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Identification potential biomarkers and therapeutic agents in multiple myeloma based on bioinformatics analysis

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Abstract. – OBJECTIVE: The study aimed to identify potential therapeutic biomarkers and agents in multiple myeloma (MM) based on bioinformatics analysis.

MATERIALS AND METHODS: The microarray data of GSE36474 were downloaded from Gene Expression Omnibus database. A total of 4 MM and 3 normal bone marrow mesenchymal stromal cells (BM-MSCs) samples were used to identify the differentially expressed genes (DEGs). The hierarchical clustering analysis and functional enrichment analysis of DEGs were performed. Furthermore, co-expression network was constructed by Cytoscape software. The potential small molecular agents were identified with Connectivity Map (cMap) database.

RESULTS: A total of 573 DEGs were identified in MM samples comparing with normal samples, including 322 down- and 251 up-regulated genes. The DEGs were separated into two clusters. Down-regulated genes were mainly enriched in cell cycle function, while up-regulated genes were related to immune response. Down-regulated genes such as checkpoint kinase 1 (CHEK1), MAD2 mitotic arrest deficient-like 1 (MAD2L1) and DBF4 zinc finger (DBF4) were identified in cell cycle-related co-expression network. Up-regulated gene of guanylate binding protein 1, interferon-inducible (GBP1) was a hub node in immune response-related co-expression network. Additionally, the small molecular agent vinblastine was identified in this study.

CONCLUSIONS: The genes such as CHEK1, MAD2L1, DBF4 and GBP1 may be potential therapeutic biomarkers in MM. Vinblastine may be a potential therapeutic agent in MM.

Key Words:
Multiple myeloma, Bioinformatics analysis, Differentially expressed genes, Biomarker, Therapeutic agent.

Introduction

Multiple myeloma (MM) is a malignancy of plasma cells characterized by unlimited proliferation of abnormal plasma cells in bone marrow and high levels of monoclonal protein in the blood. MM accounts for approximately 10% of hematologic malignancies and 1% of all cancers[1,2]. Myeloma patients usually have bone lesions, hypercalcemia, severe immunodeficiency and susceptibility to bacterial infections[3]. MM is the second most common hematologic malignancy in the United States[3]. The 5-year survival rate of MM after surgery is only 44.9%[4]. Therefore, uncovering therapeutic biomarkers and agents in MM would supply new insights for the diagnosis and treatment of MM.

Numerous studies have been done to prevent and treat myeloma in MM patients. The B-lymphocyte stimulator is determined to be a biomarker for the diagnosis and treatment of MM[5]. A high free light chain ratio is a predictor of imminent progression in smoldering MM[6]. It has been reported[7] that gene B-cell CLL/lymphoma 2 (*BCL2*) is a diagnostic biomarker in MM due to controlling the NF-κB activation-signaling pathway. Currently, some agents have been used in MM therapy. Bortezomib is a selective proteasome inhibitor that has shown encouraging results in patients with MM and other malignant diseases[8]. Lund et al[9] reported that addition of thalidomide to melphalan and prednisone treatment prolonged survival in MM. However, they may cause side effects, such as polyneuropathy, skin rash, fatigue, and venous thromboembolism[10]. Thus, it is essential to develop a safer drug for the treatment of MM. Given the limitation of these studies, we have taken into consideration all differentially expressed genes (DEGs) to explore new potential therapeutic biomarkers in MM.

The microarray data (GSE36474) were analyzed with Biometric Research Branch ArrayTools (National Cancer Institute, Bethesda, MA, USA) to identify DEGs involved in MM by André et al[11]. In this study, we downloaded this data and used limma package to identify the DEGs in bone marrow mesenchymal stromal cells (BM-

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MSCs) between MM and normal samples. Additionally, other methods were performed to analyze this data, including hierarchical clustering analysis, functional enrichment analysis, co-expression network construction and identification of small molecular agents. The purpose of this study was to identify new potential therapeutic biomarkers and agents in MM.

Materials and Methods

Affymetrix Microarray Data

The gene expression profile data of GSE36474 based on the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) were obtained from Gene Expression Omnibus (GEO) database in National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/), which was deposited by André et al[11]. GSE36474 datasets detected the transcript of BM-MSCs from 4 untreated MM patients (47, 52, 56, 65 years) and 3 healthy donors (48, 50, 58 years).

