Identification of aberrantly methylated differentially expressed genes in melanoma

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Abstract

Background: Skin Cutaneous Melanoma (SKCM) is known as an aggressive malignant cancer, which could be directly derived from melanocytic nevi. However, the molecular mechanisms underlying malignant transformation of melanocytes and melanoma tumor progression still remain unclear. Increasing researches showed significant roles of epigenetic modifications, especially DNA methylation, in melanoma. This study focused on identification and analysis of methylation-regulated differentially expressed genes (MeDEGs) between melanocytic nevus and malignant melanoma in genome-wide profiles. Methods: The gene expression profiling datasets (GSE3189 and GSE114445) and gene methylation profiling datasets (GSE86355 and GSE120878) were downloaded from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) and differentially methylated genes (DMGs) were identified via GEO2R. MeDEGs were obtained by integrating the DEGs and DMGs. Then, functional enrichment analysis of MeDEGs were performed. STRING and Cytoscape were used to describe protein-protein interaction(PPI) network. Furthermore, survival analysis was implemented to select the prognostic hub genes. Finally, we conducted gene set enrichment analysis (GSEA) of hub genes. Results: We identified 237 hypomethylated, upregulated genes and 182 hypermethylated, downregulated genes. Hypomethylation-upregulated genes were enriched in biological processes of the oxidation-reduction process, cell proliferation, cell division, phosphorylation, extracellular matrix disassembly and protein sumoylation. Pathway enrichment showed selenocompound metabolism, small cell lung cancer and lysosome. Hypermethylation-downregulated genes were enriched in biological processes of positive regulation of transcription from RNA polymerase II promoter, cell adhesion, cell proliferation, positive regulation of transcription, DNA-templated and angiogenesis. The most significantly enriched pathways involved the transcriptional misregulation in cancer, circadian rhythm, tight junction, protein digestion and absorption and Hippo signaling pathway. After PPI establishment and survival analysis, seven prognostic hub genes were CKS2, DTL, KIF2C, KPNA2, MYBL2, TPX2 and FBL. Moreover, the most involved hallmarks obtained by GSEA were E2F targets, G2M checkpoint and mitotic spindle. Conclusions: Our study identified potential aberrantly methylated-differentially expressed genes participating in the process of malignant transformation from nevus to melanoma tissues based on comprehensive genomic profiles. Transcription profiles of CKS2, DTL, KIF2C, KPNA2, MYBL2, TPX2 and FBL provided clues of aberrantly methylation-based biomarkers, which might improve the development of precise medicine.

Background

Skin cutaneous melanoma (SKCM) is an aggressive tumor that is the fifth and sixth most common malignant tumor of men and women respectively [1]. Each year, melanoma accounts for over 80% of skin cancer-related deaths in the world [2]. According to the Clark model, the pathogenesis of melanoma assumes that numerous steps are required for the progression from melanocytes to malignant melanoma, including formation of banal nevi, dysplastic nevi, melanoma in situ, and invasive melanoma [3]. However, the molecular mechanisms underlying malignant transformation of melanocytes and

melanoma tumor progression still remain unclear. Nowadays, as for the primary tumors, surgical resection is usually preferred, while metastatic melanoma is much more difficult to treat with radiotherapy and chemotherapy, which means that early diagnosis is essential [4]. Recently developed immunotherapies and targeted therapies show promise for improving the prognosis of patients with advanced melanoma [5]. Identification of melanoma-associated oncogenes informs different therapeutic strategies, and small molecule inhibitors are available to target specific proteins involved in the pathogenesis of melanoma [6]. Unfortunately, most patients with melanoma, which are initially diagnosed with highly aggressive and progressive disease, are not candidates for curative therapies [2].

DNA methylation is known as a central epigenetic modification, and a significant regulator of gene expression, which can inhibit the binding of transcription factors or the recruitment of repression proteins [7]. Aberrant promoter methylation of genes that control cell cycle and apoptosis can contribute to disruption of normal cell division and carcinogenesis [8]. Importantly, aberrant DNA methylation is regarded as an epigenetic hallmark of melanoma, and plays a significant part in formation as well as progression in melanoma [8, 9]. Methylation of CpG islands appears early in tumorigenesis and the epigenetic changes can be identified in serum, sputum, and urine samples which means it might contribute to the development of molecular strategies for cancer detection as well as function as a biomarker of cancer recurrence after excision [10]. Furthermore, it was reported that hypermethylation correlated with worse prognosis as well as drug resistance [11]. Increasing evidence showed a vital role for both global hypomethylation of oncogenes and hypermethylation of tumor suppressor genes in tumor development and progression, including in melanoma. For example, methylation silencing of PTEN, an inhibitor of PI3K signaling, was closely related to worse prognosis in melanoma patients [12]. In addition, the hypermethylation of WIF1, TFPI2, RASSF1A, and SOCS1 has been considered as significant participants in the melanoma initiation and progression [13]. Although researches on the identification of separate genes with specific hypermethylation or hypomethylation in SKCM are available, comprehensive network studies based on gene expression, methylation profiles and associated pathways have been greatly insufficient.

Over the last decade, bioinformatics technology have emerged as an indispensable tool for tumor research. It mainly focuses on genomics and proteomics to identify genotypes and phenotypes associated with immune infiltration, tumorigenesis and progression of melanoma to guide the development of targeted therapy [14-16]. For example, Cai et al. identified many differentially methylated genes (DMGs) related to lymph node metastasis in melanoma and were closely associated with the prognosis of melanoma patients [17]. Duan et al. found three methylated genes (*ARX*, *DDB2*, and *MBP*) that may be closely associated with the underlying mechanism in melanoma progression [18]. Although methylation changes in SKCM was studied in many researches, countless issues are still unclear.

Here, we performed an integrated bioinformatics analysis based on gene expression profiling by high-throughput sequencing (GSE3189 and GSE114445) and gene methylation profiling microarray (GSE86355 and GSE120878). The methylation-regulated differentially expressed genes (MeDEGs) were screened and performed functional enrichment analysis. Furthermore, protein–protein interaction (PPI)

networks and survival analysis were used to identify new prognostic biomarkers and therapeutic targets for future research in melanoma.

Methods

Acquisition and standardization of raw microarray dataset

We downloaded the gene expression profiling datasets generated by high-throughput sequencing (GSE3189 and GSE114445) and the microarray-based gene methylation profiling datasets (GSE120878 and GSE86355) from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/). Totally 18 nevi and 45 melanoma samples were included in GSE3189 (platform: GPL96 Affymetrix Human Genome U133A Array) while 5 nevi and 16 melanoma samples were enrolled in GSE114445 (platform: GPL570 Affymetrix Human Genome U133 Plus 2.0 Array). As for the DNA methylation datasets, GSE120878 included a total of 73 nevi and 89 primary SKCM tissues, while GSE86355 included altogether 14 nevi and 33 primary SKCM tissues. Both of these two methylation microarray were based on the GPL13534 platform (Illumina HumanMethylation450 BeadChip).

Identification of MeDEGs

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) is a web tool to make comparison of two or more groups of samples in a GEO Series to screen genes that are differentially expressed across specific experimental conditions. In the present study, GEO2R was used to identify the differentially expressed genes (DEGs) as well as the differentially methylated genes (DMGs). $|t|>2$ and $P<0.05$ were considered statistically significant. Then, hypomethylation-high expression genes were obtained after overlap of upregulated and hypomethylated genes, and hypermethylation-low expression genes were obtained after overlap of downregulated and hypermethylated genes. The hypomethylation-high expression genes and hypermethylation-low expression genes were identified as methylation-regulated differentially expressed genes (MeDEGs).

Functional enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) is a straight-forward web tool that can provide integrative and systematic annotation for users to unravel biological interactions of multiple genes. It was utilized to perform functional and pathway enrichment analyses. Gene ontology (GO) analysis including the biological process(BP), cellular component(CC), molecular function(MF) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted for the selected MeDEGs by DAVID[19, 20]. P-value<0.05 was considered statistically significant.

PPI network construction and identification of hub genes

In this study, STRING (http://string-db.org; version 11.0) was adopted to describe protein co-regulation of hypomethylation-high expression genes and hypermethylation-low expression genes repectively and

measure functional interactions among nodes [21]. The interaction specificity score > 0.4 (the default threshold in the STRING database) was considered statistically significant. Cytoscape (version 3.6.0) was used to visualize interaction networks obtained from STRING [22]. MCODE (version 1.4.2) of Cytoscape is a plug-in to cluster a given network to identify densely connected regions based on topology [23]. It was utilized to find the most related module network with selection threshold as follows: MCODE scores >5, degree cutoff=2, node score cut-off=0.2, Max depth=100 and k-score=2.

Survival analysis

Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) is an online tool that can provide customizable functionalities based on data from The Cancer Genome Atlas (TCGA; https://tcga-data.nci.nih.gov/tcga/) and the Genotype-Tissue Expression project(GTEx; https://www.gtexportal.org/home/index.html)[24]. GEPIA performs survival analysis based on gene expression levels, using log-rank test for the hypothesis evaluation. The horizontal axis (x-axis) represented time in days, and the vertical axis (y-axis) showed the probability of surviving or the proportion of people surviving. The lines presented survival curves of two groups.

Validation of hub genes

Oncomine (https://www.oncomine.org) is an online database that allow users to collect, normalize, and analyze gene expression profiling data for tumor samples [25]. Oncomine database was utilized to validate the differential expression of hub genes between SKCM and nevus samples. After choosing the catalog of SKCM and nevus tissue, comparison of mRNA expression levels were made. The cBioPortal (http://cbioportal.org) is an open-access resource for users to search for multidimensional cancer genomics datasets which provides access to data for over 5000 tumor samples from 20 cancer studies [26]. We used cBioPortal to investigate the genetic alterations of hub genes as well as the correlation between methylation status and gene expression.

Transcription factor network and data processing of gene set enrichment analysis (GSEA)

Transcription factor regulation networks of hub genes were constructed by using R software (Version 3.3.2). Significant nodes involved in co-regulation of *CKS2, DTL, KIF2C, KPNA2, MYBL2, TPX2* and *FBL* were described in circle plots (including transcription factor regulation-DNA binding, related lncRNA, targeted miRNA and protein-protein interaction). Based on data from the TCGA database, GSEA tool(version 2.10.1 package) was used to predict associated up- and down-regulated genes and their significantly involved hallmarks pathways [27]. Student's-t-test statistical score was implemented in consistent pathways and the mean of the differentially expressed genes was calculated for each analysis. A permutation test with 1000 times was utilized to recognize the greatly involved pathways. The adj. P using Benjamini and Hochberg (BH) and false discovery rate (FDR) method by default were used to correct for the occurrence of false positive results. Significant related genes were defined with an adj. $P < 0.01$ and $FDR < 0.25$.

