Prediction of a potential drug target based on protein druggability for thyroid cancer

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Prediction of a potential drug target based on protein druggability for thyroid cancer

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Abstract

Background: Thyroid cancer is a common endocrine malignancy; however, its treatment is still surgical. With the development and application of targeted therapy in cancer treatment, there are great development prospects in researching targeted drugs for thyroid cancer.

Methods: Differentially expressed mRNAs between thyroid cancerous tissue and normal thyroid tissues were screened from The Cancer Genome Atlas (TCGA) database. Using weighted gene coexpression network analysis (WGCNA) to build co-expression modules and combined with differentially methylated gene (DMG) analysis. The druggability was analyzed by PockDrug-Server. Due to drug repositioning to seek targeted drugs to treat thyroid cancer we constructed a protein-protein interaction (PPI) network, and screened out a drug target of thyroid cancer. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) were used to analysis the protein enrichment of PPI network.

Results: In the present study, the red module was significantly correlated with thyroid cancer. With DMG analysis, we screened out three genes: *HEY2*, *TNIK* and *LRP4*. These three genes were hypomethylation in tumors. The druggability based on PockDrug-Server predicted that only TNIK had protein pocket druggability. With PPI model for TNIK, there were ten genes interacted with TNIK. These genes were enriched in the MAPK and Wnt pathways, which are correlated with tumor proliferation, differentiation, and development. Upon searching for drugs against these 10 genes in Drugbank, it was determined that the targeted drug Binimetinib which is MEK1/2 inhibition. Therefore, we hypothesized that Binimetinib can be used as a targeted drug and TNIK can be regard as drug target for thyroid cancer therapy.

Conclusion: Our research provides a bioinformatics method for screening drugs target and provides a theoretical basis for targeted therapy for thyroid cancer.

Key words: thyroid cancer, WGCNA, drug target, druggability, drug repositioning, informatics

Background

Thyroid cancer (TC) is a common type of endocrine malignancy that primarily consists of papillary, follicular, medullary and anaplastic carcinomas. Thyroid cancer is one of the fastest growing cancers. In the past few years, the rate of thyroid cancer incidence has increased observably, with 56,460 new diagnoses of thyroid cancer in the USA, at an approximate 5% growth rate [1]. The morbidity has increased from ~10/1,000,000 to 30-40/1,000,000 in China [2]. Although the major treatments, such as thyroidectomy, radioiodine therapy and

thyroid-stimulating hormone inhibition therapy, have achieved great advancements, the targeted drugs for thyroid cancer are unsatisfactory [3]. Therefore, it is essential to explore novel drug targets for treatment.

Epigenetic changes are regarded as significant contributors to tumor progression [4]. DNA methylation is one of the most important epigenetic modifications, as it does not alter the DNA sequence but changes gene expression. Alterations of its status can lead to the development and progression of various cancers. For example, *BRCA1* is methylated and associated with the inactivation of gene expression in breast cancers [5,6]; glutathione S-transferase methylation has a strong correlation with the mortality of prostate cancer patients [7]. Methylation can be used as a prognostic biomarker and therapeutic target for cancer species and has great potential in cancer therapy [8–10]. Roy and Kandimalla et al. researched lymph node metastasis and built a comprehensive methylation signature for predicting the prognosis of esophageal squamous cell carcinoma patients [11]. Some studies also indicate that methylation serves as cancer therapeutic target. For instance, Hegi et al determined that glioblastomas with *MGMT* methylation showed sensitivity to temozolomide (TMZ) [12]. Fujii et al. found that *HSD17B4* methylation showed sensitivity to trastuzumab combined with chemotherapy in HER2-positive breast cancer patients [13]. The therapy for thyroid cancer is closely associated with aberrant DNA methylation. In recent years, more and more thyroid cancer therapy management in patients is shifting toward personalized medicine to avoid the overdiagnosis and overtreatment of tumors [14]. However, there have been few studies on the abnormal methylation of thyroid cancer patients.

Drug research and design is a complex and a lengthy period that requires a lot of investment. Shortening the time and reducing the cost for drug development becomes a tricky problem. Drug

repositioning is to confirm a new use of the drug which have approved [15]. This method greatly reduces the time and cost for people to develop drugs. Protein–protein interaction (PPI) network is one of methods to identify drug repositioning. Constructing PPIs for drug targets or differentially expressed genes, and determine the possible drugs for diseases through their interactions with known drug targets or proteins with indirect effects [16]. In recent years, more and more academic researchers are interested in drug repositioning. Wang et al. detected 3 drug targets and 15 candidate drugs by WGCNA and PPI for treating melanoma [17]. Islam et al. identified 238 gene signatures as therapeutic targets from PPI analysis and 37 novel drugs as potential anticancer drugs for low-grade glioma [18]. We used druggability analyze and PPI to provide novel drug target and agents for treatment and improve the survival rate of thyroid cancer patients.

In the current study, we identified three hub genes of thyroid cancer by constructing a coexpression network of WGCNA combined with differential methylation analysis, employing PockDrug druggability and protein-protein interaction network analyses to find drug target. Furthermore, our study might offer new insight and therapeutic options for the treatment of thyroid cancer.

