Research Square

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GHRL as a Prognostic Biomarker Correlated With Immune Infiltrates and Progression of Precancerous Lesions in Gastric Cancer

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Abstract

Objective

ghrelin is a protein that regulate appetite and energy balance in the human body, which is encoded by the ghrelin prepropeptide gene (GHRL). GHRL is linked with carcinogenesis and immune regulation. However, the correlation of GHRL to prognosis and tumor-infiltrating lymphocytes in gastric cancer remains unclear.

Materials and methods

In this study, we assessed the transcriptional expression, protein expression, prognosis, different clinicopathological features and co-expressed genes about GHRL, the correlation between GHRL and tumor infiltration immune cells in GC patients based on the data published in the following databases: TIMER, GEPIA, GEO, UALCAN, TISIDB, Kaplan–Meier Plotter and HPA databases.

Results

We found that GHRL expression in GC samples was lower than in normal samples. However, sample type, cancer stage, and worse survival were correlated to high GHRL expression. We also found that the expression of GHRL in dysplasia was significantly lower than that in CNAG and in GC. A total of 10 hub genes were identified from the co-expressed genes, including GHRH, GHSR, CCK, GPR39, MBOAT4, UCN, LEP, INS, GH1 and IGF1. High GHRL expression was connected with immunomodulators, chemokines, and infiltrating levels of B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells in GC.

Conclusions

GHRL is a prognostic biomarker for GC patients, and it is correlated with progression of precancerous lesions in GC. It might lead to poor prognosis by regulating tumor immune microenvironment. Studies are important to explore therapeutic targeting GHRL in the future.

Introduction

Gastric cancer is the fifth most common cancer and the third most common cause of cancer death globally[1]. Gastric cancer patients are usually at late stages when diagnosed, thus losing the opportunity for surgery and with the poor prognosis[2]. Currently, immunotherapy has become an effective treatment in gastric cancer(GC)[1, 3–7]. However, not all GC patients benefit from immunotherapy, because of the different immune microenvironment of tumor[8, 9], it is urgent to seek immune-related biomarkers in GC.

Ghrelin was discovered and named by Kojima in 1999[10], which is a 28-amino acid polypeptide secreted by the stomach and released into the blood, acting on the growth hormone secretagogue receptor(GHSR), thereby, promoting the release of growth hormone. Ghrelin exists in the human body in two forms, active(acylated-ghrelin) and inactive(deacylated ghrelin). Ghrelin was encoded by the ghrelin prepropeptide gene(GHRL)[11]. Dixit et al found that ghrelin and its receptor (GHSR)were expressed in T cells and monocytes, ghrelin inhibited the expression of proinflammatory cytokines TNF-a and IL-6, and inhibited inflammatory reactions[12]. It has been confirmed that the stomach was indeed not the only source of ghrelin[13], hypothalamus, hippocampus, pituitary gland, cortex, small intestine, and pancreas can also secrete a small amount of ghrelin[14–16], GHRL plays important roles in the development of tumors, and aberrant GHRL expression has been found in various cancers, such as breast cancer[17], lung cancer[18], bladder cancer[19], and adrenal cancer[20]. At present, there were some researchers who found that ghrelin affects cancer cell proliferation, migration, and invasion through different signaling pathways[21–24]. However, The possible mechanisms of GHRL about tumor development, the GHRL expression in different stages of gastric cancer Correa's cascade, and immune engagement in GC has not been well understood before.

In this study, the GHRL expression and its connection to prognosis in GC were presented via diverse databases including the Gene Expression Profiling Interaction Analysis2.0(GEPIA2), UALCAN, STRING, Human Protein Atlas(HPA), Kaplan–Meier (KM) plotter, and TISIDB datasets. Furthermore, we used R software to further analyze the dynamic changes of GHRL in different pathological stages of GC Correa's cascade[25], the Tumor Immune Estimation Resource2.0(TIMER2.0) was used to investigate the relationship of GHRL with immune-related cells in the tumor microenvironments. In addition, the hub genes related to GHRL expression in GC were investigated as well. This study uncovered the critical involvement of GHRL in GC and the possible mechanisms via which GHRL may regulate tumor-infiltrating immune cells.

Results

1. GHRL expression in GC

According to the GEPIA database, we discovered that GHRL expression level was increased in Diffuse Large B-cell Lymphoma and Acute Myeloid Leukemia, but was decreased in stomach adenocarcinoma (STAD) compared with healthy tissues, Fig. 1A, B shows the findings. Furthermore, to verify the difference of GHRL protein expression in STAD, we downloaded immunohistochemical maps of GHRL in GC and normal gastric mucosa tissue in the Human protein atlas, we also found that low expression of GHRL in STAD(Fig. 1C).

2. Relationship between GHRL expression and GC patient clinical pathology

We examined GHRL expression in relation to sample type (healthy/primary tumor), tumor stage (stages 1, 2, 3, and 4), and H. pylori status by applying UALCAN. As shown in Fig. 2A, GC samples had lower GHRL expression than that in healthy samples (p = 0.0036). However, there was no significant difference between tumor *H. pylori* positive and *H. pylori* negative (Fig. 2B). A study of tumor stages revealed that GHRL in the middle and late-stage cancers was significantly higher expressed than in the early stages, suggesting a potential function for GHRL in cancer development and migration (Fig. 2C).

To further analyze the dynamic changes of GHRL in different pathological stages of GC Correa cascade, we used R software to analyze the GEO dataset. We found that the expression of GHRL in GC was higher than that in dysplasia(LGIN + HGIN), and the expression of GHRL in dysplasia was significantly lower than that in CNAG (Fig. 3A). However, there was no significant difference in GHRL expression among CAG, CNAG, and NC (Fig. 3B).

3. GHRL expression is linked to poor overall survival in GC patients

In this research, we evaluate whether GHRL can be used as a prognostic biomarker in GC via Kaplan–Meier survival curves. This study showed that increased GHRL expression was linked with poorer OS and DFS in GC (Fig. 4). To study the significance and possible molecular mechanisms of GHRL expression in tumor development, we found the correlation between the GHRL expression and clinical–pathological features of GC in the KM plotter. High GHRL expression was linked to poorer OS in female patients and poorer PPS in male patients. Specifically, increased GHRL expression was associated with poorer PPS in stage 3(PPS HR = 0.61, p = 0.046) of GC patients (Table 1). we also discovered that OS and PPS at stage T3 (OS, HR = 0.62, p = 0.0077) and N1 + 2 + 3 (OS, HR = 0.75, p = 0.042) were related to GHRL expression. Surely, we found that PPS in Intestinal of Lauren classification was related to high GHRL expression. These findings suggested that the prognostic significance of GHRL in GC patients was determined by their clinical features.

Table 1

Correlation of GHRL mRNA expression and clinical prognosis in gastric cancer with different clinicopathological factors by Kaplan–Meier plotter.