Data Preprocessing and Differential Expression Analysis

The probe-level data in CEL files were converted into expression measures. The missing data were imputed. Then, the data were quantile normalized with the Affy package[12] in R. The limma package[13] in R was used to identify DEGs between MM and normal samples. Log_2-fold change (log_2FC) was calculated to identify genes with expression-level differences. The p-value < 0.01 and $|log_2FC| > 1$ were used as the cutoff criteria. For hierarchical clustering of samples and identified DEGs, clustering analysis was performed to create a clustering graph of samples and genes with pheatmap package[14] in R using Euclidean distance measure.

Gene Ontology Enrichment Analysis

GO[15] analysis has become a commonly used method for functional studies of large-scale transcriptomic or genomic data. Database for Annotation, Visualization and Integrated Discovery (DAVID)[16] is a gene functional enrichment analysis tool to understand the biological meaning for investigators. GO categories are divided into three systems: molecular function (MF), biological process (BP), and cellular component (CC). In this study, DAVID was used to identify GO categories for BP with false discovery rate (FDR) < 0.05.

Construction of Co-Expression Network

The genes with similar expression profiles are considered to have the common regulatory function and a high degree of correlation[17]. Search Tool for the Retrieval of Interacting Genes (STRING)[18] is an online database which collects comprehensive information of proteins. Genes in the most significant GO terms were used to construct the co-expression network. The STRING online tool was applied to analyze the co-expressed gene pairs. In the network, genes represent nodes and the interactions between the nodes represent edges. Connectivity degree represents the number of edges linked to a given node. The important nodes with high degree in the network were obtained, namely hub nodes. The co-expression value > 0.4 was used as the cutoff criterion. If the co-expressed pairs over 2000, the co-expresses value > 0.8 was used as the cutoff criterion. The co-expression network was constructed with Cytoscape software[19].

Network-Based Pathway Analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) knowledge database[20] is applied to identify the functional and metabolic pathway. KEGG Orthology-Based Annotation System (KOBAS)[21] is a software tool for automated annotation and pathway identification. DEGs in co-expression networks were analyzed in this step. KOBAS was used to identify pathways based on the hypergeometric distribution with p-value < 0.05.

Identification of Small Molecular Agents

Connectivity Map (cMap)[22] is a public database (www.broad.mit.edu/cmap/) which contains 453 Affymetrix gene expression signatures of 164 bioactive molecules. DEGs in co-expression network were compared with the data from the cMap database. The query small molecules were output with a connectivity score from +1 to −1. The high positive connectivity score indicated that the query small molecular induced the disease, while a high negative connectivity score indicated corresponding molecule suppressed disease. The |connectivity score| > 0.8 was used as the cutoff criterion.

Results

Identification of DEGs

As shown in Figure 1, the raw expression data were normalized after preprocessed. Compared

