

Identification of key genes and associated pathways in KIT/PDGFR α wild-type gastrointestinal stromal tumors through bioinformatics analysis

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Received February 21, 2018; Accepted August 23, 2018

DOI: 10.3892/mmr.2018.9457

Abstract. Gastrointestinal stromal tumors (GISTs) are the most common type of mesenchymal tumor in the gastrointestinal tract. The present study aimed to identify the potential candidate biomarkers that may be involved in the pathogenesis and progression of v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT)/platelet-derived growth factor receptor α (PDGFR α) wild-type GISTs. A joint bioinformatics analysis was performed to identify the differentially expressed genes (DEGs) in wild-type GIST samples compared with KIT/PDGFR α mutant GIST samples. Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs was conducted using Database for Annotation, Visualization and Integrated Discovery and KEGG Orthology-Based Annotation System (KOBAS) online tools, respectively. Protein-protein interaction (PPI) networks of the DEGs were constructed using Search Tool for the Retrieval of Interacting Genes online tool and Cytoscape, and divided into sub-networks using the Molecular Complex Detection (MCODE) plug-in. Furthermore, enrichment analysis of DEGs in the modules was analyzed with

KOBAS. In total, 546 DEGs were identified, including 238 upregulated genes primarily enriched in 'cell adhesion', 'biological adhesion', 'cell-cell signaling', 'PI3K-Akt signaling pathway' and 'ECM-receptor interaction', while the 308 downregulated genes were predominantly involved in 'inflammatory response', 'sterol metabolic process' and 'fatty acid metabolic process', 'small GTPase mediated signal transduction', 'cAMP signaling pathway' and 'proteoglycans in cancer'. A total of 25 hub genes were obtained and four modules were mined from the PPI network, and sub-networks also revealed these genes were primarily involved in significant pathways, including 'PI3K-Akt signaling pathway', 'proteoglycans in cancer', 'pathways in cancer', 'Rap1 signaling pathway', 'ECM-receptor interaction', 'phospholipase D signaling pathway', 'ras signaling pathway' and 'cGMP-PKG signaling pathway'. These results suggested that several key hub DEGs may serve as potential candidate biomarkers for wild-type GISTs, including phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit γ , insulin like growth factor 1 receptor, hepatocyte growth factor, thrombospondin 1, Erb-B2 receptor tyrosine kinase 2 and matrix metalloproteinase 2. However, further experiments are required to confirm these results.

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Key words: gastrointestinal stromal tumors, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, PDGFR α , wild type, bioinformatics analysis, microarray

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common type of mesenchymal tumor in the gastrointestinal tract, which account for 20% of all soft-tissue sarcomas (1,2). GISTs originate from the interstitial cells of Cajal, and may occur in any part of the gastrointestinal tract; the most frequent sites of origin are the stomach (50-60%), followed by the small intestine (30-35%), the colon and rectum (5%), and finally the esophagus (<1%) (3-5). The number of newly diagnosed GISTs is increasing yearly, since the identification of v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) and platelet-derived growth factor receptor α (PDGFR α) proteins as reliable biomarkers of these tumors (6,7).

Approximately 75-80% of GISTs have KIT gene mutations in exons 9, 11, 13, 14 and 17. Of the remaining GISTs

with no detected KIT mutations, $\sim 1/3$ have mutations in the PDGFR α gene, in exons 12, 14 and 18 (8). While the majority of GISTs are characterized by KIT/PDGFR α gene mutations, 10-15% of GISTs lack such mutations and are defined as KIT/PDGFR α wild-type GISTs (9,10). KIT-also known as CD117 or C-kit receptor-encodes the c-KIT type III receptor tyrosine kinase, which is a cytokine receptor located on the surface of hematopoietic stem cells, as well as other cell types (11). PDGFR α -also known as PDGFR α - is also a type III receptor tyrosine kinase that is expressed on the surfaces of a wide range of cell types (12). Mutations in the PDGFR α gene may induce activation of constitutive ligand-independent kinases and are mutually exclusive with KIT gene mutations, i.e., KIT and PDGFR α mutations do not coexist in patients with GISTs (8). Imatinib, a small molecule selective tyrosine kinase inhibitor, has been used to treat KIT/PDGFR α mutated GISTs (1); however, the efficacy of imatinib depends on the mutated domains of KIT/PDGFR α . It has been reported that $\sim 10\%$ of patients with GISTs are resistant to imatinib, and 40-50% of imatinib-sensitive patients will develop secondary resistance in 2 years (13). In addition, wild-type GISTs are resistant to imatinib treatment and the genetic alterations in wild-type GISTs remain unclear (8). Therefore, it is necessary to identify new target molecules that may be involved in the development and progression of wild-type GISTs.

Currently, gene profiling is widely used in the field of cancer genetics research, which is particularly suitable for the differentially expressed gene (DEG) screening. A large amount of gene profile data has been generated, and most of the data has been shared in public databases. Reintegrating these public data may provide valuable clues for further research. Although many gene profile studies have been performed on GISTs in recent years, research regarding wild-type GISTs is limited and the results are not consistent. Therefore, a joint bioinformatics analysis will be innovative and may provide valuable clues for further research.

In the present study, a joint bioinformatics analysis of two gene expression profiles was performed, in order to identify potential genetic changes in wild-type GIST samples compared to KIT/PDGFR α -mutant GIST samples. Subsequently, functional and pathway enrichment analyses were performed on the DEGs to identify potential biological functions and signaling pathways. Furthermore, a protein-protein interaction (PPI) network was constructed to identify key hub genes. The aim of the current study was to investigate the underlying biological functions and pathways involved in the development and progression of GISTs, and to identify potential candidate biomarkers for these tumors.

Materials and methods

Microarray data. The raw gene expression profiles (GSE17743 and GSE20708) were downloaded from the public Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo), which were based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and were submitted by Ostrowski *et al* (14) and Astolfi *et al* (15), respectively. The GSE17743 dataset contained 29 GIST samples, including

15 with KIT mutations detected, 11 with PDGFR α mutations detected, and three with no mutations detected. The GSE20708 dataset included 22 GIST tumor samples, including 13 with KIT mutations detected, five with PDGFR α mutations detected, and four with no mutations detected. Thus, a total of 51 GIST tumor samples were used for further analysis in the present study.

Data processing. Samples (n=51) were divided into two groups, including wild-type GIST groups (n=7) and KIT/PDGFR α mutant GIST groups (n=44). The CEL files were first converted into probe expression values and were preprocessed for background adjustment and quantile normalization by robust multiarray average algorithm using the 'affy' package in R (version 3.4.2) (16,17). The 'sva' package in R was used to remove batch effects between two gene expression profiles (18). The 'Hclust' method of R was used to perform cluster analysis for gene expression alterations at two batch levels (19). Following this, the probe-level data were transformed to the expression values of genes according to the latest version of annotation file (HG-U133_Plus_2; release 35) for Affymetrix Human Genome U133 Plus 2.0 Array, which was obtained from the official website (www.affymetrix.com/support/technical/byproduct.affx?product=hg-ul33-plus). If one gene symbol was matched by multiple probes, then the average expression value was calculated for this gene.

Identification of DEGs. The 'limma' package (version 3.26.9) in R language was used to identify DEGs between two groups (20). Fold change (FC) of the gene expression was also observed and $\log_2$ FC was calculated. The threshold was defined as a $\log_2$ FCI of >1 and an adjusted P-value of <0.05 . Hierarchical clustering analysis was subsequently performed using the 'pheatmap' package in R (21).

Functional and pathway enrichment analysis. Gene Ontology (GO) enrichment analysis and functional annotation of DEGs were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) network software version 6.8 (<https://david.ncifcrf.gov/>) (22), and enriched GO terms were visualized using the BiNGO plug-in of Cytoscape software (version 3.5.1) (23). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis and functional annotation were processed by KEGG Orthology-Based Annotation System (KOBAS) network software version 3.0 (kobas.cbi.pku.edu.cn/download.php) (24). An adjusted P-value <0.05 was set as the cut-off criterion.

PPI network construction and modules selection. The PPI networks of DEGs were identified using the Search Tool for the Retrieval of Interacting Genes (STRING) database (string-db.org; release 10) (25). Interactions with confidence scores of ≥ 0.4 were selected as significant and visualized using Cytoscape software (www.cytoscape.org) (26). The hub genes were selected by the cytoHubba plug-in, with ≥ 10 degrees for each gene (27), and also mapped into ClueGO to visualize functionally grouped GO terms and KEGG pathway annotation networks (28). The Molecular Complex Detection (MCODE) plug-in was applied to screen modules of the PPI network with degree cutoff=2, node score cutoff=0.2, k-core=2, and max.

depth=100 (29). Subsequently, the functional and pathway enrichment analysis of genes in each module (MCODE score ≥ 6 and number of nodes ≥ 6) was performed by KOBAS.

Results

Identification of DEGs. As presented in Fig. 1, the batch effects between two gene expression profiles datasets were removed. The data was normalized prior to further analysis (Fig. 2A and B). In total, 546 DEGs (238 upregulated DEGs and 308 downregulated DEGs) were identified in wild-type GIST samples, compared with KIT/PDGFR α mutant GIST samples, based on the cut-off criteria. The volcano plot (Fig. 2C) and heatmap (Fig. 3) demonstrated the distribution and cluster of DEGs, respectively.

Functional and pathway enrichment analysis. The directed acyclic graph of GO enrichment analysis and functional annotation of DEGs is depicted in Fig. 4. For biological processes (BP), the upregulated DEGs were primarily enriched in 'cell adhesion', 'biological adhesion' and 'synapse organization' (Table I; Fig. 5A and B); the downregulated DEGs were primarily enriched in 'sterol metabolic process', 'inflammatory response', and 'integrin-mediated signaling pathway' (Table I; Fig. 5C and D). For cellular component (CC), the upregulated DEGs were primarily enriched in 'plasma membrane part', 'synapse' and 'integral to plasma membrane' (Table I; Fig. 5A and B); the downregulated DEGs were primarily enriched in 'internal side of plasma membrane', 'lipid particle', and 'plasma membrane part' (Table I; Fig. 5C and D). For molecular function (MF), the upregulated DEGs were primarily enriched in 'transmembrane receptor protein tyrosine kinase activity', 'calcium ion binding' and 'transmembrane receptor protein tyrosine kinase signaling pathway' (Table I; Fig. 5A and B); the downregulated DEGs were primarily enriched in 'GTPase regulator activity', 'nucleoside-triphosphatase regulator activity', and 'calcium ion binding' (Table I; Fig. 5C and D).

