RESEARCH ARTICLE

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Identification of Hub Genes and Potential Pathogenesis in Gastric Cancer Based on Integrated Gene Expression Profile Analysis

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Abstract

Objective: Gastric cancer (GC) is one of the most common malignancies and ranks third in terms of cancer-related mortality. This study aims to identify the hub genes and potential mechanisms in GC using a bioinformatics approach. **Methods:** Microarray data GSE54129, GSE79973, GSE55696 were extracted from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) was identified using Benjamini-Hochberg method in the limma package. GO and KEGG pathway enrichment analyses of the DEGs were conducted. Furthermore, protein–protein interaction network was constructed the STRING platform, and the hub genes were discovered using Maximal Clique Centrality method via cytoHubba. The predictive significance of hub genes was evaluated through GSE15459 dataset. **Results:** A total of 73 genes was identified as DEGs in GC. Volcano plots and heatmaps of DEGs were visualized. Functional enrichment analysis revealed that the genes were mostly enriched in response to xenobiotic stimulus, digestion, cellular hormone metabolic process, extracellular matrix structural constituent, calcium-dependent cysteine-type endopeptidase activity, aromatase activity, apical part of cell, basal part of cell, and apical plasma membrane. Regarding KEGG pathway-enrichment, the genes were mainly involved in Drug metabolism-cytochrome P450, Retinol metabolism, Chemical carcinogenesis-DNA adducts, Gastric acid secretion, and Metabolism of xenobiotics by cytochrome P450. By combining the results of Cytohubba, the top five intersecting genes identified were SPP1, INHBA, MMP7, THBS2 and FAP. Kapplan-Meier analysis results showed that these 5 hub genes were highly related to the overall survival of patients. **Conclusion:** SPP1, INHBA, MMP7, THBS2, and FAP were identified as prospective biomarkers and therapeutic targets for GC that might be utilized for prognostic evaluation and scheme selection.

Keywords: Gastric cancer- differentially expressed gene- prognosis- survival- gene expression omnibus

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Introduction

With 768,793 deaths worldwide from gastric cancer (GC) in 2020, it is one of the most common malignancies and ranks third in terms of cancer-related mortality [1]. Each year, GC is diagnosed in more than 1 million individuals globally, and the total amount of cases may rise in the future as the older population increases [2]. With several origins and potential carcinogenesis processes, GC is a molecularly and phenotypically very variable illness, which causes disparities in GC incidence rates among genders, races, and geographic regions [3]. Due to a significant number of patients who receive diagnoses at an advanced stage, radical surgery is frequently ineffective shortly after diagnosis, which leads to an unfavorable outcome [4]. Understanding the condition's pathophysiology is therefore essential to find the related molecular indicators for early detection and effective management.

Microarrays have quickly advanced to serve as the most efficient tool for studying disease processes in the genomic age, shedding fresh insight into the pathogenesis at the molecular level. With lots of applications in medicine, including genetic tumor classification, cancer response estimation, prognostic prediction, genetic testing, identification of novel therapeutic targets, and patient classification, microarray analysis is increasingly used as a fundamental technique in oncology. The amount of data that has been in-depth evaluated is still missing despite the enormous number of microarray datasets that have been released. Additionally, a significant difficulty still remains in figuring out how to use classic differential expression analysis to translate the microarray data into a deeper knowledge of biology.

Therefore, the current study aims to identify the hub genes and potential mechanisms in GC using a bioinformatics approach and a number of functional evaluations to serve as a guide for future research into

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this condition.

Materials and Methods

MicroArray Data Collection

The present study used four human gene expression matrix files of GC containing GSE54129, GSE79973, GSE55696 and GSE15459 from the Gene Expression Omnibus (GEO) database. Table 1 displays the key characteristics of these four datasets.

Identification of differentially expressed genes (DEGs)

The DEGs between the normal group and the GC group were compared using the Limma package. The false discovery rate (FDR) was controlled using the Benjamini-Hochberg analysis, and DEGs were chosen using the cutoffs of FDR p-value <0.05 and absolute log2 fold-change (FC) >1.5. The R language packages ffplot2 and pheatmap were used to create the volcano plot and heatmap of DEGs, respectively.

Functional and pathway enrichment analysis

To determine the distinctive biological properties of genes, gene products, and sequences, such as biological processes, cell components, and molecular function, Gene Ontology (GO) analysis is frequently utilized [5]. An extensive collection of biointerpretations of genomic sequences and information on protein interaction networks are available thanks to the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. These technologies have been used to examine DEGs in a large number of recent research [6]. ClusterProfiler V3.14.0, enrichplot V1.10.2, and GOplot V1.0.2 packages were employed to conduct the GO functional enrichment and KEGG pathway enrichment analyses in this study. Significant enrichment was defined as p-value<0.05 and q-value<0.05.