Results

Identification of MeDEGs in SKCM

GEO2R were adopted to identify the DEGs and DMGs, respectively. For DEGs of gene expression microarray, 554 overlapping up-regulated genes (1,088 in GSE3189, 4,096 in GSE114445) and 462 overlapping down-regulated genes (1,224 in GSE3189, 4,152 in GSE114445) were screened. For DMGs of gene methylation microarray, 15,052 overlapping hypermethylation genes (17,016 in GSE86355, 22,767 in GSE120878) and 17,888 overlapping hypomethylation genes (18,944 in GSE86355, 25,934 in GSE120878) were found. As shown in **Fig.2**, we identified 237 hypomethylated, upregulated genes and 182 hypermethylated, downregulated genes after integrating the DEGs and DMGs. The flowchart was illustrated in **Fig. 1**.The representative heat map of the MeDEGs of GSE3189 (including top 50 up-regulated and down-regulated genes) was present in **Fig.3**.

Functional enrichment analysis of MeDEGs

The results of the GO enrichment analysis for the MeDEGs were shown in **Table 1** and **Table 2**. For hypomethylation-upregulated genes, changes in biologic processes were mostly enriched in the oxidation-reduction process, cell proliferation, cell division, phosphorylation, extracellular matrix disassembly and protein sumoylation. The hypermethylation-downregulated genes were mainly enriched in positive regulation of transcription from RNA polymerase II promoter, cell adhesion, cell proliferation, positive regulation of transcription, DNA-templated and angiogenesis. We also found that the hypomethylated, upregulated genes were related to cytosol, extracellular exosome, membrane and nucleoplasm , while hypermethylated, downregulated genes to cytoplasm, plasma membrane, cytosol and cell junction in the cellular component group. For hypomethylated, upregulated genes, changes in molecular function were primarily enriched in protein binding, ATP binding, enzyme binding and GTPase activity, and for hypermethylated, downregulated genes, changes were significantly enriched in protein binding, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding and transcription factor activity, sequence-specific DNA binding. Pathway enrichment was also performed using KEGG, and the results were shown in **Table 3**. We found that hypomethylated genes predominantly participated in selenocompound metabolism, small cell lung cancer and lysosome. For hypermethylated genes, the most significantly enriched pathways involved the transcriptional misregulation in cancer, circadian rhythm, tight junction, protein digestion and absorption and Hippo signaling pathway.

PPI network establishment and hub genes selection

The PPI network of hypomethylation-upregulated genes and hypermethylation-downregulated genes was visualized by Cytoscape(version 3.6.0) [22]. MCODE (version 1.4.2) is a plug-in of Cytoscape to cluster a given network to select densely connected regions based on topology [23]. The results were presented in **Fig.4**. Therefore, PBK, TK1, TACC3, MYBL2, TPX2, DTL, KPNA2, KIF2C, CKS2, ASF1B, SPAG5 and NCAPH were verified as hub genes in hypomethylation-upregulated genes module. And EIF4B, EIF3L, EIF3A, RPS7,

RPL22, RSL1D1, RPS23, RPL11 and FBL were selected as hub genes in hypermethylation-downregulated genes module.

Survival analysis

Significant survival outcomes of hub genes in PPI network were displayed in **Fig.5**. According to the expression of each gene, overall survival for SKCM patients was acquired. We found that high mRNA expression of CKS2 (P= 0.033) was closely related to worse prognosis for SKCM as well as DTL(P=0.00096), KIF2C (P=0.01), KPNA2 (P=0.0017), MYBL2 (P=0.0022), TPX2 (P=0.0074), FBL (P=0.0013).

Hub genes verification

Subsequently, we used Oncomine database to further validate the expression of seven hub genes. The different expression levels of six hypomethylated upregulated hub genes and one hypermethylated downregulated hub genes between melanoma and nevus samples were significantly obvious (**Fig.6**), which were consistent with the results we obtained.

In addition, we used the cBioPortal tool to explore the genetic alterations of seven hub genes and discovered that DTL (17%) and KIF2C (13%) were the most frequently altered genes among the seven hub genes, including amplification, fusion, and missense mutations (**Fig. 7A and 7B**). The alterations of the seven hub genes were 192 (43.24%) of 444 sequenced cases/patients. The correlations between mRNA and DNA methylation of the seven genes in the TCGA SKCM patients were demonstrated in **Fig.7C**. We found that the correlation was negative, indicating that methylation regulated the mRNA expression of these genes. This illustrated that methylation played an important role in the expression of these genes.

Significant genes and pathway obtained by GSEA

Transcriptional regulation networks among *CKS2, DTL, KIF2C, KPNA2, MYBL2, TPX2* and *FBL* were displayed in **Fig. 8**. Significantly involved nodes(including transcription factor regulation-DNA binding, related lncRNA, targeted miRNA and protein-protein interaction) were marked in different colors. Subsequently, a total of 100 significant genes were obtained from GSEA, and the genes with positive correlation were plotted. GSEA analysis, including *CKS2, DTL, KIF2C, KPNA2, MYBL2* and *TPX2* indicated that the most involved hallmarks pathways were E2F targets, G2M checkpoint and mitotic spindle. The details were illustrated in **Fig. 9**.

Discussion

Melanoma is an aggressive and devastating cancer that can directly derived from melanocytic nevus. Nowadays, surgery of tumor resection before metastasis still remain the most effective treatment [4]. Thus, it is important to diagnose the high-risk nevus in the early stage. Identification of novel biomarkers will be greatly helpful to improve diagnosis, and even better understanding of mechanism involved in

melanocytic tumorigenesis that potentially contribute to novel therapy. Hence, highly effective biomarkers for diagnosis and treatment are urgently required.

The initiation and progression involved in melanoma is a complicated and multistage process regulated by both genetic and epigenetic alterations. Increasing evidence has showed the essential roles of epigenetic modifications, especially DNA methylation, in SKCM [8, 9, 28]. However, most of these studies are limited to melanoma metastases and lack primary melanomas, which made it difficult to identify early biological progresses during melanoma development [17]. Furthermore, separate analysis of gene expression and methylation from one study are limited [29], while integrating multiple available datasets may help us find more accurate and reliable evidence through comprehensive bioinformatics analysis. Yet, conjoint analysis including both gene expression and methylation profiling microarray datasets are largely insufficient in SKCM. Therefore, we conducted an integrated bioinformatics analysis based on both gene expression and gene methylation profiling to identify the new prognostic biomarkers and therapeutic targets in SKCM for future research.

In the present study, we identified a total of 237 hypomethylated, upregulated genes and 182 hypermethylated, downregulated genes by overlapping the DEGs and DMGs. For the hypomethylation-upregulated genes, functional enrichment analysis indicated that changes in the biological processes were mainly enriched in the oxidation-reduction process, cell proliferation, cell division, phosphorylation, extracellular matrix disassembly and protein sumoylation. GO cell component analysis showed that the upregulated genes were significantly enriched in cytosol, extracellular exosome, membrane and nucleoplasm. In addition, for molecular function, the hypomethylation-upregulated genes were significantly enriched in protein binding, ATP binding, enzyme binding and GTPase activity. KEGG pathway enrichment analysis suggested significant enrichment in pathways including selenocompound metabolism, small cell lung cancer and lysosome. Notably, GSEA results showed that E2F targets, G2M checkpoint and mitotic spindle were the most involved hallmarks in SKCM. These findings are reasonable because it is universally acknowledged that the above processes are closely related to tumor initiation and progression, including melanoma [30].

PPI network of hypomethylation-high expression genes illustrated the protein-protein interactome of the hub genes, and then GEPIA was adopted to select the most prognostic hub genes, named *CKS2, DTL, KIF2C, KPNA2, MYBL2* and *TPX2*, which may provide new clues for the therapeutic strategy in SKCM.

Cyclin-dependent kinases regulatory subunit 2 (CKS2), a cyclin-dependent kinase-interacting protein, is critical for cell cycle regulation. Overexpression of CKS2 has been reported to be associated with several types of cancer, including colorectal cancer and cervical cancer [31, 32]. de Wit NJW et al. [33] reported that CKS2 could be considered as a candidate player in melanocytic tumor progression and facilitate early diagnosis of melanocytic lesions before metastasis. We found that the expression of CKS2 in SKCM tissues was higher in different datasets, and survival analysis revealed that the upregulation of CKS2 was related to a worse prognosis in SKCM, which was consistent with results of previous studies.

Denticleless E3 ubiquitin protein ligase homologue (DTL) , also known as DNA replication factor 2, can regulate the expression of various cell cycle regulatory proteins and maintain the integrity of DNA replication and repair [34]. Elevated expression of DTL has been found to be related to a variety of cancers, such as breast cancer, Ewing sarcoma and ovarian cancer [35-37]. Yang et al. [38] suggested that DTL can be regarded as an indicator of poor prognosis in acral melanoma patients. In the present study, we found that overexpressed DTL was closely associated with worse survival outcome in cutaneous melanoma patients. The rate of DTL mutation was 17%, and this higher mutation rate may lead to abnormal methylation or deregulation of DTL.

As a member of the kinesin-13 family, kinesin family member 2C(KIF2C*)* uses microtubule depolymerizing activity to correct improper microtubule attachments at kinetochores, which plays significant roles during mitosis process [39, 40]. KIF2C is likely to be the essential gene for carcinogenesis, and may be closely involved in tumor-infiltrating lymphocytes of cancer immunotherapy for patients with metastatic melanoma [41]. In our study, we found that the expression of KIF2C was significantly elevated in SKCM tissues compared to nevus tissues and associated with poor prognosis in melanoma patients. We assumed that abnormal methylation may lead to the high expression of KIF2C, the occurrence as well as the development of SKCM.

KPNA2, a member of karyopherin (KPNA) protein family, is considered as a key role in the malignant transformation of cells through transport of tumor suppressors, regulation of DNA repair proteins as well as activation of apoptosis pathways [42]. Elevated KPNA levels have been found to predict poor prognosis for multiple tumors, including breast and cervical cancer [43, 44]. Winnepenninckx et al. [45] reported that KPNA2 is closely associated with poor prognosis and tumor progression in melanoma. This finding is consistent with our results, suggesting the possibility of the aberrant methylation of the KPNA2 promoter.