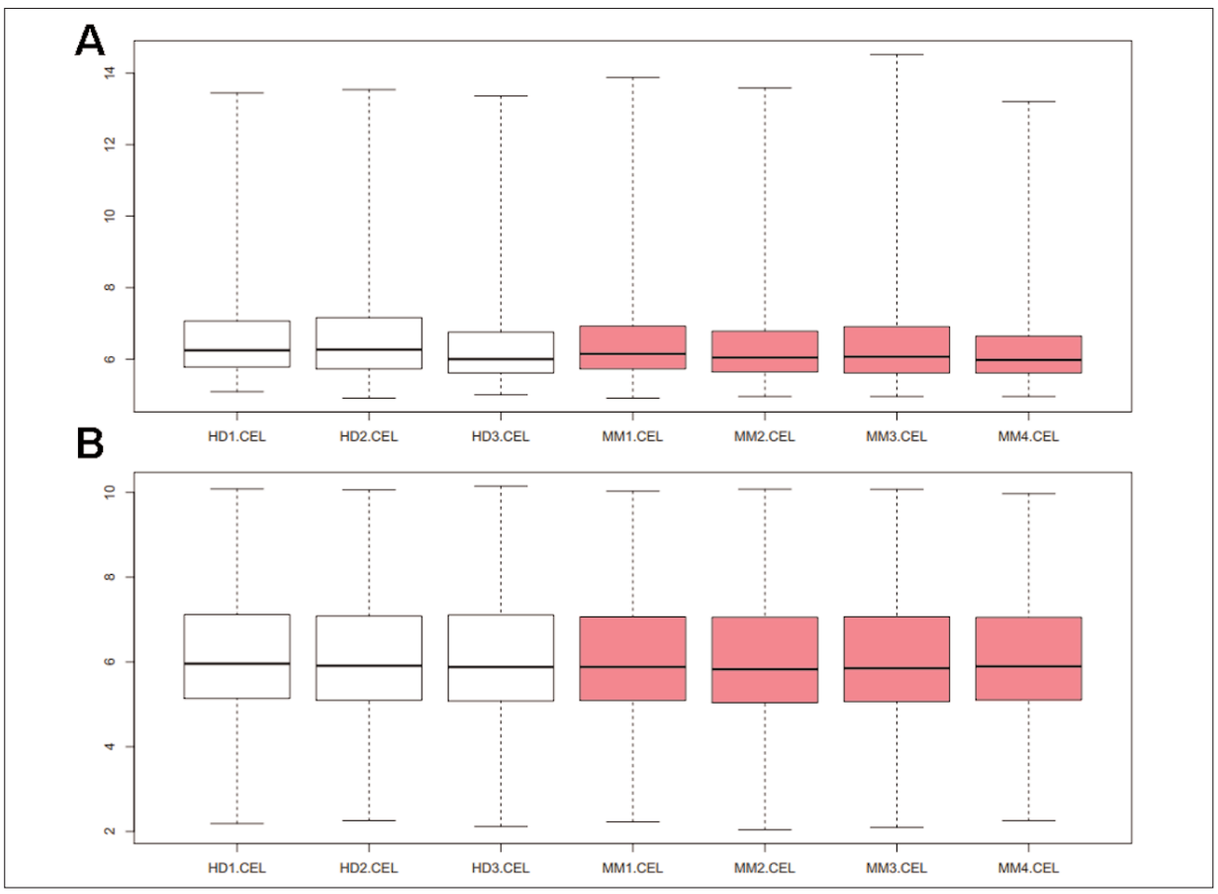

Figure 1. Box plots of data normalization. The x-coordinate represents samples; y-coordinate represents gene expression values. The white box stands for normal sample. The pink box stands for multiple myeloma sample.

with normal samples, a total of 573 genes were differentially expressed in MM samples, including 322 down- and 251 up-regulated genes.

The heat map for DEGs was shown in Figure 2. The clustering analysis results showed that DEGs were classified into 2 clusters. Genes in cluster 1 (red bar in Figure 2) were up-regulated in MM samples, while genes in cluster 2 (blue bar in Figure 2) were down-regulated in MM samples.

Gene Ontology Enrichment Analysis

The GO BP terms of down- and up-regulated genes were shown in Table I. The down-regulated genes were significantly related to cell cycle, M phase and cell cycle phase. A total of 125 DEGs were enriched in the GO term of cell cycle. On the other hand, the up-regulated genes were mainly enriched in immune response and response to the virus. A total of 23 up-regulated genes were identified in immune response function.

Construction of Co-Expression Network

As shown in Figure 3, the cell cycle-related network (A) was constructed with 35 nodes and 213 edges (co-expresses value > 0.8). The immune response-related network (B) was constructed with 13 nodes and 23 edges and up-regulated gene guanylate binding protein 1, interferon-inducible (*GBP1*, degree = 8) was a hub node in this network.

Network-Based Pathway Analysis

A total of 2 pathways enriched by down-regulated genes were obtained in this analysis (Table II), including cell cycle and oocyte meiosis pathways. Genes such as checkpoint kinase 1 (*CHEK1*), MAD2 mitotic arrest deficient-like 1 (*MAD2L1*), DBF4 zinc finger (*DBF4*), TTK protein kinase (*TTK*, alias *MSP1*), extra spindle pole bodies homolog 1 (*ESPL1*) and cyclin A2 (*CCNA2*) were identified in cell cycle pathway.

