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 (PDGFRA) wild-type GISTs. A joint bioinformatics analysis was performed to identify the differentially expressed genes (DEGs) in wild-type GIST samples compared with KIT/PDGFRA 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 metallopeptidase 2. However, further experiments are required to confirm these results.

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 (PDGFRA) 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, ~1/3 have mutations in the PDGFRA gene, in exons 12, 14 and 18 (8). While the majority of GISTs are characterized by KIT/PDGFRA gene mutations, 10-15% of GISTs lack such mutations and are defined as KIT/PDGFRA 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). PDGFRA-also known as PDGFRα-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 PDGFRA gene may induce activation of constitutive ligand-independent kinases and are mutually exclusive with KIT gene mutations, i.e., KIT and PDGFRA mutations do not coexist in patients with GISTs (8). Imatinib, a small molecule selective tyrosine kinase inhibitor, has been used to treat KIT/PDGFRA mutated GISTs (1); however, the efficacy of imatinib depends on the mutated domains of KIT/PDGFRA. It has been reported that ~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/PDGFRA-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 PDGFRA mutations detected, and three with no mutations detected. The GSE20708 dataset included 22 GIST tumor samples, including 13 with KIT mutations detected, five with PDGFRA 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/PDGFRA 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. affxi?product=mg-u133-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 llog_{2} FC 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.
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/PDGFRA 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/PDGFRα 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. However, other genetic alterations may also be associated with the development and progression of GISTs. Currently, little is known about differences in gene expression levels between wild-type GISTs and KIT/PDGFRα 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. 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/PDGFRA 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/PDGFRA mutant GIST samples, respectively. In (C), the red plot represents statistically significant DEGs (FC≥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; PDGFRA, 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.
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-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 the present study, IGF1R was upregulated in wild-type GIST samples and was associated with fibroblast growth factor receptor (FGFR), 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/PDGFRA 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, FG3, FG10, IGF1R, FG4, PDGFC                              |
| hsa04151   | PI3K-Akt signaling pathway                | 12    | 1.53x10^-6   | CDK6, HGF, FG3, MYB, EFNA2, FG10, IGF1R, FG4, ITGA8, PPP2R2B, PDGFC, THBS4 |
| hsa04514   | Cell adhesion molecules (CAMs)            | 8     | 4.06x10^-6   | PVRL3, NRXN1, CDH2, NRCAM, CNTNAP2, ITGA8, NGN4X, CADM1              |
| hsa04014   | Ras signaling pathway                    | 9     | 1.30x10^-5   | HGF, SHC3, FG3, EFNA2, FG10, IGF1R, HTR7, FG4, PDGFC                |
| hsa1521    | 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, FG3, EFNA2, FG10, IGF1R, FG4, 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, FG3, FG10, IGF1R, FZD2, FG4, 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, OPK1, 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^-3   | PI3CG, SOCS1, HLA-DPA1, MYD88, CD40, LDLR, MAPK10                    |
| hsa04925   | Aldosterone synthesis and secretion      | 6     | 5.68x10^-3   | KCNK3, PDE2A, CREB3L1, LDLR, ORA11, CACNA1H                         |
| hsa05144   | Malaria                                  | 5     | 5.85x10^-3   | VCAM1, THBS1, GYPC, MYD88, CD40                                      |
| hsa04810   | Regulation of actin cytoskeleton         | 9     | 6.24x10^-3   | ITGA4, PIK3CG, CD14, F2R, FGFR4, MRAS, SSSH3, 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, ORA11, MAPK10, VAV2             |
| hsa05205   | Proteoglycans in cancer                  | 8     | 2.52x10^-4   | MMP2, CTS1, 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, CYP27A                                   |
| hsa00071   | Fatty acid degradation                   | 4     | 4.98x10^-4   | ACA2, CPT1A, ACSL5, ALDH7A                                         |
| hsa01212   | Fatty acid metabolism                    | 4     | 6.77x10^-4   | PTPLAD2, ACA2, 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            |
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.

Table II. Continued.

| Pathway ID | Description       | Count | P-value        | Genes                                |
|------------|-------------------|-------|----------------|--------------------------------------|
| hsa04510   | Focal adhesion    | 7     | 1.22x10^{-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.
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...
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
identified that THBS1 was associated with angiogenesis, protein binding, cell adhesion, regulation of transmembrane transport and inflammatory response. Kashihara 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

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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.
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.