According to the KEGG pathway enrichment analysis, upregulated genes were primarily enriched in 'PI3K-Akt signaling pathway', 'ras signaling pathway', 'Rap1 signaling pathway', 'calcium signaling pathway' and 'ErbB signaling pathway' (Table II; Fig. 5E). Downregulated genes were primarily enriched in 'insulin signaling pathway', 'cAMP signaling pathway', 'PPAR signaling pathway', and 'NF-kappa B signaling pathway' (Table II; Fig. 5F).

PPI network construction and modules selection. To investigate the interactions between DEGs, a PPI network for the DEGs was constructed. The PPI network consisted of 338 nodes and 628 edges with a confidence score of ≥ 0.4 (Fig. 6). A total of 25 hub genes were selected from the PPI network with a degree of ≥ 10 (Table III), including leucine-rich repeat kinase 2 (LRRK2), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit γ (PIK3CG), CD44, vascular cell adhesion molecule 1 (VCAM1) and hepatocyte growth factor (HGF). GO enrichment analysis revealed that hub genes were primarily enriched in 'plasma membrane region', 'extracellular exosome', 'angiogenesis', 'regulation of transmembrane transport' and 'protein binding' (Fig. 7A; Table IV). The

KEGG analysis indicated that hub genes were predominantly enriched in 'PI3K-Akt signaling pathway', 'proteoglycans in cancer', 'focal adhesion', 'Rap1 signaling pathway', 'pathways in cancer' and 'ECM-receptor interaction' (Fig. 7B; Table IV).

Four significant modules were screened from the PPI network of DEGs using MCODE plug-in, and enrichment analysis revealed that the module genes in the sub-networks were mainly associated with 'PI3K-Akt signaling pathway', 'Rap1 signaling pathway', 'proteoglycans in cancer' and 'pathways in cancer' (Table IV).

Discussion

KIT/PDGFR α mutations are the major genetic alterations that occur in the development and progression of GISTs, and have been the only targets of molecular-based therapies in the last decade (4,30). However, other genetic alterations may also be associated with the development and progression of GISTs (15). Currently, little is known about differences in gene expression levels between wild-type GISTs and KIT/PDGFR α mutant GISTs. Therefore, joint bioinformatics analysis was performed in the present study, to obtain other potential candidate biomarkers that may be involved in the development and progression of wild-type GISTs. Ultimately, a total of 546 DEGs were identified, including 238 upregulated DEGs and 308 downregulated DEGs. GO functional and KEGG pathway enrichment analysis of DEGs was subsequently performed. Traditionally, distant metastasis is determined to be the leading cause of morbidity and mortality in patients with cancer, and genes encoding adhesion proteins, inflammatory factors, cytokines, growth factors and transduction molecules are considered major mediators of metastasis (31,32). Enrichment analysis in the present study revealed that the identified DEGs may be involved in the aforementioned process and signaling pathway, and were associated with proliferation, differentiation, apoptosis and distant metastasis in GISTs.

Furthermore, PPI networks of DEGs were constructed and 25 hub genes with degrees of ≥ 10 were identified. Functional enrichment analysis of hub genes determined that these genes were significantly associated with heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules, regulation of transmembrane transport, cardiovascular system development and angiogenesis. Additionally, pathway analysis indicated that these genes were primarily associated with the following pathways: 'PI3K-Akt signaling pathway', 'proteoglycans in cancer', 'focal adhesion', 'pathways in cancer', 'Rap1 signaling pathway', 'ECM-receptor interaction', 'cell adhesion molecules', 'phospholipase D signaling pathway', 'cAMP signaling pathway', 'Ras signaling pathway' and 'cGMP-PKG signaling pathway'. Furthermore, pathway analyses of four significant modules filtered from the PPI network was performed, and the results revealed that the genes in these modules were also primarily involved in the aforementioned pathways. Specifically, several high-frequency hub genes were identified that may be involved in the progression of GISTs by combining these results, including PIK3CG, insulin like growth factor 1 receptor (IGF1R), HGF, thrombospondin 1 (THBS1), Erb-B2 receptor tyrosine kinase (ERBB) 2 and matrix metalloproteinase 2 (MMP2).

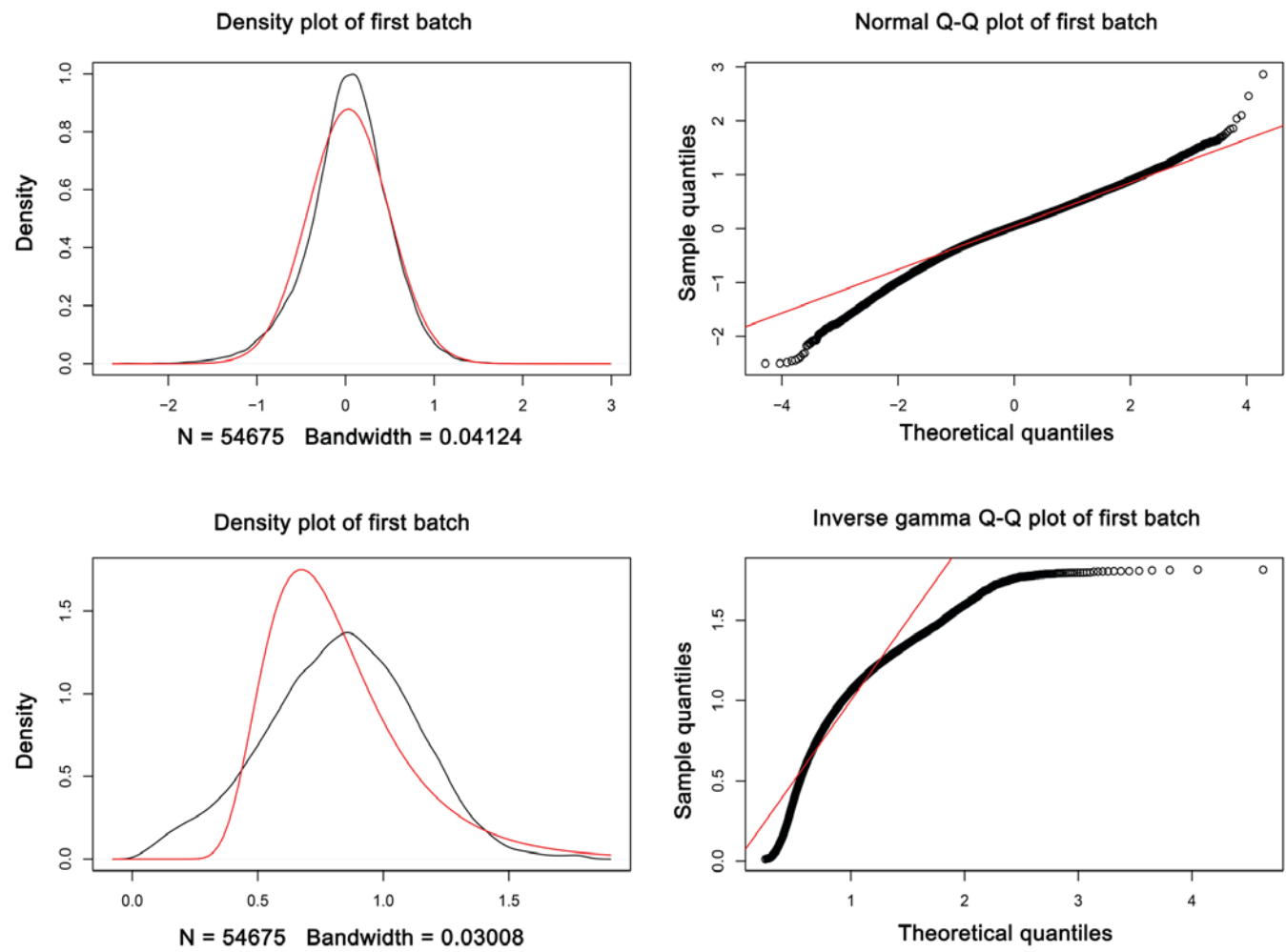


Figure 1. Batch effect plots between the two gene expression profile datasets.