Construction of Protein-protein Interaction (PPI) network and identification of hub genes

Functional connections between proteins encoded by DEGs were discovered using the STRING platform. In STRING, we chose 0.6 as the cut-off value of the combined score to establish PPI network. Then, the number of nodes of all the related proteins in the PPI network was counted using the R software and visualized using Cytoscape 3.8.2. Added to that, the hub genes of GC were discovered via the Cytoscape plugin cytoHubba. Maximal Clique Centrality (MCC), one of the topological analysis techniques included in cytoHubba, performs best at accurately determining important nodes from a PPI network. To assess the significance of DEGs in the GC biological network, we chose MCC as the topological analysis approach in this study.

Evaluating the prognostic value of hub genes

The predictive significance of hub genes was evaluated through GSE15459 dataset. The GSE15459 dataset, which is based on GPL570 platform (Affymetrix GeneChip Human Genome U133 Plus 2.0 Array), includes genome-wide mRNA expression profiles of 196 primary gastric tumors from the Singapore patient cohort. Kaplan-Meier plotter platform was used to conduct the analysis. As a benchmark for analysis, the lower and higher 50% of gene expression were used. Based on the median expression level of hub genes, the objects were divided into 2 groups in the current study. Results of the log-rank test with p<0.01 were considered statistically significant.

Results

Identification of DEGs in GC

Differential gene expression analysis was performed based on the screening criteria after normalization of the GSE79973, GSE55696, and GSE54129 datasets. Figure 1 depicts volcano plots of the DEGs. 894 DEGs were present in GSE79973, of which 240 were up-regulated and 654 were down-regulated. GSE55696 included 866 DEGs with 629 up-regulated genes and 237 down-regulated genes. Regarding GSE54129, 1065 DEGs with 471 up-regulated and 594 down-regulated were identified. The plots also indicated 5 up-regulated genes and 5 down-regulated genes with the lowest p-value. In addition, heatmaps showing the expression changes of 50 representative DEGs with the highest absolute value of FC are presented in Figure 2. The overlapping DEGs from the three databases were screened using the Venn diagram. As shown in Figure 3, 73 genes with high reliability were finally identified for further analysis. The details of these genes are represented in Table 2 and Table S1.

Functional Enrichment Analysis

Using the clusterProfiler package, GO functional annotation was carried out. Figure 4 depicts the outcomes of the major enriched analyses, encompassing biological processes, cell constituents, and molecular function.

Regarding biological processes, the genes were mostly enriched in response to xenobiotic stimulus, digestion, cellular hormone metabolic process, xenobiotic metabolic process, and cellular response to xenobiotic stimulus. Molecular function analysis predominantly indicated extracellular matrix (ECM) structural constituent, calcium-dependent cysteine-type endopeptidase activity, aromatase activity, endopeptidase activity, and steroid hydroxylase activity. Additionally, DEGs-related cell

Table 1. Details of GEO Datasets Used in This Study

Dataset	Platform	Samples
GSE54129	GPL570	111 GC and 21 normal gastric mucosa tissue samples
GSE79973	GPL570	10 pairs of stomach adenocarcinoma tissue and adjacent non-tumor mucosa
GSE55696	GPL6480	19 gastric early-stage carcinoma and 19 chronic gastritis tissue samples
GSE15459	GPL570	192 GC samples