Myb proto-oncogene like 2(MYBL2*),* located on chromosome 20q13, acts as a transcription factor that plays a significant role in cell-cycle progression. In the previous study, overexpression of MYBL2 has been found to be related to poor prognosis in various cancers, such as prostate and gallbladder cancer [46, 47]. Koynova et al. [48] found a higher frequency of low-level increase of the copy numbers of MYBL2 rather than amplification in melanoma. Taken together, these evidence indicated that MYBL2 was involved in cell proliferation and tumorigenesis in melanoma, which is consistent with our present findings. However, further research is needed to confirm our hypothesis.

Targeting protein for xenopus kinesin-like protein 2 (TPX2, also known as REPP86) located on chromosome 20q11.2 in humans. TPX2 is a mitotic microtubule-associated protein that is strictly regulated by the cell cycle and diffusely distributed during the S and G2 phases, which help spindle stability [49]. Wei et al. [50] demonstrated that upregulation of TPX2 was associated with the clinical stage, invasion and metastasis in colon cancer, participating in the P13K/Akt signaling pathway to reduce the occurrence as well as the proliferation of colon cancer cells. Increased expression of TPX2 was also observed in lung squamous cell carcinoma, ovarian cancer and giant-cell tumor of the bone [51-

53]. Yao et al. [54] showed that TPX2 improved the proliferative ability of melanoma cell lines and functioned as an oncogene in melanoma. In our study, TPX2 was found to be a hypomethylated, upregulated gene in melanoma, and associated with the poor prognostic of melanoma patients, which suggested that TPX2 may be used as a novel prognostic marker for the development and progression of SKCM. Our results were consistent with the roles of TPX2 in various tumors reported in previous studies.

As for the hypermethylation-downregulated genes, functional enrichment analysis indicated that changes in biological processes were mainly enriched in the positive regulation of transcription from RNA polymerase II promoter, cell adhesion, cell proliferation, positive regulation of transcription, DNA-templated and angiogenesis. GO cell component analysis showed that the downregulated genes were significantly enriched in the cytoplasm, plasma membrane, cytosol and cell junction. Besides, for molecular function, the hypermethylation-downregulated genes were significantly enriched in protein binding, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding and transcription factor activity, sequence-specific DNA binding. KEGG pathway enrichment analysis suggested significant enrichment in pathways including transcriptional misregulation in cancer, circadian rhythm, tight junction, protein digestion and absorption and Hippo signaling pathway. Importantly, growing evidence showed that numerous genetic changes in melanoma may be linked to in Hippo signaling pathway [55, 56]. Moreover, Hippo pathway was found to be correlated with the mitogen-activated protein kinase (MAPK) signaling pathway which is well known as a vital role in the pathogenesis of melanoma [56].

Then, we performed PPI network and survival analysis to identify the prognostic hub gene among the hypermethylation-downregulated genes. Fibrillarin (FBL) is an indispensable, highly conserved protein essential in the processing of pre-rRNAs [57]. In the previous study, expression of FBL can be regulated by p53 in multiple tumors and was considered as an ideal target to inhibit the ribosome biogenesis process in cancer therapy [58, 59]. Overexpression of FBL was found in breast, prostate cancers and squamous cell cervical carcinoma [60-62]. Thus, FBL could have a role in tumor progression and could affect the clinical outcome of patients through alteration of translational regulation in melanoma. In our study, we found that the low expression of FBL in SKCM tissues compared to nevus tissues was observed in multiple datasets, and survival analysis showed that the high expression of FBL was related to a worse prognosis in SKCM.

The present study is the first to our knowledge to construct a comprehensive network between nevus and melanoma and identify the prognostic significance of these hub genes, which may serve as valuable prognostic indicators of SKCM.

Conclusions

In summary, this study identified methylation-regulated differentially expressed genes and related pathways and functions in SKCM by using an integrated bioinformatics analysis. In addition, we constructed PPI networks and performed survival analysis that identified seven prognostic hub genes.

Our findings may deepen the understanding of the methylation-mediated regulatory mechanisms underlying carcinogenesis possibility of melanocytic nevus and melanoma and provide novel biomarkers and therapeutic targets for further research.

List Of Abbreviations

SKCM skin cutaneous melanoma

DEGs differentially expressed genes

DMGs differentially methylated genes

MeDEGs methylation-regulated differentially expressed genes

DAVID the Database for Annotation, Visualization and Integrated Discovery

GEPIA Gene Expression Profiling Interactive Analysis

GO gene ontology

BP biological process

CC cellular component

MF molecular function

KEGG Kyoto Encyclopedia of Genes and Genomes

PPI protein-protein interaction

TCGA the Cancer Genome Atlas

GTEx Genotype-Tissue Expression project

OS overall survival

GSEA gene set enrichment analysis

MARK mitogen-activated protein kinase

Declarations

Ethics approval and consent to participate: No.

Consent for publication: Not applicable.

Availability of data and material: The datasets analyzed for this study can be found in the GEO(http://www.ncbi.nlm.nih.gov/geo) and TCGA (https://www.cancer.gov).

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Authors' contributions: The work presented here was carried out in collaboration among all authors. SGL defined the research theme, discussed analyses, interpretation, and presentation. HW drafted the manuscript, analyzed the data, developed the algorithm and interpreted the results. All authors read and approved the final manuscript.

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References

1. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2020**. *CA Cancer J Clin* 2020, **70**(1):7-30.
2. Schadendorf ea: **Melanoma**. *The Lancet* 2018, **392**(10151):971-984.
3. WALLACE H ea: **A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma.** *HUMAN PATHOLOGY* 1984, **Volume 15.No. 12** 1147-1165.
4. Gadeliya Goodson A, Grossman D: **Strategies for early melanoma detection: Approaches to the patient with nevi**. *Journal of the American Academy of Dermatology* 2009, **60**(5):719-735.
5. Flaherty KT, Hodi FS, Fisher DE: **From genes to drugs: targeted strategies for melanoma**. *Nat Rev Cancer* 2012, **12**(5):349-361.
6. Mohammadpour A, Derakhshan M, Darabi H, Hedayat P, Momeni M: **Melanoma: Where we are and where we go**. *J Cell Physiol* 2019, **234**(4):3307-3320.
7. Moore LD, Le T, Fan G: **DNA methylation and its basic function**. *Neuropsychopharmacology* 2013, **38**(1):23-38.
8. Schinke C, Mo Y, Yu Y, Amiri K, Sosman J, Greally J, Verma A: **Aberrant DNA methylation in malignant melanoma**. *Melanoma Res* 2010, **20**(4):253-265.
9. Goran Micevic NT, Marcus Bosenberg: **Aberrant DNA methylation in melanoma: biomarker and therapeutic opportunities**. *Clinical Epigenetics* 2017, **9**:1-15.
10. James G. Herman SBB: **Gene Silencing in Cancer in Association with Promoter Hypermethylation**. *The new england journal of medicine* 2003, **349**:2042-2054.
11. Mori T, O'Day SJ, Umetani N, Martinez SR, Kitago M, Koyanagi K, Kuo C, Takeshima T-L, Milford R, Wang H-J *et al*: **Predictive Utility of Circulating Methylated DNA in Serum of Melanoma Patients Receiving Biochemotherapy**. *Journal of Clinical Oncology* 2005, **23**(36):9351-9358.
12. Lahtz C, Stranzenbach R, Fiedler E, Helmbold P, Dammann RH: **Methylation of PTEN as a prognostic factor in malignant melanoma of the skin**. *J Invest Dermatol* 2010, **130**(2):620-622.

13. Tanemura A, Terando AM, Sim M-S, van Hoesel AQ, de Maat MFG, Morton DL, Hoon DSB: **CpG Island Methylator Phenotype Predicts Progression of Malignant Melanoma**. *Clinical Cancer Research* 2009, **15**(5):1801-1807.
14. Xu WH, Xu Y, Wang J, Wan FN, Wang HK, Cao DL, Shi GH, Qu YY, Zhang HL, Ye DW: **Prognostic value and immune infiltration of novel signatures in clear cell renal cell carcinoma microenvironment**. *Aging (Albany NY)* 2019, **11**(17):6999-7020.
15. Zhang C, Zhang B, Meng D, Ge C: **Comprehensive analysis of DNA methylation and gene expression profiles in cholangiocarcinoma**. *Cancer Cell Int* 2019, **19**:352.
16. Fan G, Tu Y, Chen C, Sun H, Wan C, Cai X: **DNA methylation biomarkers for hepatocellular carcinoma**. *Cancer Cell Int* 2018, **18**:140.
17. Weiyang Cai XD, Jingjing Li: **Methylation analysis highlights novel prognostic criteria in human-metastasized melanoma**. *Journal of Cellular Biochemistry* 2018, **2019**:1-12.
18. Honghao Duan KJ, Dengke Wei: **Identification of epigenetically altered genes and potential gene targets in melanoma using bioinformatic methods**. *OncoTargets and Therapy* 2018, **11**:9-15.
19. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA: **The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists**. *Genome Biol* 2007, **8**(9):R183.
20. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT *et al*: **Gene ontology: tool for the unification of biology. The Gene Ontology Consortium**. *Nat Genet* 2000, **25**(1):25-29.
21. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Minguez P, Bork P, von Mering C *et al*: **STRING v9.1: protein-protein interaction networks, with increased coverage and integration**. *Nucleic Acids Res* 2013, **41**(Database issue):D808-815.
22. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T: **Cytoscape 2.8: new features for data integration and network visualization**. *Bioinformatics* 2011, **27**(3):431-432.
23. Bandettini WP, Kellman P, Mancini C, Booker OJ, Vasu S, Leung SW, Wilson JR, Shanbhag SM, Chen MY, Arai AE: **MultiContrast Delayed Enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: a clinical validation study**. *J Cardiovasc Magn Reson* 2012, **14**:83.
24. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: **GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses**. *Nucleic Acids Res* 2017, **45**(W1):W98-W102.
25. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kincead-Beal C, Kulkarni P *et al*: **Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles**. *Neoplasia* 2007, **9**(2):166-180.
26. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E *et al*: **The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data: Figure 1**. *Cancer Discovery* 2012, **2**(5):401-404.

27. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles**. *Proc Natl Acad Sci U S A* 2005, **102**(43):15545-15550.
28. Berger MF, Hodis E, Heffernan TP, Deribe YL, Lawrence MS, Protopopov A, Ivanova E, Watson IR, Nickerson E, Ghosh P *et al*: **Melanoma genome sequencing reveals frequent PREX2 mutations**. *Nature* 2012, **485**(7399):502-506.
29. Wouters J, Vizoso M, Martinez-Cardus A, Carmona FJ, Govaere O, Laguna T, Joseph J, Dynoodt P, Aura C, Foth M *et al*: **Comprehensive DNA methylation study identifies novel progression-related and prognostic markers for cutaneous melanoma**. *BMC Med* 2017, **15**(1):101.
30. Hanahan D, Weinberg Robert A: **Hallmarks of Cancer: The Next Generation**. *Cell* 2011, **144**(5):646-674.
31. Jonsson M, Fjeldbo CS, Holm R, Stokke T, Kristensen GB, Lyng H: **Mitochondrial Function of CKS2 Oncoprotein Links Oxidative Phosphorylation with Cell Division in Chemoradioresistant Cervical Cancer**. *Neoplasia* 2019, **21**(4):353-362.
32. Min-Hao Yu YL, Shao-Lan Qin, Zheng-Shi Wang, Yi-Fei Mu, Ming Zhong: **Up-regulated CKS2 promotes tumor progression and predicts a poor prognosis in human colorectal cancer**. *Am J Cancer Res* 2015, **5**:2708-2718.
33. de Wit NJW, Rijntjes J, Diepstra JHS, van Kuppevelt TH, Weidle UH, Ruiter DJ, van Muijen GNP: **Analysis of differential gene expression in human melanocytic tumour lesions by custom made oligonucleotide arrays**. *British Journal of Cancer* 2005, **92**(12):2249-2261.
34. Pan HW, Chou HY, Liu SH, Peng SY, Liu CL, Hsu HC: **Role of L2DTL, cell cycle-regulated nuclear and centrosome protein, in aggressive hepatocellular carcinoma**. *Cell Cycle* 2006, **5**(22):2676-2687.
35. Mackintosh C, Ordonez JL, Garcia-Dominguez DJ, Sevillano V, Llombart-Bosch A, Szuhai K, Scotlandi K, Alberghini M, Sciot R, Sinnaeve F *et al*: **1q gain and CDT2 overexpression underlie an aggressive and highly proliferative form of Ewing sarcoma**. *Oncogene* 2012, **31**(10):1287-1298.
36. Ueki T, Nishidate T, Park JH, Lin ML, Shimo A, Hirata K, Nakamura Y, Katagiri T: **Involvement of elevated expression of multiple cell-cycle regulator, DTL/RAMP (denticleless/RA-regulated nuclear matrix associated protein), in the growth of breast cancer cells**. *Oncogene* 2008, **27**(43):5672-5683.
37. Pan W-W, Zhou J-J, Yu C, Xu Y, Guo L-J, Zhang H-Y, Zhou D, Song F-Z, Fan H-Y: **Ubiquitin E3 Ligase CRL4CDT2/DCAF2as a Potential Chemotherapeutic Target for Ovarian Surface Epithelial Cancer**. *Journal of Biological Chemistry* 2013, **288**(41):29680-29691.
38. Yang L, Dai J, Ma M, Mao L, Si L, Cui C, Sheng X, Chi Z, Yu S, Xu T *et al*: **Identification of a functional polymorphism within the 3'-untranslated region of denticleless E3 ubiquitin protein ligase homolog associated with survival in acral melanoma**. *European Journal of Cancer* 2019, **118**:70-81.
39. Lawrence CJ, Dawe RK, Christie KR, Cleveland DW, Dawson SC, Endow SA, Goldstein LSB, Goodson HV, Hirokawa N, Howard J *et al*: **A standardized kinesin nomenclature**. *The Journal of Cell Biology* 2004, **167**(1):19-22.

40. Gardner Melissa K, Zanic M, Gell C, Bormuth V, Howard J: **Depolymerizing Kinesins Kip3 and MCAK Shape Cellular Microtubule Architecture by Differential Control of Catastrophe**. *Cell* 2011, **147**(5):1092-1103.
41. Lu YC, Yao X, Crystal JS, Li YF, El-Gamil M, Gross C, Davis L, Dudley ME, Yang JC, Samuels Y *et al*: **Efficient Identification of Mutated Cancer Antigens Recognized by T Cells Associated with Durable Tumor Regressions**. *Clinical Cancer Research* 2014, **20**(13):3401-3410.
42. Shun-Fu Tseng C-YC, Kou-Juey Wu, Shu-Chun Teng: **Importin KPNA2 Is Required for Proper Nuclear Localization and Multiple Functions of NBS1**. *THE JOURNAL OF BIOLOGICAL CHEMISTRY* 2005, **280**(47):39594-39600.
43. Dahl E, Kristiansen G, Gottlob K, Klaman I, Ebner E, Hinzmann B, Hermann K, Pilarsky C, Durst M, Klinkhammer-Schalke M *et al*: **Molecular profiling of laser-microdissected matched tumor and normal breast tissue identifies karyopherin alpha2 as a potential novel prognostic marker in breast cancer**. *Clin Cancer Res* 2006, **12**(13):3950-3960.
44. van der Watt PJ, Maske CP, Hendricks DT, Parker MI, Denny L, Govender D, Birrer MJ, Leaner VD: **The Karyopherin proteins, Crm1 and Karyopherin beta1, are overexpressed in cervical cancer and are critical for cancer cell survival and proliferation**. *Int J Cancer* 2009, **124**(8):1829-1840.
45. Winnepenninckx V, Lazar V, Michiels S, Dessen P, Stas M, Alonso SR, Avril M-F, Ortiz Romero PL, Robert T, Balacescu O *et al*: **Gene Expression Profiling of Primary Cutaneous Melanoma and Clinical Outcome**. *JNCI: Journal of the National Cancer Institute* 2006, **98**(7):472-482.
46. Anat Bar-Shira JHP, Uri Rozovsky, Myriam Goldstein, William R. Sellers, Yuval Yaron, Zelig Eshhar, and Avi Orr-Urtreger: **Multiple Genes in Human 20q13 Chromosomal Region Are Involved in an Advanced Prostate Cancer Xenograft**. *Cancer Research* 2002, **62**:6803-6807.
47. Liang HB, Cao Y, Ma Q, Shu YJ, Wang Z, Zhang F, Ye YY, Li HF, Xiang SS, Song XL *et al*: **MYBL2 is a Potential Prognostic Marker that Promotes Cell Proliferation in Gallbladder Cancer**. *Cell Physiol Biochem* 2017, **41**(5):2117-2131.
48. Denitsa K. Koynovaa ESJ, Angel D. Milevb,, Remco Dijkmane KSK, Draga I. Tonchevaa and Nelleke A. Gruise: **Gene-specific fluorescence in-situ hybridization analysis on tissue microarray to refine the region of chromosome 20q amplification in melanoma**. *Melanoma Research* 2007, **17**:37-41.
49. Alfaro-Aco R, Petry S: **How TPX2 helps microtubules branch out**. *Cell Cycle* 2017, **16**(17):1560-1561.
50. Ping Wei NZ, Ye Xu, Xinxiang Li, Debing Shi, Yuwei Wang, Dawei Li, Sanjun Cai: **TPX2 is a novel prognostic marker for the growth and metastasis of colon cancer**. *Journal of Translational Medicine* 2013, **11**:1-11.
51. Ignacio Blanco KK, Daniel Cuadras: **Assessing Associations between the AURKA- HMMR-TPX2-TUBG1 Functional Module and Breast Cancer Risk in BRCA1:2 Mutation Carriers**. *Plos One* 2015:1-18.
52. Laura T. Smith JM, Norma J. Nowak: **20q11.1 Amplification in Giant-Cell Tumor of Bone: Array CGH, FISH, and Association with Outcome**. *GENES, CHROMOSOMES & CANCER* 2006, **45**:957-966.

53. Aguirre-Portoles C, Bird AW, Hyman A, Canamero M, Perez de Castro I, Malumbres M: **Tpx2 Controls Spindle Integrity, Genome Stability, and Tumor Development**. *Cancer Research* 2012, **72**(6):1518-1528.
54. Yao Y, Zuo J, Wei Y: **Targeting of TRX2 by miR-330-3p in melanoma inhibits proliferation**. *Biomedicine & Pharmacotherapy* 2018, **107**:1020-1029.
55. Kim JE, Finlay GJ, Baguley BC: **The role of the hippo pathway in melanocytes and melanoma**. *Front Oncol* 2013, **3**:123.
56. Feng R, Gong J, Wu L, Wang L, Zhang B, Liang G, Zheng H, Xiao H: **MAPK and Hippo signaling pathways crosstalk via the RAF-1/MST-2 interaction in malignant melanoma**. *Oncol Rep* 2017, **38**(2):1199-1205.
57. Newton K, Petfalski E, Tollervey D, Caceres JF: **Fibrillarin Is Essential for Early Development and Required for Accumulation of an Intron-Encoded Small Nucleolar RNA in the Mouse**. *Molecular and Cellular Biology* 2003, **23**(23):8519-8527.
58. Marcel V, Ghayad Sandra E, Belin S, Therizols G, Morel A-P, Solano-Gonzàlez E, Vendrell Julie A, Hacot S, Mertani Hichem C, Albaret Marie A *et al*: **p53 Acts as a Safeguard of Translational Control by Regulating Fibrillarin and rRNA Methylation in Cancer**. *Cancer Cell* 2013, **24**(3):318-330.
59. El Hassouni B, Sarkisjan D, Vos JC, Giovannetti E, Peters GJ: **Targeting the Ribosome Biogenesis Key Molecule Fibrillarin to Avoid Chemoresistance**. *Current Medicinal Chemistry* 2019, **26**(33):6020-6032.
60. Su H, Xu T, Ganapathy S, Shadfan M, Long M, Huang TH, Thompson I, Yuan ZM: **Elevated snoRNA biogenesis is essential in breast cancer**. *Oncogene* 2014, **33**(11):1348-1358.
61. Koh CM, Gurel B, Sutcliffe S, Aryee MJ, Schultz D, Iwata T, Uemura M, Zeller KI, Anele U, Zheng Q *et al*: **Alterations in Nucleolar Structure and Gene Expression Programs in Prostatic Neoplasia Are Driven by the MYC Oncogene**. *The American Journal of Pathology* 2011, **178**(4):1824-1834.
62. Choi YW, Kim YW, Bae SM, Kwak SY, Chun HJ, Tong SY, Lee HN, Shin JC, Kim KT, Kim YJ *et al*: **Identification of differentially expressed genes using annealing control primer-based GeneFishing in human squamous cell cervical carcinoma**. *Clin Oncol (R Coll Radiol)* 2007, **19**(5):308-318.

