

An integrated bioinformatical analysis of miR-19a target genes in multiple myeloma

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Abstract. MicroRNA (miR)-19a, as an oncomiR, has been studied in several types of cancer; however, its role in the development and progression of multiple myeloma (MM) remains unclear. The present study used a bioinformatics approach to investigate the involvement of miR-19a in MM. miR-19a targets were predicted using target prediction programs, followed by screening for differentially expressed genes in MM. The function of these genes was then annotated using gene ontology term enrichment, signaling pathway enrichment and protein-protein interaction (PPI) analysis. In addition, natural language processing (NLP) was performed to identify genes associated with MM. A total of 715 putative targets of miR-19a were identified in the present study, of which 40 were experimentally validated. A total of 121 genes were identified to be differentially expressed in MM, including 80 upregulated genes and 41 downregulated genes. Among the differentially expressed genes, ras homolog family member B, clathrin heavy chain, prosaposin and protein phosphatase 6 regulatory subunit 2 were predicted target genes of miR-19a. The results of NLP revealed that 2 of the differentially expressed genes, Y-box binding protein 1 and TP53 regulated inhibitor of apoptosis 1, were reported to be associated with MM. In addition, 41 target genes of miR-19a were identified to be associated with the development and progression of MM. These results may aid in understanding the molecular mechanisms of miR-19a in the development and progression of MM. In addition, the results of the present study indicate that target genes of miR-19a are potential candidate biomarkers for MM.

Introduction

Multiple myeloma (MM) is a B-cell malignancy characterized by the aberrant clonal expansion of plasma cells (PCs) within the bone marrow, and, as a consequence, osteolytic bone destruction with hypercalcemia, anemia, immunosuppression and end organ damage frequently occurs (1). Recent advances in molecular and genetic research into MM have led to the discovery that although MM is defined histologically as a single entity, it encompasses a wide range of genomic abnormalities, including numerical and structural chromosomal abnormalities, gene mutations and epigenetic alterations (2-4), which differ in their molecular pathogenesis and prognostic significance (5).

MicroRNAs (miRNAs/miRs) are small non-coding single-stranded RNAs of ~22 nucleotides in length, which control gene expression at a post-transcriptional level by degrading or repressing target mRNAs, resulting in translational repression or mRNA degradation. miRNAs serve roles in essential biological processes, including cellular growth, differentiation and proliferation. In addition, miRNAs regulate the expression of >30% of protein-coding genes, and >50% of miRNA target genes are located in cancer-associated genomic regions, suggesting that miRNAs serve an important role in the pathogenesis of human cancer (6,7). It is well known that the dysregulation of miRNAs is associated with the pathogenesis of cancer, and that miRNA expression profiles have prognostic implications in numerous types of cancer. Thus, inhibiting specific miRNAs is a therapeutic strategy for the treatment of cancer (8).

Numerous previous studies have detected miRNA expression in MM via microarray profiling and reverse transcription-quantitative polymerase chain reaction analysis (9-11), with results suggesting that miRNAs serve an important role in the molecular pathogenesis, progression and prognosis of MM. Lionetti *et al* (9) evaluated the influence of allelic imbalances on miRNA expression in MM, and identified that differential miRNA expression patterns were associated with the cytogenetic abnormalities in MM, particularly with immunoglobulin heavy locus translocations. Furthermore, Wu *et al* (10) was able to develop an 'outcome classifier' in patients newly diagnosed with myeloma based on their expression of specific miRNAs.

The miR-17-92 cluster, located in an intron of miR-17-92a-1 cluster host gene on chromosome 13q31.3, was originally reported

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to be implicated in B-cell neoplasms, including MM (11). Later, the miR-17-92 cluster was identified as an oncomiR due to its oncogenic activity in several types of cancer (12). Mendell (11) identified that the deletion of miR-17-92 inhibited B-cell proliferation and development, whereas its overexpression induced B-cell hyperproliferation and autoimmune diseases. Another study revealed that members of the miR-17-92 cluster, particularly miR-19a and b, were upregulated in MM, but not in healthy cases or monoclonal gammopathy of undetermined significance (MGUS), suggesting a potential role of the cluster in the progression from MGUS to MM, likely representing MM-specific genetic changes (2).

miR-19a, a key component of miR-17-92 cluster, has been directly implicated in myeloma pathogenesis (13,14). In addition, miR-19a has been demonstrated to be upregulated in patients with MM, and in MM cell lines compared with normal plasma cells (13,15). Furthermore, miR-19a was more highly expressed in patients with MM with 13q14 deletions compared with those without these deletions (5,16,17). Additionally, miR-19a antagonists have been revealed to suppress MM tumor growth in nude mice (14). miR-19a can modulate the expression of proteins that are essential in myeloma pathogenesis, including suppressors of cytokine signaling (SOCS), a gene that is frequently silenced in MM, releasing inhibition of interleukin 6 and leading to pro-growth signaling (14). These results highlight the contribution of miR-19a to the pathogenesis of MM and its potential application as a molecular biomarker for MM.