Clinicopathological characteristics	Overall survival (n = 881)			Post-progression survival (n = 503)		
	N	Hazard ratio	P	N	Hazard ratio	P
Sex						
Female	187	1.77(1.07-2.92)	**0.025**	127	1.53(0.9-2.59)	0.11
Male	349	1.27(0.9-1.78)	0.17	256	0.66(0.45-0.97)	**0.035**
Stage						
1	62	2.21(0.68-7.21)	0.18	31	5.12(0.61-42.56)	0.092
2	135	0.65(0.33-1.3)	0.22	105	1.42(0.74-2.74)	0.29
3	197	0.78(0.53-1.14)	0.19	142	0.61(0.38-1)	**0.046**
4	140	0.75(0.5-1.14)	0.18	104	1.48(0.85-2.56)	0.16
Stage T						
2	241	1.45(0.85-2.47)	0.17	196	1.44(0.91-2.28)	0.12
3	204	0.62(0.44-0.88)	**0.0077**	150	0.68(0.46-1.01)	0.057
4	38	0.64(0.28-1.48)	0.29	29	3.19(0.9-11.36)	0.061
Stage N						
0	74	2.05(0.76-5.53)	0.15	41	3.37(0.74-15.42)	0.096
1+2+3	422	0.75(0.57-0.99)	**0.042**	337	0.79(0.58-1.09)	0.15
1	225	0.8(0.53-1.22)	0.3	169	1.39(0.86-2.24)	0.18
2	121	0.65(0.41-1.03)	0.067	105	0.63(0.38-1.07)	0.086
3	76	0.65(0.36-1.15)	0.14	63	1.57(0.75-3.26)	0.23
Stage M						
0	444	0.79(0.59-1.05)	0.1	342	0.78(0.56-1.09)	0.14
1	56	0.69(0.37-1.31)	0.26	36	0.69(0.33-1.4)	0.3
Lauren classification						
Intestinal	269	1.57(1-2.49)	0.051	192	1.79(1.04-3.07)	**0.034**
Diffuse	240	0.74(0.52-1.04)	0.085	176	0.77(0.53-1.14)	0.19
Mixed	29	2.36(0.74-7.52)	0.13	-	-	-

HER2						
Positive	202	1.33(0.89-2)	0.16	101	0.58(0.32-1.07)	0.079
Negative	429	1.29(0.94-1.78)	0.12	283	1.29(0.87-1.92)	0.21
Note. Bold values indicate p < 0.05.						

4. Protein–protein interaction (PPI) network construction

A total of 11 genes (GHRL, GHRH, GHSR, CCK, GPR39, MBOAT4, UCN, LEP, INS, GH1, IGF1) were filtered into the target genes PPI network complex, containing 11 nodes and 38 edges, as shown in Fig. 5.

5. Correlation between immune infiltration and GHRL expression in GC

Immune infiltration is an important factor associated with tumor development[26]. In this study, TISIDB and TIMER platforms were used to assess GHRL expression in connection with immune cell infiltration levels in GC. GHRL expression were adversely correlated with the purity of STAD (rho = -0.236, p < 3.22e − 6). Our results also discovered that GHRL had a correlation with the abundance of TILs (Fig. 6A). High expression level of GHRL was positively correlated with infiltrating degree of B cells (rho = 0.217), CD8 + T cells (rho = 0.239), CD4 + T cells (rho = 0.345), macrophages (rho = 0.27), neutrophils (rho = 0.183), and dendritic cells (rho = 0.238) (Fig. 6B). These results indicate that GHRL plays an important function in the immune infiltration of GC. TIMER and GEPIA databases were used to study the association between GHRL and different biomarkers of TILs (CD8 + T cells, B cells, T cells, NK cells, monocytes, DCs, TAMs, M1macrophages, M2 macrophages, neutrophils, and related subtypes) in STAD. We found that GHRL was associated with the majority of TILs markers in STAD. GHRL was linked to the majority of immune marker sets of TILs in STAD (Table 2). Indeed, GHRL had a significant association with the majority of marker sets of CD8 + T cells, B cells, monocytes, M2 macrophages, DCs, Th2, Th17, Treg, and T cell exhaustion in STAD (Table 2). including CD8A, CD8B, CD19, CD86, CD163, BDCA-1(CD1C), GATA3, STAT6, STAT3, FOXP3, CCR8, CTLA4, LAG3(P<0.01). Furthermore, according to the GEPIA database, we further assessed the connection of GHRL expression with the markers of M1 macrophages, M2 macrophages, monocytes, TAMs, and T-cell exhaustion (Table 3). Therefore, GHRL may regulate T-cell exhaustion and macrophage polarization in GC.

Table 2
Correlation analysis between GHRL and related genes and markers of immune cells in Tumor Immune Estimation Resource (TIMER2.0).

Description	Gene markers	STAD	
		Cor	P
$CD8^+$ T cell	CD8A	0.271	□□□
	CD8B	0.251	□□□
B cell	CD19	0.439	□□□
Monocyte	CD86	0.224	□□
TAM	CD68	0.154	0.003
M1 Macrophage	INOS(NOS2)	0.024	0.64
M2 Macrophage	CD163	0.15	□
Natural killer cell	KIR3DL1	-0.028	0.58
Dendritic cell	BDCA-1(CD1C)	0.525	□□□
Th2	GATA3	0.267	□□□
	STAT6	0.132	□□
Th17	STAT3	0.14	□
Treg	FOXP3	0.233	□□□
	CCR8	0.23	□□□
Tcell exhaustion	CTLA4	0.148	□
	LAG3	0.16	□

Note. STAD, stomach adenocarcinoma; TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; R, R value of Spearman's correlation. * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$.

Table 3
Correlation analysis between GHRL and related genes and markers of immune cells in Gene Expression Profiling Interaction Analysis (GEPIA).

Description	Gene markers	STAD			
		Tumor		Normal	
		R	P	R	P
Monocyte	CD86	0.17	□□	0.64	□□□
	CD115 (CSF1R)	0.25	□□□	0.42	0.012
TAM	CCL2	0.12	0.015	-0.051	0.77
	CD68	0.11	0.031	0.37	0.028
	IL10	0.18	□□	0.53	□□
M1 Macrophage	INOS(NOS2)	0.023	0.64	0.25	0.14
	IRF5	0.19	□□□	0.071	0.68
	COX2(PTGS2)	0.05	0.32	-0.17	0.31
M2 Macrophage	CD163	0.033	0.51	-0.25	0.14
	VSIG4	0.053	0.28	0.05	0.77
	MS4A4A	0.15	□	0.094	0.59
Tcell exhaustion	PD-1(PDCD1)	0.17	□□	0.74	□□□
	PDL1	-0.0093	0.85	0.08	0.64
	CTLA4	0.1	0.038	0.68	□□□
	LAG3	0.12	0.013	0.65	□□□
	TIM-3(HAVCR2)	0.16	□	0.53	□□
	GZMB	-0.016	0.75	0.69	□□□

Note. STAD, stomach adenocarcinoma; TAM, tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; R, R value of Spearman's correlation. * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$.