Tables

Table 1. Gene ontology enrichment analysis of hypomethylated upregulated genes

Category	Term	Description	Count	P.Value
BP	GO:0001887	selenium compound metabolic process	3	1.68E-03
BP	GO:0016310	phosphorylation	7	2.07E-03
BP	GO:0022617	extracellular matrix disassembly	6	3.27E-03
BP	GO:0000059	protein import into nucleus, docking	3	4.58E-03
BP	GO:0060236	regulation of mitotic spindle organization	3	4.58E-03
BP	GO:0006606	protein import into nucleus	5	7.11E-03
BP	GO:0016925	protein sumoylation	6	1.93E-02
BP	GO:0008283	cell proliferation	11	2.33E-02
BP	GO:0051301	cell division	10	4.25E-02
BP	GO:0055114	oxidation-reduction process	14	4.98E-02
CC	GO:0016020	membrane	59	2.73E-08
CC	GO:0070062	extracellular exosome	67	1.96E-07
CC	GO:0005829	cytosol	75	2.01E-07
CC	GO:0031012	extracellular matrix	15	2.53E-05
CC	GO:0005654	nucleoplasm	54	1.16E-03
CC	GO:0005783	endoplasmic reticulum	22	1.90E-03
CC	GO:0031965	nuclear membrane	10	2.60E-03
CC	GO:0005925	focal adhesion	13	4.18E-03
CC	GO:0001772	immunological synapse	4	8.90E-03
CC	GO:0042470	melanosome	6	9.11E-03
CC	GO:0043231	intracellular membrane-bounded organelle	15	1.16E-02
CC	GO:0005789	endoplasmic reticulum membrane	19	2.54E-02
CC	GO:0005737	cytoplasm	79	4.60E-02
CC	GO:0005794	Golgi apparatus	18	4.67E-02
CC	GO:0005739	mitochondrion	25	4.91E-02
MF	GO:0005515	protein binding	149	7.15E-05
MF	GO:0008139	nuclear localization sequence binding	4	6.28E-03
MF	GO:0005524	ATP binding	32	1.15E-02
MF	GO:0003924	GTPase activity	9	1.47E-02
MF	GO:0019899	enzyme binding	11	1.58E-02
MF	GO:0051015	actin filament binding	6	3.40E-02

Table 2. Gene ontology enrichment analysis of hypermethylated downregulated genes

Category	Term	Description	Count	P.Value
BP	GO:0007155	cell adhesion	15	2.30E-04
BP	GO:0001954	positive regulation of cell-matrix adhesion	4	1.34E-03
BP	GO:0045944	positive regulation of transcription from RNA polymerase II promoter	21	2.07E-03
BP	GO:0050680	negative regulation of epithelial cell proliferation	5	2.42E-03
BP	GO:0006366	transcription from RNA polymerase II promoter	13	5.80E-03
BP	GO:0090162	establishment of epithelial cell polarity	3	6.22E-03
BP	GO:0001942	hair follicle development	4	6.52E-03
BP	GO:0001525	angiogenesis	8	7.46E-03
BP	GO:0008284	positive regulation of cell proliferation	12	7.63E-03
BP	GO:0016477	cell migration	7	7.97E-03
BP	GO:0008285	negative regulation of cell proliferation	10	1.90E-02
BP	GO:0090090	negative regulation of canonical Wnt signaling pathway	6	2.46E-02
BP	GO:0008283	cell proliferation	9	3.21E-02
BP	GO:0042493	response to drug	8	3.44E-02
BP	GO:0045893	positive regulation of transcription, DNA-templated	11	3.57E-02
CC	GO:0016324	apical plasma membrane	13	3.17E-05
CC	GO:0005911	cell-cell junction	8	1.57E-03
CC	GO:0005737	cytoplasm	70	2.63E-03
CC	GO:0005856	cytoskeleton	10	1.12E-02
CC	GO:0005829	cytosol	46	1.22E-02
CC	GO:0030054	cell junction	11	1.55E-02
CC	GO:0031012	extracellular matrix	8	2.73E-02
CC	GO:0005886	plasma membrane	52	3.85E-02
CC	GO:0005925	focal adhesion	9	3.96E-02
MF	GO:0001077	transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	10	5.79E-04
MF	GO:0005515	protein binding	106	2.94E-03
MF	GO:0000989	transcription factor activity, transcription factor binding	3	8.19E-03
MF	GO:0008013	beta-catenin binding	5	8.89E-03

MF	GO:0043565	sequence-specific DNA binding	12	1.41E-02
MF	GO:0003700	transcription factor activity, sequence-specific DNA binding	17	2.88E-02

Table 3. Pathway enrichment analysis of MeDEGs

Category	Term	Count	P.Value
Hypomethylated upregulated genes			
KEGG_PATHWAY	hsa00450:Selenocompound metabolism	4	2.42E-03
KEGG_PATHWAY	hsa05222:Small cell lung cancer	6	1.22E-02
KEGG_PATHWAY	hsa04142:Lysosome	6	4.70E-02
Hypermethylated downregulated genes			
KEGG_PATHWAY	hsa05202:Transcriptional misregulation in cancer	6	3.51E-02
KEGG_PATHWAY	hsa04710:Circadian rhythm	3	4.45E-02
KEGG_PATHWAY	hsa04530:Tight junction	4	6.89E-02
KEGG_PATHWAY	hsa04974:Protein digestion and absorption	4	7.08E-02
KEGG_PATHWAY	hsa04390:Hippo signaling pathway	5	8.21E-02

Figures

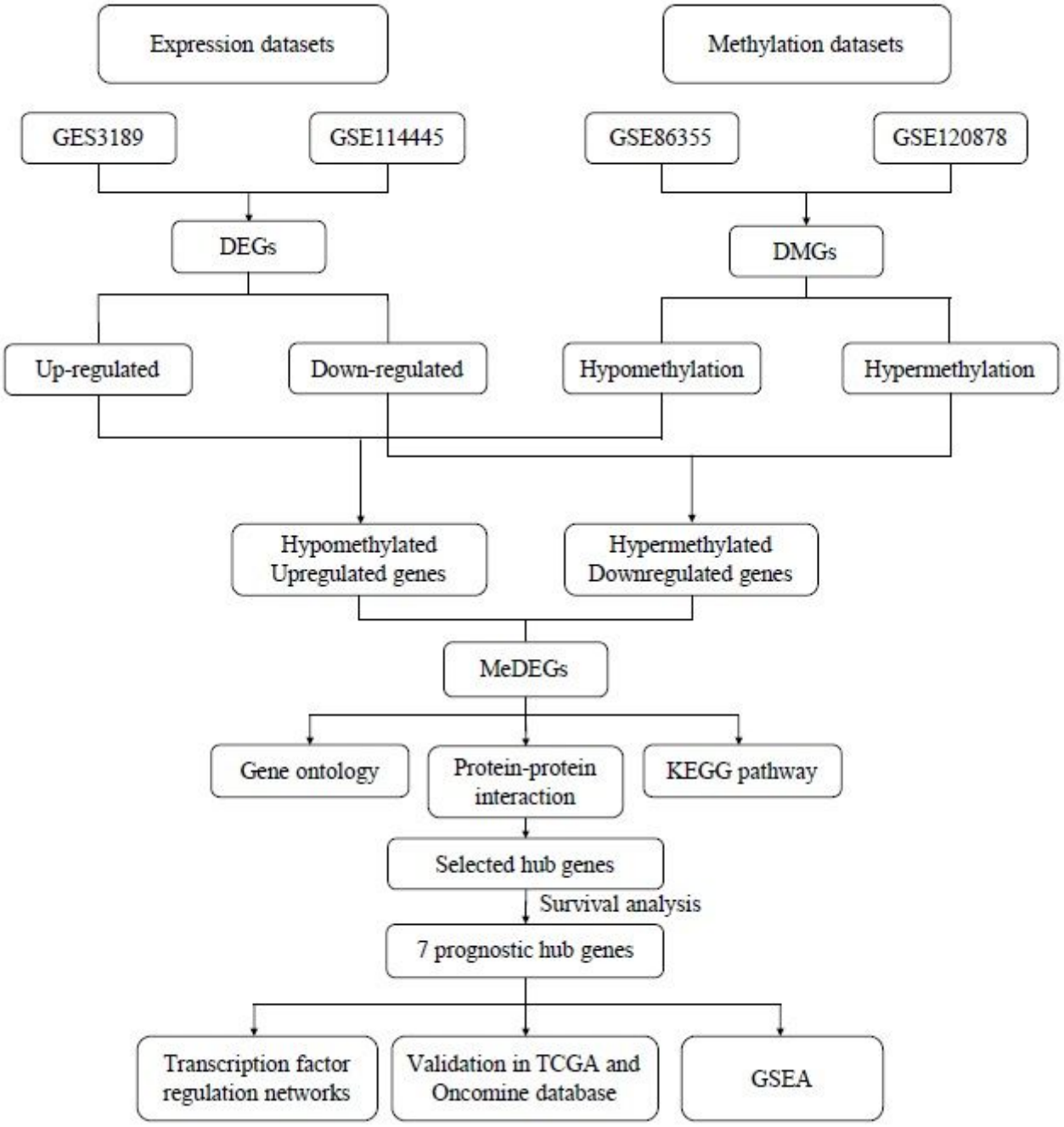


Figure 1

Flowchart of bioinformatics analysis. DEGs: Differentially expressed genes; DMGs: differentially methylated genes; MeDEGs: methylation-regulated differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; GSEA: gene set enrichment analysis.

