Identification of key genes in relapsed multiple myeloma by weighted gene co-expression network analysis

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Research

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Abstract

Background

Multiple myeloma is a hematologic disorder of abnormal plasma cell proliferation. Although there are some agents with different mechanisms in the clinic, the treatment of multiple myeloma is still challenging for the reason that its recurrence and progression are common. Therefore, it is critical to determine novel biomarkers to improve the prognosis of patients.

Methods

Firstly, raw data of GSE82307 was collected from the Gene Expression Omnibus database. Secondly, the top 50% of most variant genes were employed to construct a gene co-expression network in the R.WGCNA algorithm, and module significance and module membership were utilized to identify hub modules and hub genes respectively. The gene ontology enrichment and Kyoto encyclopedia of genes and genomes pathway analysis were carried out to assess biological characteristics. Then a protein-protein interaction network was conducted based on the STRING website and Cytoscape software. Next, differentially expressed genes were analyzed using the limma R package. Finally, survival analysis was performed by Kaplan–Meier plotter to evaluate prognosis.

Results

10826 genes were used to construct a co-expression network. In this network, the blue module was identified as a hub module in which 68 genes were identified as hub genes. Furthermore, 46 differentially expressed genes were screened in samples of GSE82307. Integrating hub genes and differentially expressed genes, we determined 14 key genes. Finally, survival analysis revealed that ten genes CDCA5, CEP55, HJURP, CDC20, FOXM1, RRM2, TTK, CENPE, SKA1, NUF2 were related to the relapse and prognosis of multiple myeloma.

Conclusion

Our study suggested that CDCA5, CEP55, HJURP, CDC20, FOXM1, RRM2, TTK, CENPE, SKA1, NUF2 may be potential biomarkers for predicting the relapse and prognosis of multiple myeloma.

1. Introduction

Multiple myeloma(MM) is a systemic hematological malignancy that is characterized by the uncontrolled growth of monoclonal plasma cells in the bone marrow. The main clinical manifestations of MM are osteolytic lesions, hypercalcemia, anemia, and kidney damage. MM is the second most common hematologic malignancy, second only to non-Hodgkin's lymphoma, accounting for 13%[1]. And its median

age of onset is 63 ~ 70 years old[2]. In the past 20 years, the application of autologous stem cell transplantation after high-dose chemotherapy, immunomodulator(IMiD), proteasome inhibitor has significantly improved the prognosis of patients[3–5]. Nonetheless, MM is still an incurable biologically heterogeneous disease of plasma cells, and most patients will eventually relapse or progress. When a patient newly develops hypercalcemia, kidney damage, worsening anemia, bone damage, extramedullary disease, or a 'significant' biochemical relapse, we consider that this patient is in a state of relapse[6]. The causes of the recurrence of MM have also aroused widespread concern. Recurrence is the result of multiple factors in myeloma. These factors mainly contain acquired drug resistance and continued acceleration of tumor cell proliferation which are caused by additional genetic mutations or genetic changes[7]. Therefore, the remission time after each salvage treatment is gradually shortened, eventually developing into relapsed MM. However, there is a lack of biomarkers for recurrence or progression of myeloma. Revealing candidate targets will provide tremendous help to the diagnosis, treatment, and prognosis of myeloma patients.

A large amount of genomic information is accumulated exponentially, with the continuous advancement of biological research techniques, especially the gradual development of sequencing technology and biological information algorithms[8, 9]. Weighted gene co-expression network analysis(WGCNA) is a systematic biological approach for appraising gene association modes among different samples[10]. WGCNA has been successfully used not only to explore the connection between genetic databases and clinical characterization but also to identify candidate biomarkers. However, the genetic difference between stable and relapsed MM patients remains unclear. Moreover, we need new biomarkers to better understand mechanisms of MM relapse and predict prognosis. Besides, Gene Expression Omnibus(GEO) is a public functional genomic database composing of an array- and sequence-based data. Therefore, we collected a GSE82307 gene expression profile submitted by Weinhold N et al[11] from GEO(http://www.ncbi.nlm.nih.gov/geo). Furthermore, we elucidated correlation patterns between genes through WGCNA-based systematic biology methods and identified new biomarkers related to relapsed MM.

2. Methods

2.1 Acquisition of microarray data

GSE82307 is an array- and sequence-based data profile dependent on the GPL570 platform(Affymetrix Human Genome U133 Plus 2.0 Array) including 66 gene expression datasets from 33 patients' samples collected at presentation and relapse respectively. By employing Affy and Limma packages in Bioconductor(http://www.bioconductor.org) in R software to read the array spectrum and standardize, as well as using the Robust Multi-Array Average algorithm, we acquired 21,653 genes for subsequent analysis.

2.2 Construction of co-expression network

According to variance analysis, the top 50% (10826 genes) of most mutated genes were selected for further WGCNA. We utilized the WGCNA package to build a co-expression network of 10826 genes in R software. The objective of the co-expression network was to reveal the correlation between genes and to identify highly relevant gene modules. Firstly, the Pearson correlation matrix was constructed by using the absolute value of the correlation coefficient between genes. Then we used the power function to construct a weighted adjacency matrix. An appropriate soft threshold power β was selected to convert the adjacency matrix to a topological overlap matrix. At last, we employed hierarchical clustering to generate a hierarchical clustering tree of genes.

2.3 Identification of hub modules and hub genes

Gene significance referred to the absolute value of the correlation between a gene and a clinical feature[12]. The module significance referred to the average value of gene significance of all genes in a module. These related values were displayed within a heatmap. Statistically significant modules were defined as p-value < 0.05. In WGCNA, a module with the highest module significance score was generally defined as a hub module and chosen for further analysis[10, 13].

Hub genes were defined as genes with the highest correlation with clinical characteristics in a hub module. Module membership referred to the correlation between module eigengenes and gene expression profile[12]. Measured by Pearson correlation, the module membership score of hub genes was considered to be higher.

2.4 Analysis of functional enrichment and pathway

To better explore the biological significance of genes in hub modules, enrichment of functions and pathways was analyzed using the cluster profiler. Cluster profiler is a package with an analysis and visualization function to provide valuable information on gene ontology(GO) enrichment and Kyoto encyclopedia of genes and genomes(KEGG) pathway analyses[14, 15]. Uploading gene list of hub modules, we obtained results of biological process(BP), cellular component(CC), molecular function(MF), as well as KEGG pathway enrichment analysis. A p-value < 0.05 was considered to be significantly enriched.

2.5 Construction of protein-protein interaction (PPI) network

After uploading hub genes to Interaction Gene/Protein Search Tool (STRING) online database, a network of correlation between proteins encoded by hub genes was constructed.