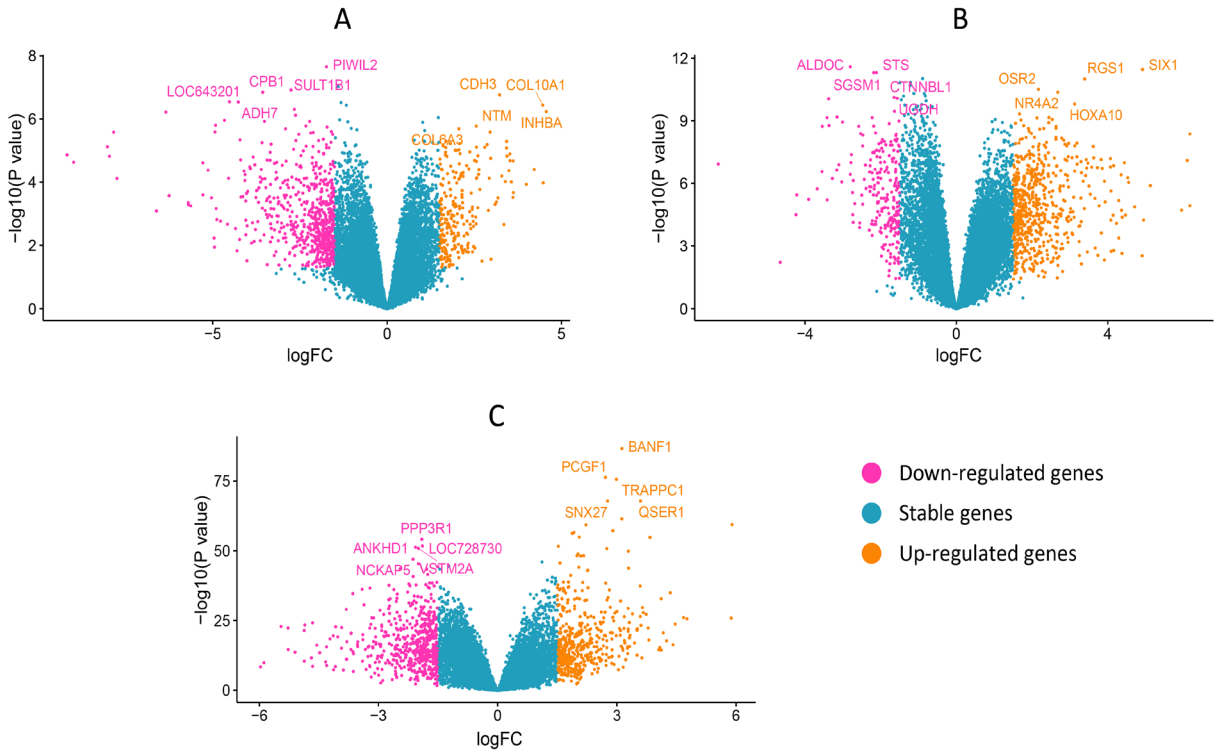

Figure 1. Volcano Plots of Differentially Expressed Genes of GSE54129 (A), GSE79973 (B), GSE55696 (C) DataSets.

components included apical part of cell, basal part of cell, apical plasma membrane, basal plasma membrane, and basolateral plasma membrane.

Pathway Enrichment Analysis

KEGG pathway-enrichment result was illustrated in Figure 5, in which GC-specific DEGs were mainly involved in Drug metabolism-cytochrome P450, Retinol metabolism, Chemical carcinogenesis-DNA adducts, Gastric acid secretion, Metabolism of xenobiotics by cytochrome P450, Steroid hormone biosynthesis, ECM-receptor interaction, Linoleic acid metabolism, Salivary secretion, and cAMP signaling pathway. The detailed information of functional enrichment analysis were shown in Table S2 and S3.

PPI Network Construction and Hub Gene Selection

Figure 6A illustrated the PPI network of GC-related DEGs made using data from the STRING database, which revealed 73 nodes and 215 edges in total.

