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Original Article

Significant target genes and signaling for miR-34a in gastrointestinal stromal tumors: a study of GEO-data mining and bioinformatics approaches

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Abstract: Objective: The study intent is to conduct an enquiry into the probable mechanism of miR-34a in the onset of gastrointestinal stromal tumors (GISTs) and to clarify the clinical role of miR-34a in GIST. Methods: Datasets relevant to miR-34a were collected from the Gene Expression Omnibus (GEO). The correlation between miR-34a levels and clinical features was identified via independent samples t-test and analysis of variance (ANOVA). In addition to a miR-34a microarray dataset GSE68743 on GIST cells transfected with a miR-34a mimic, which was downloaded from GEO, gene prediction by 10 online programs and literature screening was also performed to gather potential miR-34a targets. Then, functional enrichment analysis was conducted, and a protein-protein interaction network was constructed using STRING10.0. Results: Significantly higher levels of miR-34a were found in the large intestine group (7.50±1.59) than in the small intestine group (5.58±0.59, ANOVA: P=0.042; t-test: P=0.04) in GSE36087. We obtained 62 potential targets of miR-34a by assembling genes from 10 databases, microarray datasets and literature screening. Functional annotation of the 62 genes revealed overrepresented gene ontology (GO) terms and the most significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways: pathways in cancer and endometrial cancer. In the protein-protein interaction (PPI) network, *TP53* and *MYC* may be the prospective hub genes of miR-34a. Conclusion: In general, our study suggests that miR-34a may regulate the tumorigenesis of GIST via prospective genes (*TP53, MYC,* and *PDGFRA*) and several key pathways. However, the clinical role of miR-34a as a biomarker for GIST still needs further investigation with a larger sample size.

Keywords: miR-34a, gastrointestinal stromal tumors, GEO, gene ontology, KEGG pathway

Introduction

Gastrointestinal stromal tumors (GISTs) are recognized as the most common mesenchymal tumors of the gastrointestinal tract, with an estimated incidence of 0.32 per 100,000 in the United States [1]. The invasion mostly occurs in the stomach, which constitutes approximately 45.0% of all invasion, followed by the small intestine, colon/rectum and esophagus [2, 3]. For primary GISTs measuring >2 cm, the principle treatment strategy is surgical resection; however, recurrence after surgery is common [4, 5]. At present, administration of imatinib mesylate for three or more years is recommended due to the risk of recurrence [6, 7]. In a recent study, imatinib 600 mg/d dose escalation therapy was also demonstrated to provide further survival benefit for Chinese patients who suffer treatment failure with imatinib 400 mg/d [8]. Accordingly, imatinib mesylate has become the first choice for patients with unresectable, recurrent or metastatic GISTs [9, 10].

microRNA (miRNAs) molecules are small noncoding RNAs that post-transcriptionally modulate gene expression and control cell growth via regulating multiple gene products and cellular pathways [11-13]. The dysregulation of miRNAs has become recognized as a ubiquitous feature of malignancies [14-17]. Among miRNAs, miR-34a, whose down-regulation has been confirmed in numerous types of tumors [18-20], is

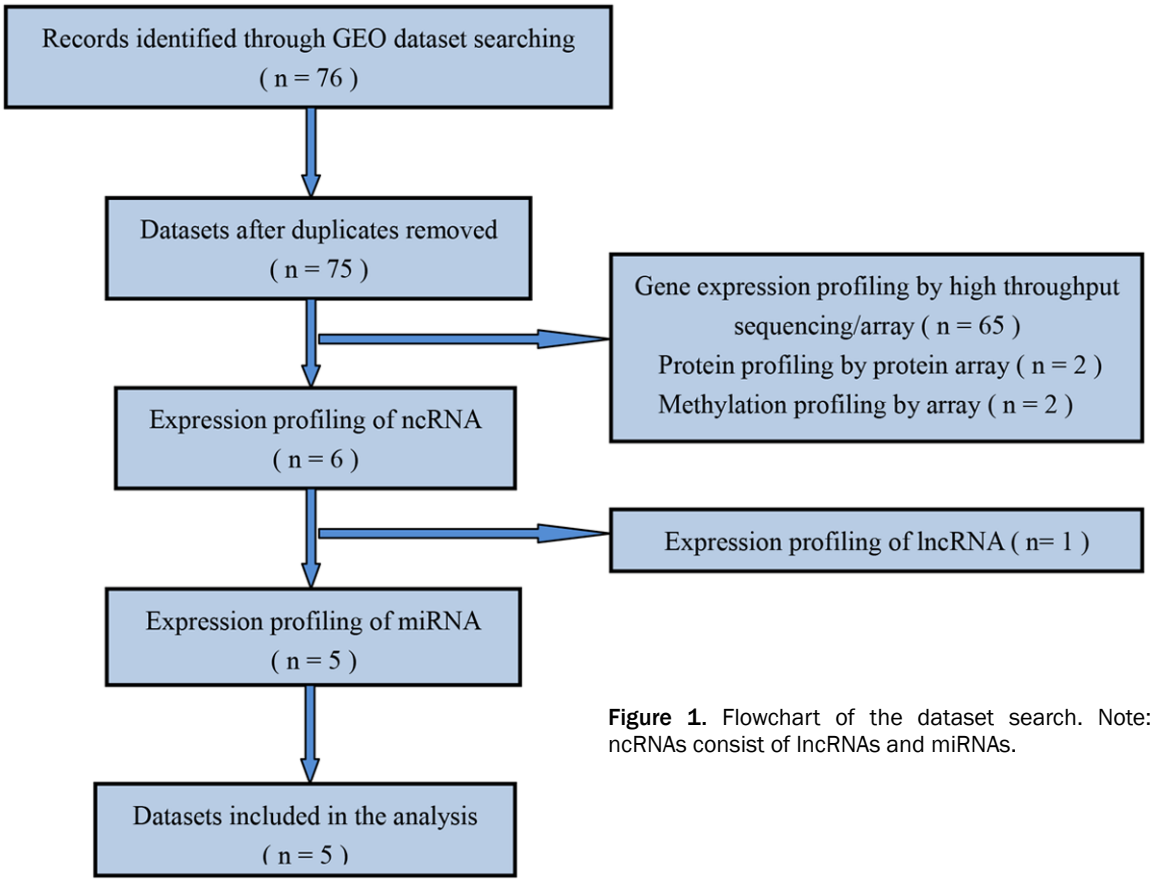

Figure 1. Flowchart of the dataset search. Note: ncRNAs consist of lncRNAs and miRNAs.