2.6 Screening of differentially expressed genes(DEGs)

Using the Limma R package[16], we filtered DEGs between 33 MM patients at two treatment time points in GSE82307. Genes with |log2 Fold Change|value > 1 and adj.P.Value < 0.05 were defined as DEGs.

2.8 Survival analysis

The key genes are cross genes of hub genes obtained by the WGCNA module and DEGs. To further investigate the prognostic value of key genes for MM, we performed Kaplan-Meier curves of key genes by the R survival package. A p-value < 0.05 was considered to be statistically significant.

3. Results

3.1 Construction of co-expression network and identification of hub modules and hub genes

The appropriate soft threshold power was chosen to be 9, which was based on the criterion of an approximate scale-free topology fit index 0.93(Fig. 1). We applied the R package of “WGCNA” to construct a weighted co-expression network. A total of 27 modules were identified, and the gene clustering was displayed as a dendrogram in Fig. 2. The identification of hub modules was based on module significance. Modules with the highest module significance score were usually defined as hub modules. According to the analysis of the module-feature relationship, the blue module(score.ModuleSignificance = 0.37, p = 0.003) had the strongest correlation with recurrent MM(Fig. 3). Furthermore, 68 highly connected genes in the blue module were recognized as representative genes exhibiting potential critical functions in relapsed MM (score.ModuleMembership > 0.8, score.GeneSignificance > 0.2)(Fig. 4).

3.2 Analysis of functional enrichment and pathway and construction of PPI network

We performed GO enrichment and KEGG pathway analysis to comprehend the biological characteristics of the above-mentioned 68 genes. A p-value < 0.05 was considered to be significantly enriched. The top ten GO enrichment and KEGG pathway analysis were enumerated below(Fig. 5). In the GO enrichment analysis, 68 genes were mainly concentrated on organelle fission, chromosomal region, and catalytic activity, acting on DNA. In the KEGG pathway analysis, these genes were mainly enriched in the cell cycle, Human T-cell leukemia virus 1 infection, and DNA replication pathways. Through these dramatically rich biological functions and pathways, we can further investigate the effects of these genes on MM.

Then we uploaded 68 genes to Interaction Gene/Protein Search Tool(STRING) online database and utilized Cytoscape software to structure a PPI network for further analysis. The relationship network between proteins encoded by 68 hub genes was shown in the figure below(Fig. 6).

3.3 Screening of DEGs

We employed the R software package and limma package to filter out DEGs in 33 MM patients at presentation and relapse in GSE82307(Fig. 7A). With a threshold at |log2 Fold Change|>1 and adj.P.Value < 0.05, a total of 46 DEGs were screened and consisted of 16 down-regulated genes and 30 up-regulated genes(Fig. 7B).

3.4 Identification of key genes and survival analysis

WGCNA identified 68 hub genes. Subsequently, DEGs analysis revealed 46 DEGs in 33 MM patients at presentation and relapse. In order to better capture valuable clues from these results, key genes were defined as cross genes of hub genes obtained by the WGCNA module and DEGs. Among a total of 97 genes, 14 genes BIRC5, CDCA5, CENPE, RRM2, TTK, FOXM1, HJURP, CEP55, PBK, UHRF1, SKA1, NUF2, CDC20, TACC3 were filtered out as key genes(Fig. 8). To further investigate the influences of key genes on overall survival(OS) and event-free survival(EFS) of MM, we adopted a Kaplan-Meier plotter for prognostic analysis(with a P-value□0.05). As a result, patients whose tissues revealed a lower expression of CDC20, CDCA5, CENPE, CEP55, FOXM1, HJURP, NUF2, RRM2, SKA1, TTK showed significantly both longer OS and EFS compared with those with a higher expression(Fig. 9). To sum it up, we considered that the above-mentioned ten key genes could serve as recurrence and prognosis biomarkers of MM.

4. Discussion

Nearly all myeloma patients eventually relapse, even those who experienced a complete response to previous treatment[17]. Therefore, clarifying underlying mechanisms that result in disease recrudesce may turn on new therapeutic approaches for MM. In the study, we firstly constructed a co-expression network of 10826 genes by the WGCNA algorithm based on GSE82307. Through analyzing module significance, module membership, and gene significance, 68 genes were recognized as representative hub genes in the blue hub module. Then performing GO enrichment and KEGG pathway analysis and constructing a PPI network, we comprehended the biological characteristics of hub genes and the relationship among proteins expressed by hub genes. Furthermore, a total of 46 DEGs including 16 down-regulated genes and 30 up-regulated genes were screened in 33 MM patients. At last, 14 cross genes BIRC5, CDCA5, CENPE, RRM2, TTK, FOXM1, HJURP, CEP55, PBK, UHRF1, SKA1, NUF2, CDC20, TACC3 of hub genes obtained by WGCNA and DEGs were filtered out as key genes. Survival analysis indicated patients with a lower expression of CDC20, CDCA5, CENPE, CEP55, FOXM1, HJURP, NUF2, RRM2, SKA1, TTK showed significantly both longer OS and EFS. Thus, the ten key genes might be potential biomarkers for the diagnosis and treatment of MM. The three genes CDCA5, CEP55, HJURP are usually overexpressed in several cancers and strongly correlated with the poor prognosis of patients. Notably, there is no relevant research on the effects of the three genes on MM so far. Apart from that, the remaining seven genes have been studied to varying degrees in MM.

CDCA5 encodes sororin which is a regulatory protein during mitosis of eukaryotic cells. Sororin is pivotal for embryo development, maintenance of cohesion between sister chromatids, and correct chromosomal separation[18]. Sororin gene CDCA5 has been associated with the development and progression of several types of human cancers. In hepatocellular carcinoma, knockdown of CDCA5 by lentivirus-mediated shRNA resulted in cell cycle arrest at the G2/M phase and cell apoptosis [19]. Moreover, higher expression of CDCA5 in hepatocellular carcinoma was associated with increased tumor size, microvascular infiltration, as well as poor prognosis of OS and DFS[20, 21]. Likewise, CDCA5 knockdown could inhibit the growth of esophageal squamous cell carcinoma cells. And the expression of CDCA5 promoted tumor cell proliferation, distant metastasis, drug resistance. The high expression of CDCA5 was related to tumor progression and shorter OS in esophageal squamous cell carcinoma[22]. Guanghou Fu et al. found that overexpression of CDCA5 in UMUC3 cells(bladder cancer cell line) could hasten cell

proliferation and hinder cell apoptosis[23]. In an additional experiment, they also found overexpression of CDCA5 in bladder cancer cells activated PI3K/AKT pathway to promote cell proliferation and inhibited mitochondrial-mediated cell apoptosis through reducing expression of downstream proteins, such as cleaved caspase-3, caspase-9, and cleaved PARP. The results of our study demonstrated that CDCA5 was up-regulated in relapsed MM. And a higher expression of CDCA5 heralded a poor prognosis.