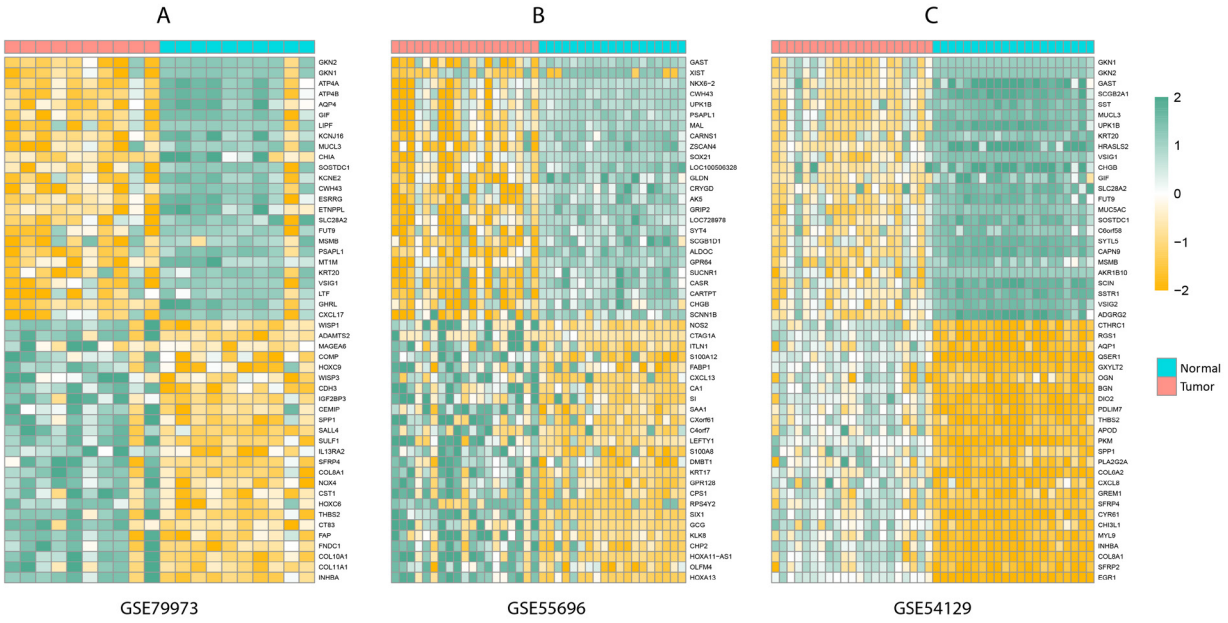

Figure 2. Heatmaps of of 50 Representative DEGs with the Highest Absolute Value of Fold Change. A, GSE54129; B, GSE79973; C, GSE55696.

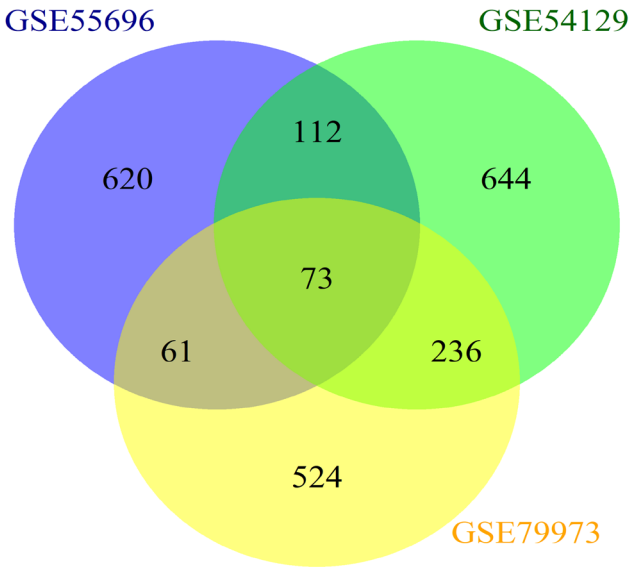

Figure 3. Venn Diagram of Common Differentially Expressed Genes from the Three Datasets

Subsequently, the cytoHubba analysis was performed using MCC module. Due to degree connectivity of the genes, the top five intersecting genes identified were Secreted Phosphoprotein 1 (SPP1), Inhibin Subunit Beta A (INHBA), Matrix Metallopeptidase 7 (MMP7), Thrombospondin 2 (THBS2) and Fibroblast Activation Protein Alpha (FAP) (Figure 6B).

Prognostic Value of the Hub Genes

Using the Kaplan-Meier approach and the GSE15459 dataset, the predictive value of hub genes was examined. All 5 hub genes were highly related to the patients' OS, as seen in Figure 7. A worse prognosis was observed in GC patients who expressed greater levels of SPP1 [HR=1.98 (1.26–3.11), P=0.002], INHBA [HR=1.95 (1.32–2.89), P<0.001], MMP7 [HR=1.91 (1.30–2.81), P<0.001], THBS2 [HR=2.21 (1.51–3.25), P<0.001], and FAP [HR=1.67 (1.13–2.47), P=0.009].

Discussion

As a complicated medical condition, GC continues to have a high mortality due to its heterogeneity. Even while surgery is still the most popular form of treatment, there are other options as well, including radiation therapy, chemotherapy, gene therapy, and targeted therapy, however, the five-year survival rate is still around 30% [7]. To prevent GC advance, improve therapy efficacy, and improve patient survival rates, it is essential to look into the underlying causes of GC progress. Understanding the molecular pathophysiology of the condition would thus benefit from identifying the key genes and pathways