Although the function of miR-19a has been relatively well studied, its exact role in the development and progression of MM remains unclear. Systematic analyses of miR-19a-associated malignant cell behavior is required. Since miR-19a's function is mediated through its target genes, the exploration of its target genes is also essential. The inverse correlation between miRNA-mRNA interactions may aid in the identification of target genes regulated by miR-19a in the pathogenesis of MM. Combined with the MM gene expression profiling data generated by high-throughput technology in a previous study (18), the present study performed a systematic analysis of miR-19a predicted target genes associated with the carcinogenesis, prognosis and chemoresistance of MM in order to further investigate the potential involvement of miR-19a in MM.

Materials and methods

Prediction of miRNA target genes. miR-19a target prediction was performed using the online tool miRWalk (<http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk>) (19) with a combination of three currently available independent target prediction programs, including PicTar (version 2005; http://pictar.mdc-berlin.de/cgi-bin/PicTar_vertibrate.cgi), miRanda (version 5; <http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5>) and TargetScan (version 5.1; <http://www.targetscan.org>). Only the target genes that were confirmed by all of the above prediction programs or experimentally validated according to the miRWalk database were considered putative targets of miR-19a.

Identifying gene expression profiles. The Gene Expression Omnibus database (GEO, <http://www.ncbi.nlm.nih.gov/geo/>),

a public repository for high-throughput gene expression datasets, was searched for MM gene expression profiling studies. Expression profiling studies of peripheral blood mononuclear cells from patients with MM were obtained from previous studies (20-22).

Differential analysis of genes in MM. The raw gene expression data of each study was downloaded from the GEO database, and preprocessed for background correction and Z-score normalization. The Bioconductor limma package (version 1.9.6) in R (23) was used to perform differential analysis of genes between MM and controls using a two-tailed Student's t-test. The p-value of individual microarray studies were combined using Fisher's exact test. Differently expressed genes with a false discovery rate (FDR) <0.01 were selected.

Functional classification. GeneCodis (<http://genecodis.cnb.csic.es/>) was used to perform Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, in order to annotate the function of the selected genes. The functional GO terms were classified into three groups, biological processes, cellular components and molecular functions. Genes were mapped to corresponding signaling pathways according to KEGG signaling pathway database, and the enrichment FDR was calculated for each pathway, and the criteria of FDR <0.05 was used as the threshold for significance.

Protein-protein interaction (PPI) network analysis. To explore the function of genes at the protein level, PPI analysis was performed as previously described (24). The Biological General Repository for Interaction Datasets (BioGRID; <http://thebiogrid.org>) was used to construct the PPI network. BIOGRID is an online interaction repository, confirmed by existing high-throughput experiments. Cytoscape version 3.3.0 software was used to display the PPI network, as previously described (25). In the PPI network produced, nodes indicate proteins and edges indicate interactions between these nodes. The nodes that contain the most connectivity degrees are defined as significant hub proteins.

Natural language processing (NLP) analysis of MM. Document searching and formatting were performed in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) using the keywords 'multiple myeloma' and 'resistance or prognosis or carcinogenesis or tumorigenesis'. All of the genes and proteins associated with these keywords were extracted, followed by gene mention tagging using A Biomedical Named Entity Recognizer software, version 1.5 (<http://pages.cs.wisc.edu/~bsettles/abner>). For the conditions, multiple genes were described in a word, such as 'STAT3/5 gene', and these were translated manually to 'STAT3 gene', and 'STAT5 gene'. Gene names were normalized based on the Entrez database (<https://www.ncbi.nlm.nih.gov/gene>). Gene names were normalized based on the Entrez database (<https://www.ncbi.nlm.nih.gov/gene>).

The frequency of the occurrence of each gene was calculated. The higher the frequency of the gene, the greater the likelihood of the association between MM and the gene. The total number of studies in PubMed database was recorded as 'N'. The frequency of the genes and diseases associated

with these in the PubMed database were denoted as 'm' and 'n', respectively. It was hypothesized that subtracting the disease co-occurrent from the actual frequency of the gene would equal 'k'. Then, by using hypergeometric distribution, the probability of a frequency greater than k co-citation at completely random conditions was calculated as follows:

$$p = 1 - \sum_{i=0}^{k-1} p(i | n, m, N)$$

$$p(i | n, m, N) = \frac{n!(N-n)!m!(N-m)!}{(n-i)!i!(n-m)!(N-n-m+i)!N!}$$

Results

Predicted target genes of miR-19a. Target genes of miR-19a were predicted using three target prediction programs. A total of 715 putative targets of miR-19a were identified using these three programs, among which 40 were experimentally validated in miRWalk (data not shown).