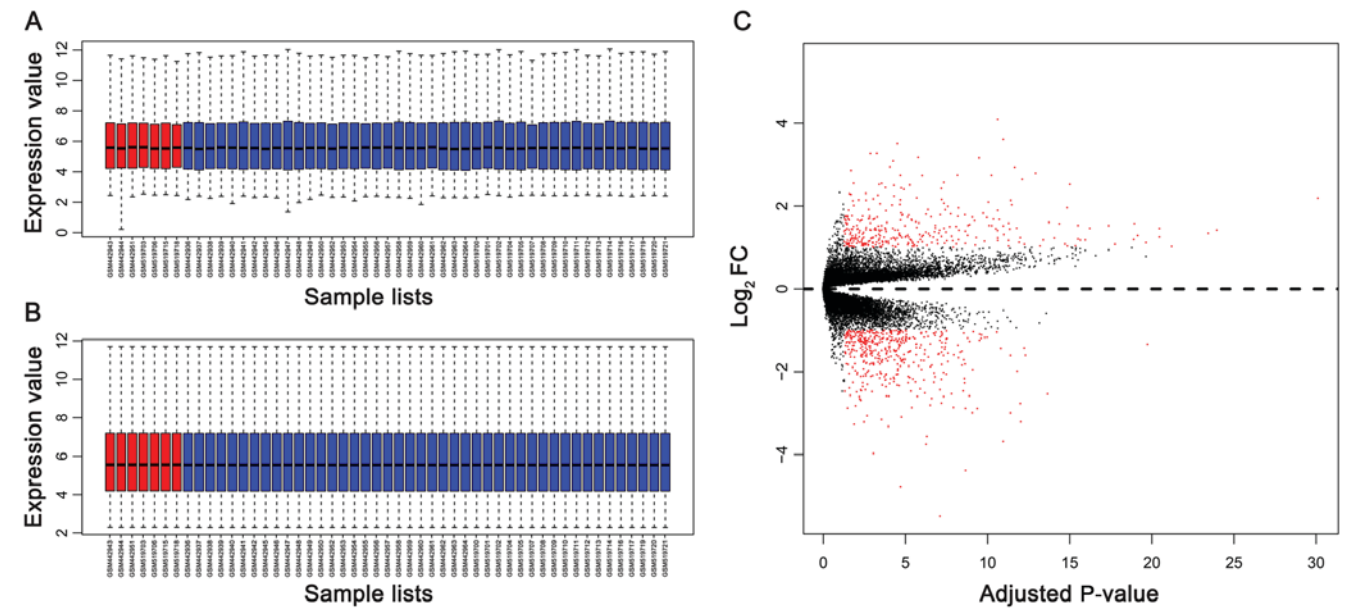


Figure 2. Box plots of gene expression profiles of GIST samples and the distribution of DEGs. (A) Gene expression profile of each sample prior to data normalization. (B) Gene expression profile of each sample following data normalization. (C) Volcano plot of gene distribution in wild-type GIST samples compared with KIT/PDGFR α mutant GIST samples. In (A) and (B), the horizontal axis and vertical axis represent samples and gene expression values, respectively; the red boxes and the blue boxes represent the wild-type GIST samples and the KIT/PDGFR α mutant GIST samples, respectively. In (C), the red plot represents statistically significant DEGs (FC $\geq$ 1 and P<0.05). The black plot represents genes with no significant expression changes. DEGs, differentially expressed genes; GISTs, gastrointestinal stromal tumors; FC, fold change; KIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; PDGFR α , platelet-derived growth factor receptor α .

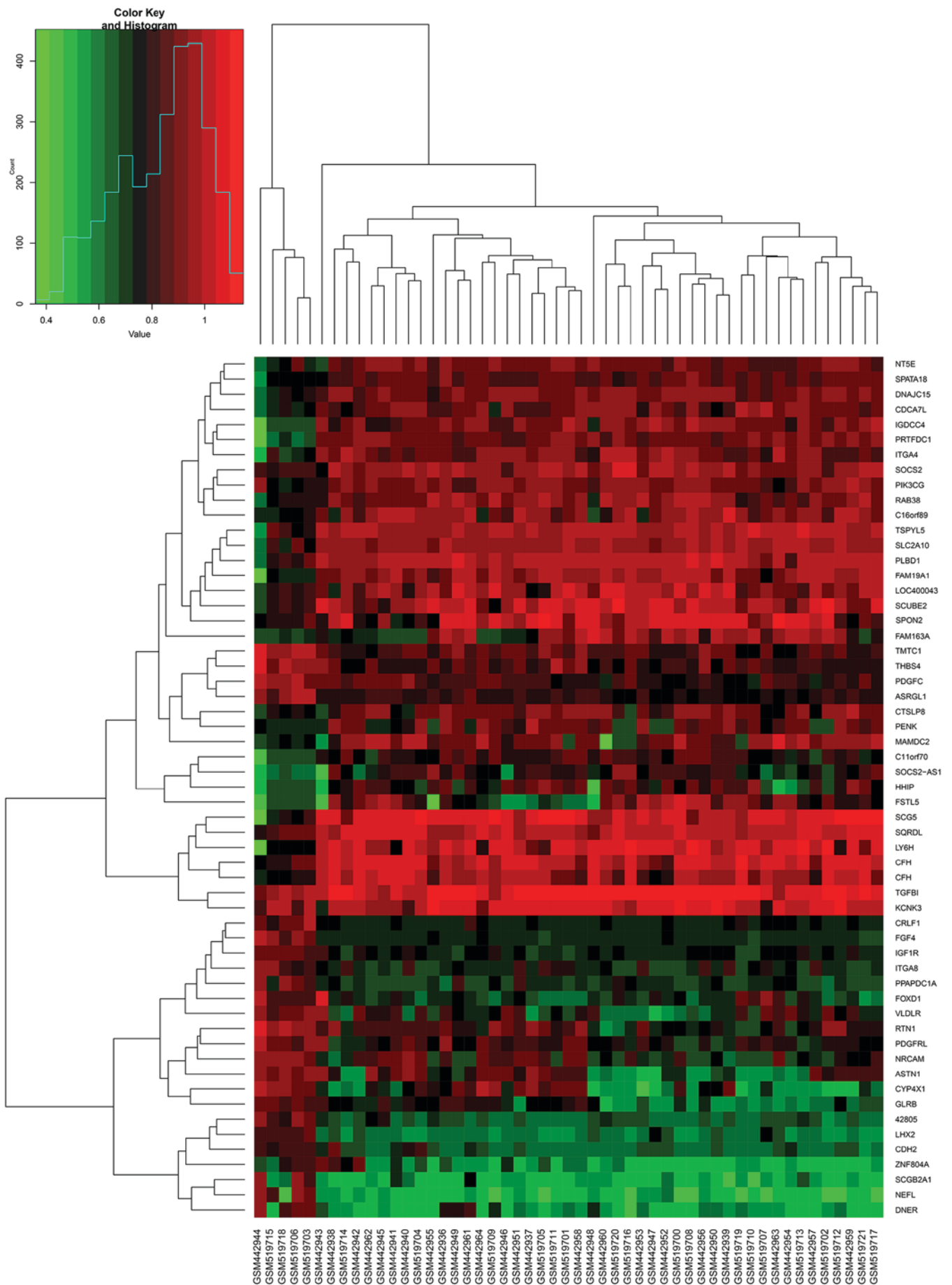


Figure 3. Hierarchical clustering heatmap of fractional DEGs. The horizontal axis indicates the sample, and the vertical axis indicates the DEGs. Red represents the upregulated DEGs and green represents the downregulated DEGs. DEGs, differentially expressed genes.

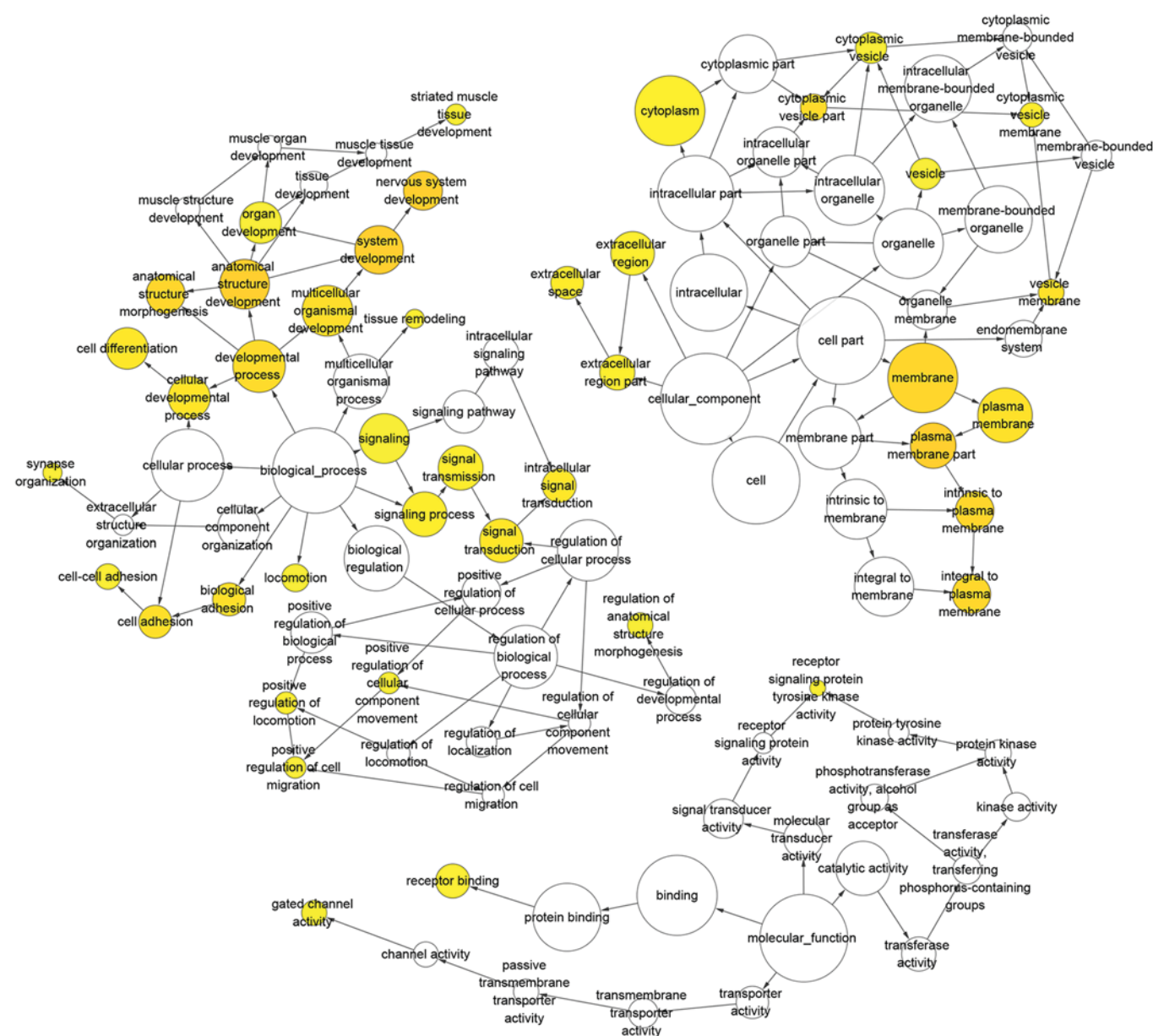


Figure 4. Directed acyclic graph of GO enrichment analysis and DEG functional annotation. Circles represent GO function, the upstream GO function includes the downstream GO function (arrows) and the color intensity represents the degree of GO function enrichment. DEGs, differentially expressed genes; GO, gene ontology.