Methods

Data and Sources

RNA-seq and DNA methylation data of thyroid cancer tissue and corresponding normal tissue were downloaded from The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/). We selected the top 5000 mRNAs for 568 samples of differentially expressed genes (DEGs) for a Weighted Gene Co-Expression Network Analysis (WGCNA). The mRNA sequencing data were normalized by the edgeR package in R language and CMP to screen out the expression of more

than one in three samples [19]. The cut-off criteria of adjusted |log2 fold change (FC)| > 1 and FDR < 0.05 were considered to be statistically significant.

Weighted Gene Co-Expression Network Analysis (WGCNA)

The "WGCNA" R package was used to construct a coexpression network for the screened out genes [20]. First, the DEGs and the Pearson correlations were used to confirm the most correlated genes and to exclude weakly correlated genes. We calculated the soft thresholding power (β) by network topology analysis and converted the adjacency to the topological overlap matrix (TOM) [21]. Second, a gene dendrogram was constructed by hierarchical clustering. We used a dynamic tree-cut algorithm to separate branches of a gene dendrogram from modules of coexpressed genes into different colors [22]. The deepsplit = 2 and minimal module size = 30. Third, we estimated the similarity of modules and merged the highly coexpression genes. Setting of 0.25 as the threshold was chosen as the dissimilarity between the modules and the merged highly coexpressed modules. Then, the module-traits relationship (MTR) was constructed by measuring the relevance between the module eigengenes and thyroid cancer tissues [22]. For the correlations and to calculate the gene significance and module membership, p-value<0.05.

Differentially Methylated Genes Analysis

DNA methylation data were based on the annotations by the TCGA. The differentially methylated genes between thyroid cancer tissues and normal adjacent tissues were analyzed with the "minfi" package in R software. First, we conducted a t-test and screened out the data for FDR<0.05. Then, to calculate the different beta-values of the screened data, we considered the beta-value differences greater than 0.3 as hypermethylation and less than 0.3 as hypomethylation between thyroid cancer tissues and normal adjacent tissues.

PockDrug druggability and Protein and Protein Interaction Analyses

PockDrug (http://pockdrug.rpbs.univ-paris-diderot.fr/) is a robust, pocket druggability prediction server with references to pocket boundary uncertainties and can queried for a protein or a set of proteins [23]. Therefore, according to PockDrug, we predicted the druggability of the genes in both the hub module and the DMGs. A druggability probability greater than 0.5 was considered a druggable pocket.

Due to drug repositioning, we identified the interaction proteins regarding the druggability of TNIK. To analyze proteins using the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/), which calculates protein–protein association data for a great amount of organisms and can uncover direct (physical) and indirect (functional) relationships to construct a PPI network for TNIK, with a minimum required interaction score of 0.7. The data were visualized by Cytoscape v3.3.0 software to construct the network [24–26].

Functional Enrichment Analysis

Functional enrichment analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v.6.8 (https://david.ncifcrf.gov/), a web-accessible program that integrates functional genomic annotations by intuitive graphical summaries [27], annotates genes and carries out Gene Ontology (GO) analysis, including biological process (BP), molecular function (MF) and cellular component (CC) [28]. Kyoto Encyclopedia of Genes and Genomes (KEGG, version 90.0; www.kegg.jp) is one of the most common online resources for interpreting biological systems from molecular level data [29]. Thus, DAVID was used to annotate genes in PPI, and GO and KEGG term enrichments were analyzed. $P<0.05$ was regarded as a statistically significant difference.

Results

WCGNA analysis and module significance calculation

The top 5000 DEGs of RNA-seq data for thyroid cancer were from TCGA, which was used to construct the coexpression network. We chosed the best value for the scale independence property of the network when the soft threshold power was set at 3 and the scale-free R2 reached 0.9 to construct the coexpression module (Fig. 1). A total of 10 modules were identified, and these gene clusters were displayed as a dendrogram and shown in different colors in Fig. 2a. To smoke out the connections between genes in each module and thyroid cancer samples, the correlations of the module eigengenes were correlated with cancer tissues and normal tissues, as shown in Fig. 2b. The eigengene dendrogram and adjacency heatmap were provided in Fig. 2c. The red module (r = 0.51; p = 3e-38) and the blue module (r = 0.51; p = 5e-39) showed the greatest positive associations and the green module (r = -0.53; p = 2e-24) showed the greatest negative associations with thyroid tumorigenesis. According to the correlation between module membership and gene significance (Fig. 2d), the red module had a high correlation (cor = 0.73; p = 9.7e-26), and this module was selected for further analysis.

DMGs in the Red Module

We used methylation data from TCGA thyroid cancer. Methylated genes identified by t-test and FDR<0.05 were regarded as discriminative methylation. We found 445 genes identified as DMGs. Meanwhile, intersection analysis between DMGs and genes in the red module screened three genes, *HEY2*, *TNIK* and *LRP4* (Fig. 3a), as candidates to be further analyzed for druggability. The heatmap of these three genes is shown in Fig. 3b. These three genes were hypomethylated in thyroid cancer tissues.

Pocket Druggability prediction of HEY2, TNIK, and LRP4 proteins

Druggability is the capacity for a protein to bind drug-like molecules with a high affinity [30]. Therefore, it is necessary to assess druggability as the first step of drug discovery. Study of the protein pocket druggability behavior is a key step in the target identification stage of drug discovery. In our study, we used the PockDrug-Server to predict the pocket druggability of HEY2, TNIK and LRP4 proteins. Following our prediction, only TNIK, which consists of 538 residues, had eight protein pockets (P0-P7, respectively) (Fig. 4). The protein pockets with an average druggability probability greater than 0.5 were considered as druggable pockets. Table 1 shows the parameters of the eight protein pockets, and the result indicated that P0 (0.9, p = 0.01), P2 (0.82, p = 0.05), P3 (0.86, p= 0.0) and P6 (0.92, p = 0.02) had the highest druggability probability. Accordingly, we considered TNIK as a possible drug target for thyroid cancer.