Differentially expressed genes in MM. Following searching the GEO database, three gene expression profiling studies of MM were collected (GSE23832, GSE21942 and GSE17048; Table I). The raw data was downloaded and processed, and 121 genes were identified to be differentially expressed in MM with an FDR <0.01, including 80 upregulated genes and 41 downregulated genes (Fig. 1). The top 10 most significantly upregulated or downregulated genes are listed in Table II. Interestingly, four putative targets of miR-19a, ras homolog family member B (RHOB), clathrin heavy chain (CLTC), prosaposin (PSAP) and protein phosphatase 6 regulatory subunit 2 (PPP6R2), were identified to be differentially expressed.

Functional classification of differentially expressed genes. For the differentially expressed genes in MM, GO term enrichment analysis was performed. For biological processes, 38 GO terms were significantly enriched, and the most significantly enriched GO terms were translation (GO:0006412; FDR, 3.14×10^{-21}) and viral transcription (GO:0019083; FDR, 3.59×10^{-18}) (Fig. 2A). For cellular components, 29 GO terms were significantly enriched, and the most significantly enriched GO terms were ribosome (GO:0005840; FDR, 5.12×10^{-15}) and cytosolic small ribosomal subunit (GO:0022627; FDR, 2.60×10^{-11}) (Fig. 2B). For molecular functions, 9 GO terms were significantly enriched, and the most significantly enriched GO terms were structural constituent of ribosome (GO:0003735; FDR, 1.40×10^{-20}) and protein binding (GO:0005515; FDR, 1.82×10^{-10}) (Fig. 2C).

When performing the KEGG pathway enrichment analysis, 7 signaling pathways were significantly enriched with the criteria of FDR <0.05 (Table III). The most significant pathway was ribosome (FDR, 2.25×10^{-16}). Furthermore, oxidative phosphorylation (FDR, 0.0277), bacterial invasion of epithelial cells (FDR, 0.0286), lysosome (FDR, 0.0318), the Wnt signaling pathway (FDR, 0.0343), tuberculosis (FDR, 0.0385) and collecting duct acid secretion (FDR, 0.0407) were also significantly enriched.

PPI network. A PPI network including all of the differentially expressed genes identified was constructed. The PPI network

produced included 2,355 nodes and 3,707 edges (Fig. 3). Highly connected proteins in the network are called hub proteins, which are the core of regulation and serve an important role in the stability of the network. The significant hub proteins were identified, including COP9 signalosome complex subunit 5 (COPS5; connectivity degree, 791), CLTC (connectivity degree, 172) and 60S ribosomal protein L11 (connectivity degree, 167).

NLP results. The abstracts of 6,795 primary studies were identified using the aforementioned search strategy and a total of 662 MM-associated genes were obtained (data not shown). Integrated analysis was performed to identify the overlap between the 715 miR-19a target genes previously identified and the 662 MM-associated genes obtained from NLP analysis. This revealed that there were 41 overlapping genes (Table IV and Fig. 4), which were associated with the development and progression of MM, and were putative miR-19a target genes. In addition, 2 of the differentially expressed genes, Y-box binding protein 1 (YBX1) and TP53 regulated inhibitor of apoptosis 1 (TRIAPI) were identified to be associated with MM (Fig. 4).

Discussion

Several previous studies (2,13-15) have demonstrated that miR-19a is deregulated in MM as an oncomiR, suggesting it serves an important role in MM. Considering that the biological significance of miRNA deregulation relies on the effect upon target protein-coding genes, predicted target genes of miR-19a that were associated with the carcinogenesis, prognosis and chemoresistance of MM were systematically analyzed in the present study in order to further investigate the potential involvement of miR-19a in MM. Strategies to determine miRNA targets include bioinformatical prediction and experimental assays. The present study utilized three common computational algorithms, miRanda, PicTar and TargetScan, to identify 715 putative target genes of miR-19a, among which 40 were experimentally validated in miRWalk. In addition, NLP analysis was performed in the current study, which identified 662 MM-associated genes. Then, integrated analysis revealed 41 predicted target genes of miR-19a that were associated with the development and progression of MM, including Kirsten rat sarcoma viral oncogene homolog (KRAS), SOCS and CCND1. Several of these MM-associated putative miR-19a targets, including SOCS and CCND1, have already been verified by miRNA functional experiments (14). Previous studies have demonstrated that oncogenic mutations of RAS occur in 30-40% of patients with MM and are rarely found in MGUS (26-28). The occurrence of RAS mutation appears independent of clinical stage, but is associated with disease progression, an aggressive phenotype, resistance to therapy and poor patient survival (26,27,29). Steinbrunn *et al* (27) also reported that the ectopic overexpression of oncogenic RAS induces MM cell proliferation and lowers drug efficacy.