CEP55 is a centrosome- and midbody-associated protein and a key regulator of cytokinesis. Over-expressed CEP55 promotes proliferation, survival, and metastasis of multiple cancer cells at several levels[24]. Some characteristic components of CEP55 are also present in cell cycle and proliferation genes whose overexpression is also detrimental to clinical outcome[24]. CEP55 has been studied in the context of cancers including breast cancer, hepatocellular carcinoma, osteosarcoma et al. In breast cancer, the high CEP55 expression blocked cancer cell apoptosis and contributed to chemotherapy resistance, associated with genomic instability. For breast tumors exhibiting high CEP55 expression, blocking MEK1/2-PLK1 signaling reduced outgrowth of tumors[25]. In hepatocellular carcinoma, CEP55 promoted activation of the JAK2/STAT3 signaling pathway and induced expression of matrix metalloproteinases via the same signaling, which led to disease deterioration. Moreover, CEP55 was highly expressed in hepatocellular carcinoma cells, which heralded a poor prognosis[26]. In osteosarcoma, CEP55 was significantly overexpressed in tumor tissues, which was associated with tumor size, tumor metastasis, and reduced OS. Meanwhile, knockdown of CEP55 inhibited cell proliferation, invasion, and migration[27]. In the data set GSE82307, CEP55 was identified as one of the key genes related to relapsed MM, and the ROC curve indicated that CEP55 was a sign of poor prognosis for MM.

HJURP is a histone chaperone of nucleosomes that plays a dual role in coordinating the recruitment of CENP-A and CENP-C. HJURP assists in the assembly of functional centromeres and mediates chromosome separation and cell division[28]. The dysfunction of HJURP has been confirmed in kind of cancers. HJURP was highly expressed in liver cancer cells and tissues, which was a sign of poor prognosis. The high expression of HJURP promoted the proliferation of liver cancer cells by down-regulating p21 through MAPK/ERK1/2 and AKT/GSK3β pathways[29]. Besides, the up-regulation of HJURP enhanced migration and invasion of liver cancer cells by interacting with sphingosine kinase 1[30]. In glioblastoma, HJURP knockout caused severe clonal ability and survival damage of glioblastoma cell lines. At the same time, these cell lines became more sensitive to radiation therapy after HJURP was reduced[31]. Moreover, HJURP was highly expressed in cells and tissues of pancreatic ductal cell carcinoma. HJURP may contribute to tumor cell growth, migration, invasion, and metastasis via the MDM2/p53 signaling pathway in pancreatic ductal cell carcinoma[32].

In order to further verify the carcinogenic ability and molecular roles of CDCA5, CEP55, HJURP in MM, we will continue to conduct in vivo, in vitro, and clinical research. The remaining seven genes CDC20, FOXM1, RRM2, TTK, CENPE, SKA1, NUF2 have been researched in MM to different depths. In the high-risk group of MM patients, the expression of CDC20 was significantly increased, accompanied by a shorter os and enrichment of genes related to proliferation. Besides, CDC20 knockout in myeloma cell lines could

weaken cell apoptosis[33]. In MM patients showing high FOXM1 expression, both their DFS and OS were reduced. In vivo and in vitro experiments, down-regulation of FOXM1 inhibited the growth of myeloma cells, while up-regulation of FOXM1 resulted in the opposite[34]. RRM2 was significantly up-regulated. In terms of possible mechanisms, RRM2 participated in the proliferation and apoptosis of MM cells through the Wnt/β-catenin signaling pathway[35]. TTK was a kinase related to the prognosis of MM, and its expression was associated with the high-risk group of MM. Besides, TTK inhibitors could significantly undermine the viability and proliferation capacity of myeloma cell lines and could be combined with Melphalan or IMiD as a new treatment strategy[36]. CENPE and SKA1 were upregulated in side population cells of MM, encoding for proteins associated with cell cycle and mitosis[37]. At last, during the culture of the fresh bone marrow of myeloma, down-regulation of NUF2 expression regulating mitosis and transcription was observed, which could reveal ensuing proliferation arrest[38]. However, the three genes CENPE, SKA1, NUF2 are initially found in MM. And their roles remain to be fully elucidated.

In summary, we adopted the WGCNA analysis to determine ten novel biomarkers CDCA5, CEP55, HJURP, CDC20, FOXM1, RRM2, TTK, CENPE, SKA1, NUF2 for recurrence and prognosis of MM. The results are beneficial to shed light on molecular mechanisms of progression and filter out targeted agents.

Abbreviations

MM: Multiple myeloma; IMiD: immunomodulator; WGCNA: Weighted gene co-expression network analysis; GEO: Gene Expression Omnibus; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; BP: biological process; CC: cellular component; MF: molecular function; PPI: protein-protein interaction; DEGs: differentially expressed genes; OS: overall survival; EFS: event-free survival.

Declarations

Acknowledgment

We appreciate the GEO database(GSE82307) for free use.

Authors' contributions

LC and YM drew related charts and co-wrote this article. RH and XW collected biological data. HQ participated in the modifying and proofreading of this article. All authors read and approved the final manuscript.

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Availability of data and materials

The profiles of GSE82307 can download in GEO datasets(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE82307). The algorithm can be obtained by the corresponding author Hui Qin via Email (qinhui6957@sina.com).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