The PIK3CG gene is located on chromosome 7 long arm q22.3, contains 12 exons, and encodes phosphoinositide 3-kinase (PI3K) γ , which phosphorylates inositol lipids and is involved in the immune response (33). Semba *et al* (34) reported that the PIK3CG gene is downregulated in colorectal cancer and is involved in tumorigenesis and progression, mainly through the PI3K-protein kinase B (Akt) signaling pathway. Li *et al* (35) determined that the PI3K-Akt signaling pathway is partially activated following imatinib secondary resistance, and PI3K activation may occur at an early stage of secondary resistance. In the present study, PIK3CG was downregulated in wild-type GISTs. PIK3CG was identified as a hub gene with 29 degrees in the PPI network, and as the core gene of module 2. The enrichment analyses demonstrated that PIK3CG was associated with cardiovascular and vasculature development, regulation of transmembrane transport,

angiogenesis and anatomical structure morphogenesis, as well as the PI3K-Akt signaling pathway, phospholipase D signaling pathway and cAMP signaling pathway. This suggested that PIK3CG may be a key molecule associated with wild-type GISTs.

IGF1R, a transmembrane receptor tyrosine kinase, serves a critical role in tumor transformation and malignant cell survival. IGF1R is predominantly involved in two signaling pathways: The PI3K-Akt pathway and the Ras-mitogen-activated protein kinase pathway (36). Ludovini *et al* (37) identified that activation of IGF1R is a necessary condition for mediating tumor cell proliferation and invasion, and is an independent poor prognostic factor in early stage non-small cell lung cancer. In addition, previous studies have demonstrated that IGF1R is overexpressed in wild-type GISTs, and inhibition of IGF1R signaling may be an effective therapeutic strategy (38-41). In

Table I. GO enrichment analysis and functional annotation of DEGs.

A, Upregulated					
Category	Term	Description	Count	%	P-value
GOTERM_BP_FAT	GO:0007155	Cell adhesion	25	11.1	7.08x10 ⁻⁶
GOTERM_BP_FAT	GO:0022610	Biological adhesion	25	11.1	7.26x10 ⁻⁶
GOTERM_BP_FAT	GO:0050808	Synapse organization	7	3.1	1.13x10 ⁻⁴
GOTERM_BP_FAT	GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	12	5.3	1.24x10 ⁻⁴
GOTERM_BP_FAT	GO:0007267	Cell-cell signaling	19	8.4	5.50x10 ⁻⁴
GOTERM_CC_FAT	GO:0044459	Plasma membrane part	55	24.4	7.52x10 ⁻⁶
GOTERM_CC_FAT	GO:0045202	Synapse	18	8.0	8.07x10 ⁻⁶
GOTERM_CC_FAT	GO:0005887	Integral to plasma membrane	35	15.6	2.90x10 ⁻⁵
GOTERM_CC_FAT	GO:0030054	Cell junction	21	9.3	3.00x10 ⁻⁵
GOTERM_CC_FAT	GO:0031226	Intrinsic to plasma membrane	35	15.6	4.59x10 ⁻⁵
GOTERM_MF_FAT	GO:0004714	Transmembrane receptor protein tyrosine kinase activity	6	2.7	1.36x10 ⁻³
GOTERM_MF_FAT	GO:0005509	Calcium ion binding	22	9.8	4.10x10 ⁻³
GOTERM_MF_FAT	GO:0022843	Voltage-gated cation channel activity	7	3.1	9.44x10 ⁻³
GOTERM_MF_FAT	GO:0022836	Gated channel activity	10	4.4	1.41x10 ⁻²
GOTERM_MF_FAT	GO:0008083	Growth factor activity	7	3.1	1.43x10 ⁻²
B, Downregulated					
GOTERM_BP_FAT	GO:0016125	Sterol metabolic process	6	2.2	1.22x10 ⁻²
GOTERM_BP_FAT	GO:0006954	Inflammatory response	11	4.1	1.34x10 ⁻²
GOTERM_BP_FAT	GO:0007229	Integrin-mediated signaling pathway	5	1.9	1.51x10 ⁻²
GOTERM_BP_FAT	GO:0006631	Fatty acid metabolic process	8	3.0	1.87x10 ⁻²
GOTERM_BP_FAT	GO:0007264	Small GTPase mediated signal transduction	10	3.7	2.38x10 ⁻²
GOTERM_CC_FAT	GO:0009898	Internal side of plasma membrane	12	4.5	4.55x10 ⁻²
GOTERM_CC_FAT	GO:0005811	Lipid particle	3	1.1	1.78x10 ⁻²
GOTERM_CC_FAT	GO:0044459	Plasma membrane part	42	15.7	2.57x10 ⁻²
GOTERM_CC_FAT	GO:0005739	Mitochondrion	24	9.0	2.68x10 ⁻²
GOTERM_CC_FAT	GO:0031090	Organelle membrane	24	9.0	2.91x10 ⁻²
GOTERM_MF_FAT	GO:0030695	GTPase regulator activity	14	5.2	3.60x10 ⁻³
GOTERM_MF_FAT	GO:0060589	Nucleoside-triphosphatase regulator activity	14	5.2	4.34x10 ⁻³
GOTERM_MF_FAT	GO:0005509	Calcium ion binding	23	8.6	6.95x10 ⁻³
GOTERM_MF_FAT	GO:0005178	Integrin binding	5	1.9	8.49x10 ⁻³
GOTERM_MF_FAT	GO:0005096	GTPase activator activity	8	3.0	3.12x10 ⁻²

Count indicates the enriched gene number in the category. GO, gene ontology; DEGs, differentially expressed genes BP, biological process; CC, cellular component; MF, molecular function.

the present study, IGF1R was upregulated in wild-type GIST samples and was associated with fibroblast growth factor receptor (FGFR) 4, Janus kinase 3 and tyrosine-protein kinase SYK in module 1. Pathway analysis revealed that IGF1R was associated with the PI3K-Akt signaling pathway, proteoglycans in cancer, and ras signaling pathway. These findings were consistent with the results of the aforementioned studies and indicated that IGF1R overexpression may act as an alternative genetic alteration event to the KIT/PDGFR mutations in GISTs. Therefore, further research is necessary to clarify underlying mechanisms of IGF1R in wild-type GISTs.

HGF, also known as scatter factor, is a member of the endothelium-specific growth factor family, and regulates cell growth and motility, migration, and angiogenesis through binding to its receptor c-Met (42). Hack *et al* (43) revealed that aberrant activation of the HGF/MET signaling pathway occurs in the malignant transformation and progression of gastroesophageal cancer, and consistently correlates with an aggressive metastatic phenotype and poor prognosis. However, there is a lack of research on the role of HGF in GISTs. The results of enrichment analysis in the present study demonstrated that HGF was associated with angiogenesis,

Table II. KEGG pathways analysis results of DEGs.

