



Identification of candidate biomarkers associated with gastric cancer prognosis based on an integrated bioinformatics analysis

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Background: This study sought to identify candidate biomarkers associated with gastric cancer (GC) prognosis based on an integrated bioinformatics analysis.

Methods: First, the GSE54129 and GSE79973 data sets were downloaded from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) identified between the 2 data sets were screened using the limma software package in R, and the intersection DEGs were obtained by a Venn analysis. Subsequently, gene clustering and a functional analysis were performed to explore the roles of the DEGs. The protein-protein interaction (PPI) network of the genes in clusters was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins. A survival analysis evaluated the associations between the candidate genes and the overall survival of GC patients. A drug-gene interaction analysis and an external data set analysis were conducted using The Cancer Genome Atlas-Stomach Adenocarcinoma (TCGA-STAD) data set to validate the prognostic genes.

Results: We extracted 421 intersection DEGs from the 2 GEO data sets. There were 5 gene clusters, and the functional analysis revealed that they were mainly associated with the extracellular matrix-receptor interaction pathway. The PPI interaction analysis identified the top 36 hub genes. The survival analysis revealed that 7 upregulated genes [i.e., platelet-derived growth factor receptor beta (*PDGFRB*), angiotensin 2 (*ANGPT2*), vascular endothelial growth factor C (*VEGFC*), collagen type IV alpha 2 chain (*COL4A2*), collagen type IV alpha 1 chain (*COL4A1*), thrombospondin 1 (*THBS1*), and fibronectin 1 (*FNI*)] were associated with the survival prognosis of GC patients. The 20 drug-gene interaction pairs among the 4 genes and 18 drugs were obtained. Finally, TCGA-STAD data set was used to validate the expression levels of *COL4A1*, *PDGFRB*, and *FNI*.

Conclusions: We found that 7 upregulated genes (i.e., *PDGFRB*, *ANGPT2*, *VEGFC*, *COL4A2*, *COL4A1*, *THBS1*, and *FNI*) were promising markers of prognosis in GC patients.

Keywords: Gastric cancer (GC); differentially expressed gene; protein-protein interaction analysis; survival analysis

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Introduction

Gastric cancer (GC) is the 4th most common cancer worldwide, and is characterized by increasing incidence and mortality rates, especially in China (1). Standard treatments, such as surgical resection, chemotherapy, and radiotherapy, have greatly increased the survival outcomes of GC patients. However, the global 5-year overall survival (OS) rate remains <15%, which is mainly due to the late diagnosis and lack of early detection of GC (2,3). A high recurrence rate and tendency to metastasize also lead to poor clinical outcomes (4,5).

Previous studies based on multivariate regression analyses have identified some prognostic biomarkers in GC. For example, Jin *et al.* (6) found that increased spondin-2 in GC tissues was significantly related to lymph node metastasis and advanced tumor, node, metastasis stages, and that the high expression of spondin-2 leads to a poor prognosis in GC patients. This regression analytic strategy may enable the identification of important prognostic targets for cancer management. Du *et al.* (7) recently identified adenoma polyposis coli (APC) as a new prognostic factor for patients with T4 GC based on APC expression and a methylation profile analysis.

Over the decades, gene chips have been proven to be a reliable technology. They can rapidly screen for differentially expressed genes (DEGs) and generate large amounts of genomic information for public databases. Some prognostic biomarkers have been identified and applied in clinical treatment (8,9); different from the above studies, our study not only identified novel biomarkers related to GC, but also we have predicted the new drugs for patients with GC based on the GSE54129 and GSE79973 data sets.

We downloaded the GSE54129 and GSE79973 data sets from the Gene Expression Omnibus (GEO) database. The DEGs between the 2 data sets were distinguished using R's limma package and Venn diagram software. We further explored the function of these DEGs, including the Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). Next, we constructed a protein-protein interaction (PPI) network to analyze the DEGs further. Kaplan-Meier (KM) curves revealed an association between the DEGs and OS in GC patients. Further, a gene-drug interaction analysis was conducted to explore the association between drugs and the DEGs. Finally, we selected a data

set from The Cancer Genome Atlas (TCGA) to validate these key prognostic genes. Our results will promote the discovery of novel prognostic markers for GC patients.

This study sought to screen candidate biomarkers associated with GC prognosis based on an integrated bioinformatics analysis. We present the following article in accordance with the REMARK reporting checklist (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-651/rc>).