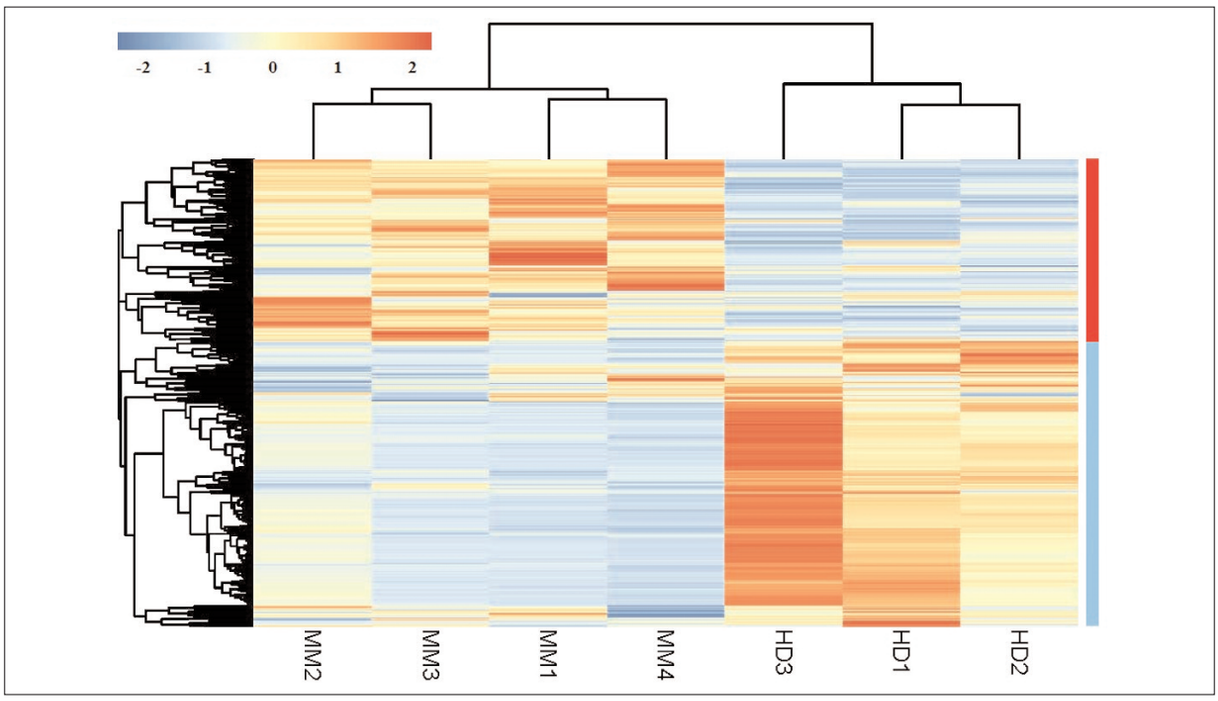

Figure 2. Heat map and clustering analysis of the differentially expressed genes (DEGs) between multiple myeloma and normal samples. The dendrogram to the left of the heat map shows clustering of the DEGs. The dendrogram above the heat map shows clustering of the samples. Red color represents high expression level, and blue color represents low expression level. The DEGs were mainly classified into 2 clusters. The red bar represents the cluster 1 and the blue bar represents the cluster 2.

Table I. The Gene Ontology (GO) biological process terms enriched by down- and up-regulated differentially expressed genes (DEGs).

GO category	GO term	Count	*p*-value	FDR
Down-regulated DEGs				
GO:0007049	Cell cycle	125	2.65E-85	4.43E-82
GO:0000279	M phase	89	9.32E-79	1.56E-75
GO:0022403	Cell cycle phase	95	3.06E-77	5.12E-74
GO:0022402	Cell cycle process	103	9.49E-74	1.59E-70
GO:0007067	Mitosis	68	2.70E-63	4.51E-60
GO:0000280	Nuclear division	68	2.70E-63	4.51E-60
GO:0000087	M phase of mitotic cell cycle	68	1.07E-62	1.78E-59
GO:0048285	Organelle fission	68	5.70E-62	9.53E-59
GO:0051301	Cell division	74	7.07E-62	1.18E-58
GO:0000278	Mitotic cell cycle	80	8.10E-62	1.35E-58
GO:0006259	DNA metabolic process	71	4.65E-41	7.77E-38
GO:0007049	Cell cycle	125	2.65E-85	4.43E-82
GO:0000279	M phase	89	9.32E-79	1.56E-75
GO:0022403	Cell cycle phase	95	3.06E-77	5.12E-74
GO:0022402	Cell cycle process	103	9.49E-74	1.59E-70
GO:0007067	Mitosis	68	2.70E-63	4.51E-60
GO:0000280	Nuclear division	68	2.70E-63	4.51E-60
Up-regulated DEGs				
GO:0006955	Immune response	23	6.95E-05	0.011488
GO:0009615	Response to virus	10	1.06E-05	0.017506
GO:0001568	Blood vessel development	11	0.001215	0.019902
GO:0001944	Vasculature development	11	0.001465	0.023955
GO:0007389	Pattern specification process	11	0.002301	0.037382

Count: enriched gene number in the GO category; FDR: false discovery rate.