a tumor suppressor inhibiting the proliferation and invasion of cells via its targets [21, 22]. However, few studies have revealed the role of miR-34a in the onset of GIST. Recently, *Isosaka et al* observed the suppressive effect of miR-34a mimetic molecules on the proliferation and migration of GIST cells. In addition, platelet-derived growth factor receptor alpha (*PDGFRA*) was found to be a miR-34a target responsible for the tumor-suppressive effect of miR-34a [14]. Based on these findings, miR-34a might function as a tumor suppressive miRNA affecting the tumorigenesis of GIST.

There are few available studies investigating the clinical role and target genes of miR-34a in GIST patients. More potential target genes of miR-34a in GIST must be further explored. Therefore, to further investigate whether miR-34a could be a clinical biomarker in GIST, in this study, we analyzed the correlation between miR-34a expression and clinical parameters by collecting data from GEO (http: //www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (http://www.ebi.ac.uk/arrayexpress/). Additionally, to reveal the underlying target genes of miR-34a involved in GIST development, target gene prediction was used and the altered genes found in a microarray after transfection of a miR-34a mimic into GIST-T1 cells was studied. Then, functional enrichment analyses were conducted to identify the prospective miR-34a targets and pathways related to GIST development. This study may provide a comprehensive understanding of the involvement of miR-34a in the early events and progression of GIST.

Materials and methods

GIST microRNA microarray collection

First, GEO and ArrayExpress were searched for datasets providing miR-34a expression in GIST patients. The search terms were “gastrointestinal stromal tumors” or “gastrointestinal stromal neoplasm” or “GIST” with the selection of “human [organism]” and the entry type of series. In total, 76 studies were initially identified. Studies were excluded using the following criteria: the dataset type was gene/protein/lncRNA/

Table 1. Relationship between the expression of miR-34a and clinical parameters in GIST

Accession number	Clinical parameter	n	miR-34a relative expression ($2^{-\Delta Cq}$) Mean ± SD	P*	P^
GSE31741	Tumor risk grade			0.149	-
	Low	10	9.472±1.9144		
	Intermediate	8	8.0438±1.1084		
	High	14	8.4507±1.4398		
	Tumor location			0.170	-
	Stomach	25	8.9496±1.6544		
	Omentum	1	-		
	Small intestine	6	1.2647±1.2647		
	Age			-	0.599
	<65	13	8.4581±1.6532		
	≥65	18	8.7841±1.7097		
	Gender			-	0.764
	Male	15	8.5522±1.7284		
	Female	16	8.7367±1.6573		
GSE36087	Tumor risk grade			0.816	-
	Low	4	5.8231±1.2769		
	Intermediate	4	6.2916±1.0780		
	High	11	6.0809±0.9436		
	Tumor location			0.042	0.04
	Stomach	10	6.1262±2.0881		
	Small intestine	7	5.5820±0.5882		
	Large intestine	2	7.5063±1.5879		
GSE63159	Treatment protocol			-	0.338
	Imatinib-treated	17	0.0194±0.0964		
	Non-treated	17	0.0591±0.1377		
GSE45901	Drug sensitivity			-	0.251
	Imatinib-resistant	7	0.0465±0.0441		
	Imatinib-sensitive	10	0.0781±0.0593		
GSE63453	Tumor location			-	-
	Stomach	8	11.796±1.7152		
	Rectum	1	-		

Note: P*, *p* value of ANOVA analysis; P^: *p* value of independent sample t-test. Abbreviations: GIST: gastrointestinal stromal tumors; ANOVA: analysis of variance.

methylation expression profiling. Finally, we included five studies (GSE31741, GSE36087, GSE45901, GSE63159, and GSE63453) from the GEO. GSE36087 and GSE63453 were based on the 3D-Gene Human miRNA platform. The GSE45901 and GSE63159 datasets used the same Agilent-029297 Human miRNA Microarray platform. For the GSE31741, the Agilent-021827 Human miRNA Microarray platform was used. The search process is shown in **Figure 1**, and the expression level of miR-34a could be extracted from the above five microarray datasets.

RNA isolation and data processing

Total RNA was extracted from GIST samples using the mirVana miRNA Isolation Kit (Applied Biosystems/Ambion, Austin, TX) according to the manufacturer's protocol. For both the GSE36087 and GSE63453 datasets, the RNA was extracted using the miRNeasy mini kit (QIAGEN, Valencia, CA). The downloaded data from these studies were normalized and imputed with missing values below 20% across the samples. For convenience, all of the normalized data were transformed into log base 2.

Relationship between miR-34a and clinical parameters

Among the datasets (GSE31741, GSE36087, GSE45901, GSE63159, and GSE63453), the number of GIST samples and the corresponding clinical parameters are summarized in **Table 1**. Of note, in the extraction of data from GSE36087, the clinical characteristics of the GIST sample involved the size of the tumor and mitoses of the tumor cells. For further investigation, the two characteristics were transformed into the parameter of risk grades (low/intermediate/high risk) referring to the risk definition rules proposed by Fletcher et al [23]. To conduct the subsequent statistical analysis, the expression values of miR-34a were then mapped to the corresponding patients. According to the clinical features, GIST patients were divided into different populations or groups, such as an imatinib-treated group and a non-treated group, and the expression values of miR-34a were sequentially mapped. Then, the means and standard deviations of the groups were calculated. Groups were analyzed by independent samples t-tests and analysis of variance (one-way ANOVA) via