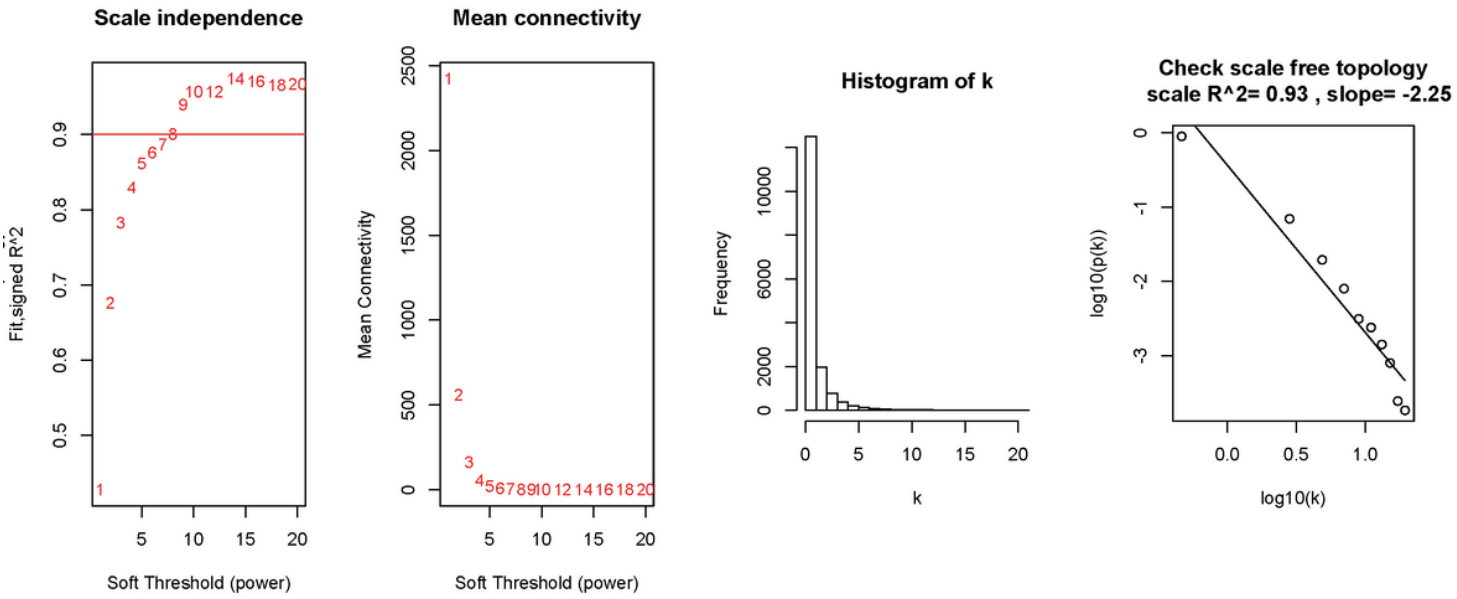


Figure 1

Determination of soft-thresholding power in weighted gene co-expression network analysis. (A) Analysis of scale-free fit index for different soft-thresholding powers (β). Numbers in plots represent corresponding soft-thresholding powers. The approximate scale-free topology can be obtained at a soft-thresholding power of 9. Analysis of mean connectivity for different soft-thresholding powers. (B) Histogram of connectivity distribution when β=9. Checking scale-free topology when β=9.

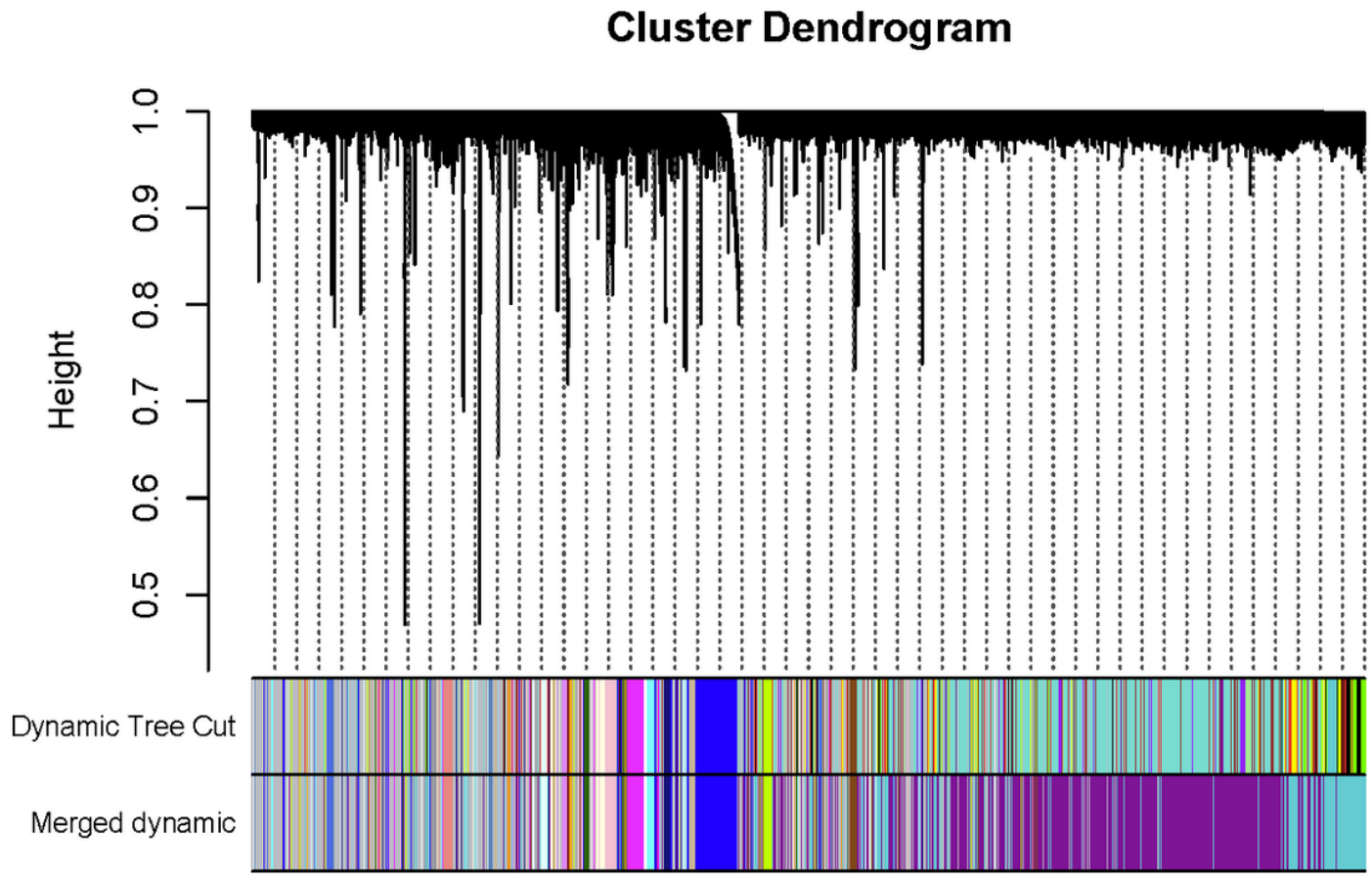


Figure 2

Dendrogram of modules identified by WGCNA.