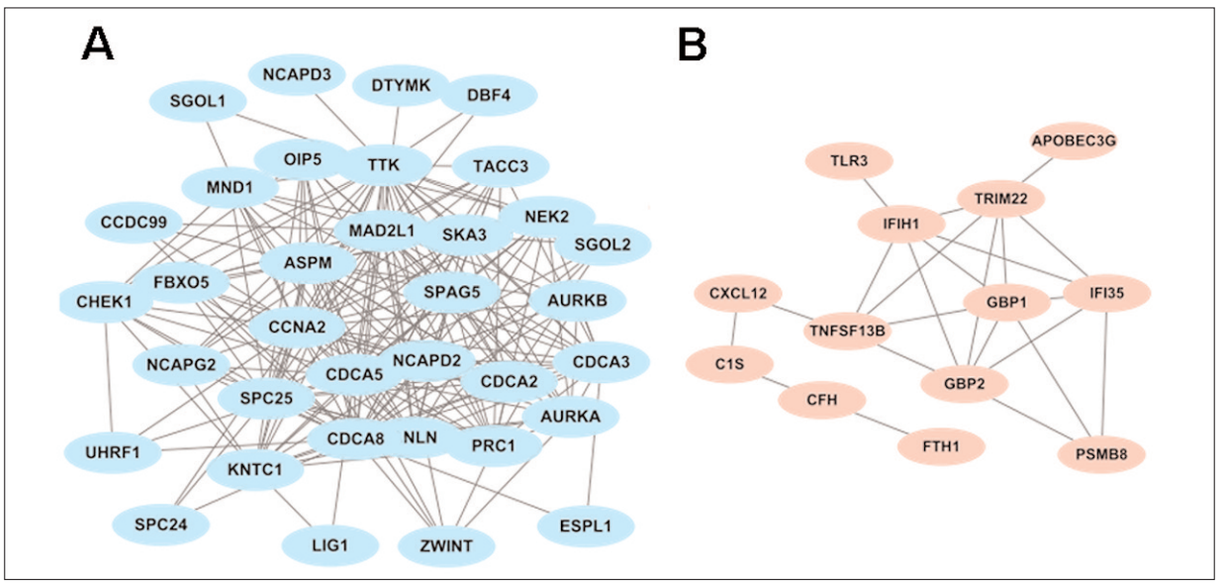

Figure 3. The co-expression networks. ***A,*** Cell cycle-related network. Blue node represents down-regulated genes. ***B,*** Immune response-related network. Red node represents up-regulated genes.

Table II. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes in the co-expression networks.

KEGG pathway term	Genes	*p*-value	FDR
Cell cycle	MAD2L1, DBF4, TTK, CHEK1, ESPL1, CCNA2	1.89E-06	0.001089
Oocyte meiosis	MAD2L1, SGOL1, FBXO5, ESPL1, AURKA	3.94E-05	0.022668

FDR: false discovery rate.

Identification of Small Molecular Agents

A total of 11 small molecular agents were obtained, such as vinblastine, MS-275, menadione and quinostatin. As shown in Table III, the small molecular agent vinblastine (connectivity score = -0.922) had the highest negative score.

Discussion

In this study, the gene expression profile data of GSE36474 were downloaded from GEO database to identify DEGs between MM and normal samples using bioinformatics analysis. In total, 573 DEGs including 322 down- and 251 up-regulated genes were selected in MM samples. Down-regulated genes were related to cell cycle, while up-regulated genes were mainly enriched in the immune response. In order to better understand the interactions of DEGs, we established the co-expression network with significant gene pairs. The KEGG pathway analysis indicated that cell cycle was the most enriched pathway, which was responding to the results of GO analysis. Down-regulated genes such as *CHEK1*, *MAD2L1* and *DBF4* were identified in this function. Moreover, up-regulated gene *GBP1* was a hub node in

Table III. The list of small molecular agents.

cMap name	Score	*p*-value
Vinblastine	-0.922	0.00004
Irinotecan	0.904	0.00176
Withaferin A	0.905	0.00008
Puromycin	0.906	0.00008
Rottlerin	0.921	0.00112
1,4-chrysenequinone	0.935	0.00817
Piperlongumine	0.955	0.00374
Quinostatin	0.962	0.00256
0297417-0002B	0.969	0.00004
Menadione	0.972	0.00133
MS-275	1	0

the immune response-related network. In addition, small molecular agent vinblastine was identified as one important potential therapeutic agent in MM.

Uncontrolled cell proliferation is the hallmark of MM, and tumor cell has typically acquired damage to genes that directly regulate their cell cycles. *CHEK1* has been reported to regulate the G_0/G_1 phase in human MM cells[23]. *CHEK1* (aka *CHK1*) encodes the serine/threonine protein kinase. Inhibition of *CHEK1* diminishes the S phase arrest and enhances the apoptosis in MM[24]. In this study, we found that *CHEK1* was decreased in MM samples and it was related to cell cycle function. The above studies demonstrated that *CHEK1*, as a negative regulator of apoptosis, might be a molecular target for MM therapeutics.