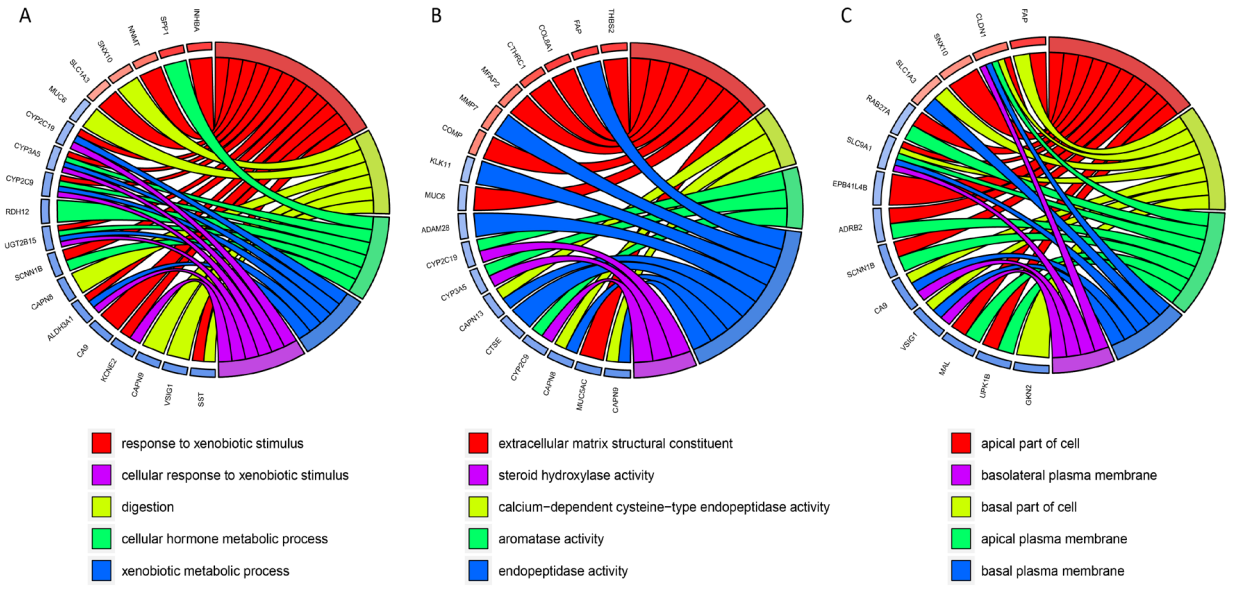

Figure 4. Gene Ontology Enrichment Analysis of Differentially Expressed Genes. A, Biological processes; B, Molecular function; C, Cell components.

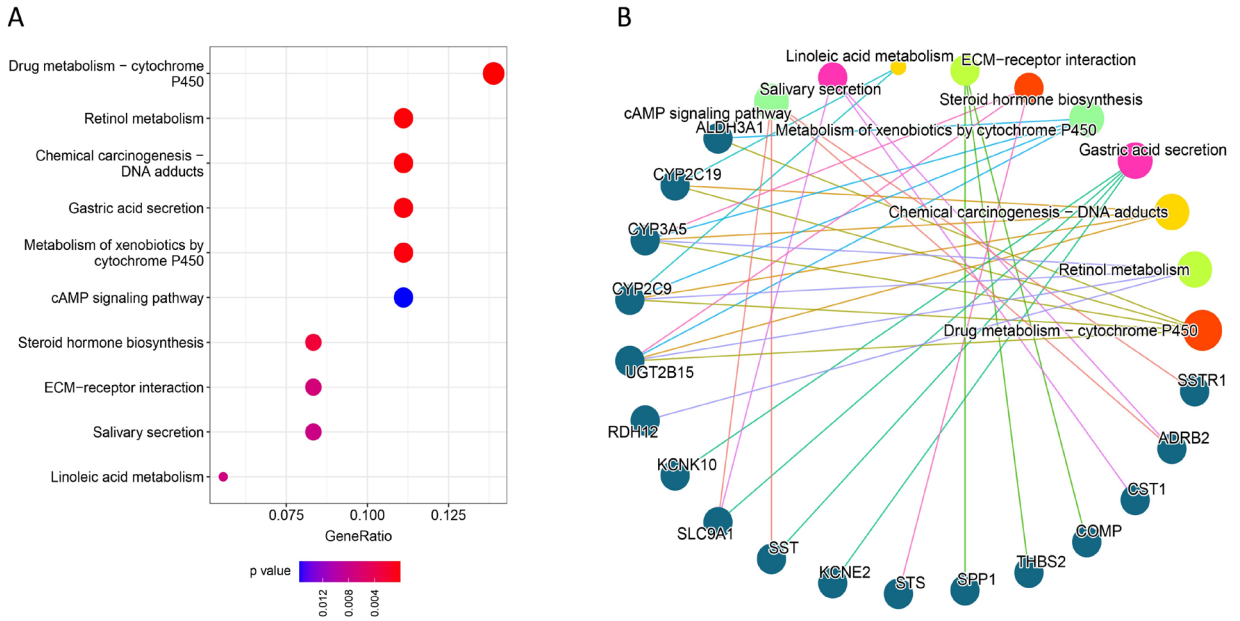

Figure 5. Kyoto Encyclopaedia of Genes and Genomes Pathway Enrichment Analysis of Differentially Expressed genes (DEGs). A, Bubble plot illustrate enrichment of DEGs in signalling pathways; B, Cluego network diagram illustrate the relationship between the DEGs and signalling pathways.

involved in GC.