PPI of TNIK and targeted drug analysis

To seek out thyroid cancer-targeted drugs, we identified Fostamatinib as a targeted drug of TNIK through Drugbank (https://www.drugbank.ca/). But Fostamatinib has been confirmed to have effects on rheumatoid arthritis and immune thrombocytopenic purpura (ITP). Due to drug repositioning, we constructed a network of PPI for TNIK using Cytoscape, which consisted of 11 nodes and 39 edges from the STRING database. Ten proteins interacted with TNIK (Fig. 5). To determine the biological functions of genes in the PPI, we performed GO and KEGG pathway enrichment analysis. The genes were enriched in positive regulation of protein phosphorylation, positive regulation of MAPK cascade, beta-catenin-TCF7L2 complex, kinase binding and protein phosphatase binding (Fig. 6). According to KEGG analysis, there was enrichment in the MAPK signaling pathway, the TNF signaling pathway and amyotrophic lateral sclerosis (ALS), among

other pathways (Table 2). Drugbank found a targeted drug for these 10 proteins, Binimetinib, which targeted MAP3K1. Thereby, Binimetinib may serve as targeted drug in future therapy of thyroid cancer.

Discussion

Thyroid cancer is a common endocrine malignancy; however, there are few targeted therapeutic drugs available. In this study, we constructed a coexpression network based on mRNA profiles in thyroid cancer and then used the PockDrug-Server in combination with PPI analysis to identify genes that could be targeted to thyroid cancer for treatment. A total of 10 coexpression modules were structured based on the top 5000 DEGs between thyroid cancer and normal samples by WGCNA, and the red module was determined to have the greatest correlation with thyroid cancer. Based on interactions with the red module and DMGs, we screened out three hub genes, *HEY2*, *TNIK* and *LRP4*. Our heatmap results showed that these three genes were hypomethylated in neoplasm tissue (Figure 3B). DNA hypomethylation can cause normally silenced genes to be harmfully expressed, leading to genomic instability [31,32]. DNA hypomethylation is associated with neoplastic progression or postoperative recurrence of disease [33,34]. Therefore DNA hypomethylation can serve as a cancer biomarker. It is estimated that *HEY2*, *TNIK* and *LRP4* play important physiologic roles in the occurrence of thyroid cancer. *HEY2* has been confirmed that correlated with the proliferation of papillary thyroid cancer [35]. *LRP4* has been validated as anovel oncogene in papillary thyroid carcinoma [36].

Protein pocket druggability is a tool for predicting the high affinity of a protein pocket's ability to bind drug-like molecules and is a major mark in the identification phase of drug target discovery [37]. To confirm whether HEY2, TNIK, or LRP4 have druggable protein pockets, we predicted

them using PockDrug-Server. PockDrug-Server is a new bioinformatics tool for efficiently measuring the druggability of holo- and apoprotein pockets. Compared with other druggability models, it can effectively distinguish the degree of the protein pocket's druggability through a unique bioinformatics algorithm [38]. A large number of studies have shown that PockDrug-Server is an optimal predictive model of druggability for apoprotein pockets. Muhammad Hamza et al. ascertained P53 Ser121 and Val122 mutations as drug targets using PockDrug-Server [39]. Trigueiro-Louro et al. used PockDrug-Server to predict the druggable sites in the effector domain of NS1 protein and then identified 3 druggable pockets and 8 new potential hot spot residues in the NS1 protein [40]. In this study, we used PockDrug-Server to predict druggable pockets of HEY2, TNIK and LRP4. It showed that only TNIK had protein pockets and indicated that it had 4 druggable protein pockets (Table 1). Consequently, we hypothesized that TNIK may be a drug target in thyroid cancer.

TNIK (Traf2- and Nck-interacting kinase) is a member of the Ste20 superfamily and has previously been shown to regulate a variety of cellular processes, including apoptosis, morphogenesis and the cytoskeletal regulation of cell shape and motility [41]. Abnormal expression or mutation of TNIK can cause cells to become cancerous. Studies have shown that high expression of TNIK may accelerate tumor progression and invasion by activating the Wnt signaling pathways in terminal colorectal cancer patients [42]. Shitashige et al. suggested that TNIK as a feasible drug target for colorectal cancer because it can affect the proliferation of colorectal cancer cells [43]. Moreover, TNIK is also a novel therapeutic target in gastric cancer, as its amplification could activate the PI3K/Akt pathway to promote tumorigenesis [44]. In the present study, we considered that TNIK might promote thyroid cancer development by

hypomethylation and could influence the treatment of thyroid cancer.