Methods

Data source and pre-processing

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). We obtained the GSE54129 data set (comprising 111 GC and 21 normal control samples) and GSE79973 data set (comprising 10 GC and 10 normal control samples) from the GEO (<http://www.ncbi.nlm.nih.gov/geo/>) database using the keywords "gastric cancer, Homo sapiens and tissue" based on the GPL570 (HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array. TCGA-Stomach Adenocarcinoma (TCGA-STAD) data set, comprising 415 tumor samples and 35 adjacent non-tumor samples, was obtained from TCGA database (<https://cancergenome.nih.gov/>). There was relevant clinical information in 408 samples. Next, the R affy package (version 1.58.1; <http://bioconductor.org/help/search/index.html?q=affy/>) was used to normalize the data by, for example, format conversion or the correction of missing data (10). The MicroArray Suite algorithm and the quantiles method were used for the data standardization. Subsequently, the probes were mapped onto the corresponding genes. The mean value of multiple probes was taken as the final expression value of the gene when the probes were mapped to the same gene.

Identification of DEGs

We used the R limma package (version 3.10.3; <http://www.bioconductor.org/packages/2.9/bioc/html/limma.html>) to screen for DEGs between the tumor and control samples (11). The P value was calculated and adjusted using the Benjamini-Hochberg method (12). The thresholds of an adjusted P value <0.05 and a $|\log_2 \text{fold change (FC)}| >1$ were set as the screening criteria for DEG identification. Additionally, a Venn analysis was conducted to extract the intersection

DEGs between the GSE54129 and GSE79973 data sets. These DEGs were regarded as the candidate targets and used in the following analysis.

Clustering and functional analyses

A clustering analysis identified the gene clusters with similar functions. The ConsensusClusterPlus algorithm (version 1.44.0; <http://bioconductor.org/packages/release/bioc/html/ConsensusClusterPlus.html>) was used to identify gene clusters based on the expression values of the intersection DEGs between the GSE54129 and GSE79973 data sets (13). The cumulative distribution function (CDF) was used to determine the optimal number of clusters (14). Subsequently, a KEGG pathway enrichment analysis of the genes in the extracted clusters was performed using the online analytic tool DAVID (15,16). Gene counts ≥ 2 and a P value < 0.05 indicated a statistically significant difference.

PPI analysis

We evaluated the relationships between the protein products and genes in the KEGG enriched pathways based on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (17). The PPI score was 0.4, and the species was *Homo sapiens*. Cytoscape software (version 3.2.0; <https://cytoscape.org/>) was used to construct the PPI network (18). Further, the topology relation of the PPI network was analyzed by Cytoscape, and the node scores were obtained. Gene nodes with a degree ≥ 5 were regarded as the hub genes in the PPI network. The KEGG analysis of these hub genes was carried out based on the DAVID (19).

Analysis of the candidate genes using KM curves

All the genes in the significant clusters were used for the survival analysis. First, we collected the gene expression matrix data and corresponding clinical information from TCGA-STAD data set. All the candidate genes were classified into either the high- or low-expression group using the R survival package (version 2.42-6; <https://cran.r-project.org/web/packages/survival/index.html>). Finally, the survival curve was constructed using the KM method. A correlation coefficient P value < 0.05 was set as the significance threshold for the survival analysis of the prognosis-related genes. Additionally, the overlapping genes between the hub genes in the PPI network and the

prognosis-associated genes were further extracted and served as the hub genes for the survival prognosis of GC patients.

Prediction of drug-gene interaction

Drug development has benefited from the Drug-Gene Interaction database (DGIdb), which is widely used to identify the drugs that target genes. The potential relationships between drugs and prognosis-related hub genes were predicted by the DGIdb using the following preset filter parameters: Food and Drug Administration (FDA) approved and antineoplastic. Next, the drug-gene interaction network was constructed using Cytoscape software.

Data validation

To further validate the candidate genes associated with GC prognosis, we performed a gene differential expression analysis for TCGA-STAD data set. The screening cutoffs for the DEGs were set as an adjusted P value < 0.05 and a $|\log_2FC| > 1.0$. Finally, a Venn analysis was conducted for the DEGs and the intersection DEGs based on the GEO data sets.

Results

Identification of DEGs

A total of 1,815 (830 upregulated and 985 downregulated) DEGs were screened between the GC and normal tissues in GSE54129. There were 704 (356 upregulated and 348 downregulated) DEGs in the GSE79973 data set. Additionally, the hierarchical clustering analyses revealed that the DEGs in the GSE54129 (see *Figure 1A*) and GSE79973 (see *Figure 1B*) data sets could significantly discriminate between the GC and normal samples. We further extracted 421 intersection DEGs between these 2 data sets by a Venn analysis (see *Figure 1C*).