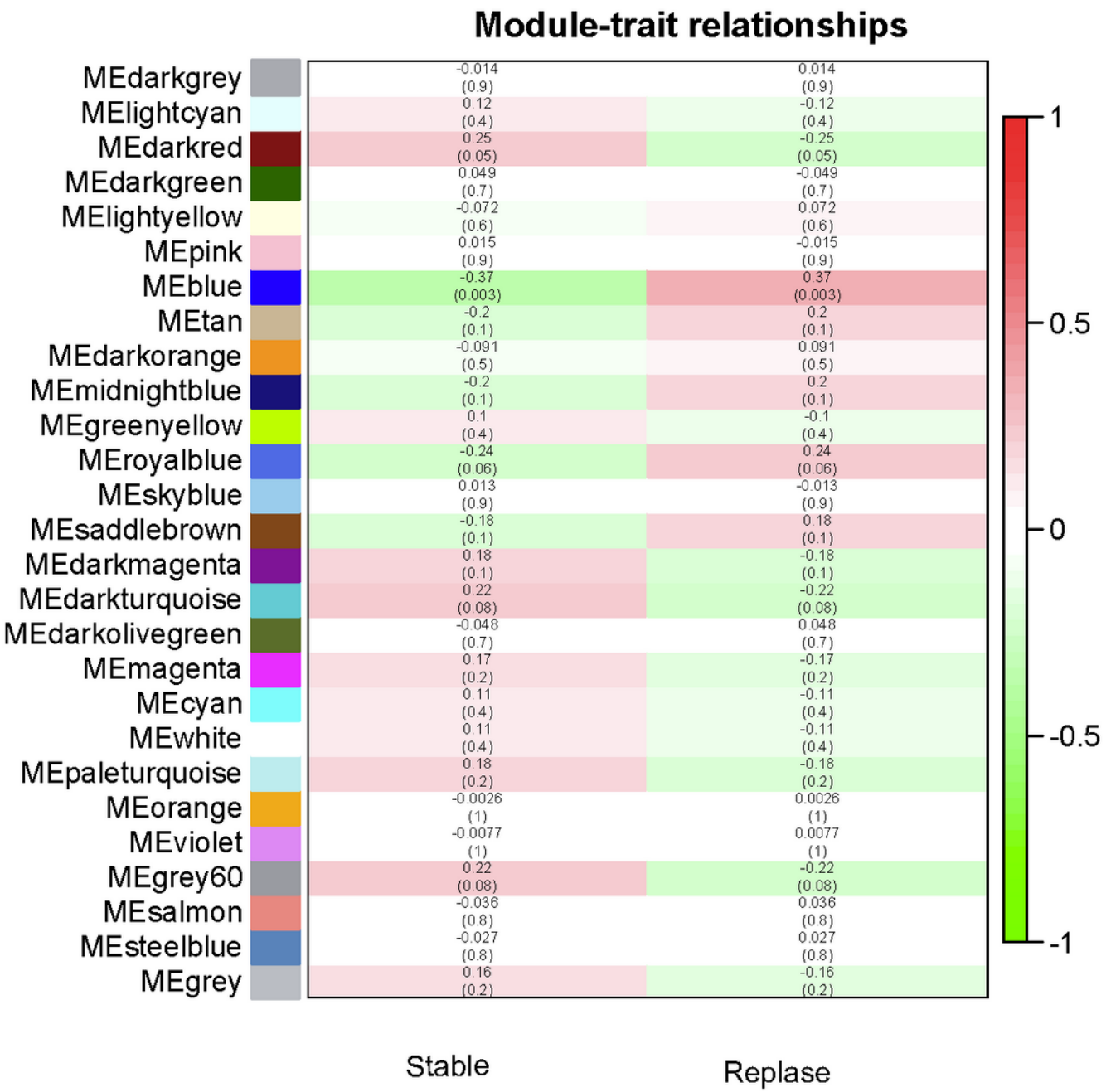


Figure 3

Heatmap of correlation between module genes and the therapeutic reaction of MM. Each module is based on the pattern of their co-expression.