We structured the PPI of TNIK and found that a total of 10 genes were related to TNIK. Enrichment analysis revealed that they were primarily enriched in intracellular signal transduction, positive regulation of the MAPK cascade and the Wnt signaling pathway. KEGG pathway enrichment indicated that most genes were concentrated in the Mitogen-activated protein kinases (MAPKs) signaling pathway and the Tumor necrosis factor (TNF) signaling pathway. The MAPKs and TNF signaling pathways are common pathogenic signaling networks that influence cellular proliferation, differentiation, development, inflammatory responses and apoptosis in tumors [45,46]. There are some small inhibitors can suppress MAPKs pathway effectors to affect the growth of thyroid cancer cells. Of these 10 genes, TNF, TNFRSF1A, TRAF2 and TRADD are the key effector molecules in the TNF signaling pathway [47–50], of which TRAF2 can activate the MAPK pathway, and TCF7L2 with CTNNB1 can activate WNT/β-catenin signaling [51,52]. The expression of CTNNB1 is related to thyroid tumor aggressiveness [53]. MAP3K1, MAP2K6, MAP2K3 and MAP2K5 are the effectors of the MAPKs pathway. MAP3K1 is a member of the MAP3K family and the STE superfamily and plays a vital part in the MAPKs pathway [46,54]. MAP3K1 and TNIK are important kinases in the activation of Wnt/β-catenin signaling in cancer cell lines [55]. Research has shown that MAP3K1 promotes Wnt/β-catenin signaling, and TNIK has been found to be primarily involved in β-catenin-dependent transcriptional activation in colon cancer cell lines [56,57]. Based on these interactions with TNIK, we looked up the targeted drugs on Drugbank and found that only MAP3K1 has a targeted drug, Binimetinib, which is a potent and selective oral mitogen-activated protein kinase 1/2 (MEK 1/2) inhibitor.

In recent years, with the growth of targeted therapy, it has become a most attractive strategy of

cancer therapy. However, surgery is still the main method for thyroid cancer treatment, as less is known about targeted therapies for thyroid cancer. However, drug research is a complex, long process and requires a great deal of investment. Drugs usually have a specific effect on one or more target proteins [58]. Drug repositioning is a good selection step to overcome the limitations of traditional methods. Drug repositioning can find novel uses for existing drugs through PPI to reduce the costs and risks of drug development [59]. We predicted that Binimetinib may be a potential targeted drug for thyroid cancer based on the PPI network of TNIK. The FDA (Food and Drug Administration) approved the combination of Binimetinib and Encorafenib for patients with unresectable or metastatic melanoma with $BRAF^{V600E}$ or^{-V600K} mutation on June 27, 2018. There has also been new clinical research on Binimetinib in the treatment of thyroid cancer with $BRAF^{V600E}$ mutation (NCT04061980). Therefore, Binimetinib is likely to have potential effects in treating thyroid cancer.

Due to the restriction of information for PPI collection regarding detailed interaction dynamics, the prediction results are not comprehensive. Binimetinib for thyroid cancer remains on clinical trial; therefore, its efficacy and safety still require experimentation for verification. Our predicted results provide a theoretical basis for future targeted therapy of thyroid cancer.

Conclusions

In conclusion, using WGCNA, PockDrug druggability and PPI analyses for thyroid cancer-related genes, we revealed that TNIK could serve as a potential drug target correlated with the treatment of thyroid cancer. Further studies that provide a method to screen the targeted gene, which has druggability and to explore the potential drug target on thyroid cancer are imperative. We hope these findings will contribute to the research on new targeted therapeutic drugs for thyroid cancer.

Abbreviations

TCGA: The Cancer Genome Atlas; WGCNA: Weighted Gene Coexpression Network Analysis; DEGs: Differentially Expressed Genes; DMGs: Differentially Methylated Genes; PPI: Protein-Protein Interaction; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes pathway; MAPK: Mitogen-activated protein kinases; MEK1/2: Mitogen-activated protein kinase 1/2.

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Authors' contributions

YFY and BY conceived and designed the study; BY, XXZ and YHZ carried out data collection and performed data analysis; YFY wrote the paper; BY, XXZ and YHZ dited the manuscript and provided critical comments. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and during the current study are available in the TCGA repository, https://cancergenome.nih.gov/.