Intersection DEG clustering and functional analyses

We carried out a clustering analysis of the intersection DEGs to identify the gene sets with a similar function. The Consensus Cluster Plus algorithm was used for the gene clustering analysis, and the CDF was calculated to determine the optimal cluster number. From the CDF curve and CDF delta area curve, we found that $k=5$ represented

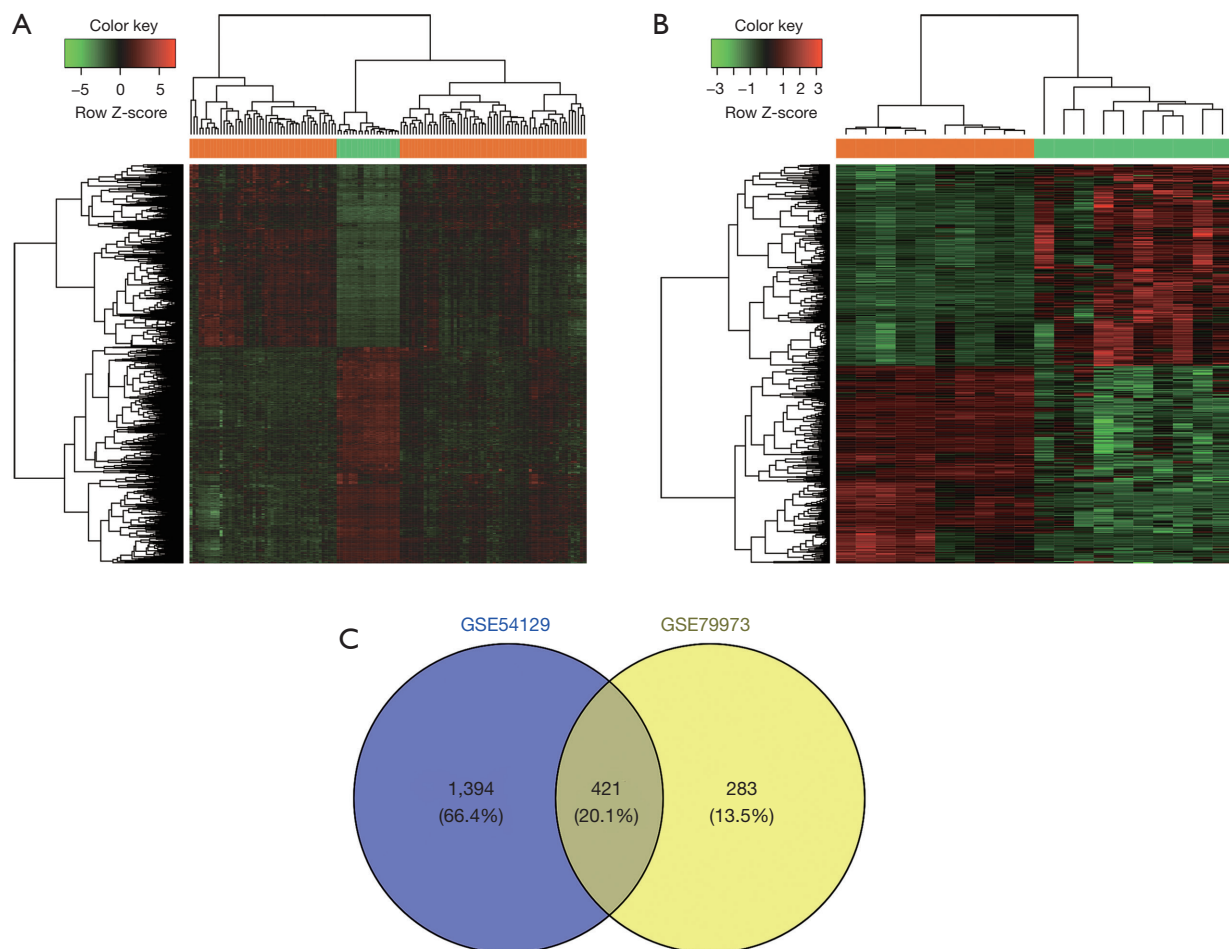


Figure 1 Gene differential expression analysis. (A) Heatmap of the DEGs in the GSE54129 data set. (B) Heatmap of the DEGs in the GSE79973 data set. (C) The Venn diagram shows the intersection DEGs between the GSE54129 and GSE79973 data sets. The orange bars indicated the GC samples, and the green bars indicated the controls. GC, gastric cancer; DEGs, differentially expressed genes.

the most stable clustering outcome (*Figure 2A,2B*). Thus, these intersection DEGs were grouped into 5 clusters (see *Figure 2C*). Our results also showed that there were 178 genes in cluster 1, 63 in cluster 2, 174 in cluster 3, 5 in cluster 4, and 1 in cluster 5. Subsequently, we conducted functional analyses of the genes in the 5 clusters. We found that the genes in clusters 1 and 3 were all significantly enriched in 9 KEGG pathways (see *Figure 2D*). For example, the genes in cluster 1 mainly participated in the xenobiotics metabolism by cytochrome P450 and chemical carcinogenesis pathways. The genes in cluster 3 were primarily associated with the extracellular matrix (ECM)-receptor interaction and focal adhesion pathways. The genes in cluster 2 were strongly related to the gastric

acid secretion and retinol metabolism pathways. The genes in clusters 4 and 5 were not enriched in any pathway.