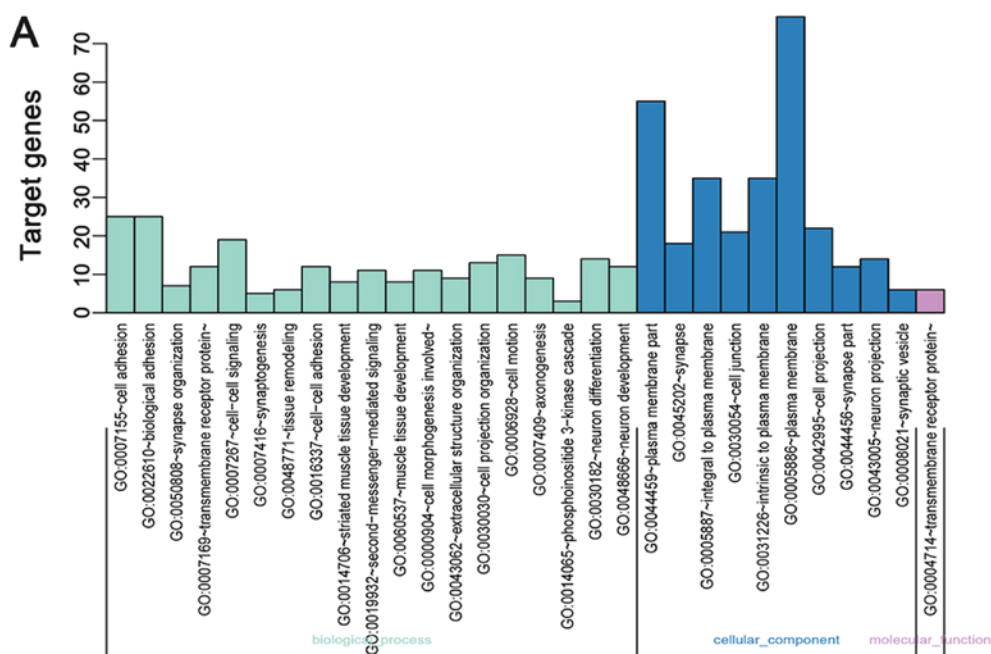
A, Upregulated				
Pathway ID	Description	Count	P-value	Genes
hsa05218	Melanoma	7	4.22x10 ⁻⁷	CDK6, HGF, FGF3, FGF10, IGF1R, FGF4, PDGFC
hsa04151	PI3K-Akt signaling pathway	12	1.53x10 ⁻⁶	CDK6, HGF, FGF3, MYB, EFNA2, FGF10, IGF1R, FGF4, ITGA8, PPP2R2B, PDGFC, THBS4
hsa04514	Cell adhesion molecules (CAMs)	8	4.06x10 ⁻⁶	PVRL3, NRXN1, CDH2, NRCAM, CNTNAP2, ITGA8, NLGN4X, CADM1
hsa04014	Ras signaling pathway	9	1.30x10 ⁻⁵	HGF, SHC3, FGF3, EFNA2, FGF10, IGF1R, HTR7, FGF4, PDGFC
hsa01521	EGFR tyrosine kinase inhibitor resistance	6	1.34x10 ⁻⁵	HGF, SHC3, ERBB3, ERBB2, IGF1R, PDGFC
hsa04512	ECM-receptor interaction	6	1.43x10 ⁻⁵	CD44, SV2B, CD36, ITGA8, THBS4, SV2C
hsa04510	Focal adhesion	8	3.98x10 ⁻⁵	ARHGAP5, HGF, SHC3, ERBB2, IGF1R, ITGA8, PDGFC, THBS4
hsa04015	Rap1 signaling pathway	8	5.17x10 ⁻⁵	HGF, FGF3, EFNA2, FGF10, IGF1R, FGF4, ADCY2, PDGFC
hsa04020	Calcium signaling pathway	7	1.31x10 ⁻⁴	ATP2B1, ERBB3, ERBB2, HTR7, TACR1, CAMK4, ADCY2
hsa05200	Pathways in cancer	10	1.70x10 ⁻⁴	CDK6, HGF, DAPK1, ERBB2, FGF3, FGF10, IGF1R, FZD2, FGF4, ADCY2
hsa05014	Amyotrophic lateral sclerosis (ALS)	4	3.18x10 ⁻⁴	GRIA2, MAP3K5, GRIA1, NEFL
hsa04080	Neuroactive ligand-receptor interaction	8	3.22x10 ⁻⁴	GRIA2, PRLHR, PTH1R, HTR7, TACR1, GRIA1, OPRK1, GLRB
hsa04730	Long-term depression	4	5.69x10 ⁻⁴	GRIA2, IGF1R, GRIA1, PPP1R17
hsa05205	Proteoglycans in cancer	6	1.66x10 ⁻³	HGF, CD44, ERBB3, ERBB2, IGF1R, FZD2
hsa04012	ErbB signaling pathway	4	2.20x10 ⁻³	SHC3, ERBB3, ERBB2, NRG3
B, Downregulated				
Pathway ID	Description	Count	P-value	Genes
hsa05145	Toxoplasmosis	7	5.50x10 ⁻⁵	PIK3CG, SOCS1, HLA-DPA1, MYD88, CD40, LDLR, MAPK10
hsa04925	Aldosterone synthesis and secretion	6	5.68x10 ⁻⁵	KCNK3, PDE2A, CREB3L1, LDLR, ORAI1, CACNA1H
hsa05144	Malaria	5	5.85x10 ⁻⁵	VCAM1, THBS1, GYPC, MYD88, CD40
hsa04810	Regulation of actin cytoskeleton	9	6.24x10 ⁻⁵	ITGA4, PIK3CG, CD14, F2R, FGFR4, MRAS, SSH3, VAV2, ITGAE
hsa04910	Insulin signaling pathway	7	1.39x10 ⁻⁴	PIK3CG, SOCS2, SOCS1, SREBF1, PYGM, PDE3B, MAPK10
hsa04024	cAMP signaling pathway	8	2.08x10 ⁻⁴	HHIP, PIK3CG, F2R, CREB3L1, PDE3B, ORAI1, MAPK10, VAV2
hsa05205	Proteoglycans in cancer	8	2.52x10 ⁻⁴	MMP2, CTSL, PIK3CG, THBS1, WNT5B, WNT9A, MRAS, VAV2
hsa04931	Insulin resistance	6	2.65x10 ⁻⁴	PIK3CG, CREB3L1, SREBF1, PYGM, CPT1A, MAPK10
hsa03320	PPAR signaling pathway	5	3.17x10 ⁻⁴	ME1, PLIN2, CPT1A, ACSL5, CYP27A1
hsa00071	Fatty acid degradation	4	4.98x10 ⁻⁴	ACAA2, CPT1A, ACSL5, ALDH7A1
hsa01212	Fatty acid metabolism	4	6.77x10 ⁻⁴	PTPLAD2, ACAA2, CPT1A, ACSL5
hsa04930	Type II diabetes mellitus	4	6.77x10 ⁻⁴	PIK3CG, SOCS2, SOCS1, MAPK10
hsa04064	NF-kappa B signaling pathway	5	9.57x10 ⁻⁴	VCAM1, CD14, SYK, MYD88, CD40
hsa05166	HTLV-I infection	8	1.11x10 ⁻³	JAK3, PIK3CG, VCAM1, WNT5B, HLA-DPA1, WNT9A, MRAS, CD40

Table II. Continued.

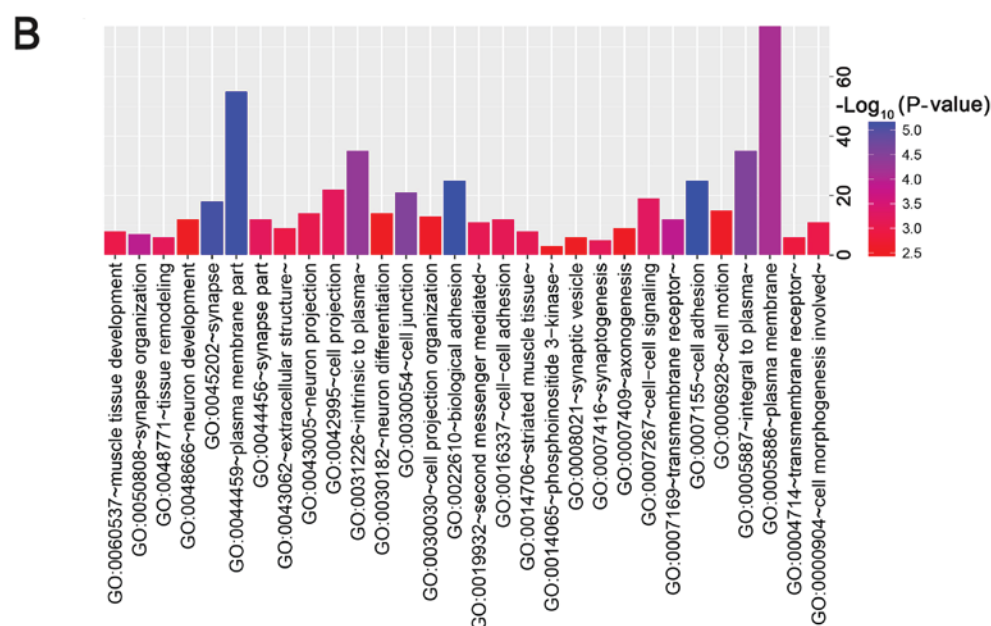
B, Downregulated

Pathway ID	Description	Count	P-value	Genes
hsa04510	Focal adhesion	7	1.22×10^{-3}	ITGA4, PIK3CG, THBS1, PARVA, MAPK10, VAV2, PARVB

Count indicates the enriched gene number in the pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

