by PDGF, (C) collagen formation, (D) glycosaminoglycan metabolism, (E) extracellular matrix organization, and (F) ECM proteoglycans in GC.

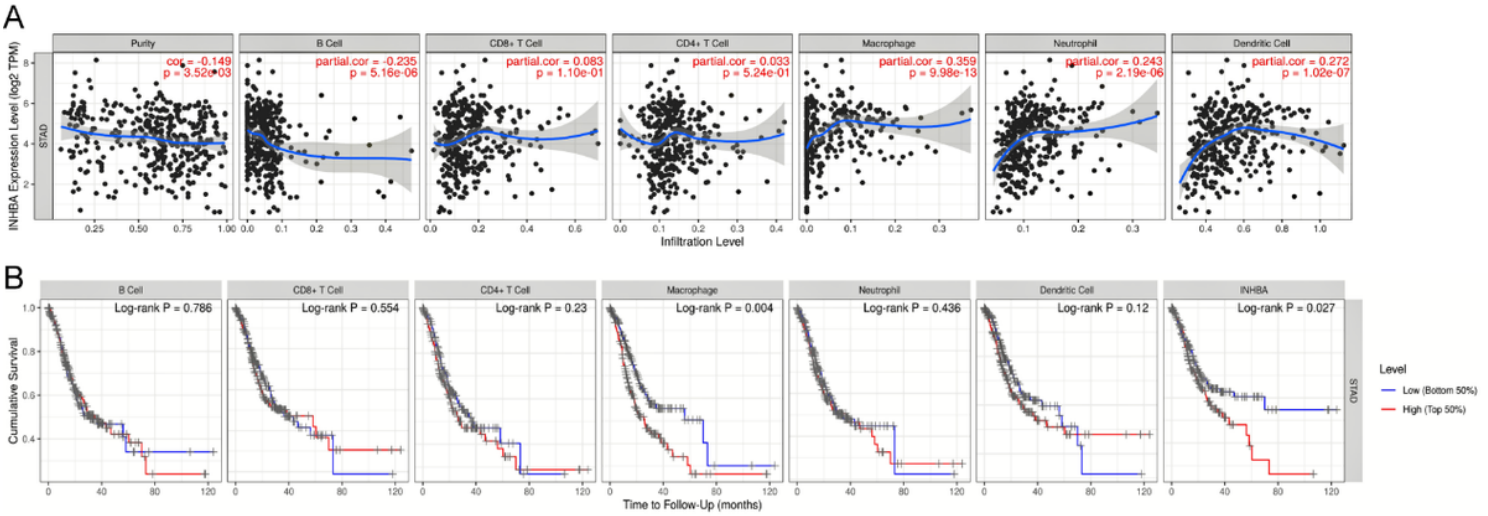

Figure 8

INHBA expression in gastric cancer is associated with (A) immune infiltration level, (B) Kaplan-Meier plotter of immune infiltration.

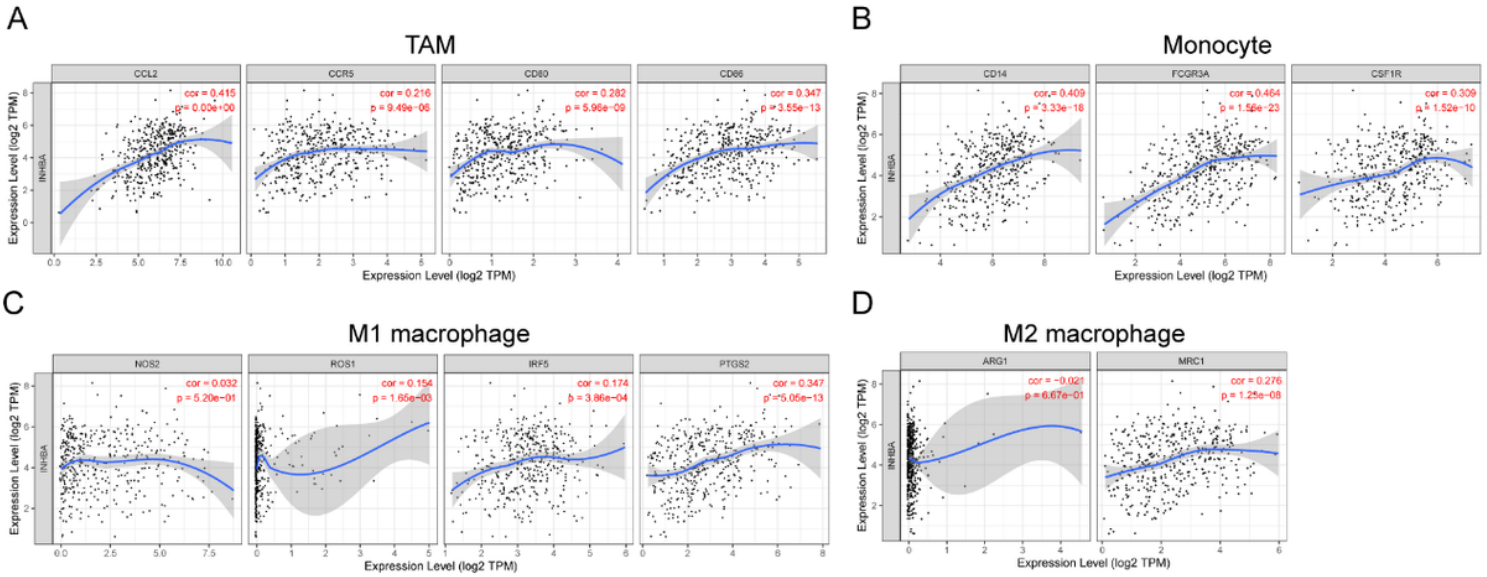

Figure 9

Association between INHBA expression and macrophage-associated markers.(A)TAM, (B)Monocyte, (C)M1 macrophage, (D)M2 macrophage.