PPI network construction and functional analysis

The PPI network based on the intersection DEGs was constructed. It contained 58 nodes and 211 interaction pairs. Notably, there were 22 genes in cluster 1, 7 in cluster 2, and 29 in cluster 3 (see *Figure 3A*). Further, there were 36 hub genes with a degree ≥ 5 (see *Table 1*). Additionally, we performed a KEGG enrichment analysis for these hub genes. The results suggested that they were predominantly enriched in 18 KEGG pathways, such as the ECM-receptor interaction and focal adhesion pathways (see *Figure 3B*).

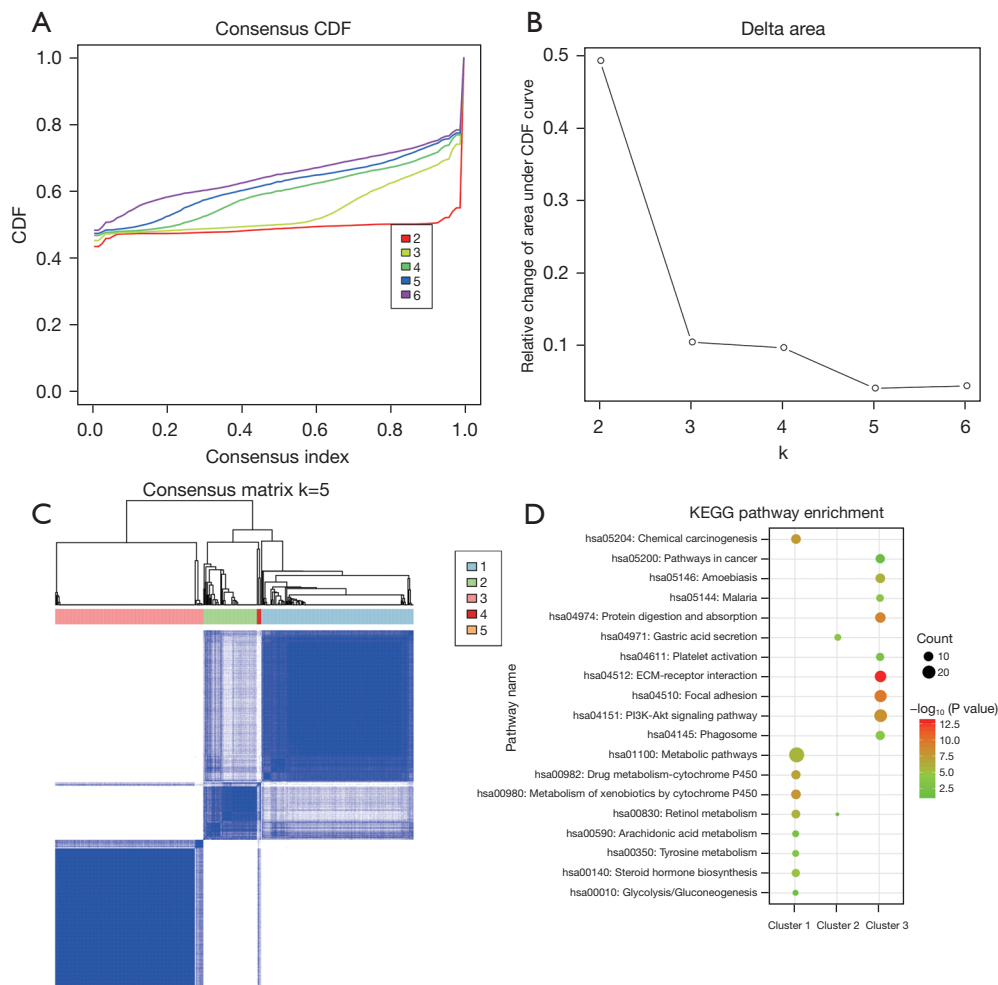


Figure 2 Gene clustering and functional analyses. (A) The consensus CDF curve; (B) the CDF delta area curve, which represents the area difference between the area of CDF under k_i and horizontal axis and CDF under $k_i + 1$ and horizontal axis; (C) the consensus cluster under $k=5$; (D) The KEGG pathway enrichment analysis of genes in clusters 1–3. k represents the number of gene clusters. CDF, cumulative distribution function, KEGG, Kyoto Encyclopedia of Genes and Genomes.