6. The expression of GHRL is associated with immunomodulators in GC

This research indicated that GHRL was significantly connected with immunoinhibitors ($p < 0.0001$), such as ADORA2A (rho = 0.347), BTLA (rho = 0.417), CD244 (rho = 0.262), and CSF1R (rho = 0.279) (Fig. 7A).

The expression of GHRL was also closely associated with immunostimulators ($p < 0.0001$), including CD27(rho = 0.447), CD28 (rho = 0.409), CD40LG (rho = 0.466), CD48 (rho = 0.414), CXCR4 (rho = 0.375), IL6R (rho = 0.342), LTA (rho = 0.324), TMIGD2(rho = 0.309), TNFRSF13C (rho = 0.335) and TNFRSF17(rho = 0.438) (Fig. 7B). These results suggested that GHRL is engaged in the regulation of immune interaction and may modulate tumor immune escape.

7. Correlation between GHRL expression and chemokines in GC

This research also implicated the association between GHRL expression with chemokines. For instance, GHRL expression was significantly linked to CCL14 (rho = 0.316), CCL19 (rho = 0.438), CCL21(rho = 0.365), CCL22 (rho = 0.319), CXCL14(rho = 0.308), CXCL17(rho = 0.404), CCR9 (rho = 0.375), and TNFRSF13B (rho = 0.518) (Fig. 8A).Meanwhile, we demonstrated that GHRL expression was also correlated with chemokine receptors ($p < 0.001$), including CCR2 (rho = 0.355),CCR4 (rho = 0.427), CCR6 (rho = 0.395),CCR7 (rho = 0.483), CXCR4 (rho = 0.375),CXCR5 (rho = 0.503), and CX3CR1(rho = 0.407) (Fig. 8B). These results demonstrated that GHRL may function as an immunoregulatory factor in GC.

Discussion

In the present study, a comprehensive bioinformatics investigation was performed to analyze the clinical significance and expression level of GHRL in GC. Our results revealed poor prognosis was consistent with high expression of GHRL in GC. Furthermore, our data also indicated that GHRL expression had a close association with the infiltration degrees of different immune cells, immunostimulators, immunoinhibitors, chemokines, and receptors in GC. Therefore, our research revealed new insights in understanding the function of GHRL, and it may be a prognostic biomarker linked to immune infiltration of GC.

Ghrelin, a widely distributed peptide hormone, participates in a series of cancer progression[27], GHRL plays a vital role in carcinogenic potential, which was correlated with breast cancer, lung cancer, bladder cancer, and adrenal cancer[17–20]. There is an increasing evidence implicating an immunoregulatory role for ghrelin. Ghrelin mainly acts on the innate and adaptive immune systems to suppress inflammation and induce an anti-inflammatory profile[28]. However, the possible function of GHRL in regulating tumor immunity and its clinical significance in GC are still unknown.

Therefore, we evaluated GHRL expression in GC by databases including GEPIA, TIMER, GEO, HPA, and UALCAN. We discovered that GHRL was clearly decreased in GC compared with normal samples. We also found that the GHRL mRNA and protein levels were decreased in GC samples compared with normal samples. These results showed that the level of GHRL expression may serve as a potential diagnostic biomarker in GC. Furthermore, to confirm whether GHRL can be used as a prognostic biomarker, we used the KM plotter database to analyze the correlation between GHRL expression and OS and PPS. However, the results indicated that the higher GHRL expression correlated with worse OS and poor PPS of GC. In addition, high GHRL expression had a significant correlation with a worse prognosis of GC in stages T3

and N1 + 2 + 3 for worse OS and in stage 3 for worse PPS. These observations support our hypothesis that GHRL may as an anti-oncogene in GC. For low expression of GHRL in GC and high expression of GHRL linked with poor prognosis in GC, our explanation may be that the higher the malignancy, the more anti-oncogene GHRL was needed in GC. In addition, we first discovered that the expression of GHRL in dysplasia was significantly lower than that in CNAG and in GC, so we speculated that ghrelin may be associated with the progression of precancerous lesions, GHRL played different roles in different pathological stages. Previously, a large number of scholars studied the relationship among ghrelin, gastric mucosal atrophy, and *H. pylori* status, but the results were controversial[29–34]. Our study first discovered that GHRL was not related to gastric mucosal atrophy and *H. pylori* status via bioinformatics investigation, probably because of the small sample size of GEO database on gastric mucosal atrophy, therefore, large sample clinical studies and fundamental experiment may be needed to validate in the future.

Additionally, this study discovered that GHRL was related to immune infiltration in GC. In the tumor microenvironment, it has been demonstrated that immune cell infiltration played vital roles in the development and progression of cancer[35, 36]. GHRL played a role in inflammatory and immune responses[37]. Recent studies have revealed that GHRL was involved in the regulation of metabolism, energy balance, and the immune, cardiovascular, and reproductive systems[38–40]. However, whether GHRL expression was linked to immune infiltration in GC remains unknown. Therefore, we systematically examined the association between GHRL expression and the degree of immune infiltration in GC. In our study, this was the first time that GHRL regulating immune infiltration in GC was identified. Our study showed that GHRL expression had a correlation with TILs including B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells. At the same time, decreased GHRL expression was associated with immunostimulators, immunoinhibitors, chemokines, and receptors. In addition, this study also demonstrated the association between GHRL expression and the TIL marker genes of GC. Indeed, GHRL expression had an association with M1 macrophage markers, like IRF5, and M2 macrophage markers, such as MS4A4A and CD163, correlated with GHRL expression. These findings indicate that GHRL has a potential function to regulate macrophage polarization.

Furthermore, Our results revealed that GHRL expression was significantly correlated to cell response to chemokines according to the TISIDB databases. These results reflected that it may be a direction for enhancing immunotherapy effectiveness by targeting GHRL. Above all, GHRL played a key function in recruiting and modulating TILs in GC, and it is worth to continue investigating the molecular mechanism and function of GHRL in modulating the tumor microenvironment.

Unfortunately, we are yet to conduct experimental studies validating the function of GHRL in the development of GC and the molecular mechanism of GHRL in GC immunity, but in the future, we guarantee that experiments will be performed to further validate the projected results.

Conclusion

The high GHRL expression is closely correlated with poor prognosis, GHRL may be associated with the progression of precancerous lesions in GC. The low GHRL expression enhanced immune infiltration degree including B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils, and dendritic cells in GC. Moreover, the expression of GHRL contributes to the regulation of CD8 + T cells, B cells, monocytes, M2 macrophages, DCs, Th2, Th17, Treg, and T cell exhaustion. Therefore, this study suggests that GHRL may serve as a useful biomarker and therapeutic target for patients with GC.