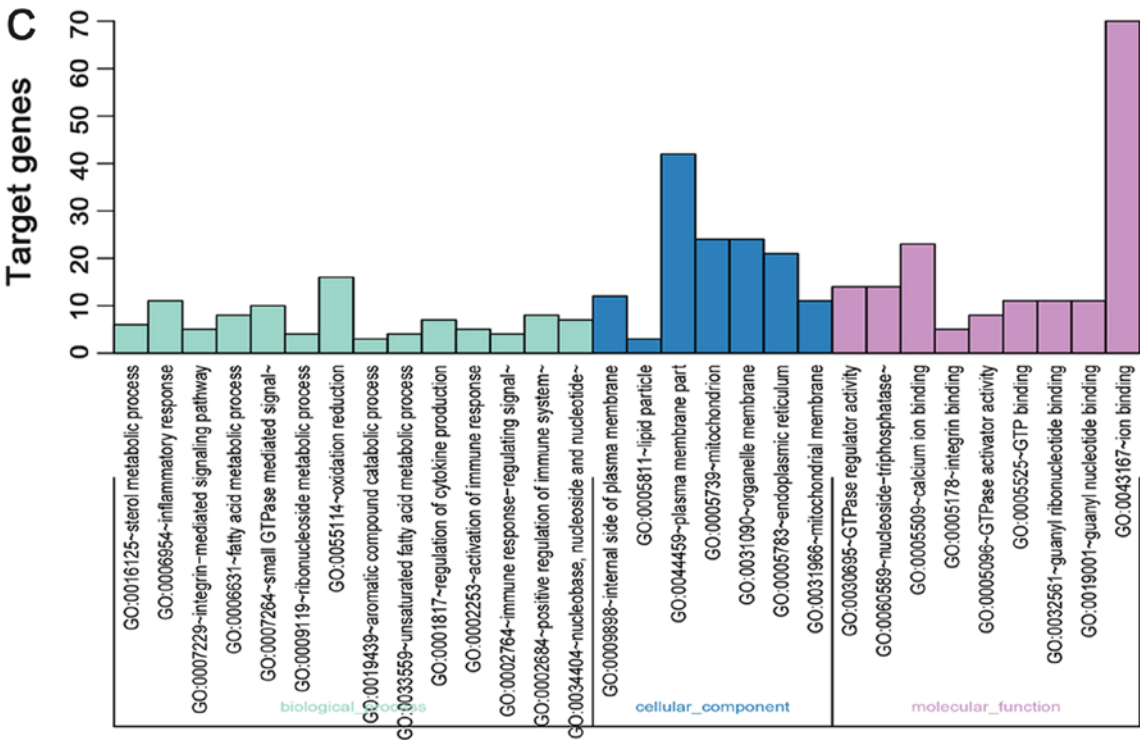


Upregulated GO terms

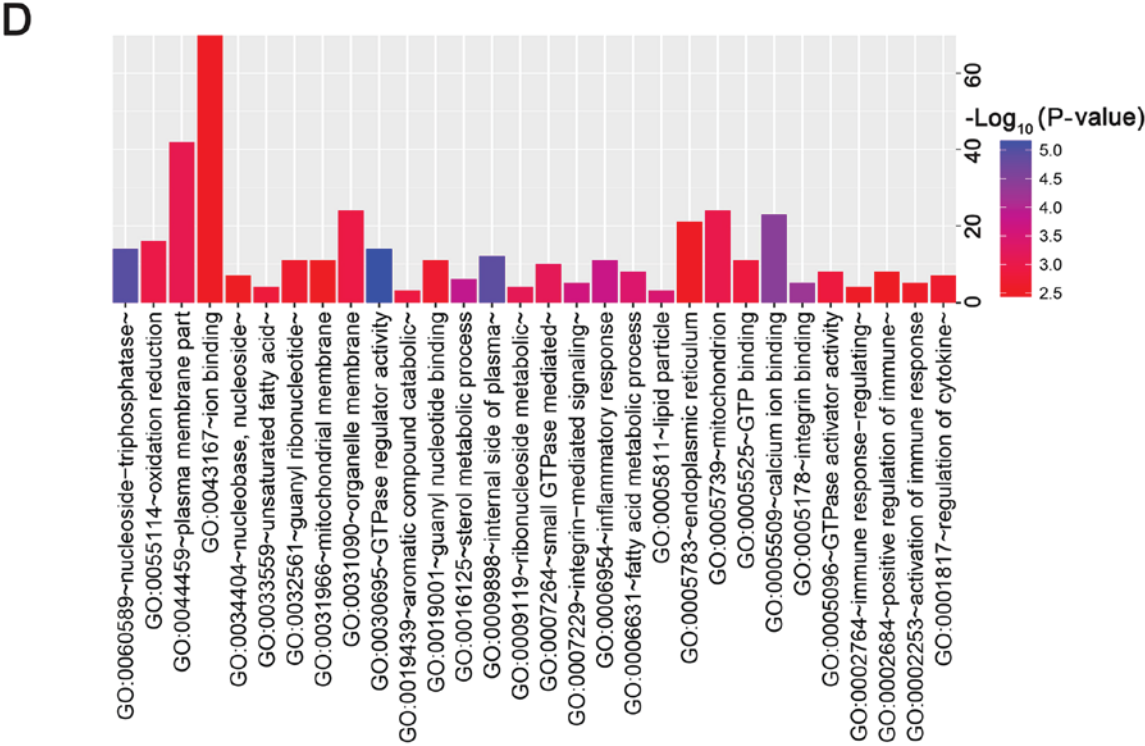


Upregulated GO terms

Figure 5. GO functional and KEGG pathway enrichment analysis of DEGs. (A) GO functional enrichment analysis of upregulated DEGs based on the three types of sub-ontologies and (B) P-value.



Downregulated GO terms



Downregulated GO terms

Figure 5. Continued. (C) GO functional enrichment analysis of downregulated DEGs (top 30 terms) based on the three types of sub-ontologies and (D) P-value. The horizontal axis and vertical axis indicate the names of GO terms and the number of target genes, respectively.

cardiovascular and vasculature development, proteoglycans in cancer, focal adhesion, pathways in cancer, and PI3K-Akt signaling pathway. Furthermore, it was associated with Wnt family member (WNT) 5B, ERBB3, and WNT9A in

module 4, revealing that HGF may be involved in progression of wild-type GISTS.

THBS1, also known as TSP-1, encodes an adhesive glycoprotein that is involved in platelet aggregation, angiogenesis

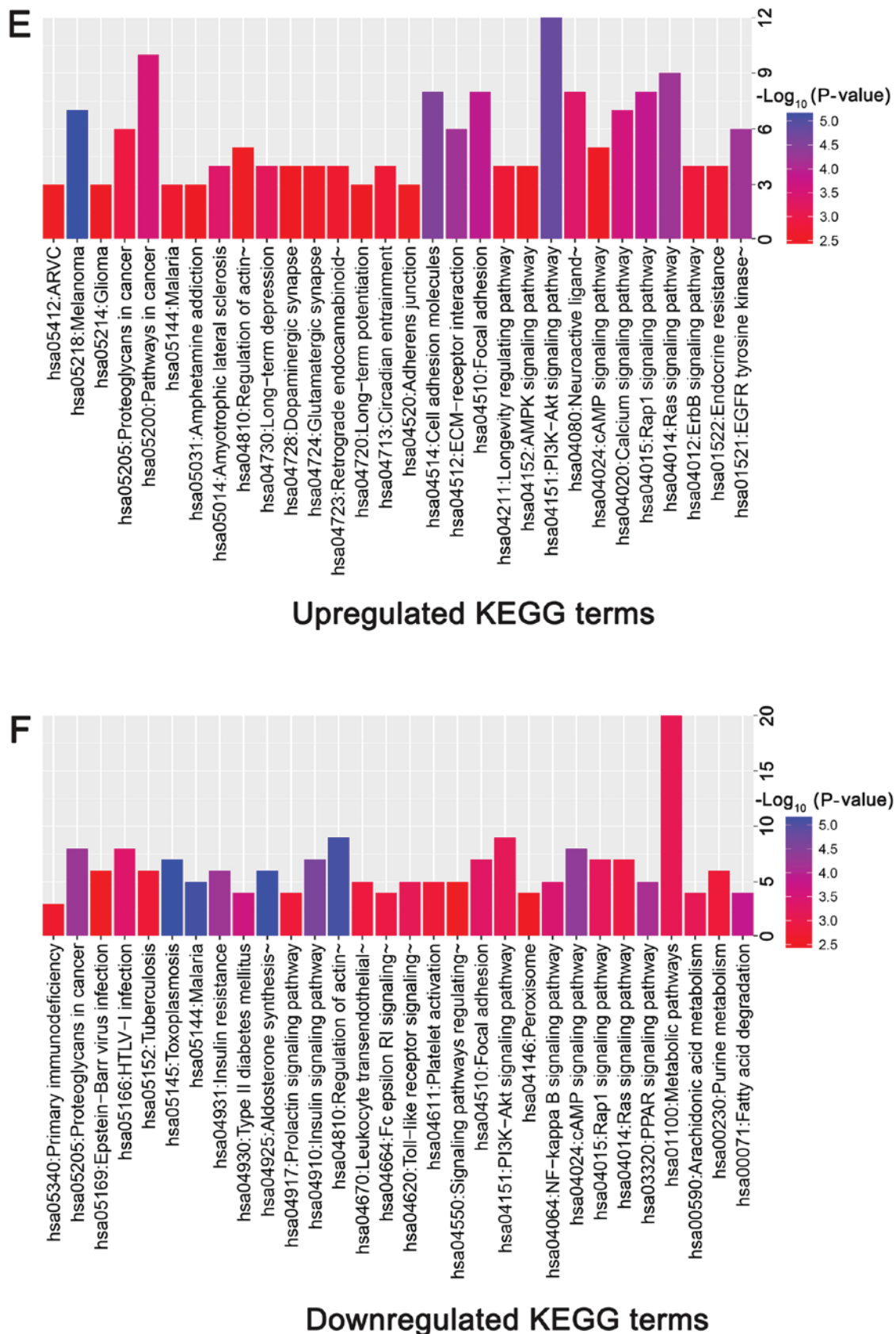


Figure 5. Continued. KEGG functional enrichment analysis of (E) upregulated and (F) downregulated DEGs (top 30 terms) are presented, based on P-value. The color of the pillars represents the size of the P-value, with the red color representing the smallest P-value. DEGs, differentially expressed genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

and tumorigenesis (44). In the present study, THBS1 was associated with proteoglycans in cancer, ECM-receptor

interaction, PI3K-Akt signaling pathway, and rap1 signaling pathway. In addition, functional enrichment analysis