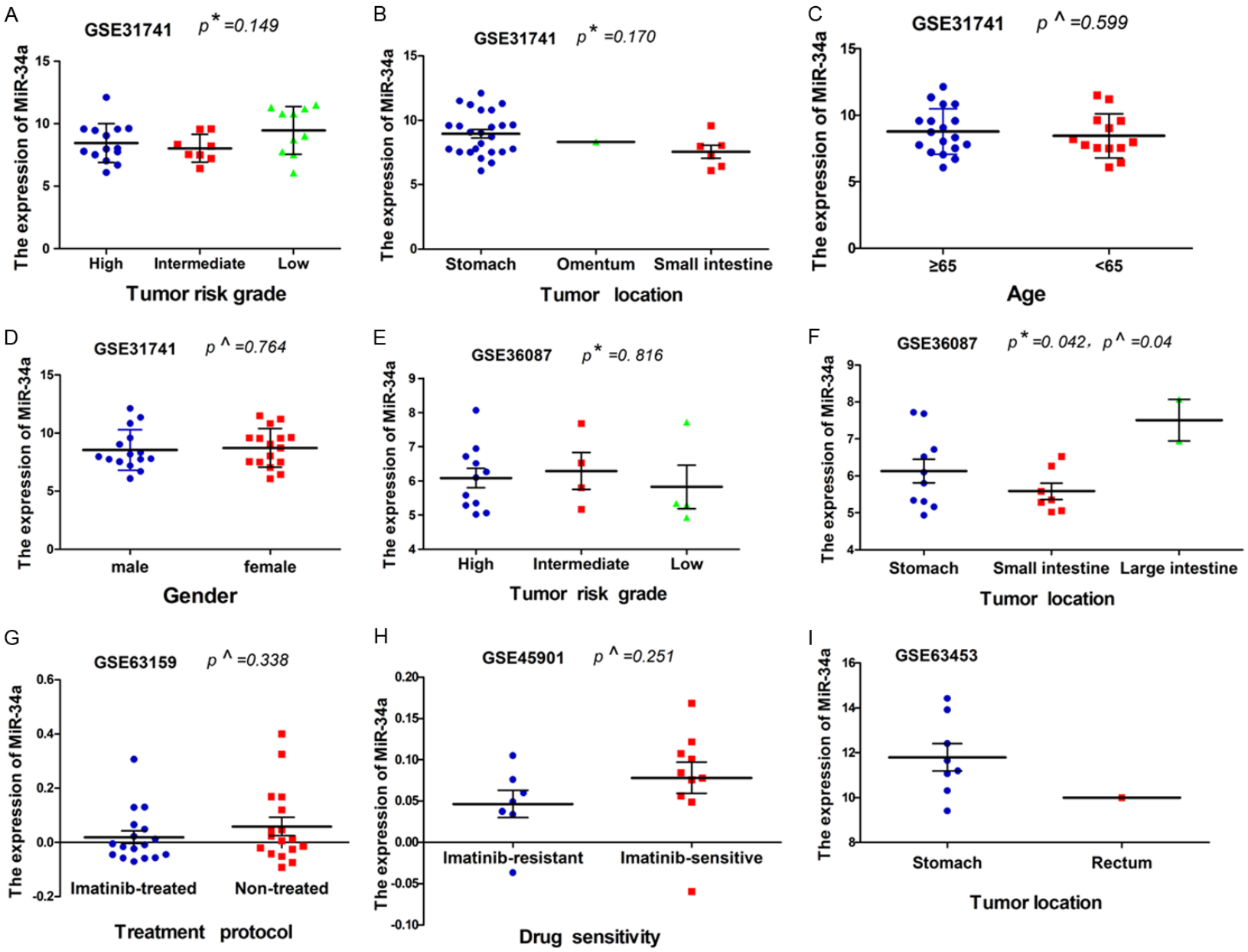
A
GSE31741
p* =0.149
The expression of MiR-34a
High
Intermediate
Low
Tumor risk grade
B
GSE31741
p* =0.170
The expression of MiR-34a
Stomach
Omentum
Small intestine
Tumor location
C
GSE31741
p ^ =0.599
The expression of MiR-34a
≥65
<65
Age
D
GSE31741
p ^ =0.764
The expression of MiR-34a
male
female
Gender
E
GSE36087
p* =0. 816
The expression of MiR-34a
High
Intermediate
Low
Tumor risk grade
F
GSE36087
p* =0. 042, p ^ =0.04
The expression of MiR-34a
Stomach
Small intestine
Large intestine
Tumor location
G
GSE63159
p ^ =0.338
The expression of MiR-34a
Imatinib-treated
Non-treated
Treatment protocol
H
GSE45901
p ^ =0.251
The expression of MiR-34a
Imatinib-resistant
Imatinib-sensitive
Drug sensitivity
I
GSE63453
The expression of MiR-34a
Stomach
Rectum
Tumor location

Figure 2. Scatter plots of miR-34a expression in GIST samples. Note: The x-axis represents clinical parameters, and the y-axis represents the expression of miR-34a. Each dot corresponds to a sample with its expression value; P*, *p* value of ANOVA; P^: *p* value of independent sample t-test. Abbreviations: GIST: gastrointestinal stromal tumors; ANOVA: analysis of variance.