Methods

We followed the methods of Junchang Zhang et al. 2022[41]. Furthermore, STRING, Human Protein Atlas(HPA) and GEO analysis were also involved in this study for comprehensive bioinformatics analysis.

Tumor immune estimation resource database analysis

The Tumor Immune Estimation Resource (TIMER2.0) is a platform to analyze immune infiltration in various cancers(https://timer.cistrome.org/)[42]. We investigated GHRL expression in GC and the relationship between the expression of GHRL and TILs. Furthermore, the relationship between GHRL expression with gene markers of TILs, including markers of CD8+/CD4 + T cells, B cells, monocytes, TAMs, M1macrophages, M2 macrophages, natural killer (NK) cells, neutrophils, and dendritic cells (DCs) has been analyzed.

Gene expression profiling interaction analysis

The Gene Expression Profiling Interactive Analysis2.0 (GEPIA2) database (http://gepia.cancer-pku.cn/index.html) is a platform that obtains the data from TCGA and The Genotype–Tissue Expression (GTEx) databases[43]. In this study, we used the GEPIA data source to analyze the expression and survival of GHRL in GC.

UALCAN analysis

In this study, we use the UALCAN website[44] to examine the GHRL expression level from major clinical features such as tissue type (healthy/tumor), *H. pylori* status, and GC stage (stages 1, 2, 3, and 4).

GEO analysis

To further validate the expression of GHRL in GC, GEO microarray series (GSE55696, GSE130823, GSE87666, GSE116312, GSE106656, GSE160116, GSE5081, GSE78523) containing STAD tumor and non-tumor samples were obtained from the National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/).

Kaplan–Meier Plotter

The prognostic significance of GHRL in GC, including overall survival (OS) and postprogression survival(PPS), was investigated using the KM plotter[45] (http://kmplot.com/analysis/).

TISIDB

TISIDB (http://cis.hku.hk/TISIDB/index.php) is an online platform that combines various heterogeneous data to find tumor and immune system interactions[46]. In this research, TISIDB was used to investigate the association of GHRL with TILs, immunostimulators, immunoinhibitors, chemokines, and receptors in GC.

Protein–protein interaction (PPI) network construction

STRING (http://string-db.org) is an online database for the prediction of protein interaction relationships[47]. The GHRL-related genes were displayed in the STRING website.

The Human Protein Atlas (HPA) database

The HPA database (https://www.proteinatlas.org/) is a platform that includes lots of transcriptomic and proteomic data in health and disease[48]. Immunohistochemistry images of GHRL between GC and normal tissues were downloaded from the HPA database.

Statistical analysis

HR and p values were described using log-rank test. Spearman's correlation coefficient was used to analyze the connection of GHRL expression with immune infiltration levels, immunostimulators, immunoinhibitors, chemokines, and receptors. Statistical significance was defined as $p < 0.05$.

Declarations

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Not Applicable.

AUTHOR CONTRIBUTIONS

YX designed the experiment and supervised the study; DWL contributed to formal analysis, JYW wrote the manuscript; YX reviewed and revised the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

ETHICS AND CONCENT TO PARTICIPATEE

Not Applicable.

CONSENT FOR PUBLICATION

Not Applicable.

CONFLICTS OF INTEREST

The authors declared no conflict of interest.