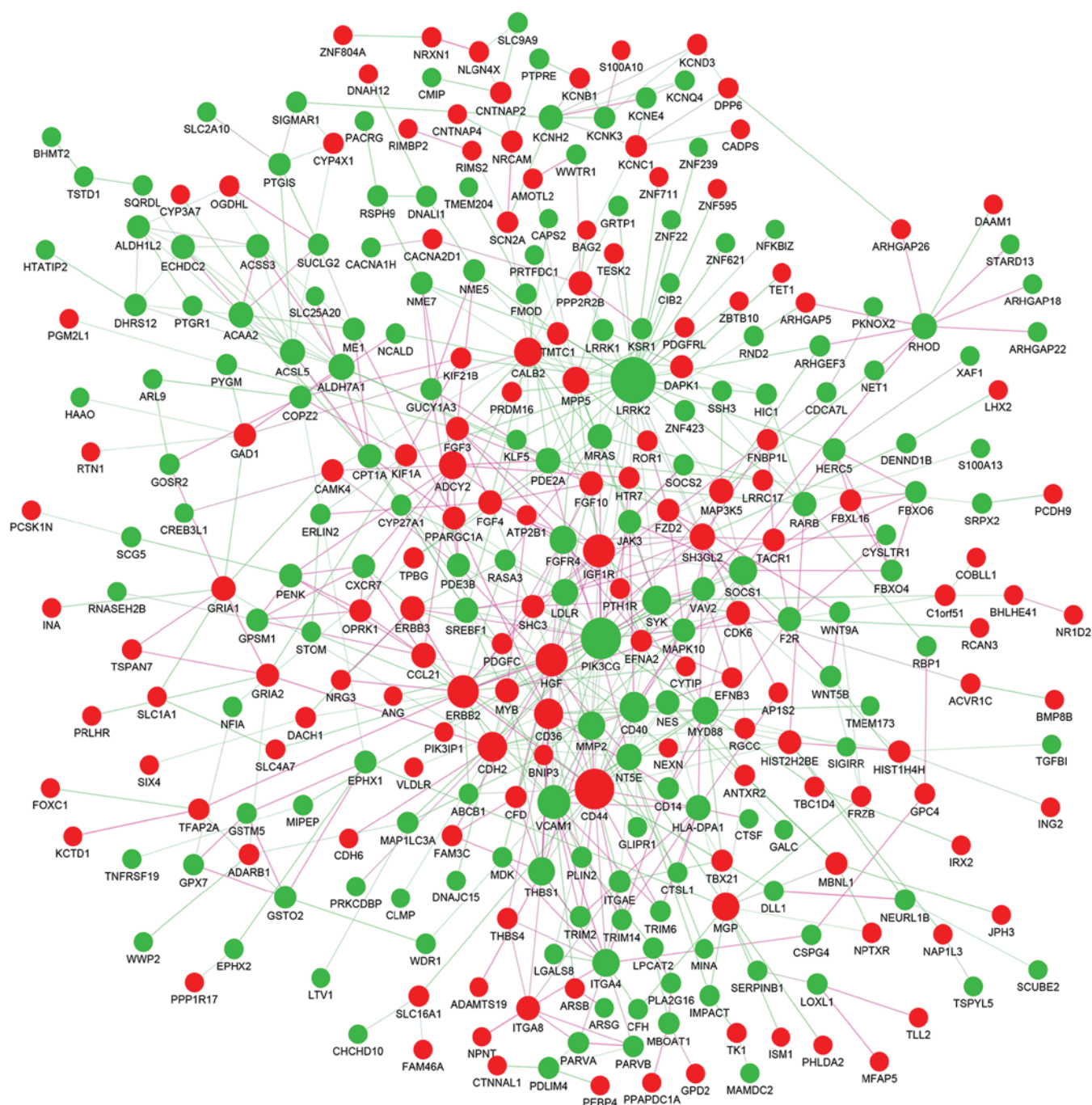


Figure 6. Protein-protein interaction network of identified differentially expressed genes. Red nodes represent the upregulated genes, while green nodes represent the downregulated genes. The magnitude of each node represents the extent to which each gene is linked, and the lines represent the interaction relationship between nodes.

identified that THBS1 was associated with angiogenesis, protein binding, cell adhesion, regulation of transmembrane transport and inflammatory response. Kashiwara *et al* (45) revealed that THBS1 is associated with carcinogenesis occurring in patients with intestinal inflammation, and may serve an important role in gastric carcinogenesis. Huang *et al* (46) reported that upregulation of THBS1 is induced by FGF7/FGFR2 via the PI3K/Akt/mechanistic target of rapamycin signaling pathway, and is associated with the regulation of invasion and migration in gastric cancer. However, no studies have elucidated the mechanism of THBS1 in GISTs. In module 3, THBS1 was associated with

adenyl cyclase type 2 (ADCY2), FGF10, FGF3, FGF4, CD36 and CD44, indicating that THBS1 may also be involved in GISTs by mediating these genes. Therefore, further research is necessary to clarify the underlying mechanism of THBS1 in wild-type GISTs.

ERBB2, also known as Her2 or Neu, is a member of the ERBB family of receptor tyrosine kinases. It encodes a transmembrane tyrosine kinase receptor of the ERBB family that has important roles in many aspects of various human cancers (47,48). However, the oncogenic role and clinical significance of ERBB2 in GISTs has not been investigated in detail. In the present study, ERBB2 was upregulated and

Table III. Top 25 hub genes identified in PPI network of DEGs.

Affy ID	Gene symbol	Gene name	Degree	log FC	P-value	Regulation
229584_at	LRRK2	Leucine rich repeat kinase 2	36	-1.78	1.04x10 ⁻⁴	Down
206369_s_at	PIK3CG	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	29	-1.89	5.37x10 ⁻⁶	Down
204489_s_at	CD44	CD44 molecule (Indian blood group)	28	1.11	1.14x10 ⁻³	Up
203868_s_at	VCAM1	Vascular cell adhesion molecule 1	20	-1.76	8.36x10 ⁻⁴	Down
209960_at	HGF	Hepatocyte growth factor	19	1.05	2.25x10 ⁻³	Up
216836_s_at	ERBB2	Erb-b2 receptor tyrosine kinase 2	18	1.21	6.69x10 ⁻⁵	Up
243358_at	IGF1R	Insulin-like growth factor 1 receptor	18	1.39	1.72x10 ⁻¹¹	Up
203441_s_at	CDH2	Cadherin 2	15	1.41	2.63x10 ⁻⁸	Up
206488_s_at	CD36	CD36 molecule	15	1.49	2.97x10 ⁻³	Up
205153_s_at	CD40	CD40 molecule	15	-1.12	5.87x10 ⁻⁴	Down
207540_s_at	SYK	Spleen associated tyrosine kinase	14	-1.48	2.52x10 ⁻³	Down
205428_s_at	CALB2	Calbindin 2	13	1.25	3.93x10 ⁻⁶	Up
210001_s_at	SOCS1	Suppressor of cytokine signaling 1	13	-1.46	4.87x10 ⁻⁵	Down
202291_s_at	MGP	Matrix Gla protein	12	1.52	5.01x10 ⁻⁶	Up
213217_at	ADCY2	Adenylate cyclase 2	12	1.96	7.75x10 ⁻⁴	Up
201069_at	MMP2	Matrix metalloproteinase 2	12	-2.29	2.25x10 ⁻⁴	Down
205884_at	ITGA4	Integrin subunit alpha 4	12	-1.94	6.88x10 ⁻⁴	Down
201108_s_at	THBS1	Thrombospondin 1	12	-1.65	2.30x10 ⁻³	Down
204579_at	FGFR4	Fibroblast growth factor receptor 4	12	-1.49	2.41x10 ⁻³	Down
219321_at	MPP5	Membrane protein, palmitoylated 5	11	1.07	6.57x10 ⁻⁵	Up
209124_at	MYD88	Myeloid differentiation primary response 88	11	-1.15	9.07x10 ⁻⁶	Down
217173_s_at	LDLR	Low density lipoprotein receptor	11	-1.06	8.12x10 ⁻⁵	Down
227486_at	NT5E	5'-nucleotidase ecto	10	-1.64	1.19x10 ⁻⁴	Down
205751_at	SH3GL2	SH3-domain GRB2-like 2	10	1.48	1.32x10 ⁻³	Up
208951_at	ALDH7A1	Aldehyde dehydrogenase 7 family, member A1	10	-1.21	5.77x10 ⁻⁵	Down

PPI, protein-protein interaction; DEGs, differentially expressed genes; FC, fold change.

identified as the core gene of module 3. Enrichment analyses revealed that ERBB2 was associated with plasma membrane region, cardiovascular system development, angiogenesis, focal adhesion and proteoglycans in cancer. Furthermore, it was associated with ADCY2, FGF10, frizzled-2 (FZD2), FGF3, FGF4, CD44, THBS1, and MMP2, revealing these genes may have joint function in GISTs. Hence, further studies are also required to determine the role of ERBB2 in wild-type GISTs.

MMP2, a member of the matrix metalloproteinase (MMP) gene family, is involved in many cancer pathways and exists in several proteoglycans in cancer. MMP family proteins are zinc-dependent enzymes capable of cleaving components of the extracellular matrix and molecules involved in signal transduction. MMP activity has been implicated in a number of key pathological processes, including tumor growth, progression, metastasis and dysregulated angiogenesis (49). Sebastiano *et al* (50) reported that MMP2 increases platelet activation by cleaving PAR1 at a noncanonical extracellular site, which induces biased receptor signaling through certain signaling pathways, usually only activated by full PAR1 agonism. Furthermore, it has been reported that the gene interaction

between MMP2 and PARP1 may increase the incidence of gastric cancer development and lymph node metastasis (51). In the current study, MMP2 was identified as a downregulated hub gene, enriched in pathways and proteoglycans in cancer of module 3 and associated with ADCY2, ERBB2, FGF10, FZD2, FGF3, FGF4, CD44 and THBS1.

There are several limitations of the present study that require acknowledgement. First, due to the limitations of the gene chip itself, the differentially expressed genes between GIST tissue and normal gastrointestinal tract tissue could not be identified. Second, all predicted results still require confirmation by laboratory data. Finally, a limited number of samples were used in the present study, which should be increased in future studies to improve the reliability of the conclusions drawn.

In conclusion, 546 DEGs were identified in wild-type GISTs, compared with the mutant GIST samples, which may be closely associated with GIST progression. In addition, several key hub DEGs were selected as potential candidate biomarkers for wild-type GISTs, including PIK3CG, IGF1R, HGF, THBS1, ERBB2 and MMP2. However, further verification experiments are required to confirm these results.

Table IV. Enriched function and pathways of hub genes and selected modules of PPI network.