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

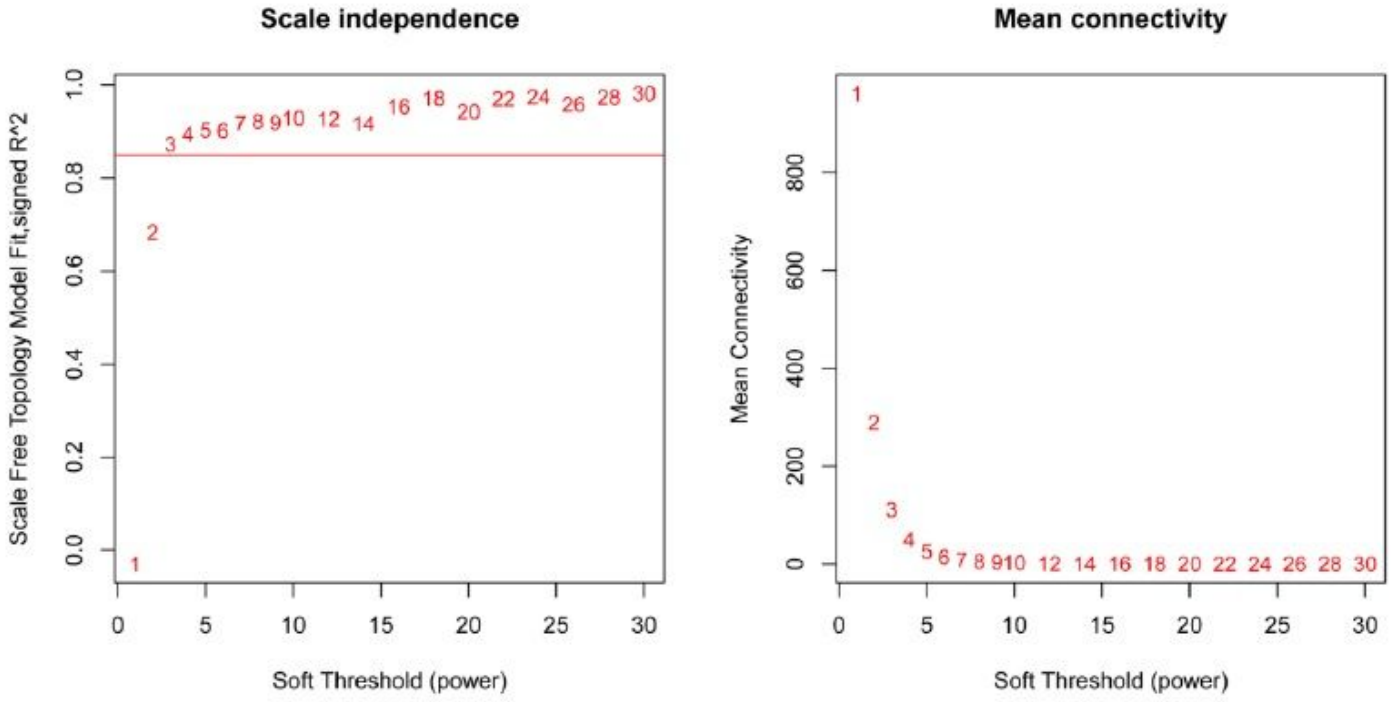

Figure 1

Determining the soft threshold power for the network topology. Analysis of the scale free fit index for various soft threshold powers (β) on the left. Analysis of the mean connectivity for various soft threshold powers in the right.