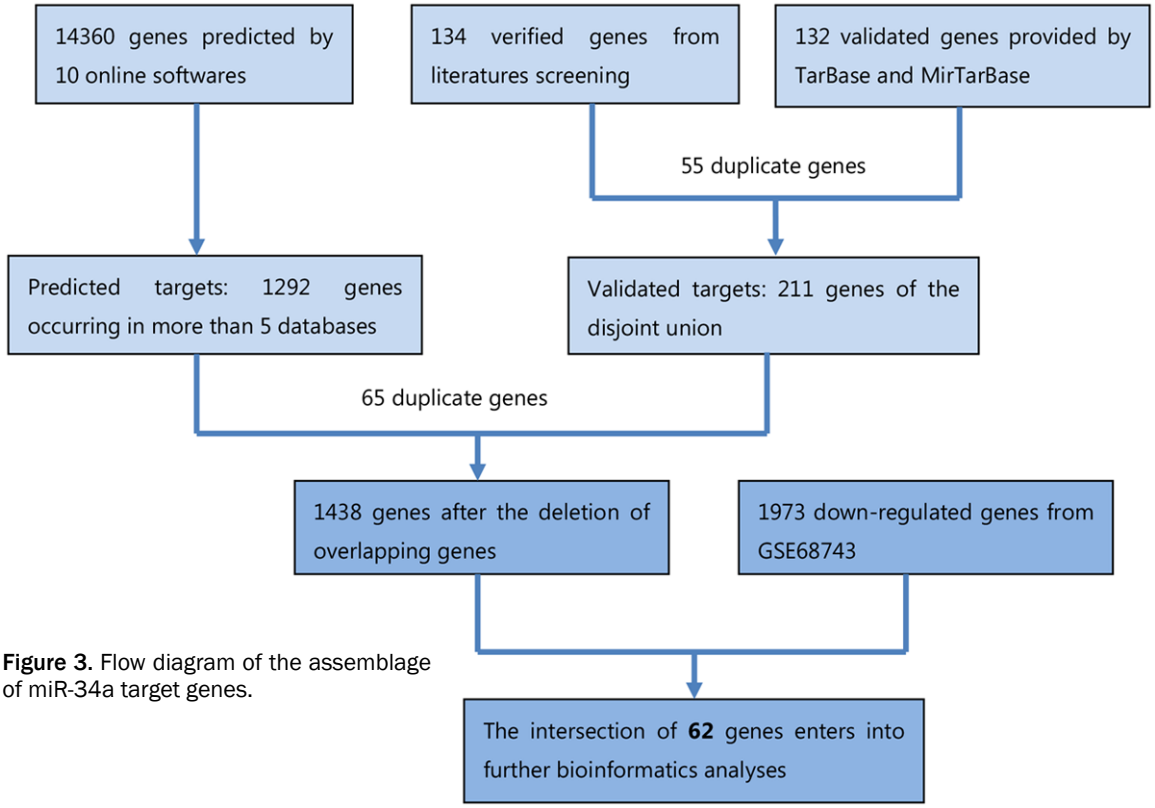

Figure 3. Flow diagram of the assemblage of miR-34a target genes.

SPSS software version 22.0. Notably, the t-test was used for comparing significant differences in the means between two populations, and the ANOVA was used for three or more populations. Among these tests, $P < 0.05$ was considered significant. To further investigate the underlying correlation between miR-34a expression and the clinical parameters of GIST, the software GraphPad Prism (version 6.0) was applied to generate scatter plots for intuitive display.

Microarray dataset for GIST-related target genes of miR-34a

To compile the miR-34a-related genes associated with GIST, in the present study, microarray dataset GSE68743 was downloaded from GEO, which provided gene expression signatures using miR-34a-overexpressing GIST-T1 cells that had been transfected with miR-34a mimetic molecules. A total of four samples in GSE68743 were deposited by Isosaka M et al [14]. These samples were divided into two pairs, including a pair of GIST-T1 cell samples containing the miR-34a mimic molecule and a pair of GIST-T1 cells containing a negative control molecule. The raw data were based on the GPL16699 Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 platform (Feature Number version).

Data preprocessing and analysis of differentially expressed genes (DEGs)

With the microarray dataset downloaded, the original data and the annotation files from GSE68743 were then downloaded and normalized. Next, the R Affy package (https://www.r-project.org/) was employed to correct the background and normalize and calculate the expression values [24]. Samples of GIST-T1 cells with the miR-34a mimic molecule and negative control molecule were assigned as cases and controls, respectively. Then, the DEGs associated with miR-34a between the cases and controls were estimated using Limma package in R [25],

and we set |logFC|>1 and a false discovery rate (FDR) <0.01 as the cutoffs for DEG screening.

Collection of predicted and validated miR-34a targets

A bioinformatics analysis to identify putative miR-34a target genes was performed for this study. Targets of miR-34a were predicted by prediction tools among the frequently used miRNA databases, namely miRDB, MirTarBase, miRanda, PITA, PicTar, PolymiRTS, RNA22, TargetScan, TargetMiner, and TarBase. To obtain more reliable prediction results, genes of miR-34a predicted by at least five of the above-mentioned 10 miRNA databases were eventually considered as potential predicted target genes. Next, we searched and collected miR-34a targets verified by more than one of the following methods: qPCR, western blot and luciferase reporter assay. This determination was made by screening the relevant literature and several target databases, including TarBase and MirTarBase. The search formulas used for collecting targets from the literature screening in PubMed were as follows: (miR-34a or miRNA-34a or microRNA-34a or miR34a or miRNA34a or microRNA34a or 'miR 34a' or 'miRNA 34a' or 'microRNA34a' or miR-34a-5p or miRNA-34a-5p or microRNA-34a-5p) and (cancer or carcinoma or neoplasm or tumor) and (target*). The overlap was finally determined by assembling genes from 10 databases, microarray datasets and literature screening (**Figure 3**).

Functional enrichment analysis

The DAVID Bioinformatics Resources, which are freely available to the public, not only provide gene-term enrichment analysis but also allow users to interpret and extract biological mechanisms related to large gene lists by developing a set of advanced analytic tools, such as Functional Annotation Clustering, Gene Functional Classification, Gene ID Conversion, Gene Name Batch Viewer, etc [26]. In this paper, gene ontology (GO) analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to analyze and predict the function of the identified miR-34a target genes. The overrepresented KEGG and GO categories were identified with P<0.05. With these 62 genes analyzed by the BiNGO plugin, the overrepresented GO terms of biological process (BP), cellular component (CC), molecular function (MF) were ultimately visualized as images in Cytoscape 3.0.

Construction of a protein-protein interaction (PPI) network

To further understand the potential mechanism of miR-34a in GIST development, a PPI network was constructed for the promising genes that miR-34a might target using STRING10.0. Notably, the gene list used for network construction came from the genes enriched in the top KEGG pathways.