Given that the altered expression of miR-19a in MM would cause changes in target gene expression, differentially expressed genes between MM and normal controls were assessed in current study using gene expression data. This revealed that 121 genes were differentially expressed in MM,

Table I. Characteristics of the three gene expression profiling datasets for multiple myeloma downloaded for integrated analysis.

Author, year	GEO dataset ID	Platform for detection	Samples (N:M)	(Refs.)
Zhang <i>et al</i> , 2011	GSE23832	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	4:8	(20)
Kemppinen <i>et al</i> , 2011	GSE21942	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	15:12	(21)
Gandhi <i>et al</i> , 2010	GSE17048	GPL6947 Illumina HumanHT-12 V3.0 expression beadchip	45:99	(22)

GEO, Gene Expression Omnibus; N:M, normal:multiple myeloma.

Table II. Top 10 significantly upregulated and downregulated DEGs identified in multiple myeloma. All gene ID's were taken from the Entrez database on NCBI.

A, Upregulated DEGs

Entrez gene ID	Abbreviation	Name	FDR
84265	POLR3GL	Polymerase (RNA) III (DNA directed) polypeptide G (32kD)-like	0.0001793
8364	HIST1H4C	Histone cluster 1, H4c	0.0002646
6170	RPL39	Ribosomal protein L39	0.0013368
5880	RAC2	Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	0.0013368
29080	CCDC59	Coiled-coil domain containing 59	0.0013368
521	ATP5I	ATP synthase, H ⁺ transporting, mitochondrial Fo complex, subunit E	0.0013773
9991	PTBP3	Polypyrimidine tract binding protein 3	0.001458
79023	NUP37	Nucleoporin 37kDa	0.001458
64801	ARV1	ARV1 homolog, fatty acid homeostasis modulator	0.001458
3700	ITIH4	Inter- α -trypsin inhibitor heavy chain family, member 4	0.001458

B, Downregulated DEGs

Entrez gene ID	Abbreviation	Name	FDR
387	RHOA	Ras homolog family member A	0.0004738
23256	SCFD1	Sec1 family domain containing 1	0.0013368
92241	RCSD1	RCSD domain containing 1	0.0014580
126364	LRRC25	Leucine rich repeat containing 25	0.0017804
5226	PGD	Phosphogluconate dehydrogenase	0.0026507
129531	MITD1	MIT, microtubule interacting and transport, domain containing 1	0.0027447
10023	FRAT1	Frequently rearranged in advanced T-cell lymphomas 1	0.0027447
8883	NAE1	NEDD8 activating enzyme E1 subunit 1	0.0028332
81631	MAP1LC3B	Microtubule-associated protein 1 light chain 3 β	0.0028998
4904	YBX1	Y box binding protein 1	0.0028998

DEGs, differentially expressed genes; FDR, false discovery rate. Entrez database: <https://www.ncbi.nlm.nih.gov/gene>.

Table III. Significantly enriched KEGG signaling pathways of the differentially expressed genes identified in multiple myeloma.

KEGG ID	KEGG term	No. of enriched genes	FDR	Genes
hsa03010	Ribosome	13	2.25x10 ⁻¹⁶	RPS15A, RPS27A, RPS25, RPS13, RPL26, RPL39, RPL21, RPS29, RPS14, RPS27, RPL11, RPL27, RPS10
hsa00190	Oxidative phosphorylation	4	2.77x10 ⁻⁰²	NDUFS4, ATP6V1A, ATP5I, ATP6V0C
hsa05100	Bacterial invasion of epithelial cells	3	2.86x10 ⁻⁰²	CLTC, RHOA, CRKL
hsa04142	Lysosome	4	3.18x10 ⁻⁰²	CLTC, PSAP, ATP6V0C, LAMP2
hsa04310	Wnt signaling pathway	4	3.43x10 ⁻⁰²	SIAH1, RAC2, RHOA, FRAT1
hsa05152	Tuberculosis	4	3.85x10 ⁻⁰²	APAF1, RHOA, ATP6V0C, LAMP2
hsa04966	Collecting duct acid secretion	2	4.07x10 ⁻⁰²	ATP6V1A, ATP6V0C

KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

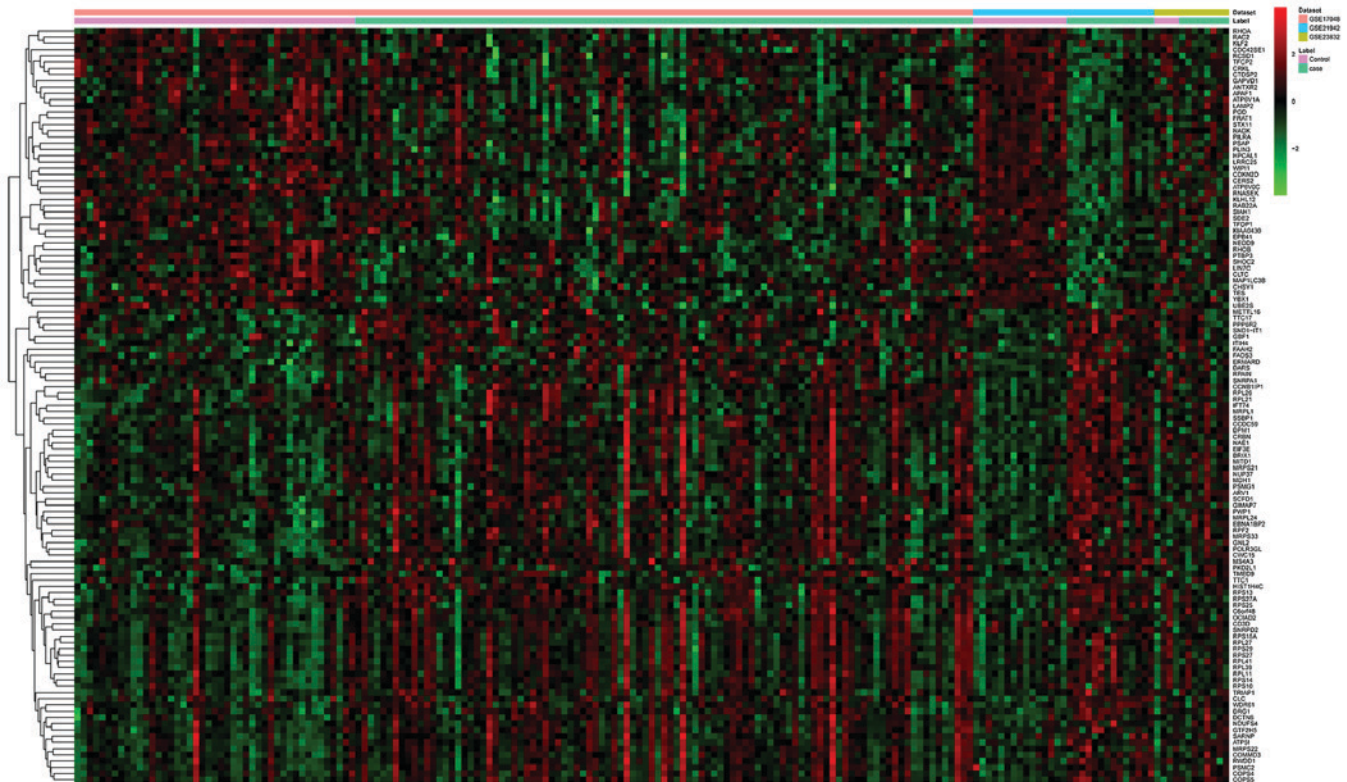


Figure 1. Heatmap of the 121 differentially expressed genes identified in multiple myeloma from the three datasets.

including 80 upregulated genes and 41 downregulated genes. In addition, 2 of the differentially expressed genes, YBX1 and TRIAP1, were identified to be associated with MM in the present study. YBX1, a member of the cold-shock domain protein superfamily, is involved in a wide range of cellular functions, including DNA transcription, replication and repair, and environmental stress and chromatin remodeling, in addition to pre-mRNA splicing (30). Chatterjee *et al* (31) demonstrated that YBX1 was overexpressed in immature

and anaplastic MM cells, but not expressed in normal PCs, MGUS PCs or the majority of MM specimens, suggesting it serves a role in dedifferentiation as part of the malignant transformation process. Furthermore, other studies have reported that the aberrant expression of YBX1 is associated with tumorigenesis, and cancer cell proliferation, survival and drug resistance (32,33).

Interestingly, four putative targets of miR-19a, RHOA, CLTC, PSAP and PPP6R2, were identified to be differentially