Analyses of candidate genes using KM curves

To further study the association between the hub genes and the OS of GC patients, survival analyses for all the genes in the 5 clusters were carried out. The results suggested that 66 genes were significantly correlated with the clinical outcomes of GC patients. Subsequently, the 7 overlapping genes between the prognosis-related genes and hub genes in the PPI network were extracted. They included angiopoietin 2 (*ANGPT2*) (see Figure 4A), collagen type IV alpha 1 chain (*COL4A1*) (see Figure 4B), collagen type IV alpha 2 chain (*COL4A2*) (see Figure 4C), fibronectin 1 (*FN1*) (see Figure 4D), platelet-derived growth factor receptor beta

(*PDGFRB*) (see Figure 4E), thrombospondin 1 (*THBS1*) (see Figure 4F), and vascular endothelial growth factor C (*VEGFC*) (see Figure 4G). These genes were all upregulated, and their high expression levels were associated with a poor prognosis.

Prediction of drug-gene interactions

The correlations between the 7 prognosis-associated genes and drugs were predicted using the DGIdb database. There were 20 drug-gene interaction pairs, including 4 upregulated genes (i.e., *PDGFRB*, *ANGPT2*, *VEGFC*, and *THBS1*) and 18 drugs (see Figure 5).

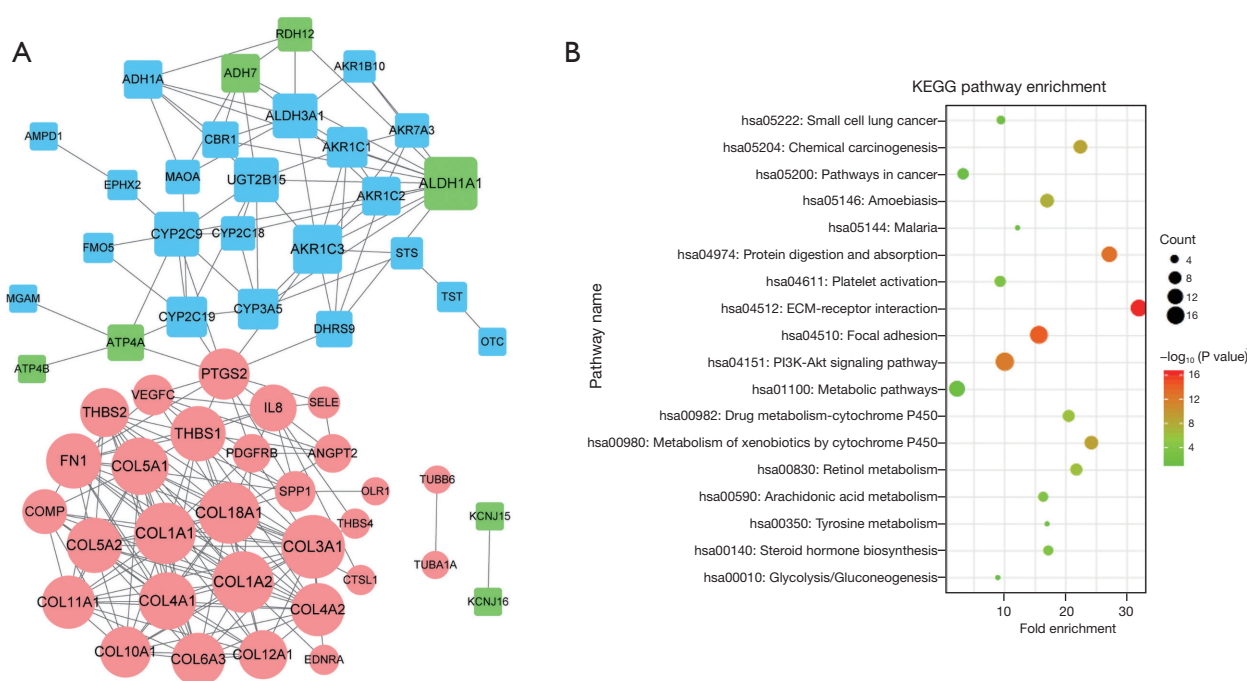


Figure 3 PPI and functional analyses. (A) The PPI network. The circular nodes represent the upregulated genes, and the square nodes represent the downregulated genes. The blue nodes show the genes in cluster 1, the green nodes show the genes in cluster 2, and the pink nodes show the genes in cluster 3. The size of each node represents the degree value. (B) The KEGG pathway enrichment analysis of the hub genes in the PPI network. The hub genes were those with a degree ≥ 5 . PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Data validation

A differential expression analysis was conducted on TCGA-STAD data set to verify our GEO data set findings. In total, 1,178 DEGs were identified between the GC and normal samples, including 737 upregulated genes and 441 downregulated genes. Next, a Venn analysis was conducted with the intersection DEGs from the GEO data set and the DEGs from TCGA-STAD data set (see *Figure 6*). We identified 131 overlapping genes, including 3 prognosis-related genes (i.e., *COL4A1*, *PDGFRB*, and *FN1*).