Results

Correlation of miR-34a expression with clinical parameters

In this study, clinical features were extracted from the five datasets according to **Table 1**. Patients in GSE31741 were divided into three groups based on tumor risk grades (low/intermediate/high), three groups based on tumor location (stomach/omentum/small intestine), two groups based on age (≥65, >65) and two groups based on gender (male/female). In GSE36087, there were three groups based on tumor risk grades (low/intermediate/high grades) and three groups based on tumor location (stomach/small intestine/large intestine). GSE63159 was divided into imatinib-treated/non-treated groups according to the treatment protocol, and GSE45901 had imatinib-resistant/imatinib-sensitive groups in terms of drug sensitivity. In GSE63453, patients with different tumor locations were divided into stomach and rectum groups. The results of the independent t-test and ANOVA showed no significant difference between these groups. However, among the groups for tumor location in GSE36087, the level of miR-34a was obviously higher in the large intestine group (7.50±1.59) than in the small intestine group (5.58±0.59). The result of the ANOVA showed statistical significance (P_{ANOVA}=0.042). Therefore, t-tests were then performed, which showed a significant difference between the small intestine and large intestine groups ($P_{t\text{-}test}$=0.04). To exhibit these results and miR-34a levels in a direct, simple and visual manner, scatter plots were generated and displayed with the *P* values of the t-test and the ANOVA (**Figure 2**).

Table 2. The overlapping genes isolated from the predicted genes of miR-34a

Category	Genes	n
Set 1	Bcl2, ACSL1, ACSL4, AR, AREG, Axin2, AXL, CCND1, CCND3, CD44, CDK4, CDK6, c-MET, cyclin D1, cyclin E2, DLL1, E2F1, E2F3, FOSL1, FOXP1, FUT8, GALNT7, GAS1, HDAC1, HNF4A, IL6R, L1CAM, LDHA, LEF1, MAGEA12, MAGEA2, MAGEA3, MAGEA6, MCM2, MCM5, MDM4, MMP9, MTA2, MYB, myc, MYCN, Notch1, Notch2, PDGFRA, PDGFRB, PIK3R2, PLK1, SIRT1, Smad4, SOX2, Src, P53, ULBP2, Wnt1, YY1	55
Set 2	ABLIM1, ACSL1, ACSL4, ARHGAP1, Axin2, AXL, Bcl2, CCL22, CCND1, CCNE2, CD44, CDH1, CDK6, CDKN1A, CEBPB, CLOCK, CTCF, DOCK3, E2F1, E2F5, FGFR1, FOXP1, FUT8, GALNT7, HDAC1, HNF4A, INHBB, JAG1, KLF4, LDHA, LEF1, MAP2K1, MAP3K9, Msi1, MTA2, MYB, MYCN, NAMPT, NCOA1, NOTCH1, Notch2, NOTCH3, PDGFRAPDGFRB, PEA15, PIK3R2, PRKCB, PRKCQ, PSMD5, RICTOR, SFRP1, SIRT1, SMAD4, SNAT1, Src, STX1A, SYT1, TCF7, Tgif2, TOM1, TPD52, VAMP2, Vcl, VEGFA, Wnt1	65

Note: Set 1, the intersection of genes found by literature screening, MirTarBase and TarBase; Set 2, genes found among both validated genes and predicted genes.

Table 3. Top ten gene ontology terms for biological process

Category	Term	Count	*P* Value
BP_FAT	Enzyme linked receptor protein signaling pathway	8	1.42E-04
BP_FAT	Regulation of peptidase activity	5	2.03E-04
BP_FAT	Response to hormone stimulus	8	2.20E-04
BP_FAT	Regulation of cell proliferation	11	2.52E-04
BP_FAT	Pattern specification process	7	2.70E-04
BP_FAT	Response to endogenous stimulus	8	3.99E-04
BP_FAT	Response to estrogen stimulus	5	4.35E-04
BP_FAT	Response to steroid hormone stimulus	6	4.67E-04
BP_FAT	Positive regulation of macromolecule metabolic process	11	4.98E-04
BP_FAT	Response to endogenous stimulus	8	1.42E-04

Abbreviation: BP: biological process.

Table 4. Top six gene ontology terms for cellular component

Category	Term	Count	*P* Value
CC_FAT	Anchoring junction	5	0.003598803
CC_FAT	Microtubule cytoskeleton	7	0.014700263
CC_FAT	Adherens junction	4	0.01921287
CC_FAT	Axon	4	0.020539706
CC_FAT	Neuron projection	5	0.03645593
CC_FAT	Cell junction	6	0.04063375

Abbreviation: CC: cellular component.

Screening the down-regulated genes

After screening out the GIST-related DEGs of miR-34a between the case and control samples, we eventually identified 1973 down-regulated genes among those DEGs associated with miR-34a overexpression according to the theory of complementarity between targets and miRNAs.

Collection of prospective miR-34a target genes associated with GIST

In the study, we performed literature screening and database searches to obtain miR-34a target genes, which were validated by qPCR, western blot or luciferase reporter assays. In total, we gathered 134 validated targets through literature screening and 132 verified miR-34a target genes from databases, including TarBase and MirTarBase. After the removal of 55 genes present both in the literature and the databases mentioned above (**Table 2**), the remaining 211 genes were regarded as the "validated set". For the predicted genes, 10 available databases were searched for the prospective target genes of miR-34a, yielding 14, 360 results. Among those genes, we screened out 1292 that appeared in more than 5 databases as the "predicted set". Next, the union of the 211 "val-

Table 5. Top seven gene ontology terms for molecular function

Category	Term	Count	*P* Value
MF_FAT	Hormone binding	3	0.016409894
MF_FAT	Steroid hormone receptor activity	3	0.016409894
MF_FAT	Ligand-dependent nuclear receptor activity	3	0.02254552
MF_FAT	Growth factor activity	4	0.026561171
MF_FAT	Steroid binding	3	0.027082235
MF_FAT	Sequence-specific DNA binding	7	0.033490283
MF_FAT	Kinase binding	4	0.03479456

Abbreviation: MF: molecular function.

Table 6. Top four significant pathways regulated by GIST-related target genes of miR-34a

Category	Term	Count	*P* Value
KEGG_PATHWAY	Pathways in cancer	7	0.006403046
KEGG_PATHWAY	Endometrial cancer	3	0.030587425
KEGG_PATHWAY	Basal cell carcinoma	3	0.033924019
KEGG_PATHWAY	Acute myeloid leukemia	3	0.037397183

Abbreviation: GIST: gastrointestinal stromal tumor.