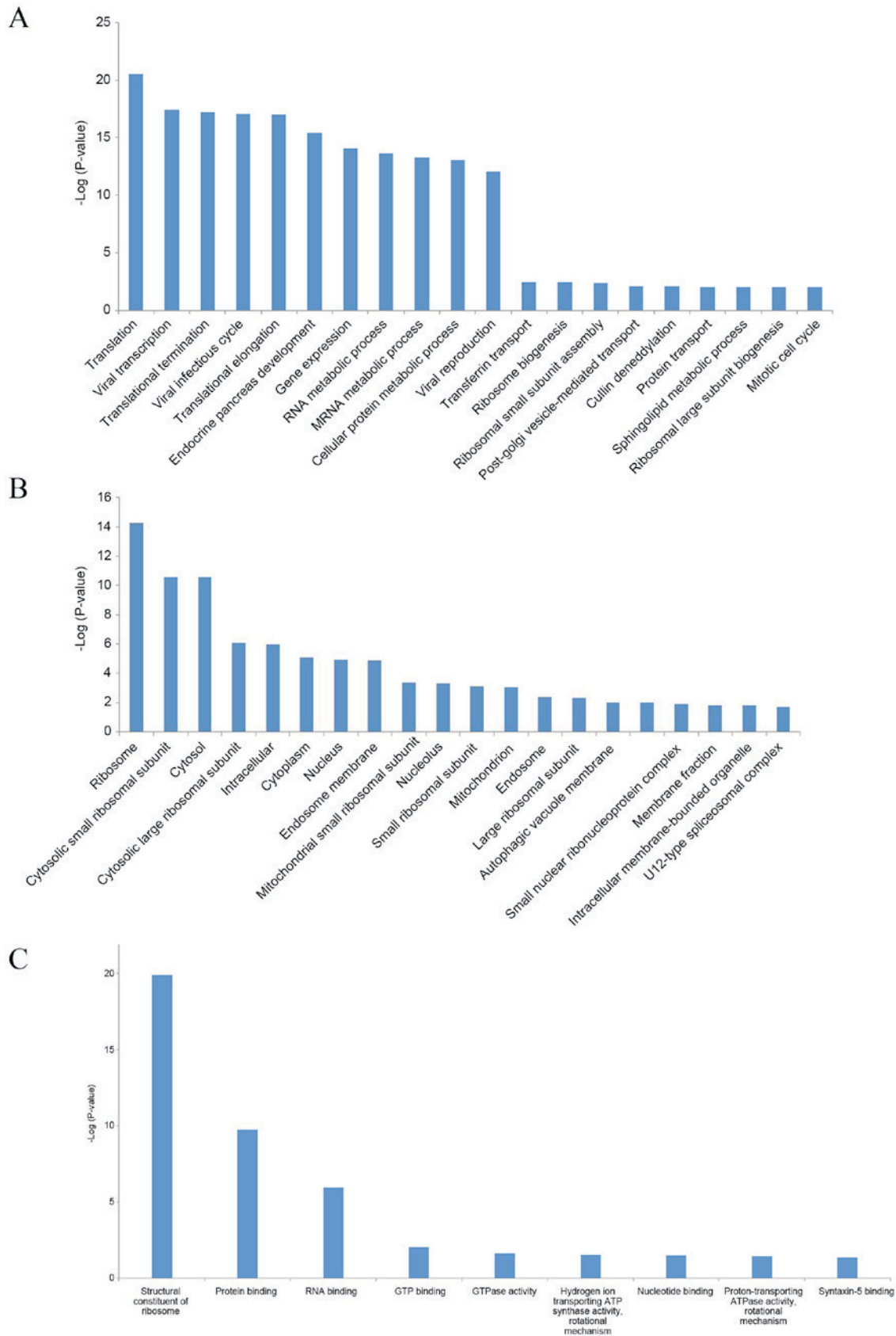


Figure 2. GO term enrichment analysis of the differentially expressed genes identified in multiple myeloma. GO analysis under the category of (A) biological processes, (B) cellular components and (C) molecular functions. GO, gene ontology.

expressed in MM in the present study. The tumor suppressor RHOB has been demonstrated to downregulated in various

types of cancer (34,35), which is in accord with the findings of the present study. Notably, Tan *et al* (36) revealed