MAD2L1 plays an important role in maintaining the mitotic spindle checkpoint function, which is corresponding to the GO term of the cell cycle in our study. Genetic variant in *MAD2L1* confers susceptibility in lung cancer, which results from reduced spindle checkpoint function due to attenuated function of *MAD2L1*[25]. Decreased expression of *MAD2L1* is reported in breast cancer cell lines exhibiting chromosome instability and aneuploidy[26,27]. The work of Shaughnessy et al[28] found that *MAD2L1* was a significant gene related to chromosome segregation (a phase of the cell cycle) in MM. In this study, *MAD2L1* was a down-regulated gene in MM and enriched in the most significant function of cell cycle. Thus, we inferred that *MAD2L1* might be associated with MM development via mediating cell cycle. It may be a potential therapeutic biomarker in MM.

DBF4 encoding a serine-threonine kinase has been reported to interact with cell division cycle 7-related protein kinase (*CDC7*)[29]. The *CDC7-DBF4* complex is an essential kinase for regulating initiation of DNA replication and plays a central role in cell proliferation[29,30]. Bonte et al[31] reported that *CDC7-DBF4* overexpression in multiple cancers and tumor cell lines was correlated with *p53* inactivation. *P53*, a tumor suppressor, plays a key role in inhibiting the tumor growth[32]. Inactivation of *p53* has been reported to be related to MM development or progression[33]. In the present study, *DBF4* was a down-regulated gene, suggesting that reduced expression of *CDC7-DBF4* might lead to *p53* activation. Therefore, we inferred that *DBF4* might be effective in controlling tumor growth and cell proliferation in MM.

In addition to cell cycle-related genes known to be down-regulated in MM, up-regulated gene *GBP1* was a hub node in the immune response-related network. *GBP1* encodes an interferon-inducible GTPase that belongs to the dynamin family of guanine nucleotide binding proteins[34]. The immune response gene *GBP1* is the key mediator of the inhibitory effects of inflammatory cytokines (e.g., interleukin-6, transforming growth factor-beta and interleukin-17) on endothelial cells proliferation and invasiveness[35]. Elevated interleukin-17 promotes myeloma cell growth and inhibits immune function in MM[36]. It also has been reported that *GBP1* expression is inhibited by vascular endothelial growth factor (*VEGF*)[37]. *VEGF* stimulates proliferation and migration of MM cells in both autocrine and paracrine mechanisms[38]. *GBP1* was found to be up-regulated in MM and closely associated with immune response. Overexpressed *GBP1* appeared to decrease MM cell proliferation and growth to some extent.

Furthermore, vinblastine was identified to be the significant small molecular agent in the development of MM. Vinblastine, an antimicrotubule drug, belongs to *vinca* alkaloids. It has been reported that *vinca* alkaloids inhibit cell proliferation by altering the dynamics of tubulin addition and loss at the ends of mitotic spindle microtubules[39]. Vinblastine has been widely used as an antineoplastic drug to treat certain kinds of cancer, including breast cancer[40], Hodgkin's lymphoma[41], non-small cell lung cancer[42], head and neck cancer[43], and testicular cancer[44]. However, the evidence concerning the drug application of vinblastine in MM is rare[45]. In our study, the small molecular drug vinblastine had the highest negative score, implying the capable of reversing MM. Therefore, it may be a potential novel agent for the treatment of MM. Further animal experiments and clinical trials are needed to investigate the usage and dosage of vinblastine in MM.