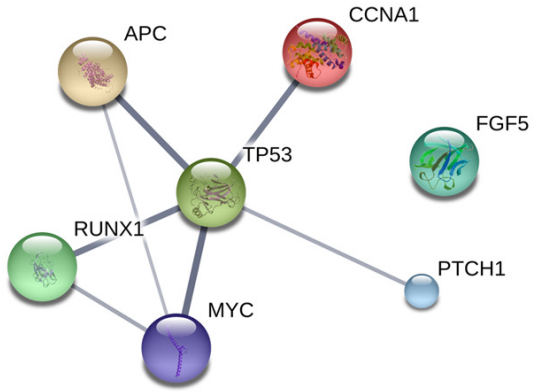

Figure 4. PPI network of the prospective targets of miR-34a. Note: A node indicates a gene, and an edge is representative of interactions between any two genes. Line thickness indicates the strength of data support. Abbreviation: PPI, protein-protein interaction.

idated target" genes and the 1292 "predicted target" genes was generated, and there was a total of 1438 nonoverlapping genes and 65 overlapping genes between the two sets (**Table 2**). To further obtain a complete picture of the putative miR-34a target genes associated with GIST, the 1438 genes were then intersected with 1973 down-regulated genes from dataset GSE68743. Consequently, there were 62 potential target genes for the functional enrichment analysis (**Figure 3**).

Functional analysis of miR-34a targets

To further explore the biological mechanism of putative miR-34a genes, GO analysis and KEGG pathway analysis were both performed. The top four pathways and GO terms identified are displayed in **Tables 3-6**. From **Table 6**, we found that miR-34a targets mainly affected pathways associated with cancer. Seven genes (FGF5, TP53, PTCH1, CCNA1, RUNX1, MYC, and APC) were enriched in the most significant pathway, hsa05200, with the term of pathways in cancer. Among these seven genes, TP53, MYC, and APC were enriched in the pathway of endometrial cancer. Moreover, basal cell carcinoma and acute myeloid leukemia were the next two most significant pathways. In **Tables 3-5**, miR-34a target genes are mainly annotated in three GO categories, including BP, MF, CC. The top ten GO terms associated with BP were enzyme-linked receptor protein signaling pathway, regulation of peptidase activity, response to hormone stimulus, regulation of cell proliferation, pattern specification process, response to endogenous stimulus, response to estrogen stimulus, response to steroid hormone stimulus, positive regulation of macromolecule metabolic process and negative regulation of cell differentiation. Six GO terms relevant to CC were identified as significant, including anchoring junction, microtubule cytoskeleton, adherens junction, axon, neuron projection and cell junction. The most significant GO terms for MF were associated with molecular binding and receptor activity (hormone binding, steroid hormone receptor activity, ligand-dependent nuclear receptor activity, growth factor activity, steroid binding, sequence-specific DNA binding and kinase binding). The overrepresentation of GO categories were then assessed and visualized as networks in Cytoscape 3.0 (**Figures 5-7**).

Prospective genes from the PPI network

As revealed in **Figure 4**, a network consisting of 7 proteins (nodes) encoded by genes and 7 interactions (edges) was generated to identify