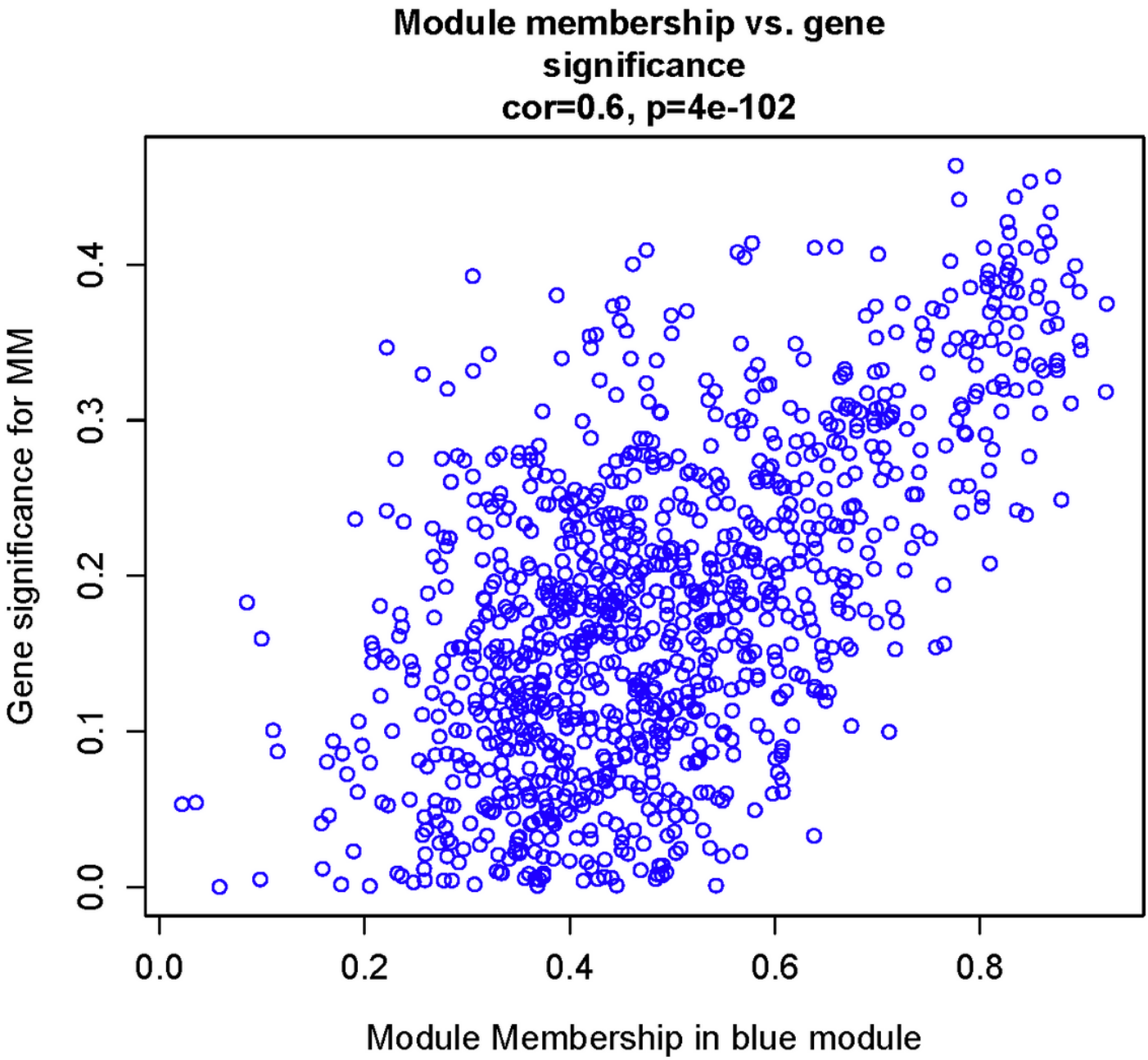
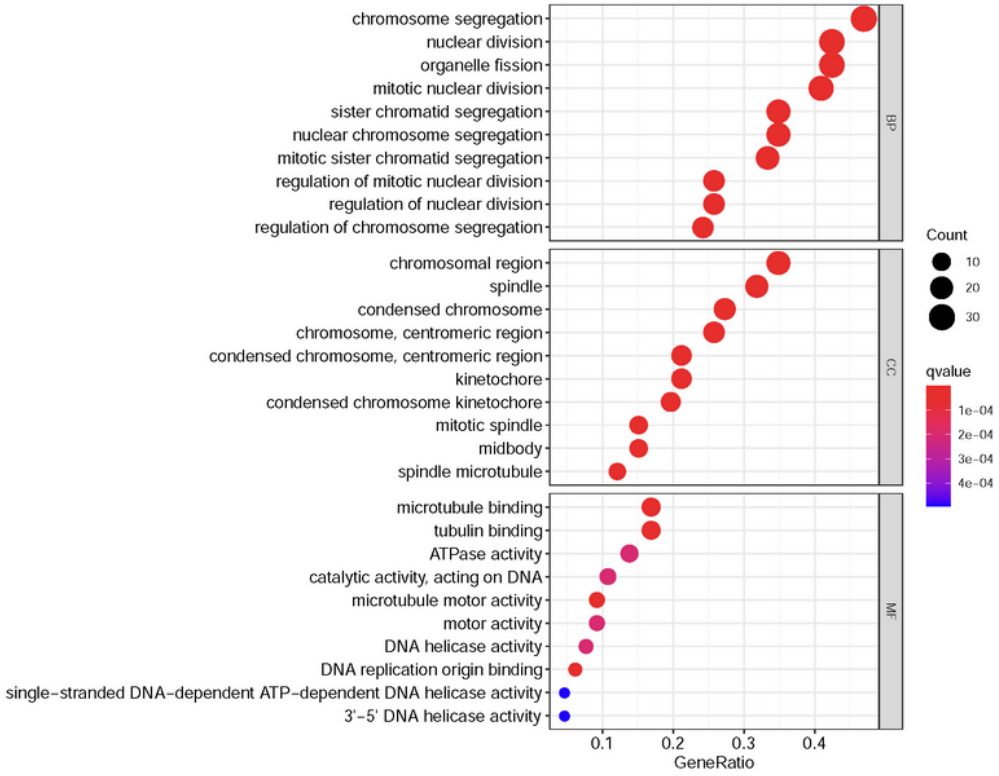


Figure 4

The scatter plot between blue module membership and gene significance for disease recurrence.



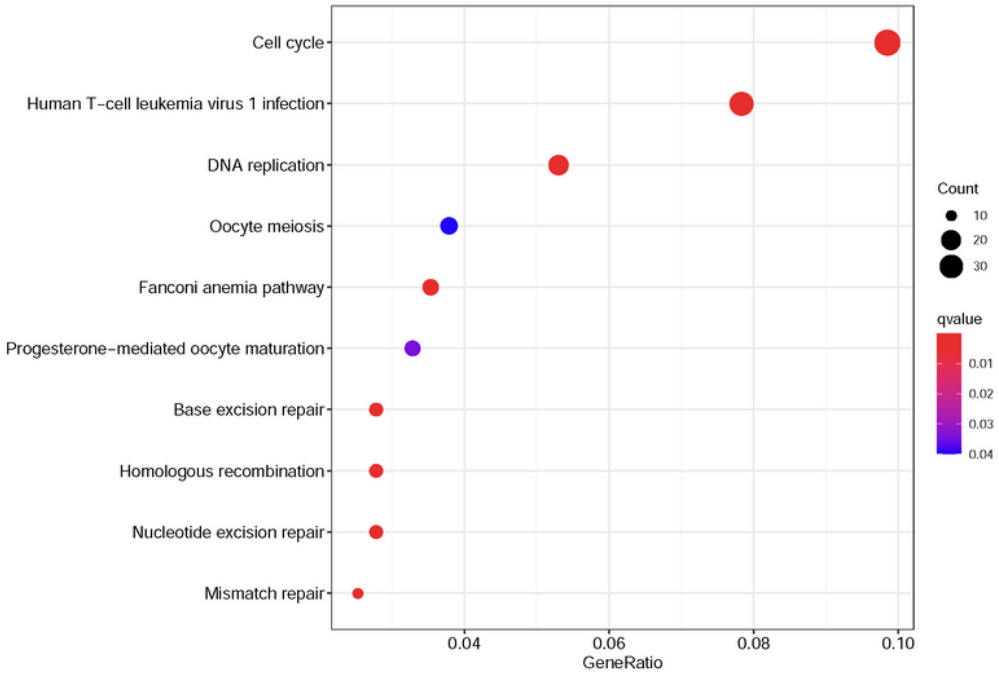


Figure 5

GO enrichment and KEGG pathway analysis in co-expression modules. (A): The results of BP, CC, MF in GO enrichment analysis. (B): The results of KEGG pathway analysis.