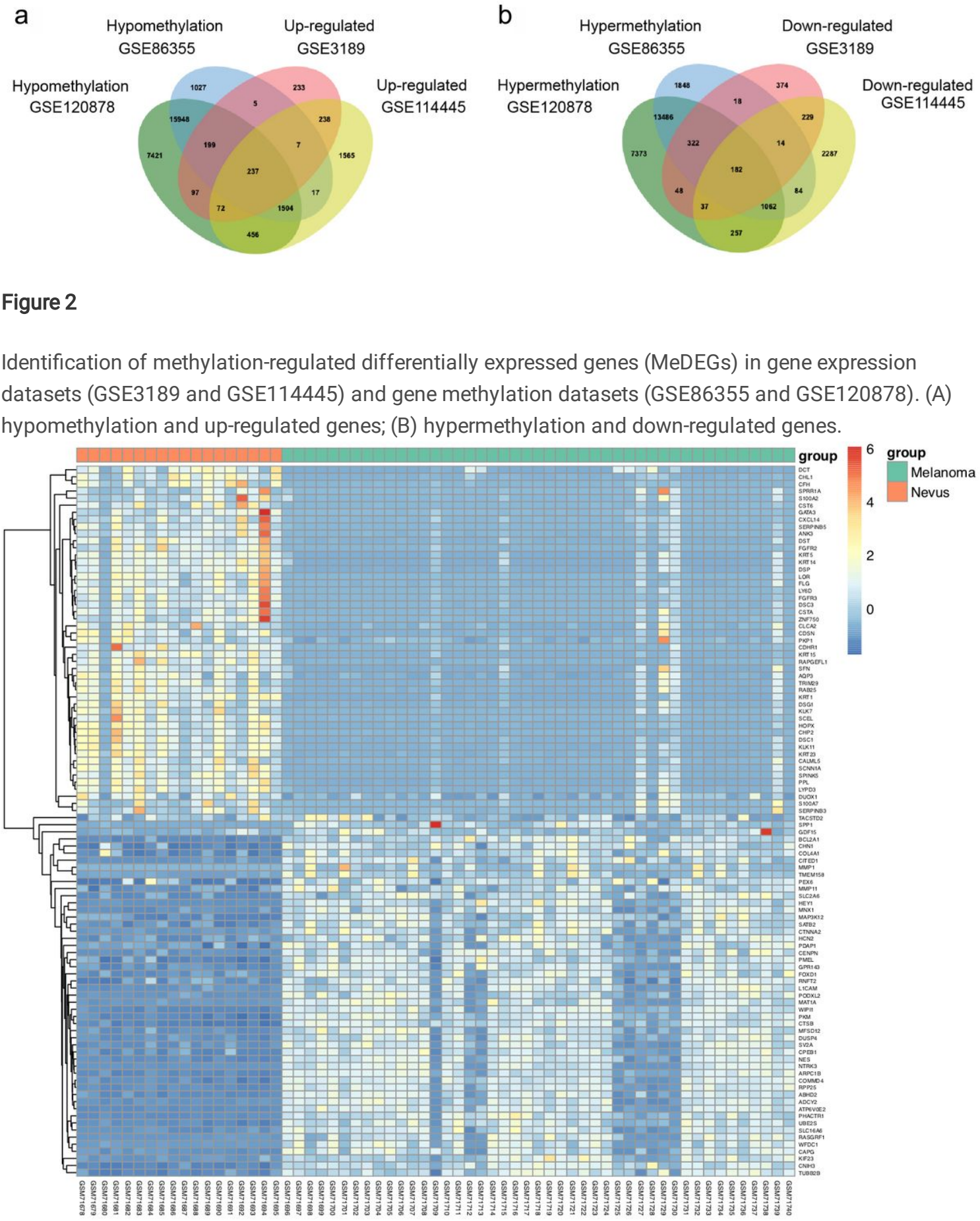
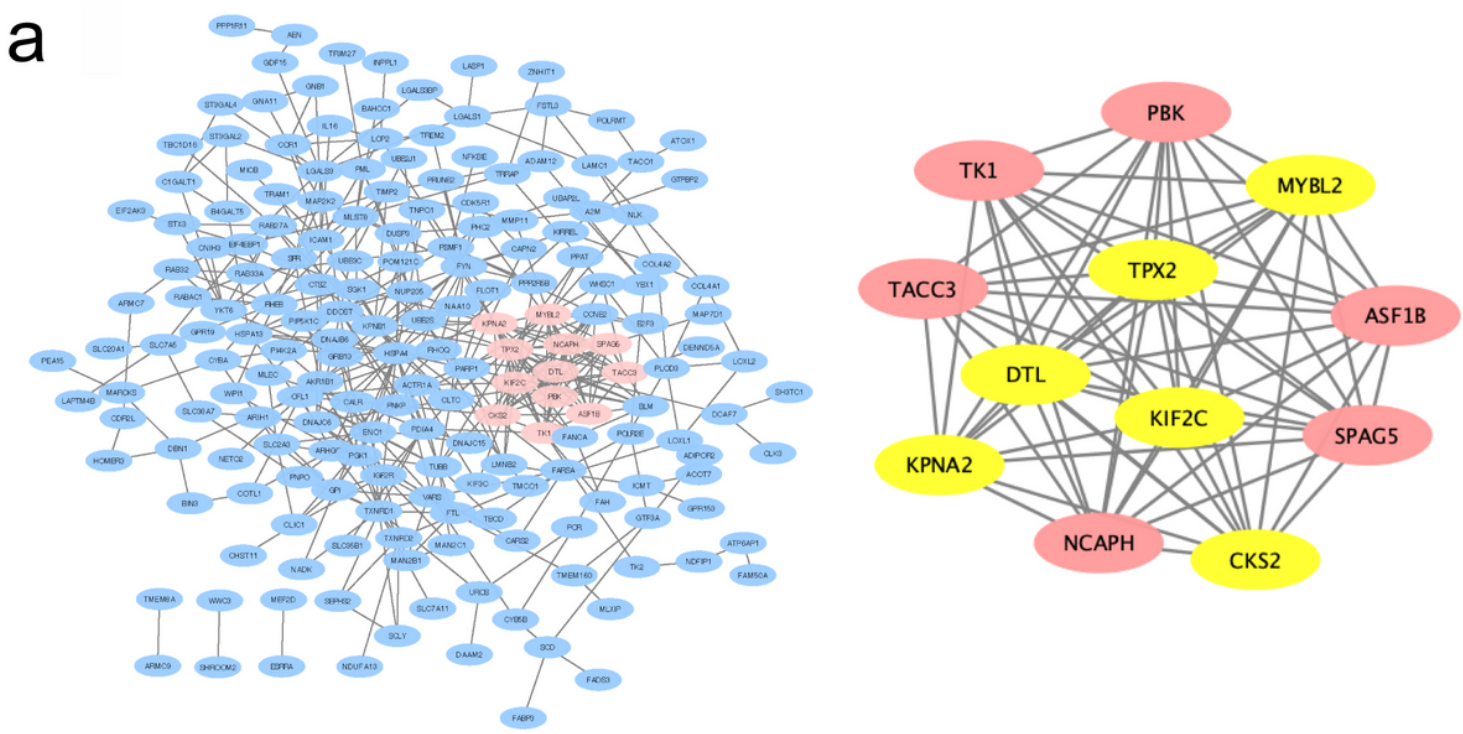


Figure 2

Identification of methylation-regulated differentially expressed genes (MeDEGs) in gene expression datasets (GSE3189 and GSE114445) and gene methylation datasets (GSE86355 and GSE120878). (A) hypomethylation and up-regulated genes; (B) hypermethylation and down-regulated genes.

Figure 3

Representative heat map of the top 100 differentially expressed genes in dataset GSE3189 (50 up-regulated genes and 50 down-regulated genes). Red: up-regulation; blue: down-regulation



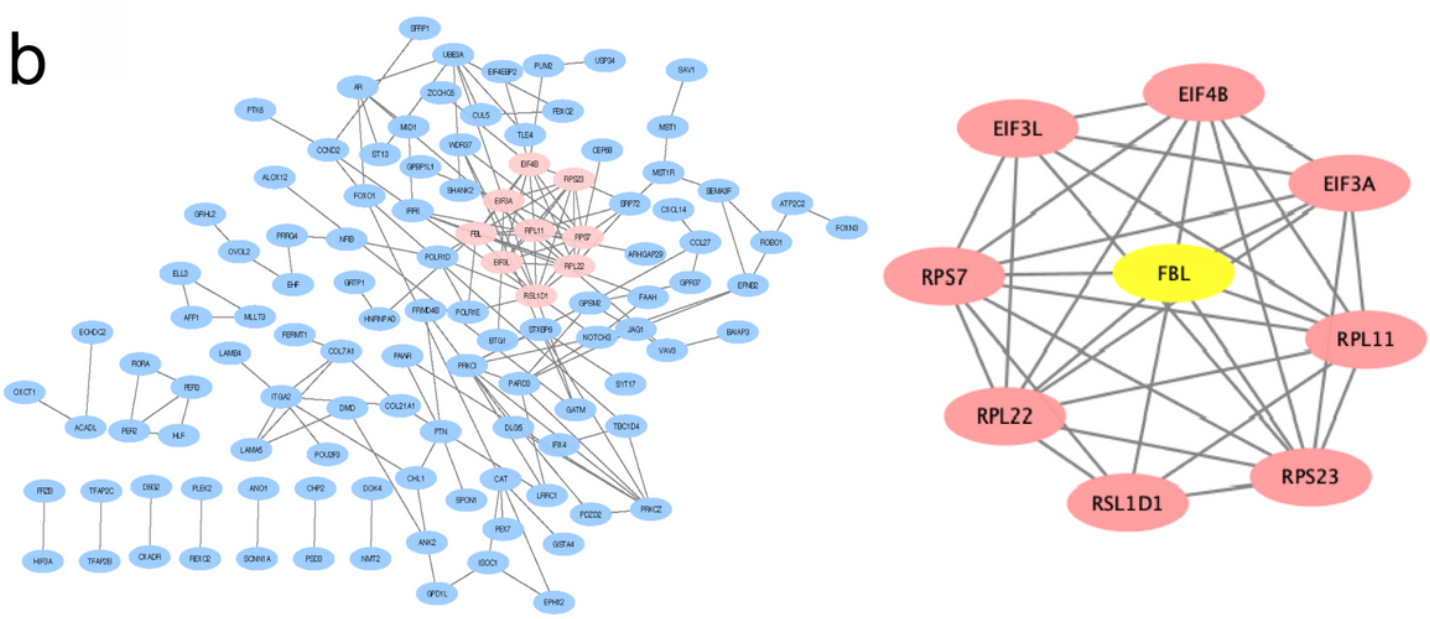


Figure 4

PPI network and most related modules of methylation-regulated differentially expressed genes. (A) Hypomethylated upregulated genes. (B) Hypermethylated downregulated genes.