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Figures

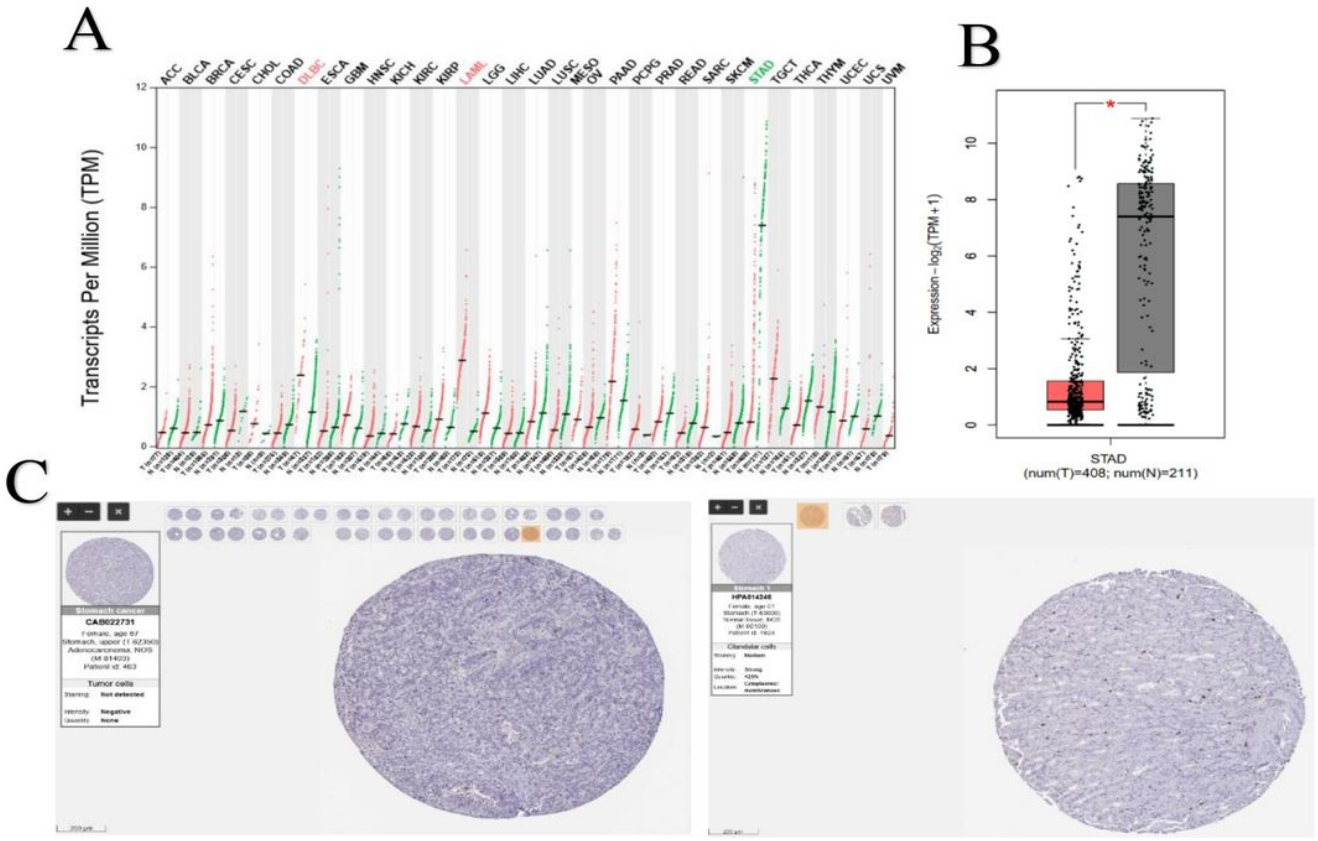
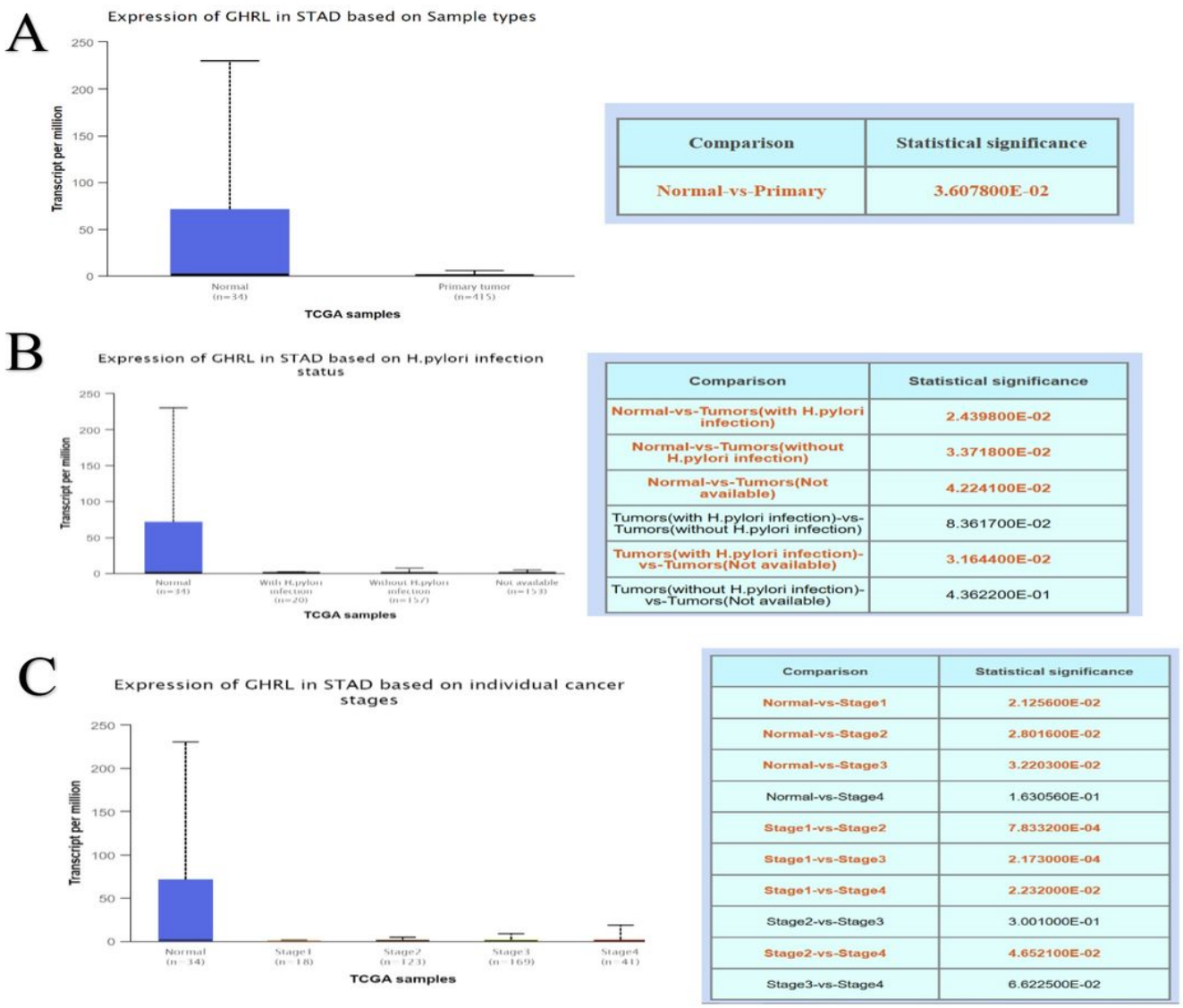


Figure 1

Difference of GHRL expression in pan-carcinoma (GEPIA), low expression in STAD at RNA level (B) and protein level(C).



Comparison	Statistical significance
Normal-vs-Primary	3.607800E-02

Comparison	Statistical significance
Normal-vs-Tumors(with H.pylori infection)	2.439800E-02
Normal-vs-Tumors(without H.pylori infection)	3.371800E-02
Normal-vs-Tumors(Not available)	4.224100E-02
Tumors(with H.pylori infection)-vs-Tumors(without H.pylori infection)	8.361700E-02
Tumors(with H.pylori infection)-vs-Tumors(Not available)	3.164400E-02
Tumors(without H.pylori infection)-vs-Tumors(Not available)	4.362200E-01

Comparison	Statistical significance
Normal-vs-Stage1	2.125600E-02
Normal-vs-Stage2	2.801600E-02
Normal-vs-Stage3	3.220300E-02
Normal-vs-Stage4	1.630560E-01
Stage1-vs-Stage2	7.833200E-04
Stage1-vs-Stage3	2.173000E-04
Stage1-vs-Stage4	2.232000E-02
Stage2-vs-Stage3	3.001000E-01
Stage2-vs-Stage4	4.652100E-02
Stage3-vs-Stage4	6.622500E-02

Figure 2

Correlation between GHRL mRNA expression level and clinicopathological parameters of GC through the UALCAN database. (A) Sample type (normal/primary tumor). (B) H. pylori status. (C) Cancer stage (stage 1, 2, 3, and 4). N, normal; P, primary tumor; S1, stage 1; S2, stage 2; S3, stage 3; S4, stage 4; STAD, stomach adenocarcinoma.