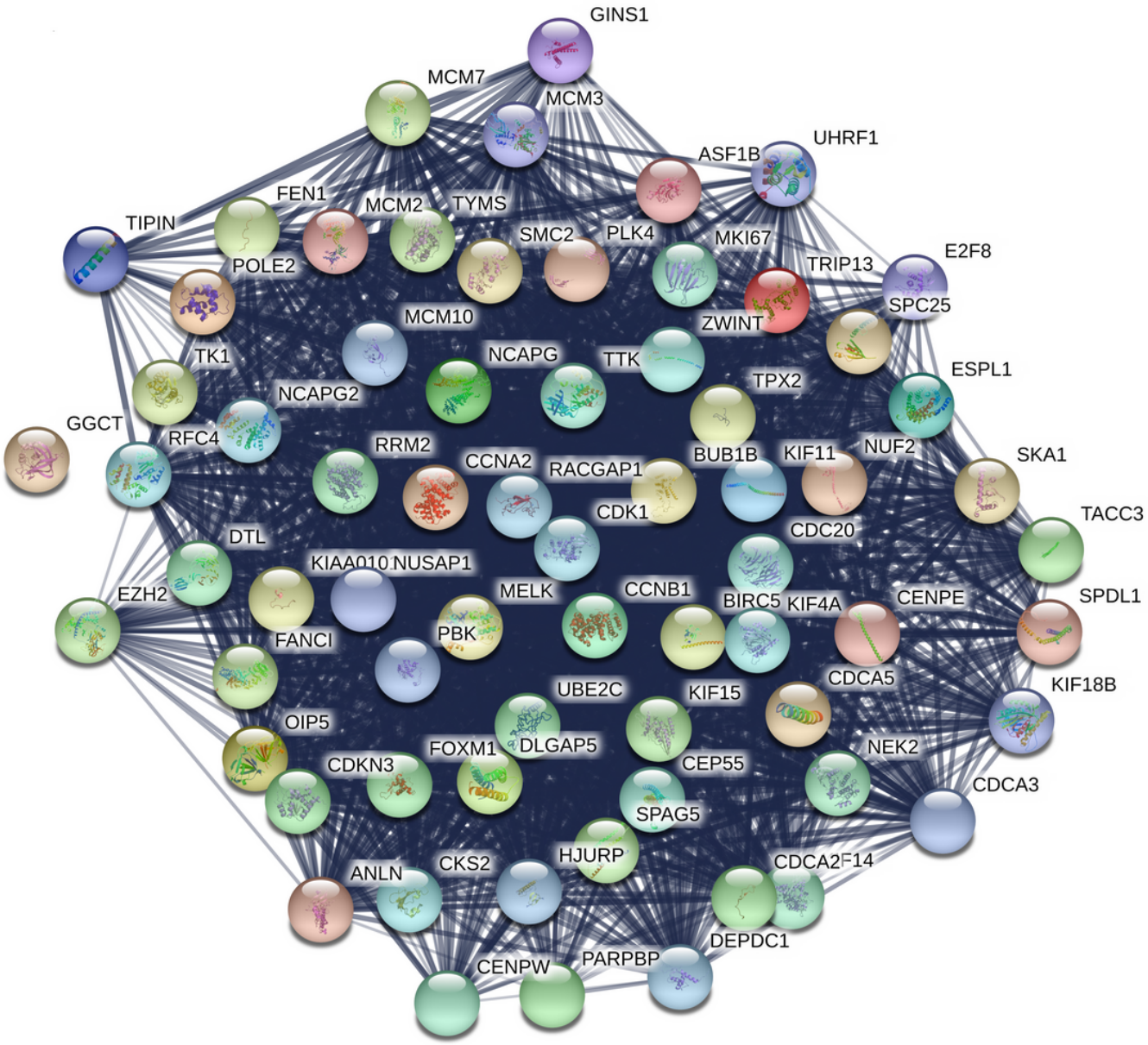


Figure 6

PPI network of 68 most connected genes. Nodes and lines represent genes and the correlation between genes.

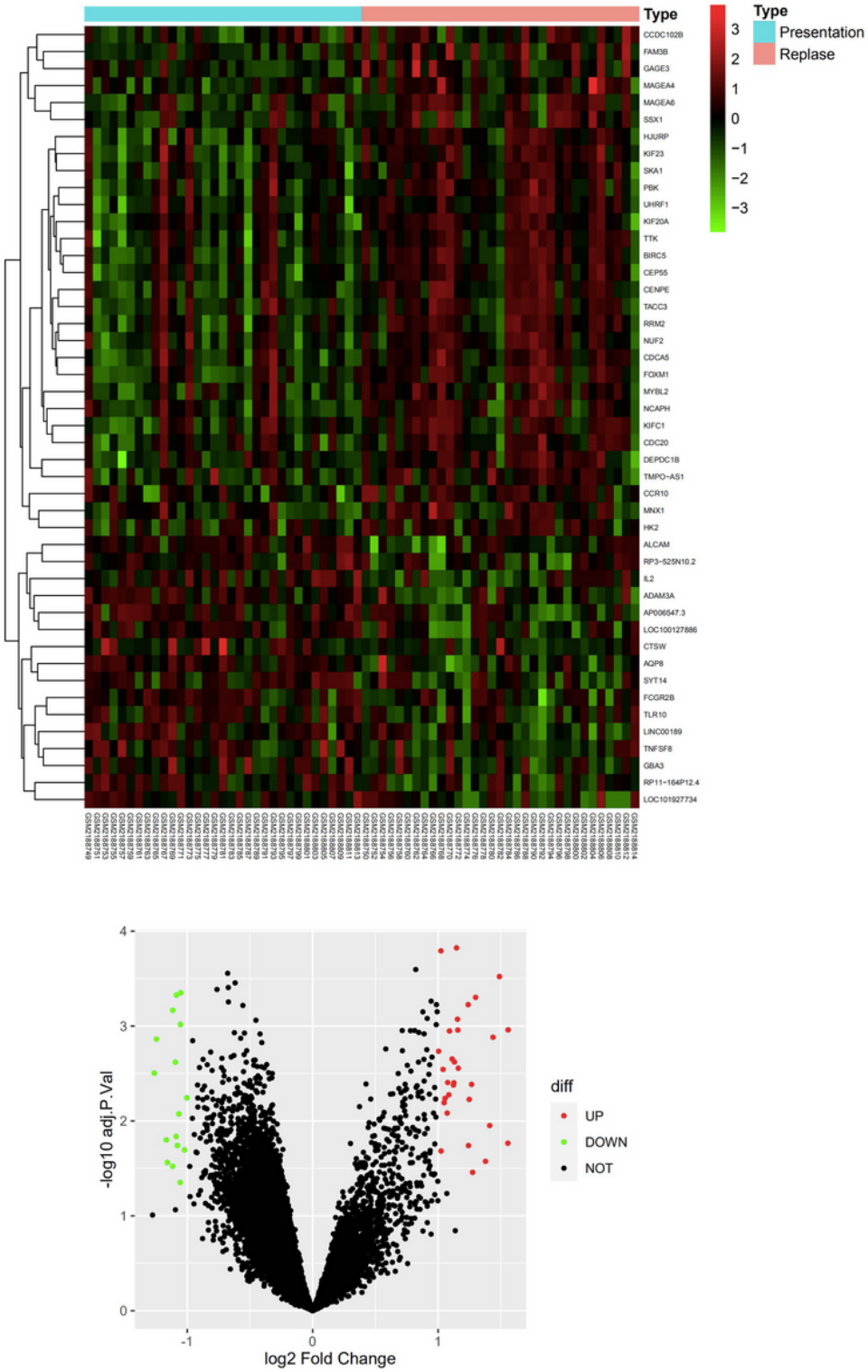


Figure 7

(A): Heatmap of all samples. The diagram presents the result of a two-way hierarchical clustering of all samples. (B):X-axis represents log2(Fold change) and Y-axis represents −log10(adj.P.Val).