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^-1 | 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 metallopeptide 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.
### A. Hub genes

| Term            | Description                  | Count | P-value       | Hub genes                                                                 |
|-----------------|------------------------------|-------|---------------|---------------------------------------------------------------------------|
| GO:0005886      | Plasma membrane              | 19    | 7.42x10⁻⁸    | PIK3CG, FGFR4, ADCY2, LDLR, ERBB2, MPP5, CD40, ITGA4, CDH2, MMP2, VCAM1, IGF1R, MYD88, CD36, CD44, LRRK2, NT5E, SH3GL2, SYK |
| GO:0016020      | Membrane                     | 11    | 2.53x10⁻⁴    | PIK3CG, VCAM1, IGF1R, ADCY2, CD36, LDLR, ERBB2, ITGA4, HGF, CDH2, NT5E    |
| GO:0070062      | Extracellular exosome        | 12    | 4.07x10⁻⁴    | VCAM1, ALDH7A1, CD44, MPP5, MGP, ITGA4, CD40, CDH2, THBS1, LRRK2, NT5E, SH3GL2 |
| GO:0005737      | Cytoplasm                    | 16    | 5.71x10⁻⁴    | PIK3CG, FGFR4, ADCY2, ERBB2, SOCS1, MPP5, CD40, CDH2, CALB2, ALDH7A1, MYD88, CD44, LRRK2, NT5E, SH3GL2, SYK |
| GO:0005515      | Protein binding              | 21    | 1.47x10⁻⁷    | PIK3CG, FGFR4, LDLR, ERBB2, SOCS1, MPP5, MGP, HGF, CD44, ITGA4, CDH2, MMP2, IGF1R, ALDH7A1, MYD88, CD36, CD44, THBS1, LRRK2, SH3GL2, SYK |

### B. Module 1

| Term            | Description                          | Count | P-value       | Genes                                                                 |
|-----------------|---------------------------------------|-------|---------------|------------------------------------------------------------------------|
| hsa04151        | PI3K-Akt signaling pathway            | 4     | 6.16x10⁻⁵    | IGF1R, FGFR4, JAK3, SYK                                               |
| hsa04550        | Signaling pathways regulating pluripotency of stem cells | 3     | 1.03x10⁻⁴    | IGF1R, FGFR4, JAK3                                                   |
| hsa05005        | Viral carcinogenesis                  | 2     | 7.47x10⁻³    | FGFR4, SYK                                                            |
| hsa04015        | Rap1 signaling pathway                | 2     | 7.89x10⁻³    | IGF1R, JAK3                                                           |
| hsa04014        | Ras signaling pathway                 | 2     | 9.15x10⁻³    | IGF1R, JAK3                                                           |

### C. Module 2

| Term            | Description                          | Count | P-value       | Genes                                                                 |
|-----------------|---------------------------------------|-------|---------------|------------------------------------------------------------------------|
| hsa04668        | TNF signaling pathway                 | 2     | 5.09x10⁻⁴    | PIK3CG, MAP3K5                                                         |
| hsa04071        | Sphingolipid signaling pathway        | 2     | 6.14x10⁻⁴    | PIK3CG, MAP3K5                                                         |
| hsa04210        | Apoptosis                             | 2     | 8.16x10⁻⁴    | PIK3CG, MAP3K5                                                         |
| hsa04072        | Phospholipase D signaling pathway     | 2     | 8.62x10⁻⁴    | MRAS, PIK3CG                                                          |
| hsa05205        | Proteoglycans in cancer               | 2     | 1.72x10⁻³    | MRAS, PIK3CG                                                          |

### D. Module 3

| Term            | Description                          | Count | P-value       | Genes                                                                 |
|-----------------|---------------------------------------|-------|---------------|------------------------------------------------------------------------|
| hsa05200        | Pathways in cancer                    | 7     | 5.55x10⁻⁸    | ADCY2, ERBB2, FG10, FZD2, MMP2, FGF3, FGF4                               |
| hsa05205        | Proteoglycans in cancer               | 5     | 1.11x10⁻⁶    | CD44, ERBB2, FZD2, THBS1, MMP2                                       |
| hsa04015        | Rap1 signaling pathway                | 5     | 1.28x10⁻⁶    | ADCY2, FG10, THBS1, FGF3, FGF4                                       |
| hsa04512        | ECM-receptor interaction              | 3     | 5.86x10⁻⁵    | CD36, CD44, THBS1                                                      |
| hsa05166        | HTLV-I infection                     | 4     | 8.31x10⁻⁵    | VCAM1, ADCY2, FZD2, CD40                                              |
Table IV. Continued.