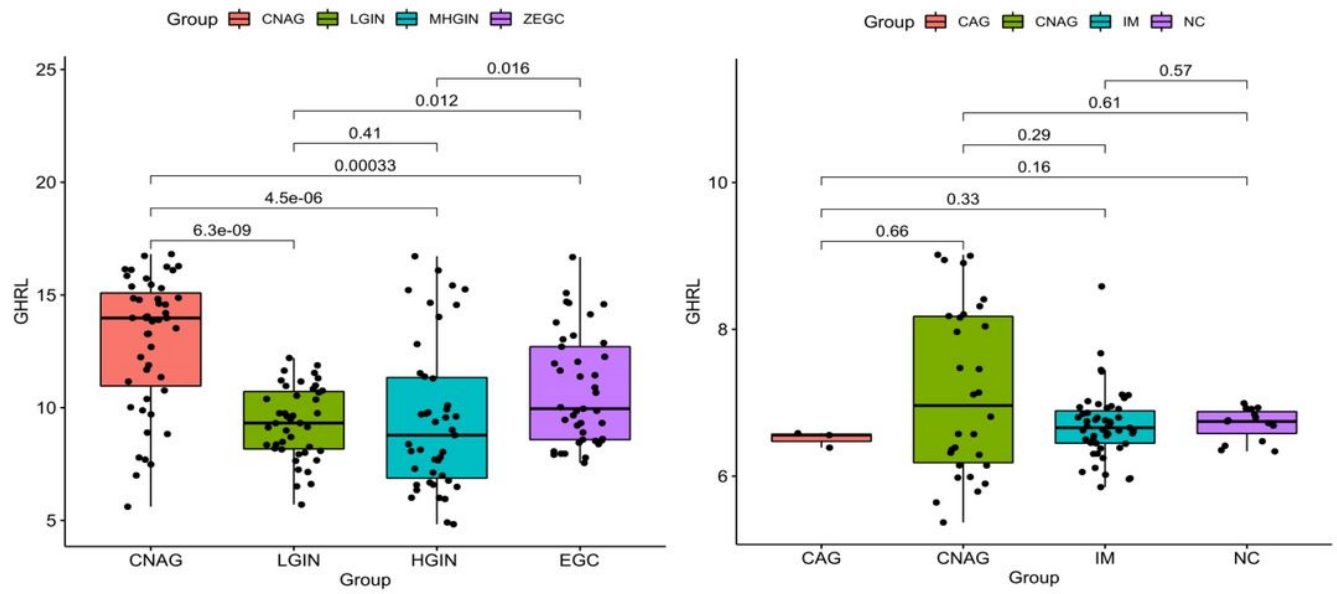


Figure 3

The GHRL expression in different stages of GC correa cascade. CNAG, chronic non-atrophic gastritis; LGIN, low grade intraepithelial neoplasia; HGIN, high grade intraepithelial neoplasia; EGC, early GC; CAG, chronic atrophic gastritis; IM, intestinal metaplasia; NC, normal control.

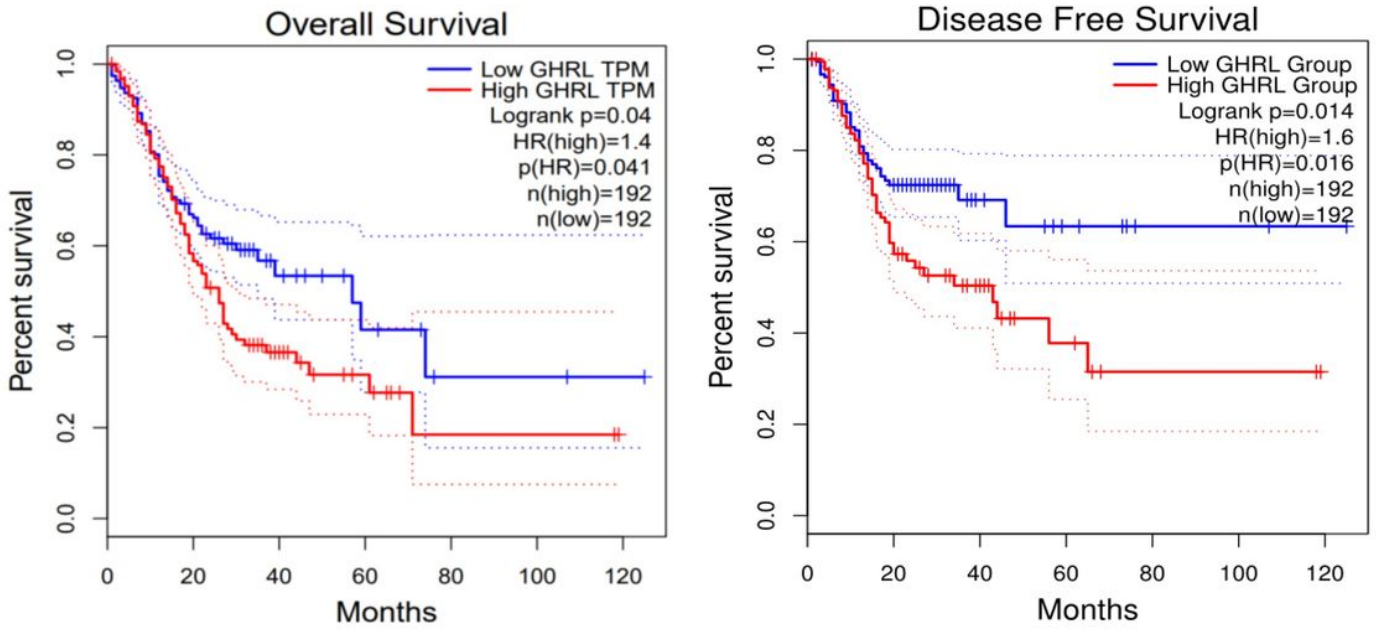


Figure 4

Kaplan–Meier survival curves comparing the high and low expression of GHRL in GC in GEPIA databases.