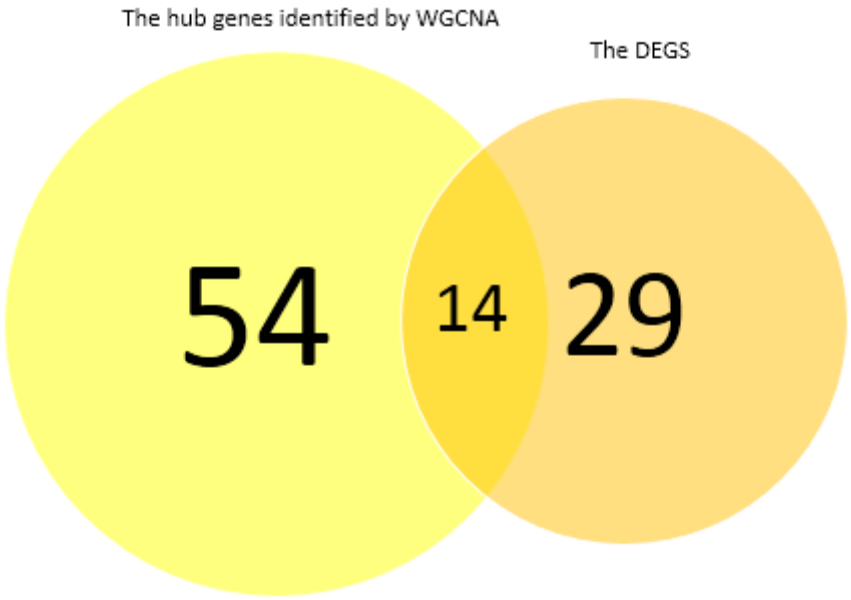


Figure 8

The Venn diagram of DEGs and hub genes. The overlapping area represents key genes.

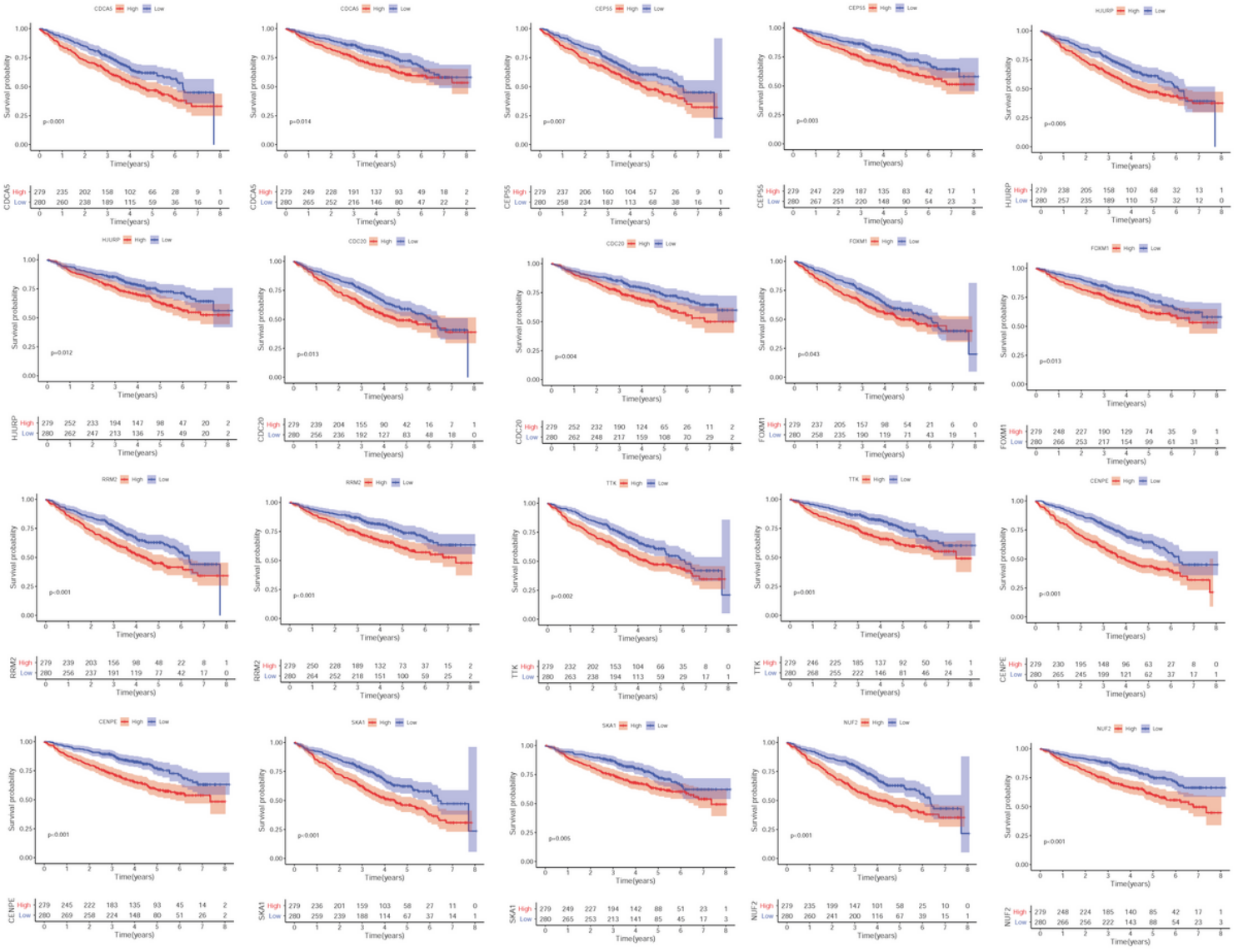


Figure 9

Survival analysis(EFS and OS, respectively) of key genes.