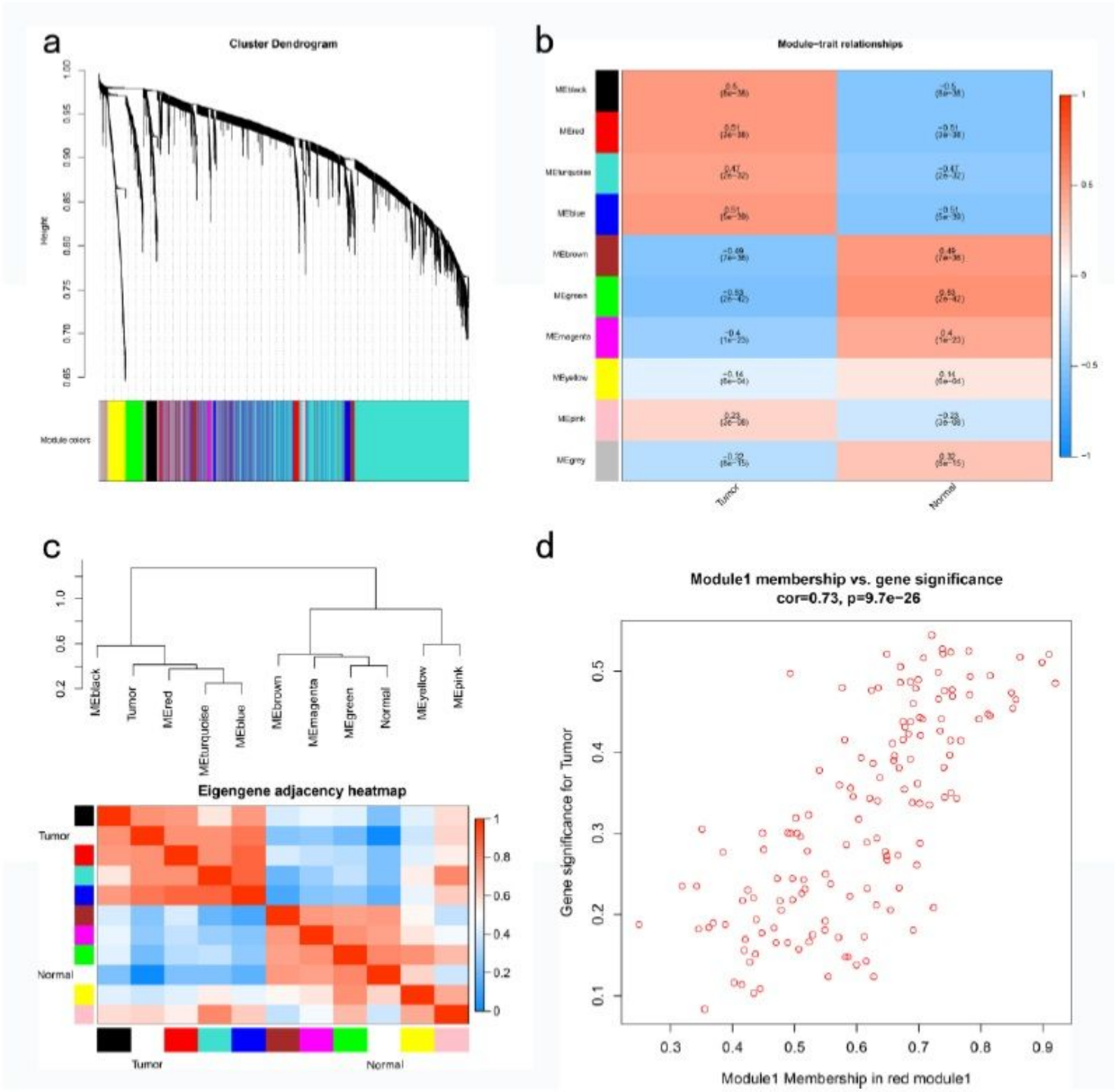

Figure 2

Gene enrichment and modules identified by Weighted gene coexpression network analysis (WGCNA). a Clustering dendrograms of screened genes. Different cluster modules were in different colors; b Heatmap of correlations between module eigengenes (ME) and thyroid cancer tissues. Each row represents a module eigengene, each column represents a thyroid cancer tissue and a normal thyroid tissue, and each cell contains the corresponding correlation and p-value. The matrices are color-coded by correlation

according to the color legend; c Dendrogram tree and adjacency heatmap of the eigengenes; d Scatterplot of the module membership and gene significance in the red module

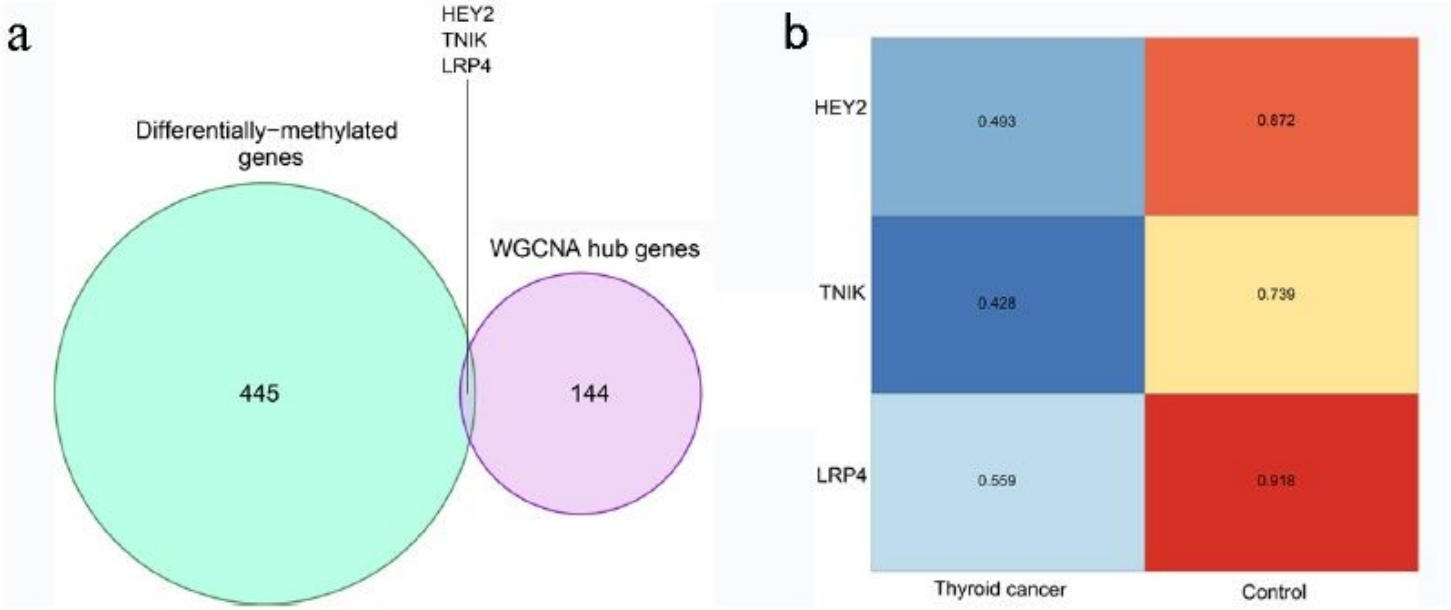

Figure 3

Screening out three genes from the intersection of DMGs and the red module. a Venn diagram between DMGs and hub genes in the red module showing the overlapping of three genes, HEY2, TNIK, LRP4; b Heatmap of DNA methylation in HEY2, TNIK, LRP4 between thyroid cancer tissues and normal thyroid tissue