In the present research, we conducted an integrative analysis of 4 microarray datasets from the GEO database to explore the expression level of important genes implicated in GC. A total of 73 DEGs were determined. Enrichment analysis revealed that they were involved in a variety of biological processes, molecular functions and cell components, such as response to xenobiotic stimulus, cellular hormone metabolic process, xenobiotic metabolic process, ECM structural constituent. Using KEGG analysis, we found that Drug metabolism-cytochrome P450, Retinol metabolism, Chemical carcinogenesis-DNA adducts, Gastric acid secretion, and Metabolism of xenobiotics by cytochrome P450 were the main pathways the genes are involved. The findings are in line with what is already known, which is that aberrant activation of these functions and pathways is a key factor in cancer development and progression [8-12]. SPP1, INHBA, MMP7, THBS2 and FAP were subsequently determined as hub genes for GC due to their degree connectivity in the MCC analysis.

Secreted Phosphoprotein 1 (SPP1), also known as

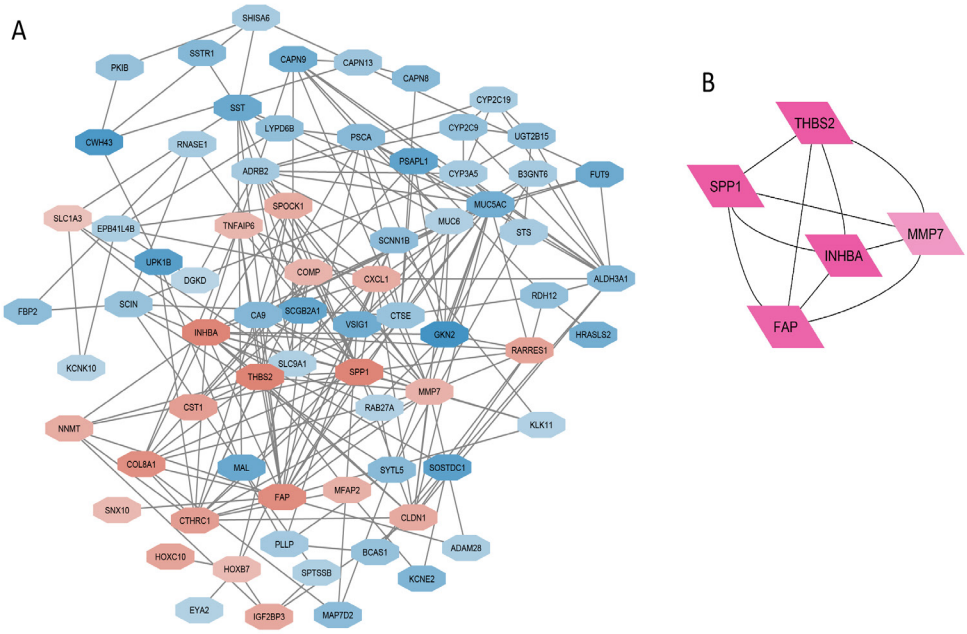

Figure 6. Protein-Protein Interaction (PPI) Network. A, PPI network of 73 differentially expressed genes (DEGs); B, Network of top 5 hub genes from the PPI network due to the Maximal Clique Centrality analysis.