Conclusions

Our study shows that cell cycle may be closely associated with MM development. The genes such as *CHEK1*, *MAD2L1*, *DBF4* and *GBP1* may be the potential therapeutic biomarkers in MM. Vinblastine may be a potential therapeutic agent in MM. These findings would supply new insights for the diagnosis and treatment of MM. However, further experiments with larger sample size are still needed to confirm our results.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Kyle R, Rajkumar S. Multiple myeloma. Blood 2008; 111: 2962-2972.
2) Rajkumar SV. Multiple myeloma: 2011 update on diagnosis, risk-stratification, and management. Am J Hematol 2011; 86: 57-65.
3) Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. Lancet 2009; 374: 324-339.
4) Institute NC. Seer stat fact sheets: Myeloma 2014.
5) Jiang P, Yueguo W, Huiming H, Hongxiang Y, Mei W, Ju S. B-lymphocyte stimulator: a new biomarker for multiple myeloma. Eur J Haematol 2009; 82: 267-276.
6) Larsen JT, Kumar SK, Dispenzieri A, Kyle RA, Katzmann JA, Rajkumar SV. Serum free light chain ratio as a biomarker for high-risk smoldering multiple myeloma. Leukemia 2013; 27: 941-946.
7) Feinman R, Koury J, Thames M, Barlogie B, Epstein J, Siegel DS. Role of NF-kappaB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. Blood 1999; 93: 3044-3052.
8) Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, Harousseau JL, Ben-Yehuda D, Lonial S, Goldschmidt H, Reece D, San-Miguel JF, Bladé J, Boccadoro M, Cavenagh J, Dalton WS, Boral AL, Esseltine DL, Porter JB, Schenkein D, Anderson KC; Assessment of Proteasome Inhibition for Extending Remissions (APEX) Investigators. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005; 352: 2487-2498.
9) Lund J, Uttervall K, Liwing J, Gahrton G, Alici E, Aschan J, Holmberg E, Nahi H. Addition of thalidomide to melphalan and prednisone treatment prolongs survival in multiple myeloma–a retrospective population based study of 1162 patients. Eur J Haematol 2014; 92: 19-25.
10) Haas PS, Denz U, Ihorst G, Engelhardt M. Thalidomide in consecutive multiple myeloma patients: single-center analysis on practical aspects, efficacy, side effects and prognostic factors with lower thalidomide doses. Eur J Haematol 2008; 80: 303-309.
11) André T, Meuleman N, Stamatopoulos B, De Bruyn C, Pieters K, Bron D, Lagneaux L. Evidences of early senescence in multiple myeloma bone marrow mesenchymal stromal cells. PLoS One 2013; 8: e59756.
12) Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2012.
13) Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol 2004; 3: Article3.
14) Wang L, Cao C, Ma Q, Zeng Q, Wang H, Cheng Z, Zhu G, Qi J, Ma H, Nian H, Wang Y. RNA-seq analyses of multiple meristems of soybean: novel and alternative transcripts, evolutionary and functional implications. BMC Plant Biol 2014; 14: 169.
15) Hulsegge I, Kommadath A, Smits MA. Globaltest and GOEAST: two different approaches for Gene Ontology analysis. BMC Proc 2009; 3 Suppl 4: S10
16) Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.
17) Fox AD, Hescott BJ, Blumer AC, Slonim DK. Connectedness of PPI network neighborhoods identifies regulatory hub proteins. Bioinformatics 2011; 27: 1135-1142.
18) Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen LJ, von Mering C. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 2011; 39: D561-D568.
19) Tanzer A, Stadler PF. Molecular evolution of a microRNA cluster. J Mol Biol 2004; 339: 327-335.
20) Altermann E, Klaenhammer TR. PathwayVoyager: pathway mapping using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. BMC Genomics 2005; 6: 60.
21) Wu J, Mao X, Cai T, Luo J, Wei L. KOBAS server: a web-based platform for automated annotation and pathway identification. Nucleic Acids Res 2006; 34: W720-W724.
22) Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, Wrobel MJ, Lerner J, Brunet JP, Subramanian A, Ross KN, Reich M, Hieronymus H, Wei G, Armstrong SA, Haggarty SJ, Clemons PA, Wei R, Carr SA, Lander ES, Golub TR. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science 2006; 313: 1929-1935.
23) Pei X-Y, Dai Y, Youssefian LE, Chen S, Bodie WW, Takabatake Y, Felthousen J, Almenara JA, Kramer LB, Dent P, Grant S. Cytokinetically quiescent (G0/G1) human multiple myeloma cells are susceptible to simultaneous inhibition of Chk1 and MEK1/2. Blood 2011; 118: 5189-5200.
24) Tu YS, Kang XL, Zhou JG, Lv XF, Tang YB, Guan YY. Involvement of Chk1-Cdc25A-cyclin A/CDK2 pathway in simvastatin induced S-phase cell cycle arrest and apoptosis in multiple myeloma cells. Eur J Pharm 2011; 670: 356-364.
25) Guo Y, Zhang X, Yang M, Miao X, Shi Y, Yao J, Tan W, Sun T, Zhao D, Yu D, Liu J, Lin D. Functional evaluation of missense variations in the human MAD1L1 and MAD2L1 genes and their impact on susceptibility to lung cancer. J Med Genet 2010; 47: 616-622.

26) Percy MJ, Myrie KA, Neeley CK, Azim JN, Ethier SP, Petty EM. Expression and mutational analyses of the human MAD2L1 gene in breast cancer cells. Genes Chromosomes Cancer 2000; 29: 356-362.

27) Li Y, Benezra R. Identification of a human mitotic checkpoint gene: hsMAD2. Science 1996; 274: 246-248.

28) Shaughnessy J Jr. Primer on medical genomics. Part IX: scientific and clinical applications of DNA microarrays--multiple myeloma as a disease model. Mayo Clinic Proc 2003; 78: 1098-1109.