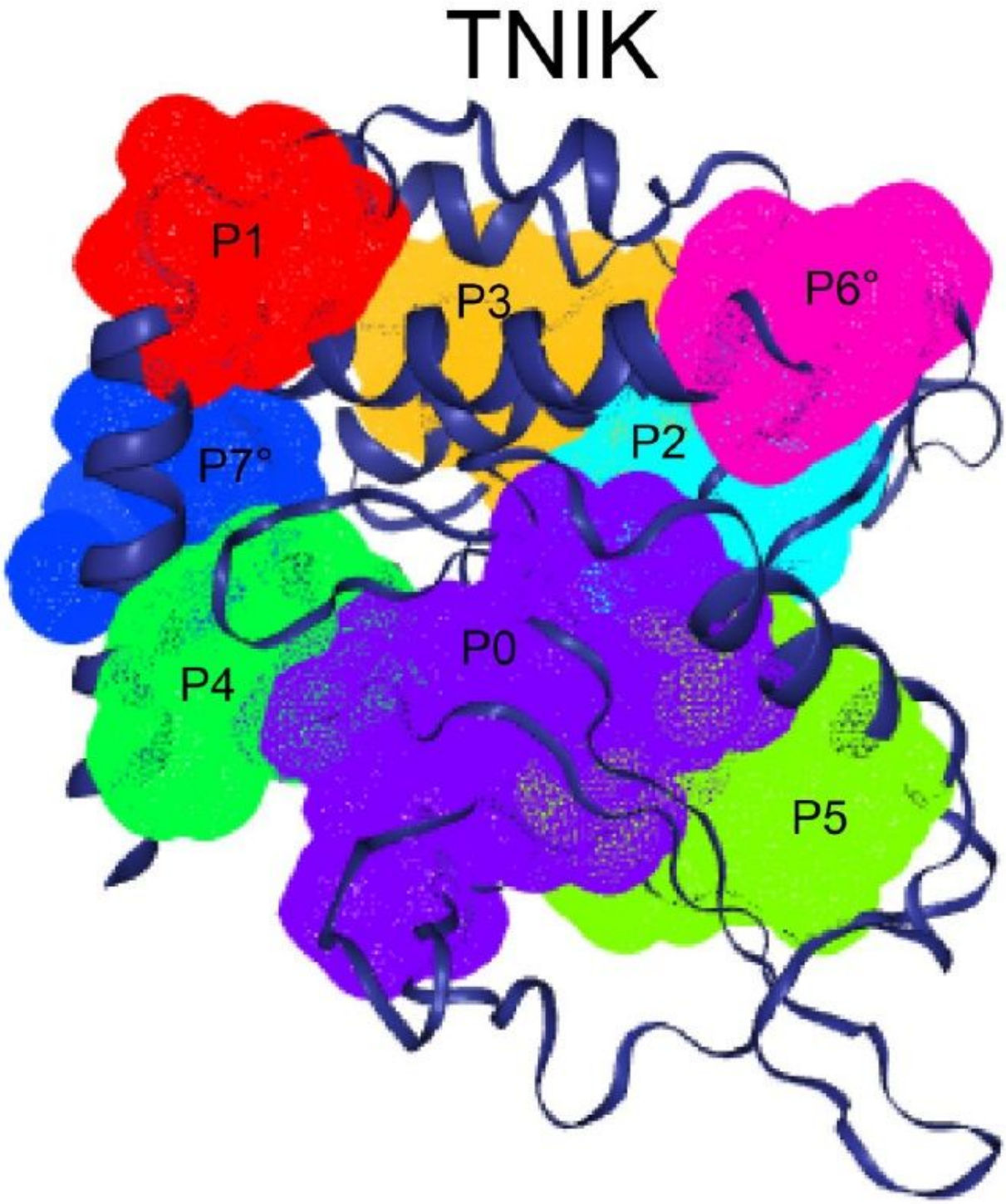

Figure 4

The protein pocket of TNIK. P0-P7 represent different protein pockets in TNIK

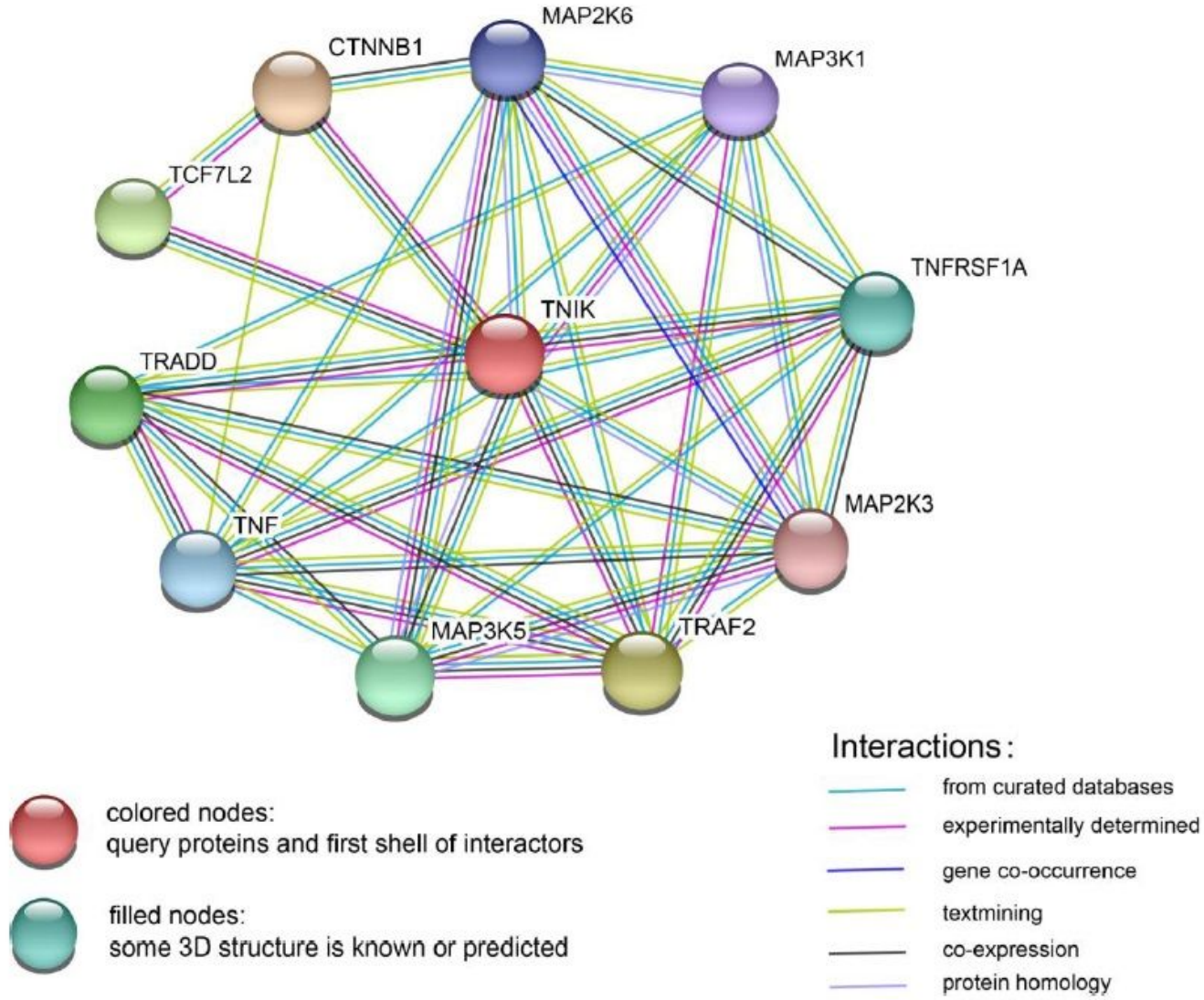

Figure 5

PPI network map for TNIK to identify targeted drug genes