Discussion

In this study, the differential expression analysis identified 421 intersection DEGs between the GSE54129 and GSE79973 data sets. These were divided into 5 gene clusters, and the corresponding genes in these clusters mainly participated in the ECM-receptor interaction pathway. The survival analyses revealed that 7 upregulated genes (i.e., *PDGFRB*, *ANGPT2*, *VEGFC*, *COL4A1*,

COL4A2, *THBS1*, and *FN1*) were strongly associated with the OS of GC patients, and these genes were also hub genes in the PPI network. There were close relationships between numerous drugs and 4 prognosis-related genes (i.e., *PDGFRB*, *ANGPT2*, *VEGFC*, and *THBS1*). Finally, the expression levels of *COL4A1*, *PDGFRB*, and *FN1* were validated with TCGA data set.

Functional analyses have revealed that most DEGs are predominantly involved in the ECM-receptor interaction pathway (20,21). Notably, Liu *et al.* previously performed a graph-based clustering analysis and found that the ECM-receptor interaction pathway was correlated with the underlying molecular mechanisms of GC development (22). Several recent studies have implicated that many DEGs in GC tissues may regulate the progression of GC via this signaling pathway (23–25). However, the detailed mechanisms of the effects of this pathway on GC progression require clarification.

PDGFRB is a member of the *PDGF* family and encodes a tyrosine kinase receptor. Our results indicated that this gene was upregulated in the GC samples, and its overexpression

Table 1 Hub genes with a degree ≥ 5 in the PPI network

Nodes	Regulation	Cluster	Degree
COL3A1	Up	Cluster 3	18
COL1A2	Up	Cluster 3	17
COL1A1	Up	Cluster 3	17
COL18A1	Up	Cluster 3	17
COL4A1	Up	Cluster 3	15
COL5A1	Up	Cluster 3	15
COL5A2	Up	Cluster 3	14
THBS1	Up	Cluster 3	14
FN1	Up	Cluster 3	14
ALDH1A1	Down	Cluster 2	13
COL4A2	Up	Cluster 3	13
COL6A3	Up	Cluster 3	13
COL11A1	Up	Cluster 3	13
COL10A1	Up	Cluster 3	12
PTGS2	Up	Cluster 3	12
AKR1C3	Down	Cluster 1	11
COL12A1	Up	Cluster 3	11
THBS2	Up	Cluster 3	11
IL8	Up	Cluster 3	10
ALDH3A1	Down	Cluster 1	9
UGT2B15	Down	Cluster 1	9
CYP2C9	Down	Cluster 1	9
COMP	Up	Cluster 3	9
AKR1C1	Down	Cluster 1	7
CYP3A5	Down	Cluster 1	7
CYP2C19	Down	Cluster 1	7
VEGFC	Up	Cluster 3	7
SPP1	Up	Cluster 3	7
ADH1A	Down	Cluster 1	6
AKR1C2	Down	Cluster 1	6
ADH7	Down	Cluster 2	6
PDGFRB	Up	Cluster 3	6
ANGPT2	Up	Cluster 3	6
DHRS9	Down	Cluster 1	5
CBR1	Down	Cluster 1	5
ATP4A	Down	Cluster 2	5

PPI, protein-protein interaction.

indicated an unfavorable prognosis. Further, the expression of this gene was verified by TCGA-STAD data set analysis. Early research reported that *PDGFB* is more highly expressed in GC tissues than normal tissues (26). Subsequently, a number of studies have suggested that *PDGFB* is involved in developing GC via different biological processes (27,28). Recently, Wang *et al.* pointed out that *PDGFB* is closely associated with neuropilin 1 (*NRP1*). Increased levels of *NRP1* and *PDGFRB* are strongly related to the many malignant phenotypes in GC patients (29). Further, the risk of death is approximately 2-fold higher in GC patients with a higher expression of *NRP1* and *PDGFRB* than others. This provides support for our finding that an enhanced *PDGFRB* level signals a poor prognosis for GC patients.