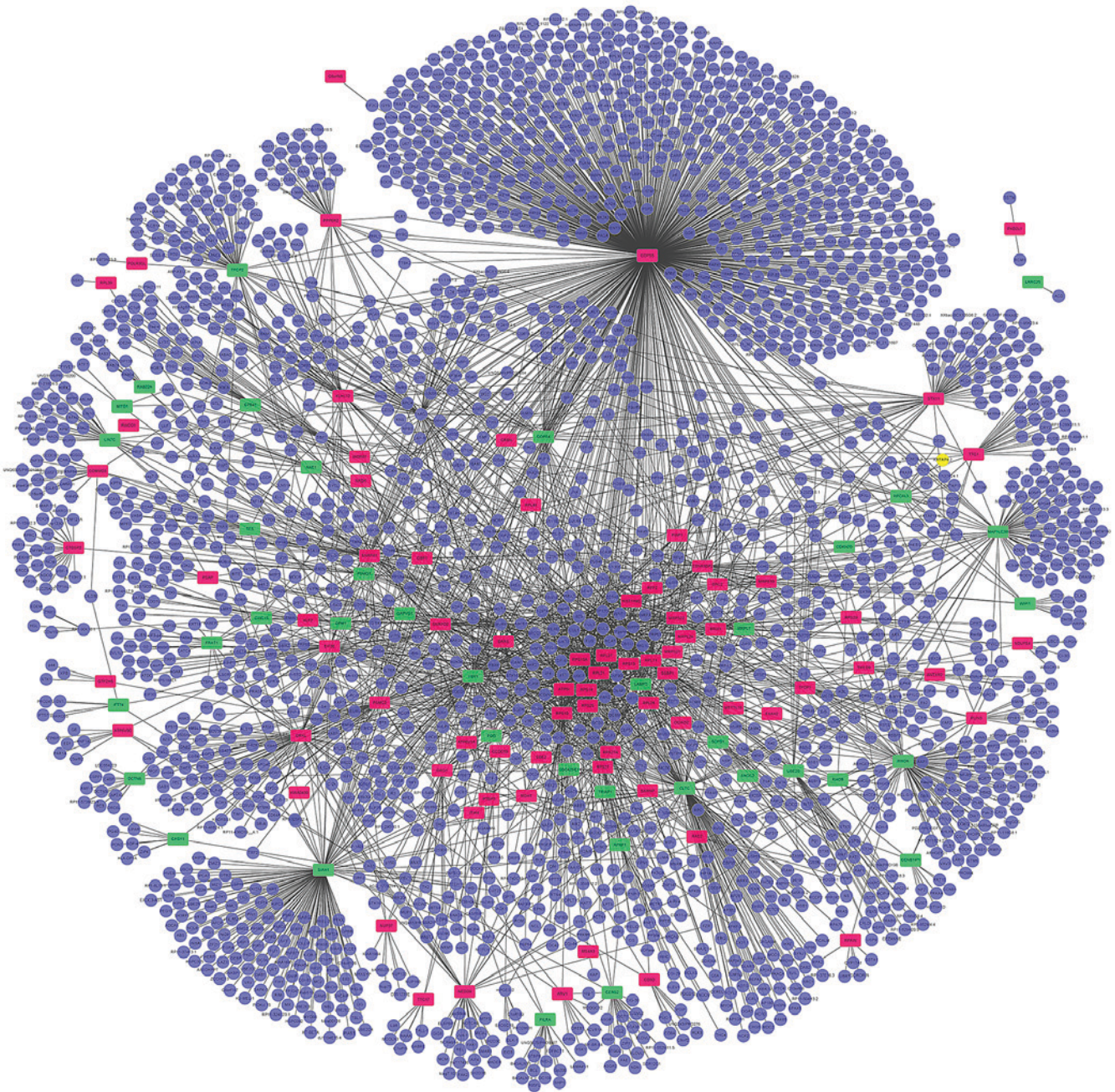


Figure 3. Protein-protein interaction analysis of the differentially expressed genes identified in multiple myeloma. Red, upregulated; green, downregulated; rectangles, differentially expressed genes; circular, genes interacting with differentially expressed genes.

that RHOB induced apoptosis, and inhibited proliferation and migration in pancreatic cancer as a direct target of miR-19a. Chromosomal and genomic analyses have revealed that the ALK receptor tyrosine kinase gene is fused to CLTC in inflammatory myofibroblastic tumors and B-cell lymphoma (37,38). However, the exact role of CLTC in MM has not yet been reported. PSAP, a highly conserved glycoprotein, is overexpressed in prostate cancer and esophageal squamous cell carcinoma (39,40). A similar expression trend for PSAP was observed in the present study, indicating that it may be a candidate biomarker for MM.

In the PPI network of differentially expressed genes produced in the present study, COPS5 had the highest connectivity degree, suggesting that it serves an important

role in MM progression. COPS5, one of the eight subunits of the COP9 signalosome, is overexpressed in a variety of types of human cancer (41). COPS5 was identified to be overexpressed in MM in the present study. A previous study demonstrated that the specific knockdown of COPS5 inhibits the proliferation of colorectal cancer cells (42), and that COPS5-transgenic mice developed a phenotype similar to that of myeloproliferative disorders (43). In addition, COPS5 is involved in Ras-mediated cell transformation by inhibiting premature senescence (44).

All of the differentially expressed genes in MM identified in the present study underwent GO term and signaling pathway enrichment analysis, in addition to PPI network construction, in order to understand their function. This revealed functions in

Table IV. Overlapping genes that were associated with the development and progression of multiple myeloma, and were putative target genes of microRNA-19a (n=41).