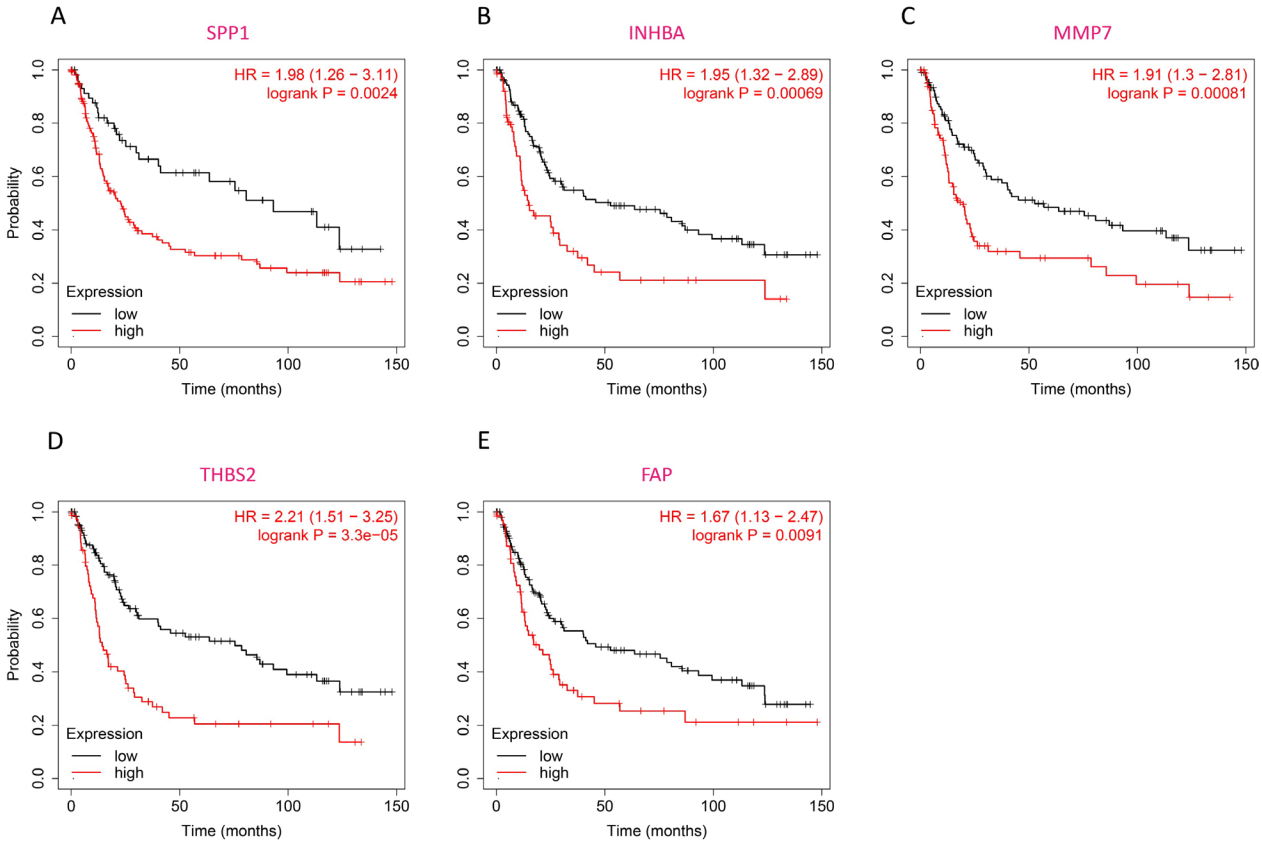

Figure 7. Kaplan-Meier Analysis for Overall Survival Based on the Expression of 5 hub Genes Using the GSE15459 Dataset.

Table 2. Differentially Expressed Genes in Gastric Cancer

DEGs	Gene symbol
Up-regulated	CXCL1 RARRES1 SNX10 HOXB7 CTHRC1 HOXC10 SPP1 CST1 SLC1A3 NNMT THBS2 INHBA MFAP2 COMP FAP CLDN1 COL8A1 TNFAIP6 SPOCK1 MMP7 IGF2BP3
Down-regulated	STS CAPN8 KCNK10 SLC9A1 DGKD CAPN13 EPB41L4B RAB27A PLLP ADRB2 CTSE MAL BCAS1 ALDH3A1 FER1L4 SST MAP7D2 MUC5AC CYP2C19 PSAPL1 CWH43 SOSTDC1 CYP3A5 UPK1B SSTR1 CAPN9 SCIN CYP2C9 SYTL5 SPTSSB CA9 SCNN1B FBP2 PKIB RNASE1 SCGB2A1 ADAM28 VSIG1 SHISA6 LYPD6B KLK11 PSCA FUT9 RDH12 EYA2 C16orf89 UGT2B15 B3GNT6 HRASLS2 MUC6 GKN2 KCNE2

Osteopontin (OPN), is a SIBLING family member that encourages growth of tumors by promoting cell migration in the ECM and interacting with CD44 and integrin receptors [13-15]. SPP1 upregulation in gastric cancer has been found to be associated with tumor cell proliferation, tumor metastases, and poor prognosis [16, 17]. The injury caused by Helicobacter pylori (H. pylori) is responsible for more than 70% of the worldwide burden of GC. Elevated SPP1 level is correlated with the severity of H. pylori infection, and inhibiting expression of SPP1 may alleviate GC development. Chang et al. [18] found that activation of SPP1 by CagA-positive H. pylori accelerated stomach tumorigenesis through IL-8 and β-catenin pathways. On the contrary, through down-regulation of uPA and MMP2 levels and suppression of Akt phosphorylation, decrease of SPP1 expression is able to inhibit the development and invasion of GC cells [19].