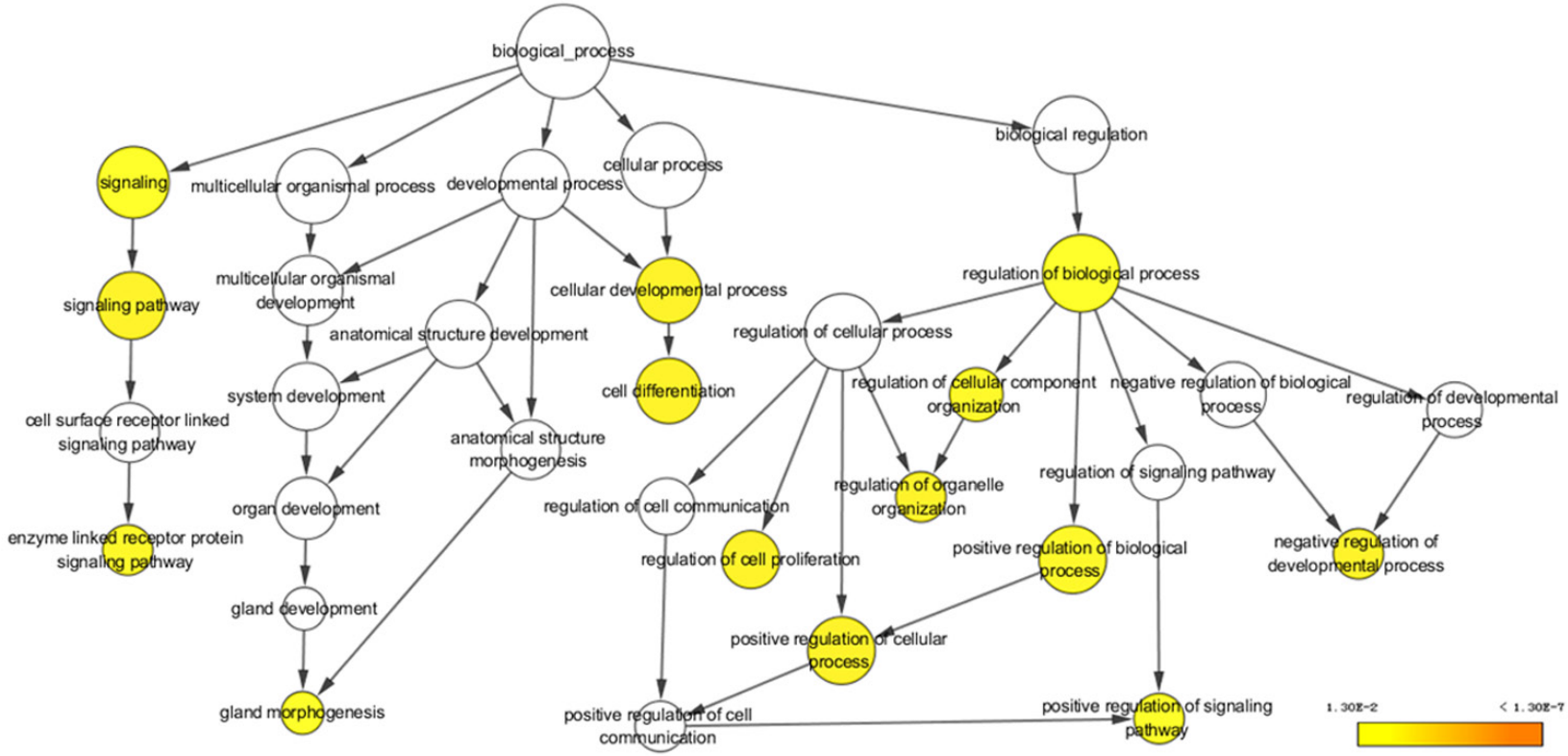

Figure 5. Network analysis of the potential target genes of miR-34a associated with BP. Note: A node represents a pathway, and the node size and color intensity indicate the number of genes involved and the *p* value, respectively. Nodes: 32, edges: 42, P=0.013. Abbreviation: BP, biological process.

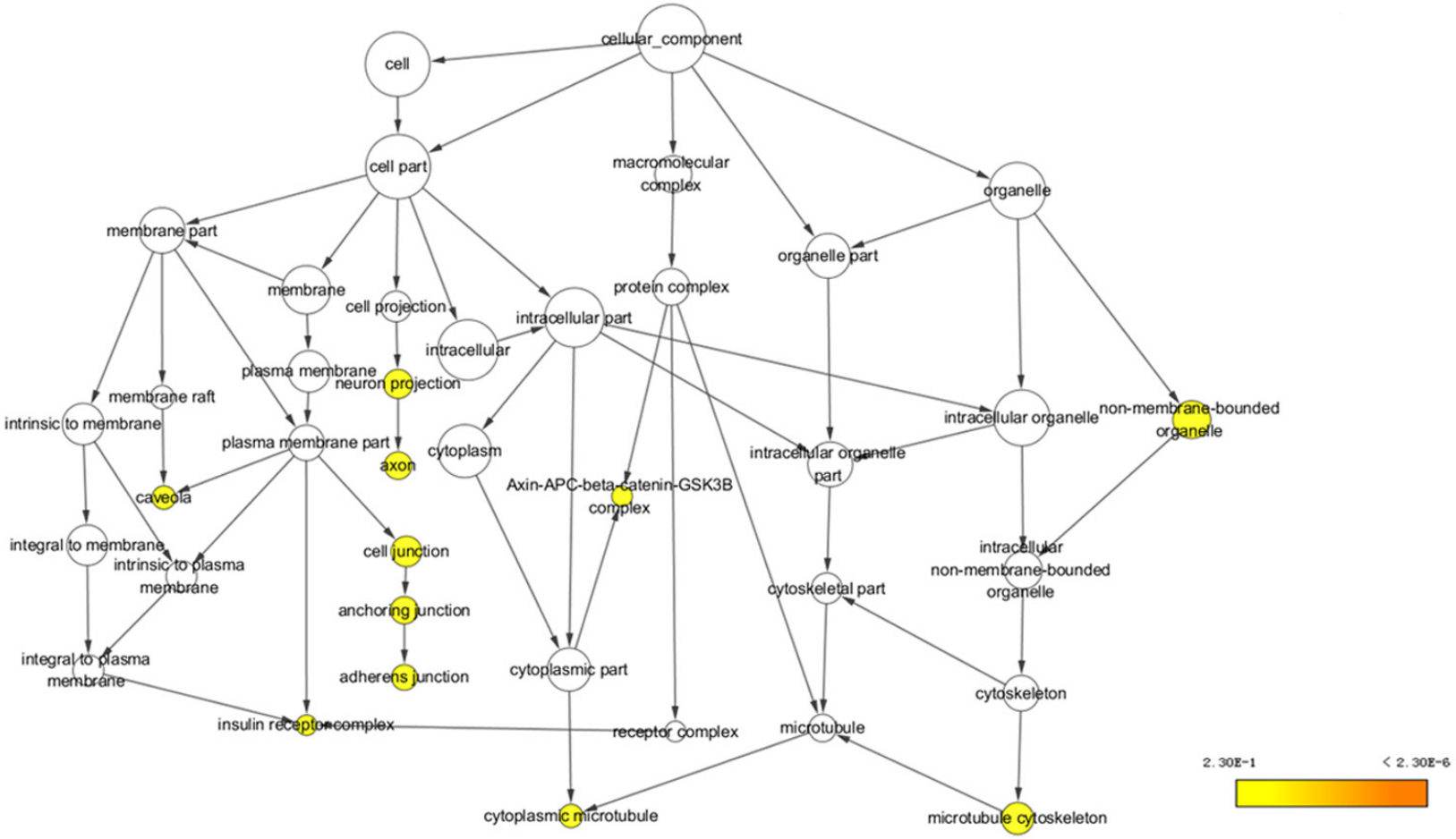

Figure 6. Network analysis of the potential target genes of miR-34a associated with CC. Note: A node represents a pathway, and the node size and color intensity indicate the number of genes involved and the *p* value, respectively. Nodes: 39, edges: 58, P=0.23. Abbreviation: CC, cellular component.

the prospective targets of miR-34a. The "degree" of a node was assessed according to the number of edges linked to it. The most highly linked genes (nodes with a high degree) were identified as "hubs". In the network, genes that were linked with more than 3 lines were chosen as "hub genes", which presented a high probability of being targets of miR-34a involved in the pathogenesis of GIST. Consequently, TP53, with a high degree of 5, and MYC, with a degree of 3, were ultimately considered as "hub genes".