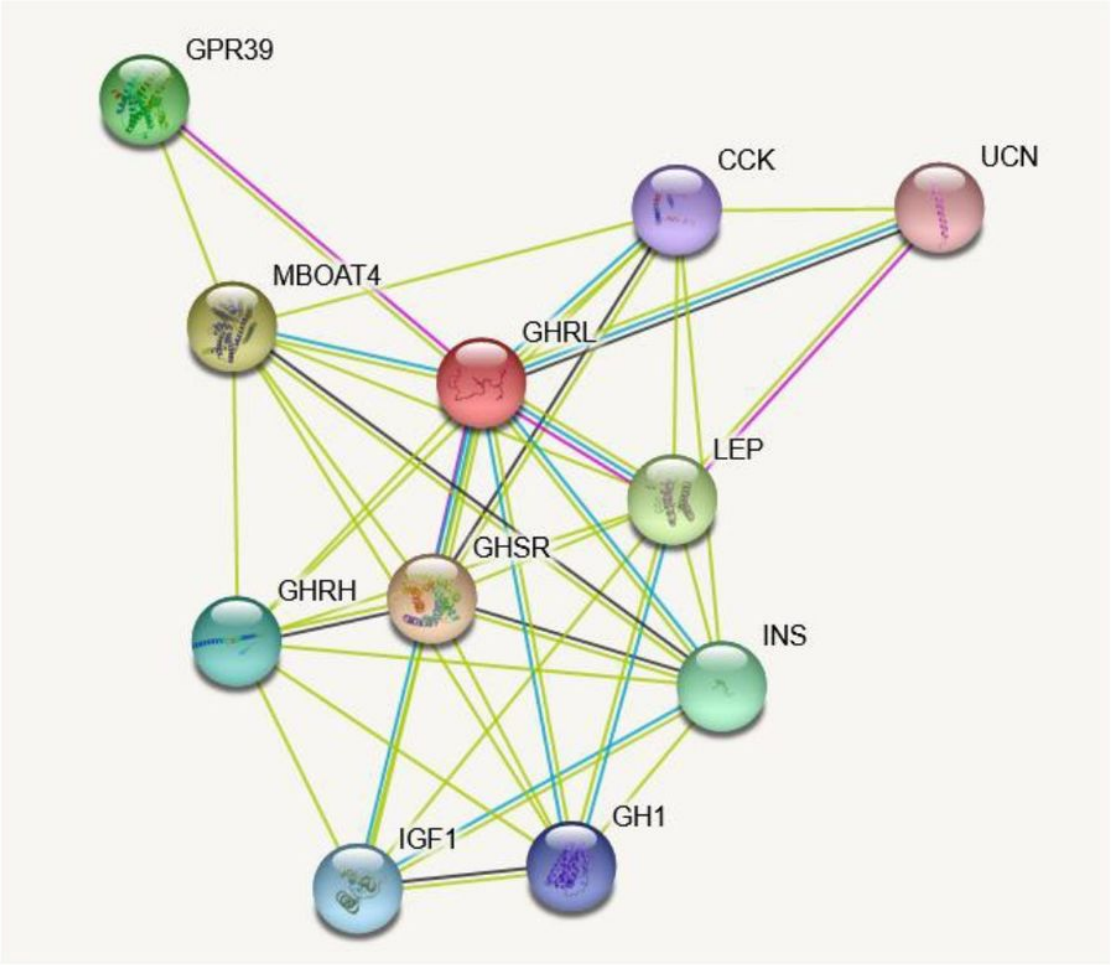


Figure 5

PPI network based on string database.

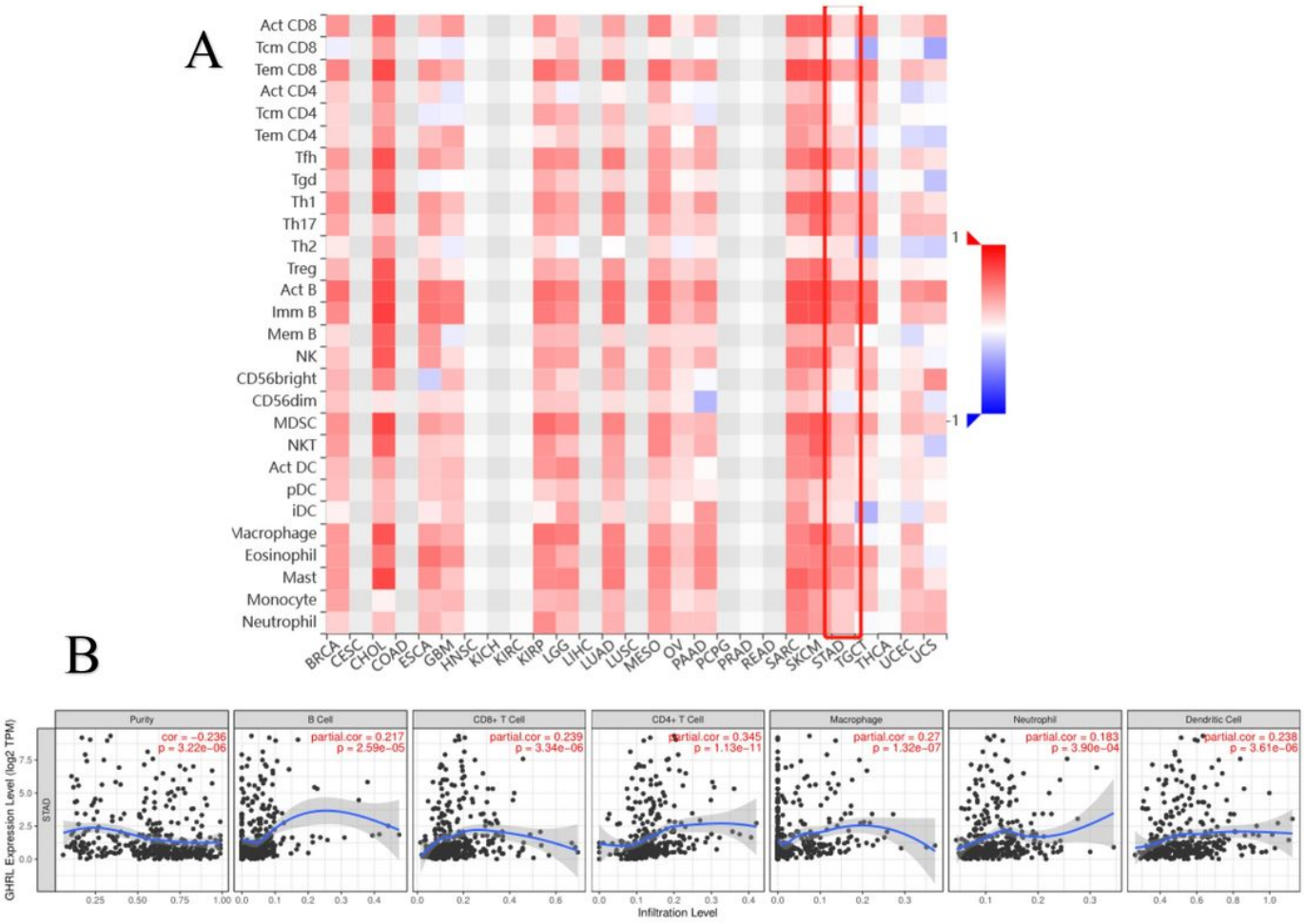


Figure 6

Correlation of GHRL expression with immune infiltration in GC. (A) Correlation between the expression of GHRL and the abundance of TILs in GC available at TISIDB database. (B) Correlation of ITGAL expression with infiltration levels of B cell, CD8 + T cell, CD4 + T cell, macrophage, neutrophil, and dendritic cell in GC available at TIMER2.0 database. TILs, tumor-infiltrating lymphocytes