A, Hub genes				
Term	Description	Count	P-value	Hub genes
GO:0005886	Plasma membrane	19	7.42×10^{-8}	PIK3CG, FGFR4, ADCY2, LDLR, ERBB2, MPP5, CD40, ITGA4, CDH2, MMP2, VCAM1, IGF1R, MYD88, CD36, CD44, LRRK2, NT5E, SH3GL2, SYK
GO:0016020	Membrane	11	2.53×10^{-4}	PIK3CG, VCAM1, IGF1R, ADCY2, CD36, LDLR, ERBB2, ITGA4, HGF, CDH2, NT5E
GO:0070062	Extracellular exosome	12	4.07×10^{-4}	VCAM1, ALDH7A1, CD44, MPP5, MGP, ITGA4, CD40, CDH2, THBS1, LRRK2, NT5E, SH3GL2
GO:0005737	Cytoplasm	16	5.71×10^{-4}	PIK3CG, FGFR4, ADCY2, ERBB2, SOCS1, MPP5, CD40, CDH2, CALB2, ALDH7A1, MYD88, CD44, LRRK2, NT5E, SH3GL2, SYK
GO:0005515	Protein binding	21	1.47×10^{-3}	PIK3CG, FGFR4, LDLR, ERBB2, SOCS1, MPP5, MGP, HGF, CD40, ITGA4, CDH2, MMP2, IGF1R, ALDH7A1, MYD88, CD36, CD44, THBS1, LRRK2, SH3GL2, SYK
hsa05205	Proteoglycans in cancer	7	4.89×10^{-11}	PIK3CG, IGF1R, CD44, ERBB2, HGF, THBS1, MMP2
hsa04151	PI3K-Akt signaling pathway	7	1.58×10^{-9}	PIK3CG, IGF1R, FGFR4, ITGA4, HGF, THBS1, SYK
hsa04510	Focal adhesion	6	3.19×10^{-9}	PIK3CG, IGF1R, ERBB2, ITGA4, HGF, THBS1
hsa04015	Rap1 signaling pathway	6	3.99×10^{-9}	PIK3CG, IGF1R, FGFR4, ADCY2, HGF, THBS1
hsa05200	Pathways in cancer	6	1.57×10^{-7}	PIK3CG, IGF1R, ADCY2, ERBB2, HGF, MMP2
B, Module 1				
Term	Description	Count	P-value	Genes
hsa04151	PI3K-Akt signaling pathway	4	6.16×10^{-5}	IGF1R, FGFR4, JAK3, SYK
hsa04550	Signaling pathways regulating pluripotency of stem cells	3	1.03×10^{-4}	IGF1R, FGFR4, JAK3
hsa05203	Viral carcinogenesis	2	7.47×10^{-3}	FGFR4, SYK
hsa04015	Rap1 signaling pathway	2	7.89×10^{-3}	IGF1R, JAK3
hsa04014	Ras signaling pathway	2	9.15×10^{-3}	IGF1R, JAK3
C, Module 2				
Term	Description	Count	P-value	Genes
hsa04668	TNF signaling pathway	2	5.09×10^{-4}	PIK3CG, MAP3K5
hsa04071	Sphingolipid signaling pathway	2	6.14×10^{-4}	PIK3CG, MAP3K5
hsa04210	Apoptosis	2	8.16×10^{-4}	PIK3CG, MAP3K5
hsa04072	Phospholipase D signaling pathway	2	8.62×10^{-4}	MRAS, PIK3CG
hsa05205	Proteoglycans in cancer	2	1.72×10^{-3}	MRAS, PIK3CG
D, Module 3				
Term	Description	Count	P-value	Genes
hsa05200	Pathways in cancer	7	5.55×10^{-8}	ADCY2, ERBB2, FGF10, FZD2, MMP2, FGF3, FGF4
hsa05205	Proteoglycans in cancer	5	1.11×10^{-6}	CD44, ERBB2, FZD2, THBS1, MMP2
hsa04015	Rap1 signaling pathway	5	1.28×10^{-6}	ADCY2, FGF10, THBS1, FGF3, FGF4
hsa04512	ECM-receptor interaction	3	5.86×10^{-5}	CD36, CD44, THBS1
hsa05166	HTLV-I infection	4	8.31×10^{-5}	VCAM1, ADCY2, FZD2, CD40

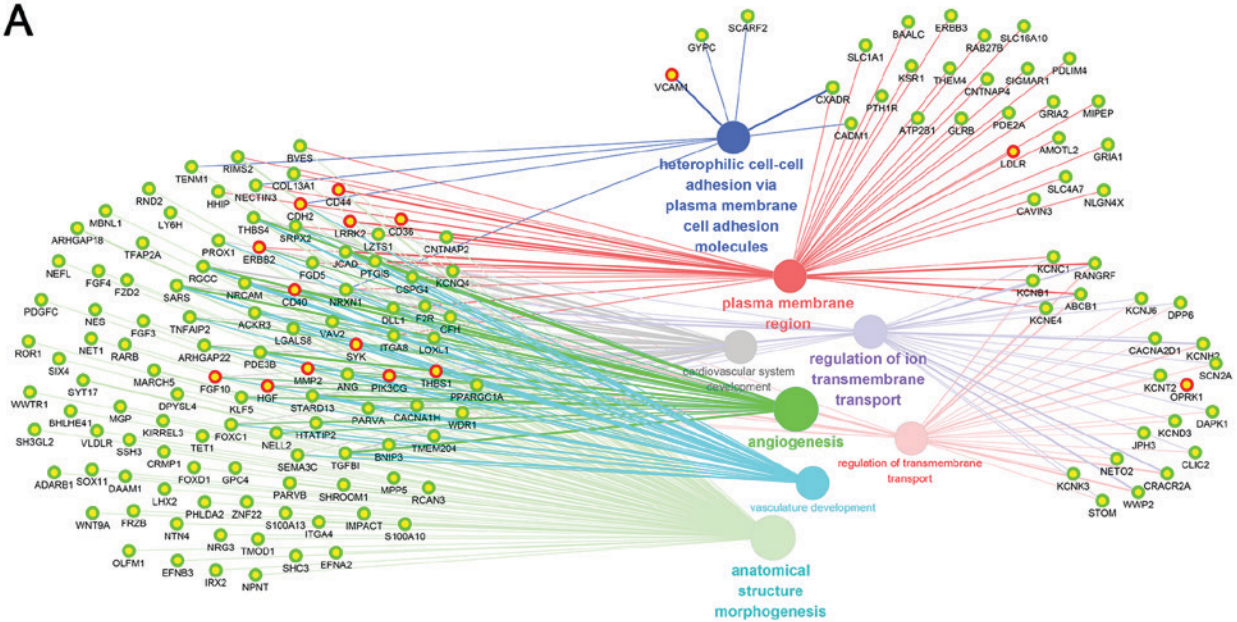
Table IV. Continued.

E, Module 4

Term	Description	Count	P-value	Genes
hsa05205	Proteoglycans in cancer	4	3.03×10^{-5}	WNT5B, ERBB3, WNT9A, HGF
hsa04810	Regulation of actin cytoskeleton	4	3.64×10^{-5}	ITGAE, ITGA4, CD14, F2R
hsa04151	PI3K-Akt signaling pathway	4	2.12×10^{-4}	MYB, ITGA4, CD14, WNT5B
hsa05200	Pathways in cancer	4	3.71×10^{-4}	F2R, ERBB3, WNT9A, WNT5B
hsa01100	Metabolic pathways	6	5.98×10^{-4}	CYP3A7, ACSL5, NT5E, PTGIS, ACSS3, ACAA2

Count indicates the enriched gene number in the category. PPI, protein-protein interaction; GO, gene ontology.

A



B

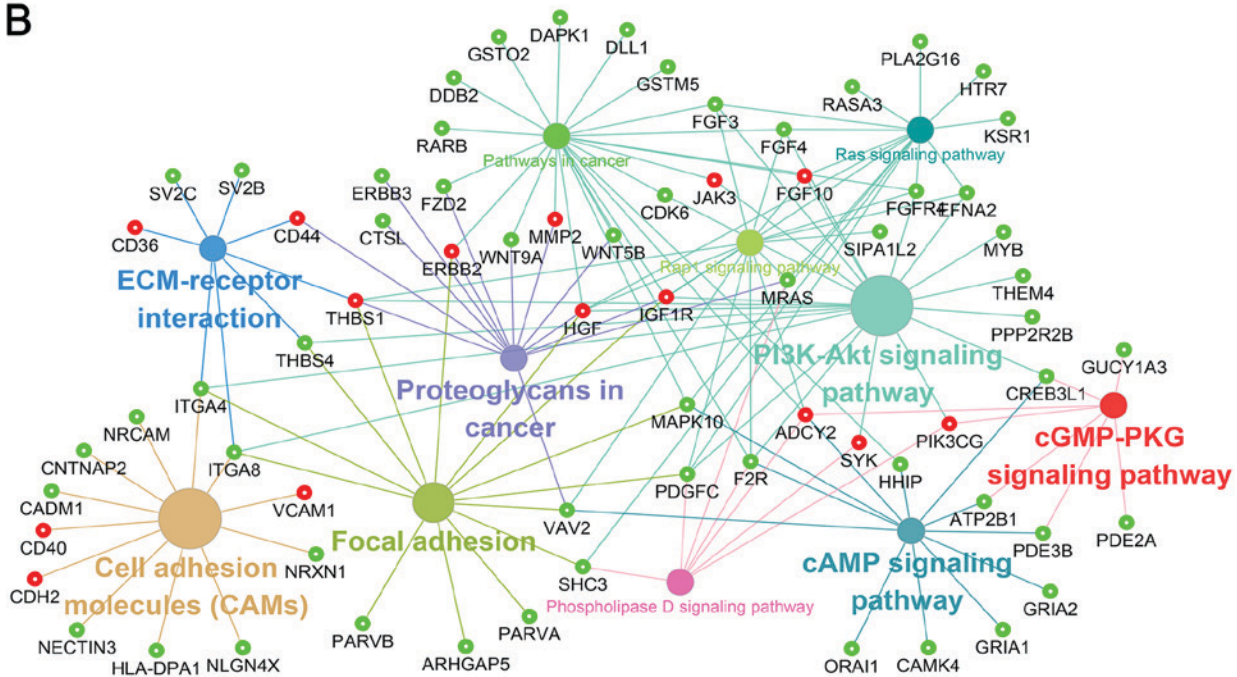


Figure 7. Nodes linking the enriched (A) Gene Ontology function and (B) Kyoto Encyclopedia of Genes and Genomes pathway by hub genes. Solid circles and hollow circles represent each annotation and the DEGs, respectively. Red hollow circles represent hub genes in the protein-protein interaction network.

Acknowledgements

The authors are grateful to medical staff of Department of General Surgery, Lanzhou General Hospital of Chinese People's Liberation Army for their support.

Funding

This study was supported by the Huimin Plan of Ministry of Science and Technology & Ministry of Finance, P.R. China (grant no. 2012GS620101), the Major Projects of Science and Technology in Gansu Province of P.R. China (grant no. 2011GS04390), the Natural Science Foundation of in Gansu Province of P.R. China (grant no. 1506RJZA309), and the Postdoctoral Research Foundation of P.R. China (grant no. 2015M572710).

Availability of data and materials

The datasets analyzed in the present study are available from the GEO repository, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17743> and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20708>.

Authors' contributions

The original study design was undertaken by HBL, WJW and ZYJ. Data collection was undertaken JPY and YML. Microarray data were analyzed by WJW and HTL, and appraised by XPH, PC and WWY. Functional and pathway enrichment analysis was undertaken by XPH, PC, WWY and WKC. The draft manuscript was written by WJW, and was reviewed and edited by HTL, JPY, WKC, ZYJ and HBL. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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