29) Jiang W, McDonald D, Hope TJ, Hunter T. Mammalian Cdc7–Dbf4 protein kinase complex is essential for initiation of DNA replication. EMBO J 1999; 18: 5703-5713.

30) Wan L, Niu H, Futcher B, Zhang C, Shokat KM, Boulton SJ, Hollingsworth NM. Cdc28-Clb5 (CDK-S) and Cdc7-Dbf4 (DDK) collaborate to initiate meiotic recombination in yeast. Gene Dev 2008; 22: 386-397.

31) Bonte D, Lindvall C, Liu H, Dykema K, Furge K, Weinreich M. Cdc7-Dbf4 kinase overexpression in multiple cancers and tumor cell lines is correlated with p53 inactivation. Neoplasia 2008; 10: 920-931.

32) Harris CC. Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J Natl Cancer Inst 1996; 88: 1442-1455.

33) Drach J, Ackermann J, Fritz E, Krömer E, Schuster R, Gisslinger H, DeSantis M, Zojer N, Fiegl M, Roka S. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. Blood 1998; 92: 802-809.

34) Prakash B, Praefcke GJ, Renault L, Wittinghofer A, Herrmann C. Structure of human guanylate-binding protein 1 representing a unique class of GTP-binding proteins. Nature 2000; 403: 567-571.

35) Guenzi E, Töpolt K, Lubeseder-Martellato C, Jörg A, Naschberger E, Benelli R, Albini A, Stürzl M. The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. EMBO J 2003; 22: 3772-3782.

36) Prabhala RH, Pelluru D, Fulciniti M, Prabhala HK, Nanjappa P, Song W, Pai C, Amin S, Tai YT, Richardson PG, Ghobrial IM, Treon SP, Daley JF, Anderson KC, Kutok JL, Munshi NC. Elevated IL-17 produced by TH17 cells promotes myeloma cell growth and inhibits immune function in multiple myeloma. Blood 2010; 115: 5385-5392.

37) Britzen-Laurent N, Stürzl M. Gene section. http://AtlasGeneticsOncology. org 2010: 761.

38) Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T, Lin BK, Gupta D, Shima Y, Chauhan D, Mitsiades C, Raje N, Richardson P, Anderson KC. Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. Blood 2001; 98: 428-435.

39) Jordan MA, Thrower D, Wilson L. Mechanism of inhibition of cell proliferation by vinca alkaloids. Cancer Res 1991; 51: 2212-2222.

40) Ghanbari P, Samadi N, Tabasinezhad M, Mohseni M, Yousefi B, Rashidi M. Adjuvant therapy improves the chemotherapeutic effect of docetaxel and vinblastine in breast cancer cells. Eur J Cancer 2014; 50: S209-S209

41) Engert A, Franklin J, Eich HT, Brillant C, Sehlen S, Cartoni C, Herrmann R, Pfreundschuh M, Sieber M, Tesch H, Franke A, Koch P, de Wit M, Paulus U, Hasenclever D, Loeffler M, Müller RP, Müller-Hermelink HK, Dühmke E, Diehl V. Two cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine plus extended-field radiotherapy is superior to radiotherapy alone in early favorable Hodgkin's lymphoma: final results of the GHSG HD7 trial. J Clin Oncol 2007; 25: 3495-3502.

42) Smith IE, O'Brien ME, Talbot DC, Nicolson MC, Mansi JL, Hickish TF, Norton A, Ashley S. Duration of chemotherapy in advanced non–small-cell lung cancer: A randomized trial of three versus six courses of mitomycin, vinblastine, and cisplatin. J Clin Oncol 2001; 19: 1336-1343.

43) Brown AW, Blom J, Butler WM, Garcia-Guerrero G, Richardson MF, Henderson RL. Combination chemotherapy with vinblastine, bleomycin, and cis-diamminedichloroplatinum (II) in squamous cell carcinoma of the head and neck. Cancer 1980; 45: 2830-2835.

44) Stoter G, Koopman A, Vendrik C, Struyvenberg A, Sleyfer DT, Willemse P, Koops HS, van Oosterom AT, ten Bokkel Huinink WW, Pinedo HM. Ten-year survival and late sequelae in testicular cancer patients treated with cisplatin, vinblastine, and bleomycin. J Clin Oncol 1989; 7: 1099-1104.

45) Costa G, Carbone PP, Gold GL, Owens AH Jr, Miller SP, Krant MJ, Bono VH Jr. Clinical trial of vinblastine in multiple myeloma. Cancer Chemother Rep 1963; 27: 87-89.