Notably, we also found that *PDGFRB* was closely correlated with numerous drugs, including imatinib, sunitinib, regorafenib, sorafenib, pazopanib, axitinib, nilotinib, lenvatinib, imatinib mesylate, and dasatinib. A previous study showed that combining fluorouracil, leucovorin, and imatinib mesylate targeting *PDGFRB* was safe and effective for GC patients (30). Additionally, Qian *et al.* suggested that 9 drug molecules (i.e., imatinib, sunitinib, regorafenib, sorafenib, pazopanib, axitinib, dasatinib, nilotinib, and lenvatinib) were *PDGFR* tyrosine kinase inhibitors. They discussed the underlying roles of these drugs in signal transduction pathways based on pharmacogenetics (31). Overall, *PDGFRB* is not a potential prognostic gene for GC, but it may be a promising therapeutic target for GC treatment.

Our analysis validated the suggestion that another prognostic gene, *COL4A1*, was upregulated in GC patients. Currently, there is overwhelming evidence that this gene is associated with the possible mechanisms of GC (1,32). Further, Li *et al.* conducted a bioinformatics analysis and found that *COL4A1* has an important prognostic value in the survival of GC patients (1). Consistent with our results, Li *et al.* found that *COL4A1* is overexpressed in GC tissues compared to normal tissues, and a higher expression level of *COL4A1* is associated with poorer overall survival for GC patients (33). Similarly, upregulated *FN1* is a prognostic marker for GC. Many research groups have suggested that *FN1* is highly expressed in GC tissues, and high levels are related to a poor prognosis for GC patients (34,35).

According to our bioinformatics analyses, the other 4 upregulated genes (i.e., *ANGPT2*, *THBS1*, *COL4A2*, and *VEGFC*) are prognostic markers for GC patients. Xu *et al.* previously argued that *THBS1* and *ANGPT2* are strong

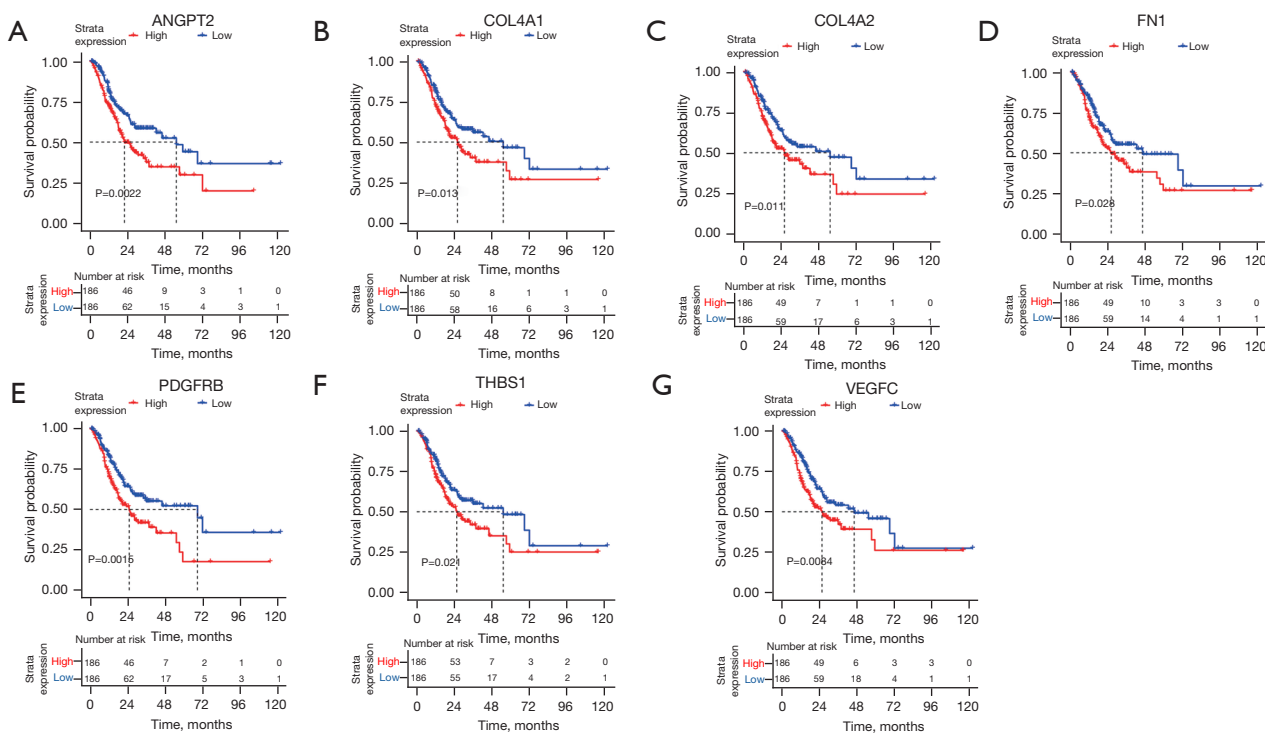


Figure 4 The Kaplan-Meier survival curves for (A) *ANGPT2*; (B) *COL4A1*; (C) *COL4A2*; (D) *FN1*; (E) *PDGFRB*; (F) *THBS1*; and (G) *VEGFC*.