E, Module 4

| Term                        | Description                                      | Count | P-value       | Genes                                    |
|-----------------------------|--------------------------------------------------|-------|---------------|------------------------------------------|
| hsa05205                    | Proteoglycans in cancer                          | 4     | 3.03x10^-5    | WNT5B, ERBB3, WNT9A, HGF                 |
| hsa04810                    | Regulation of actin cytoskeleton                 | 4     | 3.64x10^-5    | ITGAE, ITGA4, CD14, F2R                  |
| hsa04151                    | PI3K-Akt signaling pathway                       | 4     | 2.12x10^-4    | MYB, ITGA4, CD14, WNT5B                  |
| hsa05200                    | Pathways in cancer                               | 4     | 3.71x10^-4    | F2R, ERBB3, WNT9A, WNT5B                 |
| hsa01100                    | Metabolic pathways                              | 6     | 5.98x10^-4    | CYP3A7, ACSL5, NT5E, PTGIS, ACSS3, ACA2  |

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

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.
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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=GSE20708 and 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.

References

1. Valsangkar N, Sehdev A, Misra S, Zimmers TA, O’Neil BH and Koniaris LG: Current management of gastrointestinal stromal tumors: Surgery, current biomarkers, mutations, and therapy. Surgery 158: 1149-1164, 2013.
2. Ducimetière F, Lukin A, Ranchère-Vinc D, Decouvelaere AV, Péoc'h M, Istier L, Chalabreysse P, Muller C, Alberti L, Bringuer PP, et al: Incidence of sarcoma histotypes and molecular subtypes in a prospective epidemiological study with central pathology review and molecular testing. PLoS One 6: e20294, 2011.
3. Patil DT and Rubin BP: Gastrointestinal stromal tumor: Advances in diagnosis and management. Arch Pathol Lab Med 135: 1298-1310, 2011.
4. Joensuu H, Hohenberger P and Corless CL: Gastrointestinal stromal tumour. Lancet 382: 973-983, 2013.
5. Joensuu H, Vehtari A, Rihimäki J, Nishida T, Steigen SE, Brabec P, Plank L, Nilsson B, Cirilli C, Bracconi C, et al: Risk of recurrence of gastrointestinal stromal tumour after surgery: An analysis of pooled population-based cohorts. Lancet Oncol 13: 265-274, 2012.
6. Pedroso FE, Raut CP, Xiao H, Yeo CJ and Koniaris LG: Has the survival rate for surgically resected gastric gastrointestinal stromal tumors improved in the tyrosine kinase inhibitor era? Ann Surg Oncol 19: 1748-1758, 2012.
7. Artinayan A, Kim J, Soriano P, Chow W, Bhatia S and Ellenhorn JD: Metastatic gastrointestinal stromal tumours in the era of imatinib: Improved survival and elimination of socioeconomic survival disparities. Cancer Epidemiol Biomarkers Prev 17: 2194-2200, 2008.
8. Corless CL, Barnett CM and Heinrich MC: Gastrointestinal stromal tumours: Origin and molecular oncology. Nat Rev Cancer 11: 865-878, 2011.
9. Vernuccio F, Taibbi A, Picone D, LA Grutta L, Midiri M, Lagalla R, Lo Re G and Bartolotta TV: Imaging of gastrointestinal stromal tumours: From diagnosis to evaluation of therapeutic response. Anticancer Res 36: 2639-2648, 2016.
10. Heinrich MC, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, et al: PDGFRα activating mutations in gastrointestinal stromal tumours. Science 299: 708-710, 2003.
11. Ricci R: Syndromic gastrointestinal stromal tumours. Hered Cancer Clin Pract 14: 15, 2016.
12. Joensuu H and DeMatteo RP: The management of gastrointestinal stromal tumours and multi-visceral: A model for targeted and multidisciplinary therapy of malignancy. Annu Rev Med 63: 247-258, 2012.
13. Gramaz AW, Corless CL and Heinrich MC: Resistance to tyrosine kinase inhibitors in gastrointestinal stromal tumours. Clin Cancer Res 15: 7510-7518, 2009.
14. Ostrowski J, Polkowski M, Prazewska A, Skrzyczczak M, Goryca K, Rubel T, Kokoszyńska K, Rutkowski P, Nowecki ZI, Vel Dobosz AJ, et al: Functional features of gene expression profiles differentiating gastrointestinal stromal tumours according to KIT mutations and expression. BMC Cancer 9: 439, 2009.
15. Astolfi A, Nannini M, Pantaleo MA, Di Battista M, Heinrich MC, Santini D, Catena F, Corless CL, Maedu R, Saponara M, et al: A molecular portrait of gastrointestinal stromal tumours: An integrative analysis of gene expression profiling and high-resolution genomic copy number. Lab Invest 90: 1285-1294, 2010.
16. Erizarrary RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonelis KJ, Scherf U and Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4: 249-264, 2003.
17. R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
18. Leek JT, Johnson WE, Parker HS, Jaffe AE and Storey JD: The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 28: 457-465, 2012.
19. Speed TP: Exploratory, normalization, and summaries of high density oligonucleotide array probe level data. Bioinformatics 26: 249-264, 2003.
20. Smyth GK, Michaud J and Scott HS: Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 21: 2067-2075, 2005.
21. Metsalu T and Vilo J: ClustVis: A web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. Nucleic Acids Res 43: W566-W570, 2015.
22. Jiang Z, Conzen SD, Yu W, Chen Z, Brown PO, et al: Using expression profiles to identify mechanism of hormonal response in breast cancer. Bioinformatics 23: 1762-1770, 2007.
23. Antczak N and Giraldo AS: A Functional Assay Pipeline for the Identification of Cancer-Related Molecular Interactions. PloS One 7: e30204, 2012.
24. Smyth GK, Michaud J and Scott HS: Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics 21: 2067-2075, 2005.
25. Chang J and Smyth GK: Using within-array replicates to identify differentially expressed genes. Bioinformatics 23: 2512-2519, 2007.
26. Metsalu T and Vilo J: ClustVis: A web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. Nucleic Acids Res 43: W566-W570, 2015.
27. Lagerlof G, Sandgren M, Månsson A, Skålar A, Gyllensten U, et al: Imatinib resistance and cancer stem cell characteristics are associated with overall survival and disease-free survival in patients with metastatic gastrointestinal stromal tumors. Br J Cancer 108: 1755-1763, 2013.
ZNF overexpression are predictors of poor response to imatinib
Sohn TS, Kim S and Kim KM: Lee EJ, Kang G, Kang SW, Jang KT, Lee J, Park JO, Park CK, Sohn TS, Kim S and Kim KM: GSTT1 copy number gain and ZNF overexpression are predictors of poor response to imatinib in gastrointestinal stromal tumors. PLoS One 8: e77219, 2013.