Gene abbreviation	Gene name	Count	P-value
CCND1	Cyclin D1	78	<0.0001
CCNA2	Cyclin A2	1	0.2128
CCND2	Cyclin D2	16	<0.0001
CD69	CD69 molecule	1	0.0818
CTGF	Connective tissue growth factor	1	0.2861
S1PR1	Sphingosine-1-phosphate receptor 1	1	0.1044
EREG	Epiregulin	1	0.0388
ESR1	Estrogen receptor 1	3	0.4207
F3	Coagulation factor III (thromboplastin, tissue factor)	2	0.0853
GJA1	Gap junction protein, α 1, 43kDa	1	0.2950
GRK6	G protein-coupled receptor kinase 6	1	0.0625
ID2	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	1	0.1265
IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	2	0.0231
ITGA6	Integrin, α 6	1	0.1979
KIT	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	10	<0.0001
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	1	0.5071
LIF	leukemia inhibitory factor (cholinergic differentiation factor)	1	0.1516
SMAD4	SMAD family member 4	1	0.3981
MDM4	Mdm4 p53 binding protein homolog (mouse)	1	0.1155
PTEN	Phosphatase and tensin homolog	5	0.0038
ATXN1	Ataxin 1	1	0.1155
SDC1	Syndecan 1	46	<0.0001
TGFBR2	Transforming growth factor, β receptor II (70/80kDa)	1	0.3081
THBS1	Thrombospondin 1	1	0.3418
KLF10	Kruppel-like factor 10	1	0.0487
TNFAIP3	Tumor necrosis factor, α -induced protein 3	2	0.0064
SOCS1	Suppressor of cytokine signaling 1	8	<0.0001
SOCS3	Suppressor of cytokine signaling 3	3	0.0022
HDAC4	Histone deacetylase 4	1	0.1463
FOXP1	Forkhead box P1	2	0.0016
MIB1	Mindbomb homolog 1 (<i>Drosophila</i>)	1	0.0267
PCDH10	Protocadherin 10	1	0.0226
CYLD	Cylindromatosis (turban tumor syndrome)	1	0.1007
PTK2B	PTK2B protein tyrosine kinase 2 β	1	0.2757
IGF1	Insulin-like growth factor 1 (somatomedin C)	14	<0.0001
MAPK10	Mitogen-activated protein kinase 10	3	0.0001
RAF1	V-raf-1 murine leukemia viral oncogene homolog 1	1	0.4154
SGK1	Serum/glucocorticoid regulated kinase 1	2	0.0173
TSC1	Tuberous sclerosis 1	1	0.1861
BCL2L11	BCL2-like 11 (apoptosis facilitator)	4	0.0002
TLR2	Toll-like receptor 2	1	0.5613

viral transcription, the viral infectious cycle, viral reproduction and the bacterial invasion of epithelial cells. A previous clinical investigation revealed that the risk of bacterial and viral infections was seven times higher in MM patients compared with matched controls due to MM-associated immunodeficiency resulting from PC disorders, including B-cell dysfunction, and T-cell, dendritic cell and NK cell abnormalities (45).

In conclusion, the present study identified and systematically analyzed predicted MM-associated target genes of miR-19a. A total of 121 differentially expressed genes in MM were identified, including 80 upregulated genes and 41 down-regulated genes. Among these differentially expressed genes, RHOB, CLTC, PSAP and PPP6R2, were predicted target genes of miR-19a. The results of NLP analysis revealed that

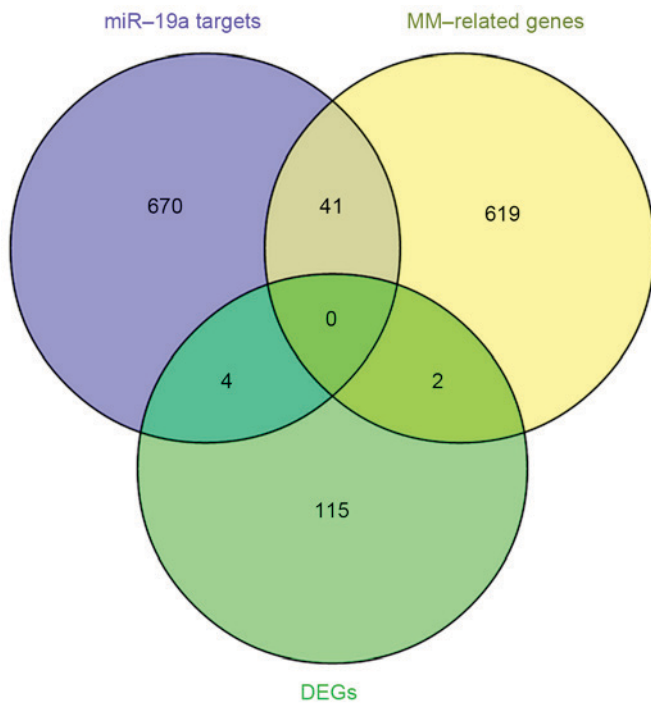


Figure 4. Overlap of the predicted target genes of miR-19a, DEGs in MM and MM-associated genes identified in natural language processing analysis. miR, microRNA; DEGs, differentially expressed genes; MM, multiple myeloma.

2 of the differentially expressed genes, YBX1 and TRIAP1, were associated with MM. In addition, 41 target genes of miR-19a were associated with the development and progression of MM. The combined examination of gene expression and bioinformatical prediction for miR-19a target genes may provide new insights into carcinogenic mechanisms of MM, in addition to highlighting potential areas for the development of novel personalized therapies. Further studies are required to confirm the results of the present study in patients with MM.

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