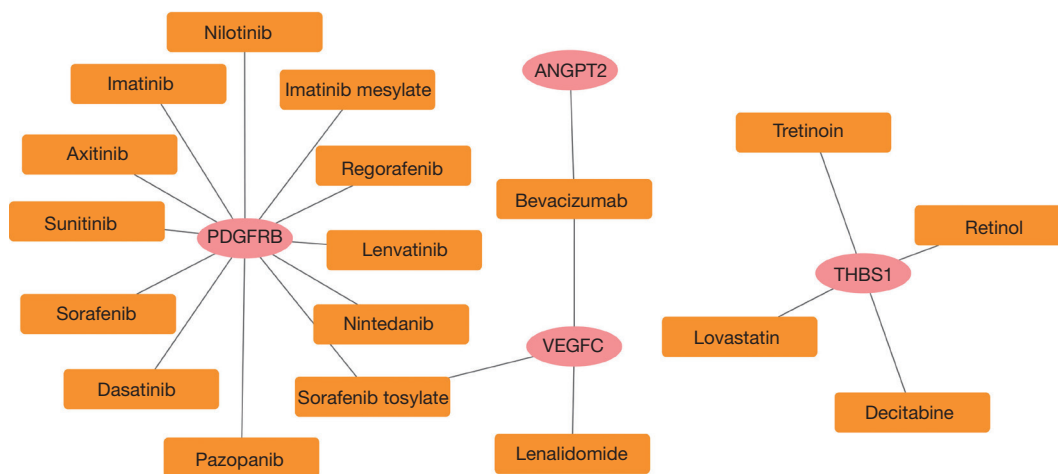


Figure 5 Drug-gene interactions based on the DGIdb database. The pink circles represent the upregulated genes in cluster 3, and the yellow squares represent the drugs.

predictors of the survival of GC patients (35). A multivariate analysis by Eto *et al.* suggested that the negative expression of *THBS1* is an independent prognostic indicator (36). Furthermore, the current targets for gastric cancer mainly

include TP53, EGFR, HER-2, VEGF, VEGFR, MET, FGFR2, mTOR, etc. The protein encoded by *VEGFC* is a member of the platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) family. The

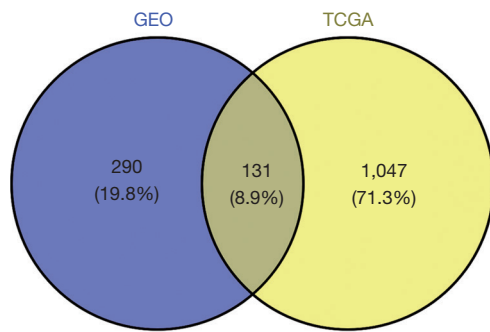


Figure 6 The Venn analysis. The overlapping genes were extracted between the intersection DEGs from the GEO data set and the DEGs from TCGA-STAD data set. DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; TCGA-STAD, The Cancer Genome Atlas-stomach adenocarcinoma.

encoded protein promotes angiogenesis and endothelial cell growth, and can also affect the permeability of blood vessels. We inferred that *VEGFC* might be a novel target for GC. Also, numerous studies have revealed that *VEGFC* acts as a promising marker in determining the prognosis of GC patients (37). Dai *et al.* stated that there were higher messenger ribonucleic acid and protein levels of *VEGFC* in GC samples and linked the expression of this gene with GC lymph node metastasis (38). There is extensive evidence implicating *COL4A2* in the development of GC; however, few reports have investigated the effect of this gene on GC prognosis.

This work had some limitations. First, an integrated bioinformatics analysis based on a larger sample size needs to be conducted to validate our results. After that, functional experiments on the main targets need to be carried out to improve the meaning of this study. Second, the detailed regulatory mechanisms of the significant signaling pathways also need to be deciphered. Finally, the *in vivo* and *in vitro* experimental validation need to be performed to validate the results of our study, which might be performed in the future.

In conclusion, our study suggests that 7 key genes (*PDGFRB*, *ANGPT2*, *VEGFC*, *COL4A2*, *COL4A1*, *THBS1*, and *FNI*) can be used to predict the survival outcomes of GC patients, and these promising prognostic markers of GC may contribute to improving risk management and clinical outcomes of GC patients.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-651/rc>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-651/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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