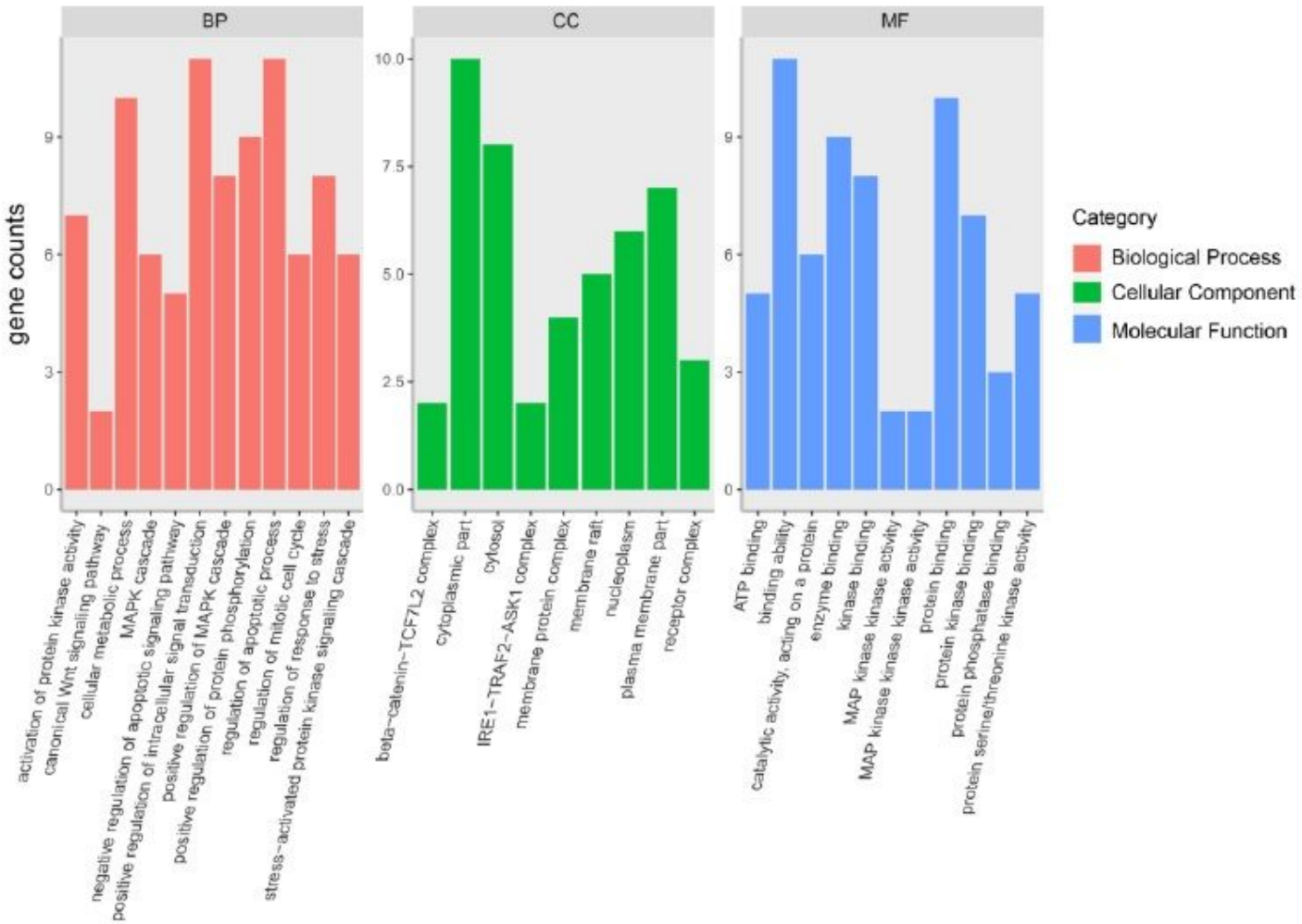

Figure 6

Gene Ontology (GO) enrichment of the genes in the PPI, including biological process (BP), molecular function (MF) and cellular component (CC)