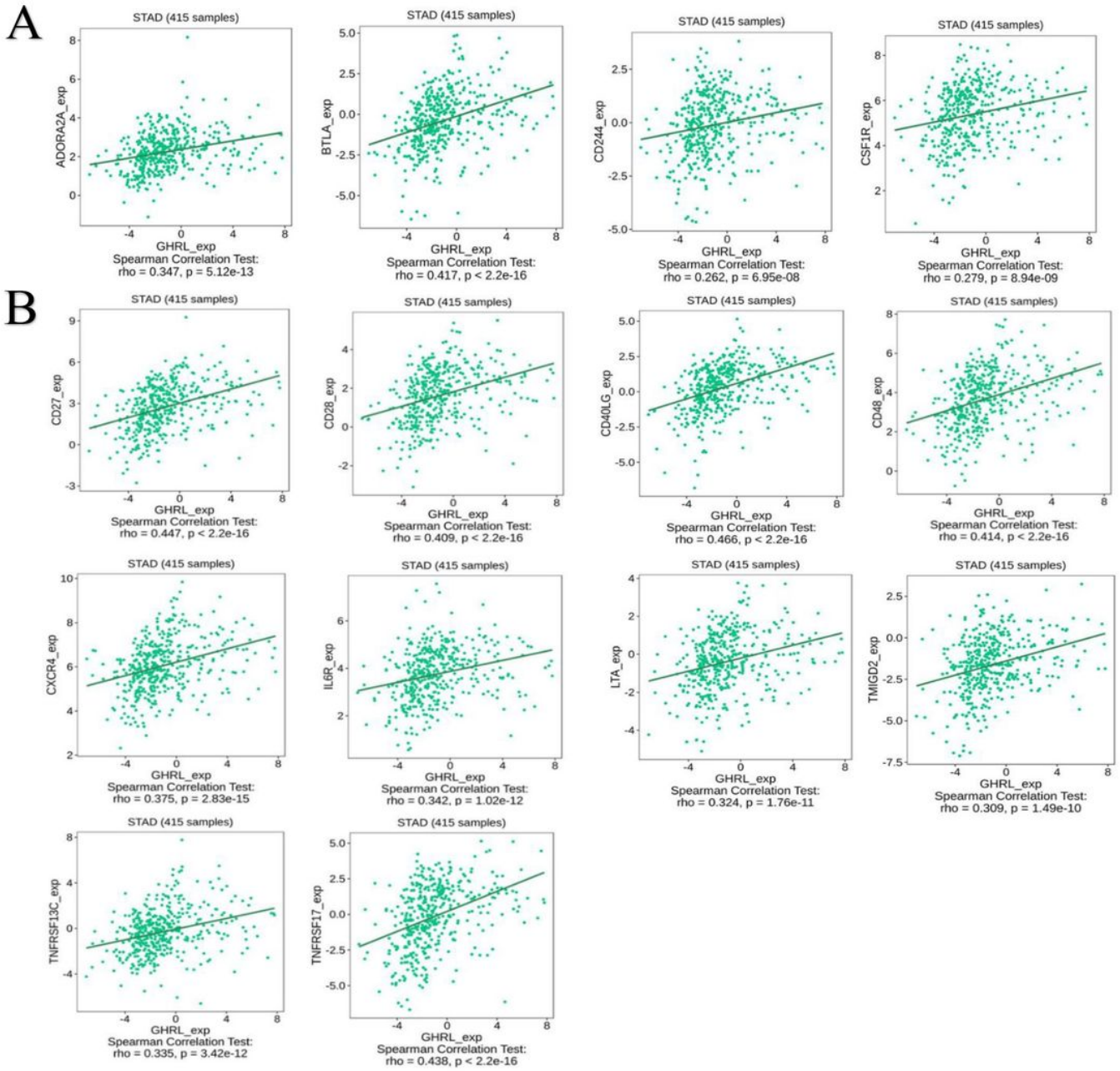


Figure 7

The expression of GHRL is associated with immunomodulators in GC. (A) Correlation between GHRL expression and immunoinhibitors in GCavailable at TISIDB database. (B) Correlation between GHRL expression and immunostimulators in GC available at TISIDB database.

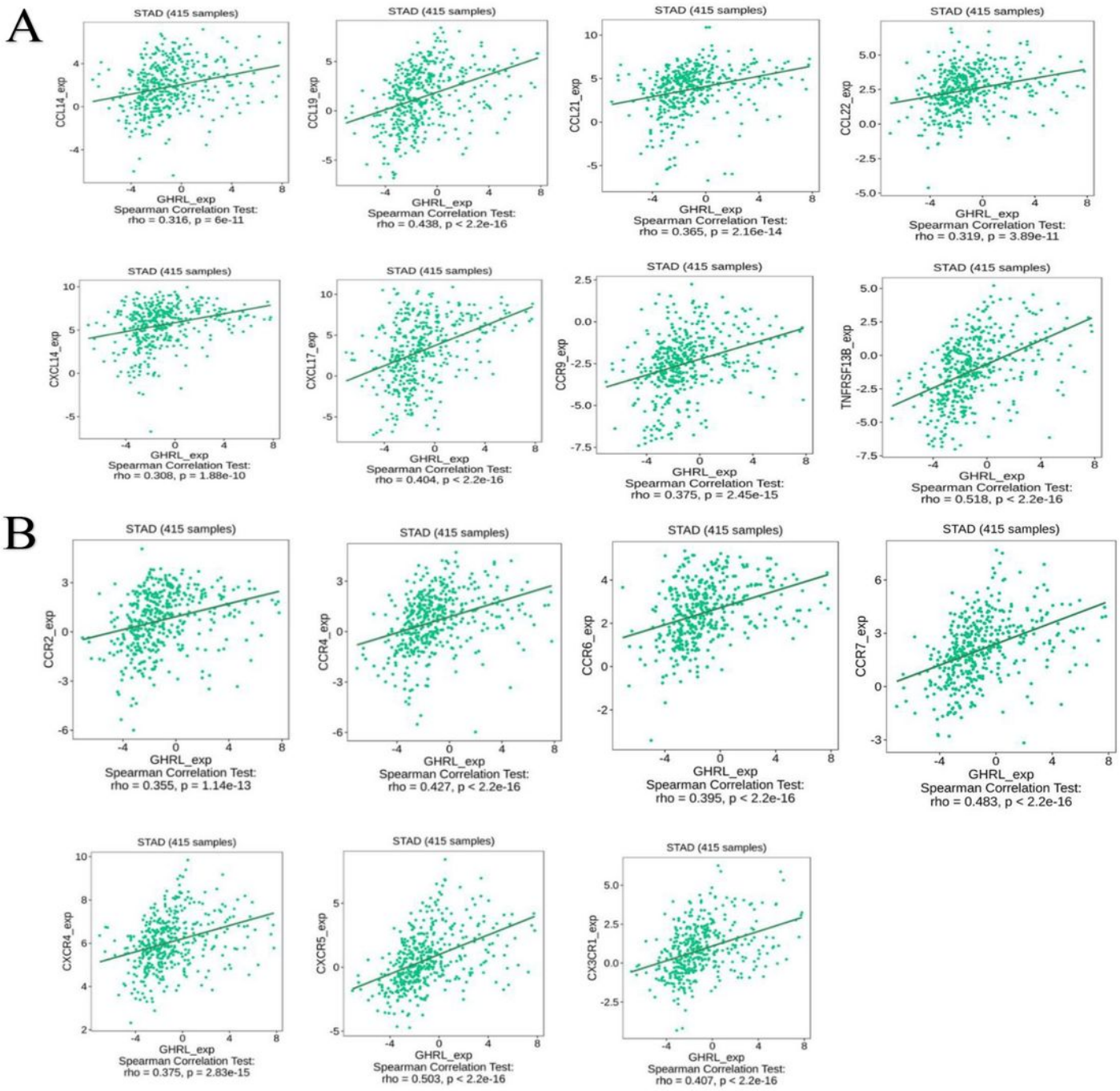


Figure 8

Correlation between the expression of GHRL and chemokines in GC. (A) Correlation between GHRL expression and chemokines in GC available at TISIDB database. (B) Correlation between GHRL expression and chemokine receptors in GC available at TISIDB database.