INHBA encodes a portion of the activin-inhibin protein complexes, which has been linked to a variety of aspects of physiology as well as pathophysiology [20]. It was discovered that elevated INHBA expression promoted the production of Activin A and the activation of activin receptors such as ACVR1, which play a well-known role in the development of tumors [21]. In line with previous studies [22, 23], we found that patients who had increased INHBA-expressing GC had worse prognosis. Cancer-associated fibroblasts (CAFs) may be a key factor in INHBA's promotion of GC. Using bulk RNA-seq technique, Grunberg et al.[24] found that INHBA was secreted in extracellular vesicles by CAFs in a manner that encourages the progression of aggressive GC subtypes. On the other hand, a recent research employing scRNA-seq analysis revealed different populations of fibroblasts and the INHBA-FAP axis as a CAF regulator in GC [25]. These findings suggest INHBA pathway is a possible target to interfere with CAF function and call for drug modality testing in pertinent model systems.

FAP is a prolyl peptidase family member and a type II

membrane-bound protease. Numerous medical conditions in humans, including fibrosis, arthritis, atherosclerosis, autoimmune disorders, metabolic disorders, and malignancies, have been connected to FAP [26]. FAP is typically linked to the disease's development and escalating severity. FAP is frequently highly expressed in the stroma in tumors, making it a recognized biomarker of CAFs. As mentioned above, the INHBA-FAP axis serves as a potential regulator of CAF in GC [25]. Besides, a GC model research revealed that FAP-positive CAF increased tumor progression in vitro, accelerated tumor growth in vivo, and inhibited T-cell activation and infiltration [27].

MMP7 is the smallest protein in the MMP family. Structurally, MMP7 varies from other MMP members in that it does not have a C-terminal hemoglobin region [28]. MMP7 is crucial in the regulation of a number of processes, including aging, wound repair, bone development, and pathways that regulate angiogenesis, inflammation, and cell proliferation. In GC, MMP7 performs an oncogenic role. It was observed that MMP7 was markedly elevated in GC tissue in comparison to the surrounding normal mucosa [29]. The upregulated level of MMP7 is significantly correlated with GC invasion, peritoneal dissemination, lymph node metastasis, distant metastasis, and poor prognosis [30].

THBS2 is a member of the secreted calcium-binding glycoprotein family that is released from different types of cells. By binding to cell membrane receptors, ECM proteins, and growth factors, THBS2 performs biological roles such as angiogenesis, cell adhesion, cytoskeletal organization, apoptosis, and cell motility. Zhang et al. [31] reported that THBS2 was significantly upregulated in GC tissue compared with cancer-adjacent tissue, and the high expression of the gene was highly correlated with macrophages enrichment, tumor grade, tumor subtype, T stage, and poor prognosis. A pan-cancer study revealed that THBS2 served as a crutial marker between ECM and immune infiltration in GC [32]. THBS2 may accelerate the development of cancer by altering the tumor microenvironment, influencing CD47-mediated pathway, triggering the oncogenic activities of a disintegrin and metalloproteinase with thrombospondin motifs, and increasing MMP-2 level. In addition, THBS2 was also released by bone marrow-derived mesenchymal stem cells to promote the development of H. pylori-associated GC [33].

The present study has several limitations. First, only GEO was used to investigate and validate the diagnostic effectiveness and prognostic value of the hub genes. In addition, this study was conducted by an integrative bioinformatics approach, further in vivo and in vitro experiments are required to confirm the findings.

In conclusion, in summary, SPP1, INHBA, MMP7, THBS2 and FAP were screened as the GC-related hub genes, and the aberrant expression of these genes is strongly related to patient survival, which may help us understand how to develop more effective therapy approaches that target these molecules. The results of the current research would serve as a basis of theory for subsequent studies into possible indicators for GC patient diagnosis and prognosis prediction.

Author Contribution Statement

Conception and design: LTTH, TMH, HVH; Methodology: LTTH, TMH, HVH; Data collection, formal analysis and investigation: LTTH, TMH, HVH; Writing - original draft preparation: LTTH, TMH; Writing - review and editing: HVH; Supervision: HVH. All authors read and approved the final manuscript.

Acknowledgements

Ethical Declaration

Since the data from the Gene Expression Omnibus (GEO) database were de-identified and publicly available, no institutional review board approval was necessary and no informed consent was signed for this study. This research was NOT part of a student thesis.

Availability of data

Data was obtained from the Gene Expression Omnibus (GEO), a freely accessible database available to the public.

Conflict of Interest

The authors have no conflicts of interest to declare.

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