Tarn C, Rink L, Merkel E, Flieder D, Pathak H, Koumbi D, Testa JR, Eisenberg B, von Mehren M and Godwin AK: Insulin-like growth factor 1 receptor is a potential therapeutic target for gastrointestinal stromal tumors. Proc Natl Acad Sci USA 105: 8387-8392, 2008.

Belinsky MG, Rink L, Flieder DB, Jahromi MS, Schiffman JD, Godwin AK and Mehren Mv: Overexpression of insulin-like growth factor 1 receptor and frequent mutational inactivation of SDHA in wild-type SDHB-negative gastrointestinal stromal tumors. Genes Chromosomes Cancer 52: 214-224, 2013.

Pantaleo MA, Ravegini G, Astolfi A, Simeon V, Nannini M, Saponara M, Urbini M, Gatto L, Indio V, Sammarini G, et al: Integrating miRNA and gene expression profiling analysis revealed regulatory networks in gastrointestinal stromal tumors. Epigenomics 8: 1347-1366, 2016.

Patel MB, Pothula SP, Xu Z, Lee AK, Goldstein D, Pirola RC, Apte MV and Wilson JS: The role of the hepatocyte growth factor/MET pathway in pancreatic stellate cell-endothelial cell interactions: Antiangiogenic implications in pancreatic cancer. Carcinogenesis 35: 1891-1900, 2014.

Hack SP, Bruye JM and Koeppen H: HGF/MET-directed therapeutics in gastrointestinal cancer: A review of clinical and biomarker development. Oncotarget 5: 2866-2880, 2014.

Soto-Pantoja DR, Sipes JM, Martin-Manso G, Westwood B, Morris NL, Ghosh A, Emenaker NJ and Roberts DD: Dietary fat overcomes the protective activity of thrombospondin-1 signaling in the Apc(Min/+) model of colon cancer. Oncogenesis 5: e230, 2016.

Kashiwara H, Shimada M, Yoshikawa K, Higashijima J, Tokunaga T, Nishi M, Takasu C and Ishikawa D: Correlation between thrombospondin-1 expression in non-cancer tissue and metastasis of gastric cancer is associated with the interaction of poly(ADP-ribose) polymerase 1 and matrix metalloproteinase 2. DNA Cell Biol 30: 1011-1017, 2011.

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