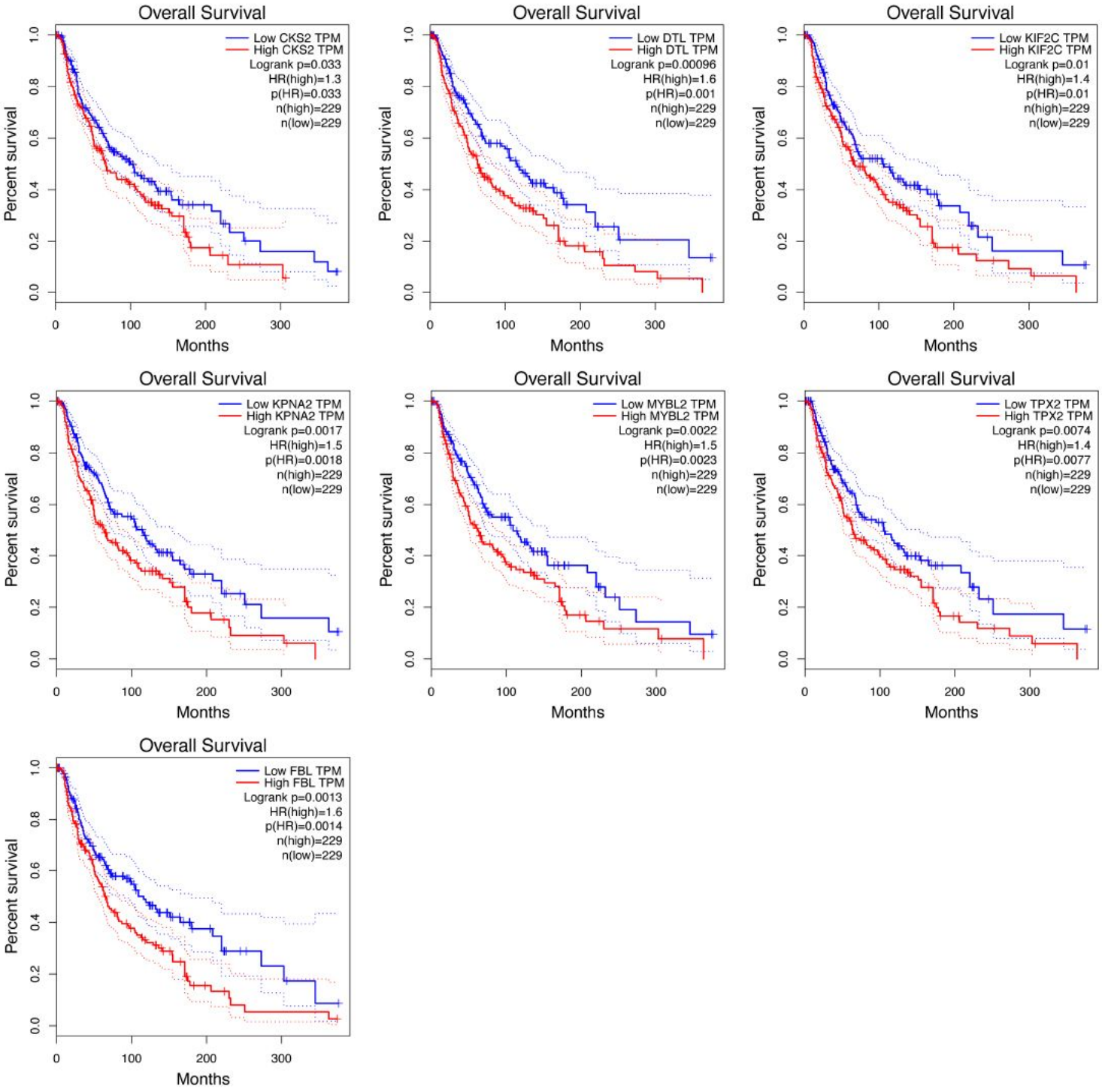


Figure 5

Survival analysis of the hub genes was performed using Kaplan-Meier curve. Each elevated expression in seven hub genes showed markedly significant worse OS in melanoma samples (P<0.05).

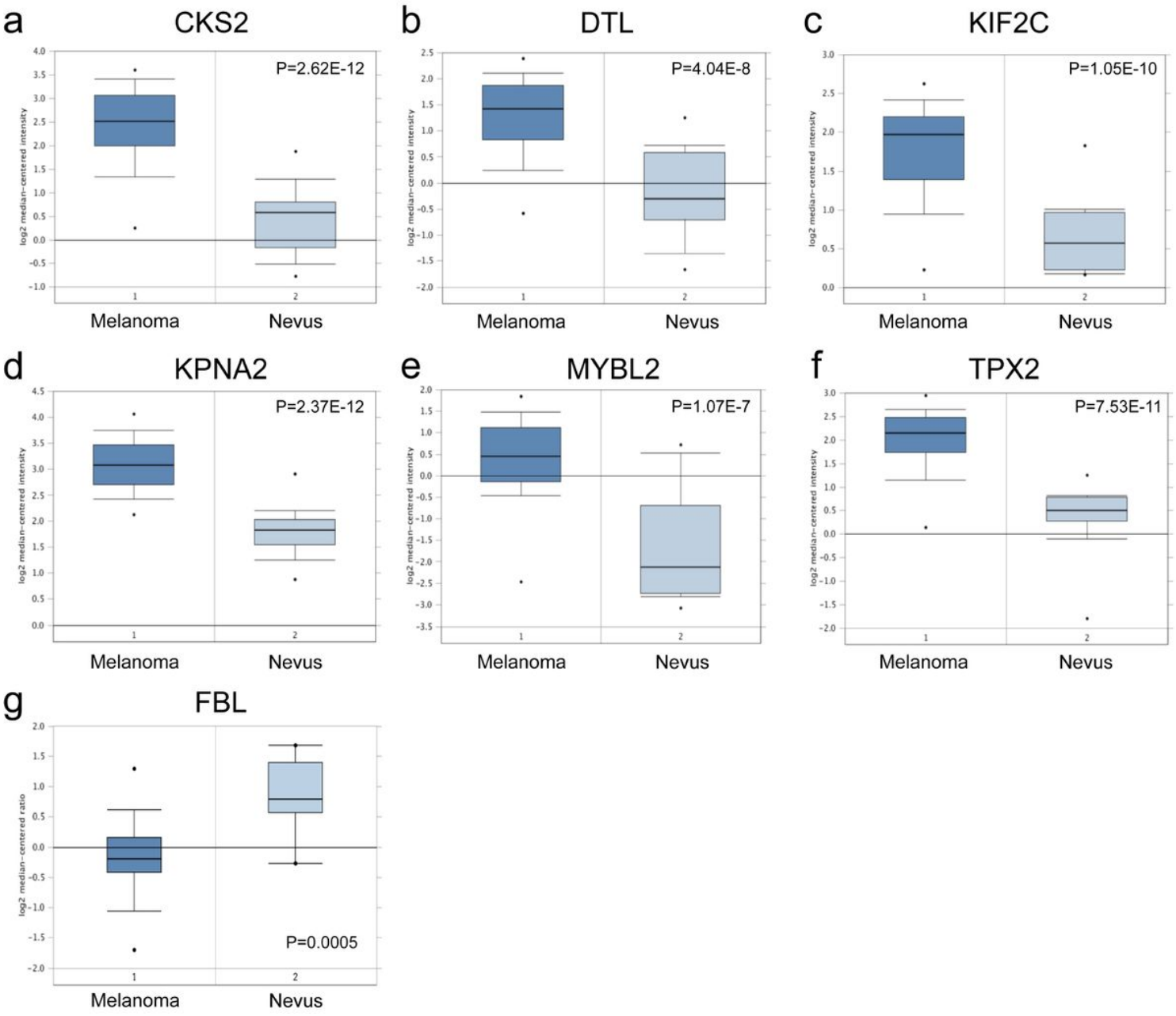


Figure 6

Validation of the expression of hub genes in Oncomine database. The expression level of CKS2, DTL, KIF2C, KPNA2, MYBL2, TPX2 and FBL were detected in Oncomine database.