Discussion

As a tumor suppressor, miR-34a participates in the biological process of multiple cancers, including GIST. Our study explored the link between miR-34a expression profiling and clinical variables associated with GIST. According to t-test and ANOVA analyses, no significant difference was detected between groups except the small intestine/large intestine groups in GSE36087, which may be caused by the small sample size of each dataset. In addition, a union of 62 potential miR-34a target genes was obtained by the combination of predicted target genes and genes from microarray datasets of GIST cells transfected with a miR-34a mimic. In KEGG pathway analysis, the results showed that among the top four pathways, two (pathways in cancer and endometrial cancer) were significant due to their respective involved genes (pathways in cancer: *FGF5, TP53, PTCH1, CCNA1, RUNX1, MYC, APC*; endometrial cancer: *TP53, MYC, APC*). A PPI network revealed genes *TP53* and *MYC* as the prospective target genes of miR-34a.

In the t-test and ANOVA analyses, there was no statistical significance except between the small intestine and large intestine groups in GSE36087. The results may be due to the limited number of patients in each group, which was on average between 10 and 20. Insufficient datasets were also a limitation in our study. Thus, more studies with large populations are required to further identify the clinical role of miR-34a in GIST.

Concerning the potential molecular mechanism of miR-34a in GIST, *TP53* may act as a pivotal target, as revealed by signaling pathway and PPI analyses. As a vital transcription factor regulating the cell cycle and apoptosis, overexpression of *TP53* occurs in the majority of GIST cases [27, 28]. Some studies have also identified the high expression of *TP53* as an independent prognostic factor of poor overall survival and relapse-free survival [28-30]. At present, the studies focusing on the regulatory mechanism of *TP53* in GIST are scarce, though it is known that *TP53* exerts tumor-suppressive effects in human tumor pathogenesis through relevant pathways. In one study, *Chou et al* demonstrated for the first time that the alteration of the *TP53/p21WAF1* pathway significantly correlates with the proliferation and progression of GIST [31]. Furthermore, *Samuel et al* suggested that *TP53* cooperates with miR-34a to mediate the cell cycle in the G2/M phase through *TP53* cellular pathways [32], which indicated that a *miR-34a-TP53* axis might be an essential mechanism of transcriptional regulation in GIST. Nevertheless, the *TP53* gene still requires further investigation to identify the underlying mechanism in the tumorigenesis of GIST.

Following KEGG pathway analysis, seven genes, including *TP53, MYC, APC* and *PATCH 1*, were found to affect pathways related to cancer. In addition to *TP53* mentioned above, the amplification of the *MYC* gene has been extensively described in GIST despite the unclarified contributions of *MYC* to GIST pathogenesis [33, 34]. The *MYC* gene is well known for its key regulation of the cell cycle and apoptosis as a transcription factor [35]. Interestingly, *MYC* is regulated by β-catenin/*TCF* signaling, whose activation through the mutant *APC* gene or β-catenin underlies intestinal tumor initiation [36]. Thus, *MYC* and *APC* are likely to be the potential targets that are responsible for GIST development. Additionally, the tumor suppressor *PATCH 1* has a key role in activating the Hh pathway, which may be an early event promoting the onset of GIST. Previous studies have shown deletion of *PATCH 1* and aberrant activity of Hh signaling in most GIST patients [37, 38]. Taken together, these findings suggest that the mutations in the genes above are related to cancer pathways and contribute to the disease.

Additionally, the KEGG analysis suggested that endometrial cancer is also a key pathway related to gastrointestinal stromal tumors. Previous studies revealed that neoplasms occurring in the uterus and ovaries frequently express high levels of *PDGFRA*, whose mutations are also

Figure 7. Network analysis of the potential target genes of miR-34a associated with MF. Note: A node represents a pathway, and the node size and color intensity refer to the number of genes involved and the *p* value, respectively. Nodes: 20, edges: 20, P=0.04. Abbreviation: MF, molecular function.

commonly found in GISTs [39, 40]. Furthermore, *Munson et al* suggested that *PDGFRA* is a mitogen that signals cell replication and is involved in the pathological proliferation of endometrial cancer [41]. Accordingly, we assume that the *PDGFRA* gene may also have a crucial role in the progression of GIST when harboring *PDGFRA* mutations. Recent studies have verified that in GIST, the *PDGFRA* gene frequently contains activating mutations [42, 43]. *Zhu et al* found that to a great extent, the consequential mechanisms of GIST may be the result of the active form of *PDGFRA* mediated by KIT [44]. There are various *PDGFRA* mutations, and *Li et al* identified for the first time a novel *PDGFRA* mutation in exon 8, exon 11, and exon 14 in addition to the previously recognized mutational site in exon 12 and the D842V mutation in *PDGFRA* exon 18 [42]. Based on these findings, accumulating studies have focused on the prognostic role of *PDGFRA* mutational status. As suggested by a recent meta-analysis, patients with *PDGFRA*-mutant GIST have favorable recurrence-free survival with surgery alone [45]. In another study investigating the response to imatinib in patients with *PDGFRA* mutations, *Yoo et al* confirmed that D842V *PDGFRA*-mutant GISTs are mostly resistant to imatinib, whereas GISTs without D842V mutations actively respond to imatinib [43]. According to these findings, *PDGFRA* mutations might be a promising factor for predicting the risk of recurrence and response to therapy with the *PDGFRA* inhibitor imatinib. A recent study presented the cooperativity between the *PDGFRA* and Hh pathways in GIST patients [46]. Therefore, it is speculated that in addition to imatinib, an Hh antagonist might serve as a novel treatment protocol for patients with GIST.

In conclusion, the genes *TP53*, *MYC*, and *PDGFRA*, which are potential targets of miR-34a, were identified as playing a critical role in GIST cell proliferation and invasion. These pathways, including pathways in cancer and endometrial cancer, could also induce GIST oncogenesis and progression. Accordingly, we speculate that miR-34a may regulate the tumorigenesis of GIST via these genes and pathways. Whether miR-34a could be utilized as a predictor for GIST still needs to be investigated by more prospective studies with a larger number of sam-

ples. In light of these findings, our study clarifies the potential regulatory mechanism of miR-34a in GIST, which lays the foundation of and highlights the direction for future investigations.

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Disclosure of conflict of interest

None.

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