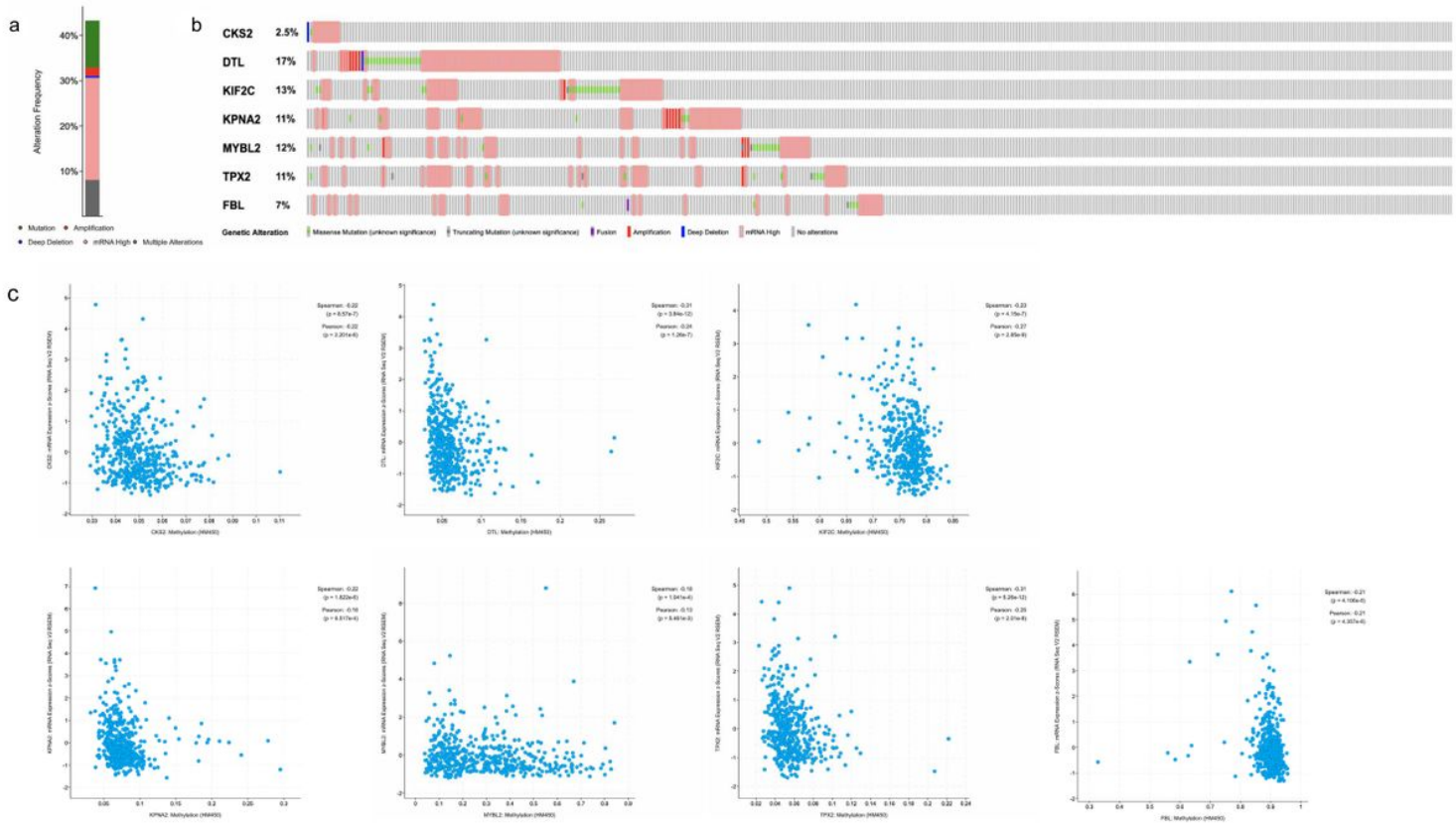


Figure 7

Genetic alteration of seven hub genes and the relationship between mRNA expression and DNA methylation in the TCGA SKCM study using the cBioPortal database. (A) Alteration frequency of hub genes. (B)A visual summary of alteration based on a query of seven hub genes, which was altered in 192 (43.24%) of 444 sequenced cases/patients. (C) The relationship between mRNA expression and DNA methylation in the seven hub genes.

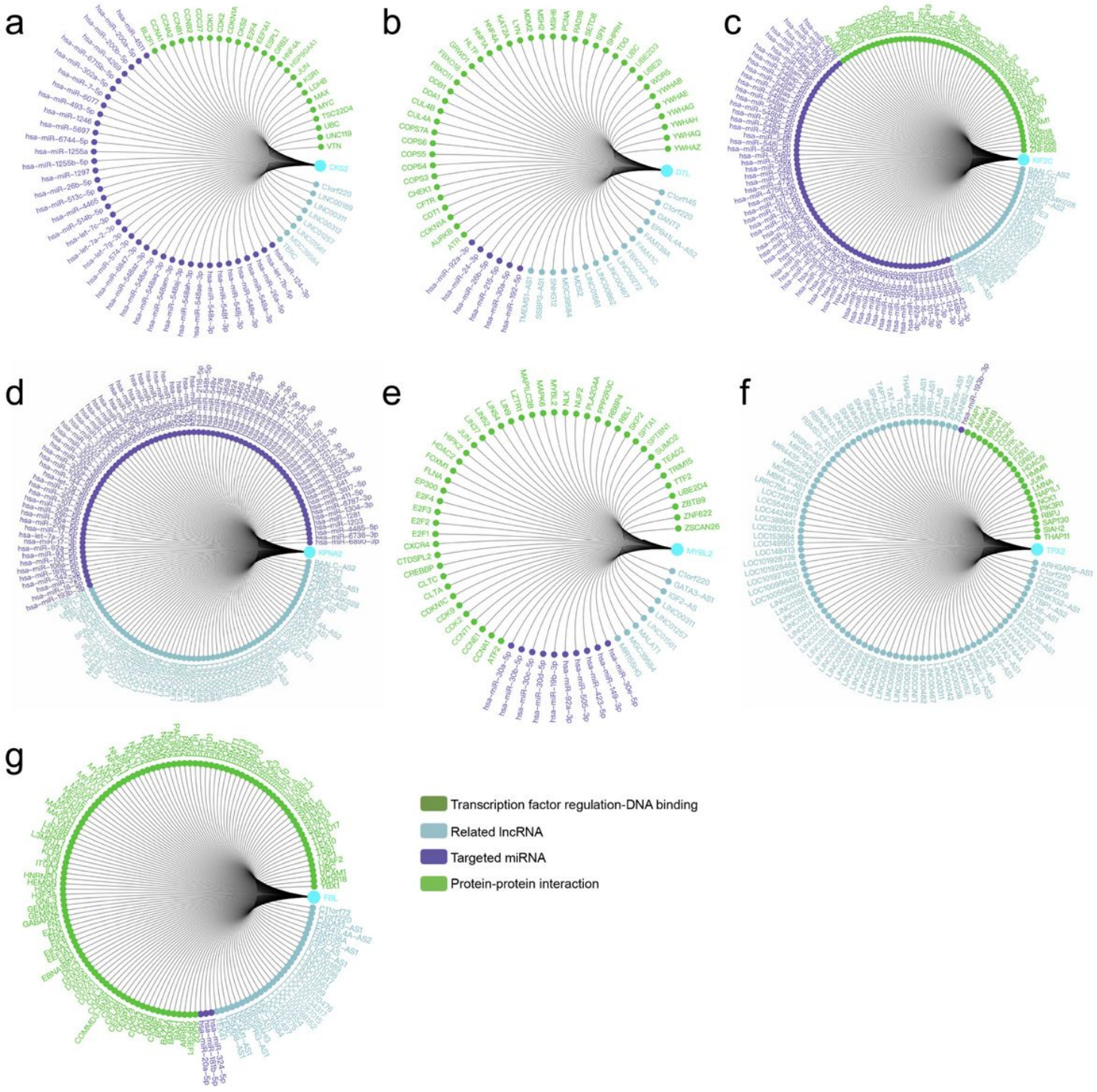


Figure 8

Transcription factor regulation network was constructed in CKS2, DTL, KIF2C, KPNA2, MYBL2, TPX2 and FBL. Significant nodes were marked in different colors in line with hub genes (Transcription factor regulation-DNA binding, Related lncRNA, Targeted miRNA and Protein-protein interaction).

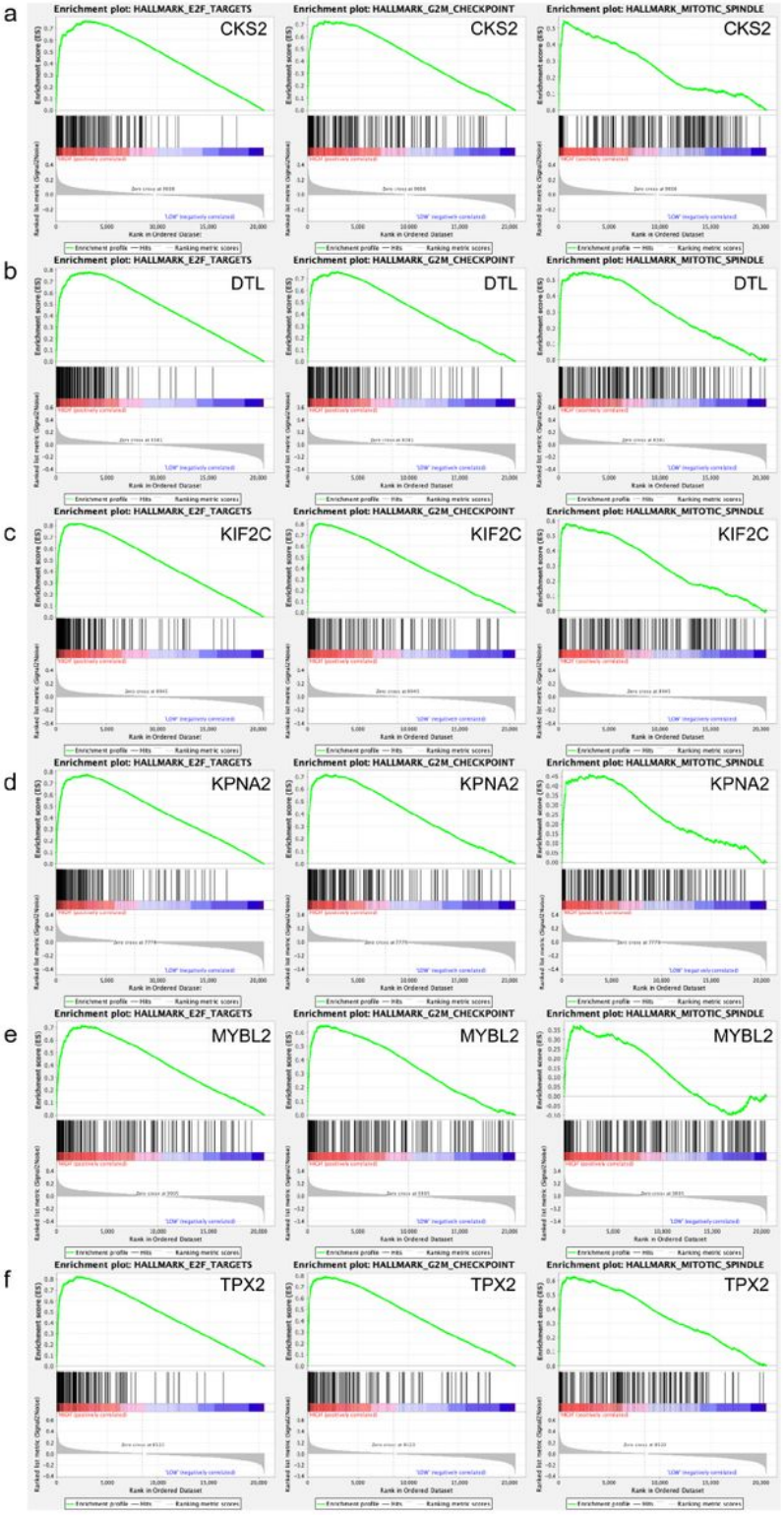


Figure 9

A total of 100 significant genes were obtained from GSEA with positive and negative correlation. GSEA was used to perform hallmark analyses in CKS2, DTL, KIF2C, KPNA2, MYBL2 and TPX2, respectively. The most involved significant pathways included E2F targets, G2M checkpoint and mitotic spindle.